## 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 test—chlorophyll 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 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.

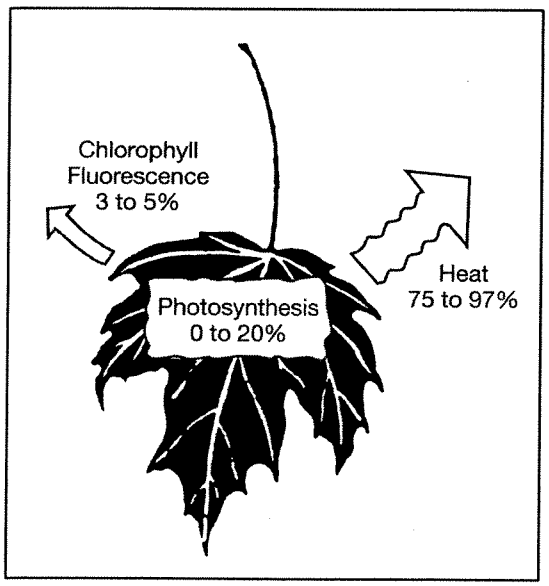


*Figure 1 - Only a small amount of photosynthetically active radiation is absorbed by leaves and actually used (quenched) by photosynthesis. The surplus energy is quenched as heat or in fluorescence.*

**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 light-sensitive pigments including chlorophyll in the leaves.

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



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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, $Q_A$ and $Q_B$. 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 $CO_2$ 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 fluorometer measures and quantifies the nature of this 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.

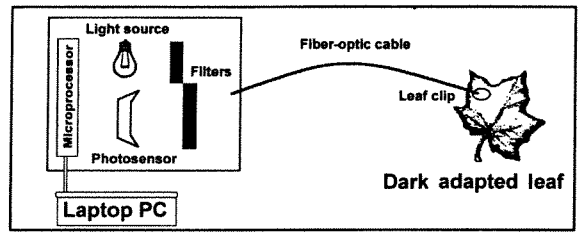


*Figure 2 - A Kautsky fluorometer consists of a light source, two filters, a photosensor, a microprocessor, and a fiber-optic cable that attaches to a leaf. Instructions are sent to the fluorometer from a laptop computer.*

**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 $F_v$

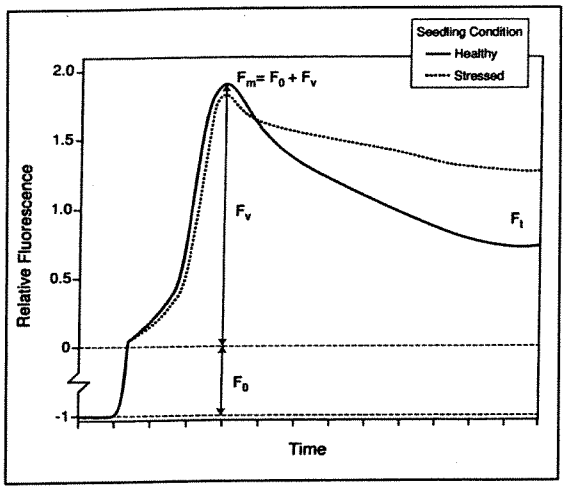


*Figure 3 - A typical quenching curve generated by a "Kautsky" fluorometer that occurs after a light pulse is delivered to a dark-adapted leaf. These curves are diagnostic because healthy and stressed plants differ in the amount and duration of their fluorescence. For example, the ratio of variable to 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).

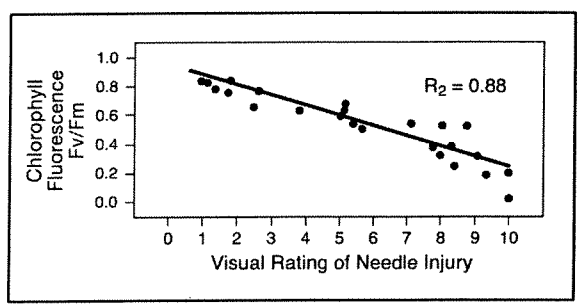


*Figure 4 - The chlorophyll fluorescence index (Fv/Fm) was an accurate predictor of cold injury and highly correlated to visible needle damage to Douglas-fir seedlings (Modified from Perks and others 2004).*

**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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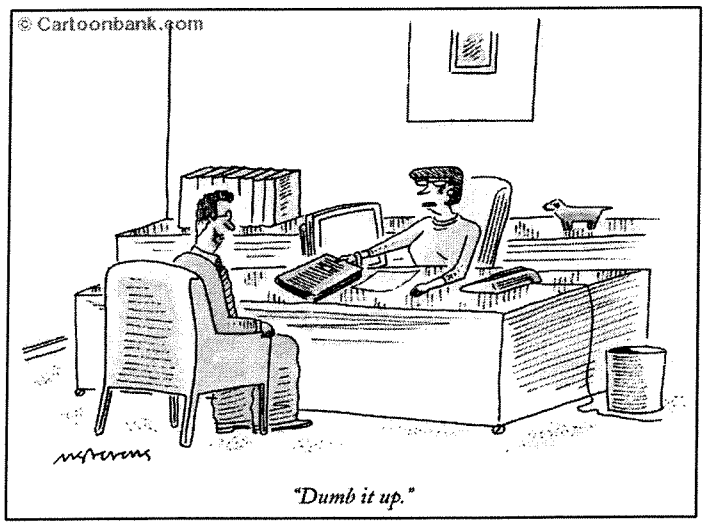


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