UTILIZATION OF CHLOROPHYLL FLUORESCENCE IN STORAGE AND VEGETATIVE PROPAGATION OF *TAXUS*

By

Sarah Eleni Bruce

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

1999

ABSTRACT

UTILIZATION OF CHLOROPHYLL FLUORESCENCE IN STORAGE AND VEGETATIVE PROPAGATION OF *TAXUS*

By

Sarah Eleni Bruce

Propagation failures in *Taxus* are often attributed to cutting collection from stock plants of poor quality. If a quick, reliable method of determining potential rooting of cuttings based on the condition of a specific stock plant was available for propagators, rooting success could be predicted prior to an investment in time, labor, and resources. These studies examined chlorophyll fluorescence (F_v/F_m) as a potential tool for stock plant selection, assessment of storage conditions, and measurement of stress over the course of propagation. Ten cultivars of *Taxus xmedia* (*Taxus baccata* L. x *T. cuspidata* Sieb. & Zucc.) were used: Brownii, Dark Green Pyramidalis, Dark Green Spreader, Densiformis, Densiformis Gem, Hicksii, L.C. Bobbink, Runyan, Tauntoni, and Wardii. Storage condition treatments consisted of desiccation (low, medium, high), duration (34, 70, 107 days), and temperature (-30, -2.5, 0, 2.5, 5, 10, and 20 °C). Cultivars differed in F_v/F_m initially, as well as over time. Correlations were not found between initial stock plant F_v/F_m and rooting percentage, root number, root dry weight, or root length, indicating that F_v/F_m is not a reliable indicator of stock plant propagation potential. Short storage duration at -2.5 to 2.5 °C was found to be ideal. F_v/F_m could detect substandard storage conditions only at temperature and desiccation extremes.

ACKNOWLEDGMENTS

I am thankful for the guidance and support of my major advisor, Brad Rowe. Mike Corbett, Tom Todor, and others at Zelenka Nursery were extremely helpful sharing their experience in propagation, and generous with their time and resources. I would also like to thank my committee members, Jim Flore and Don Dickmann, for their time and helpful criticisms. Lastly, I thank Emily Clough for her friendship, support, and advice.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

CHAPTER TWO

LIST OF FIGURES

FIGURE PAGE

CHAPTER TWO

SYMBOLS AND ABBREVIATIONS

F_o	.....	initial dark-adapted chlorophyll fluorescence
F_m	.....	maximum chlorophyll fluorescence
F_s	.....	stable chlorophyll fluorescence
F_v	.....	variable chlorophyll fluorescence (Fm – Fo)
PSII	.....	photosystem II
PSI	.....	photosystem I
IBA	.....	Indole – 3 – butyric acid
NAA	.....	1 Naphthalene acetic acid
MSU	.....	Michigan State University

LITERATURE REVIEW

Chlorophyll Fluorescence and the Propagation of *Taxus*

CHLOROPHYLL FLUORESCENCE

Chlorophyll fluorescence is a product of the photosynthetic process. It is energy that is re-emitted from all green plants. In tracing the path of that energy, we begin with sunlight, the energy's origin.

As light strikes a leaf, it travels through cellular layers to the chloroplasts, cellular organelles which house chlorophyll, the cell's light-energy absorbing pigment molecules. Blue (~420 nm) and red (~660 nm) light absorption occurs across the thylakoid membranes of a chloroplast where antennae chlorophyll molecules are arranged in arrays of several hundred each. These array chlorophyll molecules funnel energy, via resonance energy transfer, to a photochemically active chlorophyll molecule in the center of the array. This central pigment molecule is actually a chlorophyll a dimer situated within a specialized protein complex. In Photosystem II, the first light absorption mechanism in photosynthesis, it is called P_{680}, the '680' referring to the wavelength of light at or above which it absorbs. Light energy transferred to P_{680} raises the molecule's energy level to an excited state. At this point the collected energy faces four possible fates: energy transduction, resonance energy transfer, loss as heat, or loss as light.

Energy transduction is the process that enables the energy in the excited P_{680} molecule to be used for the biochemistry of photosynthesis. The excitement energy collected in P_{680} is passed down an electron transport chain in the form of an electron. The first step in this chain is pheophytin, a molecule similar to chlorophyll a. From pheophytin the electron is passed through a series of at

least two plastoquinones, Q_A and Q_B, which are lipid soluble and thus transfer the electron across the thylakoid membrane to an iron-sulfur complex which releases the electron into a plastocyanin pool in the thylakoid lumen space (to the inside of the membrane). The now oxidized pigment, P_{680}, is quickly reduced with an electron from the D1 protein which originates in the splitting of water (Krause and Weis, 1991). Photosystem I, the secondary light absorbing step of photosynthesis, draws its excitement electrons from the plastocyanin pool. Therefore, PSI uses energy transferred from PSII in addition to light absorbed by its reaction center pigment, P_{700}. From PSI, energy travels to the photophosphorylation processes of photosynthesis and the production of NADPH, which eventually leads to glucose production.

PSII has been shown to consist of two types, $PSII_\alpha$ and $PSII_\beta$, which differ in terms of antennae array size and photosynthetic capacity, $PSII_\beta$ being smaller and having lower activity levels (Holzwarth, 1988). Chlorophyll Fluorescence is therefore largely a result of $PSII_\alpha$ centers. Melis et al. (1988) suggest that these forms of PSII may simply reflect a photocenter's stage of maturity in an assembly growth process.

About 90% of the energy absorbed by P_{680} is used for photosynthesis via the electron transport processes reviewed above (Bjorkman and Demmig, 1987). The remainder is 'lost' in a number of ways. Some is transferred to non-photochemically active molecules (resonance energy transfer). Some is lost as heat. Some is lost as re-emitted light, termed chlorophyll fluorescence. It is this light, emitted at a slightly longer wavelength than absorbed (~690 nm, red and

far-red light) due to heat dissipation (termed "stokes shift"), that we measure. At physiological temperatures, almost all fluorescence is emitted from chlorophyll a molecules associated with PSII since chlorophyll b molecules transfer their excitement energy to chlorophyll a molecules (Lichtenthaler, 1988b). The exact source of re-emittance -- P_{680}, array chlorophylls, accessory pigments, etc., remains unknown (Schreiber and Bilger, 1993), although a general consensus has been formed that it is likely the array chlorophylls which are responsible (Lichtenthaler, 1988a).

CHLOROPHYLL FLUORESCENCE IMPLICATIONS

The amount of chlorophyll fluorescence emitted from a particular sample of photosynthetically-active tissue is dependent on the light energy utilization potential of that tissue. At best, chlorophyll fluorescence represents only 3 - 5% of excitation energy *in vivo* (Coombs et al., 1985; Lichtenthaler, 1988a estimates it at 0.5 - 3%). *In vitro*, up to 30% of the energy has been found to fluoresce (Coombs et al., 1985). Chlorophyll fluorescence can be considered a product of excess light energy –that light energy that exceeds the absorption potential of the electron transport process of PSII. This makes chlorophyll fluorescence levels indicative of the energy processing potential of PSII.

Photosystem II, itself, is an indirect indicator of the condition of the photosynthetic apparatus components at large (Georgieva and Yordanov, 1993). Being the primary light absorption process in photosynthesis and lying across thylakoid membranes, a region sensitive to environmental stresses, PSII electron

transport is believed to play a key role in the response of leaf photosynthesis to environmental perturbations. Chlorophyll fluorescence measurements have been have been used extensively in stress physiology studies. Chlorophyll fluorescence has been found to show a broad inverse relationship to photosynthetic carbon assimilation (Kautsky and Frank, 1943 in Lichtenthaler, 1990) and reflects the quantum yield of electron transport in PSII (Genty et al., 1989; Adams et al., 1990).

A CLOSER LOOK AT CHLOROPHYLL FLUORESCENCE MEASUREMENTS

Historically, the chlorophyll fluorescence signal pattern has been difficult to interpret. In 1977 Lavorell and Etienne described it as being "rich and ambiguous". Today we have a better understanding of the signal, achieved through a very broad base of research. However, many different measurements are used, a tribute to the difficulty of making point comparisons from fluctuating signals.

The fluorescence induction signal was first described by Kautsky in 1931 and is known as the Kautsky Effect. Depending on degree of resolution, the signal is referred to as "complex" or "simple" fluorescence kinetics. Complex fluorescence kinetics involve several minor peaks and dips in the fluorescence signal that remain unexamined in the simple fluorescence induction curve described here (for more information see Papageorgiou, 1975). In measurement, leaves are dark adapted (kept in darkness) for a certain amount of time (usually under 30 minutes) depending on the species, until the fluorescence

level is brought down to a minimum operating level, F_o. Then the leaf is brightly illuminated, sufficiently to saturate PSII, and the level of chlorophyll fluorescence immediately rises to a peak level, F_m. This "fast rise" in chlorophyll fluorescence reflects the saturation of the initial Q_A electron acceptors in the electron transport chain of PSII (Krause and Weis, 1991; Coombs et al., 1985). It takes a minimum of 200 ms for F_m to be reached (Krause and Weis, 1991), with times usually within the 300 – 400 ms range (Lichtenthaler, 1988b). Then as electron transport begins, the energy is transported to proceeding electron carriers (Q_B, plastocyanins...) and the initial electron acceptors become available carriers of energy from P_{680} again. In this way, energy moves through PSII. Chlorophyll fluorescence is reduced from F_m and, over a period of minutes, reaches a steady state fluorescence level, F_s, close to the original F_o level. This reduction of the chlorophyll fluorescence level by photochemical processes, mainly the re-oxidation of Q_A, is termed "photochemical quenching".

Chlorophyll Fluorescence Quenching is a broad title which refers to a number of processes that lower the fluorescence level from the maximum, F_m. This includes the photochemical quenching mentioned above, but also a number of non-photochemical quenching means. One of these is energy-dependent quenching, the major non-photochemical quenching mechanism (Lichtenthaler 1988a). It is a little understood process whereby the pH gradient caused by light-driven transportation of protons across the thylakoid membrane causes a reduction in fluorescence (Krause and Weis, 1991). Lichtenthaler (1988a) speculates that an increase in the rate constant of thermal deactivation of PSII

may have occurred, or structural changes that lower PSII's efficiency. Energy dissipation switches away from fluorescence and tends to be lost as heat (Coombs et al., 1985). Another type of quenching is caused by a state transition in the P_{680} photocenter due to phosphorylation (Schreiber and Bilger, 1993). This may serve to balance excitation energy distribution between the two photo systems since PSII excitation is reduced relative to PSI (Lichtenthaler, 1988a).

Photoinhibition has been the most studied of the non-photochemical quenching mechanisms. It is the increased deactivation of reaction centers, P_{680} and P_{700}, as the result of bright illumination. Two processes are involved: a process of PSII reaction center deactivation and repair involving the D1 protein, and avoidance of over-excitation by increased thermal energy dissipation, probably via the xanthophyll cycle (Long et al., 1994; Krause et al., 1990). The xanthophyll cycle is a quenching mechanism that acts as the major initial response to light that exceeds PSII photochemical capacity (reviewed in: Long et al., 1994). Diepoxide violaxanthin is converted to epoxide-free zeaxanthin, which distributes excess light energy as heat in antennae chlorophyll molecules. The conversion is gradually reversed in darkness or low light. Photoinhibition caused by excessive photon flux densities is a main cause for reduction of F_v/F_m under natural conditions (Long et al., 1994). Schreiber suggests that energy dependent quenching may actually be a mechanism to deal with excess light so as to avoid photoinhibition (Krause and Weis, 1991). However, Huner et al., (1993) argue that photoinhibition should be viewed as "the capacity of plants to adjust photosynthetically to the prevailing environmental conditions" rather than an

injury.

Although early research tended to focus on using chlorophyll fluorescence to study photochemical quenching processes, now it is the non-photochemical processes that receive the most attention. Lichtenthaler (1990) points out that it is mostly non-photochemical quenching and not photochemical quenching that is affected by environmental stress.

The values used to describe chlorophyll fluorescence, and how they are measured, have varied widely according to researcher, field, and precedent. This makes comparisons between studies often difficult. An attempt at a nomenclatural key has been published by van Kooten and Snel (1990), but they caution that it is too early for rigorous definitions. Confounding issues is the sensitivity of PSII to varying environmental conditions and their interactions. For instance, the upper and lower surfaces of a leaf often show different chlorophyll fluorescence levels, which may, in part, be caused by a much denser arrangement of chlorophyll on the upper surface leading to much re-absorption of emitted fluorescence as compared with the underside of the leaf (Rinderle and Lichtenthaler, 1988).

Measurements of the fast rise induction kinetics are perhaps the most common, probably due to the ease and speed with which data can be taken. F_o and F_m have frequently been used, often in conjunction with ratios derived from them. F_o tends to be affected by stresses that cause configuration changes in thylakoid structure, such as heat stress (Hansatech manual). F_m is most affected by non-photochemical quenching mechanisms. The variable fluorescence, F_v, is

calculated by subtracting F_o from F_m. It represents the initial light absorption ability of PSII, the number of quinone-type electron acceptors that are available in a dark-adapted state. Commonly the ratio F_v/ F_m is used. It has been shown to be a reliable indicator of the quantum yield of PSII (Adams et al., 1990), provided that the sample is dark-adapted and therefor operating at minimal fluorescence levels initially, and that the light source is sufficient to saturate PSII. If PSII is not light saturated, F_m values obtained are inaccurate. It is the measurement most often used to test the effects of environmental stresses. Bjorkman and Demmig (1987) found that although there was considerable fluorescence variation at 692 and 734 nm among different species, upper and lower leaf surfaces, and sun and shade leaves, F_v/ F_m ratios varied little. The 44 species, measured under ideal conditions, had an overall F_v/ F_m average of .832 +/- .004. Conifers averaged .853 +/- .004. Less commonly, the F_v/ F_o ratio has been used (Ruter, 1993) and was found helpful in identifying optimal growth temps in woody plants. It has been shown to be closely linked to leaf water potential (*Hansatech manual*).

However, Lichtenthaler (1990) argues that F_o, F_m, F_v, and ratios derived from them, are inappropriate measurements when dealing with stress and ecophysiology due to their being measured in the dark-adapted, non-functional state of photosynthesis. In this state, measurements would not fully reflect the actual physiological condition of the photosynthetic apparatus and its functionality. He suggests a 'vitality index', Rfd, consisting of the ratio of the fluorescence signal decrease to steady state fluorescence, or $(F_m- F_s)/ F_s$, as a

better indicator of actual photosynthetic functioning. Rfd values have been used in research involving spruce (Hagg et al., 1992) and Scotch pine (Saarinen and Liski, 1993). Using a variation of F_v/F_m measured under illuminated conditions (F_v/F_m'), Genty et al. (1989) found a clear relationship between it and the quantum yield of carbon dioxide assimilation in a wide variety of plant species.

As chlorophyll fluorometers have become more advanced, the possible measurements have increased. Fluorometers are available that can measure different wavelength ranges and thus chlorophyll fluorescence readings can be taken for PSII (~690 nm) and PSI (~ 735 nm) individually as well as combined. The P_{690}/P_{735} ratio has been used as an indicator of the reciprocal relationship of *in vivo* chlorophyll content of leaves and needles (Hagg et al., 1992). It may be especially suited for measurement of long term stress conditions in plants, and, due to the speed with which it can be measured, it has potential for aerial forest surveys (Lichtenthaler, 1988c).

Quenching measurements are often expressed in terms of 'quenching coefficients' which represent the quenched proportion of F_v. Quenching origins have been identified with the study of delayed chlorophyll fluorescence, or luminescence. The delay time can be as short as 0.3 seconds and luminescence can last up to several minutes (Schmidt, 1988). Millisecond-long luminescence is often the result of membrane energization, while luminescence of ~50 microseconds involves a slow-down of PSII donors (Schreiber and Bilger, 1993). Schmidt (1988) identifies the origin of luminescence energy as a back-transfer of charges built-up in the photosynthetic electron transport chain. Luminescence is

therefore very closely linked with photosynthetic processes. It also provides high contrast between damaged and undamaged leaves. However, measurement of luminescence requires complete darkness due to its weak signal and thus requires bulky devices (Blaich, 1988).

A calculation of the electron transport rate (ETR) through PSII has also been used (Brodribb and Hill, 1997). Edwards and Baker (1993) developed the formula for such which multiplies the photochemical efficiency of PSII (Gentry et al., 1989) by the incident photosynthetic photon flux density and the average fluorescence, halved.

Lastly, chlorophyll fluorescence lifetimes are being measured in growing numbers of experiments. The lifetime of fluorescence is analyzed by measuring the fluorescence decline after brief exciting flashes of light. This shows the decline in excitation density of chlorophyll (Krause and Weis, 1991). Chlorophyll fluorescence lifetimes are good for observing the kinetics of primary photosynthetic processes.

CHLOROPHYLL FLUOROMETERS

The technology involved in chlorophyll fluorometers has changed greatly since their first appearance on the scene in the 1930's.

Modern fluorometers vary with the uses they were designed for. Lightweight portable versions are the most common. These usually consist of a microprocessor, with some type of display and control panel, attached to a fiber optic tube. There may be some type of actinic light source with a shutter in the

control box or a light source such as light emitting diodes may be attached to the opposite end of the fiber optic. One new model uses an He/Ne-laser (Daley, 1995; Lichtenthaler, 1990). A variety of plastic clip designs exist which are clamped onto the portion of the sample to be measured. These allow for dark adaptation of the sample in a lighted lab or out of doors, as well as hold the photodiode, located at the end of the fiber optic, in place for the chlorophyll fluorescence measurement. Photodiodes measure photons by the electric current triggered when photons hitting the photodiode cause photochemical reactions, causing the excitation of an electron and leaving an empty, positively charged 'blank' where the electron used to be. In order to measure only fluoresced light and not reflected actinic light (actinic light is light meant for use in photosynthesis), optical filters are used to sort out the different wavelengths, or, more rarely, only lightwaves of less than 620 nm are used for the actinic light source (Bolhar-Nordenkampf et al., 1989). After F_o is measured, the sample is brightly illuminated by the light source and fluorescence measurements are taken, often as quickly as 100,000 readings per second for the fast rise and then gradually slowing to somewhere around 10 readings per second as the signal stabilizes to F_s (Hansatech and Morgan manuals). The quantity of fluorescence is normally measured in arbitrary units set by the manufacturer. These are often referred to as "relative units" in the literature.

As mentioned, some chlorophyll fluorometers are being designed to measure the fluorescence individually at two different wavelengths. These wavelengths correspond to the two chlorophyll fluorescence spectra peaks, 690

and 735 nm, emitted from PSII and PSI, respectively. This gives the researcher fluorescence information from both photocenters, as well as making an estimate of chlorophyll content possible (Hagg et al., 1992; Lichtenthaler, 1990). Although stress detection is possible (Lichtenthaler, 1988b), at physiological temperatures, changes in chlorophyll fluorescence due to stress are largely dominated by emissions from PSII (Bolar-Nordenkampf et al., 1989).

A relatively new modification is the Pulse Amplitude Fluorometer, PAM, also sometimes called a Pulse Modulation Fluorometer. After F_o, F_m, and F_s measurements are taken, an actinic light is turned on and the sample is subjected to additional saturating light pulses every 10 seconds or so. This allows the measurement of photochemical and non-photochemical quenching coefficients, non-photochemical quenching being that which doesn't change during a saturation pulse of light (Schreiber and Bilger, 1993). These pulse-modulation measurements can be taken in daylight conditions, an important development for photoinhibition experiments and field studies.

Also new is the advent of fluorescence video imaging. Daley (1995) describes a portable fluorescence imaging system which records video images and then is able to use digitized versions of the images for analytical purposes. Mapping of the spatial distribution of photosynthetic activity becomes possible.

Aerial sensing of large-scale vegetation fluorescence is under study (Lichtenthaler, 1990). Instead of focusing on individual leaf chlorophyll fluorescence, this instead deals with the overall reflection signature of a large group of plants at once. Stressed or dying trees tend to have lower chlorophyll

contents and this affects their reflection signal in two ways. First, visible light (800-900nm) reflection is increased because less photosynthesis is going on (less photochemical quenching). Second, the amount of infrared light reflected is decreased due to structural changes in the leaf cell arrangement. Combining these two, we get what is termed a "blue shift" in the reflected light from stressed plants. However, this blue shift is little understood in terms of what determines when it will occur and how different stresses are involved.

CHLOROPHYLL FLUORESCENCE USE

Much of early chlorophyll fluorescence research dealt with understanding the mechanism behind fluorescence itself and getting a better understanding of what it implied. It was used as a "probe of photosynthesis" (Papageorgiou, 1975) and was involved mainly in basic photosynthesis research. By the 1980's however, numerous experiments were being done comparing indicators of photosynthesis rates and various chlorophyll fluorescence measurements. Bjorkman and Demmig (1987) showed that the quantum yield of PSII, as determined by oxygen evolution, correlates linearly to F_v/F_m measurements under various stress conditions in a wide variety of plants. Genty et al. (1989) found chlorophyll fluorescence measurements representative of the photoeffeciency of PSII, as measured by carbon dioxide assimilation. Krause et al., (1990) found PSII electron transport capacity to be linearly related to F_v/F_m in spinach leaves subjected to photoinhibitory treatments. Edwards and Baker (1993) found that chlorophyll fluorescence parameters can be used under a wide

range of conditions to accurately predict carbon dioxide assimilation rates in maize. From studies like these (for review see Lichtenthaler, 1990) the precedent was set for the use of chlorophyll fluorescence as a stress detection and quantification tool.

Numerous studies have been done involving heat stress and its affect on photosynthesis. Georgieva and Yordanov (1993) used F_v/F_m values to study heat tolerance in pea. The effects of heat stress on cation-induced chlorophyll fluorescence rises in pea have been studied by Velitchkova and Ivanov (1993). Bilger et al. (1984) found heat-induced fluorescence increase to be a suitable indicator of heat dosage accumulation to lethal levels in a variety of vascular plants. Havaux (1992) used chlorophyll fluorescence to study the combined and separate effects of heat and water stress and found the combination of stresses to have less damaging effects on PSII than heat stress alone. Ruter has shown F_v/F_m to be an effective measurement of heat stress in various holly cultivars during heat tolerance experiments (Ranney and Ruter, 1997; Ruter, 1993). Likewise, Ranney and Peet (1994) used chlorophyll fluorescence to examine heat tolerance in five birch taxa.

Chlorophyll fluorescence studies have also dealt with cold stresses, including tolerance, frost effects, acclimation, and photoinhibition interactions. One possible way of measuring freezing tolerance, in terms of photosynthetic functionality, is the reduction of fluorescence parameters after short duration exposure to freezing temperatures. Frost tolerance in different birch subspecies has been examined using chlorophyll fluorescence (Hallgren et al., 1982) and

Smillie and Hetherington (1983) used fluorescence values to examine chilling tolerance in wheats and citrus crops. During et al. (1990) found F_v/F_m measurement of frost injury in grapevine buds to be comparable to visual estimation of injury. With an acclimation period, readings remained at a much healthier level and injury was reduced. In plants adjusted to low temperatures, it seems to be the proportion of PSII reaction centers operating that is reduced rather than a change in the reaction centers themselves (Huner et al., 1993). Grey et al. (1997) found that, in cold acclimated plants, the increased excitation pressure on PSII itself was enough to influence the expression of a nuclear gene involved in cold acclimation. During acclimation of rape to 2.5 °C , F_m and F_o values were found to drop dramatically in a two-day period, and then level off and recover when removed to 20 °C for 2-3 days (Maciejewska and Bauer, 1992). Fisker et al. (1995) found chlorophyll fluorescence to be an accurate estimate of needle freezing damage and seedling survival in Douglas fir and could detect non-visible damage, however, fluorescence measurements were unable to predict cold hardiness prior to the temperature treatments. Likewise, Welander et al. (1994) were unable to use chlorophyll fluorescence to predict growth response after a night of frost followed by high irradiation. Lindgren and Hallgren (1993) found chlorophyll fluorescence to be an effective method for the detection of freezing injury and ranking of cold acclimation in lodgepole pine and Scotch pine, and could be done one week earlier than a visual assessment. Cold storage has been studied in white spruce (Vidaver et al., 1989) and low temperature effects on Scotch pine (Hallgren et al, 1990; reviewed in Lindgren

and Hallgren, 1993).

Photoinhibition studies frequently overlap with cold stress studies. A number of studies found decreasing temperature to lead to a decrease in amount of light needed to saturate photosynthesis (F_v) (Hallgren et al., 1982). F_v / F_m values were found to decrease (increasing fluorescence) in Scotch pine (Hallgren et al., 1990) and spruce (Welander et al., 1994; Westin et al., 1995) the day after a night frost. Cold temperatures have been found to specifically predispose a plant to photoinhibition and Greer's studies with combinations of dark and light cold treatments suggest that photoinhibition may be the actual mechanism of cold stress effects on photosynthesis (Greer, 1990). Bolhar-Nordenkampf and Lechner (1988) identify photoinhibition, along with membrane disintegration at temperatures below –4 °C, as the two actual mechanisms of cold damage to photosynthesis. Cold acclimation was found to increase resistance to photoinhibition in spinach, limiting photoinhibition to high light intensities (Somersalo and Krause, 1988).

Although normally associated with cold stress, photoinhibition can occur in otherwise unstressed plants (i.e. *Salix* in this study) on sunny summer days (Ogren and Oquist, 1988). Bilger et al. (1995) used chlorophyll fluorescence based calculations of the quantum efficiency of PSII and electron transport rate to study the daily response of beech and cucumber leaves to changing photon flux densities. Photoinhibition due to continuous illumination combined with defoliation in sour cherry was examined by Layne and Flore (1993). Despite the reduction in photosynthetic processes, recovery potential of photoinhibition-

impaired photosynthesis has been demonstrated in numerous studies (Somersalo and Krause, 1988; Bilger et al., 1995; . . .).

Study of the seasonal changes in chlorophyll fluorescence can help identify acclimation and dormancy periods. F_v / F_m values have been used to assess the dormancy of Douglas fir (reviewed in Lindgren and Hallgren, 1993). Scotch and Lodgepole pines were found to have Fv/Fm values which decreased from .83 in August, to .77 in September, and .75 in October, and finally descended to .21 in December (Lindgren and Hallgren, 1993). In a study by Westin et al. (1995), Norway spruce F_v / F_m values were found to remain high in early fall, decline from November to April to about .35 - .5, experience a sharp drop in April presumably due to increasing photoinhibition, and then quickly rise to high values in May. Winter values were found to fluctuate with local temperature changes.

Many other environmental stresses have been studied with chlorophyll fluorescence to a lesser degree than the temperature and light effects described above. Spruce under limited mineral nutrition showed no affect on Rfd values over a ten-month study period (Hagg et al., 1992). Saarinen (1993) found low Fv/Fm values to be an indicator of pollution in Scots pine in sites with heavy vehicular traffic and trees located near oil refineries (Saarinen and Liski, 1993). However, no change in F_v / F_m was found in Norway spruce with elevated CO_2 and O_3 levels, and potassium deficiency (Barnes et al., 1995). Clorophyll fluorescence has been used to study forest decline in Norway spruce (Lichtenthaler and Rinderle, 1988) and Bukhov et al. (1990) used it to examine

leaf dehydration.

There has been some study of low temperature injury to fruit using chlorophyll fluorescence. Studies include banana, mango, cucumber, eggplant, and green bell peppers. F_v / F_m measurements were found ineffective at predicting apple scald susceptibility (Mir et al., 1998a; Mir et al., 1998b).

Little studied is the relationship between chlorophyll fluorescence and propagation. Van Huylenbroech and Debergh (1992) used fluorescence as a tool to examine stress levels and stages during the acclimatization period of micropropagated Transvaal daisy (*Gerbera jamesonii* Bol. ex Adlam). I have found no research involving chlorophyll fluorescence and stem propagation.

CHLOROPHYLL FLUORESCENCE POTENTIAL

Chlorophyll fluorescence is developing as valuable tool in the estimation and measurement of photosynthetic health. Its non-destructive, rapid, portable, and objective nature make it useful in scientific and commercial spheres. Scientific interest has shifted from interest in primary reactions and induction kinetics to overall electron transport efficiency and steady state reactions (Schreiber and Bilger, 1993). Its role in stress physiology measurements will continue to grow. Schreiber and Bilger (1993) emphasize its potential for remote sensing of photosynthetic health.

During et al., (1990) suggest chlorophyll fluorescence measurements as an objective tool for estimating frost injury of buds in grapevine.

Fiskar et al., (1995) propose chlorophyll fluorescence as a tool for rapidly

identifying cold-damaged seedlings in nurseries.

Edwards and Baker (1993) point to the time and labor that could be saved by estimating carbon dioxide assimilation using chlorophyll fluorescence values. The technique would also have advantages of measuring the plants in their normal environment instead of a gas chamber.

Daley (1994) describes a recently completed chlorophyll fluorescence video system. Using a camcorder and LED illumination, fluorescence can be visualized over a small leaf area. This allows for the detection of virus lesions before they are visible to the eye, as well as low virulence strains which otherwise may never be seen.

Chlorophyll fluorescence may have some use as a fruit-grading tool. Beaudry et al. (1997 in Mir et al., 1998a) suggest its use as a tool for quality measurement of stored apples. Mir et al. (1998b) find a potential use in the sorting of fruit having superficial defects.

TAXUS HISTORY

The genus *Taxus* has a worldwide distribution in the Northern Hemisphere. Three to four species are recognized, but the distinctions between such are more geographical than morphological. English yews belong to *T. baccata* L., Japanese yews to *T. cuspidata* Siebold and Zuccarini, and North American yews to *T. canadensis* Marshall (Chadwick and Keen, 1976). A western North American species is also referred to, *T. brevifolia* (Heinstein and Chang, 1994).

Morphologically, these conifers are usually small trees (20 to 40 ft.) or shrubs. Extremely slow growing and long-lived, their average lifespan in the wild is around 500 years, but many live to be well over 1000 years old (Tittensor, 1980). Needles tend to be a dark and glossy green (except in some cultivated varieties) and bark is fibrous and reddish. Most plants are dioecious (Chadwick and Keen, 1976). Pollen cones open in early spring. The arils ripen in early winter, single-seeded berries with fleshy, scarlet seed coats for bird-dispersal. *Taxus* may take up to 70 years to reach sexual maturity (Hulme, 1996). The needles and seeds are known to be toxic to humans (Chadwick and Keen, 1976), and have caused problems in cattle (Hulme, 1996).

In medieval Britain, yews were originally used for archery bows, due to their strong and flexible wood (Chadwick and Keen, 1976), and as land markers on property lines due to their longevity (Tittensor, 1980). The first mention of a cultivated yew comes from an English garden description in 1686 (Chadwick and Keen, 1976). Since then, yews have become a mainstay of gardens everywhere, with upright, globe, and spreading varieties.

Taxus nomenclature in America is notoriously confused. Cultivated *Taxus* is usually of *T. baccata* or *T. cuspidata* origin, or belongs to a hybrid species of the two, *Taxus xmedia* Rehder. Nomenclatural confusion can be traced back to the enactment of Quarantine 37 by the U.S. government in 1918. These laws prohibited importation of nursery stock, and growers suddenly faced with a scarcity of stock or profitable cultivars, resorted to taking cuttings from less that desirable material and often applied salable cultivar names to such (Chadwick

and Keen, 1976). The confusion created still exists today. The Living Herbarium of Taxus, at the Secrest Arboretum (Ohio Agricultural Research Experiment Station) was set up in 1942 to try to sort out some of the misnomers, and by 1976 had 141 accessions (Chadwick and Keen, 1976).

One of the studies conducted using material from Secrest, attempted to use isozyme electrophoresis to distinguish cultivar differences (Greer et al., 1993). Looking at 51 plants belonging to 21 cultivars, results often showed different electrophoretic fingerprints for members of the same cultivar and identical fingerprints for different cultivars. Extensive nomenclatural problems exist.

Aside from its traditional value as an ornamental, *Taxus* was discovered to harbor great pharmaceutical value in the 1980's. Taxol, a diterpene extraction from *Taxus* bark, needles, twigs, and roots, has been identified as one of the most promising anti-cancer drugs from a plant source (Heinstein and Chang, 1994). It has been shown to have anti-cancer activity in ovarian, breast, lung, head, and neck cancers. A complicated chemical structure has stumped the development of any synthesis process, however, semi-synthesis of taxol can be performed using another, more abundant *Taxus* extract, baccatin II. Due to the slow growth of *Taxus* cell cultures, and rarity of *T. brevifolia*, the *Taxus* bark with highest taxol content, Heinstein and Chang (1994) identify commercial ornamental nursery stock as the most economical source of taxol and baccatin II at this time.

PROPAGATION OF *TAXUS*

Propagation of Taxus from seed is possible but slow and would not conserve the cultivar traits we value today. For seed propagation, Chadwick and Keen (1976) advise stratifying cleaned seeds at 35 - 50 °F (2 - 10 °C) until the following October, or planting directly in protected beds for spring germination.

Today Taxus is commercially propagated via stem cuttings. Chadwick and Keen (1976) suggest four to eight inch cuttings taken in August and placed in cold storage or cuttings taken in March. Gerald Verkade (1976) of Verkade Nurseries, New London, Conn., describes eight inch cuttings taken in November or December, striped of needles on the bottom 2.5 inches for an auxin dip, and placed in a 1:2 perlite/ coarse sand medium. He reports 90 - 95% rooting. Joseph P. Von Kornya (1976) of Bobbink Nurseries, Freehold, N.J., reports using six inch cuttings for spreading yews and eight inch cuttings for upright yews. These cuttings are taken after two to four killing frosts and placed in 100% perlite at 65-68 °F. He stresses the importance of bottom heat.

The difficulty of rooting certain cultivars has been a persistent problem. A 1964 study attempted to link ease of rooting differences in cultivars to sex differences between cultivars without success (Davidson, 1964). The highest *Taxus* rooting percentages in an Italian study (Eccher, 1988) were achieved with apical cuttings at 20 °C and no bottom heat. Rooting media did not affect rooting percentages, however, sand/peat mixtures led to thinner, more fibrous roots than agriperlite. IBA increased rooting speed in most cultivars but did little to raise long-term rooting percentages. Long propagation times were key.

PURPOSE OF THE EXPERIMENTS

If a quick, reliable method of determining potential rooting of cuttings based on the condition of a specific stock plant was available for propagators, then rooting success could be predicted prior to an investment in time, labor, and resources. A reduction in production costs could be realized.

Chlorophyll fluorescence measurement is a tool in plant stress detection with high potential for commercial application. It has been shown effective in the detection of environmental and other stresses on a broad range of plant species, including many conifers.

Two different experiments were performed. The first was a cultivar study designed to look at the differences in chlorophyll fluorescence among ten cultivars of *Taxus*, to study the changes in chlorophyll fluorescence over the course of propagation, and to correlate chlorophyll fluorescence and rooting among cultivars.

The second study examined different storage conditions, quantifying stress in relation to subsequent rooting by using chlorophyll fluorescence measurements. Seven different temperatures, three desiccation levels, and three time durations were studied.

LITERATURE CITED

Adams, W.W. III, B. Demmig-Adams, K. Winter, and U. Schreiber. 1990. The ratio of variable to maximum fluorescence from photosystem II, measured in leaves at ambient temperature and at 77K, as an indicator of the photon yield of photosynthesis. Planta 180:166-174.

Barnes, J.D., T. Pfirrmann , K. Steiner , C. Lutz , U. Busch , H. Kuchenhoff , and H.D. Payer. 1995. Effects of elevated CO2 and potassium deficiency in Norway Spruce (*Picea abies* (L.)Karst.): Seasonal changes in photosynthesis and non-structural carbohydrate content. Plant, Cell, and Environ. 18:1345-1357.

Berry, J. and O. Bjorkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol. 31:491-543.

Bilger, W., U. Schreiber, and M. Bock. 1995. Determination of the quantum efficiency of photosystem II and of non-photochemical quenching of chlorophyll fluorescence in the field. Oecologia 102:425-432.

Bilger, H.W., U. Schreiber, and O. Lange. 1984. Determination of leaf heat resistance: comparative investigation of chlorophyll fluorescence changes and tissue necrosis methods. Oecologia 63:256-262.

Bjorkman, O. and B. Demmig. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta 170:489-504.

Blaich, R. 1988. Early detection of damage conditions in plants by delayed chlorophyll fluorescence, p. 223-228. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Bolhar-Nordenkampf, H.R. and E. Lechner. 1988. Winter stress and chlorophyll fluorescence in Norway spruce, p. 173-180. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Bolhar-Nordenkampf, H.R., S.P. Long, N.R. Baker, G. Oquist, U. Schreibers, and E.G. Lechner. 1989. Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. Functional Ecol. 3:491-514.

Brodribb, T.M. and R.S. Hill. 1997. Light response characteristics of a morphologically diverse group of Southern Hemisphere conifers as measured by chlorophyll fluorescence. Oecologia 110:10-17.

Bukhov, N.G., P. Mohanty, and S.S. Sabat. 1990. Effect of leaf rehydration on kinetics of variable chlorophyll fluorescence. Doklady Adademii Nauk SSR Sept 1989, 308(1):251-255. In: Plant Physiology, Plenum Press, p. 73-76.

Chadwick, L.C. and R.A. Keen. 1976. A Study of the genus *Taxus*. Ohio Agr. Res. Dev. Ctr. Mar. Bul. 1086.

Coombs, J., D.O. Hall, S.P. Long, and J.M.O. Scurlock (eds.). 1985. Techniques in Bioproductivity and Photosynthesis. 2nd ed., Pergamon Press, N.Y.

Daley, P.F. 1995. Chlorophyll Fluorescence analysis and imaging in plant stress and disease. Can. J. Plant Pathol. 17:167-173.

Davidson, H. and A Olney. 1964. Clonal and sexual differences in the propagation of *Taxus*, p. 156-161. In: International Plant Propagator's Society Combined Proceedings, vol. 14.

During, H., T.V. Ortoidze, and B. Bushnell. 1990. Effects of subzero temperatures on chlorophyll fluorescence of grapevine buds. J. Plant Physiol. 136:758-760.

Eccher, T. 1988. Response of cuttings of 16 *Taxus* cultivars to rooting treatments. Acta Horticulturae 227:251-253.

Edwards, G.E. and N.R. Baker. 1993. Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? Photosyn. Res. 37:89-102.

Fisker, S., R. Rose, and D.L. Haase. 1995. Chlorophyll fluorescence as a measure of cold hardiness and freezing stress in 1+1 Douglas-Fir seedlings. Forest Sci. 41(3):564-575.

Garrett, R.H. and C.M. Grisham. 1995. Biochemistry. Sounders College Publ., Fort Worth, T.X.

Genty, B., J.M. Briantais, and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophy. Acta 990:87-92.

Georgieva, K. and I. Yordanov. 1993. Temperature dependence of chlorophyll fluorescence parameters of pea seedlings. J. Plant Physiol. 142:151-155.

Gray, G.R.; L.P. Chauvin, F. Sarhan, and N.P.A. Huner. 1997. Cold acclimation and freezing tolerance: A complex interaction of light and temperature. Plant Physiol. 114:467-474.

Greer, C.E., R.E. Schutzki, A. Fernandez, and J.F. Hancock. 1993. Electrophoretic characterization of *Taxus* cultivars. HortTechnol. 3(4):430-433.

Greer, D.H. 1990. The combined effects of chilling and light stress on photoinhibition of photosynthesis and its subsequent recovery. Plant Physiol. Biochem. 28(4):447-455.

Hagg, C., F. Stober, and H.K. Lichtenthaler. 1992. Pigment content, chlorophyll fluorescence and photosynthetic activity of spruce clones under normal and limited mineral nutrition. Photosynthetica 27(3):385-400.

Hallgren, J.E., T. Lundmark, and M. Strand. 1990. Photosynthesis of Scots pine in the field after night frosts during summer. Plant Physiol. Biochem. 28(4):437-445.

Hallgren, J.E., E. Sundbom, and M. Strand. 1982. Photosynthetic responses to low temperature in *Betula pubescens* and *Betula tortuosa*. Physiol. Plant. 54:275-282.

Hansatech manual. 1996. Hansatech Instruments Ltd., Norfolk, England, U.K.

Havaux, M. 1992. Stress tolerance of photosystem II in vivo: Antagonistic effects of water, heat, and photoinhibition stresses. Plant Physiol. 100:424-432.

Heinstein, P.F. and C.J. Chang. 1994. Taxol. Annu. Rev. Plant Physiol. Plant Mol. Biol. 45:663-674.

Holzwarth, A.R. 1988 Time resolved chlorophyll fluorescence, p. 21-31. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Hulme, P.E. 1996. Natural regeneration of yew (*Taxus baccata* L.): microsite, seed, or herbivore limitation? J. Ecol. 84:853-861.

Huner, N.P., G. Oquist, V.M. Hurry, M. Krol, S. Falk, and M. Griffith. 1993. Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. Photosyn. Res. 37:19-39.

Krause, G.H., S. Somersalo, E. Zumbusch, B. Weyers, and H. Laasch. 1990. On the mechanism of photoinhibition in chloroplasts: Relationship between changes in fluorescence and activity of photosystem II. J. Plant Physiol. 136:472-479.

Krause, G.H. and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis: The basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:313-349.

Lavorell, J. and A.L. Ettiene. 1977. In vivo chlorophyll fluorescence, p. 203-268.

In: J. Barber (ed.). Primary Processes in photosynthesis. Elsevier/ North Holland Biomedical Press, Amsterdam, The Netherlands.

Layne, D.R. and J.A. Flore. 1993. Physiological responses of *Prunus cerasus* to whole-plant source manipulation, leaf gas exchange, chlorophyll fluorescence, water relations, and carbohydrate concentrations. Physiol. Plant. 88:44-51.

Lichtenthaler, H.K. (Ed.) 1988a. Applications of Chlorophyll Fluorescence, p. 1-12. Kluwer Academic Publ., Dordrecht, The Netherlands.

Lichtenthaler, H.K. 1988b. In vivo chlorophyll fluorescence as a tool for stress detection in plants, p. 129-142. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Lichtenthaler, H.K. 1988c. Remote sensing of chlorophyll fluorescence in oceanography and in terrestrial vegetation: an introduction, p. 287-297. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Lichtenthaler, H.K. 1990. Applications of chlorophyll fluorescence in stress physiology and remote sensing, p. 278-305. In: M.O. Steven and J.A. Clark (eds.). Applications of Remote Sensing in Agriculture. Butterworths, London.

Lichtenthaler, H.K. and U. Rinderle. 1988. Chlorophyll fluorescence signatures as vitality indicator in forest decline research, p. 143-149. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Lindgren, K. and J.E. Hallgren. 1993. Cold acclimation of *Pinus contorta* and *Pinus sylvestris* assessed by chlorophyll fluorescence. Tree Physiol. 13:97-106.

Long, S.P., S. Humphries, and P.G. Falkowski. 1994. Photoinhibition of photosynthesis in nature. Annu. Rev. Plant Physiol. Plant Mol. Biol. 45:633-662.

Maciejewska, U. and H. Bauer. 1992. Effects of cold acclimation on chlorophyll fluorescence in winter rape leaves. Photosynthetica 27(4):559-562.

Melis, A., G.E. Guenther, P.J. Morrissey, and M.L. Ghirardi. 1988. Photosystem II heterogeneity in chloroplasts, p. 33-43. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Mir, N., M. Wendorf, R. Perez, and R.M. Beaudry. 1998a. Chlorophyll fluorescence in relation to superficial scald development in apple. Amer. Soc. Hort. Sci. 123(5):887-892.

Mir, N., M. Wendorf, R. Perez, and R.M. Beaudry. 1998b. Chlorophyll fluorescence as affected by some superficial defects in stored apples. J. Hort. Sci. Biotech. 73(6):846-850.

Morgan CF-1000 manual. 1992. P.K. Morgan Instruments, Inc., Andover, MA, USA.

Ogren, E. and G. Oquist. 1988. Screening for photoinhibition of photosynthesis in the field using a portable fluorometer, p. 165-172. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Papageorgiou, G. 1975. Chlorophyll fluorescence: An intrinsic prove of photosynthesis, p. 320-366. In: Govindjee (ed.). Bioenergetics of Photosynthesis. Academic Press, N.Y.

Ranney, T.G. and M.M. Peet. 1994. Heat tolerance of five taxa of birch (*Betula*): Physiological responses to supraoptimal leaf temperatures. J. Amer. Soc. Hort. Sci. 119(2):243-248.

Ranney, T.G. and J.M. Ruter. 1997. Foliar heat tolerance of three holly species (*Ilex* spp.): Responses of chlorophyll fluorescence and leaf gas exchange in supraoptimal leaf temperatures. J. Amer. Soc. Hort. Sci. 122(4):499-503.

Rinderle, U., and H.K. Lichtenthaler. 1988. The chlorophyll fluorescence ratio F_{690}/F_{735} as a possible stress indicator, p. 189-196. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Ruter, J.M. 1993. Foliar heat tolerance of two hybrid hollies. HortSci. 28(6):650-652.

Saarinen, T. 1993. Chlorophyll fluorescence, and nitrogen and pigment content of Scots pine (*Pinus sylvestris*) needles in polluted urban habitats. Ann. Bot. Fennici 30:1-7.

Saarinen, T. and J. Liski. 1993. The effect of industrial air pollution on chlorophyll fluorescence and pigment contents of Scots pine (*Pinus sylvestris*) needles. Euro. J. For. Pathol. 23:353-361.

Schmidt, W. 1988. Long term delayed luminescence in green organisms, p. 217-221. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Schreiber, U. and W. Bilger. 1993. Progress in chlorophyll fluorescence research: Major developments during the past years in retrospect, p. 151-169. In: Progress

in Botany, vol. 54. Springer-Verlag, Berlin.

Smillie, R.M. and S.E. Hetherington. 1983. Stress tolerance and stress-induced injury in crop plants measured by chlorophyll fluorescence *in vivo*: Chilling, freezing, ice cover, heat, and high light. Plant Physiol. 72:1043-1050.

Somersalo, S., and G.H. Krause. 1988. Changes in chlorophyll fluorescence related to photoinhibition of photosynthesis and cold acclimation in green plants, p. 157-164. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Tittensor, R. 1980. Ecological history of yew, *Taxus baccata* L., in southern England. Biol. Conserv. 17:243-265.

Van Huylenbroech, J. and P. Degergh. 1992. Acclimation of micropropagated *Gerbera jamesonii*; Use of chlorophyll fluorescence. Forum for Applied Biotechnology. Brugge (Belgium). 24-25 Sept. Mededelingen-van-de-Faculteit-Landbouwwetenschappen-Rijksuniversiteit-Gent (Belgium). 57(4a):1595-1579.

Van Kooten, O. and J.F.H. Snel. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. Photosyn. Res. 25:147-150.

Velitchkova, M.Y. and A.G. Ivanov. 1993. Effects of short-time heat stress on the parameters of cation induced increase in chlorophyll fluorescence in pea thylakoid membranes. J. Plant Physiol. 142:144-150.

Verkade, G. 1976. Propagation of Taxus by cuttings, p. 177. In: International Plant Propagator's Society Combined Proceedings, vol 26.

Vidaver, E., W. Binder, R. Brooke, G. Lister, and P. Toivonen. 1989. Assessment of photosynthetic activity of nursery grown *Picea glauca* seedlings using an integrating fluorometer to monitor variable chlorophyll fluorescence. Can. J. For. Res. 19:1478-1482.

Von Kornya, J.P. 1976. Propagation of Taxus cuttings, pp. 178-179. In: International Plant Propagator's Society Combined Proceedings, vol. 26.

Welander, N.T., P. Gemmel, O. Hellgren, and B. Ottosson. 1994. The consequences of freezing temperatures followed by high irradiance on *in vivo* chlorophyll fluorescence and growth in *Picea abies*. Physiol. Plant. 91:121-127.

Westin, J., L.G. Sundblad, and J.E. Hallgren. 1995. Seasonal variation in photochemical activity and hardiness in clones of Norway spruce (*Picea abies*). Tree Physiol. 15:685-689.

Chapter One

Chlorophyll Fluorescence and Vegetative Propagation of *Taxus*

(Formatted according to publication guidelines of the American Society of Horticultural Science)

Chlorophyll Fluorescence and Vegetative Propagation of *Taxus*

S. E. Bruce[1] and D. B. Rowe[2]
Department of Horticulture, Michigan State University, East Lansing, MI 48824

Received for publication . Accepted for publication . This paper is a portion of a M.S. thesis submitted by S. E. Bruce.

Acknowledgement: This experiment was a Michigan State University study funded by: Zelenka Nursery in Grand Haven, MI, International Plant Propagator's Society, Michigan Nursery and Landscape Association, and Michigan Agricultural Experiment Station.

[1]Graduate research assistant
[2]Assistant professor

Propagation and Tissue Culture

Chlorophyll Fluorescence and Vegetative Propagation of *Taxus*

Additional index words. Yew, stem cuttings

Abstract. Chlorophyll fluorescence (F_v/F_m) over the course of stem cutting propagation was examined in ten cultivars of *Taxus xmedia* (*Taxus baccata* L. x *T. cuspidata* Sieb. & Zucc.) including Brownii, Dark Green Pyramidalis, Dark Green Spreader, Densiformis, Densiformis Gem, Hicksii, L.C. Bobbink, Runyan, Tauntoni, and Wardii. The study's objective was to examine chlorophyll fluorescence as a method for stock plant selection and monitoring of propagation in the various cultivars. Differences were found in F_v/F_m among cultivars, initially and over time, however, there was significant overlapping between some cultivars. F_v/F_m was found to decrease dramatically during cold storage, but usually returned to original levels after several weeks in the propagation beds. This seemed to be more a reflection of ambient temperatures than actual rooting. Correlations were not found between initial stock plant F_v/F_m and rooting percentage, root number, root dry weight, or root length, indicating that F_v/F_m is not a reliable indicator of stock plant propagation potential.

Yew (*Taxus* sp. L.) is a mainstay of ornamental gardens with its dark, evergreen needles and red winter arils. In the mid 80's great pharmaceutical value was added to its list of virtues with the discovery of taxol, a diterpene found in its bark, needles, twigs, and roots, and identified as one of the most promising anti-cancer drugs from a plant source (Heinstein and Chang, 1994). The nursery industry supplies *Taxus* for both ornamental and pharmaceutical industries and growers face great propagation pressures. Propagation is usually done via stem cuttings, taken from stock plants in early fall, and kept in cold storage until placed in propagation beds in mid-winter. Adventitious rooting can be unpredictable, with some cultivars persistently difficult to root. Davidson (1964) unsuccessfully attempted to link ease of rooting differences in cultivars to sex differences (*Taxus* spp. are largely dioecious). Eccher (1988) reported long propagation times to be key to rooting of *Taxus*. Since nursery propagation failures are often related to stock plant material, interest arose in a method for evaluating stock plant quality prior to collection of cuttings.

Chlorophyll fluorescence measurements may be a potential method for evaluating stock plants. Chlorophyll fluorescence is created when light energy, absorbed by chlorophyll a, exceeds the photochemical processing capacity of photosystem II (PSII). One way this 'extra' energy is dissipated is by being re-emitted as light, which we call chlorophyll fluorescence. The fluorescence measured at physiological temperatures is largely a product of chlorophyll a molecules involved in PSII, although other light capturing pigments do fluoresce, and PSI fluorescence can be measured. Since chlorophyll fluorescence levels

are tied to the amount of light energy *not* used for photosynthetic processes, they are inversely related to the amount of energy that *is* used for photosynthesis, and serve as indicators of plant photosynthetic potential.

Chlorophyll fluorescence measurement is increasingly used as a quantitative measure of photosynthetic health. The emitted light signal follows a general intensity pattern known as the Kautsky Effect. Pre-darkened samples, with a minimum fluorescence level (F_o), show a 'fast rise' in fluorescence to a maximum value (F_m) upon exposure to a light source. As photochemical processing of the light energy increases, fluorescence values are gradually reduced to a steady state (F_s), somewhere between F_o and F_m. A common parameter used in stress studies is F_v/F_m, F_v being the variable fluorescence, calculated by subtracting F_o from F_m. Numerous studies have shown F_v/F_m, and other chlorophyll fluorescence parameters, to be effective measurements of the photoefficiency of PSII. Bjorkman and Demmig (1987) linearly correlated F_v/F_m with the quantum yield of PSII, as determined by oxygen evolution, in a variety of stressed plants.

Studies utilizing chlorophyll fluorescence to quantify plant stress have dealt with heat and cold stress, especially tolerance studies, photoinhibition, mineral nutrition, pollution, and water stress. Studies on conifers have found effects of vehicle and oil refinery pollution on Scotch pine (*Pinus sylvestris* L.) (Saarinen, 1993; Saarinen and Liski, 1993), photoinhibition effects in Scotch pine (Hallgren et al., 1990), and forest decline and photoinhibition effects in Norway spruce (*Picea abies* (L.) Karst.)(Lichtenthaler and Rinderle, 1988; Welander et

al., 1994). Seasonal changes in F_v/F_m values have been used to assess dormancy in Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) (Hawkins and Lister, 1985), Scotch and lodgepole pines (*Pinus contorta* Dougl. ex Loud.) (Lindgren and Hallgren, 1993), and Norway spruce (Westin et al., 1995). Fisker et al. (1995) found chlorophyll fluorescence to be an accurate estimate of freeze damage in needles and seedling survival in Douglas fir, and could detect non-visible damage. However, fluorescence measurements were unable to predict cold hardiness prior to the temperature treatments. Likewise, Welander et al. (1994) were unable to use chlorophyll fluorescence to predict growth response after a night frost followed by high irradiation.

The only fluorescence study involving propagation examines stress levels during the acclimatization of micropropagated Transvaal daisy (*Gerbera jamesonii* Bol. ex Adlam) (Van Huylenbroech and Debergh, 1992). To our knowledge, no studies of *Taxus* or stem cutting propagation have involved chlorophyll fluorescence. Therefore, the objective of this study was to examine the differences in chlorophyll fluorescence among ten cultivars of Taxus x*media* (*Taxus baccata* L. x *T. cuspidata* Sieb. & Zucc.), to study changes in chlorophyll fluorescence over the course of propagation in these cultivars, and to correlate initial chlorophyll fluorescence values with rooting.

MATERIALS AND METHODS

Ten cultivars of *Taxus* x*media* were selected for this two year study: Brownii, Dark Green Pyramidalis (first year only), Dark Green Spreader,

Densiformis, Densiformis Gem, Hicksii, L.C. Bobbink, Runyan, Tauntoni, and Wardii. Trials were performed at two locations, Michigan State University, East Lansing, MI (MSU) and Zelenka Nursery, Grand Haven, MI, for the first year of the study, and solely at Zelenka Nursery the second year.

Propagation procedures were similar for all trials. In early fall, 15 - 20 cm cuttings were taken from field grown plants at Zelenka Nursery. Cuttings were bagged in plastic and placed in cold storage at various temperatures. Needles were taken for initial chlorophyll fluorescence readings prior to placement in cold storage. After 4 - 5 weeks in cold storage, cuttings were cut to 11.4 cm (apical and basal portions removed), treated with Woods Rooting Hormone (IBA 1.03%; NAA 0.66%) at 2800 ppm (1:5 ratio), and placed into 100% perlite. Cuttings were placed in rows 3.8 cm apart with 1.3 cm between individual cuttings within each row. Cuttings were watered as needed until harvest in the spring, at which time they were gently uprooted, and root lengths rated (0=less than 2.54 cm [1 inch], 1=less than 5.08 cm [2 inch], 2=less than 7.62 cm [3 inch], 3=greater than 7.62 cm), and root numbers recorded. Roots were dried at 28 °C for five days and weighed (Explorer Balance, Ohaus Corp., Florham Park, NJ). Chlorophyll fluorescence measurements were recorded periodically throughout the propagation process.

Greenhouse conditions differed between the two locations. Zelenka propagation benches were 15 cm deep and received bottom heat. MSU growth trays were 7.6 cm deep and received no bottom heat. Greenhouse temperatures at Zelenka were maintained at 18 °C, while MSU temperatures were difficult to

control, often exceeding 21 °C and sometimes reaching 32 °C during a sunny day.

The 1997-1998 trial at MSU consisted of a randomized complete block design of six blocks and ten cultivars, for a total of 600 cuttings. Cuttings were taken Oct. 14 and stored in cold storage at MSU (5 °C) until stuck on Nov. 22. Harvest took place May 21. Chlorophyll fluorescence readings were taken, ten per cultivar, on Oct. 14, Oct. 30, and Nov. 18. Four readings were taken per cultivar, per block on Dec. 3, Dec. 18, Jan. 6, Jan. 29, Mar. 3, April 6, and May 6.

The 1997-1998 trial at Zelenka Nursery was designed similarly to the MSU trial, however, only four of the six blocks were harvested. Cuttings were taken Oct. 14, stored in cold storage at Zelenka in open buckets at 2.5 °C, and stuck Nov. 20. Harvest took place May 27. Chlorophyll fluorescence readings were taken, ten per cultivar, on Oct. 14, Oct. 30, and Nov. 20. Four readings were taken per cultivar, per block, on Dec. 4, Jan. 8, Jan. 30, Feb. 27, April 3, and May 7.

The 1997-1998 chlorophyll fluorescence measurements were taken with a Morgan CF-1000 (P.K. Morgan Instruments, Inc., Andover, MA). For each measurement (F_v/F_m), the underside of a single, randomly selected needle was measured at a light level of 700 μmol /m^2/s. Samples were dark adapted for 15 minutes before measurement in the manufacturer's plastic/foam clips. Chlorophyll fluorescence measurements were usually taken the same day as the needles were collected, although occasionally logistics required needles to be stored overnight. This was accomplished in a germination tray covered with moist

paper towels and stored at 5 °C. Needles were acclimated to room temperature at least one hour prior to fluorescence measurements. No distortion of values was observed as a result of overnight storage.

The 1998-1999 trial at Zelenka was performed similarly to the previous trials with a few exceptions. Cuttings were taken Oct. 29 and a fluorescence reading taken for each individual cutting. Cuttings were rolled in plastic, to preserve their order, and placed in MSU cold storage (5 °C) until Dec. 1, when they were stuck at Zelenka. The experimental design was a randomized complete block with four blocks and nine cultivars, using 540 cuttings total. Dark Green Pyramidalis was not tested due to a shortage of material. Chlorophyll fluorescence readings were taken on each individual cutting on Oct. 30, Nov. 30, and Mar. 10, and a sample of 6 readings per cultivar, per block, was taken Jan. 7 and Feb. 3. Needles for all tests were randomly selected. Cuttings were harvested Mar. 10, which was three months earlier than the previous year in efforts to measure greater differences in rooting percentage between cuttings.

The 1998-1999 chlorophyll fluorescence measurements were taken with a Plant Efficiency Analyzer (PEA) (Hansatech Instruments Ltd., Norfolk, England, U.K.). Samples were dark adapted for 15 minutes in the manufacturer's plastic/foam clips, and a fluorometer light level of 1200 $\mu mol/m^2/s$ (40% of maximum capacity) was determined sufficient to saturate PSII, according to the manufacturer's instructions.

Statistical Analysis. Data was subjected to statistical analysis consisting of ANOVA and LSD tests performed on each trial of the harvest and chlorophyll

fluorescence data. Correlation calculations were done between individual cutting chlorophyll fluorescence and harvest data, overall and within cultivars, for the 1998 -1999 season (proc anova, proc corr, SAS/PC software, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Initial Chlorophyll Fluorescence

Differences were found in F_v/F_m values among cultivars for the 1997-1998 data (Table 1), however no individual mean was different from all others. Values ranged from .730 (relative units) for Wardii to .873 for Dark Green Pyramidalis. Subsequent readings for the 1997-1998 year never exceeded these initial values.

Similarly, differences were found among cultivars for the 1998-1999 season, but cultivar ranges overlapped extensively (Table 2). Values ranged from .778 for Brownii to .833 for Dark Green Spreader. Numbers are higher than for the 1997-1998 season, however, between-year comparisons are dangerous because different fluorometers were used for each season.

These readings suggest relatively healthy cutting material. Bjorkman and Demmig (1987) found the F_v/F_m ratio of unstressed, healthy conifers to be about .853 +/- .004. It is possible that larger cultivar differences may have been found if a non-ratio parameter had been used to measure chlorophyll fluorescence, such as F_o or F_m.

Chlorophyll Fluorescence Over the Course of Propagation

Changes in F_v/F_m over the course of propagation were measured biweekly and then monthly in the first year trials at Zelenka (Fig. 1) and MSU (Fig. 2), and monthly in the second year trial at Zelenka (Fig. 3). Generally, F_v/F_m starts out high, declines over the cold storage duration and sticking, and then increases as rooting occurs and growth resumes.

The 1997-1998 Zelenka data (Figure 1) represents the most ideal environmental conditions of the three trials. There is a clear decline in F_v/F_m over the 37 days of storage as the cuttings enter dormancy. This decline continues for more than 13 days after sticking, pointing to the creation of new stresses in the planting process. Rooting occurred approximately 75 days after severance from the stock plant, at which point an increase in F_v/F_m is observed. This increase continues as a general trend overall, until harvest when there is a slight drop in F_v/F_m. This drop could be caused by any number of factors associated with the warm, sunny May weather (photoinhibition, heat stress) or transplanting preparation (water stress).

The 1997-1998 MSU data shows a different picture (Fig. 2). There is a decrease in F_v/F_m over cold storage (39 days), however, it is not as consistent as in the Zelenka trial, perhaps due to the warmer storage conditions at MSU (5 °C vs. 2.5 °C). Readings increased quickly after sticking and peaked by day 50, long before rooting occurred. Readings then show a slight, gradual decline until harvest. This pattern may be a reflection of the warmer greenhouse conditions at

MSU, where days were commonly above 21 °C. As a result, cuttings resumed needle growth before rooting, which occurred over a month later than at Zelenka.

Although there were fewer data collection dates in the 1998 -1999 Zelenka trial, it shows a new picture as well (Fig. 3). A clear decrease in F_v/F_m can be seen during the 32 storage days. At sticking, we see the most pronounced F_v/F_m differences in cultivars of any time during the trial (Table 3). In contrast to the other trials, which started out with their highest chlorophyll fluorescence levels, these F_v/F_m values are higher 38 days after sticking than at any other point measured in the trial. A slight decline in values continues until harvest, when cultivar differences are, once again, indistinct (Table 4). The rapid increase in photosynthetic efficiency after sticking may be a result of the unseasonably warm and sunny weather during the 1998-1999 spring, disabling Zelenka from keeping greenhouse temperatures as cool as desired.

Rooting Data

Root dry weight, number, and length data provide information on the quality of the rooted cutting, which could influence future growth when planted in the field as liners. Root dry weight, root number, and root length were correlated so only data for root dry weight are presented.

The 1997-1998 MSU harvest data presented here includes rooting percentages (Table 5), and root dry weights (Table 6). Differences in these measurements were seen among cultivars, however, individual cultivar means were generally indistinct. Rooting percentages ranged from a very low L.C.

Bobbink (31.7%) to Densiformis (96.7%), with much variation within cultivars. Root dry weight values ranged from .042 g (L.C. Bobbink) to .225 g (Densiformis Gem) and root numbers from 2.5 (L.C. Bobbink) to 15 (Densiformis).

The 1997-1998 Zelenka harvest resulted in extremely high rooting percentages. There were no detectable differences in rooting percentages among cultivars, which ranged from 97.5 to 100% rooting (data not presented). Cultivar differences were found with dry weight values, and root length ratings, which were both slightly higher than at MSU, and root numbers, which were twice as high as at MSU (data not presented). These results may be a response to the more favorable environmental conditions at Zelenka, where the lack of environmental stress did not allow for separation among cultivars in regards to tolerance as it did at MSU. Increases may reflect the much earlier rooting and depth of the propagation beds at Zelenka Nursery.

The 1998-1999 Zelenka cuttings were harvested 132 days after cutting, instead of 225 as in the previous year, in an attempt to find larger differences in the harvest data. Rooting percentages ranged from 45.0% (Densiformis) to 96.6% (Brownii) (Table 7). Dry weights (Table 8), root numbers, and root lengths (data not presented) were lower than previously, likely due to the earlier harvest date. Even though Dark Green Spreader (48.5%), Runyan (46.7%), and Densiformis (45.0%) rooted poorly, the results from the previous year suggest that with sufficient time, they would have rooted in high percentages.

Unexpectedly, the relationships between cultivars differed greatly in all three trials. The lowest rooting cultivars at MSU during 1997 - 1998 were some of

the highest rooting cultivars at Zelenka during the 1998 - 1999 trial. Environmental or environmental/cultivar interactions seem to play greater roles determining chlorophyll fluorescence and harvest characteristics than cultivar genetics. One can only speculate on the many environmental factors which may have differed between the trials, years, and locations. Differing greenhouse conditions were surely a factor between the two locations and the two years were accompanied by seasonal weather differences. Cultivar traits affecting timing of rooting may have come into play in the early harvest imposed the second year and results may not be representative of what final May harvest results would have been.

Correlations

For the 1998-1999 data, each individual cutting is associated with a specific collection, sticking, and harvest F_v/F_m reading, and specific harvest data. No strong correlations were found, experiment-wide (Table 9) or within-cultivar (Table 10) between any of the F_v/F_m measurements and any of the harvest data. However, some very weak correlations existed at the P<= .05 level. In the experiment as a whole, correlations existed between initial chlorophyll fluorescence and root number (-.142) and root rating (-.208), chlorophyll fluorescence at time of sticking and dry weight (.103), and harvest chlorophyll fluorescence and rooting percentage (.127). At the cultivar level, L.C. Bobbink showed a correlation of initial chlorophyll fluorescence and root rating (-.269). Chlorophyll fluorescence at sticking was found correlated with root number in

Hicksii (.326). Harvest chlorophyll fluorescence was found correlated with rooting percentage (.395) and root number (-.796) in Dark Green Spreader, rooting percentage (.430) in Hicksii, and rooting percentage (.274) in Tauntoni. These correlations, although statistically significant, are too small to be useful.

Conclusions

Although differences in F_v/F_m exist among cultivars, they are not sufficiently distinct to enable chlorophyll fluorescence to be used as a cultivar identification tool. Since all cuttings showed initially relatively healthy readings, perhaps chlorophyll fluorescence is not a good tool for identifying unhealthy stock (or perhaps there was no unhealthy stock).

Trends in F_v/F_m values traced over time seem to be highly affected by local environmental conditions. This makes comparisons between years and locations difficult because of the multitude of different factors to consider. It also complicates attempts to use chlorophyll fluorescence as a stock plant quality measure, since each field will have its own unique set of local conditions.

There is a lack of correlation between chlorophyll fluorescence, measured as F_v/F_m, and rooting percentage, root number, root dry weight, and root length in the ten *Taxus* cultivars examined. This may indicate that photosynthetic stress is not a significant factor in determining *Taxus* rooting characteristics or that F_v/F_m is not an accurate measurement of stress in *Taxus*. Regardless, we would not consider chlorophyll fluorescence measurements a practical method for determining stock plant rooting ability in *Taxus*.

Inadequate control of unwanted variation is a potential source of error. Chlorophyll fluorescence measurements have been shown to be affected by temperature differences, photoinhibition, and other seasonal environmental effects, as well as top and bottom leaf surfaces, sun and shade leaves, needle age, storage time before measurement, and dehydration. Chlorophyll fluorescence values may be far too sensitive to these immediate conditions for the general type of health rating that we're looking for.

Only one chlorophyll fluorescence parameter was used, F_v/F_m. This may not have been the best parameter for our purposes. Perhaps F_o or F_m individually would have served our purposes better or a measurement which included quenching effects such as an Rfd value ($F_m - F_s / F_m$).

A useful approach for further studies might be one which determines the amount of variation present in a branch, individual plant, or field of *Taxus*. This would enable estimations of necessary sample size and help determine the feasibility of potential chlorophyll fluorescence uses, in both logistical and cost/benefit terms.

Unknown are the daily and seasonal fluctuations of F_v/ F_m in *Taxus*. Information on these is vital to the appropriate evaluation of stand-alone measurements taken in different years or under different climatic conditions. Is F_v/ F_m affected by stress in *Taxus*, and at what stress intensities? What stresses affect F_v/ F_m the most in *Taxus*? Cold stress? Dormancy? Photoinhibition? How does this stress detection compare with visual observation?

Although common in stress studies, F_v/F_m may not be the best chlorophyll fluorescence parameter for examining *Taxus*. Other parameters may be more effective and should be at least minimally investigated.

LITERATURE CITED

Bjorkman, O. and B. Demmig. 1987. Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta 170:489-504.

Davidson, H. and A Olney. 1964. Clonal and sexual differences in the propagation of *Taxus*, p. 156-161. In: International Plant Propagator's Society Combined Proceedings, vol. 14.

Eccher, T. 1988. Response of cuttings of 16 *Taxus* cultivars to rooting treatments. Acta Horticulturae 227:251-253.

Fisker, S., R. Rose, and D.L. Haase. 1995. Chlorophyll fluorescence as a measure of cold hardiness and freezing stress in 1+1 Douglas-Fir seedlings. Forest Sci. 41(3):564-575.

Hallgren, J.E., T. Lundmark, and M. Strand. 1990. Photosynthesis of Scots pine in the field after night frosts during summer. Plant Physiol. Biochem. 28(4):437-445.

Hawkins, C.D.B. and G.R. Lister. 1985. *In vitro* chlorophyll fluorescence as a possible indicator of the dormancy state in Douglas-fir seedlings. Can. J. For. Res. 15:607-612.

Heinstein, P.F. and C.J. Chang. 1994. Taxol. Annu. Rev. Plant Physiol. Plant Mol. Biol. 45:663-674.

Lichtenthaler, H.K. and U. Rinderle. 1988. Chlorophyll fluorescence signatures as vitality indicator in forest decline research, p. 143-149. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Lindgren, K. and J.E. Hallgren. 1993. Cold acclimation of *Pinus contorta* and *Pinus sylvestris* assessed by chlorophyll fluorescence. Tree Physiol. 13:97-106.

Saarinen, T. 1993. Chlorophyll fluorescence, and nitrogen and pigment content of Scots pine (*Pinus sylvestris*) needles in polluted urban habitats. Ann. Bot. Fennici 30:1-7.

Saarinen, T. and J. Liski. 1993. The effect of industrial air pollution on chlorophyll fluorescence and pigment contents of Scots pine (*Pinus sylvestris*) needles. Euro. J. For. Pathol. 23:353-361.

Van Huylenbroech, J. and P. Degergh. 1992. Acclimation of micropropagated

Gerbera jamesonii; Use of chlorophyll fluorescence. Forum for Applied Biotechnology. Brugge (Belgium). 24-25 Sept. Mededelingen-van-de-Faculteit-Landbouwwetenschappen-Rijksuniversiteit-Gent (Belgium). 57(4a):1595-1579.

Welander, N.T., P. Gemmel, O. Hellgren, and B. Ottosson. 1994. The consequences of freezing temperatures followed by high irradiance on *in vivo* chlorophyll fluorescence and growth in *Picea abies*. Physiol. Plant. 91:121-127.

Westin, J., L.G. Sundblad, and J.E. Hallgren. 1995. Seasonal variation in photochemical activity and hardiness in clones of Norway spruce (*Picea abies*). Tree Physiol. 15:685-689.

TABLE 1. 1997 -1998 Initial chlorophyll fluorescence (F_v/F_m) of ten cultivars of *Taxus xmedia* from stock plant material at Zelenka Nursery

Cultivar	F_v/F_m	
Dark Green Pyramidalis	0.873	a
Tauntoni	0.851	a b
Densiformis	0.826	b c
Hicksii	0.819	b c
Runyan	0.817	b c
Dark Green Spreader	0.815	b c
Densiformis Gem	0.791	c d
Brownii	0.772	d
L.C. Bobbink	0.760	d e
Wardii	0.730	e

Mean separation among cultivars by LSD, P 0.05.

TABLE 2. 1998-1999 Initial chlorophyll fluorescence (F_v/F_m) of nine cultivars of *Taxus xmedia* from stock plant material at Zelenka Nursery

Cultivar	F_v/F_m	
Dark Green Spreader	0.833	a
Hicksii	0.832	a
Densiformis Gem	0.828	a b
L.C. Bobbink	0.825	a b c
Runyan	0.822	a b c d
Densiformis	0.821	b c d
Wardii	0.814	c d
Tauntoni	0.811	d
Brownii	0.778	e

Mean separation among cultivars by LSD, P 0.05.

TABLE 3. 1998 -1999 Chlorophyll fluorescence (F_v/F_m) of stem cuttings at sticking (after 32 days of cold storage at 5 °C).

Nine cultivars of *Taxus xmedia* at Zelenka Nursery.

Cultivar	F_v/F_m					
Hicksii	0.732	a				
Dark Green Spreader	0.719	a	b			
L.C. Bobbink	0.700		b	c		
Wardii	0.693			c		
Runyan	0.659				d	
Densiformis Gem	0.653				d	
Tauntoni	0.611					e
Brownii	0.563					f
Densiformis	0.553					f

Mean separation among cultivars by LSD, P 0.05.

TABLE 4. 1998 -1999 Chlorophyll fluorescence (F_v/F_m) of stem cuttings at harvest (after 32 days of cold storage at 5 °C and 100 days in the propagation bed).

Nine cultivars of *Taxus xmedia* at Zelenka Nursery.

Cultivar	F_v/F_m	
Hicksii	0.848	a
Dark Green Spreader	0.843	a b
Runyan	0.840	a b
L.C. Bobbink	0.835	a b
Brownii	0.835	a b
Densiformis Gem	0.834	a b
Densiformis	0.832	b
Wardii	0.828	b
Tauntoni	0.807	c

Mean separation among cultivars by LSD, P 0.05.

TABLE 5. 1997 -1998 Rooting percentage of ten cultivars of *Taxus xmedia* at Michigan State University.

Cultivar	Rooting %	
Densiformis	96.7	a
Wardii	88.3	a b
Densiformis Gem	85.0	a b c
Dark Green Pyramidalis	76.3	b c d
Runyan	70.0	c d e
Hicksii	65.0	d e
Dark Green Spreader	61.7	d e f
Tauntoni	56.7	e f
Brownii	46.7	f g
L.C. Bobbink	31.7	g

Mean separation among cultivars by LSD, P 0.05.

TABLE 6. 1997 -1998 Mean root dry weights of ten cultivars of *Taxus xmedia* at Michigan State University.

Cultivar	Dry Weight (g)	
Densiformis Gem	0.225	a
Wardii	0.223	a
Densiformis	0.202	a b
Runyan	0.184	a b
Brownii	0.166	b c d
Tauntoni	0.133	c d
Hicksii	0.131	c d e
Dark Green Spreader	0.117	d e
Dark Green Pyramidalis	0.087	e
L.C. Bobbink	0.042	f

Mean separation among cultivars by LSD, P 0.05.

TABLE 7. 1998 -1999 Rooting percentage of nine cultivars of *Taxus xmedia* at Zelenka Nursery.

Cultivar	Rooting %	
Brownii	96.6	a
L.C. Bobbink	95.0	a
Hicksii	86.7	a b
Densiformis Gem	83.3	a b c
Tauntoni	75.0	b c
Wardii	71.7	c
Dark Green Spreader	48.5	d
Runyan	46.7	d
Densiformis	45.0	d

Mean separation among cultivars by LSD, P 0.05.

TABLE 8. 1998 -1999 Mean root dry weights of nine cultivars of *Taxus xmedia* at Zelenka Nursery.

Cultivar	Dry Weight (g)	
L.C. Bobbink	0.087	a
Brownii	0.084	a
Hicksii	0.075	a
Wardii	0.058	b
Densiformis Gem	0.057	b
Runyan	0.053	b
Dark Green Spreader	0.045	b c
Densiformis	0.033	c d
Tauntoni	0.023	d

Mean separation among cultivars by LSD, P 0.05.

TABLE 9. 1998 -1999 *Taxus xmedia* chlorophyll fluorescence (F_v/F_m) correlated with harvest data at Zelenka Nursery at the whole-experiment level. Initial (F_v/F_m) was taken at cutting collection, sticking (F_v/F_m) after 32 days cold storage at 5 °C, and harvest (F_v/F_m) after 100 days in the propagation bed.

	Pearson correlation coefficients			
Chlorophyll fluorescence	Rooting percentage	Root dry weight	Root number	Root length rating
Initial (F_v/F_m)	-0.029	-0.079	-0.142*	-0.208**
Sticking (F_v/F_m)	0.029	0.103*	0.096	-0.013
Harvest (F_v/F_m)	0.127**	0.040	0.007	0.038

*, ** Significant at P <= 0.05 or 0.01, respectively

TABLE 10. 1998 -1999 *Taxus xmedia* chlorophyll fluorescence (F_v/F_m) correlated with harvest data at Zelenka Nursery at the cultivar level. Initial (F_v/F_m) was taken at cutting collection, sticking (F_v/F_m) after 32 days cold storage at 5 °C, and harvest (F_v/F_m) after 100 days in the propagation bed.

Cultivar	Chlorophyll fluorescence (F_v/F_m)	Rooting percentage	Root dry weight	Root number	Root length rating
Brownii	initial	0.135	-0.053	0.140	-0.066
	sticking	-0.127	0.039	-0.039	0.174
	harvest	-0.063	-0.089	-0.142	-0.238
Dark Green Spreader	initial	0.136	0.034	-0.086	-0.012
	sticking	0.112	-0.150	-0.132	-0.185
	harvest	0.395**	0.017	-0.796*	0.172
Densiformis	initial	0.052	-0.020	0.779	0.005
	sticking	0.000	-0.015	0.529	0.138
	harvest	0.240	-0.130	0.393	-0.134
Densiformis Gem	initial	0.124	0.091	0.240	0.194
	sticking	-0.068	0.048	0.224	0.105
	harvest	-0.029	0.009	-0.043	0.112

Hicksii	initial	0.091	-0.066	0.059	-0.068
	sticking	0.067	0.130	0.326*	-0.036
	harvest	0.430**	0.109	0.137	0.090
L.C. Bobbink	initial	-0.151	-0.144	-0.025	-0.269*
	sticking	-0.140	0.085	-0.139	0.026
	harvest	0.159	-0.179	-0.019	-0.075
Runyan	initial	-0.014	-0.091	0.154	0.096
	sticking	0.186	0.176	-0.372	0.193
	harvest	0.189	0.246	0.035	0.005
Tauntoni	initial	0.060	-0.055	-0.737	0.058
	sticking	-0.080	-0.134	-0.773	-0.006
	harvest	0.274*	-0.108	0.898	0.093
Wardii	initial	0.125	0.156	-0.454	0.137
	sticking	-0.161	-0.059	-0.082	-0.064
	harvest	0.228	0.185	0.190	0.215

*, ** Significant at P <= 0.05 or 0.01, respectively

LIST OF FIGURES

Figure 1. Chlorophyll Fluorescence in *Taxus* over the course of Propagation at Zelenka Nursery 1997-1998 Season

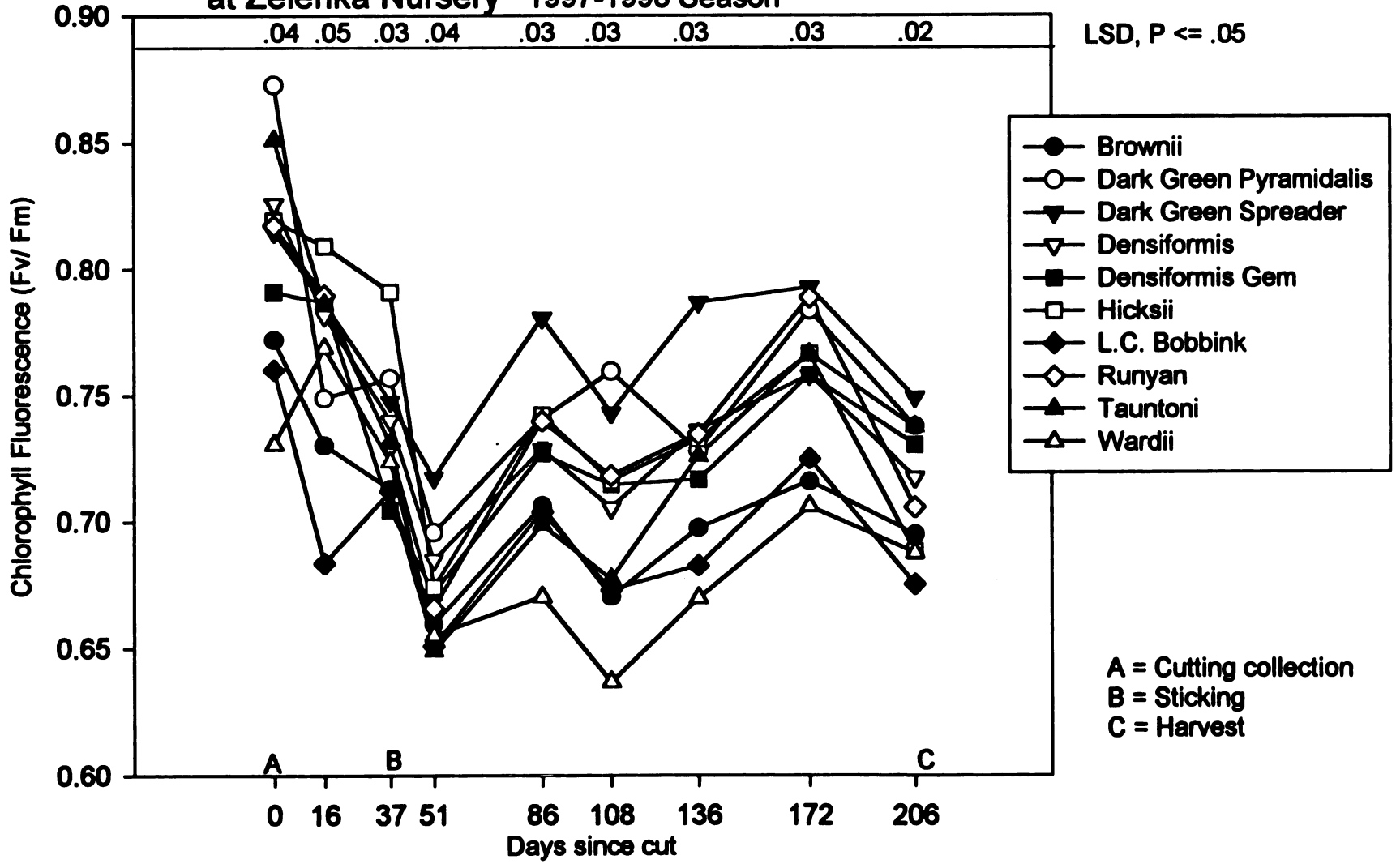


Figure 2. Chlorophyll Fluorescence in *Taxus* over the course of Propagation at MSU 1997-1998 Season

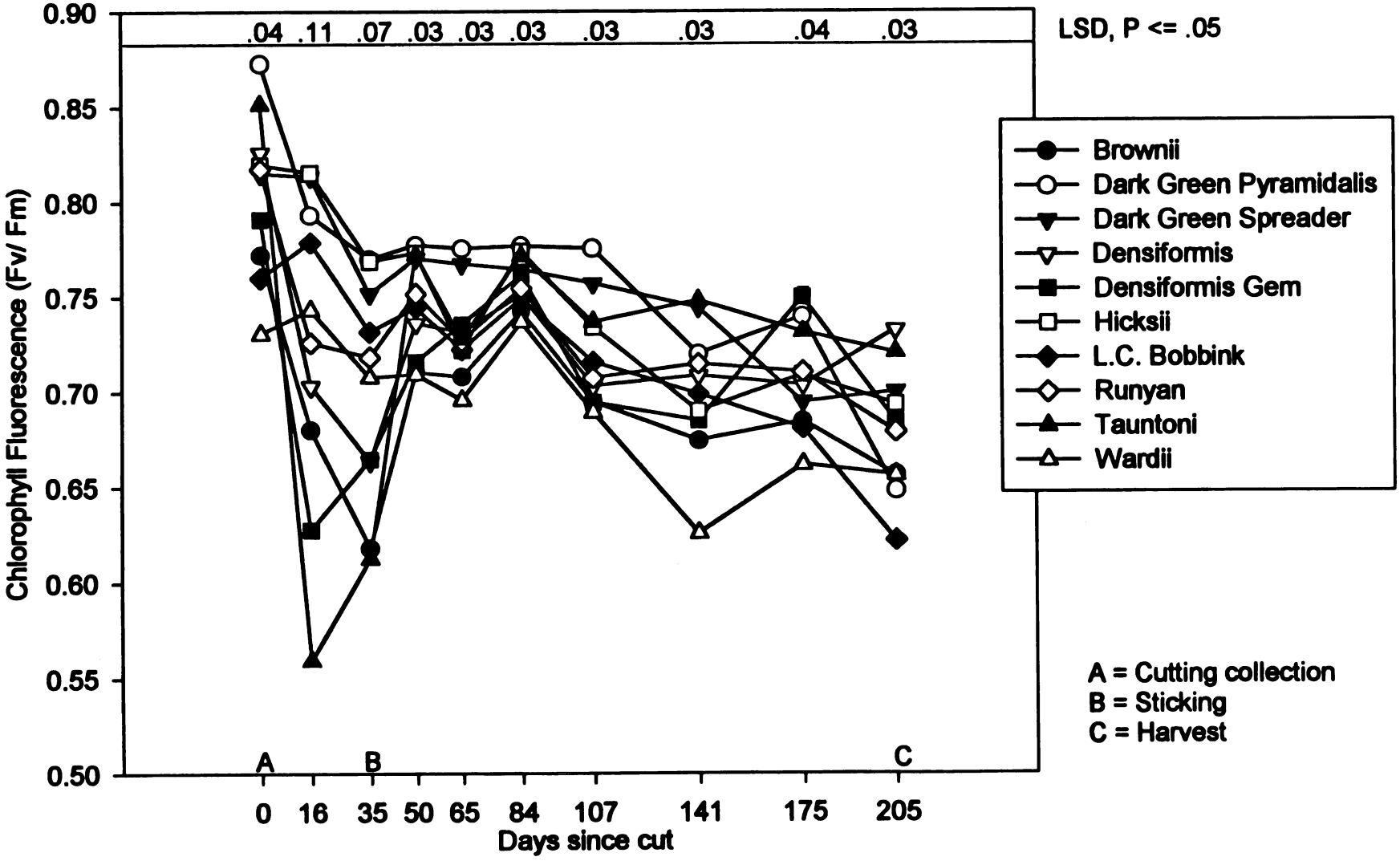
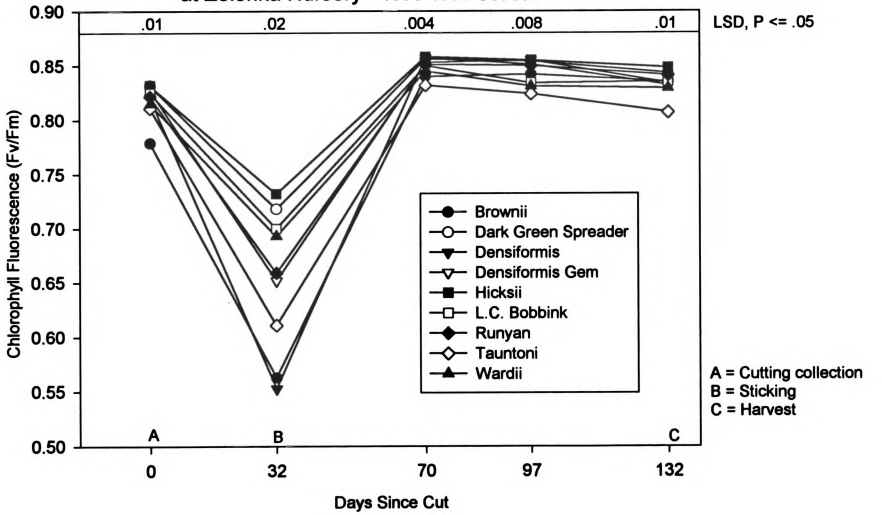


Figure 3. Chlorophyll Fluorescence in *Taxus* over the course of Propagation at Zelenka Nursery 1998-1999 Season



Chapter Two

Chlorophyll Fluorescence and Cold Storage of *Taxus* Cuttings

(Formatted according to publication guidelines of the American Society of Horticultural Science)

Chlorophyll Fluorescence and Cold Storage of *Taxus* Cuttings

S. E. Bruce[1] and D. B. Rowe[2]
Department of Horticulture, Michigan State University, East Lansing, MI 48824

Received for publication . Accepted for publication . This paper is a portion of a M.S. thesis submitted by S. E. Bruce.

Acknowledgement: This experiment was a Michigan State University study funded by: Zelenka Nursery in Grand Haven, MI, International Plant Propagator's Society, Michigan Nursery and Landscape Association, and Michigan Agricultural Experiment Station.

[1]Graduate research assistant
[2]Assistant professor

Propagation and Tissue Culture

Chlorophyll Fluorescence and Cold Storage of *Taxus* Cuttings

Additional index words. Yew, vegetative propagation, stem cuttings

Abstract. Propagators routinely use a cold storage treatment in the propagation of *Taxus* via stem cuttings. Since storage conditions are a major factor in cutting quality and subsequent rooting, the objective of this study was to examine the effect of different storage temperatures, desiccations, and durations in four cultivars of *Taxus xmedia* (*Taxus baccata* L. x *T. cuspidata* Sieb. & Zucc.), quantifying stress in relation to subsequent rooting using chlorophyll fluorescence measurements (F_v/F_m). Storage temperatures used included -30, -2.5, 0, 2.5, 5, 10, and 20 °C. Desiccation levels were created with closed, perforated, and open plastic bags, and storage durations consisted of 34, 70, 107 days. Cultivars Brownii, Dark Green Spreader, Hicksii, and Wardii were used. Chlorophyll fluorescence readings were taken initially, at sticking, and at harvest and rooting percentage, root dry weight, root number, and root length were measured. Temperatures of -2.5 to 2.5 were found to be ideal, and desiccation was not found to influence rooting at these temperatures. Longer storage durations (70 and 107 days) had negative effects on all rooting data. Some differences were found to exist between cultivars in response to the various treatments. Chlorophyll fluorescence measurements could detect substandard storage conditions only at temperature and desiccation extremes.

Propagation of *Taxus* is achieved primarily through vegetative stem cuttings. Traditionally, 15 - 20 cm cuttings are taken between November and February, after natural exposure to cold temperatures (Sabo, 1976; Scheer, 1976; Verkade, 1976). Cuttings are stripped at their base, treated with an auxin-based rooting compound, and placed in a sand, perlite, or sand/perlite media under mist (Hartman et al., 1990). Nurseries involved in large-scale production today have adjusted this basic process to make it more efficient and cost effective. Smaller cuttings tend to be used, often 10 - 15 cm, and stripping is rare (Richey, 1986). Ideal hormone treatments are relatively low (~0.25 mM) IBA (Nandi et al., 1996) and some research indicates that a 0.08% thiamine addition may be beneficial (Chee, 1995). Although impractical for most nurseries, studies indicate that apical cuttings produce the highest rooting percentages (Eccher, 1988). Perhaps the most significant change has been the use of a cold storage treatment to ensure dormancy instead of mid-winter harvest of cuttings.

In order to tap into a large seasonal work force, harvest cuttings in hospitable weather conditions, and provide for a longer rooting period in the propagation beds, nurseries collect cuttings in early fall (September or October), often before the first frost, and store them in a cooler at 2 - 5 °C (Richey, 1986). Humidity is kept at 85 - 90% (Richey, 1986). Sticking is done a month or so later. This process allows propagators to control cold exposure cuttings receive, ensuring sufficient cold duration and consistency among cuttings.

Chlorophyll Fluorescence

Chlorophyll fluorescence measurements were utilized to measure stress because of their potential as a quantitative and objective method for evaluating storage conditions.

Chlorophyll fluorescence is created when light energy, absorbed by chlorophyll a, exceeds the photochemical processing capacity of photosystem II (PSII). One way this 'extra' energy is dissipated is by being re-emitted as light, which we call chlorophyll fluorescence. The fluorescence measured at physiological temperatures is largely a product of chlorophyll a molecules involved in PSII, although other light capturing pigments do fluoresce, and PSI fluorescence can be measured. Since chlorophyll fluorescence levels are tied to the amount of light energy *not* used for photosynthetic processes, they are inversely related to the amount of energy that *is* used for photosynthesis, and serve as indicators of plant photosynthetic potential.

Chlorophyll fluorescence measurement is increasingly used as an estimate of photosynthetic health. The emitted light signal follows a general intensity pattern known as the Kautsky Effect. Pre-darkened samples, with a minimum fluorescence level (F_o), show a 'fast rise' in fluorescence to a maximum value (F_m) upon exposure to a light source. As photochemical processing of the light energy increases, fluorescence values are gradually reduced to a steady state (F_s), somewhere between F_o and F_m. A common parameter used in stress studies is F_v/F_m, F_v being the variable fluorescence, calculated by subtracting F_o from F_m. Numerous studies have shown F_v/F_m, and other chlorophyll

fluorescence parameters, to be effective measurements of the photoefficiency of PSII. Bjorkman and Demmig (1987) linearly correlated F_v/F_m with the quantum yield of PSII, as determined by oxygen evolution, in a variety of stressed plants.

Studies utilizing chlorophyll fluorescence to quantify plant stresses include heat and cold stress, especially tolerance studies, photoinhibition, mineral nutrition, pollution, and water stress. Studies on conifers have found effects of vehicle and oil refinery pollution on Scotch pine (*Pinus sylvestris* L.) (Saarinen, 1993; Saarinen and Liski, 1993), photoinhibition effects in Scotch pine (Hallgren et al., 1990), and forest decline and photoinhibition effects in Norway spruce (*Picea abies* (L.) Karst.)(Lichtenthaler and Rinderle, 1988; Welander et al., 1994). Seasonal changes in F_v/F_m values have been used to assess dormancy in Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco) (Hawkins and Lister, 1985), Scotch and lodgepole pines (*Pinus contorta* Dougl. ex Loud.) (Lindgren and Hallgren, 1993), and Norway spruce (Westin et al., 1995). Fisker et al. (1995) found chlorophyll fluorescence to be an accurate estimate of freeze damage in needles and seedling survival in Douglas fir, and could detect non-visible damage. However, fluorescence measurements were unable to predict cold hardiness prior to the temperature treatments. Likewise, Welander et al. (1994) were unable to use chlorophyll fluorescence to predict growth response after a night frost followed by high irradiation. Camm and Lavender (1993) found cold-storage light levels to affect Fv/Fm in white spruce seedlings (*Picea glauca* [Moench] Voss) while jack pine seedlings (*Pinus banksiana* Lamb.) remained unaffected.

The only fluorescence study involving propagation examined stress levels during the acclimatization of micropropagated Transvaal daisy (*Gerbera jamesonii* Bol. ex Adlam) (Van Huylenbroech and Debergh, 1992). To our knowledge, no studies of *Taxus* or stem cutting storage have involved chlorophyll fluorescence. Since storage conditions are a major factor in cutting quality and subsequent rooting, the objective of this study was to examine the effect of different storage temperatures, desiccations, and durations in four cultivars of *Taxus xmedia* (*Taxus baccata* L. x *T. cuspidata* Sieb. & Zucc.), quantifying stress in relation to subsequent rooting using chlorophyll fluorescence measurements.

MATERIALS AND METHODS

Studies were conducted over the 1997-98 and 1998-99 propagation seasons. Although similar in many respects, test condition and treatment differences were such that the studies must be considered separate experiments.

The 1997-98 study examined four factors: cultivar, storage duration, storage desiccation, and storage temperature. Brownii, Dark Green Spreader, Hicksii, and Wardii cultivars of *Taxus xmedia* were used. Seven hundred and twenty, 10 – 15 cm cuttings were collected from each cultivar on Oct. 14, 1997, from fields at Zelenka Nursery, Grand Haven, MI. Chlorophyll fluorescence readings (F_v/F_m), were taken, 10 per cultivar, and cuttings were randomly divided into the desiccation and temperature treatments. The three desiccation treatments consisted of sealed plastic bags (low desiccation), sealed plastic bags with holes punched in them (medium desiccation), and open plastic bags (high

desiccation). These were then further divided into 5 coolers at Michigan State University, East Lansing, MI, set at 0, 2.5, 5, 10, and 20 °C. After 34 days in storage, half the cuttings from each treatment were removed to a Michigan State University greenhouse where they were cut to 11.4 cm (apical and basal portions removed), treated with Woods Rooting Hormone (IBA 1.03%; NAA 0.66%) at 2800 ppm (1:5 ratio), and placed in 100% perlite media in 7.6 cm deep growth trays. The experimental layout consisted of a split-plot design with three splits (duration, cultivar, and desiccation) and four blocks. Cuttings were stuck in rows 1.3 cm apart and the rows spaced 3.8 cm apart. Sixty-five days after the original cutting collection (Dec. 18), the remainder of the cuttings were removed from storage and planted similarly to the first set (rooting data from the 65 day duration not included in this analysis). All cuttings were watered as needed until spring. Greenhouse temperatures tended to range higher than desired, often rising above 21 °C on sunny days. At harvest, June 11-18, cuttings were gently uprooted, and root lengths rated (0=less than 2.54 cm [1 inch], 1=less than 5.08 cm [2 inch], 2=less than 7.62 cm [3 inch], 3=greater than 7.62 cm) and root numbers recorded. Roots were dried at 28 °C for 3 days and weighed (Explorer Balance, Ohaus Corp., Florham Park, NJ). Chlorophyll fluorescence measurements were taken of storage material at cutting collection, 10 per cultivar, and then 5 per treatment at 24, 35, 50, and 65 day storage duration (Oct. 14, Nov. 7, Nov. 18, Dec. 3, and Dec. 18, respectively).

The 1997-98 chlorophyll fluorescence measurements were taken with a Morgan CF-1000 (P.K. Morgan Instruments, Inc., Andover, MA). For each

measurement (F_v/F_m), the underside of a single, randomly selected needle was measured at a light level of 700 $\mu mol/m^2/s$. Samples were dark adapted for 15 minutes before measurement in the manufacturer's plastic/foam clips. Chlorophyll fluorescence measurements were usually taken the same day as the needles were collected, although occasionally logistics required needles to be stored overnight. This was accomplished in a germination tray covered with moist paper towels and stored at 5 °C. Needles were acclimated to room temperature at least one hour prior to fluorescence measurements. No distortion of values was observed as a result of overnight storage.

The 1998-99 study examined three factors, cultivar, storage duration, and storage temperature. Cultivars were the same as in the 1997-98 study. Two hundred eighty-eight cuttings per cultivar were collected, Oct. 29, 1998, from Zelenka Nursery. Cuttings were randomly divided into 3 duration treatments (34, 70, and 107 days in storage), 4 temperatures (-30, -2.5, 0, and 2.5 °C), and 4 blocks. A sample of 3 chlorophyll fluorescence (Fv/Fm) readings per treatment, per block was taken, and cuttings bagged in plastic and placed in their respective cold storage temperatures at MSU. After the determined amount of cold storage, cuttings were placed at Zelenka Nursery, Grand Haven, MI, in 15 cm deep propagation beds of 100% perlite. Propagation procedures were the same as in the 1997-98 study. The experimental layout consisted of a split-plot design with two splits (storage duration and cultivar) and four blocks. Sticking occurred on Dec. 3 (34 days cold storage), Jan. 7 (70 days cold storage), and Feb. 3 (107 days cold storage). Greenhouse temperatures were held at 18 °C with bottom

heat (21 °C) provided in the benches. Cuttings were watered as needed. The staggered planting dates led to staggered harvest dates of Mar. 8, April 15, and May 13, respectively, resulting in 96 - 99 days in the propagation bed for each storage duration level. The propagation season was shortened from the previous year in efforts to measure greater treatment differences. Harvest was conducted similarly to the 1997-98 season. In addition to the initial measurement, chlorophyll fluorescence readings were taken of the material in storage at 34, 70, and 107 days storage duration (Dec. 2, Jan. 5, and Feb. 3), and their respective harvest dates (5 samples per block per treatment).

The 1998-99 chlorophyll fluorescence measurements were taken with a Plant Efficiency Analyzer (PEA) (Hansatech Instruments Ltd., Norfolk, England, U.K.). Samples were dark adapted for 15 minutes in the manufacturer's plastic/foam clips, and a fluorometer light level of 1200 $\mu mol/m^2/s$ (40% of maximum capacity) was determined to be ideal, according to the manufacturer's instructions.

Statistical Analysis. Harvest and chlorophyll fluorescence data were subjected to statistical analysis consisting of an analysis of variance (ANOVA) and LSD tests (least significant difference) performed overall and at the individual cultivar level. Correlation calculations were done between the harvest data and fluorescence data (all dates) for each season (Proc anova, proc corr, SAS/PC software, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

The storage treatments, cultivar, duration, desiccation (1997-1998 season only), and temperature, combine with one another to create unique yet inseparable effects. Due to the interactions between all treatments, analysis is difficult. This report will examine each treatment individually with respect to its interaction with other treatments.

Storage Desiccation

Low, medium, and high storage desiccation treatments were performed in 1997-1998 (Figs. 1, 2). Results for root number and root length rating tended to correlate with root dry weight, so only root dry weight data are presented. Few desiccation effects were found in Brownii due to its low rooting percentages. Dark Green Spreader showed a decrease in harvest data as a result of desiccation at 0, 5, and 10 °C, with rooting percentage only being affected at 5 and 10 °C. Hicksii was little affected by desiccation, showing a decrease in root dry weight and length at 0 °C (data not presented) and rooting percent at 10 and 20 °C. Low desiccation tended to decrease the negative effects of higher temperatures, so that it was only at low desiccation that some rooting occurred at 20 °C. The sole effect of desiccation in Wardii was a decrease in rooting percentage at 10 and 20 °C. Wardii appeared more resistant to high temperatures at low desiccation than other cultivars, showing rooting percentages in the 10 and 20 °C treatments. Other cultivars succumbed to fungal rot in these treatments.

Effects of medium and high desiccation treatments were generally indistinct, suggesting that the actual desiccation levels may have been similar. Medium and high desiccations combined with 10 and 20 °C temperatures were universally lethal. Low desiccation allows minimal rooting at higher temperatures, especially in rot-resistant cultivars such as Wardii. At lower temperatures (0 to 5 °C) desiccation treatments had little effect.

Storage Duration

Storage durations of 34, 70, and 107 days were tested in the 1998-1999 study. Decreases in rooting percentage (Fig. 3), root dry weight (Fig. 4), root number (Fig. 5), and root length (Fig. 6) were found with each increase of storage duration. Decreases in rooting percentages were found in all cultivars at -2.5, 0, and 2.5 °C. The -30 °C treatment was lethal to all cuttings stored at that temperature. Brownii rooting percentages dropped in the 70 day extension of storage, but 70 and 107 day duration effects remained indistinguishable. Wardii, however, showed no significant difference in rooting percentages between the 34 and 70 day durations, only to decline with the 107 day duration. Dark Green Spreader and Hicksii showed a decrease in rooting percentage with each extension of duration. Root dry weight and root length decreased with duration in all cultivars, except Dark Green Spreader, where treatment effects were not significant. Root number showed an overall decrease with extended storage duration.

Preliminary studies showed storage duration effects to be of little consequence under unsuitable conditions such as higher desiccation and 10 and 20 °C temperatures. At low desiccation and cooler temperatures (-2.5 to 2.5 °C), increasing duration, from 34 to 70 days and 70 to 107 days, had negative effects on rooting percentage, root dry weight, root number, and root length.

Storage Temperature

Storage treatment temperatures in the 1997-1998 study consisted of 0, 2.5, 5, 10, and 20 °C (Figs. 1, 2). Brownii rooting percentages were so low that few effects of temperature could be seen. Dark Green Spreader rooting percentages showed little difference between 0 and 10 °C at low desiccation, however decreases in dry weight (Fig. 2), root number, and root length were observed (data not presented). At medium desiccation, 0 to 5 °C temperatures produced indistinguishable rooting percentages. At high desiccation, 0 and 2.5 °C produced the highest rooting data. Dry weight, root number, and root length showed no clear trend in most treatments. In Hicksii, decreases in dry weight, root number, and root length are seen with increasing temperature. Wardii dry weight, root number, and root length were little affected by temperature treatments. Rooting percentages decreased with increasing temperatures at medium and high desiccations. Overall, the highest root numbers and dry weights were produced at 0 °C. The clearest effect of temperature is seen in the high desiccation treatments.

Storage temperature was negatively correlated with rooting percent in Brownii (-0.535), Dark Green Spreader (-0.842), and Hicksii (-0.766) (Table 1). In Hicksii, it was also correlated with root length (-0.584), root number (-0.709), and root dry weight (-0.722).

The 1998-1999 storage temperature treatments consisted of -30, -2.5, 0 and 2.5 °C. No rooting was seen in the -30 °C treatments. Brownii rooting percentages peaked at increasing temperatures with increasing storage duration: in 34 day duration, -2.5 and 0 °C were ideal; in 70 day duration -2.5, 0, and 2.5 °C were ideal; in 107 day duration 2.5 °C was ideal. Rooting percentage was the only harvest parameter with significant temperature effects. In Dark Green Spreader, Hicksii, and Wardii, rooting data at -2.5, 0, and 2.5 °C was not significantly different.

Storage temperatures were negatively correlated with root number at the 34 day storage duration in Dark Green Spreader (-0.727), and root dry weight in the 70 day storage duration of Dark Green Spreader (-0.898) and 107 day storage duration of Hicksii (-0.632).

Little rooting occurred with the 10 and 20 °C treatments, except at low desiccation and short duration, and no rooting occurred with the -30 °C treatments. Slight freezing (-2.5 °C) showed no detrimental effects in most cases, however, the Brownii data suggests that slightly warmer temperatures may be best for longer term storage (107 day). Ideal storage temperatures fall between -2.5 and 2.5 °C, with 5 °C treatments often showing high rooting in low desiccation as well.

Chlorophyll Fluorescence during Storage

Data for chlorophyll fluorescence in 1997-1998 is pooled for the four cultivars (Fig. 7). At low desiccations, a decrease in chlorophyll fluorescence values is seen only in the 20 °C treatment. This decrease was visible by day 35. At medium and high desiccations, however, a decrease in chlorophyll fluorescence in the 20 °C treatment can be detected by day 24, and by day 35 or day 50, 10 °C shows a decrease in values as well. In general, 0, 2.5, and 5 °C showed almost no difference in chlorophyll fluorescence readings during storage. Wardii, as a cultivar, showed the most resilient chlorophyll fluorescence levels.

In the 1997-1998 data, chlorophyll fluorescence readings correlated with temperature from day 24 in all cultivars (Table 1). Correlations also existed with rooting percentages in Dark Green Spreader (day 24 = 0.539, day 35 = 0.709), Hicksii (day 24 = 0.660, day 35 = 0.723), and at day 35 in Wardii (0.556). These correlations grew stronger with increased duration in storage. In Hicksii, the day 35 chlorophyll fluorescence values were also correlated with root length (0.619) and dry weight (0.562).

The 1998-1999 storage study chlorophyll fluorescence data combines temperatures (-2.5, 0, and 2.5 °C) where no significant differences in chlorophyll fluorescence values were found (Fig. 8). Data for –30 °C treatments was eliminated due to 0 percent rooting (chlorophyll fluorescence levels declined sharply in storage, reaching the fluorometer detectable minimum by 107 days).

No significant decline in chlorophyll fluorescence levels over storage was found in any cultivar at -2.5, 0, or 2.5 °C.

In the 1998-1999 data, Brownii, Dark Green Spreader, Hicksii, and Wardii chlorophyll fluorescence values are sporadically correlated with temperature and the harvest parameters. Strangely, correlations made at day 34 or day 70 often are not found at day 107 or harvest. No correlation continues in time from the point of its appearance until harvest. Correlations found with the rooting data exist at harvest in Brownii (34 day storage root length rating [-0.618], 107 day storage rooting percent [0.805], root length rating [0.759], and root dry weight [0.918]), Dark Green Spreader (70 day storage rooting percent [0.701] and root length rating [-0.972]), and Hicksii (70 day storage rooting percent [0.643] and 107 day storage root number [-0.844]). Wardii plant material was apparently not randomly distributed, since initial chlorophyll fluorescence (before cuttings placed into storage) is correlated with storage temperature.

Potential error in these studies may lie in the wide temperature ranges used and the frequency of chlorophyll fluorescence measurements. An excess of interacting treatments complicated analysis and reduced the extent of possible conclusions. The majority of treatments showed non-significant trends in means, which suggests larger sample sizes be used in the future.

CONCLUSIONS

Grower storage conditions usually consist of a month of 2.5 to 5 °C temperatures with low desiccation. These conditions are ideal. Slightly cooler temperatures may be desirable (-2.5 to 2.5) although they may not be cost effective. A shorter, colder storage treatment is more desirable than a longer, warmer one, although slight increases in temperature or duration may have negligible effects, especially in certain cultivars. Cultivars Hicksii and Wardii are more resilient in undesirable storage conditions than cultivars Brownii and Dark Green Spreader. If warmer temperatures are necessary (5 to 10 °C) normal rooting percentages may be preserved with high humidity. Cuttings are unaffected by desiccation at 2.5 °C and below. Longer storage durations are to be avoided, as rooting percentages are often almost halved with each extra month of storage. Rooting percentages and root quality show large decreases with time in storage exceeding 34 days in ideal conditions, indicating that the negative effects of extended duration are not purely effects of plant material desiccation.

This study shows chlorophyll fluorescence measurements can detect high or low storage temperatures (20 and -30 °C) and desiccation effects in *Taxus* cutting material by 3 weeks into storage. Since temperature and desiccation closely affect rooting, chlorophyll fluorescence can serve as an indicator of rooting in extreme temperature and desiccation conditions. However, at low desiccation, chlorophyll fluorescence failed to consistently distinguish between -2.5, 0, 2.5, 5, and 10 °C treatments, a range within which there are clear rooting

differences. Chlorophyll fluorescence readings also failed to detect longer storage durations which lead to decreased rooting potential. Factors causing decreases in rooting of *Taxus* as a result of these stresses do not affect plant photosynthetic efficiency in a comparative manner. Chlorophyll fluorescence values, as measured by F_v/F_m ratios, are not useful indicators of storage conditions in *Taxus*.

LITERATURE CITED

Bjorkman, O. and B. Demmig. 1987. Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. Planta 170:489-504.

Camm, E.L. and D.P. Lavender. 1993. Photosynthetic apparatus in cold-stored conifer seedlings is affected by nursery and storage photoperiod. Forest Sci. 39(3):546-560.

Chee, P.P. 1995. Stimulation of adventitious rooting of *Taxus* species by thiamine. Plant Cell Reports 14:753-757.

Eccher, T. 1988. Response of cuttings of 16 *Taxus* cultivars to rooting treatments. Acta Horticulturae 227:251-253.

Fisker, S., R. Rose, and D.L. Haase. 1995. Chlorophyll fluorescence as a measure of cold hardiness and freezing stress in 1+1 Douglas-Fir seedlings. Forest Sci. 41(3):564-575.

Hallgren, J.E., T. Lundmark, and M. Strand. 1990. Photosynthesis of Scots pine in the field after night frosts during summer. Plant Physiol. Biochem. 28(4):437-445.

Hartman, H.T., D.E. Kester, and F.T. Davies, Jr. 1990. Plant Propagation: principles and practices, p. 600-601. Prentice Hall: Englewood Cliffs, NJ.

Hawkins, C.D.B. and G.R. Lister. 1985. *In vitro* chlorophyll fluorescence as a possible indicator of the dormancy state in Douglas-fir seedlings. Can. J. For. Res. 15:607-612.

Lichtenthaler, H.K. and U. Rinderle. 1988. Chlorophyll fluorescence signatures as vitality indicator in forest decline research, p. 143-149. In: H.K. Lichtenthaler (ed.). Applications of Chlorophyll Fluorescence. Kluwer Academic Publ., Dordrecht, The Netherlands.

Lindgren, K. and J.E. Hallgren. 1993. Cold acclimation of *Pinus contorta* and *Pinus sylvestris* assessed by chlorophyll fluorescence. Tree Physiol. 13:97-106.

Nandi, S.K., L.M.S. Palni, and H.C. Rikhari. 1996. Chemical induction of adventitious root formation in *Taxus baccata* cuttings. Plant Growth Regulation 19:117-122.

Richey, M. 1986. Sticking *Taxus* as unstripped cuttings, an update, p. 597-599. In: International Plant Propagator's Society Combined Proceedings, vol. 36.

Saarinen, T. 1993. Chlorophyll fluorescence, and nitrogen and pigment content of Scots pine (*Pinus sylvestris*) needles in polluted urban habitats. Ann. Bot. Fennici 30:1-7.

Saarinen, T. and J. Liski. 1993. The effect of industrial air pollution on chlorophyll fluorescence and pigment contents of Scots pine (*Pinus sylvestris*) needles. Euro. J. For. Pathol. 23:353-361.

Sabo, J.E. 1976. Propagation of *Taxus* in northern Ohio, p. 174-176. In: International Plant Propagator's Society Combined Proceedings, vol. 26.

Scheer, C. 1976. *Taxus* propagation by cuttings, p. 173-174. In: International Plant Propagator's Society Combined Proceedings, vol. 26.

Van Huylenbroech, J. and P. Degergh. 1992. Acclimation of micropropagated *Gerbera jamesonii*; Use of chlorophyll fluorescence. Forum for Applied Biotechnology. Brugge (Belgium). 24-25 Sept. Mededelingen-van-de-Faculteit-Landbouwwetenschappen-Rijksuniversiteit-Gent (Belgium). 57(4a):1595-1579.

Verkade, G. 1976. Propagation of *Taxus* by cuttings, p. 177. In: International Plant Propagator's Society Combined Proceedings, vol. 26.

Von Kornya, J.P. 1976. Propagation of *Taxus* cuttings, p.178-179. In: International Plant Propagator's Society Combined Proceedings, vol. 26.

Welander, N.T., P. Gemmel, O. Hellgren, and B. Ottosson. 1994. The consequences of freezing temperatures followed by high irradiance on *in vivo* chlorophyll fluorescence and growth in *Picea abies*. Physiol. Plant. 91:121-127.

Westin, J., L.G. Sundblad, and J.E. Hallgren. 1995. Seasonal variation in photochemical activity and hardiness in clones of Norway spruce (*Picea abies*). Tree Physiol. 15:685-689.

TABLE 1. 1997 - 1998 *Taxus xmedia* chlorophyll fluorescence (F_v/F_m) and storage temperature treatments (0, 2.5, 5, 10, and 20 °C) correlated with harvest data (Pearson correlation coeffecients). Storage duration = 34 days.

Brownii

	Temperature °C	Rooting Percent	Root Length Rating	Root Number	Root Dry Weight
F_v/F_m Day 24	-0.765**	0.286	-0.178	-0.048	-0.421
F_v/F_m Day 35	-0.811**	0.361	-0.584	0.148	-0.685
Temperature		-0.535*	-0.015	-0.127	-0.240

Dark Green Spreader

	Temperature °C	Rooting Percent	Root Length Rating	Root Number	Root Dry Weight
F_v/F_m Day 24	-0.714**	0.539*	-0.307	-0.499	-0.402
F_v/F_m Day 35	-0.787**	0.709**	-0.398	-0.536	-0.556
Temperature		-0.842**	-0.412	-0.204	-0.547

*, ** Significant at P <= 0.05 or 0.01, respectively

Hicksii

	Temperature °C	Rooting Percent	Root Length Rating	Root Number	Root Dry Weight
F_v/F_m Day 24	-0.736**	0.660**	0.306	0.200	0.347
F_v/F_m Day 35	-0.786**	0.723**	0.619*	0.532	0.562*
Temperature		-0.766**	-0.584*	-0.709**	-0.722**

Wardii

	Temperature °C	Rooting Percent	Root Length Rating	Root Number	Root Dry Weight
F_v/F_m Day 24	-0.666**	0.502	0.248	-0.182	0.464
F_v/F_m Day 35	-0.770**	0.556*	0.215	-0.326	0.204
Temperature		-0.494	0.168	0.443	0.137

*, ** Significant at P <= 0.05 or 0.01, respectively

LIST OF FIGURES

Fig. 1 Effect of storage temperature and desiccation on rooting of four cultivars of *Taxus* (1997 – 1998). Storage duration = 34 days. Stem cuttings were collected from stock plants in the field, placed in cold storage treatments, and then placed in perlite propagation beds. Rooting percentages were measured 96 - 99 days after sticking. Points represent means of 24 readings. Vertical bars represent +/- SE.

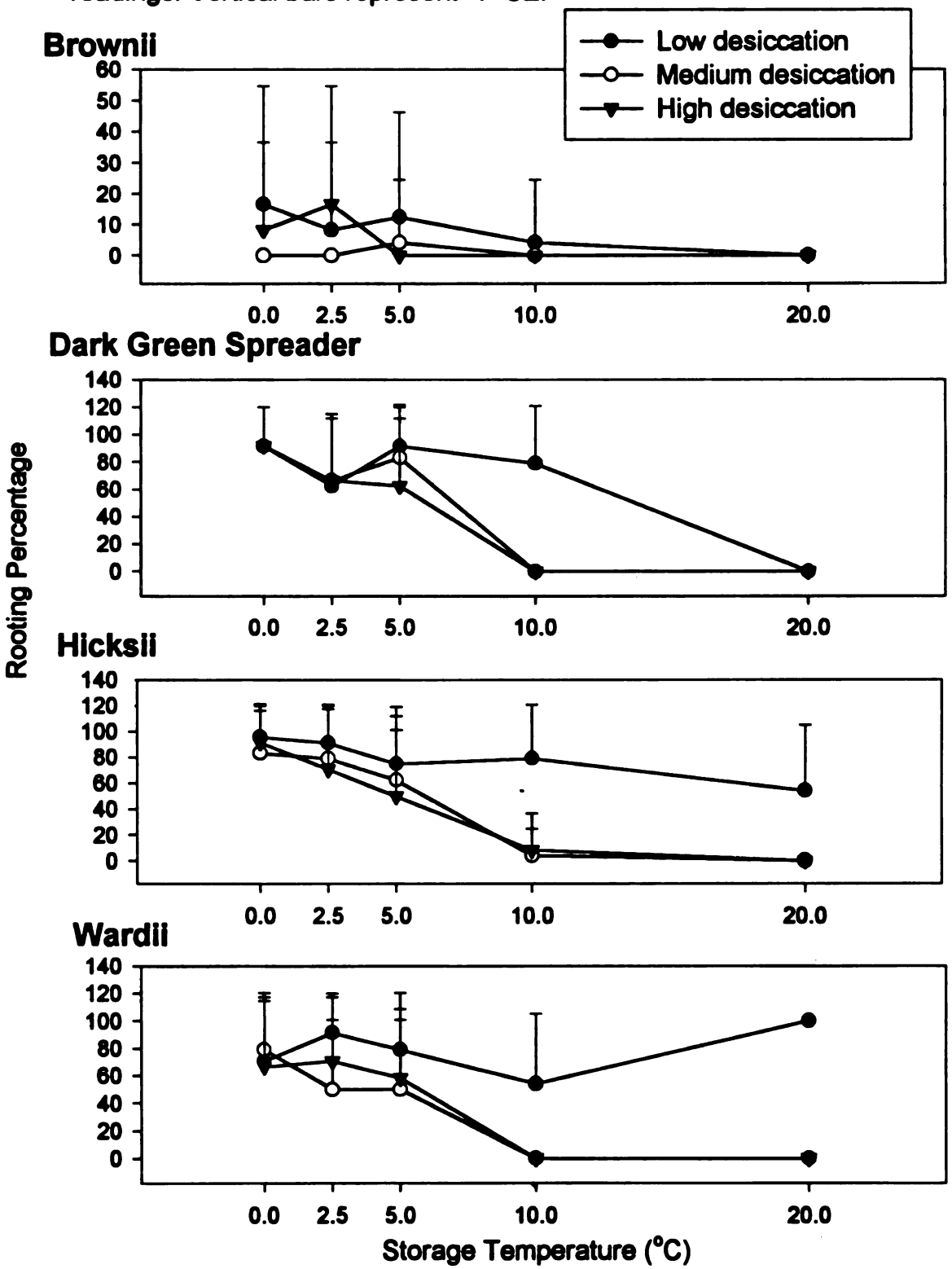


Fig. 2 Effect of storage temperature and desiccation on root dry weight of four cultivars of *Taxus* (1997 – 1998). Storage duration = 34 days. Stem cuttings were collected from stock plants in the field, placed in cold storage treatments, and then placed in perlite propagation beds. Roots were harvested 96 - 99 days after sticking, dried at 28 °C for 3 days and weighted. Points represent mean of 24 readings. Vertical bars represent + SE.

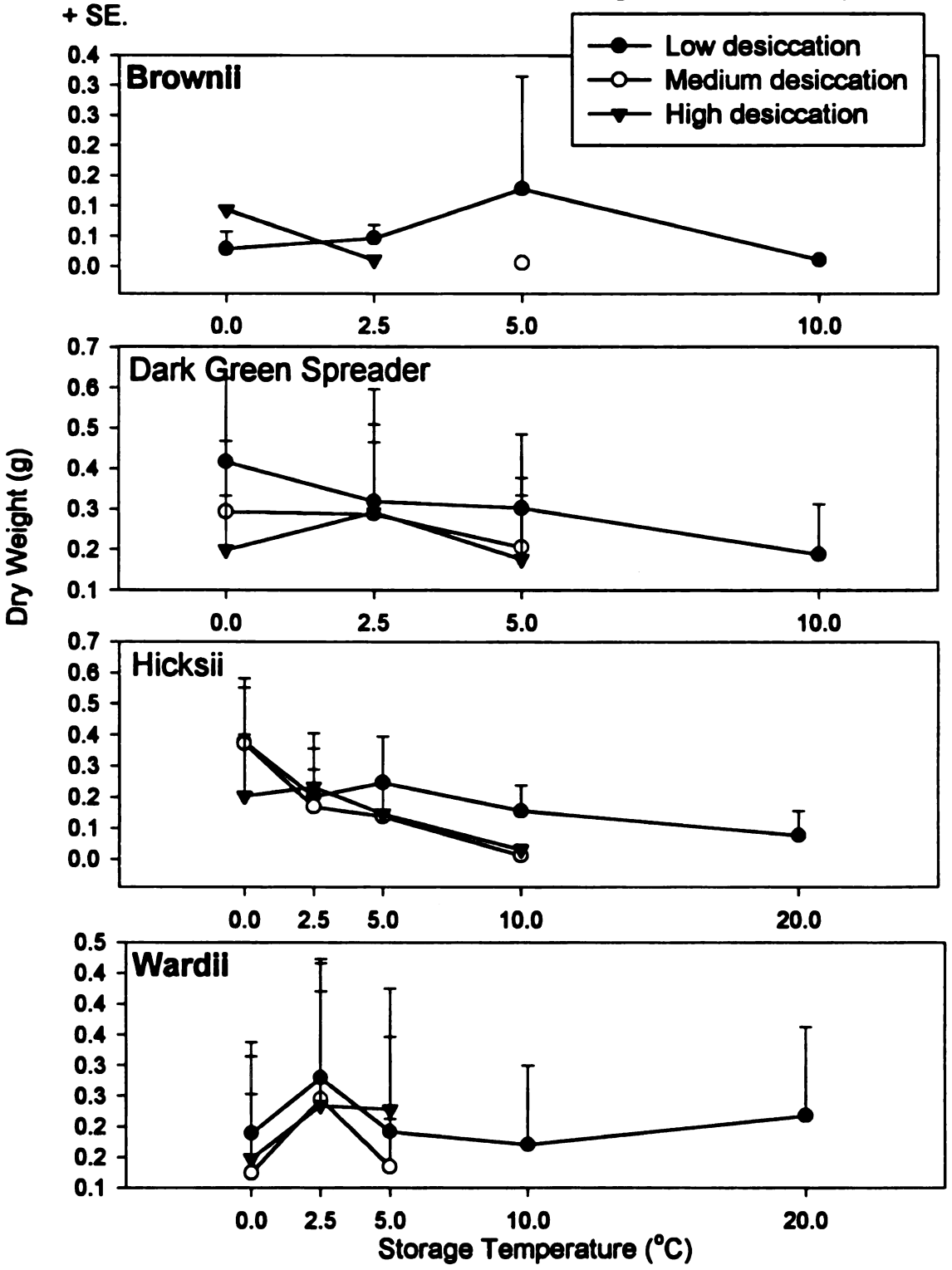


Fig 3. Effect of storage duration on rooting of four cultivars of *Taxus* (1998 – 1999). Storage temperature = -2.5 – 2.5 °C. Stem cuttings were collected from stock plants in the field, placed in cold storage for treatment duration, and then placed in perlite propagation beds at 18 °C with bottom heat (21 °C). Rooting percentages were measured 96 - 99 days after sticking. Points represent means of 72 readings. Vertical bars represent + SE.

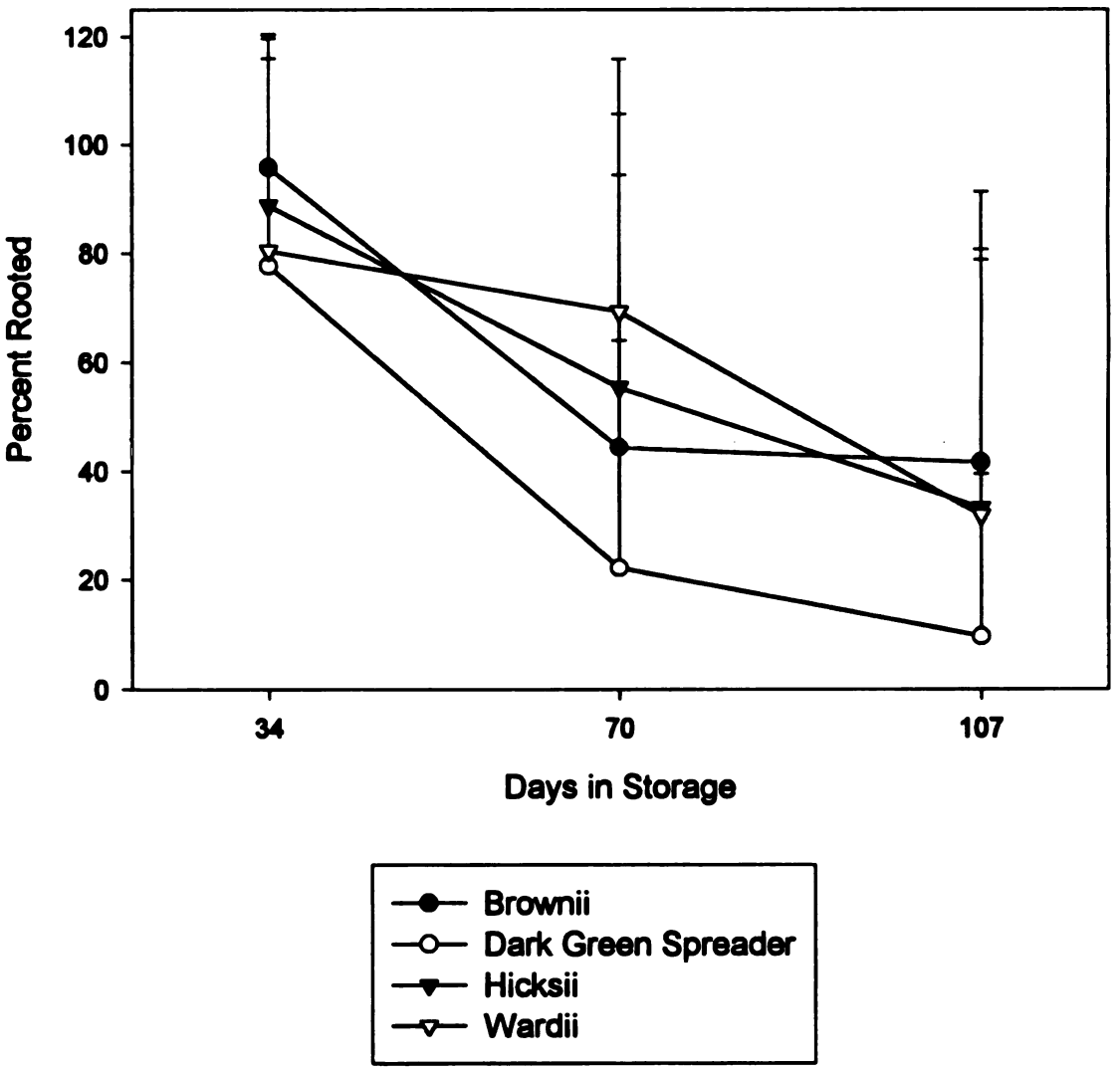


Fig. 4 Effect of storage duration on root dry weight of four cultivars of *Taxus* (1998 – 1999). Storage temperature = -2.5 – 2.5 °C. Stem cuttings were collected from stock plants in the field, placed in cold storage for treatment duration, and then placed in perlite propagation beds at 18 °C with bottom heat (21 °C). Roots were harvested 96 - 99 days after sticking, dried at 28 °C for 3 days and weighted. Points represent means of 72 readings. Vertical bars represent + SE.

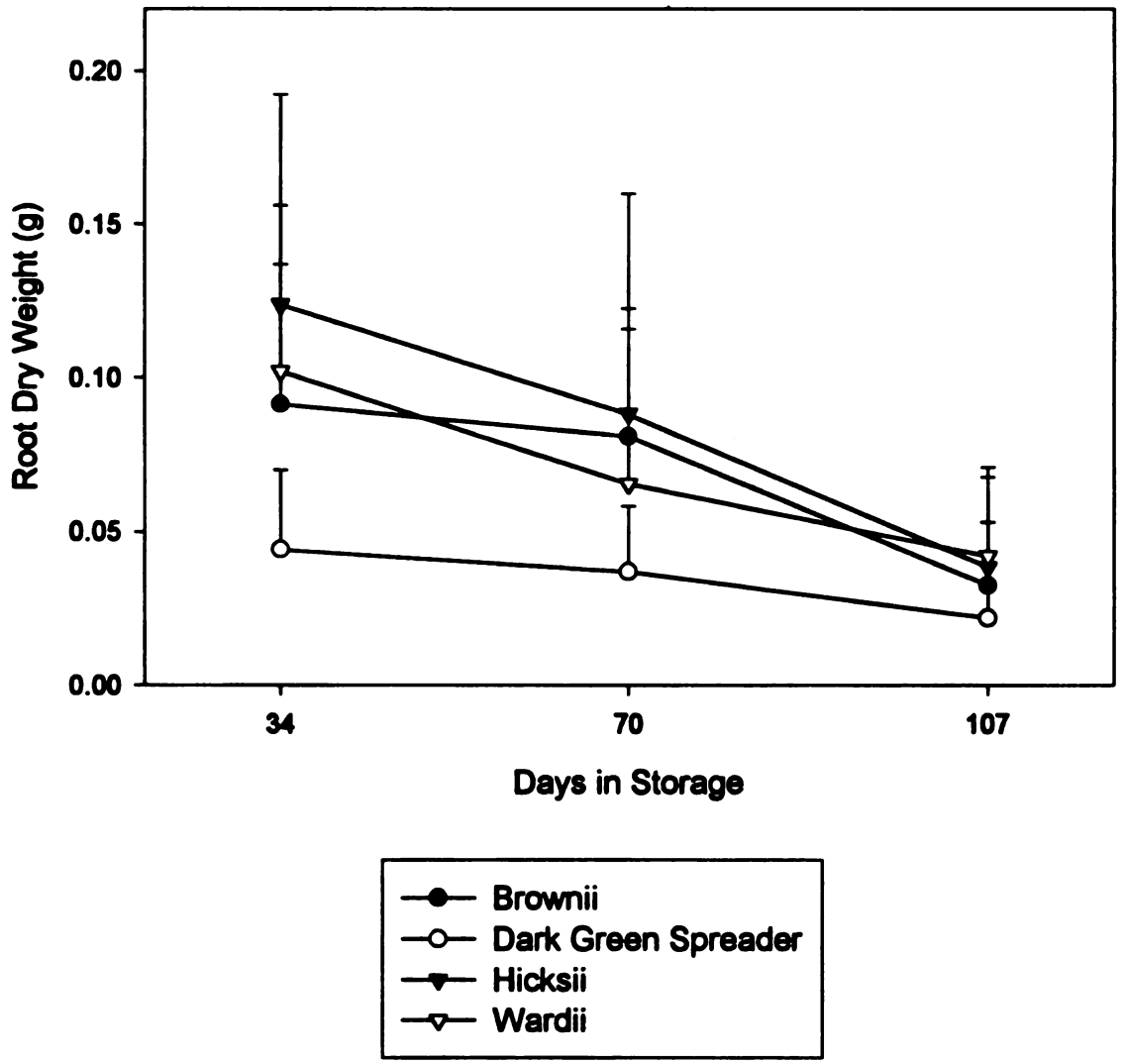


Fig. 5 Effect of storage duration on root number of four cultivars of *Taxus* (1998 – 1999). Storage temperature = -2.5 – 2.5 °C. Stem cuttings were collected from stock plants in the field, placed in cold storage for treatment duration, and then placed in perlite propagation beds at 18 °C with bottom heat (21 °C). Root numbers were measured 96 - 99 days after sticking. Points represent means of 72 readings. Vertical bars represent + SE.

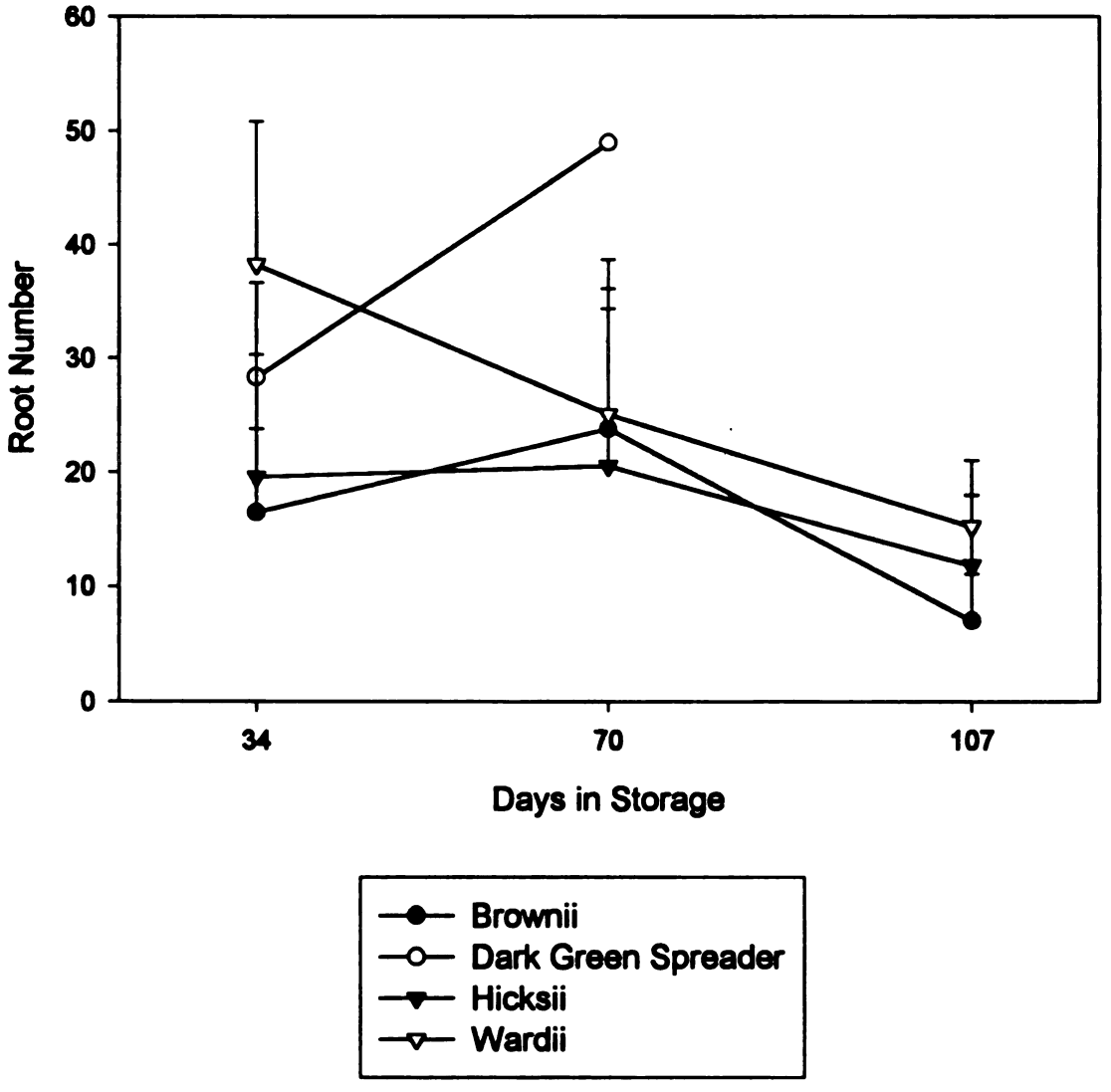


Fig. 6 Effect of storage duration on root length rating of four cultivars of *Taxus* (1998 – 1999). Storage temperature = -2.5 – 2.5 °C. Stem cuttings were collected from stock plants in the field, placed in cold storage for treatment duration, and then placed in perlite propagation beds at 18 °C with bottom heat (21 °C). Root length ratings were measured 96 - 99 days after sticking. (0=less than 2.54 cm [1 inch], 1=less than 5.08 cm [2 inch], 2=less than 7.62 cm [3 inch], 3=greater than 7.62 cm) Points represent means of 72 readings. Vertical bars represent + SE.

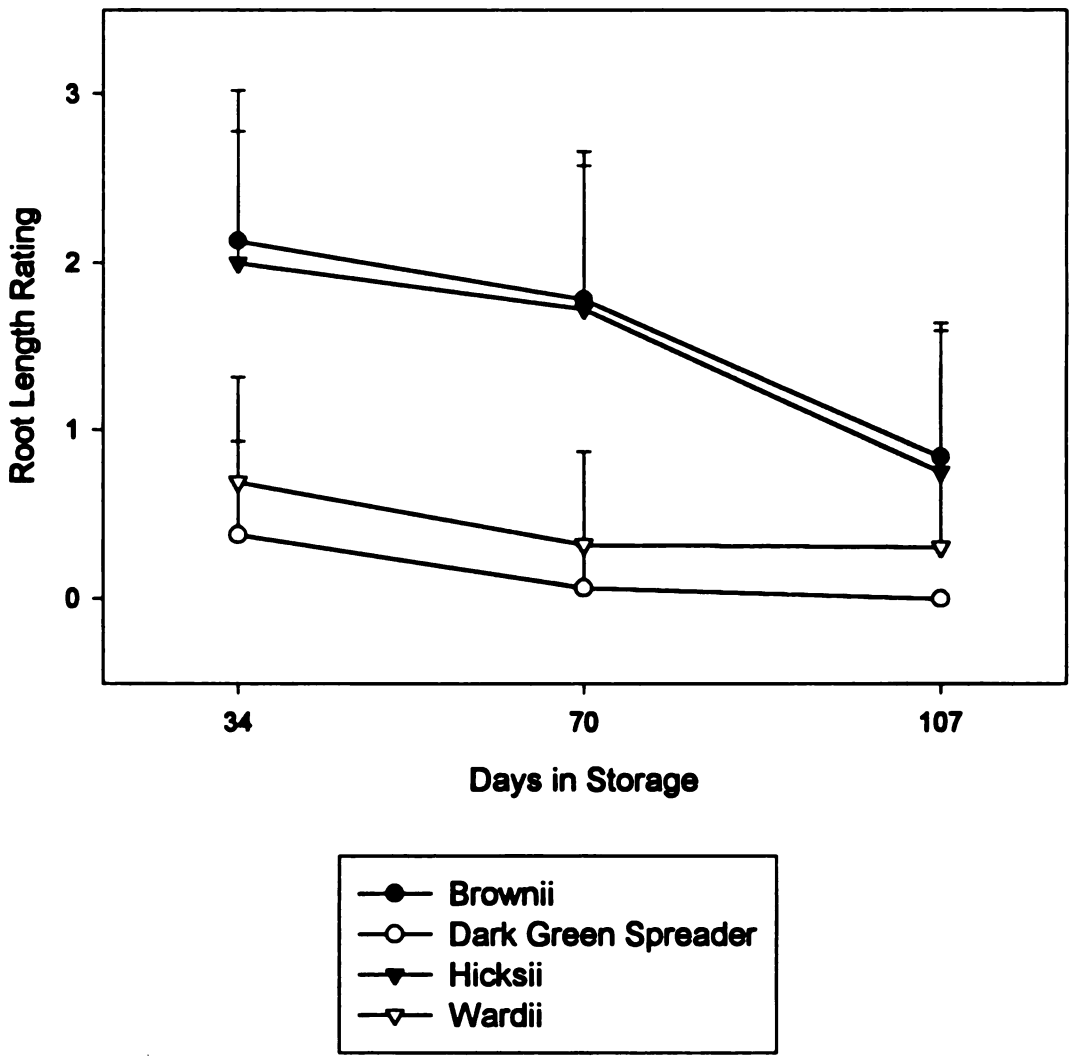


Fig. 7 Effect of storage temperature and desiccation on chlorophyll fluorescence of cuttings of *Taxus* (1997 – 1998). Chlorophyll fluorescence (F_v/F_m) was measured on the lower surface of individual dark-adapted (15 minutes) needles with fluorometer (Morgan CF-1000) light level set at 700 $\mu mol/m^2/s$. The first point of each line represents a mean of 40 readings, all other points represent means of 80 readings. Vertical bars represent + SE.

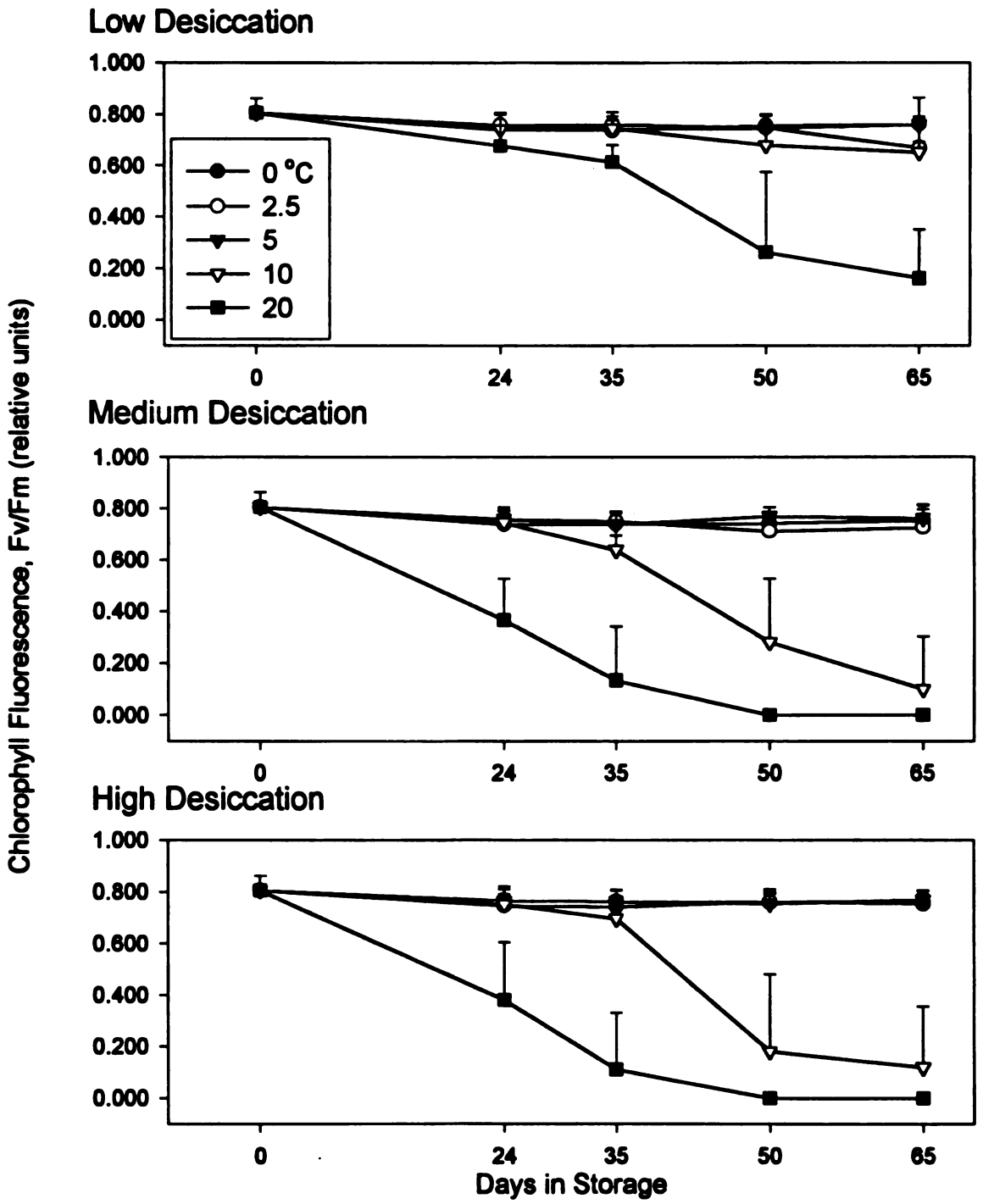


Fig. 8 Effect of storage duration on chlorophyll fluorescence of cuttings of *Taxus* (1998 –1999). Storage temperature = -2.5 – 2.5 °C. Chlorophyll fluorescence (F_v/F_m) was measured on the lower surface of individual dark-adapted (15 minutes) needles with fluorometer (PEA, Hansatech Instruments Ltd.) light level set at 1200 μmol/m^2/s. Points represent means of 20 readings. Vertical bars represent + SE.