Seedling Quality Tests: Plant Moisture Stress
By Gary Ritchie and Thomas D. Landis

Introduction

This is the fifth installment in our review of seedling quality tests. Here we focus on what is commonly known as "plant moisture stress" or PMS. Although PMS is not routinely used for seedling quality testing *per se*, it is nevertheless the most common physiological measurement made on reforestation stock. This is because the measurement itself is simple and robust, and the equipment needed to perform it is reasonably priced and readily available. However, while measurements of PMS are easily made, their interpretation is not always straightforward. In this article we will discuss the meaning and definition of PMS, how it is measured, how the measurements are interpreted and what, if any, value they have as indicators of "seedling quality."

What is Plant Moisture Stress?

It is axiomatic that water is essential for plant growth. Without copious quantities of water, plants will cease growing and ultimately die. If plants simply absorbed water from the soil to meet only metabolic needs, water requirements would be quite low. But plants also manufacture food through photosynthesis during which carbon dioxide (CO_2) from the atmosphere diffuses into leaves through tiny pores called stomata. Once inside the leaf, the CO_2 is converted to sugars. Photosynthesis is a "leaky" process, however. While CO_2 is diffusing into the leaves, water is diffusing out – this loss of water is called transpiration. Plants can reduce transpiration and conserve water by closing stomata, but this also impedes photosynthesis. So, in order to grow, plants must also transpire.

Transpiration generates a "stress," due to water's high cohesion. This stress is transmitted from the leaf down the stem and into the roots. During daylight, when stomata tend to be open, water loss exceeds the plant's ability to extract water from the soil. So plants are almost always subjected to some level of water stress during the day. This stress is normal and is not injurious unless it persists at a high level for a prolonged period of time.

In very simple terms, plant moisture stress can be modeled as:

$$PMS = A - T + S$$

Where A is the absorption of water from the soil, T is transpirational loss, and S is storage of water in the plant stem and roots, which is negligible in seedlings but important in large trees. Just as discussed, during daylight, T almost always exceeds A.

Water potential. The fundamental equation that describes the water relations of a plant cell or tissue is:

$$\psi_w = \psi_p + \psi_o$$

where ψ_w is the total water potential, a measure of the free energy or chemical potential of water. ψ_w in the plant is made up of two component potentials. ψ_p, the pressure potential, can be either positive or negative, whereas ψ_o, the osmotic potential, is always negative. Potentials are expressed in units of pressure, and although MegaPascals are the official SI unit, bars are most commonly used in nurseries. By definition, the ψ_w of pure water at standard temperature and pressure equals 0 bars. ψ_p and ψ_o are continually changing as transpiration and osmosis cause water to move across membranes, in and out of cells, and up the transpiration stream. In nursery situations, ψ_w is always negative so plants are always under some level of water deficit, or stress.

The interrelationships between ψ_p and ψ_o, and how they affect ψ_w, are illustrated in a Höfler diagram, named for the German scientist Karl Höfler, who

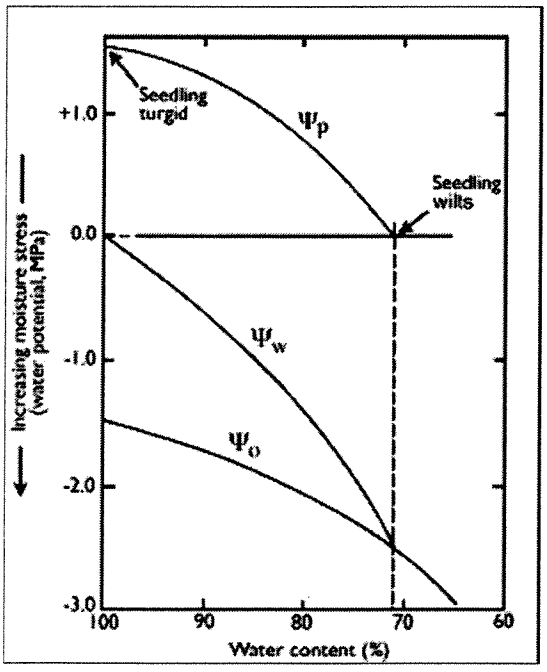

Figure 1- A modified Höfler diagram depicting the interplay of the components of water potential in a plant cell as they change with cell water content (Ritchie 1984).

Ritchie, Gary; Landis, Thomas D. 2005. Seedling quality tests: Plant moisture stress. In: Dumroese, R. Kasten; Landis, Tom D.; Watson, Rae. Forest Nursery Notes. R6-CP-TP-06-2005. Portland, OR: USDA Forest Service, Pacific Northwest Region, State and Private Forestry, Cooperative Programs: 6-12.

devised it in the 1920s (Figure 1). The X-axis is the water content of the cell expressed as a percentage and the Y-axis is in units of water potential. This diagram also shows the relationship between potential units and the common nursery terms of turgidity and wilting. At full hydration (100% water content), the positive turgor pressure of the cell walls (? $_P$) balances the negative osmotic potential (? $_O$) in the cell contents so that ? $_W$ = 0 MPa. As the cell loses water, ? $_P$ falls and ? $_O$ becomes more negative as concentration of solutes in the cell increases. This causes ? $_W$ to decrease until ? $_P$ reaches 0 MPa and cells collapse. The value of ? $_W$ at which this occurs is known as the "zero turgor point" or, as it is more commonly known, the "wilting point."

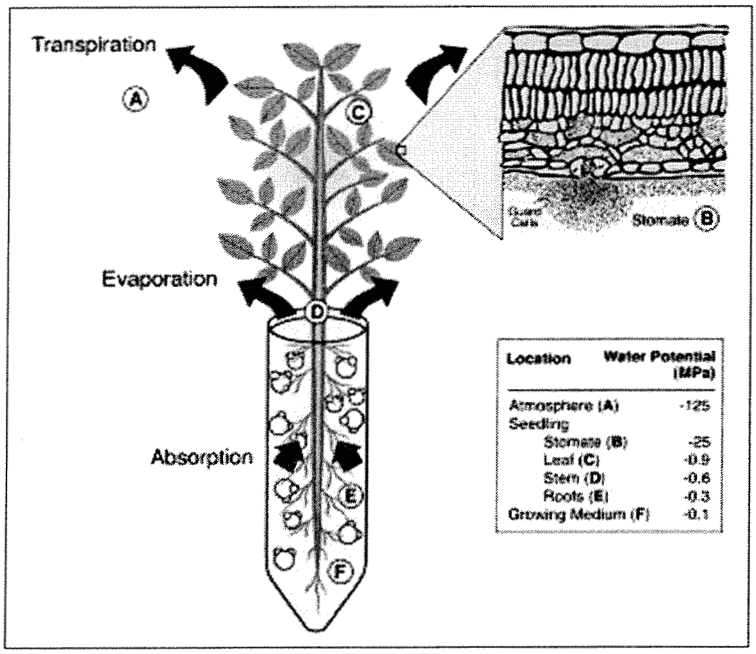

Figure 2 - Water is drawn along a gradient of water potential that is driven by transpirational losses, from higher (less negative) levels in the growing medium, through the seedling to the low (more negative) levels in the atmosphere (Landis and others 1989, modified from Mac-Donald and Running 1979).

Units of water potential—Thermodynamic water potential terminology (Slatyer 1967) has always been troublesome for growers because negative values are hard to visualize and tricky to manipulate algebraically. Fortunately, someone somewhere had the idea to express water potential as a positive value and call it "Plant Moisture Stress" (PMS). From a

Table 1. Comparison of units and descriptive terms for plant water potential (? $_W$) and plant moisture stress (PMS). ? $_W$ and PMS have the same value, but ? $_W$ is expressed as a negative value whereas PMS values are positive (Landis and others 1989).

Plant water potential (? $_W$)				Plant moisture stress (PMS)		
Units*		Relative rating	Relative moisture content	Units*		Relative rating
MPa	Bars			MPa	Bars	
0.0	0.0	High	Wet	0.0	0.0	Low
-0.5	-5.0			0.5	5.0	
-1.0	-10.0	Moderate	Moderate	1.0	10.0	Moderate
-1.5	-15.0			1.5	15.0	
-2.0	-20.0			2.0	20.0	
-2.5	-25.0	Low	Dry	2.5	25.0	High

*? $_W$ and PMS are commonly expressed in bars but have been replaced in the published literature by MegaPascals (Mpa) to conform to SI conventions.

practical standpoint, however, water potential terminology is useful because it is consistent from the soil or growing medium through the seedling and into the atmosphere (Figure 2).

Fortunately, water potential and PMS values are directly convertible simply by changing signs. This relationship and some examples are shown in Table 1. For example, a PMS value of 10 bars indicates a "moderate" level of stress and is equivalent to ? $_W$ of -10.0 bars.

Diurnal changes of plant water potential—As we have already mentioned, ? $_W$ is dynamic and this affects its usefulness as an index of seedling quality. Consider, for example, a container seedling whose growing medium is fully saturated with water (Figure 2). During the day, while stomata are open, low humidity (high vapor pressure deficit) draws moisture from the leaves. This creates an imbalance between transpiration and water absorption resulting in the development of PMS (? $_W$ decreases). At night, stomata tend to close, relative humidity rises to nearly 100% and transpiration ceases. The negative ? $_W$ in the plant pulls water from the growing medium relieving the stress. By early the next

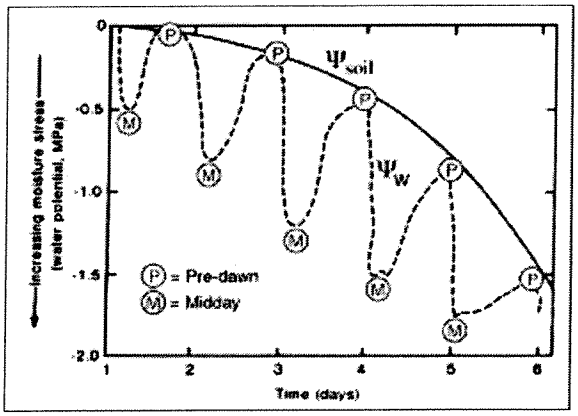

Figure 3 - Changes in plant water potential (? $_W$) and growing medium water potential (? $_{soil}$) of a tree seedling growing in a container. The container is initially watered to saturation then allowed to dry (Landis et al 1989, modified from Slatyer 1967).

morning ? $_W$ will have reached a dynamic equilibrium with soil moisture potential (? $_W$ ~ ? $_{soil}$). Assume that no water is added to the container so the growing medium is allowed to dry out. As this occurs, the pre-dawn stress and the mid-day plant moisture stress will both increase daily as ? $_{soil}$ decreases (Figure 3). After a few days the seedling will close its stomata during midday to retard transpiration. This can be seen occurring in days 4 and 5 on Figure 3. This will result in a moderating of the midday PMS. ? $_{soil}$ will eventually become so negative that the plant will be unable to equilibrate during the night. Throughout this time, the mid-day stress will continue to increase. When re-watered, the system will return to the initial state shown in Day 1.

Note that the ability to track moisture stress levels of both soil and plant in Figure 3 shows the advantage of using water potential units rather than PMS, which reflects only seedling stress.

Measurement of Plant Moisture Stress

Over the years, as plant physiologists labored to understand the dynamics of plant water relations, many attempts were made to develop methods of measuring ? $_W$ (Lopushinsky 1990). As far as nursery work goes, the most significant development was when Per Scholander and Howard Hammel at the Scripps Institute of Oceanography invented the "Scholander Pressure Chamber" (Scholander and others 1965). This device was adapted from a glass pressure chamber reported by Dixon (1914) and was further modified for trees and seedlings by Wareing and Cleary (1967), who outlined basic measurement procedures.

The modern pressure chamber consists of a metal pressure vessel that is connected to a nitrogen gas source by way of a pressure regulator (Figure 4). To measure PMS, a seedling's stem is cut and inserted through a rubber gasket. The shoot is then sealed into a hole in the chamber lid with the foliage inside the chamber and the cut stem protruding (Figure 4). Nitrogen gas is slowly bled into the chamber while the cut stem is closely observed. When a droplet of water appears at the end of the stem the chamber pressure is noted. The gas pressure required to force the drop of water to the surface is equal to the moisture stress of the seedling. For a detailed theoretical description and procedural guide see Ritchie and Hinckley (1975).

The pressure chamber has become the standard technique used for measuring PMS in forest nurseries, ecophysiology laboratories, and other plant research facilities. For example, the JH Stone Nursery in Central Point, OR uses pressure chambers to measure predawn PMS and schedule bareroot seedling irrigation. They are also used to detect dangerous PMS levels during the lifting and packing operations (JH Stone Nursery1996).

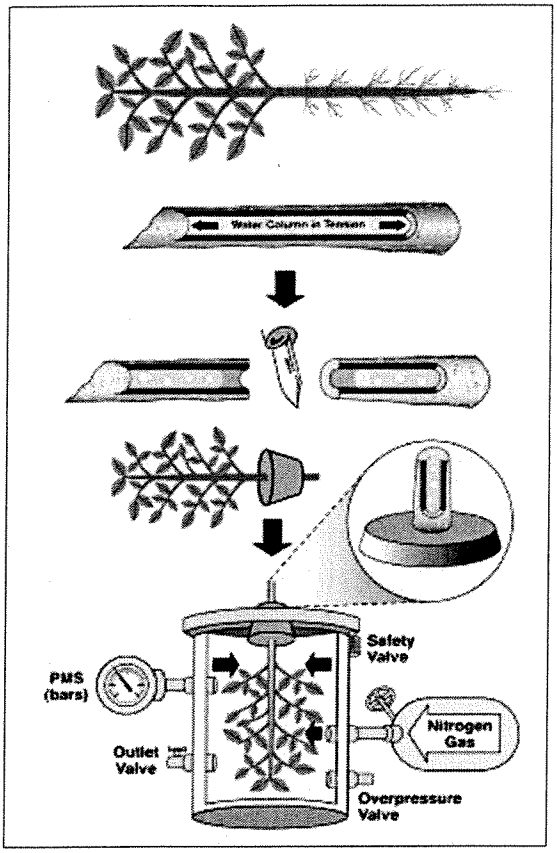

Figure 4 - Diagram showing the steps involved in measuring PMS with a Scholander Pressure Chamber. A stem is severed, and the cut end forced through a hole in the center of a rubber gland, which is then inserted into the lid of the chamber. Nitrogen gas is slowly introduced into the chamber until a drop of water is forced to the surface of the cut stem. The gauge pressure at which this occurs is equal and opposite the forces holding the water in the stem, a.k.a. the plant moisture stress (Landis and others 1989, modified from PMS Instrument Co.).

A variety of pressure chambers and supplies are available from:

PMS Instrument Company
1725 Geary Street SE
Albany, OR 97322 USA
Phone: 541.704.2299
Fax: 541.704.2388
E-mail: info@pmsinstrument.com
Website: http://pmsinstrument.com/

Interpretation of PMS values. The ease and robustness of PMS measurement has led to its extensive use in plant water studies. Interpretation of PMS values, however, is not always as straightforward as one might expect. This is partly because PMS, as an estimate of ψ_w, integrates two variables into one reading and therefore much information is lost. In addition, because the components of water potential change seasonally, a given value of PMS might have a different interpretation if taken in, say, April as opposed to, say, January. For example, Figure 5 shows how the "zero turgor point" changes seasonally in roots and stems of Douglas-fir seedlings (Ritchie and Shula 1984). In April, a stem PMS reading of 25 bars (-2.5 MPa) would be a potentially lethal value because it would be near the zero turgor point. But the same value, if measured in January, would be of little concern. Similarly, root systems with PMS near 20 bars (-2.0 MPa) would be suspect most of the year.

More importantly, there is the issue of diurnal variability. As we show in Figure 3, PMS can vary sharply from day to day and during the day. Typically, the highest values of PMS occur during midday and lowest values in early morning. Daytime PMS values can fluctuate wildly on days with intermittent clouds and sun. So, they often provide only brief "snap shots" of PMS that have little diagnostic value.

Probably the most useful PMS value is what is known as the "pre-dawn PMS." This is the PMS that obtains just before sunrise when ψ_w is in dynamic equilibrium with ψ_{soil} (Figure 3) and provides an estimate of the minimum stress the plant would experience that day. If this minimum value is high, it may be cause for concern. With the above caveats in mind, we present some suggested guidelines for interpretation of pre-dawn PMS measurements as they relate to plant growth and cultural implications (Table 2).

As a footnote, it is not necessary to travel to the field before sunup to take a pre-dawn PMS value. Instead, you can place a dark plastic bag or bucket over a seedling in the evening. This will maintain the relative humidity near 100%. During the night, PMS will reach the pre-dawn value and will tend to hold this value under the high humidity until the covering is removed the following morning.

Is PMS an Indicator of Seedling Quality?

As pointed out by Lopushinsky (1990), the properties of seedlings that are useful as plant quality indicators (root growth potential, cold hardiness, stress resistance, dormancy intensity, carbohydrate content) are not

Table 2. Growth response and cultural implications of inducing moisture stress in conifer seedlings in northwest nurseries (modified from Landis and others 1989).		
Pre-dawn PMS value (bars)	**Moisture stress rating**	**Seedling response/cultural**
0 to 5	Slight	Rapid growth
5 to 10	Moderate	Reduced growth/best for overall hardening
10 to 15	High	Restricted growth/variable hardening results
15 to 25	Severe	Potential for injury
Below 25	Extreme	Injury or mortality

correlated with PMS. Therefore, PMS cannot be used as a proxy indicator of any of these. We should also point out that dead seedlings can exhibit very low PMS values because dead roots retain the ability to absorb water. So, as you can see, low PMS values are not necessarily indicators of healthy stock.

Therefore, the question is: is PMS a useful indicator of seedling quality on its own? In our opinion, PMS indicates seedling quality only when stress is extremely high. For example, nursery seedlings with *pre-dawn* PMS values up in the 15 to 25 bar range should be suspect – especially if these high values persist after irrigation (Table 2). PMS is also operationally used to monitor seedling condition during the lifting-grading-storage process. For example, stock that has a PMS value of, say, 30 bars coming out of storage would certainly be cause for concern.

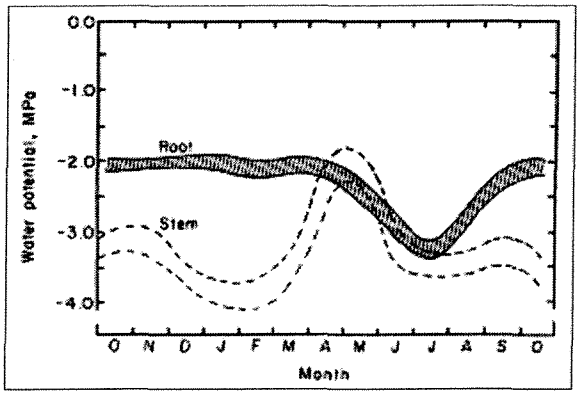

Figure 5 - Seasonal changes in water potential at zero turgor for root systems and stems of Douglas-fir seedlings (Modified from Ritchie and Shula 1984).

Two laboratory procedures exist, however, in which pressure chamber values can be used to measure some aspects of seedling quality:

Pressure-volume (PV) Analysis. PV analysis can be used to generate Höfler diagrams (Figure 1), which are useful for many purposes including identification of seedling water potential at zero turgor. The data in Figure 5 were developed using this technique. But this is a very laborious and difficult procedure and we know of no labs that currently offer it as a service.

Pressure Weight Loss. This pressure chamber technique can be used to identify cold damaged root systems (Ritchie 1990). In this procedure, a seedling root system is submerged in water overnight to assure full hydration. After weighing, it is held in a pressure chamber at 1.5 MPa pressure for 5 minutes. The sample is then removed and re-weighed. Douglas-fir seedlings that lost =7% of their weight had reduced vigor and survival three months later in field and pot trials. It is possible that tests based on this principle could be developed to detect tissue damage in other species and tissues.

PMS as a Snapshot of Seedling Water Status

The fact that PMS is not a good *predictor* of seedling quality should not be interpreted to mean that monitoring PMS is a waste of time. Pressure chambers should be used to check on plant moisture status at several times during nursery tenure. Using pre-dawn PMS readings to fine-tune nursery irrigation practices is a good idea because pressure chamber measurements are the only way to truly know the water status of seedlings at a given time.

PMS measurements during lifting can alert nursery managers to dangerously dry conditions, or excessive seedling exposure. Seedling users can use PMS to check the moisture status of their stock immediately before outplanting. In one recent study, the PMS of Radiata pine (*Pinus radiata*) seedlings was taken immediately after storage and a very strong relationship was found

between moisture stress and root growth after outplanting (Mena-Petite and others 2001). They concluded that post-storage water potentials below 1.5 MPa reduced root growth by 90% (Figure 6).

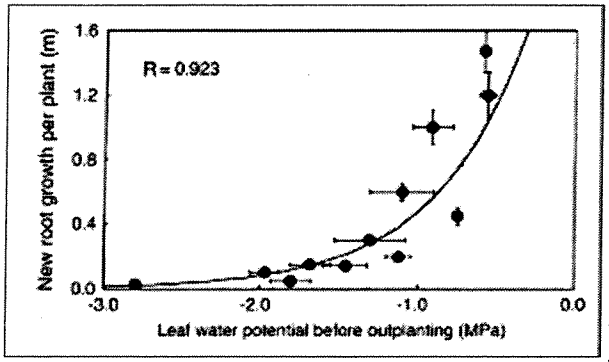

Figure 6 - A strong correlation was found between PMS readings taken after storage and new root growth after outplanting (Mena-Petite and others 2001).

Conclusions and Recommendations

Plants normally lose water more rapidly through transpiration than they absorb from the soil, so they are almost always under some level of water stress. This is often called plant moisture stress (PMS). PMS is numerically equal to, but differs in sign from, plant water potential (? $_w$). PMS shows strong diurnal variations as transpiration rates change in response to changes in temperature, vapor pressure deficit and stomatal aperture. The most useful value of PMS is that which occurs just before dawn, when ? $_w$ is near equilibrium with ? $_{soil}$. This is called the pre-dawn PMS. The Scholander pressure chamber remains the most robust and useful method for measuring PMS. Here, a stem is severed from a plant and sealed in a pressure chamber with the cut end protruding from a hole in the chamber lid. Gas pressure is introduced into the chamber until a water drop forms at the base of the stem. The pressure at which this occurs is equal and opposite to the forces holding the water in the stem and provides an estimate of PMS. Although there are seasonal variations in critical PMS values, readings in the range of 5 to 15 bars are normal whereas those above 15 bars are cause for concern.

PMS is not directly correlated with any of the classical seedling quality indicators (root growth potential, cold hardiness, stress resistance, dormancy intensity and carbohydrate concentration). Therefore its use as a seedling quality indicator is limited to only a couple of laboratory procedures, neither of which are currently available commercially. PMS readings, however should still be used as a snapshot of overall seedling water status.

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