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United States
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**Rocky Mountain
Forest and Range
Experiment Station**

Fort Collins,
Colorado 80526

**General Technical
Report RM-200**

TARGET SEEDLING SYMPOSIUM:

Proceedings, Combined Meeting of the Western Forest Nursery Associations

AUGUST 13-17, 1990
ROSEBURG, OREGON

WESTERN FOREST NURSERY COUNCIL
TARGET SEEDLINGS

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Abstract

This publication, a compilation of 28 technical articles on various aspects of forest nursery management in western North America, consists of two sections. The first 10 papers comprise the Target Seedling Symposium, and discuss the latest methods of describing and measuring the ideal seedling for reforestation purposes. Morphological characteristics such as height, diameter, stocktype, root system size, and mycorrhizae are covered. Considerable attention is paid to physiological tests such as root growth potential, hydraulic conductivity, cold-hardiness, mineral nutrition, seedling moisture status, and mitotic index. Chlorophyll fluorescence (Fvar), stress-induced volatile emissions (SIVE), and electrolyte conductivity (EC) are also discussed. The remaining papers deal with operational aspects of growing forest tree seedlings in bareroot or container nurseries.

Note

The Target Seedling Symposium papers in the first part of this publication have been refereed (reviewed by subject experts), and reviewed by the Symposium editors as well as a contract editor for the Rocky Mountain Forest and Range Experiment Station. The general nursery papers, found in the second part of this publication, were submitted camera-ready by the authors and, as such, received no review or editing. Consequently, you may find some typographical errors and slight differences in format. The views expressed in each paper are those of the author and not necessarily those of the sponsoring organizations or the USDA Forest Service. Trade names are used for the information and convenience of the reader, and do not imply endorsement or preferential treatment by the sponsoring organizations or the USDA Forest Service.

USDA Forest Service
General Technical Report RM-200

December, 1990

Target Seedling Symposium: Proceedings, Combined Meeting of the Western Forest Nursery Associations

August 13-17, 1990
Roseburg, Oregon

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Preface

Foresters have complained for years about poor seedling survival and growth, often with little understanding of why a specific reforestation effort failed. In some cases it was stock of inherently low quality due to poor nursery cultural practices, or seedling storage and handling conditions. In other cases the stock was in top notch shape, but inappropriate for specific site conditions, such as dense competing vegetation, early fall frosts, or high soil temperatures. Sometimes it was all of the above! In trying to solve this problem we too often tried to compartimentalize it and fix each piece . . . one at a time, often unsuccessfully.

The development and articulation of what is called the "Target Seedling Concept" is an effort to incorporate all the pieces into a whole that works. The Target Seedling Concept does include specific quality characteristics as targets for seedlings as they leave the nursery. But equally, it incorporates the specific physiology and morphology targets of the seedling geared to success in reforestation at a specific site. This general idea has been around for some time, but the scientific concepts and the practice have not been clearly articulated until now. The objective of the concept is to improve the overall quality of planted stock by providing qualitative measures (targets) of seedling characteristics which seedling growers and users alike can use to optimize success in reforestation.

The purpose of this symposium was to provide a sound basis for improving the field performance of tree seedlings. The goal was to equip the symposium participants with enough information, both theoretical and applied, that they can effectively use both the literature and specialists in improving the quality of seedlings intended for use at specific sites, thereby enhancing reforestation success.

With increasing costs in seedling production, site preparation, planting and early stand tending, it is important that we increase the efficiency (i.e. minimize the cost per unit of success) of the reforestation process. Planting seedlings of optimum quality for the site is one important factor. Having site specific quantifiable criteria of seedling quality will help us reach that goal.

Oregon State University has three primary functions: Education, Research, and Public Service. All three of these functions are apparent in this Symposium and Proceedings. They educate and extend public service and, in the process, the results of research are presented and made useful to practitioners at all levels. Robin Rose has assembled the best talents in this field for this purpose.

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Target Seedling Symposium

Chapter 1
The Target Seedling Concept

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ABSTRACT

The target seedling concept means to target specific physiological and morphological seedling characteristics that can be quantitatively linked with reforestation success. For decades foresters have relied on the stocktype designation, height, and caliper to grade seedlings. Nursery technology has advanced to the point where it is possible to achieve greater predictability in how seedlings will perform after outplanting. This paper highlights the concept of target seedlings and their importance to reforestation.

1.1 Introduction

A target seedling embodies those structural and physiological traits that can be quantitatively linked to successful reforestation. For many years reforestation specialists have searched for the characteristics that increase seedling survival and growth after outplanting. Only within the past three decades have they realized that height and diameter are not the only seedling traits affecting field performance. The target seedling concept is based on the premise that numerous seedling traits must work together to produce the desired field response. This paper highlights the concept of target seedlings and their importance to reforestation.

1.2 Development of the Concept

Technological advances in crop management and increased knowledge of seedling establishment have improved nursery crop quality. Now reforestation specialists realize that cultural practices in the nursery affect how well seedlings perform in the field. For example, undercutting and wrenching can have the dramatic effect of increasing root system size, which has long been linked to improved survival. Top clipping can improve field survival of excessively tall seedlings by lowering the shoot/root ratio. Altering fertilizer and irrigation schedules to encourage bud set and induce dormancy can greatly improve frost hardiness in the fall, winter storability, and stress resistance during and following planting. Currently, many nursery personnel emphasize culling standards strongly weighted toward height and diameter, because these are easily judged in the packing shed and are broadly correlated with other factors of seedling quality. Attention is often on maximizing the number of seedlings that can be shipped because they exceed the culling standards rather than on maximizing the number of seedlings that will survive and grow well.

Target seedlings go a step beyond. The standard for target seedlings is achieved by supplementing culling standards with information on such physiological and morphological characteristics as root volume, plant moisture stress, and frost hardiness. Other targeted traits include the presence of secondary needles and a firm bud, as well as presence of the proper nutrient levels and dormancy characteristics. Knowledge of these traits is used to improve the cultural techniques that tailor seedlings in the nursery.

Several years ago, Weyerhaeuser Company defined a target seedling for its southern pine operations by asking regeneration foresters to observe the morphology of the seedlings that consistently survived and grew well. Over a two-year period, foresters from each operating region described their target seedlings after June estimates of survival and at the end of the first year after planting. The results are shown in Figure 1.1. Weyerhaeuser has since

TARGET SEEDLING: LOBLOLLY PINE

Height 20-25 cm
Diameter >4mm
Mostly secondary needles
A single dominant stem
possessing a well-developed
terminal bud with resinous
bud scales
A minimum of six first-order
lateral roots, fiberous in
character and mycorrhizal
Root volume = >3.5 ml
High root growth potential

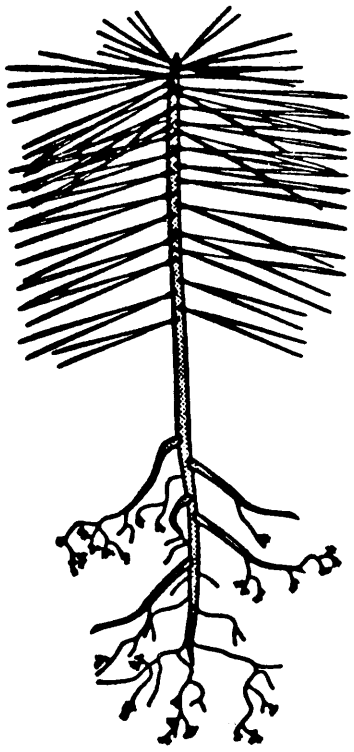

Figure 1.1—*Weyerhaeuser Company's loblolly pine target seedling.*

used this seedling not only as the goal for its cultural practices in the nursery, but also as a standard for rating its nursery crops. There is, of course, no such thing as a universal target seedling; adoption of this concept can, however, lead to crops of higher quality, especially if targets are established both by talking to users and by conducting physiological tests to determine the effects of cultural practices.

How, then, are cultural practices in the nursery affected by knowledge of target seedling morphology? The morphological traits that describe seedlings have normal population distributions (Figure 1.2). Cultural practices shift the distributions to the right or the left of the mean. Some of the target morphological traits are specified as minimums, some as maximums, and some as ranges. These limits appear at different places in the population distributions, depending on management practices. Research can be conducted to develop cultural practices that shift the population distribution in the desired direction. For instance, at the cost of losing a well-developed bud, top clipping can shift the shoot/root ratios of individual seedlings downward, thus moving a poorly managed nursery crop's unfavorable 4:1 ratio to a favorable target range below 2:1. If, however, customers differ in the desired seedling morphologies, then separate areas of the nursery should be managed differently. Similarly, genetic families of seedlings often differ in their responses to cultural practices and should be grouped accordingly in the nursery's management areas. Mixed family seedlots are difficult to culture because they usually contain families with different cultural responses.

Cultural practices are thus governed by the target traits decided upon. Certain traits have proved more satisfactory than others as indicators of seedling quality.

1.3 Seedling Traits to Consider

It should be recalled that a key element in the target seedling concept is that many seedling traits operate together to produce the desired field response. Thus, each of these traits affects many others.

1.3.1 Height

The greater the height of a seedling, the greater the leaf area available for photosynthesis and transpiration and the greater the seedling's weight and bulk. Greater weight and bulk, of course, decrease the number of seedlings that can be carried by an individual during planting. Height affects the shoot/root ratio of seedlings. The limiting factor in setting a practical height is actually the amount of root that can be planted properly.

1.3.2 Diameter

Diameter is closely related to seedling vigor, partly because average diameter of a seedling population at any one time is correlated with the average size of its root system. Furthermore, stems with larger diameters tend to have larger buds (unless they have been top-pruned). Such buds contain larger numbers of pre-formed leaf primordia that will elongate to become the first flush of growth after planting. Seedlings with larger diameters also have larger xylem cross-sectional areas for water transport, although during establishment the size of the root system is the limiting factor for this process (Carlson 1986).

1.3.3 Size of root system

In addition to increasing the potential for water uptake, larger root systems within a single genetic source also have a higher root growth potential. The size of a root system can also affect the rate of transpiration and gas exchange. Small-rooted seedlings are water-stressed because not enough water is absorbed by the roots to balance transpiration losses from the needles. If this condition is chronic, then currently available photosynthate can become the limiting factor for root growth. High root volume has been shown to improve growth after planting (Rose et al., in review).

1.3.4 Cold hardiness

Nursery managers have long known that a seedling's dormancy status and cold hardiness affect when it should be lifted and handled (Lavender 1984). Changes in such phenological traits as date of bud set, bud size, needle color, and degree of root suberization are now being used to

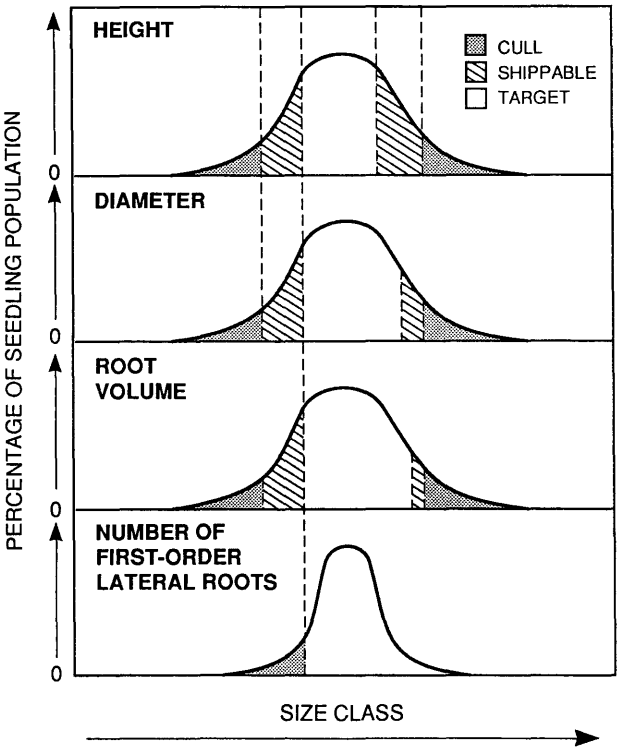

Figure 1.2—*Normal population distributions for several morphological traits of tree seedlings.*

estimate the dormancy status of seedlings prior to lifting them in the fall and spring for transplanting or outplanting. Unlike morphological measures, however, dormancy and cold hardiness have not often been considered as operationally useful target characteristics.

By putting seedlings through a pre-set freezing cycle, one can quantify their LT_{50}—the lethal temperature at which 50 percent of them sustain some sort of bud, cambium, or needle damage. It is thus possible to determine at any particular time the cold hardiness of seedlings and, therefore, when to lift and store them. The targeted LT_{50} depends on the intended use of the seedlings and the species. A low LT_{50} is less important for seedlings about to be transplanted in the fall than for those going into long-term freezer storage in late winter.

1.3.5 Mitotic index

Mitotic Index or MI (number of dividing cells/total number of cells) is used by researchers to investigate bud dormancy (Carlson et al. 1980). It has also been used successfully on roots (Dunsworth and Kumi 1982). A squash mount of a bud or root observed through a microscope at 400X magnification allows the number of dividing cells to be counted. MI tends to decrease rapidly in the fall in some species. In Douglas-fir, it remains at zero from early December until mid-March—the period when transplanting is most successful. It has potential as a target characteristic, although first the effects of cultural practices on MI, and of various MI values on seedling quality, must be determined.

1.3.6 Days to bud break

Terminal and lateral buds of seedlings are now viewed as potentially useful indicators of whether a seedling has had its chilling requirement met. Seedlings require chilling to break dormancy in the spring. The number of days before terminal and lateral buds break is being used successfully to target the best time to lift seedlings (Ritchie 1983).

1.3.7 Plant moisture stress

Plant moisture stress is used as a target characteristic. As moisture stress in a seedling increases, there is a corresponding degradation of the photosynthesis mechanism and an impairment of future growth. Most nurseries try to lift their seedlings when the water potential of stems, branches, or needles is below -10 bars. It is equally important to plant seedlings when stress levels are low.

1.4 Setting Up a Target System

Making the concept of a target seedling work requires considerable attention to detail from seed selection and sowing all the way through to planting. To achieve the desired end product it is a matter of applying cultural treatments to the seedlings and recording the responses. All of this is done in the context of the growing cycle of the seedlings.

A workable system that allows for the keeping of detailed records year to year requires effort and expense to set up and maintain, but this is outweighed by the rewards that accrue. The example below comes from a large white spruce containerized nursery and shows how the necessary information to track seedling growth relative to cultural practices can be recorded and used. Only a small portion of the information is presented here.

Table 1.1 shows the Growth Component Sheets for the month of March, which covers growing weeks 6, 7, 8, and 9. The management practice for each growth component (e.g., growth stage of seedlings, light, temperature) are described. Separate detail sheets are also used for the fertilizer schedule and growth measurements.

Examples of the Detail Sheets are shown in Tables 1.2 and 1.3 for the month of April and cover weeks 10, 11, 12, and 13. The Fertilizer Schedule contains the information on the fertilizer formulations, target fertilizer solution versus actual, and target versus actual values for needle nutrients. The Growth Measurements sheet compares target versus actual values for 18 physiological and morphological parameters.

Figures 1.3 and 1.4 show how effectively all of this information can be integrated and used operationally with regard to height and plant dry weight. Ultimately, all measurable parameters can be tracked and, when looked at together, give a total picture of the target seedling. In this kind of system it is possible to seed the cultural practice work in relation to the growth of the seedlings. If, at any time, more information is needed, it is a simple process to add another growth component to track and, if necessary, add a new detail sheet for it. This system lends itself readily to computer spreadsheets and data analyses.

1.5 Who Sets the Target?

Different targets are established for different reasons—as a public service, for profit through the sale of seedlings, or for profit at final harvest. Various cultural practices are applied to achieve the desired target. As these practices can result in a wide range of seedling morphologies and physiological conditions, nurseries must decide what practices to employ to achieve their goal. Ideally, no matter what goal, every nursery should be growing seedlings which survive and grow well after outplanting. The proper place to rate the quality of cultured seedlings is in the forest plantation. High-quality seedlings survive well and become established rapidly enough to show substantial height growth the year of planting, and are thus enabled to express their full genetic potential. Definition of the target seedling should reside with the person who sets the

Table 1.1—*Example of growing regime for white spruce containerized seedlings showing growth component's criteria. Shows transition from juvenile development stage to acceleration growing period.*

[GRTHCOMP.1] GROWTH COMPONENTS	Timing Week Number 1 Weeks from Seeding (6)	2 (7)	3 (8)	4 (9)
	JUVENILE DEVELOPMENT (Juvenile Stage Ends at Approx. 42 Days	ACCELERATION GROWING PERIOD (Acceleration Stage Starts at Approx. 43 days)		
GROWTH STAGE Soil Medium	pH 5.0 to 5.5 Elec. Conductivity: 0 to 1200 µS/cm = Low 1201 to 2500 µS/cm = Normal 2501 to 3000 µS/cm = High 3001 to 4000 µS/cm = Excessive 4001 = Lethal - pH & EC levels are monitored starting 8th week and every week after until Sept.	- This phase aims at pushing seedling height, root collar diameter, root development and initiate stem lignification. - Rapid shoot elongation - Few if any of the seedlings will have a visible terminal bud - This growth stage ends when a acceptable percentage of seedlings reach 80% of the desired shoot target.		
GROWTH MEASUREMENTS (See Detail Sheet GM #)	Weekly growth measurements are started 8 weeks after sowing until Bud Set and biweekly thereafter plotted against previous growth curves.			
DAY TEMPERATURE Optimum Min. → Max.		21° C 17° to 25° C		
NIGHT TEMPERATURE Optimum Min. → Max.		16° C 12° to 20° C		
ENVIRONMENTAL CONTROLS		N/A		
RELATIVE HUMIDITY Optimum Min. → Max.		60% 50% to 70%		
LIGHT Natural Supplemental		Outside Conditions For photoperiod extension (as in juvenile stage): - Monitor light intensities in greenhouse to ensure 400 lux min. is being maintained - This lighting allows the seedling to take full advantage of normal daylight. Extends growing hours by approx. 2 hrs.		
FERTILIZER SCHEDULE (See Detail Sheet)		Again depending on growth of seedlings would in most cases occur around 7-8th wk Schedule 8 (ppm): N P K Iron Chelate 125 60 159 5.5 ppm (1 appl/wk for 6-7 wks)		

[____March_____]-11

[GRTHCOMP.2] GROWTH COMPONENTS	Timing Week Number 1 Weeks from Seeding (6)	2 (7)	3 (8)	4 (9)
	JUVENILE DEVELOPMENT (Juvenile Stage Ends at Approx. 42 Days)	ACCELERATION GROWING PERIOD (Acceleration Stage Starts at Approx. 43 days)		
IRRIGATION pH Electrical Conductivity	- Monitor and record irrigation applied in time and amounts.	As before in Juvenile Stage Plus; - General watering (approx. 1/2 hr) 24-48 hrs prior to scheduled fertilizer application - Flush out trays for 1 to 1 1/2 hrs every 3 wks to purge any salt buildup (flush thoroughly)		
CO_2 LEVELS		- CO_2 production reduced to 6 hrs (0600 to 1200 hrs) - Same ppm as Juvenile		
INSECT AND DISEASE CONTROL		Continue monitoring		
PESTICIDE APPLICATION		One Benlate preventative soil drench @ _____		
RECORD KEEPING	- Irrigation applied in (ml) greenhouses	- Monitor crop daily - Keep records updated: - fertilizer - growth measurements - irrigation - nutrient analysis - pH & EC - temps and RH - photoperiod		
OPERATIONS properly for photoperiod		- Routine maintenance and operational greenhouse checks - Checks on calibration of all grenhouse equipment, i.e., lights on timer are working		

Table 1.2—*Example of fertilizer schedule detail sheet showing target and actual amounts found in various solutions and needles.*

[FERTSCH.3]
FERTILIZER SCHEDULE
Detail Sheet FS #

[April]-III

		FORMULATION & TYPE (Percentage %)			RAW WATER Range ppm	RAW WATER Actual	STOCK SOLUTION Target ppm	STOCK SOLUTION Actual	GH FERTIGATION Target ppm	GH FERTIGATION Actual	NEEDLE NUTRIENTS Target % and ppm	NEEDLE NUTRIENTS Actual
NITROGEN	N	20	10	0	< 6	1.79	25,000	19,820.50	125	148.85	1.50/2.50%	2.57%
Nitrate	N_3											
Ammonium	N_{HX}											
PHOSPHORUS	P	0	52	0	< 6	0.52	12,000	9,670.00	60	52.40	0.18/0.32%	0.35%
Phosphate	H_2PO_4											
POTASSIUM	K	25	10	62	< 6	3.20	31,800	27,650.00	159	153.50	0.45/0.80%	1.30%
CALCIUM	Ca				< 121	43.55		52.00		52.65	0.10/0.20%	0.40%
MAGNESIUM	Mg				< 26	14.40		49.05		15.90	0.15/0.40%	0.16%
SULFUR	S				< 201	45.10		2,175.00		205.00		
Sulfate	SO_4^2											
IRON	Fe	Chelated Iron			< 6	0.08	1,100	1,230.00	5.5	6.85	60/200 ppm	
ZINC	Zn				< 0.6	0.12		23.00		0.64	30/150 ppm	
COPPER	Cu				< 0.3	1.08		12.20		0.31	4/ 20 ppm	
BORON	B				< 0.6	0.01		23.40		0.09	20/100 ppm	
MOLYBDENUM	Mo				< 0.03	--		4.56		0.02	0.25/5.00 ppm	
OTHER: CHLORINE	Cl				< 101	--		11,400.00		60.50	--	
MANGANESE	Mn				< 1.0	0.01		33.85		0.33	100/250 ppm	
pH					3.0-8.0 (0 pt. 5.5)	6.72						
ELECTRICAL CONDUCTIVITY					< 750 µS	385 µS						

APPLICATION PROCEDURES AND TIMING

20-0-25 = 4.5 kg
10-52-10 = 24 kg
0-0-62 = 0.6 kg
Chelated Iron = 0.5 kg
Mixed in 10 gals.

Table 1.3—*Example of growth measurements detail sheet showing target and actual values for the seedlings.*

[GRTHMEAS.4]
GROWTH MEASUREMENTS

[April]

Week Number	1		2		3		4	
Weeks from Seeding	(10)		(11)		(12)		(13)	
	TARGET	**ACTUAL (1989)**	**TARGET**	**ACTUAL (1989)**	**TARGET**	**ACTUAL (1989)**	**TARGET**	**ACTUAL (1989)**
GERMINATION								
Seedling Emerged								
Cavities Filled & %								
WEIGHT (cm)	5.00	5.17	6.50	6.50	7.50	7.38	9.00	8.86
ROOT COLLAR DIAMETER (mm)	0.95	0.77	1.25	0.87	1.45	1.19	1.55	1.22
Sturdiness Quotient	(5.26)	(6.71)	(7.47)	(7.47)	(5.17)	(6.20)	(5.81)	(7.26)
ROOT INTENSITY (mg/cc)	0.46	0.23	0.62	0.30	0.77	0.42	0.92	0.59
ROOT VOLUME (cc)	--	--	--	--	--	--	--	--
SHOOT DRY WEIGHT (mg)	125.00	74.19	175.00	103.04	225.00	146.91	275.00	196.80
ROOT DRY WEIGHT (mg)	30.00	15.24	40.00	19.60	50.00	27.57	60.00	38.31
PLANT DRY WEIGHT (mg)	155.00	89.43	215.00	122.64	275.00	174.48	335.00	235.11
Shoot/Root Ratio	(4.17)	(4.87)	(4.38)	(5.26)	(4.50)	(5.33)	(4.58)	(5.14)
TERMINAL BUD DEVELOPMENT								
Bud Elongating %								
Bud Initiation %								
Bud Set %								
Bud Burst %								
Bud Reflush %								
(Lammas Growth)								
P.M.S. (bars)								
(Drought Stressing)								
OPTIONAL								
Frost Hardiness Tests								
Root Growth Capacity								
Days to Budbreak								
(Freezer stored seedlings only)								

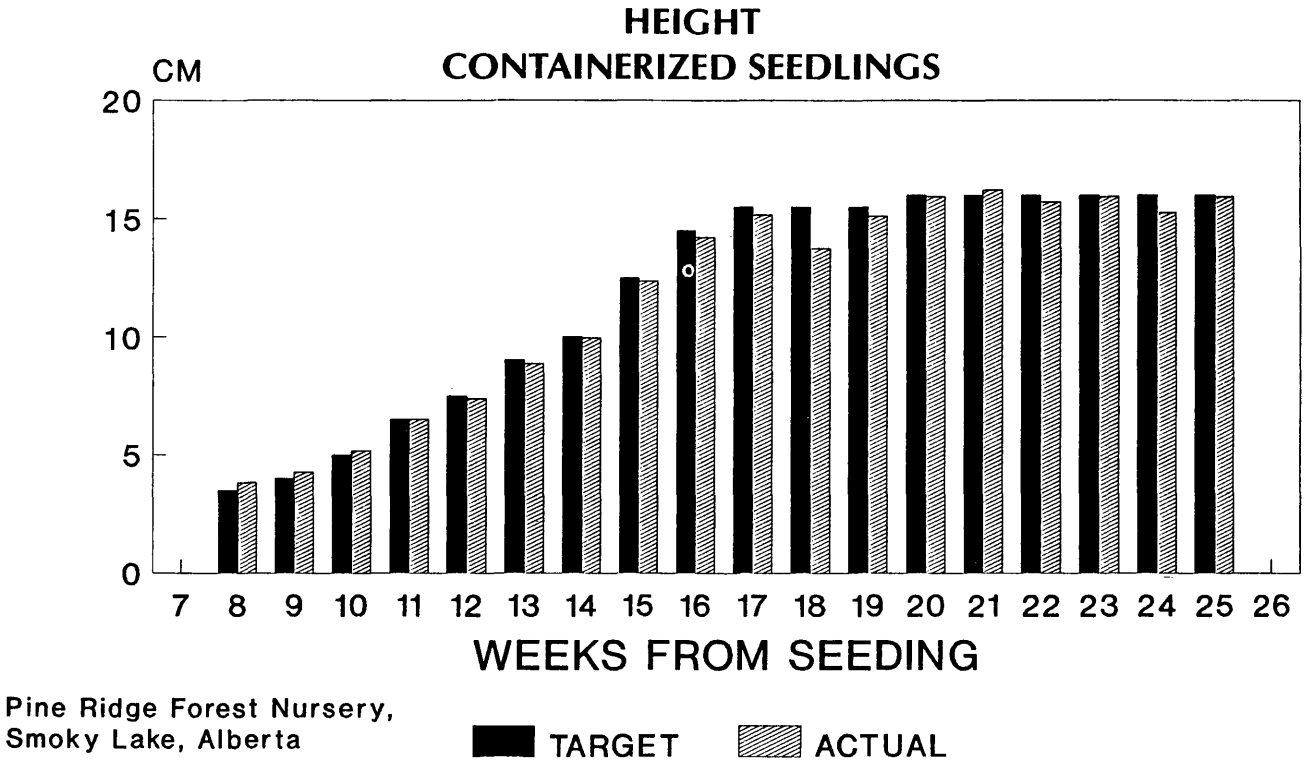

Figure 1.3—*Examples of target versus actual values for height and plant dry weight of white spruce containerized seedlings from week 8 to week 25.*

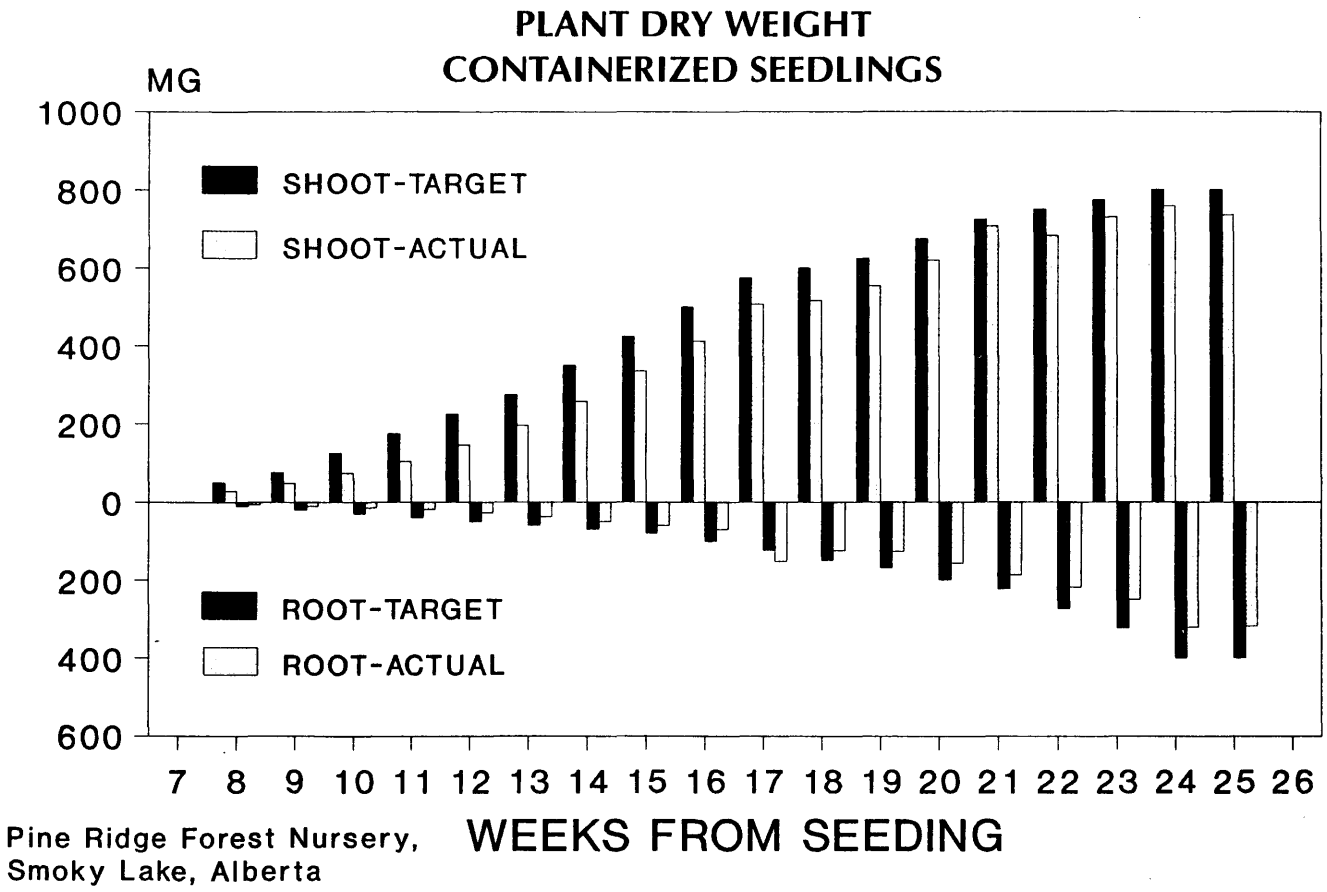

Figure 1.4—*Example of target versus actual values for plant dry weight of white spruce containerized seedlings from week 8 to week 25.*

standard for plantation performance. Target standards should be worked out in close cooperation with the nursery manager to ensure that observations of field performance result in a steady flow of improvements in quality of seedlings coming from the nursery.

1.6 The Future

The chapters that follow contain a great deal of information on some old and some very new seedling quality assessment techniques available to us. While height and caliper have served us well for decades, it is clear that we must continue to learn all we can about predicting future performance. It is no longer acceptable to look at morphology at lifting and expect that to always be a safe indicator of field performance.

Height, caliper, frost hardiness (LT_{50}), plant moisture stress (PMS), and root growth potential (RGP) will serve most grower and user needs (Chapters 3, 4, 5, 7, 8, 9). Target heights and calipers have been with us for a long time. In areas where the equipment is available, LT_{50} targets have been set to ensure that the seedlings are dormant and stress resistant enough to lift. Plant moisture stress is used extensively to monitor irrigation to achieve bud set without damaging seedling physiology and to control stress at lifting. Root growth potential has been used to target the best time to lift and plant seedlings.

Even with all that is known about the parameters that can be measured in seedlings, there is still very little that has been learned about what constitutes the best combination of traits. Parameters like root volume, fibrosity, shoot/root ratio, height/caliper ratio, nutrient levels and ratios, and others play a vital role in the survival and growth of a seedling after outplanting. Implicit in the target seedling concept is establishing minimum, maximum, and standard values for as many seedling parameters as possible and learning how to integrate and use the information in order to achieve a crop of target seedlings.

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Target Seedling Symposium

Chapter 2
Target Seedling Specifications: Are Stocktype Designations Useful?

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ABSTRACT

A stocktype designation identifies a seedling's age and the basic method by which it was produced. The designation inexactly implies seedlings' relative size and conveys very little information about their critically important physiological condition. Although designations for the primary types of seedlings have not changed much over the years, size and quality of most types have been improved significantly. Comparisons of field performance in the Pacific Northwest indicate that survival is often not greatly different whether a seedling was produced in a container, in a bareroot seedbed, or had been transplanted. On the other hand, seedling height after three to five years in the field tends to be somewhat greater for stocktypes that usually consist of larger seedlings; increased growth probably relates more to initial seedling size than to seedling age and production method. For most sites and situations, foresters should prescribe seedlings of the size and physiological condition that are most appropriate ecologically and economically. Nursery managers should use the cultural and economic options available to them to meet those client needs. Choosing the type of seedling to produce is just one of the decisions to be made in accomplishing that goal.

2.1 Introduction

A seedling's "stocktype" tells us its age and by what general method it was produced (e.g., bareroot, container-grown, transplant, or a combination of methods). Stocktype designation, per se, relates only inexactly to seedling size and even less to physiological condition. Production of different stocktypes, however, was the first attempt at growing seedlings targeted for specific sites. Foresters of earlier generations knew the species and size of seedlings they wanted for the sites on which they were planting them.

Furthermore, they knew from experience approximately what size of seedling they would get by specifying species and stocktype. Times have changed. More stocktypes are available; seedling sizes for a given type have increased markedly as technology has improved; and economic realities demand refinements to achieve even better seedling performance than obtained in the past. Most of the other papers in this symposium indicate, at least by inference, that specifying stocktype is not sufficient to target seedlings for specific sites, and I agree. Furthermore, I believe that results of empirical field comparisons of stocktypes are primarily applicable to the particular combinations of nurseries, stock, and sites tested.

I believe, however, that stocktype designation is useful—it is a good communication tool; the basic types have some general characteristics that affect use and performance; and comparisons in the field are useful for specific, localized situations.

2.2 Terminology

Development of new stocktypes in recent years has resulted in confusing terminology. Thus, for this paper, I will define the basic terms that I will be using:

Seedling—a very young tree regardless of where and how it is growing.

Nursery stock and planting stock—synonymous terms denoting seedlings being grown or having been grown for outplanting on forest sites.

Stocktype—a class of nursery stock produced by one or more of the basic production methods—bareroot, container, transplant, and so forth—for a particular length of time. Special treatments used in production are not considered part of the designation. For example, seedlings inoculated with mycorrhizal fungi are not a separate stocktype. Nor is species considered part of the designation.

Bareroot seedlings—seedlings grown in soil in traditional outdoor nursery beds and lifted from the beds for packing and shipping with their roots essentially bare of soil. I consider a transplant to be a type of bareroot seedling.

Container seedlings—those grown in individual pots and usually, but not necessarily, in greenhouse or shadehouse nurseries.

Plug seedlings—container seedlings that are extracted from their containers and planted with a plug of roots and potting mixture. Since this is, by far, the most common technique, the terms "container" and "plug" seedling are often used synonymously.

Transplants—seedlings that were started from seed in either a bed of soil or in some type of container and then transplanted into an outdoor bed for subsequent lifting as a bareroot plant.

Miniplugs—seedlings grown in very small containers (about one cubic-inch volume) of several configurations (cubical or tubular). They are usually grown in the container for only three to six months and are produced solely for transplanting into a nursery bed and later lifting as bareroot plants. Thus, they warrant a designation separate from standard plugs.

Stocktype is usually expressed as a two-part code, with the parts separated by a plus sign (e.g., 1+0, 2+0, P+1, MP+1) or a dash (e.g., 1-0, 2-0, P-1 MP-1). If the first part is a digit, the stock was grown in a traditional outdoor seedbed; the digit represents the number of years (i.e., growing seasons) it grew in the bed in which its seed was sown. The second digit represents years in a transplant bed. Thus, a tree to be outplanted as a two-year-old bareroot seedling directly from its original seedbed is termed a 2+0 seedling; a seedling transplanted for the second year is a 1+1. If the first part of the stocktype designation is a letter, the stock was started in a container. Since standard container seedlings are normally grown for one year (season), I designate them as P+0's (P for plug with a one year growing time understood). A seedling grown one year in a container and then put into a transplant bed for a second year is termed a P+1. Miniplug transplants are designated as MP+1.

One suggestion I have for the industry is not to devise a new stocktype designation for every variation of similar practices—at least not for industry-wide use. Detailed designations are fine for individual organizations, but a complicated system probably will not be widely accepted.

One proposal for designation by type of production, size, and intended season of planting was proposed at the Western Forest Nursery Meeting over 15 years ago (Nicholson 1974). It seemed like an efficient, useful system; but it has never been widely used.

Let's keep basic stocktype designation as a simple communication tool for all of us to use and readily understand.

2.3 Stocktype Characteristics and Uses

2.3.1 Common stocktypes

Stocktypes can be conveniently grouped into bareroot, container, combination, and minor types. The most commonly used stocktypes are relatively few in number, and their production and use tend to vary by region (Table 2.1).

Stocktypes also have some differences in basic characteristics that influence where they are used (Table 2.2). Although these differences relate to targeting in a general sense, I want to restate my introductory comment that stocktype does not define a target seedling with the accuracy and detail that current practices require.

Table 2.1—Common stocktypes.

STOCK TYPE	REGION(S) OF PRIMARY PRODUCTION AND USE
Bareroot	
1+0	temperate zones, mostly warmer parts
2+0	temperate zones, mostly cooler parts
3+0	temperate zones, mostly cooler parts
1+1	temperate zones, mostly cooler parts
2+1	temperate zones, mostly cooler parts
2+2	temperate zones, mostly cooler parts
Container	
P+0, large container	tropics
P+0, small container	temperate and boreal zones
Combination	
P+1	temperate zones, cooler parts
MP+1	temperate zones (exclusively for transplanting)

Table 2.2—Relative stocktype characteristics.

Stocktype	Bareroot	Size	Plantability	Cost
1+0	Yes	Small	Easy	Low
2+0	Yes	Average	Average	Average
1+1	Yes	Av. to Large	Av. to Difficult	High
2+1	Yes	Large	Difficult	High
P+0	No	Small	Easy	Av. to High
P+1	Yes	Large	Difficult	Highest

2.3.1.1 Bareroot stocktypes

Sizes of any plants are influenced strongly by the length of time and amount of space in which they grow. Limitations in a nursery are primarily economic—individual seedlings for the large-scale plantings characteristic of reforestation programs cannot be so large that nurseries do not have room to grow required numbers or that handling and planting are too costly for economic realities. The "reforestation culture" in much of the temperate world has been built around the production and planting of 1+0 seedlings in warm areas and 2+0 seedlings in relatively cool temperate regions. Resulting seedling sizes fit reasonably well with the logistic capabilities and economic realities of most reforestation programs. Furthermore, they have been reasonably good performers on typical reforestation sites. Other stocktypes are invariably spoken of in terms of how they compare with non-transplanted, bareroot seedlings.

The most obvious characteristic of bareroot stocktypes is their lack of root contact with the soil between the time they are lifted and planted. Such exposure makes it imperative that the seedlings be dormant when they are lifted from the seedbed, handled, stored, and planted. Succulent, growing tissues are easily killed by desiccation or damaged by mechanical forces and, thus, cannot withstand the exposure and handling that occur in normal operations.

Another characteristic of bareroot seedlings is that part of their root systems are cut off in the lifting process and may be further trimmed to facilitate planting. This mechanically increases the ratio of tops to roots when it would be better for survival if the ratio were decreased!

In the western United States, 2+0's are commonly grown at densities of 215 to 325 per square meter (20 to 30 per sq. ft.) and to sizes of 15 to 46 cm (6 to 18 in.) in height and about 4 to 7 millimeters (0.2-0.3 in.) in stem diameter at the root collar. Their root systems are usually trimmed in the nursery at 20 to 30 cm (8 to 12 in.) below the root collar to facilitate planting.

For harsh sites, foresters prefer using 1+1, 2+1, or even 2+2 transplants that have sturdier stems and more fibrous root systems than 2+0's. To produce these stocktypes, 1- or 2-year-old bareroot seedlings are lifted from their seedbeds and transplanted into beds at less dense spacing—commonly 130 to 170 per square meter (12 to 16 per sq. ft.). In addition to the morphological advantages of sturdier stems and more fibrous root systems, cull factors for transplants can be lower than for 2+0 seedlings grown at higher densities.

Thus, transplanting can make economic sense when using very costly seed, despite higher production costs. A trend towards production of larger stock was noted in the early '80s (Iverson 1984), and relatively large stock is common-

ly used now. Informal discussions with nursery managers and reforestation specialists in the Pacific Northwest indicate that the 1+1 transplant has been rapidly gaining favor.

In warmer parts of the temperate zones, where species such as loblolly pine (*Pinus taeda* L.) in the southern United States grow at faster rates than species in cooler regions, 1+0 seedlings are commonly planted. Seedling sizes approach or even exceed those of 2+0 seedlings grown in cooler climates. In cooler part of temperate zones, improved nursery practices in the past 15 to 20 years have led to increasing use of 1+0 stock of faster growing species—ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) grown in California and southwestern Oregon nurseries is a prime example. Sizes of this stock also approach or exceed that of 2+0 seedlings grown further north or in the interior West.

Use of 1+0 stock is attractive because of its short production cycle and lower costs. Thus, it has been tested and used even when the stock is smaller than average 2+0 seedlings (Jenkinson and Nelson 1983). Successful use of these smaller 1+0's is usually restricted to sites that are only low to moderately stressful or to situations where the stock can be given protection from environmental stresses of drought, competition, ravel, high radiation, animal damage, and so forth. As nursery technology keeps improving, the use of 1+0's will probably move northward as this stocktype more closely resembles current-day 2+0's and performs similarly.

2.3.1.2 Container stocktypes

In the tropics, where seedlings do not experience true dormancy, use of P+0 stock grown in large containers such as polyethylene bags has been common practice for many years. The lack of disturbance to the root systems allows planting of such stock while trees are not dormant. More recently, technology was brought to bear on development of small container systems for use in temperate and boreal regions (Tinus and Owston 1984). In these regions, containers with volumes of 65 to 165 cubic cm (4 to 10 cu. in.) are commonly used for one-year production schedules in greenhouses.

Use of relatively small seedling containers in the western United States began in earnest in the early 1970s. The main impetus was the attraction, for some, of a more automated and economical system of reforestation. For others, the attraction was the perceived biological advantage of an undisturbed root system. It was also believed possible that, because of production in a relatively controlled environment, container seedlings could be lifted on demand. This would allow for a somewhat extended planting season compared to bareroot stock.

One characteristic of this seedlings, when grown in containers of realistic size for large-scale programs, is that they tend to be smaller than 2+0 bareroot stock. Thus, they often need more protection from environmental factors such as solar radiation and animal damage than larger seedlings.

Although the plugs of potting mixture and roots make container-grown seedlings somewhat bulky to ship and handle, the relatively small size of the individual seedlings and their compact root systems make them easier to plant than bareroot stocktypes. This characteristic is particularly useful for planting where the soil is rocky or shallow or when planters are inexperienced.

Minor species, which are usually grown in relatively small quantities and often grow more slowly than the major tree species, are well-suited to container production. Small seedlots can be readily handled in greenhouses, and the controlled environment usually results in more rapid growth than in outdoor beds. This is particularly true in the western United States for species such as western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and true firs (Abies spp.), which tend to benefit from extended photoperiods and long growing seasons that are possible in many greenhouses. The controlled environment of greenhouses also allows such specialized production as, for example, growing trees in Oregon, Washington, or Idaho for planting in Alaska (Zasada and Owston 1990).

2.3.1.3 Combination stocktypes

Development of the P+1 stocktype was an effort to take advantage of the high growth potential of plug seedlings when placed in good growing environments (Hahn 1984). Transplanting of plug seedlings creates the largest reasonable seedlings with the most fibrous root mass possible in two growing seasons—presumably for the very toughest sites. Plug transplants are particularly useful to organizations or regions that have developed a plug-oriented nursery and planting system but find it necessary to have larger stock for some sites. British Columbia is a good example (Van Eerden and Gates 1990).

The miniplug-transplant technology takes the concept further by producing smaller but readily plantable stock in one year (Hahn, this volume; Hee et al. 1988; and Tanaka et al. 1988). Production in one year reduces costs and increases flexibility in reforestation planning (Tanaka 1988). Another alternative is to produce stock comparable in size to 2+1's in about 1.5 years (Hee et al. 1988).

2.3.1.4 Minor stocktypes

Other stocktypes that have not been used enough to be given common designations are:

Bedhouse seedlings—those grown in outdoor seedbeds under a greenhouse cover (Hansen 1983).

Seedlings grown in raised beds—e.g., the Dunneman process of using litter or duff (Maurer et al. 1986).

Wildlings—young, naturally regenerated seedlings dug from roadsides or forest sites.

Cuttings—either rooted (also termed stecklings (Russell and Ferguson 1990)) or, as in the case of *Populus spp.*, unrooted.

Someday, it may be common to use TC+1's; i.e., transplants from tissue cultures (Ritchie and Long 1986).

2.4 Cost Comparisons

The cost of nursery stock is only a small part of reforestation economics. Data from the Siuslaw National Forest in the Oregon Coast Range, for example, show that planting stock comprises only 10 percent of their total reforestation cost per hectare (Owston and Turpin, in press). Nevertheless, cost of stock should not be overlooked in planning a planting project. Table 2.2 indicates relative costs by stocktype, and Table 2.3 contains some specific values as an example of the widely varying costs of different stocktypes.

Reforestation planners have to use production costs along with estimated costs of handling, planting, protection, necessary replanting, and so forth to arrive at actual reforestation costs. A specific procedure for comparing alternatives has been developed for British Columbia; it takes into account costs, survival, and anticipated wood production (Tunner 1982).

***Table 2.3**—Examples of seedling costs by stocktype.*

Selling costs per thousand bareroot seedlings, USDA Forest Service nurseries in Oregon and Washington, 1990:

Stocktype	Ave. Cost per M
1+0	$125
2+0	152
1+1	233
2+1	304

Prices paid for container seedlings by some national forests in Oregon and Washington, 1990:

4-cu. in. cells	$120 - 130
10-cu. in. cells	228

2.5 Comparisons of Field Performance

Many comparisons of stocktype field performance are reported in the literature. There is even evidence that differences between types are discernible more than 20 years after planting (Krumlik and Bergerud 1985). However, after examining most of the reports, I have concluded that stocktype, by itself, makes very little difference—size and condition are the important factors. Hobbs (1984) reached a similar conclusion after reviewing the literature; i.e., he found no clear consensus favoring a particular stocktype. You will see plenty of evidence to support that conclusion in the rest of this volume.

At one time, I believed that the less-disturbed root systems and the opportunity for careful culturing of container seedlings would give them a clear performance advantage. But I have not seen that demonstrated consistently in the Pacific Northwest. What I have found instead, both from studies in which I have been personally involved and others reported in the literature, is that survival tends to be relatively similar among stocktypes and that the larger bareroot seedlings tend to maintain their initial advantage in stem height.

I recently summarized the results from almost 80 field comparisons of container-grown vs. bareroot seedlings

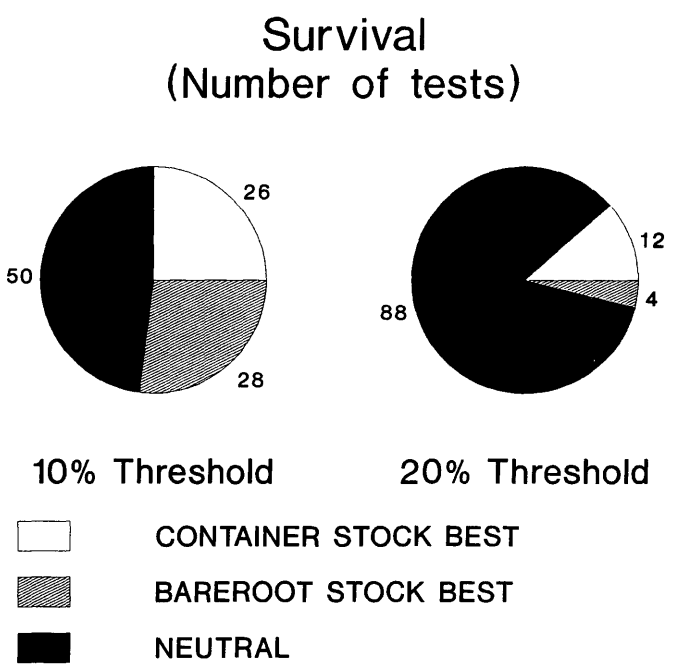

***Figure 2.1**—Number of individual tests in southwestern Oregon, northern California, and coastal Oregon and Washington in which survival between bareroot and container stocktypes did or did not differ by more than 10 or 20 percentage points. All tests were 2 to 10 years old.*

(mostly 2+0's) that were installed in southwestern Oregon and northern California in the 1970s and 1980s and 28 plots that were planted in the Oregon and Washington Coast Ranges in the 1970s. Sources used for this summary were Duddles and Owston (this volume), Helgerson et al. (1990, unpublished data), McDonald (1990, unpublished data), Owston (1990, unpublished data), and Walters (1990). All data were for trees at least two years old, and most of them were five to ten years old. Actual survival percentages varied widely for each stocktype. To see how they compared on the same sites, I developed frequency diagrams for survival and height that compared the number of individual tests in which one of the stocktypes did better, worse, or about the same as the other.

For survival, types were considered to have performed the same if their average survivals did not differ by more than 10 percentage points in one scenario or 20 percentage points in another (Figure 2.1). In about half the trials, average survivals for container and bareroot stock were within 10 percentage points of each other—very small differences given the wide variability of seedlings and microsites. Furthermore, one stocktype came out ahead just about as many times as did the other. When 20 percentage points were used as the threshold difference for survival, most of the comparisons showed no difference.

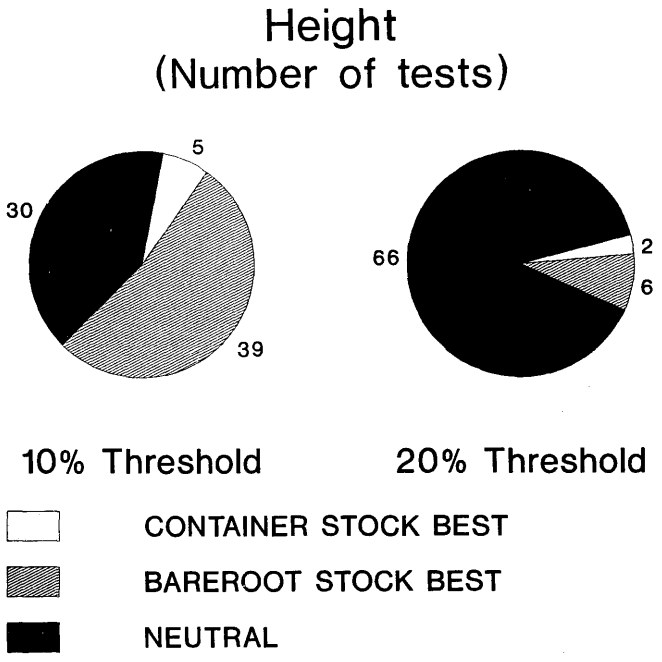

***Figure 2.2**—Number of individual tests in southwestern Oregon, northern California, and coastal Oregon and Washington in which total height between bareroot and container stocktypes did or did not differ by more than 10 or 20 percent. All trials were 3 to 10 years old.*

Although there appears to have been a slight tendency for container seedlings to perform better based on this threshold, I do not feel that the data are convincing enough to draw any conclusions.

For height, the threshold values used were 10- and 20-percent differences between the stocktypes (Figure 2.2). Height data from tests that were at least 3 years old were available from all of the coastal tests and 46 of those in southwestern Oregon and northern California. Forty-one percent of the tests showed less than ten-percent difference between stocktypes, but bareroot seedlings were taller in many more instances than were container seedlings. At the 20-percent level, where differences probably are important, less than 10 percent of the comparisons were different.

For those interested in a brief description of container vs. bareroot trials over a wider geographical area, see Sloan et al. (1987). For a wide range of sites, I believe that most of the current types will perform acceptably if they are sturdy plants that are in good physiological condition and are handled and planted with care. That includes being given protective treatments appropriate for the site onto which they are being planted. The only exception I can think of is the better plantability of small container stock in very rocky or shallow soils.

2.6 Are Stocktype Comparisons Useful?

That depends. I think the evidence is sufficient to conclude that all current stocktypes can perform well if they are grown and used properly. Thus, I see no further need for the broad-scale comparisons that were needed when container technology was in its infancy. Morphology and physiology vary so much within stocktypes that it makes little sense to make management decisions based on broad comparisons or to extrapolate from narrow ones.

I do feel, however, that empirical comparisons have their place. I believe they are appropriate for specific combinations of planting stock and nurseries and for specific types of sites or reforestation situations. But if practices or management change, do not assume that the stocktypes will perform the same as before.

One statement published long ago comes very close to matching current conventional wisdom: "The results of the experiments with western yellow and western white pine showed that, other factors being equal, large stock survived better than small stock, that transplants are usually preferable to seedlings, that stock with roots eight inches long or longer succeed better than stock with shorter roots, and that a low top-root ratio indicates better planting stock than a high ratio." (Wahlenberg 1928). Examination of Wahlenberg's data shows, however, that his stock was much smaller and the results much poorer

than would be acceptable by current standards. It is the quantitative rather than relative results that determine success or failure of reforestation programs.

Whatever comparisons are made, the morphology and physiology of the test stock should be characterized. It is those characteristics and their interaction with the environment, rather than the stocktype, per se, that will largely determine how the stock performs.

2.7 Why Bother With Stocktype Designation?

Use of stocktype won't put you in the bull's-eye, but it might point you in the general direction of the target. Here are some suggested guidelines for those responsible for plantation establishment:

1. There is no substitute for a well-planned prescription that takes numerous factors of site, logistics, and costs into account.

2. Once the seedling parameters have been established, arrange for production of that stock with a nursery that you know from experience or reputation will provide a consistently good product at a reasonable price. The stocktype will influence factors such as protection required, lead time needed for ordering, and so forth. But, unless you are under constraints such as lack of time or some specific condition mentioned below, the stocktype probably will not greatly influence performance as long as the seedlings meet size and condition specifications.

3. There are a few situations where particular stocktypes fit better than others:

    a. P+0's are the easiest stocktype to plant. Use them when the soil is too rocky or shallow to do a good job of planting bareroot stock.

    b. Consider 1+0's for use on sites where stress factors are low.

    c. If stress factors are critical (competition, ravel, temperature extremes, animals, and so forth), only use small stocktypes (P+0's or 1+0's) if the factor(s) can be mitigated.

    d. Do not plant plugs in the fall on sites prone to frost heaving. Until their roots grow into surrounding soil, plugs are more easily pushed out of the ground than bareroot seedlings.

    e. Use a large stocktype (as long as the tops and roots are well-balanced) on sites where stress factors cannot be sufficiently mitigated.

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Target Seedling Symposium

Chapter 3
Target Seedling Concepts: Height and Diameter

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ABSTRACT

The target seedling concept involves morphological and physiological seedling attributes which affect outplanting performance. Both morphological and physiological attributes are directly influenced by nursery cultural practices. Among the cultural practices which influence target seedling attributes are transplanting, growing density, and both root and shoot pruning.

Morphological features, specifically height and stem diameter, currently provide the best estimate of seedling performance after outplanting. Diameter is the best predictor of survival, while height seems to predict height growth. Parameters such as root mass or number of laterals are also useful in assessing potential performance, but their utility diminishes as stem diameter increases above 5 mm.

Seedling morphology does not always predict performance because the morphology does not indicate vitality or vigor of the seedling. In the future, nursery cultural practices will target specific morphological attributes as well as acceptable ranges of other important variables. One such approach is discussed.

3.1 Introduction

Tree planting has been the primary means of achieving artificial regeneration over the past six decades. As concerns about global deforestation increase, planting programs also will increase to mitigate potential climate changes. This emphasis on tree planting has focused renewed attention on identifying those seedling attributes in the nursery, that can predict establishment success. A simple, easy-to-measure index of these seedling attributes is needed. Nursery managers also need a seedling index to help them make cultural decisions during the growing season—particularly during the critical seedling harvesting season.

These seedling attributes necessary for reforestation success have been collectively termed "seedling quality." Perhaps the best definition of seedling quality has been "fitness for purpose" (IUFRO 1980). For reforestation purposes, seedling quality may be defined as those attributes necessary for a seedling to survive and grow after outplanting (Duryea 1985). Many seedling attributes have been studied with respect to field survival. However, much less is known about those necessary for early growth, and the question of acceptable growth following outplanting has been ignored.

Measurements of seedling quality can be categorized in several ways. Ritchie (1984) separated measures of seedling quality into two categories: material attributes and performance attributes. Material attributes, either morphological or physiological, are directly measurable, and include mineral nutrient status and seedling dimensions such as height and stem diameter. Performance attributes are physiological tests measuring a specific seedling function, such as root growth potential or cold-hardiness.

Morphological characteristics, such as seedling size, have been used traditionally to rate seedling quality in the nursery and match seedlings to the environmental conditions on the outplanting site. These morphological indices fail to account for differences in seedling physiology. As an extreme example, rating a seedling on morphological dimensions alone does not indicate whether the seedling is dead or alive.

Beginning with the investigations of Wakeley (1949) in the 1930s, forestry researchers began to search for physiologically based indices of seedling quality. Many aspects of seedling physiology have been evaluated to better understand seedling quality, including cold-hardiness and root growth potential. New techniques continue to be developed, such as the recent work on chlorophyll fluorescence (Vidaver et al. 1988). However, none of these individual physiological factors has proven to be the critical key factor to measuring seedling quality and predicting outplanting success. Physiological estimates of quality have the same limitations as traditional morphological ratings in that they provide only a narrow glimpse of the complex nature of seedling quality.

Part of the problem is that seedling quality cannot be viewed as a static parameter. It is a dynamic process that is a culmination of all the practices that have preceded and will succeed the point of measurement. Seedling quality can vary as it is estimated at specific points in time during crop growth, harvesting and storage, and shipping to the outplanting site (Duryea 1985). Consequently, the appropriate rating technique can also change over time. Also, seedling quality indices that are useful at particular stages in the nursery process are less reliable for predicting how well a seedling will perform on the outplanting site. For example, measures of seedling cold-hardiness are useful in determining proper lifting time (Faulconer 1988), but are useless for prescribing when to irrigate in the nursery, when plant moisture stress measurements are more relevant.

Root growth potential (RGP) has been widely used during seedling harvesting and shipping season to predict outplanting success. However, such predictions have limited utility because RGP and other physiologically based seedling quality indexes change considerably from lifting through storage (Landis and Skakel 1988). Seedling quality is particularly vulnerable after seedlings leave the carefully controlled and monitored nursery environment and can deteriorate rapidly during shipping and field storage. Even stock with a high quality rating may perform poorly due to unfavorable outplanting conditions (Rietveld 1989).

It may be possible to identify one specific rating index that will suffice in all situations, but this is doubtful. Some seedling quality ratings, however, have application for both the nursery manager and tree planter because they can be used during nursery culture, seedling harvesting, and also help match stock to outplanting site conditions. This paper discusses traditional morphological parameters, shoot height and stem diameter, within the context of defining the "target seedling." It discusses how these culling criteria are related to nursery and field performance, relates them to other morphological and physiological seedling quality measurements, and explains how they are affected by nursery cultural practices.

3.2 Defining the Target Seedling: Height and Caliper

3.2.1 Definitions and measurement procedures

Morphology in the classical sense is the study of external structures. For purposes of this discussion, morphology will be defined as the physical manifestation of a seedling's physiological response to the growing environ-

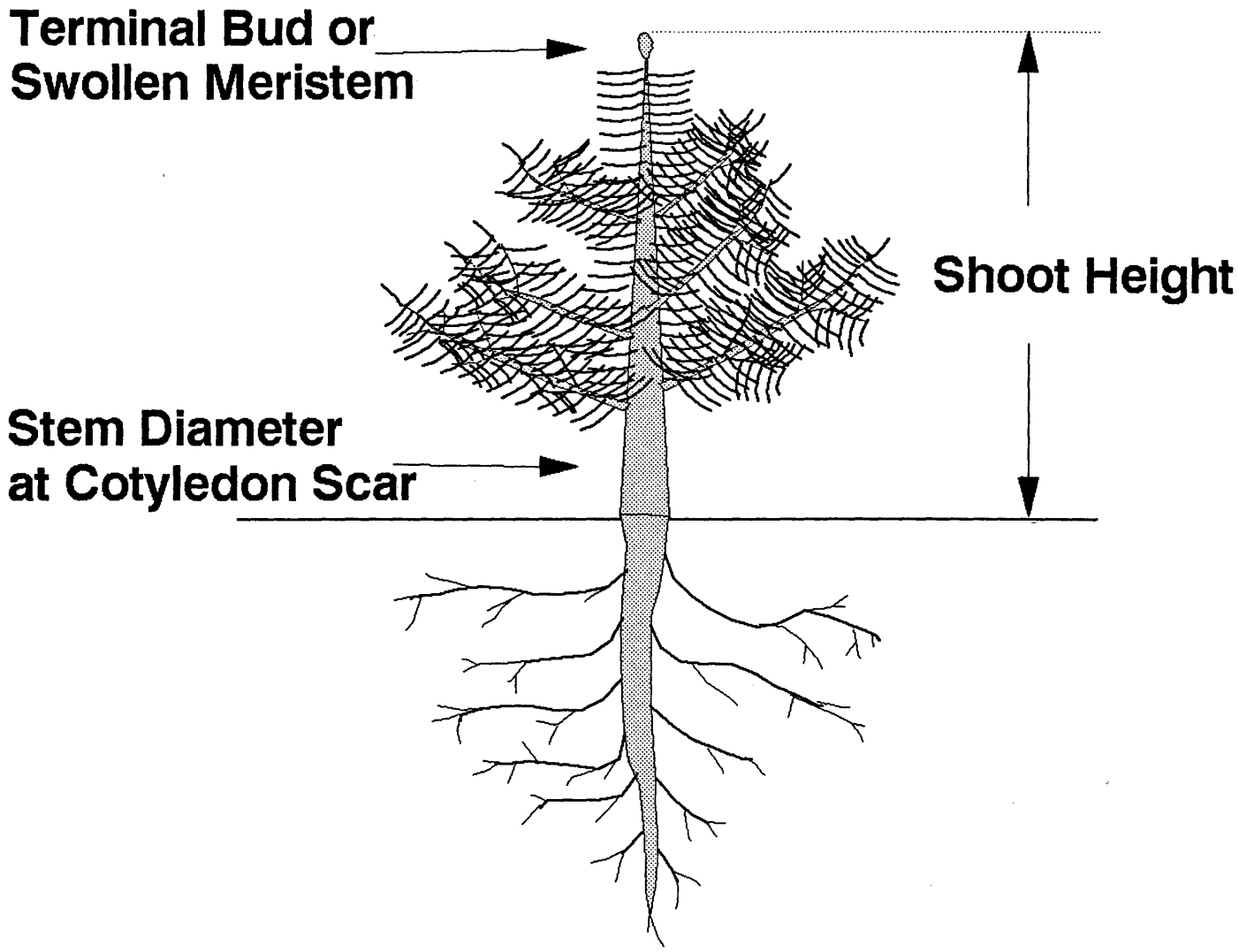


Figure 3.1—*Measurement points for shoot height and stem diameter can be determined from the cotyledon scar or original ground line.*

ment. This is an important distinction because morphological attributes of the harvested seedling are the result of a cumulative series of physiological processes reacting to resources and stresses during the nursery production phase.

Shoot height is the vertical distance from the ground line to the tip of the terminal leader (Figure 3.1). The ground line is obvious in the nursery bed but must be established on harvested stock by close observation. One physical indication of the original ground line is the point where the color of the inner bark changes from white to green when the outer bark is scraped aside. This technique is slow and destructive. Nurseries measure height either 1 cm above the uppermost lateral root (Hodgson and Donald 1980), or approximately halfway between the uppermost lateral root and the cotyledon scar.

The top of the seedling shoot can be difficult to ascertain, particularly when the seedling is actively growing. Erroneous readings occur when measurements include the highest point on a growing seedling, usually the tip of the foliage (Thompson 1985). If there is no obvious terminal bud, the measurement should be taken from the slightly swollen part of the shoot tip indicating the position of the terminal meristem (Figure 3.1).

Stem diameter, often called root collar diameter or caliper, is the diameter of the main stem of the seedling at ground line. Because the stem diameter can change significantly in this area, measurements should be made at a standardized location. Some nurseries specify that stem diameter be measured at the cotyledon scar or 1 cm above the first lateral root (Figure 3.1).

3.2.2 Target seedling specifications

A historical review of height and stem diameter grading standards may improve understanding of current applications. In one of the first U.S. nursery manuals (Tillotson 1917), seedling grading standards were only briefly dis-

Table 3.1—*Target seedling specifications from Intermountain Area forest nurseries in the early 1920s (Korstian and Baker 1925).*

Species and Stock Type	Shoot Height (cm)	Stem Diameter (mm)	Seedling Biomass	
			Shoot (g)	Roots (g)
Ponderosa Pine 2+1	10.4	3.8	4.20	1.86
Ponderosa Pine 3+0	14.2	4.3	4.00	1.17
Douglas-fir 2+2	11.9	4.1	3.64	2.12
Douglas-fir 3+1	16.8	3.6	3.82	2.54

cussed and no actual stock specifications were given. At that time, transplants were the only stock types considered suitable for outplanting in those early nurseries, and target seedlings were considerably shorter and smaller in diameter (Table 3.1) than today's standards (Table 3.2). This size difference can be attributed to improved nursery cultural practices; in the past, seedlings were grown at extremely close spacing (400 to 2000/m^2), and fertilization was not "accorded much attention" (Tillotson 1917).

Seedling grading standards were adopted by the late 1930s. Nursery manuals devoted sections to grading standards, and the importance of stem diameter as a target seedling specification was firmly established. Engstrom and Stoeckeler (1941) concluded stem diameter was "the best and most practical basis of grading deciduous nursery stock," and discussed proper measurement techniques.

Stoeckeler and Slabaugh (1965) continued to stress the importance of establishing seedling grades. They concluded stem diameter was the most important grading characteristic, followed by shoot height and root development. The classic nursery manual of Wakeley (1954) described three different seedling grades for southern pines based primarily on shoot height and stem diameter. However, he recognized the limitations of using morphological standards by themselves, and presented one of the first detailed discussions of physiological seedling characteristics.

Most modern nurseries have substituted sophisticated seedling culling systems for grading with seedling height and diameter serving as the standards. Seedling heights are typically listed as ranges between some minimum and maximum, whereas stem diameter is usually a minimum standard. Occasionally, root length or the number of primary lateral roots is specified. Other components of seedling morphology such as needle length, terminal bud

Table 3.2—*Median shoot height and stem diameter targets for conifer species and stock types from the Pacific Northwest (Iverson 1984).*

Species	Stock Type	Shoot Height (cm)	Stem Diameter (mm)
Douglas-fir	1+0	11.5	3.0
	2+0	30.5	5.0
	1+1	38.0	8.0
	Plug+1	46.0	9.0
True fir	2+0	15.0	4.5
	2+1	23.0	6.0
Spruce	2+0	18.0	4.0
	2+1	23.0	5.5
Ponderosa pine	2+0	13.0	4.0
Lodgepole pine	2+05	13.0	4.0

size, root:shoot (R:S) ratio and presence of mycorrhizas may be listed in target seedling specifications (Mexal and South 1990), but are rarely used as culling standards. Regardless, nursery managers and tree planters recognize that shoot height and stem caliper specifications vary by species, seed zone, stock type, and operational requirements, particularly the environmental conditions on the outplanting site.

The ideal shoot height for a particular planting site will depend on moisture conditions, the extent of vegetation competition and the presence of predatory animals such as deer or elk. Generally, tree planters prefer shorter, stockier seedlings for arid sites, and taller seedlings where vegetative competition or animal damage is severe. Stem diameter is not as site specific, although larger caliper seedlings have proven superior on difficult sites, where high soil temperatures or unstable soils are a problem (Iverson 1984). Normally, managers prefer seedlings with as much stem diameter as operationally possible regardless of site.

It would be impossible to provide a complete listing of seedling specifications for all species, seed sources and stock types although some typical examples are provided here. Iverson (1984) listed morphological targets for some species and stock types from the Pacific Northwest (Table 3.2). Similar targets are provided by Mason and others (1989) for Scots pine and spruce, and for the southern pines by (Mexal and South 1990). Menzies (1988) provides a comprehensive listing of both morphological and physiological specifications for radiata pine.

In actual practice, target height and stem diameter specifications for custom-grown seedlings are individually negotiated between the nursery manager and the seedling buyer when the seedling order is placed. These specifications may be adjusted, however, because of actual seedling performance later in the growing season. Those who buy speculation seedlings usually have to accept whatever stock is available for their particular species and seed zone, unless several nurseries have trees for that particular area. Therefore, target seedling specifications for a nursery can vary among customers and across years.

3.3 Factors Influencing Height

3.3.1 Transplanting

One of the first decisions that influences seedling height in the nursery is stocktype. That is, should the crop be grown as seedlings or transplants? In the western United States, seedling stocktypes range from 1.5+0 to 3+2 with dramatic effects on seedling height (Figure 3.2). In the Pacific Northwest, Douglas-fir seedlings are often transplanted after the first growing season and grown as either 1+1 or 1+2 transplants, or transplanted after the second year for 2+1 transplants (Iverson 1984). The age of the seedling at time of transplanting is important, because 1+1 and 1+2 transplants are typically taller than seedlings of similar age. On the other hand, 2+1 transplants are shorter than 3+0 seedlings. These growth differences may be due to greater transplant shock of older 2+0 seedlings as well as shade-induced height growth response of the 3+0 seedlings.

A relatively new stocktype that larger seedlings in less time is the plug + one (P+1). This is a small, containerized seedling, transplanted to the nursery bed for one addition-

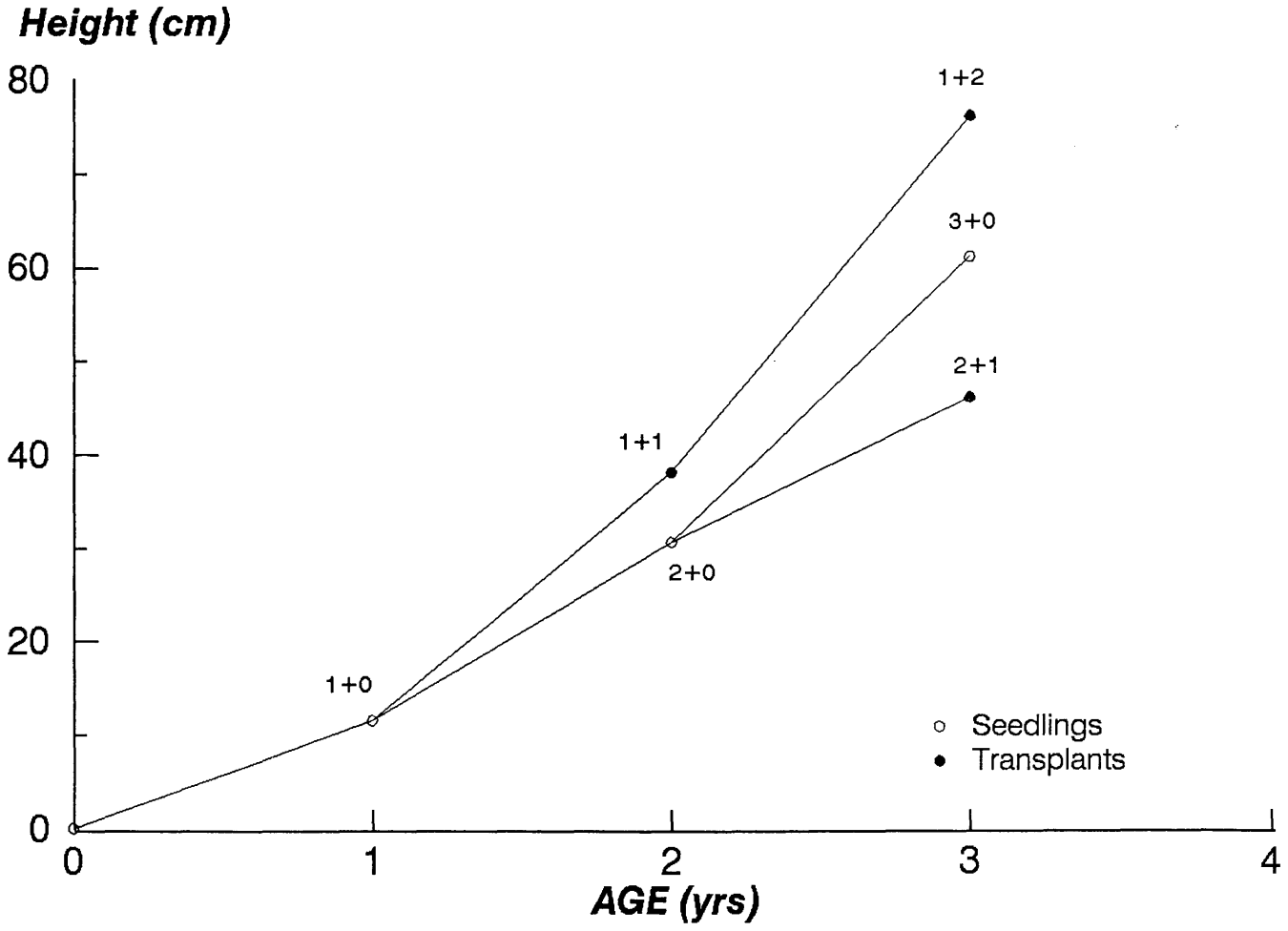


Figure 3.2—*Relationship between age and seedling height for nursery bed grown and transplanted seedlings (after Iverson 1984).*

al growing season. These seedlings are comparable in size to a 2+1 but attain the size in less than 2 years (Hahn 1984).

3.3.2 Growing density

Seedlings compete for resources necessary for growth, especially light, moisture, and nutrients. The amount of growing area afforded an individual seedling affects the growth habit and growth potential. The relationship between shoot height and growing density is complex and variable. However, the growth response can be divided into four phases based on published literature (Figure 3.3). While these four phases have been illustrated as being continuous, that is most likely not the case. It is likely that different responses are attainable over the same growing densities, depending on other variables that may become limiting. Phase A demonstrates decreasing height with increased density (van den Driessche 1982, 1984). The increased height at lower densities can be attributed to larger summer shoot or lammas growth, or simply greater resource availability. Phase B demonstrates no consistent relationship to density (Neilly 1983, van den Driessche 1984). Phase C illustrates the classic competition-induced shade-response (Brissette and Carlson 1987, Timmis and Tanaka 1976). Phase D occurs at high densities where other resources such as water and nutrients can severely limit growth (Hulten 1989). At high densities, seedling crops appear stunted and contain a high proportion of culls.

As competition increases (Phases C and D), photosynthate allocation will be driven by the response to mutual shading. That is, more carbon will be allocated to shoot extension at the expense of root growth. The R:S ratio will decrease with increasing seedling density, and outplanting performance may suffer (Mexal and Dougherty 1982). At higher growing densities, height growth should be restricted through water stress and undercutting. These practices tend to shift the allocation of photosynthates into diameter and root growth.

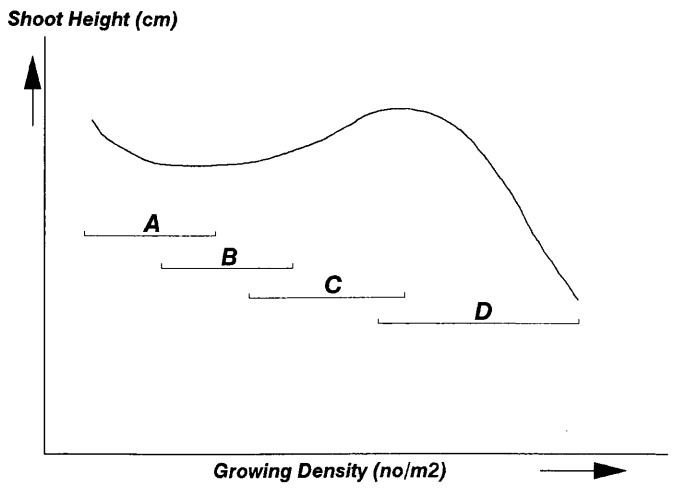


Figure 3.3—*Idealized height growth response to growing density.*

Seedling growing density can be controlled in bareroot nurseries by careful calculation of seed sowing formulae and by using precision seeders. Overly dense seedbeds can be manually thinned early in the growing season, but this is difficult to economically justify. Some nurseries use specially designed equipment to mechanically thin their seedbeds. For seedbeds that are marginally dense, height growth can be restricted with moisture stress and undercutting.

3.3.3 Fertility

In bareroot nurseries, nitrogen fertilization markedly increases seedling biomass and caliper but has only slight effects of shoot height (Armson and Sadrieka 1979, Switzer and Nelson 1963, van den Driessche 1982). Furthermore, height response to fertilizer may not be apparent until the second growing season (van den Driessche 1988). This does not infer fertilizer should not be applied; only that height growth is not greatly influenced by fertility. Other parameters are, however, dependent on level of fertility. Of course, all fertilizer amendments should be based on a regular program of soil and seedling foliar analysis, and applications should be timed to seedling phenology (Landis and Fischer 1985).

3.3.4 Irrigation

Shoot extension is more sensitive to mild water stress than are diameter or root growth (Stransky and Wilson 1964). Furthermore, there is a strong interaction between level of irrigation and fertility (Armson and Sadrieka 1979, Schomaker 1969). Maximum growth occurs at high soil water regimes and moderate to high fertility levels. At low soil water regimes, fertilizers can actually depress growth because of salt toxicity. Likewise, excessive fertilizer rates depress height (Colombo and Smith 1987).

3.3.5 Pruning

Pruning is commonly used in many nurseries to regulate height. Most western nurseries top-prune the shoots (Duryea 1984). Seedling growth response to top-pruning is a function of the stage of seedling development and the amount of shoot removed. Top-pruning typically removes only the succulent 3-7 cm of new growth. Regrowth is delayed until fascicular buds form (usually three to five weeks). Top-pruned trees grow longer into the season (Duryea and Omi 1987). Nevertheless, top-pruning reduces shoot length, diameter, and biomass. Top-pruning improves height uniformity (Mexal and Fisher 1984) and yield (South unpubl.). However, uniformity based on biomass or diameter may not be improved, and yield is not always improved (Mexal and Fisher 1984). Recent

work with southern pines indicates that top-pruning can improve survival on difficult sites (South unpubl.).

Root culturing, by undercutting, sidecutting, or the more severe wrenching is also a common nursery practice. Undercutting and wrenching effectively limit height growth (Benson and Shepherd 1977, Koon and O'Dell 1977). The crop must be actively growing for undercutting to be effective (Venator and Mexal 1981), and undercutting must be relatively shallow (<15 cm). However, Tanaka et al. (1976) did not reduce the height of either Douglas-fir or loblolly pine by undercutting. Nevertheless, R:S ratio was improved in both species. Undercutting also improves crop uniformity (Koon and O'Dell 1977, Mexal and Fisher 1984). Undercutting increases root fibrosity, which often increases seedling survival following outplanting (Tanaka et al. 1976). However, the increased root growth caused by undercutting also can increase the harvest and handling cost to the nursery.

3.4 Factors Influencing Stem Diameter

3.4.1 Transplanting

As seedlings grow, stem diameter increases concomitantly with height; however, the relationship is not absolute. It is influenced by other nursery cultural factors such as growing density, fertility and pruning. Consequently, some nurseries prescribe both height and stem diameter culling guidelines (Mason et al. 1989). These guidelines vary based on age, species, and stocktypes. However, for the species examined, the relationship is linear regardless of age or timing of transplanting. With limitations, stem diameter is a reasonable predictor of seedling height at time of lifting.

3.4.2 Growing density

Increasing growing density decreases seedling caliper, and the response is often curvilinear (Edgren 1977, Mexal 1982, van den Driessche 1982). Consequently, yield based primarily on seedling diameter may be curvilinear (Figure 3.4) and dependent on the culling standard. In these studies, less strict grading standards (3 and 2.5 mm,

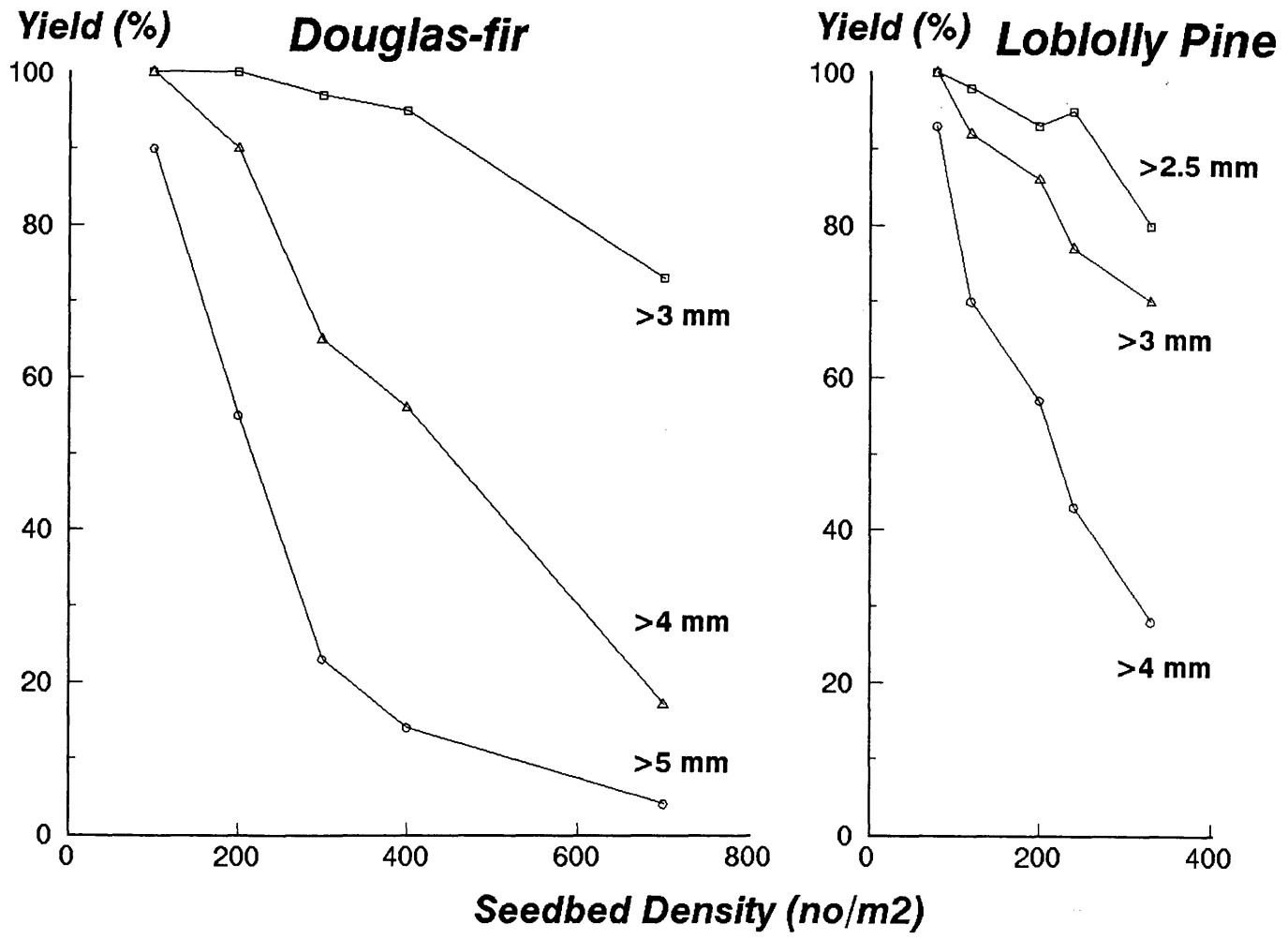


Figure 3.4—*Effect of seedbed density and culling standard on yield of Douglas-fir (Edgren 1976) and loblolly pine (Mexal 1982).*

for Douglas-fir and loblolly pine, respectively), impact yield only at the higher densities. With moderate standards (4 and 3 mm), yields fall below 80 percent at growing densities above 200/m2. With the strictest standards (>5 mm), yields exceed 80 percent only at growing densities less than 100/m^2.

While percent yield is sensitive to, and decreases with, increasing growing density, the yield per unit area usually increases as stocking increases (van den Driessche 1982). The value of the land and the associated costs of production are often greater than the costs of culling. Consequently, economics may favor growing at densities that maximize the yield per unit area, regardless of percentage culls. However, this assumes seed efficiency is of little economic importance and the performance potential is identical for comparably sized seedlings grown at different densities. In one study, this appears to be true (Burns and Brendemuehl 1971), despite changes in root morphology and R:S ratio with changes in growing density (Mexal 1982). However, other studies (e.g., Blake et al. 1989) infer density-induced changes in root morphology will translate into performance differences at time of outplanting.

3.4.3 Fertility

Nitrogen nutrition is a major determinant of seedling stem diameter and subsequent yield based on caliper. Switzer and Nelson (1963) found seedling dry weight and yield were a function of amount of nitrogen applied. Over 3 years and 4 growing densities, nitrogen (measured as amount applied per plant) accounted for 81 percent of the variation in dry weight and yield (Fisher and Mexal 1984). In spite of this relationship, many nurseries probably apply too little nitrogen over the growing season (Boyer and South 1985). The diameter of several western species increased with increasing nitrogen, up to 235 kg/ha (van den Driessche 1982). Furthermore, fertilization-induced changes in seedling size results in improved height growth, RGP, and survival following outplanting.

3.4.4 Pruning

Top-pruning and undercutting decrease seedling diameter. However, the effects on diameter are not as dramatic as on height growth. Top-pruning decreases shoot and root biomass; undercutting tends to maintain or increase root biomass while decreasing shoot biomass (Mexal and Fisher 1984). Root pruning can improve seedling quality by increasing root fibrosity, while top-pruning can only maintain quality by restricting height growth.

3.5 Relationships With Other Target Seedling Measurements

3.5.1 Morphology

Height. The relationship between height or diameter and other morphological measurements is often confounded by the cultural practices employed to attain the target height or diameter. While height is frequently highly correlated with seedling diameter, it is often weakly correlated with other parameters such as total seedling weight, root:shoot ratio, or root morphology. Factors such as growing density and fertility have a small or complex effect on seedling height, yet have such a strong impact on other parameters that any relationship between height and other parameters is tenuous at best.

One factor that logically should be correlated with height is terminal bud size. Intuitively, larger seedlings should have larger terminal buds. However, cultural practices late in the growing season can impact bud size with no appreciable effect on seedling height. Consequently, shoot height often is not an indicator of bud size (van den Driessche 1984). Seedlings that are water stressed or undercut to promote early bud set will be shorter and have larger buds than nonstressed seedlings. Conversely, top-pruned seedlings will be shorter than non-pruned seedlings, yet have a smaller bud because budset is often delayed in top-pruned seedlings. Fall fertilization will have little effect on seedling height, yet increases bud size (Hinesley and Maki 1980). Furthermore, fertility increases the number of needle primordia in the terminal bud, regardless of height development (Colombo and Smith 1987).

Seedling stem diameter is correlated with most morphological characteristics because it seems to integrate the entire seedling's morphological response to the environment. Certainly, diameter is correlated with height. It is also highly correlated with total seedling dry weight (Figure 3.5). While the absolute relationship between diameter and dry weight varies among species (van den Driessche 1982), stem diameter accounts for more than 97 percent of the variation in seedling dry weight. Diameter is equally well correlated with shoot and root weight as well as total seedling weight.

Diameter is also related to root characteristics including root weight and root morphology, when seedlings are carefully lifted. At harvest, large diameter seedlings have more primary laterals (Rowan 1986), which has been related to improved survival (Hatchell 1986). While it is possible that large diameter seedlings inherently have a more fibrous root system, it is more likely that smaller seedlings have thinner primary lateral roots that are more easily stripped during the lifting operation. The improved field performance ascribed to larger diameter may, partially, be the result of decreased root stripping.

Even though stem diameter is strongly correlated to both root and shoot weight, the relationship between diameter and R:S ratio is less clear. For southern pines, R:S ratio increases with increasing diameter (Harms and Langdon

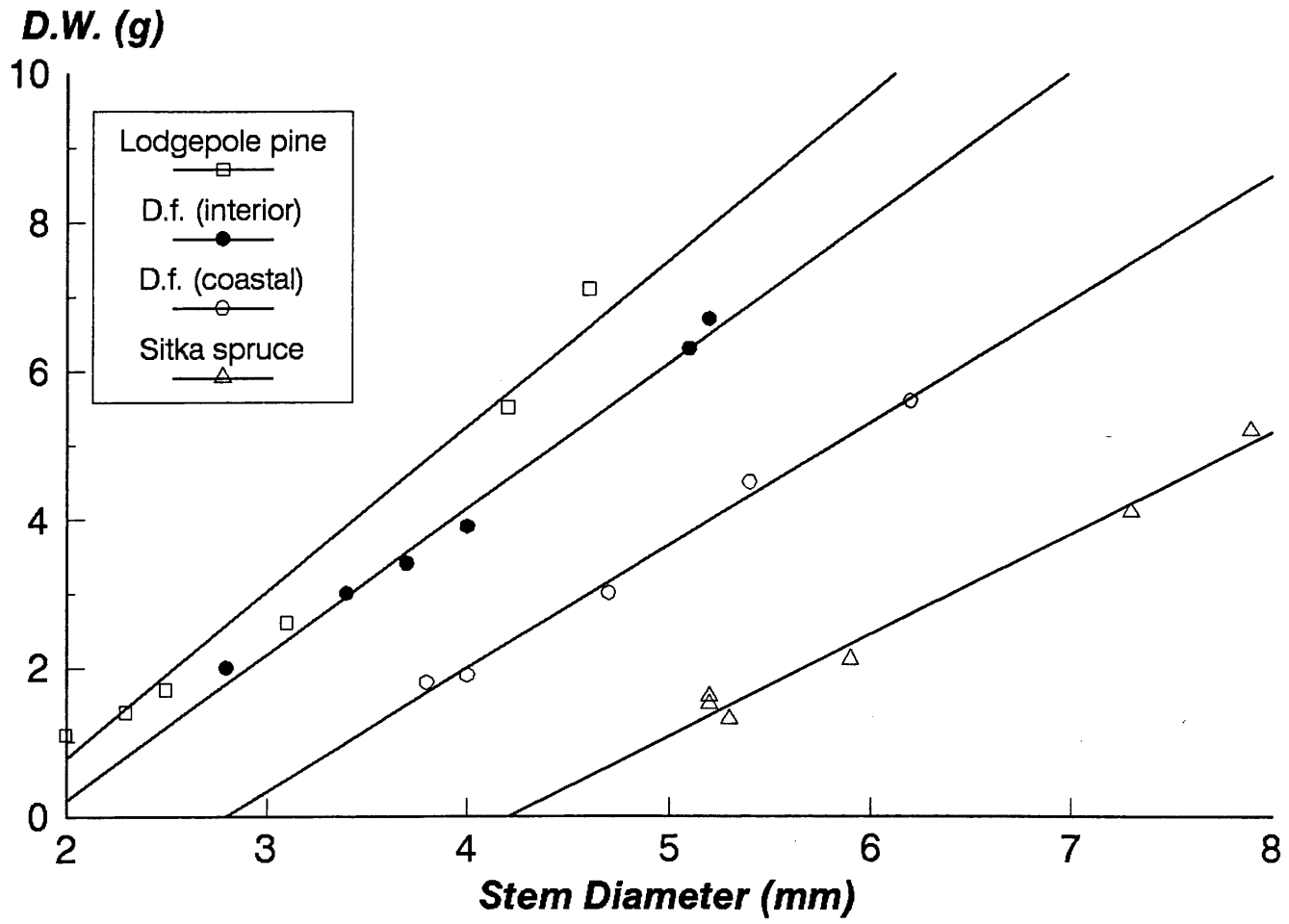


Figure 3.5—*Relationship between seedling dry weight and diameter for Douglas-fir (O,•), Sitka spruce (Δ) and lodgepole pine (□) (after van den Driessche 1982).*

1977). However, others (Mullin 1981, van den Driessche 1982) found R:S was either unrelated or negatively correlated with caliper for several species. Negative correlations held regardless of whether the increase in diameter was accomplished through increased growing area or fertility (van den Driessche 1980, 1982, 1988). This discrepancy between southern and western species is not explained.

The relationship between diameter and bud size is complex. The timing of budset largely determines final bud size. Concomitantly, early bud set corresponds to increased diameter and root growth. Seedlings that set buds early tend to have larger diameters than comparably sized seedlings that set bud later. Seedlings that invest carbon in stem elongation may not have the excess carbon to invest in diameter accretion. This relationship is transitory because seedlings with greater leaf mass can acquire more carbon. Greater leaf area through stem elongation ultimately leads to greater caliper growth. However, as nursery-grown seedlings approach harvestable size, it is desirable to have more biomass in diameter and roots than needles. Grigsby (1971) demonstrated long-term growth advantages attributable to bud morphology. Seedlings with well-formed and presumably early-formed buds performed the best in terms of ten-year volume growth. Apparently, early bud set imparted a survival and growth advantage following outplanting. However, it is possible another factor, such as simple size differences, could have accounted for the response.

3.5.2 Physiology

Root growth potential (RGP) is a measure of a seedling's ability to quickly regenerate new roots under controlled conditions. As such, it has been correlated with performance potential (Larsen et al. 1986, Ritchie and Dunlap

1980, Ritchie 1984). However, it is not well correlated with other seedling parameters, especially stem diameter (Feret et al. 1985). Factors such as undercutting tend to increase RGP while decreasing shoot size (Bacon and Bachelard 1978). However, growing density appears to have little effect on RGP compared with the strong influence it has on seedling size (van den Driessche 1984). In these studies there was more variability in RGP associated with species, year, and nursery than was associated with spacing-induced changes in seedling size (van den Driessche 1984).

While growing density per se has little or no effect on RGP, seedling stem diameter or biomass does influence RGP. Williams et al. (1988) found loblolly pine seedling weight predicted RGP (r^2 = .66). However, RGP is more a function of root architecture than absolute size (Nambiar 1980). Larger seedlings can have more root apices from which new roots originate. It may be, within a population, larger seedlings have higher RGP. However, among populations, such as a growing density experiment, the variability precludes statistical differences.

The relationship between nursery cultural practices and RGP appears complex, but no less so than between RGP and field performance. This may explain the conflicting results reported. Seedlings with low RGP can perform well because of other attributes not readily apparent such as site conditions (Burdett 1987). Many studies do not report variables tangential to the research objective, which may help explain negative results. Survival of seedlings with similar RGP values can vary by more than 40 percentage points on the same site (Binder et al. 1987, Burdett 1987), yet seedling morphology and cultural practices are often unreported in these publications. Many questions will remain unanswered until more complete morphological and physiological characterization of the stock type is reported.

3.5.3 Stress tolerance

Stress tolerance is the ability to survive exposure to low temperature (cold-hardiness), high temperature, drought and toxicants. A seedling's ability to tolerate these stresses is usually at a maximum during mid-winter upon satisfaction of the chilling requirement. This period also marks the transition between endodormancy and ecodormancy (Lang 1987), or at the end of rest (Fujigami and Nee 1987). Furthermore, RGP often reaches a maximum at this point (Ritchie 1985). While these factors are correlated, the cause of the relationship is not fully understood. Furthermore, stress tolerance and seedling morphology are probably related in an indirect manner.

Size per se should not alter the relative stress tolerance of a seedling crop, although seedlings with larger diameters and more fibrous roots may be more tolerant of physical stress, such as poor handling. However, cultural practices which influence seedling diameter are likely to also influence stress tolerance. Timmis and Tanaka (1976) found cold-hardiness of container Douglas-fir seedlings was related to growing density. Seedlings grown at lower densities were more cold-hardy and were also sturdier, heavier seedlings with lower leaf water contents. Seedlings grown at lower densities were exposed to greater environmental stresses—higher temperatures, higher incident radiation, and greater evaporative demand—as evidenced by leaf water content and potential (Timmis and Tanaka 1976). It would appear that this higher stress exposure accounted for the increased cold-hardiness, rather than the increased seedling size per se.

3.6 Utility in Performance Prediction

3.6.1 Survival

Harvested seedlings are routinely culled to remove damaged or diseased seedlings and seedlings that fail to meet specified size criteria. Typically, size standards are based on planting trials that have demonstrated smaller seedlings (especially smaller diameter) have lower survival than larger seedlings (e.g., Wakeley 1949). For example, Mullin (1959) found survival of cull seedlings was 18-23 percentage points less than survival of plantable white fir seedlings. While the percentage of the crop culled ranged from 10-30 percent over the 3 years, the relative survival advantage of plantable seedlings over cull seedlings remained similar (ca 20 percentage points). Obviously, culling does not separate trees that will live from those that will die. Rather, culling provides relative performance prediction. Smaller seedlings have lower survival potential, regardless of the environmental conditions and subsequent survival of plantable seedlings.

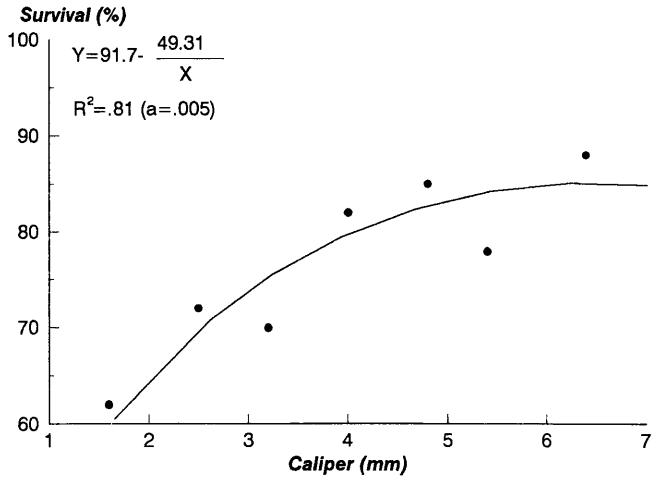


Figure 3.6—*Relationship between seedling diameter at time of lifting and outplanting survival (South and Mexal 1984).*

A major culling criterion in nursery production is shoot height. Yet, initial seedling height is not be a good predictor of seedling survival. Above a minimum size, the best seedling height is a function of outplanting site conditions. Mullin and Svaton (1972) found white spruce survival increased with increasing height up to about 20 cm and did not change between 20 and 30 cm. Tuttle et al. (1988) found survival of loblolly pine seedlings planted on adverse sites decreased if seedling height after planting exceeded 20 cm. However, on non-adverse sites, survival increased slightly with increasing height up to 35 cm. Lopushinsky and Beebe (1976) found heights ranging from 7-21 cm had no effect on survival of Douglas-fir or ponderosa pine.

The relationship between height and survival is confounded by other morphological parameters, especially R:S ratio. Thompson (1985) elegantly displayed the impact of R:S ratio on survival of seedlings in different height classes. Within the height range of 9-47 cm, seedlings with higher R:S had higher survival. The R:S ratio decreased with increasing seedling height, and above 30 cm, there was little difference in R:S ratio between seedlings with high survival and low survival. Other factors, such as site conditions, influenced seedling survival.

Stem diameter is a much better predictor of outplanting survival than shoot height. South and Mexal (1984) summarized studies dealing with loblolly pine seedling grade and survival. Seedling stem diameter predicted survival, and this relationship was curvilinearly over the range of stem diameters (Figure 3.6). They concluded, to consistently average survival above 80 percent, southern pine seedlings should have stem diameters greater than 4 mm.

Blake et al. (1989) reported a similar relationship between outplanting survival and stem diameter for Douglas-fir (Figure 3.7). He found the relationship between survival and diameter was also affected by seedling root mass, especially for smaller diameter seedlings. Seedlings with

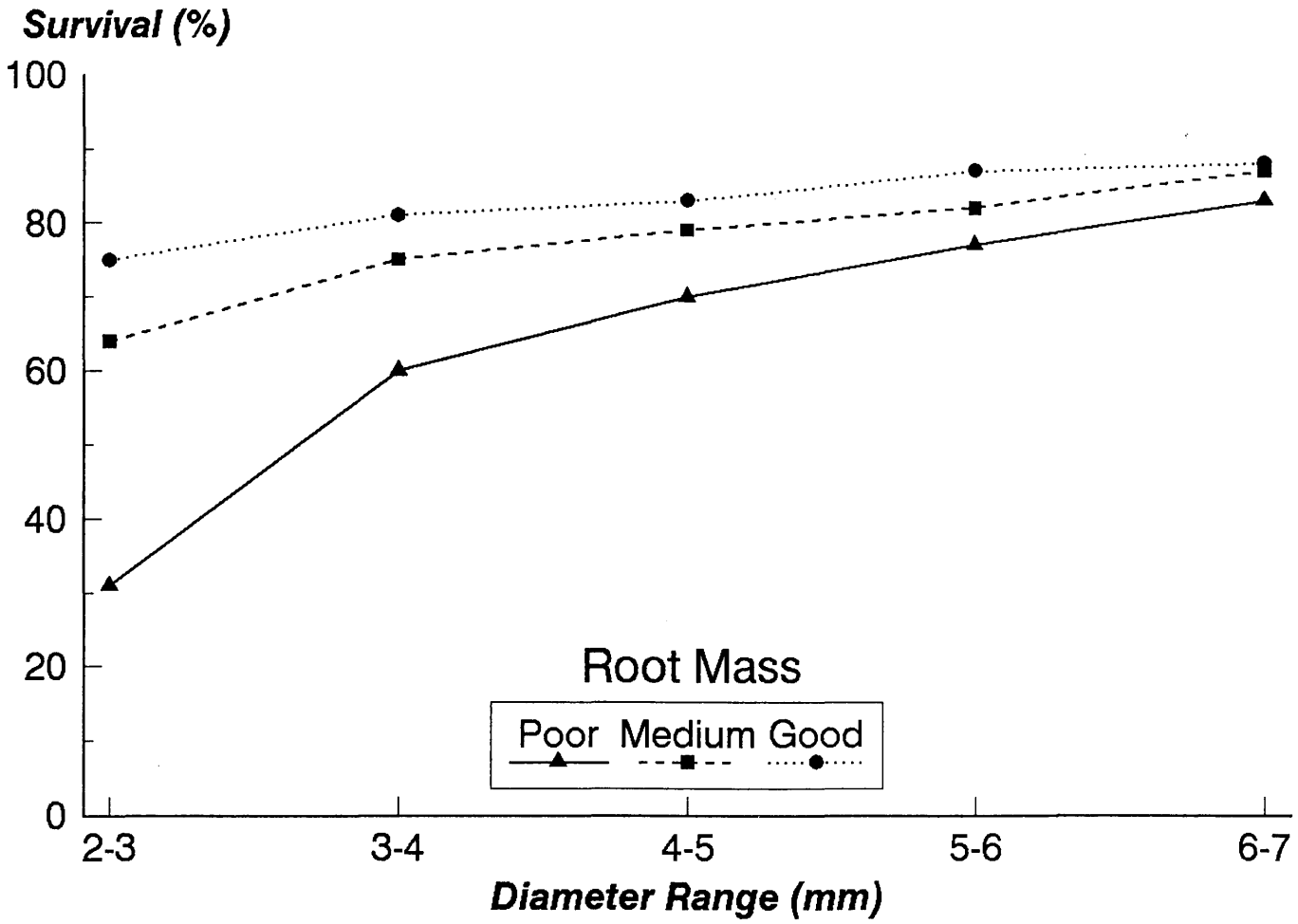


Figure 3.7—*Effect of seedling diameter on survival of Douglas-fir with good (•), medium (■), or poor (▲) root mass (after Blake et al. 1989).*

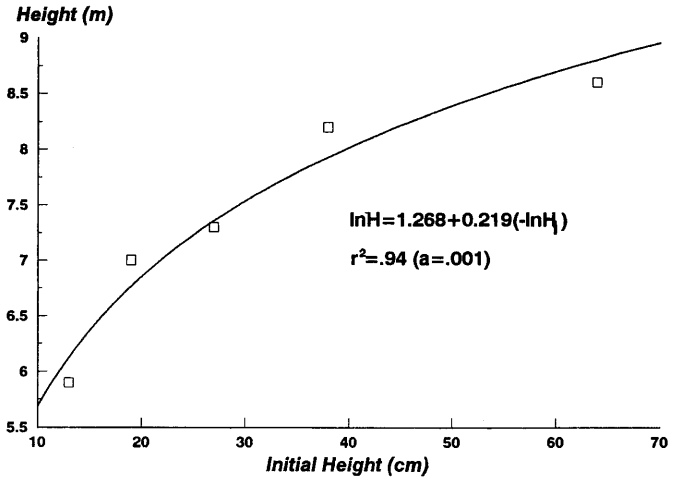
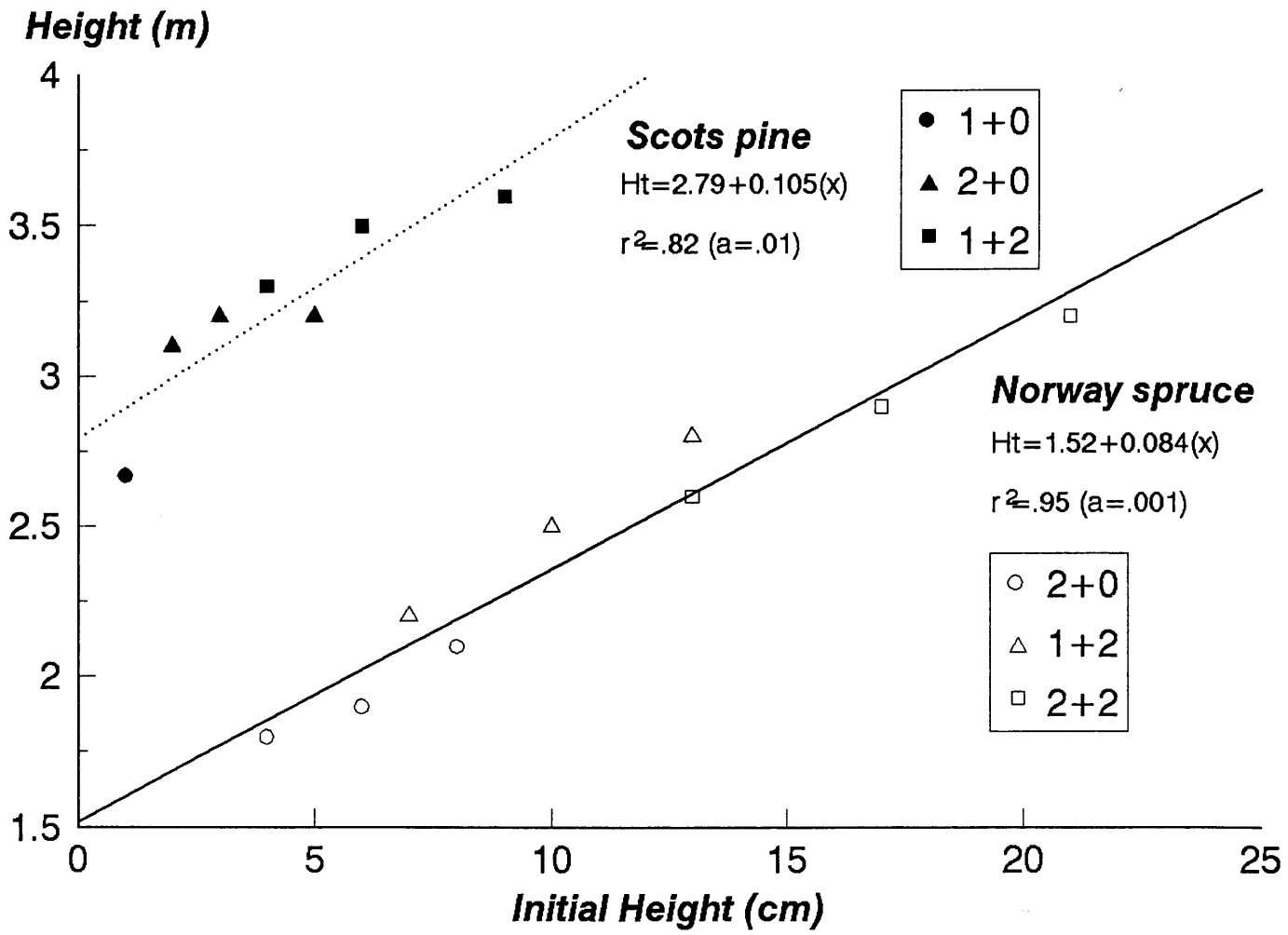


Figure 3.8—*Effect of initial seedling height on 11-year height growth of Douglas-fir (after Smith 1975).*

good root mass consistently survived better than those with poor root mass. Even seedlings normally considered culls (< 3 mm stem diameter) had high survival (> 70 percent) if they possessed a good root mass. However, only large seedlings (> 5 mm) had comparable survival potential with a poor root mass. In addition, cull seedlings with poor root mass had low survival. It would appear from these data, to ensure survival of 75 percent or greater, the nursery should provide large seedlings (> 5-6 mm), regardless of root mass, or incorporate root grades into the sorting operation.

From a practical standpoint, culturing the seedling crop to produce consistently large seedlings is the easiest choice, but it may not be the most economical. If grading based on root mass occurs, the nursery must be concerned with root stripping and exposure as a result of increased handling. This impact on performance must be considered

Figure 3.9—*(Below) Effect of initial seedling height on 15-year height of Scots pine and Norway spruce (after Mellberg and Naslund 1987).*



when weighing the benefits of growing to grade or culling to grade.

3.6.2 Growth

Growth following outplanting is more complex than initial survival and is related to the planting environment, the genetic potential of the seedling, and the physiological and morphological status at time of outplanting. Consequently, performance prediction based solely on seedling morphology may be clouded by other factors that may not be related with either morphology or nursery cultural practices, such as site conditions. Despite these confounding factors, there are many reports correlating subsequent height growth in the plantation with initial seedling height at time of planting. Smith (1975) found growth of 3+0 Douglas-fir seedlings was correlated with initial height of the seedlings (Figure 3.8). In the first growing season, height growth was not correlated with initial height. However, shoot growth in years 2 through 7 was highly correlated with initial height. For years 7-11, the growth rate among height classes was statistically different only for the shortest seedlings. Nevertheless, by year 11, a 0.5 m difference in seedling height at time of outplanting had grown to 2.7 m between the shortest and tallest seedlings.

The effect of initial seedling size on growth after outplanting appears to hold, regardless of how seedlings are cultured to attain the specified height. Mellberg and Naslund (1987) examined 15-year growth of Scots pine and Norway spruce seedlings of different stocktypes. They found height growth of different stock types was linearly related to initial seedling height (Figure 3.9). Thus, a 1+0 seedling would have the same performance potential as a 2+2 seedling if they were the same height at time of outplanting. For these species, large seedlings tend to outperform smaller seedlings, regardless of stocktype.

A similar relationship seems to hold for the effect of seedling diameter on long-term volume growth. South et

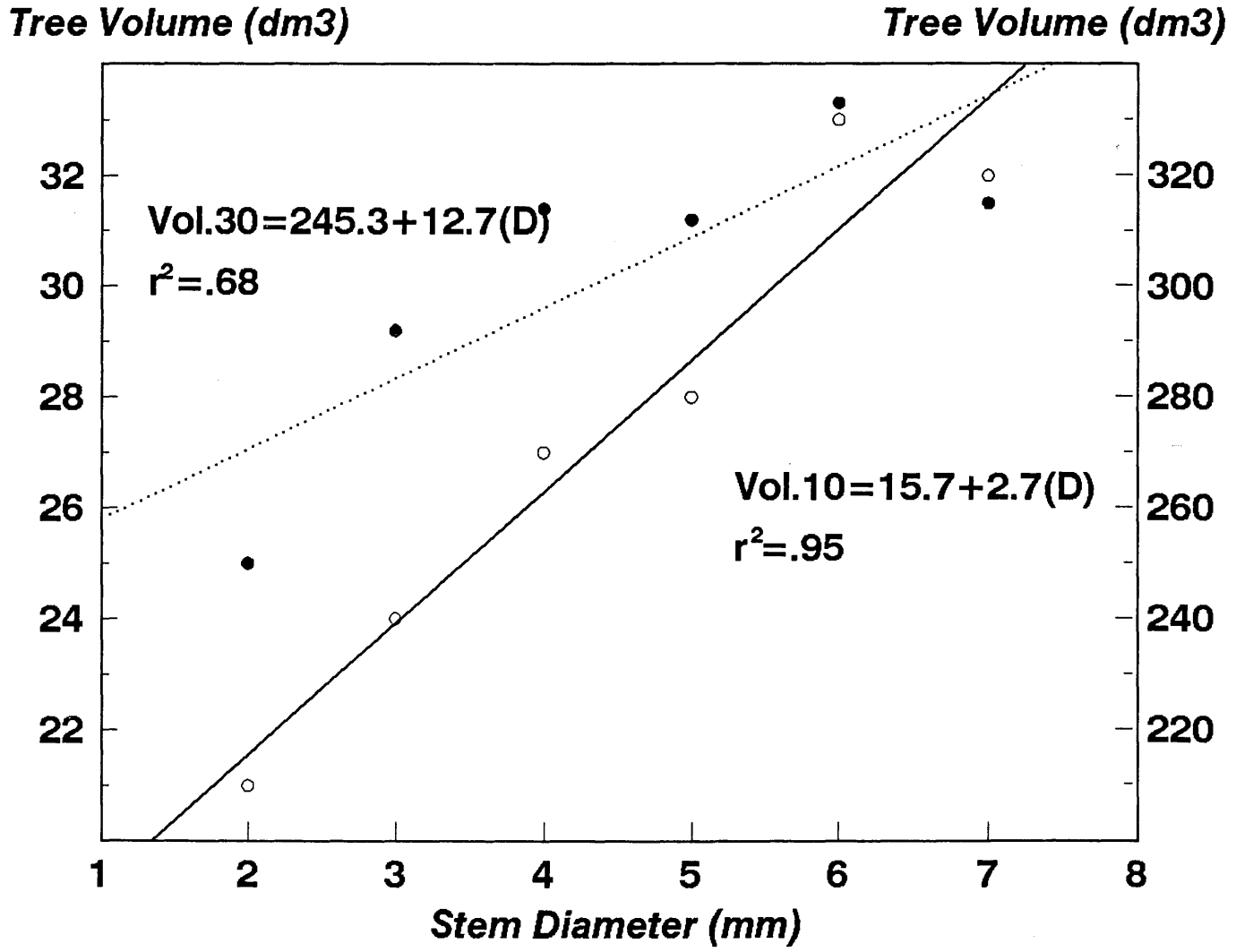


Figure 3.10—*Effect of initial seedling caliper on 10-year (O) and 30-year (•) volume of loblolly pine (South et al. 1988).*

al. (1988) examined 30-year growth of loblolly pine and found average tree volume was highly correlated with initial seedling diameter at time of planting (Figure 3.10). At 10 years of field growth, there was a 20 percent volume increase between 3 mm seedlings and 5 mm seedlings. At 30 years, the difference was 6.5 percent or 10.9 cubic decimeters/mm. The authors concluded the larger seedlings did not grow faster than smaller seedlings, but small differences in diameter at time of planting were maintained and expanded over time. This suggests small diameter seedlings are not likely to catch large diameter seedlings.

3.6.3 Outplanting site interactions

Few studies have examined the interaction between seedling size and outplanting site quality. South and Mexal (1984) felt taller seedlings may have a competitive advantage on sites with severe weed competition or slash where shading may occur. On the other hand, shorter seedlings with less transpirational surface area may have the advantage on droughty sites. Blake et al. (1989) examined the interaction among seedling diameter, root mass and site quality for Douglas-fir outplanting sites. The sites were classified into average and severe sites. Severe sites were south facing slopes greater than 15 percent, and all other sites were classified as average. They found seedling survival was high (> 70 percent) on average sites when diameter exceeded 5 mm or root mass exceeded 0.6 g (Figure 3.11A). Only seedlings with diameters less than 5 mm failed to survive well on average sites. Survival seemed to plateau when root mass exceeded 1.0 g, regardless of diameter.

On severe sites, the relationship was similar, although survival in general was lower (Figure 3.11B). Survival exceeded 70 percent only for large diameter seedlings and only if root mass exceeded 2.0 g. Furthermore, it appeared that further improvements in survival were attainable with seedlings larger than those tested.

For both sites, the relationship between survival and root mass was linear within a given stem diameter size. In general, as diameter increased, root mass increased and the advantage in survival of incremental gains in root mass decreased. Nevertheless, it appears, if the culling standards were 6 mm in diameter with a minimum root mass of 2.0 g, 7 survival percentages of 80 percent and 70 percent could be expected on average and severe sites, respectively.

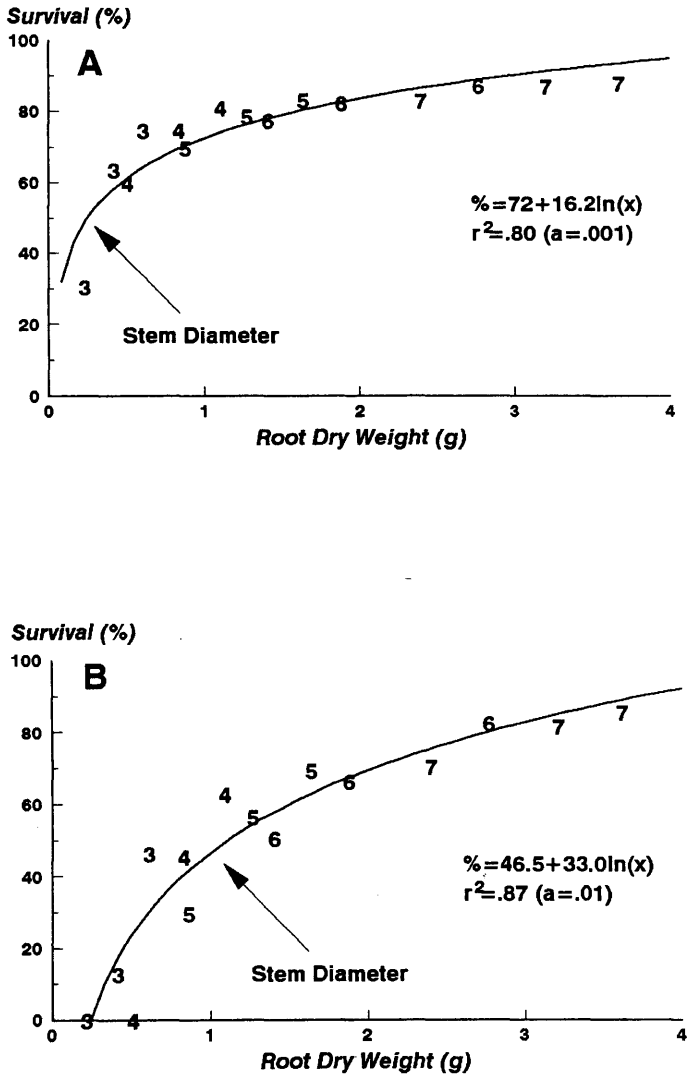


Figure 3.11—*Effect of root dry weight and seedling diameter on survival of Douglas-fir on average (A) and severe (B) planting sites (after Blake et al. 1989). Severe sites were classified as south facing with > 15% slopes.*

3.7 Future Directions

3.7.1 Current applications

The increased reliance on artificial reforestation over the past six decades has spawned an equally intense effort to identify reliable predictors of regeneration success. The term seedling quality was coined to described the attributes a seedling should possess in order to thrive following outplanting. Initially, easily measured parameters such as height and root collar diameter provided reasonable estimates of quality. However, exceptions were noted and the quest for physiologically based parameters began (Wakeley 1949).

Since 1949, many publications have focused on aspects of seedling quality. Among the many topics that are or were popular are cold-hardiness, dormancy, carbohydrate content, root growth potential, hormonal content, stress tolerance, electrical impedance, chlorophyll fluorescence and nutrient content. All of these define an important, albeit narrow, component of the myriad of factors that determine seedling performance. Consequently, none of

these individual parameters reliably predicts field survival and growth across the many reforestation systems and climates. They fall victim to the same criticisms that befell morphological parameters. That is, these parameters predict seedling performance under restricted circumstances and are, at times, even more restrictive.

Most discussions of seedling quality deal with measurable, quantifiable attributes of a seedling—the contents of the seedling. What attribute does the seedling possess that imparts success? How many new roots does it generate? How cold-hardy? How fast does it release from bud dormancy? How big is it? However, quality is not a simple, measurable parameter. It is not the content of the seedling that determines whether it will live or how rapid it will grow. It is the process of seedling production that determines the quality of the seedling. What was the growing density? What was the fertilization schedule? When were the seedlings lifted? How long were the seedlings stored? These factors determine the degree of quality. We propose the process of seedling production defines the morphological quality as well as the physiological quality of the seedling at time of lifting. This process also defines the seedling's ability to withstand the rigors of harvesting and handling. Mistreatment following lifting can be ascertained by comparing physiological test results with expected results based on the process of production. However, seedlings produced through a quality process will better withstand mistreatment. As the process of seedling production becomes more important in defining quality, so will seedling morphology become more important in assessing seedling lots.

3.7.2 Future: engineering seedling grade

Today, most nurseries can grow seedlings to certain size specifications. All nurseries can cull to any size specification. However, few nurseries know how to grow to specified quality standards. To do that, they must understand the process of quality seedling production and how environmental conditions interact with the physiological makeup of the seedling to yield the resultant seedling morphology. The seedling morphology provides an insight into past cultural practices including sowing date, growing density, fertilization, irrigation, and root or shoot pruning. However, we often fail to look at the entire morphology. To most, seedling height and caliper are the only attributes examined.

It is difficult to characterize a seedling population in relatively simple terms. A sturdiness quotient (H/D) has been proposed and adopted in some production systems, most notably in New Zealand (Menzies 1988). Various quality indices have been proposed but not widely adopted (Dickson et al. 1960). This may be the result of the lack of data relating performance to the index. It is also the result of the changing relationships among morphological parameters as growing conditions change.

A technique that may prove useful in the future is to view the seedling as a cantilever beam. As a seedling (beam) extends in length, it must expand in diameter to maintain the same relative strength properties. This relationship is described by the equation

$$d_2^3 = \frac{w_2 l_2^2 d_1^3}{w_1 l_1^2}$$

where d is diameter (mm), l is shoot length (mm) and w is the specific shoot weight (g/mm). Over a narrow range (15-30 cm), w may be considered constant. However, over larger ranges (15-60 cm), w may vary 15 percent for pines (Rikala 1989) and 20 percent for Douglas-fir (Deans et al. 1989). Regardless, d changes as the cube root of w. For most purposes w can be considered constant.

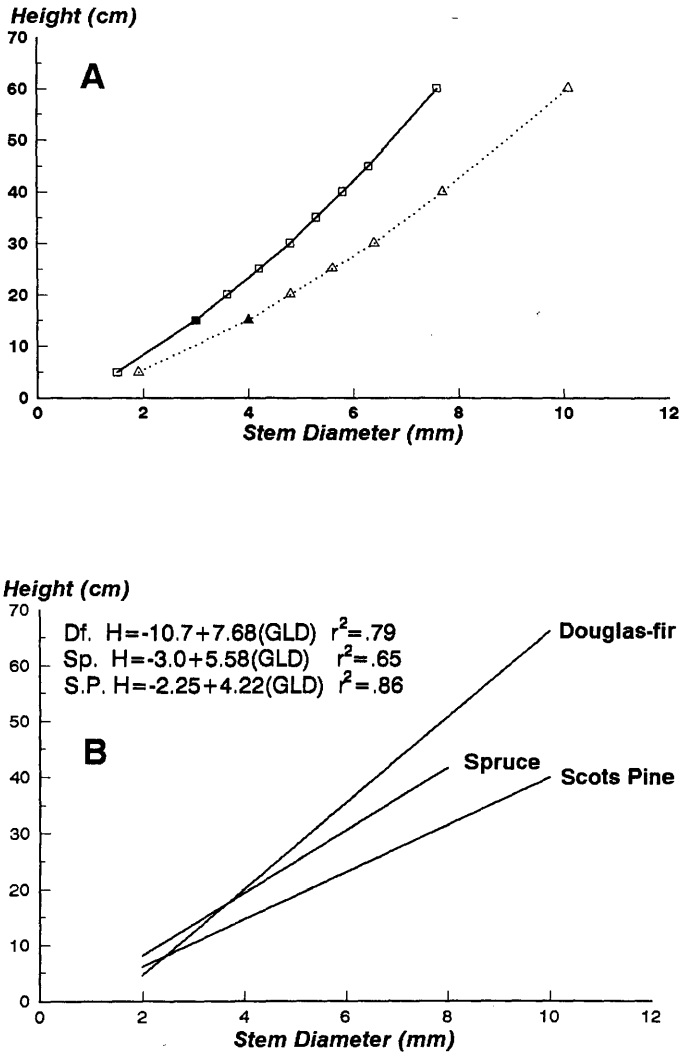


Figure 3.12—*A. Relationship between diameter and height using the cantilever beam equation and the standard height of 15 cm and diameter of 3 mm (■) or 4 mm (▲). B. Relationship between height and diameter for nursery grown seedlings (after Iverson 1984, after Mason et al. 1989).*

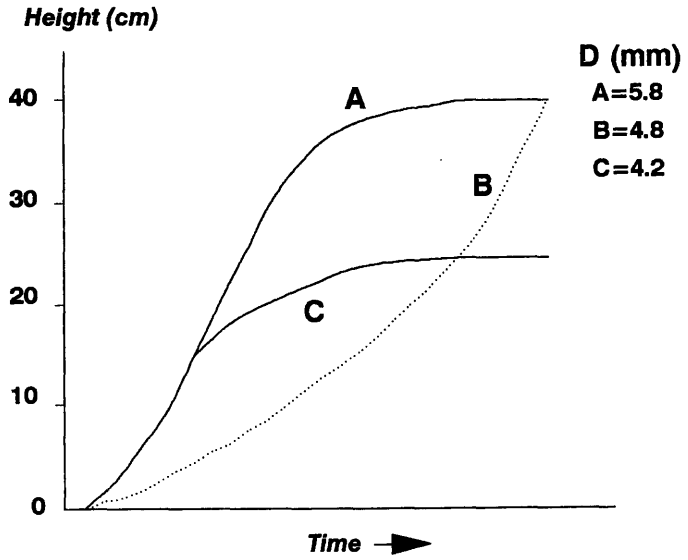


Figure 3.13—*Idealized height growth and final diameter of seedlings grown under three regimes.*

Consider a minimum size for plantable seedlings is 15 cm in length and 3 or 4 mm in diameter. Given these standards, the relationship between height and diameter can be calculated without correction for w (Figure 3.12A). For the tallest seedlings, d_2 is underestimated by about 7 percent without the correction for w. Theoretically, seedlings with height and diameter measurements falling along the curve would have similar strength properties and, therefore, similar performance attributes. Coincidently, the height-diameter relationships for Douglas-fir and spruce fall to the right of the curve developed using 15 cm and 3 mm as the standard (Figure 3.12B). Scots pine falls to the right of the curve using 15 cm and 4 mm as the standard. It appears empirical data collected over time support this hypothesis. Given minimum standards of 15 cm in height and 3 mm stem diameter, a 20 cm tall seedling would not be acceptable with a diameter of only 3 mm. This model suggests the diameter should be at least 3.6 mm. If the standards were 15 cm and 4 mm, a 20 cm seedling should have a diameter of 4.8 mm.

This relationship can be used to compare seedlings grown under different regimes. In the example in Figure 3.13, seedlings A and C have different morphologies but similar strength properties. Both seedlings fit the curve using 15 cm and 3 mm standards. Seedling B falls to the left of this curve. It is spindlier than the others and should not have the same strength properties. This seedling meets the minimum culling standards, yet the quality of this seedling is not the same as the others. Theoretically, seedlings A and C would survive better than seedling B, and seedling A would grow faster than B and C. The growth differences between B and C would depend on the severity of transplant shock for seedling B. Seedling C would suffer less transplant shock and exhibit greater absolute growth. At the end of the transplant phase, the taller seedling would expand any growth advantage.

The target seedling is not one seedling possessing specific morphological features. The target is a continuum of variables fitting the general concept of sturdiness and size. The process of achieving the target specifications is much more important than the actual attainment of those specifications. In fact, the crop may fail to reach the target height requirements, yet exceed the target diameter requirements. This seedling would have exceeded the target. The target cannot be economically attained by culling the crop to meet the standard; the crop must be grown to achieve the standard.

3.8 Conclusions

Growing the target seedling is a process that can not be easily quantified by snapshots-in-time of either the morphological or physiological features of the seedling. These provide some, but not all of the picture. It is likely no single factor will ever be found that will provide a perfect prediction of outplanting success. Stem diameter and shoot height have proven their utility over many years. These two parameters are universally accepted measures of seedling performance potential.

Both stem diameter and shoot height are affected by cultural practices in the nursery, especially growing density, transplanting, top-pruning, and root culturing. Stem diameter is a good predictor of other morphological characteristics, including height, and both shoot and root dry weight. Apparently, stem caliper reflects the entire seedling's response to the environment. However, stem diameter and shoot height may not be correlated with physiological measures of performance prediction. Reasons for this are discussed.

Stem diameter is a good predictor of outplanting survival, especially when an estimate of root mass is included. It is also correlated with long-term tree volume growth. Shoot height is not highly correlated with seedling survival, but is a good predictor of growth following outplanting. While these characteristics indicate a seedling's performance potential, they do not reflect seedling vitality or vigor. Combining morphological measurements with an appropriate measure of physiological quality may result in improved indices of outplanting performance.

Future target standards will integrate the process of producing seedlings with the content (measurements at the end of the production). Future target standards may resemble the cantilever beam equation which integrates several variables into one equation. Undoubtedly, future

standards will include information on cultural practices that produced the visible morphological features, as well as the unseen physiological parameters, both of which play a critical role in reforestation success.

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Target Seedling Symposium

Chapter 4
Root Growth Potential and the Target Seedling

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ABSTRACT

The review focuses on several key points regarding the conduct and interpretation of Root Growth Potential tests in forest regeneration. Key points are 1) RGP is developed in the nursery and is expressed after planting; 2) RGP can be accurately assessed in as little as seven days in several species; 3) RGP is a very good indicator of seedling quality but only a fair predictor of survival; 4) survival prediction is only fair because RGP indicates plant quality, not site quality or planting quality; 5) RGP can indicate when seedlings possess high stress resistance or when seedlings are damaged; 6) RGP seasonal periodicity seems to be modulated internally by (a) the intensity of shoot dormancy and (b) the strength of the carbon sink in the growing shoot; and 7) despite problems associated with lack of accuracy and precision and often unrealistic expectations, RGP testing remains a valuable tool for assessing quality of planting stock.

4.1 Introduction and Objectives

The idea of a "target seedling" brings to mind morphological targets—stem diameter, height, root/shoot ratio. In practice, nearly all conifer seedlings are grown to "target" specifications based on one or more of the above variables. And for good reason, for considerable research and experience have shown that planting seedlings which fall below or above generally accepted morphological targets increases risk of failure or accelerates planting costs.

However, the modern, sophisticated forest nursery manager is now well aware that morphological targets, while important, fall short of guaranteeing high planting stock quality. It is also critical that he or she pay attention to physiological targets such as root desiccation resistance, low temperature tolerance, and the ability to endure rough handling.

One physiological target which has come into fashion during the previous decade is high Root Growth Potential (RGP = Root Growth Capacity = Root Regeneration Potential). Root Growth Potential is a seedling *performance* attribute (Ritchie 1984a) which enjoys the considerable advantage of being easily measured. However, its interpretation and use have been the subject of sometimes heated debate as researchers and practitioners have struggled to understand this novel "bioassay" concept of seedling quality testing.

The most recent comprehensive review of RGP (Ritchie and Dunlap 1980) is now a decade old. In this chapter we will attempt to update this review focusing on key aspects of testing and interpretation with a strong view toward practical application.

4.2 Brief Review of Basic Concepts

4.2.1 Historical overview

Philip Wakeley (1948) introduced the term "physiological grade" into our lexicon of planting stock jargon. While this was a novel and powerful concept, it was not clear then how such grades were to be determined or quantified. As Wakeley himself put it: "How to recognize physiological grades before planting the seedlings . . . remains to be discovered." Soon thereafter, Edward Stone, in a series of papers (c.f. Stone 1955, Stone and Jenkinson 1970, 1971) introduced the idea of using root growth as a measure of physiological grade. His work repeatedly showed that potentially poor performing lots of planting stock could often be identified in advance by their weak response in root growth tests.

International attention was drawn to Stone's work and its importance in a IUFRO sponsored conference on Planting Stock Quality held in New Zealand in 1979 (Ritchie and Dunlap 1980). Since publication of those proceedings, interest in RGP has grown exponentially around the globe. Many private and public forestry organizations now routinely use RGP to screen nursery stock before planting (Sutton 1990, Landis and Skakel 1988). Private testing services have arisen throughout the United States and other countries. Even the landscape nursery industry has become interested in RGP testing of nursery transplant stock (Struve 1990).

Along with this surge of interest has come confusion, abuse, and misunderstanding of the technique and its interpretation. Some organizations rely heavily on RGP testing to verify stock quality while others have abandoned it, disappointed by its inability to predict field performance accurately and consistently. In this review we will discuss some of these issues toward developing a common sense understanding of what RGP measurements can and cannot do.

4.2.2 RGP development, expression, and measurement

RGP is defined as a seedling's ability to grow roots when placed into an environment which is highly favorable for root growth (i.e., warm, moist, well lighted). This is a key point—RGP is distinctly different from root growth which occurs in a natural environment, as will be seen later. This ability is *developed* in a seedling while it is growing in the nursery and can be controlled by several nursery cultural factors such as time of lifting, root culturing to stimulate root fibrosity (Deans et al. 1990), fertilization, irrigation, top pruning, and (importantly) cold storage.

RGP is *expressed* after planting but this expression rarely matches the potential for root growth. RGP expression is very strongly affected by soil temperature and moisture, and also by air temperature, handling (Tabbush 1986) and planting quality. The proper time to measure RGP is immediately before the stock is to be planted. This is because RGP is constantly changing, e.g., in cold storage. So an RGP measurement carried out on seedlings before storage may or may not reflect their RGP following storage.

RGP is measured by potting seedlings in a growing medium and placing them into a warm, well lighted environment under conditions which are standardized for that nursery or testing lab. After one month in the test environment seedlings are extracted and the amount of root growth which has occurred is somehow quantified. The main problem with the test is the excessive duration of the test period. Results are often needed immediately—not after 30 days.

4.3 RGP Measurement: Do We Have a Rapid Test Yet?

4.3.1 Testing procedures and fundamentals

The first step in RGP testing is to wash the root system of sample seedlings thoroughly and clip off any white new roots to bring all the seedlings to the same starting point. Seedlings are then planted in pots, trays, or other containers. The growing media most frequently used are mixtures of peat, perlite, and vermiculite. The main consideration is that the media have good water holding capacity and at the same time adequate drainage to avoid development of a perched water table in the pot (Whitcomb 1984).

Seedlings are then placed in a spring-like warm environment conducive to "optimum" root growth. For this purpose, temperatures of air and/or media, relative humidity and daylength are often controlled. Here it is again pointed out that RGP is to be differentiated from root growth expression in the field since the latter usually takes place under a suboptimal environment and is less than RGP.

The growing system used in evaluating RGP must provide an optimum, uniform and reasonably repeatable environment. Although most workers use a soil-less mix of some type, others have successfully used a hydroponic system (Brissette 1986, DeWald et al. 1984, Freyman et al. 1986, Johnsen and Feret 1986, Ludwig 1986, Palmer and Holden 1986, Rietveld 1989a, Ritchie 1984, Rose and Wales 1984, Williams et al. 1988) as well as aeroponic or mist systems (Brissette et al. 1988, Burr and Tinus 1988, Burr et al. 1989, Rietveld 1989a, 1989b). Each has advantages and disadvantages (Ritchie 1985, Rietveld 1989a).

Western white pine and ponderosa pine had more root growth in aerated water than in soil growing media (Ludwig 1986) while the opposite was true with loblolly pine (Brissette 1986, Freyman et al. 1986), and Douglas-fir (Ludwig 1986). Rietveld (1989a) reported that root growth of jack pine was faster and less variable in aeroponic culture than in soil or hydroponic culture. All three systems are viable alternatives and the pattern of root growth has been found to be closely related among three systems (Brissette 1986, DeWald et al. 1984, Rietveld 1989a, Ritchie 1985). The important consideration is adherence to the same method throughout a testing program with a given objective, once a system is selected.

4.3.2 Sample size

Owing to the labor intensive procedure of root counting, the following question has often been asked: how many sample seedlings does the RGP test require? There would not be one sample size that is optimum to all tests. The number depends on objectives, species, stock types, etc. The main consideration is to keep the sample as small as possible to minimize costs but yet to maintain a large enough sample to yield meaningful results. That is, to have confidence limits around means narrow enough to detect any differences among treatments, lots, lift dates, ages of trees, etc., that are being sought.

Statistically, choosing an appropriate sample size depends on: (1) the variability inherent in the population being sampled, and (2) the desired size of the differences to be detected. In general, smaller differences are more difficult to detect than large differences, especially as variability increases. A guide to determining the number of replications is offered by White (1984) in the *Forest Nursery Manual.*

Of the 32 papers we reviewed (which have been published since 1980), the sample size has ranged from 8 to 60. Six percent used fewer than 10 trees, 44 percent from 11 to 20 trees, 34 percent from 21 to 30 trees, and 16 percent over 31 trees.

4.3.3 Measurement procedures

Once the RGP test has been completed the next task is to determine how much root growth occurred. The most commonly used method is to count the number of new roots greater than a certain minimum length (0.5 cm and 1.0 cm used most frequently). Length of new roots can also be measured and summed to express total length of new roots. These two measures are generally closely correlated in many species. Index values are also used. The most notable is Burdett's Root Growth Index (RGI), which stratifies new root growth into six categories in a somewhat geometric progression (Burdett 1979). RGI is widely used in some parts of Canada and found to reduce the time required to count new roots.

Change in volume or weight of roots has also been used to quantify root growth. These are measured at the beginning and end of the test and are subtracted to estimate root growth. The weight change method is used operationally in Swedish nurseries (D. Simpson, B.C. Min. For., pers. comm.). Area changes have also been successfully measured to estimate new root growth (Rietveld 1989b). Of the 32 papers we reviewed for methodology, 84 percent used number of new roots, 44 percent used root length, 16 percent used index values, 6 percent volume, and 3 percent root area (the percent values do not sum to 100 because many workers used more than one method).

4.3.4 Reporting results of RGP tests

All too often RGP test results are stated in terms of a simple mean—e.g., RGP = 100 new roots per seedling. The fallacy of this approach can be illustrated by the following hypothetical example. Suppose RGP is measured on a sample of 20 seedlings. Ten seedlings give 200 new root tips each, the other 10 die during the test. The mean RGP value is 100 new roots despite the high probability that this stock is in very poor condition.

As much information about test results should be given as possible, including: (1) sample size, (2) mortality during the test period, (3) the mean RGP value, (4) the standard deviation around the mean, (5) the highest and lowest values, and (6) a frequency distribution. This information gives the user a far better feeling for the physiological condition of the stock sample than a simple mean.

4.3.5 Opportunities for test shortening

The RGP test is considered to be one of the more reliable methods for assessing viability and vigor of planting stock. However, one major drawback of the method is a relatively long test duration. In the standard test procedure, seedlings are grown for one month before being assessed (Ritchie 1984a). One month is too long in many situations when important management decisions need to be made quickly with respect to disposition of stock in the event of suspected stock quality problems (such as frost and desiccation damage in nursery beds, mishandling during storage or transporting, etc.).

Studies conducted during the past decade have shown that shortening the test period to 14 or even 7 days is feasible for several species including Douglas-fir (Binder et al. 1988, Burr and Tinus 1988, Burr et al. 1989, Cannell et al. 1990, Simpson et al. 1988, Tabbush 1986), Engelmann spruce (Burr et al. 1989), interior spruce (Simpson et al. 1988), Sitka spruce (Cannell et al. 1990), white spruce (Johnson-Flanagan and Owens 1985), western hemlock (Binder et al. 1988, Grossnickle et al. 1988), jack pine (Rietveld 1989a), loblolly pine (DeWald and Feret 1987, DeWald et al. 1984, Freyman et al. 1986), lodgepole pine (Burdett et al. 1983, Simpson et al. 1988), maritime pine (Donald 1983), ponderosa pine (Burr et al. 1989), radiata pine (Donald 1983, 1988), red pine (Andersen et al. 1986), Scots pine (Mattson 1986), slash pine (Donald 1983), and western redcedar (Grossnickle et al. 1988).

4.3.6 Where are we today?

It is encouraging to find a volume of papers that report RGP results based on 7-15 day tests in many species. This clearly indicates that the test duration can be shortened to two weeks, or even one week, for a majority of tree species if tests are conducted under an optimum environment for root development.

Most of the above reports have shown that the 7-15 day RGP test can be used to detect differences in stock quality as affected by nursery treatments, storage, handling, etc. These types of comparisons are relatively straightforward as the changes in RGP can be compared with that of untreated controls. In operational application of this technology to reforestation programs, RGP of untreated controls is not often available. Since RGP exhibits distinct monthly fluctuations, additional testing would be needed to establish seasonal baseline data of each species at each nursery site over several years so that the results of any future tests could be compared at any time of year.

4.4 Interpreting RGP

4.4.1 RGP and survival

Numerous articles published on RGP concern the relationships between RGP and field performance. Ritchie and Dunlap (1980) reviewed the literature and reported that, out of 26 papers they surveyed, 85 percent showed a positive correlation. The remaining articles showed poor to inverse relationships. We've examined more recent literature since the above review and found a generally similar trend with 75 percent of 12 studies showing a positive relationship and 25 percent showing poor or no relationships.

Reasons for the lack of correlation are sometimes difficult to determine. However, there appear to be at least three. One is inadequate methods and procedures, such as use of excessively wet or dry media in pot tests, or insufficient supply of oxygen in hydroponic systems due to equipment malfunction or inadequate design. This would also include inadequate sampling procedures resulting in unrepresentative results. RGP tests can lack both accuracy and precision (Binder et al. 1988).

The second reason relates to various steps after the seedlings have left nurseries and following the RGP tests. These include mishandling of stock during transport to

		RGP	
		Low	High
Field of Condition	Harsh	+	?
	Mild	?	+

Figure 4.1—*Failure of RGP always to predict field performance relates to the interaction of RGP with field conditons. Performance of poor stock planted on harsh sites or good stock on good sites is predictable. Performance of good stock on harsh sites or poor stock on mild sites is less so.*

planting sites or improper procedures such as planting trees in duff layers. Even the best stock with good RGP may not do well under such conditions. These problems could be overcome, however, by careful planning and design and by special effort on the part of everyone involved in the reforestation system.

The third possibility is the unpredictability of site and weather conditions in the field, factors over which we have little or no control (Burdett 1987). This may be explained using a matrix diagram of stock quality and field condition (Figure 4.1). Performance of poor stock planted on harsh sites and of good stock planted on mild sites is usually predictable. However, it is more difficult to predict performance of good stock planted on harsh sites or poor stock planted on forgiving sites. Because of these reasons, the correlation of RGP and field performance may not be high in some instances, as evidenced in our literature review.

4.4.2 The seed testing analog

Much of the current misunderstanding and dispute regarding RGP testing arises out of the misplaced expectation that RGP is designed to predict field performance (Binder et al. 1988), when, in fact, it is designed to evaluate seedling quality (Ritchie 1984). An important idea to keep in mind is that: *RGP testing is like seed testing.* Seed are tested under optimum conditions for germination. The report from the seed testing lab guarantees that the seed performed at a certain germination level *under* those test conditions. This does not guarantee the same level of germination after sowing in the greenhouse or nursery. Although one might expect a seedlot that tested out at 95 percent germination to give higher germination in the field than one which tested at, say, 30 percent, it is unrealistic to expect it to give 95 percent germination when sown in a cold wet nursery soil in April. This is common sense to nurserymen. It must also become common sense to foresters that RGP tests should be interpreted in the same manner. The test data guarantee that the stock was at some level of quality when tested. Nothing more; nothing less.

4.4.3 How much RGP is enough?

We ask this question having said that we hesitate to speculate on how much RGP is needed to ensure plantation success. However, since so much debate has surrounded this question and because it is so often asked, we would be remiss not to at least give it pause in this review.

A study conducted in British Columbia showed that the threshold value of interior spruce and lodgepole pine for good performance was 10 new roots greater than 1 cm in length (Simpson et al. 1988). Threshold values could also be determined for other species for which the positive relationship between RGP and field performance has been found (Burdett et al. 1983, Larsen et al. 1986,

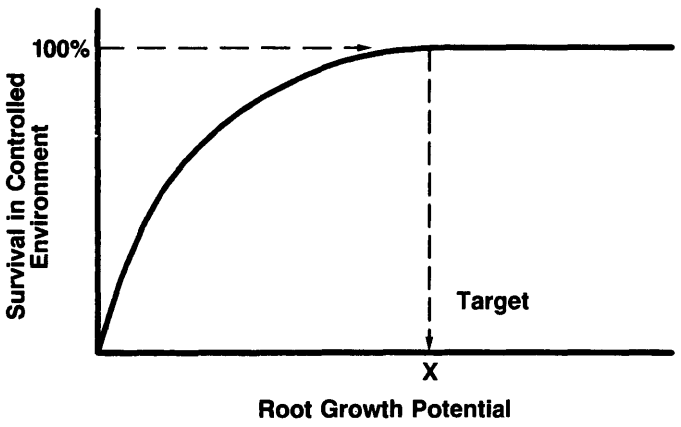


Figure 4.2—*Illustration of an approach for determining threshold RGP values for survival in controlled environment or greenhouse tests.*

McCreary and Duryea 1987). These values would be helpful as a general guide of stock quality but would not predict survival under specific field conditions because of the reasons stated earlier.

Owing to the uncertainty of weather and site conditions, threshold values are difficult to estimate. In addition, costs of field studies are high. As a shortcut, we have conducted similar studies to determine threshold values under a more controlled environment in a greenhouse. A modified Burdett's (1979) root growth index was used to establish the relationship between RGP and four-week greenhouse pot test of seedling viability. We found that there was a curvilinear relationship between these parameters (Figure 4.2). We also found that the threshold values vary according to stock type and the duration of the test period even within the same species (Table 4.1). An appropriate RGP target could perhaps be established using the threshold value approach.

Table 4.1—*Threshold RGP values for two Douglas-fir stock types tested in a greenhouse (4-week RGP) and growth chamber (14-day RGP) environment.*

Stock type	Root Growth Index* 14-day test	28-day test
1+1	3.0	4.8
2+0	2.0	4.0

* Modified Burdett's (1979) index

4.5 RGP and Dormancy: How Are They Related?

Growth of the root system in tree seedlings is under control of both the external environment and various internal factors. Environmental factors which affect root growth are soil and air temperatures, soil matric potentials, soil aeration, soil strength, and other factors. In RGP testing these variables are held constant; nevertheless, RGP exhibits strong seasonal cycles. These cycles must be modulated by internal, rather than environmental, agents. The internal drivers of these seasonal cycles have been the subject of much research and debate.

An early theory was that these seasonal changes are modulated by changes in seedling carbohydrate reserves. This theory, however, is not well supported by experimental evidence (Ritchie 1982, Duryea and McClain 1984, Cannell et al. 1990). Another theory which enjoys considerable support is that seasonal changes in RGP are driven by the annual dormancy cycle. Ritchie and Dunlap (1980) reviewed early evidence supporting this view. Here we will examine evidence from studies reported since 1980 which bear on this hypothesis.

In many (but not all, see Phillipson 1988) species, these internal factors apparently originate in the shoot. Such factors are presumably: (1) chemical or hormonal messengers which either inhibit or promote root initiation, and (2) assimilates which sustain root elongation. This has been demonstrated in girdling, decapitation, and defoliation experiments (Lavender and Hermann 1969, Zaerr and Lavender 1974) and labeling studies with $^{14}CO_2$ (van Den Driessche 1987).

Several early investigators working with deciduous hardwoods (Richardson 1958, Webb 1976, 1977, Farmer 1975) reported that seedlings exhibited very weak root growth when the shoots were in a state of intense dormancy, but exposure of these seedlings to chilling restored root growth. Similar studies with conifers suggested a strong relationship between chilling and RGP and dormancy intensity and RGP (reviewed by Ritchie and Dunlap 1980) indicating that RGP was in some way linked to shoot dormancy.

Other work with conifers in nurseries and in RGP environments has pointed to a distinct weakening of root growth when shoot activity is intense during spring and early summer (e.g., Winjum 1963, Stone et al. 1962).

These observations taken together suggest the following hypothesis for explaining the internal control of RGP:

Root growth tends to occur in a favorable environment unless impeded by:

(a) a *dormant* shoot (perhaps either by reducing the supply of promoters or increasing the supply of inhibitors to the root system), and
(b) a rapidly *expanding* shoot (by outcompeting the root for carbon).

Therefore, seasonal RGP peaks would be expected to occur during periods when: (1) dormancy intensity is weak, but (2) active shoot growth is not evident. This would usually be in late summer and early autumn, then again in late winter-early spring for most northern conifers.

4.5.1 Dormancy defined

Many of the problems of interpreting and communicating dormancy-related processes result from lack of a precise terminology and frame of reference. Recently Fuchigami et al. (1982) and Fuchigami and Cheng-Chu Nee (1987) have provided such a reference in their "Degree Growth Stage Model." Although developed mainly from work with hardwood species, this model appears to accommodate most dormancy-related observations reported for conifer seedlings. We feel that it has considerable merit and, when used in the context of seedling physiology and RGP, could make important contributions toward understanding and communicating dormancy related phenomena.

The degree Growth Stage (°GS) model portrays the annual developmental cycle of woody temperate plants as a sine wave cycling through 360°GS (Figure 4.3). The model contains five seasonal "point events" (indicated below the graph). These are:

0°GS: Spring budbreak (SBB). Defined as when bud scales part and the new leaf becomes visible. Growth rate is temperature-regulated and plants are highly susceptible to stress. This occurs around mid-March in coastal Oregon (L. Fuchigami, pers. comm.).

90°GS: Maturity induction point (MI). Between 90°GS and 180°GS plants will respond to shortening photoperiod and the state of rest will develop. However, this can be overcome if plants are artificially exposed to long days. In this stage, plants are not hardy to freezing temperatures. 90°GS occurs in early June in coastal Oregon.

180°GS: Vegetative maturity (VM). This is the onset of rest. Before this point plants are dormant due to correlative inhibition. This stage of dormancy intensifies as chilling temperatures (roughly -3°C to 12°C) accumulate (Kobayashi et al. 1982). Cold hardiness also develops during this stage and is hastened by exposures to frost conditions. 180°GS normally occurs around September 20 in coastal Oregon.

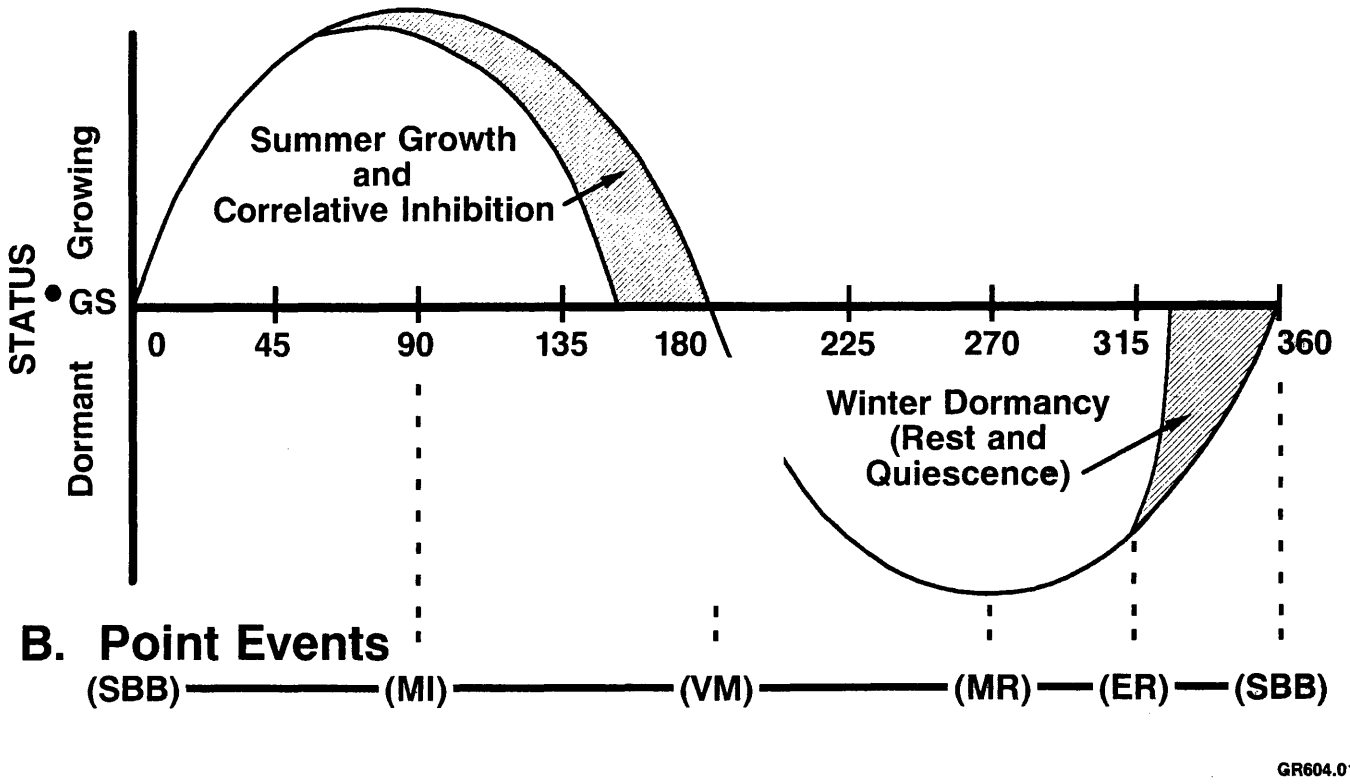


Figure 4.3—*A Degree Growth Stage (°GS) model for woody plants after Fuchigami and Cheng-Chu Nee (1987). See text for explanation. Reprinted with permission from HortScience 22:836 (1987).*

270°GS: Maximum rest. This is the point at which mitotic index (MI) reaches 0 and where plants require the maximum number of days in a warm, long day environment to force terminal budbreak. As a rule, many plants will break bud only after 200 such days. During this °GS, chilling temperatures release dormancy, rather than strengthening it, as during the previous °GS. Maximum Rest occurs around November 10 in coastal Oregon.

315°GS: End of rest. By this point, enough chilling has accumulated to complete rest but plants are held in dormancy by low temperatures. Spring budbreak (360°GS, 0°GS) is then stimulated by high temperatures, and the cycle repeats. End of Rest occurs at the end of December in coastal Oregon.

Dates provided are for the region around Corvallis, Oregon (N. lat. 44° 35'). In more northerly latitudes the period from 0°GS to 180°GS would tend to be more compressed with respect to calendar dates, and from 180°GS to 360°GS would be expanded. Moving south, the opposite would occur.

4.5.1.1 RGP and degree growth stages

The above hypothesis predicts that RGP would behave in the following manner relative to the °GS Model (Figure 4.4). At 0°GS, RGP would be decreasing rapidly because expanding shoots are becoming increasingly strong carbon sinks. As shoot expansion draws to a close, between 45°GS and 90°GS roots should regain their priority for carbon allocation and RGP should begin to increase. In species which continue to exhibit shoot elongation throughout summer (e.g. loblolly pine), this RGP peak may be modest or nonexistent.

After 180°GS as dormancy intensifies, RGP would weaken considerably to a low point between 225°GS and 270°GS. Then as chilling releases dormancy, from 270°GS to 360°GS, RGP would again rise to a peak or plateau. It would then fall as shoots elongate and again outcompete roots for carbon. Seasonal peaks and valleys

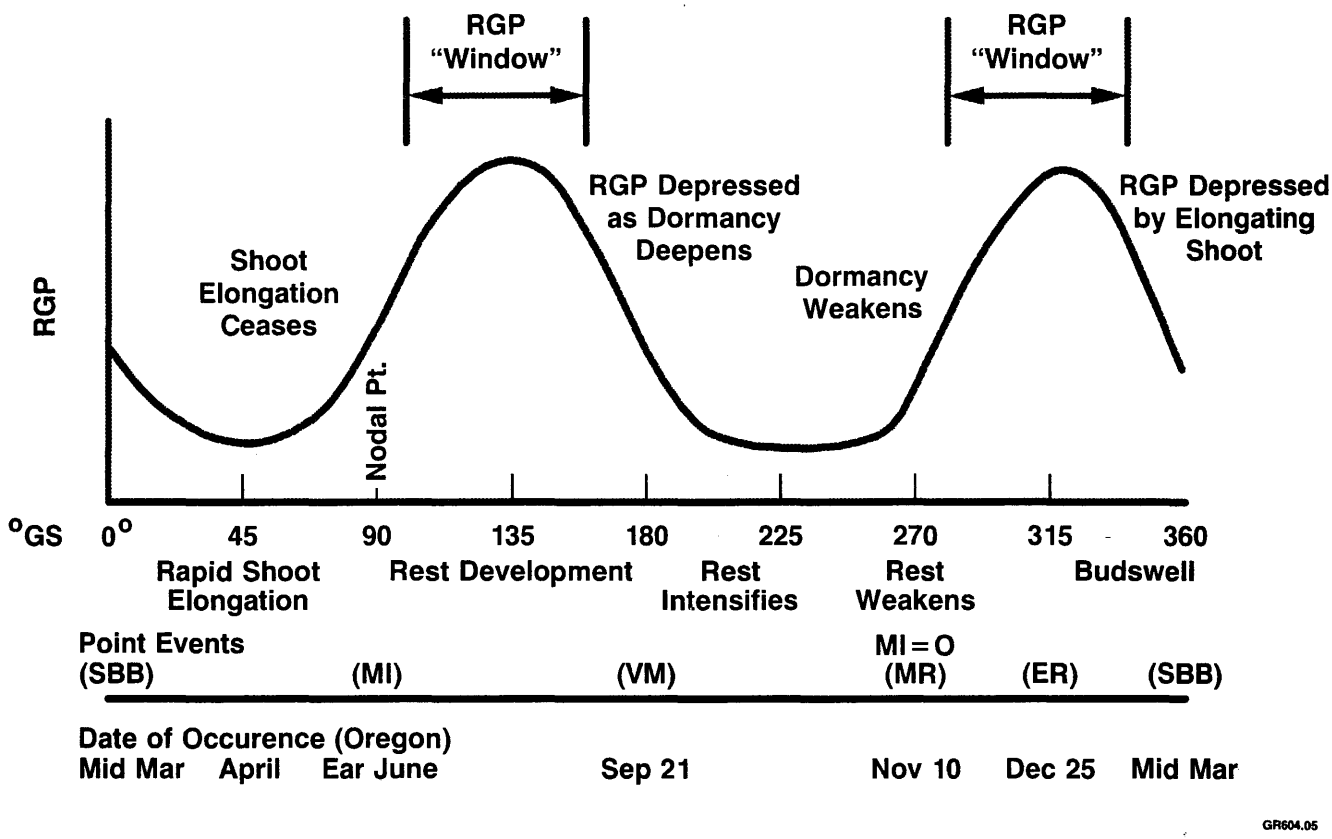


Figure 4.4—*Proposed model of seasonal changes of Root Growth Potential superimposed on Degree Growth Stages.*

of RGP, then, are modulated by changes in shoot dormancy status and sink strength.

4.5.1.2 Tests of the hypothesis

This hypothesis is suggested largely by seasonal RGP patterns reported in studies before 1980. To test the hypothesis, we will examine two case studies reported subsequent to 1980.

At least two difficulties arise in testing this hypothesis with existing data: (1) studies of RGP do not contain information on °GS, so these points must be inferred from reported calendar dates or observed phenological events, and the data calibrated accordingly, and (2) RGP studies are most often conducted during winter after the point of Maximum Rest (270°GS). Hence, only a small segment of the the seasonal pattern is available for evaluation. This is understandable because most interest in RGP is during the "lifting window" which normally begins in December in Northwest nurseries.

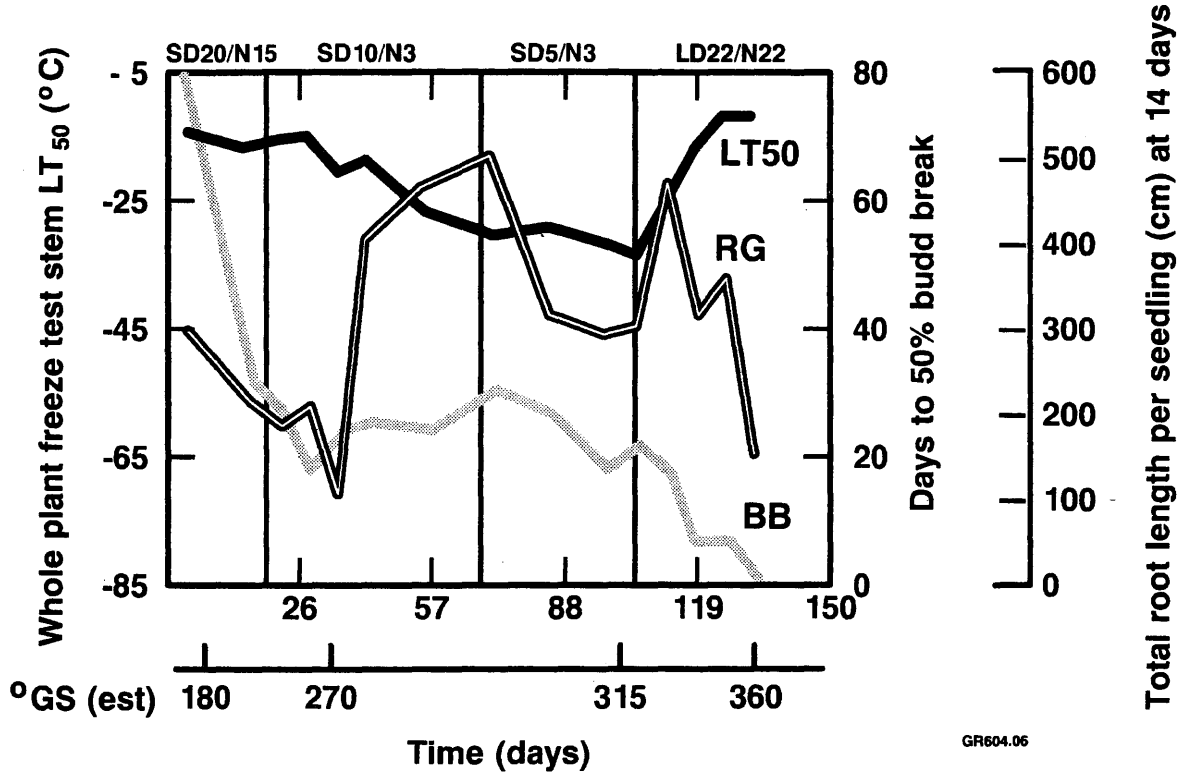
Nevertheless, two excellent recent studies have encompassed relatively broad seasonal sampling regimes and have also provided information on dormancy intensity, MI, cold hardiness, and shoot growth phenology in a range of species from diverse geographical locations. We shall now examine these studies toward gaining insight into the relationship between dormancy and RGP.

4.5.1.2.1 Ponderosa pine, Douglas-fir and Engelmann spruce in Arizona

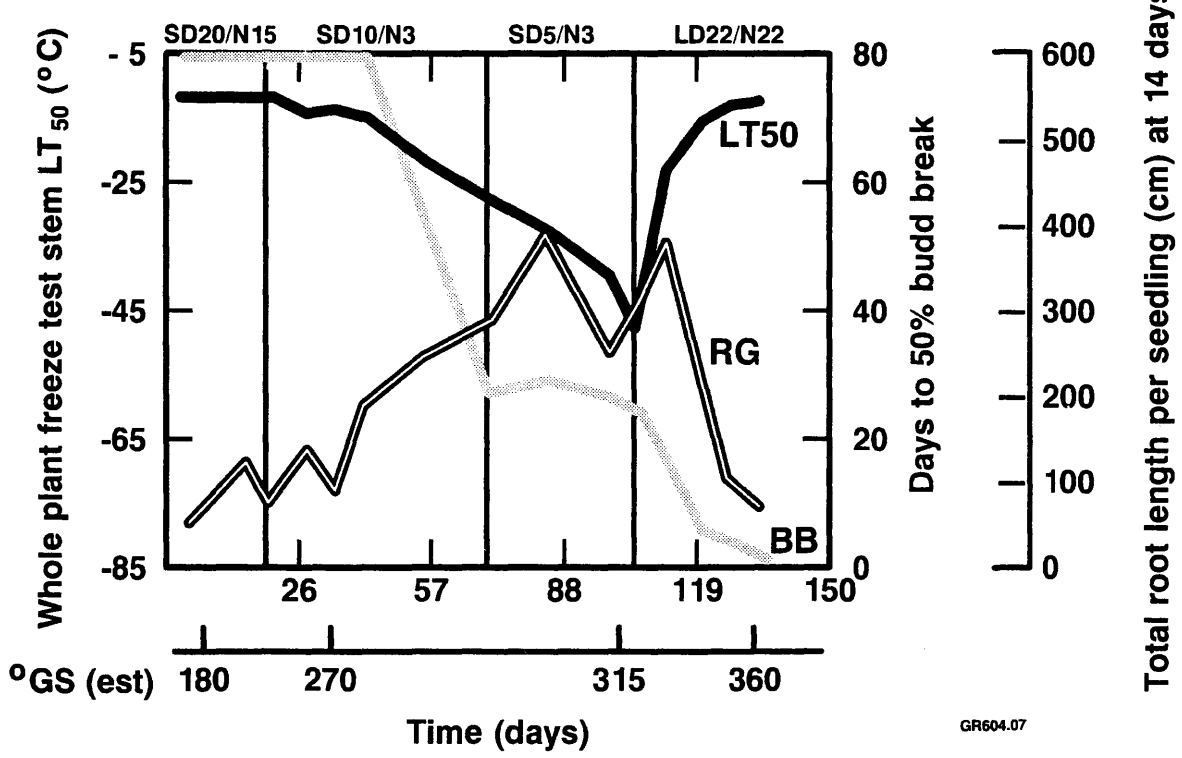
Burr et al. (1989) conducted intensive studies of RGP, dormancy intensity, and cold hardiness of ponderosa pine, interior Douglas-fir, and Engelmann spruce in controlled environment chambers. Four chamber environmental regimes were sequenced to induce dormancy and hardening, then to release dormancy and promote dehardening and budbreak. Dormancy intensity was measured with a budbreak test and hardiness was determined with whole-plant freeze tests.

Their results were calibrated against °GS from the curves of hardiness and budbreak data provided (Figures 4.5A-C). Patterns for each species were as follows. RGP was low prior to 270°GS then rose, sharply in ponderosa pine, to a peak or plateau at about 315°GS, then fell quickly as 360°GS approached. Maximum RGP coincided with maximum hardiness in all three species and this coincided

A. Ponderosa Pine

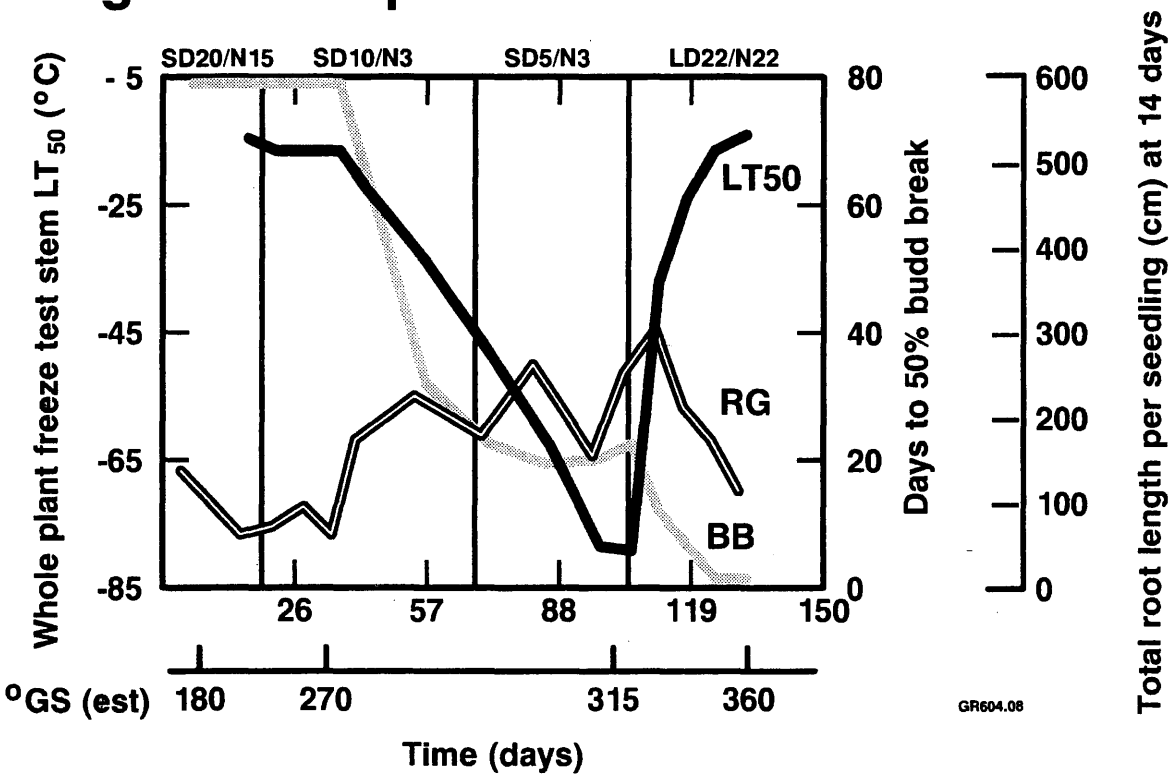


B. Douglas-fir



Figures 4.5A-B—*Changes in Root Growth Potential, cold hardiness and days to 50% budbreak in A. ponderosa pine and B. Douglas-fir in Arizona (Burr et al. 1989). °GS point events are estimated from phenological data. Reprinted with permission from Tree Physiol. 5:301 (1989).*

C. Engelmann Spruce



Figures 4.5C—*Changes in Root Growth Potential, cold hardiness and days to 50% budbreak in Engelmann spruce in Arizona (Burr et al. 1989). °GS point events are estimated from phenological data. Reprinted with permission from Tree Physiol. 5:301 (1989).*

with the period when dormancy was weakening but prior to shoot elongation. These patterns closely fit model predictions.

4.5.1.2.2 Sitka spruce in Scotland

Sitka spruce is widely planted throughout the British Isles, particularly in Scotland. Sitka spruce 2+1 transplants from the Queen Charlotte Islands (British Columbia, N. lat. 53°) were lifted from a nursery in southern Scotland (N. lat. 56°) from late September through early May and measured for RGP, and several other variables (Cannell et al. 1990). This study is particularly useful because it also provides information on several aspects of seedling growth phenology enabling close calibration with the °GS model across a ten-month period.

RGP was low in September and October then increased rapidly beginning in mid-November (Figure 4.6). It remained high until late April then fell to near 0 in early May. Mitotic Index (MI) reached zero about November 20. This establishes the date of the 270°GS point. Indeed, this point coincided precisely with peak dormancy status and the beginning of the rise in RGP. MI increased again early March and shoot expansion in May, 180°GS. These results are also in good agreement with model predictions.

4.5.1.2.3 Conclusions

The hypothesis holds up well under the above independent data sets. Granted, there is some latitude for interpretation of °GS stages in these studies and other investigators might offer different interpretations. Nevertheless, results from several diverse species in two independent studies do not deviate far from model predictions.

Direct tests of this hypothesis would be more powerful than the observational tests offered above. Such tests might involve the artificial release of dormancy between 180°GS and 270°GS to induce an RGP response. This might be achieved with any number of environmental or chemical agents (Fuchigami and Cheng-Chu Nee 1987). Another simple test would be removal of elongating shoots to eliminate their influence as carbon sinks during periods of low RGP. At the least, more detailed studies of other species in which RGP and °GS are determined on a year-round basis would provide valuable additional tests.

Sitka Spruce

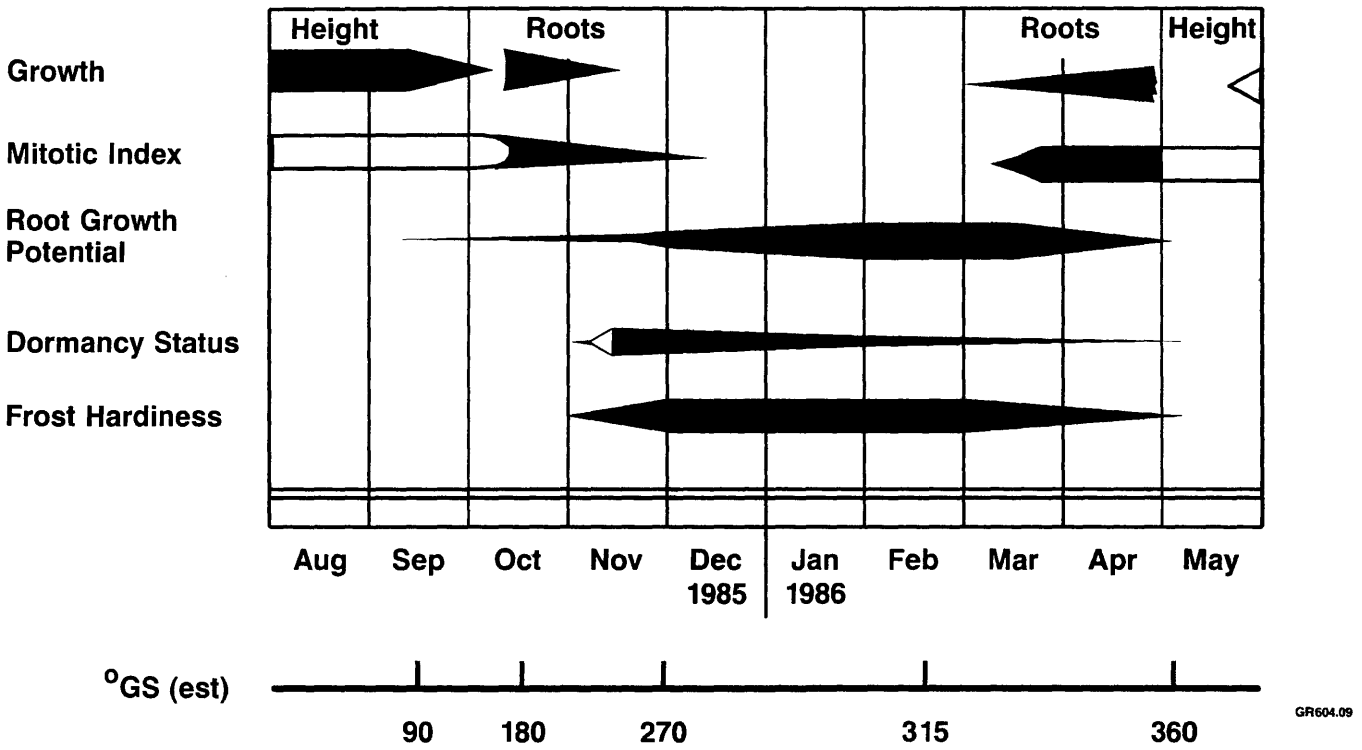


Figure 4.6—*Seasonal changes in Root Growth Potential, growth, mitotic index, dormancy status, and frost hardiness of Sitka spruce in Scotland (Cannell et al. 1990). °GS point events are estimated from phenological data. Reprinted with permission from Forestry 63:21 (1990).*

4.6 Why Does RGP Work?

When one reads the older (and even more recent) literature on RGP, one often finds statements to the effect that: "In order to become established after planting, a tree seedling must rapidly produce new roots to enable it to obtain water and minerals from the soil. Therefore seedlings with high RGP will have a better chance at survival." On the surface this logic seems sound and has pervaded the RGP literature for years. However, as pointed out by Ritchie (1985), seedlings are rarely planted into soils which are warm enough to permit roots to grow. In fact, throughout most of the Pacific Northwest, January- or February-planted seedlings must endure from two to four months before soils warm to the range in which root initiation and elongation can begin (Nambiar et al. 1979, Abod et al. 1979, Stupendick and Shepherd 1979, Ritchie 1985).

From this observation it would seem that RGP tests, conducted in 20°C soil, would have little or no bearing on what happens on the planting site (see c.f. Sutton 1983). Nevertheless, as pointed out in Section 4.4 above, RGP tests are often very good predictors of survival. One is then left with the question: Why?

There are probably two parts to the answer: the first has to do with RGP values which fall *within* normal seasonal ranges, and the second with those that fall *outside* normal seasonal ranges.

4.6.1 When RGP falls within normal seasonal ranges

As proposed in Section 4.5 above, RGP is highest when shoot dormancy is weak but when shoots are not elongating. Seasonally, this occurs during late summer into autumn, and then again in mid- to late-winter. RGP is very low in spring during shoot elongation and early winter when dormancy intensity is high.

Stress resistance and cold hardiness begin to develop at about 180°GS and peak in the range of 270°GS to 315°GS. RGP is rapidly increasing in this range. Therefore, high or rapidly increasing RGP is a signal that seedlings are at or near their seasonal peak of stress resistance and cold hardiness. Dehardening can be rapid after 315°GS and by 360°GS seedlings are completely dehardened and highly susceptible to stress. RGP is then low, denoting a seedling with low stress resistance.

By this reasoning, RGP itself does not determine survival potential, but instead indirectly indicates when seedlings

have high survival potential because they have high stress resistance. This argument has been set forth earlier (Ritchie 1985).

4.6.2 When RGP falls outside normal seasonal ranges
When RGP falls outside normal seasonal ranges it can indicate that the seedling is suffering from damage, disease, or other stresses which may portend poor performance or mortality. This logic turns on the observation (van den Driessche 1987) that short-term bursts of new root growth (hence RGP) occur at the expense of currently assimilated carbon—not stored carbon. This is a very important finding because it leads to the following line of reasoning.

If a seedling exhibits strong RGP then:

1) photosynthesis must be occurring, therefore

2) all the metabolic pathways that support photosynthesis must be functional, and

3) stomata must be open, therefore

4) transpiration, hence water uptake and transport must be occurring, therefore

5) the xylem system must be open and functional from root to shoot, and roots must be taking up water,

6) downward translocation of photoassimilate must be occurring, therefore

7) there must be an intact, functional phloem pathway from shoot to root,

8) root tips are capable of growing, therefore

9) root respiration must be occurring, therefore

10) all the metabolic pathways that support root respiration must be functional, etc.

These relationships can be demonstrated by girdling, defoliating, or holding seedlings in darkness or CO_2-free air (van den Driessche, pers. comm.) while testing RGP. In Douglas-fir each of these treatments effectively stops root growth.

It follows that if RGP falls within some "normal" range for a given species at a given time of year it is good evidence that there is nothing markedly wrong, structurally or metabolically, with that seedling. In contrast, if RGP values fall below what is known to be "normal" a red flag is thrown up and further testing is called for. The RGP test gives no clues to what the problem might be, but it does signal that a problem exists.

RGP testing is far more useful for sorting out bad or damaged seedling lots than for predicting survival.

4.7 Summary and Conclusions

In this review we have tried to focus on some key points bearing on the conduct and interpretation of RGP tests in reforestation. In our view these points are:

1. RGP is developed in the seedling during its tenure in the nursery and is expressed after the seedling is planted. The appropriate point at which to measure RGP is as soon before planting as possible because RGP can change rapidly.
2. The RGP measurement period need not be lengthy—ample evidence now exists that 15 or even 7-day tests can often be used successfully. However, it is important that environmental conditions remain consistent among tests because of the sensitivity of RGP to these conditions.

3. The primary value of RGP is its ability to characterize seedling physiological quality at a point in time, *not* to predict field performance. In this light RGP testing should be viewed as analogous to seed testing.

4. RGP is not a perfect predictor of field performance. This is because RGP test results are confounded by site and planting conditions which vary greatly.

5. However, RGP does have some predictive value because it indicates (a) when seedlings are physiologically resistant to stress, and (b) when seedlings are in some way damaged.

6. RGP periodicity seems to be modulated by two internal factors: (a) the depth, or intensity, of shoot dormancy, and (b) the strength of the carbon sink in the elongating shoot. When dormancy is weak but shoots are not actively expanding, RGP tends to be high, and vice versa.

7. Despite problems associated with lack of accuracy and precision and unrealistic expectations, when conducted and interpreted properly RGP testing remains a very valuable tool for assessing quality of planting stock.

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Target Seedling Symposium

Chapter 5
Target Seedling Root System Size, Hydraulic Conductivity, and Water Use During Seedling Establishment

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ABSTRACT

Basic survival requires that a seedling root system be large enough to supply water in amounts that cover transpirational loss. Because transpiration is an interactive phenomenon influenced by the planting environment as well as the shoot and root morphology of the tree, what might be sufficient seedling morphology for one geographic region could be inadequate for another region or another species within the same region. A target seedling morphology that has worked well for loblolly pine in the South can be described as 10-12 inches tall, 4 mm in diameter, and having 6 or more lateral roots. The net result is a seedling that has a high probability of survival. The focus of this paper is how hydraulic conductivity and seedling water use can be used to quantify relationships between morphology and function.

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5.1 Introduction

Morphological characteristics of target seedlings should be defined to a large degree by foresters observing success and failure of plantations. These characteristics are imposed by practical limitations of the planting job. These include the size of the root system that can be properly planted and the weight and size of seedlings that can be carried by a planter or placed on a planting machine. One of the factors heavily affected by the morphology of the seedling is the balance between the capacity for water uptake and water loss due to transpiration. The purpose of this paper is to discuss some of the factors affecting the capacity of the root system for taking up water immediately after planting and during the process of establishment and to relate these factors to target seedling design.

5.2 Root Tissues and Water Uptake in Seedlings

Seedlings in a bareroot nursery bed have root system development to a depth of about 15 inches in the first few months (Huberman 1940). Undercutting, root wrenching, and lateral pruning are used to promote development of the root system (Rook 1969, Tanaka et al. 1976) within the depth and lateral width that can be easily planted properly. The resulting root system is mostly woody, with some suberized and unsuberized root tips (depending on month of lifting and prevailing soil temperature), and mycorrhizal fine roots.

Kramer and coworkers have shown that suberized roots and woody root tissues can and indeed must be the location of a major proportion of water uptake in tree root systems (Kramer 1946, Kramer and Bullock 1966, Chung and Kramer 1975, MacFall et al. 1990). This uptake apparently is facilitated by the presence of lenticels on the surface of the root, and by discontinuities in the periderm (bark) plates. Magnetic resonance images of water depletion around loblolly pine seedling roots indicate that soil water depletion is uniform around the tap root rather than just around points where lateral roots disrupt the continuity of the vascular cambium (MacFall et al. 1990). These studies also showed depletion around the tap root prior to depletion around the laterals. It is clear that woody roots are areas of importance in water uptake.

Unsuberized (white) root tips that occur on growing roots are the region of highest uptake per unit of surface area (Sands et al. 1982). Suberized roots also conduct water but at a lower rate per unit area (Chung and Kramer 1975). Sands and coworkers (1982) reported the water uptake rate to be 1.95×10^{-6} cm/s/0.1 MPa pressure differential for unsuberized white root tips, compared with 7.55×10^{-7} cm/s/0.1 MPa in suberized brown root tips. Thus unsuberized roots have the potential to conduct about 2.6 times the volume of water in a given time period at a given water potential gradient than do suberized roots. The root system capacity for water uptake when water is readily available results from relatively lower permeability through the large surface areas of woody and suberized roots and higher permeability through the smaller surface area of unsuberized root tips.

5.3 Effects of Planting on Subsequent Water Relations

It is well known that seedlings should be planted into soil that is moist to the touch. The quality of the planting job determines the availability of soil moisture to the seedling. Seedling roots should be planted with tight soil contact; that is, air pockets should be forced out as the hole is closed. Most of us are familiar with the method described in Figure 5.1 but failure to follow it is still a frequent cause of plantation failures. Site preparation methods such as ripping make it easier to consistently plant seedlings correctly. Even when seedlings are well planted the relative reduction in root-soil contact due to transplanting can be a cause of reduced water uptake and transplanting stress (Sands 1984).

5.4 Hydraulic Conductivity of the Newly Planted Root System

Most planting operations take place in the dormant season when soil temperatures are cool. These cool temperatures decrease the permeability of root membranes and increase the viscosity of water (Kramer 1934, Kramer 1940, 1942, Kaufman 1975, Lopushinsky and Kaufman 1984). Rate of water uptake (hydraulic conductivity) in a loblolly pine seedling after lifting and cold storage is a function of the temperature of water surrounding the roots (Figure 5.2). Root growth is limited by low soil temperatures (Bilan 1961, Stone and Schubert 1959, Andersen et al. 1986) and the magnitude of the effect of temperature on root growth varies by genetic family (Carlson 1986, Nambiar et al. 1979). Temperature affects both the rate of uptake of available water and the rate of metabolism, and thereby influences the length of time that a newly planted seedling will remain with little or no new root growth.

It follows that there is a time period from planting until new root growth occurs when a seedling is dependent on its nursery cultured root system for water uptake. During this period the size of the planted root system determines the rate at which water will be taken up at a given water potential and temperature (Figure 5.3, Carlson 1986). As new root growth occurs, water uptake under pressure (Figure 5.4) becomes a function of the number of new roots (Carlson 1986).

5.5 Water Transport Through Seedlings

Water transport through the seedling is driven by transpiration. Water is lost through the stomatal pores in the leaf

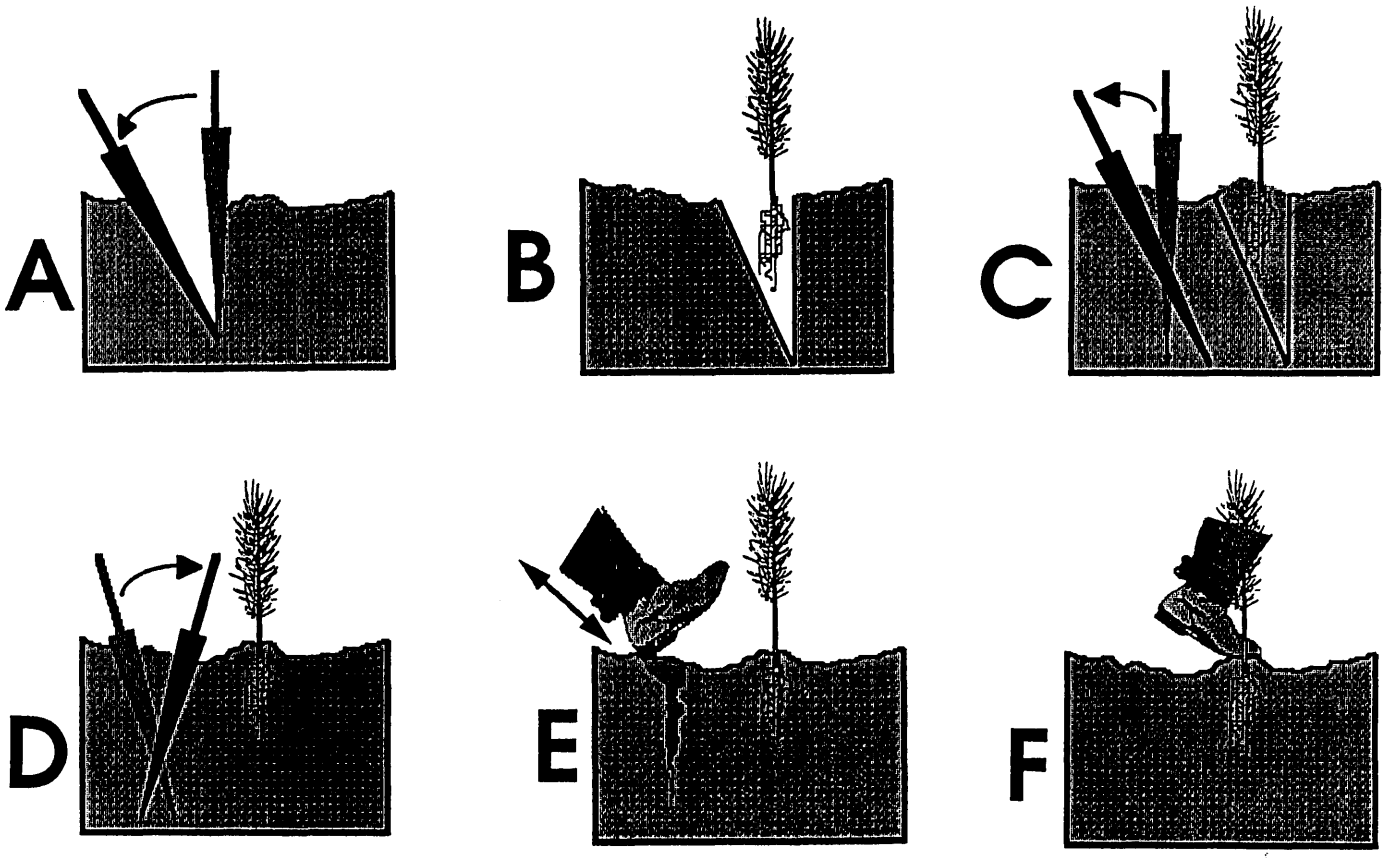


Figure 5.1—*Planting method for ensuring tight root-soil contact. A. Open the hole deeper than the tree will be planted; B. Place tree initially deeper than it will be planted, and pull upward to correct depth (3-6 cm below root collar) to straighten roots; C. Close bottom of hole avoiding air pockets; D. Close top of hole avoiding air pockets; E. Close dibble extraction hole; and F. Firm soil at base of seedling. Seedling should be firmly in the soil.*

surface and to a lesser extent through the leaf cuticle. As water is lost from the leaves, a water potential gradient builds from the leaves, down the stem, into the root system and root-soil interface causing water to move into the plant (Kramer 1939). When transpiration is severely reduced by stomatal closure, flow continues until the within-plant gradient is reduced to the point that the water potential in the root is at equilibrium with that in the soil. Figure 5.5 shows the relationship between water loss by transpiration (measured as weight loss, g/hr) and water uptake (measured as flow through the stem, ml/hr) in a loblolly pine sapling. Kramer (1937) showed similar relationships for seedlings of several species. Note that considerable water is lost by transpiration prior to the beginning of flow. This is because a water potential gradient must form to initiate flow. Such gradients have been documented in large trees (Schulze et al. 1985). As soils

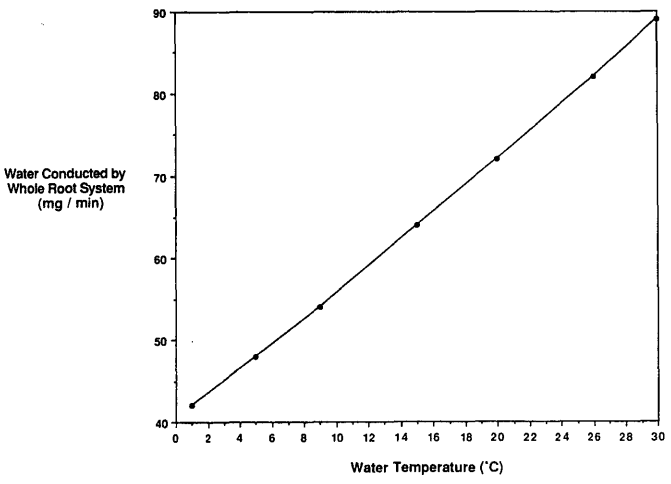


Figure 5.2—*The relationship between water uptake of a loblolly pine root system and water temperature. The apparatus used in obtaining these data is shown in Figure 5.4. Pressure was set at a constant 0.3 MPa. The seedling had a root volume of 4.5 ml and had been potted in sand in a greenhouse at 20°C for 28 days prior to measuring hydraulic conductivity.*

dry and soil and predawn plant water potentials become more negative, the maximum transpiration for the day, estimated in Figure 5.6 by leaf conductance, decreases (Teskey et al. 1986, Carlson et al. 1988). Decreased transpiration, if uptake continues, decreases the hydraulic gradient (increasing water potential) thus reducing stress (Kramer 1937, Grossnickle and Russell 1990).

Soils usually dry from the surface downward therefore it is important that roots grow downward at a rate that will keep a portion of the seedling root system in moist soil. Seedlings that have even a few roots in moist soil are able to recover from midday water stress by the following morning (Farnum 1977, Brissette 1990).

5.6 Target Seedling Morphology and Water Use

Seedling height and needle area are interrelated (Figure 5.7A). Taller seedlings have a greater surface available for both photosynthesis and for water loss by transpiration. Similarly, seedlings with large root volumes have a greater surface area available for water uptake (Figure 5.7B). When whole plant water use is monitored, it can be seen that in fact taller seedlings tend to use more water (Figure 5.8A) as do those with larger root systems (Figure 5.8B). Note the separation of data points around the regression in Figure 5.8B. This is due to some interactions between the morphological and physiological factors that we have been discussing. Specifically some seedlings form new roots sooner than others, and those that form roots more quickly have higher hydraulic conductivity (and therefore lower stress)—and as a result—greater leaf conductance and higher water use. Since trees with larger root volumes also have greater root growth potential (Carlson 1986), the effect is magnified.

Water use as related to seedling morphological balance should be considered on a whole tree basis. Transpiration measurements taken by porometer are point samples recorded on small samples of leaf area. If one multiplies transpiration per unit area by total leaf area and by the photoperiod (the maximum time period stomates are

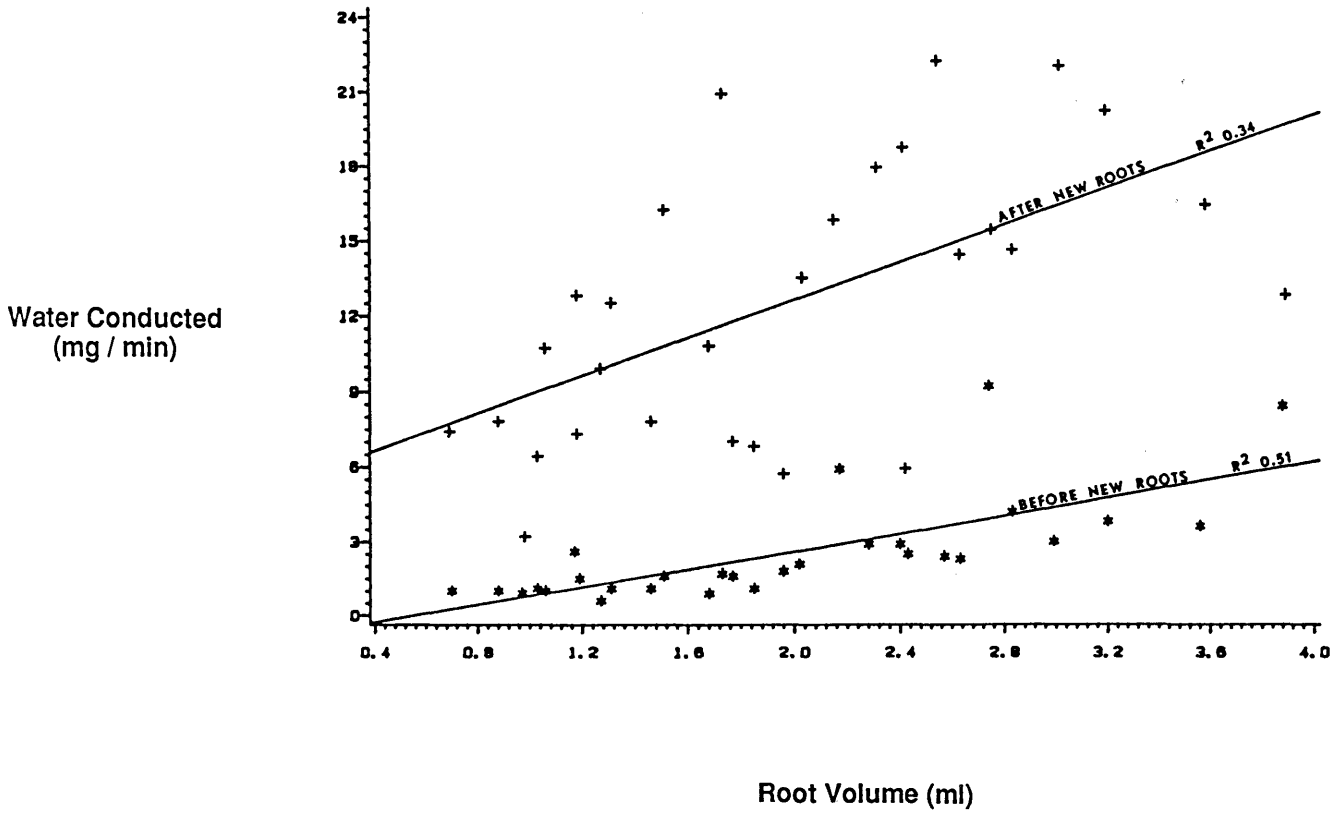


Figure 5.3—*Water conducted by whole root systems of loblolly pine as a function of their root system volume and root growth status. (Hydraulic conductivity was measured at a pressure of 0.2 MPa and a temperature of 20°C in an apparatus similar to that shown in Figure 5.4.) Seedlings in the "before-new-root-growth" group were matched in pairs for root volume to those in the "after-new-root-growth" group. The before new root growth group was tested immediately after cold storage, whereas the other group was potted in sand and held in the greenhouse for 28 days at 20°C prior to testing.*

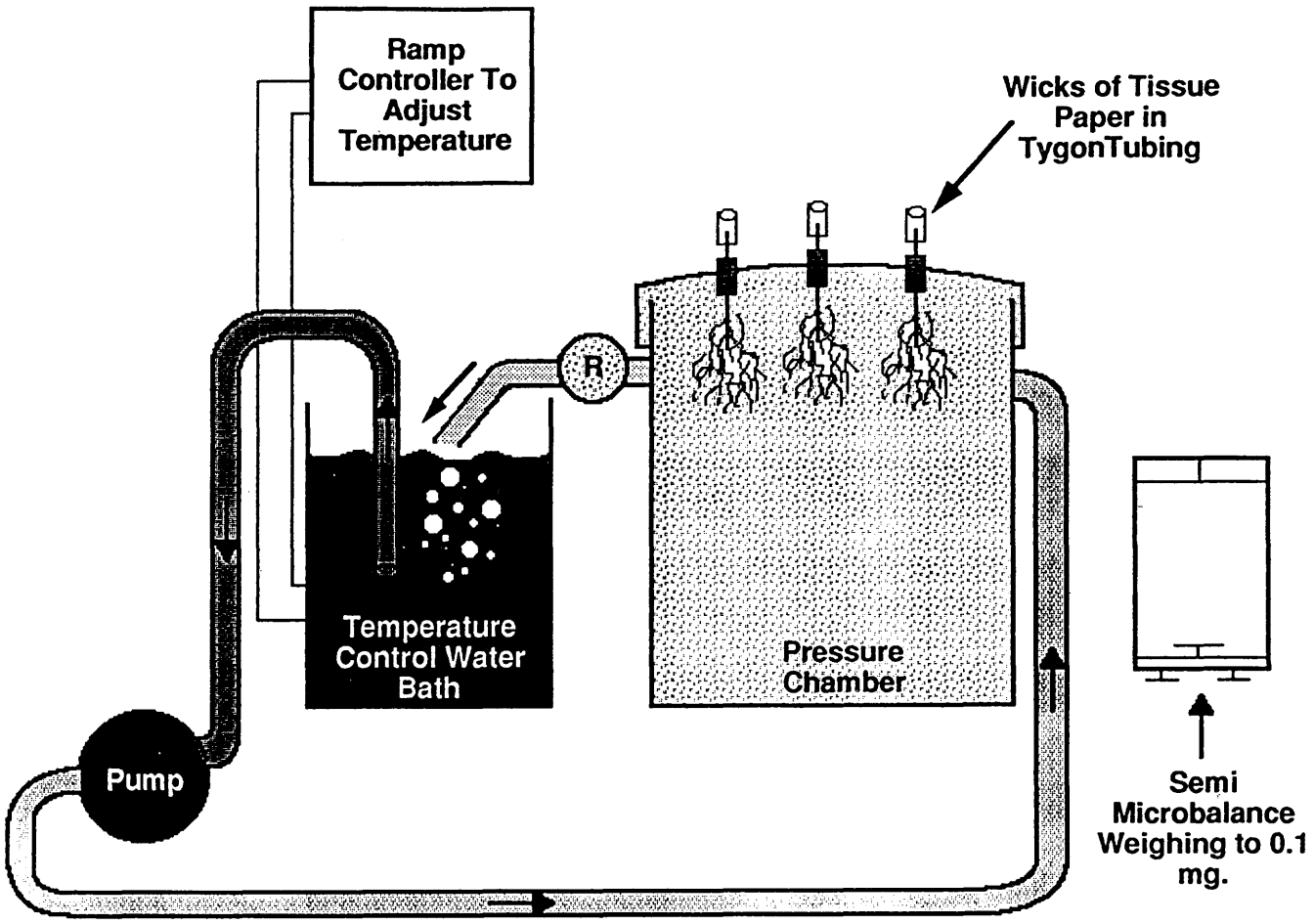


Figure 5.4—*An apparatus for assessing the hydraulic conductivity of seedling root systems. Seedling roots are washed free of soil and detopped about 5 cm above the root collar. The lower shoot is placed through a screw type pressure fitting in the lid of the pressure chamber that includes a custom made silicone rubber washer under a metal washer and cap. As the cap fitting is tightened the metal washer compresses the silicone rubber washer facilitating a water-tight seal. The pressure chamber lid is then placed in the chamber which is full of water. The pressure of the water around the roots is set at the regulator (R) on the outflow side of the chamber and is caused by pumping aerated water from a controlled temperature bath into the closed chamber. A ramp type programmable controller is used to control the temperature of the water bath, which is either cooled by refrigerant circulated through immersion coils or warmed by immersion heaters. The root is allowed to equilibrate for 15 minutes. Then the stump is wiped dry, and a preweighed wick is placed on the stump. At five minute intervals the wicks are replaced, and the wet wicks are weighed to 0.1 mg. Conducted water is determined by subtracting dry wick weight from wet wick weight. The pressure chamber used was modified from a common pressure cooker.*

open), then one can account for 68 percent of the variation in water use (Figure 5.9A). In addition, there is an important interaction with root development. Figure 5.9B shows a regression of needle area/new root area against water use. Note that on the left-hand side of the figure, where ratios favor new root area, there is maximal water usage. On the rest of the graph, water use is limited by low conductance of poorly balanced seedlings (ratio of needle area to new root area too high). Further interactions occur when reduced conductance limits currently available photosynthates and thereby new root growth.

The nature of the various morphological and physiological interactions discussed above can cause estimates of the effect of root size on the rate of water uptake under pressure (Figure 5.4) to differ from the estimate of the effect of root size on water use by intact seedlings. Thus to estimate effects of root parameters alone, studying the rate of water uptake under low pressures in an apparatus such as that shown in Figure 5.4 is appropriate. For studying interactions between transpiration and uptake, studies on intact seedlings where water use is quantified by weight loss of potted seedlings and transpiration mea-

sured by diffusion porometer (e.g., Figures 5.8-5.9) are preferable. Water flow sensors such as the Dynamax sensor used in Figure 5.5 can also be used to characterize water use in intact trees.

5.7 Seedling Carbohydrate Management During Establishment

When a seedling is under moisture stress, both leaf conductance and rate of photosynthesis are reduced. This leads to a reduction in reserve carbohydrates and currently available photosynthate, the latter of which is considered to be the primary energy source for root growth in some species (van den Driessche 1987, Phillipson 1988). This was discussed clearly in a recent review of seedling establishment by Reitveld (1989). The take home messages for purposes of this discussion are twofold: 1) the period of seedling establishment can be lengthened by moisture stress and depletion of carbohydrate reserves which limit root growth (Kaufman 1968); and 2) the period of establishment characterized by altered water and carbohydrate status can last more than one year (Baldwin and Barney 1976, Orlander 1986).

5.8 Water Availability in Drying Soils During Establishment

Without precipitation, the soil water potential around the roots will continue to decrease causing the plant's equilibrium water potential (measured predawn) to likewise become more negative. In this scenario, progressively lower root water potentials are necessary to initiate flow (Faiz and Weatherly 1978). Increasingly negative water potentials can cause roots to shrink away from the soil, causing even higher resistance to water uptake.

5.9 New Root Growth and Root System Hydraulic Conductivity

Root growth after planting improves water uptake in sev-

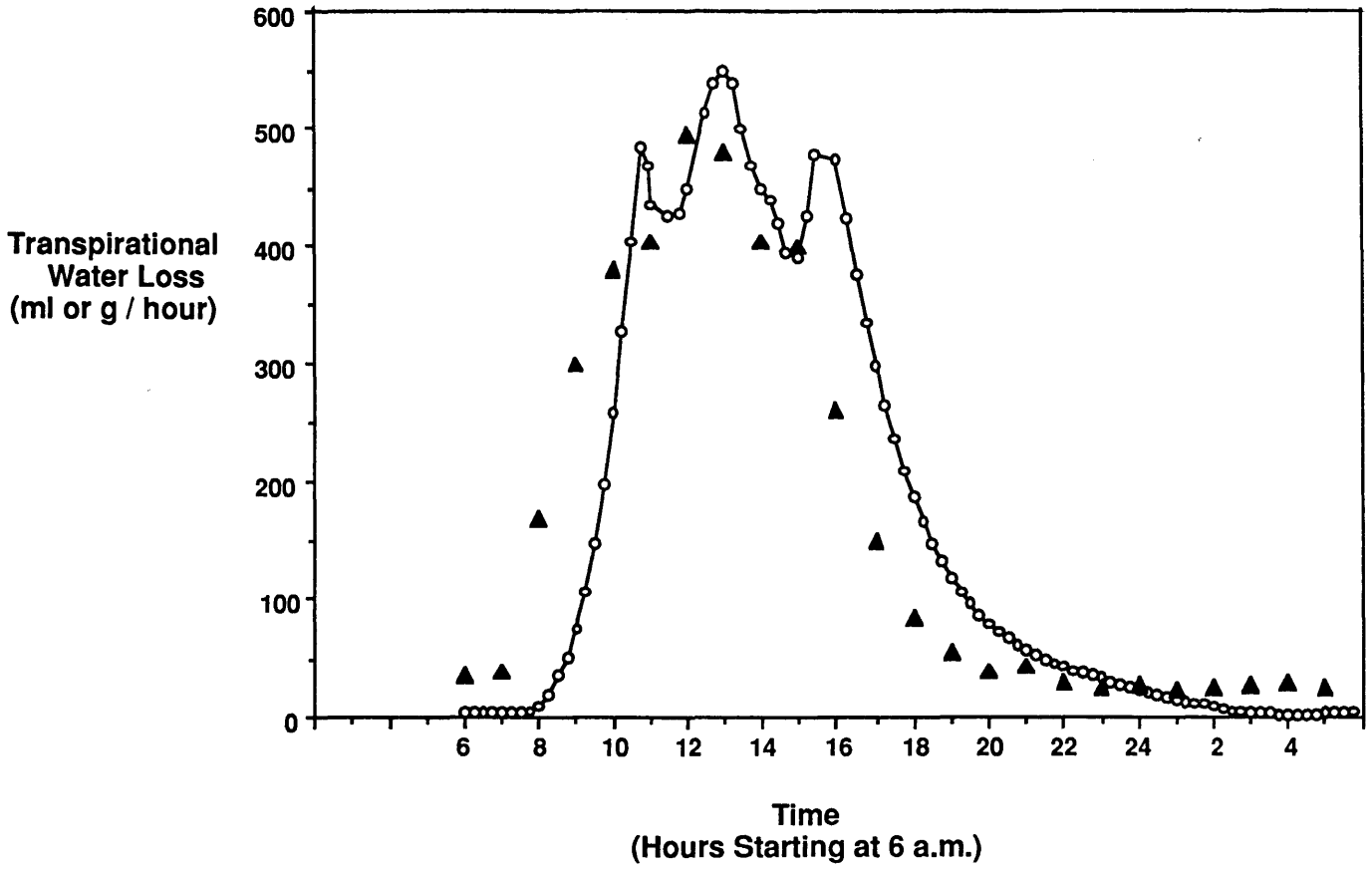


Figure 5.5.—*Transpirational water (Δ) measured as weight loss and water uptake (O) measured as flow through the main stem of a 6.0 cm dbh loblolly pine sapling. Flow through the stem was measured with a Dynamax sensor (Dynamax Inc., Houston, Texas). For this day, the sensor overestimated flow by 0.7%. In order to attain this low error level, insulating foam on the sensor was cleaned and dried weekly. The two curves are out-of-phase by approximately 2 hours; this effect is caused by transpiration building a negative hydraulic pressure gradient in the stem prior to the beginning of flow. Conversely, that gradient caused flow to continue until water potentials came to equilibrium many hours after stomatal closure.*

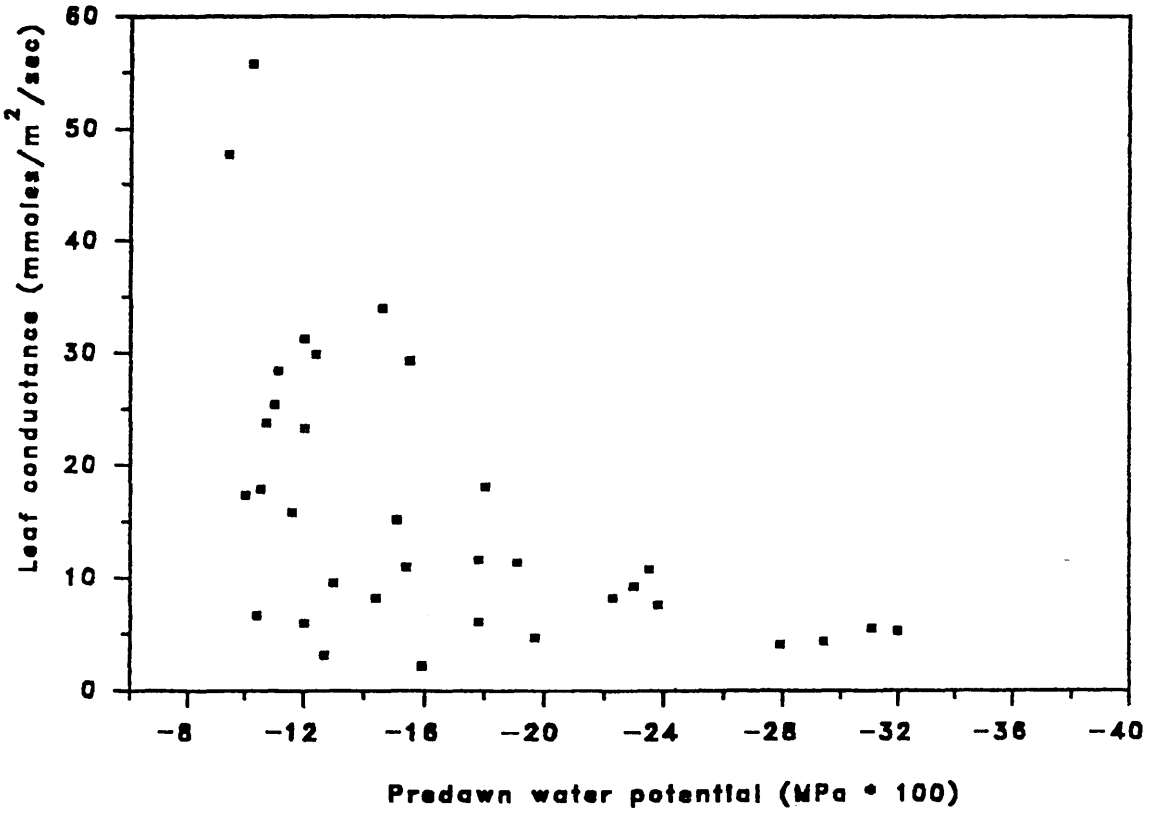
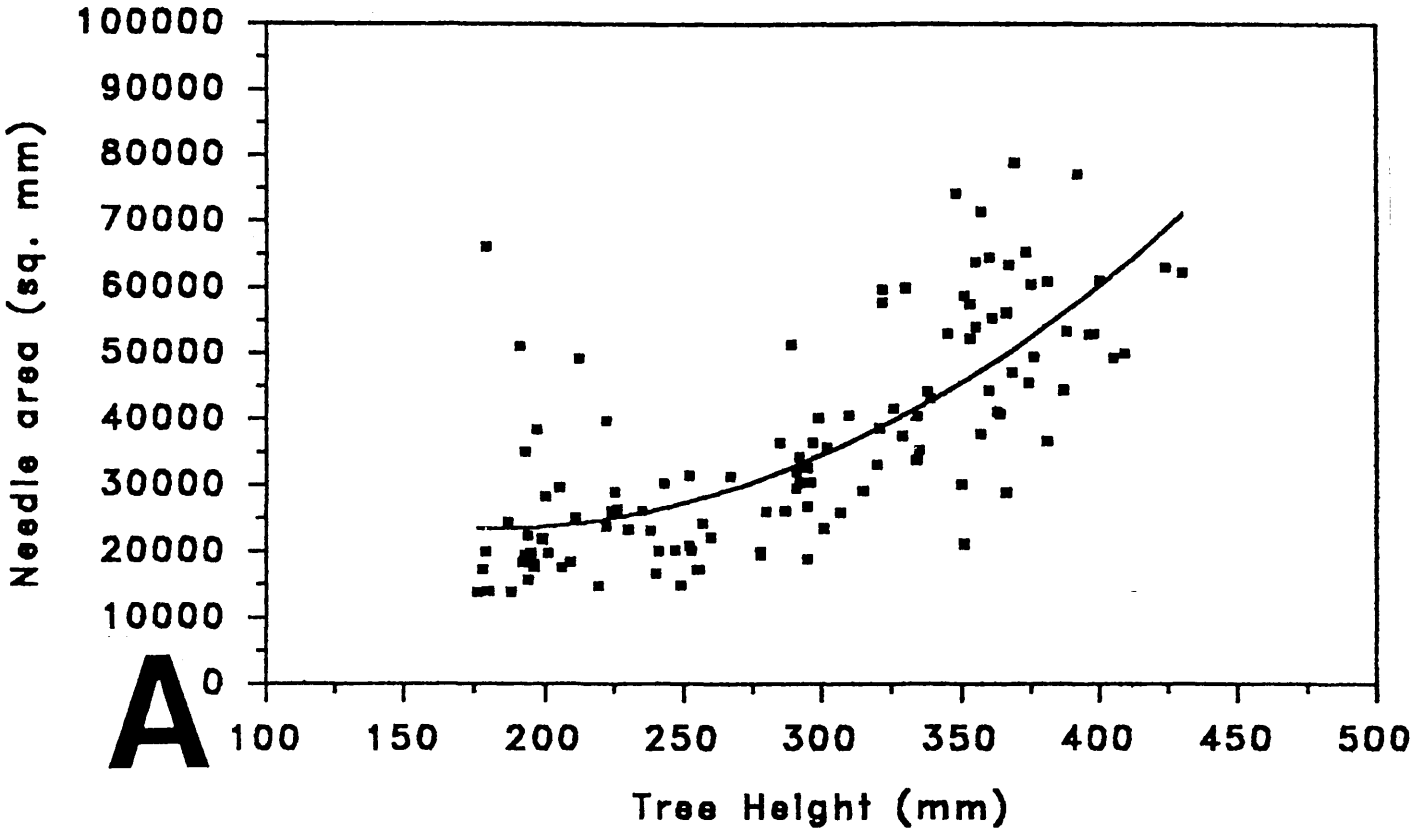


Figure 5.6—*Relationship between leaf conductance and predawn seedling water potential. In order to create a variation in stress levels, a group of seedlings was irrigated with tap water, another group with an aqueous solution of polyethylene glycol (molecular wt. 6000) at an osmotic potential of 0.3 MPa, and another group with aqueous PEG at 0.6 MPa. Seedlings were brought to field capacity, placed with their pots in plastic bags, with a rubber septum on each bag. Each day at the same time, a pot was weighed, water use calculated, and that amount of tap water added to the surface of the soil in the pot by inserting a syringe needle through the septum. Water potential was measured predawn with a pressure chamber (PMS Instruments, Corvallis, Oregon). Leaf conductance was measured midday with a LICOR 1600 diffusion porometer (LICOR Instruments, Lincoln, Nebraska).*

eral ways: 1) new root tips are unsuberized and highly conductive to water, 2) new root tissues are in tight contact with the soil, and 3) extension of the root system will increase the soil volume from which water can be extracted. Increased conductivity after root growth is demonstrated in Figure 5.3, indicating that after new root growth, whole root system conductivity is proportional to the number of new root tips (Carlson 1986). Thus for rapid establishment it is desirable to plant a seedling having a large root surface area to increase the availability of water prior to root growth, and to increase the number of growing root tips when the soil warms enough to promote such growth.

5.10 Considerations for Target Seedling Design, Culture and Use

Ensuring good hydraulic conduction by the root system involves almost all of the factors that lead to rapid seedling establishment (Figure 5.10). Many of these factors are discussed in detail because they must be integrated to obtain consistently good seedling survival and growth. In the South, Weyerhaeuser Company foresters designed a target seedling 8-10 inches (20-25 cm) tall, 4-5 mm groundline diameter, with a minimum of 6 first-order lateral roots, a well developed terminal bud, and having mostly mature secondary needles. This seedling morphology was designated after close inspection of success and failure of individual seedlings on reforestation sites over a period of a few years. Since soils and climate play major roles in seedling establishment, other land owners should determine the target seedling morphology that works best on their sites and with their species. The role of research is to create nursery cultural practices that will increase the proportion of target seedlings in the crop. Research should also clarify morphology-function relationships such as root system parameters that foresters cannot easily observe when scoring plantation success. Foresters and researchers should work with nursery managers to provide the best quality results.



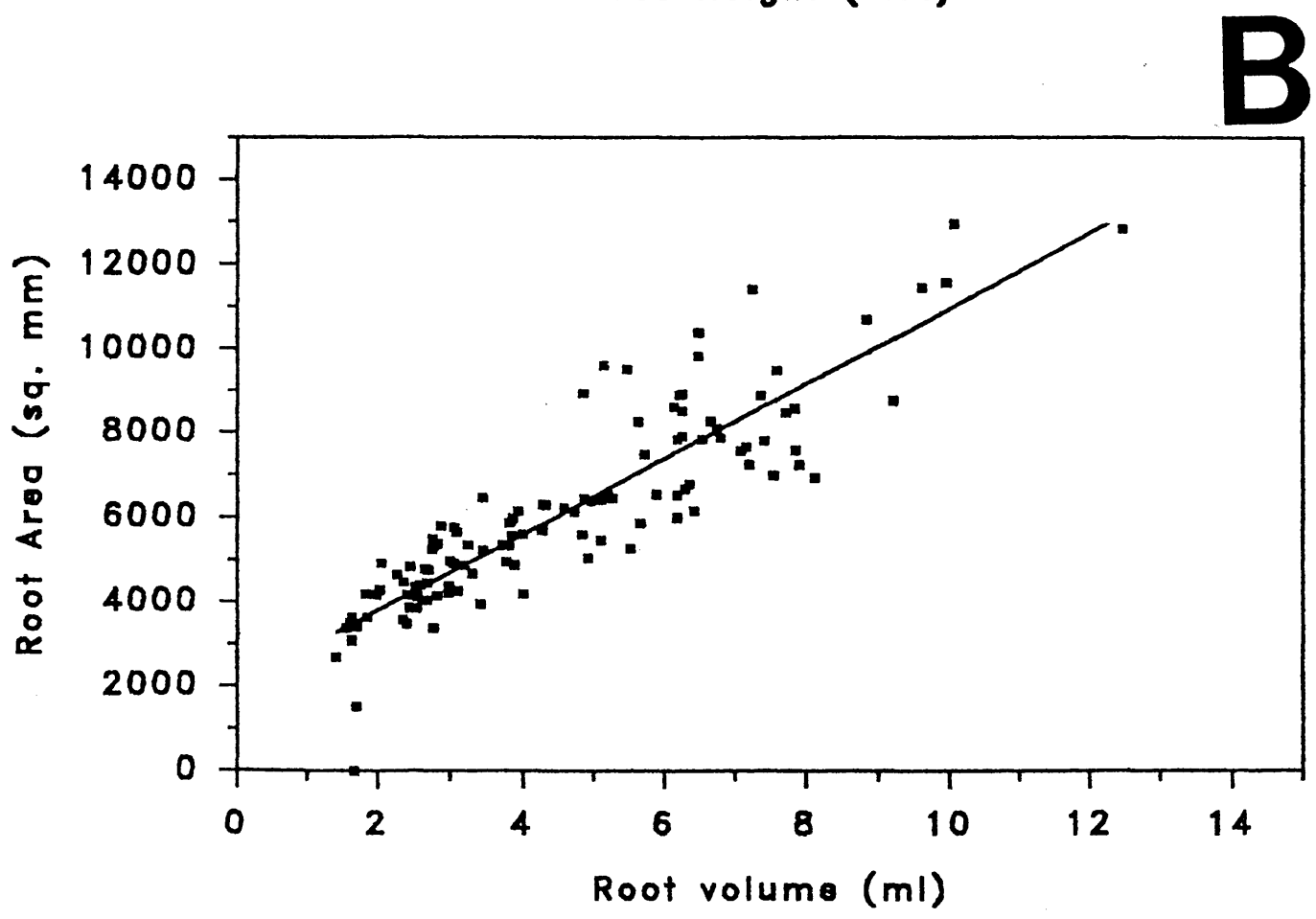


Figure 5.7—*Height was a moderately good estimator of needle surface area (R^2 = 0.71) (A) and root volume was a reasonably good estimator of root surface area (R^2 = 0.79) (B). These seedlings are from the same study as those in Figure 5.6.*

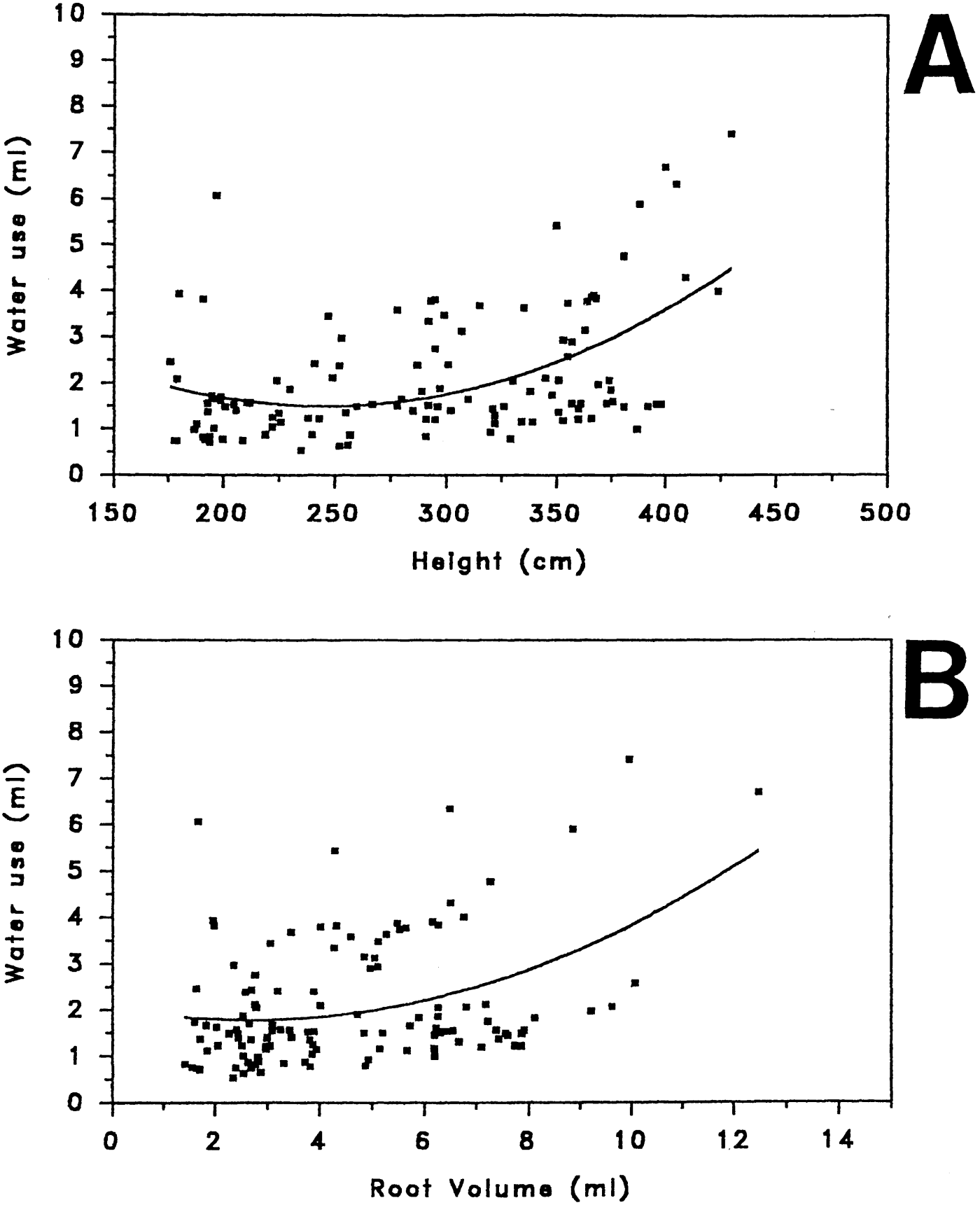


Figure 5.8—*Water use by loblolly pine seedlings kept at field capacity: (A) Effect of seedling height on water usage (R^2 = 0.80), and (B) Effect of root volume on water usage (R^2 = 0.15). Reasons for the separation of points around the regression line and thus the low R^2 are discussed in the text. Seedlings were from the study described in Figure 5.6 and were in the group irrigated with tap water only (i.e., no artificial stress was created with PEG).*

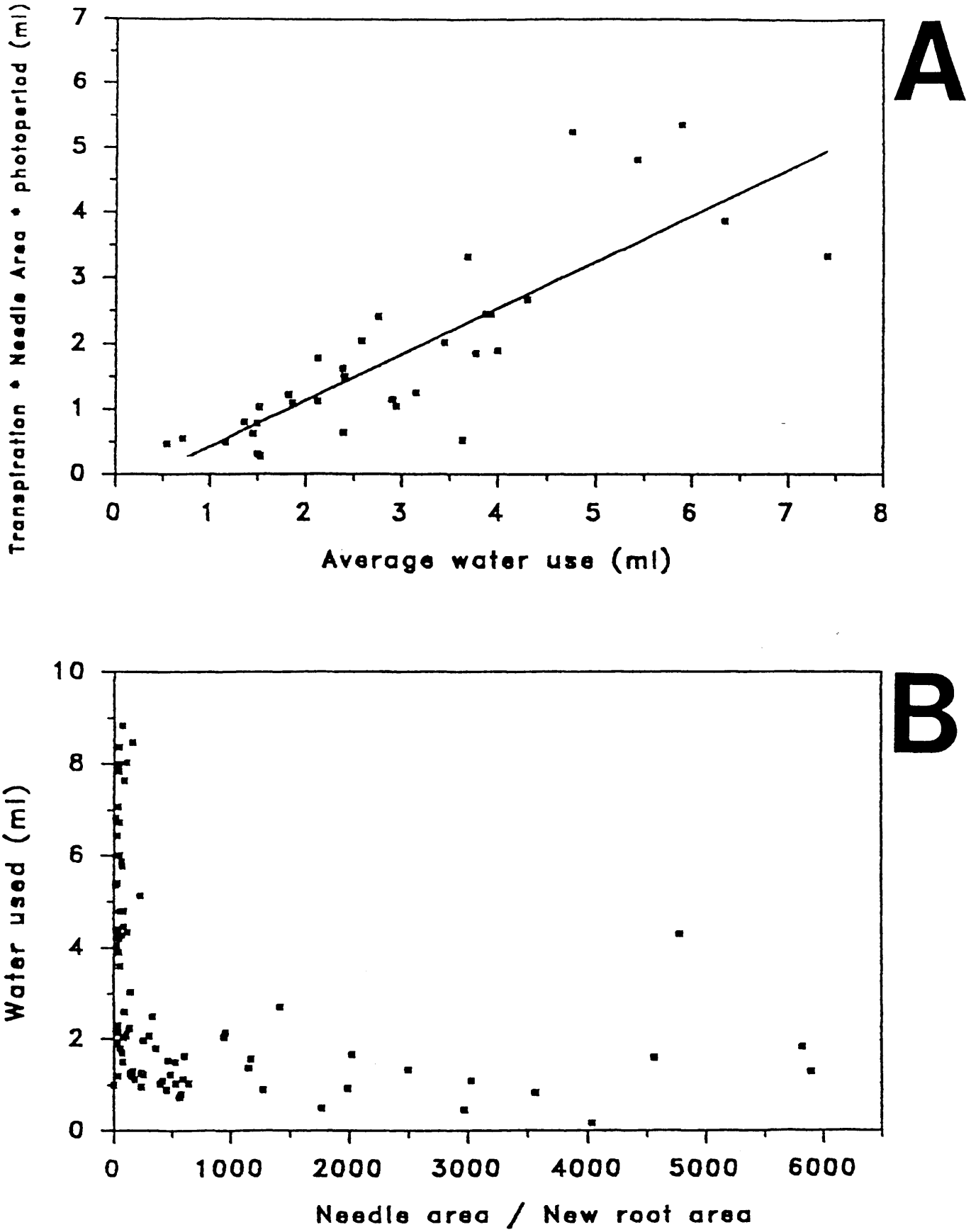


Figure 5.9—*Interactive effects of morphological and physiological factors on seedling water use per day: (A) Water use per day versus transpiration per unit area * needle area per seedling * photoperiod (R^2 = 0.69); (B) Water use per day versus needle area/new root area after 28 days. Seedlings are the same as in Figure 5.8.*

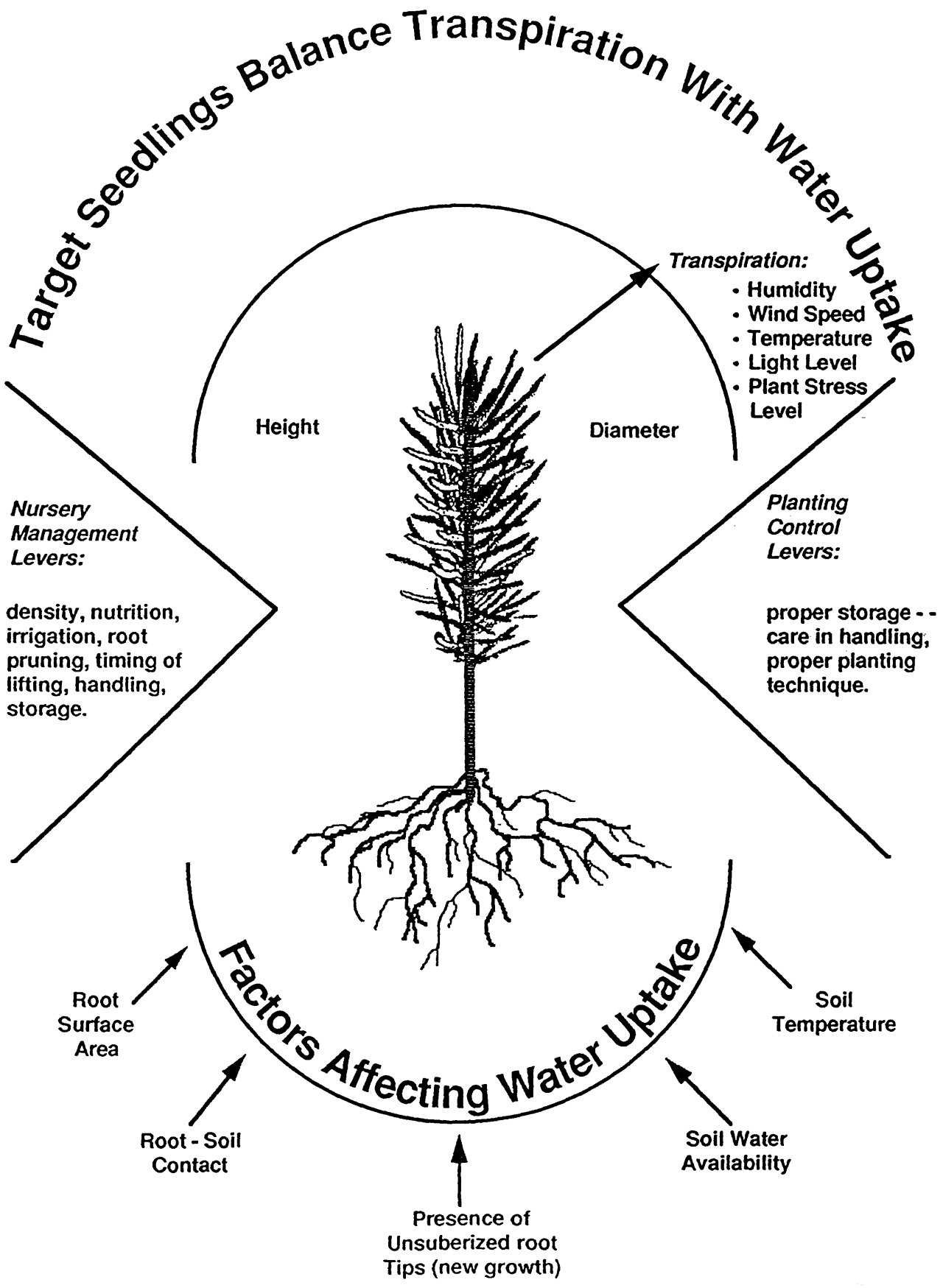


Figure 5.10—*Interactions in the Target Seedling during establishment as described in the text.*

Seedling root system surface area should be maximized within the basic dimensions that can be properly planted. This can be done by management of growing density, supplying uniform irrigation and nutrition to well aerated soils, and by root pruning. It is also important that nurseries maintain adequate mycorrhizal populations to ensure well developed mycorrhizal fine roots. Target seedling specifications usually include a description of the root system supplied from the experience of the field forester and/or research done to support reforestation efforts. Displacement of the root system in water (root volume) is a rapid method of estimating root surface area (R^2 = 0.79) . This can be done on an individually tagged population of seedlings that can then be outplanted on various sites for survival determinations. Such information can be used to develop cultural practices that induce the development of good quality root systems. Seedling culling in the packing room should include estimates of root system quality. Root surface area and root volume are not used operationally since their measurement is too labor intensive. Presence of a minimum number of first order lateral roots (six seems to be common in both loblolly and radiata pine) and an inspection for root damage are two criteria that can be estimated quickly and can be used to determine if cultural practices and lifting machinery have been applied properly.

Height is reasonably correlated (R^2 = 0.71) with needle surface area and therefore can be used to predict potential for water loss by transpiration. Seedling height and diameter should be managed to fit the observed best performance guidelines supplied by the regeneration forester to fit their environment, soil, and site preparation conditions. This means that there should be a continual dialogue between field foresters and nursery staff to set goals and to measure success in achieving these goals.

It is important that seedlings be lifted at a stage of development when carbohydrate reserves are high and when storage will promote new root growth (Carlson 1985). It is also important that seedling root systems not be physically damaged or dried out during lifting, packing, storage, transport, or planting. Storage temperature and duration should minimize respiratory loss of reserves (1-3°C for species that cannot be frozen) and should minimize impairment of photosynthetic processes (McCracken 1978).

Site preparation methods should provide an even distribution of planting spots that are free from competing vegetation, have access to mineral soil, and are easily penetrable with a planting tool. Planting should provide tight contact of the root with the soil over the full length of the root system and should avoid bending the tap root into a J or L configuration (Harrington et al. 1986).

The Target Seedling approach to nursery management and reforestation provides an opportunity to integrate the forester's data on plantation success, the nursery management practices necessary to produce the desired product, and supporting research. We believe that maintaining an ongoing interaction between these parts of the reforestation system is critical to ensure production of the highest quality seedlings.

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Target Seedling Symposium

Chapter 6
Mycorrhizae and Realistic Nursery Management

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ABSTRACT

In the Pacific Northwest, when producing target seedlings for reforestation of sites that have not been drastically disturbed, there will likely be adequate mycorrhiza development on the seedlings without the necessity of soil inoculation in the nursery. This represents the majority of seedlings being produced. However, problems in the nursery can occur through excessive use of certain pesticides, especially soil fumigants and fungicides. These may require re-establishment of mycorrhizal fungi in the nursery soil. When growing seedlings for planting on drastically disturbed or inhospitable sites, or for planting on natural grasslands, nursery inoculation may represent the difference between success or failure. For the future, there exists ample opportunity to significantly increase forest productivity through matching tree genotypes with mycorrhizal fungus genotypes. At present, however, our knowledge base is inadequate for taking full advantage of these possibilities.

6.1 Introduction

The fossil records clearly show that the early land plants appeared before roots had evolved. These plants were equipped with rhizomes, the precursors of roots. Of interest to this discussion is the fact that the rhizomes of these early land plants were generally associated with fungi in an arrangement similar to mycorrhizae. Thus when Gymnosperms, and later Angiosperms, evolved the root-fungus association was ready and waiting. The point is that mycorrhizae appear to be as old as the plants with which we are dealing. They are not of modern origin and certainly not of man's design.

The mycorrhizal condition was first described by a German forest pathologist (Frank 1885). This was followed by a 30- to 40-year period of open warfare over whether this was a pathological or beneficial relationship. As late as 1918, the pathological nature was still assumed (Rankin 1918, 82-84): "A short account of (mycorrhizas) is, however, of interest since the structures are now generally considered to represent a diseased condition of the roots and not a true type of symbiosis or mutual-advantage relation, as was previously believed by many."

For the purpose of this discussion of the implications of mycorrhizae to the production of "target seedlings," we need to learn what the practical nursery manager should know and/or do about the mycorrhizae on the seedlings being produced. More than 40 years ago, Wilde (1944) concluded that 99 percent of all practicing foresters will not have to lose any sleep over the problem of mycorrhizal inoculation. That turned out to be a bit optimistic. However, in areas where native tree seedlings are produced for outplanting on sites that are not drastically disturbed, inoculation is generally not necessary. However, the term native species must be taken carefully. In 1953, I worked with Wilde in Wisconsin, and we had to inoculate an entire nursery which was built to produce native (to Wisconsin) tree species. The problem was that the area selected for the nursery was on prairie soil and thus ectomycorrhizal fungi were entirely absent.

On a more global basis, Mikola (1980) wrote a review of the movement of mycorrhiza inoculum across international boundaries as foresters sought to plant exotics in various parts of the world. Such movements of inoculum have been absolutely essential for the successful establishment of such species in new places. Once both a tree species and its associated mycorrhizal fungus (or fungi) have been established in a favorable soil and climate, the inoculum remains viable for a long time. This is true both in bare root nurseries and field plantings. The exception is in containerized nurseries where inoculation is necessary with every crop.

6.2 Essentiality and Physiology of Mycorrhizae

The essentiality of roots is hardly in question. There are some difficult questions concerning roots, however, that warrant at least some consideration. These include: Why don't roots die? Why do roots die? And what effects do mycorrhizae have on the answers to the first two questions? Finally, then, are mycorrhizae important to seedling survival and growth? Are there quantitative and qualitative differences among mycorrhizae formed by different fungi? And if there are, what should the practical nursery manager do about the situation, if anything? Hopefully, we will address all of these questions as we progress through this discussion.

Let's start with a brief summary of the effects that have been attributed to mycorrhizae. Discussion of these will come later:

1) Uptake of poorly mobile or immobile nutrients.

2) Absorption of water and of mobile ions as soil dries and diffusion rates decrease.

3) Countering of toxicities caused by high exchangeable aluminum, strong acidity, strong alkalinity, high salts, and heavy metals.

4) Tolerance of high soil temperature.

5) Improved soil aggregation and thus protection from wind or water erosion.

6) Nutrient conservation on the planting site through very efficient recycling of nutrients that become available.

7) Root protection from disease through antibiosis, physical barrier to pathogen penetration of host tissue, improved tree nutrition which can quickly compensate for the loss of feeding roots, and avoidance of infection through enhanced suberization of fine roots.

The typical planting site is less than optimal in some respects. Thus, the problems associated with yield improvement are at least involved with improving stress tolerance (Jones 1985). There is ample literature to show that seedlings with proper mycorrhizae have increased tolerance to stresses imposed by low soil fertility, low available moisture, and root pathogens. Not all mycorrhizal fungi impart all of these benefits, but each benefit has been demonstrated as attributable to the mycorrhizal condition.

6.3 Natural Status of PNW Soils

This statement can be fairly short and to the point. There is no shortage of fungi capable of forming mycorrhizae on the roots of the native tree species in the PNW (Trappe 1977). Just as there is a decided change in the tree species east and west of the Cascade crest, so there is a change in the mycorrhizal fungi associated with those species and on those different soils. Somewhat less dramatic changes also occur east and west of the crest of the Coast Range.

6.4 Ecology of Roots, Including Mycorrhizae

6.4.1 Root and mycorrhiza physiology

There are three main parts to any tree root system. These include: 1) the large structural roots, 2) the long exploratory fine (1-2 mm in diameter) roots, and 3) the short, fine roots. It is these short, fine roots that become infected with certain specific fungi and produce the mycorrhizae. In certain tree species, it is only the ultimate short roots that become infected and form ectomycorrhizae. In all other tree species, the vescicular-arbuscular mycorrhizal (VAM) fungi infect both types of non-suberized fine roots. Typically, the fine roots average about 5 percent of the root mass but about 90 percent of the root length (Bowen 1985). The mycorrhizal hyphae greatly extend this length.

The amount of photosynthate going to roots is usually underestimated. The standing root biomass, at any given moment, is about 20-30 percent of the total tree biomass. Carbon lost from root respiration, root exudates, sloughed cells, and fine root turn-over are missed. Estimates of total photosynthate going to roots of PNW tree species have varied from a low of 8 percent to a high of 66 percent (Bowen 1984). The mycorrhizal fungi on Pacific silver fir have been estimated as using about 15 percent, of the total photosynthate (Vogt et al. 1982). In a very detailed study of 6-month-old loblolly pine seedlings, over a 12 week period, Vongkaluang (1978) showed that 30 percent of the photosynthate went to the root system while 25 percent of the seedling weight was in the root system. Details of the energy partitioning are shown in Table 6.1.

The energy and carbon cost of maintaining the root system is decreased by the sloughing-off of fine roots during times when conditions are not conducive to growth. This turnover of fine roots usually exceeds the annual turnover of foliage (Sanchez et al. 1989). Rapid production and turn-over of fine roots and mycorrhizae give the perennial plant great plasticity in dealing with environmental stress. It also suggests one reason why nursery inoculation of seedlings with mycorrhizal fungi may not last long in normal forest soil. On the other hand, in severely disturbed locations or in places where the tree species is introduced as an exotic, the fungus may perpetuate itself through many generations of mycorrhizae (see Section 6.6).

Table 6.1—*Energy partition by root systems of 6-month-old loblolly pine seedlings over a 75 day period.*

	Energy partition by root systems (small calories/day)			
Day	Root tissue	Respiration	Sloughings	Exudates
0	60	15	15	30
25	100	25	25	40
50	140	70	60	70
75	560	150	90	80

This table is adapted from Vongkaluang 1978.

At this point, it is appropriate to address the two related questions of why roots *don't* die and why roots *do* die. There are various cycles in the plant, but the place to start this discussion is at a point where the seedling has a small excess of foliage in relation to the fine roots. At that point, the foliage produces more carbohydrate than the top can use since the roots are inadequate for providing a commensurate supply of mineral nutrients and water. The result is that the excess carbohydrate is translocated to the root system. This allows the formation of new fine roots in locations in the soil where the nutrients have not been excessively utilized. The new fine roots both lose organic exudates to the rhizosphere and become invaded by mycorrhizal fungi. The exudates tend to feed a large population of saprophytic microbes in the rhizosphere and these in turn discourage the invasion of the roots by pathogenic organisms. In some cases, the mycorrhizal fungi also protect the roots from certain pathogens (Marx and Davey 1969). These new mycorrhizal roots are very efficient in the uptake of nutrients from the soil in their vicinity. They supply the foliage which then begins to form more structural material in the top. As the network of mycorrhizal fungi expands in the soil, they require increasing supplies of carbohydrate from the top to sustain their respiring biomass. At some point, the cost to the plant in carbohydrate exceeds the value in returned nutrients. At that point, the plant either reduces the carbohydrate being translocated to the fine roots or it forms an abscission layer at the base of the fine root. In either case, the fine root can no longer supply the mycorrhizal fungi or the rhizosphere saprophytes with the needed carbohydrate, and the fine root either dies from starvation or it is invaded by some weak pathogen which kills it. After this has happened to enough fine roots, we are back to the initial step and the process repeats. This is actually a very efficient strategy for the plant since new mycorrhizal roots in nonexplored soil are much more energy efficient than larger, older ones in exploited soil. It also helps explain the high rate of turnover of the fine roots. Non-mycorrhizal fine roots have much higher carbon demand per unit of nutrients taken up and thus they pass the break-even point much sooner than mycorrhizal roots. Con-

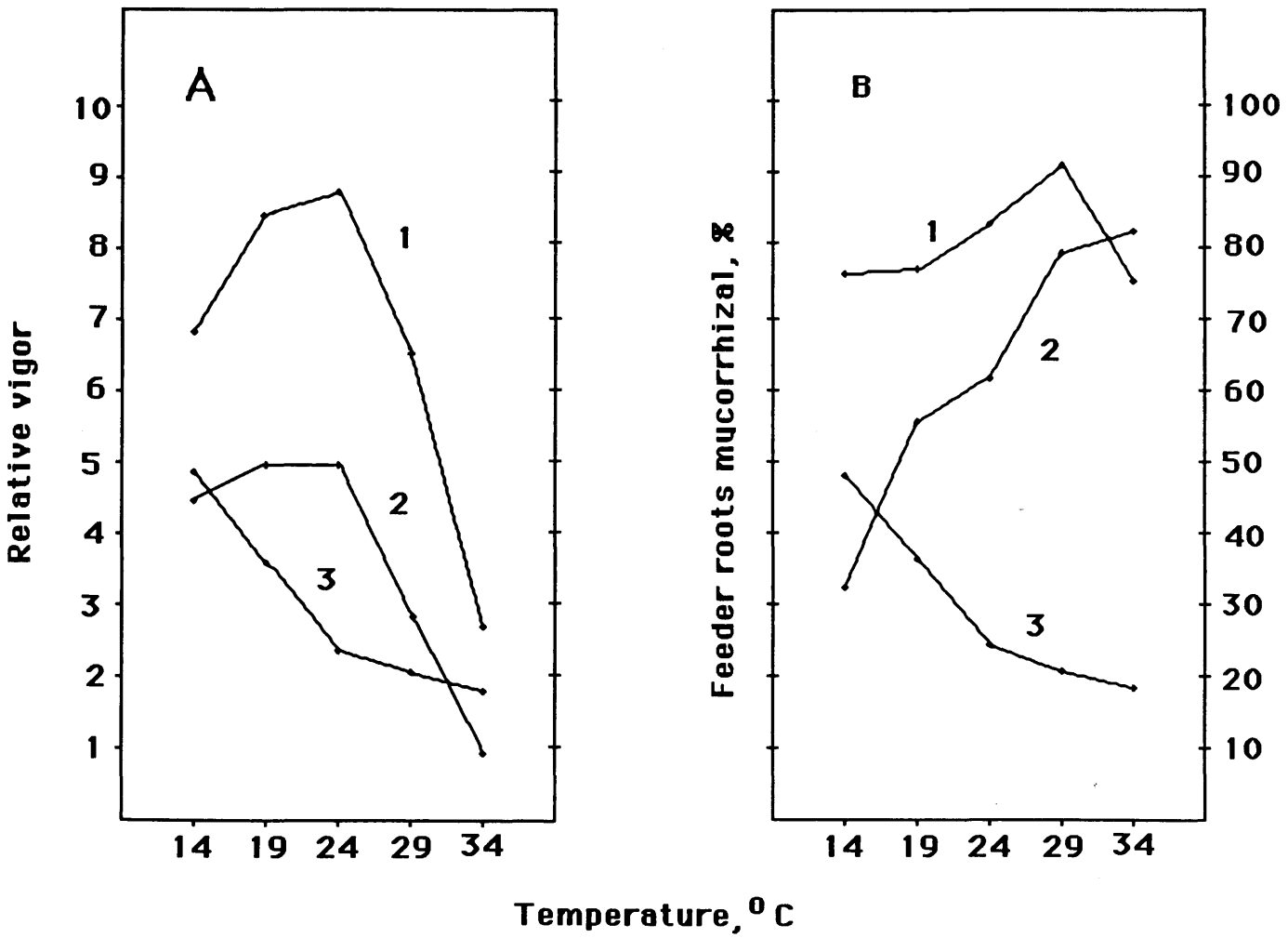

Figure 6.1—*Relation between ectomycorrhizal development (2) on loblolly pine seedlings by Thelephora terrestris (A) and Pisolithus tinctorius (B) and relative seedling vigor (1) as influenced by soil temperature. Control line (3) represents relative vigor of nonmycorrhizal seedlings (from Marx et al. 1970).*

sequently, the mycorrhizal condition actually extends the life-expectancy of the short, fine roots. Mycorrhizae have been reported to absorb P and Zn at about the same rate per unit of surface area as non-mycorrhizal, non-suberized roots (Bowen 1985). Bowen (1985) also reported that the mycorrhizal hyphae length-to-weight ratio is at least 500 times that of the fine, non-suberized roots. Thus we may conclude that the uptake of P and Zn are at least 500 times greater per unit weight of carbon devoted to the mycorrhizal hyphae than to that devoted to the fine roots. The pattern of uptake of poorly mobile nutrients, such as P, is first an intense depletion of a narrow band around the root. This is delineated by the length of the root hairs on plants where they exist. Then there is a limited depletion beyond this, depending on both the ion and the soil colloids involved. Then, with development of mycorrhizae, soil further out is explored, often intensely. This is because the hyphae not only extend much farther than the root hairs but they also branch and by virtue of their small diameter they can enter much smaller pores than root hairs. Finally, mycorrhizal hyphae produce phosphatases and phytases that permit them to obtain P directly from the humus. Roots frequently possess similar enzymes but they seldom have access to the humus layer.

Both added P and mycorrhizae caused considerable rate increases in photosynthesis and dry matter accumulation (Rousseau and Reid 1990). At low and medium levels of mycorrhizal development, net photosynthetic response was due to enhanced P uptake. At high levels of mycorrhizal development, as much as 17 percent of the growth increase was attributed to "mechanisms other than enhanced phosphorus nutrition" (Rousseau and Reid 1990). All inoculation was done at a very low (0.1 ppm) solution P level. The other mechanisms are usually assumed to be hormonal in nature.

Generally, mycorrhizal hyphae will be more important in the uptake of ammonium (NH_4^+) than nitrate (NO_3^-) since nitrate is highly mobile and will get to the roots via

mass flow (soil solution movement to roots) when transpiration is active, and by diffusion when it is not (Bowen and Smith 1981). Ammonium, being a cation, is more tightly bound to soil particles on the cation exchange (CEC) sites and thus does not move to the roots. The foraging mycorrhizal hyphae are effective at accumulating ammonium from the CEC sites and transporting it to the host plant. Also, there is some evidence that the mycorrhizal hyphae are able to accumulate simple organic nitrogen compounds directly from the humus layer. In fact, this has been used as an argument as to why the humus accumulates on some sites. The mycorrhizal hyphae appear to be more efficient at getting the nitrogen from the humifying material than the soil saprophytes. This leaves the carbonaceous residue deficient in nitrogen and hence its rate of decomposition is reduced and the thickness of the humus layer increases.

There has been considerable discussion in the literature regarding the ability of forest trees to use nitrate nitrogen. In order for any plant to utilize nitrate, it must contain an enzyme known as nitrate reductase. This enzyme converts nitrate to a form that the plant can use for growth. One possible mode of action would be for the mycorrhizal fungi to take up and reduce the nitrate and pass the reduced N on to the host plant. An investigation of nitrate reductase activity by nonmycorrhizal fine roots of Douglas-fir and by seven ectomycorrhizal fungi showed that the roots possessed more nitrate reductase than any of the fungi by at least a factor of six (Ho and Trappe 1980). However, the Douglas-fir roots do not have what would be considered high nitrate reductase activity. In comparison with a plant like wheat which does efficiently utilize nitrate, the Douglas-fir roots were only one-eighth to one-fourth as active. And the mycorrhizal fungi were as little as 1 percent as active. This research shows that for Douglas-fir at least, while nitrate may be used, it is not an efficient nitrogen source, and while the mycorrhizal fungi may help some in nitrate accumulation, they do not enhance its reduction prior to utilization by the plant.

Progeny testing in tree improvement programs has given indirect evidence to support the idea that at least one of the causes of poor performing progeny is poor roots. This allows us to speculate that either 1) genetic improvement in trees may reflect better roots and thus reduce our concern over mycorrhizae or 2) if a poor performer possesses a very desirable trait (wood property, disease resistance, etc.) we may be able to improve its performance through inoculation with appropriate mycorrhizal fungi.

6.4.2 Natural selection

In several studies, involving different techniques, it has been found that inoculum collected from beneath stands of various species of trees will often result in mycorrhiza formation on a given species that is being tested. The most successful inoculum, however, is almost always from beneath the species being inoculated. This suggests that there has already been considerable natural screening of candidate fungi by nature.

6.4.3 Interactions

The influence of soil temperature on mycorrhiza formation has been investigated under conditions of both low (Amaranthus and Perry 1989) and high (Marx et al. 1970) temperature. In soil temperature incubators, Marx et al. (1970) investigated aseptic synthesis of mycorrhizae on loblolly pine from a soil temperature of 14° to 34°C with the mycorrhizal fungi Thelephora terrestris (Tt) and Pisolithus tinctorius (Pt). With Tt, mycorrhiza abundance increased from 14° to 24°. It then decreased rapidly from 24° to 29° and was zero by 34° (Figure 6.1). With Pt, mycorrhiza development continued to increase all the way to 34° while seedling development peaked at 29°. In a second study, Marx and Bryan (1971), investigated the effects of an extreme soil temperature (4O°) for 5 weeks on the survival of loblolly pine seedlings that were mycorrhizal with Tt or Pt, or were non-mycorrhizal. Survival of non-mycorrhizal seedlings was 45 percent, of Tt-mycorrhizal seedlings it was 70 percent, and of pt-mycorrhizal seedlings it was 95 percent. These results showed that there was as much difference between mycorrhizal fungi (95 - 70 = 25 percent) as there was between mycorrhizal and non-mycorrhizal seedlings (70 - 45 = 25 percent). Subsequent work by numerous investigators in various climates and soils has confirmed that Pt offers a real benefit where the planting site is likely to be hot, but is of no particular value on cold sites (e.g., Riffle 1989).

In a detailed study of the effect of temperature on the growth of various mycorrhizal fungi in pure culture and on mycorrhiza formation with radiata pine, Theodorou and Bowen (1971) found very strong relationships. Generally, they found 25°C to be optimum for both growth of the fungi in culture and mycorrhiza formation. Growth was almost nil at 15° and very low at 30°. In a sandy soil, after 14 weeks, 36 percent of seedling roots were mycorrhizal at 25° while only 6 percent were at 15°. There were differences among the fungi tested. The authors concluded that there is a need to select fungi for mycorrhizal inoculation on the basis of the soil temperatures appropriate to the season and site, as well as to their ability to stimulate seedling growth.

Different plant responses to both ectomycorrhizal and VAM fungi may be related to their relative ease and speed of infection, differences in their rate of spread throughout the root system, and the growth pattern of the hyphae in the soil. Hyphal growth in the soil is difficult to study, but several fungi have been traced for at least 12 cm (5 inches) from the root surface. On the other hand, soil compaction has been shown to be quite adverse to hyphal penetration. Compaction from bulk density 1.2 to 1.6, which is not at all uncommon, reduced hyphal penetra-

tion by 90 percent (Bowen 1980). This would be expected to seriously reduce nutrient uptake and subsequent tree growth or even survival. This has occurred frequently on skid trails and decks and unfortunately also in nurseries. Often, ripping or ripping plus discing is all that is needed to restore the productivity of such compacted areas in the field or nursery.

When one is investigating the value of a plant being mycorrhizal, it is not correct to compare the performance of a large mycorrhizal plant with a small non-mycorrhizal one (Bowen 1980). Rather the correct comparison is between the mycorrhizal plant and a non-mycorrhizal plant that has been fertilized sufficiently to reach the same size as the mycorrhizal one. Then their behavior can be fairly compared and also their cost of production can be evaluated. This restriction should also be used when comparing plants that are mycorrhizal with differing fungi. Only then can truly equitable comparisons be made. It has been noted that mycorrhizal plants have a lower root/shoot ratio than non-mycorrhizal ones. This disappears when the non-mycorrhizal plant is fertilized up to the same size as the mycorrhizal one, and suggests that the root/shoot ratio is really a function of plant nutrition and not something peculiar to the mycorrhizal condition (Bowen 1980).

Growth depression as a consequence of a seedling being mycorrhizal, while rare, is possible. One circumstance in which this occurs is during the time that the mycorrhizal association is forming. The plant must invest much photosynthate to the association before it realizes any benefit from it. This is probably common but of such short duration as to be of little consequence. In highly fertile soil, where the mycorrhizae are of reduced value to the seedling but where their formation is not significantly inhibited, the seedling invests more than it receives in return. In some estimates, the mycorrhizal fungus accounts for up to 17 percent of the total root system weight, a significant investment if not needed or if not needed in such abundance. Imbalanced nutrition can occur and result in growth suppression. In soils with very high P levels, the mycorrhizae tend to overload the seedling with P and this upsets nutrient balances within the plant and actually depresses growth. One of the most common problems in nursery fertilization is excessive P application. Thus this type of growth suppression is probably more common than we realize. Unfortunately, the common response to less-than-desired growth is to apply more fertilizer and in some cases this may be exactly the wrong response. It is certainly counter productive.

6.5 Mycorrhizae and Nursery Practice

6.5.1 Fumigation

Fumigation of the nursery soil is the single most important operation in nursery soil management, as far as the mycorrhizal fungi are concerned (Danielson and Davey 1969). Methyl bromide and chloropicrin are both general poisons with chloropicrin more toxic to fungi than methyl bromide. Soil fumigation has been conducted safely in our nurseries for about 30 years. There have been some mistakes made, occasionally, because of improper metering of the fumigant or intentional high rates of application. Soil temperature, moisture content, and organic matter content all affect the results of any given fumigation. The damage done to mycorrhizal fungi differs between the ecto- and endomycorrhizal fungi. With a nursery that is surrounded by ectomycorrhizal trees, there is a delay in mycorrhiza formation, following an overdose of the fumigant, but the ectomycorrhizal fungus spores are air-borne and will naturally re-inoculate the soil over the first growing season. The spores of the endomycorrhizal fungi are soil-borne and do not blow around.

Consequently the adverse effects of an overdose of fumigant will persist considerably longer where endomycorrhizal seedlings are grown compared with where ectomycorrhizal seedlings are grown. An interesting example of the effect on an endomycorrhizal species was seen in the D.H. Phipps Nursery (Elkton, Oregon) several years ago. Because of high soil moisture and low soil temperature at the time of fumigation, the fumigant action was concentrated more in the top few inches than usual. The endomycorrhizal fungi were essentially eliminated. During bed shaping, a little non-fumigated soil was dragged into the first few feet of each bed from the road. The crop planted was western redcedar and except in those first few feet near the road, where those seedlings were mycorrhizal, the crop was a failure.

6.5.2 Fertilization

There has been concern that inoculum that is successful in stimulating seedling growth in the rich environment of the nursery soil may not be particularly effective in the much less fertile soil in the field. Lamar and Davey (1988) found that VAM fungi they isolated from green ash (*Fraxinus pennsylvanica*) from a low P field location was highly effective in stimulating seedling growth in a high P nursery soil. They concluded that there are at least some VAM fungi that are effective in both fertile and infertile soil in stimulating the growth of seedlings and young outplants.

In soil with less than 15 ppm available P, roots of citrus seedlings infected with the pathogen *Phytophthora parasitica* and the VAM fungus *Glomus fasciculatus* were healthier and weighed more than roots infected by the pathogen alone (Davis and Menge 1980). Above 56 ppm P in the soil, the beneficial effect of the mycorrhizal fungus was lost. The evidence strongly suggests that the tolerance to infection by the pathogen in trees with the mycorrhizal fungus is associated with improved plant

Table 6.2—*Influence of* Glomus fasciculatus, Phytophthora parasitica, *and soil phosphorus on root health and root infection by* Glomus fasciculatus.

	Healthy roots (%) Soil P (ppm)			Root tissue infected with Glomus fasciculatus (%) Soil P (ppm)		
Inoculation	6	56	600	6	56	600
Non-inoculated	100 z	100 z	100 z	0 a	0 a	0 a
Phytophthora parasitica (Pp)	33 v	74 xy	86 y	0 a	0 a	0 a
Glomus fasciculatus (Gf)	100 z	100 z	100 z	54 d	38 c	12 b
Pp plus (Gf)	50 w	72 x	79 xy	27 c	30 c	8 b

This table is adapted from Davis and Menge 1980. Values within a comparison (Healthy roots and Root tissue infected with *G. fasciculatus*) followed by the same letter are not significantly different (p = 0.05) according to Duncan's Multiple Range Test.

nutrition, especially P. Although the disease is significantly reduced in low P soil, there is no apparent direct effect of the mycorrhizal fungus on the pathogen. Rather, the effect is indirect through improved tree nutrition (Table 6.2).

6.5.3 Irrigation

Irrigation, as long as it is adequate for the seedlings and the water is of acceptable quality, is seldom a determinant of mycorrhiza formation or function. In general, the tree seedlings will suffer from improper (inadequate or excessive) irrigation or low quality water sooner and more severely than the mycorrhizal fungi.

6.5.4 Root pruning

It seems strange that we increase the number of roots by removing roots from the seedling. However, that is exactly the outcome of proper root pruning, both undercutting and lateral pruning. The removal of apical meristems of the initial roots allows several new roots to be formed in their place. These new roots appear in the zone where some mycorrhizal activity has already occurred and they become mycorrhizal and active relatively soon. The number of times any crop should be undercut has been the subject of several investigations. We can summarize them by saying that the greatest effect is caused by the first undercutting. The second one still produces significant changes. The third and subsequent undercuttings produce progressively smaller changes. There is a species factor in these results and probably soil and climatic ones as well, but in general, the effect continually decreases in intensity as the number of undercuttings increases but the fibrosity and mycorrhizal intensity do increase. The nursery manager should be able to decide when enough is enough.

6.5.5 Fungicides and other pesticides

Other than the general poisons, such as methyl bromide and chloropicrin, pesticides are more-or-less restricted to a narrow range of target organisms. In general, we can say that herbicides have little if any effect on mycorrhizal fungi. Insecticides and bacteriocides have slightly more effect. The fungicides have the most effect and the effect varies with the specific chemical. In the South, the systemic fungicide Bayleton is routinely used for control of fusiform rust on southern pine seedlings. This fungicide must be used carefully, however, since it is also toxic to several fungi that form mycorrhizae on the same tree species that are being protected from the disease. However, with proper usage, the only noticeable effect is a slight delay in the initiation of mycorrhiza formation. There is no loss in seedling quality and there is great reduction in the disease. Some fungicides, such as Subdue, have no apparent adverse effect on mycorrhiza formation.

6.6 Seedling Performance in the Field

If one is to select mycorrhizal fungi for inoculation in the nursery on the basis of improved nutrient uptake, it is better to select on the basis of uptake of nutrients from low concentrations rather than on the basis of a high rate of uptake. Most mycorrhizal fungi will be able to take up nutrients fast enough for optimum growth, but the ability to be effective in very low nutrient concentrations, which are common in forest soils, is very important (Lamar and Davey 1988). Some mycorrhizal plants appear to increase the availability of nutrients in the rhizosphere, by producing organic acids, phosphatases, and iron siderophores. Selection for these traits may also be important. The forester has both the tree and mycorrhizal fungus genomes from which to select in order to enhance tree growth and wood production (Bowen 1985). How to take advantage of these possibilities is still mostly a mystery. When one considers that approximately 2,000 individual fungal species will form mycorrhizal associations with Douglas-fir (Trappe 1977), we certainly have plenty to select from. The problem lies in the fact that we know very little about the behavior of nearly all of them. This makes realistic selection all but impossible. Making the problem even more difficult is the fact that there are large strain differences within individual genera and species. For example, there are strains of *Pisolithus tinctorius* that will form ectomycorrhizae on pines but not on eucalypts. There are other strains that will form mycorrhizae on eucalypts but not on pines. Finally, there are strains that will form mycorrhizae on both pines and eucalypts. These basic differences must be accounted for even before we consider any traits that are related to nutrient uptake or other properties.

Another confounding situation is the fact that just as there is a succession of plants on an area, so there is a succes-

sion of fungi that form mycorrhizae on roots as trees age. This fact discourages some people from even considering trying to influence the mycorrhizal fungi that will dominate the roots of their seedlings. This is quite short-sighted, however. If proper fungi are established on seedling roots and this results in improved survival and early growth of those seedlings, we will have improved the stocking of the new stand. That in itself is a major gain in productivity in most locations. Then if the early height advantage is merely maintained through the growth of the stand the wood production will be again increased. I have heard foresters say, with some scorn, "What difference does an extra five feet of non-merchantable top make?" The fact is that they are looking at the wrong end of the tree. The five extra feet are in the butt log, not in the top, and it makes a great deal of difference. The bottom line on this point is that the succession of mycorrhizal fungi which inhabit tree roots in the field should not diminish our appreciation for the positive effects that are potentially possible from nursery inoculation.

In site prepared forest soil, a mycorrhizal fungus species that infects and responds quickly is likely to give seedlings a competitive advantage over other plants on the site. They will capture the site and become the dominant vegetation in the least amount of time. Then the photosynthate that is produced on the site will be deposited as wood in the crop trees rather than in some of the competition. This can have significant economic consequences since it will either shorten the rotation or increase the wood produced.

In a recent study, it was reported that rapid new root and mycorrhiza formation occurred in Douglas-fir outplants in cold planting sites in the Klamath Mountains in response to adding soil from a good Douglas-fir plantation to the planting hole (Amaranthus and Perry 1989). Inoculated seedling survival rate was 36 percent while non-inoculated survival rate was only 11 percent. Such a technique may be useful in the reforestation of difficult sites. Possibly the transferred soil contained mycorrhizal fungi that represent different successional stages of mycorrhizae. The authors concluded, however, that more study would be needed to identify the specific microorganisms involved and their effects and interactions in the transfer soil.

Places where the succession of mycorrhizal fungi does not occur, or occurs very slowly, include drastically disturbed lands (e.g., mine spoils) and areas where the tree is being planted as an exotic. As an example, I have been working in a vast grassland in Venezuela called the llanos. There are no native ectomycorrhizal plants on the llanos. In this project, about 100,000 acres (40,000 ha) of *Pinus caribaea* have been planted annually for about 20 years. The original 54 seedlings that were brought from Trinidad were mycorrhizal with *Thelephora terrestris* (Tt). All seedlings in the Venezuelan project were inoculated from those or subsequent trees until 1982, when *Pisolithus tinctorius* (Pt) was introduced. There are four nurseries on the project and each is inoculated annually with Pt spores. The surrounding plantations supply plenty of air-borne Tt spores. A detailed assessment of both the plantations and the nurseries was made this past February. All trees and seedlings inspected were mycorrhizal and, regardless of age, Tt mycorrhizae dominated. In the younger plantations, Pt mycorrhizae formed a small percent of the total mycorrhizae. There has been no succession of the mycorrhizal fungi in the stands over the 20 year period simply because the only alternative was for the trees to be non-mycorrhizal and any that may have tried that route are no longer around to be counted.

Since an abundance of mycorrhizae requires a considerable investment in carbon and energy by the plant, the question could be asked as to how many or how much mycorrhizae is optimum. This question was investigated by Last et al. (1990) with Sitka spruce *(Picea sitchensis)* for two years in four different field soils (two peats and two mineral soils). They inoculated the seedlings with either *Laccaria proxima* (one isolate) or *Paxillus involutus* (two isolates) and found that with either fungus, the height growth was positively related to the total numbers of mycorrhizae per plant. They concluded that, irrespective of treatment, seedlings with similar numbers of mycorrhizae tended to be of similar height (Figure 6.2).

Other findings in the study by Last et al. (1990) were that the *Laccaria* isolate increased mycorrhizal numbers per plant more than either of the *Paxillus* isolates; fewer mycorrhizae were formed in peat than in mineral soil; at the end of the first year, most of the mycorrhizae in the peats came from the inoculum while there was a considerable range in the mineral soils (from 7 percent in one of the *Paxillus* isolates to 100 percent with the *Laccaria* isolate); by the end of the second year, *Laccaria* still accounted for 77 percent of the mycorrhizae but the two *Paxillus* isolates each accounted for less than 5 percent; and irrespective of treatment, seedlings with similar numbers of total mycorrhizae tended to be the same size. The authors' final conclusion was that an increase in numbers of mycorrhizae per plant from very few to some (e.g., 101 to 102) is hardly noticeable while an increase from many to a great many (e.g., 103 to 104) causes really discernable differences in yield.

Interactions between tree species and mycorrhizal fungi have been shown to influence field survival of outplanted seedlings. Richter and Bruhn (1989) inoculated 8-week-old red and jack pines with four different fungi and grew them for an additional 26 weeks, after which they were outplanted. Survival was checked after one and two growing seasons in the field (Table 6.3). Red pine inoculated with *Laccaria bicolor* survived about 20 percent better in

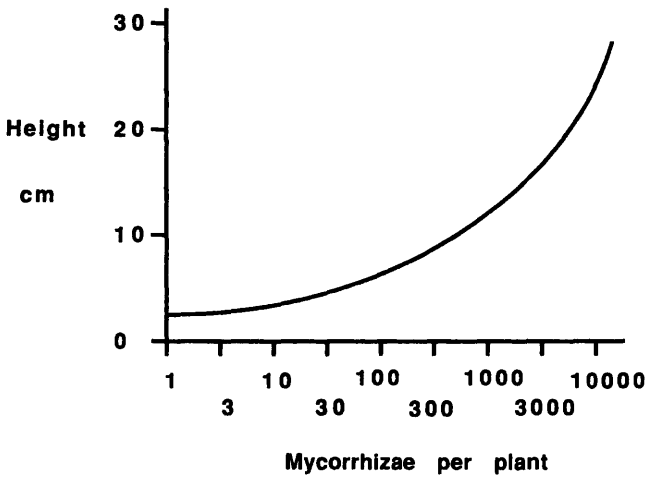

Figure 6.2—*Height of two-year-old Sitka spruce seedlings as affected by the number of mycorrhizae per plant (adapted from Last et al. 1990).*

both years than the *Thelephora terrestris* controls. Other treatments did not significantly affect survival. Data show that seedlings inoculated with *Laccaria bicolor* had significantly more mycorrhizae per plant than with other inocula. Jack pine exhibited a nonsignificant 10 percent increase in survival.

In a comparison of ponderosa, scots, and Austrian pines that were either inoculated with *Pisolithus tinctorius* or

Table 6.3—*First and second year survival of container-grown red and jack pine seedlings outplanted on an excessively drained sandy soil in Baraga County, Michigan.*

Pine species	year	Control[1]	*Laccaria bicolor*	*Scleroderma citrinum*	Isolate W31 -2B[2]
			(%)		
Red	1	52.7	74.0**	60.7	62.0
	2	35.3	54.0*	35.3	40.0
Jack	1	67.0	77.0	70.0	68.0
	2	59.0	70.0	60.0	59.0

This table adapted from Richter and Bruhn, 1989. The ** and * represent significant difference from the control treatment at the p = 0.005 and 0.05 level, respectively.

[1]The control seedlings were naturally mycorrhizal with *Thelephora terrestris*.

[2]The W31-2B isolate was a confirmed mycorrhiza former but it had not been identified.

were allowed to become mycorrhizal with indigenous fungi and outplanted in prairie soils in both Kansas and Nebraska, survival and growth were monitored for 5 years (Riffle 1989). While the Pt remained viable on all three pine species, it did not increase either survival or growth when compared with mycorrhizal, but not inoculated, stock. Possible reasons for the lack of response included mostly non-acidic soils (pH 6.8 to 7.1 in Kansas and pH 5.5 to 8.0 in Nebraska) which result in induced Fe deficiency and thus reduced carbohydrate for the mycorrhizal fungi, replacement of Pt by other mycorrhizal fungi brought to the planting site from the nursery, insufficient inoculum used in the nursery to produce seedlings with a very high percent Pt mycorrhizae, and little high temperature stress. It has been noted above that Pt is particularly valuable where there is a high soil temperature. Inadequate inoculation with Pt spores was recently reported by Marx (personal communication) to be responsible for low Pt mycorrhization on *Pinus caribaea* in Venezuela in competition with *Thelephora terrestris*. In that case, Marx suggested that the inoculation rate be increased from 20 g to 6 kg/ha (0.25 oz. to 5.5 lbs/acre).

It has been proposed that mycorrhizae may increase the tolerance of trees to contaminated soil, especially where heavy metals are concerned. Since paper birch has shown the ability to grow relatively near the large nickel smelter in Sudbury, Ontario, seedlings were inoculated with four different mycorrhizal fungi and grown at two levels (high and very high) of either nickel or copper (Jones and Hutchinson 1986). The authors did find that seedlings that were mycorrhizal with *Scleroderma flavidum* were more nickel tolerant than seedlings inoculated with the other three fungi. The authors proposed both a passive and an active mechanism for the nickel tolerance. None of the fungi increased copper tolerance. Thus, this effect appears to be highly specific.

6.7 Alternative Futures

As we look from the present to the future and ever better target seedlings, what can we discern from this review of what is known, and what are the potentials of mycorrhiza management? For the present, the practical nursery manager must remember that the mycorrhizal fungi are in the soil and should not be overly abused. This abuse may arise from over-fumigation; improper use of certain pesticides, especially some fungicides; and over fertilization, especially with phosphorus. This last point does not really represent actual abuse of the mycorrhizal fungi but it may significantly impair the ability of the tree seedling and the fungus from entering into this beneficial (essential) association.

Eventually, it may be important to know both the genotype of tree seed being planted *and* the genotype of the mycorrhizal fungus being involved in the synthesis of the

mycorrhizae. In fact, although we seldom have to consider it at present, we are not raising a single species—we are raising at least a dual species (tree+fungus) and usually a multiple species (tree+fungi). The exact combination of tree and fungus can have major consequences in silvicultural decisions. As pointed out by Bowen (1980), "Infection with an appropriate mycorrhizal fungus can radically change the estimate of the production potential of a soil and its fertilizer requirement." In other words, Site Index estimates, as determined by tree measurements, can be drastically different, depending on the mycorrhizal fungus infecting the roots. Bowen (1980) further stated, "Almost no nutritional studies in either tropical or temperate plants have ensured adequate mycorrhizal infection was present." Even that will not be the ultimate in intensive forest management. Rather than just "adequate" infection, we will need to know by what genotype of which fungus is infection caused. I mentioned this possibility to the director of our regional forest nutrition cooperative and his only comment was that he hoped he would be retired before such precision in our research were required. I suspect he will be, but that does not diminish the fact that the possibility exists and we should not be surprised when it eventually becomes the norm, rather than some researcher's dream. Certainly we will need to learn a great deal more about the fungi themselves before we can take even the first steps in the direction of this possible future. However, I would not have fulfilled my responsibility if failed to at least mention the possibilities involved.

In some distant future, we may be able to combine our knowledge of tree genetics with a vastly improved understanding of the ecology of the mycorrhizal fungi and some of the newer information on tree nutrition such as that now coming out of the laboratory of Torsten Ingestad in Sweden (Ingestad et al. 1981). Some day we may be able to spend less money, do less environmental damage, produce much more wood of excellent quality in less time on less area, and give the spotted owl rest while offending no one—neither the preservationist, the conservationist, the ecologist, the forester, the logger, nor the mill manager. Sounds nice, doesn't it? Let's work toward that future.

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Target Seedling Symposium

Chapter 7
The Target Seedling Concepts: Bud Dormancy and Cold-Hardiness

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ABSTRACT

Bud dormancy and cold-hardiness vary markedly throughout the annual growth cycle of trees in the temperate zone and have a profound impact on the ability of tree seedlings to withstand lifting, storage, and outplanting stresses. The Degree Growth Stage model is a useful tool for visualizing the changes in bud dormancy and cold-hardiness and their relationship to changes in other physiological attributes, such as root growth potential and stress resistance. Relationships among these attributes provide an opportunity to infer the status of one from another. The level of cold-hardiness can be used to infer bud dormancy status, as well as general stress resistance, at the time of lifting because all are correlated with performance, and cold-hardiness is easiest to measure. Practical approaches for measuring bud dormancy and cold-hardiness, and for routine monitoring for associated physiological targets, are discussed.

7.1 Introduction

Cyclic changes in bud dormancy and cold-hardiness have evolved in temperate zone trees in response to the stresses imposed by the annual climatic cycle. The rates of development and loss of bud dormancy and cold-hardiness of nursery-grown tree seedlings are cued in any particular year by naturally occurring changes in climatic factors, such as temperature, photoperiod, and precipitation, as well as by nursery cultural practices, such as irrigation, fertilization, and pruning. Consequently, changes in bud dormancy and cold-hardiness do not occur linearly through time, such as by a specific calendar date, but rather as a complex function of many interacting factors which can vary by year, location, and genotype. Even so, nurseries have typically established lifting, storage, and outplanting schedules for the species and ecotypes they grow based on historically successful calendar dates.

Today, measurement of cold-hardiness and bud dormancy, as well as other physiological attributes, morphological parameters, and climatic data, can establish the reasons why these historical schedules are usually successful. In addition to improving our understanding of the physiological processes behind stock performance and our ability to set physiological targets, this information can be extremely useful when atypical situations arise. Challenges may occur, for example, when unusual climatic conditions alter seedling physiology too far from the historical norm; when cultural practices alter seedling physiology so that it is no longer synchronized with the natural environment; and when lifting, storage, or outplanting must be rescheduled for operational reasons, further disrupting physiological development. In many such situations, a thorough knowledge of whole-plant physiological condition will greatly improve our ability to make decisions that will best enhance stock quality and performance (Duryea 1984, 1985, Lavender 1984).

Since bud dormancy and cold-hardiness status cannot be determined simply as a function of time, or by virtue of association with visible changes in morphology, the best approach for their accurate assessment is periodic testing in a wide variety of genotypes during the dormant period, when these two attributes best reflect stock quality. This chapter discusses the annual growth cycle of temperate zone trees and the associated changes in bud dormancy, cold-hardiness, and related physiological attributes, to provide a foundation for establishing appropriate bud dormancy and cold-hardiness targets. In addition, practical approaches for measuring bud dormancy and cold-hardiness, and for their routine monitoring, are discussed.

7.2 Annual Growth Cycle

The Degree Growth Stage model (Fuchigami and Nee 1987, Fuchigami et al. 1982) is a useful tool for visualizing the annual changes in bud dormancy and cold-hardiness and their relationship to changes in other physiological attributes (Figure 7.1). Use of such a model can improve communication about the annual growth cycle by offering a standard framework and terminology to serve as a foundation for integrating related aspects of whole-plant physiology.

Figure 7.1 is divided into five sections. Section 1 illustrates a modified Degree Growth Stage model, representing one complete annual cycle for a temperate zone woody plant growing under ambient conditions. Sections 2 through 5 describe the changes in root growth potential (RGP), shoot growth, cold-hardiness, and stress resistance during the cycle. These are idealized patterns for typical conifer seedlings, also under normal climatic conditions.

7.2.1 Degree Growth Stage model defined

The Degree Growth Stage model represents the annual growth cycle as a sine wave from 0 to 360°, with bud break at 0 and at 360° as the cycle begins again (Figure 7.1, Section 1). A sine wave is used rather than a straight line because, as mentioned, physiological changes do not proceed linearly through time. There are five specific phenological "point events" at specific degrees along the sine wave: bud break (0°), maturity induction (90°), vegetative maturity (180°), maximum rest (270°), and end of rest (315°). The months assigned to the point events were established for coastal Oregon, but will vary with location (Ritchie and Tanaka 1990). The five point events delineate the five "segment events" of the model, which will be denoted by the range in degrees over which they occur.

The model is divided into two halves—growth and dormancy—which refer to the condition of the aboveground, vegetative portion of the plant, especially the shoot meristems. **Growth** can be interpreted to mean that the shoot is getting bigger, as by elongation and production of new foliage, for example. **Dormancy** can be loosely defined as the opposite, when shoot growth is not visible, such as during the existence of terminal buds. Note that growth is not synonymous with meristematic activity, however, because there may be activity in the lateral cambium or apical meristems during dormancy. Dormancy can be divided into rest and quiescence, based on internal or external control of growth resumption, respectively (Lavender 1985). A bud is in **rest** when dormancy is maintained by agents within the bud itself (Romberger 1963). This occurs prior to the meeting of chilling requirements for bud break in late autumn or early winter. A resting bud will not elongate under favorable environmental conditions. A bud is **quiescent** when dormancy is imposed by the environment, such as by continued low temperatures after chilling requirements have been met in late winter (Samish 1954). The transition from rest to quiescence occurs under natural conditions in response to exposure to chilling temperatures (Lavender 1981).

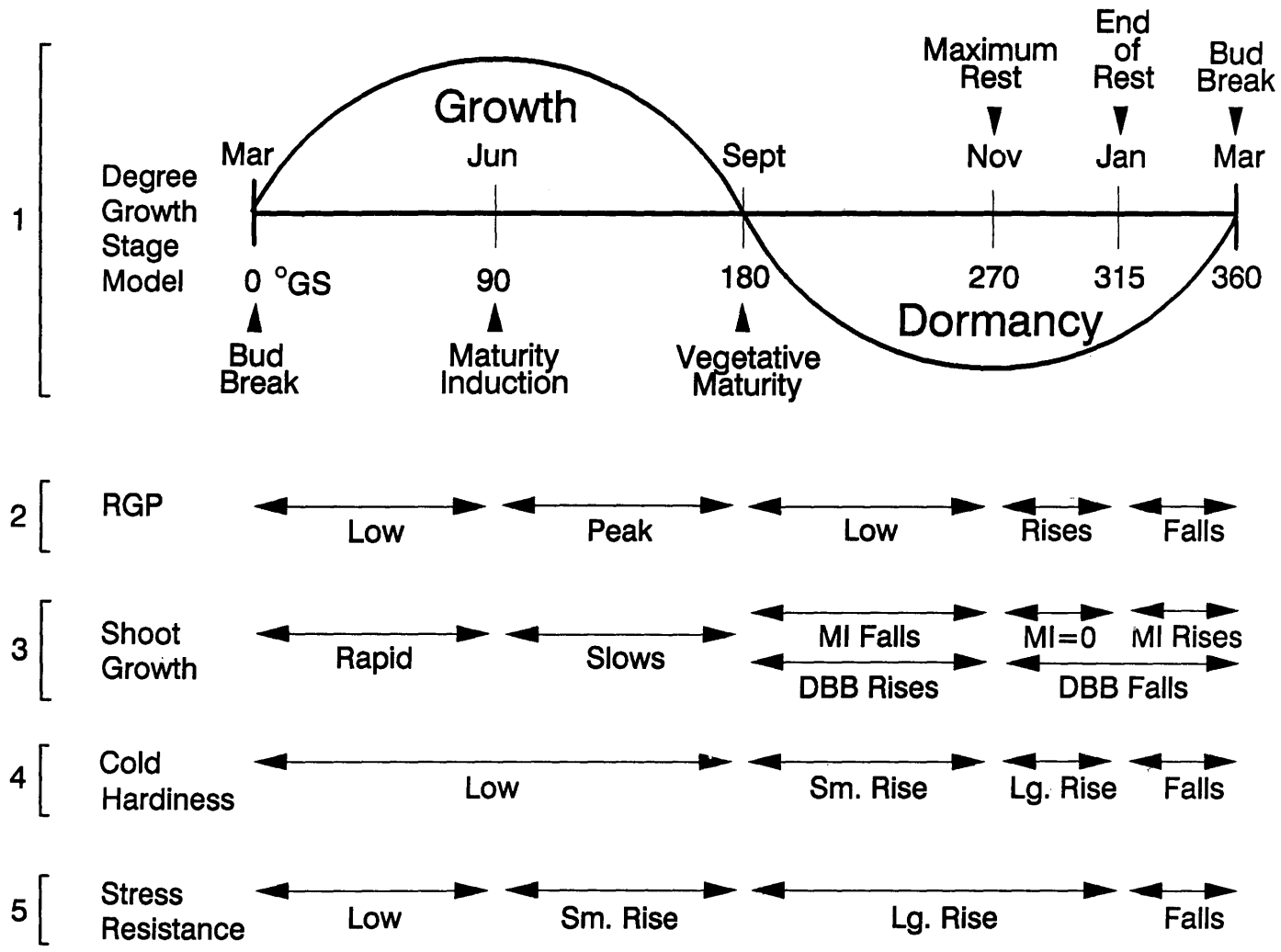

Figure 7.1—*A Degree Growth Stage model (Fuchigami and Nee 1987, Fuchigami et al. 1982) representing one complete annual cycle, with changes in root growth potential (RGP), shoot growth (MI = mitotic index, DBB = days to bud break), cold-hardiness, and stress resistance during the cycle.*

The point and segment events are described from left (0°) to right (360°). Bud break (0°) is the point at which new foliage becomes visible in the spring. Between bud break and maturity induction (0-90°), trees are temperature sensitive in that the rate of growth and development is generally temperature controlled. Growth is not inhibited by a short photoperiod. At approximately maturity induction (90°), buds are initiated. Between maturity induction and vegetative maturity (90-180°), trees are primarily photoperiod sensitive, with short days promoting budset and long days preventing or retarding budset. Drought can also be a major factor promoting budset, and may cause trees to enter a summer quiescent condition during this period (Lavender 1981, 1985). Vegetative maturity (180°) marks the onset of rest. Overwintering buds are well developed at this point.

Dormancy is maintained internally and intensifies between vegetative maturity and maximum rest (180-270°). The dormancy peak at maximum rest (270°) is characterized by an almost total absence of growth anywhere on the plant, and a chilling requirement which must be met before buds will resume rapid development (Ritchie 1984). Between maximum rest and the end of rest (270-315°), dormancy decreases in intensity as chilling requirements are met. At the end of rest (315°), buds are quiescent, with dormancy then imposed by the environment. An extended period of quiescence follows as long as environmental conditions remain unfavorable. When favorable environmental conditions for growth resume (315°), bud development renews, followed by bud break at 360°, completing the annual cycle.

Table 7.1—*Comparison of the Degree Growth Stage model segments with the four phases of dormancy presented by Lavender (1984).*

Degree Growth Stage model segment	Phase of dormancy	Physiological condition
90-180°	I - Dormancy initiation	Buds initiated and developing
180-270°	II - Deep dormancy	Dormancy intensity increasing
270-315°	III - Dormancy lifting	Dormancy intensity decreasing
315-360°	IV - Postdormancy	Quiescence to bud break

Dormancy theory has also been presented in the forestry literature in terms of four phases by Lavender (1984). The Degree Growth Stage model is quite compatible with this alternative approach (Table 7.1).

7.2.2 Root growth potential pattern

Root growth potential (RGP) is the ability of a tree seedling to initiate and elongate roots when placed into an environment favorable for root growth (Figure 7.1, Section 2) (Ritchie 1985, Ritchie and Dunlap 1980). Changes in RGP during the annual growth cycle are related to shoot dormancy and sink strength, defined as the relative ability of a plant part to compete for current photosynthate (Ritchie and Tanaka 1990). Peaks in RGP are evident when dormancy intensity is weak, but the sink strength of the shoot is also weak because rapid shoot growth is not occurring. Thus, RGP is low from 0-90° when shoots are the primary sink. From 90-180°, RGP peaks when shoot growth slows and roots become the primary sink. This corresponds to the typical surge and subsequent decline of root growth in the autumn (Lavender 1984). RGP remains low from 180-270° while dormancy intensifies. As dormancy weakens from 270-315°, RGP rises. This is the increase in RGP used to indicate fall lifting windows. RGP declines again from 315-360° as shoots regain their sink strength, with the resumption of environmental conditions favorable for growth.

7.2.3 Shoot growth pattern

Shoot growth is rapid following bud break (0-90°) with elongation and production of new foliage (Figure 7.1, Section 3). As buds develop from 90-180°, shoot growth slows. Elongation ceases by vegetative maturity (180°). During the dormant period (180-360°), the mitotic index (MI) and days to bud break (DBB) are two important measurements that indicate changes in dormancy intensity.

Mitotic index refers to the percentage of dividing cells in an apical meristem (Grob 1990b, Hawkins and Binder 1990, Owens and Molder 1973). Mitotic index falls as dormancy intensifies between 180-270°. By maximum rest (270°), the mitotic index is approximately zero, indicating that no cell divisions are occurring. This lack of mitotic activity continues until the end of rest (315°). With the onset of favorable environmental conditions assumed to occur at 315°, the mitotic index rises again between 315-360°, as buds resume development.

Days to bud break refers to the number of days required for terminal buds to break under optimum growing conditions. The seedlings must be taken from ambient conditions and placed under optimum conditions to determine days to bud break. Days to bud break increases as dormancy intensifies from 180-270°. At maximum rest (270°), seedlings require the maximum number of days under favorable conditions to break bud, or buds will not break at all, depending on species. After maximum rest, days to bud break decreases as chilling requirements are met and dormancy intensity weakens. A stable number of days to bud break will be maintained if a quiescent period occurs after the end of rest. But when bud development resumes with exposure to warm temperatures, days to bud break will continue decreasing to reach zero by 360°.

7.2.4 Cold-hardiness pattern

Cold-hardiness refers to the ability of a plant or plant tissue to survive or resist injury from exposure to freezing temperatures (Figure 7.1, Section 4). The level of cold-hardiness is frequently defined by a lethal temperature value, such as the LT_{50}, which represents the minimum temperature at which 50 percent of a group of seedlings, or 50 percent of a specified tissue, is killed.

Cold-hardiness is low during the growth period (0-180°), with trees generally unable to withstand exposure to temperatures below about -3°C without sustaining injury (Glerum 1985). Cold-hardiness increases somewhat from 180-270°, the amount varying with species. For example, a change in the LT_{50} from -5°C at vegetative maturity (180°) to -15°C at maximum rest (270°) has been observed in Southwest conifers (Burr, unpublished data). The majority of the cold hardening occurs from 270-315°, with a change in the LT_{50} from -15 to -40°C or lower, depending on the species (Sakai and Larcher 1987). After the end of rest (315-360°), with exposure to warm temperatures, cold-hardiness is rapidly lost and returns to growth period levels (LT_0 = -3°C).

7.2.5 Stress resistance pattern

There are several stresses to which temperate zone trees have developed cyclic annual patterns of increasing and decreasing resistance, such as drought stress (Lavender 1985), low temperature stress (Glerum 1985), mechanical stress (Tabbush 1986), and root exposure stress (Hermann 1967). Although these stresses affect the tree in different ways, trees develop a general stress resistance that varies

throughout the annual growth cycle (Figure 7.1, Section 5).

Stress resistance is lowest during rapid shoot growth (0-90°) and increases some as shoot growth slows (90-180°), especially with regard to drought resistance, which may parallel the development of summer quiescence (Lavender 1985). The major increase in stress resistance occurs during dormancy, with a maximum reached by the end of rest (315°) (Lavender 1985, Ritchie 1986a). Stress resistance falls with renewed development and growth after the end of rest (315-360°).

7.3 Relationships Among Physiological Attributes

The potential for highest seedling performance results when seedlings are harvested and outplanted when their resistance to stress is highest (Lavender 1985). Thus, the period of maximum stress resistance (290-315°) is the target period for fall lifting and storing. It has been proposed that one of the reasons RGP is such an effective seedling quality test is because the rise in RGP during dormancy identifies the period of maximum stress resistance (Ritchie 1985, Ritchie and Tanaka 1990). This period is approximately from December to February in the coastal Northwest. Given that the indicated relationships among these attributes exist (Figure 7.1), this period can also be identified by an extended period of low mitotic activity, by decreasing days to bud break, and by rapidly increasing cold-hardiness, as well as by the rise in RGP.

There are data in the literature to support the existence of these relationships (Cannell et al. 1990, Colombo 1990, Faulconer 1988, Glerum 1982, Ritchie 1986a). The following example illustrates the relationships among bud dormancy, cold-hardiness, and RGP in an Arizona seed source of Douglas-fir (Figure 7.2) (Burr et al. 1989). Greenhouse-cultured, container-grown, nine-month-old Douglas-fir seedlings, which had set bud and entered the

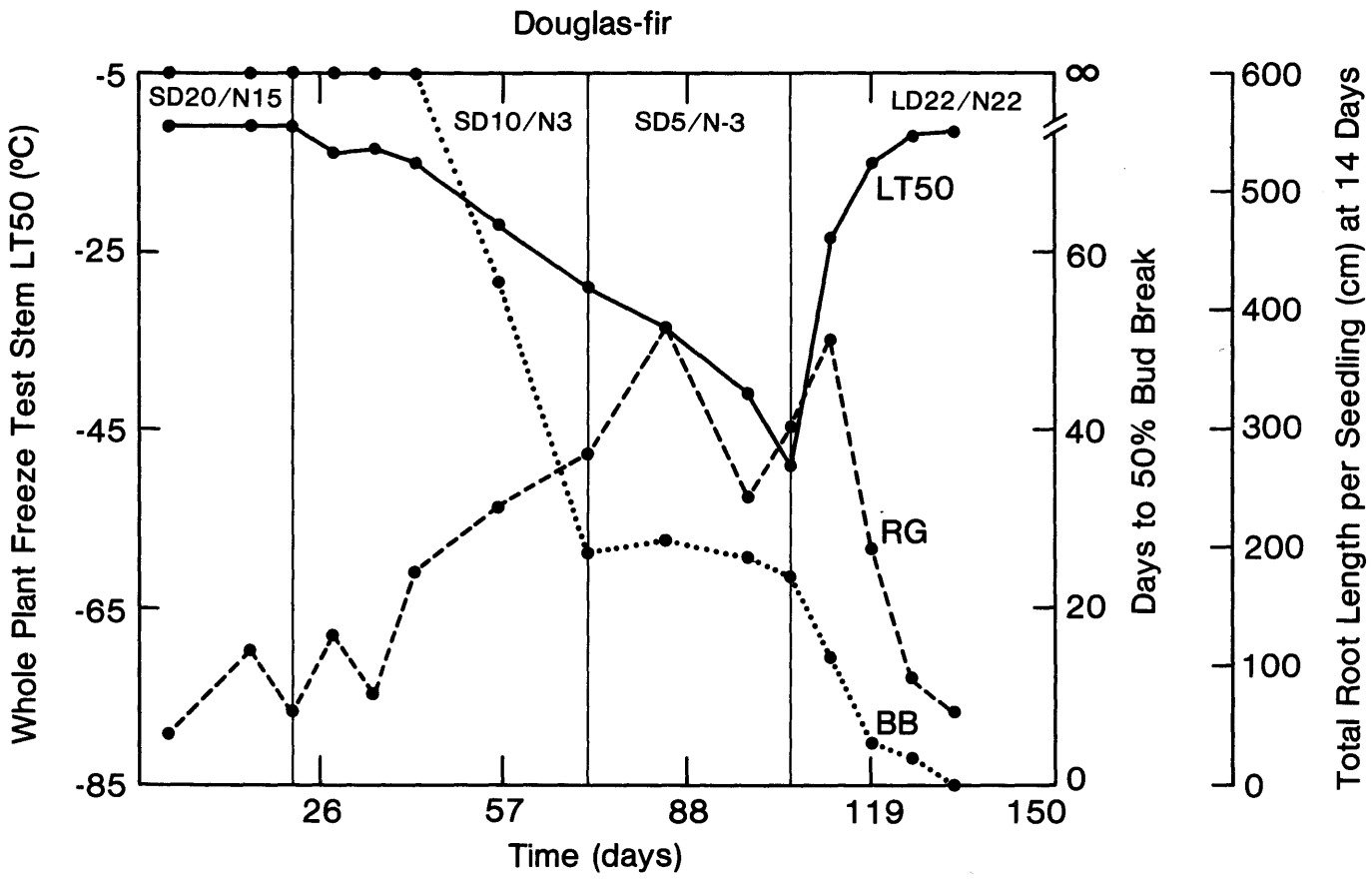

Figure 7.2—*Interior Douglas-fir stem cold-hardiness (LT_{50}), root growth potential (RG), and number of days to 50% bud break (B as a function of time under a 4-stage, growth chamber regime (Burr et al. 1989 from Tree Physiol. 5:301). Growth chamber cond tions, indicated across the top of the graph, are as follows: SD20/N15 = 10 hr (Short), 20°C Day/15°C Night; SD10/N3 = 10 hr, 1 Day/3°C Night; SD5/N-3 = 10 hr, 5°C Day/-3°C Night; LD22/N22 = 16 hr (Long), 22°C Day/22°C Nigh*

dormant period, were placed in growth chambers for a four-stage cold acclimation and deacclimation regime designed to simulate seasonal development from autumn to spring. The first three stages acclimated seedlings to cold under a short (10 hour) photoperiod and progressively colder temperatures, and the fourth stage deacclimated seedlings under a long (16 hour) photoperiod and warm temperatures. The point events on the Degree Growth Stage model can be identified from the days to bud break curve (BB). During the first 42 days of the experiment, buds did not break after 150 days under forcing conditions, indicated by infinity on the bud break axis. Following day 42 of the experiment, days to bud break declined. Thus, day 42 was the maximum rest point (270°). During the third stage, days 72 to 105 of the experiment, days to bud break stabilized at 24 to 28 days. Since the third stage chilling did not reduce the number of days to bud break, this was a period of quiescence resulting from the continued exposure to cold temperatures after chilling requirements were met. Thus, day 72 of the experiment was the end of rest point (315°). With exposure to a long photoperiod and warm temperatures in the fourth deacclimating stage, beginning day 106 of the experiment, days to bud break continued to decrease rapidly to zero on day 130, which was the bud break point at 360°.

Changes in cold-hardiness (LT_{50}), as well as RGP (RG), were related to the timing of the above sequence of changes in bud dormancy (Figure 7.2). Prior to maximum rest, day 42 of the experiment, RGP was low and cold-hardiness only increased from -11 to -15°C. (Note that an increase in cold-hardiness is represented by a decrease in the LT_{50}.) This is the small rise in cold-hardiness referred to earlier (Figure 7.1, Section 4). Hardening from about -5 to -11°C occurred prior to data collection. After maximum rest, all three attributes changed rapidly; days to bud break decreased, and RGP and cold-hardiness increased. During the quiescent period of the third stage, RGP remained high though fluctuating, and cold-hardiness continued to increase. In the deacclimating fourth stage, all three attributes changed rapidly again; days to bud break decreased as bud development resumed, and RGP and cold-hardiness declined. Similar relationships among bud dormancy, cold-hardiness, and RGP have also been observed for Arizona seed sources of ponderosa pine and Engelmann spruce (Burr et al. 1989), and are presented in Ritchie and Tanaka (1990).

If bud dormancy and cold-hardiness targets for fall lifting were set to indicate the period of rapid change during cold acclimation, such as at day 57 of the experiment, it is expected—given our present state of knowledge—that chilling requirements will be completed and rapid cold hardening will continue in storage. It can also be inferred that RGP will be rising rapidly at lifting (barring any unforeseen detrimental climatic or cultural events) because of the relationships among the attributes. In addition, the rapid approach to the period of maximum stress resistance will be identified. Lifting and storing at this time will result in improved storability, as well as improved survival and growth in the field. Similarly, outplanting targets should be set such that a decline in any of the three attributes from their levels at the end of rest will indicate resumption of development and the associated loss of stress resistance.

The above discussion does not imply that rapidly increasing cold-hardiness and decreasing days to bud break at lifting will predict good field performance, nor that maximum cold-hardiness and rapid uniform bud break at outplanting will predict good field performance. It means only that, all things being equal, these characteristics will be positively correlated with performance because they are correlated with stress resistant, high quality seedlings. Seedling quality test results only provide part of the equation needed to predict field performance. Outplanting site conditions must also be included in the equation because it is the interaction between seedling physiological condition and the field environment which will ultimately determine performance.

7.4 Relationships Between Physiological Attributes and Performance

There are data in the literature that support the hypotheses that bud dormancy and cold-hardiness are positively correlated with aspects of performance. An example dealing with each attribute follows.

7.4.1 Bud dormancy

The correlation between bud dormancy at lifting and field survival is illustrated in an experiment by Larsen, South, and Boyer (1986) (Figure 7.3). Twenty loblolly pine lots of the same seed source location, produced at 20 southern forest nurseries, were lifted in early December, stored briefly, and then outplanted in late December. At outplanting, an RGP test was conducted on a sample of 20 seedlings from each lot to determine the speed and uniformity of bud break (number of active buds), as well as RGP. Each data point in Figure 7.3 represents one lot and indicates the number of active terminal buds in the sample after 23 days in the RGP test, versus the survival of the outplanted lot 11 months after planting. Rapid, uniform bud break in the RGP test, which reflected the proportion of quiescent buds in each lot at planting, was positively correlated with increased field survival of the lot. Consistent with the relationships indicated in Figures 7.1 and 7.2, samples with a high proportion of active buds, i.e., which had reached the end of rest (315°) at outplanting, also had higher RGP than samples which had not received their full chilling requirement.

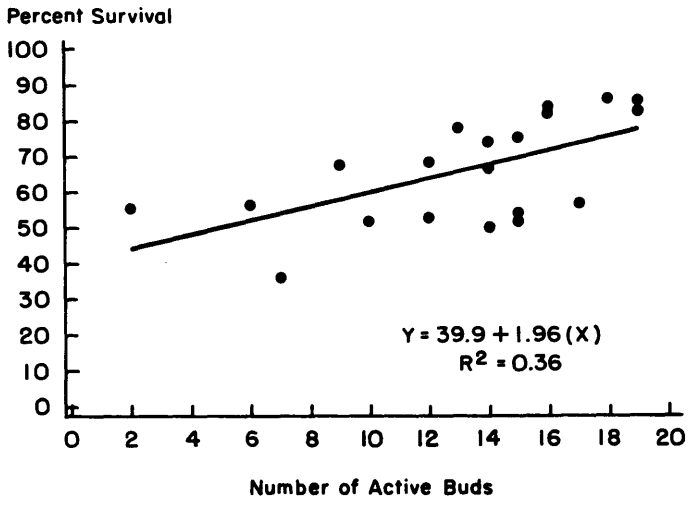

Figure 7.3—*Relationship between percent survival of 20 lots of a loblolly pine seed source 11 months after planting and terminal bud activity of samples (n = 20) taken from each lot at outplanting, after a 23-day RGP test (Larsen et al. 1986 from Tree Physiol. 1:258).*

It is not possible from the above experiment to conclude that rapid bud break directly resulted in better survival. However, the combination of quiescence, high RGP, and the inferred high stress resistance contributed to the higher performance of some lots. It should also be noted that the speed of bud break was an indicator of dormancy intensity in this experiment, and as such, was correlated with field survival. The speed of bud break does not always indicate vigor or future performance in seedlings which have all had chilling requirements fully met prior to exposure to conditions favorable for growth (Lavender 1985).

7.4.2 Cold-hardiness

The correlation between cold-hardiness and performance is illustrated in an experiment by Burdett and Simpson (1984) (Figure 7.4). There was a close relationship between cold-hardiness (frost hardiness) at lifting and storability, defined as the ability of 2+0 seedlings to maintain or improve their RGP during storage. Seedlings lifted with an LT_{50} of about -22°C had the same, or 100 percent, of the RGP after storage as at lifting, while seedlings with greater cold-hardiness had greater RGP after storage than at lifting. Even though cold-hardiness at lifting was not necessarily the sole factor affecting storability, a cold-hardiness target of -22°C could be set for these species to minimize loss of physiological quality in storage.

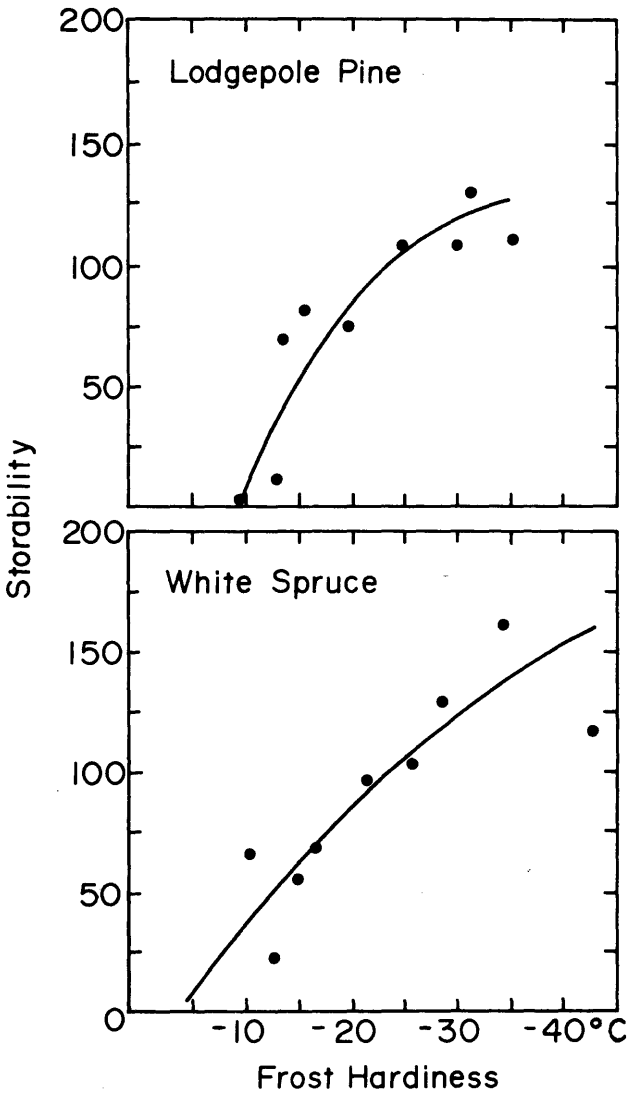

Figure 7.4—*Relationship between storability, estimated by RGP after 6 months of storage at -2°C as a percentage of RGP at lifting, and needle tissue frost hardiness (LT_{50}) in 2+0 bareroot lodgepole pine and white spruce (Burdett and Simpson 1984 from Forest Nursery Manual: Production of Bareroot Seedlings. Eds. M.L. Duryea and T.D. Landis. p. 230).*

7.5 General Considerations for Establishing Targets

While it is beneficial to monitor bud dormancy and cold-hardiness because of their correlations with various aspects of performance, and the inferences which can be made about other physiological attributes, there are a number of items to consider when using bud dormancy and cold-hardiness data to establish lifting or outplanting targets.

Lifting targets that indicate the period of rapidly decreasing days to bud break and rapidly increasing cold-hardiness are most informative. Similarly, monitoring bud

dormancy and cold-hardiness at outplanting in such a way that will detect rapid changes in these attributes in the opposite directions will be most informative. This is because the stable minimum number of days to bud break, as well as the maximum level of cold-hardiness, will vary from year to year, and from nursery to nursery, for the same seed source, when seedlings are quiescent, following the end of rest (315°) (Figure 7.2, Stage 3). For example, in an experiment in which one-season-old, container-grown, interior Douglas-fir seedlings were cold acclimated under three different day/night temperature regimes, three different stable values for days to bud break (range: 18-32 days), and three different maximum levels of cold-hardiness (LT_{50} range: -23 to -38°C) were attained (Burr and Tinus, unpublished data). Given the infinite number of nursery environments, combined with yearly differences in climate, setting discrete targets of a minimum number of days to bud break or a maximum level of cold-hardiness, is not as helpful. Instead, rates of change are more relevant.

A second concern is the uncertainty involved in predicting seedling physiological quality following storage because bud dormancy and cold-hardiness can improve, deteriorate, or remain constant in storage, as can other physiological attributes (Ritchie 1986b). Even if improvement occurs, the rate of physiological development in storage may not be the same as if the seedlings had been left in the field, which adds further difficulty to estimating the final effect of storage on physiological condition (Burr and Tinus 1988, Arnott et al. 1988). Consequently, establishing and monitoring for both lifting and outplanting targets is especially important when storage is an intermediate step. Additionally, the storage environment can also be used to advantageously alter seedling physiological development when storage itself is not the primary goal. For example, substantial reacclimation to cold (2°C/week) was possible when deacclimating (315-360°) interior Douglas-fir seedlings were placed in 1°C storage for 4 weeks (Burr and Tinus, unpublished data). Thus, when a loss in cold-hardiness is premature from a management perspective, it is possible to reverse the loss with skill in environmental manipulation of this attribute (Fuchigami et al. 1982, Sakai 1966).

As a final consideration, a single test, measuring one physiological attribute, is not necessarily enough to ensure physiological quality, especially if it requires inferring too much from bud dormancy and cold-hardiness information and the relationships among physiological attributes. For example, it is possible for RGP to be very different from that expected, based on the relationships indicated in Figures 7.1 and 7.2, when bud dormancy and cold-hardiness targets for lifting and outplanting have been met. Fully cold-hardy seedlings, as determined by a needle browning test for cold-hardiness, may produce no new roots in an RGP test. Also, seedlings may break bud rapidly in an RGP test, but produce no new roots. Both situations can occur because of the delayed response of the shoot to root system damage. Consequently, thoughtful application and interpretation of physiological quality tests is essential.

7.6 Observing and Measuring Targets

An ability to determine seedling physiological status is prerequisite to setting and meeting physiological targets. In this section, observing and measuring bud dormancy and cold-hardiness are discussed, with practical implications for use in nursery monitoring programs.

7.6.1 Bud dormancy

Bud dormancy status can be observed directly at some of the point and segment events on the Degree Growth Stage model (Figure 7.1, Section 1). Obviously, bud break at 0 and 360° would be examples. Maturity induction (90°) can be approximated by examination of the shoot apex for bud initiation. Accurate determination of maturity induction requires testing for photoperiod sensitivity (Fuchigami et al. 1982). The definitive test for vegetative maturity (180°) is most applicable to deciduous species. When defoliation no longer results in bud break (i.e., correlative inhibition has ended), vegetative maturity has been reached (Fuchigami et al. 1982). With conifers, the stop in height growth and the development of overwintering buds can be observed. From 180-270°, the decline in growth to an almost complete absence anywhere on the tree at maximum rest (270°), can be observed, for example, in the decline in root activity as indicated by decreasing numbers of white root tips. However, maximum rest can be estimated with greater precision by testing under forcing conditions to determine the point at which the most time is required for bud break. During the critical 270-315° segment, the change in dormancy intensity cannot be directly observed, nor can resumption of development between 315-360°, until bud swell.

Measurement of mitotic index (Grob 1990b, Hawkins and Binder 1990) can identify maximum rest, as well as the resumption of development after the end of rest (Figure 7.1, Section 3). Monitoring the decline in mitotic activity to zero after vegetative maturity (180°) indicates the maximum rest point (270°). Monitoring for the subsequent increase in mitotic activity indicates the conclusion of any quiescent period and the renewed apical meristem development. However, mitotic index is not useful for assessing the decrease in dormancy intensity as chilling requirements are met between maximum rest and the end of rest (270-315°) because of the complete absence of mitotic activity in most species during this period. A quick test measuring the speed of resumption of mitotic activity under forcing conditions, while the mitotic index is zero under actual conditions (270-315°), is under development to indicate this change in dormancy intensity (Grob

1990a). This test may prove to be a very useful tool to monitor for bud dormancy targets because it provides timely, accurate results by measuring the ability to resume rapid mitotic development, rather than bud break (the result of that development).

Currently, days to bud break tests are the most reliable way to monitor the change in dormancy intensity from 270-315° (Ritchie 1984). This test can be easily conducted as an extension of an RGP test by maintaining seedlings under the optimum root growth conditions until bud break, but the length of time before results are available may range from weeks to months, making days to bud break testing impractical for routine monitoring of dormancy intensity. If the relationship between the decrease in days to bud break and the increase in cold-hardiness were established for this period (270-315°), as was done for interior Douglas-fir (Figure 7.2), inferences about dormancy intensity could be made from the level of cold-hardiness. Setting a cold-hardiness target would also be setting a bud dormancy target. The status of both could then be determined quickly because of the relative speed with which cold-hardiness can be measured.

7.6.2 Cold-hardiness

There are a number of excellent testing procedures available for measuring cold-hardiness. The method of choice for nursery monitoring is the whole-plant freeze test (WPFT), also known as the browning test (Glerum 1985, Ritchie 1984). Entire plants, with root systems insulated, are exposed to a series of sub-freezing temperatures at defined rates of cooling and rewarming. The plants are then maintained under optimum growing conditions until visible evidence of injury develops in about 7 to 14 days (Rietveld and Tinus 1987). Exposure to a range of test temperatures will permit determination of the actual level of cold-hardiness. Once a cold-hardiness target has been set, repeated testing at that temperature until no injury results is a time-saving modification to the standard procedure. Use of stem sections, with needles and buds attached, can be useful if plant material is limited. Root system cold-hardiness can also be measured.

The great advantage of the WPFT is its accuracy resulting from the exposure of entire plants to actual stress temperatures. The test is also easy and inexpensive to conduct, and injured tissue is readily distinguishable. There are disadvantages, however. A week is usually required before the low-temperature injury is evident, though this is much less time than typically required for bud dormancy testing. Additionally, the WPFT does not estimate cold-hardiness with precision because of variability between seedlings. Thus, it may be difficult to detect small (1-2°C) changes in cold-hardiness, and sample sizes of 50-60 are often recommended (Burr et al. 1990, Owston 1988).

Once cold-hardiness targets are set, and seedlings at the target can be identified with the WPFT, it is not difficult to minimize the disadvantages of the WPFT by converting to a faster, more precise, tissue test. Such tests assess cold-hardiness by indirect methods and often use only a single tissue, e.g., electrolyte leakage from needles, differential thermal analysis of buds, and electrical impedance of stems (Glerum 1985). To convert to one of these quick tests, both the WPFT and the tissue test should be conducted on seedlings at the target cold-hardiness level. The tissue test results can then be calibrated to the WPFT results to determine the correct target tissue test result. This is necessary because actual cold-hardiness may be inaccurately estimated by a tissue test, depending on the methodology used (Burr et al. 1990).

The tissue test of preference is the freeze-induced electrolyte leakage test because results are available in 2 days. The test is very precise, it requires less plant material than the WPFT, and it has been operationally tested (Burr et al. 1990, Glerum 1985, Hawkins and Binder 1990). Though the cost of equipment is higher than for the WPFT, a great many samples can be processed at once. Procedures for using this test in nursery applications (Colombo et al. 1984) and in research (Burr et al. 1990) are available.

7.7 Practical Approach

Though there may be a diversity of opinion on how and when to use physiological targets, the following discussion presents one approach for incorporating bud dormancy and cold-hardiness targets into an existing forest nursery operation. The intent is not to suggest that the historical lifting and outplanting schedules should be abandoned, but rather that they be supported and enhanced by the additional information physiological monitoring provides.

The first step toward incorporation of physiological targets into nursery practice is the establishment of a solid foundation of data to use in setting appropriate targets (Owston 1988, Rietveld et al. 1987). By routine, periodic monitoring of bud dormancy, cold-hardiness, RGP, root activity, etc., as well as morphological, cultural, storage, and climatic variables, the relationships among the physiological attributes and the nursery environment can be determined. Ideally, this should be done intensively over several years, from sowing through outplanting and establishment, with a spectrum of genotypes representative of the stock produced at a given nursery. While only continual tracking of physiological attributes provides the detailed level of understanding desired, considerable progress can be achieved by measuring physiological status at lifting and outplanting with simple procedures and inexpensive equipment, depending on the resources of the individual nursery (Burr et al. 1987, Faulconer 1988, Rietveld and Tinus 1987, Ritchie 1984, Simpson 1986).

Additionally, physiological measurements can be made by the nursery, or by sending seedling samples to organizations offering testing services (Munson 1986). In any case, the status of the physiological attributes can then be compared with the successful lifting and outplanting schedules in order to set target values.

Annual air and soil temperature patterns, compiled during intensively monitored years, can be compared with the historical climate at the nursery and with the climate in future years. These comparisons will indicate how representative the monitored years were, and aid in determining the patterns of physiological attributes in future years with considerably less intensive monitoring. Additionally, the probability of damaging low-temperature events on any given date can be determined from historical weather data. This information can aid in making freeze protection decisions in the nursery (James Bryan, Weyerhaeuser Mima Forest Nursery, 1990, pers. comm.).

Targets can be tested by lifting seedlings at several times, before and after the actual lift date. The performance of those seedlings varying in physiological quality at lifting can be monitored at outplanting, and at intervals thereafter in the field. This provides an opportunity to compare quality and refine the target values at both lifting and outplanting.

The physiological attribute(s) best used for lifting and outplanting targets must be decided once information is available on the relationships among physiological attributes, morphological development, and climate. For example, bud dormancy or cold-hardiness targets could be used in a seedling monitoring system, or these could be omitted in favor of RGP testing. An excellent approach is the two-part testing program in use in British Columbia forest nurseries (Simpson 1990). The lifting target is a specific level of cold-hardiness, defined as an LT_{25} measured with a WPFT to -18°C. The outplanting target is a minimum RGP level, tailored to species, stock type, and planting site.

The idea of a monitoring program in which cold-hardiness is measured at the time of lifting, and RGP is measured immediately before planting, is not a new one (Duryea 1985, Johnson 1986). There are many advantages to this testing program.

1. Both a lifting and an outplanting target are used to allow for changes taking place in storage.
2. Two different physiological attributes are used in the event that one test is not enough to ensure quality.
3. Cold-hardiness is ideal as a lifting target attribute because it reflects physiological development well, permits inference about bud dormancy and stress resistance, fluctuates minimally while increasing, and is quick and easy to measure (Faulconer 1988).
4. RGP is ideal as an outplanting target attribute because it best reflects whole-plant performance potential (Ritchie and Tanaka 1990), and is also easy to measure.
5. Two strong relationships are used in series, the first between cold-hardiness at lifting and post-storage RGP, and the second between post-storage RGP and field performance.
6. The cold-hardiness lifting target is set to indicate the period of rapidly increasing cold-hardiness, rather than maximum cold-hardiness.

7.8 Conclusions and Recommendations

1. A considerable amount of information is known about the annual growth cycle of temperate zone trees and the associated patterns of change in various physiological attributes. The Degree Growth Stage Model is an effective tool for communicating this information.
2. Relationships among physiological attributes such as bud dormancy, cold-hardiness, and RGP permit inferring the status of one from another. The period of maximum stress resistance during which lifting, storage, and outplanting procedures should be conducted can thus be identified by measuring any of these attributes.
3. Lifting targets should be set to identify the period of rapid change in bud dormancy, cold-hardiness, and RGP during cold acclimation, while outplanting targets should be set to detect a decline in the three from their levels at the end of rest.
4. Bud dormancy and cold-hardiness status are **correlated** with performance, but this information must be combined with data on field conditions to **predict** performance.
5. Both lifting and outplanting targets are necessary when storage is an intermediate step.
6. During much of the annual growth cycle, bud dormancy status can be observed or quickly measured by determining the mitotic index. However, to measure the decline in dormancy intensity during the critical lifting period between maximum rest and the end of rest, lengthy bud break tests must be performed. With knowledge of the relationship between bud dormancy and cold-hardiness, cold-hardiness targets will incorporate bud dormancy status, and cold-hardiness is much quicker to measure.
7. Cold-hardiness can be measured with the accuracy of the whole-plant freeze test, or faster and with greater precision using the electrolyte leakage test, once results of the two tests are calibrated.
8. Annual base-line data on the relationships among physiological attributes, morphological parameters, and cultural and climatic conditions are needed to establish why lifting and outplanting schedules are (or are not) successful so that appropriate targets can be set for the many nursery-genotype combinations.
9. A practical approach to pursue is a testing program

with lifting and outplanting targets based on solid relationships among physiological attributes and measured by quick, straightforward, non-labor-intensive testing procedures.

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Chapter 8
State of the Art Seedling Stock Quality Tests Based on Seedling Physiology

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ABSTRACT

This chapter describes the methodology and potential application of two physiology-based seedling assessment tests under development (variable chlorophyll fluorescence and stress-induced volatile emissions); the application of two established seedling assessment tests which are in limited operational use (mitotic index and electrolyte leakage); and a short description of three other physiological assessments (triphenyl tetrazolium chloride, days to bud break and the phytogram). The advantages and liabilities of the individual physiology-based tests are discussed.

An argument is presented for the use of an integrated battery of stock quality measures under varied environmental conditions, so that probability based predictions of future seedling performance can be generated. Finally, a variety of seedling assessment technologies are rated individually (sum and product) according to nine criteria. This procedure reinforces the hypothesis that no one test will be able to predict seedling stock quality or move forest regeneration toward the target seedling concept.

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8.1 Introduction

The 1984 Oregon State University Workshop on Evaluating Seedling Quality (Duryea 1985a) was the first major North American attempt to provide a sound basis for evaluating the various past, present, and projected methods of evaluating forest seedling stock quality. Traditionally, morphological specifications have been important grading criteria. However, we stress the theme of others (Wakely 1948, Kramer 1956, Sutton 1979, Bunting 1980, Ritchie 1984, Glerum 1985, Navratil et al. 1986, Sutton 1988, Lavender 1989, Puttonen 1989, Ritchie 1989) that, although seedling morphology is an important management standard (Sutton 1979, Puttonen 1989), it is not what the tree looks like before planting but how it performs after planting that is important to its future performance (Wakely 1948, Sutton 1979).

Morphological seedling grading for height and root collar diameter is rapid but it is unchanging, whereas stresses occurring between grading and planting significantly change physiology without altering morphological grade (Duryea 1985b). This has been aptly summed by Norris (in Duryea 1985a) that seedlings are not of equal physiological quality when planted. Physiology is critical. Its interaction with the environment and its morphological package determine the success or failure of every plantation (Wakely 1948). Seedling physiological assessments should not be used in isolation because there is no one effective method of measuring seedling vigor (Lavender 1989). Rather, they should be done in concert so that a composite of physiological evidence, as to the health of the seedling, is generated. Therefore to ensure plantation success, it is imperative that the physiological condition or vigor of forestry seedlings be monitored from sowing in the nursery through to planting at the reforestation site.

The intent of this review is to report on new and improved seedling physiological assessments with operational potential and novel methods of their integration, rather than reviewing all possible physiological tests. We include theory, methodology, and data interpretation at different levels (depending on the test's historical use in conifer regeneration) for promising operational stock quality assessments. It is hoped this information will aid operational practitioners in understanding and selecting an appropriate series of physiological-based assessments. The expansion and integration of useful stock quality assessments will promote, by definition, the management-driven target seedling stock quality concept.

8.2 Variable Chlorophyll Fluorescence (F_{VAR})

8.2.1 Background and theory

In a living plant, some of the light energy absorbed by green chloroplast pigments used to drive photosynthesis is re-emitted as long-wave infra-red light (Kautsky and Hirsch 1931a,b, Kautsky and Frank 1943). This phenomenon has been coined the Kautsky effect and abbreviated F_{VAR} (Hipkins and Baker 1986, Goedheer 1972, Bose 1982, Geacintov and Breton 1987, see Figure 8.1). The basic principles that govern the yield of fluorescence in the photosynthetic system of plants is complex and have been reviewed elsewhere (Butler 1977, Krause and Weis 1984, Briantais et al. 1986, Krause and Weis 1988). In general, the red light emitted from the plant chloroplast thylakoid membrane reflects the primary processes of photosynthesis including light absorption, excitation ener-

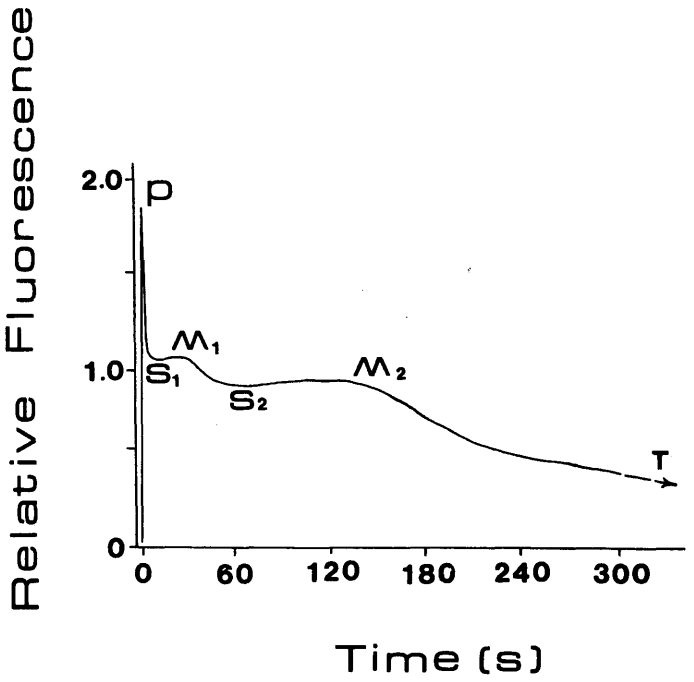

Figure 8.1—*Generalized F_{VAR} curve from 0 to 5 minutes of a typical Douglas-fir seedling. Fast-rise from 'O' to 'P': 'O' to 'I', rapid reduction of Q_A associated with water-splitting charge accumulation; 'I' to 'D', oxidation of Q_A as Q_B is reduced; 'D' to 'P', Q_A and the plastoquinone pool are highly reduced and the photochemical quenching of fluorescence approaches zero (Papegeorgiou 1975, Bothar-Nordenkampf et al. 1989). Slow change decline from 'P' through S_1: re-oxidation of Q by electron transport as CO_2 reduction to carbohydrate level activity increases. Rate through M1 interaction between build up of membrane proton potential, rate of non-cyclic electron flow ATP synthesis, and CO_2 reduction. S_2 and M_2: slow equilibrium. M_2 to T: attainment of steady state equilibrium between proton gradient, ATP synthesis and CO_2 reduction to carbohydrate. After Vidaver et al. (1990).*

gy transfer, and the photochemical reactions in photosystem II (Schreiber and Vidaver 1976, Schreiber et al. 1976, Holzwarth 1988, Krause and Weis 1988, Walker 1988, Vanselow et al. 1989ab). The basis of the chlorophyll fluorescence assessment is that the F_{VAR} response, which is an indicator of the plant's photochemical activity, varies with plant species, season of the year, changes in environmental conditions, previous history of the sample and other factors which may have physiological effects (Vidaver et al. 1990).

In recent years, the measurement of fluorescence has been increasingly applied to various fields in plant physiology. Pertinent reviews on the subject include that of Papageorgiou (1975), Schreiber (1983), Krause and Weis (1984), and Lichtenthaler and Rinderle (1988b). Fluorescence has been studied in relation to chilling injury (Hetherington and Öquist 1988), as a screening method for cold tolerance (Schapendink et al. 1989, Serrano et al. 1988), in relation to the effects of different water regimes (Mugnozza et al. 1988), for detection of stress conditions in plants (Lichtenthaler and Rinderle 1988b, Lichtenthaler 1988b), and in ecophysiological investigations (Lichtenthaler et al. 1986). The first monograph devoted to the applications of fluorescence was published in 1988 (Lichtenthaler 1988a).

With reference to conifers, fluorescence has been studied in relation to seasonal variations in photosynthetic activity (Lichtenthaler et al. 1988, Vidaver et al. 1988, 1989, 1990, Brooke et al. 1989), stress evaluation (Toivonen 1985, Bothar-Nordenkampf and Lechner 1988), forest decline (Lichtenthaler and Rinderle 1988a), frost hardening and dehardening (Strand and Öquist 1988a, Öquist and Malmberg 1989), water stress (Toivonen and Vidaver 1988), nutrient deficiency (Baillon et al. 1988), light stress (Öquist and Malmberg 1989), and provenance differences (Vidaver et al. 1989, 1990).

8.2.1.1 Instrumentation

A portable probe for *in vivo* detection of plant chlorophyll a fluorescence has been commercially available since 1979 (Richard Branker Research Ltd., Ottawa, Ontario). Other units using fiberoptics (Schreiber 1983) with microprocessor (Öquist and Wass 1988) or laser-equipped portable field systems (Lichtenthaler and Rinderle 1988a) have also been described. These include the PSM (BioMonitor), MFMS (Hansetech Limited) and PAM 100 (Heinz Walz). All fluorometers have their unique assets and liabilities. These systems are all useful in determining the state of the plant's photosynthetic membrane. However, they cannot assess large samples or entire seedlings and therefore have low utility in conifer applications. Toivonen and Vidaver (1984) constructed a fluorometer to make F_{VAR} measurements on whole conifer seedlings.

The system constructed by Toivonen and Vidaver (1984) uniquely incorporates an integrating sphere, light source, photographic shutter, optical fibers, and a photodetector. These components are arranged in an appropriate housing and interfaced to a microcomputer which triggers the shutter opening and acquires and stores the fluorescence emission data at onset of shutter opening (Figure 8.2).

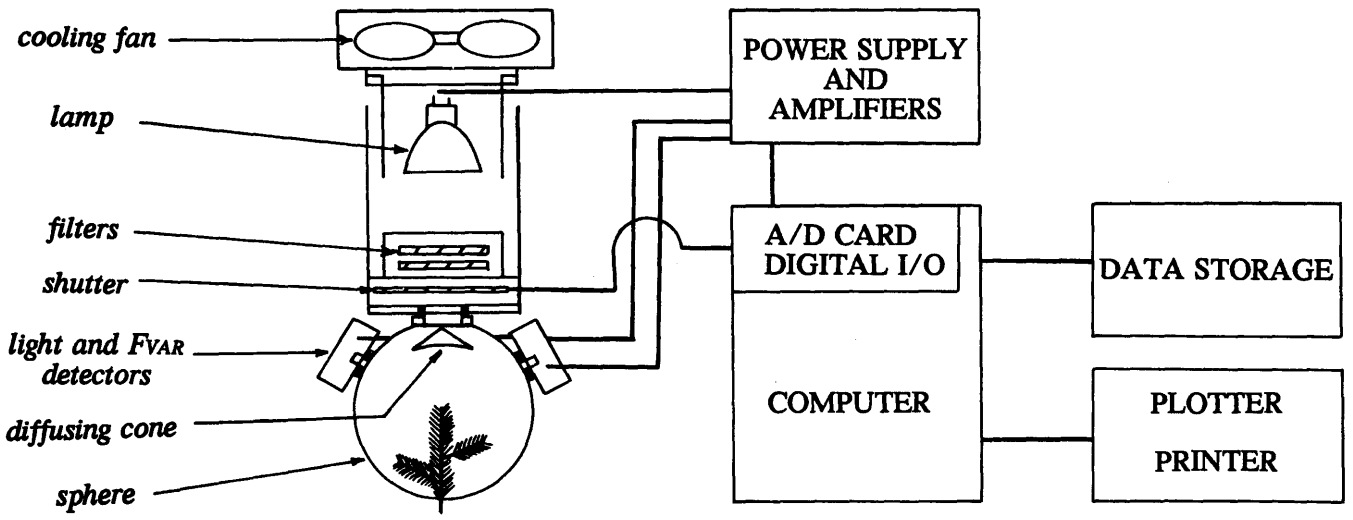

Figure 8.2—*Diagram of the integrating fluorometer showing major mechanical and electrical components for detecting, converting, and storing fluorescence events. From Vidaver et al. (1990); with permission.*

Chlorophyll fluorescence emission from the plant has a peak wavelength of approximately 680-685 nm with a secondary shoulder at 740 nm. Measurement durations can vary from milliseconds (fast change) to five minutes or longer. The data of the completed F_{VAR} time courses can be normalized. This removes the effect of sample size (fluorescence emission amplitude) when comparing data from different samples or when averaging the responses of more than one sample. In practice, any number of samples can be added. A complete description of the system and operation is given in Vidaver et al. (1990).

8.2.2 Applied data

Variable chlorophyll fluorescence induction analysis is a direct measure of the physiological status of the thylakoid membrane (photosynthesis). It can be used in conjunction with other types of physiological assessments such as electrical conductivity, root growth potential, and stress-induced volatile emissions. After placing the plant material in the dark for 20 minutes, the technique requires only a few minutes for measurement. It is also reliable, provides an immediate response, and is completely non-destructive of the sample, so the sample can be remeasured as many times as required by a trained technician or outplanted for future reference.

8.2.2.1 Stress-induced photosynthetic inactivation

Fluorescence is useful as a stress indicator (Conroy et al. 1986, Lichtenthaler 1988b). Conifers and other evergreen perennials possess an unknown mechanism which causes the reversible inactivation of photosynthesis when the needles are exposed to low temperature (Hawkins and Lister 1985) or experience water stress (Brooke et al. 1989). This mechanism apparently prevents the pigments from becoming damaged (photodamage or photoinhibition) under the above conditions, when CO_2 assimilation rates are minimal (Plaut and Bravdo 1973, Boyer 1976, Kaiser et al. 1981). Annual and deciduous plants which are easily damaged by light and chilling temperatures appear not to have this protective mechanism.

The capacity for photosynthetic inactivation enables inactive conifer needles to withstand relatively long periods of drought or subfreezing temperatures even when exposed to high light intensities. Inactivation in conifers is completed within a few hours and probably involves a change or reorganization of the chloroplast thylakoid membrane (Parker and Philpott 1963, Perry and Baldwin 1966, Kimball and Salisbury 1973, Senser et al. 1975, Senser and Beck 1977). The onset of freezing or water stress can cause damage if it occurs more rapidly than the time required for the chloroplast to become inactivated. Fluorescence time courses are distinctly different in damaged and undamaged needles. It is not difficult to distinguish between them. In inactivated but undamaged needles, F_{VAR} is absent but gradually reappears when the stress is relieved (Fink 1976, Hawkins and Lister 1985).

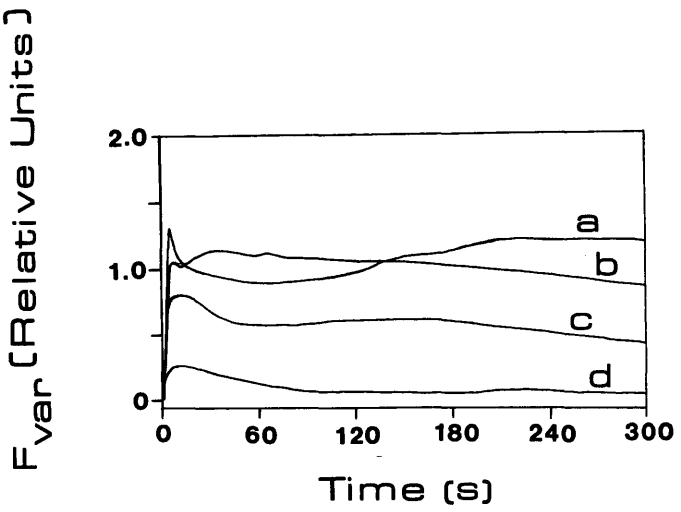

Figure 8.3—*Progression toward seasonal inactivation of F_{VAR} for 2+0 white spruce seedlings monitored during 1986 in a container nursery. Modified from Vidaver et al. (1989); with permission.*

Recovery of F_{VAR} requires adequate root function for needle rehydration, and failure of recovery can reflect root damage.

For example, data obtained on white spruce show that photosynthetic (photochemical) inactivation occurs in parallel with bud set and progression toward dormancy (Vidaver et al. 1988, 1989, 1990; Figure 8.3). This inactivation is apparently a long-term cued event induced by shortening daylength, beginning in mid-August. Daylength-dependent photochemical inactivation may therefore be an adaptation which protects against shoot photodamage from sunlight during winter (c.f. Hawkins and Lister 1985). Because of the ease of obtaining variable chlorophyll fluorescence data on whole seedlings with the integrating fluorometer and its reliability, F_{VAR} assessment could become the method of choice for determining fall nursery lifting dates for interior spruce (Vidaver et al. 1989). The sensitivity of F_{VAR} assessment is demonstrated by its ability to distinguish between provenance types. Seedlings from more northerly seedlots begin inactivation at an earlier time than more southerly seedlots even though they are grown at the same nursery under, as near as possible, identical conditions (Vidaver et al. 1989). The ability to distinguish between provenance types could be useful not only to nursery growers but could also be of great value in coniferous tree improvement and genetic studies.

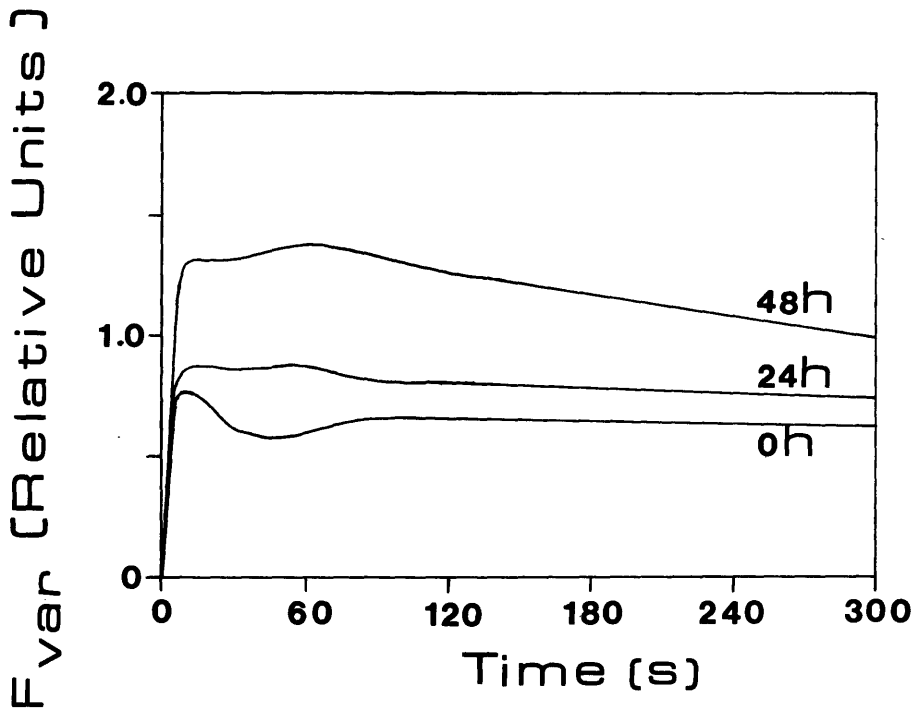

Figure 8.4—*Recovery of F_{VAR} activity in 2+0 white spruce seedlings upon removal from cold dark storage. The F_{VAR} response at 48h is near normal and indicates efficient root function. From Vidaver et al. (1989); with permission.*

8.2.2.2 F_{VAR} and other physiological responses

Since the high quality of seedlings is a critical factor to plantation success, reliable assessments of seedling quality are badly needed. Present tests, such as Root Growth Potential or Capacity (RGP or RGC), tend to be time consuming and controversial (Burdett 1987, Binder et al. 1988, Landis and Skakel 1988). Fluorescence data indicate the technique has the potential to become an extremely valuable tool for determining post-storage seedling quality. For example, seedlings that have high RGP values and optimum field performance in early farm-field tests show near pre-storage levels of F_{VAR} within 24 hours of removal from cold storage and complete recovery within 48 hours (Vidaver et al. 1989, Figure 8.4). Slower or incomplete recovery appears to indicate that the seedlings are physiologically impaired. After cold storage, white spruce seedlings were assessed for photosynthetic capacity (B.C. Ministry of Forests, Research Branch EP 737, Victoria, British Columbia). There was little. Within an hour of severing the roots and providing the shoot with ample water (base of shoot cut under water and immersed in a vial of water), photosynthetic capacity increased tenfold to about 5 mg $CO_2h^{-1}g^{-1}$ (W.D. Binder and G.R. Lister, unpublished results). This is evidence that in some cases poor root function may be the cause of incomplete photosynthetic recovery.

In addition to the long-term daylength-dependent photochemical inactivation observed in spruce species, seedlings of all conifer species tested have shown that photosynthetic activity can be influenced by environmental changes (Vidaver et al. 1988, 1989, Brooke et al. 1989). Both exposure to water deficits, especially at high summer temperatures, and exposure to low temperatures will both induce inactivation. The extent of inactivation and subsequent recovery is dependent upon severity and duration of exposure (Brooke et al. 1989). In the case of spruce, the short-term inactivation was superimposable on the long-term daylength-dependent inactivation. Fluorescence monitoring of a crop could, therefore, warn when remedial measures should be taken to protect against the physiological stress which causes reduced growth potential and decreased seedling vigor.

Daylength-dependent fall inactivation has been observed in white and Engelmann spruce, but not in coastal Douglas-fir or any of the pines so far examined (Vidaver et al. 1990). In coastal Douglas-fir, in response to low temperature, provenance type elevational differences have been observed using F_{VAR} analysis (Brooke et al. 1989, Vidaver et al. 1989). Fluorescence inactivation was induced at lower temperatures on high elevation seedlings than on low elevation seedlings growing under the same conditions at the same nursery site. Such results provide evidence F_{VAR} could be useful in tree improvement as well as in nursery operations.

8.2.3 Test potential (pros and cons)

Variable chlorophyll fluorescence data, obtained to date, strongly indicate that such analyses could be highly useful to the conifer seedling industry. There is strong evidence in the literature suggesting that F_{VAR} measurements serve to indicate the physiological condition of conifer seedling shoots and roots, the status of chlorophyll, and other parts

of the photosynthetic apparatus necessary for carbon assimilation. In the shoot, such information is useful to growth and dry matter production. In the roots, it reflects the ability to provide water and nutrients to the needles, thereby optimizing conditions for photosynthetic processes.

Potential uses of F_{VAR} include:

1) determination of winter lifting window for white spruce and probably Engelmann spruce;
2) assessing post-cold storage seedling vigor in white spruce and probably other conifer species;
3) monitoring effects of environmental factors on photochemical activities (i.e., stress) in most, if not all, conifer species; and
4) in all conifer species, detection of provenance photochemical differences.

To date, the most reliable F_{VAR} results have been observed in species which are strongly cued by photoperiod, such as the white and Engelmann spruces. Typically, "P" values greater than 1.0 indicate high photosynthetic capacity while "P" values less than 0.25 indicate photosynthetic inactivation. The seasonal data interpretations are not as clear cut for temperature-cued species, such as Douglas-fir, western redcedar and hemlock. Their "P" values in the fall fluctuate, using the apparatus described by Toivonen and Vidaver (1984), between 0.5 and 1.0 depending on the environment. The paucity of F_{VAR} data on these species must be overcome prior to F_{VAR} becoming a high utility, stock quality assessment tool. It should be noted that, while understanding of much of the physiological basis and scientific principles which underlie the changes in F_{VAR} are becoming clearer, more work is required before the technique can be readily extended to any wide variety of applications in operational forestry situations, either alone or in conjunction with other assessments.

8.3 Stress-Induced Volatile Emissions (SIVE)

8.3.1 Background and theory

Most reforestation workers are familiar with the sweet odor emitted when a box of "not so good" seedlings is opened. The stress-induced volatile emissions (SIVE) test takes the "smell" several steps further and quantifies the odor. The test is based on low molecular weight hydrocarbons given off in response to stress events by conifer seedlings (Drakeford and Hawkins 1989, Hawkins and DeYoe 1990). The SIVE work is based, in part, on recent stress physiology research on *Pinus resinosa* and *Betula papyifera* (Kimmerer and Kozlowski 1982). Woody plants produce the gases ethylene, ethane, acetaldehyde and ethanol, among others, in response to stresses such as air pollutants (NO_X, O_3, SO_2), water deficits, and freezing (Kimmerer and Kozlowski 1982). The amplitude of gas production is a function of the stress intensity.

The idea of using volatile gas production to determine levels of seedling stress and injury is not original. Ethylene, a gaseous plant growth regulator, is an integral part of the seedling's stress response mechanism (Abeles and Abeles 1972, Jaffe and Telewiski 1984). The gas ethane is a sensitive indicator of cell injury, and more specifically membrane breakdown (Riely and Cohen 1974, Chia et al. 1984, Johnson and Gagnon 1988). The two gases have also been used in concert to describe stress and injury (Elstner and Konze 1976, Konze and Elstner 1978, Kobayashi et al. 1981). Ethanol and its biochemical precursor, acetaldehyde, have also been investigated as a stress response pair (Kimmerer and MacDonald 1987). All four gases have been used simultaneously to assess stress events in pine and birch (Kimmerer and Kozlowski 1982).

Of the four documented stress response gases, it appears that ethanol and acetaldehyde are the best for rapid screening of stress resistance or quality of plant tissue (Kimmerer 1987, Kimmerer and MacDonald 1987, Hawkins and DeYoe 1990). If a test is developed using a single gas, ethanol would be the gas of choice because it is produced continually in woody plants (Kimmerer and Stringer 1988) under both aerobic and anaerobic conditions (Kimmerer and MacDonald 1987, MacDonald et al. 1989).

SIVE testing may have two major advantages over most of the presently used tests: speed and preventive maintenance. After the incubation time of one to two hours, the results are available immediately after the gas chromatograph (GC) run—minutes rather than hours, days or weeks after testing. SIVE distinguishes between stress and injury. This would allow remedial cultural corrections to be made prior to a crop stress becoming a crop injury. Crop injuries decrease the value and performance potential of nursery seedlings.

8.3.1.1 SIVE and other stock quality assessments

Stock quality or seedling assessment is important at all phases of the regeneration continuum (Sutton 1988, Puttonen 1989, Ritchie 1989). High levels of seedling stress resistance correlate well to seedling survival and growth (Ritchie 1984ab, 1986, 1989, Glerum 1985) and can also be used to gauge when to lift and plant stock (Burdett and Simpson 1984). Functionally, cold hardiness induction or frost resistance, mechanisms separate but parallel to dormancy induction, prepares tissues to withstand stresses inherent to winter (Weiser et al. 1979, Levitt 1980). This intensifies the resistance of the seedling to a number of stresses (Levitt 1980, Lavender 1984, Ritchie 1984b, 1989, Glerum 1985), but does not preclude it from being stressed. Clearly, knowledge of the level of seedling stress resistance and a rapid means of determining it would aid in operational decision making.

Operationally, two techniques are used for assessing frost resistance (even though many techniques are available, Keates 1990): one which is qualitative and the other which is quantitative. Visual evaluation (browning) is the qualitative assessment. This assessment takes from 5 to 10 days to complete depending on the time of year. The quantitative assessment is electrical conductivity (leakage), see Section 8.5. Conductivity is either expressed as the ratio of fresh to killed or as an index of injury (Flint et al. 1967, Section 8.5.2). This assessment takes at least three days. Correlation of SIVE data to these assessments would promote its utility.

Seedlings are exposed to handling (mechanical and physical) stresses between the nursery and the planting site which can have deleterious effects on seedling performance (Tabbush 1986). An immediate means of determining the severity of such stresses has yet to be established. If mechanical and physical stresses alter volatile gas production, SIVE has the potential to be used for such assessments.

8.3.2 Applied data

Tests and their application described to this point, for the most part, have been done individually under standard defined conditions. The results from such tests provide the present health of the seedling but to date correlations allowing predictions have not been forthcoming. To move from tests which assess the past and present, to forward projections, requires that the batteries of tests be done under a range of environmental conditions. A stress test will allow the generation of a response surface for a variety of performance attributes, and from this a probability-based projection of seedling performance could be made.

This section will present individual assessment results and the results of a stress test.

8.3.2.1 SIVE and freezing stress

Starting in early August, Douglas-fir seedlings, half of which had been subjected to four weeks of nursery drought stress (Hawkins and DeYoe 1990), were transported from the nursery to the laboratory. They were subjected to five levels of temperature stress (Hawkins and DeYoe 1990).

The season can be divided into four phases in terms of frost tolerance as indicated by ethanol and acetaldehyde production and visual damage assessments (Hawkins and DeYoe 1990). The phases are late summer, early fall, late fall, and winter.

In late summer (August, September), during initiation of bud scales and filling of buds, exposure to lower temperatures resulted in unchanged or decreased ethanol production and increased acetaldehyde production (Figure 8.5). Ethanol production peaked around the LT_{25} (temperature

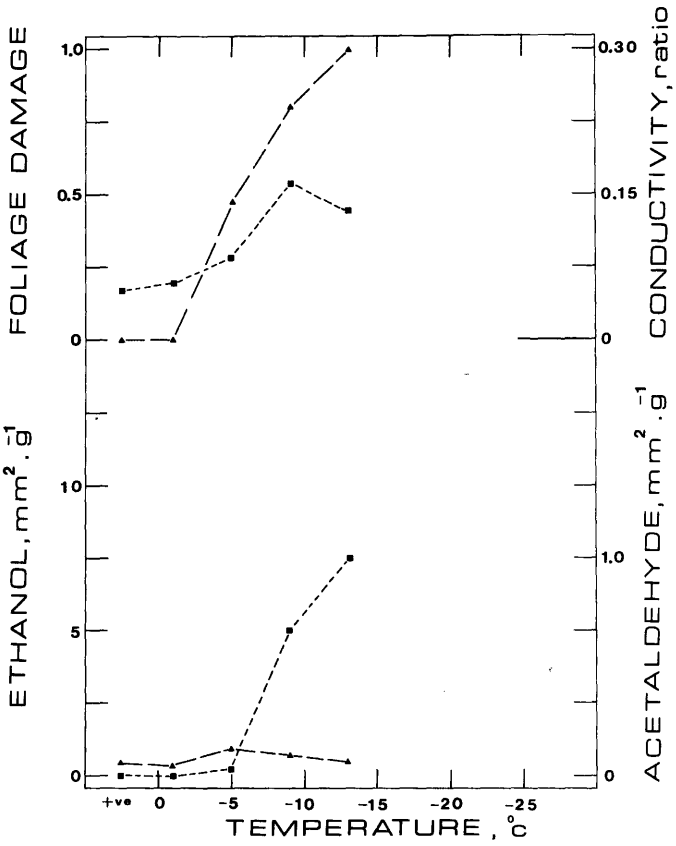

Figure 8.5—*Typical August, September pattern of foliage damage as a proportion of total foliage (▲—▲), conductivity damage ratio (■—■), ethanol (▲—▲) and acetaldehyde (■—■) production in response to decreasing temperature. Control and drought-treated stock were not separated during this time period. Modified from Hawkins and DeYoe (1990).*

resulting in damage to 25 percent of the sample) and acetaldehyde production peaked at the lowest temperature. Visual foliage damage and the electrical conductivity damage ratio both increased with decreased temperature (Figure 8.5). During this period, the LT_{25} of foliage damage ranged from about -3 to -6.5°C.

During late September and October (early fall), both ethanol and acetaldehyde production increased with decreased temperature (Figure 8.6), though ethanol production tended to plateau. The conductivity damage ratio increased with decreased temperature and the LT_{25} of foliage ranged from -4 to -6°C. Generally, nursery drought-treated stock had lower levels of damage, regardless of the variable being examined.

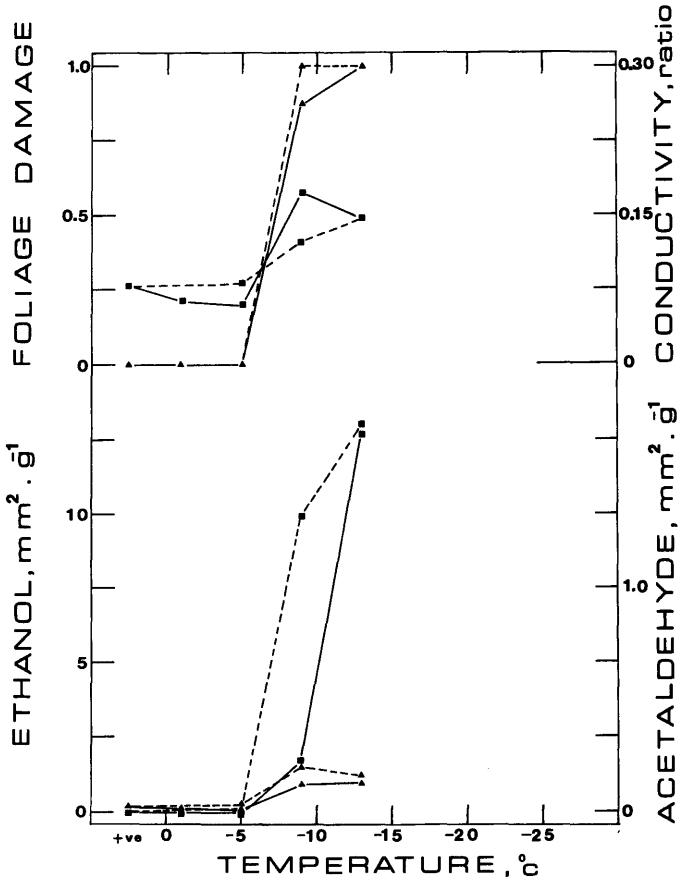

Figure 8.6—*Typical September, October pattern of foliage damage (▲), conductivity damage ratio (■), ethanol (▲) and acetaldehyde (■) production in control (▲—▲ or ■—■) and drought- (▲—▲ or ■—■) treated Douglas-fir seedlings in response to decreasing temperature. Modified from Hawkins and DeYoe (1990).*

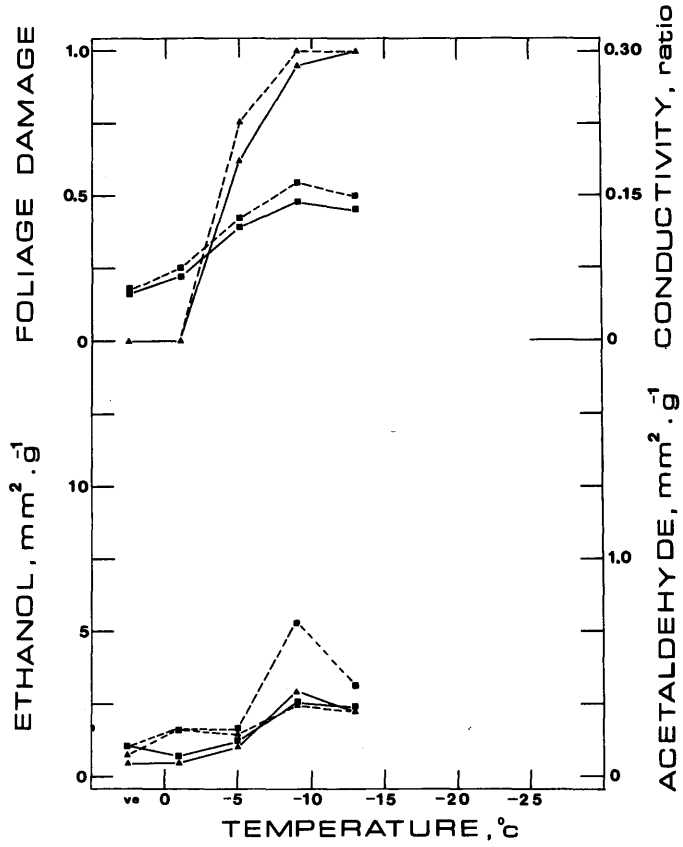

Figure 8.7—*Typical October and early November pattern of foliage damage (▲), conductivity damage ratio (■), ethanol (▲) and acetaldehyde (■) production in control (▲—▲ or ■—■) and drought- (▲—▲ or ■—■) treated Douglas-fir seedlings in response to decreasing temperature. Modified from Hawkins and DeYoe (1990).*

Ethanol production increased and acetaldehyde production decreased during late fall (October-early November) compared with the preceding period (Figure 8.7). Drought-treated stock generally had greater levels of damage. Interestingly, the foliage LT_{25} increased from that previously observed, and ranged from -2.5 to -4.7°C during the period. Again, the conductivity damage ratio increased with decreased temperature.

From late November through early February, maximum winter frost hardiness was achieved. Gas production and conductivity damage ratio were greatest in control stock while there was little difference between nursery treatments for foliage damage (Figure 8.8). Ethanol and acetaldehyde production increased throughout this period. The foliage LT_{25} ranged from -9 to -18°C.

Measurements were terminated when the stock was placed in storage.

Mathematical correlations have been done between gas production, visual damage and electrical conductivity damage ratio (Hawkins and DeYoe 1990). Correlations of ethanol and acetaldehyde production to the other variables were significant during the winter period. These data suggest that SIVE can be used in conjunction with and instead of slower, more established tests for the assessment of frost injury/stress resistance and for the prediction of optimum lifting windows.

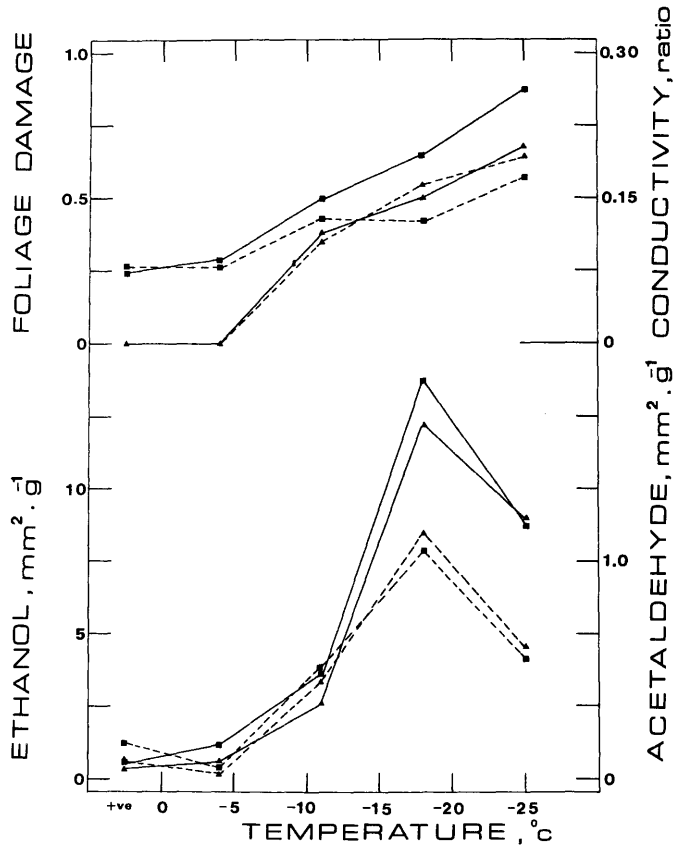

Figure 8.8—*Typical late November through early February pattern of foliage damage (▲), conductivity damage ratio (■), ethanol (▲) and acetaldehyde (■) production in control (▲—▲ or ■—■) and drought- (▲—▲ or ■—■) treated Douglas-fir seedlings in response to decreasing temperature. Modified from Hawkins and DeYoe (1990).*

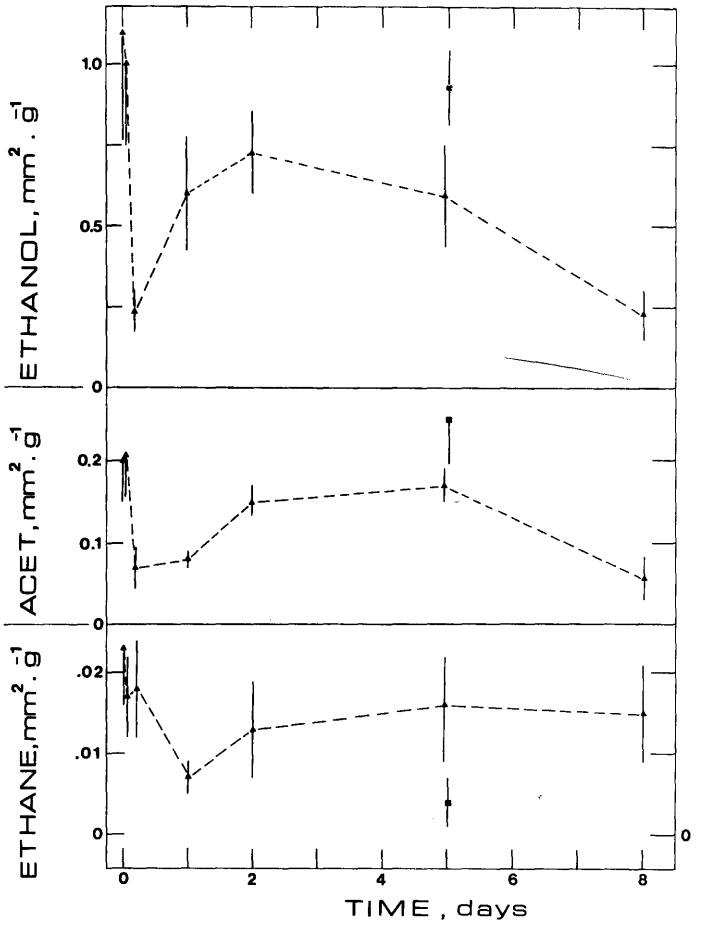

Figure 8.9—*Mean ± SE Douglas-fir seedling ethanol, acetaldehyde (ACET) and ethane production with time from sampling in the nursery through eight days in the laboratory (▲—▲). Mean ± SE gas production of seedlings subjected to physical-mechanical stress (■) on day 5 (the outlier). Modified from Hawkins and DeYoe (1990).*

8.3.2.2 SIVE and handling stress

Douglas-fir seedlings were sampled in the nursery as well as upon return of the seedlings to the laboratory for baseline ethanol production. Laboratory baseline samples were taken for several days. Seedlings were then moved within a styroblock (simulate a handling event) and left to stand for one hour prior to sampling and incubation.

Initially upon sampling and returning the seedlings from the nursery to the laboratory (Hawkins, unpublished results), gas emissions were high (Figure 8.9). A stable but gradually declining level of gas production was reached after 24 to 48 hours in the laboratory. After five days in the laboratory, a sub-sample was moved within the styroblock and this resulted in increased ethanol and acetaldehyde and decreased ethane production (Figure 8.9).

The effect of movement of the seedlings on day five was detected by SIVE. Based on this, it is hypothesized that the rapid decline in gas production observed during the first few hours in the laboratory is the recovery from the stress of sampling and transporting the seedlings to the laboratory. The observed stress response could have a short- and a long-term component. Within hours, levels of gas production have returned to what they were in the nursery. However, due to the warm, long day, low humidity laboratory conditions (as opposed to greenhouse conditions in December and January), there is a slow, one week acclimation to the laboratory conditions until low levels of gas production are again observed.

These data, though preliminary, suggest that SIVE has the sensitivity to detect low levels of physical-mechanical stress. SIVE could prove a valuable tool in screening for

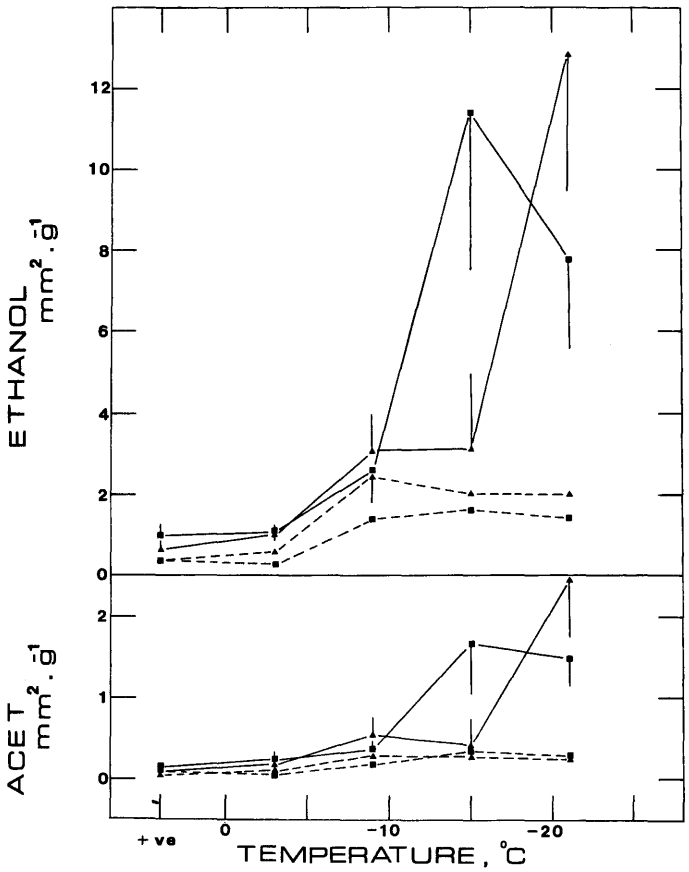

Figure 8.10—*Mean ± SE ethanol and acetaldehyde production in Douglas-fir seedlings after one (▲—▲) and two (■—■) exposures to the various temperatures. Mean (no SE because of small n) ethanol and acetaldehyde production (recovery) three (▲—▲) and five (■—■) days after exposure to the initial temperatures. Modified from Hawkins and DeYoe (1990).*

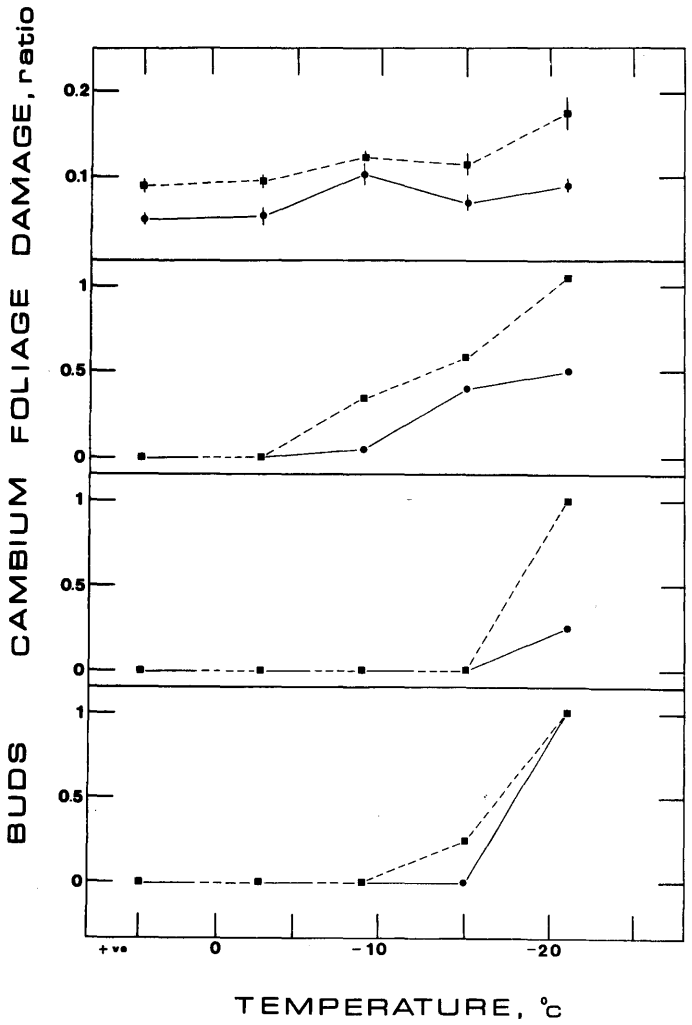

Figure 8.11—*Mean ± SE conductivity damage ratio and mean proportion of foliage, cambia and bud damage in Douglas-fir seedlings after one (•—•) and two (■—■) exposures to the various temperatures. Modified from Hawkins and DeYoe (1990).*

stress events between the nursery and the planting site once species' baseline values are established.

8.3.2.3 SIVE as a "stress" test

Douglas-fir seedlings were brought from the nursery to the laboratory, placed in the freezer, exposed to five temperatures, and subsamples at each temperature assessed for SIVE, EC, and visual damage after freezing. The remaining stock from each temperature exposure was placed under ambient climatic conditions, the temperature range being 0 to 9°C. Seventy-two hours after the initial temperature exposure, recovery gas production was determined. Two days later, seedlings from each test temperature were assessed for further recovery. The next day, the remaining seedlings from each temperature were placed in the freezer and re-exposed to the same temperatures as on day zero. Seedlings were reassessed after the second freezing and root production was also determined.

Ethanol and acetaldehyde production increased significantly (Hawkins, unpublished results) between -15 and -21°C after the first freeze (Figure 8.10). Recovery at all temperatures was greatest after five days (Figure 8.10). Recovery ethanol production was three to four times greater in stock exposed to temperatures of -9°C or less, indicating that while recovery occurs, it is not to pre-stressed levels. The similar level of recovery for seedlings exposed to the three lowest temperatures probably indicates that none of the temperatures constituted a lethal stress even though damage had been done.

Table 8.1—*Mean number of roots (± SE of mean) produced after one week in a misting (aeroponic) tank under continuous, full spectrum light (≈ 200 μmols • m^{-2} • s^{-1}) at 23°C for 1+0 Douglas-fir seedlings after two exposures to the temperatures noted.*

Temperature °C	Roots #	SE
+ve	13.3	1.2
-3	11.7	2.4
-9	7.0	2.0
-15	8.0	2.5
-21		0

There was a significant increase in ethanol and acetaldehyde production between -9 and -15°C after the second freeze (Figure 8.10). A similar result was also observed for the other damage assessments (Hawkins, unpublished results) except on the cambium (Figure 8.11).

A large decrease in ethanol production with decreased temperature (as seen between -15 and -21°C) indicates significant lethal damage to the seedling. Foliage, buds, and cambium were completely damaged and conductivity ratio had its greatest value at -21°C. Root growth indicated the same (Table 8.1). No root growth was observed in seedlings exposed twice to -21°C while good root growth was observed at all other temperatures.

While preliminary, the assessment results are encouraging. It appears that once correlations are established with standard tests and the species baseline is increased, a stress test with predictive capabilities can be successfully developed using the SIVE technique and ethanol production as its backbone and other tests, such as F_{VAR} and EC, as adjuncts.

8.3.3 Comparison of the assessment methodologies

While untested operationally, SIVE has shown strong correlations to other established stock quality tests (Hawkins and DeYoe 1990, Hawkins unpublished results). The major advantage of SIVE over the tests with which it was compared is the speed with which results are obtained. Another potential advantage is that it can detect minor physical-mechanical stress events prior to symptoms appearing. Combined, the speed and sensitivity of the SIVE technique make it an excellent candidate for a remedial cultural program in the nursery. SIVE would also be a good screening assessment for seedlings during the storage transportation phase.

To date, the majority of SIVE analyses have been done destructively and this limits the utility of the test. However, this was for technical convenience rather than necessity. In the future in our laboratory, there will be a gradual move from destructive to non-destructive sampling. The present gas chromatograph (GC) program limits the number of samples which can be done in a day (four per hour), thereby increasing sample costs. Alternative column types and GC programs, and detection systems, are being investigated to overcome this problem. Once done, the major hurdle to SIVE testing will be in the capital cost of a GC. This may, in the short-term, restrict the SIVE test to fee for service, seedling quality assessment laboratories.

Regardless, SIVE is a test with great potential. If coupled to other tests, the SIVE technique could be developed during the next decade to become an important member of

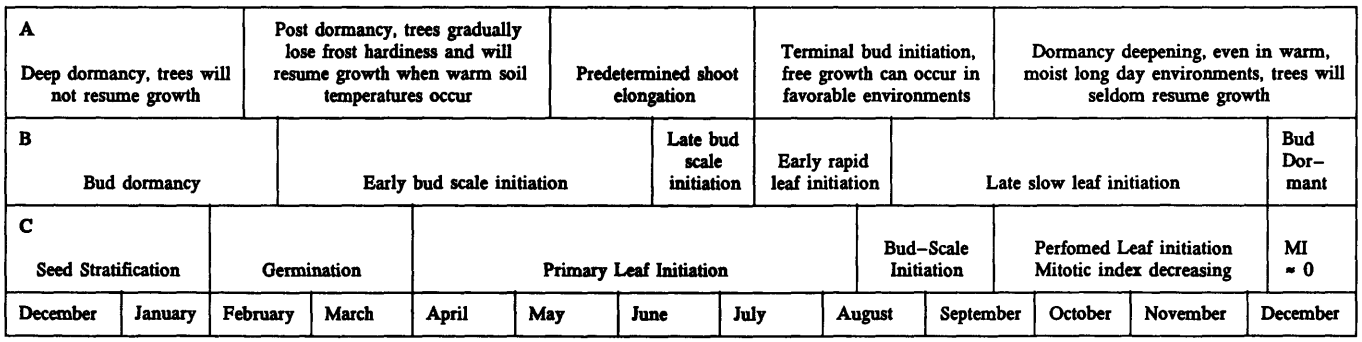

Figure 8.12—*A general comparison of annual physiological events (A), growth patterns of young and mature trees (B), and events of growth and development in first year seedlings (C). Also shown in (C) is the mitotic index state in the terminal bud. Modified from Carlson et al. (1980) and Fielder & Owens (1989).*

the battery of predictive tests which will ensure seedling quality and plantation success.

8.4 Mitotic Index (MI)

8.4.1 Historical theory

There has been considerable literature devoted to both the definition of dormancy and its developmental stages (see Carlson et al. 1980 for review). In conifers, dormancy is generally defined as any case in which elongation does not take place in a tissue predisposed to do so (Doorenbos 1953, Cleary et al. 1978). Owens and Molder (1973), in describing the annual growth cycle of mature Douglas-fir buds, defined dormancy as the absence of cell divisions in the apex. The work of Owens (1968) and Owens and Molder (1973) shows that the mitotic frequency (percentage of dividing cells in ten percent of apical volume of five median sections) clearly decreases with the onset of dormancy and becomes approximately zero (no cell divisions). This corresponds to the period of deep dormancy (Lavender and Cleary 1974) and lasts several months (Figure 8.12) and appears to be closely correlated with seedling resistance to stress (Lavender 1985).

Because the methodology of Owens (1968) for determining mitotic frequency was lengthy and fairly complex, Carlson et al. (1980) developed a bud squash method which is a comparatively rapid assessment of bud nuclear activity. In this method, buds are squashed on a microscope slide, stained, and the number of cells in division expressed as a percentage of all cells counted. This has been coined the "bud squash" method (Table 8.2) and has been used extensively since its development.

Table 8.2—*The bud squash method for determining mitotic activity in conifer seedlings. After Carlson et al. (1980).*

Step	Description of Step
A	Remove buds from seedlings, remove bud scales.
B	Fix buds in McClintoks [Conc. acetic acid: 100% ETOH; 1:3 v/v] for a minimum of 6h.
C	Hydrolyse buds in Warmk's solution [95% ETOH: Conc. HCL; 1:1 v/v] for 10-25 min[1].
D	Place buds into Carnoy's [100% ETOH: Conc.acetic acid: Chloroform; 6:1:3 v/v/v] solution for 5-20 min[2].
E	Stain buds with orcein or acetocarmin[3] 10-20 min. Halfway through the staining place a cover slip over the bud and press firmly down to flatten the bud to a near single layer on the slide. Heat the slide over an open flame but not to boiling.
F	Count the number of cells using a microscope with a 12.5X ocular equipped with a counting grid and a 40X objective lens[4].

1 To dissolve material between cell walls.

2 To counteract softening.

3 Preparation of 0.5% acetocarmin solution. 550mg in 45% acetic acid. Heat to boiling point, remove from heat, add dye, stir, and cool. After solution has cooled, filter through Whatman #1 or similar type filter paper.

4 To determine the onset of bud dormancy precisely the most mitotically active area of the squash should be counted.

8.4.2 Applications of mitotic index

Using the bud squash technique, Carlson et al. (1980) viewed a steady decrease in meristematic cell activity of coastal Douglas-fir during the fall. Cell divisions became essentially zero by December 15. Binder (1983) observed that for coastal Douglas-fir, cell divisions or MI became zero on about December 15 while in interior seedlots, activity ceased one month earlier, even though the seedlots were grown under similar conditions at the same location (Figure 8.13). Activity rapidly increased about the middle of February in greenhouse container stock and in early March for bareroot stock (Figure 8.13). Fielder and Owens (1989) in a detailed developmental study using sectioned embryonic shoots of coastal and interior Douglas-fir (Figure 8.14), confirmed the findings of Binder (1983). Carlson (1985) found that MI on loblolly pine ranged from 17 percent in midsummer to 3 percent in midwinter. Comparative studies of three, open-pollinated, loblolly pine families showed significant differences in MI at several points between late September and early March. These results are discussed in terms of loblolly pine bud dormancy (Carlson 1985). An MI study comparing western hemlock seedlings lifted and placed in cold storage in mid-November against seedlings that remained in the greenhouse was conducted by O'Reilly and Owens (1989). They observed that MI was zero by December 23 in the former, while MI of the latter went to zero on January 13 and for less than one month.

O'Reilly and Owens (1987) did an extensive study on morphology, including MI, on seven provenances of lodgepole pine from 50 to 60° N latitude and planted at one interior location near Prince George, British Columbia. (53° 46' N latitude.). Their data indicate that terminal apices of six of seven provenances were active mitotically by the end of March and began to decline in

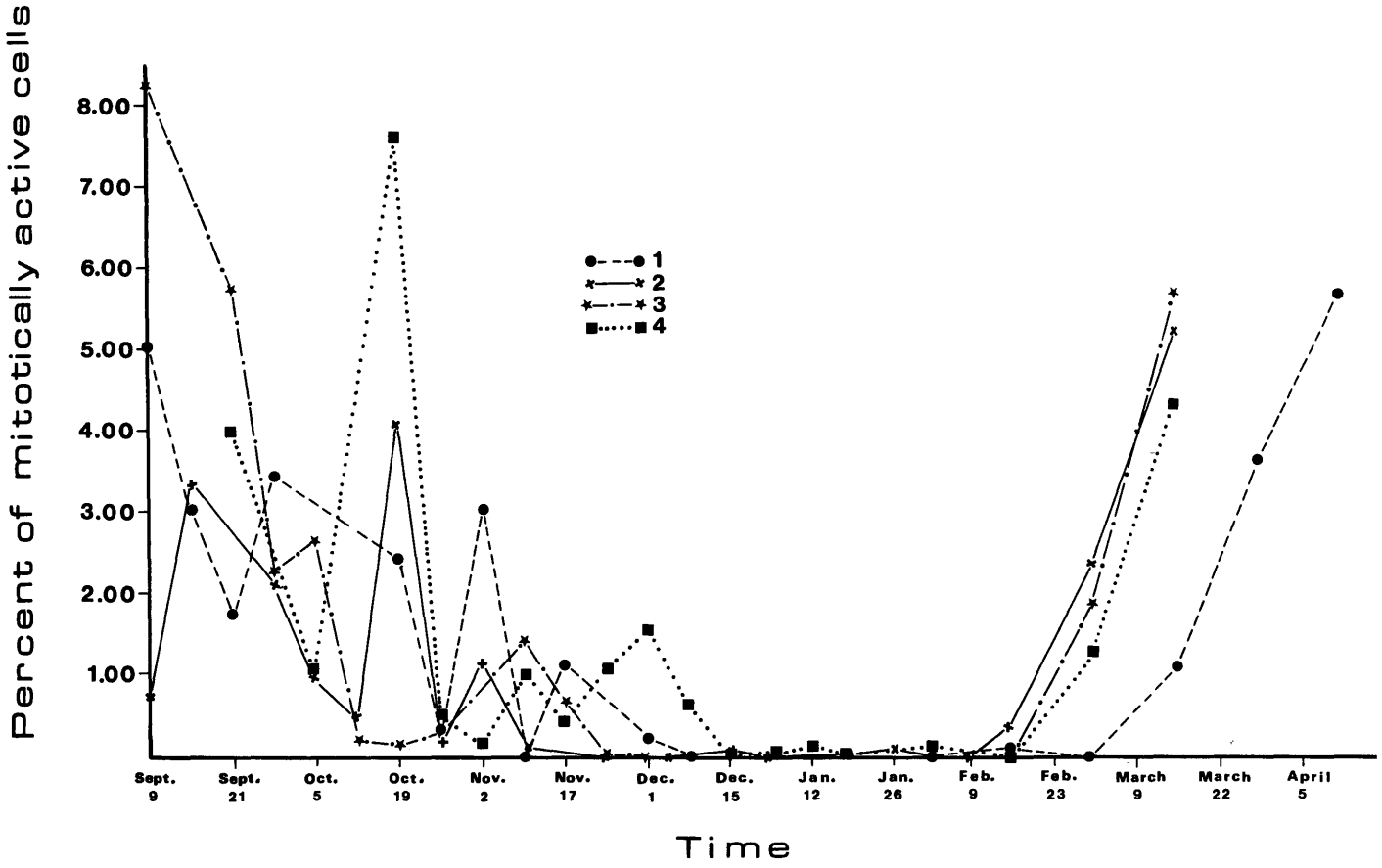

Figure 8.13—*Mitotic activity of two coastal (1 = bareroot, 4 = container) and two interior (2 = drybelt and 3 = wetbelt) Douglas-fir seedlots from September through April. All seedlings were held either in the greenhouse or the field under ambient conditions during the test period.*

mid-August. They were bud dormant by mid- to late-September. Differences among provenances in this regard were significant.

Macey (1982) reported that MI correlated well with frost hardiness in white spruce and that the technique may be used to predict the ability of seedlings to withstand cold storage. Because seedlings exposed to short days and warm temperatures into early winter formed mitotically inactive buds but flushed when exposed to favorable conditions, this worker suggests that MI does not reflect bud dormancy status. Therefore, MI cannot be used to predict lifting date. While this would appear to compromise the test's predictive capability, this is not so from an operational point of view. Operationally grown stock would never be exposed to such conditions. Therefore, the situation should never arise. Also, frost hardiness of black spruce seedlings exposed to different environments has been correlated with MI of the embryonic shoot (Colombo et al. 1989).

Dunsworth and Hartt (1987) found MI sensitive enough to discriminate among a variety of Douglas-fir seedlots. They indicate further study is required to correlate mitotic indexing with other physiological parameters in order to determine optimal lifting and storage times with respect to bud dormancy.

Dunsworth and Kumi (1982) applied the concept of MI to study root activity. They found the technique was sensitive enough to detect seasonal variability in root activity of both Douglas-fir and amabilis fir. Their data indicate that high elevation natural amabilis fir was considerably more active and reached highest activity two weeks before natural Douglas-fir from low elevation. The technique may be useful to discriminate among stocktypes but this requires further study. Using mitotic indexing, Dunsworth (1989) also demonstrated that peak activity in both natural Douglas-fir and western hemlock for both spring and fall could be bracketed by soil climate conditions above -1 bar soil tension and 4°C. Based on mitotic

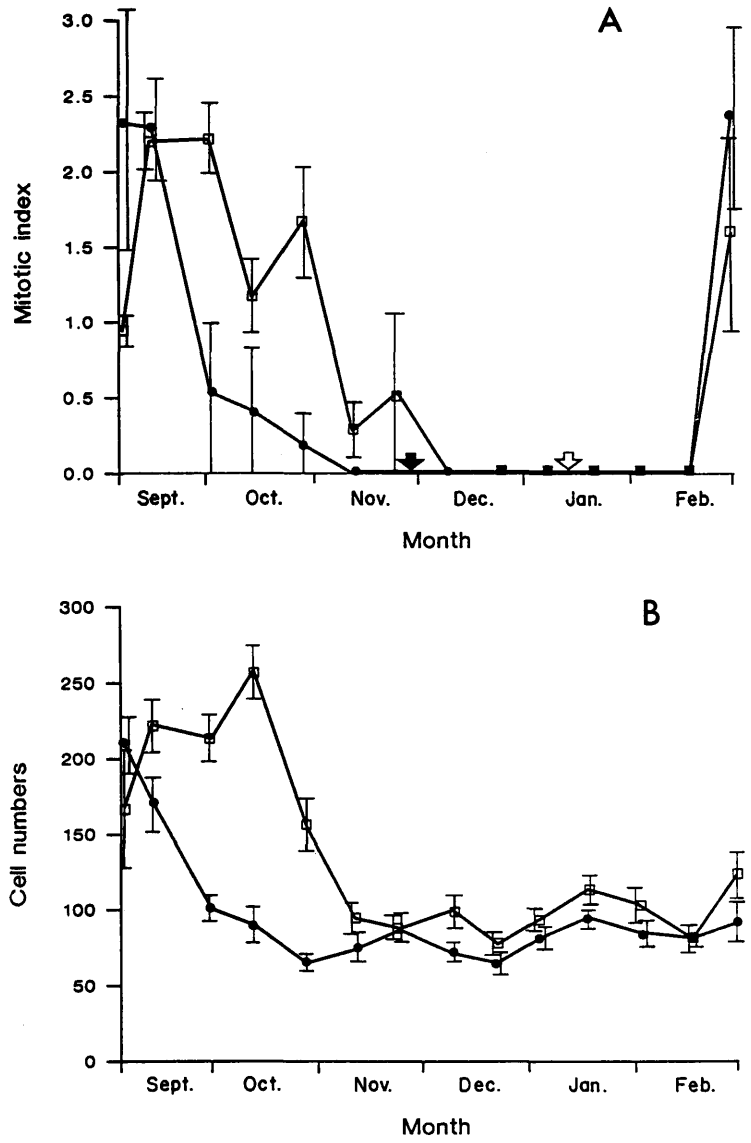

Figure 8.14—*Average ± 1 SE mitotic index (A) and average number of cells (B) per median section, based on four to nine apices per collection of coastal (■) and interior (•) Douglas-fir. End of cell division in subtending leaf primordia indicated by an open and a closed arrow for coastal and interior varieties, respectively. From Fielder and Owens (1989); with permission.*

activity of roots, a survival and growth advantage of 10 percent to 15 percent can be gained by planting within this hypothetical window.

8.4.3 Summary of MI application

After the initial purchase of the microscope, MI becomes a relatively inexpensive test to be done by a trained individual. The lack of wide operational use is probably due to its apparent complexity and lack of applied operational publications (method outlined in Table 8.2). However, this should not detract from the test. There are sufficient data to suggest that MI could play an important role in the optimization of stock quality during the bridging phase (lifting to planting hole), in conjunction with testing of seedling stress resistance. For example in Douglas-fir, MI should remain at or near 0 for 7 consecutive days prior to lifting and storage and the seedlot at -18°C should have less than 25 percent foliage damage (i.e., LT_{25}).

8.5 Electrolyte Conductivity (EC)

8.5.1 Historical theory

The measurement of electrolyte conductivity (leakage) from stressed plant tissue to assess viability was developed by Dexter et al. (1930, 1932). The technique is based on the assumption that whatever the cause of injury to the plant, the result is always a loss of semipermeability of the protoplasmic membrane (Wilner 1960). This results in ion (electrolytes) flow out of the cells and it can be measured using a good quality conductivity meter. The amount of electrolytes which diffuse is assumed to be proportional to the injury. It should be noted here, in caution,

that cell rupture and loss of semipermeable characteristics are generally inferred to be synonymous (see Palta and Li 1978). This, however, is not the case. The loss of plasma membrane semipermeability, as opposed to mechanical rupture, can be affected by other means (Steponkus 1984).

8.5.2 Electrolyte conductivity and cold-hardiness

Most studies that use electrolyte leakage as an indicator of stress damage have used it to measure, for example, relative ratings of cold hardiness of both shoots and roots in several woody species (Wilner 1955, 1959, Wilner and Vaartaja 1958). Flint et al. (1967) improved the technique and developed the equation now known as the Index of Injury (I_t). See also Colombo and Glerum (1984), Colombo et al. (1984), and Glerum (1985).

$$I_t = 100[(R_t-R_0)/(1-R_0)]$$

where: $R_t = L_t/L_{k2}$ and $R_0 = L_0/L_{k1}$, and

I_t = Index of injury resulting from exposure to freezing temperatures.
R_t = Fractional release of total electrolytes from sample exposed to freezing temperature (t°C).
R_0 = Fractional release of electrolytes from unfrozen sample.
L_t = Specific conductivity of leachate from sample frozen to temperature (t°C).
L_{k2} = Specific conductivity of leachate from sample frozen to temperature (t°C) and then heat killed.
L_0 = Specific conductivity of leachate from unfrozen sample.
L_{k1} = Specific conductivity of leachate from unfrozen sample after heat killing.

Early estimates of cold-hardiness using leakage were done in Douglas-fir (van den Driessche 1969, 1976), Scots pine (Aronsson and Eliasson 1970), Monterey pine (Green and Warrington 1978), and black and white spruce (Colombo et al. 1981, 1989). Burr et al. (1986) found freeze-induced electrolyte leakage of needle tissue to be a better predictor of cold hardiness than differential thermal analysis and even the whole plant test (visual damage) in Douglas-fir, ponderosa pine, and Engelmann spruce. According to Burr et al. (1986) the electrolyte leakage test, with the exception of the last week of deacclimation, tends to be somewhat more conservative than the whole plant freeze test. The LT_{50} (temperature at which 50 percent of the samples are killed) occurs at a higher temperature. Nevertheless, they (Burr et al. 1986) state that the EC test is the most precise of the three and detects slight changes in tissue cold-hardiness. Berrang and Steiner (1986) found they could detect seasonal differences in cold tolerance of needles, stems, and male and female strobili in pitch pine using this technique. However, van den Driessche (1976) found that hardiness level prediction of mean conductivity percent did not fully agree with controlled-environment survival results obtained from whole Douglas-fir seedlings after freezing tests.

Freeze-induced electrolyte leakage of shoot tips is used operationally for monitoring frost-hardiness of stock in extended greenhouse culture in Ontario (Colombo et al. 1984, Colombo and Cameron 1986).

In general, the electrolyte leakage method works well to detect tissue cold hardiness either as a direct test of cold hardiness or as a reaction to cold stress (Flint et al. 1967, Colombo et al. 1984). Two important advantages of the technique are: it is useful for measurement of all conifers, and a great many samples can be measured concurrently with no increase in equipment (Burr et al. 1986).

In this regard, the results of Zhang and Willison (1987) are very interesting. Using cultures of brome grass to measure cold-hardiness they found that electrolyte leakage always underestimated the frost hardiness by comparison to fluorescein diacetate (FD) vital staining. Fluorescein diacetate tests for metabolic activity, that is, the capacity of cells to display esterase activity. They found that there was a difference in ions leaked after 18 hours in deionized water and leakage after 1 hour in deionized water. They termed this differential percent leakage (DPL). They found that one-half the maximum DPL (DPL_{max}) was very similar to LT_{50} estimates of frost damage using the FD method. The value of the correlation between LT_{50} by DPL_{max} and LT_{50} by FD is just over 0.97. The physiological basis of the DPL_{max} effect apparently is that frost-killed cells, on thawing, leak electrolytes rapidly while living cells with intact plasma membranes leak ions slowly. The rationale is that as more cells are damaged, the difference in leakage in relation to deionized water immersion time will decrease. Thus, if the maximum difference (DPL_{max}) corresponds to 100 percent living cells, then one-half of this difference corresponds to 50 percent living cells. To our knowledge, this version of electrolyte leakage has not yet been applied to conifers and if used, may yet further improve the sensitivity of the test.

8.5.3 Electrolyte conductivity and other assessments

Electrolyte leakage has been used to assess genetic variations in cold tolerance (Kolb et al. 1985, Raymond et al. 1986) of tree species. It has also been used to assess damage to trees from air pollutants (Keller 1986, Leith et al. 1989) and other stresses such as leaf desiccation (Leopold et al. 1981). The technique has worked fairly well in such studies. Keller (1986), for example, reports that sulphur dioxide fumigations increased measurable leachate conductivity even at concentrations causing no visible symptoms of injury. Kolb et al. (1985) also commented that the technique may have practical value for tree improvement programs if differences remain reasonably consistent between years, as their results indicate.

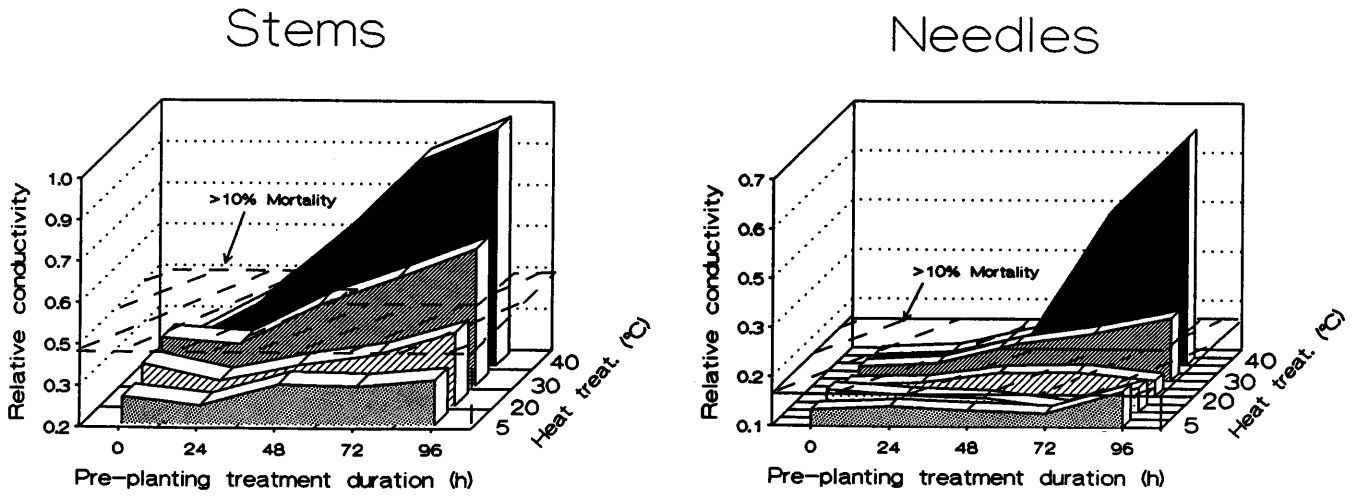

Figure 8.15—*Relative conductivity of white spruce stems and needles after 5, 20, 30 and 40°C heat treatments for 0, 24, 48, 72 and 96h. The 10 percent mortality rate from outplanted test plots is indicated. Modified from Binder and Fielder (1990).*

8.5.4 Electrolyte conductivity for stress evaluation in conifers

Electrical conductivity has been recently used to evaluate heat stress resistance in white spruce seedlings prior to planting (Binder and Fielder 1990).

8.5.4.1 Heat-treated stem and needle segments

After removal from cold storage, boxed seedlings were heated to 5, 10, 20, 30 and 40°C for 0, 24, 48, 72 and 96 hours. The conductivity of leachates were determined for untreated and killed controls, and frozen and frozen/killed treatments after incubation for 24 hours at 25°C. The I_t was calculated after Flint et al. (1967).

Temperature treatment and duration affected the fractional release of electrolytes from stem and needle segments (Figure 8.15). The rate of increase of mortality over time was dependent upon the treatment temperature. The results for needle and stem segments were similar except the response was greater and conformed closer to the expression of mortality at lifting time in the case of stem segments (Figure 8.15). Electrolyte leakage from needle segments was strongly correlated with field needle damage after 14 days (Figure 8.15). Fractional release of electrolytes above 0.5 for stem segments indicated a potential for mortality >10 percent for all temperature treatments. There appears to be close agreement between electrolyte leakage from stem and needle segments to high post-cold storage temperatures, and field results. This is opposed to the findings of van den Driessche's (1976) growth chamber survival assessment where changes in EC did not fully reflect field survival.

The development of damage over 48 hours at 40°C (Figure 8.15) indicates this was not due to direct primary heat injury (i.e., heatshock). It would suggest temperature and time of exposure were interacting to intensify indirect heat injury. Direct heat injury is manifested in the order of minutes or hours following the exposure (Levitt 1980). Field results show that needle damage was evident after 72 hours at 40°C, but not after 48 hours. Needle damage after the 48 hours at 40°C treatment increased over the season, this being consistent with the long-term effects of indirect heat injury.

The difference in response of stem and needle segments to post-cold storage heating also indicates the most probable chief cause of mortality. Electrolyte leakage from needle segments corresponded most closely to percent field needle damage after 14 days. On the other hand, electrolyte leakage from stem segments corresponded most closely with mortality at lifting time. These results suggest that membrane damage to stem tissues (i.e., cambium and conducting tissues) may be more important to eventual survival than damage to needle cells. Survival is dependent upon healthy conducting tissues.

8.5.4.2 Frost-hardiness testing by index of injury method

The hardiness of stems and needles can be determined by electrolyte conductivity. Binder and Fielder (1990) determined the temperature increase (i.e., to less negative value) to I_{50} (50 percent index of injury) for stem and needle segments of boxed, post-cold stored white spruce exposed to various heat treatments for up to 96 hours (Table 8.3). The extrapolated I_{50} for control stems and needles was -100°C and -79°C, respectively. Results sug-

Table 8.3—*Average percent temperature increase (i.e., less negative) in the index of injury of 50% (I_{50}) to stem and needle segments as a result of exposure of boxed white spruce seedlings to 5, 10, 20, 30 and 40°C temperature for up to 96 h*. Freezing temperatures were determined from interpolation and extrapolation from regressions of index of injury with freezing treatments to -12, -20, -28 and -36°C. Regressions were fitted through 4 points with 3 measurements at each point (modified from Binder and Fielder 1990).*

Temperature Treatment °C	% of Control in I_{50} over 96 h Stems	Needles
5	100	100
10	59	58
20	36	44
30	26	32
40	35 (to 48 h)**	58 (to 48 h)

* Seedlings received 8 days of thawing at 5°C before being heat treated

**There were no live seedlings after 48h at 40°C.

gest that for both stems and needles, temperatures in excess of +5°C applied to stock thawed from the -2°C of storage reduced frost-hardiness. The magnitude of the reduction depends on the amount of heat and its duration. The reduction in I_{50} at 40°C after 48 hours was similar to 20°C and 10°C for 96 hours for stems and needles, respectively. Differences in frost hardiness between stems and needles after 48 hours at 40°C may point to a greater thermotolerance of needles (needles are subjected to more direct thermal heating than stems), at least initially. Evidence suggests (Levitt 1980) that thermotolerance and winter freezing tolerance are related since high reduction capacity of the membrane is important to both.

8.5.5 Assessment of electrolyte conductivity

Electrolyte leakage has a wide range of potential applications in stock quality testing. Some of its advantages are that large numbers of samples can be done in a short period of time, it is statistically valid, a small tissue sample is required, and it is highly sensitive. However, disadvantages are that it is destructive and seasonal baseline trends have not been described for nursery monitoring programs.

Because of its mechanical simplicity, statistical rigor, and low initial cost of equipment, EC is a test that could be used increasingly in nursery and field diagnostic situations. EC provides good support data to almost all physiological tests. This test should be a part of any integrated assessment program.

8.6 Other Tests

8.6.1 Triphenyl tetrazolium chloride (TTC)

The earliest reports of triphenyl tetrazolium chloride (TTC) as a test of tissue viability were by Roberts (1951, 1957), Parker (1951, 1953), Larcher and Eggarter (1960) and Purcell and Young (1963). These tests were generally of a qualitative nature. If color was observed, the tissue was considered to be viable. Steponkus and Lanphear (1967) refined the technique so that it was quantitative and could be analyzed statistically. This allowed testing of small

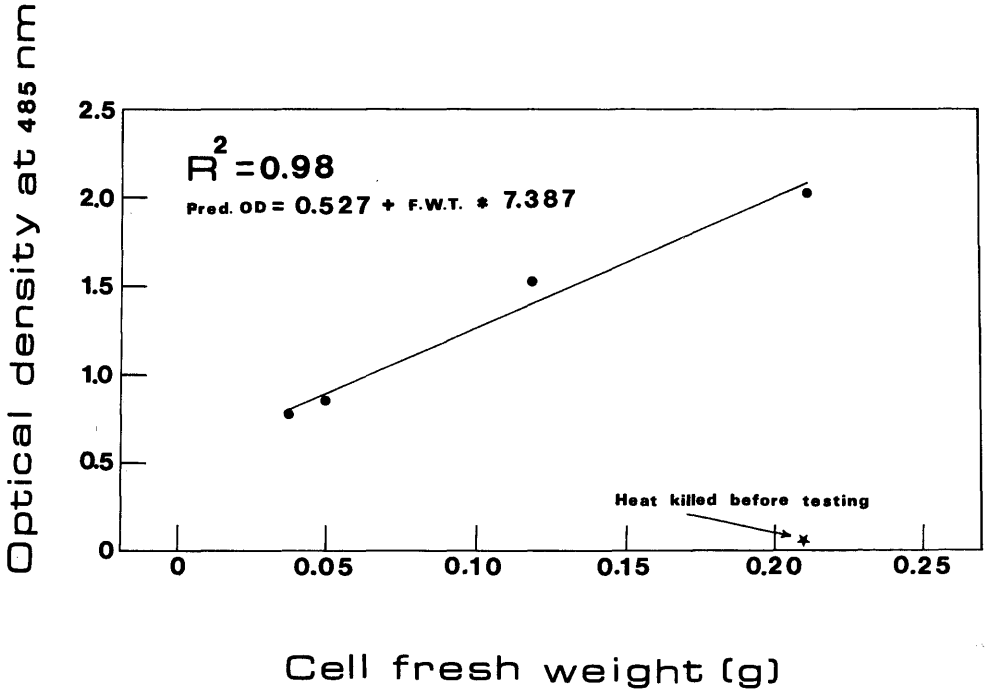

Figure 8.16—*The regression correlation (r^2 = 0.97) between TTC reduction and Douglas-fir callus tissue fresh weight.*

pieces of tissue, the results of which were used to predict the future viability of the whole plant.

The technique estimates the activity of live tissue which has the capacity to display dehydrogenase activity. The test is, therefore, one of metabolic viability and is similar to the fluorescein diacetate method (Section 8.5.2). Dehydrogenase class enzymes are able to alter TTC, which is colorless and soluble in water, to its derivative, formesan, which is red and soluble in alcohol and can be boiled out of the tissue and measured optically in a spectrophotometer (Steponkus and Lanphear 1967). The procedure is outlined in Table 8.4.

There is a good correlation (r^2) between the amount of TTC reduced and tissue fresh weight using this method (Figure 8.16). Using this method, Sugawara and Sakai (1978) measured cold acclimation of callus cultures of Jerusalem artichoke. When calluses were hardened at 0°C for 18 days and then frozen to temperatures ranging from -3 to -20°C, they found a very good reciprocal correlation between TTC reduction rate and amino acid releases, and a parallel correlation between TTC reduction rate and regrowth after freezing. Chen and Gusta (1983) found good agreement between the TTC test, the fluorescein diacetate test (enzymatic and membrane permeability) and a regrowth test after freezing of cell suspension cultures of winter wheat and a winter rye. Zhang and Willison (1986) used the fluorescein diacetate method and adjusted electrolyte leakage method (see Section 8.5.2) to assess freeze damage and found close agreement between the two tests. Altmann (1969) used a variation of the TTC test to study freeze stress. The substrate is nitroblue tetrazolium and the product of the dehydrogenase activity is the green dimethyl-formamide molecule (c.f. Timmis 1976).

Table 8.4—*The TTC procedure as modified from the findings of Steponkus and Lanphear (1967), Withers (1978) and Binder (1981).*

Step	Description
A	X% Buffered TTC[1] in 78% $NaHPO_4$+ 22%KH_2PO_4 + Wetting agent.
B	Add above solution to small pieces of tissue (mg).
C	Vacuum infiltrate.
D	Incubate at 30 °C for 15h.
E	Drain off TTC.
F	Wash with distilled water.
G	Extract with hot ethanol (95%) for 30 min. in water bath.
H	Bring to volume with 95% ethanol[2].
I	Read in spectrophotometer[3].

1 Use between 0.1 and 0.7% TTC depending on the amount of tissue.
2 Amount of tissue should be enough to produce optical density readings below 2 absorbance units at 485 nm.
3 Use 430 nm (Steponkus and Lanphear 1967) or 485 nm (Withers 1978; Binder 1981)

There are few reports in the literature which directly use TTC as a stress test in conifers. Timmis (1976) compared leaf segment flotation, seedling water stress, photosynthesis, impedance ratio, and needle dehydrogenase activity (TTC) to detect live and dead Douglas-fir seedlings after freezing stress during five different frost hardening stages. He found that TTC was the second best method of detecting live and dead seedlings (76 percent on average; the impedance test was first at 87 percent on average). However, the TTC test could not distinguish between dead and live groups when these seedlings were exposed to night frosts (i.e., quiescence after rest) when frost hardiness was maximum. The TTC test was not predictive of frost damage at any stage of frost hardiness if stem segments were used. The method has been used to estimate cell viability of Douglas-fir suspension cultures after freezing to -196°C (Binder 1981). The method has also been used to estimate the seasonal variation in root hardiness of container-grown Norway spruce, Scots pine, and lodgepole pine (Lindström and Nyström 1987). The test was able to distinguish between fall hardening and spring dehardening of roots, as well as distinguishing species differences in this regard. The test can also detect differences between cold hardiness of mature and young roots of the same species (Lindström and Mattsson 1989).

Binder and Fielder (1990) used TTC as an indicator of sensitivity to heat stress in white spruce buds after cold storage (Figure 8.17). Enzyme activity was significantly reduced after 12 hours at 40 and 60°C but not at 20 and 30°C. Activity was reduced after 24 and 48 hours at 20 and 30°C but this was not significant compared to 12 hours. Respiration measurements on these buds were erratic. Only buds treated at 60°C showed no respiration after 12 hours. Respiration was also significantly depressed after a 40°C treatment for 48 hours. Apparently the TTC test is a better diagnostic measure of heat stress in buds than is respiration. However, what possible correlation there is between the buds increasing lack of ability to reduce TTC with temperature and time and ability to flush, extend needles or elongate is not known at this time.

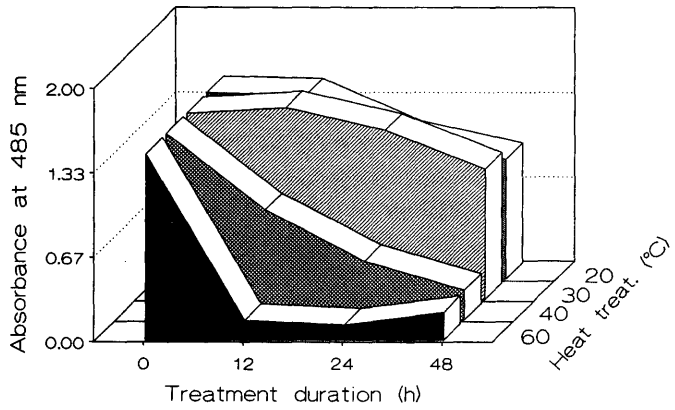

Figure 8.17—*TTC reduction in white spruce after 20, 30, 40 and 60°C heat treatments for 12, 24 and 48h. Results based on three replicates, each of six buds. At 20 and 30°C, the results are not significantly different. Results at 40 and 60°C after 12 and 24h were significantly different from each other and 20 and 30°C. After 48h, there was no difference between 40 and 60°C.*

Even though laboratory-based, TTC could be incorporated into a stress test to assess for functional levels of enzyme activity. Historically, this test has not been used to its fullest extent. During the planting of stock from storage, it could be an important aid in discriminating between viable, moribund, and dead stock. Clearly, there is a need to define the range and conditions under which this test can be used and which tests are best used in conjunction with it.

8.6.2 Days to bud break (DBB)

Days to bud break can be used to assess the level of dormancy in conifer seedlings (Ritchie 1984a, Ritchie et al. 1985). In the simplest terms, the longer it takes for buds to break, the greater the level of dormancy (Campbell 1978, Ritchie 1984a, Lavender 1985).

Table 8.5—*Days to budbreak at lift, during storage and at planting in a white spruce seedlot exposed to four different photoperiod durations to control height growth during the nursery cultural phase. Modified from Hawkins and Draper (1990).*

Photoperiod h	DBB November 88 lift	DBB January 89 store	DBB May 89 plant
13	15.1	10.8	7.9
15	20.2	11.7	9.5
17	20.0	12.6	9.7
19$	24.1	13.7	9.7

$ Ambient photoperiod at Red Rock Research Station, near Prince George, B.C., ≈ 54° N. latitude.

To assess DBB, stock is placed in an optimizing environment, similar to that described for RGC (c.f. Binder et al. 1990) and the number of days required for the terminal bud scales to part and expose new, green needles is recorded. Ritchie (1986) used the days to bud break to develop a linearized "dormancy release index" (DRI). This index is calculated as the ratio of the number of days required to force bud burst in a fully chilled seedling over that in the seedling of interest. According to Hermann (1967) and Ritchie (1984a), a fully chilled coastal Douglas-fir seedling can be force-flushed in a minimum of 10 days. Therefore in Washington state, the Douglas-fir DRI is written as: DRI = 10/DBB.

However, for other geographic locales, the DRI numerator must be redefined. The DBB assessment, though time-consuming, can provide valuable information about the effect of nursery cultural treatments on seedling dormancy intensity. Days to bud break has been used to define the relationship between bud dormancy, cold hardiness and stress resistance (Ritchie 1986) as well as root growth potential (Burr et al. 1986, 1989) in some western conifers. For example, according to Ritchie (1986), in Douglas-fir, maximum stress resistance and hence survival potential falls somewhat beyond the peak of dormancy after several hundred hours of chilling exposure.

When photoperiod is used to control height growth (photoperiod is often viewed as a "non-stress" method of achieving seedling height control) in white spruce, it can have a significant impact on DBB between nursery treatments (Table 8.5). This phenomenon has been reported for other spruce species and the result can be a significant perturbation on bud phenology and diminished field performance (Hawkins and Hooge 1988, Odlum and

Colombo 1988). The range of DBB at planting presented (Table 8.5) is much smaller than previously described (Hawkins and Hooge 1988). Whether the smaller range in DBB is of significance, under field conditions, has yet to be determined.

As dormancy intensity plays a crucial role in seedling establishment (Ritchie 1984b), nursery cultural modifications should be assessed for their impact on DBB, especially considering that cultural modifications, such as blackout, can elicit such marked performance responses. Douglas-fir seedlings in mid-dormancy release (i.e., that region between maximum dormancy [buds do not flush] and quiescence [buds flush] when placed in a growth permissive environment), apparently, are most resistant to stress (Ritchie 1986).

While DBB is inexpensive, simple and straightforward to conduct and assess, it seldom is done on an operational basis—presumedly because of the time involved (a minimum of 30 to 80 days depending on the species and the requirement to assess bud development at regular intervals). Perhaps, in the future, the time factor may be overcome by the establishment of correlations between DBB and some of the more rapid tests. Ritchie (1989) has shown that accumulation of about 1,400 natural chilling hours (air temperature below 6°C) equates with both DBB and DRI. Regardless of the time aspect, DBB is a test which should be encouraged operationally because of the valuable information it yields. Recently, Ritchie (1989) has outlined a strategy in which freeze or cold storage of conifer stock can be used to manipulate the release of dormancy and hence maximize seedling physiological quality at the time of planting. As a target in Douglas-fir, the DRI value should be between 0.25 and 0.40 (Ritchie 1989).

8.6.3 Phytogram

A protocol which may prove to be of immediate utility to bare root nurserymen and field foresters is the phytogram response. A noble metal (palladium) electrode is placed in the stem or lateral branch of the tree and a reference electrode is placed in the soil (Gensler 1980, 1986, 1988, 1990). The two electrodes yield a dynamic extracellular electropotential for the tree. A phytogram is a plot of the continuous measure of the extracellular electropotentials obtained from the tree.

Three zones of electropotential are found in plants (Gensler 1989ab). The normal range is from 300 to 700 mV. In this range a diurnal pattern is exhibited, rising in the morning to an afternoon plateau and then declining until the morning rise. The second zone is from 0 to 300 mV and is termed the hypo-potential range (Gensler 1989a). This range is characteristic of wet soil conditions. Time spent in this range is usually short but under prolonged saturated conditions, the potential will remain in this range. Seven hundred mV up to 1,400 mV is the hyper-potential range, and movement into and out of this range is very rapid and is termed "spiking" (Gensler 1990). This range is entered only under relatively specific combinations of temperature and light (high stress). Not only are the dynamic potentials valuable, but daily, seasonal, and annual activity or vigor indices can be constructed (Gensler 1990). This provides the user with a number that can be used to compare two treatments, sites, or species separated in space and time.

Hypotheses have been put forward and related to empirical results for the three ranges of electropotential discussed above. In the hypo-potential range, the hypothetical causative reactant is the ethanol/acetaldehyde couple (Gensler 1989a). This assumes anaerobic root zone conditions. The oxygen hypothesis has been developed to deal with potentials in the normal range (Gensler 1986, 1988). The electropotential is a measure of the extracellular electrolyte concentration in this range. Oxygen diffuses to the extracellular spaces during active photosynthesis and away from the spaces during active respiration, thus accounting for the diurnal pattern. Hydrogen peroxide is the hypothetical causative reactant for the hyper-potential range (Gensler 1989a). Due to excess energy, a superoxide radical is formed and is then converted to hydrogen peroxide, preventing physical damage to the plant.

Regardless of what causes the potential to be generated, this technique has been successfully used as a water management tool for cotton crops in Arizona (Gensler 1983, 1988, 1989b). This indicates the technique has the ability to be used as an aid in monitoring the "physiological" impact and time line of droughting in bare root seedlings. The phytogram approach has also been used to distinguish between field site types and levels of tree vigor in established Douglas-fir plantations (Gensler 1990). This suggests the technique may also have a place in assisting the field forester with his silvicultural decision-making. However, these areas require an expanded understanding and application of the phytogram.

Because of its inherent diurnal periodicity, the phytogram technique could play a vital role is in container nursery crop management. This area is a must for research because it offers the opportunity of assigning phytogram (physiological) indices to nursery crops. Nursery phytogram indices in conjunction with other physiological assessments would allow stock to be better matched to its planting site, resulting in enhanced performance and reinforcing the target seedling concept.

8.7 Toward 2000

The importance of the physiological state of a seedling as a component of "quality" is accepted today without ques-

Table 8.6—*Description and rating scale of the nine criteria used for evaluating seedling stock quality tests. This is based on the conceptual framework proposed by Zaerr (1985). Two criteria added to Zaerr's list are basis of the test, that is what is it measuring, and predictiveness of the test, because of its relationship to plantation success.*

Criterion	Scale	Description
BASIS:		What is the test based on?
	0	Non-physiological
	1	Physiology is inferred
	2	Physiology is directly measured
RAPID:		Time with which results are available.
	1	> 1 week
	2	1-7 days
	3	2-24 h
	4	< 2 h
SIMP:		Simple, Ease of understanding/use, all levels of operation.
	1	Requires a researcher
	2	Requires a forestry/nursery professional
	3	Requires a technician
	4	No formal training required
CHEAP:		Cheap and accessible to all potential users.
	1	Available only in a research laboratory
	2	> $1,000 and available in the marketplace
	3	$100 - $1,000 and available in the marketplace
	4	< $100 and available in the marketplace
RELI:		Reliable, the test works every time.
	1	It works every time but is seasonally limited
	2	It works every time
NON-D:		The test is non-destructive.
	1	A non-tested sample is outplanted (destructive test)
	2	A portion of the plant is removed for testing and the plant outplanted
	3	Same plant tested and outplanted
QUANT:		The test is quantitative not qualitative.
	1	The test is neither precise or repeatable
	2	Precise or repeatable
	3	Precise and repeatable
DIAG:		Diagnostic, cause of any past seedling damage is indicated.
	1	No specific diagnostic ability
	2	Physiological system specific diagnostic ability
	3	Multisystem diagnostic ability
	4	Cause of any seedling damage is indicated
PRED:		Predictive, future performance of the seedling indicated.
	1	No indication of performance potential
	2	Potential indicator of performance
	3	Predictor of performance

tion (Ritchie 1984b, Duryea 1985a, Puttonen 1989). However, as recently as 1989, Ritchie (1989) pointed out there still is no consensus among workers as to what "physiological quality" means—even though the major concepts and principles of physiological quality are well documented (Duryea 1985a, Puttonen 1989). Therefore, one is forced to conclude that cohesive guidelines for the application of these concepts and principles to forest regeneration management are still required. We must define what management value is in the test results. In practical terms, why do we want to do the test and what do we expect to gain from it?

Zaerr's (1985) astute characterization that an ideal vigor test must be rapid, simple, accessible, reliable, non-destructive, quantitative, and diagnostic is even more crucial today. He (Zaerr 1985) aptly stated ". . . these characteristics can serve as goals for developing new methods and as benchmarks for judging existing techniques."

Several of the tests described in previous sections, as well as others (see Puttonen [1989] for a more detailed list), are evaluated using a modification of Zaerr's (1985) criteria (Table 8.6). Whether all nine criteria and the rating scales used are valid is open to debate. The criteria guidelines suggested here are meant only as a starting point to evolve from, not an end point. For instance, compared to a $25,000 regeneration program, "simple" and "cheap" (Table 8.6) become less important when a $1,000,000 program is at issue.

Clearly, based on our modified criteria, none of the tests, alone, go very far toward achieving high scores or maximum sum/products of 29/27648 (Table 8.7). There are three major surprises in Table 8.7: how poorly standard tests such as RGC rate, how well the EC technique rates compared to those in widespread operational use, and how the "up and coming" tests still have a distance to go to reach the level of the "ideal" single test. For simplicity, none of the tests were evaluated as a battery. Seedling stock quality tests must be evaluated, both alone and in conjunction with other tests that are frequently used with it. All seedling stock quality assessments must be evaluated using the same criteria, not different criteria for different tests.

Since seedling quality must potentially be capable of being evaluated at any stage from nursery tenure through planting, the utility and applicability of specific tests must be rigorously defined. The development and expression of root growth potential by Ritchie and Dunlap (1980) is an excellent example. A compiled list of specific physiological tests in relation to diagnostic utility at various cultural or lifting phases would be useful to forest nursery personnel, field foresters, and academics alike. This process has, in part, been initiated with *Evaluating Seedling Quality*

Table 8.7—*Sum and product rankings (maximum sum and product are 29 and 27648) of various stock quality tests using the nine criteria and their rating scales outlined in Table 8.6. The greater the sum and the product the greater the utility of the individual test. Evaluations are based on technology at the time of writing, not where it looks to be headed. RGC and morphology were the benchmarks for this work.*

TEST	BASIS	RAPID	SIMP	CHEAP	RELI	NON-D	QUANT	DIAG	PRED	SUM	PRODUCT
Morph[a]	0	4	4	4	2	3^{σ}	3	1	1	22	0
RGC	1	$1^{ß}$	4	3	1	1^{Σ}	1	1	2	15	24
MI	1	3	3	3	2	2	2	1	1	18	216
F_{VAR}	2	4	2	1^{π}	2	3	2^{τ}	2	2	20	768
EC	1	2	3	3	2	2	3	2	2	20	864
DBB	1	1	4	3	1	1^{Σ}	2	1	2	16	48
SIVE	2	4	2	1	2	1^{μ}	3	2	2	18	384
Phytogram	1	2^{Γ}	2	2	2	3	2	2	2	18	384
TTC	2	2	3	2	2	2	3	2	1	19	576

a, Abbreviations: Morph, morphology; RGC, root growth capacity; MI, mitotic index; F_{VAR}, variable chlorophyll fluorescence; EC, electrical conductivity; DBB, days to bud break; SIVE, stress-induced volatile emissions; and TTC, triphenyl tetrazolium chloride.

ß, Test can be done in seven days but generally is longer.

Γ, About 3-7 days for wound to heal around electrode and for the signal to stabilize, then it is instantaneous, a 4; sum/product would then be 20/768.

π, There are a limited supply of instruments in operational use, could be viewed as a 2, resulting in a sum/product of 22/2304.

σ, If masses are done, this value becomes a 1, sum/product of 20/0.

Σ, After the assessment is done the stock can be outplanted but this is not done operationally.

μ, This can be done non-destructively too, resulting in a sum/product of 19/576.

τ, F_{VAR} transient quantification under analytical review.

(Duryea 1985a) and Puttonen's (1989) review. We propose, over the next ten years, that a handbook of rigorously defined tests displaying their utility and applicability be compiled. A periodic update of this handbook would serve two important functions. First, it would provide a useful dictionary of current tests; second, it would suggest areas, either with a test, its intent, or a particular species, in which gaps exist and more information is required.

In the preceding sections, we have generally viewed the physiology-based tests on an individual basis. This is, however, a misrepresentation of how they should be used. Tests looking at different seedling systems must be incorporated into a test battery so as to allow overall seedling health and vigor to be established. Because of the application of individual tests, to date, we have no stock quality test(s) which can predict actual field performance (c.f. Lavender 1989). In Proser's (1958) terminology, acclimation is plant adaptation to a single factor while acclimatization is adaptation to a complex of environmental factors. To date, what we do have is a series of tests which indicate seedling potential of performance or

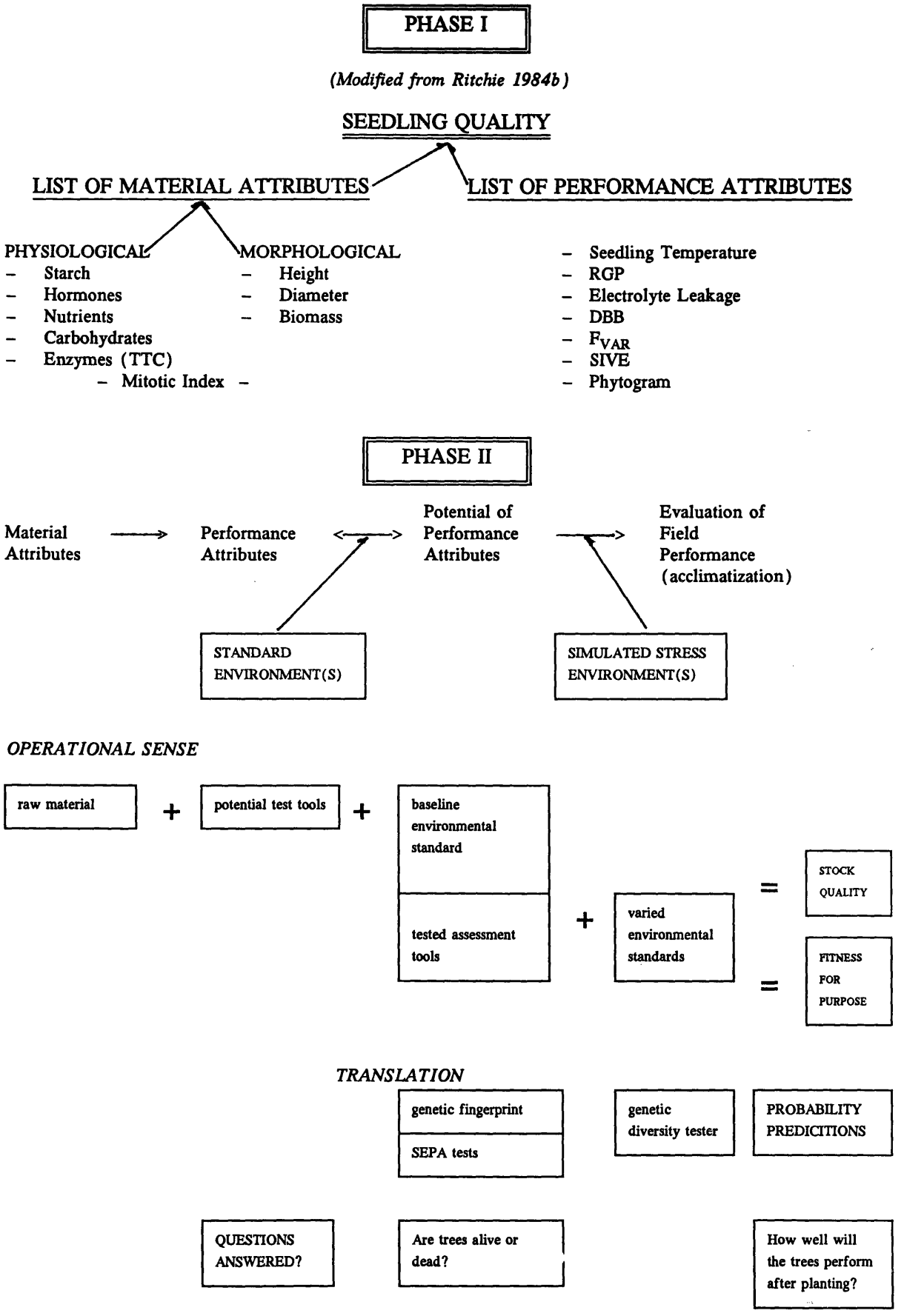

Figure 8.18—*A model for testing or determining seedling quality starting with a static phase I based on Ritchie's (1984b) conceptual framework and evolving to phase II which is predictive or dynamic in nature.*

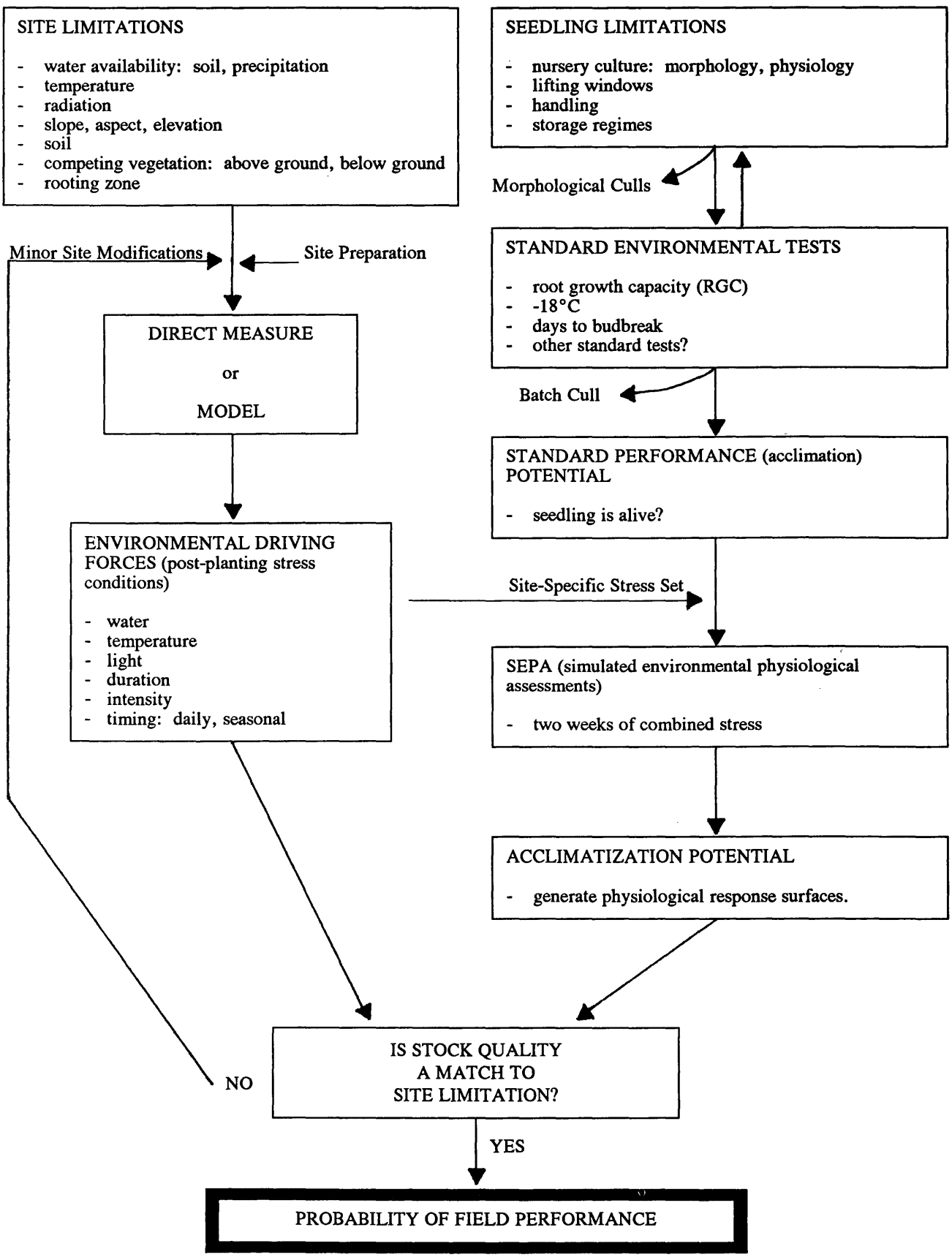

Figure 8.19—*A generalized operational model of the Phase II concept (presented in Figure 8.18). Specific SEPA (simulated environmental physiological assessments) tests and environmental parameters depend on management objectives. Glen Dunsworth is thanked for his contributions to this Figure.*

acclimation (c.f. Sutton 1979, Ritchie 1984b, Puttonen 1989). However, as Puttonen (1989) indicated, prediction of seedling performance in the field (acclimatization) is the ultimate objective of seedling testing. The goal in the coming decade is to extend the potential of performance indicator assessments to prediction of acclimatization.

Based on Ritchie's (1984b) conceptual development of seedling material and performance attributes (Figure 8.18, Phase I), we propose to extend the use of this model from Phase I, a potential performance indicator (i.e., acclimation), to Phase II, a performance predictor (i.e., acclimatization). The largest difference between Phase I and Phase II is that Phase II relies on a sound basic, theoretical understanding of what is being measured. By definition, Phase I assessments must be carried out in strictly defined standardized environments and are unbiased. Phase II measurements will be conducted in a variety of environments and the specific physiological assessments will be based on management objectives, e.g., stock allocation and reallocation. In the Phase II context, Ritchie's (1984b) performance attributes are viewed as a potential of performance, under standard defined conditions. While responses derived under standard conditions allow system performance function to be ascertained (put simply, the seedling is alive and performs under standard conditions, or it is dead), the ability to predict actual field performance of the tested stock is marginal at best. Witness the RGC controversy. Despite the vast sums riding on RGC test results, there still is uncertainty as to what is being measured and how RGC results relate to field performance (Burdett 1987, Binder et al. 1988, Landis and Skakel 1988). We propose that a series of simulated, environmental-physiological assessments (SEPA) tests be conducted under varied environmental, perhaps stressful, conditions, so that seedling response surfaces can be generated. The response surfaces can then be used in probability based projections to answer that very important question: *How will the trees perform after planting?* This amounts to developing genetic-environment interaction performance ratings for a range of species. Clearly, realization of the completion of Phase II will require a comprehensive, well planned, multidisciplinary team approach.

In Figure 8.18 (Phase II), we present a hypothetical scheme using the SEPA approach to give an indication of how battery assessments could be done. The choice of assessments and environmental conditions used will depend on the management objectives for that stock (Figure 8.19). For example, Grossnickle et al. (1988) used a battery approach when looking at material and performance attributes—specifically, drought avoidance, drought tolerance, and cold tolerance. Environmental conditions could simulate specific low, moderate, or high stress sites. A high stress site would be a steep southerly aspect and the environmental variables could be high temperature, high insolation, and low soil water potential (due to drought or low soil temperatures). Stock would undergo a two week acclimatization under these conditions with SIVE/F_{VAR} monitoring throughout, followed by evaluation. Post environmental stress evaluations could include RGC, F_{VAR}, EC, TTC, etc. In the short term, this would provide a response surface on which to base reforestation establishment decisions. In the long term, such tests performed in conjunction with field growth measurements will provide base values that ensure field performance of specific stocktypes. It must be demonstrated (cost effectiveness) to both producers and consumers of seedlings that the cost of ensuring seedling health is minor compared to the cost of plantation failure.

The mandated mission of stock quality physiologists for the next decade should be to move stock quality tests toward a greater score on all nine points (Tables 8.6 and 8.7) and to redefine rating criteria. This will be accomplished by test refinement, by developing and establishing new tests which meet the specific rating criteria, but above all, by integrating tests together so that seedling fitness (acclimatization) rather than plant system health (acclimation) is evaluated. When predictive test batteries with their corresponding goals and implications are achieved, the target seedling will no longer be a management concept but a physiological fact.

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Target Seedling Symposium

Chapter 9
Seedling Moisture Status

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ABSTRACT

The water status of nursery tree seedlings can be determined by measuring seedling water content, and by liquid equilibration, psychrometric, and pressure chamber techniques. The latter two techniques measure water potential, an expression of the free energy of water which is closely related to physiological functions. Liquid equilibration methods are laborious, time consuming, and imprecise. Water potential can be measured very accurately with thermocouple psychrometers, but long equilibration times and other technical requirements make this method best suited for laboratory use. The hydraulic leaf press is easy to use and economical; however, endpoints vary with the type of tissue and with the level of water potential. The best choice for nursery work is the pressure chamber. With it, measurements are fast, simple, and accurate. It can be used to obtain estimates of osmotic and turgor potential, measure the hydraulic conductivity of root systems, and detect cold injury in roots. The pressure chamber is being used to schedule irrigation and, in some cases, to monitor water stress during lifting and packing. During seedling growth, predawn water potentials should be maintained above -0.5 MPa. Cold and drought hardiness can be increased by exposure to moderate water stresses (-0.5 to -1.0 MPa), but conditioning procedures and responses have not been studied extensively in northwest conifers. Available data indicate that seedling water potentials down to -2.0 MPa during lifting will not adversely affect seedlings, provided they are moistened prior to storage. Interpretation of seedling water potentials requires that consideration be given to the magnitude of the water stresses, their duration, stage of seedling growth or dormancy, the species involved, and seedling vigor.

9.1 Introduction

The growth of plants probably is reduced more often by water deficits than by any other factor. In plants rooted in soil or other media, water deficits occur when water loss by transpiration exceeds water absorption through roots. In the case of bare-root nursery stock, water deficits can occur at any time from lifting to outplanting as a result of water loss from both shoots and roots. Whether in the nursery, cold storage or the field, conifer seedlings experience water deficits all the time, because moisture recharge never is complete. Thus water deficits are normal occurrences and become important only when they are large enough to adversely affect physiological processes, growth, or survival. Water deficits can affect practically every aspect of plant growth including anatomy, morphology, physiology and biochemistry (Kozlowski 1972, Hsiao 1973). Moderate water deficits can result in stomatal closure and reduced photosynthesis, while more severe deficits can damage the photosynthetic apparatus. Water deficits can affect respiratory and translocation processes, disrupt carbohydrate and protein metabolism, damage membrane structures of cells, and cause changes in enzyme activity. Also, water deficits often increase susceptibility to attacks by pathogens and insect—and severe desiccation, as a result of inadequate soil moisture, is a major cause of mortality of planted seedlings in the western United States. Currently, increased attention is being focused on all aspects of nursery culture of tree seedlings in attempts to improve seedling quality, and this has increased interest in the water relations of tree seedlings. This paper discusses water relations concepts and terminology, describes various methods of measuring and expressing water status in plants, and evaluates their usefulness for assessing the water status of nursery seedlings. For other reviews dealing with the water status of nursery seedlings, readers are referred to papers by Ritchie (1984), Joly (1985) and Landis et al. (1989).

9.2 Concepts and Terminology

9.2.1 Water content

The water status of a plant can be measured and expressed in a number of ways, all of which are useful for particular applications. The simplest method of determining water content involves measuring the fresh and ovendry weights of a plant part, and expressing the weight of water lost as a percent of ovendry weight. Dry weight, however, can undergo both short- and long-term changes, so attempts have been made to express leaf water content as a percentage of turgid or saturated weight. A commonly used version of this approach is Weatherley's (1950) Relative Water Content (RWC). The procedure involves weighing a leaf to obtain fresh weight, floating the leaf on water in the dark until it ceases to gain weight, and then weighing it to obtain turgid weight. The leaf is then ovendried, weighed again, and RWC calculated as:

$$RWC = \frac{\text{fresh wt. - ovendry wt.}}{\text{turgid wt. - ovendry wt.}} \times 100 \quad (1)$$

In a fully turgid sample, RWC is 100%. A related method employing the same measurements can be used to express water content as water deficit (WD). Water deficit is calculated as:

$$WD = \frac{\text{turgid wt. - fresh wt}}{\text{turgid wt. - ovendry wt.}} \times 100 \quad (2)$$

WD and RWC are related; RWC = 100 - WD, or RWC + WD = 100%. RWC and WD are more meaningful expressions of plant water status than water content as percent of dry weight because they relate field water content of foliage to the fully turgid condition, and thus provide a better correlation with physiological functions. Procedures most likely to give reliable results vary with species. A problem sometimes experienced with conifers is bringing the sample to full turgidity. Clausen and Kozlowski (1965) and Harms and McGregor (1962) found the use of entire needles satisfactory for several species of conifers. With proper calibration, RWC and WD can be related to plant water stress or water potential (explained below), but a calibration must be made for each species. With some species the calibration may be useful for only short-term studies, because the relationships can change with age of leaves and habitat (Knipling 1967).

9.2.2 Water potential

A meaningful assessment and expression of plant water deficit requires a quantitative measurement of water status that is directly related to physiological processes. The single most useful measurement is that of water potential because it is a measure of the chemical potential or free energy of water, it controls water movement in the soil-plant-atmosphere system, and it can be measured in plants and soil. Water potential is defined thermodynamically as the ability of water to do work in comparison to free pure water at standard pressure and temperature, whose water potential is zero. Units of water potential are equivalent to pressure units; however, in SI (Systeme International) units (Incoll et al. 1977), pressure is expressed in pascals and 1 MPa (megapascal) = 10 bars, 10 atm. or 150 psi. In this paper I will use the unit MPa which, in plant research, has largely supplanted the term "bars."

The water potential (Ψ_w) of the cells in a tree seedling is the sum of osmotic (Ψ_s), pressure or turgor (Ψ_p), matric (Ψ_m), and gravitational (Ψ_g) potentials. The influence of matric potentials is negligible and the gravitational potential becomes important only in tall trees, so that the equation for Ψ_w usually is expressed as:

$$\Psi_w = \Psi_s + \Psi_p \quad (3)$$

Table 9.1—*A comparison of units and descriptive terms for plant water potential (Ψ_w) and plant moisture stress (PMS). Ψ_w and PMS have the same value, but Ψ_w is expressed as a negative value, whereas PMS values are positive (Landis et al. 1989).*

Plant water potential Ψ_w				Plant moisture stress (PMS)		
Units		Relative	Relative	Units		Relative
MPa	Bars	rating	moisture	MPa	Bars	rating
0.0	0.0	High	Wet	0.0	0.0	Low
−0.5	−5.0			0.5	5.0	
−1.0	−10.0	Moderate		1.0	10.0	Moderate
−1.5	−15.0			1.5	15.0	
−2.0	−20.0	Low	Dry	2.0	20.0	High

where Ψ_s is a negative number and Ψ_p, in turgid plants, is positive, so that Ψ_w in most situations is a negative number. Plant water potential becomes lower (more negative) as plants lose water and water deficit increases, and water movement both in plants and soils occurs along a gradient from high to low water potential. An in-depth discussion of water absorption and translocation processes in plants, which is beyond the scope of this paper, is adequately covered elsewhere for plants in general (Kramer 1983) and for containerized nursery seedlings (Landis et. al. 1989).

The pressure potential (Ψ_p) or turgor pressure portion of Equation 3 is very important because of its direct influence on cell enlargement, guard cell movements and other processes dependent on changes in cell volume. It is usually assumed to be the difference between Ψ_w and Ψ_s and varies from zero in a flaccid cell to a value equal to that of the Ψ_s in fully turgid cells. The interrelationships of these factors can be illustrated in a Höfler (1920) diagram (Figure 9.1) which shows how the components of water potential shift with a change with seedling water content. When a seedling is fully turgid, Ψ_w is zero and Ψ_p is equal and opposite in sign to the value of Ψ_s. When water content decreases sufficiently to cause Ψ_p to decline to the zero turgor point, Ψ_w equals Ψ_s. The point of zero turgor, sometimes called the "wilting point," can be physiologically detrimental to the seedling; growth stops and if the conditions persists, cellular damage and death may occur.

Another term used to describe seedling water status, "plant moisture stress" (PMS), is so well established in the nursery literature and everyday jargon that there is little doubt that it will continue to be used. This presents no

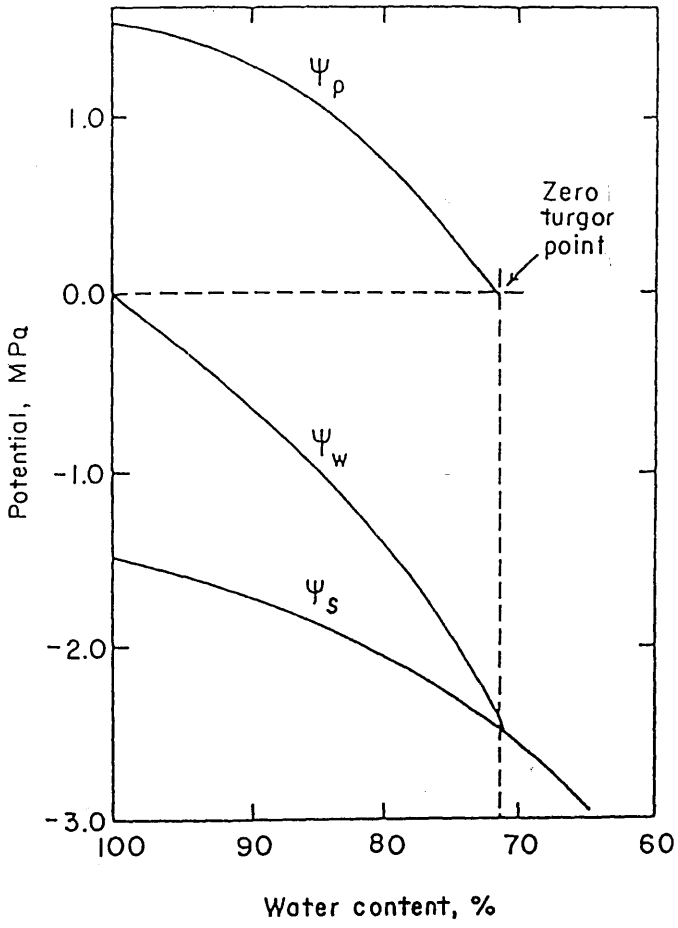

Figure 9.1—*A Höfler diagram showing the relationship between water potential (Ψ_w), osmotic potential (Ψ_s), and turgor (Ψ_p) over a range of water contents from full turgidity to the wilting point (Ritchie 1984).*

real problem since Ψ_w and PMS are dimensionally equivalent and differ only in sign (Table 9.1). Thus, as Ψ_w decreases (becomes more negative), PMS increases, i.e., a low Ψ_w of —2.0 MPa (-20 bars) is equivalent to a high PMS of 20 bars.

9.3 Water Potential Measurement Techniques

9.3.1 Liquid equilibration

This technique involves immersing weighed pieces of plant material in a series of solutions of known osmotic potentials (which in an unconfined solution equals Ψ_w) made using sucrose, mannitol or polyethylene glycol of high molecular weight. After a suitable time period, the samples are removed, blotted and reweighed. Theoretically, the osmotic potential at which the sample neither gains nor loses water is equal to its water potential. Actually, weights are plotted over osmotic potentials

of the solutions and the water potential is taken as the value of osmotic potential where weight intersects the zero line.

A variation of the liquid equilibration method that avoids the need to weigh the sample involves measuring changes in density of the test solutions. The sample loses water to solutions with a lower water potential, diluting them, and absorbs water from solutions with a high water potential, concentrating them. The water potential of the sample is assumed to be equal to the osmotic potential of the solution which undergoes no change in density. Changes in solution concentration can be measured with a refractometer (Gaff and Carr 1964) or by observing the rise or fall of drops of dyed control solutions carefully introduced into the middle of test solutions from which samples have been removed. The dye method, first described in Russian by Shardakov (1948) and discussed in detail by Slavik (1974), has been used to measure needle water potential in several species of conifers (Brix 1966, Knipling and Kramer 1967, Cunningham and Fritts 1970). The dye method is simple, does not require expensive equipment, and can be used in both the laboratory and field, but problems can occur because of contamination of test solutions by cell sap and leaf surface residues. Its best use is to provide estimates of water potential rather than precise measurements. Leakage of solutes can be avoided by allowing weighed samples to equilibrate in air over solutions of known osmotic potentials, thereby avoiding direct contact with the solution (Slayter, 1958). While useful for some laboratory and field research, liquid and vapor equilibration techniques are too laborious and time consuming for operational nursery use.

9.3.2 Psychrometric methods

With the psychrometric method, a plant sample is enclosed in a small airtight chamber containing a fine wire chromel-constantan thermocouple and the chamber is brought to a constant temperature. The Spanner (1951) psychrometer (Figure 9.2) requires that sufficient time be allowed for both temperature equilibration and equilibration of vapor pressure of water in the air with water potential of the plant sample. A small current then is passed through the measuring junction cooling it (Peltier effect) sufficiently to condense water on the junction. After the cooling current is stopped, the rate of water evaporation from the measuring junction, and the magnitude of the resulting temperature depression, are functions of the humidity in the chamber. The voltage output from the thermocouple, recorded with a microvoltmeter, is a measure of the water potential of the sample.

The Richards and Ogata (1958) psychrometer originally was developed to measure the water potential of soil samples, but it quickly was adopted for measurement of plant water potential. A drop of water is placed on a small silver ring at the measuring junction, and voltage readings are taken when the rate of evaporation from the water droplet reaches a steady value indicated by a constant temperature depression of the thermocouple. Calibration with both types of psychrometers is performed by taking readings with salt solutions of known water potential in the chamber. Theoretical considerations for thermocouple psychrometers are discussed in detail by Rawlins (1966) and Dalton and Rawlins (1968), and much information is available in a review by Barrs (1965) and from books edited by Kozlowski (1968), Brown and Van Haveren (1972) and Slavik (1974).

The original versions of the Spanner and Richard and Ogata psychrometers have been modified in various ways to improve accuracy and reduce temperature sensitivity (Hsieh and Hungate 1970). Boyer and Knipling (1965), using a Richards and Ogata psychrometer, devised an isopiestic technique to avoid the problem of leaf resistance to diffusion of water vapor. A measurement is first made with water on the thermocouple, followed by another measurement with a solution whose water potential is close to that of the leaf sample. Voltage outputs then are graphed to determine the solution potential (equal to the sample potential) at which voltage output would be zero.

A significant innovation is the dew point hygrometer described by Campbell et al. (1973). It features an electronically maintained, constantly wet junction that produces a somewhat greater thermocouple output. Also, the very precise temperature control formerly considered necessary, now generally is not required so long as temperature remains constant during the time the measurement is being taken. Various forms of psychrometers have been used to measure water potential in conifers in detached needles (Brix 1962, Kaufmann 1968, Dosskey and Ballard 1980), attached roots (Nnyamah and Black 1977), and in tree trunks (Wiebe et al. 1970). Thermocouple psychrometers also have been modified to make *in situ* measurement of leaf water potential in aspen (*Populus tremuloides* Michx.) and in herbaceous plants (Hoffman and Hall 1976, Brown and McDonough 1977), but *in situ* leaf methods have not been used with conifers.

The psychrometer method has some distinct advantages. It is capable of making very accurate measurements of water potential, readings can be made with a small sample consisting of only one or two needles, and the system can be automated with data loggers (Stevens and Acock 1976). Also, the method permits assessment of the osmotic and turgor components of water potential. To accomplish this, a measurement of water potential is first made with an intact sample. The sample is then frozen and thawed to disrupt cell membranes and release cell sap, and another measurement is made to determine osmotic potential. Turgor potential is calculated as the difference between the water and osmotic potentials.

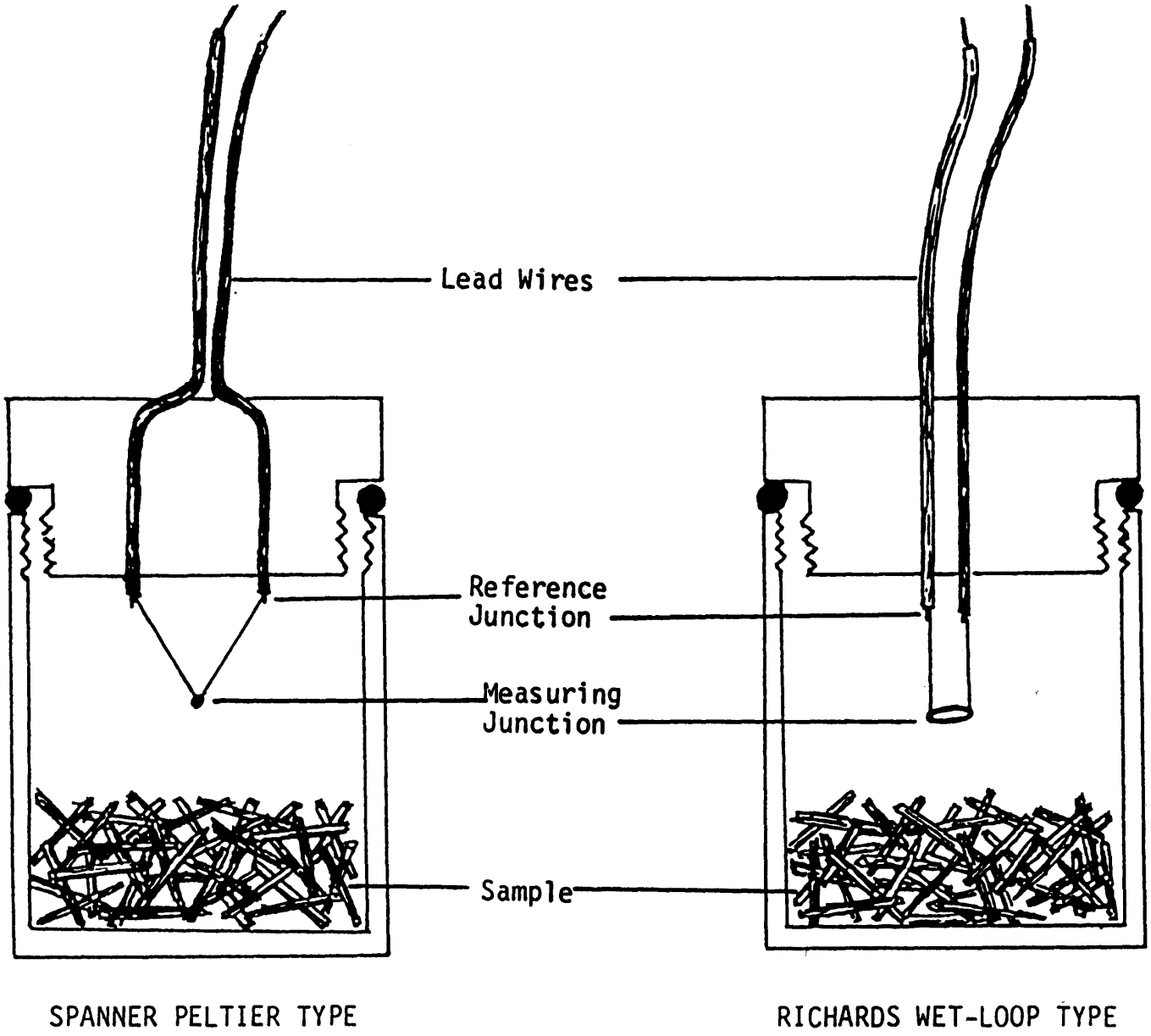

Figure 9.2—*Comparison of a Spanner and a Richards and Ogata thermocouple psychrometer. With the Spanner psychrometer, water is condensed on the measuring junction by Peltier cooling, whereas with the Richards and Ogata psychrometer, a drop of water is placed on the ring at the measuring junction.*

The psychrometric method has been very successful in the laboratory; however, certain considerations limit its usefulness in forest nurseries. Leaf surfaces and interiors of sample chambers must be kept clean, otherwise they tend to act as moisture sinks (Boyer 1972, Dixon and Grace 1982). Psychrometers need to be recalibrated periodically, and ambient temperature must be maintained fairly constant during measurements. Also, humidity equilibration with heavily cutinized conifer needles takes several hours, and cutting needles into segments can release resins (which tend to gum up the chamber) and extracellular water which could result in erroneously high values of water potential. These problems have largely restricted the technique to laboratory use; however, further refinements may provide procedures applicable to some aspects of nursery research. For example, a unique temperature-corrected psychrometer now is available to continuously monitor water potential in intact plant stems (Dixon and Tyree 1984, Dixon et al. 1984). This psychrometer, which can be used with stem diameters down to about 7 mm (0.28 in), may provide a means of follow

ing changes in water potential in nursery seedlings for a period of days or weeks.

9.3.3 Hydraulic press

The J-14 hydraulic press was designed to provide a portable and inexpensive method for measuring plant water potential without the need for compressed gas. Thus it has some logistical and safety advantages over the pressure chamber. Hydraulic pressure beneath a flexible membrane is used to press a leaf or other tissue against a thick Plexiglass window until water appears at the cut edges or certain color changes occur. The pressure at this point is taken to be equal to the leaf water potential. Mixed results have been reported with the hydraulic leaf press. Cox and Hughes (1982), working with perennial grasses, found that predawn measurements with the leaf press correlated well with the pressure chamber under conditions of optimum soil moisture. Comparisons became erratic during periods of increased water stress, and large changes in water potential measured with the pressure chambers were measured as small changes with the leaf press. Shayo-Ngowi and Campbell (1980) reported that measurements of matric potential made using the hydraulic press with frozen tissue, including ponderosa pine, showed good agreement with matric potentials measured with the pressure chamber. Brown et al. (1975) compared values obtained with thermocouple psychrometers and the leaf press for various plant parts including leaves and seeds, and found a poor correlation between the two methods. Sojka et al. (1990) compared measurement of water status made with the J-14 leaf press and a pressure chamber for tomato, rapeseed, corn, and soybean. The leaf press performed well with soybean but not with the other species, leading the authors to conclude that J-14 measurements are at best a relative indicator of water status in the absence of species-related calibrations. Grant et al. (1981) also obtained good results with the hydraulic press and soybeans.

Relatively few comparisons of the hydraulic press with other methods of measuring water potential have been made for conifers. The most extensive test of the hydraulic press with conifers appears to be the work done by Childs (1980) with Douglas-fir (*Pseuedotsuga menziesii* var. glauca [Beissn] Franco) seedlings. He found reasonable correlations with pressure chamber measurements, but satisfactory results required using several different endpoints depending on the water potential of the sample, and calibrations with large numbers of samples. A similar comparison by Cleary and Zaerr (1980) with Douglas-fir produced poor results. A troublesome problem with the leaf press is correctly identifying the endpoint. Another is that the underlying theory is not as well established for the leaf press as it is for the pressure chamber method. Further work is needed before the leaf press can be recommended for nursery use, but because of its low cost and simplicity it deserves further evaluation.

9.3.4 Pressure chamber

Since the description of the pressure chamber method by Scholander et al. (1965), and Waring and Cleary (1967), it has become the most widely used technique for measuring water potential in plants. It has been used to measure water potential in a wide variety of herbaceous and woody plants, including conifers, using samples of whole shoots and roots, individual leaves, fascicles of needles and single needles. Several types of pressure chambers are available commercially, and custom-built chambers or special methods of sealing the sample in the lid have been designed for use with conifer needles (Johnson and Nielsen 1969, Gifford 1972); wheat (Powell and Goggins 1985); sorghum (Blum et al.1973) and irregularly-shaped succulent samples (Simonelli and Spomer 1980).

Determinations made with the pressure chamber are rapid and simple, and measurement procedures have been described by numerous authors (Waring and Cleary 1967, Boyer 1967, Ritchie and Hinckley 1975, Cleary and Zaerr 1980). To make a measurement, a twig or shoot is cut from a plant, and if a conifer or hardwood is used, the bark and phloem are peeled back far enough to allow the twig to be inserted through a rubber stopper or similar type of compression seal. The sample is placed in the chamber with the cut end of the shoot protruding through the lid of the chamber and exposed to atmospheric pressure (Figure 9.3). Chamber pressure is slowly increased with nitrogen from a high pressure tank until water is forced back to the cut surface. That pressure, indicated on a pressure gauge, is taken as the water potential of the sample. A bleed-off valve allows nitrogen to be exhausted rapidly from the system following a determination. Certain precautions are required to obtain reliable results with the pressure chamber. These are discussed in detail by Ritchie and Hinckley (1975) and will not be repeated here, other than to emphasize that readings should be made quickly to avoid sample desiccation, needle removal should be kept at a minimum so that a large proportion of the foliage is enclosed in the chamber, and pressure should be increased at a moderate rate (about 0.07 MPa sec^{-1}). Recognizing the endpoint (the point at which water is observed on the cut surface) can be a problem with some species, particularly pines, because resin exuding from the cut surface may be mistaken for water. One solution is to wipe away the resin. McGilvray and Barnett (1988) suggest holding a small piece of brown paper towel against the cut stem, so that water exuding from the cut surface can be detected as a wet spot darkening the paper.

When a twig is cut from an intact branch, negative pressure or tension in the water conducting element is released, and water retreats from the cut surfaces. The general assumption is that the positive pressure required to force water back to the cut surface is equal to the negative pressure which existed in the intact twig prior to exci-

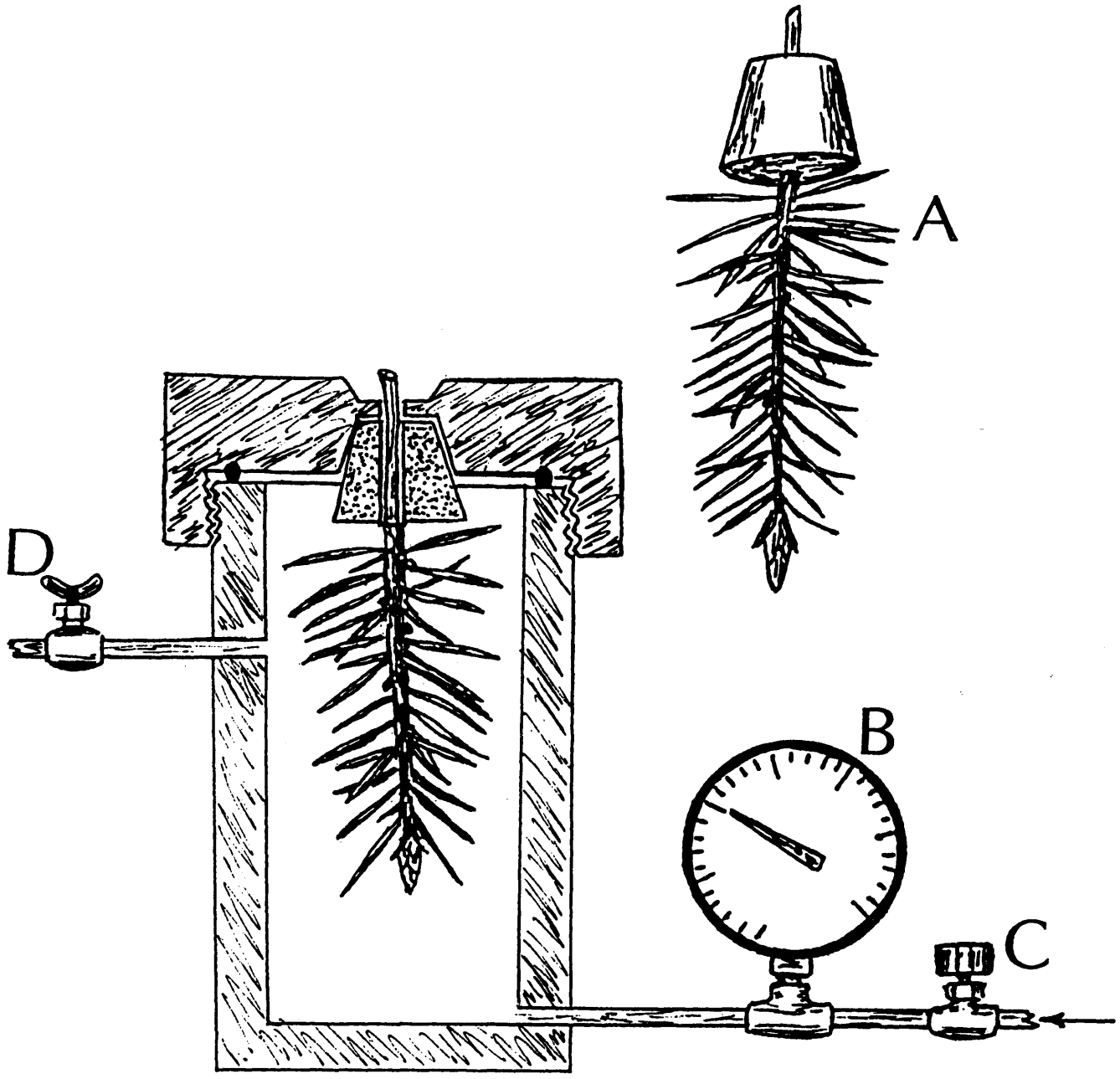

Figure 9.3—*Diagram of a pressure chamber showing (A) a conifer twig with the cut end protruding through a rubber stopper, (B) pressure gauge, (C) pressure increase needle valve, and (D) pressure release valve.*

sion. Theoretical considerations of forces involved in water movement during a measurement with a pressure chamber are discussed by Boyer (1967) and Ritchie and Hinckley (1975). In brief, the pressure chamber method measures the pressure necessary to raise the potential of water in the leaf cells to the point at which it equals or slightly exceeds the potential of the xylem sap at atmospheric pressure.

Pressure chamber measurements, however, do not include the osmotic component in the xylem sap; therefore, the values obtained are only estimates (rather than actual values) of leaf water potential and are referred to by most researchers as "xylem pressure potentials," although again, the more general term "plant moisture stress" is acceptable. Because the osmotic component usually is negligible, it is assumed that pressure chamber readings approximate leaf water potential in many species.

In spite of the considerable literature on the pressure chamber method, it is difficult to precisely assess the accuracy of measurements made with a pressure chamber. Early investigators (Boyer 1967, Kaufmann 1968), comparing pressure chamber readings in conifers with those made with thermocouple psychrometers, found that at low water potentials, pressure chamber values could be as much as 0.5 MPa more negative than those obtained with psychrometers. The closest agreement occurs at high and moderate water potentials. Roberts (1977) found good agreement between pressure chamber and psychrometer readings for needles of Scots pine (*Pinus sylvestris* L.). Surprisingly, there appear to be only two such comparisons for western conifers. One was by Waring and Cleary (1967) with Douglas-fir in which pressure chamber readings were found to agree within + 0.1 MPa of those determined with a vapor equilibrium technique. In a more recent test (Hardegree 1987) with ponderosa pine (*Pinus ponderosa* Doug. ex Laws), values obtained with a pressure chamber were about -0.5 MPa lower than those measured with a Richards and Ogata-type psychrometer. In any case, absolute accuracy is not a prerequisite for nursery work so long as standard guidelines for relative values are recognized and reasonably reflect seedling condition.

Pressure chamber measurements can easily be made with fascicles of needles from long-needled species such as ponderosa pine and lodgepole pine (*Pinus contorta* Dougl. ex Loud.). The advantages of needle measurements are that repeated measurements can be made on small seedlings, gas consumption is reduced and, theoretically at least, readings with needles should more closely approximate needle water potential than measurements with shoots. Johnson and Nielson (1969) found that needle water potential was nearly identical to that measured on the branch from which needles were taken in several species of pines. They also found that if the needle fascicle is stripped off so that the xylem trace remains attached, there is no problem with resin obscuring the endpoint. Resin exudation was a problem, however, if a single pine needle was used. Ritchie and Hinckley (1971) also found similar water potentials in needle fascicle and shoots of lodgepole pine and Jeffrey pine (*Pinus jeffreyi* Grev. and Balf.) seedlings, but in Douglas-fir, Pacific silver fir (*Abies amabilis* [Dougl.] Forbes), and noble fir (*Abies procera* Rehd), needle values were up to 0.4 MPa higher than equivalent branch values. On the other hand, Kelliher et al. (1984), working in a young Douglas-fir stand, found that values of needle xylem water pressure potential obtained with a pressure chamber were similar to twig xylem water pressure potential. Measurements with individual small needles such as those of Douglas-fir require that the needle be held in a rubber stopper modified in such a way to assure that a large portion of the needle remains exposed within the chamber (Ritchie and Hinckley 1971). Kelliher et al. (1984) reported that breakage of needles and the minute size of the needle xylem make measurement of needle xylem potential quite difficult. Only about 40 percent of their measurements were successful. While useful for research studies, single-needle measurements normally are not needed in nursery work.

Pressure chamber guidelines usually specify that samples be measured quickly after detachment to avoid desiccation; however, with proper precautions excised conifer foliage can be stored for several hours with minimal change in xylem pressure potential. Kaufmann and Thor (1982) found that excised branch tips of Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) and subalpine fir (*Abies lasiocarpa* [Hook] Nutt.), and fascicles of lodgepole pine needles stored in cool, humid vials exhibited very little change in xylem pressure potential over a four-hour period. Myers (1988), employing a similar technique, harvested fascicles of radiata pine (*Pinus radiata* D. Don) before dawn, stored them in test tubes on ice, and measured xylem pressure potential two or three hours later. Samples stored for measurement later should be placed quickly in sealed containers kept humid and cool, and the cut ends of the samples should not be allowed to contact and absorb free water.

A valuable feature of the pressure chamber is that it can be used to estimate osmotic and turgor potential by the "pressure-volume" method (Tyree and Hammel 1972, Roberts and Knoerr 1977, Ritchie and Roden 1985, Schulte and Hinckley 1985). A cut twig is placed in a pressure chamber and subjected to increasing increments of pressure, and the volume of sap exuded with each increment is measured, usually by weighing the expressed sap. Finally, the branch is weighed, dried and reweighed. The procedure is described in detail by Ritchie (1984). Ritchie and Shula (1984), using the pressure-volume method, showed that considerable seasonal changes in tissue water relations occur in Douglas-fir seedlings, particularly in the shoots. In a modified version of this method, tissue water content is reduced by allowing the foliage to transpire between successive measurements with the pressure chamber (Ritchie and Roden 1985). From these data a "pressure-volume" (P-V) curve representing the relationship of reciprocal water potential ($1/\Psi_w$) with water content can be plotted (Figure 9.4). The upper portion of the line is curvilinear for a small decrease in water content, while the lower portion becomes linear with further decrease in water content. The osmotic potential at full turgor can be determined by extrapolating the linear portion of the curve back to point A on the y-axis. The osmotic potential at zero turgor, which occurs where the curvilinear and linear regions meet, can be determined by extrapolating horizontally to point B. This value is the same as the water potential since at zero turgor (the wilting point), water potential equals the osmotic potential. Colombo et al. (1984) sug-

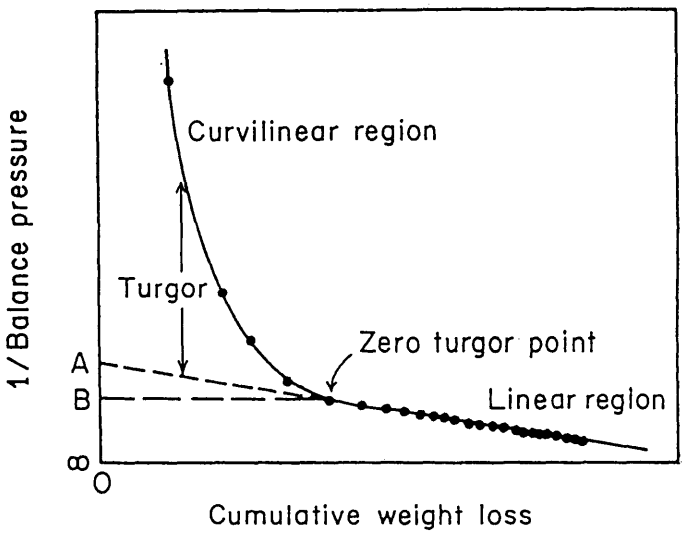

Figure 9.4—*A pressure-volume (P-V) curve showing extrapolation of the linear region to point A to obtain an estimate of the osmotic potential at full turgor, and horizontally to point B for an estimate of the osmotic potential and water potential at zero turgor.*

gested that since cell expansion ceases at zero turgor, the water potential at the wilting point, determined from a P-V curve, is a critical water potential for growth and thus could be used as an index of seedling quality. Thus, the water potential at zero turgor can be considered a "target" in that seedling water potentials should be kept above this point to maintain normal seedling functioning and growth. This "critical water potential," however, is not fixed, but varies seasonably (Ritchie, 1984). It should also be noted that while the osmotic component does influence seedling hardiness, it is only one of the factors determining seedling quality. Osmotic and turgor potentials also can be obtained with a pressure chamber used in combination with a thermocouple psychrometer (Livingston and Black 1987). Water potential is measured with a pressure chamber, osmotic potential of frozen and thawed tissue or expressed sap measured with a psychrometer, and turgor pressure is calculated as the difference between the water and osmotic potentials.

The pressure chamber also has several other interesting applications. These include measuring the hydraulic conductance of roots (Johnson et al. 1988, Smit and Stachowiak 1988), and detecting some types of seedling damage such as cold injury in conifer roots which damages cell membranes. To measure hydraulic conductance, a root system is immersed in water in a pressure chamber with the cut stump protruding through the lid. Pressure in the chamber is raised to create a pressure gradient from the root surface to the cut stump forcing water through the roots. Rates of water movement per unit of pressure per unit of root material (surface, weight) are then used to calculate hydraulic conductance. Procedures are discussed in detail by Markhart and Smit (1990). The application to cold injury is based on the observation that under pressure more water can be expressed from cold damaged tissue than from healthy tissue (Ritchie 1990). A recent review of various applications of pressure chambers, thermocouple psychrometers, and other methods of measuring plant water status is that by Hanks and Brown (1987).

9.4 Operational Applications

It should be remembered that seedling water relations are by nature dynamic, and that a single measurement of water potential, by whatever method, represents only the water potential present at the time the measurement was taken. It does not provide any information on the magnitude or duration of previous moisture stresses. If severe and of long duration, such previous stresses could affect present growth behavior. Also, tree seedlings typically exhibit diurnal variations in water potential (Figure 9.5) related to environmental conditions (McDonald and Running 1979), thus timing of measurements needs to be considered. If measurements are being taken to follow seedling drying trends in nursery beds, then predawn measurements are preferred because water potentials at that time approach equilibrium with soil water potentials, and thus provide the most stable basis for day-to-day comparisons. For some purposes, a midday or early afternoon measurement also is useful because it provides an indication of maximum water stress, which together with predawn values shows daily minimum and maximum water stresses experienced by seedlings.

The pressure chamber can be used to schedule irrigation, but there is little information available on the effects of plant water deficits on the growth of seedlings of western conifers. Consequently, there are few published water potential standards for seedlings available to guide nursery managers. One study with Douglas-fir indicates that shoot elongation in Douglas-fir seedlings can occur at plant water potentials more negative than -0.5 MPa (Zaerr and Holbo 1976). In any case, because of the dynamic nature of water relations, it is impractical to specify what seedling water potentials or osmotic potentials ought to be (i.e., "targets") at any given time. Instead, there are general guidelines, based on studies with two species (Douglas-fir and ponderosa pine) that suggest stress limits that should not be exceeded. Some general criteria for containerized seedlings based on predawn water potentials are given in Table 9.2. A detailed description of procedures recommended for maintaining non-stressful water potentials in containerized seedlings and growing media is presented by Landis et al. (1989). According to these authors, a general rule for container seedlings is to irrigate

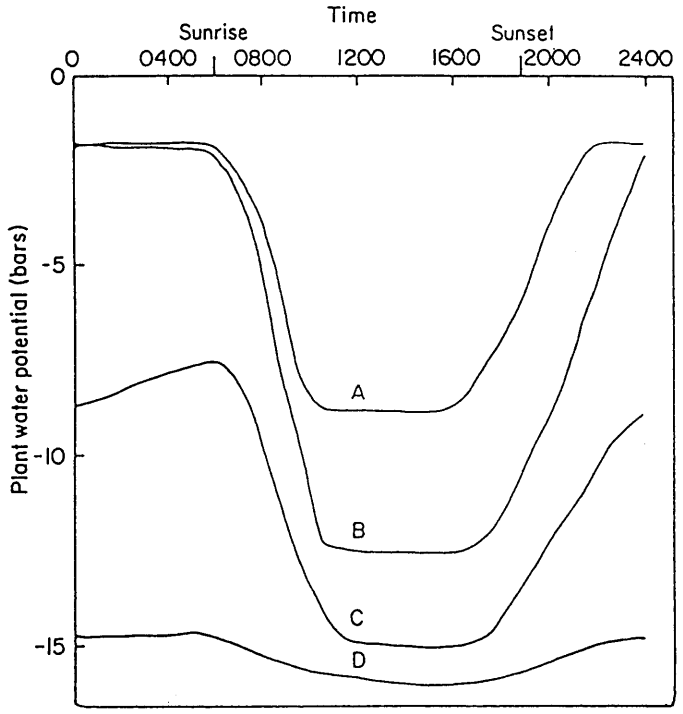

Figure 9.5—*Diurnal patterns of plant water potential for a nursery seedling under varying conditions of soil and atmospheric water stress. A - high soil water potential and low evaporative demand; B - high soil water potential and high evaporative demand; C - low soil water potential and high evaporative demand; D - extreme plant water stress (McDonald and Running 1979).*

when predawn water potential drops below -0.5 MPa, and water potential should not be allowed to decrease below -1.0 MPa unless reduced growth or dormancy induction is desired. The same guidelines apply to bareroot stock growing in nursery beds, although water potentials can be expected to decrease more slowly in bareroot seedlings because the roots of these seedlings exploit a greater mass of soil than container seedlings.

Several investigators have shown that controlled water potentials can be used to condition seedlings to better tolerate adverse conditions following planting. Many species acclimate morphologically and physiologically when exposed to sublethal water stress. Increased moisture stress can be used to induce seedling dormancy during the summer (Zaerr et al. 1981). Blake et al. (1979) found that exposing Douglas-fir seedlings to a mild stress of -0.5 to -1.0 MPa during late summer improves cold hardiness, while a moderate stress (-1.0 to -1.5 MPa) retarded lammas growth and reduced cold hardiness. Timmis and Tanaka (1976), working with container-grown Douglas-fir seedlings, also found that moisture stress increased cold

Table 9.2—*Growth response and cultural implications of inducing moisture stress in conifer seedlings in northwest nurseries (Landis et al. 1989).*

Plant water potential (predawn) MPA	Moisture stress rating	Seedling response/ cultural implications
0.0 to -0.5	Slight	Rapid growth
-0.5 to -1.0	Moderate	Reduced growth/ best for overal hardening
-1.0 to -1.5	High	Restricted growth/ variable hardening results
-1.5 to -2.5	Severe	Potential for injury
< -2.5	Extreme	Injury or mortality

hardiness. Seedlings also can be conditioned for increased drought hardiness. Christersson (1976) showed that subjecting pot-grown Scots pine (*Pinus silvestris* L.) and Norway spruce (*Picea abies* [L] Karst.) seedlings to a period of moisture stress enabled seedlings to tolerate a drought stress of -3.5 MPa, compared to a drought stress of -2.5 MPa for unhardened seedlings. Other effects of moisture-stress conditioning also have been noted. Seiler and Johnson (1985, 1988) reported that moisture-stress conditioning of loblolly pine (*Pinus taeda* L.) seedlings resulted in acclimation of photosynthesis to low water potentials, lowered osmotic potential, reduced transpiration, and increased water-use efficiency. Results, however, varied with species. For example, red spruce (*Picea rubens* Sarg.) seedlings exposed to sublethal water stress did not become more drought tolerant, undergo osmotic adjustment, or show photosynthetic or stomatal acclimation to water stress (Seiler and Cazell 1990).

Some nurseries measure seedling water potential during lifting and packing operations. Low water potentials can occur during lifting of seedlings because of low soil moisture content, cold soils (Lopushinsky and Kaufmann 1984, Lopushinsky and Max 1990), or high evaporative demand. These concerns have led to the establishment of guidelines based on pressure chamber readings (Day and Walsh 1980, Scholtes 1989) in an attempt to avoid lifting and packing seedlings with low water potentials. Generally, seedlings are not lifted when water potentials drop below -1.5 or -2.0 MPa, and water potentials are not allowed to fall below -0.5 MPa during grading and packing. These limits appear to be arbitrarily set because little is known about the relationship of water potentials in seedlings during lifting and processing to subsequent field survival and growth. Cleary (1971) found that in Douglas-fir and ponderosa pine seedlings, photosynthesis drops at water potentials between -1.0 and -2.0 MPa, and below -2.0 MPa vigor presumably continues to decline. But

these criteria, or those listed in Table 9.2, cannot be used to predict responses of seedlings which have been allowed to recover from water stress, kept in cold storage, and in many cases, planted months later.

Occasionally, bare-root seedlings in storage bags dry out during cold storage. Depending on the extent and duration of moisture stress, such drying may or may not affect seedling performance after planting. Daniels (1978) found that when bare-root Douglas-fir seedlings with a water potential of -2.0 MPa were lifted and cold stored for 55 days, field survival and growth declined. But he also found that the adverse effects of low water potentials at lifting were eliminated by spraying the trees with water immediately after lifting. In another study, water potentials as low as -1.7 MPa during storage of Douglas-fir seedlings were found to have no effect on subsequent survival (Hermann et al. 1972), and in spruce survival decreased only when water potentials were less than -2.0 MPa at the time seedlings were planted (Ruetz 1976). In a recent study with white spruce (*Picea glauca* [Moench] Voss), seedlings lifted in October at two levels of water potentials, above -0.1 MPa and below -0.11 MPa, and freezer stored for seven months, showed no adverse effects of pre-storage moisture stress on timing of budburst or height growth (Rose 1990). The absence of adverse effects probably can be explained by the fact that the "high stress" treatment was relatively mild, i.e., an average water potential of only -0.135 MPa, and that the roots of seedlings in both stress treatments were dipped in water prior to storage.

9.5 Interpretation of Water Potential Values

Probably the most difficult problem associated with measurement of seedling water potentials is interpreting the significance of lowered water potentials for seedling growth and survival, particularly with mid-range values from about -1.0 to -2.5 MPa. When assessing seedling responses, consideration must be given not only to the magnitude of water stresses, but also to their duration, the stage of growth or dormancy at which stresses occur, the species involved, and seedling vigor. Certainly, seedlings which have desiccated to water potentials below -4.0 MPa for prolonged periods of time very likely will exhibit reduced growth and survival, but what about seedlings with a water potential of -2.0 MPa? A water potential of -2.0 MPa measured at midday during the summer in nursery beds which show high predawn potentials (0 to -0.5 MPa) will, with most species, have little or no effect on seedling growth in the nursery or subsequently in the field. A water potential of -2.0 MPa measured before dawn, on the other hand, is a cause for concern. Low predawn seedling water potentials develop as the result of a gradual increase in soil water stress over a considerable period of time. Thus, the seedlings would have been subjected to a low water potential, during both nighttime and daytime periods, for an extended period of time. A predawn potential of -2.0 MPa is not likely to result in seedling mortality, however, it will prevent normal stomatal opening during the daytime, greatly reduce photosynthesis, and severely suppress or stop seedling growth. Following irrigation, seedling water potentials will increase. Normal growth rates may or may not resume, however, depending on the duration of the water stress, the sensitivity to stress of the species involved, and other factors. A water potential of -2.0 MPa measured predawn or in early morning hours during lifting also is also a cause for some concern, but in a different sense. Since the seedlings are dormant, suppression of current growth is not a problem. Also, it has been shown that during winter and early spring, Douglas-fir seedlings are at their highest level of resistance to water stress (Hermann 1967, Ritchie 1984, Lavender 1985). If the moisture stresses are only temporarily high, or can be relieved by delaying the lifting or by moistening the seedlings after lifting, it is unlikely that measurable survival or growth effects will be observed. On the other hand, unmoistened seedlings with a water potential of -2.0 MPa at the time they enter storage, or seedlings which have desiccated to -2.0 MPa during storage, very likely will experience some reduction in survival and growth.

A factor that needs to be taken into account is the relative sensitivity of different species to water stress. Differences in drought resistance are recognized, but it is not known, for example, to what extent the elongation of terminal shoots in Douglas-fir seedlings is reduced by a given water stress, compared to bud elongation in ponderosa pine or lodgepole pine. Finally, the overall vigor status of seedlings also needs to be taken into account, because it is likely that seedlings low in vigor from other causes will be affected to a greater degree by water stress than seedlings with high vigor.

A related issue which deserves consideration here is the extent to which measurements of water potential or PMS can be used to assess seedling quality. The importance of plant water status to seedling growth and survival, and the ease with which measurements of water potential now can be made with pressure chambers, have tended to foster the belief that a measurement of water potential or PMS can be used as an index of seedling quality. In a very limited sense it can, as for example, in the case of seedlings with extremely high stresses, or those subjected to prolonged desiccation during storage. And, as mentioned earlier, the pressure chamber can be used to check for cold injury in roots. Generally, however, factors known to influence seedling quality such as root growth potential, stored carbohydrate level, cold resistance, size of seedlings, and size of root systems, have no direct relationship to water potential. Clearly, seedlings can be so deficient in some or a combination of the above attributes

that prospects of good growth and survival are poor, yet can be moist enough to exhibit a low water stress.

9.5.1 Allowable water potential limits

Given the above considerations, what then are allowable water potential limits (targets) for nursery seedlings during the growth stage, and during lifting and storage? For seedlings during the growth stage, appropriate stress limits are those shown in Table 9.2, i.e., predawn water potentials should be kept above -0.5 MPa to maintain growth, and in the range of -0.5 to -1.0 MPa to limit growth, induce dormancy or increase cold-hardiness. During lifting and processing, seedling water potentials ought to be maintained above -1.0 MPa, with seedlings moistened as required to reduce stresses to this level. Seedlings about to be placed in storage also should have water potentials above -1.0 MPa. Seedlings with water potentials between -1.0 and -2.0 MPa that have been moistened before being placed in storage probably will not experience significantly reduced survival and growth, mainly because in sealed bags, the seedlings will equilibrate to higher water potentials. On the other hand, placing unmoistened seedlings with a water potential of -2.0 MPa or less in cold storage has been shown to result in reduced seedling performance. The actual falldown in performance will vary for different lots, depending on the influence of other factors that also affect seedling vigor.

During cold storage, seedlings kept in sealed storage bags typically will have water potentials above -0.7 MPa (most often above -0.5 MPa), and will not exhibit problems related to water stress. Water potentials in the range of -0.7 to -2.0 MPa increase the likelihood of adverse effects. Moistening such seedlings, and allowing time for water stress to decline will reduce, but may not entirely eliminate, adverse effects. Stored seedlings with water potentials below -2.0 MPa can be expected to show reduced field performance. Again, moistening such seedlings will reduce the water stress, but probably not restore seedling performance to normal levels. Actual performance will vary, depending on the duration of the exposure to water stress, and the influence of other vigor-related seedling factors.

The foregoing discussions emphasize that, properly used, measurements of seedling water potential can provide valuable information that will help nursery personnel produce high quality stock. Conversely, improper measurements and interpretations of water potentials can result in unnecessary work and precautions and can lead to less than effective nursery management.

9.6 Summary

Information on the water status of nursery seedlings is important because water deficits affect practically every aspect of plant growth. The water status of tree seedlings can be determined by measuring seedling water content, and by liquid equilibration, psychrometric, and pressure chamber techniques. The last three methods are preferred because they measure water potential, an expression of the free energy of water, which is more directly related to physiological functions in plants than is water content. Liquid equilibration methods are laborious, time consuming, and yield estimates rather than precise values of water potential. Water potentials can be measured most accurately with thermocouple psychrometers which also can be used to measure osmotic potentials, but long equilibration times, temperature sensitivity and other technical considerations make this method better suited for use in the laboratory than in forest nurseries. The J-14 hydraulic leaf press is easy to use and economical, but endpoints vary with the type of tissue and with the level of water potential. So far it has not found wide acceptance for use with conifers.

The method of choice for nursery work is the pressure chamber because it is fast, simple and accurate. It can provide estimates of osmotic and turgor potential, and it also can be used to measure the hydraulic conductivity of root systems and to detect cold injury in roots. The pressure chamber also is useful for scheduling irrigation. To maintain growth, seedlings should be irrigated when predawn water potential drops below -0.5 MPa. Conditioning seedlings in the nursery by exposure to moderate moisture stresses can cause osmotic adjustments and other physiological changes that increase cold and drought hardiness in seedlings, but conditioning procedures and effects have not been thoroughly studied in northwest conifers. In some nurseries, the pressure chamber also is being used to monitor seedling water potentials during lifting and packing. Limited data indicate that during lifting, seedling water potentials down to -2.0 MPa will not adversely affect seedlings, provided that seedlings are moistened to relieve stresses prior to storage. Storing seedlings with a water potential of -2.0 MPa or less, however, likely will result in reduced survival and growth after outplanting.

9.7 Research Needs

Additional research related to the water status of nursery seedlings is needed in several areas. More research is needed on the effects of plant water deficits on all aspects of seedling growth, including bud and shoot extension, needle elongation, stem diameter, and root growth. Seedling water status is a major determinant of seedling growth, yet water potential guidelines presently available are only general in nature, and do not adequately reflect stress-related growth responses for many important species or provenances of species. Better information in this area is needed to permit nursery managers to tailor irrigation schedules more closely to the requirements of specific species.

More research also is needed to determine how moisture-stress conditioning can be used to acclimate seedlings to better tolerate adverse conditions. Such conditioning may be particularly feasible with container-grown seedlings since environmental factors can be closely controlled in container facilities (greenhouses). Pressure-volume curves obtained with a pressure chamber provide a means of monitoring osmotic adjustments during such conditioning.

Another research need is related to the concern about seedling water potentials during lifting and packing. Research is needed to determine what, if any, relationship exists between low water potentials during lifting and packing of seedlings and subsequent performance in the field. Seedling water potential generally increases in the precooler during processing and during cold storage (J. Scholtes 1990, R. Rose 1990, personal communication). These observations, and the ability to eliminate moisture stresses by moistening seedlings prior to storage, suggests that a temporary low water potential during lifting and processing is not a serious problem, but data in this area are lacking.

More information is needed about the ways in which moisture stress and seedling vigor interact, and how these interactions affect seedling performance. It is well known that seedling vigor can vary considerably as a result of different lifting dates, time in storage, and other factors. So the question arises, "To what extent do low water potentials affect survival and growth of seedlings of low vigor compared to those with high vigor?"

Additional research also is needed to determine whether the hydraulic leaf press can be used to measure water potentials in nursery conifer seedlings. There are indications that the endpoint is easily observed at high water potentials (Childs 1980), suggesting that the method may provide a quick and easy way to check seedling moisture stress during grading and packing when moisture stresses usually are relatively low.

Finally, though not directly applicable to routine nursery operation, more research is needed on the effects of water stress at the molecular level in tree seedlings. It is known, for example, that water stress can cause changes in the kinds and concentrations of growth substances in the root that affect shoot metabolism and growth (Itai and Vaadia 1965, Livne and Vaadia 1972). To better understand water stress-growth interactions, more emphasis needs to be placed on the effects of water stress on the balance of growth regulators and on other enzyme-mediated processes because the effects of water deficits cannot be explained fully by decrease in water content or water potential.

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Target Seedling Symposium

Chapter 10
Mineral Nutrition and the Target Seedling

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ABSTRACT

Containerized Douglas-fir seedlings were grown in a greenhouse for seven months. Treatments were started in June 1989 by modifying a standard nutrient solution to give three levels each of nitrogen and phosphorus in a complete factorial design. Both nutrients were supplied at one-third of control, control, and three times control. Foliar nitrogen, phosphorus, potassium, total dry weight, and root growth capacity after four weeks were measured in late December 1989. These data were used to compare three methods of assessing plant nutritional status: critical concentration, vector diagnosis, and DRIS (diagnosis and recommendation integrated system). Unlike critical concentration, both vector diagnosis and DRIS identify relative, not absolute, differences between treatments.

Both nitrogen and phosphorus were found to limit growth when compared to the standard nutrient solution. Dry weight was most influenced by nitrogen and RGC was most influenced by phosphorus. Data suggest that the level portion (luxury consumption) of a critical concentration curve is caused by deficiencies in other nutrients. Also, critical concentration was found to be of little value in making nutrient recommendations. Both vector diagnosis and DRIS were more useful in identifying and ranking limiting nutrients.

10.1 Introduction and Objectives

Good mineral nutrition is fundamental to producing the target seedling. It is as basic as light and water. And just like these other factors, mineral nutrition is more or less taken for granted. A mental picture of the ideal seedling is a summary statement of the effects of good mineral nutrition. Among other details, this picture includes good color, height, and caliper. It is equally easy to visualize a seedling that has a mineral nutrient deficiency. That mental picture can be as vivid as the first and is highlighted by poor growth and color.

Most forest nursery managers would acknowledge that good mineral nutrition is a basic part of producing the target seedling. In spite of this fact, many nursery managers do not have the tools needed to gather information about nutrient imbalances or deficiencies before damage has been done. Many managers find interpretation to be as difficult as gathering the information.

This primary goal of this chapter is to describe some relatively new methods of evaluating the nutrient status of plants. These methods will be compared with conventional methods. The focus will be on the practical application of these new methods and detail the technical aspects of the methods. The core of this paper will be principles and not specific prescriptions.

10.2 Basic Principles of Mineral Nutrition

10.2.1 Uses of mineral nutrients

The emphasis of this brief review of basic mineral nutrition is to place scientific facts into a practical perspective. It is beyond the scope of this chapter to enter a detailed discussion of the biochemical or cellular level actions and interactions of the different mineral nutrients. Discussions at this level can be found in several readily available textbooks. Among these are: Kramer and Kozlowski 1979, Epstein 1972, Hewitt and Smith 1974, and Gauch 1972. A recent publication by Landis et al. (1989) is a readable overview of mineral nutrition in forest nurseries.

In the mid part of the nineteenth century, agricultural chemists began to understand that mineral elements used by plants were taken up from the soil (Hall 1905). The obvious extension of this idea was to use the analysis of plant material to describe the nutrient supply of the soil. For many years plant analysis was seen as a biological method of soil analysis. Only in the past 30 years or so has the emphasis changed to using the analysis of plant material to evaluate the nutrient status of the plant (Bouma 1983).

It is important to emphasize the limitations of plant nutrient analysis. A thorough examination of a plant's nutrient content can show an imbalance, a deficiency, or an excess of certain nutrients. It cannot prescribe a particular amount of fertilizer to be added nor can it predict the response to a given amount of fertilizer. It must be remembered that the soil type, environmental conditions (temperature, humidity, etc.), and the plant itself will regulate uptake and utilization of a mineral nutrient. Knowledge of the nutrient content of a plant is useful because of the relationship between nutrients and physiological processes. For example, the analysis of nitrogen in a leaf is useful primarily because nitrogen in a leaf is correlated to the relative rate of photosynthesis. However, many other factors can change photosynthesis. Consequently, the measurement of nutrient content is only useful because the overall nutrient picture of a seedling is a reflection of the overall vigor of that seedling. Knowing something about an item that is relatively easy to measure (nutrient content) is valuable when that information is strongly related to something that is not as easy to measure (physiological condition).

Like all plants, tree seedlings have definite, well defined mineral nutrient requirements. There are 16 commonly

Table 10.1—*Elements essential to plant growth. Ranked by quantity found in oven dry tissue and listed by major role in plant tissue (Modified after Salisbury and Ross 1978).*

Element	Rank	%	Role in plant
Carbon	1	45.0	Carbohydrate
Oxygen	2	45.0	Carbohydrate
Hydrogen	3	6.0	Carbohydrate
MACRONUTRIENTS			
Nitrogen	4	1.5	Amino acids, protein, nucleic acids, chlorophyll
Potassium	5	1.0	Enzymes, osmotic control, pH balance
Calcium	6	0.5	Enzymes, membrane stability, middle lamella
Magnesium	7	0.2	Enzymes, chlorophyll
Phosphorus	8	0.2	Energy transfer, nucleic acids, phosphorylated sugars.
Sulfur	9	0.1	Amino acids, protein, enzymes
MICRONUTRIENTS		ppm	
Iron	10	100	Enzymes, electron transport
Chlorine	11	100	Photosynthesis, non-essential role in osmotic control
Manganese	12	50	Enzymes, oxidation-reduction
Zinc	13	20	Enzymes
Boron	14	20	Carbohydrate translocation
Copper	15	6	Enzymes
Molybdenum	16	0.1	Enzymes

accepted elements that make up the essential mineral nutrients (Table 10.1). These elements are usually placed in broad groups based on relative concentration in the plant. Macronutrients are more abundant than micronutrients. It is a common mistake to believe that a macronutrient is more important to a plant than a micronutrient. While it is certainly true that macronutrients are required in greater quantity than micronutrients, all are required for the plant to function normally. An absence of any essential element will have serious consequences.

An element is judged essential if it meets three criteria (Arnon and Stout 1939):

1) Absence of the element will cause abnormal growth or in severe cases cause the plant to be unable to complete its life cycle.

2) It must be a part of some compound needed by the plant for normal metabolism. The effect of the element must come about by an internal function and not an external function.

3) The element cannot be completely replaced by another element.

These criteria are so basic that they have become a part of the litany and grown transparent. There are two basic principles involved that are worth restating. First, it would be unusual in a nursery situation for a plant to be grown long enough that problems with the life cycle would become apparent. In contrast, abnormal or poor growth is relatively common. Unfortunately the definition of poor growth is often subjective and hence is not always immediately apparent. Second, elements that are not essential can alter how a seedling grows. These elements can either be beneficial or toxic. An example of a beneficial element would be sodium which can partially replace potassium in some roles within the plant. Partial substitution can make a moderate deficiency less apparent at first. In contrast, lead is an example of an element which has a harmful effect and can stop some enzymes from functioning. By interacting with essential elements, some toxic elements may mimic deficiencies.

10.2.2 Symptoms of deficiency

Nutrient deficiencies have been the subject of many studies. Two studies have been done on western conifers (Murison 1960, van den Driessche 1989). Both studies have color plates and descriptions of the deficiencies. Most deficiency studies are done by removing the element in question from a nutrient solution and then evaluating the appearance of the plants (Table 10.2). This produces a plant that would not usually be seen in an operational nursery. Even though a nursery may not supply enough nitrogen for optimum growth, it is unlikely that a nursery would not supply any nitrogen.

***Table 10.2**—Generalized symptoms of mineral nutrient deficiency of selected elements. More detailed descriptions can be found in Landis et al. (1989) and van den Driessche (1989). Note: In conifer leaves, the symptoms will usually appear at the tips first. This may or may not be followed by the entire leaf.*

NITROGEN	Nitrogen is used as a constituent of chlorophyll and one of the first symptoms of nitrogen deficiency is pale green, short needles. Nitrogen is mobile within the plant and the symptoms may appear on older foliage first, but because nitrogen is used in so many important compounds (enzymes, nucleic acids), deficiency will cause plant-wide symptoms.
POTASSIUM	Potassium is used to balance osmotic potentials and help regulate pH. The symptoms are variable, but usually include browning of the leaves. Potassium is mobile so the symptoms are usually on the older leaves first. Potassium is used throughout the plant so overall the plant will be stunted.
CALCIUM	Calcium is used in the middle lamella and cell walls. Calcium is not mobile within the plant. The usual symptoms include distorted leaves, poor meristem elongation and yellowing of newer leaves. A recent calcium deficiency will only be shown in the new leaves.
MAGNESIUM	Magnesium is used in chlorophyll. Leaves usually become yellow from a lack of chlorophyll.
PHOSPHORUS	Phosphorus deficiency will usually be shown as dull green-gray leaves. In some plants the leaves become dark green or purple. The leaf size tends to be normal, but the plant becomes stunted.
SULFUR	Sulfur is used in amino acids. Because nitrogen is also used in amino acid, the symptoms are similar. Pale green to yellow leaves that are stunted will usually be the first symptom. Most often appears in the younger leaves first.
IRON	Iron is used in the formation of chlorophyll. The first symptoms are yellowing foliage. Because iron is immobile the younger leaves are usually the first to show symptoms.

Consequently the symptoms, if any, are commonly less dramatic.

Ingestad has developed theories relating growth rate and nutrient concentration (Ingestad 1977, Ingestad 1982). These theories are explained in mathematical equations that relate growth rate, uptake rate, and other related processes. This work is important to discussion of deficiencies because it shows the relationship between nutrient

supply, growth rate, and development of deficiency symptoms.

Nutrients are consumed at a rate that is dependent on growth. Faster growing plants need, and consume, more nutrients. The problem faced by nurseries and those doing nutrient experiments is that the supply of nutrients is lumpy while growth is smooth. Nutrients are supplied in large, infrequent doses and growth is an ongoing process with a more or less constant rate. At the beginning of a growing season the plants are small and need relatively little nutrients. Plant growth is exponential and as the season progresses the addition of nutrients must greatly increase. If the nutrient supply is inadequate for the growth rate, then deficiency symptoms appear. This is often the case at the beginning of a new season when plants break bud and quickly add new growth. These deficiency symptoms are usually transient and disappear when the growth rate adjusts to the nutrient situation. Ingestad (1982) has shown that ". . . under natural conditions with marked nitrogen deficiency, vegetation is normally green, independent of plant species. It is to be expected that plants in their natural environment attain a steady state because growth adjusts to the nutritional resources of the site." This means the only dependable symptom of a nutrient deficiency will be a reduction in growth. Other visible symptoms may or may not appear. As a consequence, a nutrient analysis of the plant tissues will be required if a deficiency is to be detected and maximum growth maintained. It should be pointed out that maximum growth is not always the goal of the nursery. Inducing dormancy or relocating growth may be the goal at different times of the year.

10.3 Measuring Mineral Nutrient Content

10.3.1 Review of statistics

An understanding of five ideas from basic statistics will be useful in the following sections. Two of these are mathematically based (mean and variance) and three are conceptual (sample, normal distribution, and equality). Both mean and variance are easily determined with a hand calculator. In fact, many hand calculators have these functions preprogrammed and report the results at the push of a button. The textbook *Elementary Statistics* by Khazanie (1990) is recommended for a review of basic statistics. It is outside the scope of this paper to deal with statistical principles beyond this brief review.

Biological data, such as nutrient concentrations, will usually be more or less bell-shaped when it is plotted (Figure 10.1). Most commonly this kind of data will also be skewed to the right (Samuels 1989). Imagine a piece of graph paper with a horizontal line drawn across the bottom. This line represents the range of numbers that the data points have assumed and one square has been filled in each time a data point was measured. The more frequently occurring values form taller and taller stacks of filled in squares. If enough data points are measured and the population is normally distributed, then the curve will be perfectly bell-shaped.

If the data is normally distributed, then the mean will be at the top of the bell or the center of the distribution. The mean is the arithmetic average, or the sum of all data points divided by the number of data points (sum of X/n). The main use of the mean is to locate the center of the data distribution.

The variance determines the shape of the bell. The bell will be wide and flat (platykurtic) if the data is highly variable. In contrast, the bell will be tall and narrow (leptokurtic) if the data has little variation. Variance is calculated by subtracting each data point from the mean and squaring the result. All of these subtracted and squared numbers are added and divided by the number of data points minus 1 (sum of X minus mean of X squared divided by n-1). Variance does not have any hidden significance. It is simply one method of answering the question, "How variable is this data set?" The most common method of expressing variance is standard deviation. Standard deviation is the square root of variance and is used because it is in the same units as the mean (variance is in units squared). After the mean and variance have been calculated it is possible to estimate the shape and middle of the normal curve.

The coefficient of variation is frequently used in nutrient analysis. The c.v. is the standard deviation divided by the

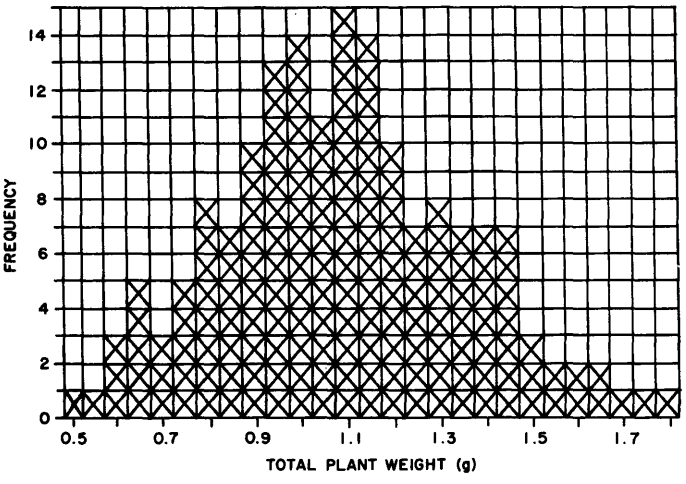

Figure 10.1—*Total weight of seedlings from the December harvest of the comparison experiment. Each X represents one plant. The distribution of the data approximates a normal distribution.*

mean times 100 and expresses variation as a percentage of the mean.

A sample is a part of a population. Without worrying about rigorous definitions, a sample is just a small part of a larger group. The major problem with a sample is it may not represent the population. The two most common errors are: 1) too few individuals are chosen for the sample or 2) the sample has been in some way biased. In this context, bias means that one part of the population has been over- or under-represented by the way in which the sample was chosen. An example would be choosing plants next to the road because they are easier to collect. The most important thing to understand about samples is that they are subsets of populations. From a practical viewpoint this means if the sample were to be repeated a second time, the mean and variance that were obtained the first time would be different from those of the second sample.

The final concept is that of equality. It is fairly straightforward that three does not equal four. However, in statistics three may well equal four. Mathematically equal means four equals four, but statistically equal does not. Much of inductive statistics is concerned with procedures to determine if the means of two or more samples are statistically equal. In general, it is more likely two samples will be judged statistically different if their means are far apart on the number line and their variances are small. The major difficulty in nutrient analysis is in determining when a value being compared to a standard is statistically equal or statistically different.

10.3.2 Sampling and determination of chemical composition

A few general ideas summarize some of the important aspects of sampling. First, the goals of sampling should reflect the goals of the experiment. Quite often the goals of an experiment done by a production nursery are more general than those for a scientific study. The major differences are usually seen in purpose, use of the information, and sampling intensity. If only general, record keeping information about the nutrient status of a stock type is desired, then infrequent samples may be taken on fewer populations. However, in all cases the sampling must encompass the full variation in the population being evaluated. This means that plants must be included from as many beds or benches as the stock type occupies. Many times a section of a nursery bed will show obvious reduced growth or other symptoms of difficulty. These areas can be identified and separated from other beds before sampling. Poor growth areas should still be sampled. In all cases the sample size should be large enough to identify meaningful, statistically significant differences. If the sample is too small, no significant differences will be detected. Procedures for determining sample size are detailed in virtually all statistic textbooks.

Second, plants should be randomly chosen. The easiest way to ensure a random sample is to use some form of a random starting point. A random number generator or table will help with this step of the process. From the starting point, some systematic pattern can be followed. Remember that, in general studies, the major problem is to overcome bias. A nursery manager needs to be careful to collect trees that are truly representative of their nursery. It is easy to systematically choose plants that are above average and not like most of the nursery. A truly unbiased sample will be valuable if the goal is to run an ongoing evaluation. In some of the following nutrient evaluation procedures it is useful to have samples from both the better trees and the cull trees.

When designing a nutrition experiment, a control group must be identified. In a bareroot nursery, an unfertilized plot may be used as a control treatment. Alternatively, a plot treated with a standard fertilizer system could be used. A container nursery may want to use a standard nutrient solution as a control and formulate different nutrient solutions for treatments.

The plant part sampled and timing of sampling can have a large effect on the usefulness of tissue analysis results. Plant tissue samples may be taken from whole plants, the shoot, or foliage only. The nutrient content of each plant part will be quite different. A review of Table 10.1 shows that some nutrients, like nitrogen, would be present in high quantities in the metabolically active parts of the plant. In contrast, calcium would be present in all parts of the plant. This is not unrelated to age. Consider the effect of the stem on an analysis. In very young seedlings the stem is a relatively small part of the whole plant. As the plant ages, the stem becomes more and more of the biomass. By the time a 2-0, 1-1, or 2-1 plant were sampled, the stem would be the major portion of the biomass. The most useful procedure is to use an easily identifiable part of the plant in the sample. Using the last fully expanded, mature leaves will solve the problems of physiological age, and identification of a plant part. Repeatable, useful information will be obtained if the same plant part is sampled at the same physiological time each year.

Collected samples should be clean and placed in plastic bags along with an identification tag. Samples cannot be over identified. If the sample cannot be quickly dried, it should be kept cold in an ice chest to slow metabolic activity. After collection, the samples are usually oxidized to remove carbon, hydrogen, and oxygen by the Kjeldahl acid digestion method. The nutrient content is then determined by titration, specific-ion electrode, atomic absorbtion or spectrophotometry. Many of these procedures are discussed more in depth by Landis (1985). A useful handbook for these and other procedures is Chemical Analysis of Ecological Materials (Allen 1974).

10.4 Description of Comparison Experiment

Non-mycorrhizal Douglas-fir seedlings were grown in a heated, ventilated greenhouse in 5-in^3 leach tubes with a standard nutrient solution (Ingestad and Lund 1986). After seven months, treatments designed to bring about a range of nutrient conditions were started. The nitrogen and phosphorus levels of the standard nutrient solution were modified. These nutrients were supplied at one-third of control, control, and three times control (Table 10.3). This created a two-way factorial design with nine treatments. Other nutrients continued to be supplied at the levels described by Ingestad and Lund.

The treatment that received nutrients with the original levels of nitrogen and phosphorus will be referred to as the control. The exception is in the DRIS section where the convention of other authors will be followed and the control treatment will be referred to as the norm. Throughout the paper this treatment will be abbreviated as Nn Pn (nitrogen normal-phosphorus normal). Similarly the treatment which had one-third the control nitrogen level and three times control phosphorus level would be referred to as N- P+.

This experiment had five harvests: June 5, July 2, July 31, August 28, and December 27, 1989. On each harvest date, ten seedlings per treatment were lifted. Among the variables measured on each tree were: root growth capacity at two weeks, root growth capacity at four weeks, height, caliper, leaf, stem and root dry weight, number of buds, number of branches, and net photosynthesis. A micro-Kjeldahl digest was done on the foliage. Phosphorus was determined with a spectrophotometer, while nitrogen and potassium levels were done by specific ion electrode.

Table 10.3—*Nitrogen and phosphorus levels in ppm in nutrient solution for each of the nine treatments. Minus (-) treatments are 1/3 of the normal (n) level, while the plus treatments are 3 times the normal level. The minus treatments were intended to induce deficiency and the plus treatments were intended to show the luxury consumption phase. All other essential nutrients were held constant at the normal level.*

	N_-	N_n	N_+
	N, P	N, P	N, P
P_-	8.3, 1.1	25, 1.1	75, 1.1
P_n	8.3, 3.25	25, 3.25	75, 3.25
P_+	8.3, 9.75	25, 9.75	75, 9.75

10.5 Interpretation of Values

10.5.1 Critical nutrient concentration/range

The most common method of diagnosing mineral nutrient problems is determining critical nutrient concentration. In practice the mineral nutrient content of a specific plant part is determined in the laboratory. These values are adjusted with experience and used as guides to compare how well other plants are supplied with the same mineral nutrients. This concept is based on a predictable and repeatable relationship between yield and the concentration of any single mineral nutrient. The dependability of the method depends on how comparable the experimental plants were to the plants used to establish the critical values (Armson 1973). This relationship has been defined in several different ways:

1) The concentration that is just deficient for maximum growth (Ulrich 1952).

2) The concentration that is just adequate for maximum growth (Ulrich op. cit.).

3) The concentration within the transition zone at the breaking point of the curve, or mathematically when dx/dy = 0 (Ulrich 1976).

4) The concentration beyond which further application of nutrient does not return a profit (Bates 1971).

These definitions are similar, but the differences are based on the criteria being used to determine yield. In some crops maximum dry matter production does not necessarily correspond to either the better plant or to optimum economic yield. Instead some combination of quantity, quality, and plant performance is used to define the better plant. This is probably the case in forestry.

Critical nutrient concentration can be viewed in two ways. It can be seen as a minimum value below which production is inadequate or as a maximum value above which production is unsatisfactory. This may seem a belaboring of a relative minor point. However, the most difficult part of evaluating mineral nutrition is defining yield, or setting an optimum value that is to be attained. Probably the most useful definition of critical nutrient concentration is "the level of a nutrient below which crop yield, quality, or performance is unsatisfactory" (Tisdale et al. 1985).

The relationship between yield and nutrient concentration has been illustrated in several different ways (Figure 10.2). The most commonly used curve is drawn without the dilution or toxic areas defined (Ulrich and Hills 1967). Using this simpler curve makes sense from a practical application point of view. Neither the dilution or toxic phases commonly occur in an operational forest nursery.

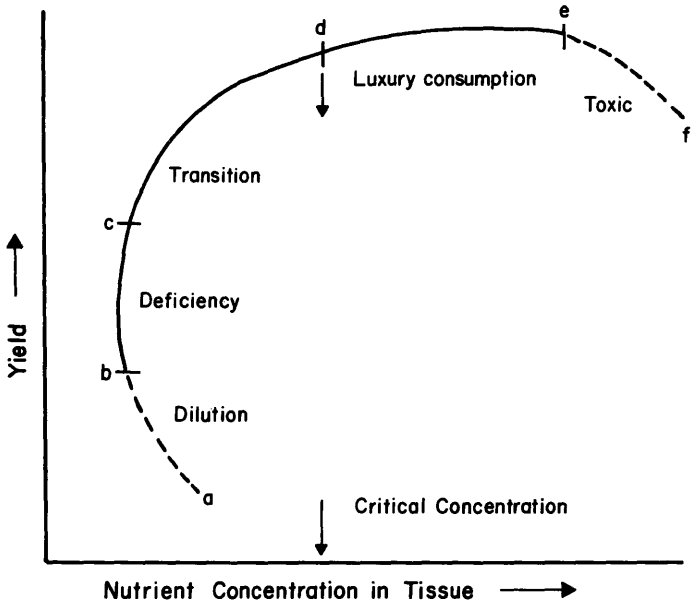

Figure 10.2—*Relationship between yield and nutrient concentration. With the exception of the dilution and toxic phases (dashed lines), the trend is for greater yield as nutrient concentration increases. The critical concentration is loosely defined as the middle part of the critical nutrient range. This range includes part of the transition and luxury consumption phases. Lowercase letters indicate the start and end points of the different phases.*

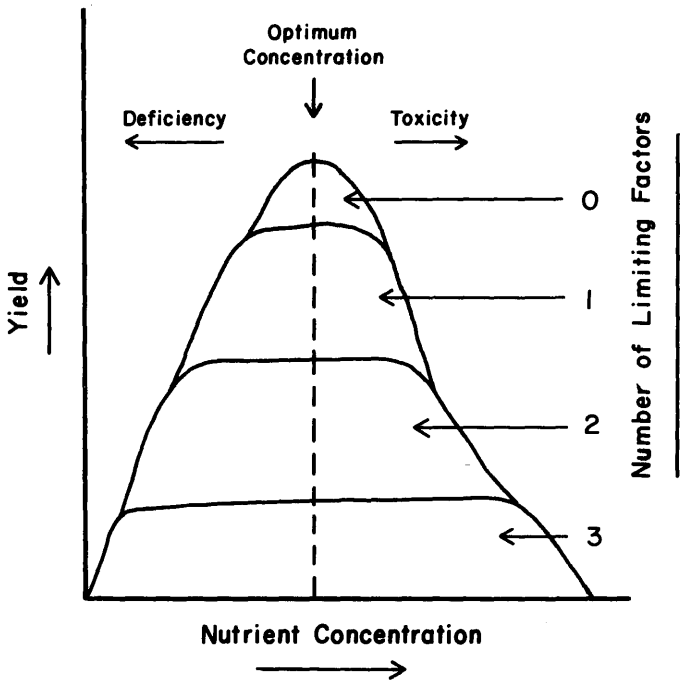

Figure 10.3—*Relationship between yield and the number of factors that limit yield. The optimum concentration is similar to the mean of a normal distribution and represents the concentration of nutrient in the plants with the greatest yield. (Adapted from Sumner and Farina 1986.)*

However, both are useful in building an understanding of the overall processes involved. The dilution phase was first described by Piper (1942) and was later described in more detail by Steenbjerg (1951). In this phase, biomass increases while nutrient concentration goes down. This is usually viewed as a constant amount of nutrient being diluted by greater growth. The exact cause of this phase has been the subject of debate. It is usually explained as either being caused by a variation in physiological age (Bates 1971) or by a change in element mobility in deficient plants (Loneragan 1978). Similarly, the toxic phase can be explained in two ways. First, a simple concentration effect where so much of the nutrient has been applied as to cause cell damage. Second, the element being supplied has an antagonistic effect on a second element which is in relatively short supply. An example would be precipitation of phosphorus by calcium. Both ways would cause a decrease in yield.

A flat luxury consumption phase is most commonly illustrated. If all other elements are present in optimal supply this portion of the curve will not be flat; rather, it will be curved (Bouma 1983). If an element other than the one being tested for is in low supply, then the luxury consumption phase will be flat and relatively long. Imagine an experiment done to evaluate the effects of nitrogen on the plant dry weight. In this experiment phosphorus, potassium, and calcium were inadvertently supplied at suboptimal levels. With all three of these elements limiting growth, the critical concentration curve would look like the bottom most curve in Figure 10.3. Assume that the phosphorus deficiency was corrected and the experiment was repeated. The curve would now resemble the second curve in Figure 10.3. The outermost, bell-shaped curve would be evident only if all elements were supplied at optimal levels. This curve illustrates three important points:

1) The optimum concentration for one element cannot be determined if other elements are deficient.

2) The yield curve is nearly statistically normal when all factors are optimum.

3) A flat-topped curve is probably an indication that a factor other than the one being tested is deficient.

This is in fact a graphic representation of Mitscherlich's Law of the Minimum (1921). His law states, "The increase in crop production by unit increment of *any* lacking fac-

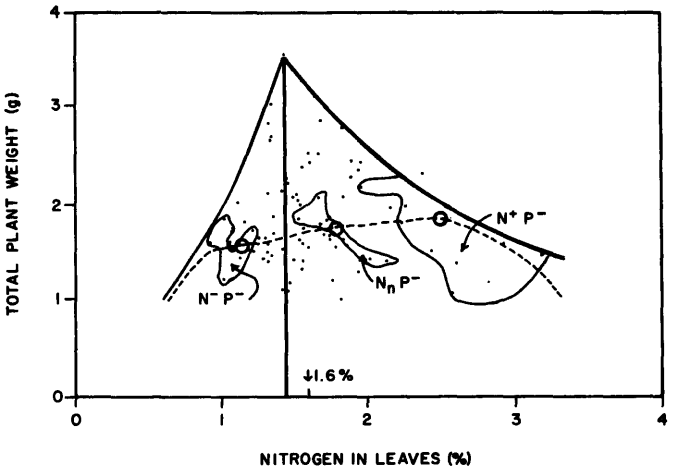

Figure 10.4—*Total plant weight and nitrogen concentration in leaves of plants from the comparison experiment that were harvested in December. The outer heavy lines enclose the data in a roughly bell-shaped curve. The vertical line shows the nitrogen concentration of the largest plants to be about 1.5%. The average nitrogen concentration for all plants in the experiment was 1.6% (arrow). The light lines inside the heavy lines surround all plants in each of three treatments (N- P-, Nn P-, N+ P-). The open circles are means from each of these groups. The dashed line connecting the means approximates a critical concentration curve.*

tor is proportional to its decrement from the optimum." In most nursery situations the deficiency picture is not black and white. Many factors are limiting growth, but none are totally lacking. For example, nitrogen and phosphorus might be deficient, but neither may be stopping growth. The complication arises if it is arbitrarily decided that nitrogen is the most lacking factor when in fact the most lacking factor is phosphorus. Mitscherlich's law says there will be a growth response to increased nitrogen, but not as much as there would have been if phosphorus had been added. The obvious answer to the problem is to examine several factors simultaneously. It is equally obvious that economics, and not biology, will dictate exactly how many factors will define the word several.

Figure 10.4 shows coordinate pairs for weight and nitrogen concentration for plants from all treatments, harvested in December in the comparison experiment. The dashed line goes through the means (open circles) for the phosphorus deficient treatments. This line approximates the lower curve in Figure 10.3. If this experiment had been done to establish the nitrogen critical concentration, and the low phosphorus concentration has been used, then the critical concentration curve would have been the dashed line. The dashed line drops quickly at about 1 percent nitrogen. That value would probably be chosen as the critical concentration. Compare this value to the average nitrogen content for all plants in all treatments (1.6 percent). However, because the luxury consumption phase was wide (1 percent to 2.6 percent nitrogen) and relatively flat (1.5 to 1.7 g), the deficiency of another nutrient was indicated. Success in determining critical values is dependent on all other factors being at optimum levels.

A quick study of the curve in Figure 10.2 shows that the placement of the critical concentration value is arbitrary. Dow and Roberts (1982) argue that establishing a single point on a curve to serve as the critical nutrient concentration is mostly an academic question. This is because the same critical value would not be obtained in successive experiments. Indeed if the experiment was repeated as exactly as possible it is unlikely that a mathematically equal value would be seen. The alternative is a critical nutrient range. Dow and Robert's definition is "that range of nutrient concentration at a specified growth stage above which we are reasonably confident the crop is amply supplied and below which we are reasonably confident the crop is deficient." They also note that Ulrich (1976) had earlier stated that critical nutrient concentration "as determined experimentally is not a point as the word concentration implies, but a narrow range of concentrations, above which the plant is amply supplied and below which the plant is deficient." From a practical point of view it is the transition zone of Figure 10.2. The sharper the break in the curve between deficiency and luxury consumption, the narrower the transition zone and the narrower the critical nutrient range.

The major advantage of using either critical nutrient range concentration or critical nutrient range is they are fairly simple to apply, *if the critical values are known.* There are at least two disadvantages. First, critical values have to be determined for each situation. Values for one species would be different from those for other species. Furthermore, there would be differences for plants of the same species and seed origin when grown under different conditions. Only when environment, genetics, sampling, analytical methods, etc., are similar will pre-determined critical values be accurate (Leaf 1973). Second, it is hard to tell if other nutrients are limiting the plant's response to a given nutrient. It will not always be possible in a single test to determine which nutrient is limiting growth. This is the fundamental difficulty with critical nutrient concentration. The technique is based on the principle that nutrients other than the limiting one will be present at optimum levels. In practice this is seldom the case. Consequently, multiple deficiencies will be particularly difficult to unravel and the determination of critical values will require large experiments.

Table 10.4—*Total dry weight (g), week 4 root growth capacity (cm), and concentrations (%) of nitrogen, phosphorus, and potassium in leaves for all treatments in the comparison experiment.*

Treatment	T.D.W.	R.G.C.	N%	P%	K%
N- P-	1.57	29	1.14	0.09	0.78
N- Pn	1.44	81	1.34	0.20	0.86
N- P+	1.46	95	1.27	0.38	0.94
Nn P-	1.74	47	1.77	0.07	0.83
Nn Pn	1.71	101	1.61	0.13	0.75
Nn P+	2.17	160	1.48	0.21	0.76
N+ P-	1.84	43	2.48	0.07	0.75
N+ Pn	2.46	128	1.68	0.10	0.80
N+ P+	2.29	165	1.59	0.21	0.93

Table 10.4 shows some of the results of the comparison experiment. By excluding plants in the P- and P+ treatments it is possible to determine an approximate critical value for nitrogen of 1.6 percent. Likewise by excluding plants in the N- and N+ treatments, the critical value for phosphorus can be seen to be 0.13 percent. Using these values as reference points it is seen that all of the trees from the low nitrogen treatments have low leaf nitrogen. Similarly all plants in the low phosphorus treatments have phosphorus levels below the critical concentration. However, there are some other relationships that are not as clear. For example, the Nn P+ treatment has a nitrogen value which is below the critical concentration (1.48 percent) with the plants being among the largest from the experiment.

The clearest example of the need for other nutrients to be optimum is in the low phosphorus treatments. A nitrogen range experiment using just these three treatments (N- P-, Nn P-, N+ P-) would have seemed successful. These three treatments have plants in the deficiency, transition, and luxury consumption ranges. Naturally when seen in the context of the whole experiment, it is plain these plants are phosphorus deficient. However, it would not have been obvious if treatments using different levels of phosphorus had not been used. To determine critical values, all nutrients must be present in adequate, but not toxic amounts. Suppose the critical values for nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and iron were to be determined. If just three levels of nutrient were to be added for each of these elements, and if a factorial experiment were done, then the experiment would have 2,187 treatments. When this is coupled with the number of species/seed zone combinations that most nurseries work with, the experiment becomes unmanageably large.

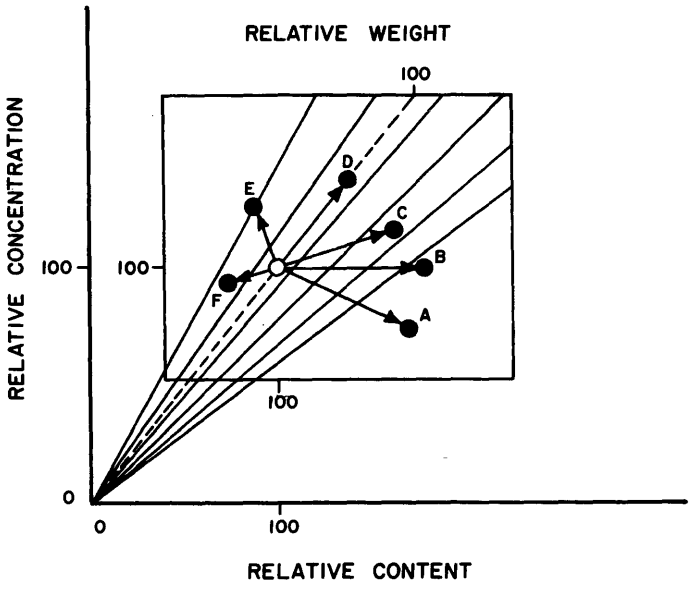

Figure 10.5—*Explanation of vectors in relative weight, nutrient content, and concentration between plants from different nutrient treatments. The open circle represents the control treatment after adjustment to 100. Vectors described by lettered points are interpreted in Table 10.5. (Adapted from Timmer and Armstrong 1989.)*

10.5.2 Vector analysis

One of the problems associated with using critical nutrient concentrations is determining the correct values for each nutrient-species-nursery combination. A re-examination of Figure 10.2 shows that this may not always be necessary. If at least two groups of plants with different levels of fertilizer can be compared, then the critical value does not need to be established. Instead it is possible to diagnose the change in nutrient status by examining the directional changes in yield and nutrient concentration. As an example, consider the following hypothetical experiment using nitrogen fertilizer. The results showed that when plants in the control or beginning treatment were compared to plants in the experimental or added fertilizer treatment there was no change in yield, but the nitrogen concentration in leaves increased. This is as if the control treatment was at point d on Figure 10.2 and the experimental treatment at point e on Figure 10.2 (increased nitrogen in leaves with no increase in yield). This could be interpreted as luxury consumption. Table 10.5 summarizes the directional changes in yield and concentration. This is the starting point for the vector analysis approach to analyzing plant nutrition.

Vector analysis or vector diagnosis has been developed by V.R. Timmer and his associates (Timmer and Stone 1978, Timmer and Morrow 1984, Timmer 1985, Timmer

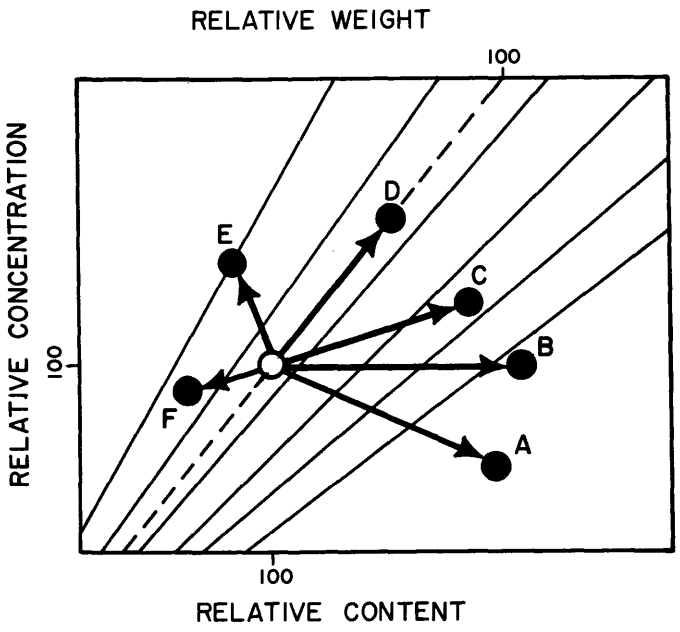

Figure 10.6—*Enlargement of Figure 10.5 showing the role of the origin in the placement of relative weight isolines. (Adapted from Timmer and Morrow 1984.)*

Table 10.5—*Interpretation of relationships between yield and nutrient concentration as described in Figure 10.2.*

		Directional change in	
Area of curve	Location	Yield	Concentration
DILUTION	A to B	INCREASE	DECREASE
DEFICIENCY	B to C	INCREASE	NO CHANGE
TRANSITION	C to D	INCREASE	INCREASE
LUXURY CONSUMPTION	D to E	NO CHANGE	INCREASE
TOXICITY	E to F	DECREASE	INCREASE

and Armstrong 1987, Timmer and Ray 1988, Timmer and Armstrong 1989, Munson and Timmer 1989a, Munson and Timmer 1989b). Vector analysis is done by using a nomograph (Figure 10.5 and Table 10.6). This analysis is slightly different from what which was just explained in that nutrient content is added to the analysis. Nutrient content is simply the absolute amount of a mineral nutrient found in a needle. In practice, a given amount of needles are collected and the nutrient content analyzed and the result is expressed on a single needle basis. Thirty needles per seedling were used in the comparison experiment.

Nutrient content is added to the analysis to help clarify a problem with using concentrations. Concentration can remain equal in three situations: when weight and content go up equally, go down equally, or remain the same. It is usual for the addition of an element to cause the leaves to become larger. If content also increases, then the concentration will remain the same. This is more of a problem when evaluating a nutrient other than the target nutrient. For example, fertilizing with one nutrient may cause the concentration of a second nutrient to go down. This is a dilution effect. Larger leaves with the same content of the second nutrient would have a lower concentration. In this case it is useful to know what has happened to the content. If it has gone down, then the addition of first nutrient has caused antagonism. On the other hand, if content has remained the same or gone up, then it is a simple dilution of the second nutrient. This can also occur with luxury consumption (Timmer and Stone 1978). Adding content to the analysis helps prevent these misinterpretations.

Nutrient content and nutrient concentration form the axes of the graph and weight is added as diagonal lines starting at the origin. Figure 10.5 does not show the origin, rather it is a section of a graph with truncated axes (Figure 10.6). The window is drawn so it that includes just the part where the points have been plotted. Relative, not absolute, values are used for weight, concentration, and content. In addition to simplifying the graph, it also makes the analysis/graphing procedure fairly easy to do with a spreadsheet.

Table 10.6—*Interpretation of relationships between weight, nutrient concentration, and nutrient content as described in Figure 10.5 (after Timmer and Armstrong 1989).*

	Change in relative nutrient			
Direction of shift	Weight	Conc.	Content	Possible interpretation of vector
A	INCREASE	DECREASE	INCREASE	CAUSED DILUTION
B	INCREASE	NO CHANGE	INCREASE	WAS JUST SUFFICIENT
C	INCREASE	INCREASE	INCREASE	WAS DEFICIENT
D	NO CHANGE	INCREASE	INCREASE	CAUSED LUXURY CONSUMPTION
E	DECREASE	INCREASE	EITHER	CAUSED TOXICITY
F	DECREASE	DECREASE	DECREASE	CAUSED ANTAGONISM

It should be noted that this is not the only way to do a vector analysis. Valentine and Allen (1990) do a similar analysis using concentration and weight as the axes and content as isolines on the graph. The underlying principles are the same, but the picture is different.

Vector analysis is done by comparing the vector shift between the control and experimental treatments. The following points refer to Figure 10.5 and may make the figure easier to read:

1) Values below the 100 line indicate more weight. Those above the 100 line show less weight.

2) Horizontal vectors to the right of the 100,100 point indicate a higher nutrient content. Those to the left of this point mark a lower nutrient content.

3) Vertical vectors above the 100,100 point indicate a higher concentration. Those below this point signify a lower concentration. All possible shifts are summarized and interpreted in Table 10.6.

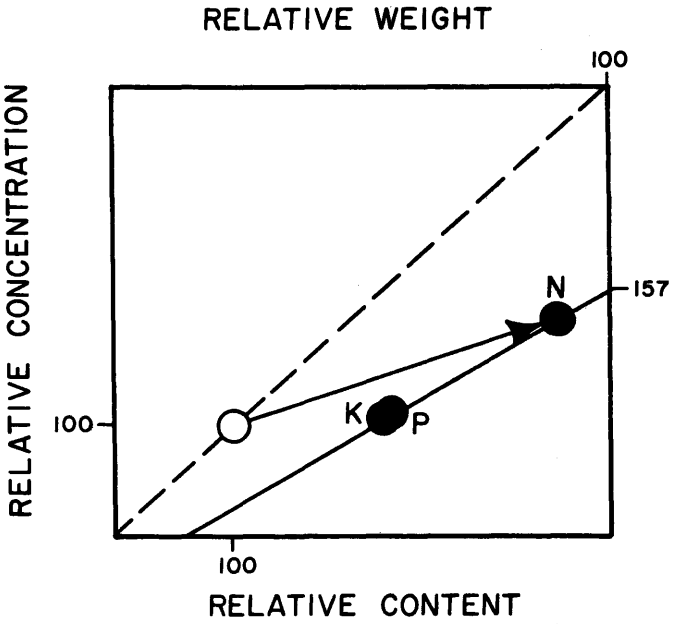

Figure 10.7—*Results of the comparison experiment. Plants in the December harvest in the N- P- treatment were compared with plants in the N+ Pn treatment. Relative N, P, and K for N- P- treatment are all represented by the open circle on the 100 weight line. Relative N, P, and K for the N+ Pn treatment are shown on the line representing the relative weight (157%). The longest vector is in C direction (Figure 10.5 and Table 10.5) which leads to the interpretation of a nitrogen being most deficient in the control treatment. Details of the calculations are presented in Appendix Table A1.*

Figure 10.7 shows some of the results of the comparison experiment as interpreted by vector analysis. The N- P- treatment was compared to the N+ Pn treatment. The convention for vector analysis is that the biomass of the treatment with the lower fertility is represented by the 100 line and all nutrients being evaluated are drawn at the 100,100 point (see Appendix Table A1 for detailed calculations). In this case the N- P- treatment had the lower fertility. Because relative values were used in constructing the graph, this point (100,100) is the same for nitrogen, phosphorus, and potassium. Similar to the control plant being represented by a single relative point, the weight, nitrogen, potassium, and phosphorus values of the N+ Pn plants can be represented on a single weight line. In this case the biomass of N+ Pn plants was 157 percent of the N- P- plants and the weight line is labelled with a 157. All that remains is to locate the coordinates for each nutrient being evaluated along the weight line. Consequently, all four variables can be interpreted at the same time on a single graph. Vectors are then drawn to each nutrient point. The longest vector is considered to be the most limiting nutrient. In Figure 10.7 only one vector has been drawn in order to simplify the drawing. In this case, nitrogen was most limiting and the vector was interpreted as being most like vector C in Figure 10.5. The lower fertility treatment can be considered to have been deficient when weight, concentration and content all increase (Table 10.6). If a vector had been drawn to the potassium and phosphorus points it would have corresponded to vector B. The concentration has remained constant, while weight and content have increased. In this case greater growth and greater uptake of phosphorus and potassium have kept pace with each other and the concentration has remained unchanged.

Timmer's vector analysis has several advantages when compared to critical nutrient concentrations. Perhaps the greatest of these is the elimination of the determination of the critical concentration for each nutrient. It is also simple to do and fairly easy to interpret. Figures have been used in this paper to illustrate the results. However, in practice it would be less time consuming to compare the results to the description of vectors in Table 10.6. Both the graphic presentation and comparison to table values can be adapted to spreadsheets.

Vector analysis has two disadvantages. First, representing several treatments on the same graph can make the interpretation difficult. An obvious solution to this problem is to make several graphs and do each interpretation separately. However, there are times when the relationship between several treatments is as important as the relationship of each treatment to the control. Second, there is no simple way to account for the differences in magnitude between treatment responses. In the comparison experiment the phosphorus concentration ranged from 75 percent to 418 percent. This is a problem in statistics. Two

Table 10.7—*Results of the comparison experiment. Values given are relative to the N- P- treatment. Least significant differences (L.S.D.) were done after a two-way analysis of variance was done on the data.*

Treatment	Nitrogen		Phosphorus		Potassium		Weight
	Conc.	Cont.	Conc.	Cont.	Conc.	Cont.	
N- P-	100	100	100	100	100	100	100
N- Pn	118	111	216	204	111	106	92
N- P+	111	99	418	366	122	107	93
Nn P-	155	189	80	96	106	128	111
Nn Pn	141	184	138	178	96	124	109
Nn P+	130	226	227	399	98	168	138
N+ P-	218	298	75	96	97	129	117
N+ Pn	147	311	111	223	103	217	157
N+ P+	139	256	225	406	120	216	146
L.S.D.	19	42	33	55	14	35	21

Table 10.8—*Results of the comparison experiment. The N- P- treatment was used as control. A zero in the table indicates the value was not statistically different from the control treatment. A plus means the value is statistically larger.*

Treatment	Nitrogen		Phosphorus		Potassium		Weight
	Conc.	Cont.	Conc.	Cont.	Conc.	Cont.	
N- P-	100	100	100	100	100	100	100
N- Pn	0	0	+	+	0	0	0
N- P+	0	0	+	+	+	0	0
Nn P-	+	+	0	0	0	0	0
Nn Pn	+	+	+	+	0	0	0
Nn P+	+	+	+	+	0	+	+
N+ P-	+	+	0	0	0	0	0
N+ Pn	+	+	0	+	0	+	+
N+ P+	+	+	+	+	+	+	+

values may be mathematically different, but statistically equal. This problem can be solved by subjecting the data to either a t-test or an analysis of variance before interpreting the vectors. Any means that were statistically equal would either not be used in the vector analysis or would mean no difference between treatments. Adding the statistical analysis adds complexity to the results. Tables 10.8 and 10.9 show the results of the comparison experiment after a statistical analysis. Some of the treatments have given clear, unambiguous results. Many have not. Unfortunately, doing the interpretation without statistically separating the means leads to even more questionable results. For example, in the N- Pn treatment, there

Table 10.9—*Results of the comparison experiment. The N- P- treatment was used as control. The vector letter refers to those listed in Figure 10.5 and Table 10.5. A zero indicates the treatment was not statistically different from control. A letter within parentheses indicates two of the variables were statistically different, but the third was not.*

Treatment	Nitrogen	Phosphorus	Potassium
N- P-	control	control	control
N- Pn	0	(C)	0
N- P+	0	(C)	0
Nn P-	(C)	0	0
Nn Pn	(C)	(C)	0
Nn P+	C	C	B
N+ P-	(C)	0	0
N+ Pn	C	(A)	(A)
N+ P+	C	C	C

was an increase in the concentration and content of N, P, K, and a decrease in weight (Table 10.7). This is interpreted as Vector E; adding phosphorus has caused toxicity. However, only the phosphorus values were statistically different from the N- P- means. This could be interpreted as nothing happened to the nitrogen content when phosphorus was added.

There is a practical solution to this problem; recognition of the fact that interpretation is never absolute. Simple statistics need to be used with this procedure and followed by a careful, reasoned interpretation of what happened to the plants. It is worth remembering the advantages of this method before dwelling on what may seem to be sizable problems. Critical concentrations do not have to be known and the results of the analysis are usually clear when used with statistics.

10.5.3 DRIS

The diagnosis and recommendation integrated system (DRIS) was conceived by Beaufils in the 1950's (Beaufils 1957). Originally called physiological diagnosis, it has primarily been used on agricultural crops like rubber and maize (Beaufils 1971, Beaufils 1973). Ideally DRIS uses all factors known to contribute to yield. However, DRIS can be effective with just a few factors being evaluated. The more factors that are evaluated the more effective the method becomes. To simplify the discussion, only mineral nutrients will be considered. Factors other than nutrients (e.g., water or light) could be have easily been used in DRIS. Several reviews of the DRIS method are available (Sumner 1978, Sumner 1982, Sumner and Farina 1986, Walworth and Sumner 1987, Walworth and Sumner 1988).

One of the fundamental principles behind DRIS is the evaluation of nutrient ratios. It is important that it be clear these ratios are not physiologically based. While there are physiologically important ratios (like calcium/potassium), DRIS ratios have no physiological base. This particular section is long and is divided into three subsections. First is a general introduction to the DRIS system and some of the underlying principles. Next is a subsection that deals with the calculation and application of DRIS indices and functions. This part may seem mathematically complex. However, these indices become less complex when a personal computer and a spreadsheet are used to do the arithmetic. Finally, a graphic method using DRIS charts is presented.

10.5.3.1 Introduction and principles of DRIS

One of the fundamental problems with nutrient analysis is a lack of a consistent correlation between nutrient content and yield. A re- examination of Figure 10.4 shows that the observations with the largest and smallest yields had the same nitrogen concentration. Surely this has a simple explanation. Most likely the small plant was deficient in another element, or had a disease, or damaged roots, or any one of number of possible problems. While all this may be true, they still had the same nitrogen concentration. Figure 10.8 is an illustration of what any nutrient analysis needs to accomplish. In this diagram the lowest yield is at the outside rim of the circle (arrow) and the greatest yield is in the middle at the intersection of the three circles. Yield can go from large to small when the level of any single nutrient is optimum. Two things happen when two nutrients are at optimum levels. First, yield is somewhat higher (intersection of two circles, like P + K). Second, the range of nutrient concentrations is smaller. Ultimately, when all three nutrients are optimum, the yield is maximized and falls within the N + K + P area of the figure. This is a restatement of the principle shown in Figure 10.3; the more nutrients that are optimum the greater the yield. DRIS is based on simultaneous analysis of several nutrients. At least three nutrients must be used for DRIS to work.

10.5.3.2 Calculation and application of DRIS indices

The DRIS system is based on a comparison between a high yielding population called the norm (control) and an experimental group. Figure 10.9 shows a cutoff between culls and usable plants which would in practice be determined by experience. This is nothing new to nursery managers. A cutoff like this is used every year when a forester asks for a minimum caliper or height. If a full range of heights were evaluated, a full range of nitrogen values would be found. Three facts can be seen in this illustration:

1) The tallest trees have to have close to the optimum amount of nitrogen. These trees do not have high or low levels of nitrogen (trees in the shaded areas).

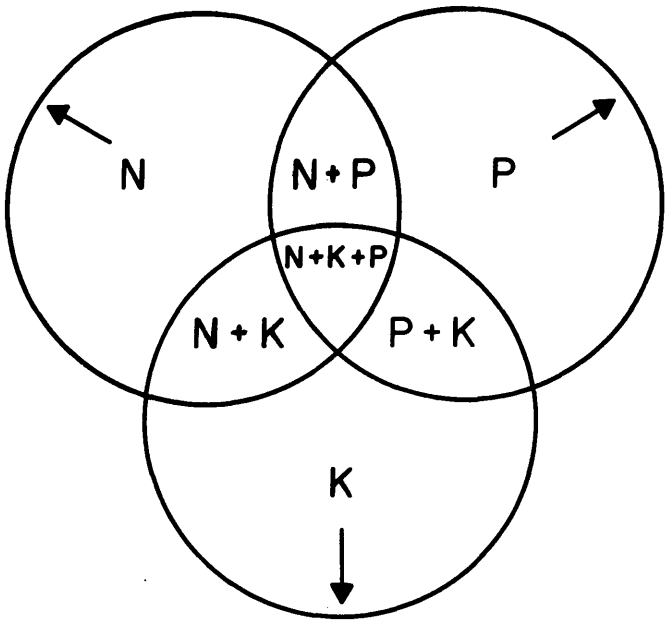

Figure 10.8—*Venn diagram illustrating the relationship between optimum yield and nutrient concentration. Arrows point to minimum yield for nitrogen, phosphorus, and potassium. Maximum yield is in the middle of the N + K + P area on the diagram. As more nutrients are present in optimum quantity, the yield is increased.*

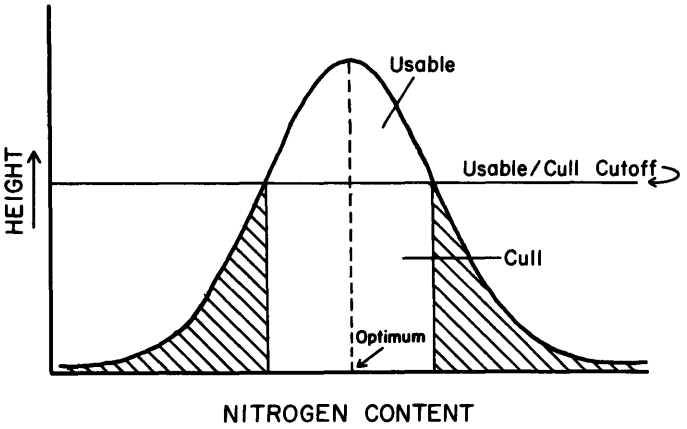

Figure 10.9—*Representation of how DRIS norms are derived. The usable/cull cutoff is determined by experience or prescription. Based solely on nitrogen content, plants in the unshaded area cannot be determined to be usable or cull. Plants in the tail areas of the curve represent those that are definitely culls by virtue of nitrogen content being too high (toxicity) or too low (deficiency).*

2) Short trees can also have the optimum amount of nitrogen because some other unknown factor has influenced growth. These trees can have high and low levels of nitrogen.

3) The plants in the tails of the curve (shaded area) can be eliminated solely on the basis of nitrogen being too low or too high. For each seedling represented by this illustration there were two values: nitrogen content and height. A normal curve results from a plot of these values if the sample size was large enough. It would be possible to treat the data as coming from two populations by cutting the curve in half at the usable/cull cutoff. Only the mean and variance for the usable group will be used in the calculation of the DRIS norm.

If the analysis were expanded to include phosphorus and potassium, there would be four values for each seedling (height, nitrogen, potassium, and phosphorus). As a first step in the application of the DRIS system the seedlings in the comparison or experimental group will be compared to the norm or control group. Rather than use N percent or P percent, DRIS uses the ratio of each pair of nutrients. Ratios such as N/P, P/K, etc., are calculated and their variances determined. Any one pair of nutrients such as nitrogen and phosphorus could be expressed in three different ways (N/P, P/N, or P times N). Which of the three expressions used is based on the ratio of the variances of between plants from the norm or high yielding sample to the experimental plants. The variance ratio calculation is done for each different expression of the ratio (Appendix Table A3). The expression with the highest variance is used in further calculations. This procedure gives greater separation between norm and treatment groups. In the case of the comparison experiment N/P had a ratio of 6.23, P/N was 3.24, and N*P was 0.20. Therefore, the ratio of N/P was chosen to be used in the analysis. Similarly K/N and K/P were picked for further use.

(**NOTE:** Throughout this section each step will be highlighted and numbered so that the process can be repeated without reviewing the text explanation. Detailed calculations are provided in Appendix Tables A2, A3, A4, and A5 at the end of the chapter.)

STEP 1: Establish the norm (control). Ideally this will be the best plant. It is recognized that best is a subjective term. In this experiment the Nn Pn treatments were chosen as the norm.
There are two ways to approach the DRIS norm. The ideal is to establish a norm for the species that is applicable in most situations. This approach requires a considerable amount of time and effort. However, once established this type of norm is very useful. A more short-term approach can be used. In this case one group of plants is simply assumed to be the norm. Remember the DRIS process is relative and the goal is to compare two or more treatments to each other. Once this concept is accepted, the relatively uncomfortable idea of it not being important which group is the norm (or control) becomes more palatable.

STEP 2: Calculate the variance ratio for each possible expression for each nutrient pair (N/P, P/N, P*N) (Appendix Tables A2 and A3).

STEP 3: Divide the variance of the comparison group (in this case N- P-) by the variance of the norm group. Determine which expression has the highest variance ratio (Appendix Table A3).
The determination of the expression form, variance ratios, and development of norms is the starting point for the calculation of DRIS indices. The expression with the highest variance ratio is used in the calculation of DRIS indices.

STEP 4: Determine DRIS functions using the function formula.
The first step in calculating the indices is the determination of the DRIS function for each pair of elements in the experiment. The mean of the ratio is used, not the variance. In the following equations the cv is the coefficient of variation for the norm (usable) population, n/p is the ratio of nitrogen to phosphorus for the norm population, and N/P is the ratio of nitrogen to phosphorus for the comparison population.

$$f(N/P) = \frac{N/P}{n/p} - 1 \quad \frac{1000}{cv} \quad \text{when } N/P > n/p$$

$$f(n/p) = 1 - \frac{n/p}{N/P} \quad \frac{1000}{cv} \quad \text{when } N/P < n/p$$

STEP 5: Determine the DRIS indices using the formulas.
The functions are combined in equations to used to calculate the indices. Indices for nitrogen, phosphorus, and potassium were needed for the comparison experiment.

$$N \text{ index} = \frac{f(N/P) - f(K/N)}{2}$$

$$P \text{ index} = \frac{f(N/P) - f(K/P)}{2}$$

$$K \text{ index} = \frac{f(K/N) + f(K/P)}{2}$$

In general, the indices can be determined for as many elements as were evaluated in the experiment. The DRIS functions are added and divided by the number of comparisons. The sign is minus if the element being evaluated appears in the denominator of the function and positive if

it is in the numerator. So N/P is positive in the N index, but is negative in the P index. The only unusual circumstance arises when the product (N*P) is used instead of some form of division (N/P). In this case the 1/P is redefined as a new element arbitrarily designated as Q. Then N*P = N/Q and the calculations are done as described above. When the Q index is determined the sign is changed and it becomes the P index. DRIS indices are unitless and represent relative abundance of nutrients in the plant.

STEP 6: Evaluate the indices.
An interesting extension of the method allows a comparison between mineral nutrients and the amount of C, H, and O that have been accumulated (Walworth and Sumner 1988). The authors caution this idea needs further support from experimental data. The concept is the relationship between dry matter and mineral nutrients is what defines deficiency. If there is too little nitrogen relative to the amount of tissue produced, then nitrogen would be considered deficient. The three expressions to be evaluated are nutrient divided by dry matter (N/DM = N%), dry matter divided by nutrient (DM/N = 1/N%), and nutrient times dry matter (N*DM). DRIS indices are then calculated and placed in ascending order. Any nutrient index that has a more negative value than the dry matter index is considered deficient.

Results of the comparison experiment are shown is Table 10.10 and summarized in Tables 10.11 and 10.12. All nutrients with a DRIS index lower than dry matter were considered deficient. In the N- P- treatment, both nitrogen and phosphorus were deficient. Adding phosphorus in the N- Pn and N- P+ treatments left only nitrogen deficient. In the N+ P- and N+ Pn treatments only phosphorus was deficient. Similar evaluations can be made for the other treatments. A close examination of Table 10.10 shows that as a deficiency is removed the index values become more positive. Thus the DRIS index is an indicator of the magnitude of the deficiency. For example, the phosphorus values for the N- P-, N- Pn, N- P+ treatments were -13, 27, and 92. When these values were used in a simple linear regression with the amount of phosphorus supplied in each treatment (P- = 1.1, Pn = 3.25, P+ = 9.75, Table 10.3), the r^2 value was 0.98. Similar values for the coefficient of determination can be obtained by using data from the literature. Table 10.3 in van den Driessche's (1989) paper on nutrient deficiency lists values for plant N percent, P percent, K percent, dry weight, and level of nutrient supplied (among other things). Because variance was not listed, an arbitrary coefficient of variation of 20 percent was used in the calculation of DRIS indices. When the resulting indices were correlated to the amount of nutrient supplied, the r^2 values were 0.98 for nitrogen, 0.87 for phosphorus, and 1.00 for potassium.

Like many procedures, the advantages and disadvantages of the DRIS method are reflections of one another. The advantages are the results are easy to interpret, variation within the sample is considered, and the results are quantitative. The disadvantages are the amount of calculation required, larger sample sizes are needed, and the calibration of the norm or control group.

DRIS indices are easy to interpret. This is particularly true when the dry matter index is included. More deficient nutrients have a larger, more negative index. Including the variance in the calculation of a DRIS index helps solve the problem of what is statistically valid.

Unlike other methods the results are quantitative and related to the relative abundance of the nutrient in the tissue. This holds true until the nutrient reaches the luxury consumption range. In the comparison experiment the nitrogen levels (N-, Nn and N+) and DRIS indices were compared within each phosphorus treatment. Within the P- treatment the r^2 value for nitrogen level and DRIS index was 0.91. As nitrogen level increased, the DRIS index became more positive. For nitrogen levels within the Pn treatment the r^2 value was 0.67 and was 0.52 for the P+ treatment. The indices showed less change once the plants reached luxury consumption levels of nitrogen. This was most noticeable between the Nn and N+ treatments. A threefold addition of nitrogen did not show a similar change in the DRIS index.

The amount of calculation required may seem the greatest disadvantage. However, the calculations are quick and relatively easy to do using a personal computer and a spreadsheet. Once the spreadsheet is completed, it can be used for other analyses. It is better to do two spreadsheets. One is used for the calculation of the variance for each nutrient expression and one for the calculation of the DRIS indices.

An examination of the DRIS function equations shows that the coefficient of variation is used as a divisor. Because DRIS uses variance in this calculation, larger sample sizes may be required. It is difficult to argue this as a disadvantage. Larger sample sizes virtually always mean more accurate, precise estimates of populations values. From an economic point of view it may be a disadvantage, but from a scientific point of view it is not.

The most frequent criticism of DRIS concerns establishing DRIS norms. From a practical view this is not a problem. DRIS norms can be used as species standards or as a comparison in an experiment. DRIS norms can be established and used as benchmarks against which all other crops of the same species are evaluated. DRIS norms have been established for many crops, including: maize, soybeans, sorghum, potatoes, wheat, rubber, sugarcane, sunflower, alfalfa (Letzsch and Sumner 1983), Populus deltoides

Table 10.10—*Nutrient concentrations, DRIS indices, and yields for the comparison experiment. Nn Pn was arbitrarily chosen as the norm or control treatment. DRIS indices were calculated using the expressions with the highest variance ratios shown in Appendix Table A3.*

Treatment	Tot. DW.	N%	P%	K%	Indices N	P	K	DM
N- P-	1.57	1.14	0.09	0.78	-19	-13	16	16
N- Pn	1.44	1.34	0.20	0.86	-27	27	6	-6
N- P+	1.45	1.27	0.38	0.94	-65	92	-1	-25
Nn P-	1.74	1.77	0.07	0.84	21	-44	18	6
Nn Pn	1.71	1.61	0.13	0.75	0	0	0	0
Nn P+	2.16	1.48	0.21	0.76	-18	30	-6	-6
N+ P-	1.84	2.48	0.07	0.75	57	-56	2	-3
N+ Pn	2.46	1.68	0.10	0.80	7	-16	7	1
N+ P+	2.28	1.59	0.21	0.93	-16	24	7	-14

Table 10.11—*Factors evaluated in the comparison experiment listed in ascending order for each treatment. DRIS indices in Table 10.10 were used to determine rankings. Nn Pn was arbitrarily chosen as the norm or control treatment.*

Treatment	Ranking
N- P-	N < P < DM = K
N- Pn	N < DM < K < P
N- P+	N < DM < K < P
Nn P-	P < DM < K < N
Nn Pn	CONTROL OR NORM
Nn P+	N < DM = K < P
N+ P-	P < DM < K < N
N+ Pn	P < DM < K = N
N+ P+	N < DM < K < P

Table 10.12—*DRIS indices and nutrient ranking for the comparison experiment. In this comparison, the group with the highest total dry weight (N+ Pn) was designated as the norm or control group.*

Treatment	DRIS Indices N	P	K	DM	Ranking
N- P-	-28	8	9	12	N < P < K < DM
N- Pn	-47	68	-7	-14	N < DM < K < P
N- P+	-110	182	-26	-46	N < DM < K < P
Nn P-	9	-19	8	2	P < DM < K < N
Nn Pn	-9	21	-10	-3	K < N < DM < P
Nn P+	-36	73	-22	-15	N < K < DM < P
N+ P-	45	-35	-8	-2	P < K < DM < N
N+ Pn	0	0	0	0	CONTROL OR NORM
N+ P+	-33	61	-6	-21	N < DM < K < P

(Leech and Kim 1981, Kim and Leech 1986), and *Pinus radiata* (Svenson and Kimberly 1988). Some of these norms have been established using worldwide databanks and are remarkably uniform throughout the world. While the developing this sort of norm is an admirable goal, it is not required for the use of DRIS on a individual nursery basis. However, if the experience gained with other crops is an indication of what could be expected with trees, then the norms could be established fairly quickly.

At the beginning of the comparison experiment it was decided to use the Nn Pn plants as the control or norm. These plants did not produce the greatest dry matter. An examination of the dry weight data in Table 10.10 shows that while Nn Pn treatment averaged 1.71 grams, the N+ Pn treatment averaged 2.46 grams. Table 10.12 was done after the DRIS norm was changed from Nn Pn to N+ Pn. A comparison of the relative rankings of the data in Tables 10.11 and 10.12 shows that very few were altered by this change. None of the changes are substantial enough to have caused a change in the prescription to correct deficiencies. The major change that redesignating the norm group brings about is a comparison of the previous norm group becomes possible (see Nn Pn data in Tables 10.11 and 10.12).

10.5.3.3 Construction and application of DRIS charts

The mathematical approach in the preceding section is more complicated than many people would like. The DRIS chart is a simpler, less accurate, alternative method that is somewhat easier to develop. This alternative is successful if no more than three or four factors are being evaluated. Beyond that the charts become difficult to read. More than four factors will require the use of DRIS indices.

DRIS charts consist of an axis for each nutrient ratio and two concentric circles (Figure 10.10). The diameter of the inner circle is set at the mean plus and minus 4/3 times the standard deviation of the norm or control group. Likewise the outer circle diameter is mean plus and minus 8/3 standard deviation (Table 10.13). Plants in a treatment are considered to have balanced nutrition if the ratio falls within the inner circle. If the ratio falls between the two circles there is a moderate imbalance and beyond the outer circle is considered to indicate marked imbalance (Walworth and Sumner 1987). When conflicting answers are obtained in two subsections it is considered to indicate a slight to moderate imbalance. By convention only insufficiencies are recorded during the analysis. The rationale for this convention is that in terms of balance, a deficiency in one element corresponds to an excess of the other element in the ratio. A comparison of the N- P- treatment (Table 10.14) to the graph in Figure 10.10 would be done as follows:

1) The ratio of N/P was 12.67 which is within the inner circle (N→ P→).

2) K/N was 0.68 which is between the two circles (N↘ K↗).

3) For K/P the ratio was 8.67 which is also between the two circles (P↘ K↗). At this point there should be an arrow by each element. If this is not the case, then a horizontal arrow is placed next to the element without an arrow. At the end of this analysis, the individual steps are added together. If a horizontal and a diagonal or vertical arrow are shown for a given element, then the horizontal arrow is discarded. If there are two vertical or diagonal arrows, they are retained to indicate greater magnitude. For the above analysis, N has→↘ arrows, P has→↘ arrows and K has ↗↗ arrows. The summary statement would be N↘ P↘ K↗.

4) The interpretation for this treatment is N = P < K. Nitrogen and phosphorus are more limiting than potassium.

This does not mean that nitrogen and phosphorus are certain to be deficient. Rather this gives a relative ranking for the nutrients in the study, the answer being that nitrogen and phosphorus are more limiting than is potassium. More extensive discussions on the preparation of DRIS charts can be found in Sumner (1982) and Walworth and Sumner (1987).

DRIS charts have the advantage of being easily prepared and quickly interpreted. There are three disadvantages. If the sample size is small, the standard deviation will tend to be large and most of the values will fall within the inner circle. Although there may be some indication of relative abundance, the information will be less useful than that from the DRIS indices. Second, the method is not quantitative. The rankings are strictly relative and do not indicate more than general magnitude of deficiency.

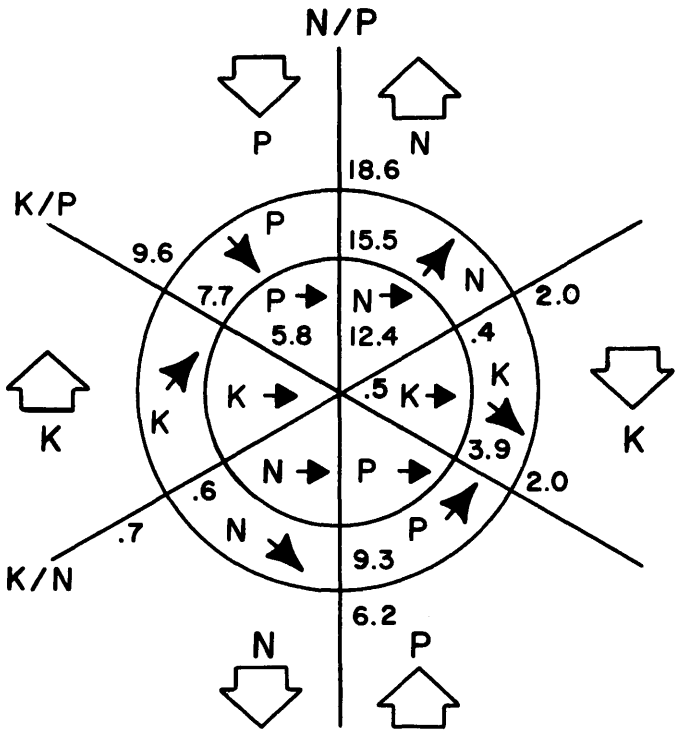

Figure 10.10—*DRIS chart for qualitative determination of nitrogen, phosphorus and potassium requirements of plants in the comparison experiment (see Table 10.13). Seedlings in the Nn Pn treatment were used as norms. Values for the N/P line as displayed from top to bottom were 18.6, 15.5, 12.4, 9.3, and 6.2. The mean value for the Nn Pn treatment was 12.4. Values greater than 9.3 and less than 15.5 (inner circle) were considered normal. Values less than 9.3 and greater than 6.2, or greater than 15.5 and less than 18.6 (area between the two circles) represented a moderate imbalance in the N/P ratio. Values greater than 18.6 or less than 6.2 represented a marked imbalance in the N/P ratio. Similar interpretations can be done with K/P and K/N. (Adapted from Sumner 1982.)*

Table 10.13—*Means, standard deviations, and circle diameter sizes used to draw DRIS chart using Nn Pn as the control or norm (see Figure 10.10). Circle diameters were set at the mean plus and minus 4/3 standard deviation for the inner circle and mean plus and minus 8/3 standard deviation for the outer circle.*

			Inner circle		Outer circle	
Ratio	Mean	s	+	-	+	-
N/P	12.38	2.33	15.5	9.3	18.6	6.2
K/N	0.47	0.09	0.6	0.4	0.7	0.2
K/P	5.77	1.43	7.7	3.9	9.6	2.0

Table 10.14—*Values for nutrient ratios for each of the treatments in the comparison experiment.*

Treatment	N/P	K/N	K/P
N- P-	12.67	0.68	8.67
N- Pn	6.70	0.64	4.30
N- P+	3.34	0.74	2.47
Nn P-	25.29	0.48	12.00
Nn Pn	12.38	0.47	5.77
Nn P+	7.05	0.51	3.62
N+ P-	35.43	0.30	10.71
N+ Pn	16.80	0.48	8.00
N+ P+	7.57	0.59	4.43

In contrast, the absolute size of a DRIS index is a good indication of how abundant or lacking a nutrient is within a given system. Finally, the means and standard deviations still have to be calculated. The hard work has been done. If a spreadsheet is being used to analyze the experiment, then the calculation of the indices is faster than the construction of the DRIS chart.

10.6 Conclusions

Both vector analysis and DRIS are improvements over the use of critical nutrient concentrations. Both simplify the process of gathering and interpreting the information. Either method gives a clear, unambiguous answer to the question, "What is wrong with my trees?" The authors of this paper prefer DRIS because of the more quantitative nature of the information. It is very likely that others will dislike DRIS for exactly the same reason.

The real problem with nutrient analysis has not been brought up and will not (cannot) be answered in this paper. Vector analysis and DRIS both require a control against which other trees can be evaluated. Analysis is a simple problem. The real challenge is in defining the perfect tree which is to serve as the control. Table 10.15 illustrates the problem. This is the result of a DRIS analysis that used the treatment with maximum root growth capacity as the norm. A comparison of Table 10.15 to Tables 10.11 and 10.12 highlights the problem. In Tables 10.11 and 10.12, nitrogen is shown as the most deficient nutrient five out of eight times. In contrast, phosphorus is shown as most deficient five out of eight times in Table 10.15. Note the effect of phosphorus on root growth capacity. Within each nitrogen grouping as phosphorus increases, so does root growth capacity. This would seem to indicate that if the goal is greater root growth, then more phosphorus needs to be added. In contrast, if maximum biomass is the goal, then more nitrogen will be required.

Defining the perfect tree is a rubber cookie question. The perfect tree is conditional. Preparing a tree for some field conditions might require a high root growth capacity, or height, or caliper, or frost tolerance, or—? Most of these goals are conflicting. The nutrient prescription for one goal will not meet another goal. Furthermore, the prescription will differ by nursery and species. It seems likely that the perfect tree will be defined as a combination of goals. The norm against which other trees are compared will reflect this combination. A procedure like DRIS or vector analysis would work as well with an arbitrary tree score. This score might be defined as 40 percent height, 30 percent caliper, 20 percent root growth capacity, and 10 percent frost tolerance. A tree score would be adaptable to different nurseries and field conditions. Using the principles of nutrient analysis can help reach the goals implicit in the definition of the perfect tree.

The authors gratefully acknowledge the contributions of Professors Malcom Sumner and Vic Timmer. Discussions with each person were helpful in understanding methods and principles.

Table 10.15—*DRIS indices and nutrient ranking for the comparison experiment. The group with the highest root growth capacity (N+ P+) was chosen as the norm or control group. RGC 4 is the root growth capacity (cm) measured after four weeks growing time.*

	DRIS Indices					
Treatment	N	P	K	DM	Ranking	RGC 4
N- P-	-8	-39	17	29	P < N < K < DM	29
N- Pn	-10	2	1	7	N < K < P < DM	81
N- P+	-31	42	-7	-4	N < K < DM < P	95
Nn P-	36	-77	21	21	P < DM = K < N	47
Nn Pn	16	-21	-8	13	P < K < DM < N	101
Nn P+	1	6	-14	7	K < N < P < DM	160
N+ P-	88	-96	-6	15	P < K < DM < N	43
N+ Pn	24	-46	5	16	P < K < DM < N	128
N+ P+	0	0	0	0	CONTROL OR NORM	165

Appendix Table A1—*Results of the comparison experiment used in the construction of Figure 10.7. Content was determined by evaluating the nitrogen content of the leaves on the plant. Concentration is the percent nutrient contained in all needles. The lower fertility treatment was considered the control and had a relative value of 100 % concentration and 100% content. The relative values for the N+ Pn treatment were calculated by dividing the N+ Pn value by the N-P- value and multiplying by 100. For example, relative N content was 14.93 / 4.80 * 100 = 311, which means the nitrogen content of the N+ Pn plants was 311% of N- P- plants. The total dry weight of the N- P- plants was 1.57 g and that of the N+ Pn plants was 2.46. This gives a relative plant weight value of 157 for the N+ Pn treatment (see Figure 10.7).*

	N- P-		N+ Pn	
	Concentration	Content	Concentration	Content
Nitrogen	1.14	4.80	1.68	14.93
Phosphorus	0.09	0.39	0.10	0.87
Potassium	0.78	3.33	0.80	7.24
Relative N	100	100	147	311
Relative P	100	100	111	223
Relative K	100	100	103	217

Appendix Table A2—*Raw data, treatment means and standard deviations (s.d.) for N%, P% and K% from the December harvest of the comparison experiment. Only the N- P- and Nn Pn treatments are listed.*

	N- P-			Nn Pn		
	N%	P%	K%	N%	P%	K%
	1.11	0.10	0.67	1.55	0.10	0.75
	1.17	0.14	0.83	1.35	0.12	0.87
	1.00	0.10	0.92	1.77	0.13	0.60
	1.00	0.11	0.64	1.35	0.09	0.75
	0.99	0.10	1.02	1.55	0.14	0.87
	0.91	0.08	0.81	1.59	0.17	0.74
	1.24	0.08	0.63	2.01	0.17	0.85
	1.75	0.07	0.86	1.64	0.11	0.68
	1.06	0.05	0.67	1.73	0.13	0.67
	1.20	0.08	0.71	1.53	0.09	0.69
mean	1.14	0.09	0.78	1.61	0.13	0.75
s.d.	0.238	0.025	0.133	0.197	0.029	0.092

Appendix Table A3—*Determination of variance ratios for the different ways of expressing DRIS ratios. Data is for the December harvest of the comparison experiment and only the N- P- and Nn Pn treatments have been used. The variance ratio is calculated by squaring the standard deviation (s.d.) for each treatment and then dividing. Expressions with the highest variance ratios are marked with an asterisk. All expressions are in concentration (percent), which are derived by dividing nutrient content by dry weight.*

Form of	N- P-		Nn Pn		Variance Ratio
expression	mean	s.d.	mean	s.d.	(N- P-)/(Nn Pn)
N	1.14	0.238	1.61	0.197	1.46
1/N	0.88	0.148	0.62	0.076	3.79*
N*DW	1.97	0.351	2.59	0.330	1.31
P	0.09	0.025	0.13	0.029	0.74
1/P	11.11	4.116	7.69	1.910	4.64*
P*DW	0.12	0.033	0.16	0.039	0.716
K	0.78	0.133	0.75	0.092	2.09*
1/K	1.28	0.215	1.33	0.173	1.54
K*DW	1.02	0.181	0.95	0.142	1.63
N/P	12.67	5.815	12.38	2.329	6.23*
P/N	0.08	0.027	0.08	0.015	3.24
NP	0.10	0.030	0.21	0.067	0.20
P/K	0.12	0.035	0.17	0.040	0.77
K/P	8.67	2.826	5.77	1.438	3.86*
PK	0.07	0.026	0.10	0.028	0.86
N/K	1.46	0.361	2.15	0.420	0.74
K/N	0.68	0.184	0.47	0.093	3.91*
NK	0.89	0.25	01.21	0.207	1.46

Appendix Table A4—*Calculation of DRIS functions for the comparison experiment. In the following equations, N/P is the nitrogen/phosphorus ratio in the N- P- treatment; n/p is the nitrogen/phosphorus ratio in the Nn Pn treatment, and cv is the coefficient of variation for the Nn Pn treatment. By convention, the treatment being used as the DRIS norm (control) is denoted by lowercase letters. The cv was calculated by dividing the standard deviation by the mean and multiplying by 100. Means and standard deviations are from Appendix Table A2.*

DRIS functions are calculated by the formula:

$$f(N/P) = \frac{N/P}{n/p} - \frac{1000}{cv}$$ when N/P is greater than n/p and by:

$$f(N/P) = 1 - \frac{n/p}{N/P}\,\frac{1000}{cv}$$ when n/p is greater than N/P.

Other nutrient ratios are done in the same manner.

N/P (cv) = 2.329/12.38 * 100 = 18.81
K/P (cv) = 1.438/5.77 * 100 = 24.92
K/N (cv) = 0.093/0.47 * 100 = 19.79
1/N (cv) = 0.076/0.62 * 100 = 12.26
1/P (cv) = 1.910/7.69 * 100 = 24.84
K (cv) = 0.092/0.75 * 100 = 12.27

f (N/P) = ((12.67 / 12.38) - 1) * 1000 / 18.81 = 1.2
f (K/P) = ((8.67 / 5.77) - 1) * 1000 / 24.92 = 20.2
f (K/N) = ((0.68 / 0.47) - 1) * 1000 / 19.79 = 22.6
f (1/N) = ((0.88 / 0.62) - 1) * 1000 / 12.26 = 34.2
f (1/P) = ((11.11 / 7.69) - 1) * 1000 / 24.84 = 17.9
f (K) = ((0.78 / 0.75) - 1) * 1000 / 12.27 = 3.3

Appendix Table A5—*Calculation of DRIS indices for the comparison experiment. A DRIS index is calculated by adding all of the functions that contain the element being evaluated and dividing by the number of functions used. The N index was calculated by adding f (N/P), f (K/N) and f (1/N%) and dividing by 3. By convention, a minus sign is given to the function if the element being evaluated appears in the bottom of the ratio fraction. Values of DRIS functions are from Appendix Table A3.*

$$\text{N index} = \frac{f(N/P) - f(K/N) - f(1/N\%)}{3} = \frac{1.2 - 22.6 - 34.2}{3} = -19$$

$$\text{P index} = \frac{-f(N/P) - f(K/P) - f(1/P\%)}{3} = \frac{-1.2 - 20.2 - 17.9}{3} = -13$$

$$\text{K index} = \frac{f(K/P) + f(K/N) + f(K\%)}{3} = \frac{20.2 + 22.6 + 3.3}{3} = 16$$

$$\text{DM index} = \frac{f(1/N\%) + f(1/P\%) - f(K\%)}{3} = \frac{34.2 + 17.9 - 3.3}{3} = 16$$

Any element with an index more negative than the Dry Matter (DM) index would be considered to be deficient. In the above comparison (N- P- to Nn Pn), nitrogen was most limiting. Phosphorus was nearly as deficient, while potassium was not limiting.

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General Papers

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Comparison of the Summit Precision Seeder with the Oyjord Seeder[1]

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Abstract.--The Summit Precision Seeder was compared with the Oyjord Seeder at five Forest Service nurseries, using nine conifer species and 26 seedlots. Results varied with seed characteristics, but the Summit did not prove to be a big improvement over the Oyjord. Plots oversown and thinned to target spacings produced less clumping and fewer gaps between seedlings but showed little improvement in seedling size and cull rates over either seeder.

BACKGROUND

Uniform spacing between seedlings is important to the successful and efficient operation of forest tree nurseries. The value of tree seed continues to increase due to dwindling amounts of some seed sources, growth of genetically improved seed, and an increased emphasis on nondestructive seed collection from high quality stands and trees. In the nursery, discarding cull seedlings is becoming more and more costly. Through more uniform spacing of trees, nursery managers hope to better utilize precious seed supplies, reduce the proportion of nonshippable trees, and produce a more uniform seedling size that could possibly improve field performance.

To obtain more uniform spacings between seedlings in the nursery bed, we must achieve a more precise and consistent placement of the seed during sowing.

Most of the Forest Service, U.S. Department of Agriculture, nurseries in the western United States sow seed with a Love/Oyjord seeder (fig. 1). The Oyjord seeder was developed in Norway in the early 1970s. It was tested by the Forest Service's Equipment Development Center at Missoula, MT, in 1975. Lott and Lowman (1976 and 1978) and Lott and Casavan (1978) reported that the Oyjord was simple, reliable, well designed and constructed, and versatile.

Although Lott and Lowman (1976 and 1978) found the Oyjord seeder to be clearly the most accurate of eight seeders tested at that time, the Oyjord is not a precision seeder. Average seed spacings were close to targets, but the actual placement of the seed was random and therefore not evenly spaced. Boyer and others (1985) said that the Oyjord gave a high proportion of doubles, had variable spacing, and varied in the number of seeds per 0.6 m row. However, it gave narrow drills to facilitate lateral root pruning, was easy to calibrate, is capable of sowing seed lots with low germination rates, and has a higher operation speed than other seeders.

The Summit Precision Seeder (fig. 2) was designed and manufactured in New Zealand. Lafleur (1987), Boyer and others (1985), and Huber (1985) have all tested the precision seeder. The Summit Seeder performed well but still did not achieve perfect spacing. The Summit Seeder also gives narrow drills but is easier to calibrate than the Oyjord, does not waste seed at the ends of the seedbeds, and provides control of seed depth. Disadvantages include its slow speed, the necessity for high germination seed, and its price.

The Summit Precision Seeder works using a vacuum sowing head, which places seeds individually in seven rows. It works especially well with large seeded species. Machine travel speed is important to the accuracy of the Summit. As speed increases, accuracy decreases. Seed

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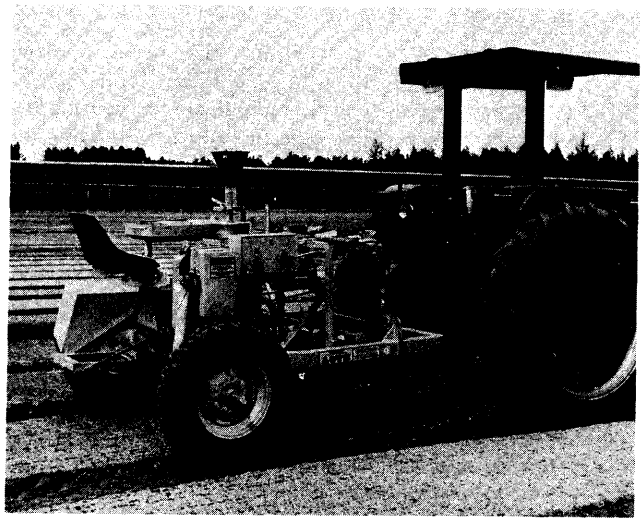

Figure 1.--The Oyjord Seeder.

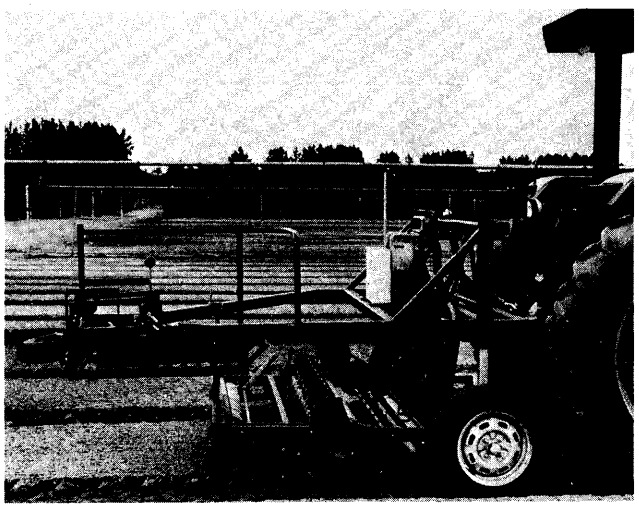

Figure 2.--The Summit Precision Seeder.

spacing is controlled by changing drive sprockets, so the closer the desired spacing, the slower the seeder will move to do its task properly.

The primary objective of this study was to compare seed placement by the Summit Precision Seeder with that of the Oyjord seeder. We also wanted to compare seedlings sown by the two seeding machines with seedlings grown at the same density but at precise spacing. This was done at five Forest Service nurseries, each with different soils and weather conditions. We used seed of nine species. Our interest was in seed delivery performance, seedling emergence and the uniformity of seedling spacing in relation to seed placement, the resulting seedling morphological characteristics, and the number of shippable trees per unit area of bed.

MATERIALS AND METHODS

Sowing of all 26 seedlots chosen for this study took place in the spring of 1985. The five Forest Service nurseries which participated were Coeur d'Alene Nursery, Coeur d'Alene, ID; Lucky Peak Nursery, Boise, ID; J. Herbert Stone Nursery, Central Point, OR; J. W. Toumey Nursery, Watersmeet, MI; and Wind River Nursery, Carson, WA. The study was a cooperative effort between the nurseries, the Missoula Equipment Development Center, and the U.S. Intermountain Research Station, Forest Service.

The Summit Precision Seeder was calibrated to sow at the same rate as each nursery's target rate for the Oyjord seeder for a given seedlot. Seeds were covered with aluminum powder before sowing to make them readily visible and facilitate smooth flow through the seeder.

We tested the seeders using seed from nine conifer species. Douglas-fir (Pseudotsuga menziesii var. menziesii and var. glauca [Beissn.] Franco) (coastal and inland varieties) was sown at three nurseries. Engelmann spruce (Picea engelmannii Parry) and ponderosa pine (Pinus ponderosa Dougl. ex Laws) were each sown at two nurseries. White spruce (Picea glauca [Moench] Voss.), noble fir (Abies procera Rehder), jack pine (Pinus banksiana Lamb.), lodgepole pine (Pinus contorta var. latifolia Engelm.), red pine (Pinus resinosa Ait.), and western larch (Larix occidentalis Nutt.) were each sown at one nursery (table 1).

Four species were sown at Lucky Peak Nursery, three at Coeur d'Alene and J. W. Toumey, two at J. Herbert Stone, and only noble fir at Wind River (table 1).

Each species at a nursery was treated as a separate test. Within each test, two seedlots were sown. The first had a germination rate near 95 percent; the second had a germination rate between 75 and 85 percent. Within each of three blocks, six plots were arranged randomly. The six plots consisted of three treatments replicated two times for both seedlots: (1) seed sown using the Summit Precision seeder calibrated to the target spacing, (2) seed sown using the Oyjord seeder calibrated to the target spacing, and (3) seed oversown using the Oyjord seeder and hand thinned to the target spacing after seedling emergence.

All plots were six m long and a 1-m buffer separated the plots. Within each plot there were three 0.5 m sample plots. After sowing, a meter stick was laid along side each row in a sample plot, and the position of every seed was recorded in millimeters. As soon as the measurements were made, the seed was covered.

Five weeks after sowing, the oversown plots were hand thinned to the target spacing. Afterward, actual positions of the seedlings were measured on all sample plots.

At three of the nurseries, several measurements were taken at lifting time. Each sample plot was measured separately, and all were graded and counted by an experienced grader. Grading specifications were set by nursery

Table 1.-- Actual and target seed densities for the Summit and Oyjord Seeders using nine tree species at five nurseries. Significant differences are indicated by "**" (α = 0.1) and "*" (α = 0.5). "NS" indicates no significant difference.

Species	Nursery	Seeds per foot of row			Significance
		Summit	Oyjord	Target	
		---(seeds/ft of row)---			
Douglas-fir	CDA	19.8	18.9	20.6	ns
	LPN	14.5	25.8	22.9	**
	JHS	12.9	12.8	17.5	ns
Engelmann spruce	CDA	16.2	20.9	20.6	**
	LPN	18.0	23.3	21.9	**
White spruce	JWT	29.4	22.8	29.4	*
Noble fir	WRN	11.7	24.2	21.9	**
Jack pine	JWT	21.9	14.0	18.8	**
Lodgepole pine	LPN	14.4	18.0	19.9	**
Ponderosa pine	LPN	11.6	18.9	18.1	**
	JHS	11.4	11.6	16.2	ns
Red pine	JWT	20.4	14.3	17.9	**
Western larch	CDA	22.6	22.0	20.6	ns

Nursery coding: CDA - Coeur d'Alene JHS - J. Herbert Stone
JWT - J.W. Toumey - LPN - Lucky Peak
WRN - Wind River

Table 2.--Percentage of target seed densities obtained using the Summit Precision Seeder and the Oyjord Seeder. Tests are grouped by Nursery: Coeur d'Alene (CDA), Lucky Peak (LPN), J. Herbert Stone (JHS), J. W. Toumey (JWT), and Wind River (WRN). Species code: Douglas-fir (DF), Englemann Spruce (ES), western larch (WL), lodgepole pine (LP), ponderosa pine (PP), white spruce (WS), jack pine (JP), red pine (RP), and noble fir (NF). Significant differences are indicated by "**" (α = 0.1) and "*" (α = 0.1). "NS" indicates no significant differences.

Nursery	Species	Summit	Oyjord	Significant
		(% of target)	(% of target)	
CDA	DF[1]	96	92	ns
	ES	79	101	ns
	WL	110	107	ns
LPN	DF[1]	63	113	**
	ES	82	106	**
	LP	72	90	**
	PP	64	104	**
JHS	DF	74	73	ns
	PP	70	72	ns
JWT	WS	100	78	*
	JP	116	74	**
	RP	114	80	**
WRN	NF	53	111	**

[1] Inland variety of Douglas-fir.

personnel and were unique for each species and nursery. For all sample plots, we measured total number of trees lifted, number of trees meeting specifications, and number of nonshippable trees. From each sample plot, 10 trees were randomly selected for morphological measurements: top height, stem caliper, top dry weight, and root dry weight.

RESULTS AND DISCUSSION

Data summaries and analyses are presented in tables 1 through 6. Within some of the tests mean values appear to show obvious differences. However, in a few cases the variation in the data is so great that means that are visually different are not statistically different.

Seed Placement

Seeding Rates

Overall, both machines sowed less seed than the target rates. The Oyjord was under by an average of 1.5 seeds per foot of row, and the Summit was under the target by an average of 3.4 seeds when we combine all the data from all the nurseries (table 1).

When evaluated by the average number of seeds per foot of row, the Oyjord seeder was more consistent than the Summit seeder. Of the 13 tests sown, the Oyjord was within 20 percent of the target in nine of them. The Summit seeder was within 20 percent of the target in only six tests. However, the number of seeds per foot of row, expressed as a percentage of the target, was inconsistent. In fact, table 2 shows that there is more consistency within the nurseries than within species. At Coeur d' Alene, both machines sowed close to the target. At J. Herbert Stone, both machines sowed about the same but were almost 30 percent below target. At Lucky Peak and Wind River, the Oyjord sowed close to the target but,the Summit sowed significantly fewer seeds. At J.W. Toumey, the Summit sowed close to the target and the Oyjord significantly fewer.

Reasons for the differences were not identified, but we suspected several factors. The Summit's accuracy is speed sensitive, and some of the tractors were more difficult to regulate than others. Though speed is not as important for the Oyjord, the machine must be properly calibrated and set up. Where both seeders delivered the same number of seeds but missed the targets, the seed calculations or weight measurements may have been incorrect.

Seed Deltas

A Seed delta is a calculation used by the manufacturers of seed sowers to evaluate seeder operation. It measures the consistency of sowing rates between rows for a length of seedbed. The seed delta is figured through a simple equation:

$$S.D. = \frac{\text{Maximum seeds/row - Minimum seeds/row}}{\text{Mean seeds per row}} \times 100$$

The only information required is the actual seed delivery rates for each row of the seedbed for a given length, in this case the 0.5-m sample plots. The higher the variability in number of seeds delivered between sample rows, the higher the seed delta.

From table 3 we see that seed deltas varied greatly between species and nurseries. In only five of 13 tests were there significant differences between the two seeders (α = 0.05). Twice, the Summit Precision Seeder had a higher mean seed delta. The Oyjord produced a higher mean seed delta in three tests. This would suggest that there is little difference between the consistency of the seeders.

Seed Spacing

Definitions of important seed spacing terms are:

Doubles - seeds (or seedlings) closer than half the average spacing.

Blanks - seeds (or seedlings) spaced greater than 1.5 times the average spacing.

Singles - between seed (or between seedling) distances of 0.5 to 1.5 times the average spacing.

Seed Deltas-calculations used to evaluate consistency between rows.

Average number of seeds can be misleading. More helpful, table 3 summarizes the seed spacing figures for the two seeders. Rows sown with the Oyjord seeder had more doubles, seeds closer than half the average spacing, in seven out of 13 tests. However, in two of the three tests at the J. W. Toumey Nursery we found opposite results. Here, the Summit seeder produced more doubles. There was no difference in doubles at the J. Herbert Stone Nursery and in one test each at the J. W. Toumey and Coeur d'Alene.

The blank space results were similar to the "doubles" analysis (table 3).

The most important measurement of seed placement is the mean number of seeds that met the target spacing. Neither the Summit Precision Seeder nor the Oyjord Seeder is consistently more accurate in placing single seed in these tests (table 3).

Seed Characteristics

We observed some relationships between seed characteristics and the performance of the seeders. The Summit Precision Seeder seemed to show improvement over the Oyjord when large, symmetrical, and rounded seeds were sown. When small or angled seed was used, the Summit sower had problems in placing one seed at a time consistently.

Of the seeds tested in this study, the spruces were the smallest and white spruce was much smaller than the moderately sized Engelmann spruce. Black spruce seed at the J.W. Toumey Nursery was so small that it could not be successfully sown by the Summit Precision Seeder. The western larch was also small. Ponderosa pine seed was the largest, and for that reason produced the best spacing. Lodgepole pine seed was smaller than the ponderosa, red pine was smaller yet, and jack pine had the littlest seed of the pine species. Douglas-fir seed was large but variable in shape. The coastal variety planted at the J. Herbert Stone Nursery was much more triangular in shape than the more rounded seed of the inland variety at Coeur d' Alene and Lucky Peak. The angular shape caused the Oyjord to perform better than the Summit. Noble fir seed was large, but it was also resinous, sticky, and often had pieces of

Table 3.--Seed spacing measurements on plots sown by the Summit Precision and the Oyjord Seeders. See text for definitions of blank spaces, double seeds, single seeds, and seed deltas. Values separated by a * indicate a significant difference between seeders at α = .05. ** indicates α = .01. NS indicates not statistically different at the 95 percent level of confidence. See table 1 for nursery codes.

Species	Nursery	Blank spaces			Double spaces			Single seeds			Seed deltas		
		Summit		Oyjord	Summit		Oyjord	Summit		Oyjord	Summit		Oyjord
		(mean #/ft of row)			(mean #/ft of row)			(mean #/ft of row)			(no units)		
Douglas-fir	CDA	4.5	ns	4.8	6.6	*	8.8	8.5	**	7.0	35	**	64
	LPN	3.0	**	6.9	5.2	**	9.9	6.1	*	9.7	46	ns	47
	JHS	5.3	ns	5.9	9.2	ns	10.4	12.0	ns	9.8	100	ns	50
Engelmann spruce	CDA	2.4	**	5.4	4.7	**	8.2	4.4	ns	8.2	133	**	45
	LPN	3.8	**	5.9	5.9	**	9.0	7.9	ns	8.8	58	ns	70
White spruce	JWT	7.3	ns	6.0	11.3	ns	9.2	11.8	**	8.4	78	ns	78
Noble fir	WRN	3.0	**	7.0	5.0	*	11.5	4.7	*	7.6	141	ns	112
Jack pine	JWT	5.3	**	3.3	7.1	*	5.6	9.4	**	5.1	78	*	95
Lodgepole pine	LPN	2.8	**	4.4	4.1	**	7.0	7.3	ns	6.9	96	*	67
Ponderosa pine	LPN	2.2	**	4.7	3.1	**	7.0	5.9	*	7.5	53	ns	55
	JHS	4.1	**	8.1	5.6	ns	7.4	12.9	**	9.3	47	ns	52
Red pine	JWT	4.7	**	3.4	6.5	*	5.7	9.0	**	5.3	51	**	83
Western larch	CDA	5.5	ns	5.5	8.6	ns	8.6	8.6	ns	8.3	61	ns	58

wing still attached. This created clumping problems in both seeders, especially the Summit.

Seedling Mapping

Five weeks after sowing we mapped the location of all established seedlings. Overall, the hand-thinned plots showed a lower seedling density than both the Summit and the Oyjord Seeder plots (table 4) and therefore a greater mean distance between seedlings. The exceptions to this were the Engelmann spruce tests (Coeur d' Alene and Lucky Peak) and the Douglas-fir (J. Herbert Stone) in which there were no significant differences and the noble fir test (Wind River) in which the Summit Seeder produced a lower density and wider average spacing. We could not include two tests in our analysis of seedling location: at Lucky peak the ponderosa pine seedbed was next to a shelterbelt that harbored seed-eating birds, and the Douglas-fir seedlot, with an expected 95 percent germination rate, simply failed to germinate at the expected rate.

In every case the hand-thinned treatment produced fewer blank spaces and fewer double seedlings than the Summit and Oyjord Seeders. However, there were even a few blank spaces in the hand-thinned plots where seeds were not sown or did not germinate. This shows that even the hand thinning was not precise.

The thinned plots also had more well-spaced seedlings (singles) in eight tests. In the three tests at J. W. Toumey Nursery, however, the hand-thinned plots did not have the most: in white spruce where there was no difference, and jack and red pine where the Summit Precision Seeder produced more. In most of the tests there is little difference between the number of blanks, doubles, and singles on plots sown by the two seeders, even less difference than the seed data showed.

Seedling Grade and Morphology

After two years, eight of the 13 tests were lifted and graded. At that time there were no differences in seedling height for any of the sowing treatments (table 6). At lifting time there were only two cases out of eight where mean seedling caliper (table 5) and total dry weight (table 6) differed between treatments: Engelmann spruce and lodgepole pine, both grown at Lucky Peak. In both instances, mean seedling caliper and total dry weight were less on plots sown by the Oyjord Seeder than the Summit Precision Seeder and hand-thinned plots.

Cull rates and number of shippable trees show a lack of overall trends except that the plots sown with the Oyjord Seeder produced as many or more trees that met specifications on the grading table compared to the Summit Precision Seeder and the hand-thinned plots (table 6).

CONCLUSIONS

The placement of seed by the Summit Precision Seeder and the Oyjord Seeder varied with each species and nursery. In general, the Oyjord was successful in coming close to target densities. However, the seeds were not evenly spaced. On the other hand, the Summit Seeder placed the seed at more evenly spaced intervals and seemed to be an improvement over the Oyjord when the seeds were large and rounded. The Oyjord plots tended to have more blank spaces and more clumping of seeds, but this was not the case at the J.W. Toumey Nursery where the seeds were smaller and seed placement results were just the opposite. Likewise, seed delta calculations failed to prove one seeder to be superior over the other.

Table 4.--Seedling spacing measurements on plots sown by the Summit Precision Seeder, Oyjord Seeders and hand-thinned. See text for definitions of blank spaces, double seeds, single seeds. A set of values followed by * indicates a significant difference between seeders at α = 0.05. ** indicates α = 0.01. NS indicates not statistically different at the 95 percent level of confidence. See table 1 for nursery codes.

Species	Nursery	Seedling density				Blank spaces				Double seedlings				Single seedlings			
		Summit	Oyjord	Thinned		Summit	Oyjord	Thinned		Summit	Oyjord	Thinned		Summit	Oyjord	Thinned	
		--(seedlings/ft of row)--				--(mean #/ft of row)--				--(mean #/ft of row)--				--(mean #/ft of row)--			
Douglas-fir	CDA	15.0	16.7	9.9	**	3.0	3.9	1.1	**	5.3	7.0	0.5	**	6.5	6.2	7.8	**
	JHS	11.5	11.3	8.8	ns	4.7	5.6	2.2	**	8.7	9.0	0.8	**	10.7	8.7	14.1	*
Engelmann spruce	CDA	12.4	17.1	9.7	ns	2.0	4.1	1.1	**	6.0	6.7	0.4	**	4.3	6.8	8.0	*
	LPN	11.1	12.2	8.2	ns	2.0	3.4	0.9	**	4.3	6.1	0.3	**	4.6	5.3	6.5	*
White spruce	JWT	15.9	14.3	7.2	**	3.6	3.5	0.9	**	7.5	6.8	0.5	**	5.2	4.4	5.3	ns
Noble fir	WRN	5.4	12.7	6.8	**	0.7	3.0	1.0	**	2.5	6.2	1.0	**	1.7	3.9	4.4	**
Jack pine	JWT	14.8	10.1	6.3	**	3.3	2.2	0.9	**	5.5	4.4	1.1	**	6.2	3.4	3.8	**
Lodgepole pine	LPN	10.6	13.3	8.6	**	1.8	3.1	1.0	**	3.6	5.4	0.4	**	4.9	4.9	6.6	*
Ponderosa pine	JHS	8.5	10.0	8.3	**	3.3	5.1	2.2	**	4.8	7.3	1.3	**	8.7	8.0	12.7	**
Red pine	JWT	14.1	10.9	6.8	**	3.5	2.5	0.9	**	4.9	4.7	0.7	**	5.9	3.7	4.8	**
Western larch	CDA	17.5	16.2	10.1	**	4.1	3.9	1.1	**	6.2	6.3	0.4	**	7.2	6.2	8.1	**

Table 5.--Seedling Heights and Calipers for Summit Precision Seeder, Oyjord Seeder, and hand-thinned plots . A set of values followed by * indicates a significant difference at α = 0.05. ** indicates α = 0.01. See table 1 for nursery codes.

Species	Nursery	Mean seedling heights-2nd year Summit	Oyjord	Thinned		Mean seedling caliper Summit	Oyjord	Thinned	
		---(in cm)---				---(in mm)---			
Douglas-fir	CDA	17.9	16.6	16.0	ns	3.4	3.4	3.6	ns
	LPN	--	--	--		2.8	2.8	3.0	ns
	JHS	29.5	29.9	30.8	ns	6.6	6.3	6.5	ns
Engelmann spruce	CDA	18.6	18.9	19.0	ns	3.8	3.4	4.1	ns
	LPn	5.9	5.2	5.4	ns	3.4	3.1	3.5	*
Lodgepole pine	LPN	19.3	18.9	19.0	ns	5.4	4.7	5.6	**
Ponderosa pine	JHS	22.2	22.6	22.5	ns	6.9	6.5	7.1	ns
Western larch	CDA	38.3	37.0	36.1	ns	4.0	3.9	4.2	ns

When seed was small such as white spruce or angled on one end such as coastal Douglas-fir, the Summit's vacuum system had problems in picking up and placing just one seed at a time. This is why several of the species showed little difference between the two seeders. The Summit Seeder also had difficulty in sowing the resinous and sticky noble fir seed at Wind River Nursery.

In most cases there was not much difference between seedling density with the two seeders. However, the plots that were oversown and hand thinned produced fewer seedlings per unit area, fewer blank spaces, less clumping of seedlings, and generally more well spaced seedlings. The performance of the two seeders in meeting seedling target spacings varied with species, but overall there was little difference.

Even though seedbed spacing can have great effects on seedling morphology, in none of the tests were the spacing differences large enough to have an effect on seedling heights after two years of growth. In only two tests were there differences in caliper and seedling dry weight. Both times the Oyjord plots produced seedlings with mean caliper and dry weights less than the other two treatments.

Seedbed density and spacing did not affect the percentage of total trees that met grading specifications. In fact, Oyjord plots, which often had the poorest seedling spacing, produced as many or more shippable seedlings per unit area than the other two treatments in all of the tests.

Seedling spacing is a function of seed placement and seedlot germination. This complex problem and the way it influences seedling growth and morphology depend on many factors. Many of these factors were not measured in this study. It appears that the differences in seedling spacings were not great enough to change the seedling morphology. Even when we hand thinned plots to specified seedling spacings, we did not greatly reduce the cull rates and total shippable seedling production per unit area dropped.

Table 6.--Seedling dry weights and grades for Summit Precision Seeder, Oyjord Seeder and hand-thinned plots. A set of values followed by * indicates a significant difference at α = 0.05. ** indicates α = 0.01. NS indicates no significant difference at the 95 percent level of confidence. See table 1 for nursery codes.

Species	Nursery	Total seedling dry weight Summit	Oyjord	Thinned		Shippable seedlings Summit	Oyjord	Thinned		Mean number of shippable trees Summit	Oyjord	Thinned	
		---(grams)---				--(percent of total)--				---(#/ft^2)---			
Douglas-fir	CDA	4.12	4.16	4.52	ns	74	72	74	ns	54	51	33	**
	LPN	1.35	1.33	1.41	ns	64	56	55	ns	6	10	7	--
	JHS	16.53	14.07	14.75	ns	47	65	85	ns	35	43	46	--
Engelmann spruce	CDA	5.42	4.15	6.34	ns	72	79	78	*	38	60	33	*
	LPN	1.89	1.49	1.76	*	59	64	64	ns	12	14	7	*
Lodgepole pine	LPN	10.81	7.52	11.30	**	69	76	79	**	15	24	15	**
Ponderosa pine	JHS	13.24	11.65	13.93	ns	37	45	40	ns	11	15	11	--
Western larch	CDA	3.62	4.03	4.58	ns	73	75	81	ns	35	35	24	**

The results of this study emphasize some of the major underlying problems that must first be solved if we desire evenly spaced and morphologically consistent seedlings. A machine that can place seed exactly where we would like loses its value quickly as the germination rate of our seed goes down. Even a precise and consistent seeder using a high germination seedlot is of little value if we have not determined the optimum morphological characteristics of the seedlings we are growing and the spacing that will produce those results.

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Soil Fumigation, Cover Cropping, and Organic Soil Amendments: Their Effect on Soil-Borne Pathogens and the Target Seedling[1]

Philip B. Hamm and Everett M. Hansen[2]

Abstract.--Results of two studies are reported dealing with the impact of three common cultural nursery practices (fumigation, cover cropping and organic soil amendments) on soilborne populations of Fusarium and Pythium. The influence of these practices on seedling quality, mortality, and number of seedlings meeting packing standards is included. The potential use of Brassica sp. as a cover crop to lower propagule counts of soilborne pathogens is discussed.

INTRODUCTION

Soil fumigation and cover cropping are common cultural practices in Pacific Northwest bare root forest nurseries. Soil fumigation in the fall, using metam-sodium, methyl bromide (MC33) or dazomet, kill weed seeds and reduce pathogen populations. These chemicals are very toxic and kill both wanted and unwanted organisms alike. Typically, fumigation is used in blocks that will be used as seed beds the following spring.

Cover crops are grown to replace or build soil organic matter levels, increasing soil aggregation, structure, and water-holding capacity. In addition, cover crops can help in soil stabilization, reclaim nutrients that have moved to lower soil levels, and break up hard pans when their roots penetrate these layers (McGuire and Hannaway 1984). Incorporation of soil amendments, such as sawdust, can have some of the same effects produced by cover cropping.

While these cultural practices are widely used, their impacts on seedling quality and soil borne pathogens have not been adequately documented. Spring fumigation has been extensively studied in forest nursery production but a single report by Tanaka et al. (1986) describes the impact of fall fumigation, the method of choice in the Northwest. Likewise, the influence of cover crops and other organic amendments has been addressed in other crop systems but not in Douglas fir nurseries (Wright et al. 1963, Lu 1967, Johnston and Zak 1977). For these reasons, work was initiated to better understand the interactive effects of these practices on soil borne populations of Fusarium and Pythium in nurseries that grow Douglas-fir. In addition, the influence of these practices on seedling mortality and quality of seedlings at lifting was determined. A second study was initiated to confirm the results of the first study and look further at how cover crops influence soilborne pathogen levels. This paper reports some of the information obtained during these two studies; a more complete description of the first study can be found elsewhere (Hansen et al. 1990).

MATERIALS AND METHODS

Study 1. Plots were established in three bare root nurseries; two in Oregon and one in Washington. Three or four cover crops (legume, grass, legume and grass combination, and fallow) and two fumigation treatments (with and without) each with four replications were installed in each nursery. The legume was either peas or beans, the grass either sudan or oats. Cover

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crops were sown in the spring and plowed under in August. Soil fumigation was done soon after and the area remained fallow until the following spring. Douglas-fir seed was sown in May. Seedling inventories (both alive and dead) were done in mid-summer and late fall as 1+0's and at lifting as 2+0's. Soil samples from five composited subsamples were taken at ten separate times from the center of each replication. Sample times were as follows: (1) immediately before fumigation in the fall, (2) immediately after the tarps were removed following fumigation, (3) mid-winter, (4) immediately before beds were formed prior to sowing, (5) immediately after sowing, (6) late summer, (7) late fall, (8) spring as 2+0's, (9) summer as 2+0's and, (10) just before lifting.

Soils were processed in the laboratory to determine the levels of Fusarium and Pythium. Populations were determined using a modified Komada's medium (Komada 1975) amended with 1 μg/ml Benlate for Fusarium and a V-8 agar medium for Pythium developed by Peninsu-Labs (Hansen et al. 1990). Harvest information determined for each treatment included: numbers of packable seedlings; shoot/root ratios; and Fusarium colonization of roots. All seedlings from the sample plots were graded at lifting to determine numbers of packable seedlings. Shoot/root ratios were determined by comparing dry weights of ten seedling shoots and roots per plot, 40 per treatment. Subsamples of ten seedlings from each plot (40/treatment) were selected at lifting for colonization data. Ten, 1 cm root segments from each seedling's tap root were placed on Komada's medium with Benlate.

Study 2. Following the conclusion of study 1, plots were established in two bare root nurseries (one each in Oregon and Washington) to further test the effects of cover crops, fumigation, and soil amendments. Each nursery had four blocks (replications), each including two main plots (sawdust added or no sawdust) and eight subplots. The subplots were (1) grass (Sudan or Rye) cover crop, soil fumigated and tarped, (2) grass, soil not fumigated and not tarped, (3) grass, soil not fumigated and tarped, (4) fallow, soil not fumigated, not tarped, (5) fallow, soil not fumigated, not tarped; (6) mustard, soil not fumigated, not tarped, (7) mustard, soil not fumigated, not tarped; (8) mustard, soil fumigated, tarped. Cover crop treatments were chopped and dried for two weeks before soil incorporation. Soil samples were collected to determine propagule levels of Fusarium and Pythium at three times: (1) just prior to incorporation of cover crops; (2) eight weeks following incorporation of cover crops, and (3) the following spring prior to sowing. Mustard (variety "Tellney") was grown because of recent reports that Brassica sp. had a potential to control Fusarium levels in soil (Ramirez-Villapudua and Munnecke 1987, 1988) through the break down of glycosinulate to form methyl-isothiocyanate gas (Davis 1988). Propagule levels of Fusarium and Pythium were determined as before. Data analysis for both studies use a Fischer's protected LSD, P = 0.05.

RESULTS

Study 1. For the sake of brevity, only results from two nurseries, A and B, are reported. Populations of Fusarium and Pythium before fumigation were high in all nursery soils (Table 1). Fumigation dramatically reduced populations of both fungi at all nurseries and they remained significantly lower than the unfumigated treatments through the two-year crop cycle (Table 1 and 2).

Cover cropping affected populations of both Fusarium and Pythium at Nurseries A and B. Differences in Fusarium levels due to cover cropping were significant before fumigation at both nurseries and at eight of nine (only 5 sample times listed in Table) subsequent sample times in unfumigated plots. In fumigated plots, the effects of cover cropping were significant for two of nine sample times at Nursery A and seven of nine at Nursery B. Populations of Fusarium were generally lowest in the fallow areas and highest with the legume cover crop. Differences due to cover crops persisted in the unfumigated treatments through lifting.

Pythium populations were also affected by cover cropping. Again, as with Fusarium, fallowing had the lowest number of propagules per gram of soil while the beans or pea cover crop supported the highest. Fumigation nearly eliminated Pythium propagules in the soil so cover crop effects within the fumigated areas could not be determined.

Number and quality of seedlings harvested at the end of the two-year crop cycle differed significantly among treatments only at Nursery B (Table 3). More live trees, and more trees meeting nursery size standards (packable), were present in fumigated plots than in unfumigated plots at both nurseries. On fumigated plots, the trees had greater shoot-to-root ratios. All differences were significant (p = 0.05) except for shoot/root ratio at Nursery B. Fusarium oxysporum was recovered significantly less frequently from roots of seedlings harvested from fumigated plots than from nonfumigated plots at Nurseries A and B. Very little Pythium was recovered from seedlings of any treatment at any nursery.

Little, if any, disease was evident at Nursery A during the first growing season regardless of whether the plots were fumigated. Fusarium hypocotyl rot caused serious losses at Nursery B, however, as evidenced by differences in seedling count between June and August (Table 3). Mortality in unfumigated beds (45%) was significantly greater than that in fumigated beds (25%).

At Nursery B, more packable trees, with less Fusarium infection, were produced with fallowing than with either cover crop, regardless of whether the plots were fumigated. There was also less hypocotyl rot after fallowing than after either type of cover-cropping.

Study 2. Fusarium and Pythium levels before fumigation were high (Table 4) at both nurseries, as they were in Study 1. Fumigation (Rye plus Fumigation) significantly reduced propagule counts when measured 12 weeks later (Table 5) and these levels remained low through eight months (Table 6).

The addition of sawdust as a soil amendment reduced soil populations at all sample times but means were not always significantly different. Fusarium numbers before fumigation were halved (26,529 versus 13,044) at Nursery D and reduced by 1/3 (9,260 versus 6,089) at Nursery E (Table 4). Pythium levels were also lower (152 versus 120) at Nursery D and were significantly reduced (P = 0.05) at Nursery E (292 versus 5). These differences persisted for 12 weeks (Table 5) and eight months (Table 6) following fumigation at the two nurseries.

Cover crops also had a significant impact in some cases. As in Study 1, fallow treatments in unfumigated plots had nearly always the fewest propagules of Fusarium at all sample times, and differences were often significant. Pythium numbers were also reduced in fallow areas, but not as dramatic or consistant as that which occurred with Fusarium. Highest levels of Fusarium were generally where mustard was grown before treatment (Table 4). Mustard incorporation, however, reduced Fusarium levels at both nurseries over the rye cover crop, but only significantly so at Nursery E (Table 5). Pythium numbers were variable following incorporation of the mustard cover crop at both nurseries.

DISCUSSION

The dramatic reduction of soil populations of Fusarium oxysporum and Pythium spp. following fall fumigration was not surprising, although it has only been documented in one other study involving western conifer nurseries and current nursery practices. The duration of the effect

Table 1. Fusarium populations (colony-forming units per gram dry weight of soil) at various times during the two-year crop cycle in soils of two Douglas-fir seedling nurseries subjected to various combination of cover crops and fumigation treatments[1]

Nursery and Treatment	Sampling Time: Pre-fumigation Sept. 1985	Post-fumigation Nov. 1985	Presowing June 1986	Aug. 1986	Aug. 1987
Nursery A					
Fumigated					
Fallow	1670a	15a	40a	1170a	6390a
Oats	10570b	0a	40a	1130a	2910a
Peas & Oats	5700b	0a	20a	820a	3510a
Peas	3750b	3a	40a	1000a	5400a
Unfumigated					
Fallow	1820A	1260A	430A	1260A	17060A
Oats	6909B	10040B	2460BC	6570B	53440A
Peas & Oats	5820B	8270B	3640B	9100B	43340A
Peas	11420B	9550B	1920B	6720B	39910A
Nursery B					
Fumigated					
Fallow	13690a	90a	40a	80a	760a
Sudan	32910b	1a	330b	530b	2710b
Beans	48340b	160a	1170c	1670c	9180b
Unfumigated					
Fallow	1920A	8510A	1370A	3590A	2460A
Sudan	17120B	8710A	4520B	11820B	8990A
Beans	31660B	8680A	13139C	18030C	33780A

[1]Within a column segment for a single nursery and fumigation treatment, populations followed by the same letter (lower case letter = fumigated areas, uppercase letter = unfumigated areas) are not significantly different by Fischer's protected LSD (P = 0.05).

was surprising, however. Not only were populations low at the time of sowing eight months after fumigation (Study 1 and 2), but also they increased very slowly and remained significantly lower than in unfumigated beds through the entire two-year crop cycle (Study 1). Population differences were maintained despite the immediate proximity of unfumigated beds and the repeated movement of tractors and irrigation water across the plots. Not until a new cover crop was plowed under nearly three years later did populations approach prefumigation levels (Hansen et al. 1990).

Although more seedlings were produced in fumigated beds during Study 1 than in unfumigated ones, there were no real differences in root growth potential of the trees, as measured by the standard test (data not shown). Seedlings from unfumigated beds were smaller and more variable in size than those from fumigated beds, and more of them did not meet packing standards for this reason.

The effect of the preceding cover crop in determining populations of both Fusarium and Pythium was evident in the fall of the first year, even before the ground was fumigated (Study 1 and 2). Differences persisted through the entire crop cycle in unfumigated treatments (Study 1). Although legume cover crops tended to support higher populations than did grass cover crops, the most significant differences were between no cover crop (fallowing) and the other treatments. These differences were still present 30 months after the cover crop was plowed under in unfumigated plots. Fusarium populations in fallow, unfumigated plots were often within the range found among fumigated plots with cover crops. There is very little experimental basis for the practice of cover cropping in the Northwest (McGuire and Hannaway 1984). Benefits cited include disease control from crop rotation, soil stabilization, and increased levels of soil organic matter with supposed improvements in soil structure. Actual species used for cover cropping vary from nursery to nursery, depending on the experience of local managers.

The potential use of mustard to lower soil population levels of Fusarium and Pythium needs further investigation. While Fusarium numbers decreased substantially in mustard plots

Table 2. Pythium populations (colony-forming units per gram dry weight of soil at various times during the two-year crop cycle in soils of two Douglas-fir seedling nurseries subjected to various combinations of cover crops and fumigation[1]

	Sampling Time				
Nursery and Treatment	Pre-fumigation Sept. 1985	Post-fumigation Nov. 1985	Presowing June 1986	Aug. 1986	Aug. 1987
Nursery A					
Fumigated					
Fallow	280a[1]	4a	4a	2a	10a
Oats	640a	0a	4a	6a	20a
Peas & Oats	680a	0a	30a	10a	10a
Peas	940b	1a	2a	20a	30a
Unfumigated					
Fallow	160A	260B	150A	130A	100A
Oats	780B	790B	200A	150AB	230A
Peas & Oats	630B	580B	400A	170AB	250A
Peas	1060B	1100B	240A	530B	380A
Nursery B					
Fumigated					
Fallow	2a	0a	0a	0a	0a
Sudan	20b	1a	0a	0a	0a
Beans	80b	0a	0a	0a	0a
Unfumigated					
Fallow	0A	100A	10A	0A	4A
Sudan	30B	60A	10A	20B	20B
Beans	80B	110A	30A	20B	20B

[1]Within a column segment for a single nursery and fumigation treatment, population followed by the same letter (lower case letter = fumigated areas, uppercase letter = unfumigated areas) are not significantly different by Fischer's protected LSD (P = 0.05).

following incorporation compared to grass, these levels were still much higher than the standard grass and fumigation treatment and generally higher than the fallow areas. This may be partially due to the higher levels of Fusarium found in the mustard treatments prior to incorporation and/or fumigation (Table 4). Data are not yet available on whether these higher Fusarium levels affect seedling survival or quality in Douglas-fir grown from seed sown into these areas.

The addition of sawdust reduced levels of these pathogens in the soil. Apparently the benefits of adding organic matter are greater than those limited to improving the physical properties of the soil. Whether this benefit of lowering propagule levels transfers to higher seedling survival and quality is unknown. Previous reports dealing with pine in the northwest would indicate this is likely to happen (Wright et al. 1963, Lu 1968, Johnston and Zak 1977). Additional field plots have been established during the spring of 1990 to further investigate the use of cover crops and soil amendments to lessen soil borne propagule counts and future disease losses.

These studies confirm the value of fumigating forest tree nursery beds before sowing. As long as fumigation is the standard practice, there is little practical significance to the results about cover cropping or soil amendments without fumigation. Fumigation is a costly procedure, however, and the chemicals used are extremely toxic. Both economic and environmental pressures are stimulating interest in alternative strategies for disease control. The influence of cover crops and soil amendment on pathogen populations will be an important factor in proposed programs of integrated biological and cultural control.

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Table 3. Number and quality of Douglas-fir seedlings grown in two nurseries with and without cover cropping and fumigation[1]

Nursery and Treatment	Packable Seedlings[2]	Shoot/Root Ratio	Fusarium Isolation[3]	Seedling Count[4] June 1986	Aug. 1986
Nursery A					
Fumigated					
Fallow	195a	2.7a	3a	28.3a	27.1a
Oats	200a	--	--	28.7a	26.3a
Peas & Oats	193a	2.7a	3a	29.7a	26.9a
Peas	204a	--	--	28.8a	27.5a
Unfumigated					
Fallow	186A	2.2A	11A	28.3A	26.9A
Oats	180A	--	--	28.1A	25.3A
Peas & Oats	205A	2.5A	27B	28.5A	26.3A
Peas	197A	--	--	26.4A	24.9A
Nursery B					
Fumigated					
Fallow	313a	1.5a	7a	38.0a	32.0a
Sudan	283b	1.8a	17a	32.8a	26.3ab
Beans	223b	1.9a	48b	34.8a	23.3b
Unfumigated					
Fallow	197A	1.6A	44A	29.5A	18.1A
Sudan	161B	1.6A	73B	28.8A	15.4AB
Beans	143B	1.4A	73B	30.5A	14.6B

[1]Within a column segment for a single nursery and fumigation treatment, populations followed by the same letter (lower case letter = fumigated areas, uppercase letters = unfumigated areas) are not significantly different by Fischer's protected LSD ($p = 0.05$).
[2]Average n;umber of seedlings (per 1- x 1.2 m plot) meeting nursery standards at final harvest.
[3]Average frequency (%) of isolations of Fusarium from 10, 1 cm sections of tap root on Komada's medium.
[4]Number of healthy seedlings in 0.93 m^2 of bed at the indicated dates.

Table 4. Numbers of *Fusarium* and *Pythium* propagules before fumigation and/or incorporating cover crops into soil[1]

	Sawdust			
	Nursery D		Nursery E	
Treatment	*Fusarium*	*Pythium*	*Fusarium*	*Pythium*
Fallow	8297a[2]	98a	2133a	36a
Fallow & Tarp	1708a	112a	6290b	22a
Mustard	11201bc	153a	11333b	398b
Mustard & Tarp	30341c	136a	5733ab	253b
Grass & Fumigation	19885c	117a	5947ab	15a
Grass	7834ab	108a	4858ab	55b
Overall	13044	120	6089	5[3]
	No Sawdust Added			
Fallow	6184a	140a	16161b	35a
Fallow & Tarp	17017a	305a	13501b	102a
Mustard	48417bc	127a	7274ab	503b
Mustard & Tarp	30989c	96a	7678ab	313b
Grass & Fumigation	42500c	141a	3122a	121a
Grass	14067ab	103a	7823ab	677b
Overall	26529	152	9260	292[3]

[1]Propagules per gram of dry soil
[2]Numbers in a single column followed by the same letter not significantly different at P = 0.05.
[3]Significantly different at P = 0.05.

Table 5. Numbers of *Fusarium* and *Pythium* propagules 12 weeks following fumigation and/or cover crops incorporation[1]

	Sawdust			
	Nursery D		Nursery E	
Treatment	*Fusarium*	*Pythium*	*Fusarium*	*Pythium*
Fallow	19426a[2]	687c	6636b	28b
Fallow & Tarp	7639b	127b	10746bc	12b
Mustard	24946b	705cd	23056c	247c
Mustard & Tarp	41138b	257b	18357c	274c
Rye & Fumigation	1232a	0a	0a	0a
Rye	54561b	843d	76240d	365c
Overall	24824	437	22506	154[3]
	No Sawdust Added			
Fallow	38650b	455c	10606b	84b
Fallow & Tarp	59019b	229b	27230bc	236bc
Mustard	111750b	561cd	42610c	816d
Mustard & Tarp	138305b	301b	44434c	444cd
Rye & Fumigation	337a	0a	0a	0a
Rye	168823b	1129d	63328d	192bc
Overall	56147	446	31368	295[3]

[1]Propagules per gram of dry soil
[2]Numbers in a single column followed by the same letter not significantly different at P = 0.05.
[3]Significantly different at P = 0.05.

Table 6. Numbers of Fusarium and Pythium propagules eight months following fumigation and/or cover crop incorporation (prior to sowing)[1]

	Sawdust			
	Nursery D		Nursery E	
Treatment	Fusarium	Pythium	Fusarium	Pythium
Fallow	4935bc[2]	203b	1616ab	52b
Fallow & Tarp	324a	103b	1768ab	18b
Mustard	5676bc	334b	5225b	163c
Mustard & Tarp	8390c	424bc	4606b	176c
Grass & Fumigation	4529b	19a	3518a	8a
Grass	9421c	1060c	13196c	81c
Overall	5546[3]	357	4988	83
	No Sawdust Added			
Fallow	7620b	305b	4920ab	41b
Fallow & Tarp	6702ab	542b	5765ab	103b
Mustard	24660c	404b	4361b	554c
Mustard & Tarp	12280bc	604bc	5503b	342c
Grass & Fumigation	2990a	76a	1927a	2a
Grass	11715bc	791c	15933c	228c
Overall	10994[3]	454	6402	212

[1]Propagules per gram of dry soil
[2]Numbers in a single column followed by the same letter not significantly different at P = 0.05.
[3]Significantly different at P = 0.05.

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The Balsam Woolly Adelgid and Pine Needle Mite as Potential Pests of Reforestation Nurseries in British Columbia[1]

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Abstract.--The Balsam Woolly adelgid, Adelges piceae, an introduced pest to B.C., is currently controlled by quarantine regulations. Life history, damage, pest potential in nurseries, and control trials are described. Life history, damage, pest potential and control of the pine needle mite, Trisetacus campnodus, a newly discovered pest, are also discussed.

THE BALSAM WOOLLY ADELGID: ADELGES PICEAE, (RATZ.)

The Balsam Woolly adelgid (BWA), of European origin, was initially discovered in B.C. near Vancouver in 1958. It is now known to be distributed over 10,000 sq. km. on Southern Vancouver Island and in the Fraser River Valley.

This adelgid infests the twigs and stems of all Abies spp. Alpine fir A. lasiocarpa is the most susceptible to damage although A. amabilis and grand firs A. grandis are most frequently infested in coastal B.C. The insect inserts its mouthparts into the living cells of the bark introducing substances that produce an interaction with the tree causing twigs to swell or "gout" at the nodes. Repeated gouting of the main terminal may produce a stunted top. Persistent crown infestation results in visible thinning of the foliage, top-killing and broken tops. Heavy attacks on the bole or stem often result in tree death after two or three years. Mortality in mature Abies stands is highly variable and patchy, and ranges from 5% to 95%.

Adult BWA's are wingless purplish-black insects less than 2mm long. During the summer, they are covered in white woolly wax threads. During the winter, they are black and flattened with little or no wool. All adults are females which may lay as many as 100 red-brown eggs. These hatch into tiny red-brown first stage nymphs or crawlers, the only mobile stage. The nymphs crawl to a new part of the stem or blow in the wind. Evidence suggests they were able to travel from mainland Canada to Newfoundland, a distance of 260 km. After selecting a feeding location on thin bark, branch nodes, or leaf and cone buds, the crawlers insert their mouth parts and remain at this location for the rest of their lives. After three moults they become adults and begin egg laying. There are two to three generations each year. Eggs and young crawlers are present from late April to October (Harris 1978).

In 1966, the British Columbia BWA regulations were drafted to prevent the spread of this imported pest throughout the range of its Abies hosts. Under the existing regulations all Abies spp. must be grown under permit regardless of nursery location. Nurseries located within the infested zone are not permitted to ship seedlings outside the zone. When stock is moved inside the zone, a spray program using Safers Insecticidal Soap at 1-2% is mandatory. Stock moved between April 1 and October 31, when reinfestation by crawlers is possible, must be treated twice. Cones and seeds, cut Christmas trees, boughs or wreaths when moved between Nov. 1 and Jan. 1, and logs when transported in water and promptly processed, are exempt from the regulations.

Privatization of B.C. reforestation nurseries has created a more competitive atmosphere within the industry. Nurseries inside the regulation area have expressed a desire to grow Abies for areas outside the zone. To develop a treatment that would permit growers to ship stock outside the quarantine zone a sequence of potential control methods was evaluated. Also, as this adelgid had never been detected on nursery stock, the potential for this species to infest seedlings, needed to be established.

In 1987, a trial was established to determine the ability of the BWA to infest and survive on seedlings; and if various insecticides could eradicate established BWA from seedlings. Two year old Abies amabilis seedlings were artificially infested with adelgids. In November, the following insecticides were applied to the overwintering stages using the recommended label rates for aphids: permethrin (100 ml in 1,000 L water/ha/), dimethoate (mix 4 ml in 1 L water, spray for good

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[1]Paper presented at the Western Forest Nursery Council Conference, Roseburg, Oregon, August 13 - 17, 1990

[2]Gwen Shrimpton, Nursery Pest Management Officer, B.C. Forest Service, Silviculture Branch.

coverage) oxydemeton-methyl (3.75 L in 1,000 L water/ha),potassium salts of fatty acids or soap (mix a 2% solution and spray to run-off), soap plus pyrethroids or sap (500 ml in 11 L water and spray).

Each insecticide was applied to five 313 A container blocks half filled with Abies seedlings and interspersed with ten aphid infested trees. Sprays were applied using a specialized pesticide applicator designed to simulate operational conditions while applying small amounts of pesticides for trial use. Due to the high density of nursery stock and the small size and cryptic nature of the aphids, high volumes and pressures were used. Permethrin, oxydemeton-methyl and dimethoate were applied at a volume of 3,000 L water per ha, the soap and sap were applied at 5,000 L water per ha. All sprays were applied using D2-23 nozzles and pressures of 150 PSI. Three weeks after the application, whole seedlings were carefully inspected for the presence of live aphids using a dissecting microscope. The legs of overwintering aphids atrophy and no movement is detectable. Aphids were considered to be alive if a drop of purplish fluid was exuded after they were squashed.

Treatments with soap were repeated on a further series of infested seedlings in August 1988, to determine potential control of the summer populations when eggs and crawlers are present. Applications of soap against the overwintering stages were repeated a second time in March 1989 in order to confirm the results of the 1987 trial. Also, the effectiveness of an extra application of soap two weeks later was evaluated.

Results of all three trials are presented in Table 1. Cygon and Metasystox-R failed to provide acceptable control. Both are organophosphates which become deactivated as the temperature drops. Also, at the time of their application there would have been little or no action of these systemics as the seedlings are dormant during the winter and not actively translocating. Although Permethrin provided the best control it was deemed unsuitable for nursery use because the quarantine regulations require applications just prior to seedling lift. Residues from this product would be too high for nursery workers to handle the stock during the lift safely. Applications of the soap during the summer provided very little control, probably because they were not effective against the eggs and crawlers. When applied during the winter, soap consistently provided about 80% control. However, this level of control was determined unacceptable for assuring that the BWA would not be transported on infested nursery stock.

Table 1.-Control of the Balsalm Woolly Adelgid in Conifer Seedling Nurseries

Treatment	# of trees Assessed	Avg # Adelgids per tree	% Reduction over Control
November 1987 Trial			
permethrin	49	.9	95.6
dimethoate	50	18.5	6.6
oxydemeton-methyl	48	7.8	62.0
soap spray	50	2.8	85.7
sap	50	2.3	90.5
control	50	19.9	0
overwintering counts	50	4.4	57.3
August 1988 Trial			
1 soap spray	49	34.3	13.9
control	25	77.6	0
2 soap sprays 2 weeks apart	50	18.5	33.5
control	25	55.6	0
March 1989 Trial			
1 soap spray	50	2.8	67.6
2 soap sprays 2 weeks apart	50	1.8	79.1
control	50	8.7	0

To date, the BWA has not been found infesting Abies seedlings in reforestation nurseries in B.C. The ability to establish this adelgid on 2+0 container seedlings indicates that they could potentially become a nursery pest; however, survivorship was poor. Counts of adelgids on seedlings held over until March showed there was a 57% reduction in numbers compared to the control seedlings in November. This is possibly because this species is adapted to infesting the stems and twigs of mature trees surviving under humid protected conditions with little direct sunlight. On small seedlings the aphids would be exposed to much harsher environmental conditions.

In spite of the unsuccessful attempts at chemical control of the BWA, we are continuing to work with this pest. A program to gain information necessary for developing a nursery certification program has been initiated. Work to develop a passive trapping technique for the mobile crawler stage, to determine the potential for inoculation of seedlings from mature trees, to assess the level and risk of BWA populations surrounding nurseries within the regulation area, and to develop reliable survey techniques to determine presence or absence of the BWA on nursery stock has been initiated.

THE PINE NEEDLE MITE: TRISETACUS CAMPNODUS (Keifer)

In 1987, the small pine needle mite Trisetacus campnodus was identified on pine seedlings from several B.C. reforestation nurseries. Mites have been collected from outplanting and bare-root production stock at Chilliwack River Nursery, outplantings and bare-root production stock at Surrey Nursery, and outplantings at Green Timbers and Skimikin Nurseries.

T. campnodus occurs on scots pine, Pinus sylvestris, and lodgepole or shore pine, Pinus contorta. It has been a major pest in scots pine Christmas tree plantations, and is distributed throughout Washington, Oregon, and B.C.

This mite is probably a native pest of shore pine where it is not a large problem. However, it is a relatively new pest of lodgepole pine. Interior lodgepole pine planted on the coast is readily attacked and the mite can become a major debilitating problem. R. Hunt (1981) made observations of 70 provenances of P. contorta, ranging from California to the Yukon, growing in a five year old plantation near Cowichan, B.C. and found that damage varied according to provenance. Damage to coastal provenances was slight compared with interior provenances, and within the coastal provenances northern ones were damaged more than southern ones.

In B.C. reforestation nurseries this mite is of concern because interior lodgepole pine seedlings have been grown at coastal nurseries and then planted back in interior native habitats. It is possible that the mite could spread from native shore pine in and around the nursery site to the lodgepole pine in the nurseries, and then from the seedlings in reforestation sites to native lodgepole pine stands.

T. campnodus are extremely small mites that appear only as specks with the naked eye. A hand lens or microscope are necessary to see them, and they must be identified under high magnification. The mites are less than .3 mm long, light yellowish -white, translucent, wormlike & elongate. They are usually sedentary, but can move very slowly with their four legs.

Mite infestations occur at the base of the needles beneath the sheath. At first they occur at the interface where the needles meet, but as the population increases, the entire needle base covered by the sheath may be invaded. In heavy infestations, there can be up to 200 mites per needle base, but 10-20 can cause permanent damage. Eventually, the epidermis of the entire needle base is destroyed and appears necrotic, browned, and sometimes calloused.

Mite damage is often easily detected by the presence of discoloured and distorted needles. The needles become chlorotic, pale yellow, blotched, stippled or mottled. Needle growth can be reduced by up to 70%, and the needles are twisted or hooked, with the new growth being crinkled. Twigs where needles are attacked for several years may become twisted and deformed. Mites also cause premature needle drop. Severely infested trees retain only the current years needles, and in some instances even the current needles are sparse and greatly shortened.

Repeated infestation reduces vigour and may kill trees within a few years. Infested trees are chlorotic and generally appear unthrifty; they can be spotted by their thinner crown of paler foliage. Most pines infested for any length of time are noticably stunted, and there can be a decrease in annual increment of up to 20%. There is some evidence that infested pines may be predisposed to bark beetle attack.

The opportunistic secondary imperfect fungus, Sclerophoma pithyophila, is commonly found fruiting on necrotic foliage and shoots. Dieback associated with the fungus may occur, resulting in bushy, stunted and broomy trees with an exceptional number of buds on each shoot.

The damage causes symptoms sometimes referred to as kinky disease. It is often misidentified by growers as the effects of poor site, needle cast diseases, air pollution, herbicides, poor drainage, and lack of fertility. Magnesium deficiency can produce similar symptoms, but this can be easily rectified with applications of magnesium sulfate. Kinky disease trees are not adversely low in magnesium, nor do applications solve the problem.

The mite population overwinters as both adults and eggs within the needle sheaths. During the time of candle elongation in the spring the mites move to the new growth, and lay several overlapping generations of eggs. It is at this time they cause considerable damage to the new needles, producing the symptoms of kinky disease. During the summer, as necrotic tissue begins to develop at the needle base, the mites often disappear; presumably they move on to other healthier needles.

Infested trees are often erratically distributed with a healthy tree growing next to a badly infested one. Also, the distribution doesn't seem to follow wind patterns because there are often as many infested trees to the windward as there are in more sheltered places. Possibly these tiny mites are carried by birds, squirrels, or insects. Several species of mites attach themselves to insects for transportation.

T. campnodus does not seem to have a large number of natural parasites and predators. Due to its small size it is free from internal parasites, and its inaccessible hiding place protects it from most predators. When the needle sheath becomes loosened with age, or the mites are migrating to new needles they can be subject to predators of which the large mite Seius seems to be important.

Chemical control is also difficult. Pesticides will not readily penetrate into the base of the needle sheath in which the mites are enclosed. Studies have shown that the most effective time to control this pest is from April to early June, during the period after candle elongation but prior to needle elongation. The specific time when the mites migrate onto the new growth depends on the location and species of pine.

Carbaryl and oil has shown to provide the best control over other pesticides tested in several studies. It is recommended that growers use Carbaryl 80% WP at 0.55 - 1.2 kg (1.25 - 2.5 lbs) product with 7.5 liters (2 gals.) of 60 - 70 sec. superior oil per 375 liters (100 gals.) of water. A second application should be repeated 10 - 14 days later to kill newly hatched mites. The oil is necessary to penetrate through or around the sheath to the infested area. The length and tightness of the needle sheath can vary considerably and will affect the control achieved (Adams 1986).

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The Nursery Program at Missoula Technology and Development Center[1]

Ben J. Lowman[2]

Abstract. This paper highlights the various projects in the Nursery and Reforestation Program at the Missoula Technology and Development Center. Projects discussed are: The Root Pruner, Machine Vision, the Tree Seedling Counter, the Progeny Seeder, and Portable Field Storage. Other projects, including the Pollen Collector and the Bracke Scarifier Seeder will be discussed in less detail. Development efforts, field tests, and documentation will be described.

INTRODUCTION

The Missoula Technology and Development Center (MTDC) has provided improved equipment and techniques to Forest Service nurseries for more than 20 years. The goal is to continue to contribute to the efficiency, safety, and production of the Forest Service Nursery Program.

Ben Lowman manages the Center's Nursery Program. If you have questions or need information, contact him at: USDA Forest Service
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PROJECT STATUS REPORTS

Nursery Technical Services--TE02E12

MTDC's Nursery Technical Service project allows Center personnel to attend meetings and provide technical advise on request. The project allows us to provide technical services to Forest Service nurseries and respond to requests from State and Private personnel. Current work underway in this project includes a major update of the "Nursery Equipment Catalog," originally published in 1975. Work on this update is 70 percent complete and publication is planned for December of 1990. In addition, MTDC has drawings of various nursery equipment available on request. Additional drawings will be added as resources become available. A list of drawings and publications will be distributed in December, and the list is also periodically published in Tree Planters' Notes. Examples of projects that are responses to requests from the field include: MTDC modified and built small root growth chambers for Forest Service Research laboratories. These are now being used by nurseries. MTDC designed and built electrical surge protection for 43 Forest Service weather stations used in the Reforestation Improvement Program. MTDC made drawings of the gravity table modification designed and built at the California Division of Forestry, L.A. Moran Regeneration Center in Davis. New applicable technology is continually monitored under this project.

Isozome Laboratory--0E02E33

A National Forest Genetics Electrophoresis Laboratory (NFGEL) was founded by the Forest Service in 1988 for starch gel testing of forest plant material. Since existing equipment was not designed for use on a production basis, problems with efficiency and accuracy were immediately evident. MTDC was asked to identify the problems and design an efficient system of equipment geared toward a production rather than a research environment.

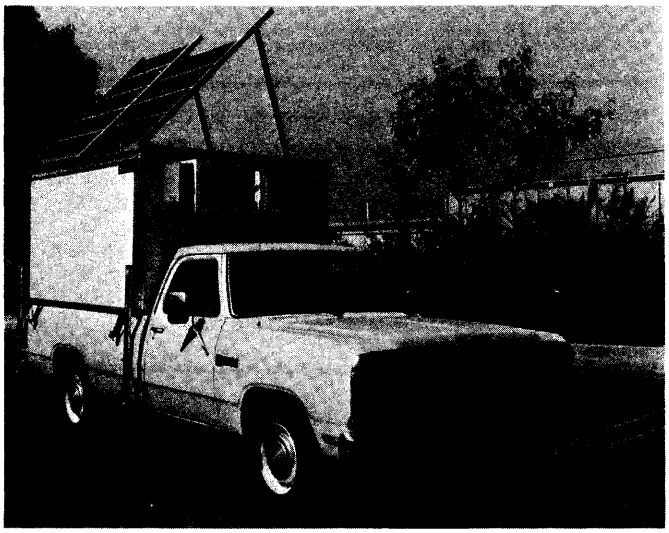
MTDC engineers and NFGEL genetists met in November of 1989 and identified the problems and explored possible solutions. MTDC agreed to design and build buffer trays and gel molds, a gel slicer, and a grinding block, wick combs, and jig. Each piece of equipment would be part of an integrated system that would eliminate much of the hand work and provide consistant operating conditions.

Prototypes of the equipment were designed and built by MTDC. The prototypes were then sent for testing. All equipment provided good results, and it was agreed that the equipment offered a more efficient testing procedure.

MTDC is currently searching for a plastics manufacturer to build the equipment required to fully equip NFGEL. Drawings are available upon request.

[1] Paper presented at the Combined Meeting of the Western Forest Nursery Council and Intermountain Forest Nursery Association, Roseburg, OR, Aug. 1990.

[2] Ben Lowman is Program Manager, Missoula Equipment Development Center, Missoula, MT.



Field Storage Refrigeration Unit

Automated Tree Seedling Counter

Field Storage--6E62E11

A portable pick-up sized refrigeration unit was developed under contract by Polar Products of Torrence, California. The 12-volt system operates from the 12-volt vehicle electrical system, a photo-voltaic array with a backup battery or 110-VAC through a battery charger.

The unit was field tested in Region 6 during spring planting. Reviews were mixed. Illinois Valley Ranger District, on the Siskiyou National Forest, reported in-bag temperatures were held below 35°F during a 6 to 10 hour day. Walla Walla Ranger District found the cooling capacity of the reefer to be adequate. The tree hauling capacity was insufficient since this District has fairly large planting sites. Suggested improvements were an easier method of inclining the PV array, simplifying the AC (battery charger) hookup, and reducing the overall weight.

A random survey of the field conducted by Dick Miller, WO Timber staff, suggested the demand for this unit varies. Regions 5 and 8 were the most enthusiastic. Many respondents were reluctant to use anything more mechanical than an insulated box and ice.

In FY91, MTDC is considering developing a 110-VAC storage unit. This unit would have a more simple electrical system and would require less maintenance.

Seedling Counter--5E52E28

To meet the demand for seedlings for national reforestation efforts, Forest Service nursery managers must have accurate cultural and inventory data. Much of this information is obtained by seedling counts. Such counts are labor-intensive and expensive.

An automated tree seedling counter was developed by MTDC with the aid of two contracts to Dr. Glenn Kranzler of Oklahoma State university.

The counter consists of light emitter and receiver circuitry housed in sealed aluminum enclosures and mounted on a skid. There is a magnetic pick up for determining distance traveled and to provide a reference for measuring stem diameter. A computer allows data storage and retrieval and interfaces with a personal computer. Along with a battery, all these components are mounted on a cart that attaches to a tractor with a three-point hitch. The heart of the machine is its unique opto-electronics that permit accurate seedling counts. A count is made each time a seedling interrupts the invisible light beam between the emitter and the receiver. Once a run is completed, the on-board computer can provide a diameter range of seedlings in the bed and distance traveled. It also produces a profile of seedling stem diameter versus quantity, from one-eighth millimeter to 30 millimeters in one-eighth millimeter increments. This data gives nursery managers an indication of how many seedlings won't meet their grading criteria.

A video, "The Seedling Counter" is available on request from MTDC.

Progeny Test--7E72E27

A seeder for exact placement of tree seeds for progeny testing has been developed. Two units were built and delivered to Bend Pine Nursery in Oregon and Wind River Nursery in Washington.

Seeds are individually placed into a "shutter box" apparatus in the seed laboratory using "air" tweezers. Each shutter box container holds 96 seeds. The shutter boxes are then taken to the field and sown with the seeder.

Two units were built and field tested. A publication and drawings will be prepared in FY 1991 and may be requested.

Pollen Collector--8E82E18

The Pollen Collection and Dispenser Project actually consists of two efforts, one to develop equipment to collect pollen for subsequent mass pollination uses and the other to develop equipment to apply pollen in controlled pollination work. The pollen collection equipment work is being done with Don Copes of the Pacific Northwest Forestry Sciences Laboratory in Corvallis, Oregon, on West Coast Douglas-fir. The pollen application equipment work is being done with Floyd Bridwater of the Southern Forestry Sciences Laboratory at Raleigh, North Carolina, on Loblolly Pine.

Several devices were evaluated for collecting pollen including: enclosing the lower two-thirds of the tree with canvas and shaking the tree; blowing through the tree and collecting on the opposite side; and vacuuming the tree and collecting electrostatically or with a cyclone separator. All of these methods work to some degree, but all have drawbacks. The cyclone separator was selected as the most promising and its development is continuing.

For application of pollen several air injector devices were evaluated for metering pollen into the airstream and several emitters for directing the pollen onto the individual cones were also evaluated. The equipment developed is light weight for application from an aerial bucket. We are in the process of making drawings of the applicator and looking at equipment for controlled pollen application.

Bracke Scarifier--9E92E18

The Seminole Ranger District on the Ocala National Forest asked MTDC to improve the Bracke seeder/scarifier machine to obtain better stocking for direct seeding of sand pine.

MTDC added an air planter to the unit as well as a Dickey John planter monitor. Drag chains were added to both units to cover seeds placed in the scarified spots. The seed drop location on the old planter unit was modified to improve it's performance with an optional plate to restore it to the original configuration. The Bracke drive chain boxes were repaired, cleaned, and re-lubed.

The new air planter unit distributed the seeds out longer along the patch and delivered 25 seeds per patch. The Seminole Ranger District used the machine for 300 to 400 acres and had no problems with the machine. On inspecting the fields planted, the seeds were adequately covered, but the ground was still loose and had not packed around the seed. It appears that a packing mechanism would provide a more optimum seed bed.

MTDC considered a BC drag chain scarifier as an alternative to the Bracke Seeder Scarifier. The scarifier was assembled with the drawbar pulling three double-chain assemblies equally spaced. The scarifier was pulled with a D-4 crawler tractor. One test plot was broadcast seeded and then chain scarified and a second plot was first scarified and then broadcast seeded. Seedling germination will be monitored on these plots.

Although the three double-chain configuration was adequate, the double-chain caused more soil disturbance or ridging than desired, so the scarifier was re-configured with five single chain assemblies. About 400 acres were seeded with the BC drag chain scarifier in the five single chain configuration. A cyclone broadcast seeder was mounted to the front of a skidder that pulled the scarifier. The seed is placed over the ground by the seeder and then covered by the drag chain scarifier. Other seeders may be used in this system to better control the quantity and placement of the seed before covering. Further modifications will be tested in FY 91.

Machine Vision--9E92E19

Tree seedlings are grown in Forest Service bare-root nurseries based on specifications tailored to specific Forest and District needs. After lifting, seedlings are delivered to packing sheds for grading and packing. Each Forest Service nursery has developed its own quality control standards for the seedlings they deliver to field units for outplanting.

The current quality control and grading process is unacceptably labor-intensive and expensive. The graders cull seedlings that do not meet field specifications, count seedlings that do meet specifications, and place them on a packing belt for final processing and packaging. The grader sorts the seedlings by stem diameter, top length, root area and overall quality. Quality control is maintained by checkers who sample graded seedlings and monitor grader's performance.

MTDC was asked to determine the feasibility of automating the quality control and grading process in an effort to reduce costs.

Work was done on contract to Oklahoma State University by Dr. Glenn Kranzler and Mike Rigby. They investigated using machine vision to perform the grading or quality control measurements needed in seedling grading operations. Machine vision and image processing were used to measure various morphological properties of seedlings. A grading scheme was integrated into the software to accept or cull each seedling, depending on morphological characteristics.

OSU's study proves that machine vision can measure morphological features more consistently

than current methods. OSU has demonstrated the feasibility of using machine vision to measure, record, and grade individual seedlings. As a result, MTDC recommends that technology to automatic quality control in the grading process should be pursued. When automated quality control has been established, automating machine grading should be explored.

Packing Shed Root Pruner--9E92E20

Tree seedlings are pruned in the nursery packing shed to provide tree planters seedlings with a uniform root length. This process, currently done with a hand-operated paper cutter, requires additional personnel, is unsafe, and is often a bottleneck in the packing shed operation. MTDC is currently developing a root pruner prototype that will automate this process to increase packing shed efficiency. Safety will be improved.

MTDC first developed several preliminary concepts of root pruners. Conceptual drawings were reviewed by Forest Service Nursery managers and a prototype was developed. This design, a conveyor system with wire baskets that close around seedling roots and present them to the cutter, provided a safe and efficient method of root pruning. The prototype was equipped with three cutter heads for testing three types of cutters: (1) a high-speed circular saw, (2) reciprocating blades, and (3) a trigger air shear.

The prototype was tested by four Forest Service nurseries in the Northwest for comments and review. All nurseries were pleased with the concept and would like to see development continue. Modifications will be made and production testing should begin this winter.

Computer Vision: A Nursery Management Tool[1]

Michael P. Rigney and Glenn A. Kranzler[2]

Abstract.--Computer vision provides quality control for many manufacturing and agricultural processing industries. Objective assessment, high measurement precision, increased inspection rates, and comprehensive production statistics are among the advantages provided by the technology. A brief overview of computer vision technology is presented. Applied research and potential applications for the forest nursery industry are described.

COMPUTER VISION

Computer vision is the integration of image sensors with digital computers to obtain useful information. Physical dimensions, surface features, and color may be quantified. Computer vision systems are used to inspect a wide variety of manufactured and agricultural products. Agricultural applications are generally more challenging due to greater product variation (size, color, and types of defects) and high production rates. In this section we present a brief overview of vision system components. Detailed discussions may be found in Ballard and Brown (1982), Chin and Harlow (1982), Jain (1989), Novini (1985), and Pratt (1978).

Image Sensors

Solid-state television cameras, incorporating a rectangular grid of discrete photosensitive elements (200 to 600 each direction), are the most commonly used image sensors. These cameras are generally rugged, free from geometric distortion, and tolerant of intense illumination and magnetic fields. Images are typically transmitted from the camera to a vision computer as an analog signal defined by television broadcast standards. This signal is digitized into a rectangular array of picture elements (pixels) in the vision computer. Image resolution (amount of detail) is limited by broadcast standards as well as sensor manufacturing constraints. Color and monochrome cameras are available in a variety of spatial resolutions.

Line-scan cameras contain a single row of sensing elements (128 to 4096). Rectangular images are constructed line-by-line as an object moves past the camera or as the camera moves past an object. These cameras allow independent selection of horizontal and vertical spatial resolution.

Lighting

Selection of illumination technique can be critical to system performance. Front lighting allows inspection of surface color and texture. Backlighting provides a silhouette image of opaque objects, useful for dimensional measurement. Structured lighting allows depth to be measured. Strobe illumination enables sharp images of moving objects to be acquired. Illumination wavelength can also be controlled to advantage. For example, ultraviolet light, invisible to the camera, can give high contrast to objects which fluoresce.

Optical Filters

Contrast between the object(s) of interest and the background or neighboring objects can be increased by exploiting spectral reflectance differences. Band-pass and cut-off filters limit the bandwidth of light reaching the image sensor. Sensitivity to selected wavelengths is thereby increased, allowing object color to be evaluated with a monochrome camera. Infrared (IR) cut-off filters are often used because solid-state cameras have high sensitivity to near-infrared wavelengths which otherwise overwhelm the visible light image. In some cases the IR image is desired, and IR-pass filters are used. Polarizing filters can reduce glare from spectral reflection.

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[1]Paper presented at 1990 Conference of the Western Forest Nursery Council. [Roseburg, Oregon, August 13-17, 1990.] Paper No. 3334 of the Oklahoma Agricultural Experiment Station.

[2]The authors are: Michael P. Rigney, Research Engineer and Glenn A. Kranzler, Professor, Agricultural Engineering Dept., Oklahoma State University, Stillwater, OK.

Image Processing Computers

Analog video signals from the image sensor must be digitized before a computer can process the image. Some interfaces accept digital data directly from a camera. The digitizer generates a rectangular array of pixels, each having an intensity or gray level between 0 (black) and 255 (white). A frame buffer is typically available for temporary storage of one or more new, intermediate, or processed images. The digitizer, frame buffer, and other image processing boards are linked by a high-speed image data buss supplementing the host computer buss.

Images as large as 256 K-bytes may be digitized at rates up to 30 per second, far exceeding the computational capabilities of a typical central processing unit (CPU). Specialized computer hardware is required for most inspection applications. Although all image processing operations may be implemented in software by a computer's (CPU), real-time processing constraints require hardware implementation of frequently used and computationally intensive operations.

Hardware implementations of image processing functions may reside with the digitizer or be placed on separate processing boards. Typical hardware functions include histogram computation (counting pixels at each gray level), and thresholding, which transforms a gray-level image into a black-and-white image. Digital filtering hardware allows noise reduction and edge enhancement. Region-of-interest processing, runlength encoding, and color space transformation are useful hardware capabilities.

Many vendors offer modular architectures so that a system may be configured with the desired functionality. Low-level image processing functions are performed by the hardware discussed above. High-level image understanding algorithms are programmed by the user (or applications engineer) and implemented on the host CPU.

NURSERY MANAGEMENT OPPORTUNITIES

Seedling Inspection and Grading

Current Nursery Practice

Seedling grading is a labor intensive, seasonal operation, performed in an environment optimized for seedling viability, not human comfort. Graders identify culls by visually evaluating several morphological characteristics. It is not feasible to manually inspect individual seedlings or sort into more than two classes (acceptable and cull). Classification is subjective and susceptible to human error. Increasing labor cost and personnel injuries have become a nursery management concern.

Prototype Grading System

The feasibility of grading bare root pine seedlings with computer vision has been demonstrated (Rigney and Kranzler, 1988a,b) and a prototype system tested at a commercial nursery (Rigney and Kranzler, 1989). A specialized computer vision system inspected singulated seedlings moving along a conveyor at a rate of 2 per second. Two low-resolution (256H x 240V) cameras and strobe lamps were used for image acquisition (Fig. 1).

Two images of each seedling were acquired and processed. An image of the entire seedling and close-up of the root collar zone had a spatial resolutions of 2 mm and 0.5 mm, respectively. These resolutions were coarse, considering that many roots have diameters less than 2

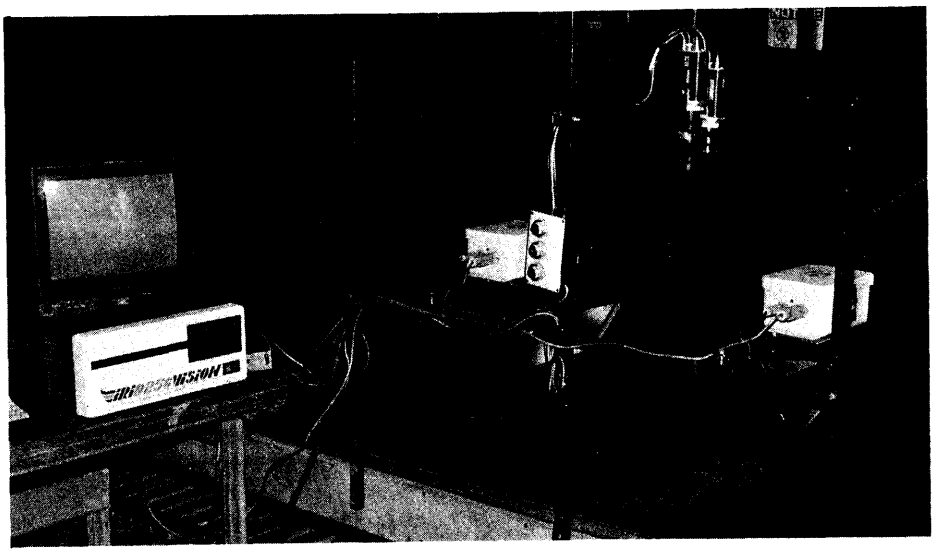

Figure 1.--Vision computer, cameras, strobe lamps, conveyor, and seedlings.

mm, and that 3-mm diameter stems were only six pixels wide. Special image processing operations were implemented to enhance measurement accuracy in spite of limited spatial resolution.

Image processing consisted of several tasks. First, each seedling was detected and images acquired. Seedling orientation on the belt was determined for use in diameter and height computations. Root collar location and stem diameter at the root collar were determined from the close-up image. Seedling height, projected root area, and projected foliage area were extracted from the second image. Sturdiness ratio and shoot/root ratio were computed from primary measurements. Finally, each seedling was classified as acceptable or cull, based on programmable feature setpoints. System performance equaled or exceeded that of manual graders.

Although projected root area cannot be determined for container grown seedlings, plug integrity can be verified. Additional features which may be inspected include stem straightness and multiple leaders. For bare root seedlings, root length and number of root laterals may be quantified.

Line-scan Concept

Conceptual designs for grading systems providing increased measurement accuracy and/or inspection rates have been developed. A concept for a line-scan camera based seedling grader is presented in Figure 2. Line-scan cameras can provide five to ten times the spatial resolution (0.05 mm) of our first prototype, enabling precise measurement of stem diameter and projected root area. Line scan rate may be selected to provide a lower resolution for measurement of shoot height (1.0 mm). High-contrast silhouette images may be acquired by passing seedlings between the line-scan camera and a backlight, increasing measurement precision while reducing computational requirements. Image data may be processed line-by-line, as opposed to acquisition of an entire frame before beginning processing on conventional systems. Grading rates as high as 15 seedlings per second are feasible. Advantages of line-scan processing for many applications have prompted several equipment suppliers to introduce new hardware for line-scan support.

The requirement of seedling singulation means that automated grading has greater implementation potential at container nurseries than at bare root nurseries. Container-grown seedlings are inherently separated, whereas bare root seedlings may require manual singulation. Fast inspection capability necessitates automated material handling.

Automated Grading Benefits

Automated seedling grading offers the opportunity to sort seedlings into multiple classes defined for optimal performance at various planting sites. Customer specifications may easily be keyed into the system and seedlings sorted accordingly. Increased yields may be realized by sorting and marketing alternate grades, using seedlings normally culled under current practice. These capabilities can increase both nursery yields and seedling value.

Customers can be provided with a statistical description of the seedlings purchased. Accurate seedling package counts are an important additional benefit. Further, package count can set by customer specification, as opposed to the standard single package size currently marketed.

Comprehensive production statistics are often cited as the most valuable benefit of computer vision inspection systems. Applied to nursery management, morphology statistics can be correlated with seed source, weather, cultural practices, and field performance.

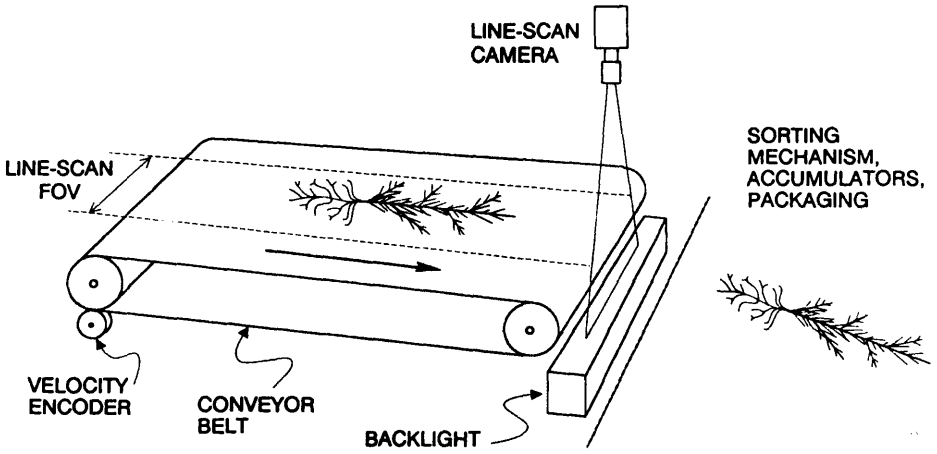

Figure 2.--Seedling grader concept based on line-scan camera and backlighting.

Cost/benefit analysis indicates that implementation of automated grading is economically justified for most large nurseries (Kranzler and Rigney, 1989). Figure 3 illustrates the relationship between manual grading expense and the number of seedlings to be graded in order to recover computer vision equipment costs. We estimate computer vision equipment costs to be $30,000 to $40,000 (vision computer, camera, and lighting). This amount does not include one-time inspection software development cost or seedling feeding and sorting equipment. If we assume $40,000 for equipment and a grading cost of $4.00/1000 seedlings, Figure 3 indicates that 10 million seedlings must be graded to break even.

Alternatively, a quality control station could provide a low-cost introduction to computer vision benefits. Quality of manually graded seedlings can be evaluated on a sampling basis. A system inspecting one seedling every 2 seconds might be configured for as little as $10,000.

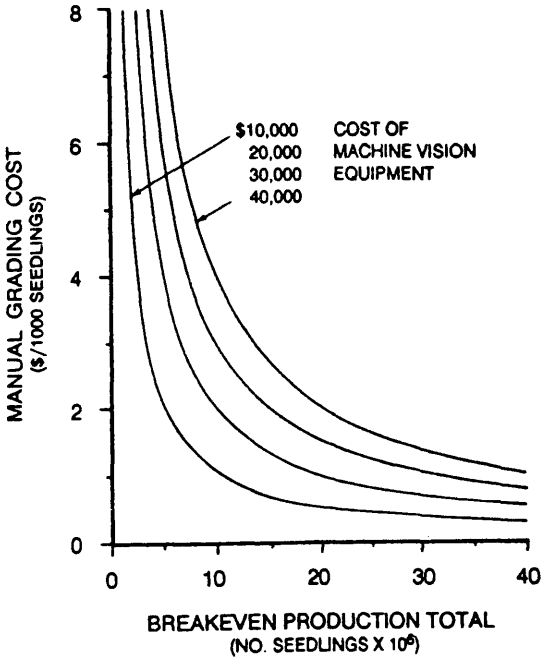

Figure 3.--Seedling production required to recover machine vision equipment costs.

Quantification of New Root Growth

Computer vision systems are currently used to quantify root system morphology for measurement of root growth potential (RGP). Projected root area and total root length are commonly measured (Johnsen et al., 1988; Rietveld, 1989). Current implementations, however, have limited ability to distinguish between new and old root tissue. Root systems are placed on a backlighted diffuser to obtain silhouette images. New root growth is semi-translucent and shows little or no contrast in backlighted images.

Root system morphology is typically measured before and after a test period during which seedlings are held in an environment favorable to new root growth. New growth tips are removed prior to test initiation, and root system morphology is measured. New root growth is stained after the growth period to enhance contrast, and the root system is measured again. The difference between projected area or total length before and after the growth period is attributed to new root growth.

Rigney and Kranzler (1990) have investigated two techniques allowing quantification of new root growth in a single operation after the growth period. Dark-field illumination and crossed polarizing filters are optical techniques which exploit the translucence of the new root tissue. Existing root measurement systems may be modified to incorporate these techniques. Available software can be used to extract desired features of the new root growth.

Precision of root system measurements can be improved by utilizing a line-scan camera, as discussed above for seedling grading. An early seedling measurement device used a linear 1024-element sensing array to measure projected root area (Buckley et al., 1978). Line-scan cameras with up to 4096 pixels are now available. A simple transport mechanism will be required to either move the camera and backlight past the root system or move the root system between the camera and backlight. A system may be configured at relatively low cost, since seedling handling precludes high inspection rates.

In-Bed Inventory and Emergence Count Bare Root Seedlings

Computer vision may be utilized to improve seedling inventory estimates. Instead of hand counting seedlings in sampling frames, a video camera could be used to acquire images. Seedlings could then be identified and counted by computer vision. Sections or complete beds could be recorded on video tape and later processed automatically by a vision system.

Work by Kranzler et al. (1984) and DeVoe (1987) has shown the feasibility of counting newly emerged and young seedlings with computer vision. Under ideal conditions, simple image thresholding techniques can be used to segment individual seedlings in nursery bed images. DeVoe developed four seedling detection algorithms of varying complexity. These exploited the contrast between the seedling and background, the lines formed by individual needles, and the radial distribution of the needles. A variation on the Fourier transform, although computationally expensive, was able to identify seedlings in noisy images.

Sowing, Emergence Count, and Thinning Container Seedlings

Seedling identification techniques may also be used in the container seedling industry. Here, the problem is much simpler because the environment is highly structured. Expected seedling locations are known. Each cell may be inspected individually.

Computer vision offers the opportunity to sow a single germinated seed in each container. A singulated stream of germinated seeds may be viewed by a vision system programmed to detect newly sprouted roots. Each sprouted seed would be diverted to a waiting cell and sown. Each container block would hold seedlings which sprouted at approximately the same time. This concept provides efficient use of premium seed and eliminates thinning operations.

Algorithms and techniques supporting sprouted seed identification have already been developed. Ling et al. (1990) investigated spectral features for distinguishing between the seed coat and root of germinated tomato seeds. Techniques described by Rigney and Kranzler (1990) for segmentation of new root tissue are also applicable.

Propagation via tissue culture and rooted cuttings are technologies receiving increasing attention. Although currently labor intensive, computer vision guided robots will be key components of future commercial systems (Deleplanque, 1985). Simonton (1989) has developed a computer vision and robotic workcell for vegetative propagation of geranium cuttings.

Under the current practice of sowing multiple seeds per cell, computer vision could determine how many, if any, seedlings have emerged in each cell. A more challenging, but technically feasible task, would be to determine the locations of each of the new seedlings, enabling robotic thinning. Further, the same robot could transplant new seedlings into empty cells.

SUMMARY

Computer vision is a powerful technology offering enhanced product quality and reduced production cost through automation of many nursery operations. Objective assessment, high measurement precision, comprehensive production statistics, and high inspection rates are capabilities of the technology.

Automated tree seedling grading has been demonstrated and is ready for commercialization. Commercial systems are available for measuring root system morphology. Emergence count and bare root seedling inventory applications have been investigated. Computer vision guided robotic sowing, inspection, and thinning of container seedlings have been researched. The versatility and power of computer vision is available to nursery managers as a tool for growing the target seedling.

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Target Root Starch Concentrations Before Storage: Testing the Model[1]

Steven K. Omi and Robin Rose[2]

Abstract.--Because carbohydrate reserves decline with long-term storage, it is important to know whether this depletion will affect subsequent survival and growth after planting. Sufficient target starch concentrations which enable the seedling to buffer itself against reserve depletion during storage, have not been defined. We found little evidence to support the model of a target (pre-storage) root starch concentration. No seedling target can be viewed alone. If the seedling is not preconditioned to grow but reserves are plentiful, a poor correlation between carbohydrate reserves and survival or growth is to be expected.

INTRODUCTION

Carbohydrate reserves have essential functions in trees. Reserves are utilized for maintaining living tissue (maintenance respiration) as well as providing substrates for growth (growth respiration). During certain periods of the year, trees may rely heavily on stored reserves for growth or for buffering against environmental stress and injury (Waring and Schlesinger 1985).

Roots generally contain the largest concentration of nonstructural carbohydrates and are often considered the primary storage organ (Loescher et al. 1990). However, the mechanisms responsible for causing root reserves to be mobilized and how they are translocated are not well understood; additionally, specific relationships between reserve carbohydrates and tree survival or growth have not been clearly established (Duryea and McClain 1984, Loescher et al. 1990).

Carbohydrate reserves occur primarily in the form of starch and sugars, with starch generally being the most abundant form of carbohydrate reserve in tree species (Little 1970, Glerum 1980). Accumulated root starch reserves may supplement spring root growth (Wargo 1979). At the time of initiation, starch concentration in fine roots determines how long the fine roots survive (Marshall and Waring 1985).

The notion of achieving or maintaining a target or optimum amount of stored carbohydrates in nursery seedlings comes from the concept that cultural practices which reduce reserves may ultimately decrease field survival, root growth, and shoot growth (Duryea and McClain 1984, Marshall 1985). Nursery practices that could deplete reserves are those which influence photosynthesis and respiration. These include: a) growing seedlings at high seedbed densities (reducing light), b) inducing dormancy by decreasing irrigation (reducing available soil moisture and increasing leaf temperature), and c) altering fertilizer regime (increasing available nutrients causing large respiratory costs) (Marshall 1985, McNabb 1985).

The potential for depletion of carbohydrate reserves is especially high with long-term cold storage in the dark (McCracken 1979, Ritchie 1982). The fixation of carbon is halted, but respiration continues even at storage temperatures slightly below freezing. Additionally, the photosynthetic machinery may be damaged in storage (McCracken 1978), necessitating recovery and repair of photosynthetic mechanisms after planting. With the popularity of fall lifting and long-term freezer storage in the Northwest (Hee 1986), we ask the question: should seedlings be cultured to achieve target reserve concentrations prior to storage?

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[1]Paper presented at the Combined Meeting of the Western Forest Nursery Council and Intermountain Forest Nursery Association, Roseburg, Oregon, August 13-17, 1990.

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THE MODEL

Marshall (1985) presented a hypothetical situation comparing the carbohydrate reserve concentration of two seedlings at lifting, during storage, and after planting (fig. 1). Both seedlings decline in carbohydrate concentration with storage, but the seedling that survives and grows is the one with sufficient pre-storage reserves (upper line). These reserves provide an adequate buffer for losses due to: maintenance respiration (during storage), re-organization of the photosynthetic apparatus (after planting), and using reserves in preparation for shoot elongation (prior to starch accumulation).

The appropriateness of this model can be examined by determining if there is a relationship between carbohydrate reserves and outplanting growth or survival with and without storage. If different storage treatments create classes of seedlings with different reserve concentrations, we would expect seedlings with very low reserves to die or grow poorly.

TEST OF THE MODEL

Methods

In an ongoing investigation into the effects of fall lifting and long-term storage on seedling physiology (Omi and Schuch 1987), ponderosa pine (Pinus ponderosa) seedlings were lifted 3 times in the fall (Sept., Oct., and Nov., 1987), stored overwinter at -1.5 °C, and compared with seedlings lifted and handled conventionally (Mar., 1988, cold storage, 2-4 °C for 2 weeks). Seed for the bareroot seedlings were sown in 1986 and grown with standard cultural regimes used at the USDA Forest Service Bend Pine Nursery in central Oregon (44° 5' N, 121° 16' W, 1100 m elevation).

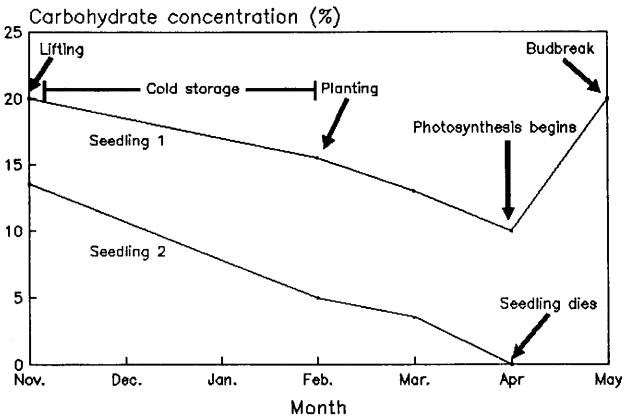

Figure 1.--Hypothetical carbohydrate reserve concentration (% dry weight) of two seedlings at lifting, through storage, and after planting. One seedling (top) survives; the other (bottom), with inadequate reserves, dies (Adapted from Marshall 1985).

Root starch concentration (% dry weight) was determined at lifting and after storage using an enzymatic (α-amylase and amyloglucosidase) digestion method (Rose et al. 1990).

Seedlings were outplanted on a vacant field at the nursery in March 1988. We measured several responses at the end of the growing season, including survival (%), height (cm), growth (cm, final height - initial height), and fascicle length (mm). We also planted seedlings at a forest site in April 1988; however, the ranking of treatments was similar so only the Bend data is included in this paper. Coincident with outplanting, a 30-day root growth potential test was initiated in a greenhouse. We measured the percent of seedlings that initiated new roots, and the dry weight (mg) of new roots.

Data analysis is not complete, so the means and significance of the differences among means should be considered preliminary. To determine the relationship between initial root starch and subsequent field performance, field response variables were regressed against initial root starch.

Results

Root starch concentration declined in storage so that at the time of planting (after storage), there was a significant difference in starch among the treatments (table 1). However, there appeared to be little relationship between initial root starch and subsequent root initiation or field survival and growth. September-lifted seedlings had low root starch, the lowest root initiation and dry weight of new roots, and the poorest field performance. This would be consistent with the model.

On the other hand, November-lifted seedlings had low root starch, high root initiation, and the highest survival and growth (table 1). This result conflicted with the model.

All correlations between initial root starch and field response variable were nonsignificant ($P>0.05$) with the exception of survival. However, initial root starch accounted for only 21 percent of the variation in first-year survival (fig. 2). Field survival and growth appeared to be more closely related to the capacity of seedlings to grow new roots and not initial starch concentration (table 1).

DISCUSSION

In terms of field performance, our ability to create a precise model failed. Carbohydrate reserve status has been qualitatively associated with tree survival or growth (Hellmers 1962, Winjum 1963, Puttonen 1980), but strong quantitative relationships have not been

Table 1.--Root starch concentration (% dry weight) before and after storage, new root initiation (% of seedlings with new roots after 30 days, mg dry weight of new roots), first-year field survival (%), and first-year growth (cm) of ponderosa pine seedlings after four lifting and storage treatments. Means are averaged over 2 seed sources and 4 replications per seed source. Means down a column with different letters are significantly different ($p<0.05$).

Lift date	Root starch (%) after:		Root initiation after 30 days[1]		First-year:	
	lifting[2]	storage	% with new roots	mg new root weight	Survival (%)	Growth (cm)
Sept.	2.3 a	0.04 b	15 c	2 a	24 c	2 c
Oct.	1.8 b	0.06 b	47 b	7 a	66 b	4 b
Nov.	0.9 c	0.05 b	78 a	9 a	84 a	5 a
Mar.	--	1.70 a	80 a	8 a	80 ab	4 b

[1]Root initiation in a 30-day greenhouse test, coincident with outplanting.

[2]After lifting=before storage.

reported (Ronco 1973, Little 1974, Ritchie 1982). Factors such as storage condition, site condition at planting, method of carbohydrate analysis, and reserve carbohydrate quantified (e.g., starch, sugar, or total) probably influence the range of results reported in the literature (Marshall 1985).

Furthermore, carbohydrate reserves and new root growth are generally poorly related (van den Driessche 1978, Ritchie and Dunlap 1980, Ritchie 1982, Rose and Whiles 1984, McNabb 1985, Reid 1986). Current photosynthates or other shoot factors may control new root growth in conifers (Shiroya et al. 1966, Gordon and Larson 1970, Marshall and Waring 1985, van den Driessche 1987), although there are likely to be differences in species response (Philipson 1988).

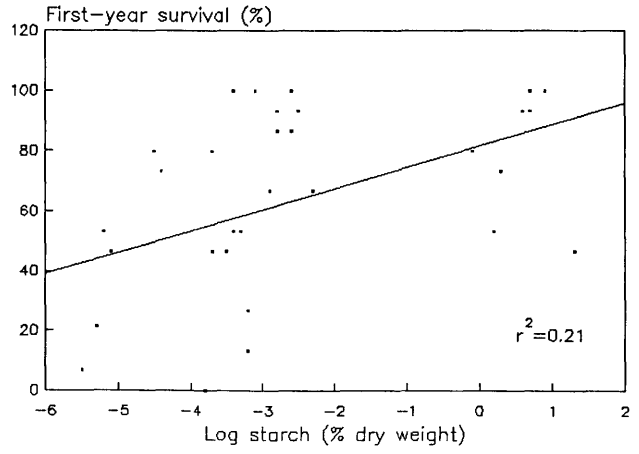

Figure 2.--Relationship between first-year field survival (%) and initial root starch concentration (log % dry weight).

Other factors which could influence root growth include auxin or other plant growth regulators. Auxin stimulates root primordia in tree roots (Coutts 1987). In ponderosa pine, exogenous applications of auxin to seedlings positively affected new root growth, but not the elongation of old roots (Zaerr 1967); however, Lavender and Hermann (1970) could find no positive effect on root growth from external application of growth regulatory compounds. They concluded that a translocatable substance from foliage was necessary for root growth. Zaerr and Lavender (1974) concluded that the substance controlling root growth was not carbohydrate alone.

Therefore, if carbohydrate reserves are available, but the root or shoot is not preconditioned to grow (e.g., having the right balance of growth regulators), then a poor correlation between reserves and growth is to be expected. Douglas-fir (Pseudotsuga menziesii) seedling roots are highly sensitive to exposure in the fall (Hermann 1967); therefore, if any root damage occurs with fall lifting, it will likely alter future performance, irrespective of carbohydrate status. Similarly, if the plant is ready to grow and environmental conditions allow a positive carbon balance, new root growth may be more reliant on current photosynthate (van den Driessche 1987), resulting once again with a

poor correlation. Stored reserves are more important if photosynthesis cannot keep up with respiratory demands (e.g., poor site conditions, van den Driessche 1987).

We only measured starch concentration; yet, sugars can make up a large fraction of the total nonstructural carbohydrate pool (McCracken 1979, Ritchie 1982). Interconversion among carbohydrates is rapid and much more needs to be learned about function and allocation of carbohydrates before we categorize them as metabolically active versus storage (McCracken 1979).

In a current study, we found that seedlings with new roots consistently had less moisture stress and higher root starch content relative to seedlings that do not initiate new roots. Thus, root starch may indicate overall seedling vitality (functioning root system and high water use efficiency) even though its predictive value was questionable in this study. Bigg (1990), however, has preliminary evidence that suggests the doubling of winter root starch concentration in Douglas-fir coincides with the lifting window and the end of dormancy.

CONCLUSIONS

No seedling target can be viewed alone. Plentiful starch reserves are insignificant if the seedling is not ready to grow, or has been damaged. A stressed seedling may accumulate starch if growth is slowed more than photosynthesis (Marshall 1985). Target starch concentrations, in combination with other factors (e.g., nutrients and root volume) will affect performance depending on site conditions. On a favorable site, seedlings with low starch may do as well as seedlings with high reserves. Using starch as a predictor, therefore, has the same problems as using root growth potential alone (Landis and Skakel 1988).

In the study discussed in this paper, there was little evidence to support the model of a target root starch concentration to enhance survival and growth after planting. However, this does not diminish the importance of maintaining reserves. Cultural practices that cause stress could reduce photosynthetic capacity or increase respiratory losses. Inadequate reserves could create nitrogen deficiency because of insufficient carbon substrates for root growth (Loescher et al. 1990). The mobilization of sugars is important for maintaining favorable water relations (Levitt 1980) and may be related to frost hardiness (Sakai and Yoshida 1968, Levitt 1978). Future research for using starch as a target should account for other biochemical or physiological conditions of the seedling, as well as site conditions.

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Moisture Stress and Root Volume Influence Transplant Shock: Preliminary Results[1]

Diane L. Haase[2] and Robin Rose[3]

Abstract--Despite evidence of its economic impact, very little is known about transplant shock. This study was designed to evaluate transplant shock in relation to root volume and soil water content for two year-old Douglas-fir seedlings. Preliminary results found that new growth decreased and days to budbreak increased with higher moisture stress. This effect was most pronounced for high root volume seedlings in the driest soil. Forthcoming results are expected to further implicate moisture stress as an influencing factor in transplant shock.

INTRODUCTION

Transplant shock can be a serious problem to reforestation efforts. A seedling in shock is characterized by "bottle brushing" symptoms (stunted terminal growth with a greater number of needles per unit of leader), browning or loss of needles, cessation of growth, or even death.

This can have quite an economic impact. Mullin (1964) found transplant shock to reduce seedling leader length of white spruce by about 50% in the first year after outplanting. Smith and Walters (1963) found similar results in Douglas-fir. This slow growth, combined with the stressed condition of a seedling in shock, can result in a longer stand rotation age and even plantation failure. Despite the quantitative evidence of its effects, relatively few studies have been published which specifically examine transplant shock. This may be because of the difficulty in assessing such a transient problem.

Although no studies have offered proven causes for transplant shock, most have indicated that the root system's ability to take up water is a most important factor. Following transplanting, a seedling must recover from any damage, reestablish root to soil contact, and resume water and nutrient uptake in a new environment. During this adjustment period, the seedling continues to transpire, resulting in a stressed condition of physiological drought (Rietveld 1989). One study suggests that transplant shock is due primarily to poor root to soil contact after planting when air gaps form at the root-soil interface (Sands 1984). Other studies cite damage to the root system during lifting and handling procedures as a significant factor (Mullin 1963; Stoneham and Thoday 1985). Soil drought further contributes to the stressed condition of the seedling. Kaufmann (1977) found that dry soils significantly reduced growth of Monterey pine (Pinus radiata) seedlings.

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Nursery cultural practices such as fertilization, packing and irrigation have also been examined as possible factors in seedling field performance (Darbyshire 1984; Jopson and Paul 1984; Mellor et al. 1970). However, even seedlings grown under optimum cultural practices commonly go into shock.

A few experiments have been done to attempt to increase drought resistance in planted seedlings by pre-conditioning (Kaushal and Aussenac 1989; Unterscheutz 1974). Although these studies found that drought pre-conditioned seedlings had lower transpiration rates, transplanted seedlings still had reduced terminal shoot growth. Other studies have indicated that cold storage may reduce transplant shock. Jenkinson and Nelson (1984) found that survival potential, growth capacity, and field performance of seedlings stressed after storage approximated that of unstressed seedlings. Blake (1983) found that cold stored seedlings appear better able to avoid transplant shock and early drought despite delayed root growth.

It is unlikely that transplant shock can be entirely eliminated. However, it would be useful to target specific seedling characteristics which are correlated with minimum transplant shock symptoms. These targeted characteristics could be used to supplement current seedling grading criteria. Burdettt (1983) and Sutton (1979) both emphasize the importance of a quality grading system which ensures that stock is best adapted to the planting site.

The objective of our current research is to induce transplant shock in 2+0 Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco.) seedlings by applying moisture stress treatments in a controlled greenhouse environment. The data will be used to better understand the causes of transplant shock and to establish relationships among initial seedling morphological parameters, (specifically root volume), moisture stress, and transplant shock. These relationships could be applied to nursery grading standards in order to select seedlings which are least likely to go into transplant shock following transplanting to a specific site. This paper is a report of our preliminary results and future plans.

PROCEDURES

Plant material

Two year-old (2+0) Douglas-fir seedlings from a BLM northwestern Oregon provenance (seedlot 261-20-01, Western Forest Tree Seed Council, State of Oregon Tree Seed Zone) were grown under standard nursery cultural practices at International Paper's Kellogg Nursery located in western Oregon approximately 10 km south of Elkton. A live tree count before lifting, on January 18, 1990, gave a count of 25 seedlings per square foot.

Following lifting, seedlings were graded to operational specifications. Each tree was measured for height (cm) from bud scar to base of terminal bud, caliper (mm) just below the bud scar, root volume (cm^3) measured by water displacement, and total fresh weight (g).

Treatments

Following measurement, seedlings were divided into four root volume categories (table 1). These categories were determined from the root volume distribution shown in figure 1. Each seedling was then randomly assigned to a moisture stress treatment (table 2) and experimental block.

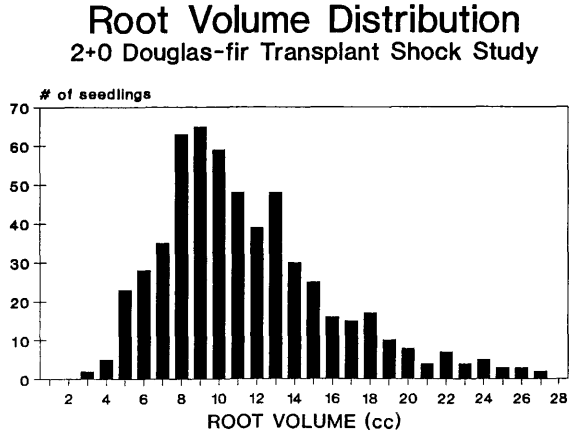

Figure 1.--Root volume distribution of 2+0 Douglas-fir seedlings in transplant shock study.

Table 1.--The four root volume categories used in this transplant shock study.

CATEGORY	ROOT VOLUME (CC)
1	5-8
2	9-10
3	11-13
4	14-20

Table 2.--Moisture stress treatments applied to seedlings in terms of soil water content (%) and soil water potential (MPa).

TREATMENT	WATER CONTENT (%)	WATER POTENTIAL (MPa)
1	6	-1.60
2	12	-0.80
3	18	-0.10
4	24	-0.01

(Field capacity = 42 %)

Seedlings were transplanted into 15 liter plastic pots (five seedlings per pot). The same weight of sterilized soil mix was put in each pot. All pots were thoroughly watered after planting and placed in a controlled greenhouse environment. Fans were used daily for 6 hours to lower greenhouse humidity, encourage normal seedling transpiration, and better simulate a true transplant environment.

Moisture stress treatments consisted of watering all pots to field capacity and letting them dry down to a predetermined soil moisture content and then rewatering to field capacity over a period of 120 days. Moisture stress treatments were selected based on earlier trials and represent a wide range of soil water potentials (table 2).

Soil water content was monitored by weighing the pots and using the following equation:

$$TW = (WC * DS) + DS + P$$

where

TW = Total weight (soil + water + pot)
WC = Water content of desired treatment.
DS = Average weight of dry soil in each pot
P = Weight of pot

(Weight of seedlings was considered negligible)

Pots were weighed two to three times per week to assess water content and were rewatered once they had dried down to their specified water content. Sixty days after transplanting, new terminal length (cm) and lateral length were measured. Days to terminal and lateral budbreak were also monitored.

PRELIMINARY RESULTS AND DISCUSSION

Growth

New terminal and lateral length depended on soil moisture content (fig. 2). Under more moist conditions (18-24% water content), terminal length stayed relatively constant over all root volume categories. On the other hand, with the drier soils (6-12% water content), leader length tended to decline as root volume increased. The effect was most pronounced for the driest soil treatment where seedlings had the greatest reduction in growth at high root volume (fig. 3).

It was not surprising to find that under well-irrigated treatments, root volume had little effect on growth. However, we hypothesized that the highest root volume should have the greatest growth in drier soils because of higher root growth potential and greater absorption capacity (Carlson and Miller 1990). We found the exact opposite.

This apparent inconsistency may be explained by the fact that seedlings with higher root volumes were observed to have a greater number of branches. Therefore, selecting for higher root volume may also be selecting for higher leaf area and hence greater surface area for transpiration. Pots with higher root volumes needed to be watered more often indicating relatively higher water uptake (roots) and demand (leaves). We speculate that demand exceeds uptake by a greater margin in the higher root volume trees resulting in reduced growth.

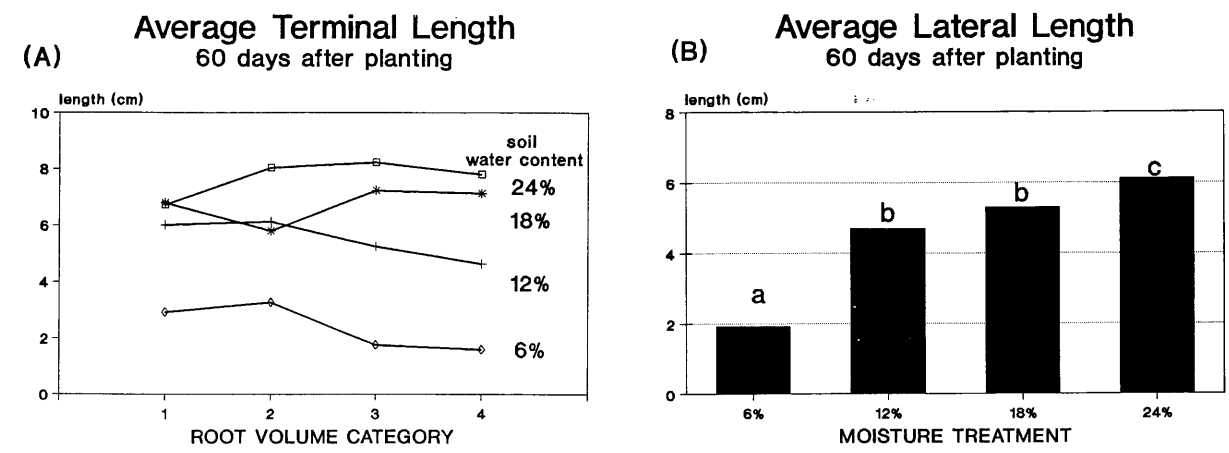

Figure 2.--Growth of seedlings 60 days after planting depended on soil water content: (A) terminal length decreased with higher moisture stress especially for high root volume seedlings; (B) lateral length also decreased with stress, however, there was no root volume interaction.

(A) (B)

Figure 3.--Photographic comparison of moisture treatment effect on high root volume seedlings (14-20 cm^3): (A) treatment 1 (6% water content) exhibiting transplant shock symptoms; (B) treatment 4 (24% water content) showing no evidence of shock.

Budbreak

Similar to the growth measurements, days to terminal and lateral budbreak depended on soil water content (fig. 4). At relatively high water content (18-24%), days to budbreak was generally constant, irrespective of root volume. However, at the drier water content (6-12%), days to budbreak tended to increase with increasing root volume. Once again, the effect was most pronounced with the driest soil (6%), where days to budbreak increased about 30% from root volume category 1 (5-8 cm^3) to category 4 (14-20 cm^3)

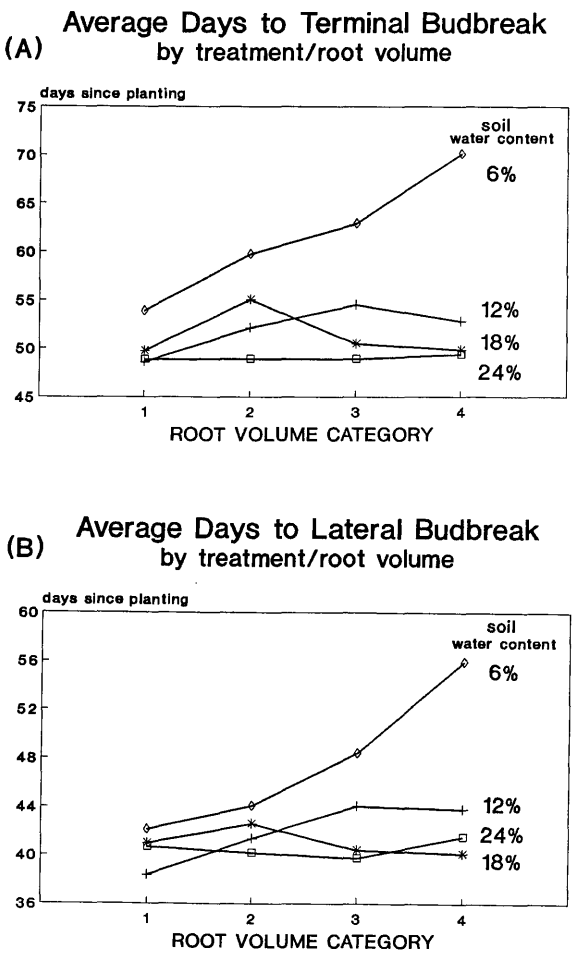

Figure 4.--Average days to budbreak increased with increasing moisture stress, particularly for seedlings in the high root volume category, for both (A) terminal budbreak and (B) lateral budbreak.

Delayed budbreak with increasing water stress has been found in another ongoing study with the Nursery Technology Cooperative (unpublished data) and was expected in this study. Seedlings with large root volumes were expected to be most vigorous (i.e. initiate rapid budburst). However, this was not the case, especially in the driest treatment. As with growth, we speculate that the high root volume seedlings may actually be under greater transpirational stress, despite a high capacity for water uptake.

FURTHER PLANS

Seedlings were harvested in late May, 1990 and measured for transplant shock symptoms such as new terminal and lateral growth, needle length, needles per centimeter on the terminal, and dry weights. Although the data have not been analyzed yet, it appears that both root volume and moisture treatments are significant influences on transplant shock. It is expected that moisture stress will be further implicated as an important causal factor of transplant shock and that the relationship between initial root volume and transplant shock will be better defined.

This study is being repeated with seedlings from the same seedlot which were cold stored for 120 days following lifting. Since this second study is being conducted at a different time, it cannot be statistically compared to the study with unstored seedlings. However, observational differences will be noted. It is expected that the moisture stress or root volume effects may differ between the two studies since the stored seedlings flushed much earlier in the experiment before soil dried to treatment levels.

A complete report of these studies will be prepared for publication in 1991.

ACKNOWLEDGEMENTS

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Discrete Proteins Associated with Overwintering of Spruce and Douglas-fir Seedlings[1]

Dane R. Roberts[2], Peter Toivonen[3], and Stephanie M. McInnis[2]

Abstract.–Seasonal protein changes were followed in seedlings of interior spruce (a mixture of Picea glauca and P. engelmannii) and Douglas-fir (Pseudostuga menziessi) by SDS-PAGE. In seedlings of Douglas-fir a 30 kD protein and interior spruce a 30 and 27 kD protein that were not detected in the late summer, accumulated in seedling tissues during the fall. These proteins remained present throughout the winter, but declined rapidly in seedlings during the initial flush of spring growth. There was an increase in the total protein content of interior spruce seedling tissues during the fall, however, the accumulation of the 30 and 27 kD protein was tissue-specific since it increased in the apical bud, shoot and root tissue but not in the leaves. By late fall these proteins represented approximately 15% of the total seedling protein. These results suggest that conifer seedlings may utilize proteins as a storage reserve during overwintering. The potential of utilizing these "vegetative storage" proteins as biochemical markers of seedling quality is discussed.

INTRODUCTION

Seasonal changes in the nitrogen content of deciduous trees suggests that nitrogen is translocated from the leaves in the fall into the woody tissues, stored in these tissues during the winter and utilized for the first flush of growth in the spring (Kang and Titus 1980; Nelson et al. 1970). Specific proteins have been identified in the phloem tissue of several deciduous trees which accumulate in parallel with seasonal nitrogen fluctuations and are believed to be a storage form of nitrogen. These vegetative storage proteins can represent up to 30% of the total bark protein in overwintering trees and maybe an important source of nitrogen nutrition (Wetzel et al. 1989). Soluble bark protein has been found to increase in the fall in some species of conifers and this has been associated with the development of

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frost hardiness (Pomeroy and Siminovitch, 1970). We report that conifer seedlings accumulate specific proteins in the fall and utilize these proteins during flushing.

MATERIALS AND METHODS

Plant Material

Interior spruce seedlings (seedlot 8534) used in this study were grown as 2-0 container stock by British Columbia Ministry of Forests Nursery at Surrey, British Columbia. For flushing experiments seedlings were considered "overwintered" in early February and brought into the laboratory. These seedlings were kept at room temperature under a 19 hr photoperiod at a light intensity of 70 einsteins/M2/sec. Douglas-fir seedlings were grown as 2-0 container stock by Peltons Reforestation, Maple Ridge, British Columbia. For the study of seasonal changes these seedlings were grown outdoors at B.C. Research Corporation. For protein analysis seedlings were divided into leaf, shoot, apical bud and root tissues and stored at -80oC.

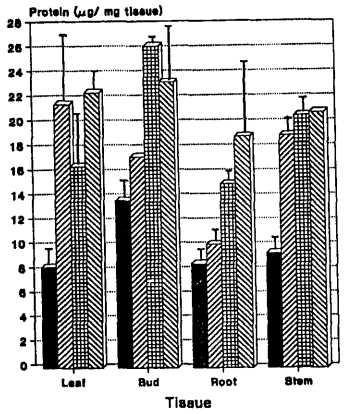

Figure 1. Changes in total protein for different tissues of interior spruce seedlings during the fall of 1988.

Sept. 08 Sept. 29 Oct. 28 Nov. 29

Protein Analysis

Seedling tissues (approx. 200 mg) were ground in a mortar and pestle with liquid nitrogen until a fine powder was achieved. Approximately 20-40 mg of tis-

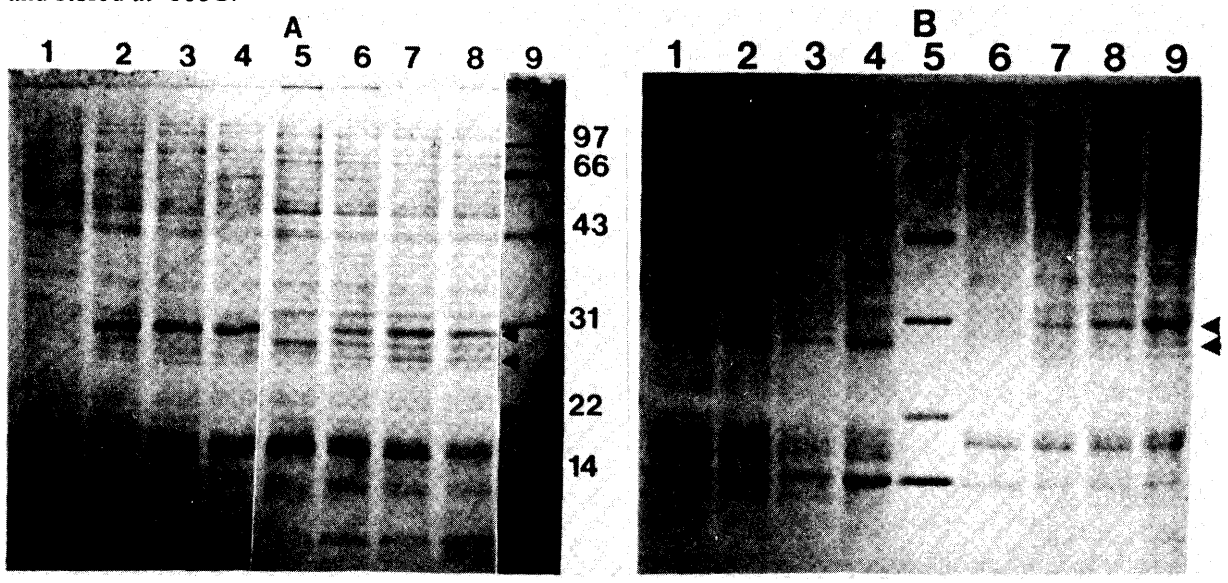

Figure 2. Changes in the protein profile for different tissues of interior spruce seedlings during the fall of 1988. A) lanes 1-4 apical bud; lanes 5-8 shoot; lane 9 mw standards. Lanes 1,5 Sept 8; lanes 2,6 Sept 29; lanes 3,7 Oct 28; lanes 4,8 Nov 29. B) lanes 1-4 leaves; lane 5 mw standards; lanes 6-9 roots. Lanes 1,6 Sept 8; lanes 2,7 Sept 29; lanes 3,8 Oct 28; lanes 4,9 Nov 29. (arrows denote discrete proteins).

sue was placed in a pre-weighed microfuge tube, 5 l/mg tissue of solubilizing buffer (0.125 M Tris-HCl pH 6.8 containing 22.5% mercaptoethanol, 9% sodium dodecylsulfate and 22.5% glycerol) was added and the sample was boiled for 6 - 7 minutes. The homogenate was centrifuged at 16 000 x g for 10 min, the supernatant removed and the sample was stored at -70oC. Sample protein was determined using the method of Ghosh et al. (1988). For analysis of specific protein changes, samples were fractionated by SDS-PAGE on 12% polyacrylamide gels with a 5% stacking gel (Laemmli, 1970).

RESULTS

Interior Spruce

The protein content of the apical bud, shoot, leaf and root tissue of interior spruce seedlings increased during the fall (fig. 1). SDS-PAGE analysis revealed that two specific proteins of apparent molecular weights 30 and 27 kD accumulated during this period (fig. 2). These proteins were not detected in seedlings sampled in early September, began to accumulate in late September and reached a maximum level by the end of October. Apical bud, shoot and root tissue contained equivalent levels of the proteins but they were not detected in the leaves. The temporal appearance of these proteins in the fall was similar among all the individuals tested although there was some variation in the relative amounts of the two proteins in different seedlings. The level of the 30 and 27 kD proteins relative to total protein increased throughout the fall and by late October represented approximately 15% of the tissue protein (table 1).

Table 1. The percentage of total protein represented by vegatative storage proteins of interior spruce during the fall of 1988.

Collection Date	Protein Concentration (% of total protein)
September 8	n.d.[1]
September 29	7.92±3.58[2]
October 28	11.87±6.91
November 29	15.65±5.78

[1]n.d. = not detectable
[2]mean S.D.

When overwintered seedlings were placed in a favorable environment for growth (day 0) the buds expanded by day 7 and a flush of new growth occurred by the end of the three week sampling period. The levels of 30 and 27 kD protein in these seedlings declined to undetectable levels by day 7 in the apical buds, day 12 in the shoot tissue and day 21 in the roots (fig. 3)

Douglas-fir

Seasonal changes in the morphology and protein profiles of the apical bud were followed in seedlings of Douglas-fir (fig. 4). Bud scales began to develop on seedlings in early September and by late September visual inspection suggested that bud development was complete since no further morphological changes were noted until spring. A 30 kD protein began to accumulate in the bud tissue in early November and by late November this protein had reached its maximum level and remained at this level throughout the winter. The apical bud began to swell in early April and by the middle of the month, needles were protruding from the bud scales. The levels of the 30 kD protein had declined to undetectable levels in seedlings by early April.

DISCUSSION

Recently, specific storage proteins have been identified in vegetative tissues of deciduous trees such as elderberry and poplar (Greenwood et al. 1986; Sauter et al. 1988; Wetzel et al. 1989). These vegetative storage proteins can represent up to 30% of the tissue protein in the overwintering trees and it is believed that they contribute significantly towards nitrogen nutrition during spring flush (J. Greenwood, Univ. of Guelph, Ontario, Personal communication). Proteins are classified as storage molecules based on their accumulation during the fall in preparation for overwintering, their high concentration in dormant seedlings, and their rapid decline during flushing of overwintered seedlings. Based on the

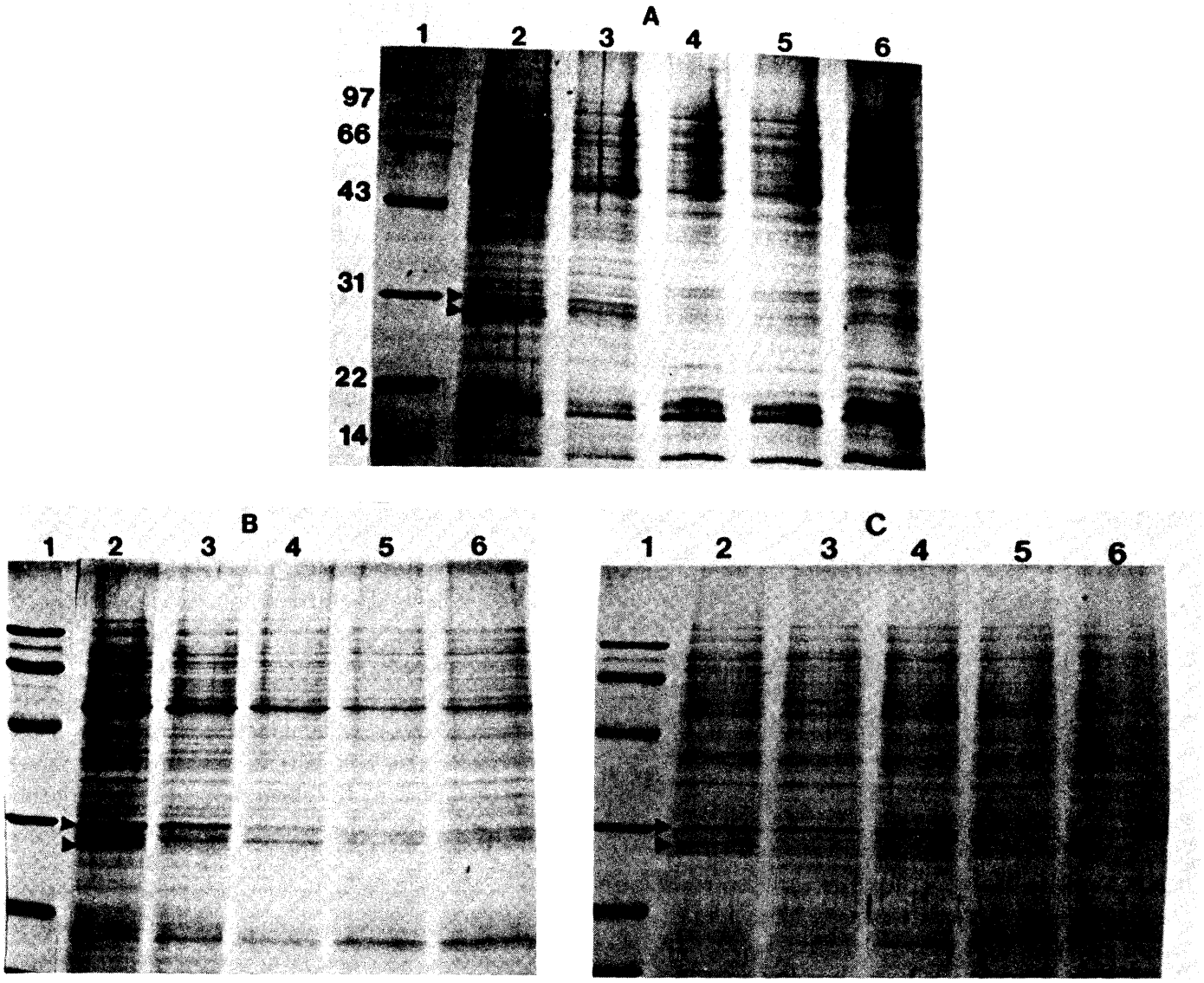

Figure 3. Changes in the protein profile for different tissues of interior spruce seedlings during flushing of overwintered seedlings. A) apical bud; B) shoot; C) roots; For A, B & C lane 1 mw standards; lane 2 day 0; lane 3 day 3; lane 4 day 7; lane 5 day 12; lane 6 day 21. (arrows denote discrete proteins).

seasonal changes in the 30 kD protein of Douglas-fir and the 30 kD and 27 kD proteins of interior spruce, it is possible that these proteins are accumulated for overwintering and used as a source of nutrition during early spring growth. Furthermore, that fact that the same protein (based on molecular weight) shows similar seasonal changes in two different species suggests an important role for this protein during the overwintering process.

Conifer seedlings utilized for forest regeneration are generally grown in the nursery, lifted in the fall, overwintered in cold storage and planted in the spring. Perhaps the most crucial time to evaluate seedling quality is to determine the lifting date for cold storage, since lifting date can have a dramatic effect on seedling quality (Burdett and Simpson, 1984; Cannell et al. 1990). However, this is also a difficult time to evaluate seedling quality since the seedling is in various stages of quiescence and dormancy.

To date it has proven difficult to identify morphological or biochemical attributes of forest seedlings that can be used to evaluate their potential performance.

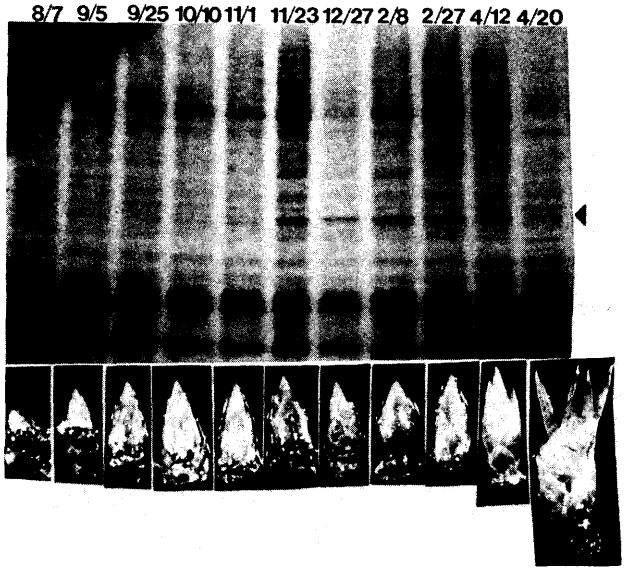

Figure 4. Seasonal changes in apical bud morphology and protein profiles of Douglas-fir seedlings. Numbers above each lane denote the collection date and photographs below each lane show the bud morphology of that sample.

The nutritional status is one attribute that can be intrinsically related to seedling growth potential. The use of macro/micro nutrients and carbohydrate reserves to evaluate seedling quality appears to be limited by fluctuations of these compounds that occur throughout the growth season due to stress and diurnal changes (Marshall, 1985; Landis, 1985). In contrast, the vegetative storage proteins only accumulate during the stage of seedling development associated with bud formation and acquisition of dormancy. Studies are underway to determine the relationship between the accumulation of the vegetative storage proteins, dormancy and seedling quality of interior spruce seedlings.

Their also appears to be a relationship between the development of frost hardiness during the fall, lifting date and seedling quality (Burr et al. 1989). Currently, nursery growers rely on a frost hardiness test to determine the time to lift seedlings, but this technique can take up to two weeks. Pomeroy and Siminovitch (1970) found that soluble protein increased in bark and needles of mature red pine during the winter and that this increase was associated with the acquisition of frost hardiness. The accumulation of 30 kD proteins during the fall may also be associated with the development of frost hardiness and preparation for overwintering.

We believe that the possible role of vegetative storage proteins as storage reserves and in frost hardiness make them potential biochemical markers for seedling quality. Their use as biochemical markers is facilitated by the fact that they only accumulate during the stage of seedling development associated with dormancy, frost hardiness and the preparation for overwintering. If we can establish the relationship between the vegetative storage proteins and seedling quality an enzyme-linked immunosorbant assay (ELISA) can be developed so that nursery growers can perform a simple and rapid colorometric assay to determine the amount of protein present in nursery grown seedlings.

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Mitotic Index of Conifer Shoot Tips: Processing, Sampling, and Data Interpretation[1]

James Grob[2]

Abstract.--A standardized methodology does not exist for determining the mitotic index (MI) of conifer shoot tips. A Feulgen staining procedure coupled with horizontal scanning at fixed vertical intervals is proposed as a reliable and repeatable method to determine MI. Anatomical and cell cycle factors which affect mitotic activity and influence interpretation of MI data are discussed.

INTRODUCTION

Mitotic index (MI), the percentage of cells in mitosis, has been successfully used to observe changes in seedling shoot apices of conifers under natural and experimental growth conditions using squash preparations (Carlson et al. 1980, Carlson 1985, Colombo et al. 1989, O'Reilly et al. 1989). However, the anatomical region(s) of tissue squashed, as well as the staining and sampling procedure has varied. This has made comparison between studies difficult, especially in terms of the magnitude of MI. As use of MI increases a standardized methodology to determine and interpret MI data is needed.

A practical squash technique should allow many samples to be processed easily and quickly, allow short term storage at certain stages, and consistently produce high quality permanent preparations. A practical sampling procedure should allow rapid and objective sample selection of a minimum number of cells per squash, adequately represent the mitotic activity of the whole apex, allow sampling of large and small apices without major procedural modification, and produce a small standard error. This was accomplished with improved Feulgen staining techniques and fixed interval horizontal scanning.

MATERIALS AND METHODS

Preparation of Shoot Tips

1. Dissect down to last 2-3 primordia or bud scales covering the apical dome.

2. Fix in cold, 4° C 10% neutral formalin for a minimum of 24 hours (storage stage). Sampling apices for fixation should be done at a constant time of the day such as predawn (Carlson 1985) in order to avoid diurnal variability.

3. Wash in cold distilled water for 24 hours, changing the water 3 times.

4. Hydrolyze in 5N HCl at 20° C for 50-60 minutes (optimum hydrolysis duration may vary slightly between species).

5. Stain in Schiff's reagent (use basic Fuchsin) for 2 hours in the dark.

6. Wash 3 times in SO_2 water for 30 minutes total.

7. Store in 4° C distilled water until needed (storage stage).

Dissection and Squash Preparation:

1. Remove remaining bud scales and/or primordia. Use a triple 0 insect pin or microscalpel (Beaver Microsharp 7511) to cut across the base of the apical dome

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at point of insertion of the last primordium.

2. Use the microscalpel to lift apex away from remaining tissue and place in a drop of 45% acetic acid on a frosted slide.

3. Place a 22 X 22 mm coverslip over dome. Use the eraser end of a pencil to gently squash the dome without causing lateral movement of the coverslip (which can cause cell shear).

4. Place slide face up on a block of flat dry ice until frozen (approximately 30 seconds).

5. While the slide is still on the dry ice, use a fine, double edge razor blade to pry the coverslip from the slide. The squash will remain on the slide.

6. Quickly place the slide into 95% alcohol for 2-3 minutes, then 100% alcohol for 2-3 minutes.

7. While still wet with 100% alcohol mount with a new coverslip in euparal (Carolina Biological Supply).

Sampling the Squash Preparation

1. Use a square ocular counting grid with defined median vertical and horizontal lines which produce a sampling point (Fig. 1). Determine the vertical distance from the top and bottom horizontal lines to the sampling point under 40X magnification (this should be around 100-200 microns). This is your vertical interval.

2. Place the top horizontal line at top of the squash and move the sampling point to left of the squash (Fig. 2).

3. Scan from left to right counting any nuclei or chromosomes which make contact with the sampling point. Do not sample brown, tannin containing cells of the pith which are often found in the center of the squash or elongate nuclei which are from the procambium. Count only one metaphase, anaphase or telophase figure per pair since both figures represent one mitosis.

4. At the end of a scan find a distinctive nucleus or mitotic figure which intersects the bottom horizontal line. Move the sampling point to this position then move it back to the left side of the squash and begin the second scan. Continue scans until the squash is completely sampled (Fig. 2).

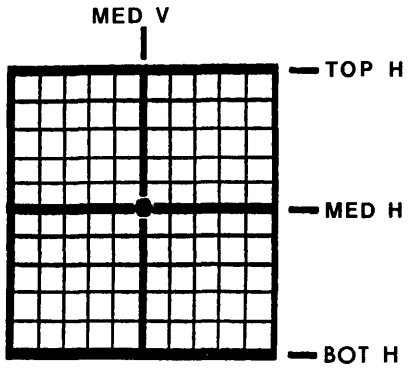

Figure 1.--Diagrammatic representation of ocular counting grid used in horizontal scanning. The sampling point is created by the cross section of the median horizontal (MED H) and median vertical (MED V) lines of the grid. A constant vertical interval between scans is maintained by using the distance from the top or bottom horizontal lines (TOP H or BOT H) to the median horizontal line (MED H).

5. Determine MI by the following equation:

MI=(# mitotic figures counted/ total cells counted) X 100.

6. When analyzing MI data statistically use the arcsin transformation to normalize the data. Do not multiply by 100 when using the arcsin transformation.

To test this sampling technique 10 squash preparations of loblolly pine seedling shoot apices in free growth were sampled using a vertical sampling interval of 200 microns and then resampled at a 40 micron interval. The 40 micron interval did not sample every cell in the squash but was sufficiently intensive to determine if horizontal scanning at 200 microns was an accurate measure of MI. The effect of vertical interval on mean MI was compared using a paired t-test after arcsin transformation.

RESULTS

Squash preparations using the Feulgen reaction produce visually excellent, permanent preparations of nuclei and mitotic figures (Fox 1969, Greilhuber 1986). This visual clarity allows early prophase and late telophase figures to be identified. Prophase figures are identified by nuclei with visible chromosomes which in early stages appear granular with a lobed perimeter, while

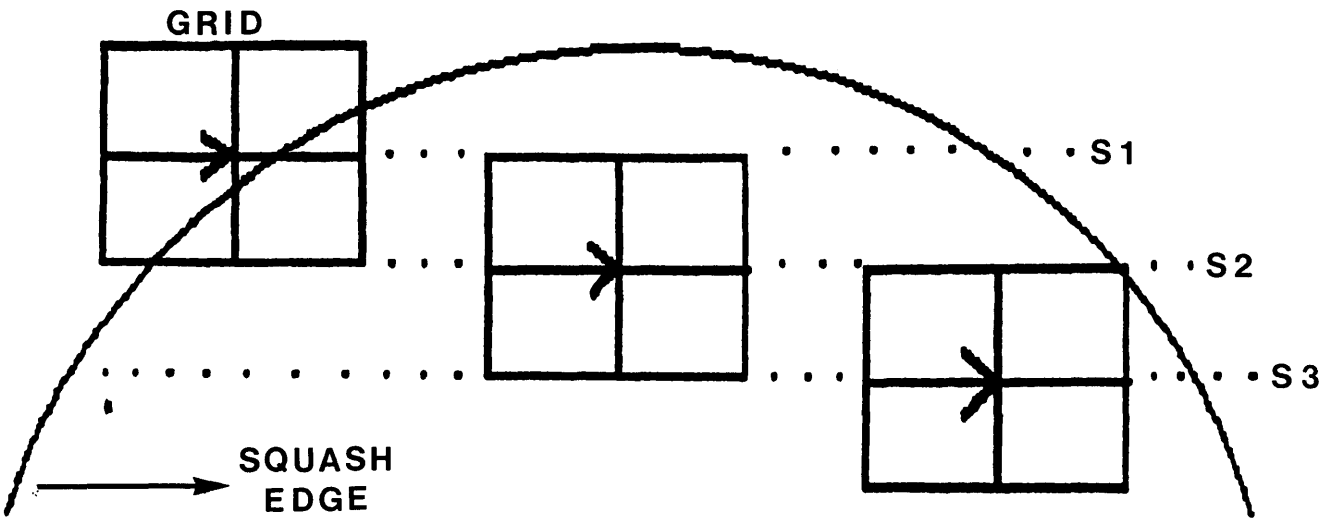

Figure 2.--Diagrammatic representation of sampling for mitotic index using a series of horizontal scans (S1,S2,S3) at a fixed vertical interval.

telophase figures are identified by paired asymmetrically shaped, reforming nuclei which often have a convoluted perimeter. When more than one observer is measuring MI, observers should be in visual agreement on what constitutes prophase and telophase figures.

Since preparations are made permanent (Conger and Fairchild 1953), sampling to determine MI does not have to take place at the same time as squash preparation. This feature in conjunction with the two storage stages during the staining process offer considerable flexibility for preparing and sampling squashes when time is available. This is advantageous in seedling studies where other physiological parameters may require more immediate measurement. With practice, dissection and squash preparation becomes rapid and routine.

Horizontal scanning at 200 micron intervals is both a rapid and objective method to determine MI. With practice a squash preparation can be measured in 5-8 minutes. The intensive 40 micron interval was time consuming, increased the number of cells counted five-fold and resulted in a insignificant difference in mean MI (Table 1). Horizontal scanning at 200 micron intervals represents an accurate method to determine the MI of loblolly pine shoot apices. An appropriate vertical interval should be determined in this way for each conifer species since differences in cell size and apex size are likely.

Table 1.--Mitotic index assessed by horizontal scanning at 2 vertical intervals.

Vertical interval (microns)	Mean cells counted/apex	Mean MI	Standard Error
200	430	6.42	.40
40	2199	5.99	.23
Paired t-test		t=1.41	p=.192

DISCUSSION

The anatomical components of the shoot tip must be recognized when preparing squash preparations. These include the shoot apex, foliar primordia, bud scales and the shoot axis. These regions vary in the magnitude and timing of their mitotic activity during the annual cycle of growth (Owens and Simpson 1988, Fielder and Owens 1989). Preparation of specific anatomical regions will avoid these difficulties. The apical dome represents the most appropriate region for squash preparation since it generally lacks the high levels of cellular tannins and vascular tissue present in other regions. Measurement of apical dome height and width

on freshly dissected apices before fixation provides additional information since apex size correlates with stage of development and dome activity (Fielder and Owens 1989).

Mitotic index determined by horizontal scanning provides an accurate measure of the mitotic activity of the entire apex (Grob 1990). However, as MI approaches zero in winter, horizontal scanning will not be an accurate measure of when mitosis ceases. Previous studies (Carlson et al. 1980, Carlson 1985, Colombo et al. 1989) have measured MI in the most active region of the apex. This was a conservative measure of when mitosis began or ceased, but represented an inflated measure of the mitotic activity of the apex.

The objectives for examining mitotic activity will determine the measurement method used. To determine when mitosis ceases, the scanning method is not necessary. The visual presence or absence of mitosis in the entire squash without determining MI is adequate. However, where relative changes in MI are required or in species such as loblolly pine which never reach a MI of zero during winter (Carlson 1985), the scanning method should be used. If desired, both MI and the presence/absence of mitosis can be determined concurrently.

The cell cycle is a fundamental concept at the cellular level. The cell cycle consists of 4 stages; two "gap" stages (G1 and G2), a period of DNA synthesis (S), and mitosis (M). Mitotic index is a useful measure because it is responsive to changes in two cell cycle parameters; the duration of the cell cycle (the time taken to proceed through G1, S, G2 and M) and the growth fraction (the proportion of the cell population proceeding through the cell cycle).

Changes in cell cycle duration and the growth fraction alter interphase stages in relation to mitosis. This changes the proportion of cells in mitosis at any time t, and therefore MI (Walker 1954). G1 is a particularly important interphase stage since it is the longest cell cycle stage (Miksche 1967), and because most cells in the shoot apex accumulate in this stage during the fall and winter as MI reaches zero (Owens and Molder 1973, Cottignies 1979).

This close relationship between MI, interphase stages and cell cycle status is the rationale for using MI as a measure of seedling dormancy. When interpreting MI data these relationships should be considered. Measurement of MI complements current physiological and developmental tests of seedling quality, and will provide a useful cellular parameter to correlate future tests which use biochemical and molecular methods.

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Effect of Styroblock Design and Copper Treatment on Morphology of Conifer Seedlings[1]

Gary A. Hunt[2]

Abstract.--Interior Douglas-fir, lodgepole pine, and white spruce were used to determine the effects of cavity volume, styroblocks modified with vertical ventilation holes, and copper coating on seedling morphology.

Decreasing cavity volume from 60 to 50 ml resulted in smaller shoots and heavier roots in Douglas-fir and spruce and could be an aid to limiting height growth.

Venting did not affect morphology greatly, but modestly increased height, diameter, and total seedling weight in Douglas-fir. In pine grown in 39 ml cavities, venting was detrimental to overall balance

Copper treatment stimulated shoot growth in Douglas-fir, but had little effect on growth of pine shoots. Copper increased root fibrosity and stimulated growth of the mycorrhizal fungus *Thelephora terrestris* in pine.

Judicious selection of container type can help nursery managers obtain desired morphology, minimize cull, and improve the potential for good field performance.

INTRODUCTION

Morphological characteristics of seedlings often affect field performance. The primary goal of manipulating seedling morphology is to produce stock capable of tolerating stresses likely to be encountered on planting sites. In the Interior of British Columbia (B.C.), the primary stresses are frost, drought, heat, and mechanical damage.

Changing the spacing and volume of cavities can be used to alter seedling morphology (Tanaka and Timmis 1974). Growth data from seedlings grown in styroblocks with varying density and volume can be used to determine which block types have the greatest potential to optimize specific parameters such as height, diameter, or root weight. The objectives of cavity volume experiments were to find alternative block types that (1) improve height control in spruce and (2) improve root weight and overall balance in Douglas-fir.

Venting is a recent addition to styroblock design. Holes a few millimeters in diameter extend through the body of the styroblock at every intercavity intersection. The holes increase ventilation in the seedling canopy by allowing air to circulate between the top and bottom of the block. Studies have demonstrated that incidence and severity of gray mold (*Botrytis cinerea*) are reduced by venting (Peterson and Sutherland 1990). The objective of this experiment was to determine if venting alters morphology in Douglas-fir or lodgepole pine.

Coating containers with latex paint containing cupric carbonate ($CuC0_3$) is an effective way to increase the number of fine roots and root growth, especially in the upper part of the root plug (Burdett and Martin 1982, Wenny and Woollen 1989). However, these changes have not generally been reported to improve field performance (Wenny 1988). The objective of this experiment was to determine if copper coating altered root weight or shoot growth in Douglas-fir or lodgepole pine.

Nursery managers can take advantage of the diverse types of styroblocks available commercially and use them as an additional tool to obtain desired morphology.

METHODS

Seeds were sown at the Heffley Reforestation Centre Ltd., Kamloops, B.C. (Lat. 50° 51' N, Long. 120° 16' W). Studies were conducted during 1989 except the cavity volume experiment with white spruce which was done in 1988. The growing medium was composed of peat and vermiculite (4:1, v:v) with 1.4 Kg/m^3 dolomite lime. Micromax Micronutrients (Sierra Chemical Co.) was incorporated at 385 g $/m^3$.

Table 1 lists the types of styroblocks (Beaver Plastics, Edmonton, AB) tested. Type 198/50 was used in the copper treatment experiment. Comparison of vented and nonvented blocks was done in type 198/50 for Douglas-fir and type 240/39 for lodgepole pine.

Seedlings were reared in a greenhouse until mid-June, when the greenhouse cover was removed. Seedlings were lifted in November. Photoperiod was extended to 18 hours until 29 June using high pressure sodium lamps.

Soluble fertilizer was applied in all experiments according to the following sequential schedule: Peters Conifer Starter (7-

[1]Paper presented at the Western Forest Nursery Council 1990 Conference. [Umpqua Community College, Roseburg, Oregon, August 13-17, 1990].

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40-17, W. R. Grace Co.) was applied over 5 weeks, beginning 4 weeks after sowing, at an average rate of 28 ppm N; Peters Conifer Grower (20-17-19) for 8 weeks at 80-100 ppm N; and Plant-Prod Finisher (8-20-30, Plant Products Co. Ltd.) for 12 weeks at 50 ppm N.

Table 1. Types of styroblocks used in this study

Metric	US	Cavities/ Block	Cavity Volume (ml)	Density Cav./m^2
240/39 (211A)	2A	240	39	1238
198/60 (313A)	4A	198	62	1000
198/50 (312)	---	198	50	1012
112/106 (415B)	6	112	106	571

To assess growth, 30 trees (5 from each of 6 blocks) were selected randomly from each treatment. The outer two rows of trees in a sampled container were excluded to remove edge effects. Shoot length and root collar diameter were recorded. Seedlings were separated at the root collar, dried at 100°C for 24 hours, and weighed.

Growth was compared to B.C. Forest Service standards for each species which indicate cull and target values for height, root collar diameter, and root weight (Table 2). Standards have not been established for the other parameters listed in the growth tables. The Dickson Quality Index (Dickson *et al.* 1960) is a measure of seedling balance and is calculated as: dry weight / [height-diameter ratio + shoot-root ratio].

Table 2. B.C. Forest Service target and cull specifications for stock types used in this study

Tree Species	Stock Type	Height (cm)	Diameter (mm)	Root Dry Wt. (g)
Douglas-fir	[1]198/60	18 [2][12 & 25]	3.0 [2.2]	0.6 [0.4]
	112/106	20 [15 & 30]	3.5 [2.8]	0.8 [0.5]
Lodgepole Pine	240/39	12 [7 & 17]	2.5 [2.2]	0.5 [0.3]
	198/60	15 [7 & 20]	3.0 [2.5]	0.7 [0.5]
White Spruce	198/60	17 [12 & 25]	3.0 [2.4]	0.7 [0.5]

[1]Standards for 50 ml stock types are the same as 60 ml for the respective species. [2] Numbers in brackets are cull specifications; heights are minimum and maximum.

To determine recovery, the average percentage of acceptable seedlings (according to B.C. Forest Service standards) from five randomly selected blocks per treatment was calculated.

Data were subjected to analysis of variance and the F-test (P = 0.05) used to separate treatment means except in the Douglas-fir cavity volume experiment where significant differences were indicated by the Scheffe' method of multiple comparisons (P = 0.05).

RESULTS

Cavity Volume

Douglas-fir reared in 50 or 106 ml cavities differed substantially from 60 ml controls for most measured parameters (Table 3). Seedlings from 50 ml cavities had similar overall balance and recovery, but smaller shoots, more root mass and lower shoot-root ratios than controls. Stock from 106 ml cavities differed from 60 ml controls in all parameters except shoot-root ratio. Quality Index of the larger trees was more than twice the controls while recovery improved by about 30%.

Table 3. Growth of Douglas-fir reared in 50 ml, 60 ml (control), or 106 ml containers

	60 ml	50 ml	106 ml
Shoot Height (cm)	26.4a	17.9b	30.1c
Diameter (mm)	2.87a	2.65b	3.95c
Shoot Weight (g)	1.60a	1.01b	2.77c
Root Weight (g)	0.65a	0.72a	1.23c
Shoot:Root Ratio	2.5a	1.4b	2.4a
Height:Diameter Ratio	9.3a	6.8b	7.8c
Total Weight (g)	2.26a	1.73b	4.00c
Quality Index	0.20a	0.22a	0.42b
Recovery (%)	66a	67a	86b

Reading across, means within rows followed by a different letter are significantly different by the Scheffe' method (P = 0.05).

Compared to controls, spruce grown in 50 ml cavities (Table 4) had shorter shoots, a substantially greater root mass, and somewhat better balance. Diameter and total weight did not differ significantly between treatments.

Table 4. Growth of white spruce reared in 50 ml or 60 ml containers

	60 ml	50 ml
Shoot Height (cm)	19.6a	17.5b
Diameter (mm)	2.99a	2.84a
Shoot Weight (g)	1.78a	1.62a
Root Weight (g)	0.71a	0.92b
Shoot:Root Ratio	2.6a	1.9b
Height:Diameter Ratio	6.6a	6.2b
Total Weight (g)	2.49a	2.53a
Quality Index	0.27a	0.32b

Reading across, means within rows followed by a different letter are significantly different by the F-test (P = 0.05).

Vented Styroblocks

Venting affected growth of Douglas-fir and lodgepole pine (Tables 5, 6). Venting significantly increased shoot height, diameter, total weight,and recovery in Douglas-fir. In pine, venting increased height, but reduced diameter, root weight, and Quality Index.

Table 5. Growth of Douglas-fir reared in vented or nonvented 50 ml containers

	Nonvented	Vented
Shoot Height (cm)	17.9a	20.3b
Diameter (mm)	2.65a	2.83b
Shoot Weight (g)	1.01a	1.25b
Root Weight (g)	0.72a	0.77a
Shoot:Root Ratio	1.4a	1.6b
Height:Diameter Ratio	6.8a	7.2a
Total Weight (g)	1.73a	2.02b
Quality Index	0.22a	0.23a
Recovery (%)	67a	83b

Reading across, means within rows followed by a different letter are significantly different by the F-test (P = 0.05).

Table 6. Growth of lodgepole pine reared in vented or nonvented 39 ml containers

	Nonvented	Vented
Shoot Height (cm)	18.3a	20.5b
Diameter (mm)	2.96a	2.50b
Shoot Weight (g)	1.11a	1.11a
Root Weight (g)	0.58a	0.46b
Shoot:Root Ratio	2.0a	2.5b
Height:Diameter Ratio	6.4a	8.2b
Total Weight (g)	1.57a	1.69a
Quality Index	0.21a	0.15b
Recovery (%)	69a	70a

Reading across, means within rows followed by a different letter are significantly different by the F-test (P = 0.05).

Copper Treated Styroblocks

Copper treatment produced larger shoots, greater dry weight, and higher recovery in Douglas-fir compared to controls (Table 7). Root weight and Quality Index were not affected by copper treatment.

In lodgepole pine, copper treatment had little effect on morphology or recovery (Table 8). The treatment increased diameter and decreased root weight somewhat compared to controls.

DISCUSSION AND CONCLUSIONS

Conclusions about the effect of seedling morphology on performance after outplanting are valid only if it is known that the seedlings tested had approximately the same physiological condition. Because physiological condition is generally not reported in published studies comparing morphology, it is often difficult to separate the influence of morphology and physiology on performance.

In general, seedlings with suitable diameter and good shoot-root balance can best avoid or tolerate stresses common on plantation sites in B.C. (Mitchell *et al.* 1990, Chavasse 1980, Thompson 1985).

Table 7. Effect of copper treated styroblocks on growth of Douglas-fir reared in 50 ml containers

	Control	Copper
Shoot Height (cm)	17.9a	19.8b
Diameter (mm)	2.65a	2.86b
Shoot Weight (g)	1.01a	1.26b
Root Weight (g)	0.72a	0.79a
Shoot:Root Ratio	1.4a	1.7b
Height:Diameter Ratio	6.8a	6.9a
Total Weight (g)	1.73a	2.04b
Quality Index	0.22a	0.24a
Recovery (%)	67a	81b

Reading across, means within rows followed by a different letter are significantly different by the F-test (P = 0.05).

Table 8. Effect of copper treated styroblocks on growth of lodgepole pine reared in 50 ml containers

	Control	Copper
Shoot Height (cm)	20.2a	19.7a
Diameter (mm)	2.76a	3.04b
Shoot Weight (g)	1.32a	1.42a
Root Weight (g)	0.66a	0.57b
Shoot:Root Ratio	2.0a	2.6b
Height:Diameter Ratio	7.4a	6.5b
Total Weight (g)	1.98a	1.98a
Quality Index	0.21a	0.22a
Recovery (%)	72a	70a

Reading across, means within rows followed by a different letter are significantly different by the F-test (P = 0.05).

Cavity Volume

Douglas-fir 50 and 60 ml Cavities

The smaller shoots of seedlings grown in 50 compared to 60 ml containers resulted in greater sturdiness as indicated by the smaller height-diameter ratio. Generally, for sites in B.C., height-diameter ratio should be less than 8 (Mitchell *et al.* 1990). Stockier trees could be an advantage on some sites; they provide better support and resist bending from debris, snow, or trampling.

A shift in biomass distribution from shoots to roots is expected when the ratio of cavity density to soil volume is increased (Tanaka and Timmis 1974). Data comparing 50 and 60 ml cavities for both Douglas-fir and spruce (Tables 3,4) conform to this principle, i. e., shoot weight decreased while mean root weight increased in 50 ml cavities. Other factors that may have influenced growth include reduced nutrient availability in 50 ml cavities due to smaller root volume and reduced height of 50 ml cavities. The cavity size and density provided by 198/50 containers appears to be an effective way to keep height closer to target level and reduce shoot-root ratio.

Overall balance, as indicated by the Quality Index, was not affected by reducing cavity volume from 60 to 50 ml. The

positive effect of improved shoot sturdiness in 50 ml cavities was offset by reduced total weight.

Douglas-fir 60 and 106 ml Cavities

Based on data from the Heffley Reforestation Centre and other sources (Dickson *et al.* 1960, Payandeh and Wood 1988, Roller 1977), we have established a minimum value of 0.20 for the Quality Index of Douglas-fir and spruce grown in 50 or 60 ml cavities. The substantially greater Quality Index of the 106 ml stock (Table 3) may be significant for plantation performance (Ritchie 1984). Payandeh and Wood (1988) found that Quality Index was a significant factor in predicting performance over a variety of site conditions in northern Ontario.

Where high temperature is a problem, large diameter stock is more resistant to damage because of better heat dissipation away from the stem (Cleary *et al.* 1978). Given equal physiological condition, seedlings grown in larger containers have better performance potential than those grown in small containers (Cleary *et al.* 1978). However, improved performance of larger stock may not offset the higher cost. Douglas-fir 112/106 seedlings sell for 8 to 10 cents Canadian (50-63%) more than 198/60 stock in B.C. Additional tests are needed to establish the field conditions under which large stock types are cost effective.

The 20% greater recovery in larger stock is enough to be a significant cost factor to nursery operations. Analysis of culls indicated that the major reason for improved recovery was fewer over-height/under-diameter trees.

White Spruce 50 and 60 ml Cavities

Although shoot-root ratio, height-diameter ratio, and Quality Index of 50 ml stock were statistically improved compared to 60 ml stock, the differences are not likely to be biologically significant. Measurements of both treatments were well within acceptable standards. As in Douglas-fir, it may be easier to limit height and boost root weight by use of the smaller cavity size.

Vented Styroblocks

Douglas-fir

Taller shoots of Douglas-fir and pine stock grown in vented blocks was not expected. Vented blocks dried out more rapidly than unmodified blocks (up to 30% faster, G. Hunt unpublished data) and presumably were under greater moisture stress during some of the growing season. The unusually cloudy and rainy summer weather experienced in the southern Interior in 1989 may have minimized this drying effect. Greater dry weight of seedlings in vented blocks suggests that improved air circulation resulted in a higher rate of photosynthesis or that growth was suppressed in nonvented blocks. Slower drying of nonvented blocks following irrigation may have resulted in longer periods of saturation accompanied by anaerobic conditions in the root zone; perhaps this reduced root respiration and nutrient uptake.

Reasons for the increased recovery from vented blocks is not clear. Analysis of culls was not conducted on these treatments. Because Douglas-fir did not have a high incidence of gray mold at the nursery last year (losses ranged from 0 to 18% in other stock types), it is unlikely that this accounted for the recorded increased recovery. This study is being repeated in 1990 for clarification.

Lodgepole Pine

Stock from vented blocks was not of high quality (Table 6). Increased height and reduced diameter resulted in greater losses due to over-height/under-diameter. Analysis showed that 35% of culls from vented blocks were defective due to over-height/ under-diameter compared to 2% for stock from unmodified blocks. In addition, vented stock had an average root weight below target (0.5 g) and a height-diameter ratio exceeding the recommended value of 8 (Mitchell *et al.* 1990). Data on Quality Index for pine (Hunt, unpublished) indicate that the score of 0.15 recorded for vented stock is quite low. Stock grown at this relatively high density and small cavity size sometimes does not achieve a Quality Index of 2.0, but scores below 0.18 are not common.

The incidence of gray mold did not differ significantly between block types and resulted in losses of about 10% in both treatments. This contrasted with stock from 198/50 vented and nonvented blocks where venting resulted in an 8% reduction in loss due to gray mold (data not shown). Perhaps the denser canopy in 240/39 blocks did not permit significantly increased air flow in spite of venting.

Copper Treated Styroblocks

Douglas-fir

Copper treatment clearly altered root morphology by increasing root branching and the number of fine roots. Perhaps the increased absorption area improved nutrient uptake resulting in larger seedlings. It is unlikely that higher levels of copper in the tissue stimulated growth. Nontreated seedlings were not copper deficient and contained 11 ppm copper (4-20 ppm is the optimum range) at season's end. This compared to 22.3 ppm copper in treated seedlings.

The altered root morphology may have contributed to improved recovery, but data were not recorded to confirm this. Inadequate root development, particularly in the upper part of the root plug, is a common problem in Interior Douglas-fir.

Lodgepole Pine

Copper treatment substantially increased root branching and root system fibrosity. Because the tap root is usually pruned and fewer large diameter lateral roots are present, treated root plugs are more flimsy and lack the rigidity of nontreated plugs. This may make them more susceptible to "J rooting" from improper planting. Copper treatment did not reduce cull due to poor roots; 36% of culls in both treatments had inadequate root development.

Tissue copper level increased somewhat in treated seedlings. Levels for control and copper treated stock were 12 and 17 ppm, respectively.

Growth of the mycorrhizal fungus *Thelephora terrestris* was substantially better in copper treated plugs. Although more than 90% of roots were colonized in both treatments, the amount of

extramatrical mycelium and mycelial strands was far greater in treated plugs. The larger number of root tips available for colonization or altered physical environment of the plugs produced by increased fibrosity may have stimulated fungal growth. Growth hormones produced by mycorrhizal fungi stimulate root branching (Slankis 1973) resulting in additional root fibrosity. Increased root fibrosity improves root growth capacity and therefore may be important in outplanting success (Deans *et al.* 1990).

ACKNOWLEDGMENTS

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The Use of Styroblock 1 & 2 Containers for P+1 Transplant Stock Production[1]

Philip F. Hahn[2]

Abstract.--The well known Plug+1 and the recently developed Miniplug+1 seedlings begin their life as typical plugs in a container nursery. Each is grown on a slightly different schedule but at the end of their container growing phase both are transplanted in a bareroot nursery. In the bareroot nursery they continue their development for another growing season or until they are outplanted to a reforestation site. Both of these stock types develop bushy tops and fibrous root masses. Such attributes are needed for high survival and good growth on typical Northwest reforestation sites.

INTRODUCTION

The Plug+1 and Miniplug+1 seedling types are the newest among a variety of seedling types used for reforestation. They are hybrids derived from merging recently developed containerized seedling production methods with age-old bareroot methods. Due to this, it utilizes two different nurseries (greenhouse and open field) and two distinctly different growing schedules and growing regimes. Good coordination between the two nursery types is essential to harmonize the two production phases.

Plug+1 seedlings have been produced in large scale quantities for over a decade. Georgia-Pacific Corporation (container nursery), with the cooperation of Tyee Tree Nursery (bareroot), was instrumental in developing this seedling type. The process is described by Philip Hahn in 1984.[3] Therefore, this report will concentrate mostly on the development of the Miniplug+1 seedling type as carried out by Georgia-Pacific Corporation and cooperating bareroot nurseries, primarily the International Paper Company nursery at Kellogg, Oregon.

A BRIEF DESCRIPTION OF THE MINIPLUG+1 SEEDLING TYPE

The "Miniplug+1" is an outgrowth of the "Plug+1" production. Both spend their first growing phase in a container nursery as a Miniplug or Plug and the second growing phase in a bareroot nursery as a Miniplug+1 or Plug+1. While they are similar, they are also different.

The major differences between the two are the following:

1. The container size for the Miniplug is 1 cu. inch (Styro-1) and for the Plug 2.5 cu. inches (Styro-2).

2. The seedling density in the greenhouse for the Miniplug is 210 seedlings/sq. ft. and for the Plug 100.

3. The greenhouse space utilization is further increased by the Miniplugs because two crops/year are raised instead of only one Plug crop on the same greenhouse space.

4. The Miniplugs also utilize the bareroot space better because they are transplanted in a closer spacing than the Plugs.

Miniplug production has a short history. Therefore, it is still in its development stage. In spite of this, the production of the seedling type *is showing good success*. Just like in all containerized production, there are also several approaches used for producing Miniplugs.

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[1]Paper presented at the Western Forest Nursery Council 1990 Conference held in Roseburg, Oregon on August 13-16, 1990.

[2]Philip F. Hahn is Director of Forestry Research at Georgia-Pacific Corporation's Forestry Research Center, Cottage Grove, Oregon.

[3]Hahn, Philip F. Forest Nursery Manual, 1984 (Edited by Duryea, Landis), pp 165.

To satisfy the tree seedlings unique root requirement, Georgia-Pacific Corporation stuck to the proven container configuration and developed a container type highly suitable for tree seedling production. The container block size is 4"x14"x20". Each block has 408 one cubic inch size cavities. The slightly tapered container cavities have root guiding ribs. There are holes among the cavities to help air circulation among the densely grown seedlings. Each block consists of four segments to aid seedling extraction. This container is commonly known as the Styro-1 (1 cu. inch cavity size) block (fig. 1) or HAHN 408.

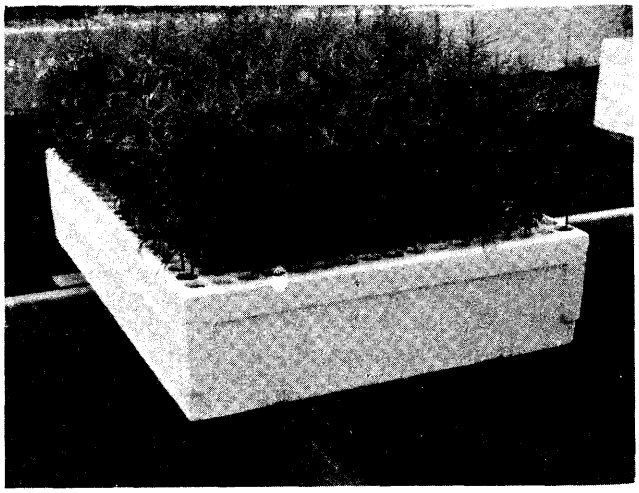

Figure 1.--Hahn 408 container with fall sown Miniplugs.

The HAHN 408 container produces seedlings with good roots and stems suitable for transplanting with a slightly modified conventional transplant machine (see fig. 1).

CONTAINERIZED GROWING PHASE

Growing Facility

Georgia Pacific Corporation uses a shelterhouse type growing facility (fig. 2). These houses have permanent roof covers with double full length roof vents. They have heaters and wall vents, removable sidewall covers, photo period extension lights, ceiling fans. All environment control equipment is motorized and thermostatically controlled. Since the houses provide good natural ventilation through wall and roof vent openings, there is no need for cooling pads and large air moving fans to cool the houses. The seedlings are grown in these houses as close to natural growing conditions as possible. This

Figure 2--Georgia-Pacific's Shelterhouse growing facility.

provides less troublesome growing and helps in developing the required morphological and physiological traits for good transplant quality.

Containers

The shape and size of the HAHN 408 container were described earlier. Other

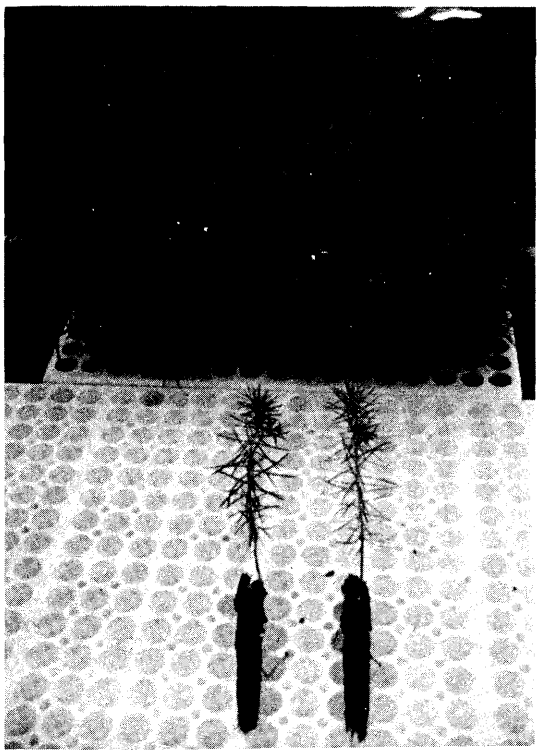

Figure 3--Fall sown Miniplugs raised in Hahn 408 container ready for spring transplanting.

container types may also be used for such seedling production but we found the HAHN 408 to be the most desirable when used in a shelterhouse system (fig. 3). Beside the container's attributes to produce the desired root configuration, it also aids in producing a hardy seedling when used in a shelterhouse facility.

Crop Growing Schedule

Before starting the Miniplugs, their production is timed so that they reach optimum size and condition on the desired spring or fall target transplant dates. Pre-established sowing dates, target height, and diameter charts aid in this process (figs. 4 & 5).

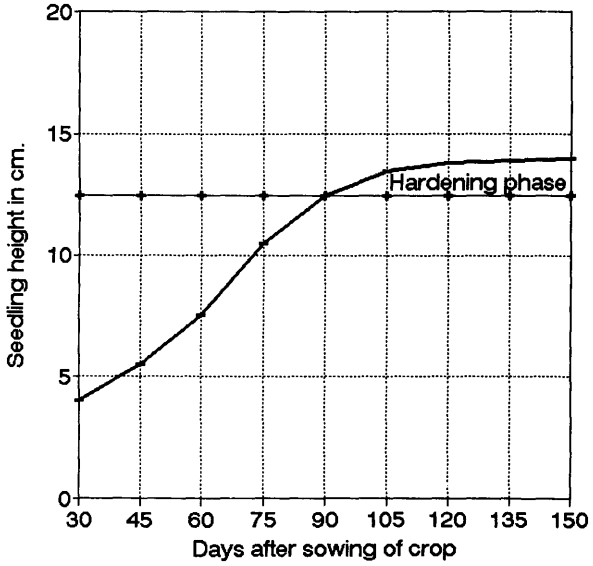

Figure 4--Target height for fall sown Miniplugs.

Miniplug Production for Spring Transplanting

Sowing for the spring transplant growing schedule takes place in August or early September. Warm weather conditions during this time of year aids rapid germination. Soon after germination the activated photoperiod extension lights help prevent premature budsetting, thus promoting continuous seedling growth. During growing, height and diameter growth are closely monitored and matched to the predesignated growing charts shown in Figures 4 and 5.

For optimum growth, the climate in the greenhouse is maintained at 70-75°F during daytime and 60-65°F during the night. For nurturing the seedlings, an appropriate

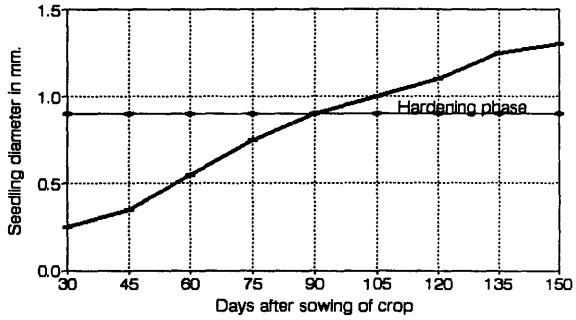

Figure 5--Target diameter for fall sown Miniplugs.

irrigation and fertilization schedule is followed.

Table 1 shows a typical nutrient requirement for liquid feed application. The recommended rates are only suggested guidelines. They are altered to accommodate specific nursery conditions and nutrient requirements of the seedlings. Periodic testing of soil and foliar nutrient contents aid in balancing the nutrient needs of the crop. Tables 2 and 3 show the foliar nutrient indicators for optimum seedling growth.

When growing is done by following the growing schedule and the predesignated growth charts, the seedlings generally reach the proper size for hardening around late November. For hardening, the greenhouse temperature is maintained around 65°F, the lights are turned off, and the seedlings are exposed to several nutrient and water stresses to initiate budsetting. After budset initiation, normal irrigation and fertilization resumes as needed. The fertilizer applications favor high K and low N mixes to promote stem lignification and strong bud development. When budsetting is taking place the greenhouse temperature is allowed to cool to 26-28°F. Seedlings exposed to short and light freezes helps the hardening process. Avoid freezing the root plugs. The insulating capacity of the styroblock container will aid in this effort. During the entire growing season,

TABLE 1 - General nutrient requirements for a liquid feed application to grow Douglas-fir containerized seedlings

	Macronutrients (ppm)						Micronutrients (ppm)						
NUTRIENTS	N	P	K	Ca	Mf	S	Cu	Zn	Mn	Fe	B	Mo	Cl
Establishment Phase	70	100	90	30	10	10	0.20	0.20	0.10	7.00	0.20	0.05	0.50
Rapid Growth Phase	160	90	100	50	15	15	0.50	0.50	0.30	12.00	0.30	0.06	0.60
Hardening Phase	50	60	140	20	5	7	0.30	0.40	0.20	5.00	0.15	0.03	0.50

TABLE 2 - Desirable Soil Fertility Levels for Containerized Seedling Production. The rates are based on Soil and Plant Laboratory, Inc.'s testing method.

				Macronutrients (ppm)						Micronutrients (ppm)				Saturated Extract	
	Half Sat. %	pH	ECe	No3* N	NHA N	PO4-	K	Ca	Mg	Cu	Zn	Mn	Fe	B ppm	SO4 Me/L
DESIRABLE LEVEL	200-280	4.5-5.5	0.5-1.0	Total of 100-300		50-175	600-1000	6000-8000	800-1000	4-8	18-25	30-70	350-550	.1-.5	0.3-2.0

*Ratio of NO3:NH4 should be 2:1 or better

TABLE 3 - Desirable foliar mineral content rates. The rates are based on Soil and Plant Lab., Inc. tests.

	Percent						Parts Per Million				
NUTRIENTS	N	P	K	Mg	Na	S	Cu	Zn	Mn	Fe	B
DESIRABLE LEVEL	1.5-2.5	.4-.8	1.0-2.0	.10-.20	.01-.03	.2-.4	4-20	15-40	100-300	50-80	25-50

disease and insect control is maintained as needed.

By the end of January or early February the crop reaches the proper morphological and physiological condition for packaging and storing. The seedlings are freezer stored (28°F) until transplanting. Transplanting normally takes place from late March to early June. This depends on the work schedule and weather conditions in the bareroot nursery.

Spring Transplanting of Miniplugs

Spring transplanting has a lot of advantages. It is also traditional and well suited for containerized and bareroot crops. The seedling's generally develop their most favorable physiological condition for spring transplanting. With a spring transplant schedule, bareroot nurseries are able to rotate their nursery space better and keep their space occupied for a shorter time period. There is no need to worry about over wintering a freshly transplanted crop. In addition, late hardening and winter frost sensitive species develop better with spring than with fall transplanting. The Miniplug+1 crop production specifically adds two more advantages to the spring transplant schedule. First, the plug growing phase takes place on bonus growing space in the container nursery as a second crop. Second, the fall grown plug doesn't develop a pot-bound root system as the spring sown and held over Styro-2 plug does.

On the flip side of the above, general and specific advantages, one needs to look at some of the disadvantages for spring transplanting, too. Late summer or fall sown seedlings grow under a more artificial environment than the spring sown crop. As the days get shorter and colder during fall, the use of photoperiod extension lights and heaters are required. Using artificial means for growing makes growing cumbersome and increases the cost for the container phase production. However, most of this cost increase is compensated by lower spring transplant production costs in the bareroot operation phase.

It may be more natural to transplant in the spring than fall. However, spring transplanted seedlings will have a shorter time for growing between transplanting and lifting. For this reason, they will develop into smaller trees than the fall transplants (figs. 6 & 7).

Figure 6--Fall sown Miniplugs transplanted in April 1990 (photo: July 5, 1990).

On the other hand, the spring transplanted Miniplug+1 will still have a good mass of fibrous roots because of its many root starts and the absence of pot-boundness.

Miniplug Production for Fall Transplanting

Sowing normally takes place in early to mid-April. Due to the small container

Figure 7--Fall sown Miniplugs transplanted in April 1990 show good height and root development by early July.

cavity size and dense spacing, the seedlings are produced in short growing periods (4-5 months) (fig. 8).

The spring sown seed germinates rapidly and the seedlings also develop rapidly at near natural growing conditions. Growing during the summer, under near natural growing conditions, is less cumbersome and less expensive when compared to fall growing.

The nutrient requirement and growing schedule for this crop is similar to the fall sown crop (see Tables 1, 2 & 3 and Figs. 9 & 10).

Hardening of the seedlings for transplanting is also important. The seedlings need to have the proper morphological and physiological condition for successful transplant development. Transplanting normally takes place during September.

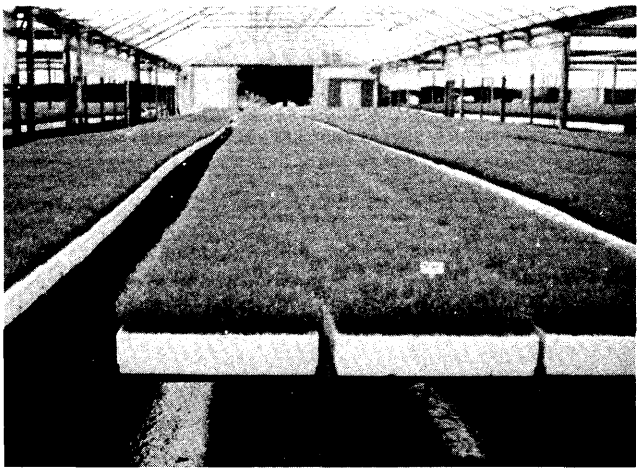

Figure 8--Spring sown Miniplugs raised in shelterhouse for fall transplanting.

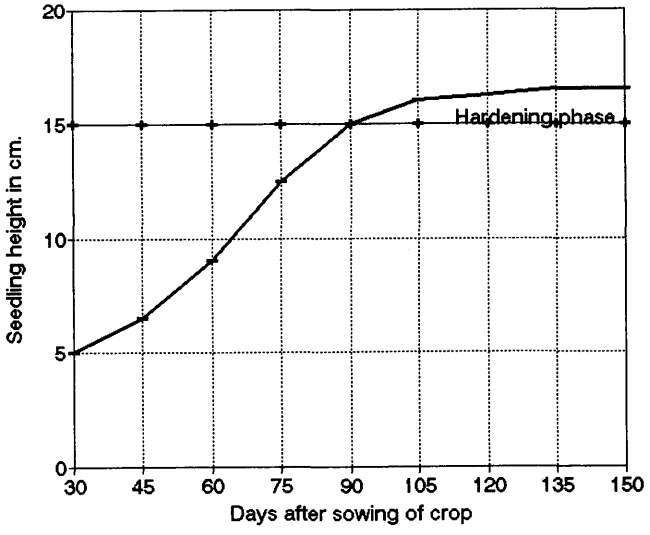

Figure 9--Target heights for spring sown Miniplugs.

Fall Transplanting of Miniplugs

Fall transplanting works well when the seedling's are conditioned for the open field over wintering requirements. Most species are suitable for this. However,

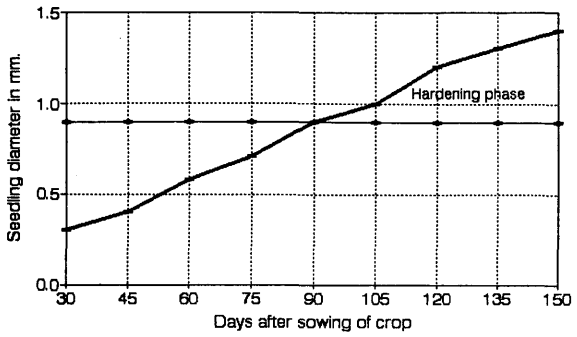

Figure 10--Target diameter for spring sown Miniplugs.

there are some species like Western Hemlock and true firs that set bud late. Therefore, they don't do well when fall transplanted.

There are several good reasons for fall transplanting Miniplugs. These are the following:

- Seedlings removed from greenhouses late August or early September provide space for a second crop.

- Fall transplanted plugs continue bud development, lignification and maintain active root and diameter growth in the bareroot nursery bed. This primes the seedlings for early budburst and more rapid growth during the following growing season (figs. 11 & 12).

- They develop into larger seedlings than the spring transplants and have a heavier and more fibrous root system.

- The husky transplants are produced at a lot lower cost than the similar size Styro-2 transplants.

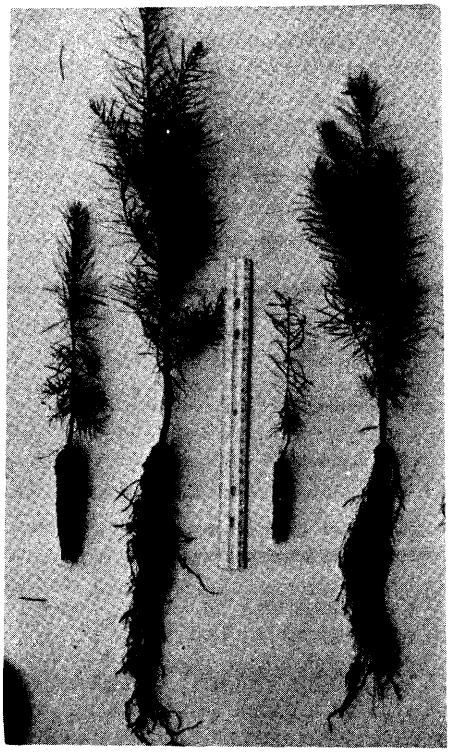

Figure 11--Fall transplanted plug comparisons. From left to right: (Plug (Styro-2), Plug+1, Miniplug (Styro-1) and Miniplug+1 (photo: June 6, 1990).

Figure 12--Fall transplanted Miniplugs almost ready for hardening (Photo July 5, 1990).

While there are some highly desirable advantages in fall transplanting there are some disadvantages, also. These include:

- The problem of over wintering a freshly transplanted crop under potential adverse weather conditions in the bareroot nursery. With good species selection and hardened crops this, however, is seldom a problem.

- The bareroot nursery space cycling is more difficult.

- The seedlings occupy the bareroot nursery space longer than the spring transplants do. Due to this the bareroot production phase for fall transplanting is higher. This higher cost is somewhat compensated by the lower container or first phase production costs.

Packaging, Handling and Shipping

The crop is ready for transplanting when the seedlings are in the proper physiological and morphological stage. At this stage, the stems are lignified enough to allow extraction of seedlings from their containers without injury. The root plugs are firm enough to hold together during shipping and transplanting.

All plugs are packaged in a pre-extracted form. Such a packaging method is a routine operation in container nurseries. Most nurseries have an assembly-line type packaging operation. This makes the process quick and cost effective. About 100 seedlings are removed from 1/4 of the HAHN 408 styroblock to form a seedling package. The root plugs of these seedlings are placed into a plastic bag to protect the roots. About 30 of these bags fill a 14"x20"x16" shipping box. The stacked boxes on pallets are easily moved into cold storage or loaded onto refer vans for shipment. One 40 foot van has the capacity to transport about 1.8 million seedlings in one shipment.

Shipping and storing large quantities of Miniplugs are convenient and inexpensive. This makes it easy to keep up with a rapid transplant operation.

BAREROOT GROWING PHASE

As soon as the Miniplug seedlings arrive at the transplant nurseries, the second or bareroot growing phase for Miniplug+1 production begins. Naturally, by this time, the bareroot nursery must be ready to schedule and carry out the transplanting.

The bareroot nursery operation to produce Miniplug+1 is similar to Plugs+1 production. However, some adjustments are needed in the equipment used and in cultural practices.

Bed Preparation

Timing transplant bed preparation is relatively easy for fall transplanting because of good climatic conditions during late summer. Fall transplanting also helps in shifting some of the workload from the heavy spring transplanting load.

Both fall and spring planting bed preparation requires similar plowing, discing, rototilling, soil-loosening and bed shaping procedures. Precise bed shaping is more important for Miniplugs, because of their smaller size, than for larger seedling types.

Planting and Handling

Mechanized transplanters with some modifications are highly suitable for Miniplug transplanting. Due to the smaller seedling size, the transplanter is equipped with smaller plating shoes. The smaller shoes are put closer on the row. This way the 6-8 row planters are converted to 9-12 row planters (fig. 13). Nine row planters are successfully used and a pilot model 12 row planter is being considered for future development.

Closer seedling spacing increases the efficiency of the expensive bed space utilization. This lowers the bedspace associated cultivation and growing cost, too. When these savings are added to the savings from the Miniplug production the overall seedling cost becomes one of the lowest among transplant seedling types. Besides its low cost, it has all the good qualities (bushy top, strong stem, fibrous roots, etc.) the Plug+1 transplants demonstrated in the past.

Figure 13--Nine row transplanter. Suitable to transplant Miniplugs.

Rearing of the Transplanted Crop

The bareroot rearing practices for Miniplug+1 production is similar to those used for Plug+1 production. The only exception might be the lack of need for top mowing. However, at the rate the fall transplanted Miniplug are growing, this may not be an exception either (see fig. 12).

Due to the detailed discussion of Plug+1 transplant production in the Forest Nursery Manual (see Chapter 16),[4] the discussion of this is omitted in this paper.

CONCLUSION

Miniplug+1 transplant seedlings are the newest and just one more seedling type among the many available for reforestation. There is no doubt that they will fill a special purpose but will not cure all reforestation problems. They show special qualities and abilities to perform well if produced and used properly. They are produced in the shortest time out of all transplants and they are the most economical.

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Hahn, Philip F. Forest Nursery Manual, 1984. (Edited by Duryea, Landis), pp 165.

[4]Ibid, Chapter 16.

The Use of Lannen RT-2 Transplanters to Transplant Containerized Seedlings at Surrey Nursery[1]

Tony Willingdon[2]

Abstract.--The use of Lannen RT-2 transplanters, rather than planting wheel type transplanters led to significant cost savings at Surrey Nursery.

Surrey Nursery is a forest seedling nursery located in the Fraser Valley in British Columbia. At Surrey Nursery seedlings are grown for planting sites located in both coastal and interior regions of B.C.

In 1989 requests to supply large white spruce planting stock primarily for the Prince George region totaled 9.85 million seedlings These requests asked for container seedlings to be started in greenhouses with transplanting to bare-root transplant beds to take place in midsummer. Approximately 6.5 million seedlings were grown in this way. An additional 3.3 million seedlings were grown in containers on an open compound and transplanted directly from the containers in April 1990.

Transplanters on hand at the nursery to do this job were manufactured by the Mechanical Transplanter Company in Holland, Michigan. The design of these machines limits production for each unit to about 1900 plants per hour with each planting unit. The operator of the unit must have seedlings readily accessible, that is, they must be extracted from the container and bundled. This is due to the fact that in the normal operation of the machine, seedlings are placed in rubber clips by the operator. The operator holds the seedling in place while the planting wheel rotates until the clip closes on the seedling. The seedling is then carried by the planting wheel to the furrow that is opened by the planting shoe of the machine.

Early in 1989 we became aware of the Lannen Transplanter system which is manufactured in Finland. Contacts with Hakmet Ltd. confirmed that this equipment would be capable of handling the type of seedlings that would be produced at Surrey.

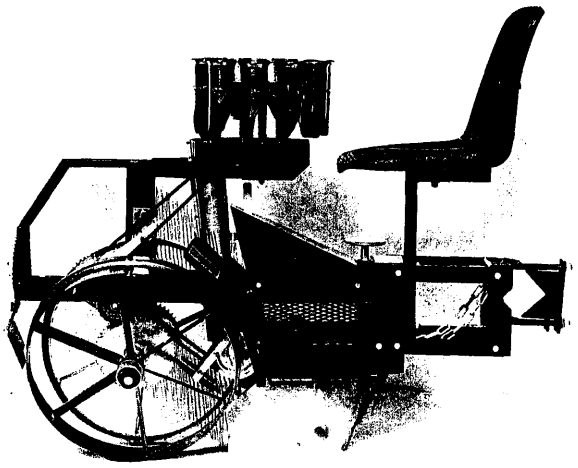

Figure 1.--Lannen RT-2 Transplanter

Estimates of the speed of the Lannen units suggested a 25 to 30 per cent saving in time transplanting. It was also apparently possible for the transplanter operators to carry out the extraction of the seedlings during the transplanting operation. Total savings in labor costs for the 1989 summer transplant program were expected to be about $60,000. The cost of the twelve machines was set at about $40,000.

The machines were ordered in February and after some delays, they arrived at the nursery in April.

Existing frames for carrying the Mechanical Transplanters were then modified to carry the Lannen Transplanters and racks to carry styroblocks were fabricated and installed for a labor and material cost of about $2500. The planting machines were mounted in the frames in the same configuration as the mechanical transplanters, that is, in a gang of six to allow the planting of six rows per bed with approximately six inches between the rows.

[1] Paper presented at the Combined Meeting of the Western Forest Nursery Council and Intermountain Forest Nurdsery Association, Roseburg, OR, Aug. 1990.

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In July transplanting with the new system was begun. Few problems were encountered. Speed and ease of use was as good as forecast. Planting quality was better than with the Mechanical Transplanter System. Operators were more comfortable on the new units and found that the job was more interesting when extracting was performed on the transplanter units. Cost savings were as predicted. Production rates per planting unit averaged about 2200 seedlings per hour. These machines have allowed significant savings in labor and dollars. (Table 1.)

Table 1.--Costs incurred using carousel type planter (actual 1989 costs) and projected costs with planting wheel type planter (based on actual 1988 costs.)

CAROUSEL TYPE PLANTER					
Costs	No. of plants (000)'s	per plant lifting costs	per plant planting costs	total lifting cost	total planting cost
Planted from blocks	4378	$0.00	$0.0189	$0.00	$82,744.20
Shipped from other nursery	2048	$0.00	$0.0189	$0.00	$38,707.20
	Total actual cost (lifting and planting)				$121,451.40
PLANTING WHEEL TYPE PLANTER					
Costs	No. of plants (000)'s	per plant lifting costs	per plant planting costs	total lifting cost	total planting cost
Planted	4378	$0.0135	$0.0212	$59,103.00	$92,813.60
Shipped from other nursery	2048	$0.00	$0.0212	$0.00	$43,417.60
	Total projected cost (lifting and planting)				$195,334.20
	Cost reduction				$73,882.80

Application of Foliar Fertilizer During Bud Initiation Treatments to Container-Grown Conifer Seedlings[1, 2]

Mark E. Montville and David L. Wenny[3]

Abstract. -- Foliar fertilizer applications to ponderosa pine and Douglas-fir seedlings during bud initiation treatments significantly impacted seedling quality. Timing of bud formation was undisturbed in ponderosa pine seedlings but delayed in Douglas-fir seedlings by foliar fertilization. Foliar fertilizer enhanced foliar nitrogen concentration, seedling caliper and height growth, bud length, and shoot-root ratio of both species. Root growth potential was unaffected by foliar fertilization, while differences in seedling cold hardiness were modest.

INTRODUCTION

Growers of conifer reforestation stock routinely moisture and nutrient stress seedlings to induce bud development after seedlings achieve desired height (Wenny and Dumroese 1987; Tinus and McDonald 1979; Owston 1974; van Eerden 1974). Since nutrients are applied through irrigation water, nursery managers are unable to separate moisture and nutrient stress. It is yet unproven that inducing nutrient stress to seedlings is required to initiate bud development (Tinus and McDonald 1979; Lavender and Cleary 1974).

Nutrient stress reduces seedling nutrient reserves. These reserves are vital for increasing seedling caliper and for root and bud development following cessation of height growth (Tinus and McDonald 1979). Moreover, seedling nutrient reserves are essential to sustain seedlings during winter storage and are used in spring for vigorous growth (Margolis and Waring 1986) which results in higher seedling field survival rates (Jopson and Paul 1984).

Growers usually apply increased rates of fertilizer after buds are set to renew nutrient reserves depleted during initiation stress, promote large bud development, and improve root collar diameter. Of course, if nutrient stress is unnecessary for bud initiation, and if seedlings could receive adequate nutrition during moisture stress, caliper growth and bud enlargement could continue uninterrupted. Foliar fertilization may provide seedling growers with an option for fertilizing seedlings while maintaining moisture stress since foliar fertilizer is only applied until runoff, thereby minimizing moisture additions to root systems. Foliar absorbed nutrients are used on woody ornamentals (Bramlage et al. 1985; Nielsen and Hoyt 1984), while coastal Douglas-fir trees (*Pseudotsuga menziesii* var. *menziesii*) (Miller 1979) and slash pine seedlings (*Pinus elliotii* Engelm.) (Eberhardt and Pritchett 1971) are also known to assimilate these nutrients. We examined foliar fertilizer application on ponderosa pine (*Pinus ponderosa* Dougl. ex Laws. var. *ponderosa*) and Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) seedlings as a means of reducing nutrient stress during bud initiation and to determine if a constant nitrogen supply was beneficial or detrimental to seedling quality.

Research described in this paper is part of a larger study which investigated impacts of different foliar fertilizer application rates, durations, and frequencies on seedling quality including foliar phosphorus and potassium concentrations.

METHODS

Ponderosa pine and Douglas-fir seeds were sown the first week of April 1988, week 0, into 24 trays each containing 200 Ray Leach® pine cells (66cm^3). Ponderosa pine seedlings during weeks 2 through 5 and Douglas-fir seedlings during weeks 3 through 6 received Peters'® Conifer Starter (7-40-17), at 42 ppm N, and micronutrients twice weekly (see Wenny and Dumroese 1987 for micronutrient rates). Following this treatment seedlings received Peters'®

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Conifer Grower (20-7-19), at 120 ppm N, micronutrients, and calcium nitrate (15.5-0-0-19), at 46 ppm N, once per week through week 9 for ponderosa pine and week 10 for Douglas-fir. At this point, seedlings had attained the target height of 12-15 cm and were subjected to two different treatments to induce bud formation.

The first bud initiation treatment employed both nutrient and moisture stress to induce bud formation. During weeks 10 through 14 ponderosa pine seedlings and during weeks 11 through 17 Douglas-fir seedlings were irrigated only after their growing media had dried to barely moist (approximately 75% of saturated tray weight) with irrigation water containing Peters'® Conifer Finisher (4-25-35), at 24 ppm N, and micronutrients.

Conversely, the second treatment relied on moisture stress alone to induce bud formation. Seedlings were irrigated with water containing micronutrients only after their growing media became barely moist (as described above). Each species received four different rates of Peters'® Foliar Feed 27-15-12 per 100 gallons of water: Control -- 0 pounds (0 gL^{-1}); Rate 1 -- 1 pound (1.2 gL^{-1}); Rate 2 -- 2 pounds (2.4 gL^{-1}); and Rate 3 -- 3 pounds (3.6 gL^{-1}) (Table 1). R-11, a spreader-activator, was added to foliar fertilizer solutions at a rate of 2 liquid ounces per 100 gallons of water (0.15 mlL^{-1}) to reduce water tension and enhance penetration of fertilizer into leaves. Both ponderosa pine and Douglas-fir seedlings received twice weekly applications for five consecutive weeks. Following bud formation all seedlings received alternating applications of Finisher (24 ppm N) with micronutrients and calcium nitrate (46 ppm N).

Table 1. Treatment rates of Peters[R] Foliar Feed (27-15-12)

RATE OF FOLIAR FEED (lbs./100 gal. H_2O)	(gL^{-1})	NITROGEN CONCENTRATION (ppm)
0	0	0
1	1.2	324
2	2.4	648
3	3.6	972

Foliar fertilizer was applied with an Ortho sprayer nozzle attached to a garden hose connected to a 1:100 injector. Using an injector enabled precise foliar fertilizer concentrations to be applied to seedlings while the Ortho sprayer provided complete and accurate coverage. Foliar Feed was applied until it ran off from seedlings, thus only minimal moisture was added to roots. Each foliar fertilizer rate was applied early in the morning to three trays of 200 seedlings, replicated twice.

Measurements

Four morphological characteristics were evaluated: root collar diameter (caliper), height, shoot-root ratio, and bud length. Growth measurements of seedling caliper and height were collected from ten seedlings of each species/treatment combination monthly from start of bud initiation treatment through growing season conclusion. Seedling growth from treatment initiation until cessation of growth was the data of interest, thus data analyzed for each collection date was the difference between respective caliper and height measurements at that date and initial caliper and height measurements gathered during week 9 for ponderosa pine and week 10 for Douglas-fir. At growing season conclusion, bud length was measured and seedlings were oven dried 24 hours at 65°C to calculate shoot-root ratio (Thompson 1985). In addition, seedling physiological traits were investigated. On three occasions, prior to bud initiation treatment, after terminal bud formation, and prior to cold storage, W.R. Grace and Company performed foliar tissue analyses to determine foliar nutrient concentrations. Root growth potential tests were performed during the storage period by growing 16 seedlings per species/treatment combination for 30 days with 16 hour photoperiod at a constant temperature of 20°C (Ritchie 1985). New roots longer than 2.5 cm for ponderosa pine (Krugman and Stone 1966) and longer than 1.3 cm for Douglas-fir (Todd 1964) were tallied. Following cold storage, cold hardiness examinations were conducted by subjecting 15 seedlings of each species/treatment combination to three different freezing temperatures and then assessing freezing damage after seedlings had been in a growing environment for seven days. Damage assessment was performed as described by Glerum (1985).

Treatment means of tests containing two or more dependent variables were first compared using multivariate analysis with Wilks' Criterion as the test for multivariate significance, while tests containing a single dependent variable were initially analyzed with a general linear model. Fisher's Protected Least Significant Difference test was implemented to separate significant means.

RESULTS AND DISCUSSION

Regardless of foliar fertilizer rate, ponderosa pine seedlings formed terminal buds concurrently with control seedlings. However shortly after bud set less than 10 percent of the seedlings experienced lammas growth and then re-formed terminal buds. Foliar nitrogen concentration of seedlings increased with foliar fertilizer application rate during bud initiation (Table 2). All foliar fertilizer rates significantly increased caliper and height growth, bud length, and shoot-root ratio (Tables 3 and 4), with Rate 3 being most beneficial. This rate improved seedling caliper, critical for survival (Duryea 1984) and necessary for vigorous growth after outplanting (Ritchie 1985), by 45% over control seedlings; final caliper for seedlings receiving the 3 pound rate of foliar fertilizer was 3.02 mm compared to 2.58 mm for control seedlings. Although height growth was also significantly

Table 2. Foliar nitrogen concentrations of ponderosa pine seedlings.[1]

TREATMENT	PRIOR TO FOLIAR FERTILIZER TREATMENTS	AT BUD SET	AT GROWING SEASON CONCLUSION
	percent		
Control	1.4	1.1	1.2
Rate 1	1.4	1.6	1.6
Rate 2	1.4	2.1	1.5
Rate 3	1.5	2.4	2.0

[1] Statistical analysis were not performed on nitrogen concentrations.

Table 3. Increases in caliper and height growth of ponderosa pine seedlings.

TREATMENT	CALIPER GROWTH		HEIGHT GROWTH	
	AT BUD SET	AT GROWING SEASON CONCLUSION	AT BUD SET	AT GROWING SEASON CONCLUSION
	(mm)		(cm)	
Control	0.53 a[1]	0.94 a	3.3 a	5.0 a
Rate 1	0.62 b	1.10 b	4.0 b	5.3 a
Rate 2	0.63 b	1.22 c	4.2 bc	5.7 b
Rate 3	0.67 b	1.36 d	4.4 c	6.1 c

[1] Values in same column with different letters are significantly different at $P < 0.05$ using Fisher's Protected LSD test.

Table 4. Bud length and shoot-root ratio measurement data for ponderosa pine seedlings.

TREATMENT	BUD LENGTH	SHOOT DRY WEIGHT	ROOT DRY WEIGHT	SHOOT-ROOT RATIO
	(mm)	(g)		
Control	9.3 a[1]	0.8 a	0.8 a	1.1 a
Rate 1	10.0 ab	1.0 b	0.9 a	1.2 b
Rate 2	11.2 c	1.3 c	0.9 a	1.4 c
Rate 3	10.4 bc	1.3 c	0.9 a	1.5 d

[1] Values in same column with different letters are significantly different at $P < 0.05$ using Fisher's Protected LSD test.

increased, the difference between final heights was only 1.3 cm, 15.8 cm for Rate 3 seedlings versus 14.5 cm for control seedlings, which is an inconsequential amount to growers. Seedlings which received Rate 3 developed buds 19.5% longer than controls, indicating an increased potential for improved early height growth in the field (Kozlowski et. al 1973; Hanover 1963). Shoot-root ratios are a good predictor of field survival (Rowan 1987; Thompson 1985), with low shoot-root ratios yielding higher survival. Although Rate 3 seedlings possessed the highest shoot-root ratio value, 1.5, this value is still considered low and will not represent biologically significant differences to field survival especially considering some of this increase was a result of improved caliper growth, a beneficial characteristic. Root growth potential was unaffected by foliar fertilizer, and cold hardiness was only slightly reduced (4°C) by the Rate 3 treatment when tested after four months of cold storage (Table 5).

Table 5. Root growth potential and cold hardiness measurements of ponderosa pine seedlings.

TREATMENT	NEW ROOTS (number)	LD_{80} TEMPERATURE (degrees Celsius)
Control	26 a[1]	-20 a
Rate 1	45 a	-17 bc
Rate 2	44 a	-18 b
Rate 3	43 a	-16 c

[1] Values in same column with different letters are significantly different at $P < 0.05$ using Fisher's Protected LSD test.

Conversely, terminal bud formation in Douglas-fir seedlings is more sensitive to foliar nitrogen concentrations. Seedlings receiving foliar fertilizer failed to form terminal buds on schedule, and consequently, foliar fertilizer applications were reduced and then suspended until bud formation. Suspension of foliar fertilization resulted in low nutrient reserves for all seedlings at bud set, however, seedlings receiving higher foliar fertilizer rates still contained greater foliar nitrogen concentrations (Tables 6 and 7). As foliar fertilizer rate increased, caliper and height growth, bud length, and shoot-root ratio significantly increased (Tables 7 and 8). Although foliar fertilizer improved some seedling quality attributes, especially caliper and bud length, treatments prevented timely bud set resulting in seedlings that exceeded target height; control seedlings final actual height was 15 cm while each foliar fertilizer rate produced seedlings between 19 and 21 cm tall. Shoot-root ratios were significantly increased because of the extra height growth gained by delayed bud formation, but shoot-root ratios for all foliar fertilized seedlings were low, below 1.5, consequently these differences in shoot-root ratio will have an insignificant impact on seedling quality. As with ponderosa pine, root growth potential was unaffected, and differences in cold hardiness were slight (Table 9).

None of the foliar fertilizer treatments resulted in needle burn due to volatilization. Foliar fertilizer treatments were visually apparent as seedlings exhibited darker green foliage in comparison to control seedlings, but two weeks passed before these color differences became evident. Furthermore, foliar fertilized ponderosa pine seedlings possessed this darker green foliage throughout the remainder of the growing season. However, since foliar fertilizer treatments were suspended in order to induce bud formation Douglas-fir seedlings lost their dark green appearance.

Table 6. Foliar nitrogen concentrations of Douglas-fir seedlings.[1]

TREATMENT	PRIOR TO FOLIAR FERTILIZER TREATMENTS	AT BUD SET	AT GROWING SEASON CONCLUSION
	percent		
Control	1.6	1.0	1.0
Rate 1	1.5	1.0	1.1
Rate 2	1.8	1.3	1.1
Rate 3	1.7	1.4	1.1

[1] Statistical analysis was not performed on nitrogen concentrations.

Table 7. Increase in caliper and height growth of Douglas-fir seedlings.

TREATMENT	CALIPER GROWTH		HEIGHT GROWTH	
	AT BUD SET	AT GROWING SEASON CONCLUSION	AT BUD SET	AT GROWING SEASON CONCLUSION
	--------(mm)---------		--------(cm)---------	
Control	0.52 a[1]	0.66 a	5.8 a	6.0 a
Rate 1	0.75 b	0.93 b	9.2 b	9.5 b
Rate 2	0.87 c	1.07 c	9.9 c	10.3 c
Rate 3	0.89 c	1.18 d	10.7 d	11.4 d

[1] Values in same column with different letters are significantly different at $P < 0.05$ using Fisher's Protected LSD test.

Table 8. Terminal bud length and shoot-root ratio measurements for Douglas-fir seedlings.

TREATMENT	BUD LENGTH (mm)	SHOOT DRY WEIGHT (g)	ROOT DRY WEIGHT (g)	SHOOT-ROOT RATIO
Control	3.8 a[1]	0.5 a	0.5 a	0.9 a
Rate 1	4.7 b	0.7 b	0.6 a	1.1 b
Rate 2	4.9 bc	0.8 c	0.7 a	1.2 c
Rate 3	5.1 c	0.8 c	0.6 a	1.4 d

[1] Values in same column with different letters are significantly different at $P < 0.05$ using Fisher's Protected LSD test.

Table 9. Root growth potential and cold hardiness measurements for Douglas-fir seedlings.

TREATMENT	NEW ROOTS (number)	LD_{50} TEMPERATURE (degrees Celsius)
Control	31 a[1]	-17 a
Rate 1	42 a	-20 b
Rate 2	40 a	-17 a
Rate 3	65 a	-17 a

[1] Values in same column with different letters are significantly different at $P < 0.05$ using Fisher's Protected LSD test.

MANAGEMENT IMPLICATIONS

Nursery managers can improve ponderosa pine seedling quality by reducing nutrient stress during bud initiation via foliar fertilization. Using 3 pounds of foliar fertilizer per 100 gallons of water, applied with conventional irrigation systems, allows timely bud formation while maintaining high nutrient reserves which improve caliper and bud growth. Results from this project, coupled with further research that examined application frequencies on ponderosa pine, enable us to recommend applying 3 pounds of foliar fertilizer per 100 gallons of water once every other week during bud initiation instead of twice weekly to reduce lammas growth caused by high nutrient concentrations. Early morning applications prevent needle damage due to volatilization.

Douglas-fir seedlings were stimulated to grow, rather than develop buds, by foliar fertilization. Improvements in Douglas-fir caliper and bud length were achieved, but foliar fertilization prevented timely bud formation resulting in seedlings exceeding target height. Based on the results of this project and further tests on Douglas-fir seedlings using a 1 pound rate of foliar fertilizer, we recommend applying 2 pounds of Foliar Feed per 100 gallons of water to Douglas-fir

seedlings every other week after terminal bud formation to increase seedling nutrient reserves and enlarge caliper development and terminal bud length. Finally, we have successfully applied the 3 pound rate of foliar fertilizer to western larch, grand fir, Engelmann spruce, Colorado blue spruce, western white pine, Scotch pine, and Austrian pine after buds are well developed to recharge depleted nitrogen reserves after bud initiation treatments.

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Approaches to Integrated Pest Management of Fusarium Root Disease in Container-Grown Conifer Seedlings[1]

R. L. James, R. K. Dumroese, and D. L. Wenny[2]

Abstract - An integrated approach to management of *Fusarium* root disease in container-grown conifer seedlings includes reducing levels of pathogen inoculum within the seedling growing environment, enhancing host resistance to infection and disease development, encouraging organisms competing with or antagonistic toward pathogenic *Fusarium* spp., and minimizing use of chemical fungicides whenever possible. Integrating these procedures into standard growing regimes should greatly reduce impact of *Fusarium* root disease.

INTRODUCTION

Diseases caused by *Fusarium* spp. are important limiting factors in the production of container-grown conifer seedlings in the western United States and Canada (James and Gilligan 1985;James and others 1987, 1989; Sutherland and others 1989). Several types of diseases associated with these pathogenic fungi have been identified, including pre- and post-emergence damping-off and cotyledon blight of young germinants, and root diseases of older seedlings (James 1986a, 1987b).

Root disease is especially difficult to control because once symptoms appear on seedlings, their root systems are usually extensively colonized with pathogenic fungi (James and others 1987). Chemical fungicide applications are largely ineffective in reducing further damage (James 1986b) and trying to save these seedlings is usually unsuccessful (James and others 1988c). A more reliable approach is to prevent infection when seedlings are young.

Several investigations (Bloomberg 1971, 1973; Hansen and Hamm 1988; James 1985c; James and others 1987) have shown that shortly after seeds germinate, germinants often become infected with *Fusarium*. Pathogen inoculum may reside on or within planted seed (James 1986a, 1987b), or on the inner walls of containers used to grow seedlings (James and Gilligan 1988b, 1988c;James and others 1988a;Sturrock and Dennis 1988). Once infected, seedlings may or may not display disease symptoms which result from decay of root systems, such as foliar chlorosis and necrosis or wilting (James and Gilligan 1988a; James and others 1987). Expression of disease symptoms in infected seedlings is enhanced by late season hardening and bud initiation stress (James and Gilligan 1985; James and others 1987).

Because of problems controlling *Fusarium* root disease with chemical fungicides (James and others 1988c), efforts have recently focused on an integrated approach to reduce disease damage using cultural, biological and chemical methods of control. This paper discusses techniques that have either proven effective or hold promise for reducing losses from *Fusarium* root disease in container-grown conifer seedlings. Four aspects of an integrated pest management program have been identified. Each will be discussed under its appropriate heading.

REDUCTION OF PATHOGEN INOCULUM

To reduce infection levels, it is important to limit pathogen inoculum within and adjacent to the seedling growing environment. Since seed is often an important inoculum source in container seedling operations (James 1986a, 1987b), steps to reduce amounts of seedborne *Fusarium* are necessary. Past evaluations have indicated that most *Fusarium* is carried externally on seedcoats (James 1984, 1985b, 1986b). Rarely does this fungus actually penetrate the seedcoat to infect seed endosperm or embryo (Bloomberg 1966). Several types of chemicals have been tested to reduce seedborne *Fusarium*. Common surface sterilants like household bleach (active ingrediant = sodium hypochlorite) and hydrogen peroxide are usually effective in reducing levels of *Fusarium* on seed (Advincula and others 1983; Barnett 1976; James and Genz 1981). However, some problems with seedling toxicity and reduced seed germination have occurred, especially with hydrogen peroxide (Edwards and Sutherland 1979; James and Genz 1981). Bleach treatments have been more successful and are often used operationally by some nurseries (Dumroese and others 1988; Wenny and Dumroese 1987). Common fungicides applied directly to seed have limited utility, partly because they may adversely affect seed germination (Dick and others 1958; Peterson 1970; Shea 1959) and young seedling growth (Cooley 1983; Lock and others 1975). Perhaps one of the most effective and least toxic treatments is standard water, either heated or applied over seed as a running water rinse (Dumroese and others 1988). Water heated with microwaves was effective in eliminating *Fusarium* on seedcoats (James and others 1988b); however, care must be taken not to exceed temperatures lethal to seeds. Running water rinses for at least 48 h have proven effective in reducing seedborne *Fusarium* without adversely affecting seed germination (Dumroese and others

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1988; James 1984, 1987a). Therefore, procedures are available for reducing seedborne *Fusarium* inoculum without adversely affecting germination or establishment of young germinants.

Seedling containers may accumulate *Fusarium* inoculum when reused several times without adequate cleaning (James and Gilligan 1988b, 1988c; James and others 1988a; Sturrock and Dennis 1988). Contaminated styroblock and Ray Leach® pine cells have been implicated as important inoculum sources for new seedling crops. Most *Fusarium* inoculum resides near the bottom of containers (James 1989b; James and others 1988a), probably existing on remaining organic debris, such as pieces of soil mix, roots and algal growth which is inadequately removed during cleaning. Most growers have used high pressure steam for cleaning their containers, sometimes followed with immersion in a bleach solution. Although such operations reduce amounts of *Fusarium*, enough inoculum usually survives to cause problems to the next crop (James and others 1988a). Recent investigations (James and Woollen 1989; Sturrock and Dennis 1988) have shown effective elimination of *Fusarium* on containers immersed in hot water (68-80°C) for 3-10 minutes. A solubilized spreader such as R-11 or standard detergent is often added to water to ensure all container surfaces come into contact with hot water. Styroblock containers probably require exposure to higher temperatures for longer durations than pine cells (James and others 1988a). Another promising treatment is immersion of containers in sodium metabisulfite, a chemical used to kill yeast organisms in brewing (Sturrock and Dennis 1988). This chemical is toxic to fungi when mixed in a water solution. Major disadvantages of this treatment are chemical toxicity to workers and problems of solution disposal after use. For these reasons, most growers are implementing some form of hot water immersion for cleaning their containers.

Another possible source of *Fusarium* inoculum in container operations is the growing media. Most pre-mixed peat/vermiculite growing media are usually pathogen free (James 1985a). This media may contain high populations of potentially antagonistic fungi, such as *Trichoderma* spp. (James 1985a, 1989a). However, if *Fusarium* spp. are introduced into growing media, they may quickly colonize it and cause severe disease problems (James 1985a; James and Gilligan 1984). Therefore, it is important to prevent pathogen introduction into the media. If contamination is suspected, steaming the media (82°C for 30 min) will usually eliminate pathogens, while allowing some beneficial microorganisms to survive (Baker and Olsen 1959; Hartmann and others 1990).

Keeping the growing environment clean is important in reducing problems from *Fusarium* and other pathogenic fungi. Greenhouse interiors, including floors, walls, ceilings, and benches should be thoroughly cleaned and sanitized between crops. Organic debris, which may harbor *Fusarium* inoculum, should be eliminated as much as possible (James and others 1987). Standard sterilants such as bleach or similar household products are usually effective in cleaning these surfaces. Unfortunately, some greenhouses have dirt or gravel floors, surfaces nearly impossible to sterilize. Higher disease losses are expected in greenhouses without concrete floors (James and others 1988c).

Another potential inoculum source in container operations is irrigation water. Pathogen propagules may readily colonize some nursery water supplies, especially those from, or exposed to ponds, streams, or ditches. Well water is usually much less contaminated (Landis and others 1989). Although *Fusarium* spp. are not important water contaminates (Burgess 1981), propagules of *Fusarium* may be carried in water and subsequently introduced into crops (Cook 1981).

Some *Fusarium* spp. pathogenic to conifer seedlings may also colonize other hosts. For example, greenhouse weeds may be infected with *Fusarium oxysporum* Schlecht., which is pathogenic to conifer seedlings (James and others 1987, 1989). Weeds just outside greenhouses may also serve as hosts to *Fusarium* spp. (Landis and others 1990). It is important that these other hosts which serve as inoculum reservoirs be eliminated.

A final way of reducing amounts of *Fusarium* inoculum within the growing environment is periodic removal of diseased seedlings. Several pathogenic *Fusarium* species produce spore-containing structures called sporodochia on above-ground portions of diseased seedlings (Nelson and others 1983). Spores released from these structures may be disseminated via irrigation splash and air currents to infect nearby seedlings (Burgess 1981; Cook 1981). Therefore, if diseased seedlings are removed before sporodochia form and release spores, threat to other seedlings is reduced. Dead seedlings left in greenhouses may also become colonized by other pathogens, such as *Botrytis*, thereby enhancing potential of these pathogens to cause greater problems (Landis and others 1990). Diseased seedlings should be carefully removed, placed in bags and removed to disposal areas that will not threaten nursery seedlings.

ENHANCE HOST RESISTANCE

Most conifer species are susceptible to *Fusarium* root infection at some level, but disease expression by infected seedlings varies greatly among different species and among individuals of a single species. For example, although ponderosa pine seedlings are often infected with *Fusarium* spp., they rarely display disease symptoms (James and Gilligan 1988a). However, Douglas-fir (James and others 1987), Engelmann spruce (James and Gilligan 1985), and western larch (James 1985c) seedlings display disease symptoms much more commonly.

Several factors probably influence level of disease expression of infected seedlings. These might include seedling moisture stress (Bloomberg 1976), greenhouse temperatures (especially extremes) (Bloomberg 1976; Tint 1945b), and nutrient levels within seedlings and the growing media (Bloomberg 1985; Tint 1945a). Seedlings infected with *Fusarium* often display disease symptoms toward the end of the growth cycle when they are water and nutrient stressed to initiate bud set (James and others 1987, 1988c). From a disease expression standpoint, it is probably important to limit both water and nutrient stress to the least amount necessary to initiate bud set. Recent work (Montville and Wenny 1990) indicates nutrient stress may be unnecessary for bud intiation, and foliar applied fertilizers can be used to reduce nutrient stress during periods of reduced moisture applications. If infected seedlings are stressed for prolonged periods, they will probably become diseased, i.e. fungi colonizing their roots become active and "pathogenic," eliciting disease expression (Bloomberg 1971). Temperature may be very important in disease expression since most pathogenic *Fusarium* spp. are considered "warm weather" fungi. That is, they grow more rapidly (Booth 1971; Nelson and others 1983) and are more pathogenic (Bloomberg 1976; Tint 1945b) when temperatures are high. Bareroot stock often displays disease symptoms when ambient temperatures exceed certain thresholds (Bloomberg 1971, 1973, 1976), particularly in July and August. Fortunately, greenhouse temperatures can be regulated during most of the growing season. However, if seedlings are moved outdoors to shade

houses, temperature control is lost. Since this usually occurs in conjuction with moisture and nutrient stress to enhance bud set (Landis and others 1989), *Fusarium* root disease often becomes most apparent after seedlings are placed outside and temperatures become warm (James and others 1987, 1988c). Keeping seedlings cool with irrigation may help alleviate this problem.

Early literature dealing with damping-off of conifer seedlings (Rathbun 1922; Rathbun-Gravatt 1925; Spaulding 1914; Tint 1945a) emphasized the importance of regulating nutrient applications during periods when young germinants are susceptible to damping-off fungi. Adding nutrients (especially nitrogen) during seedling emergence but before stem lignification enhances damping-off losses by making seedlings more succulent. Added nutrients may also promote growth of pathogenic fungi (Landis and others 1989). Therefore, it is important to regulate fertilizer during the critical stage of seedling establishment and promote rapid lignification of germinant stems.

ENCOURAGE COMPETING AND ANTAGONISTIC ORGANISMS

Fusarium spp. compete with a wide range of microorganisms in natural soil. Several different types of organisms will commonly occupy the same niches as *Fusarium*, i.e. root cortical cells and rhizospheres. If nonpathogenic organisms occupy these sites first, pathogenic *Fusarium* spp. may be excluded and therefore unable to infect and elicit disease. In addition, many soil microorganisms produce antibiotics which give them competitive advantages (Baker and Cook 1974; Brian and McGowan 1945; Papavizas 1985; Weindling and Emerson 1936). Antagonism and competition are important in the balance of organisms colonizing organic substrates in soil. If specific microorganisms that display both competitive and antagonistic properties can be introduced into nursery systems, it is possible to exert biological control on pathogenic organisms such as *Fusarium* (Baker and Cook 1974).

Several types of organisms have potential as biological control agents of pathogenic fungi. Bacteria in the genus *Pseudomonas* and actinomycetes in the genus *Streptomyces* are potentially important biocontrol agents (Baker and Cook 1974; Brown 1972). Perhaps the most widely studied group of potential biocontrol agents are fungi in the genera *Trichoderma* and *Gliocladium* (Papavizas 1985). Several of these fungi successfully compete with, are antagonistic toward, and parasitize plant pathogenic fungi. *Trichoderma* spp. are often very fast growing and rapidly colonize substrates, thus excluding pathogens such as *Fusarium* spp. Several of these fungi are also parasitic on other fungi including plant pathogens (Ayers and Adams 1981; Hubbard and others 1983; Papavizas 1985). Recently, special strains of *Trichoderma* have been genetically engineered to be more effective biocontrol agents (Stasz and others 1988). When introduced on seed or within the growing medium, these strains rapidly colonize the rhizosphere and may exclude host invasion by plant pathogens (Harman and Taylor 1988; Harman and others 1989). Unfortunately, these engineered biocontrol agents are yet to be tested for their efficacy to control *Fusarium* root disease in container-grown conifer seedlings. However, such evaluations are planned.

Another interesting possibility for biocontrol involves inoculating nursery seedlings with nonpathogenic strains of *Fusarium* (especially *F. oxysporum*) to exclude invasion of host roots by pathogenic strains. This "cross protection" has been effective in several agricultural systems (Damicone and Manning 1982; Davis 1967). The rationale behind this approach is that sites commonly colonized by pathogens can just as easily be colonized by nonpathogenic (saprophytic) strains of the fungus. Since many *Fusarium* spp. are rapid colonizers of root cortical tissues (Booth 1971; Nelson and others 1983), by introducing nonpathogenic strains, these sites can be occupied preferentially by desirable organisms. Of course, it is important that strains of *Fusarium* used for biocontrol are nonpathogenic under all potential conditions for host production. Pathogenicity tests with *Fusarium* spp. isolated from conifer seedlings have identified several nonpathogenic strains (James and others 1989), but these strains have yet to be tested for their ability to "cross protect" hosts from pathogenic strains.

Ectomycorrhizal fungi may display antagonism toward some plant pathogenic fungi (Marx 1972; Sinclair and others 1975; Stack and Sinclair 1975). Mycorrhizal symbionts usually colonize fine root tips and provide a physical barrier to pathogen colonization; these symbionts may also produce antibiotics which restrict development of some pathogens (Marx 1972; Stack and Sinclair 1975). Most young container-grown seedlings are nonmycorrhizal, but infection increases towards the end of the growth cycle, especially if seedlings are placed outside where mycorrhizal inoculum is more available. However, it is possible to inoculate seedlings with mycorrhizae a few weeks after germination (Castellano and others 1985; Sinclair 1974). Specific mycorrhizal symbionts have been developed for specific conifer species. These symbionts may improve seedling performance and be antagonistic toward potential plant pathogens. Since not all mycorrhizal fungi are equally beneficial to particular hosts, it is important to introduce those organisms best adapted to specific hosts (Castellano 1987). Although some inoculation of container-grown seedlings has been successful (Castellano and others 1985; Marx and others 1982), much more work is needed to evaluate specific responses of some conifer species and effects of mycorrhizal symbionts on plant pathogens.

MINIMIZE CHEMICAL FUNGICIDES

Previously, many growers have attempted to control *Fusarium* root disease by using chemical fungicides once disease symptoms are apparent. As mentioned earlier, such an approach has been largely unsuccessful because once disease symptoms are seen, seedling roots are often completely colonized with pathogenic fungi. In general, most fungicides are more effective in preventing infection than curing infected seedlings (Delp 1980). Although fungicides may be effective during the damping-off phase, they are usually ineffective later in the growth cycle when seedlings are several months old (James and others 1988c). For example, benomyl is commonly used against damping-off (Landis and others 1990), but is ineffective against *Fusarium* root disease later in the growing season (Shrimpton and Williams 1989). Further, most pathogen inoculum is concentrated near the bottom of plugs in container-grown seedlings (James 1989b) and it is unlikely that fungicides readily penetrate throughout the root zone in sufficient concentrations to be effective against pathogens.

Another potential problem from fungicide usage is development of resistance to specific chemicals by pathogenic fungi (Dekker 1976; Delp 1980). Resistance has been demonstrated for several plant pathogenic fungi, especially those subjected to consistently high doses of a specific fungicide. Although foliar pathogens most commonly develop resistance, some root pathogens have also become resistant to certain chemicals

(Dekker 1976; Georgopoulos and Zaracovitis 1967). By minimizing exposure of pathogenic fungi to chemical fungicides, selection pressures for fungi to develop resistance are reduced.

An integrated management program for *Fusarium* root disease should discourage indiscriminate use of fungicides for the reasons discussed above. When used, they should be for a specific purpose (such as to control damping-off if losses are relatively high). Experience has shown that much pesticide use is unnecessary and does not reduce disease (Dumroese and others 1990). Reducing fungicide use will also reduce costs of seedling production, problems with worker exposure to potentially toxic chemicals, and potential problems with contamination of nursery sites and
nearby groundwater.

CONCLUSIONS

Fusarium root disease of container-grown conifer seedlings can be satisfactorily controlled by implementing an integrated approach to disease management. Such an approach should be designed to prevent initial infection by pathogenic fungi. This can be done most effectively by reducing levels of pathogen inoculum within and adjacent to the growing environment, providing growing conditions more beneficial to the growth of host plants than pathogenic fungi, encouraging proliferation and development of competing and antagonistic organisms, and minimizing use of chemical fungicides. Integrated disease management using these approaches should help growers reduce losses from *Fusarium* root disease while placing less emphasis on chemical control.

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Regulation of Seedling Height in Container-Grown Spruce Using Photoperiod Control[1]

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Abstract.--In 1989, container-grown white x Sitka spruce (Sxs) seedlings were subjected to a 15h day, either static or dynamic method of blackout treatment, for four weeks to promote budset and regulate height. The blackout treated seedlings were shorter, and had less shoot mass compared to controls without any effect on stem diameter. Control seedlings acquired cold-hardiness slower than blackout treated seedlings, and the dynamic method acquired cold-hardiness slower than the static method. Under controlled conditions, days to budbreak in spring was three days earlier for blackout treated seedlings compared to controls. In 1988, a larger experiment with six seedlots and 12 blackout treatments, applied using dynamic method, was conducted which included the same seedlot as in 1989 and results are compared. All seedlings were lifted and frozen stored. In May 1989, all treatments for all seedlots were outplanted in nursery beds and four seedlots were also planted in their respective regions. First year field assessments indicated a reduction in height growth with 13 h daylength for Engelmann spruce, but not for white x Sitka spruce seedlings. Blackout treatments had little or no effect on phenology.

INTRODUCTION

In British Columbia we produce container-grown seedlings of white (Picea glauca (Moench), Englemann (P. engelmannii Parry), and Sitka spruce (P. sitchensis (Bong.) Carr.) and commonly we are dealing with naturally occurring hybrids of these three species. Regulating height growth at the end of the season is a common problem for nurserymen, particularly in nurseries located in northern latitudes with naturally long days during the growing season.

Nurserymen use both drought and nutrient stress to regulate seedling height (Macey & Arnott 1986). Both of these stresses appear to regulate height by slowing growth rather than promoting early budset. Shortened daylengths imposed by blackout systems promote early budset to regulate seedling height (Dormling et al. 1968). The advantages of using blackout over drought and nutrient stressing are:

1) seedlings are not stressed,
2) crop response is uniform, and
3) it is easier to impose.

Research on the use of blackout culture to promote budset and regulate height in spruce seedling production began in 1987 at the B.C. Ministry of Forests, Red Rock Research Station (RRRS), Prince George, B.C. Results from the 1987 growth chamber study (Hawkins and Hooge 1988) and preliminary results from 1988 (Hawkins and Draper 1988) were presented at the Combined Meeting of the Western Forest Nursery

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Associations held August 8-11, 1988 in Vernon, B.C. This paper presents some of the results from experiments conducted during 1988 and 1989, including first year field performance of blackout cultured seedlings.

OBJECTIVE

Experiments were conducted at RRRS, a northern latitude research facility (lat. 53° 45'N; long. 122° 41'W), to determine the effect of short day treatments on height growth of spruce seedlings in order to provide nurserymen with operational guidelines for length of day (hours) and duration (weeks) of blackout treatment. The effects of blackout culture on cold-hardiness, dormancy, and field performance were also investigated.

MATERIALS AND METHODS

Spruce seeds (Sxs) of SL3958 (Lat. 55° 00'N, Long. 128° 45'W; Elevation 400 m) from the Prince Rupert Region were stratified at 2°C for three weeks prior to sowing March 16, 1989 in BC/CFS 313 styroblocks using a 2:1 peat:vermiculite (v/v) growing mix. The styroblocks were placed in a research polyhouse at the Red Rock Research Station, Prince George, B.C. and grown using the culture described by Hawkins and Draper, 1988. Seedlings were grown under a 23h photoperiod until blackout treatments began on June 28, 1989.

The blackout treatments were ambient (control), and a 15h daylength for four weeks applied using either the static or the dynamic method of imposing short days (fig. 1). The static method is simply a constant night length for a given period of time and is the standard method used in crop production. The dynamic method parallels the natural declining day length over the treatment period so that the daylength gradually shortens during the weeks of treatment, offset from the ambient daylength by a predetermined, constant number of hours.

Following treatment, seedlings were grown in the polyhouse under ambient conditions until lift. The lift date (Nov. 24, 1989) for all seedlings was based on the results of freezer tests conducted every one to two weeks starting Sept. 15, 1989.

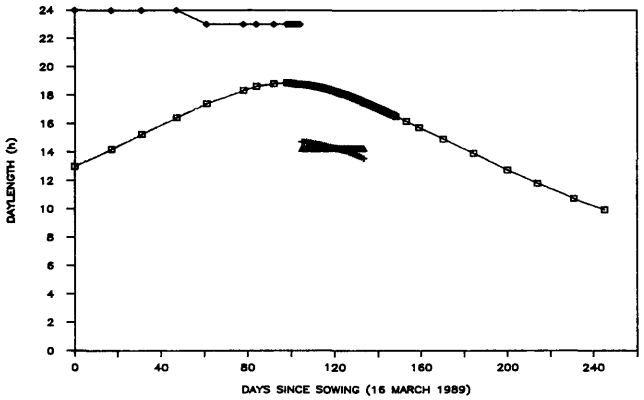

Figure 1.--The natural daylength (includes civil twilight) at RRRS (□), showing the daylengths imposed by the static (Δ) and dynamic (+) blackout treatments for four weeks from June 28 to July 27, 1989.

Ten seedlings per treatment were selected at random and exposed to three or four preset temperatures during each test with a constant 5°C h^{-1} ramp and 5 min soak at each plateau temperature. The temperatures used on each date were decreased gradually as seedlings acquired cold hardiness. Following freezing, 5 seedlings from each treatment were potted into 1-gal nursery pots for a total of two pots per treatment. Seedlings were placed in controlled environment growth chambers and assessed one week later for percent foliage browning. At lift, 150 seedlings per treatment were systematically selected and measured for height, stem diameter, shoot dry weight, and root dry weight. As well, 15 blocks for each treatment were assessed for percent of seedlings reflushing. Following lift, seedlings were stored at -2°C until spring 1990.

Twenty seedlings per treatment were selected at random from storage in May 1990, potted with five seedlings per pot and placed in controlled environment chambers for determination of days to budbreak (DBB) and root growth capacity (RGC). Two pots were placed in a chamber with day/night temperatures of 25°C/20°C, and two in a chamber with 15°C/5°C.

In 1988, spruce seedlings from six seedlots were exposed to four blackout

daylengths, each applied for three durations as described by Hawkins and Draper, 1990. All seedlings were lifted and frozen stored.

On May 13, 1989, 99 seedlings per treatment were outplanted at RRRS and terminal bud phenology assessed using the codes in Table 1. End of season height and stem diameter increment were determined on 54 seedlings per treatment.

Table 1.--Phenology coding and description of each code used during the first field season following outplanting of blackout treated spruce seedlings.

Code	Description
0	resting spring terminal bud
1	bud swell
2	bud flush/shoot elongation
3	lateral buds forming
4	terminal buds forming
5	resting fall terminal bud

As well, four seedlots were outplanted in their respective regions through the cooperation of the Regional Forest Science staff. This paper reports the terminal bud phenology, and seedling height and stem diameter increment after the first year in the field for Engelmann spruce seedlot 4311 (Lat 50° 55', Long. 120° 05'; Elevation 1435 m) from the Kamloops Region. Twenty-five seedlings per each of the seven original blackout treatments were planted May 30, 1990, at McGillivray Lake in the ESSFdc2[1]; elevation 1480 m; aspect SE; logged 1987 and blade scarified.

RESULTS

Static vs Dynamic 1989

The 15h for four week blackout treatments resulted in spruce seedlings that were shorter and had lower shoot dry weight compared to untreated seedlings (table 2). Stem diameter and root dry weight appeared unaffected by treatment, but the shoot:root ratio was lower for treated seedlings due to the decrease in shoot dry weight. The control seedlings, if graded using the B.C. stock specifications[1], would have been overheight (maximum = 300 mm) and culled for low root dry weight (cull = 0.5g).

Seedlings subjected to the static blackout treatment (constant 15h day) were significantly shorter with greater stem diameter, compared to those subjected to the dynamic blackout treatment (table 2). With both methods of applying blackout, reflushing occurred in 26-30% of the seedlings for static and dynamic respectively. The static treatment tended to have greater root dry weight and shoot dry weight but not significantly different from the dynamic treatment.

Cold-hardiness was acquired earlier in the growing season by blackout treated seedlings (fig. 2), and the static method appeared to accelerate the acquisition compared to the dynamic method. By Nov. 17, 1989 (just prior to lifting) 55% of the foliage on control seedlings was killed by a test temperature of -18°C compared to only 35% for the dynamic treatment, and 4% for the static treatment. Eleven days later (Nov. 28) the percent injury was down to only 18% for both control seedlings and those subjected to the dynamic blackout, and the seedlings from the static treatment remained at 3-4%.

After six months of frozen storage, the DBB for control seedlings was three days longer than for blackout treated seedlings (table 3) under warm temperature conditions (25°C/20°C D/N). With the cooler temperature regime of 15°C day and 5°C night, the treatment differences in DBB disappeared (table 3), and with either regime there was no difference between static and dynamic.

First year field performance

First year field height growth and stem diameter increment appeared to be unaffected by the blackout treatments they received during the nursery culture

[1]B.C. Ministry of Forests Biogeoclimatic Zones Classification System.

[1]B.C. Ministry of Forests, Silviculture Branch 1989 Stock Specifications for Sitka spruce crosses stock type PSB313A/B.

Table 2.--End of season seedling height, stem diameter, shoot dry weight (SDW), root dry weight (RDW) and root:shoot ratio following a 15 hour day blackout treatment for four weeks starting June 28, 1989 for SL3958.

Blackout Treatment	Height (mm)	Stem dia. (mm)	SDW (mg)	RDW (mg)	Ratio S:R
Control	386 ± 5.5[1]	3.2 ± 0.05	2489 ± 85	494 ± 22	5.0
Dynamic	283 ± 6.3	3.2 ± 0.06	2011 ± 77	508 ± 23	4.0
Static	265 ± 6.0	3.4 ± 0.06	2030 ± 80	546 ± 23	3.7

[1]Mean ± SE, n = 150.

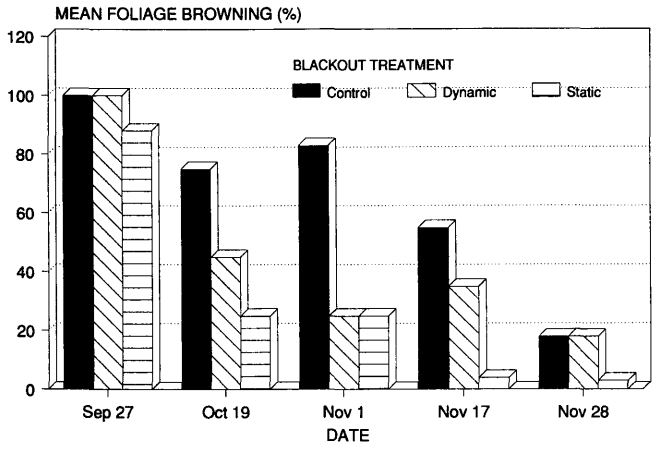

Figure 2.--Development of cold hardiness expressed as percent mean foliage browning assessed one week after frost hardiness tests for control and blackout treated spruce seedlings, SL3958. Test temperature on Sept. 27 and Oct. 19 was -13°C, on Nov. 1 was -17°C, and on Nov. 17 and Nov. 28 was -18°C.

for Engelmann spruce seedlings SL4311 (table 4). The trend for Sitka x white spruce seedlings showed an increase in first year height growth with both 13h and 15h photoperiods compared to 17h and controls. Averaging over treatment duration (weeks), the 13h photoperiod increased 149 mm in height and the 15h increased 146 mm compared to 116 mm and 91 mm for 17h and control, respectively (table 4). Stem diameter increment remained unaffected by blackout treatment.

Table 3.--Days to budbreak of spruce seedlings (SL 3958) in spring 1990 following frozen storage for control, dynamic, and static blackout treatments with the tests conducted at two temperatures (day/night)

Blackout Treatment	Days to budbreak 25°C/20°C	15°C/5°C
Control	10 ± 1.0[1]	11 ± 0.5
Dynamic	7 ± 0.4	11 ± 0.4
Static	7 ± 0.5	10 ± 0.5

[1]Mean ± SE, rep = 5 seedlings, n=2

The opposite effect was observed for SL4311 when planted in a regeneration site (table 5). The height growth for the 13h photoperiod was 111 mm (2 wk + 6 wk) compared to 130, 140, and 128 mm for 15h, 17h, and control treatments, respectively. Again, there appeared to be no effect on stem diameter growth due to blackout treatment during the nursery culture year.

Terminal bud phenology during the first year in the field was not affected by nursery blackout treatments for Engelmann spruce seedlings (table 6). On June 12, 1990 100% of all treated seedlings had flushed and 80% of the controls. However, within the next six days all the controls had flushed as well. By Aug. 22, 1990, all seedlings in all treatments had set bud with the

Table 4.--Seedling height increment and stem diameter increment following first year in the nursery bed at Red Rock Research Station for two seedlots 4311 and 3958, Engelmann spruce and sitka x white hybrid spruce, respectively.[1]

Treatment day (h)	weeks	Height increment (mm) SL 4311	Height increment (mm) SL 3958	Diameter increment (mm) SL 4311	Diameter increment (mm) SL 3958
13	2	123 ± 6[2]	138 ± 15	3.6 ± 0.6	2.5 ± 0.5
	4	140 ± 21	166 ± 18	4.4 ± 0.4	3.5 ± 0.3
	6	126 ± 10	144 ± 14	4.2 ± 0.3	3.9 ± 0.2
15	2	148 ± 4	129 ± 5	3.5 ± 0.2	3.2 ± 0.4
	4	132 ± 11	157 ± 9	3.8 ± 0.4	3.1 ± 0.3
	6	163 ± 10	153 ± 8	3.9 ± 0.1	4.0 ± 0.4
17	2	135 ± 5	97 ± 4	3.3 ± 0.2	3.4 ± 0.1
	4	126 ± 4	115 ± 1	3.1 ± 0.4	3.6 ± 0.4
	6	152 ± 12	135 ± 6	4.0 ± 0.3	3.1 ± 0.2
19	2	138 ± 4	81 ± 13	3.7 ± 0.3	2.7 ± 0.2
	4	151 ± 3	95 ± 2	3.8 ± 0.2	3.3 ± 0.1
	6	135 ± 5	98 ± 4	3.2 ± 0.6	2.6 ± 0.5

[1]Hawkins and Draper, 1990
[2]Mean ± SE, rep = 18 seedlings, n = 3.

Table 5.--Mean seedling height increment and stem diameter increment following first year outplanting in Kamloops Region for Engelmann spruce SL4311

Treatment day (h)	weeks	Height inc. (mm)	Diameter inc. (mm)
13	2	115 ± 7[1]	1.4 ± 0.06
	6	107 ± 2	1.7 ± 0.05
15	2	135 ± 6	1.4 ± 0.04
	6	125 ± 5	1.7 ± 0.07
17	2	140 ± 5	1.4 ± 0.08
	6	140 ± 5	1.5 ± 0.04
Control		128 ± 5	1.4 ± 0.08

[1]Mean ± SE, rep = 5 tree row, n=5.

exception of the 13h 2 wk treatment which had 5% of the seedlings without a resting terminal bud. Within one week 100% of the 13h 2 wk treatment seedlings had a final terminal bud, and no reflushing occurred during the rest of the growing season.

In the nursery bed at RRRS, the same seedlot showed the same phenology at budflush, i.e., no treatment effect (table 7). Terminal bud scale formation began nine weeks after planting (mid-July), and resting terminal buds developed in mid-August, 14 weeks after planting. However, in mid-August, reflushing was observed on seedlings from both the 13h, and 15h, for 2 wk and 6 wk treatments (table 7). This resulted in as much as a 14 day delay in final budset in the 13h photoperiod for the longest duration (6 wks).

The Sitka x white hybrid seedlings SL3958 continued to elongate longer into the growing season and did not start the final budset stage (stage 5) until mid-Sept. for control seedlings, and even later in Sept. and Oct. for treated seedlings (table 8). Seedlings from the 13h photoperiod appeared to set bud even later than either the 15h or 17h photoperiods.

DISCUSSION

A complete report of the 1988 experiment which included SL3958 and

Table 6.--Spruce seedling terminal bud modal phenology class during the first year in the field in Kamloops Region. Seedlings were produced in 1988 at RRRS using four daylengths and two durations of blackout culture.

Blackout Treatment day	weeks	Phenology Code[1]								
13h	2 wk	0	2	2	2	3	3	4	5	5
	6 wk	0	2	2	2	3	3	4	5	5
15h	2 wk	0	2	2	2	3	3	4	5	5
	6 wk	0	2	2	2	3	3	4	5	5
17h	2 wk	0	2	2	2	3	3	4	5	5
	6 wk	0	2	2	2	3	3	4	5	5
Control		0	2	2	2	3	3	4	5	5

Julian Day	150	163	170	182	194	205	220	234	248
Month	May	June		July			Aug.		Sept.

1989 field growing season

[1]Codes as described in Table 1.

Table 7.--Spruce seedling terminal bud modal phenology class during the first year in the nursery bed at RRRS for SL4311. Seedlings were produced in 1988 using four daylengths and three durations of blackout culture[1].

Blackout Treatment day	weeks	Phenology Code[2]											
13 h	2 wk	0	1	1	2	2	3	4	4	(4)	4	4	5
	4 wk	0	1	2	2	2	3	3	4	4	5	5	5
	6 wk	0	1	2	2	2	3	3	4	(4)	(5)	4	5
15 h	2 wk	0	1	1	2	2	3	4	4	(4)	4	5	5
	4 wk	0	1	1	2	2	3	3	4	4	4	5	5
	6 wk	0	1	1	2	3	3	3	4	(4)	5	5	5
17 h	2 wk	0	1	1	2	2	3	3	4	4	5	5	5
	4 wk	0	1	1	2	2	3	3	4	4	4	5	5
	6 wk	0	1	1	2	2	3	3	4	4	5	5	5
19 h	2 wk	0	1	1	2	2	3	3	4	4	5	5	5
	4 wk	0	1	1	2	2	3	4	4	4	5	5	5
	6 wk	0	1	1	2	2	3	4	4	4	5	5	5

Julian Day	133	139	149	157	171	180	194	209	223	235	249	275
Month	May			June			July		August		September	

1989 field growing season

[1]Adapted from Hawkins and Draper, 1990.
[2]Codes as described in table 1.
[3]Brackets indicate >20% terminal bud flushing.

Table 8.--Spruce seedling terminal bud modal phenology class during the first year in the nursery bed at RRRS for SL3958. Seedlings were produced in 1988 using four daylengths and three durations of blackout culture[1].

Blackout Treatment day	weeks	Phenology Code[2]											
13 h	2 wk	0	1	2	2	2	3	3	3	3	3	4	5
	4 wk	0	1	2	2	2	3	3	3	3	3	4	4
	6 wk	0	1	2	2	2	3	3	3	3	3	4	4
15 h	2 wk	0	1	1	2	2	2	3	3	3	4	4	5
	4 wk	0	1	2	2	2	3	3	3	3	4	4	5
	6 wk	0	1	1	2	2	3	3	3	3	3	4	4
17 h	2 wk	0	1	1	2	2	2	3	4	4	4	4	5
	4 wk	0	1	2	2	2	2	3	3	3	4	4	5
	6 wk	0	1	2	2	2	2	3	3	3	3	4	4
19 h	2 wk	0	1	1	2	2	2	3	4	4	4	5	5
	4 wk	0	1	1	2	2	2	3	3	3	3	4	5
	6 wk	0	1	1	2	2	2	3	3	4	4	4	5

Julian Day	133	139	149	157	171	180	194	209	223	235	249	275
Month		May			June		July		August		September	

1989 field growing season

[1]Adapted from Hawkins and Draper, 1990.
[2]Codes as described in Table 1.

SL4311 can be found in Hawkins and Draper (1990). The response of the vigorous Sitka x white hybrid spruce seedlings (SL3958) to a four week, 15h day blackout treatment varied in 1989 from the response in 1988 in both root dry weight and percent reflushing. The difference in mean root dry weight between controls and treated was 180 mg in 1988, but only 14 mg in 1989. Hawkins and Draper (1990) reported only 2% reflushing in this seedlot and these were lateral buds on seedlings in the 13h daylength treatment. This is minimal reflushing compared with the 26 - 30% recorded in 1989 which included terminal bud reflushing as well. The two growing seasons did differ as control seedlings in 1989 were 77mm taller than those in 1988, and had 12% less root dry weight.

Hawkins and Draper (1990) have given many reasons for using the dynamic method for applying blackout and one possible positive feature is that this method may give adequate height regulation without negatively impacting on seedling physiology. Cold hardiness development was accelerated by blackout treatments in both years, however in 1989, the dynamic method produced seedlings that behaved more like the control seedlings (figure 2).

Days to budbreak for SL3958 were 10 days in 1989 compared to seven days in 1990. Hawkins and Draper (1990) reported that DBB decreased with decreasing daylength and increasing duration. However, there was only five days difference between controls (12 days) and the severist blackout treatment (13h for 6 weeks=7 days). They observed the same trend in white and Engelmann spruce though the difference was only two days for the same comparison.

In the nursery bed there was little or no treatment effect on budflush or budset except with the 13h daylength. For SL4311 on the regeneration site, all treatments were at 100% budset by August 22, 1990 except the 13h for two week blackout treatment which was at 95%. This is in contrast to the results for black spruce in Ontario where short day seedlings remained actively growing for three to six weeks longer than untreated (Odlum and Columbo, 1988). The

difference in response between nursery bed and field in our study was the reflushing that was recorded in the nursery bed. This may be related to the fertilization and irrigation practised in the nursery compared with the drier regional site.

First year after planting terminal height growth was unaffected by the previous blackout treatments for SL4311 in nursery beds. Results from the outplanting in the region showed a reduction in height increment for seedlings receiving the 13h daylength compared to all other treatments. This same trend was observed in the three other regional outplantings (data not presented). The difference in height increment between nursery and field again may be due to the watering, fertilizing, and weeding that occurred in the nursery.

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Morphological Development of Field-Planted Western Hemlock Seedlings from Various Dormancy Induction Treatments[1]

S. C. Grossnickle, J. E. Major, and J. T. Arnott[2]

Abstract.—Western hemlock seedlings from four dormancy induction treatments (i.e. long-day dry, long-day wet, short-day dry, short-day wet) were planted on a coastal reforestation site in British Columbia and monitored for morphological development over two growing seasons. Short-day wet treated seedlings had the greatest incremental height growth and lowest stem units cm^{-1} over two growing seasons. Short-day treated seedlings had the least needle damage after two years in the field. Seedlings from all treatments had good root development and this was reflected in high survival (i.e. approx. 90%) after two field seasons.

INTRODUCTION

Performance of seedlings planted on a reforestation site is dependent on seedling quality and site environmental conditions (Timmis 1980, Burdett 1983 & 1990, Sutton 1988, Puttonen 1989). To quantify the degree of improvement from any particular stocktype requires an assessment of seedling performance both before and after field planting.

Western hemlock (Tsuga heterophylla (Raf.) Sarg.) seedlings were grown under a series of dormancy induction treatments (DIT) and tested with a stock quality assessment procedure that measured seedlings material and performance attributes (Grossnickle et al. 1988, 1990a). Material attributes measured were morphology, pressure-volume analysis and soluble sugars. Performance attributes measured were root growth capacity at high and low root temperature, seedling water movement at high and low root temperature, low root temperature response, drought stress response and frost hardiness. Seedlings from all DIT were in good physiological and morphological condition when tested under optimum environmental conditions, while short-day DIT, particularly short-day wet, showed the best establishment potential under less than ideal environmental conditions (e.g. low soil temperature and drought) (Grossnickle et al. 1988, 1990a).

In the second phase of the research program, partially reported in this paper, seedlings from these DIT were planted on a reforestation site in coastal British Columbia and morphological development monitored over two growing seasons.

MATERIALS AND METHODS

Plant Material

Western hemlock (Tsuga heterophylla (Raf.) Sarg.) seedlings were grown from seed at the Pacific Forestry Centre, Victoria, British Columbia, Canada (Lat. 48° 28" N) in BC/CFS 313A styroblocks. Nursery cultural program is described in Grossnickle et al. (1990a). On July 20, 1987, when seedling population mean shoot height was 15.8 cm, one fourth of the seedling population was treated with one of the following dormancy induction treatments (DIT):

[1]Paper presented at the Western Forest Nursery Council 1990 Conference, Umpqua Community College, Roseburg, Oregon, August 13-17, 1990.

[2]S.C. Grossnickle is a Research Scientist and J.E. Major is a Research Forester at the Forest Biotechnology Centre, B.C. Research Corporation, Vancouver, British Columbia. J.T. Arnott is a Research Scientist at Forestry Canada, Victoria, British Columbia.

1) Long-day wet (LDW); seedlings continued to grow under long (16h) photoperiod to prevent bud set and normal watering and fertilization regime.

2) Long-day dry (LDD); seedlings continued to receive an extended photoperiod, but a moisture stress treatment was initiated.

3) Short-day dry (SDD); seedlings had a moisture stress treatment initiated and photoperiod was reduced to 8 hours on August 1, 1987.

4) Short-day wet (SDW); seedlings continued to receive normal watering and fertilization regime, but had the photoperiod reduced to 8 hours on August 1, 1987.

All dormancy induction treatments were concluded on August 29, 1987 after which time fall watering, fertilization, daylength and temperature regimes were implemented. Full details are described in Grossnickle et al. (1990a). Seedlings were placed in cold storage (2°C) on January 11, 1988 and held until field planting on February 24-28, 1988.

During January and February 1988 seedlings were tested with the above mentioned stock quality assessment procedure (Grossnickle et al. 1988, 1990a).

Field Site Conditions

The test site was located at Cowichan Lake on southern Vancouver Island, British Columbia, Canada (Lat. 48° 49' N, Long. 124° 10'W). The site was logged of second growth Douglas-fir (Pseudotsugsa menziesii (Mirb.) Franco) in 1986 with no subsequent site preparation. Elevation is 165 m above sea level. Land is undulating well-drained, gravely, sandy-loam (coarse fragments 30-50-20%), Duric Humo-Ferric Podzol of the Quimper soil association (Jungen 1985). Biogeoclimatic zone is Coastal Western Hemlock and the variant is Vancouver Island Dry Maritime CWHal (Klinka et al. 1984). Vegetative competition was characterized in late June 1988 on sixteen randomly selected 1 x .5 m plots. Mean vegetation cover was 63% and ranged from 95 to 33% with an average maximum vegetation canopy height of 30 cm with a range of 50 to 10 cm. Species composition is described in Grossnickle et al. (1990b).

Seedlings were planted during late February, 1988 in a randomized block (3) design. Seedlings from each DIT (4) were represented in 10 randomly selected rows for a total of 40 rows per block with 30 seedlings planted per row in a 1 m x 1 m spacing. A total of 900 seedlings from each DIT were planted.

Morphological Assessment

In each row, selected seedlings (i.e. 1, 5, 10, 15, 20, 25, 30) were reserved for survival and permanent growth measurements. Three randomly selected seedlings in each row were planted in buried cylindrical (25 cm diameter, 30 cm length) porous felt bags. This facilitated removal of twenty seedlings from each DIT at 8 and 20 months (i.e. November 1988 and 1989, respectively) after planting to determine root and shoot development. Further discussion of the root analysis technique can be found in Grossnickle and Reid (1983). Seedling morphological parameters assessed on seedlings excavated at 8 and 20 months were: 1) shoot height, 2) root collar diameter, 3) shoot dry weight, 4) needle damage index, 5) stem units cm^{-1} of new main shoot growth, 6) root dry weight in container plug, 7) root dry weight in soil, 8) total root dry weight, 9) number of new roots, 10) total length of new roots, 11) total shoot to total root ratio (dry wt.) and 12) seedling water balance ratio (shoot dry weight/ [diameter x total root dry weight]). Needle damage index quantified the visual assessment of percent needles where: 1=100%, 2=90-99%, 3=75-89%, 4=50-74%, 5=25-49% and 6=1-24% green needles. Stem unit measurements were taken from the middle of the main shoot tip of new growth. A stem unit is defined as an internode, together with the node and nodal appendages at its distal extremity (Doak 1935). Growth data were subjected to analysis of variance and a mean separation test (p=0.05) (Steel and Torrie 1980). Statistical analysis was not conducted on field incremental diameter data because nursery data were used to determine initial field diameters.

RESULTS AND DISCUSSION

Nursery Development

Long-day wet seedlings had the biggest overall shoot system with a greater height and shoot dry weight than any other DIT (Table 1). Both LDW and SDW had a greater root collar diameter than water stressed DIT. Similar studies with western hemlock have also shown greater height growth in LDW treated seedlings and greater diameter growth in non water stressed treatments (Arnott et al. 1988, O'Reilly et al. 1989).

Root dry weight was greater in non water stressed DIT (Table 1). Reduction in root development in water stressed DIT are comparable to a similar study with western hemlock (Arnott et al. 1988). Studies have shown root dry weight to decline with seedling moisture stress (Leshman 1970, Day and MacGilvray 1975, Larson 1980). Root growth in western hemlock is

Table 1. – Morphological development of western hemlock seedlings from different dormancy induction treatments just before planting and at the end of first and second growing season on a reforestation site.

Dormancy Induction Treatment	SHOOT Height (cm)	SHOOT Root Collar Diameter cm	SHOOT Dry Weight (g)	SHOOT Needle Damage Index[1]	SHOOT Stem Units (cm^{-1})	ROOT Container Plug Dry Wt. (g)	ROOT In Soil Dry Wt. (g)	ROOT Total Dry Wt. (g)	SHOOT/ROOT RATIOS Total Shoot / Root (by Wt.)	SHOOT/ROOT RATIOS Seedling Water Balance[2]
				FEBRUARY 1988 (Before Planting)						
LDD[3]	27.18±.61b[4]	.27±.01b	1.27±.07bc	1.00[5]	-	.35±.02c	-	.35±.02c	-	14.68±.86b
LDW	31.52±.96a	.32±.01a	1.96±.10a	1.00	-	.49±.04ab	-	.49±.04ab	-	13.65b
SDD	22.18±.57c	.28±.01b	1.00±.03c	1.00	-	.40±.03bc	-	.40±.03bc	-	10.19±.76a
SDW	23.04±.52c	.31±.01a	1.28±.08b	1.00	-	.53±.04a	-	.53±.04a	-	8.69±.55a
				NOVEMBER 1988						
LDD	36.49±1.12a	.433±.016a	3.28±.15ab	3.06±.26ab	12.76±.42bc	1.19±.10a	.673±.082a	1.87±.16a	1.91±.12b	4.59±.42a
LDW	35.07±1.60a	.443±.014a	3.47±.26a	3.44±.18b	13.50±.58c	1.28±.12a	.806±.093a	2.08±.17a	1.73±.10ab	3.97±.25a
SDD	30.71±1.39b	.429±.015a	2.82±.20ab	2.50±.23a	11.68±.50b	1.08±.11a	.813±.108a	1.89±.21a	1.60±.10ab	3.87±.35a
SDW	30.72±1.46b	.425±.017a	2.75±.21b	2.89±.21a	8.86±.52a	1.06±.08a	.798±.108a	1.86±.18a	1.57±.11a	3.80±.30a
				NOVEMBER 1989						
LDD	46.14±2.72a	.58±.03a	7.90±1.03a	3.85±.22c	6.15±.53b	1.57±.18b	1.55±.32a	3.12±.48a	2.75±.16b	5.24±.58b
LDW	47.00±2.82a	.60±.03a	7.98±1.00a	3.35±.17c	5.90±.69b	1.95±.21ab	1.46±.30a	3.41±.48a	2.56±.19b	4.54±.49ab
SDD	41.56±2.41a	.61±.03a	7.98±1.12a	2.80±.19b	6.60±.73b	2.62±.40a	1.81±.36a	4.46±.74a	2.01±.18a	3.54±.45a
SDW	48.38±1.86a	.60±.02a	8.65±.89a	1.85±.17a	3.75±.31a	2.06±.25ab	1.63±.29a	3.70±.53a	2.62±.18b	4.52±.38ab

(1) Needle damage index was catagorized as: 1=100%, 2=90-99%, 3=75-89%, 4=50-74%, 5=25-49%, and 6=1-24% green needles.

(2) Seedling water balance ratio is: shoot dry weight/(diameter x total root dry weight).

(3) LDD = Long-day dry
LDW = Long-day wet
SDD = Short-day dry
SDW = Short-day wet

(4) Mean and standard error. A difference in the letter for a morphological variable within each harvest date indicates a significant difference between dormancy induction treatment at p = 0.05 as determined by ANOVA and Waller-Duncan mean separation test.

(5) No statistical analysis due to lack of variation in one or more treatment(s).

seasonal with high root growth normally occurring during early summer (Zaerr and Brown 1976). Water stress DIT, applied during early to mid summer, resulted in reduced root growth.

Short-day DIT had a lower shoot to root ratio and a better (i.e. lower) seedling water balance ratio than long-day DIT (Table 1). In newly planted seedlings, low shoot to root and seedling water balance ratios are important to ensure survival by avoiding the development of high water deficits caused when absorption lags behind transpiration (Kramer and Kozlowski 1979, Thompson 1985).

First Year Development on the Reforestation Site

Long-day DIT seedlings had the largest shoot height and dry weight, while root collar diameter was similar between all DIT (Table 1). O'Reilly (personal communication) found western hemlock long-day, compared to short-day, DIT seedlings had larger diameter growth after one field growing season. Stem units cm^{-1} was lowest to highest in SDW, SDD, LDD and LDW. Lower stem units cm^{-1} indicates greater cell elongation (O'Reilly et al. 1989) and is probably attributable to reduced stress during growth. Short-day DIT seedlings had lower needle damage index than long-day DIT. Short-day DIT seedlings, and especially SDW, had the greatest seasonal incremental height growth and LDD seedlings the greatest seasonal incremental root collar diameter growth (Fig. 1). Short-day DIT seedlings appear better able to withstand stressful reforestation site environmental conditions. Seedling quality assessment results indicated short-day, compared to long-day, DIT seedlings had a better cold and drought stress performance potential (Grossnickle et al. 1988, 1990a) and had better photosynthetic and stomatal conductance capability during summer environmental conditions on the reforestation site (Grossnickle and Arnott 1990).

Root development was similar between all DIT (Fig. 2, Table 1). Short-day, compared to long-day, DIT seedlings had a better shoot to root ratio, while all treatments had a similar seedling water balance ratio. Seedlings from all treatments were well established on the reforestation site after one growing season (Fig. 3). This contrasts work with 1+0 western hemlock seedlings planted on a dry south facing clear-cut where a greater shoot to root imbalance occurred after one growing season (Livingston and Black 1988).

Seedling survival at the end of the first growing season was between 95 and 97% for all DIT (Fig. 4). This survival is higher than previously reported for western hemlock seedlings from similar DIT grown for one field season (O'Reilly, personal communication). Non stressful environmental conditions during the first half of the growing season (i.e. moderate mean temperatures) and high monthly precipitation) allowed seedlings from all DIT to grow roots and become well established

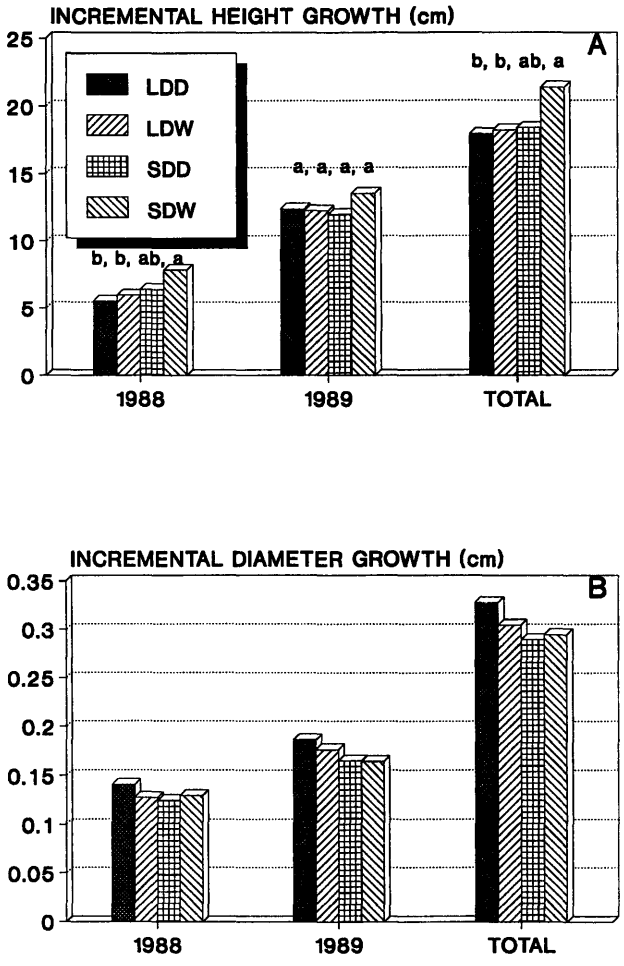

Figure 1.– Incremental height (A) and diameter (B) growth over two growing seasons (1988 & 1989) for western hemlock seedlings from dormancy induction treatments: 1) long–day dry (LDD), 2) long–day wet (LDW), 3) short–day dry (SDD) and 4) short–day wet (SDW). Means covered by the same letter are not significantly different at the 5% level.

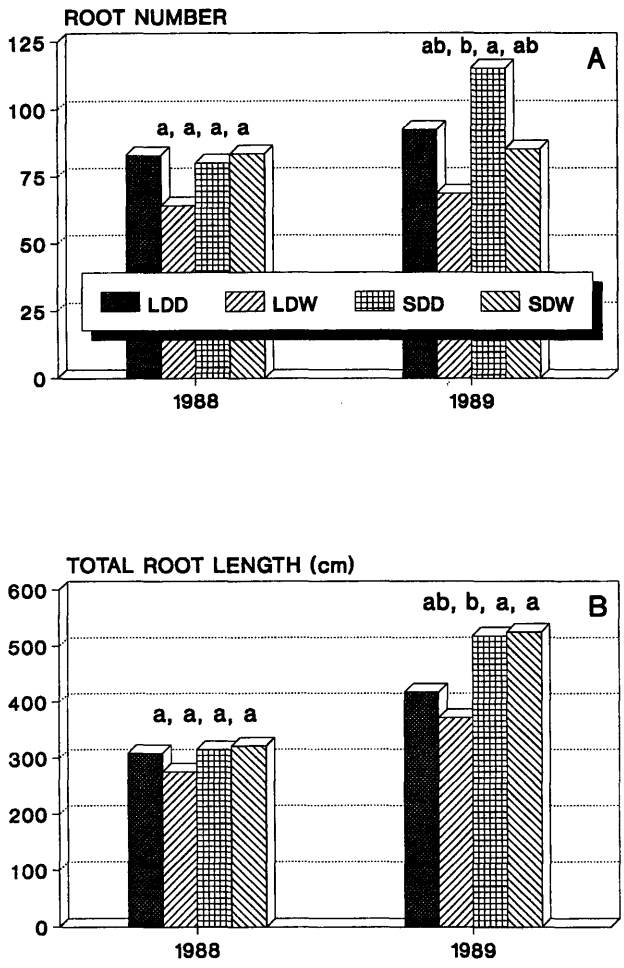

Figure 2.– Number of roots (A) and total root length (B) development outside the container plug over two growing seasons (1988 & 1989) for western hemlock seedlings from dormancy induction treatments: 1) long-day dry (LDD), 2)long-day wet (LDW), 3) short-day dry (SDD) and short-day wet (SDW). Means covered by the same letter are not significantly different at the 5% level.

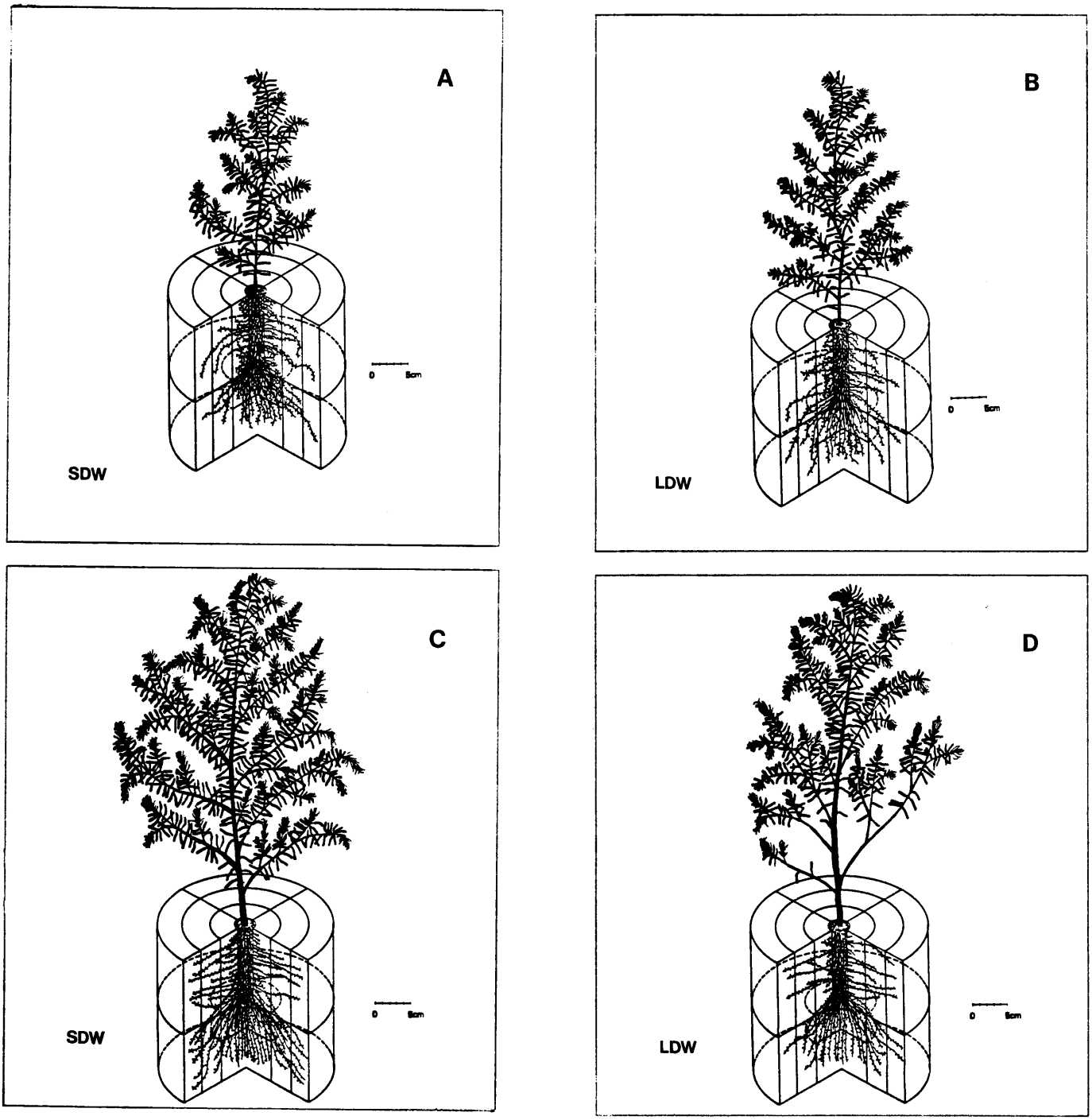

Figure 3.– Diagramatic representation of western hemlock seedling shoot and root development (n=20) from short-day wet (SDW) and long-day wet (LDW) dormancy induction treatments at eight (A and B) and twenty (C and D) months after planting on a reforestation site.

(Table 1 & Fig. 3). Thus, when drought or high evaporative demand occurred during the summer, seedlings did not experience severe levels of water stress (Grossnickle et al. 1990b, Grossnickle and Arnott 1990) because adequate root development provided the capability to extract water from a large soil volume. Previous western hemlock reforestation trials have found seedling mortality to be high when root development is restricted (Arnott 1975, Livingston and Black 1988).

Second Year Development on the Reforestation Site

Shoot height, root collar diameter and dry weight were similar between all DIT (Table 1). Short-day wet seedlings had the lowest number of stem units cm^{-1}, plus the greatest incremental height growth in 1989 and total over two growing seasons (Fig. 1). Seedlings from all DIT at least doubled their incremental shoot height growth during the second growing season. Once established in the field, western hemlock seedlings have the capability to grow very rapidly (Arnott 1975, 1976, Arnott and Burdett 1988). Incremental diameter growth was similar between DIT. SDW seedlings had the lowest needle damage index of all DIT (Table 1) and SDW, compared to LDW, seedlings showed better shoot form and less needle drop (Fig. 3). Stock quality assessment procedures predicted SDW seedlings to have the best field performance potential to adverse environmental conditions (Grossnickle et al. 1988, 1990a), and two years later in the field their shoot systems still seem to be in the best overall morphological condition.

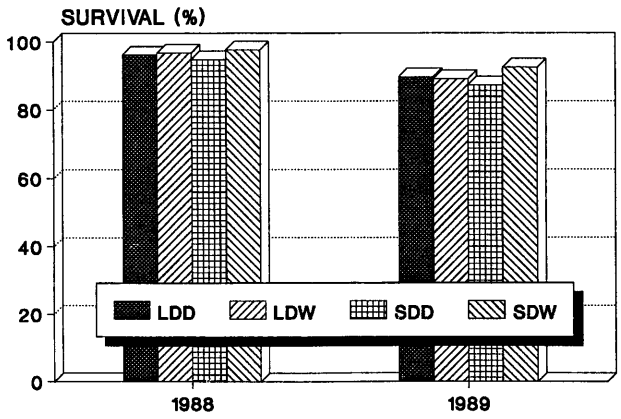

Figure 4.– Percent survival after the first (1988) and second (1989) growing seasons for western hemlock seedlings from dormancy induction treatments: 1) long-day dry (LDD), 2) long-day wet (LDW), 3) short-day dry (SDD) and 4)short-day wet (SDW).

Root dry weight measurements were similar between all DIT (Table 1). Seedlings from all DIT showed root development characteristic of an established styro-plug western hemlock seedling (Arnott 1976, 1978) with large masses of fibrous roots symmetrically distributed around the original root plug and no indication of a taproot (Fig. 3). Long (1978) found that less than 20% of planted western hemlock seedlings had well developed taproots. Long-day wet, compared to other DIT, seedlings had the least number of roots and total root length for roots developed outside the container plug (Fig. 2) and this is shown diagrammatically in figure 3. Seedlings in all DIT had more roots extend from the bottom third of the plug than from upper zones and this is comparable to previous work with styro-plug western hemlock seedlings (Long 1978, Carlson and Shaw 1981).

SDD seedlings had the lowest total shoot to total root and seedling water balance ratios (Table 1). Carlson (1981) reported comparable shoot to root ratios for 1+0 styro-plug western hemlock seedlings after two field seasons. Interestingly, shoot to root ratios for seedlings in all DIT increased by the end of the second growing season. Western hemlock is known for increasing its shoot to root ratio as tree size increases (Eis 1974) and this also occurs during seedling establishment (Long 1978). This inherent growth strategy, coupled with optimal environmental conditions over much of both growing seasons resulted in greater shoot development at the expense of root development.

Seedling survival at the end of the second growing season was between 87 and 92% with SDW treatment having the highest survival rating (Fig. 4). This second year survival is higher than reported in earlier western hemlock field trials (Arnott 1974, 1975, 1976).

SUMMARY AND CONCLUSION

Morphological development of western hemlock seedlings from a series of DIT was monitored over two growing seasons on a reforestation site. Previous stock quality assessment found seedlings from all DIT in equivalent physiological condition when exposed to optimum environmental conditions, while short-day DIT, and especially SDW, had a better capability to respond to limiting (i.e. low temperature and drought) environmental conditions (Grossnickle et al. 1988, 1990a). At the time of planting, LDW seedlings had the largest shoot system and short-day DIT had the best shoot to root balance. After two growing seasons in the field all DIT had equal height, diameter

and shoot dry weight indicating that SDW, SDD and LDD seedlings grew more than LDW. Short-day wet seedlings had the greatest incremental shoot height growth and the least number of stem units cm^{-1} over two field growing seasons. Short-day treatments also suffered less needle damage indicating that their shoot systems were better conditioned to site environmental conditions. Root development over two growing seasons was equal between DIT. Root growth was sufficient to reduce stress that occurs during seedling establishment and this was indicated by the high rate of seedling survival in all DIT.

The combination of stock quality assessment results and subsequent field trial performance provided a comprehensive information base to select the best performing stocktypes for specific reforestation site conditions. In this program, western hemlock seedlings from short-day, and especially SDW, DIT had the best performance capability in both stock quality assessment testing and field performance over two growing seasons on a coastal reforestation site.

ACKNOWLEGDEMENT

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Performance of Conifer Stocktypes on National Forests in the Oregon and Washington Coast Ranges[1]

Ralph E. Duddles and Peyton W. Owston[2]

Abstract.--During the 1970's, container and bareroot stocktypes of conifer timber species were widely tested in the Coast Ranges of Oregon and Washington. Both survival and total height varied widely in tests on national forests. After 4 to 5 years, neither stocktype survived consistently better than the other. However, on these relatively moist sites with lush development of competing vegetation and high animal populations, larger nursery stock (represented by bareroot seedlings) tended to grow taller than stock that was initially smaller. Site factors seemed to influence survival and growth more than did the original stocktype.

INTRODUCTION

The decade of the 1970's saw rapid development of forest nursery technology. High demand for seedlings, lack of good sites for expansion of bareroot nurseries, and a perceived biological advantage of protected root systems fostered creation of container nurseries. The new stocktypes were being planted operationally, and they were also being tested throughout the Pacific Northwest to determine their suitability and limitations.

From 1974 through 1976, scientists from the Pacific Northwest Research Station of the USDA Forest Service initiated extensive field tests on the national forests in the Pacific Northwest Region. These planting trials were designed to systematically compare survival and growth of container and bareroot stocktypes on a wide variety of sites and in a number of different field conditions (Owston and Stein 1974). In addition, stocktype comparisons were part of a large, integrated study of reforestation systems on the Siuslaw National Forest in western Oregon (Stein 1984, unpubl.). Examination of the 4- or 5-year results of all these trials provides insight and guidance for the use of nursery stock in current reforestation operations (age was consistent within trials). This paper focuses on two primary species, Douglas-fir (Psuedotsuga menziesii (Mirb.) Franco) and western hemlock (Tsuga heterophylla (Raf.) Sarg.). Some trials of Sitka spruce (Picea sitchensis (Bong.) Carr.) and western redcedar (Thuja plicata Donn ex D. Don) are also described.

METHODS

For the Region-wide trials, container and bareroot seedlings of one species were planted on each of numerous test sites. The sites were part of the normal reforestation programs of the participating ranger districts. Four plots, each consisting of two rows of 25 trees of each stocktype, were to be established on each site. Variations in this design occurred on some sites to meet special objectives or to accomodate site conditions (e.g., several different sizes of containers were tested in a few of the trials by adding additional rows). Plot locations were selected so that slope, aspect, and composition of associated species were representative of the area and relatively homogeneous within individual plots. Unplantable spots were skipped, and rows were extended to provide the required 25 planting spots. Generally, survival was checked and seedling heights were measured every other year.

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Several steps were taken to ensure unbiased comparisons:

1. Within each trial, the container and bareroot seedlings were from the same seed source.
2. Both stocktypes were planted concurrently to eliminate weather as a variable.
3. Trees within a plot were planted by the same person, or planters were rotated between stocktypes to minimize planting quality as a bias.
4. Animal protection measures or other treatments were applied the same to each stocktype in a trial.

Similar methods were used for the integrated study on the Siuslaw National Forest, except that site preparation treatments and more stocktypes were included in large installations on six clearcuts (Stein 1984). Douglas-fir and western hemlock were both planted.

Each of the sites in the Region-wide study was considered separately for statistical analyses (analysis of variance). The results are indicated for example sites to be described in detail. The six sites in the Siuslaw study were designed and analyzed as one experiment (Stein 1984); these results will be detailed in a later research paper.

We provide an overview of coastal results by presenting the number the individual coastal study trials in which one stocktype did better, worse, or about the same as the other. For survival, one comparison considers performance to be the same if the mean survivals of both stocktypes are within 10 percent of each other. A second comparison considers performance to be the same if the means do not differ by more than 20 percent. These are arbitrary thresholds used to indicate a general levels of comparability that the authors feel are useful to reforestation specialists.

Similar comparisons were made for total height using arbitrary threshold values of 10- and 20-percent differences between mean heights to categorize stocktypes as being the same or different from each other.

NURSERY STOCK

The majority of tests were performed using seedlings grown in relatively small containers, 2.4 to 4 cubic inches in volume, and average 2+0 bareroot seedlings (tables 1 and 2). Most of the container stock was grown at the Beaver Creek Seed Orchard of the Siuslaw National Forest, near Corvallis, Oregon. The containers used were either styrofoam blocks with cavities or individual plastic cells (RLP's). All of the container stock was planted as plug seedlings; i.e., removed from the containers before planting. The bareroot stock was produced mostly at the Humboldt Nursery in northern California or the Wind River Nursery in southwestern Washington.

Stein's (1984) study on the Siuslaw National Forest included several different classes of bareroot Douglas-fir stock, but only the medium-size class was considered in this paper. Only one size-class of western hemlock was used. Half of Stein's seedlings were protected from animal damage and half were not. We used the data from unprotected stock, because stock in the Region-wide study were also unprotected.

Of 28 trials included in our data, 13 were with Douglas-fir, 10 with western hemlock, 3 with Sitka spruce, and 2 with western redcedar.

Table 1.--Characteristics of planting stock for example trials of Douglas-fir.

	Trial no.			
Description	1	2	3	4
When Planted	12/73	3/76	11/77	7/75
Bareroot type	2+0	2+0	2+0	2+0
Ave. ht. (cm)	Unk.	30	38	35
Ave. cal.(mm)	Unk.	Unk.	5.2	3.4
Container type	Styro-2	RLP	RLP	Styro-2
Root Vol.(cc)	40	65	65	40
Ave. ht. (cm)	20[1]	19	11	21
Ave. cal.(mm)	2.5[1]	Unk	2.1	2.5

[1]Estimated from species averages for seedlings produced at the same nursery during the same time period.

Table 2.--Characteristics of planting stock for example trials of western hemlock (WH), Sitka spruce (SS), and western redcedar (WRC) on the Siuslaw National Forest.

	Trial no.			
Description	5	6	7	8
Species	WH	WH	SS	WRC
When Planted	2/75	4/74	1/74	7/75
Bareroot type	2+0	Wildling	2+0	2+0
Ave. ht. (cm)	26	Unk.	20	Unk.
Ave. cal.(mm)	3.4	Unk.	2.8	Unk.
Container type	Styro-2	Styro-2	Styro-2	Styro-2
Root vol.(cc)	40	40	40	40
Ave. ht. (cm)	15	14	27	20[1]
Ave. cal.(mm)	2.0	2.0	2.2	2.2[1]

[1]Estimated from species averages for seedlings produced at the same nursery during the same time period.

STUDY SITES

Details are given for four trials of Douglas-fir on the Olympic, Siuslaw, and Siskiyou National Forests (table 3) and trials of western hemlock, Sitka spruce, and western redcedar on the Siuslaw (table 4) to illustrate examples of variations encountered. Elevations of the sites ranged from 800 to 2,700 feet above sea level. Soil conditions varied from those typical of the Coast Ranges to shallow, rocky sites perceived or experienced to be difficult to reforest. All of them were on clearcuts and most of them had been burned for site preparation; but re-encroaching vegetative competition was variable.

Sites in the other 20 trials covered a similar range of conditions.

RESULTS

Example Sites

One readily apparent result is that differences in average survival were much greater between sites than between stocktypes within sites. Assuming little change in survival between 4th and 5th years, differences between sites ranged from 2 to 52 percent, whereas those between stocktypes within sites ranged from 0 to 12 percent (figs. 1 and 2).

Considering only the 5-year-old trials, the range of height differences between sites was 9 to 161 centimeters, whereas differences between stocktypes within sites ranged from 5 to 19 centimeters (figs. 3 and 4).

Table 3.--A summary of site conditions for example trials of Douglas-fir planted in the mid-1970's.

	Trial no.			
Description	1	2	3	4
National Forest	Siuslaw	Siskiyou	Siskiyou	Olympic
Ranger District	Mapleton	Gold Beach	Gold Beach	Soleduck
Elevation (ft.)	1,000	2,700	1,500	1,400
Aspect	South	Southeast	West	North
Site Index	IV	III	II	III
Soil Depth & Character	Shallow Gravelly	Medium Rocky	Medium Loam	Shallow Rocky
Site Preparation	Burned	Burned	Burned	Burned
Cover at Planting	Open	Brush (tanoak)	Open	Hvy. herbs & slash

Table 4.--A summary of site conditions for example trials of western hemlock (WH), Sitka spruce (SS), and western redcedar (WRC) planted in the mid-1970's on the Siuslaw National Forest.

	Trial no.			
Description	5	6	7	8
Ranger District	Alsea	Hebo	Waldport	Mapleton
Species	WH	WH	SS	WRC
Elevation (ft.)	1,200	800	750	900
Aspect	North	South	East	W to N
Site index	II	II	II	III
Soil depth & character	Medium Well-drained	Medium Well-drained	Medium Loam	Shallow Rocky
Site preparation	Unburned	Burned	Burned	Burned
Cover at planting	Medium brush	Light brush	Open	Hvy. herbs & slash

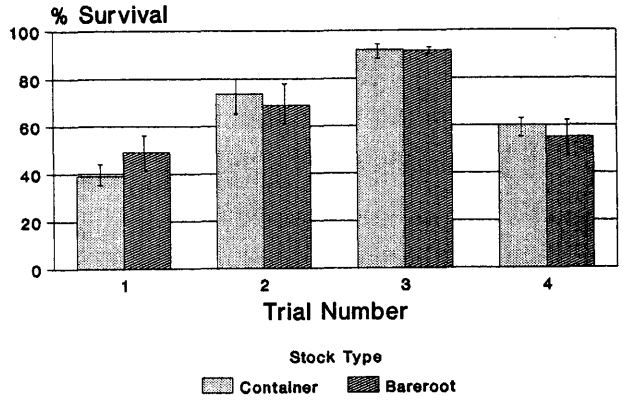

Figure 1.--Average survival 4 (trials no. 1 and 4) or 5 years after planting of Douglas-fir stocktypes in example trials on coastal sites in Oregon and Washington. Vertical bars represent 1 standard error. None of stocktype differences are significant at the 95% level.

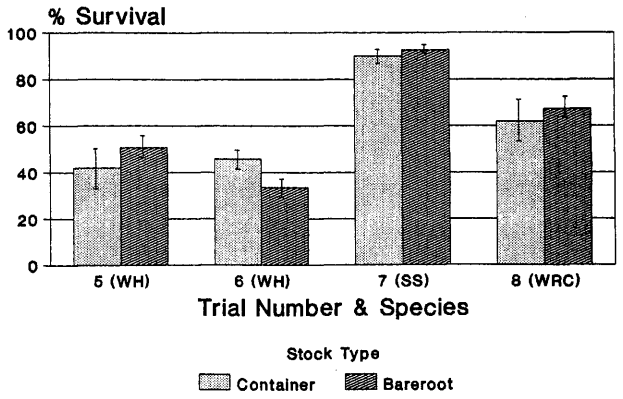

Figure 2.--Average survival 4 (trial no. 5) or 5 years after planting of western hemlock, Sitka spruce, and western redcedar stocktypes in example trials. Vertical bars represent 1 standard error. None of the stocktype differences are significant at the 95% level.

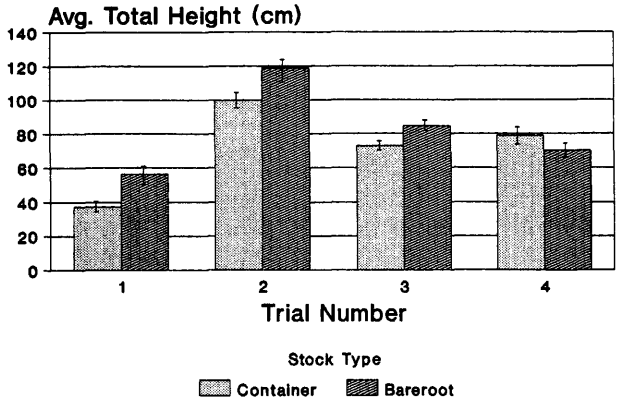

Figure 3.--Average total height 4 (trials no. 1 and 4) or 5 years after planting of Douglas-fir stocktypes in example trials. Vertical bars represent 1 standard error. Stocktype differences in trials no. 1 and 3 are significant at the 95% level.

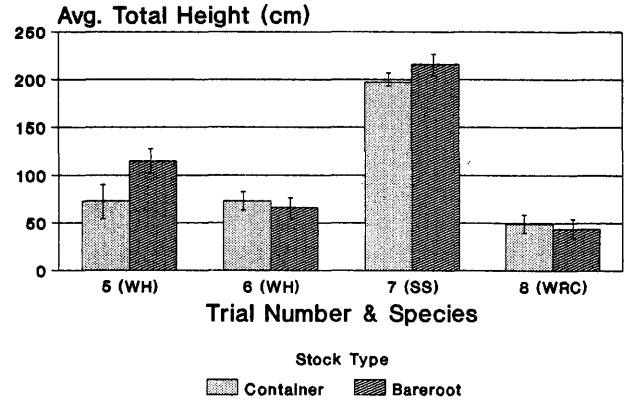

Figure 4.--Average total height 4 (trial no. 5) or 5 years after planting of western hemlock, Sitka spruce, and western redcedar stocktypes in example trials. Vertical bars represent 1 standard error. Stocktype differences in trials no. 5 and 7 are significant at the 95% level.

In two other tests where containers both 2.4 and 4.0 cubic inches in volume were used, survival differences were only 3 to 4 percent and inconsistent; height differences in the seedlings after 4 or 5 years were only 6 to 7 percent of average total height, but the larger containers yielded the tallest average seedling height in both cases. In another trial of Sitka spruce, where containers with volumes of 2.4 and 8.0 cubic inches were used, survival after 5 years was 90 percent and 100 percent, respectively; average total height was 197 and 265 centimeters, respectively.

Overall Comparisons

A total of 28 stocktype study sites were installed by the PNW Research Station on coastal sites in Oregon and Washington (counting Stein's sites twice--once for each of the two species). Figures 5 and 6 display the numbers of these sites in which one stocktype or the other does better, worse, or about the same as the other.

The most striking observations from these comparisons are:

1. Stocktype very seldom made a large difference in survival (i.e., 20% or more).
2. Even small differences in survival between stocktypes (10% to 19%) occurred in only about half of the tests, and one type was better about the same number of times as the other.

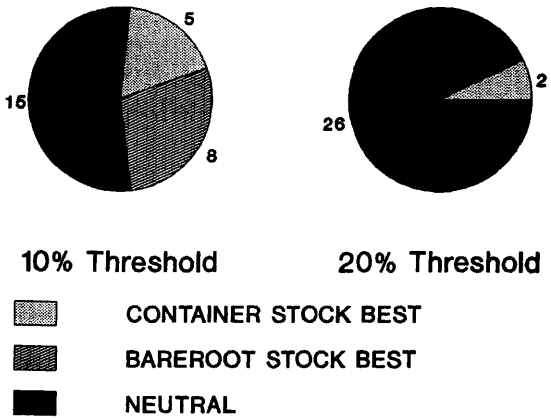

Figure 5.--Number of individual trials in which one stocktype had better, the same, or worse average survival than another.

3. In terms of total height after 4 to 5 years, bareroot seedlings showed an advantage at both the 10- and 20-percent thresholds.

DISCUSSION

In terms of survival, bareroot and container stocktypes have performed fairly similarly on coastal sites. Individual cases when one stocktype has done much better than another can sometimes be traced to a specific case of poor or mishandled stock, and we suspect that has been the case in other, untraceable situations.

Some readers might be surprised at the relatively low survival of both stocktypes in some of the examples. We ascribe this to two causes: (1) sites selected for container vs. bareroot tests were often the very toughest on the national forests, because the silviculturists selecting the sites were looking for answers to difficult problems; and (2) seedlings were not protected from animal damage in most of the tests. In situations where half of the seedlings of each type were protected and half unprotected, the protection treatment (tubing with rigid plastic mesh) significantly improved survival of both stocktypes (Stein 1984, unpubl.).

In terms of growth on coastal sites, initial seedling size seemed more important than if it was raised in a container or in a bareroot seedbed. The few tests of different container sizes in the Region-wide study and Stein's data for different size classes of bareroot stock (Stein, 1984 unpubl.) indicated that larger seedlings grew better than smaller ones. The relatively mesic environment makes top/root ratio less critical than in dry areas. Also, large seedlings have an advantage in withstanding animal damage and being able to stay ahead of the regrowth of competing vegetation.

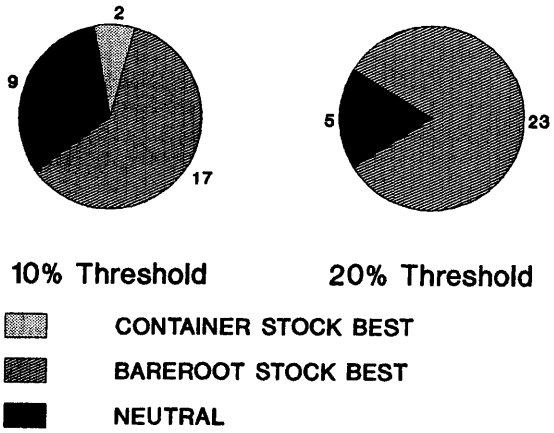

Figure 6.--Number of individual trials in which one stocktype was taller, the same, or shorter in average total height than another.

It is our opinion that factors of seedling physiological condition, size at time of planting, and environmental conditions on the planting site override differences in performance potential between stocktypes. This is based on personal experience, studying available data, and examining the literature (Owston, this volume). Empirical trials of stocktypes are probably only useful for very specific areas and situations where particular types are related directly and consistently to distinct sizes and conditions of seedlings.

It was the hope of many early proponents of container seedlings, including the authors, that the protected, relatively undamaged root systems and opportunity to fine-tune their condition in greenhouses would provide a biological advantage that would more than offset their generally smaller initial size. This has not been the experience on the coastal national forests in the Pacific Northwest. Also, a stocktype trial in the Oregon Coast Range by one Oregon paper company resulted in larger bareroot stock both surviving and growing better than smaller bareroot or container stock (Iverson and Newton 1980).

Other ownerships have had different results--probably because of size and condition factors, as already mentioned. For example, Georgia-Pacific Corporation had consistently better survival with container seedlings than with bareroot stock in plantings along the Pacific Northwest coast in the 1970's (Hahn and Hutchison 1978).

In conclusion, we recommend that reforestation specialists in the Coast Ranges of Oregon and Washington and areas of similar site conditions look first for where they can consistently obtain healthy, well-conditioned planting stock with a good balance between tops and roots. Within those choices, they should opt for the largest seedlings they can afford when considering the overall environmental and economic objectives of their organizations.

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NAA Effects on Conifer Seedlings in British Columbia[1]

David G. Simpson[2]

Abstract.- NAA (1-napthaleneacetic acid) soil drenches applied 20-40 days after sowing to container-grown Douglas-fir (20 mgl^{-1}), Ponderosa pine (10 mgl^{-1}), western larch (10 mgl^{-1}) or lodgepole pine (2 mgl^{-1}) increased lateral root formation and only slightly diminished seedling growth. After 2 field seasons, NAA treated container-grown Douglas-fir and western larch seedlings grew as well as un-treated seedlings. NAA treated (20 mgl^{-1}) bareroot-grown Douglas-fir and Ponderosa pine seedlings had greater numbers of lateral roots than untreated seedlings. Further study of application rates for bareroot nurseries is required.

INTRODUCTION

Production of forest planting stock in containers in British Columbia has increased rapidly over the past 20 years (van Eerden and Gates 1990). In 1989, of the 300 million seedlings planned for production, 260 million were to be container grown. The principal species grown in British Columbia are spruces (white, Englemann, sitka), lodgepole pine, Douglas-fir, western red cedar, hemlock (western and mountain), true firs and miscellaneous other species including Ponderosa pine, white pine, western larch and yellow cedar.

In British Columbia, container grown plants are removed from their growing trays (usually styroblock 211, 313, 415) at the nursery, culled according to morphological standards that include the presence of a "plantable root system", and packaged for cold storage, shipment and field planting. In some species, such as Douglas-fir (interior variety), Ponderosa pine, western larch and lodgepole pine the root plugs may be poorly formed, in the upper 20 mm of the plug. This lack of roots in the upper part of the plug can cause the root plug to fall apart on lifting thus making the seedling unplantable.

An earlier study (Simpson 1986) with the particularly problematic interior variety of Douglas-fir has shown that soil drenches of NAA (1-napthaleneacetic acid) applied to Douglas-fir seedlings at a rate of 18.6 mgl^{-1} some 30 days after sowing were particularly effective in stimulating the production of first order lateral roots.

In spite of apparently conclusive results obtained at three different forest nurseries, B.C. Forest nurserymen have been hesitant to use NAA soil drenches on their stock. Questions raised by the nurserymen, and considered in this paper are:

a) Are the rates and timing suggested in Simpson (1986) generally applicable to Douglas-fir at other nurseries, and how are growth rates affected ?
b) Is NAA effective on other species including western larch, Ponderosa pine and lodgepole pine ?
c) Is field performance of NAA treated stock affected ?
d) Can NAA be used in bareroot nurseries ?

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[1]Paper presented at Western Forest Nursery Council Meeting August 13-17, 1990 Roseburg, Oregon, U.S.A.

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METHODS

Data reported here were obtained from a number of trials, undertaken at various forest nurseries in B.C. (see acknowledgements). The cultural practices used in B.C. forest nurseries vary between nurseries; however, at a specific nursery the experiments were designed and undertaken such that the only aspect of nursery culture which varied was the rate and timing (days after sowing) of NAA soil drenches. In all experiments NAA treatments were applied as single applications either by hand watering or through overhead irrigation such that the blocks were saturated (2 l per styroblock) with NAA solution. The NAA solutions were prepared immediately before use from ethanolic stock solutions and the final ethanol concentration was <1%.

Sample sizes and replication varied between experiments; however, these values are indicated in figure and table captions. Statistical analyses were done using SAS-STAT™ (1985) programmes for personal computers.

RESULTS AND DISCUSSION

NAA Effects on Douglas-fir

At the Hi-Gro Silva Forest Nursery in Quesnel, B.C. four Douglas-fir seedlots (2916; 2920; 8376; 8378) received drenches of NAA at 10, 20 and 50 mgl^{-1} 30 and 45 days after sowing. Analysis of variance for the morphological variables (Table 1) suggests that although there are some significant seedlot x treatment interactions, growth of all four Douglas-fir seedlots was affected similarly by NAA treatment. There was a very clear

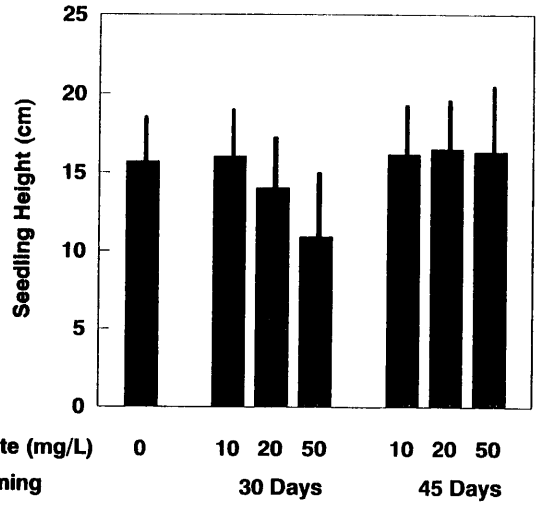

Figure 1. Height of container-grown Douglas-fir seedlings treated with NAA. Each bar is the mean height of 300 seedlings. Standard deviation is indicated.

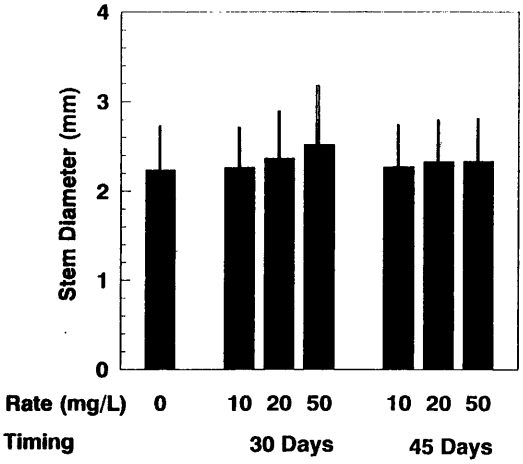

Figure 2. Stem Diameter of container-grown Douglas-fir seedlings treated with NAA. Each bar is the mean diameter of 300 seedlings. Standard deviation is indicated.

Table 1. Analyses of Variance for Douglas-fir seedling morphology: Hi-Gro Silva experiment.

Source of Variation	df	Height		Root Collar Dia.		Shoot Weight		Root Weight		Lateral Roots	
		MS	F	MS	F	MS	F	MS	F	MS	F
Seedlot (S)	3	236.5	3.83*(1)	4.776	7.83***	205.6	1.97^{NS}	612.4	15.58***	2685.3	9.31***
Treatment (T)	6	1139.6	18.12***	2.628	2.58^{NS}	568.7	4.30*	87.6	0.98^{NS}	44618.2	57.50***
Contrasts:											
Control (C)	1	140.0	2.23^{NS}	2.973	2.92^{NS}	4.1	0.03^{NS}	275.9	3.08^{NS}	42587.9	54.88***
Date (D)	1	2805.8	44.61***	2.307	2.26^{NS}	748.9	5.66^{NS}	171.9	1.92^{NS}	184004.3	237.12***
Rate (R)	2	883.5	14.05***	3.663	3.59^{NS}	724.4	5.48*	17.0	0.19^{NS}	17101.5	22.04***
D * R	2	1062.4	17.19***	1.581	2.59^{NS}	605.2	5.80**	60.6	1.54^{NS}	3457.0	11.99***
S * T = Error 1	18	62.9	1.02^{NS}	1.019	1.67^{NS}	132.2	1.27^{NS}	89.5	2.28*	776.0	2.67**
REP (S * T) = Error 2	55	61.8	6.65***	0.610	2.62***	104.3	12.72***	39.3	11.23***	288.4	3.12***
Error 3	2002	9.3	---	0.233	---	8.2	---	3.5	---	92.3	---

1) NS = $p>0.05$; * = $p\leq0.05$; ** = $p\leq0.01$; *** = $p\leq0.001$

interaction between the rate and timing (date) of NAA treatment. For seedling height (Figure 1), stem diameter at root collar (Figure 2), dry weight components (Figure 3) and lateral root number (Figure 4) it can be seen that NAA applications made 30 days from sowing had greater effect than similar treatments made 45 days from sowing. At 30 days from sowing the greatest number of lateral roots were produced at the 50 mgl^{-1} rate, however, seedling height and shoot weights were reduced at this level. Although there were effects on seedling morphology due to NAA, it is unlikely that these effects on growth were of a sufficient magnitude to increase culling losses. In fact, seedling root collar diameter (an important culling criteria in B.C.) was slightly increased by NAA treatment (Figure 2).

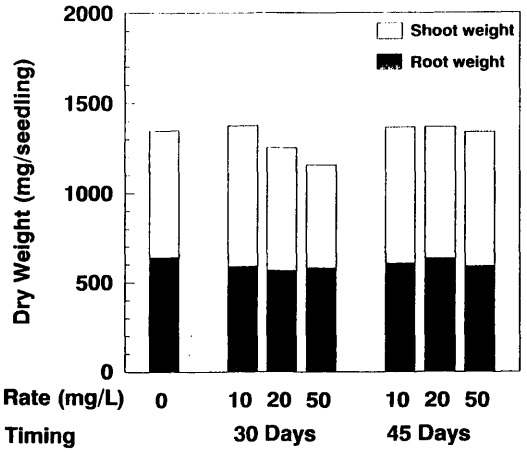

Figure 3. Shoot and root dry weight of container-grown Douglas-fir seedlings treated with NAA. Each bar is the mean weight of 300 seedlings. 5% LSD values are 84 and 51 mg for shoot and root weights, respectively.

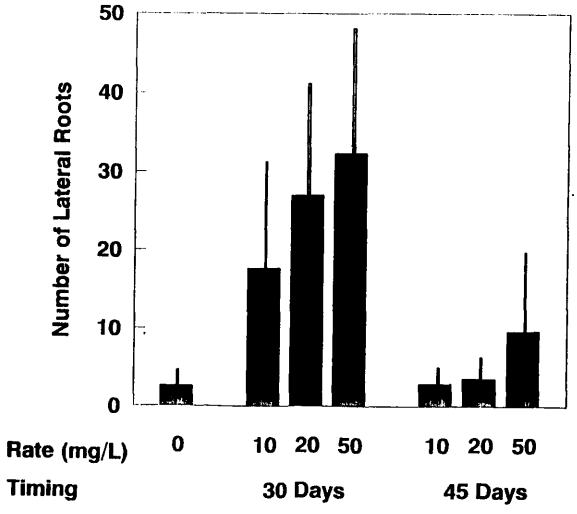

Figure 4. First order lateral root initiation by container-grown Douglas-fir seedlings treated with NAA. Each bar represents the mean number of lateral roots from 300 seedlings. Standard deviations are indicated.

It is clear that the recommended NAA application (Simpson 1986) of 18.6 Mgl^{-1} (≈20 mgl^{-1}) 30 days from sowing was an appropriate rate for Douglas-fir at this nursery.

At the Daveron Forest Nursery in Summerland, B.C. two Douglas-fir seedlots (8144; 26227) received drenches of NAA (20 mgl^{-1} 30 days from sowing). The height growth of these treated (as well as untreated) seedlings was measured periodically throughout the growing season (February - October). The results indicate that seedling height (Figure 5) was affected soon after treatment, and that subsequent growth occurred at similar rates in treated and untreated seedlings. Final heights were less in NAA treated seedlings as all seedlings ceased height growth around the same time, presumably in response to longer nights in late summer.

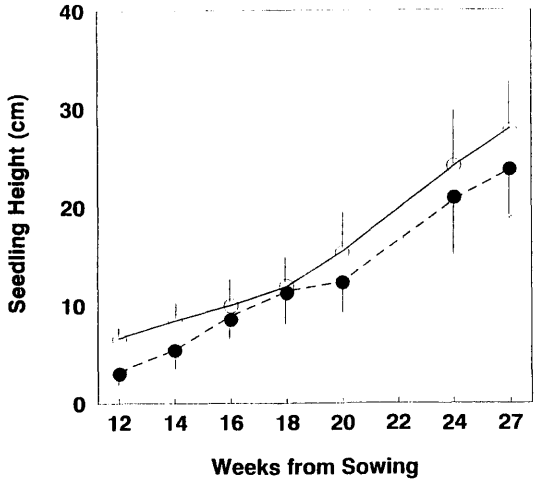

Figure 5. Effect of NAA on height growth of Douglas-fir (8144) at Daveron Forest Nursery. Each point is the mean of 50 seedlings. Solid line = untreated ; broken line = NAA treated. Standard deviation indicated

The results from this trial suggest that depression of seedling height due to NAA application could be minimized by simply growing seedlings for 2-3 weeks longer. In B.C. forest nurseries, attaining sufficiently tall container grown Douglas-fir seedlings is rarely a problem; in fact, some nurseries resort to moisture and nutrient stresses in attempts to regulate height growth.

NAA Effects on Other Species

At the Pacific Regeneration Technologies Forest Nursery in Vernon, B.C. a rate and timing study was undertaken with Ponderosa pine, western larch and lodgepole pine. Five rates of NAA (between 2 and 100 mgl^{-1}) and application dates which ranged from 17 to 67 days from sowing were considered.

For Ponderosa pine (Table 2), western larch (Table 3) and lodgepole pine (Table 4) there were significant treatment effects on seedling morphology. There were significant NAA rate and timing effects as well as significant interactions between application rate and application date. Data for the three species are presented in Tables 2 - 4; however, when considered together, these results suggest that NAA application resulted in increased numbers of lateral roots in all three species with the minimum application rate being 10 mgl^{-1} for Ponderosa pine and western larch and 2 mgl^{-1} for lodgepole pine. Application rates greater than these resulted in greater numbers of lateral roots being initiated; however, growth, particularly height and weight, was reduced at higher application rates. Timing of NAA applications was most effective between 20 and 40 days from sowing, later applications required greater application rates and often resulted in decreased growth without similarly greater numbers of lateral roots being produced.

Field Performance of NAA Treated Seedlings

Western larch seedlings from the Pacific Regeneration Technologies Nursery in Vernon, B.C. and Douglas-fir from the Daveron Forest Nursery in Summerland, B.C. were treated with 20 mgl^{-1} NAA 30 days from sowing. At the end of the growing season the seedlings were removed from their styroblock growing trays, packaged and cold (-2°C) stored overwinter as is the practice at many B.C. forest nurseries. Measurements of seedling morphology (Table 5) indicate that the NAA treatment increased lateral root numbers and had some effects on growth, particularly on height growth of western larch.

Overwinter stored seedlings were planted on forest sites at Hidden Lake (near Vernon, B.C.) and their heights measured at planting and again following the first and second growing seasons. The field results (Figure 6) indicate that height differences which existed at planting have become proportionately smaller (and less important) as the seedlings have grown larger. The annual height increment does not appear to have been affected by NAA treatment.

Table 2. NAA effects on 1+0 container-grown ponderosa pine morphology.

Date	Rate ($mg \cdot l^{-1}$)	Height (cm)	Diameter (mm)	Shoot Wt. (mg)	Root Wt. (mg)	Lateral Roots (#)
May 27	2	16.4	3.05	864	642	14
day 28	10	13.8	2.99	737	606	88
	20	11.9	2.85	652	623	96
	50	7.8	3.13	390	398	87
	100	6.5	3.45	354	347	91
June 7	2	15.3	3.00	780	611	6
day 39	10	16.2	2.91	793	632	59
	20	14.8	2.99	795	650	88
	50	7.5	3.33	358	312	84
	100	4.6	3.50	263	266	66
June 21	2	19.7	2.99	464	712	7
day 53	10	16.6	2.66	834	645	9
	20	10.4	2.38	494	654	11
	50	6.0	2.53	283	381	51
	100	4.6	3.42	245	296	39
July 5	2	16.3	2.91	894	653	5
day 67	10	14.5	2.73	770	742	7
	20	10.6	2.49	536	740	10
	50	9.0	2.67	441	674	33
	100	5.6	3.02	263	322	27
control		13.8	3.34	920	710	6
5% LSD		2.1	0.36	222	82	19

Analyses of Variance

		Height		Root Collar Dia.		Shoot Weight		Root Weight		Lateral Roots	
Source of Variation	df	MS	F	MS	F	MS	F	MS	F	MS	F
Treatments (T)	20	415.8	40.0***[1]	2.037	6.8***	124886	7.4***	57851	25.1***	24827	30.8***
Contrasts											
Control (C)	1	112	10.8***	2.866	9.5*	1008957	59.5***	820423	356.6***	27555	34.2***
Date (D)	3	5	0.5^{NS}	3.879	12.9***	113839	6.7**	32409	14.1***	81289	100.9***
Rate (R)	4	1725.4	165.9***	4.95	16.4***	408680	24.1***	212815	92.5***	37314	46.3***
D * R	12	50.7	4.9**	0.534	1.8^{NS}	22241	1.3^{NS}	9181	4.0**	6322	7.8***
Block (B)	1	13	4.8^{NS}	0.278	1.2^{NS}	3774	0.2^{NS}	20103	8.7***	1214	2.72^{NS}
Error 1 (T * B)	20	10.4	3.9***	0.301	1.3^{NS}	16960		2301		806	1.8*
Error 2	378	2.7		0.228		n/a		n/a		445	

n/a = not available; dry weights were determined on a block rather than individual tree basis.
[1] * = $p \leq .05$; ** = $p \leq .01$; *** = $p \leq 0.001$; NS = $p > .05$

Table 3. NAA effects on 1+0 container-grown western larch morphology.

Date	Rate ($mg \cdot l^{-1}$)	Height (cm)	Diameter (mm)	Shoot Wt. (mg)	Root Wt. (mg)	Lateral Roots (#)
May 27	2	14.6	2.79	639	846	13
day 17	10	13.7	2.46	503	606	41
	20	16.4	2.71	412	587	63
	50	9.0	2.23	296	417	40
	100	7.0	2.31	258	395	43
June 7	2	16.3	2.86	552	805	8
day 28	10	13.9	2.80	623	756	32
	20	15.7	2.36	523	652	41
	50	13.5	2.93	465	618	43
	100	9.1	2.55	363	560	47
June 21	2	14.2	2.50	693	836	10
day 42	10	17.5	2.77	518	707	25
	20	14.0	2.56	447	562	38
	50	11.4	2.28	378	459	44
	100	7.5	2.26	251	344	42
July 5	2	15.3	2.89	536	712	8
day 56	10	15.4	2.80	486	757	4
	20	10.4	2.65	390	675	9
	50	5.8	2.34	220	397	25
	100	4.2	2.36	148	256	38
control		21.8	2.46	685	667	2
5% LSD		3.7	0.37	114	158	10

Analyses of Variance

		Height		Root Collar Dia.		Shoot Weight		Root Weight		Lateral Roots	
Source of Variation	df	MS	F	MS	F	MS	F	MS	F	MS	F
Treatments (T)	20	569.2	18.1***[1]	1.6212	5.8***	71050	15.8***	85212	9.9***	9125	26.9***
Contrasts											
Control (C)	1	2618.2	83.1***	0.3632	1.2^{NS}	179024	29.9***	18741	1.6^{NS}	23118	93.6***
Date (D)	3	332.7	10.6***	1.6779	5.4**	58896	13.1***	44862	5.2**	14417	58.4***
Rate (R)	4	1430.8	45.4***	3.314	10.7***	239770	53.4***	336989	39.1***	21168	85.7***
D * R	12	111.2	3.5**	1.1476	3.7**	8851	2.0^{NS}	17229	2.0^{NS}	2622	10.6***
Block (B)	2	6.1	0.8^{NS}	0.3442	1.4^{NS}	107	0.02^{NS}	5452	0.6^{NS}	405	3.5^{NS}
Error 1 (T * B)	40	31.5	4.2***	0.3084	1.3^{NS}	4491		8608		247	2.1***
Error 2	567	7.5		0.2379		n/a		n/a		115	

n/a = not available; dry weights were determined on a block rather than individual tree basis.
[1] * = $p \leq .05$; ** = $p \leq 0.01$; *** = $p \leq .001$; NS = $p > .05$

Table 4. NAA effects on 1+0 container-grown Lodgepole pine morphology.

Date	Rate ($mg \cdot l^{-1}$)	Height (cm)	Diameter (mm)	Shoot Wt. (mg)	Root Wt. (mg)	Lateral Roots (#)
May 27	2	11.0	2.39	568	521	25
day 23	10	5.2	2.07	298	424	31
	20	3.3	1.50	158	262	30
	50	2.3	1.72	152	257	32
	100	2.0	1.80	130	212	30
June 7	2	12.2	2.24	595	520	27
day 34	10	10.4	2.01	422	446	34
	20	7.4	2.18	428	447	40
	50	3.3	2.62	214	374	34
	100	2.3	2.03	155	271	23
June 21	2	12.0	2.35	550	547	12
day 47	10	8.7	2.01	390	534	29
	20	4.7	2.20	269	351	26
	50	3.7	2.62	180	247	12
	100	2.9	2.98	177	255	31
July 5	2	13.0	2.18	563	514	4
day 61	10	11.0	2.32	456	577	5
	20	7.8	2.14	343	532	5
	50	3.0	1.82	220	427	25
	100	3.8	3.20	218	264	24
control		18.8	2.05	630	495	3
5% LSD		1.7	0.34	65	86	9

Analyses of Variance

		Height		Root Collar Dia.		Shoot Weight		Root Weight		Lateral Roots	
Source of Variation	df	MS	F	MS	F	MS	F	MS	F	MS	F
Treatments (T)	20	650.7	103.3***[1]	4.7467	18.3***	86403	58.9***	45409	17.6***	3816	19.5***
Contrasts											
Control (C)	1	4340.2	688.9***	0.7231	2.8^{NS}	267037	181.9***	137048	53.2***	12007	61.3***
Date (D)	3	247.2	39.2***	7.9966	30.9***	34382	23.4***	42167	16.4***	32905	167.9***
Rate (R)	4	1609.2	255.4***	4.4047	17.0***	319273	217.5***	158233	61.4***	7715	39.4***
D * R	12	36.3	5.8***	4.3836	16.9***	6733	4.6***	10192	4.0***	1975	10.1***
Block (B)	2	35.4	11.8***	0.2761	1.4^{NS}	392	0.03^{NS}	5045	2.0^{NS}	433	4.5*
Error 1 (T * B)	40	6.3	2.1***	0.259	1.3^{NS}	1468		2577		196	2.0***
Error 2	567	3		0.1953		n/a		n/a		97	

n/a = not available; dry weights were determined on a block rather than individual tree basis.
[1] * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = $p > 0.05$

Table 5. Effect of NAA on seedling morphology at Daveron (Douglas-fir) and Vernon (western larch) nurseries.

Species-Seedlot	Treatment	Height (cm)	Root Collar Diameter (mm)	Shoot Weight (mg)	Root Weight (mg)	# Lateral Roots[2] 0-20 mm	20-50 mm
D-fir 8144	Control	28.2 ± 3.8[1]	2.8 ± 0.4	1625 ± 405	880 ± 250	3 ± 1	7 ± 3
D-fir 8144	NAA	25.0 ± 3.6	2.6 ± 0.4	1331 ± 328	880 ± 250	17 ± 15	19 ± 17
D-fir 26227	Control	27.7 ± 4.2	2.6 ± 0.4	1542 ± 390	950 ± 320	3 ± 2	7 ± 4
D-fir 26227	NAA	24.1 ± 5.9	2.6 ± 0.4	1440 ± 687	867 ± 360	40 ± 19	33 ± 20
W. larch 5235	Control	27.6 ± 3.3	3.1 ± 0.5	1082 ± 247	739 ± 180	4 ± 4	7 ± 2
W. larch 5235	NAA	14.0 ± 2.2	3.0 ± 0.4	582 ± 159	762 ± 207	61 ± 25	67 ± 36

[1] mean ± standard deviation (n = 25)
[2] number of first order lateral roots originating from primary root between 0-20 mm and between 20-50 mm below soil surface.

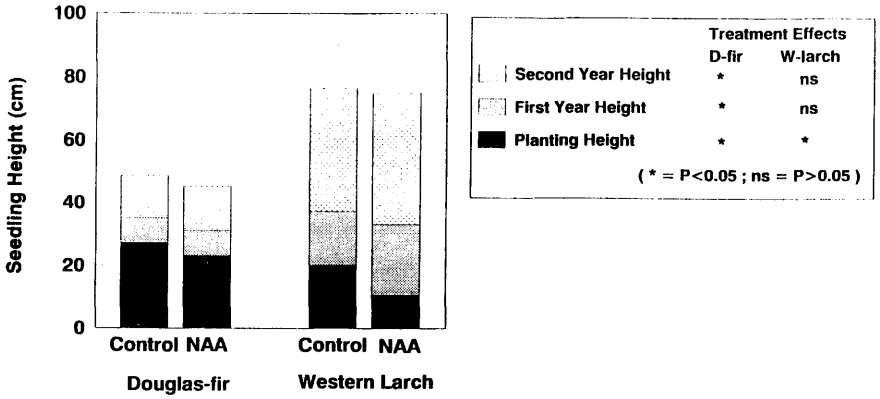

Figure 6. Mean total height at planting and after the first and second growing season for Douglas-fir and western larch container-grown seedlings treated or not with NAA. Each bar is the mean of 75 or 150 western larch or Douglas-fir seedlings, respectively.

Root form of forest planted seedlings has not been examined at the Hidden Lake plantation, however, the growth of treated seedlings in clear plastic root observation boxes has been examined. Roots of NAA treated Ponderosa pine, western larch and lodgepole pine seedlings appear to be more vigorous and more evenly distributed around the root plug compared to roots of untreated seedlings (Figure 7). If this change in seedling root form also occurred in forest planted seedlings is not known, however further study of forest planted NAA treated seedlings will indicate if this is the case.

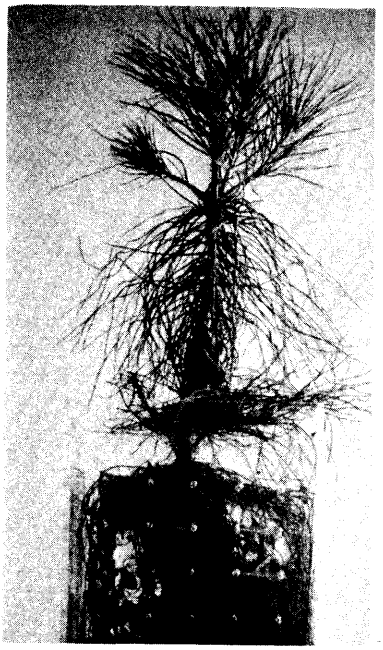

Figure 7. NAA treated lodgepole pine seedling. grown for one season in root observation box. Notice the lateral roots originating from the the upper part of the root plug.

Table 6. Effect of NAA on 1+0 bareroot Douglas-fir and western larch seedling morphology at Weyerhaeuser Canada Nursery.

Species-Seedlot	Treatment	Height (cm)	Root Collar Diameter (mm)	Shoot Weight (mg)	Root Weight (mg)	# Lateral Roots[2] 0-20 mm	20-50 mm
Douglas-fir (1377)	Control	6.5	1.67	332	237	0.3	3.4
	NAA[1]	6.8	1.80	353	244	1.5	6.1
Ponderosa pine (8260)	Control	5.5	2.29	692	407	0	1.8
	NAA	5.6	2.42	705	440	1.9	8.2

[1] 20 mg·L^{-1} NAA applied 30 days from sowing.

[2] Number of first order lateral roots originating from the primary root between 0 - 20 mm and between 20 - 50 mm below soil surface.

NAA Effects on Bareroot Nursery Stock

At the Weyerhaeuser Canada Grandview Forest Nursery near Armstrong, B.C. sections of nurserybed sown 30-days previously with Douglas-fir and Ponderosa pine were treated with NAA. The NAA was applied at mid-day such that 2.5 lm^{-2} of 20 mgl^{-1} NAA drench was applied to nurserybeds which had just been irrigated.

At the end of the first growing season, seedling morphological assessment indicated (Table 6) that the number of lateral roots in both Douglas-fir and Ponderosa pine had been increased by NAA treatment. There were slight, but not significant, effects on seedling height, root collar diameter and dry weight in both species.

Results at the end of the second growing season are not available as the experiment had to be abandoned due to an infestation of strawberry root weevil larvae (*Otiorhynchus oratus*).

CONCLUSIONS

The results from the experiments undertaken in several B.C. forest nurseries with the plant growth regulator NAA suggest the following:

- NAA will affect the number of first order lateral roots initiated by conifer seedlings such that for container grown stock better formed root plugs are produced.

- The timing of application for all species seems to be similar with applications between 20 and 40 (30 best) days from sowing most effective.

- The rate (concentration) of NAA applied as a soil drench to container grown conifers varies between species. Recommended rates which minimize negative effects on shoot growth yet promote substantial root initiation are 20 mgl^{-1} for Douglas-fir; 10 mgl^{-1} for Ponderosa pine and western larch, and 2 mgl^{-1} for lodgepole pine.

- Although the root form and vigour of container grown seedlings after outplanting may be enhanced by NAA treatment, early field performance results from Douglas-fir and western larch outplantings do not indicate enhanced field growth. Negative effects of NAA treatment on field growth have not been observed.

- NAA treatment of bareroot Douglas-fir and Ponderosa pine nursery stock may result in increased numbers of first order lateral roots. Further investigation of the rate of NAA application at 30 days from sowing in required.

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B.C. Ministry of Forests, Surrey Nursery, Surrey, B.C.
B.C. Ministry of Forests, Skimikin Nursery, Salmon Arm, B.C.
Daveron Forest Nursery, Summerland, B.C.
Hi-Gro Silva Forest Nursery, Quesnel, B.C.
Pacific Regeneration Technologies Forest Nursery, Vernon, B.C.
Weyerhaeuser Canada Forest Nursery, Armstrong, B.C.

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van Eerden, E. and J.W. Gates. 1990. Seedling production and processing: container (Chapter 17) In Regenerating British Columbia's Forests. Edited by D.P. Lavender, R. Parish, C.M. Johnson, G. Montgomery, A. Vyse, R.A. Willis and D. Winston. UBC Press pp. 226-234.

Minutes of the Annual Business Meeting

The annual business meeting of the Western Forest Nursery Council and Intermountain Forest Nursery Association was called to order at 1:00 P.M. on August 15, 1990 by Ad Hoc Chairperson Tom Landis. There was no old business, and so the floor was opened for new business.

The only agenda item was the location of future meetings. The Western Forest Nursery Council meets biennially on even-numbered years, whereas the Intermountain Forest Nursery Association meets every year. In recent years, the two groups have held joint meetings on the even-numbered years.

The 1991 meeting of the Intermountain Forest Nursery Association will be hosted by the Utah Department of State Lands and Forestry, and will be held at the Olympia Hotel in Park City, Utah on August 12-16. In addition to a tour of the Lone Peak State Forest Nursery, the technical sessions will focus on propagation of native plants for riparian habitats, and groundwater quality in forest nurseries.

The floor was opened for nominations for the 1992 Western Forest Nursery Council meeting. After discussing the location of past meetings, the members decided that it was time to meet in Northern California. Placerville Nursery was nominated by Bill Krelle and the motion was seconded by Ron Adams. The motion was unanimously approved by a voice vote. A discussion followed regarding the meeting and the following suggestions were made:

1. Visit the Glass Mountain Nursery

2. Use the University of Davis conference facility

3. Form a committee of local people to look into the best location for the meeting. The following members were nominated: Laurie Lippitt, Ron Adams, Pat Trimble, Bill Scheuner, and John Rea.

The motion was made to hold the meeting during the first full week in August (August 3-7, 1992), which is a week earlier than normal, to avoid conflict with late summer transplanting of plug + one seedlings. The motion carried.

Ron Adams noted that this was the 40th meeting of the organization. Tom Landis requested that copies of past proceedings be sent to him, so that he could maintain a complete set.

The meeting adjourned at 1:10 P.M.

Seedling Beauty Contest

On Monday night, August 13, forest tree seedlings from 19 different nurseries were displayed and evaluated for quality by a panel of judges. The 221 entries were divided into 171 categories consisting of 49 species, 33 stock types, and 29 special-attribute classes.

The awards consisted of 172 "first class", 24 "second class", and 8 "third class", as well as 12 seedlings which were awarded a special "superior class":

California red fir	2+0 BR	USDA Forest Service, Placerville Nursery
Coast redwood	P+1 BR	International Paper Company, Kellogg Forest Tree Nursery
Douglas-fir	1+1 BR	Weyerhaeuser, Aurora Forest Nursery
Douglas-fir	2+0 BR	Weyerhaeuser, Aurora Forest Nursery
Engelmann spruce	2+0 BR	USDA Forest Service, Wind River Nursery
Engelmann spruce	5 in^3 C	Green Tree Northwest Nursery
Grand fir	2+0 BR	D.L. Phipps State Forest Nursery
Hybrid pine	21 in^3 C	Fir Springs Timber Farm
Incense cedar	2+0 BR	D.L. Phipps State Forest Nursery
Noble fir	1+1 BR	Silver Mountain Conifer Nursery
Noble fir	2+0 BR	USDA Forest Service, J. H. Stone Nursery
Western Hemlock	MP+1 BR	Weyerhaeuser, Mima Forest Nursery

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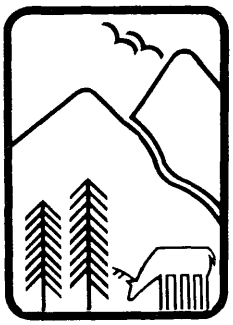
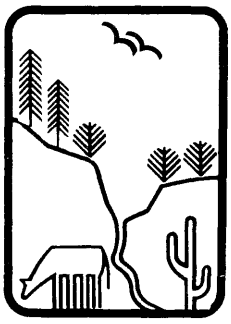
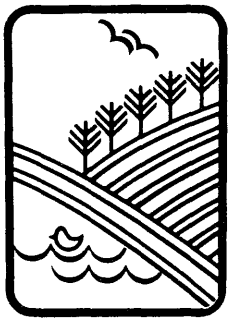


Rocky Mountains



Southwest



Great Plains

U.S. Department of Agriculture
Forest Service

Rocky Mountain Forest and Range Experiment Station

The Rocky Mountain Station is one of eight regional experiment stations, plus the Forest Products Laboratory and the Washington Office Staff, that make up the Forest Service research organization.

RESEARCH FOCUS

Research programs at the Rocky Mountain Station are coordinated with area universities and with other institutions. Many studies are conducted on a cooperative basis to accelerate solutions to problems involving range, water, wildlife and fish habitat, human and community development, timber, recreation, protection, and multiresource evaluation.

RESEARCH LOCATIONS

Research Work Units of the Rocky Mountain Station are operated in cooperation with universities in the following cities:

Albuquerque, New Mexico
Flagstaff, Arizona
Fort Collins, Colorado*
Laramie, Wyoming
Lincoln, Nebraska
Rapid City, South Dakota
Tempe, Arizona

*Station Headquarters: 240 W. Prospect Rd., Fort Collins, CO 80526