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How to Test Herbicides at Forest Tree Nurseries

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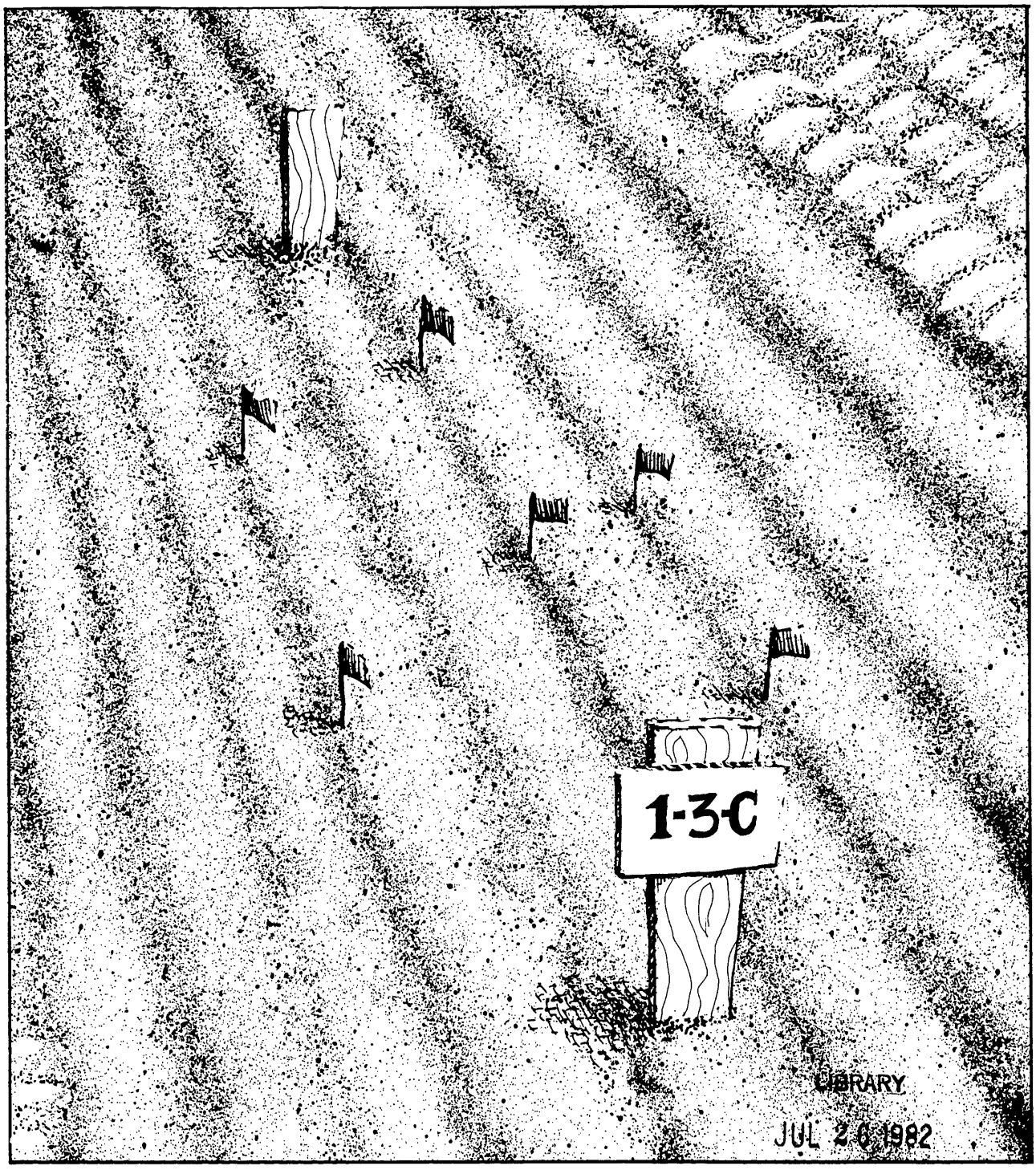

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Abstract

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Procedures developed in a cooperative westwide study of weed control in forest tree nurseries are described in a form modified for use by nursery managers. The proven, properly designed test and evaluation methods can be used to generate data needed for evaluation and registration of herbicides.

Keywords: Nursery methods, herbicides (nursery), weed control (nursery), pesticide registration.

Pesticide Precautionary Statement

Pesticides used improperly can be injurious to people, animals, and plants. Follow the directions and heed all precautions on the labels.

Store pesticides in original containers under lock and key—out of reach of children and animals, and away from food and feed.

Apply pesticides so they do not endanger humans, livestock, crops, beneficial insects, fish, and wildlife. Do not apply pesticides when there is danger of drift, when honey bees or other pollinating insects are visiting plants, or in ways that may contaminate water or leave illegal residues.

Avoid prolonged inhalation of pesticide spray or dust; wear protective clothing and use protective equipment if specified on the container.

If your hands become contaminated with a pesticide, do not eat or drink until you have washed. In case a pesticide is swallowed or gets in eyes, follow the first-aid treatment given on the label, and get prompt medical attention. If a pesticide is spilled on skin or clothing, remove clothing immediately and wash skin thoroughly.

NOTE: Some States have restrictions on the use of certain pesticides. Check your State and local regulations. Also, because registrations of pesticides are under constant review by the Federal Environmental Protection Agency, consult your county agricultural agent or State extension specialist to be sure the intended use is still registered.

Common Measures and Metric Equivalents

1 foot	0.305 meters
1 pound, avoirdupois	0.454 kilogram
1 pound/acre	1.12 kilograms/hectare
1 ounce	29.6 milliliters
1 pint	0.473 liter
1 quart	0.946 liter
1 gallon, United States	3.785 liters

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Testing Herbicides at Forest Tree Nurseries

Herbicides should be tested at forest tree nurseries for three major reasons:

1. *Effective use of herbicides in an integrated pest management program can lower tree prices by reducing costs of hand and mechanical weeding.*

In 1969, at the USDA Forest Service Nursery in Coeur d'Alene, Idaho, hand-weeding costs were reduced 75 percent by using one herbicide, diphenamid, in a marginally effective way (McDonald et al. 1974). The Cooperative Western Nursery Weed Control Study,[1/] has led to registration of three new herbicides since 1976 and shown that 80 percent or more of weeds can often be controlled. Work by the Auburn University Forest Chemicals Cooperative has led to registration of seven herbicides (Gjerstad et al. 1978), and South et al. (1978) estimate that use of effective herbicides will save $250,000 per year in weed-control costs in forest nurseries of the southeastern United States alone.

2. Use of herbicides at tree nurseries is a small market, so those who operate nurseries must often collect their own data for registration rather than depend on chemical companies.

Before they can be used, herbicides must be registered in the geographic area in which a nursery is located. Registration is based on performance data that come from well-planned and conducted field tests. Since the potential for sales in the tree nursery market is small, there is little incentive for chemical companies to develop the needed data, and the tree nursery industry will normally have to do this for itself. The data must be collected in a way that produces sound information in a standard, proven way.

Studies have isolated only a few herbicides and treatments that are safe and effective in nursery seedbeds. In the Cooperative Western Nursery Weed Control Study, for instance, herbicides were tested at only a few application rates and times. Because of limited time available, only chemicals found safe and effective by the highest standards were retained in the study; all others were quickly discarded. Much more work is needed to refine information about other potentially useful herbicides or combinations of products.

The broadest possible arsenal of herbicides should be established and maintained, as repeated applications of the same or similar chemicals may generate resistant weed populations. Also, since total use of an herbicide in tree nurseries involves small amounts compared to general agriculture, changes in the agricultural-use market can result in discontinuing production. With only a few chemicals to choose from, a nursery manager can be left without an effective alternative.

3. It is goodbusiness to determine the safety and effectiveness of an herbicide at each nursery prior to operational use because each nursery has a different mixture of tree and weed species, soil and climatic factors, and cultural practices.

Even if a potentially useful chemical is registered for herbicide use, nurseries should test it prior to use on a large scale. Each nursery situation is slightly different, and the chemical's performance may vary from nursery to nursery. On-site experience with, and confidence in, a given chemical is developed through testing. Also, testing can allow tailoring application rates and timing to more precisely fit the nursery's conditions. This paper describes a method to do the testing. It should guide those who engage in herbicide testing and encourage and expedite such work at the nurseries.

Herbicide Selection

Under the Federal Insecticide, Fungicide and Rodenticide Act, administered by the U.S. Environmental Protection Agency, it is legal to use any pesticide (herbicides are pesticides) in one of two ways. First, pesticides may be used according to their label directions and precautions. This means that use-pattern, site, and target pest appear on the label, with directions and precautions which pertain to a particular situation. *Or, the pesticide label is written in such a manner that a prudent person can interpret it to include hislher proposed use-pattern.*

Secondly, pesticides may be used under experimental permits issued by State pesticide regulatory agencies or the U.S. Environmental Protection Agency. In these instances, a pesticide is obtained from and used under the general direction of a representative of the chemical manufacturer, who is usually the person to whom the experimental permit is issued. Details and assistance can be obtained from USDA Forest Service pesticide specialists, State pesticide regulatory personnel, or technical representatives of the chemical manufacturer. The objective of testing is to find safe herbicides which control weeds economically while not adversely affecting the seedlings produced.

If restricted-use herbicides are used, the applicator or supervisor must be certified. Certification usually requires training plus passing a written test. Information on the certification of applicators is available from the USDA Forest Service or Cooperative Extension Service.

Various factors must be considered when selecting herbicides to test. These include the weed species to be controlled and the crop species to be grown. Read pertinent publications, contact colleagues in the forest and ornamental tree nursery industry to learn of their experiences with particular products. Seek the advice of chemical company technical representatives. Most companies want to be involved from the beginning, because this helps them understand your problems and objectives and how their products may be useful to you.

[1/] Stewart, R. E., S. McDonald, and L. Abrahamson. 1976. An Administrative Study for Herbicide Screening and Weed Control Demonstration in Western Forest Tree Nurseries 1976-1980. Pacific Northwest Forest and Range Experiment Station, Corvallis, Oreg. (unpublished).

Test Procedures

If problems with weed control or phytotoxicity arise, the company's familiarity with its product may hasten solutions. The company will provide information on previous screening studies, weed species controlled, and phytotoxicity and performance of the product under various climatic and soil conditions. Tests may have limited benefits if companies are uninterested or if their products are to become unavailable in the near future.

Realistically, it should not be necessary to test more than one or two new chemicals or combinations at a time. Large-scale tests such as the Cooperative Western Nursery Weed Control Study have already screened the bulk of available chemicals, and new ones should not appear on the market often. Furthermore, too many treatments can make a study too large.

General Scheme

A minimum test takes 2 years. The 1st-year's effort consists of treating small plots to carefully screen one or more chemicals, dosages, and/or application times. Although weed control is evaluated, the emphasis is on investigating phytotoxic effects. During the 2d year, herbicides showing promise are tested in somewhat larger plots to better assess economic benefits and further insure that unacceptable phytotoxicity does not occur. Also during the 2d year, further phytotoxicity data can be gathered from seedlings treated the 1st year (if seedlings are grown for 2 years). A 3d year of semi-operational testing is advisable if results after 2 years are not clear cut and/or if firmer economic data are desired.

Procedures described are for simple, randomized block experiments that can be readily analyzed for statistical significance. To be valid and free of bias, statistical principles of replication and randomization of treatments must be rigorously applied in designing the experiments: (1) A replication is a set of plots consisting of one plot of each treatment. Three replications are the minimum for reasonable statistical sensitivity, so resist any urge to reduce that number; (2) Each replication must be assigned to a specific plot by some process of randomization, such as using a random numbers table. Randomize each replication separately.

Contact a research or analytical agency, institution, or company for assistance if you want to determine such things as herbicide residue in the soil or the effects of herbicides on mycorrhizae. To save time and money, do these analyses in the 2d or 3d year if a particular treatment looked promising after the 1st year.

We have attempted to provide enough detail to permit a knowledgable nursery manager or technician to conduct the tests. Appendix 1 summarizes the procedural steps in chronological order.

First Year

Objectives.—Specific questions to ask:

1. Do the herbicide treatments (chemicals, dosage, and application variations) reduce seedling survival, growth, and damage rating at the end of the first growing season?

2. Do the herbicide treatments reduce the number of weeds?

Experimental design.—The basic treatment unit (plot) is a 3-foot-long section of nursery seedbed. Small plots such as this permit testing of a wide range of options without utilizing excessive seedbed space. This is important for minimizing the number of seedlings exposed to possible phytotoxic effects and for keeping the test small enough for a nursery to undertake.

Unless there are recommendations or experience to the contrary, test three combinations of dosage and application timing for each herbicide on each species for which data are desired:

1. Manufacturer's recommended dosage (1X) applied within 2 days after sowing (postseeding or ps).

2. Twice the recommended dosage (2X) applied postseeding (ps) to assess effects of accidental double spraying.

3. The 1.X dosage applied both postseeding and 4 to 5 weeks after emergence of tree seedlings (postgermination or pg).

In addition to the test herbicide(s) we recommend two types of control treatments for each species:

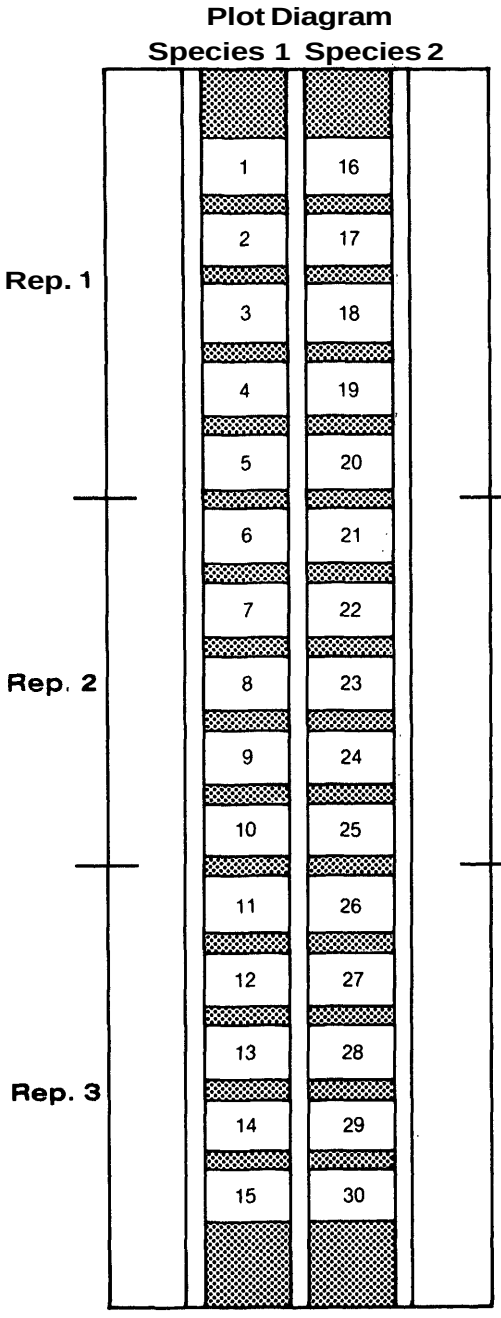
1. Untreated
2. When applicable, the standard herbicide treatment used at the nursery.

As an example, an experiment using the above five treatments with one new herbicide on two species will be described. Other combinations can be used. For instance, you might want to test more dosages and frequencies of application on a single crop species.

The experiment described below requires 30 plots [((3 dose-application combinations x 1 herbicide) + 2 control treatments)x 2 species x 3 replications]. The number of plots increases rapidly as additional herbicides and species are tested. For example, testing three new herbicides on three species would require 99 plots. Thus, give careful thought to this selection.

Plot layout.—Select two seedbeds for the test. They should be on uniform terrain and in the middle beds between irrigation or drainage lines. A weedy location is desirable. One crop species will be sown per bed, and the three 5-treatment replications will be placed in three contiguous 20-foot-long sections of seedbed. This will accommodate a 1-foot-long buffer strip between each of the 3-foot-long plots.

In the office, assign species to seedbed and treatments to plots by a process of randomization and prepare a diagram of the layout and a list of plot treatments (fig. 1). These will be useful in installing plots and maintaining treatment identity.



Treatment listing for species 1

Plot	Treatment[1]
1	Test herbicide (1X) — ps[2]
2	Standard treatment
3	Test herbicide (2X) — ps
4	Untreated
5	Test herbicide (1X) — ps + pg[3]
6	Test herbicide (2X) — ps
7	Test herbicide (1X) — ps
8	Test herbicide (1X) — ps + pg
9	Standard treatment
10	Untreated
11	Test herbicide (1X) — ps + pg
12	Standard treatment
13	Untreated
14	Test herbicide (1X) — ps
15	Test herbicide (2X) — ps

[1] List each herbicide treatment by name of chemical and rate of application (acid equivalent per acre, or ai/A)

[2] ps = postseeding application

[3] pg = postgermination application

Scale: 1/8 inch = 1 foot

Figure 1.—Example of plot diagram and randomized treatment listing for 1st-year screening test.

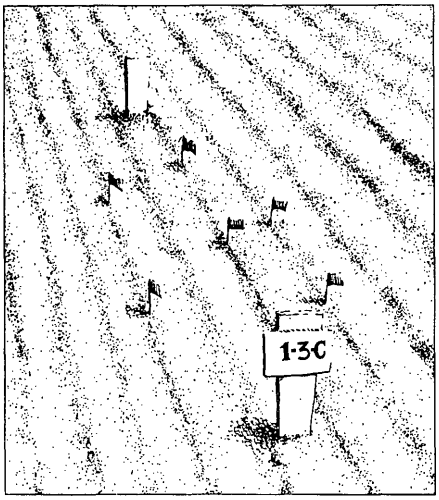


Figure 2.—Completed layout of a single 1st-year plot.

Form the seedbeds and sow the seeds. Use high quality seeds from one seed source per species or a thoroughly mixed combination of sources for each species. Lay out the plots using a steel tape for measuring and wooden stakes for marking the ends of each plot. Place stakes in the middle of beds rather than at the edges to reduce chance of their being hit by tractor wheels. Aluminum plant tags marked in advance with replication number, species, and treatment should be firmly attached to one of the stakes on each plot. Do not locate plots closer than 10 to 20 feet from either end of seedbeds. Post large signs at each end of the test area warning nursery workers not to disturb the plots.

Place two garden stakes or flagged pins 1 foot apart in each of three seeded rows per plot. Select the locations of these 1-foot subplots in a random manner, but do not place any in outside rows or within 6 inches of the ends of plots. These 1-foot subplots will be used for making seedling counts as described later.

Figure 2 illustrates the completed layout of one plot.

Herbicide application. — *Preparation of herbicide dosages.* — Prepare an herbicide mixing guide before measuring. Table 1 is an example of such a guide. Wettable powders should be weighed out in advance of treatment. Small plastic bags such as Whirl-Pak,[2/] 6-ounce size, are ideal for holding small amounts of wettable powders. All of the herbicide in such bags is easily rinsed into the spray solution during the mixing phase. Be sure to pre-label the bags with plot number, name of herbicide, formulation, rate of application, and amount of herbicide in grams.

[2/] Mention of product or trade name does not imply endorsement by the U. S. Department of Agriculture.

Table 1—Example of herbicide mixing guide for nursery weed control trials

Herbicide	Formulation[1/]	Rate	Amount Reauired		
			1 plot	3 plots	6 plots
		(lb ai/a)[2/]			
bifenox	Modown 80WP	3	0.53g	1.59g	3.18g
trifluralin	Treflan 4EC	3/4	0.2ml	0.7ml	1.3ml
napropamide	Devrinol 50WP	3	0.84g	2.52g	5.04g

[1/] Mention of product or trade name does not imply endorsement by the U.S. Department of Agriculture.

[2/] ai/a means acid equivalent per acre.

The formula for determining the amount of wettable powder in grams to treat one plot is:

$$\frac{\dfrac{\text{length of plot (feet)} \times \text{sprayer swath width (feet)}}{43{,}560\ \text{ft}^2} \times \text{pounds active ingredient per acre}}{\text{pounds active ingredient per pound of formula}} \times 458.6$$

or, for 3- x 4.5-foot plots,

$$\frac{\text{pounds active ingredient per acre}}{\text{pounds active ingredient per pound of formula}} \times 0.141.\ ^{3/}$$

Mix this amount of herbicide with 100 ml of water to obtain a spray batch equivalent to 85 gallons per acre for plots 4.5 wide x 3 feet long. Adjustments should be made if different carrier rates are recommended by product manufacturers.

The amount for all three replications of each treatment can be combined into one batch to reduce mixing and rinsing times. The amount of water would likewise be increased from 100 to 300 ml.

The small amounts of herbicide needed require a good laboratory-type balance, accurate to hundredths of a gram.

Measure out liquid herbicides at time of application using a pipet accurate to tenths of a milliliter. Use a pipet filler (not mouth suction!) for safety and convenience. The formula for determining the amount of liquid herbicide to use in milliliters is:

$$\frac{\dfrac{\text{length of plot (feet)} \times \text{sprayer swath width (feet)}}{43{,}560\ \text{ft}^2} \times \text{pounds active ingredient or acid equivalent per acre}}{\text{pounds active ingredient or acid equivalent per gallon of formula}} \times 3785$$

or, for 3- x 4.5-foot plots,

$$\frac{\text{pounds active ingredient or acid equivalent per acre}}{\text{pounds active ingredient or acid equivalent per gallon of formula}} \times 1.17.\ ^{4/}$$

3/Re-compute for other swath-widths.

/Re-compute for other swath-widths.

Have the herbicide mixing guide in front of you at the time of application (table 1).

Application equipment. — Good spray equipment is essential for accurate and uniform application of liquid formulations to small plots. During the Cooperative Western Nursery Weed Control Study, an AZ Small-Plot CO_2 Research Sprayer with a 4.5-foot swath width was used (fig. 3). The sprayer consisted of a 3.4-foot boom with four flat-fan nozzles. A 1-pint bottle held the chemical mixture, and pressure was supplied by a 2.5-pound CO_2 cylinder with a pressure regulator. Other sprayer configurations are available from the manufacturer: AZ Field Test Service (see footnote 2), Accord, New York.

Several modifications were necessary. Stainless steel strainers (50-mesh) with check valves were installed in the nozzle assemblies. The check valves are necessary if more than one plot is to be sprayed per batch. Also, smaller nozzle tips were installed (Tee Jet (see footnote 2) size 8001 or other flat-fan nozzle of same spray angle and flow rate). The small nozzle is necessary to give enough spray time to spray each plot evenly. Stainless steel nozzle tips are recommended for longer wear. It is important to disassemble and clean the strainer and nozzles periodically and to spray the check valves with a lubricant.

A small hand-pumped pressure sprayer may be modified to perform acceptably. A 1.5-gallon stainless steel tank can be fitted with a pressure gauge. A corrosion resistant pump, funnel and shutoff, a 16-inch curved brass extension tube, and a 5-foot hose are desirable. A cap nozzle providing two overlapping flat-fan spray streams should provide a good swath.

Calibration and applications of liquids. — Calibration of the sprayer is important for applying the proper amount of spray to plots. Because the small-plot pressurized sprayer has a CO_2 pressure source plus a pressure regulating device, calibration is quick and easy. Set the pressure and time delivery of the desired volume (100 ml per plot).

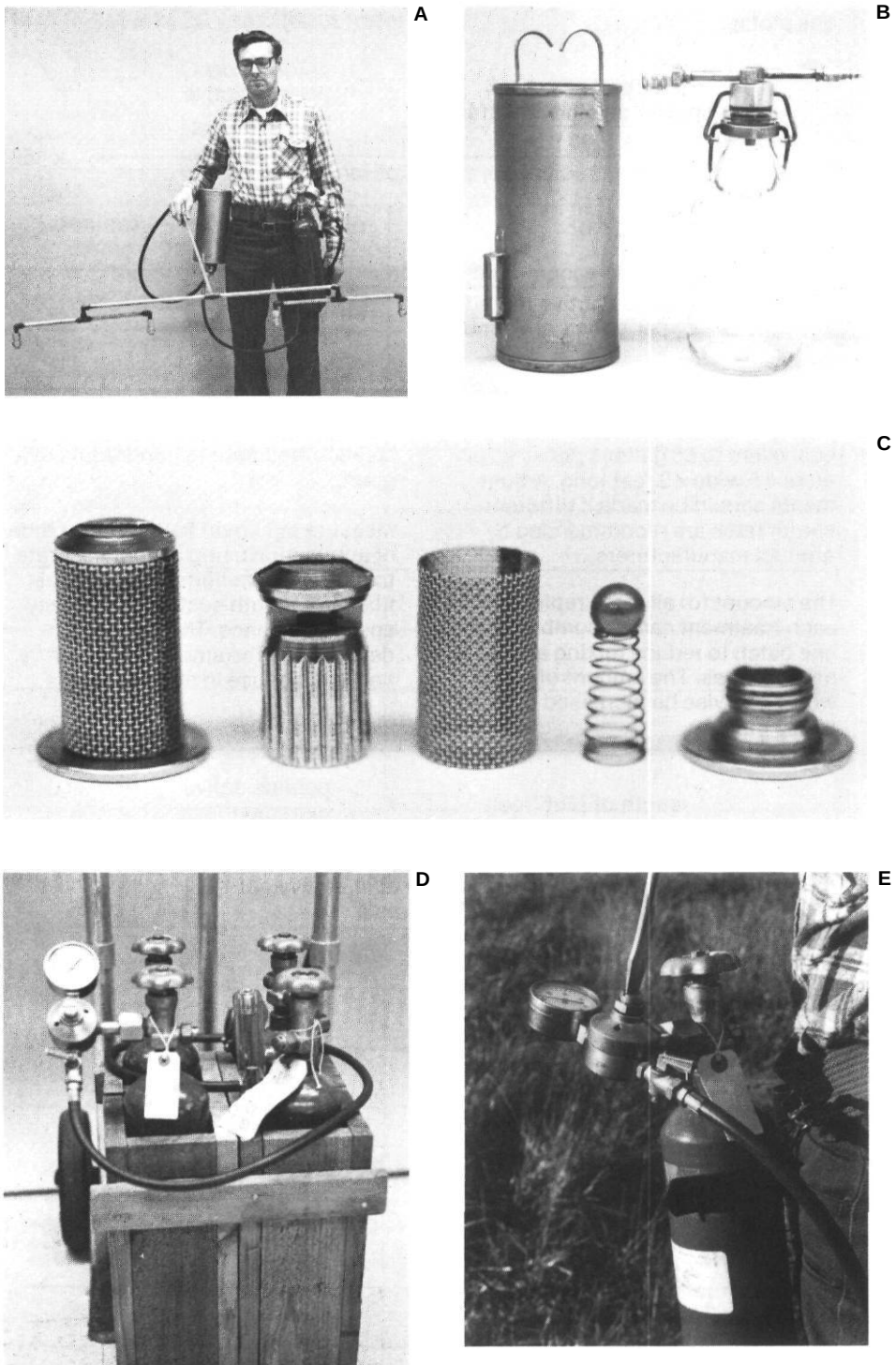


Figure 3. — Small-plot CO_2 research sprayer: **A** Complete apparatus ready for use; **B** Bottle with spray head attached and carrying case on left; **C** Stainless steel 50-mesh strainer and check valve assembly; **D** CO_2 cylinders in transporting box (cylinders must be kept upright); **E** Adjustment of pressure regulator (see text).

Use the average of several trials as delivery time for the test application. Check nozzles periodically for clogging and uniform distribution. Our experience with the small-plot sprayer equipped as described suggests that 100 ml of spray will be applied in 5.5 seconds at 20 lb/in^2 pressure. A stop watch is necessary. One person should operate the sprayer, and a second person should time the application.

When using the small plot sprayer, it is advisable to first turn the adjusting screw on the regulator out (counterclockwise) before opening the valve on the CO_2 cylinder. Otherwise, excessive pressure may occur and damage the gauge. Open the CO, valve slowly to "full", then turn the adjusting screw on the regulator in (clockwise) to obtain the desired pressure. Check the pressure frequently and readjust as necessary. Do not allow the CO_2 cylinder to lie horizontal with the valve open or liquid CO_2 will enter the system and damage the regulator. Overfilled cylinders will also cause liquid CO_2 to enter the system, even when held upright. Keep cylinders out of direct sunlight when not in use.

Calibrating a hand-pump pressure sprayer is more involved. The objectives are to fill the sprayer once with enough material to spray all plots with that herbicide and to get uniform delivery at fairly low pressure, about 20 lb/in^2. This will require putting enough herbicide in the tank to spray an extra plot before application, because you won't be able to empty the tank completely. The extra can be sprayed on seedbed outside the plots. Some experimentation is necessary to develop the technique. Put 3 liters into the sprayer and pump up to about 20 lb/in^2. Time the delivery of five 100-ml portions, pumping the sprayer between each portion. If there is little variation between the times, use the average time to deliver the 100 ml. If there is variation, practice your technique until you can consistently pump the tank to deliver the 100 ml in about the same amount of time. Pump up the sprayer. Time the delivery of two 500-ml portions, pumping the sprayer between applications. Again, if there is variation, practice your technique until you can consistently apply the same volume within a given time.

Valid tests require uniform application of specific amounts of herbicide to given areas. We suggest that two persons develop the technique and apply the herbicide, one person operating the sprayer and the other doing the timing. They should know the objectives and procedures of the study. Necessary equipment and materials should be on hand, and sufficient time should be made available to do the job correctly and precisely.

Apply emulsifiable concentrates, wettable powders, and water soluble compounds in a water carrier at a volume equivalent to 85 gallons per acre (100 ml per plot). Spray one-half the volume in each of two passes over the plot in opposite directions. Apply all herbicides on the same day under as calm conditions as possible. Irrigate thoroughly afterwards to wash chemicals into the soil. Apply post-seeding treatments within 2 days after seeding and postgermination treatments 28 to 35 days after emergence of crop seedlings. Consider emergence to be the time when most seedlings have shed their seedcoats.

In addition to insuring good calibration, it is critical to avoid contaminating herbicide treatments. The fewer times a sprayer has to be filled with different herbicides, the less chance there is for contamination or error. Thus; it is best to spray all the plots of one herbicide before turning to another.

Cleaning application equipment. — Between uses of different herbicides, rinse all parts of the sprayer completely with water, clean with a mixture of strong detergent and ammonia, and rinse again thoroughly with water. Take care not to damage the finely machined surfaces of the nozzle tip. Clean them using a soft brush, not a knife, wire, or other hard material. Replace a nozzle when it is damaged. Spray rinsings on a non-crop area, preferably where some weed control could be gained from the residual herbicide.

Choose your cleaning area with great care. It is important to discharge the cleaning water where it will not contaminate water supplies, streams, crops, or injure other plants, and where puddles will not be accessible to children, livestock, pets, or wildlife.

Application *of* granular herbicides. — Apply granular herbicides with a shaker device. Ocularly divide the plot into quarters and apply one-fourth of the herbicide on each quarter.

If the material must be incorporated into the soil prior to seeding, the plots must be laid out in advance and the chemical applied no more than 1 day before seeding. Incorporate the material into the top 2 inches of soil with a garden rake. Reshape and compact the ground surface to conform to the rest of the seedbed.

Records. — Be sure to follow the diagram and plot tags when applying treatments, and record any deviation which occurs by mistake. Record date of application, weather conditions, and any events or situations you feel may affect the results.

Plot maintenance. — Conduct all other nursery operations such as irrigation, fertilization, and undercutting as needed and as uniformly as possible. Maintain a record of the operations and dates of application.

Data collection. Weed control. — Hand-weed all plots about 26 to 34 days after tree seedling emergence but 1 to 2 days prior to application of post-germination treatments. In addition, hand-weed all plots as needed beginning 1 month after postgermination application. Base needs for weeding on the most weedy plot or plots, and weed all plots in each replication on the same day to avoid differences resulting from additional weed growth. Do not water during the 24 hours preceding weeding.

For each weeding, separate weeds from individual plots into grass and forbs, count the number in each group, and identify the major species (appendix 2). Summarize data on numbers at the end of the season (appendix 3).

Phytotoxicity.—To assess the effects of treatments on seedling emergence and survival, count the number of seedlings between the two stakes that were placed 1-foot apart in three rows of every plot. Do this about 25 days after seedling emergence and at the end of the first growing season (appendix **4).**

Rate the damage to crop seedlings at the time of postgermination application and at the end of the first growing season using the following rating system (appendix **4):**

Damage rating system for crop seedlings (adapted from Anderson **1963)**

Ratina	Description
10	No damage.
7-9	Slight damage; seedlings will recover and make near normal growth.
4-6	Moderate damage; few seedlings have died but some show chemical effects and reduced growth.
1-3	Severe damage; many seedlings have died; others are discolored and stunted.
0	All seedlings dead.

It is unlikely that different people rate seedlings exactly the same. To provide consistency, a single individual should do all the rating at a nursery. In addition, that person should briefly describe and record specific factors used to determine ratings.

At the end of the first growing season, measure height of all seedlings between the three sets of stakes used to determine survival in each plot (appendix 5). Also, dig a few seedlings from each plot and examine them closely for abnormal growth characteristics that might not appear in the quantitative data, such as stem swelling (Stewart et al. 1978) or lack of root branching.

Summarize survival, damage (appendix **6),** and height data (appendix 7).

Data analysis.—As a minimum, determine sums and means (averages) of the numerical data for each treatment: total number of weeds by type for each weeding and the season; number of crop seedlings per foot; crop seedling height; and damage rating. Pool (combine) all of the weed control data for the nursery if the same treatments were used for each species, but keep the crop measurements separated by species.

For easier comparison, convert the data to percentages of effect, using the untreated control as the base of 100 percent.

The data should also be analyzed statistically by analysis of variance and some test of individual treatment comparison to make sure that differences are due to treatment rather than chance. Although the mechanics can be done relatively easily on modern programable calculators or computers, it is not advisable to attempt an analysis without knowledge of statistical concepts. Seek the help of someone with training in statistics if such expertise is not available at the nursery. The formats for an analysis of variance of the test example using one herbicide, two species, and three replications are:

Weed control data (pooled by species)

Source of variation	Degrees of freedom
Treatments	**4**
Replications	5
Error	20
Total	29

Phytotoxicity data (for each species)

Source of variation	Degrees of freedom
Treatments	**4**
Replications	2
Error	8
Total	**14**

Use Tukey tests to compare individual treatment differences.

Subjectively examine the information on weed species to determine whether herbicides differ in their species selectivity.

Interpretation of the results involves examination of the data and analyses and consideration of test conditions and observations — in other words, a combination of facts, logic, and common sense. For assistance, study published reports of similar experiments (Steward 1977 and Stewart et al. 1978).

If one or more herbicides or specific combinations of herbicide and application rate appear promising in this initial test, conduct a larger scale trial the 2d year. Firm guidelines cannot be given for deciding what treatments warrant further testing. Each nursery manager must decide on the basis of weed control objectives, costs, and effects on crop seedlings. In the Cooperative Western Nursery Weed Control Study, further testing was generally done for treatments that gave at least 70-percent weed control and resulted in an average damage rating no more than 10 percent less than that of untreated seedlings (Steward 1977).

Second Year

Objectives.—The objectives of the 2d-year test are to further evaluate the effectiveness and safety of herbicide treatments identified as promising in a 1st-year test. Specific questions to be asked are:

1. Do the herbicide treatments reduce the time required to hand-weed 1st-year nursery beds?

2. Do the herbicide treatments reduce conifer crop seedling survival, growth, and damage rating at the end of the first growing season and shoot length and condition at the end of the second growing season?

Experimental design.—The plots will be 20 feet long. Treatments to test are those that, in the previous year's testing· equaled or exceeded results from the current standard treatment and untreated controls.

For example, assume that the previous year's test herbicide, applied post-seeding (1X dose), performed slightly better than the standard herbicide treatment. This year's test will consist of three treatments — 1X dose of test herbicide applied postseeding, standard nursery treatment, and an untreated control. This will be done on the same two species as before. Eighteen plots will be needed (three treatments x three replications x two species).

Plot layout.—Lay the plots out the same as the 1st year but increase plot size to 20 feet. This will require 188 feet of seedbed for each of two species.

Herbicide application.—Prepare and apply herbicide in the same way and with the same schedule as before, but use the amount needed to cover the larger plot size, and use a 1-quart bottle for the small-plot sprayer rather than the 1-pint size. A hand-pumped pressure sprayer is probably unworkable for this size of plot. Clean equipment as described for the 1st-year test.

Use the same formula as before. Amounts of wettable powder (grams) and liquid (milliliters), respectively, to apply to plots 4.5-feet x 20-feet are:

$$\frac{\text{lbs ai per acre}}{\text{lbs ai per lb of formula}} \times .937 = \text{grams of wettable powder}^{5/}$$

$$\frac{\text{lbs ai or ae per acre}}{\text{lbs ai or ae per gallon of formula}} \times 7.82 = \text{milliliters of solution}^{5/}$$

Records and plot maintenance.—Conduct these tasks as in the 1st year.

Data collection. ***Weed control.***—Conduct weeding as before, but, in addition, carefully measure and record the time required to weed each plot (appendix 8). Identify the weed species (appendix 2), but do not bother to count them this year.

Phytotoxicity.—Collect and record damage rating and growth data as before for the 1+0 seedlings (appendices **4-7).** In addition, lift 10 randomly selected 2+0 seedlings from each plot in the 1st-year test that received the same treatment the 2d year. Collect and record the same growth and condition information (appendices 4-7).

Data analysis.—Follow 1st-year procedures. Analysis of variance tables for the test example of two herbicide treatments and an untreated control on two species are:

Weed control data (pooled by species)

Source of variation	Degrees of freedom
Treatment	
Control vs. test herbicides + standard	1
Test vs. standard	1
Replications	5
Error	10
Total	17

Phytotoxicity data (for each species)

Source of variation	Degrees of freedom
Treatments	
Control vs. test herbicide + standard	1
Test vs. standard	1
Replications	2
Error	4
Total	8

Third Year (optional)

Objectives.—A 3d-year test may be advisable to provide a greater margin of safety, yield better economic data, and demonstrate levels of weed control under semi-operational conditions. If previous results were not clear-cut, 2-year-old seedlings may also be sampled for growth.

Semi-operational tests should be coordinated through the potential registrant of the herbicide to assure that all requirements for Experimental Use Permits and other requirements are met.

Two specific questions will be asked:

1. Do the herbicide treatments reduce the time required to hand-weed 1st-year nursery beds?

2. Do the herbicide treatments reduce crop seedling survival (optional), growth, and damage rating?

Experimental design.—The basic treatment unit (plot) is a 200-foot-long section of nursery seedbed. Plots this large permit use of regular nursery spraying equipment and provide enough space to accurately assess total crew time required for hand weeding. The first 2 years have presumably eliminated any treatments that are phytotoxic to the crop species.

Treatments to use are:

1. Applications identified as promising in the first 2 years of testing (the best or a few of the best combinations of herbicide and application timing).

2. Untreated control.

3. Standard nursery treatment (if applicable).

Again, use a minimum of three replications of each treatment in a randomized block design. That is, install a complete set of treatments (one plot of each in adjacent seedbeds) in three places in the nursery. At this point in the testing program, phytotoxicity of the herbicides should have been established. Any species for which phytotoxicity data is still wanted, however, must be represented in each plot. It is safe to assume that weed control characteristics of the herbicides will not vary with crop species.

The example will consist of three replications of three treatments: (1) postseeding plus postgermination applications of one herbicide; (2) an untreated control; and (3) the standard treatment. This will require nine 200-foot-long plots.

5/ Re-compute for other swath widths.

Plot layout.—Select three sets (replications) of three seedbeds each for the test. Use the same seedlot(s) of the crop species within a replication. As before, assign treatments within each replication to plots in a random manner and prepare a diagram of the layout.

Form the seedbeds and sow the seeds on a single day. Lay the plots out using a steel tape and wooden stakes. Avoid using the first or last 10 to 20 feet of any seedbed. Attach treatment identification to one of the stakes of each plot, and post large signs at each end of the test area.

Placing sets of wire pins, as done the 1st and **2d** years to mark subplots for seedling counts, is optional.

Herbicide application. Preparation.—It is not feasible to spray the exact amount of herbicide for the 200-foot plots; application should begin before the operator reaches the beginning of the plot and extend somewhat beyond the end of the plot to ensure uniform application over the full length. Furthermore, most sprayers can't maintain uniform pressure as the tank approaches the empty point. Thus, the approach should be to adjust the tractor speed to evenly dispense the correct amount of herbicide on the plots.

In advance of treatment, weigh or measure the amount of herbicide needed for the test plots plus one extra plot. Use the same formula as before:

$$\frac{\frac{200 \text{ ft} \times \text{spray swath width (feet)}}{43{,}560 \text{ ft}^2} \times \text{Dounds active ingredient per acre}}{\text{pounds active ingredient per pound of formula}} \times 453.6 \text{ grams of wettable powder per plot.}$$

or

$$\frac{\frac{200 \text{ ft} \times \text{spray swath width (feet)}}{43{,}450 \text{ ft}^2} \times \text{pounds per active ingredient or acid equivalent per acre}}{\text{pounds active ingredient or acid equivalent per gallon formula}} \times 3785 \text{ milliliters of liquid herbicide per plot.}$$

Application equipment.—Use the spray equipment normally employed at the nursery. Some temporary modification, such as blocking nozzles, may be necessary to adjust the swath width to cover only a single seedbed. For convenience, mix enough for four plots at one time.

Manufacturers of granular herbicides should be able to recommend equipment for spreading their products evenly.

Calibration and application *of* liquids.—Apply herbicides in water at a volume equivalent to 50 gallons per acre. This amount is equal to:

$$\frac{200 \text{ ft} \times \text{swath width (feet)}}{43{,}560 \text{ ft}^2} \times 50 = \text{gallons per plot.}$$

Using plain water, determine how much time it takes for the sprayer to dispense the required volume for a plot. Use the average delivery of nozzles to be employed, cleaning or replacing nozzles that vary more than 10 percent from the average. Spray cups may be used to catch the spray over a given period of time and compare it with the amount of water delivered. Then drive the tractor at a speed which will cover the 200 feet in that length of time (table 2). Practice on 200 feet of unsown beds or roadway, using the appropriate swath-width.

Table 2—Times needed to travel 200 feet at specific tractor speeds

Speed (m/h)	Time to travel 200 feet (s)
0.50	273
0.75	182
1.00	136
1.25	109
1.50	91
1.75	78
2.00	68
2.25	60
2.50	54
2.75	49
3.00	45
3.25	42
3.50	39
3.75	36
4.00	34
4.25	32
4.50	30
4.75	29
5.00	27

Apply all herbicides on the same day, under conditions as calm as possible. Irrigate thoroughly afterwards to wash the chemicals into the soil. Apply postseeding treatments within 2 days after seeding, and postgermination treatments should be applied 28 to 35 days after emergence of crop seedlings.

To speed operations and avoid contamination, spray all plots receiving the same herbicide before using another chemical.

Cleaning *of* equipment — As before, avoid contamination between herbicides by thoroughly rinsing the tank, boom, and nozzles. Rinse once with plenty of plain water, a second time with water and a commercial tank rinsing compound, and a third time with plain water. As before, dispose of the rinse water where it will not contaminate water supplies, streams, crops, or injure other plants, and where puddles will not be accessible to children, livestock, pets, or wildlife.

The following steps are suggested for thorough cleaning:

1. Hose down the inside of the tank completely, filling it half full of water. Then flush the cleaning water through the nozzles by operating the sprayer.
2. Repeat the procedure in step 1.
3. Remove nozzle tips and screens. Clean them in kerosene or detergent solution, using a soft brush. Do not use a knife, wire or other hard material to clean nozzle tips. The finely-machined surfaces of the tips can be easily damaged, causing distortion of the spray pattern and an increased rate of application.
4. Fill the tank about half full of water and add about 1 pound of detergent for every 50 gallons of water.
5. Operate the pump to circulate the detergent solution through the sprayer for about ½ hour, then flush it out through the boom.

If you have used 2,4-D or an organo-.phosphorous insecticide, before doing step 6, follow this additional procedure:

a. Replace the screens and nozzle tips.
b. Fill the tank about half full of water and add 1 pint of ammonia for every 25 gallons of water.
c. Operate the pump to circulate the ammonia solution through the sprayer for about 5 minutes, and discharge a small amount through the boom and nozzles.
d. Keep remaining solution in the sprayer overnight.
e. In the morning, flush out all the ammonia solution through the nozzles by operating the sprayer.

6. Fill the tank about half full of clean water while hosing down both the inside and outside, then flush out through the boom.

When finished with the sprayer for the season, remove the nozzle tips, strainers, and screens and store them in light oil. Store the sprayer in a clean, dry shed. If the pump cannot be drained completely, store it where it cannot freeze.

Records. — Be sure to follow the diagram and plot tags when applying treatments, and record any deviation occuring by mistake. Record date of application, weather conditions, and any events or situations you feel may affect the results.

Plot maintenance. — Conduct all other nursery operations such as irrigation, fertilization, and undercutting as needed and as uniformly as possible. Maintain a record of the operations and dates applied.

Data collection. Weed control. — Hand-weed all plots about 26 to 34 days after crop seedling emergence but 1 to 2 days prior to application of postgermination treatments. Hand-weed all plots as needed beginning 1 month after postgermination application. Base need for weeding on the most weedy plot or plots, and weed all plots on the same day to avoid differences in weed growth. Do not water during the 24 hours preceding weeding.

Record the total time needed to hand-weed each plot (appendix 8). Since times will be compared, have the same crew do all the weeding within each replication on the same day. If possible, keep conditions similar between replications as well. Do not include break times. Summarize the effects of herbicides on time needed for hand-weeding as in appendix 9.

Identify weed species in each plot prior to each weeding and subjectively classify them into one of three categories of abundance — low, medium, high.

How to Use the Acquired Information

Phytotoxicity. — Use the damage rating system described for the 1st-year test. At the end of the growing season make observations in 1-foot-long samples every 20 feet along the test seedbeds (appendix 10).

Also at the end of the growing season, measure length of main stems of three random groups of 10 seedlings in each plot (appendix **4).** Summarize as in appendix 7. Make seedling counts if desired (appendix 3).

Data analysis. — Follow the general procedures described for the 1st year. Numerical data for the 3d year consist of: (1) time needed for each weeding and total season, (2) crop seedling heights, (3) and damage rating.

Analysis of variance tables for the example have the following form:

Weed control and phytotoxicity

Source of variation	Degrees of freedom
Treatment	
Control vs. test herbicide + standard	1
Test vs. standard	1
Replications	2
Error	4
Total	8

As stated in the introduction, one of the reasons to test herbicides in forest tree nurseries is to obtain data to support registration of useful herbicides. To be useful, the data must meet requirements of both chemical companies and registration. The method offered here satisfies all current requirements, but changes might invalidate a subsequent study. It is important to keep in contact with representatives of the chemical company whose products you are investigating. Also, knowledge of testing that other nurseries are doing gives you and the chemical company representative a basis for comparing results if testing is done similarly. The first year's data, while not usually adequate for registration of a product, will indicate whether testing should be continued a second year. Chemical company representatives, USDA Forest Service Pesticide Specialists, Cooperative Extension Service personnel or others can assist in making these determinations. The data developed may supplement information from other nurseries and may help achieve registration.

It is important to have all data available and summarized shortly after the field season so if the data support use, the chemical company can seek registration and have the herbicide available for the next field season. It takes 3 months to a year to get a use registered once the data are available. Since the company may be working with several nurseries, progress will depend upon information from the last nursery to report. Examples of forms useful for summarizing data are found in appendices 3,6,7, and 9.

The chemical company will analyze the data to see whether it is interested in registering its product for forest tree nursery use. Standards of performance must be met before companies are interested in pursuing specialty uses. One of the prime considerations is liability. If there is not an adequate margin of safety for crop seedlings, a company may not wish to register the product. In most instances, however, if a nursery manager is satisfied the product is safe, the chemical company will be also.

We cannot stress too strongly that information developed should be given to chemical companies as soon as possible, because they are usually the ones to initiate registration of the products.

The information developed in your test should also be shared with your colleagues. This can be done informally, but presentation at nursery managers' meetings or in written publications will allow a greater dissemination of your work. Communication with others may lead to greater standardization or innovative methods to make the job easier.

Safety in Pesticide Use

Even though amounts of herbicide used in tests are small compared with operational use, follow safe practices. Be certain that persons using pesticides are competent and trained. Training assistance can be requested of pesticide or nursery specialists of the USDA Forest Service or technical representatives of the chemical companies. Training should include:

1. Description of known potential human health effects anticipated from exposure to each herbicide.

2. Procedures for mixing and applying herbicides and cleaning equipment.

3. Procedures for storing and disposing of unused herbicides and the residue in rinse materials.

Summary

This report describes a proven way of identifying herbicides that are safe and effective in controlling weeds in forest tree nursery seedbeds. The design, equipment, methodology, and analysis techniques are given for testing herbicides for postseeding and postgermination weed control and phytotoxicity. Weed control techniques in the nursery need to be refined. The approach presented here can be modified to test any number of applications of herbicides to seedbeds. Testing is encouraged, as innovation must come-from individual nurseries. Proper documentation of new approaches or new herbicides, plus timely and open communication with colleagues, pesticide specialists, and chemical companies can result in further advances in nursery weed control.

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Appendix 1

Summary of study procedures for each year in chronological order

1. Certify the applicator or supervisor.
2. Select treatments and acquire herbicide(s) and other supplies (winter).
3. Prepare dry herbicides for mixing (early spring).
4. Diagram plots in office and randomly assign treatments to plots (early spring).
5. Form seedbeds and sow seeds (spring).
6. Lay plots out on ground (spring).
7. Calibrate sprayer (spring).
8. Apply postseeding treatments (spring).
9. Hand-weed plots and record data (late spring).
10. Apply postgermination treatments (late spring).
11. Hand-weed as necessary and record data (late spring and all summer).
12. Collect seedling counts, heights, damage ratings, and weights (fall).
13. Analyze data and make plans for next year (fall and/or winter).

Appendix 2

Weed control data for nursery herbicide test — 1st and 2d years (data form)

Nursery _____ Crop species _____

Seedbed location _____ Date _____

Plot no.	Treat.	Grass			
		No.	Species[1/]	No.	Species[1/]

[1/] Amount of detail concerning individual species *is* flexible. The objective is to determine species selectivity of the herbicides.

Appendix 3

Weed control for nursery herbicide test — 1st and 2d years[1/] (summary)

Nursery _____ Year_____

Treatment	Weed numbers			
	First weeding	Subsequent weedings	Total season	Percent of untreated
Untreated				100

[1/]Weed control ratings shown are the means of all plots of each treatment. Number of weeds are expressed as percent of the untreated plots (average divided **by** average of untreated **x** 100).

Appendix 4

Crop-seedling survival and damage data for nursery herbicide test (data form)

Nursery _____ Crop species _____

Seedbed location _____ Year _____

Plot no.	Treatment	No. seedlings/foot (Date_____)			Damage rating (Date_____)
		1	2	3	

Appendix 5

Crop seedling height for nursery herbicide test (data form)

Nursery _____ Crop species _____

Seedbed location _____ Date sown _____

Treatment _____ Date sampled _____

Plot_____	Plot_____	Plot_____
mm		
Total		
mean		

Grand total _____ mean _____

Appendix 6

Crop-seedling damage and survival data (summary)

Nursery _____ Year_____

Treatment	Species_____		Species_____	
	Ave. damage rating	Survival (% of untreated)[1/]	Ave. damage rating	Survival (% ofuntreated)[1/]
Untreated		100		100

[1/]Percents are found by dividing the average rating for each treatment by the average for untreated plots and multiplying by 100.

Appendix 7

Effects of herbicides on growth of crop seedlings (summary)

Treatment	Height (mm)	
	Species_____	Species_____

Appendix 8

Time needed to weed plots — 2d and 3d years (data form)

Nursery _____ Crop species_____

Seedbed location _____ Date sown_____

Date'	Plot no.	Treatment	(1) Crew size (no.)	(2) Crew weeding time (min. & sec.)	(1 x 2) Total weeding time (min. & sec.)

Appendix 9

Effect of herbicides on hand-weeding — 2d and 3d years[1/] (summary)

Herbicide	Timing	Rate	First weeding	Subsequent weedings	Total season	Percent of[2/] untreated
		(lbs ai/acre)	***Minutes***	***Minutes***	***Minutes***	***Percent***
untreated						100

[1/] Times shown are the mean number of person-minutes to weed a 200-foot-long plot.

[2/] Percents are found by dividing the total season weeding times for each treatment by the weeding time for untreated plots and multipling by 100.

Appendix 10

Crop seedling damage rating — 3d-year (data form)

Nursery _____ Crop species _____

Seedbed location _____ Date _____

Sample point[1/]	Plot _____ Treatment _____	Plot _____ Treatment _____	Plot _____ Treatment _____	Plot _____ Treatment _____	Plot _____ Treatment _____
	Damage rating				
20					
40					
60					
80					
100					
120					
140					
160					
180					
Total					
Ave.					

[1/] Twenty-foot intervals along 200-foot plots.

Sandquist, Roger E., Peyton W. Owston, and Stephen E. McDonald.
How to test herbicides at forest tree nurseries. **USDA** For. Serv. Gen. Tech. Rep. PNW-127. Pac. Northwest For. and Range Exp. Stn., Portland, OR. 1981.

Procedures developed in a cooperative westwide study of weed control in forest tree nurseries are described in a form modified for use by nursery managers. The proven, properly designed test and evaluation methods can be used to generate data needed for evaluation and registration of herbicides.

Keywords: Nursery methods, herbicides (nursery), weed control (nursery), pesticide registration.

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