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Please send address changes to Rae Watson. You may use the Literature Order Form on page 36 to indicate changes.



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Reforestation, Nurseries, and Genetic Resources (RNGR) Web Page: http://www.rngr.net/>

The all new "Directory of Plant Material Providers" is now online and is a combination of three previous hard copy directories:

- 1) Directory of Forest and Conservation Nurseries
- 2) Commercial Seed Dealers Directory
- 3) Native Plant Materials Directory.

As you can probably imagine, it is almost impossible to keep hard copy directories up-to-date because as soon as they are printed, addresses, phone numbers, FAX numbers and E-mail addresses begin to change.

By combining three directories into one, now you can find nurseries, seed dealers, and native plant producers by location, products or services. In addition, suppliers can manage their respective information directly through the RNGR website. For more information on the directory, how to update information, or how to become a part of this powerful tool, please contact:

Bryan Jordin TEL: 706.542.1965 E-Mail: bryan@sref.info

Native Plants Journal



Hopefully, many of you already subscribe to NPJ but, if you don't, you should consider doing so. In a few short years, NPJ has established itself as one of the best journals in horticulture. Not only does it contain a wealth of technical information but the color photographs and illustrations are of the highest quality. Many people think that "native plants" doesn't mean forest trees but NPJ has featured articles on Douglas-fir and longleaf pine as well as ninebark and Nebraska sedge. Many issues also contain focus topics which have ranged from "Nasty Plants" (Poison-oak and stinging nettle) to the Salicaceae family in the latest issue. Each issue also contains a good mix of propagation protocols, nursery equipment, refereed research articles, and outplanting considerations. Starting in 2005, NPJ will be published three times per year and...are a bargain at \$35 for students and \$45 for individuals.

The Native Plants Journal can be ordered from:

Indiana University Press 601 North Morton Street Bloomington, IN 47404-3797 TEL: 800.842.6796 E-mail: pjwilson@indiana.edu Website: www.nativeplantsnetwork.org or iupjournals.org/npj

Nursery Meetings

This section lists upcoming meetings and conferences that would be of interest to nursery, reforestation, and restoration personnel. Please send us any additions or corrections as soon as possible and we will get them into the next issue.

Forest Renewal Co-op Annual General Meeting and Workshop is scheduled for **January 31 to February 1, 2005** in Thunder Bay, Ontario, Canada. A few of the topics include, *Second year results of Black and White Spruce Stock Trials, Established for Clients Throughout Manitoba, Successes and Challenges of Afforestation on Private Lands in Southwestern Ontario, and Niagara's Woodland & Fragile Land Restoration Program.* For more information please contact:

> Forest Renewal Co-op Lakehead University c/o Faculty of Forestry and the Forest Enviornment 955 Oliver Road Thunder Bay, ON P7B 5E1 TEL: 807.343.8313 FAX: 807.343.8116 E-Mail: forestrenewal@lakeheadu.ca www.forestrenewal.ca

The Western Forest and Conservation Nursery Association (WFCNA) will be meeting in Park City, UT on July 18 to 21 2005. The theme for this conference is *Watershed Restoration: From Mountain Tops to Wetlands, with People in Between.* For more information please contact:

Lee Riley Umpqua National Forest 34963 Shoreview Road Cottage Grove, OR 97424 TEL: 541.767.5723 FAX: 541.767.5709 E-Mail: leriley@fs.fed.us

Forest Renewal Co-op and the Ontario Ministry of Natural Resources are hosting **The Thin Green Line**, an international symposium on planting stock and stand establishment practices to enhance forest productivity. The symposium will be held in Thunder Bay, Ontario, Canada from **July 26 to 28, 2005**. For registration information or if you wish to submit a paper or poster please contact:

Sonia Geller KBM Forestry Consultants 349 Mooney Avenue Thunder Bay, ON P7B 5L5

Northeastern Forest and Conservation Nursery Association has scheduled the next conference in Springfield, MO on August 1 to August 4, 2005. For more information contact:

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Macronutrients - Potassium

by Thomas D. Landis

Introduction

Potassium (K) makes up about 1.5% of the earth's crust but is never found in its elemental form due to its highly reactive nature. Its chemical symbol K comes from the German term for this element (kalium) which comes from the Arabic ("the ash"). Potassium is commonly known as "potash" because people used to burn wood in pots as the first step to making soap. The ashes were rinsed and the water was allowed to evaporate, leaving a residue of potassium salts which they called "pot ashes." These salts were then boiled with animal fat to produce soap.

Potassium is most commonly found in nature as potassium chloride as the result of evaporation of prehistoric lakes and seas including the Great Salt Lake in Utah and the Dead Sea in the Middle East. These brine reserves are now a commercial source of potash. Potassium is an integral part of soil minerals such as micas and feldspars (Figure 1), and their derivative clay soils typically contain from 2% to 4% potassium, as do young volcanic soils. Highly weathered sands and organic soils, on the other hand, are typically low in potassium.

Role in Plant Nutrition

Potassium is the most abundant monovalent cation (K⁺)



Figure 1 - Potassium slowly becomes available to crops from the weathering of clay minerals but is often deficient in sandy nursery soils. Because of this low availability and because it is so readily leached, growers must supply the potassium needs of their crops as fertilizer (modified from Havlin and others 1999).

in plant tissue. Of the three "fertilizer elements" (N-P-K), the average concentration of potassium in plant tissue is only slightly below nitrogen (Table 1). Potassium is very mobile in plants and young, metabolically active tissues like new leaves have a much higher concentration than mature leaves or structural tissues.

Availability in the soil and growing media There are 4 classes of soil potassium in soils (Figure 1):

1. Minerals which are very resistant to weathering and release negligible potassium to crops.

Table 1—Potassium is the Second Most Common Mineral Nutrient in Seedling Tissue					
Element	Symbol	% of Total Mineral Nutrients in Plants	Average Concentration in Seedling Tissue %		
			Bareroot	Container	
Nitrogen	Ν	37.5	1.20 to 2.0	1.30 to 3.50	
Potassium	K	25.0	0.40 to 0.80	0.70 to 2.50	
Calcium	Ca	12.5	0.20 to 0.50	0.30 to 1.0	
Magnesium	Mg	5.0	0.10 to 0.15	0.10 to 0.30	
Phosphorus	Р	5.0	0.10 to 0.20	0.20 to 0.60	
Sulfur	S	2.5	0.10 to 0.20	0.10 to 0.20	

2. Slowly available potassium is held tightly between clay minerals and is released only slowly to plants. Vermiculite contains a significant amount of potassium, but potassium fixation can be a concern in clay soils.

3. Exchangeable potassium comprises about 1% of the total soil potassium and is available for plant uptake by the cation exchange process. Because clay soils and growing media components like *Sphagnum* peat moss and vermiculite have high cation exchange capacities (CEC), they retain potassium against leaching.

4. Solution potassium, about 0.5% of the total, is most available to plants but also susceptible to losses from leaching.

Most of the potassium in soils moves very slowly by diffusion and so, without supplemental fertilization, levels may be low in the rhizosphere. This is particular true of sandy nursery soils and organic growing media. Tropical soils, especially those containing kaolinite clays, are typically very low in potassium and heavy rains just exacerbate the problem.

Uptake by plants. Potassium is taken-up by plants as a cation (K^+) and is the only cation that can be transported against an electrochemical gradient into plant cells ("active" uptake). Once inside the roots, potassium is very mobile in plants through the phloem and towards the meristems and young leaves. In the case of a local deficiency, potassium can be quickly transported both acropetally (up) and basipetally (down) which ensures that young metabolically active leaves have enough for their high metabolism and cell growth.

Influences on Plant Growth and Development

Potassium is the only macronutrient that is not a component of any plant structure. However, it is considered the most important cation in plant physiology due to its many important functions:

Seed priming. The first effect that potassium has on the growth of native plants is on seed germination. Soaking seeds of some hard-to-germinate species in potassium hydroxide for only 1 minute was shown to increase germination and seedling emergence. This effect varies significantly by species, however, so tests should be done before using potassium hydroxide operationally.

Regulation of water relations. Because potassium can be accumulated in actively growing tissues like root tips, it enhances uptake and retention of water. This ability is the reason for its most important function - the opening and closing of stomata. During daylight, and using energy from respiration, potassium ions are "pumped" into the guard cells on either side of the stomata. The resulting increase in turgor pressure causes the stomata to open. During dark periods, the potassium is "pumped" out of the guards cells and they lose turgor and close. Also, when the plant is under moisture stress, the turgor pressure in the guard cells drops and the stomata shut down. The effect of the potassium concentration on the transpiration rate of Douglas-fir needles is shown in Figure 2A.

So, in summation, plants that are deficient in potassium have impaired stomatal activity which affects 3 important physiological processes;1) carbon dioxide



Figure 2 - Because potassium controls stomatal functioning, it has a critical effect on many important physiological process including transpiration (A), and photosynthesis and respiration (B).

uptake for photosynthesis; 2) transpiration which affects turgidity and growth; and 3) oxygen uptake for respiration (Figure 2B).

Energy relations. Plants need potassium for the production of adenosine triphosphate (ATP) which allows energy captured in photosynthesis to fuel all other physiological processes. Numerous studies have shown that potassium stimulates translocation of the products of photosynthesis. While this could be an indirect result of more carbon dioxide uptake, it appears that it is due to synthesis of the energy compound ATP.

Enzyme activation. Potassium has been shown to stimulate the activation of 50 to 80 different enzymes. These enzymes control a wide variety of physiological processes such as synthesis of starch from glucose, allowing energy to be stored for subsequent respiration and growth. Potassium is also involved in several steps in protein synthesis and a deficiency results in an accumulation of soluble nitrogen products.

Cation-anion balance. Potassium is the major cation for balancing anions in plant tissue. For example, potassium is the predominant cation to balance the negative charge of nitrate in long distance transport in the xylem.

Hardening plants against frost and pest resistance. Traditional nursery wisdom has been to apply extra potassium as part of the hardening process. For example, potassium is considered to help plants resist stresses such as cold injury or attack by fungi or insects. This perception may be due to the fact that potassium increases the strength of straw in grasses and helps prevent lodging. Potassium has also been shown to reduce the severity of several diseases of agricultural plants. However, the evidence is much weaker for woody plants. We know that high nitrogen levels stimulate rapid "soft" growth whereas adequate potassium counteracts this by promoting firmer tissues. In addition, potassium, calcium, and magnesium have been shown to increase waxes on the surface of leaves and needles which would help with the hardening process. A thicker cuticle also increases the resistance to insect feeding and penetration by fungi.

The relationship between potassium and frost hardiness has a long history and there are even potassium sprays available for "plant frost protection." However, the ability of high potassium levels to increase the frost resistance of trees was tested on Scots pine (*Pinus sylvestris*) and European birch (*Betula pendula*) seedlings. These experiments actually revealed an inverse relationship: foliage with higher levels of potassium was actually less resistant to cold injury. Because potassium has a major effect on water relations, it may be that adequate potassium fertilization helps prevent overwinter desiccation. Nevertheless, the practice of increasing potassium fertilization during the hardening phase appears to be groundless.

Monitoring Potassium

Because of its many effects on plant physiology and growth and the fact it must be supplied by fertilization, it only makes sense to carefully monitor potassium nutrition in nurseries.

Deficiency symptoms. Foliage that is deficient in potassium looks like it has been burned on the leaf margins or the tips of needles-a condition known as "scorch." Since potassium is so mobile in plants, these deficiency symptoms first occur in older tissues. The relationship between potassium and nitrogen has been mentioned earlier but overfertilization with nitrogen has been shown to induce potassium deficiency in bareroot spruce seedlings in two different studies. Typical tip burn symptoms were shown to occur when foliar potassium concentration was below 0.35% and subsequent potassium treatments did not always alleviate the problem. Experiments have shown that plant growth rate slows considerably ("hidden hunger") before symptoms develop so growers should use other means of monitoring potassium.

Toxicity symptoms. Potassium has no direct toxicity effects but overfertilization can interfere with the uptake of calcium and magnesium, especially the latter. In studies with radiata pine (*Pinus radiata*), the cause of magnesium deficiency was determined to be related to high soil levels of potassium.

Soil tests. Soil analysis is routinely run for potassium and many fertilizer recommendations are made on the basis of such analyses. Because of the many different forms of soil potassium (Figure 1), however, there is no standard extraction method that truly reflects available potassium. And, because of the wide variation in soil properties, it is difficult to develop a potassium test that is good for all soils. Therefore, the ability of soil tests to predict the potassium needs of crops is questionable.

Artificial growing media tests. Although there are several techniques available, forest and conservation nurseries do not typically analyze their growing media for potassium. Instead, most growers have developed their fertilization regimes based on seedling growth response or foliar tests (Figure 3).



Figure 3 - Although soil tests have been traditionally used, growth trials (A) or tissue tests (B) are the most reliable way to monitor potassium.

precisely measure how much potassium seedlings have taken-up, and are quick and inexpensive. However, nutrition experiments have shown that foliar potassium concentration can vary considerably between plant species. Apparently, some species like Douglas-fir have a significantly higher demand for potassium than others, like white spruce (Figure 3B). The extreme mobility of potassium within plants is another consideration. Studies with agricultural crops suggest that the ratio of potassium levels between young and old tissue was the best indicator of true potassium status.

Considering the limitations of testing soils or growing media, chemical analysis of plant tissue is one the most useful ways to monitor potassium in forest and native plant nurseries. Typical ranges for potassium in the foliage of bareroot and container plants are given in Table 1. Container plants accumulate higher potassium levels, probably due to higher fertilization rates and greater availability in artificial growing media.

Potassium Management

Plants usually absorb most of their potassium during the active growth and metabolism in the first half of the growing season. Growth curves show a steadily increasing amount of potassium in plant tissue (Figure 3B) so growers should make sure that potassium fertilizers are applied early and then regularly during the entire growing season. This is particularly important for sandy nursery soils where potassium leaching can be significant. Because of its limited availability in nursery soils and growing media, essentially all of the potassium needed for the growth of nursery crops should be supplied by fertilization.

Potassium fertilizers. Fortunately, there are many potassium fertilizers and all of them are soluble (Table

Seedling tissue analysis. Tissue tests have the ability to 2). Remember that the legal analysis of multinutrient fertilizers will list potassium as % K₂O- to convert to % K, multiply by 0.83. When formulating or applying fertilizers, the nitrogen-to-potassium ratio should be considered. As we have discussed, high nitrogen fertilization can induce potassium deficiency so try to maintain a ratio of 2 parts N to 1 part K. Note that Sul-Po-Mag (K-Mag) is a naturally occurring mineral and therefore is popular with organic growers. Because of its chemical make -up, it serves as a natural slow release source of potassium, magnesium, and sulfur.

> Fertilization in Bareroot Nurseries. The potential fixation of potassium in field soils and its high leaching potential are challenges to fertilization in bareroot nurseries. Two materials are most commonly used: potassium sulfate and potassium chloride. Although potassium sulfate is more expensive and has lower analysis (Table 2), it is more popular with growers because of concerns about the excess chloride. Potassium fertilizers are usually broadcast and incorporated into the seedbeds prior to sowing but banding may be recommended in sandy soils with high leaching potential. Based on crop response or foliar tests, another top-dressing may be required in midseason. As mentioned earlier, high nitrogen fertilization can induce potassium deficiencies so this should be monitored.

> Fertilization in Container Nurseries. Because all granular potassium fertilizers are highly soluble (Table 2), there are many choices when formulating custom fertilizer solutions. Potassium carbonate has appeal because it is a single-nutrient source but, because the carbonate ion also increases solution pH, extra acid may be required. Fertigation solutions typically contain from 150 to 200 ppm potassium. This may be excessive, however, as growth trials with container white spruce seedlings in growing media showed that between 50 and

Table 2—Types of Potassium Fertilizers Commonly Used in Forest and Native Plant Nurseries						
Fertilizer	Nutrient Analysis			Nursery	Application	Remarks
	% N	%P ₂ O ₅	% K ₂ O	Туре	Method	
Potassium chloride	0	0	60 to 62	BR or C	Top dressing or fertigation	Water soluble with moderate salt index
Potassium sulfate	0	0	50 to 62	BR or C	Top dressing or fertigation	Water soluble with low salt index; also contains sulfur
Potassium magnesium sulfate "Sul-Po-Mag" or "K-Mag"	0	0	22	BR or C	Top dressing or fertigation	Naturally occurring mineral so good for organic growers
Potassium phosphates	0	41 to 51	35 to 54	BR or C	Fertigation	Water soluble with low salt index
Potassium nitrate	13	0	44	С	Fertigation or foliar	Water soluble with low salt index
Potassium carbonate	0	0	56	С	Fertigation	Single nutrient fertilizer but raises pH of solution
Plant Products 20-20-20	20	20	20	С	Fertigation	Completely soluble with micronutrients
Scotts Excel Cal-Mag 15-5-15	15	5	15	С	Fertigation	Completely soluble, with calcium, magnesium, sulfur and micronutrients
Scotts Peters Plant Starter 9-45-15	9	45	15	С	Fertigation	Completely soluble, with high P for young plants
Scotts Peters Foliar Feed 27-15-12	27	15	12	С	Fertigation	Completely soluble
Controlled-Release Formulations						
Osmocote Fast Start; 8 to 9 month release	18	6	12	С	Incorporation	Polymeric resin- coated prills
Osmocote High N; 8 to 9 month release	24	4	8	С	Incorporation	Polymeric resin- coated prills
Polyon 25-4-12; 8 to 9 month release	25	4	12	С	Incorporation	Polyurethane-coated prills
Nutricote 270; 8 to 9 month release	18	6	8	С	Incorporation	Thermoplastic resin- coated prills

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100 ppm potassium gave the best response (Figure 3A). This is probably due to the fact the potassium ions can be held against leaching in these high CEC growing media and so less needs to be added in each fertigation. Being a type of clay mineral, vermiculite also contains a significant amount of potassium.

Foliar Fertilization. Because soil applications are more economical and provide a greater amount of available potassium, foliar fertilization is not common. If needed, however, potassium nitrate is the fertilizer of choice.

Environmental Affects of Overfertilization.

Although potassium can quickly leach from nursery soils or growing media, there are no serious environmental consequences of potassium fertilization. Nevertheless, growers should carefully plan and monitor potassium fertilization in their nurseries.

Conclusions and Recommendations

The availability of potassium in sandy nursery soils and organic growing media is naturally low. It is actively absorbed by roots and moves freely throughout the plant. Potassium is the only macronutrient that is not a component of any plant structure but has many important metabolic functions. Potassium is needed for numerous enzymatic reactions including the synthesis of starches and proteins. Perhaps its most important role is the opening and closing of stomata which controls water loss, carbon dioxide uptake, and gas exchange from respiration. Potassium has traditionally been thought to increase plant hardiness and resistance to pests but this has not been proven experimentally, at least for conifers.

Because of its critical metabolic importance, potassium should be carefully monitored in nurseries, and plant tissue analysis is most practical. Nurseries should plan to supply most of the potassium needed for seedling growth with fertilization. Bareroot nurseries should apply potassium as a preplant incorporation and then as top dressings. Because potassium fertilizers are so soluble, container growers can maintain adequate potassium throughout the growing season. High nitrogen fertilization can induce potassium deficiency so growers should consider a nitrogen to potassium ratio of 2 parts N to 1 part K. Unlike nitrogen and phosphorus, potassium fertilization does not have any potentially negative environmental consequences.

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Maybe This Will Work for Volume Seven of Container Tree Nursery Manual—TDL

Seedling Quality Tests: Chlorophyll Fluorescence

By Gary Ritchie and Thomas D. Landis

Introduction

So far in this series we have discussed the most commonly-used seedling quality tests: root growth potential, cold hardiness, and stress resistance. In this issue, we're going to talk about one of the newest testchlorophyll fluorescence (CF). The technology for measuring CF has been in place for over 50 years but has been applied to tree seedling physiology only since the late 1980s. In early trials, forestry researchers found CF to be an important research tool holding promise of many potential applications: assessing the effectiveness of irrigation and fertilization, determining the lifting window, and evaluating seedling vigor after storage. They concluded that CF was a "simple, rapid, reliable and non-destructive method of evaluating seedling physiological status during the nursery production cycle" (Vidaver and others 1988).

In the intervening years, CF has not lived up to all those early expectations and has seen very limited use as an operational seedling quality test. However, since CF terminology has been showing up in the nursery



Figure 1 - Only a small amount of photosynthetically active radiation is absorbed by leaves and actually used (quenched) by photosynthesis. The surplus energy is quenched as heat or in fluorescence.

literature and at meetings, we thought that both growers and seedling users should have a basic understanding of CF and what it can and cannot do. Before we proceed with a discussion of the test itself, let's begin with a brief review of how light energy is processed by leaves.

What is chlorophyll fluorescence? When solar radiation hits a leaf, some light energy is reflected, some is transmitted through the leaf tissue, and some energy is absorbed. Plants absorb much more light energy than they need for photosynthesis. In fact, less than 20% of the photosynthetically active radiation absorbed by a leaf is actually used for photosynthesis. The blue and red wavelengths are absorbed by chlorophyll and other pigments, but the green wavelengths are reflected giving living plants their green color. To get rid of all that excess energy, plants have developed ingenious processes known as "quenching." Three types of quenching are known. The first is photochemical quenching (qP), which refers to the energy that is used in photosynthesis. Nonphotochemical quenching (qN), is the energy that is dissipated as sensible heat, and fluorescence quenching (qF) is that which is emitted as fluorescence. The largest amount of the absorbed energy is dissipated as sensible heat (qN), and a much smaller amount is given off as fluorescent light (qF) (Figure 1). Measuring qF is the basis for the chlorophyll fluorescence test.

At high light levels, these quenching mechanisms may become overloaded, the surplus energy driving a biochemical process called the "Moehler reaction." This generates free radicals, mainly oxygen species, that are toxic to the plant. To protect themselves, leaves contain scavenging molecules that mop the free radicals up and render them harmless. The carotenoid pigments, for example, serve this function. However, when light intensity is so high that these scavenging systems are overwhelmed, photodamage occurs (Demig-Adams and Adams 1992). This often appears as leaf "scorching" and is common in nursery plants that have been moved too quickly from shade to full sun. In Pacific Northwest nurseries, we sometimes see these scorch symptoms on western hemlock seedlings if they are suddenly exposed to intense sunlight in early spring.

Photosynthesis and variable chlorophyll fluorescence. Photosynthesis consists of three sequential processes (Vidaver and others 1991):

1. Light harvesting—light energy is absorbed by lightsensitive pigments including chlorophyll in the leaves.

2. Photochemistry—this absorbed light energy is converted into chemical energy; and

3. Biochemistry—chemical energy is used to drive Calvin cycle reactions that convert atmospheric carbon into simple sugars.

CF probes the process of photochemistry. Because all three processes are intimately interconnected, a perturbation to one part of one process affects the entire set of reactions. These changes in the photosynthetic process are reflected in variations in the amount and rate of CF.

Let's look at these photochemical reactions in a little more detail. The light energy captured by the leaf pigments is funneled into two reaction centers called Photosystem I (PSI) and Photosystem II (PSII). Two water molecules are split at PSII and their electrons are accepted by an acceptor molecule which passes them on to chlorophyll-a, raising it to an excited state (the oxygen is vented to the atmosphere sustaining all life on Earth). The electrons are passed onto two acceptor pools, QA and QB. From here, they flow through a series of acceptor molecules to the PSI reaction center where the process more or less repeats. This energy transfer leads to the generation of ATP and ultimately the reduction of NADP⁺ to NADPH. The energy contained in ATP and the reducing power of NADPH contribute to the fixation of CO₂ molecules and their ultimate conversion to simple sugars in the Calvin Cycle.

This process generates fluorescence (CF), which emanates entirely from chlorophyll-a in PSII (Kraus and Weis 1991) as it decays to its ground state. This occurs when the Q_A pool is fully reduced or when the electron transport pathway is backed up. In other words, when more excited electrons are produced than can be processed, they fall back to their ground state, releasing their excitation energy in the process. This weak fluorescence emission is not visible to the naked eye but can easily be detected by an instrument called a chlorophyll fluorometer. The fluorescence emission, and forms the basis of the CF test.

How Chlorophyll Fluorescence is Measured

A German plant biochemist named Hans Kautsky first observed chlorophyll fluorescence in the late 1920s (Govindje 1995). Kautsky darkened a sample of photosynthetic tissue then excited it with a brief, intense light pulse. He noted that an emission of fluorescent light followed the light pulse. Surprisingly, he found that in healthy tissue the emission disappeared within a few minutes, but when the tissue was killed with cyanide or by freezing, the fluorescence emission persisted for a longer time. It has since been determined that poisoning

or freezing leaf tissue disables the electron flow pathway causing excited electrons to fall back to their ground state, giving off measurable fluorescence. In healthy tissue, by contrast, more electrons are quenched in the electron transport pathway leaving fewer to decay yielding lower fluorescence emissions.



Figure 2 - A Kautsky fluorometer consists of a light source, two filters, a photosensor, a microprocessor, and a fiber-optic cable that attaches to a leaf. Instructions are sent to the fluorometer from a laptop computer.

Kautsky fluorometers. Kautsky's observation led to the development of instruments called "Kautsky" fluorometers, which became a staple of photosynthesis research for many decades. While the initial machines were large and suitable only for laboratory work, Kautsky fluorometers have now evolved into small, affordable, portable, and user-friendly devices. They contain a light source, two sets of filters, a microprocessor, and a photosensor, and they typically interface with a laptop computer (Figure 2). The light source sends a pulse of photosynthetically-active light through a fiber-optic cable to the leaf surface where it activates chlorophyll-a in Photosystem II. The chlorophyll-a emission returns back through the cable and passes through a second filter that transmits fluorescent light to the photosensor, which records the emission. The process is controlled by the microprocessor which is programmed using the laptop computer.

The CF measurement process begins with "dark adapting" the leaf for about 20 minutes. This ensures that all chlorophyll is in a ground state, the Q_A pool is empty, and the electron transport pathway is clear before the light pulse is received. Following the light pulse, the Kautsky fluorometer generates a curve in which the intensity of the resulting fluorescence emission is plotted over time (Figure 3). In the Kautsky curve, F_o is the fluorescence that emanates from the light harvesting pigments in the leaf—not from Photosystem II. F_m is the maximum fluorescence, and F_v is the variable fluorescence coming from PSII. This curve has many diagnostic features, but the most useful is the ratio of F_v



Figure 3 - A typical quenching curve generated by a "Kautsky" fluorometer that occurs after a light pulse is delivered to a dark -adapted leaf. These curves are diagnostic because healthy and stressed plants differ in the amount and duration of their fluorescence. For example, the ratio of variable to maximum chlorophyll fluorescence (Fv/Fm) is a good indicator of photosynthetic efficiency (modified from Vidaver and others 1991).

to $F_m(F_v/F_m)$. This is called the optimal quantum yield and provides a direct estimate of the efficiency of the overall photosynthetic process (Genty et al 1989). F_v/F_m is the most often cited result of a CF measurement.

Pulse amplitude modulated fluorometers. A more recent development in fluorometry is an instrument called the Pulse Amplitude Modulated (PAM) Fluorometer (Schreiber et al 1995). After delivering an initial excitation light pulse, the PAM generates a rapid stream of high intensity saturating light pulses that overwhelm Q_A acceptor pools, thus canceling out photochemical quenching (qP). The fluorescence emission differences between these peaks and the fluorescence decay curve is, therefore, qN.

This powerful procedure enables measurement of the three different energy quenching components along with determination of overall process efficiency at several levels. One of these instruments, the PAM-2000, is manufactured by Heinz Walz in Germany (www.walz.com). PAMs have become an essential tool for seedling physiology research. A PAM-2000 run produces estimates of the following variables: optimal quantum yield (Fv/Fm), effective quantum yield (Y), photochemical quenching (qP), nonphotochemical quenching (qN), electron transport rate (ETR), and many

others. See Mohammed and others (1995) for the full list and a comparison of available fluorometers.

Use of CF in Seedling Quality Assessment

Dormancy. There have been attempts to use CF as an indicator of plant phenological condition or dormancy status, but we're not yet convinced that these studies are verifiable or repeatable.

Cold hardiness. The greatest value of CF currently is in detecting and assessing plant injury or stress, such as cold injury. Rather than assessing seedling response to low temperatures using visual, electrolytic, or other methods (see Ritchie and Landis 2003), the CF approach uses the response of the photosynthetic process as an index of cold injury (Figure 4). "Normal" conifer seedlings will typically have Fv/Fm values in a range between 0.70 to 0.83, or slightly lower in winter. When this value falls to < 0.60 following freezing it indicates that there has been significant damage to the photosynthetic process. Since 1994, the Seedling Quality Testing Laboratory at the Ontario Forest Research Institute has been using CF as one of four seedling quality tests. On another test with Rhododendron leaves, however, CT significantly overestimated frost resistance by 9 °F (5 °C) (Neuner and Buchner 1999).



Figure 4 - The chlorophyll fluorescence index (Fv/ Fm) was an accurate predictor of cold injury and highly correlated to visible needle damage to Douglas-fir seedlings (Modified from Perks and others 2004).

Outplanting performance. Some studies have attempted to correlate CF variables with outplanting performance. For example, measures of effective quantum yield predicted variations in survival and plant health of stored and non-stored Douglas-fir seedlings in an Irish nursery (Perks and others 2001). **Moisture stress.** CF, especially F_v/F_{m} , however, is not very sensitive to water stress. The pressure chamber offers a far more direct and useful technique for measuring this variable, and we will discuss this seedling quality assessment in the next FNN issue.

Other applications. The more powerful and versatile PAM fluorometers are capable of detecting very subtle stresses, such as those associated with certain nutrient deficiencies, foliar diseases, and cold storage. Often these stresses are not sufficient to cause reductions in Fv/Fm, but can be detected by examining changes in quenching coefficients. As stress begins to develop and the photosynthetic mechanism becomes disabled, plants resort more to nonphotochemical quenching to dissipate energy. This can be easily detected using a PAM fluorometer.

When CF was used to assess the quality of *Taxus* cuttings, the F_v/F_m index was found to be a poor indicator of propagation potential (Bruce and Rowe 1999). It also goes without saying that CF has little utility in direct studies of non-photosynthetic tissues, such as roots, although inference regarding root physiology can sometimes be drawn from measurements of photosynthetic efficiency.

Summary and Conclusions

Chlorophyll fluorescence (CF) is mainly a research tool but has potential to become a standard seedling quality test in the not too distant future. So, it is important for nursery and regeneration personnel to understand how CF works and how to interpret results.

CF provides a quantitative evaluation of the plant's photosynthetic apparatus—how efficient it is and how well it is working. It also provides insight into the plant's ability to dissipate excess light energy, which can be a sensitive indicator of certain types of stress (Kraus and Weis 1991).CF measures fluorescence emanated by electrons in Photosystem II that are decaying from a high energy state to low energy state. The pattern of these emissions can be interpreted as a barometer of the functioning of the photosynthetic mechanism.

Two types of fluorometers are available: Kautsky fluorometers and pulse amplitude modulated (PAM) fluorometers. The former are fairly limited to providing estimates of optimal quantum yield (F_v/F_m), which can be a very useful variable in studies of cold hardiness. PAM fluorometers are more expensive but far more versatile. They enable estimates of quenching coefficients, as well as of photosynthetic efficiency, and are capable of detecting stress at very low levels.

CF is most often used in assessment of cold damage. It also has applications in other areas involving photosynthetic efficiency such as nutrient deficiencies, disease, and so on.

CF is not directly useful for measuring plant moisture stress or for studies on non-photosynthetic tissues such as roots.

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How I Adapt Guest Author's Articles for FNN - TDL

Overwintering Container Plants without Refrigeration

By Thomas D. Landis

The type of overwinter storage system that you select will depend on the species that you are growing and the severity of winter conditions in your area of the country. Traditionally, container seedlings for reforestation have been stored under refrigeration but the demand for larger stock types has growers looking for other alternatives.

Open storage. In areas with freezing temperatures, open storage is the least expensive but most risky overwintering option. Select an area of the nursery that has some protection from the wind and where cold air will drain away. Utilize gravel and/or drainage tile to promote free drainage of rain or snow melt in the spring. Pack all containers together tightly and insulate the perimeter with straw bales or a berm of sawdust. With this perimeter insulation, the roots of the stored plants will be protected by the heat stored in the ground (Figure 1A).

Open storage is most successful in forested northern climates where adjacent trees create both shade and a windbreak, and continuous snow cover can be expected. If tree shelter isn't available, seedlings can be stored in narrow East-West oriented bays between vertical snowfence (Figure 1B). This orientation is critical with conifer stock to shade foliage and prevent desiccation. Snow is an ideal natural insulation for over-wintering container plants but complete and continuous snow cover is not always reliable. Some northern nurseries have had success with generating snow cover with snow making equipment (Figure 1C).

Structureless storage systems.

These are the simplest and least expensive ways to overwintering container stock. The term "structureless" means that plants are enclosed in a protective covering but without any mechanical support. Many different coverings have been used but the basic Thermal Mass In open storage, the thermal mass of soil and growing media protects sensitive roots from freezing. Therefore, the smaller the container size, the higher the risk of freezing injury.

principle is the same: to provide a protective, insulating layer of the stored plants. Clear plastic should never be used because it transmit sunlight so that temperatures







Figure 1 - Open storage can be effective when plants are blocked on the ground and surrounded by insulation (A). Plants should be protected from direct sun and wind by natural or artificial snowfences (B). Snow is an excellent insulator, and northern nurseries have augmented natural snowfall with snow-making equipment (C).

within the storage area can reach damaging levels. Because they reflect a majority of the sunlight, white or reflective coverings are preferred. All these coverings are eventually decomposed by direct sunlight so should be stored in a dry, dark location when not in use (Green and Fuchigami 1985).

White plastic sheeting - Single layer films are the most common covering in structureless systems, such as a 4mil white copolymer plastic sheeting (Figure 2A). White is preferred because it reflects sunlight and keeps temperatures from building-up under the covering. As mentioned in the last section, grouping the containers together on the ground takes advantage of the heat stored in the soil (Figure 1A).



Figure 2 - Container plants can be bunched together on the ground and covered with white plastic or Microfoam® sheeting (A). Supporting the cover with a hoop of PVC plastic pipe or heavy metal wire provides better protection (B-C).

White foam sheets and sheeting - Microfoam® is a breathable Styrofoam® -like material that is lightweight, reusable, and easily removed and stored. It is available in a variety of widths and thicknesses. Microfoam® prevents wide fluctuations in temperature, minimizes heat build up and keeps root zone to above 10 °F (-12 °C) if snow is present.

This system generally works well with hardy native species. The sheeting can be placed directly over the plants but works best if supported by wooden poles or hoops of PVC pipe or heavy metal wire (Figure 2B-C). Because Microfoam® is so lightweight, it needs to be secured well so that it does not rip or blow off the plants during windstorms. Typically, foam sheeting is secured around the edges with concrete blocks, wooden planks, or even a berm of sand. One layer of Microfoam® is reported to be insufficient in harsh climates without reliable snow cover such as northern Minnesota and

North Dakota. SM/ Perimate® is rigid sheet insulation panels that have been proven to protect conifer seedlings as well as refrigerated storage but with a significant cost savings.

Timing is Critical Any structureless storage system is only effective if it is applied after plants have developed sufficient hardiness and, most importantly, removed before plants lose dormancy in the spring.



Plastic bubble-



better insulation than regular plastic sheeting and is reported to be cheaper and more durable than Microfoam® sheets. Because it is relatively transparent however, sunlight can penetrate and cause premature warming.

Frost Fabrics - Woven and nonwoven landscape fabrics have been used for covering structureless storage systems. Because they are white in color they retard solar heating but allow infiltration of rain or snow melt and also allow stored plants to "breathe." Horticultural suppliers offer frost fabrics in a range of weights and thicknesses giving from 4 to 8 °F (2.2 to 4.4 °C) of thermal insulation. Arbor Pro is a felt-like material that has been used successfully for conifer storage in Canada.

Plastic film with layer of insulating material - In harsh, northern climates without reliable snow cover, some nurseries cover their container stock with a "sandwich" of straw or other insulating material between two layers of clear plastic sheeting. The clear plastic and straw create additional heat on clear, frigid days and provide more constant temperatures during the over-wintering period compared to other systems. The thermoblanket system consists of white plastic sheeting over a layer of Microfoam® proved effective for overwintering a variety of forbs. In general, the sandwich covers provide better insulation but cannot be removed or vented during periods of sunny warm winter weather.

Cold frames - A variety of different cold frames have been used for overwinter storage. In northern Alberta and Alaska, cold frames constructed of sideboards lined and topped with rigid Styrofoam® sheets have proven effective (Figure 3A). During warm periods in the winter or as soon as weather conditions permit in the spring, the top layer of insulation is removed so that plants can be irrigated (Figure 3B). Cold frames of wooden pallets, which are supported by cement blocks and covered by white plastic poly sheeting, are considered the most effective overwintering system for conifer seedlings at a nursery in eastern Canada.

Conclusions and Recommendations

The growing popularity of larger container stock types has made traditional refrigerated storage uneconomical, and growers are looking for alternatives. Open storage is an option where freezing temperatures are not frequent or in northern climates with predictable snow coverage. Many growers are looking to the various types of structureless storage as a low-cost but effective way to overwinter their stock. While these overwintering systems are effective at preventing desiccation, growers need to be especially vigilant during late winter when high temperature around plants can cause premature dehardening and even bud break. In many nurseries, more than one overwintering system is used to accommodate the requirements for different native plant species.

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Figure 3 - Cold frames of wood and rigid StyrofoamTM sheet insulation are used to overwinted container plants in northern climates (A). When weather conditions permit, the top layer of insulation is removed so that plants can be irrigated (B).

Horticultural Humor



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