A PHYSIOLOGICAL STUDY OF FALL DORMANCY AND SPRING REACTIVATION OF PHOTOSYNTHESIS IN WHITE SPRUCE [*PICEA GLAUCA* (MOENCH) VOSS] AND DOUGLAS-FIR [*PSEUDOTSUGA MENZIESII* (MIRB.) FRANCO]

by

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Title of Thesis/Project/Extended Essay

A physiological study of fall dormancy and spring reactivation in white spruce (*Picea glauca* (Moench) Voss) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco).

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ABSTRACT

Physiological differences were found between white spruce [Picea glauca (Moench) Voss] and Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seedlings. In the fall and winter, white spruce seedlings were found to be less sensitive to 48 hour dehardening temperatures prior to cold storage than coastal Douglas-fir from either British Columbia or Oregon. Photosynthesis as assayed by both chlorophyll fluorescence and gas exchange increased significantly and cold hardiness decreased with increasing temperature. The storability of seedlings was significantly affected by the 48 hour temperature regime prior to lifting, in particular in the Douglas-fir seedlings. Seedlings given warm dehardening temperatures prior to cold storage had the poorest root growth after storage.

Evidence was found for photochemical regulation of the photosynthetic apparatus in white spruce, where the ratio of variable fluorescence to maximal fluorescence and net photosynthesis decreased in response to day length. In contrast, net photosynthesis did not decrease with day length in the coastal Douglas-fir.

Significant differences were found in the susceptibility to damage of hardened, dehardened and newly flushed white spruce needles at sub-zero temperatures in the light and darkness. Hardened needles were not susceptible to damage from sub-zero temperatures (-2 to -22.5°C) in either the light or dark. Both dehardened and newly flushed needles suffered

damage in the dark at a lower temperature than in the light. A correlation was found between chlorophyll fluorescence measured after sub-zero temperatures, membrane leakage and visible damage to foliage. At temperatures that did not cause tissue damage, rapidly reversible decreases in the ratio of Fv/Fm seen in seedlings given sub-zero temperatures in the light were attributable to photoinhibition. Damage caused by sub-zero temperatures in darkness was attributable to freezing damage and appeared to be related to needle water content, damage in light was attributed to photooxidation caused by toxic oxygen species.

The assessment of seedling quality is important for successful reforestation. Chlorophyll fluorescence was found to the most efficacious method used to assess the physiological status of these seedlings. The main advantages of this technique were: it is rapid, easy to operate, nondestructive and useful for multiple functions throughout the growing season.

DEDICATION

To my husband, Larry

ACKNOWLEDGEMENTS

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I would like to acknowledge Hybrid Nursery in Maple Ridge and Surrey Nursery (B.C. Ministry of Forests) for providing seedlings and in particular, Bruce Morton at Hybrid Nursery for all his help.

I would like to thank my parents for their belief in me, and finally, my deepest appreciation goes to my husband, Larry, for his excellent criticisms and suggestions, for proofreading the manuscript, and for continued support and encouragement throughout my studies.

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A. GENERAL INTRODUCTION

In British Columbia, millions of conifer seedlings are grown in nurseries each year for reforesting harvested areas. Because of the high costs involved in planting, the production of high quality seedlings is of primary importance to enhance plantation establishment. Therefore, assessing seedling quality by measuring the physiological status of the seedlings is crucial.

Most containerized seedlings grown for reforestation are fall and winter lifted, then over-wintered in cold storage instead of being left to over-winter naturally. In the late spring or summer of the following year, seedlings are removed from cold storage and planted. Root growth potential tests are used to determine seedling quality at that time.

Cold storage conditions are considerably different from the natural environmental conditions seedlings would normally experience in the winter. Instead of fluctuations in temperature and light, seedlings are subjected to near 0°C in total darkness for two to six months. Selection of lifting dates for cold storage is important, and seedlings are best lifted when physiologically dormant; however, the accurate prediction of dormancy and determining the optimal lifting date for cold storage can be difficult. The current method for determining lifting dates and hence storability, is cold hardiness testing (Navratil et al. 1986): seedlings are generally considered suitable to lift and place in

storage when they achieve a minimum cold hardiness to -18°C (Colombo et al. 1984; Simpson 1985).

Classically, dormancy refers to the cessation of vegetative growth and the completion of bud set. Dormancy can be divided into rest and quiescence (Lavender 1985). A bud is in rest when the dormancy is maintained internally (Romberger 1963), and occurs prior to the acquisition of the chilling requirement for bud break. Resting buds will not elongate under favourable environmental conditions. A bud is quiescent when dormancy is maintained by low temperatures once chilling requirements have been met (Samish 1954). Bud dormancy can be assessed by either placing seedlings under optimal conditions for bud break and counting the number of days it takes for flushing to occur or by monitoring the mitotic index, the percentage of cells undergoing active cell division, of the apical bud. Buds can be considered dormant when no mitotic divisions are occurring in apical buds (Owens & Molder 1973).

The induction of dormancy may be triggered either by a decrease in photoperiod or a decrease in temperature, or both, depending upon the species (Christersson 1978, Sakai & Larcher 1987). For many conifer species, dormancy appears to have two separate stages, one which is triggered by day length, and another which is triggered by chilling temperatures (Sakai & Larcher 1987). By separating day length and chilling temperatures, it may be possible to

determine the importance of each to the dormancy and storability of the seedlings.

Increases in cold hardiness and decreases in physiological activity generally occur in the fall and winter. Declines in photosynthetic gas exchange have been seen in many conifer species in winter (Helms, 1965, Shiroya et al. 1966, Delucia 1987, Leverenz & Öquist 1987, Jurik et al. 1988). Winter declines in chlorophyll fluorescence also occur in several conifer species during frost hardening and dormancy (Martin et al. 1978, Hawkins & Lister 1985, Strand & Lundmark 1987, Bolhar-Nordenkampf & Lechner 1988, Strand & Öquist 1988, Vidaver et al. 1989a, Sundblad et al. 1990). It is unlikely that such declines in chlorophyll fluorescence and gas exchange are due only to low air temperature (Jurik et al. 1988); photochemical regulation of the photosynthetic apparatus appears to be involved as well (Vidaver et al. 1989a & b, Gillies & Vidaver 1990). Evidence for photochemical regulation of photosynthetic activity has been observed in white spruce seedlings where late summer and early fall declines in chlorophyll fluorescence were associated with decreased day length and not ambient temperature (Vidaver et al. 1989b).

My studies followed winter dormancy and spring recovery of photosynthesis in white spruce and coastal Douglas-fir seedlings. Seasonal changes in the photosynthetic activity of seedlings was assessed using both gas exchange and chlorophyll fluorescence.

Seedlings received dormancy induction treatments to test for the presence of day length-mediated photochemical regulation, and to provide a range of seedlings for cold-storage. The efficacy of a number of physiological measurements, including chlorophyll fluorescence, root growth potential, needle water potential and photosynthetic gas exchange were assessed as predictors of seedling quality after cold storage.

The objectives of this research were:

i) to compare the physiological reactivation in the spring of seedlings over-wintered under natural conditions with that of cold-stored seedlings

ii) to compare the sensitivity of these species to various 48 hour temperature regimes prior to cold storage

iii) to test for the presence of day length-mediated photochemical regulation of photosynthesis

iv) to compare the physiological differences between hardened, dehardened and newly flushed needles in white spruce seedlings.

v) to determine the efficacy of chlorophyll fluorescence, mitotic index, CO_2 gas exchange, cold hardiness testing, needle water potential, and root growth potential as methods for evaluating the physiological status of seedlings.

B. GENERAL MATERIALS AND METHODS

Plant Material

White spruce (Picea glauca [Moench] Voss) and coastal Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) seedlings (Table 1) were provided by Hybrid Nursery, Pitt Meadows and Ministry of Forests Surrey Nursery, Surrey, British Columbia. The establishment and growing conditions of the seedlings used by the nurseries were as follows. All seedlings were grown in styroblock containers (Figure 1A) containing 3:1 peat:vermiculite mixture. Two to three seeds were mechanically sown in each cavity in February and early March, styroblock containers were then placed in polyethylene covered greenhouses. In April and May, seedlings in styroblock containers were culled to one seedling per cavity.

Seedlings were watered when styroblock container weight decreased to 50% field capacity weight. Seedlings were fertilized according to the nursery practice and photoperiod was artificially extended to 16 hours using high pressure sodium lamps from the time of sowing until May.

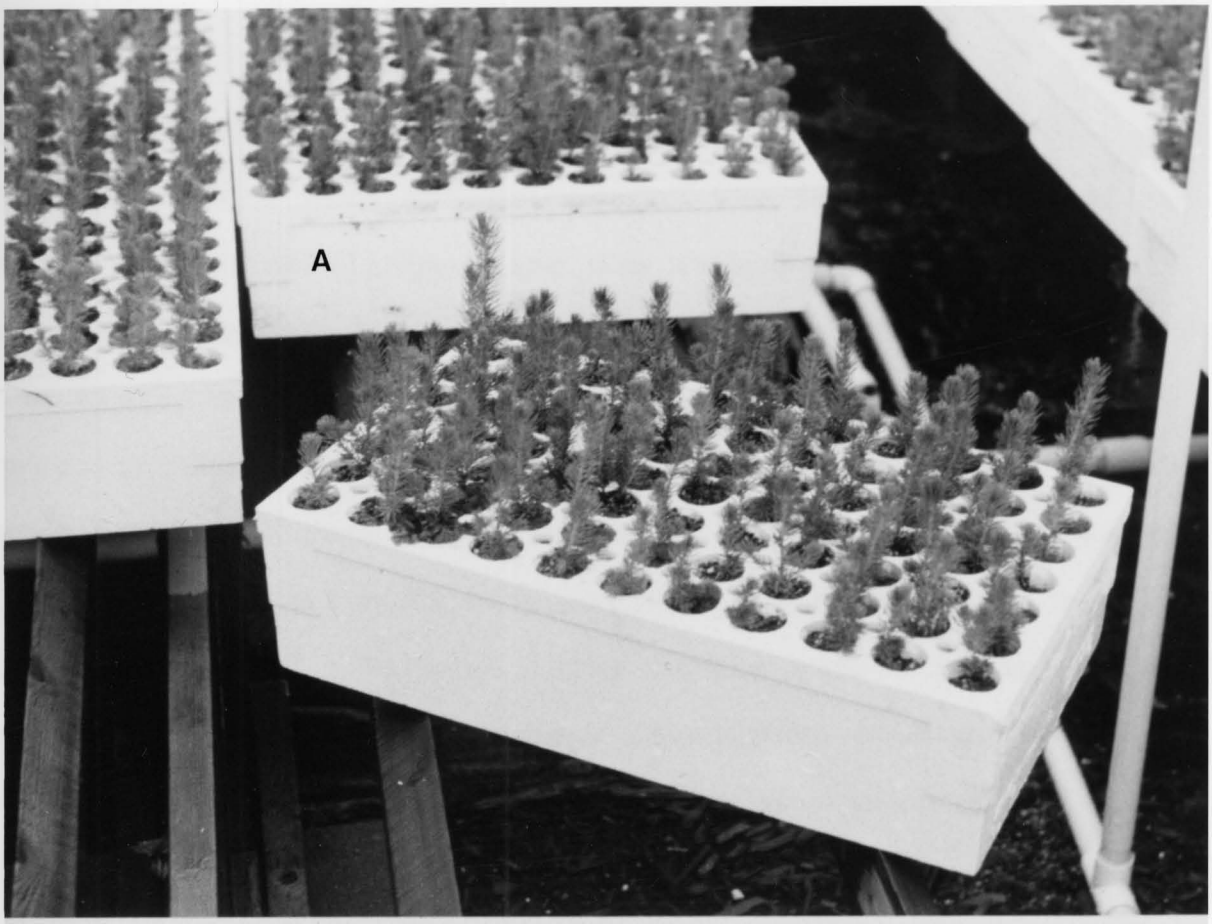
All seedlings used for fall dormancy research were sown in the spring of the same year. Styroblock containers were brought to Simon Fraser University (SFU) one month prior to the beginning of each study and placed in an unheated greenhouse or an open compound. Seedlings were well watered, but were not fertilized after being brought to SFU.

Typical white spruce and Douglas-fir seedlings are shown in Figure 1B.

Table 1. Seedlot origin, container size and cavity volume for the white spruce and Douglas-fir seedlings used in the experiments.

Species	Seedlot	Elevation (m)	Origin	Styroblock size	Cavity volume
White spruce	29135	850	56°07'N 124°49'W	313	75 mL
Douglas-fir	6399	915	49°35'N 122°30'W	415	100 mL

Figure 1. A) 313 Styroblock container with white spruce seedlings. B) Seven month old i) white spruce, and ii) Douglas-fir seedlings.



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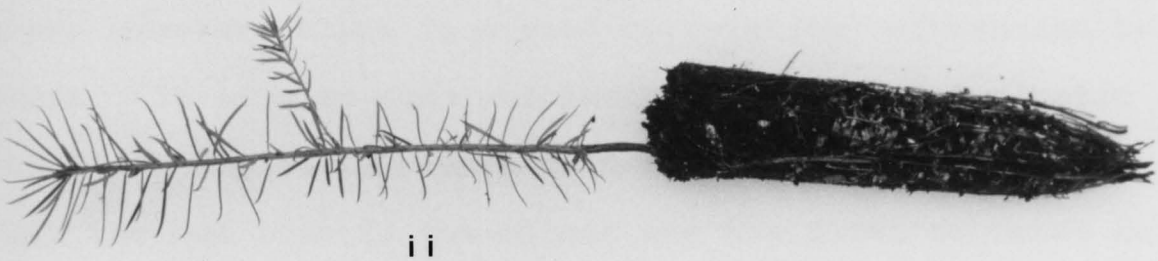

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Experimental Procedures

Mitotic Index

Mitotic indices were determined for the terminal buds of batches of 10 seedlings using the method of Grob (1990). Terminal buds were fixed in 4°C formaldehyde for 24 hours, then rinsed several times with 4°C distilled water for 24 hours, stained with Schiff's Reagent for 2 hours and rinsed for 1 hour with room temperature (22 ± 1°C) distilled water. Apices of terminal buds were excised and squashed on a microscope slide. Mitotic index is the number of apical cells in all stages of active mitosis (excluding interphase) expressed as the percentage of the total number of cells that fell on transects 200 micrometers apart.

Mitotic index can be underestimated using this transect method, mitotic indices calculated per unit area are generally higher; however, there is a good correlation between the two methods. It is also much quicker to calculate the mitotic index using transects rather than per unit area (Grob 1990). Within the bud itself, the apices are the first to cease cell division, cell division continues for some time in the needle primordia.

Cold hardiness

Two methods of determining cold hardiness were used. The first method was similar to the procedure used by the B.C. Ministry of Forests. Seedlings (n=15) were placed in styroblock containers insulated with moist peat, then placed

in a dark incubator at 15°C. The temperature was lowered at $6°C \cdot h^{-1}$ until it reached -18°C, and maintained there for two hours. Temperature was then raised at a rate of $6°C \cdot h^{-1}$ to room temperature before seedlings were placed in an incubator with a photosynthetic photon flux (PPF) of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 23/17°C day/night temperature and 14 hour photoperiod for two weeks. Seedlings were considered to be cold hardy to the Ministry of Forests standard of -18°C if <25% of the foliage showed necrosis.

The second method of determining cold hardiness testing followed the procedure of Levitt (1980). Seedlings (n=40) were placed in styroblock containers insulated with moist peat. Seedlings were placed in an incubator at 15°C and the temperature was lowered at $6°C \cdot h^{-1}$. Ten seedlings were removed at four different temperatures. The temperatures were chosen to bracket an LT_{50} temperature predicted for the particular species and time of the season. Seedlings were kept at the desired temperatures for one hour. The temperature was then raised to room temperature at a rate of $6°C \cdot h^{-1}$. After thawing, seedlings were placed in an incubator with a PPF of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 23/17°C day/night temperature and 14 hour photoperiod for two weeks.

Seedling status was assessed after two weeks, the temperature where 50% of the foliage had visible damage was determined using linear regression (LT_{50}). Data between 5-95% visible damage were used to calculate the regression.

Chlorophyll fluorescence

Chlorophyll fluorescence assay systems have been widely used to investigate the photosynthetic functioning of plants (for a review see Krause & Weis 1991). Chlorophyll fluorescence was measured using an integrating fluorometer (Toivonen & Vidaver 1984); data acquisition and processing was interfaced to a computer (Dubé and Vidaver 1990). This fluorometer utilizes an integrating sphere that allows for fluorescence measurements to be taken from whole shoots rather than from a subsample with a small surface area. This minimizes the effects of variation between subsamples, with the resultant curve representing an integrated fluorescence signal for the whole shoot.

Vidaver et al. (1991) state that seedlings moved from an outside growing area or growth chamber to a laboratory for physiological assessments, such as chlorophyll fluorescence and CO_2 gas exchange reflect the response or physiological capacity of the seedlings to a given set of laboratory conditions at the time of measurement. Therefore, measurement conditions should be kept as constant and reproducible as possible throughout an experimental series. For this reason, seedlings were brought into the laboratory, well watered and equilibrated to room temperature for 2 hours at a PPF of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to allow them to acclimate to room temperature at low light intensities. Seedlings were then placed under a PPF of 650 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 45 min to condition and standardize all seedlings to a uniform near saturating light intensity.

Seedlings were then dark pretreated for a minimum of 25 min at room temperature.

The chlorophyll fluorescence of the whole shoot was monitored for 300 s for most experiments, a total of 3740 data points were collected for each scan (Table 2). The excitation light level in the sphere had a PPF of 115 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by a quartz-iodine projection lamp (Sylvania EJL 150W 12V), as measured at the centre of the sphere by a LI-COR Model LI-185A radiometer with a quantum sensor (Li-Cor Inc. Lincoln, NE).

The typical features of such curves, seen in Figure 2, known as Kautsky or induction curves are described in Krause & Weis (1984). Unless otherwise indicated, all curves for white spruce and Douglas-fir are the average response of 10 seedlings, data are normalized to compensate for different seedling sizes according to Vidaver et al. (1989a).

Normalization is performed using the formula:

$$Fvar = (Fv-F_O) \cdot F_O^{-1}$$

where

Fvar is normalized variable fluorescence at time t

Fv is non-normalized fluorescence at time t

F_O is O-level fluorescence.

Several components of the induction curve were used for analysis: F_O, initial fluorescence; F_T, steady state fluorescence; Fv/Fm, the ratio of the induced to the maximum chlorophyll fluorescence at the time of peak fluorescence (P) (Hipkins & Baker 1986).

Initial fluorescence, F_O, is not related to the photochemistry of variable fluorescence but is dependent upon the instantaneous excitation light flux (Papageorgiou 1975). Initial fluorescence is then proportional to the total number of emitting sources of chlorophyll fluorescence in sample, which in turn is proportional to the amount of tissue and seedling size. Normalization assigns a unit value of 1 to F_O, although when normalized curves are graphed F_O appears at 0 (Vidaver et al. 1989a). This does not indicate that there is no fluorescence at all at F_O, only that there is no variable fluorescence at F_O. Normalized chlorophyll fluorescence induction curves are therefore graphed to emphasize the variable fluorescence, as shown in Figure 2.

Steady state fluorescence, F_T, was estimated after a five minute scan, and is related to CO_2 assimilation (Hipkins & Baker 1986).

The ratio of the variable to the maximum chlorophyll fluorescence, Fv/Fm, was used as an indicator of PSII photochemical efficiency, since it relates to the probability of a photon absorbed by the chlorophyll matrices being utilized to drive PSII photochemistry (Baker 1978).

The Fv/Fm ratio was calculated by the formula:

$$Fv/Fm = P/(P+1)$$

where

Fv is measured at the time of peak variable fluorescence, P

Fm is maximal fluorescence P + F_O.

The Fv/Fm ratios reported in this study were lower than ratios acquired using alternative chlorophyll fluorescence measuring systems, possibly due to lower excitation light levels in the integrating sphere; however, the ratios do appear to be consistent with those of other studies of conifers (Bolhar-Nordenkampf & Lechner 1988, Krause & Somersalo 1989) and were considered to be valid, though not directly comparable with ratios measured using higher excitation light intensities. Therefore, the ratio Fv/Fm may be considered as an indicator of PSII activity rather than efficiency when measured under such subsaturating light levels (Strand & Lundmark 1987).

Table 2 Rate, frequency and duration of 300 s chlorophyll fluorescence induction scans.

	Rate	Hz	Duration	Data points
	1	5000	10 ms	50
	2	120	1 s	120
	3	30	99 s	2970
	4	3	200 s	600
Total			300 s	3740

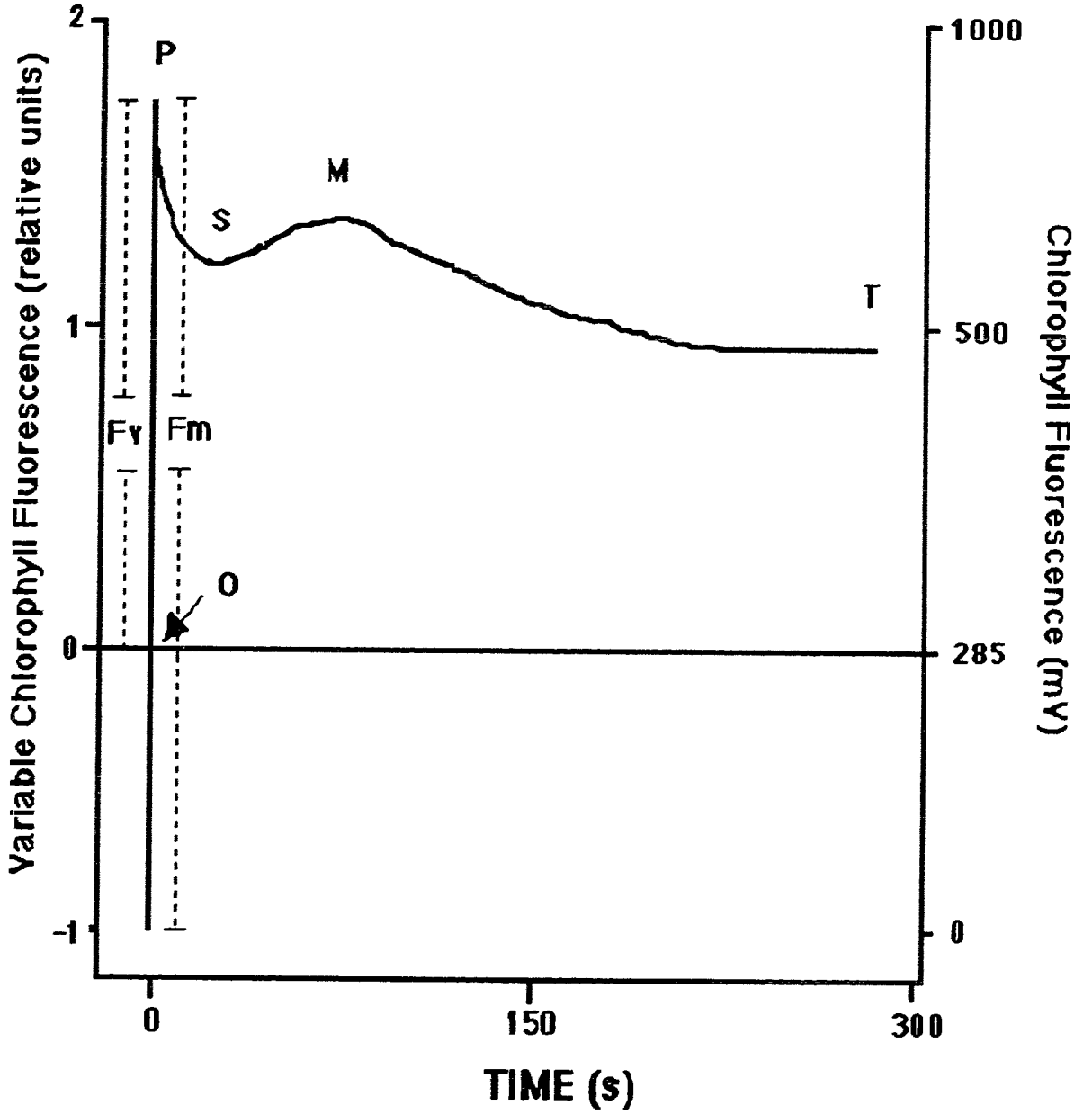

Figure 2. Typical 300 s chlorophyll fluorescence induction curve. An integrating fluorometer with an excition PPF of 115 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was used. Right axis shows normalized units where all variable fluorescence is plotted above the origin (F_O), left axis shows non-normalized values. Shown are O, P, S, M and T-level fluorescence. Variable fluorescence, Fv, and maximal fluorescence, Fm, measured at peak (P) fluorescence are indicated.

Carbon Dioxide gas exchange

Immediately following fluorescence measurements, seedlings were placed under quartz-halide lamps providing a PPF of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 15 min to acclimate seedlings to moderate light levels under laboratory conditions, seedlings were then placed for a minimum of 30 min under saturating light levels of 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Room temperature (22 ± 2°C) was maintained by placing a water bath between the light source and seedlings. Whole shoot gas exchange was measured using a method modified from Lister et al. (1961) and described by Toivonen & Vidaver (1988). Seedling shoots were sealed in a transparent Plexiglass container connected into a closed gas circulation system in which changes in CO_2 concentrations were measured by means of a model 865 Beckman infrared gas analyzer (IRGA).

The IRGA was calibrated using a standard CO_2 in air mixture, 100% nitrogen was used to set zero. Water vapour in the air stream from transpiration mimics CO_2 by absorbing infra-red radiation, and can cause errors. Therefore, transpired water vapour was removed from the circulating air by an ice jacket water vapour condenser, thus maintaining a constant relative humidity (RH) in the system.

Net photosynthesis (P_N) measurements were taken using a CO_2 range of 310 to 370 $\mu L \cdot L^{-1}$ at room temperature (22 ± 2°C) and 80-85% RH. Minimum air flow velocity in the Plexiglass chamber was 1.26 $m \cdot min^{-1}$. Plants were positioned to receive from the side 850 to 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF (90-95% light

saturation) provided by a high pressure sodium lamp (400 watt Poot Elektra, type PC 1078/N lamp, with a General Electric Lucalox LU400/40 bulb).

Dark respiration (R_D) was measured using the same system by covering the Plexiglass container to seal out all light. Measurements were taken for several minutes until a steady respiration rate was achieved.

Lifting and storage

Randomly chosen seedlings were lifted by hand and gathered into replicate batches of 10 seedlings each. A plastic bag was wrapped around the roots of each batch. Batches were placed in plasticized paper bags, then into waxed cardboard boxes and placed in cold dark storage (-2.0 ± 1°C) at SFU, Burnaby, B.C.

Root growth potential

Root growth potential (RGP) conditions followed the procedure of Stone (1955). On removal from cold storage, seedlings were allowed to warm to room temperature under low light conditions (70 $\mu mol \cdot m^{-2} \cdot s^{-1}$) prior to potting. Seedlings were potted in one liter containers (7 X 7 X 21 cm) containing moist standard potting mix (Fison's Sunshine Mix). Seedlings were then placed in a growth chamber at SFU with a PPF at canopy height of 375 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 85% RH, 24/19°C day/night, 16 hour photoperiod. Seedlings were well watered, but received no fertilizer. Root growth was assessed 28 days after potting. The number of white root tips longer than 1 cm were counted.

Bud flush

Flushing of apical buds was monitored daily, scores for different stages of bud break for the seedlings were assessed as follows:

0 tight brown bud

1 bud brown and swelling

2 bud swollen and appears green, some green needles visible below scales

3 needles free from scales and clearly visible

4 needles elongating, free of bud

5 stem elongating

Seedlings were considered to have flushed when 50% of the seedlings were at stage 3.

Dry Weights

Seedlings were divided into root and shoot, then all rooting material was washed off. Root and shoot dry weights were measured after oven drying (70°C) for 48 hours, samples were cooled in a desiccator, then weighed.

Calculations of surface area

The importance of the selection of a method for measuring leaf surface area to express gas exchange data has been reviewed by Smith et al. (1991). In particular, conifer seedlings have complex shoot patterns, where leaf geometry is not planar and orientation to the light source is not perpendicular. This study used several methods to calculate surface area. In particular, projected surface area (PSA), the area of the leaf shadow cast on a surface beneath and

parallel to the leaf plant was utilized. Projected surface area was calculated by removing all needles and photocopying them. Projected surface area of individual needles was estimated from the photocopy. This method permitted surface areas to be calculated at a later date from a permanent photocopy record, and allowed for a larger number of samples to be tested than would have otherwise been possible.

Where this method was not practical, the following formulae were developed after calibration with 100 samples. To calculate projected surface area (PSA, in dm^2):

i) White spruce:

PSA = .009 X total number of needles

ii) Douglas-fir:

PSA = .015 X total number of needles

C. Fall dormancy and spring reactivation of photosynthesis in white spruce and Douglas-fir seedlings

I. Fall dormancy and spring reactivation of photosynthesis in naturally over-wintered and cold-stored white spruce and Douglas-fir seedlings

INTRODUCTION

Seedlings over-wintered under natural conditions show a decrease in stress resistance (eg. cold hardiness) in the spring (Schuch et al. 1989); however, Lavender (1985) and Burr et al. (1989) have shown that the best seedling performance in the field occurs if they are lifted and replanted when their stress resistance is highest. For this reason, a large proportion of seedlings are over-wintered in cold storage to maintain seedlings in a dormant, hardy state for late spring planting (Ritchie & Tanaka 1990). Over-winter cold storage has been found to maintain or actually enhance spring root growth potential (RGP) (Ritchie & Tanaka 1990), providing the seedlings are both morphologically and physiologically dormant prior to being placed in cold storage (Hermann et al. 1972, Timmis 1980).

Seedlings are generally considered suitable to lift and place in cold, dark storage when they achieve a minimum cold hardiness of -18°C (Colombo et al. 1984; Simpson 1985). Cold hardiness has also been correlated to subsequent RGP in Douglas-fir seedlings (Simpson 1990) and is therefore considered a good general indicator of the capacity of seedlings to store satisfactorily.

This study followed seasonal changes in photosynthetic activity of one year old containerized white spruce (Picea glauca [Moench] Voss.) and Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco) seedlings grown for reforestation following

the nurseries' standard procedures. The objective of this study was to provide a baseline of information on seedlings grown using typical conifer nursery procedures, and also compare the spring recovery of spring-lifted seedlings over-wintered under natural environmental conditions with seedlings over-wintered in cold storage using CO_2 gas exchange and chlorophyll fluorescence. In order to determine the contribution of new foliage to recovery of photosynthetic activity in the spring, newly flushed needles were compared separately to one year old needles both over-wintered under natural conditions or cold-stored.

MATERIALS AND METHODS

Seedlings used in this experiment were grown and supplied as per General Materials and Methods. To induce bud set, the seedlings were subjected to a 12 hour photoperiod for two weeks in late July using a greenhouse with black-out facilities. Approximately 600 seedlings sown in 1989 were used for study in 1989-90, and another 600 seedlings sown in 1990 were used for study in 1990-91.

Seedlings were brought to SFU at the end of July and placed in an outside compound. Seedlings were kept well watered, but were not fertilized after this date. In November of each year, the styroblock containers were recessed 15 cm in sawdust filled potting beds at the outside compound to insulate the roots against sub-zero temperatures.

In July seedlings were randomly assigned to three groups (Figure 3):

1. BL - The first group of seedlings were left in an open compound for the duration of the study to collect baseline data on dormancy and spring reactivation of seedlings under natural conditions. This group is referred to as baseline (BL) seedlings.

2. CS - The second group of seedlings were winter-lifted and cold-stored for 13 weeks. In the spring, seedlings were removed from cold storage and placed in a growth chamber. This group is referred to as cold-stored (CS).

3. SL - The third group of seedlings were over-wintered in the outside compound, then spring-lifted in March and placed in a growth chamber. This group is referred to spring-lifted (SL).

In order to follow the natural seasonal pattern of photosynthetic activity of seedlings, BL seedlings were maintained under ambient environmental conditions in an open compound from August to the end of May in both 1990 and 1991. Once a month during this period, 10 randomly chosen BL seedlings were brought into the laboratory and warmed to room temperature before chlorophyll fluorescence and CO_2 gas exchange rates were measured (Figure 3). These seedlings experienced cultural conditions typical of those found in the conifer nursery that supplied the seedlings, data was therefore considered to representative of the typical seasonal responses of nursery grown conifers.

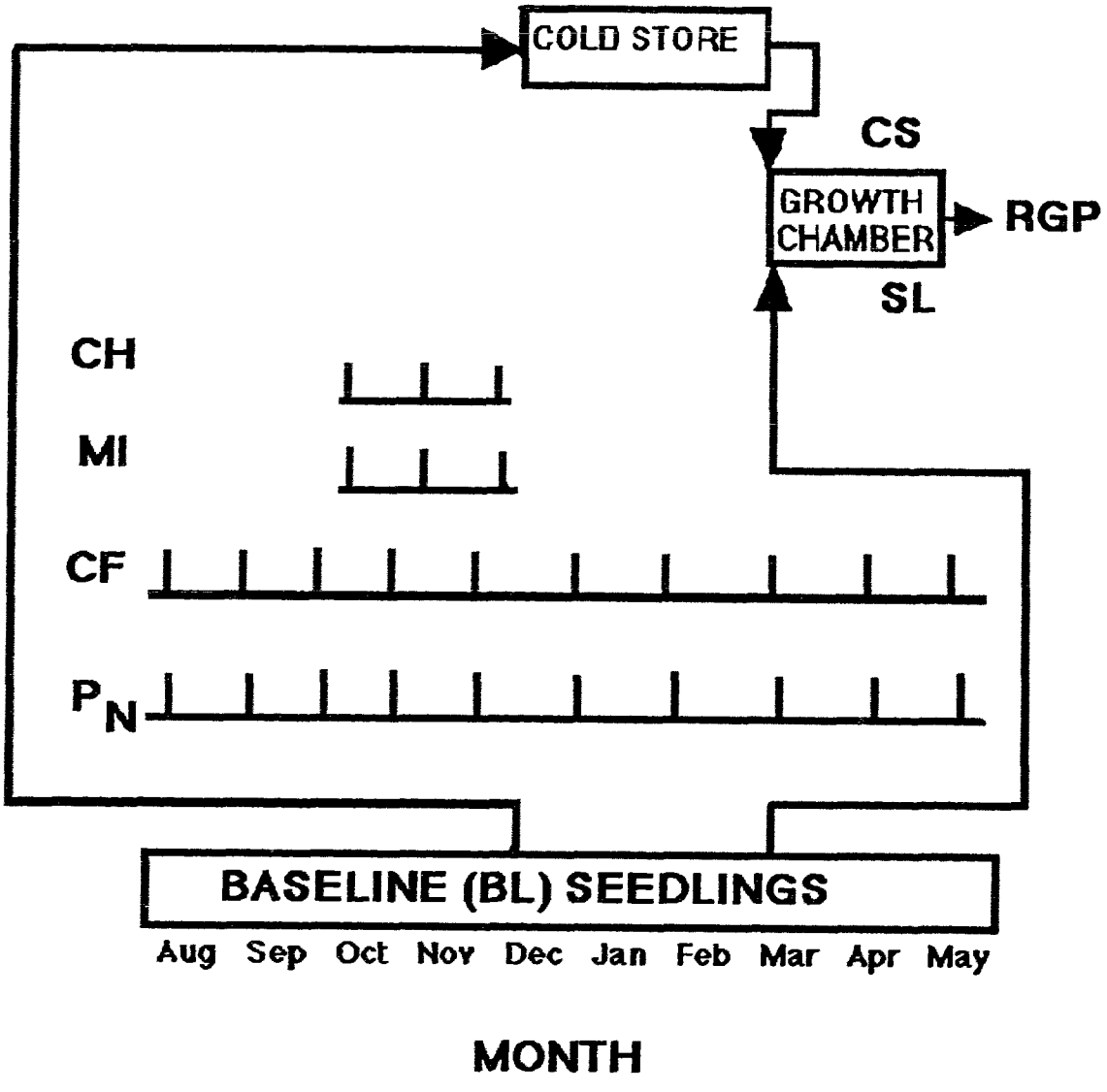

Figure 3. Time line of Experiment. Bars indicate when net photosynthesis (P_N), chlorophyll fluorescence (CF), mitotic index (MI) and cold hardiness (CH) testing were conducted. Arrows indicate when seedlings were lifted for cold storage, and when cold-stored (CS) and spring-lifted (SL) seedlings were placed into growth chamber.

Environmental conditions for the seedlings over-wintered in the outside compound were monitored and supplied by Environment Canada. The number of sunshine hours is reported in Table 3, and the five day minimum and maximum temperature reported in Figure 4.

For the BL group, the number of days to bud break was measured in December, January, February, March and April (Table 4) to assess the bud dormancy stage. Ten BL seedlings were placed in a growth chamber with a 16/8 day/night photoperiod, 85% RH, PPF of 375 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Seedlings were considered to have flushed once the terminal bud flushed on 50% of the seedlings as per General Materials and Methods. To optimize the quality of the seedlings winter lifted for cold storage, seedlings were lifted when they were both dormant and cold hardy. Bud set and mitotic indices of apical buds were used as indicators of morphological dormancy; from October to January, mitotic index was calculated biweekly on a random sample of 10 BL seedlings. Cold hardiness testing, (LT_{25} -18°C), was conducted from October through December as per General Material and Methods. The mitotic index of the apical buds was 0% and the seedlings were cold hardy to -18°C when they were lifted for cold storage. White spruce seedlings were lifted the first week of December in 1989 and in December of 1990, the coastal Douglas-fir were lifted the third week of December, 1989 and 1990. Randomly chosen seedlings of both species were lifted

Table 3. The number of bright sunshine hours per month measured at the Vancouver International Airport. Sunshine hours were calculated using the Campbell-Stokes method, supplied by Environment Canada (1989, 1990, 1991).

	Year	
Month	1989/90	1990/91
Aug	229.0	263.4
Sep	265.0	218.4
Oct	118.1	88.9
Nov	60.5	45.5
Dec	42.2	47.1
Jan	53.2	85.1
Feb	106.3	85.7
Mar	138.6	149.4
Apr	178.6	219.8
May	173.4	168.5

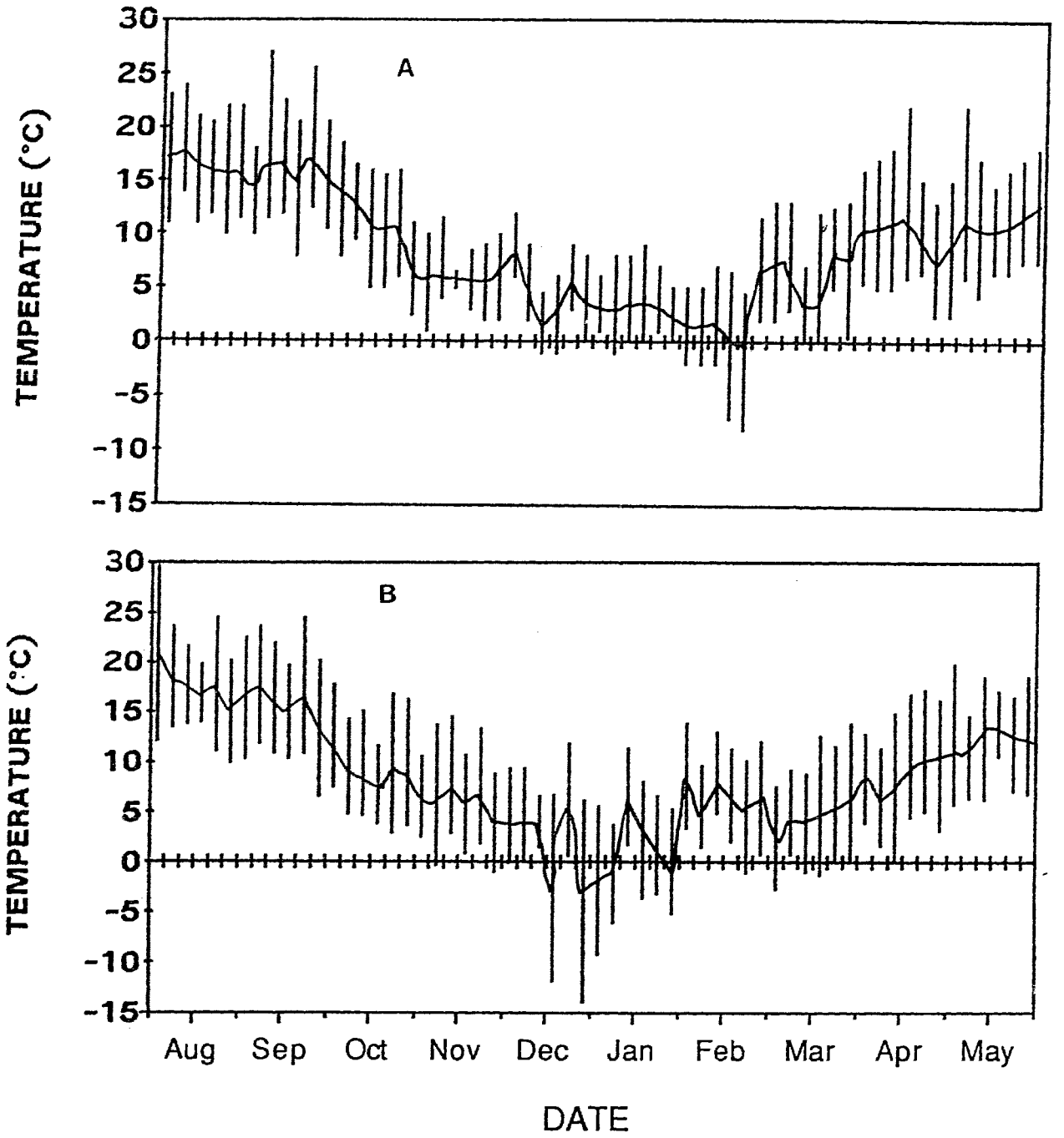

Figure 4. The five day minimum and maximum temperature measured at Simon Fraser University, A) 1989-1990 and B) 1990-1991. Average air temperature is indicated by solid line.

by hand and placed into 4 replicate batches of 10 samples in each batch and placed into cold storage.

Beginning the first week of March in 1990 (year 1) and 1991 (year 2), both CS and SL seedlings were placed in a growth chamber to induce growth. Two replicate batches of 10 randomly chosen naturally over-wintered BL seedlings were spring-lifted (SL), and one batch of 10 cold-stored (CS) seedlings was removed from cold storage each day for four consecutive days. The CS and SL seedlings were allowed to warm to room temperature (22°C) under low light conditions (70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPF) and potted as per General Materials and Methods. Seedling net photosynthesis, P_N, and chlorophyll fluorescence were measured 1, 2, 4, 10, 18 and 28 days after placing them under growth chamber conditions.

In year 1, one subsample each (n=10) of SL and CS seedlings were maintained in the growth chamber for 70 days (SL-70 and CS-70), at which time P_N, R_D and chlorophyll fluorescence were again measured.

Water potential

Needle water potential was measured using a J-14 Plant Press (Decagon, Pullman, WA) on day 1 and 28. A random sample of 5 needles was removed from each of 10 seedlings and placed on filter paper in the press window. Pressure was applied until liquid appeared on the uncut edge of the needles (Rayendrudu et al. 1983).

Statistical analysis

All statistical analyses were done on the mainframe computer at SFU using SAS (1988). Measurements of winter dormancy and spring recovery of BL seedlings were analyzed using one-way ANOVA contrasting for time. The 28 day spring recovery of the CS and SL seedlings' P_N, R_D and chlorophyll fluorescence components, Fv/Fm ratio and F_T, were analyzed using two-way ANOVA on one factor with repeated measurements and multiple comparisons (Bonferroni test) (Bellavance 1987; Winer 1977), as well, an F-protected T-test and non-overlapping 95% confidence intervals was used.

Differences in climatic conditions between year 1 and year 2 precludes data between years from being pooled (see Table 3 and Figure 4); however, when no significant difference was found between replicate groups within a given year, data were pooled. In year 1, chlorophyll fluorescence, root growth potential, P_N and R_D data for replicate groups of SL seedlings were pooled. Also in year 1, chlorophyll fluorescence, root growth potential, P_N and R_D data for replicate groups of CS seedlings were pooled. Data were pooled in a similar manner in year 2.

Simple linear correlations were used to determine relationships between factors where neither factor is assumed to be functionally dependent upon the other For these cases, the coefficient of determination, r^2, is reported as the measure of the strength of the straight-line relationship, no dependent and independent variable is implied (Zar 1984).

RESULTS

a) Baseline (BL) Seedlings

The number of days to bud break (Table 4), measured from the day seedlings were placed in the growth chamber, decreased over the winter and spring for both species. At the time of lifting for storage, time to bud break was 38 days for white spruce, 46 days for the Douglas-fir.

Both white spruce and Douglas-fir seedlings showed similar seasonal patterns of photosynthetic activity. Net photosynthesis and R_D decreased in the fall, reaching a minimum in January, then increased in the spring in both years (Figure 5).

Net photosynthesis of white spruce was positively correlated with day length (not sunshine hours) in year 1 ($r^2=.76$) and in year 2 ($r^2=.75$). Net photosynthesis was also correlated with average air temperature for the previous 5 days based on Figure 4, in year 1 ($r^2=.74$) and 2 ($r^2=.69$). Similar correlations were found for Douglas-fir, P_N correlated with day length ($r^2=.69$) in year 1 and 2 ($r^2=.72$), and average air temperature in year 1 ($r^2=.65$) and 2 ($r^2=.63$).

Table 4. Days to bud break for white spruce and Douglas-fir seedlings over-wintered outside. Bud break was measured from the time seedlings were placed in growth chamber until 50% of the terminal buds had new needles showing.

Species	Date					
	Dec 6	Dec 21	Jan 11	Feb 11	Mar 21	Apr 23
White spruce	38	27	20	10	*	*
Douglas-fir	NA	46	NA	25	20	14

* seedlings had already flushed by this date

NA data not collected for this date

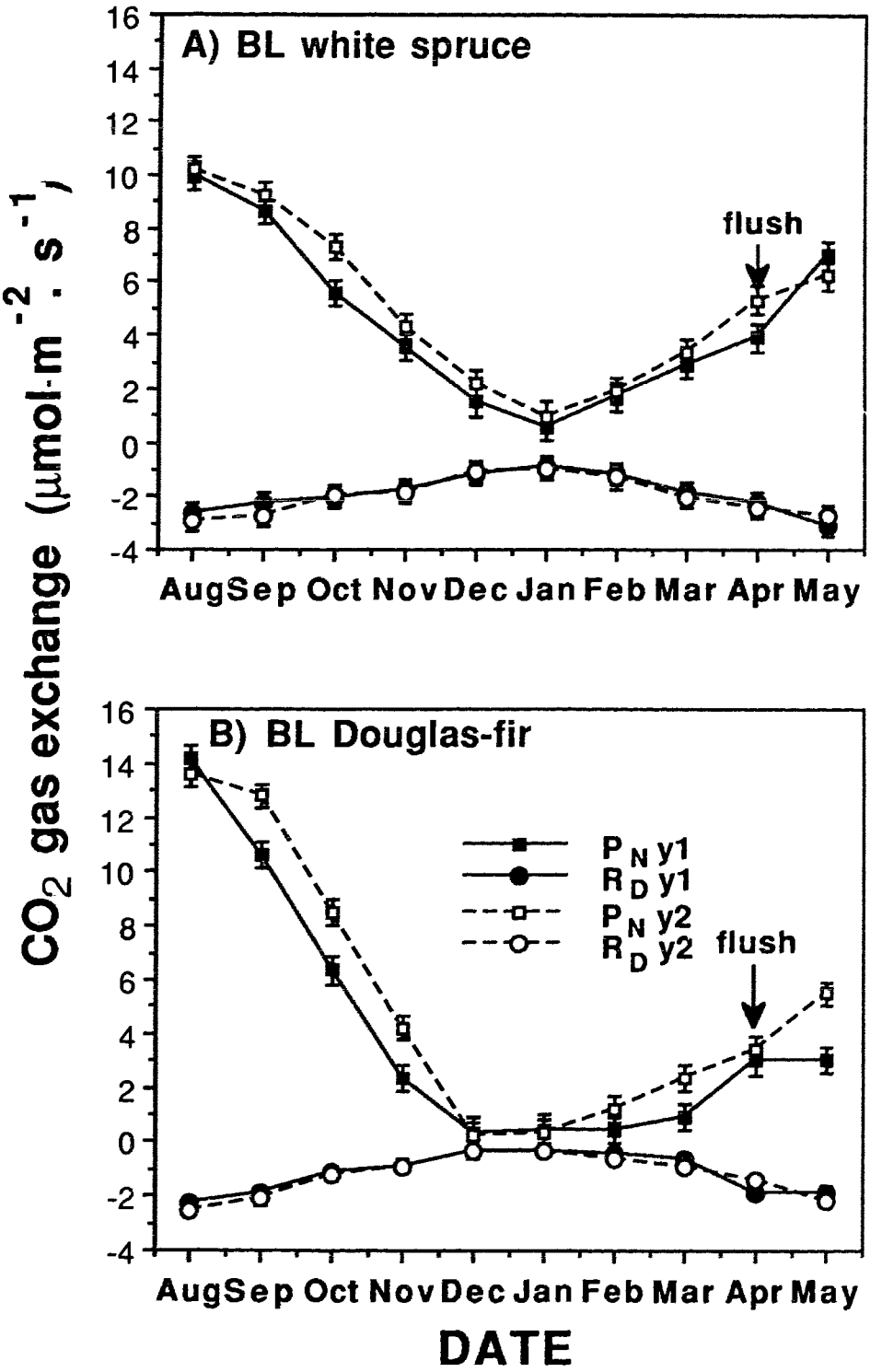

Figure 5. Seasonal CO_2 gas exchange of baseline (BL) seedlings over-wintered under natural conditions. A) white spruce, B) Douglas-fir. Arrow indicates when 50% of the terminal buds had flushed. Net photosynthesis (P_N) and dark respiration (R_D) measurements were taken mid-month. (Mean ± SE, n=10)

Chlorophyll fluorescence induction curves for BL white spruce seedlings from August to April are shown in Figure 6. The typical features of such curves, known as Kautsky or induction curves are described in Krause & Weis (1984). Each curve is an average of 10 seedlings, data have been normalized to compensate for different seedling sizes. The shape of the induction curve varied each time, and was substantially different on all four dates.

Both species showed seasonal changes in the chlorophyll fluorescence induction curve. The Fv/Fm ratio decreased from August to January for both species (Figure 7), then recovered in the spring. In year 1, the Fv/Fm ratio was positively correlated to daylength (r^2=.72) for white spruce and Douglas-fir (r^2=.76), and was similarly correlated in year 2 (r^2=.69, r^2=.73) for white spruce and Douglas-fir respectively. The Fv/Fm ratio also correlated positively with average air temperature for white spruce (r^2=.75) and Douglas-fir (r^2=.68) in year 1, and (r^2=.68) for white spruce and Douglas-fir (r^2=.76) in year 2.

Steady state fluorescence, F_T, also decreased to a minima in January, then increased over time in the spring. Steady state fluorescence correlated with average air temperature for white spruce in year 1 (r^2=.79) and 2 (r^2=72), and Douglas-fir in year 1 (r^2=.65) and 2 (r^2=.63).

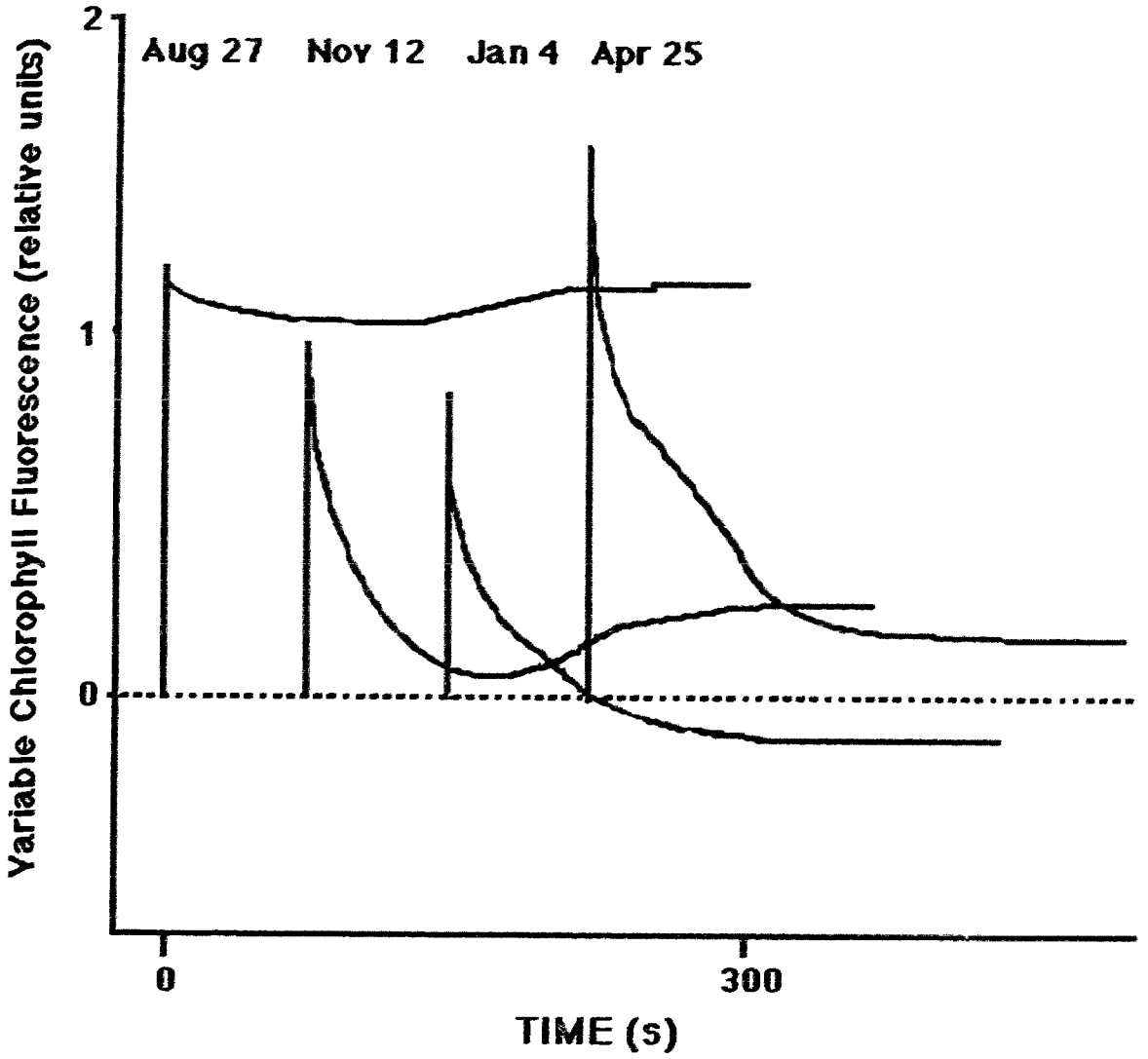

Figure 6. Normalized chlorophyll fluorescence induction curves of white spruce seedlings. Each curve represents an average of 10 naturally over-wintered seedlings assayed in August, November, January and April.

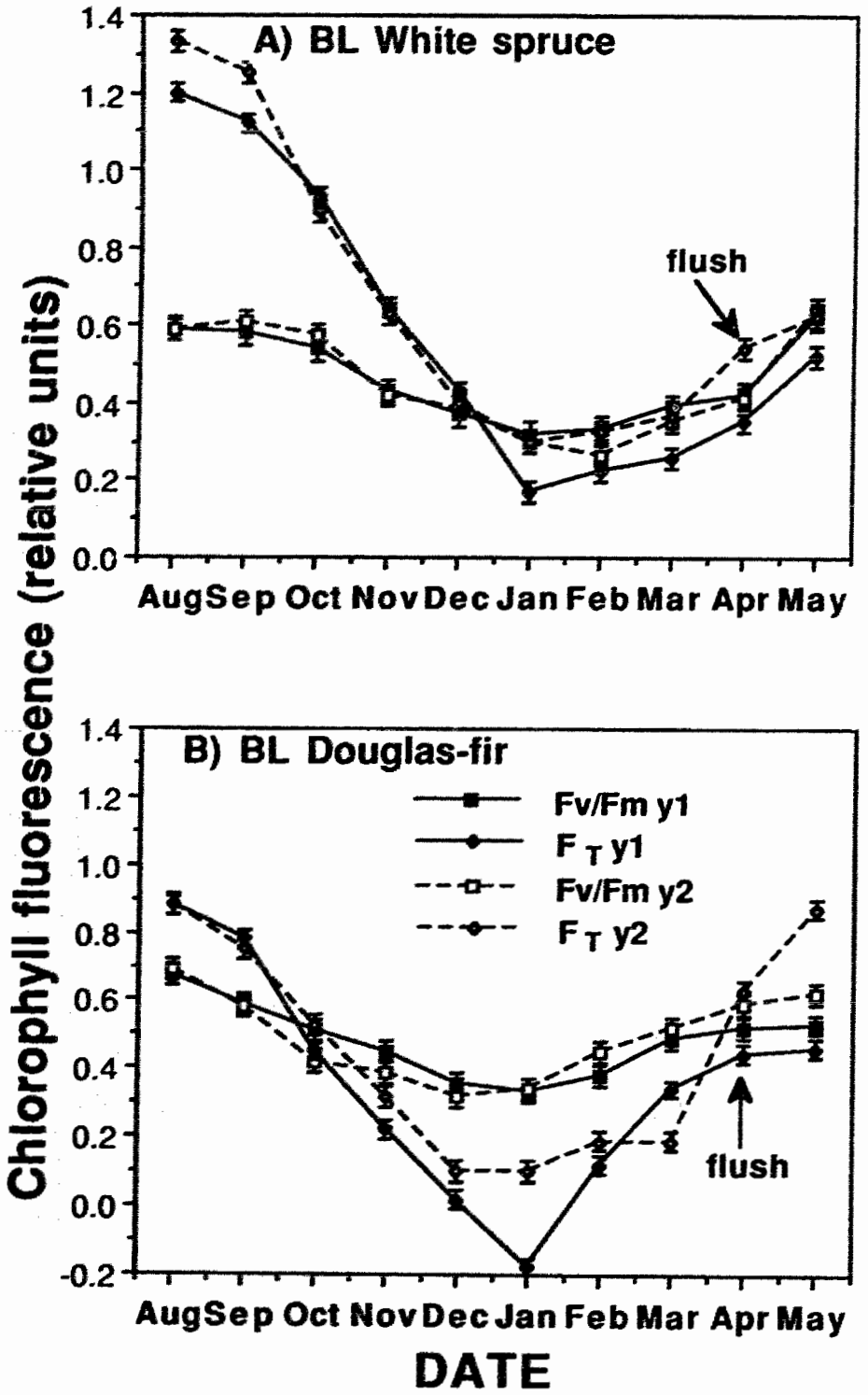

Figure 7. Ratio of variable fluorescence to maximal fluorescence, Fv/Fm, and steady state fluorescence, F_T, of baseline seedlings (BL) naturally over-wintered, A) white spruce, B) Douglas-fir. Measurements were taken mid-month. (Mean ± SE, n=10)

b) Spring recovery of SL and CS seedlings

After 28 days in the growth chamber, both the white spruce and the Douglas-fir SL and CS seedlings had all flushed. The newly flushed needles were tightly bunched and had not fully expanded on day 28. New needles were succulent and light green in colour.

Root growth potential and water potential

In year 1, the RGP of the SL white spruce seedlings was higher than in year 2 (Table 5). There was no significant difference between the RGP of the CS seedlings in year 1 and year 2. The average length per white root was significantly longer in SL seedlings (7.2 cm) than CS seedlings (1.4 cm).

In year 1, the RGP of the SL and CS Douglas-fir seedlings were not significantly different from each other; however, in year 2, the RGP of the CS seedlings was significantly higher than the SL seedlings (Table 5). The average length per white root of the SL seedlings was 8 cm, the average length per white root of CS seedlings was 1.5 cm.

For both species, the needle water potentials of both the SL and CS seedlings were significantly higher on day 28 than on day 1 (Table 6).

Table 5. Root growth potential (number of white roots greater than 1.0 cm in length) of white spruce and Douglas-fir seedlings. Root growth potential was measured after 28 days in growth chamber (mean ± SE, n=40 CS, n=20 SL) for both spring-lifted (SL) and cold-stored (CS) seedlings.

Species	treatment	Year 1	Year 2
White spruce	SL	36.5± 1.2[a]	26.1± 1.4[b]
	CS	24.6± 2.1[b]	19.7± 1.7[b]
Douglas-fir	SL	35.7± 1.5[a]	20.5± 1.5[b]
	CS	33.6± 1.6[a]	29.6± 1.8[ab]

[a,b] same letter signifies no significant difference between rows and columns.

Table 6. Needle water potentials for a) naturally over-wintered spring-lifted (SL) and, b) fall lifted and cold-stored (CS) white spruce and Douglas-fir seedlings. Needle water potential was measured with a J-14 leaf press on day 1 and day 28 after placement in a growth chamber. (Mean ± SE, n=10)

Species	Treatment	n=	time	Water potential MPa
			Year 1	
White spruce	CS	40	day 1	-1.97 ± 0.32[a]
	CS	40	day 28	-1.34 ± 0.29[b]
	SL	20	day 1	-1.72 ± 0.33[a]
	SL	20	day 28	-1.37 ± 0.31[b]
Douglas-fir	CS	40	day 1	-1.94 ± 0.35[a]
	CS	40	day 28	-1.17 ± 0.16[b]
	SL	20	day 1	-1.89 ± 0.30[a]
	SL	20	day 28	-1.18 ± 0.27[b]
			Year 2	
White spruce	CS	40	day 1	-1.89 ± 0.33[a]
	CS	40	day 28	-1.28 ± 0.27[b]
	SL	20	day 1	-1.80 ± 0.29[a]
	SL	20	day 28	-1.31 ± 0.35[b]
Douglas-fir	CS	40	day 1	-1.95 ± 0.26[a]
	CS	40	day 28	-1.18 ± 0.28[b]
	SL	20	day 1	-1.91 ± 0.31[a]
	SL	20	day 28	-1.23 ± 0.29[b]

[a,b], same letter signifies no significant difference between values in column (Bonferroni test $p<.001$)

Carbon dioxide gas exchange

After placement in the growth chamber, for most groups a rapid increase in the P_N rate from day 1 through day 5 was observed, then in most cases a less rapid but still progressive increase occurred (Figure 8). The decreased P_N rate seen from day 5 through 28 may be partly attributable to increased dark respiration perhaps related to bud swell. From day 15 to 28, rapid increases in dry weight and surface area of the newly flushed needles before they have full photosynthetic capacity and increased self shading would also decrease P_N rates.

There were no significant differences in P_N or R_D rates between SL and CS white spruce seedlings in year 1 or 2.

There was a significant difference in the P_N rates of the Douglas-fir seedlings in year 1 and year 2, and there was a significant difference between SL and CS seedlings. In year 1, the P_N rates of the SL seedlings increased significantly over time (Figure 8); however, in year 2, there was no significant increase in P_N over time.

In year 1, the P_N and R_D rates of the CS Douglas-fir seedlings increased until day 10, then was not significantly different through to day 28. In year 2 the P_N and R_D rates continued to increase over time (Figure 8).

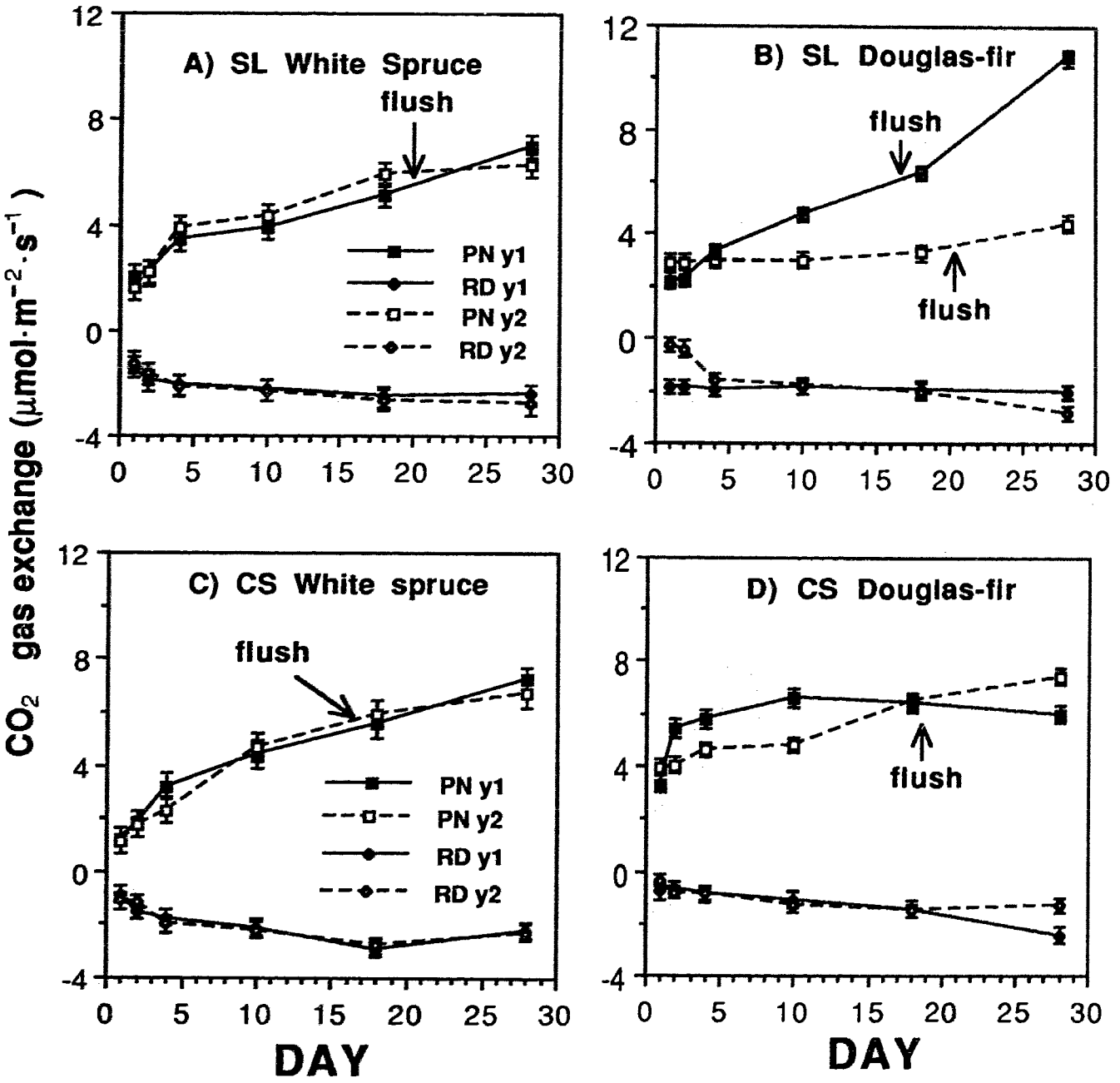

Figure 8. CO_2 gas exchange of spring-lifted (SL) and cold-stored (CS) white spruce and Douglas-fir seedlings. Net photosynthesis (P_N) and dark respiration (R_D) were measured while seedlings were in growth chamber for 28 days in March. A) SL White spruce, B) SL Douglas-fir, C) CS white spruce, D) CS Douglas-fir. (mean ± SD, n=40 CS, n=20 SL).

Chlorophyll fluorescence

There was a significant change in Fv/Fm ratio of the SL and CS white spruce seedlings over the 28 days in the growth chamber (Figure 9).

Steady state fluorescence, F_T, increased over time for both the CS and SL white spruce seedlings.

There was no significant increase in the Fv/Fm of the SL Douglas-fir seedlings from day 1 to day 28 in both year 1 and 2 (Figure 9). There was no significant change in steady state fluorescence, F_T, over time in year 1. The F_T measured in year 1 was significantly higher than in year 2. In year 2, F_T increased significantly over time (Figure 9).

Cold-stored Douglas-fir seedlings also showed no significant increase in Fv/Fm ratio from day 1 through day 28; however, year 2 was significantly higher than year 1 (Figure 9). Steady state fluorescence, F_T, of the CS seedlings increased significantly over time in year 1, but not in year 2. In year 2, F_T was below relative F_O level until the fourth day after potting (Figure 9).

There was a linear correlation between P_N measured on day 1 and RGP measured on day 28 (Figure 10A) for cold-stored seedlings. A linear correlation was found between the Fv/Fm ratio measured five hours after cold-stored seedlings were removed from storage and RGP measured after 28 days (Figure 10B).

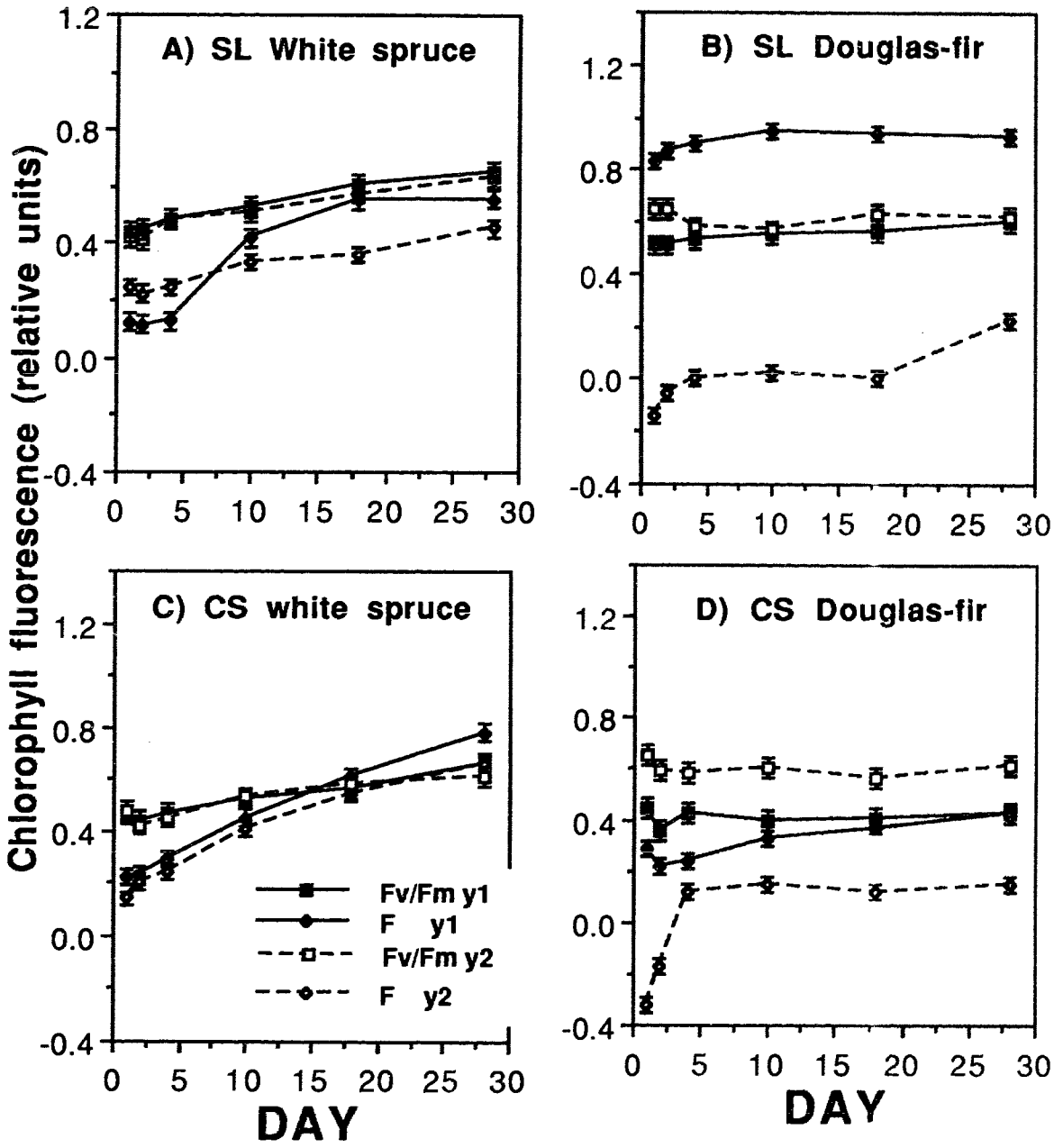

Figure 9. Ratio of variable fluorescence to maximal fluorescence, Fv/Fm, and steady state fluorescence, F_T, for spring-lifted (SL) and cold-stored (CS) white spruce and Douglas-fir seedlings. Measurements were taken while seedlings were in growth chamber for 28 days in March. (A) SL white spruce, B) SL Douglas-fir, C) CS white spruce, D) CS Douglas-fir. (mean ± SD, n=40 CS, n=20 SL).

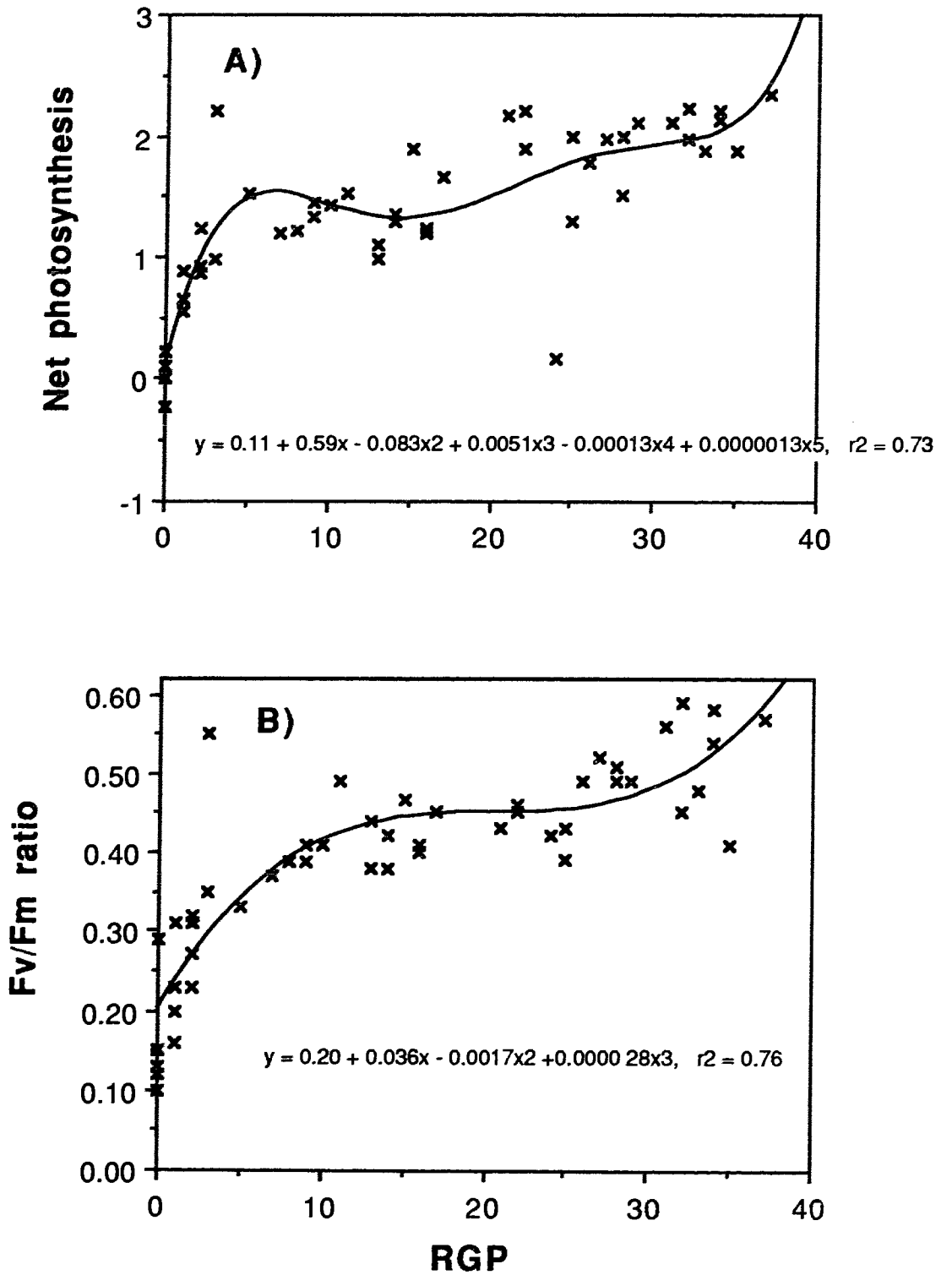

Figure 10. A) Net photosynthesis (P_N) measured five hours after seedlings removed from cold storage related to RGP of same seedlings measured after 28 days. Each point is an average of five seedlings. B) Fv/Fm ratio measured five hours after seedlings removed from cold storage related to RGP; each point is an average of five seedlings.

Table 7 summarizes the differences in net photosynthesis and Fv/Fm ratio of newly flushed and 1 year old needles after seedlings experienced 28 days in the growth chamber and 70 days in the growth chamber. It should be noted that new needles were not 28 days old; buds flushed between 15-20 days after seedlings were placed in the growth chamber.

Analysis of variance indicated that neither cold storage nor spring lifting were significant factors in affecting P_N or Fv/Fm in these seedlings. The age of the needles was a signficant factor for P_N after 28 days and Fv/Fm after 70 days. Newly flushed white spruce needles had significantly higher P_N rates after 28 days in the growth chamber than one year old needles. There were no significant differences in the P_N rates between newly flushed needles and one year old needles after 70 days in the growth chamber. Newly flushed needles had significantly higher Fv/Fm ratios than one year old needles after 70 days in the growth chamber.

Table 7. Net photosynthesis, P_N, and ratio of variable to maximal fluorescence, Fv/Fm, of newly flushed and one year old needles. Seedlings were either naturally over-wintered and spring-lifted (SL) or cold-stored (CS) seedlings. Measurements taken after 28 and 70 days in a growth chamber in year 1 (mean ± SD, n=10).

	Net photosynthesis, P_N ($\mu mol \cdot m^{-2} \cdot s^{-1}$)			
Treatment	28 days		70 days	
	New	1 y old	New	1 y old
White spruce				
SL	9.78±0.4^{a}	6.45±0.5^{b}	11.32±0.4^{c}	10.15±0.3^{ac}
CS	8.99±0.3^{a}	7.33±0.6^{ab}	11.77±0.4^{c}	10.61±0.5^{c}
Douglas-fir				
SL	10.89±0.3^{a}	10.35±0.4^{a}	13.35±0.4^{d}	12.69±0.3^{d}
CS	10.28±0.4^{a}	9.45±0.4^{a}	12.98±0.4^{d}	12.12±0.3^{cd}

	Fv/Fm ratio (relative units)			
Treatment	28 days		70 days	
	New	1 y old	New	1 y old
White spruce				
SL	0.63±0.14^{a}	0.56±0.09^{ab}	0.78±0.12^{c}	0.61±0.11^{ab}
CS	0.65±0.12^{a}	0.51±0.16^{b}	0.76±0.09^{c}	0.56±0.13^{ab}
Douglas-fir				
SL	0.66±0.13^{a}	0.57±0.11^{ab}	0.79±0.14^{c}	0.59±0.12^{ab}
CS	0.68±0.11^{a}	0.54±0.08^{b}	0.76±0.09^{c}	0.52±0.10^{b}

^{a,b,c} same letter signifies no significant difference between values in columns or rows (Bonferroni test $p<.001$)

DISCUSSION

In both white spruce and Douglas-fir, the number of days to bud break in the baseline (BL) seedlings steadily declined from December till April (Table 4). Such a decrease in the number of days to bud break over time is commonly seen in conifers, and is related to an accumulation of chilling hours (Campbell & Sugano 1975, Carlson 1985, Garber 1983, Ritchie 1984). Seedlings over-wintered in an open compound rely on natural conditions to meet their chilling requirement, whereas the cold-stored seedlings can have a portion of theirs met within storage. The days to bud break in all cases indicates buds were in the quiescent state, where environmental conditions were maintaining rest and much of the chilling requirement had already been met (Lavender 1985).

The average length of new white roots was much longer in spring-lifted (SL) seedlings than cold-stored (CS) seedlings, possibly because of the longer growing period available to the spring-lifted seedlings. Even though photosynthetic rates of the baseline seedlings over-wintered outside was low in February and March, and were likely overestimated by warming them in the laboratory prior to measuring, they could still have been capable of positive net photosynthesis during warm periods, and thus accumulated more total photosynthate for root growth sooner than the cold-stored seedlings. This possible accumulation of more total photosynthate is consistent with Fielder & Owens (1989) who found that

naturally over-wintered containerized British Columbia coastal Douglas-fir has a root growth period beginning in late January or early February and continuing through the early spring.

Similar to the results found by McCreary & Duryea (1987), the needle water potential of both cold-stored and spring-lifted seedlings was higher after 28 days in the growth chamber, and was positively correlated to RGP (Deans et al. 1990). Seedlings with high RGP values had higher water potential values on day 28; consistent with previous results found by Deans et al. (1990). Seedlings with lower RGP values had needle water potential values that did not change significantly from day 1 to day 28, indicating restricted water conduction and root function.

The chlorophyll fluorescence of naturally over-wintered white spruce (Vidaver et al. 1989a) and Douglas-fir seedlings (Hawkins 1981) has previously been found to decrease in the fall and recover in the spring. The variable chlorophyll fluorescence of baseline seedlings over-wintered outside in this study were also low in the winter and recovered over several months in the spring. This reversible decline of Fv/Fm in winter is due to a decrease in Fm, not an increase in F_O, indicating there was no damage to the photosynthetic reaction centers (Briantais et al. 1986).

The decline of Fm in winter, and its subsequent recovery in spring, has been attributed to either photochemical regulation of the photosynthetic apparatus in conifers

(Vidaver et al. 1989a) or to photoinhibition (Krause & Somersalo 1989, Öquist & Malmberg 1989) and has been treated in the literature as the same phenomenon. This decine of Fm in winter serves an adaptive function to prevent photodamage during periods of cold and high light levels (Krause & Somersalo 1989). The baseline naturally over-wintered seedlings had low Fv/Fm ratios in the winter, which increased significantly over time prior to bud flush. This would be expected if reversible photosynthetic inactivation or photochemical regulation were involved; cold-stored seedlings showed little increase in Fv/Fm prior to flushing.

Spring reactivation of photosynthesis in cold-stored white spruce seedlings has previously been monitored using chlorophyll fluorescence; Vidaver et al. (1989a) show that the first 30 seconds of the chlorophyll fluorescence curve of vigourous seedlings return to nearly normal within 48 hours of removal from storage in the spring. In this study, a high Fv/Fm ratio five hours after seedlings were removed from cold storage was indicative of rapid recovery of PSII activity. This agrees with Van den Driessche (1987), who suggests reactivation of photosynthesis may be very rapid in seedlings after removal from cold storage; seedlings removed from cold storage in the current study had positive net photosynthetic rates within 5 hours. Prior to 5 hours, there was negligible net uptake of CO_2 (data not shown), perhaps because of high stomatal resistance, or insufficient recovery of photochemical or biochemical components of photosynthesis.

The Fv/Fm ratio declined on day 2 in all cold-stored seedlings in both year 1 and year 2 (as seen in Figure 9), and in most cases did not recover to the day 1 value until day 28. This decline of Fv/Fm on day 2 may be related to the cold storage treatment; seedlings were maintained under cold, dark conditions for 13 weeks. Internal protective mechanisms to prevent damage from excess light may not be fully functional when removed from such an extended storage.

The higher Fv/Fm ratios of the newly flushed needles after 70 days may be partly attributable to newly flushed needles being fully expanded on day 70, whereas, on day 28 they were still tightly bunched. Measuring the chlorophyll fluorescence of tightly bunched needles in an integrating sphere resulted in lower fluorescence levels than for fully expanded needles possibly because of self-shading. Newly flushed needles appeared to have higher PSII photochemical efficiency than old needles, as indicated by higher Fv/Fm values, although the photosynthetic rate was not significantly different between them. This may be explained if the new needles' PSII and electron transport system have surplus capacity relative to that of reductive carbon metabolism (Öquist & Malmberg 1989).

The F_T of the baseline seedlings in year 2 was extremely low during winter, reaching levels below apparent F_O levels in January or February (Figure 7). This was also observed in spring-lifted Douglas-fir seedlings in year 2 after they were placed in the growth chamber. Such a decline in the F_T

portion of the chlorophyll fluorescence curve is likely due to F_O quenching, caused by quenching within the pigment bed or the reaction centers (Bilger & Schreiber 1986, Bilger & Bjorkman 1990). The phenomena of F_O quenching has been observed in seedlings under low CO_2 conditions and environmental stress, and has been partially attributed to xanthophyll cycle regulation of the energy flow to PSII reaction centers (Demmig et al. 1987, Demmig-Adams 1990, Adams et al. 1990). Seedlings maintained outside under natural conditions may be subject to the combination of low temperatures and bright sunlight in the winter. Therefore, along with photoinhibition and photochemical regulation, F_O quenching may allow excess energy to be dissipated harmlessly as heat.

In this study, seedlings with F_T values below apparent F_O (F_O quenching) prior to placement in cold storage, displayed F_O quenching after cold storage. The presence of F_O quenching after cold-storage was not necessarily associated with decreases in net photosynthetic rates nor a decrease in seedling vigour or quality. The amplitude of the Fv/Fm ratio after cold storage was more indicative of photosynthetic reactivation than F_T. The presence of F_O quenching may be a confounding factor in the interpretation of chlorophyll fluorescence induction curves. The persistence of F_O quenching after seedlings were placed in the growth chamber implies more than just a short term, rapidly reversible structural change in the pigment bed, the

possibility of changes in pigment bed composition must also be considered.

Spring-lifted Douglas-fir seedlings had lower photosynthetic rates in year 2, possibly as a result of exposure to environmental stresses. Conifers can suffer photodamage during winter, resulting in the loss of chlorophyll due to bleaching (Öquist 1983), although extremely low temperatures in combination with bright sunshine is rare in coastal British Columbia, as can be seen in Table 3 and Figure 4. The extremely low temperatures experienced in December of year 2 were not associated with periods of sunshine. It is more likely winter freezing damaged the roots. The roots of containerized seedlings are generally more sensitive to low temperatures than shoots, and any damage to the roots due to winter freezing results in decreased RGP in the spring (Lindström & Nyström 1987). Recovery of photosynthetic activity in the spring could therefore be highly dependent on environmental conditions experienced during the previous winter.

Root growth potential has been proposed as being indicative of whole seedling quality (Ritchie & Tanaka 1990). The relationships found between RGP and needle water potential, net photosynthesis, Fv/Fm and F_T for both the cold-stored and spring-lifted seedlings indicates a strong interaction between shoot and root functioning. Van den Driessche (1987) found new photosynthate was necessary for

new root growth in the spring, suggesting that increased photosynthetic rates could result in higher RGP values.

There appears to be a trade-off between over wintering seedlings under natural conditions and placing them in cold storage. Immediately out of storage, cold-stored seedlings may be deficient in internal protective mechanisms against high light intensity, as indicated by the decline of Fv/Fm seen in these seedlings after being placed in the growth chamber. Therefore, direct planting of cold-stored seedlings in clear cut or highly exposed areas may be detrimental to seedling survival. Cold-stored seedlings are planted in the late spring and summer, which may also hinder seedling establishment. Douglas-fir has a root growth period in the early spring and fall; cold-stored seedlings do not experience this early spring root growth period. The longer average white root length of naturally over-wintered seedlings may place them at an advantage in dry and highly competitive conditions.

Although this study suggests photochemical regulation is operating during fall dormancy induction, further research was conducted and reported in Section 3 to look at the process of photochemical regulation during fall and winter dormancy. This section provides background data on seedlings produced using typical conifer nursery procedures that were non-detrimental to seedling vigour and provides a basis of comparison for seedlings given alternative dormancy induction treatments.

The current method of determining fall and winter cold storage lifting dates is based on cold hardiness testing. Including other techniques for assessing the physiological status of seedlings may help produce consistent, high quality seedlings.

Once seedlings have been determined to be suitable for cold storage, lifting may not actually commence for several weeks. The next study looked at the physiological effects of dehardening temperatures on such seedlings, their storability and quality after cold storage.

II. Effect of temperature variations on the pre- and post -storage physiology of white spruce and Douglas-fir seedlings

INTRODUCTION

Over-winter cold storage of seedlings is an important silvicultural procedure to keep seedlings in a dormant state until late spring planting (Ritchie & Tanaka 1990). In British Columbia, many conifer nurseries are located in the Fraser Valley, where maximum temperatures in unheated greenhouses can reach over 15°C during November and December. Warming prior to lifting for storage may affect the suitability of the seedlings for storage and the quality of the seedlings after storage (Gillies & Vidaver 1991).

This study assesses the effects of short term pre-storage temperature treatments on the physiological activity of dormant seedlings, as well as the recovery of photosynthetic activity of the treated seedlings after cold storage. Seedlings grown under standard nursery practices were obtained from diverse climatic regions, ranging from northern interior British Columbia (56°N latitude) to coastal Oregon (46°N latitude), because seedling temperature responses may be related to provenance.

MATERIALS AND METHODS

The container grown 1-0 white spruce and coastal Douglas-fir seedlings from British Columbia used in this study are described in General Materials and Methods. A southern seedlot of coastal Douglas-fir from Oregon grown in 415

Styroblock containers was used as well (seedlings supplied by Pelton Reforestation, Ltd., Maple Ridge, British Columbia). Seed origin was near sea level, 44°N latitude, 124°W longitude.

British Columbia coastal Douglas-fir and Oregon Douglas-fir sown in 1989 were used for study in 1989-90, white spruce seedlings sown in 1990 were used in 1990-91. Seedlings were brought to Simon Fraser University (SFU) in August, left in the styrobocks, and placed in an unheated glass greenhouse. Seedlings were well watered but were not fertilized after this date.

In December and January of each year, seedlings were assessed for dormancy using mitotic index, cold-hardiness testing (LT_{50}), net photosynthesis (P_N), and chlorophyll fluorescence. When seedlings were considered dormant and physiologically inactive [MI=0%, CH < -18°C, Fv/Fm ratio and P_N were low] they were randomly divided into treatment groups and placed in a growth chamber at 48 hour intervals for temperature treatment. Growth chamber conditions were: 12 hour photoperiod, PPF of 375 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and 70-85% RH maintained by a container of water placed on the floor of the growth chamber. Six replicates of 10 samples each were placed under 4 different temperature regimes (day/night) for 48 hours in the growth chamber: $T_{5/1°C}$; $T_{10/5°C}$; $T_{15/10°C}$; $T_{20/15°C}$. Immediately after temperature treatment, a subsample of seedlings, 3 replicates of 5 samples each, were taken to the laboratory where cold hardiness, net photosynthesis, and

chlorophyll fluorescence were again measured. The remainder of the seedlings were lifted and placed in cold storage (-2 ± 1°C) for 15 weeks at SFU as per General Materials and Methods.

Following storage, seedlings were potted and placed in a growth chamber for 28 days as per General Materials and Methods. Gas exchange and chlorophyll fluorescence were monitored regularly (a total of six times) during the 28 days in the growth chamber.

Several morphological parameters of all seedlings were measured after 28 days. The number of white roots > 1 cm in length, the length of the newly flushed leader and the oven dry weight (48 hours at 70°C) of newly flushed leaders were measured at that time. Flushing of apical buds of all seedlings was monitored daily as per General Materials and Methods.

Post-treatment data was analysed using SAS/STAT (1988). Two way ANOVA, as well as one way ANOVA with orthogonal contrasts were done. The ambient temperature at the time of lifting the seedlings prior to treatment varied; therefore, pretreatment parameters were not included in the statistical comparison of the treatment groups. The $T_{5/1}$ group was considered the control because on the basis of my hypothesis, this temperature would reflect the ideal pre-lifting conditions.

In the spring, the same seedlings were measured repeatedly to reduce the variation in sampling. This allowed for a reduction in the number of seedlings tested. To

compensate for a lack of independence, post-storage spring measurements of P_N, Fv/Fm and F_T were analyzed independently using two-way ANOVA on each factor compensating for repeated measurements, ANOVA tested for differences between each sampling date and between the four different treatments, resulting in multiple comparisons (Bonferroni tests of differences) (Bellavance 1987; Winer 1971).

Morphological characteristics of seedlings on day 28 were analyzed using one-way ANOVA and Bonferroni tests of differences. Simple linear correlations were used to determine relationships between factors where neither factor is assumed to be functionally dependent upon the other, the coefficient of determination, r^2, is reported. Linear regressions were used to determine relationships between dependent and independent variables, the coefficient of determination, r^2, is reported as the measure of the strength of the straight-line relationship (Zar 1984). Where there was no significant difference between replicate groups, data were pooled.

RESULTS

Post treatment

Two way ANOVA, using data for all species, showed a significant treatment effect on P_N and F_T ($p=.001$). There was also a strong species*treatment interaction for all parameters, indicating each species had different responses to the treatment.

The chlorophyll fluorescence induction curves varied with temperature treatment for white spruce, as seen in Figure 11. One way ANOVA on individual species showed the treatment had a significant effect on steady state fluorescence, F_T, for all species ($p=.0001$) (Table 8). Steady state fluorescence, F_T, decreased with decreasing temperature ($r^2=.79$).

The treatment had a significant effect on P_N (Table 8), which increased with increasing temperature for the white spruce and B.C. Douglas-fir, and was neither linear nor quadratic for any of these species. Cold hardiness was also significantly affected, decreasing with temperature increases.

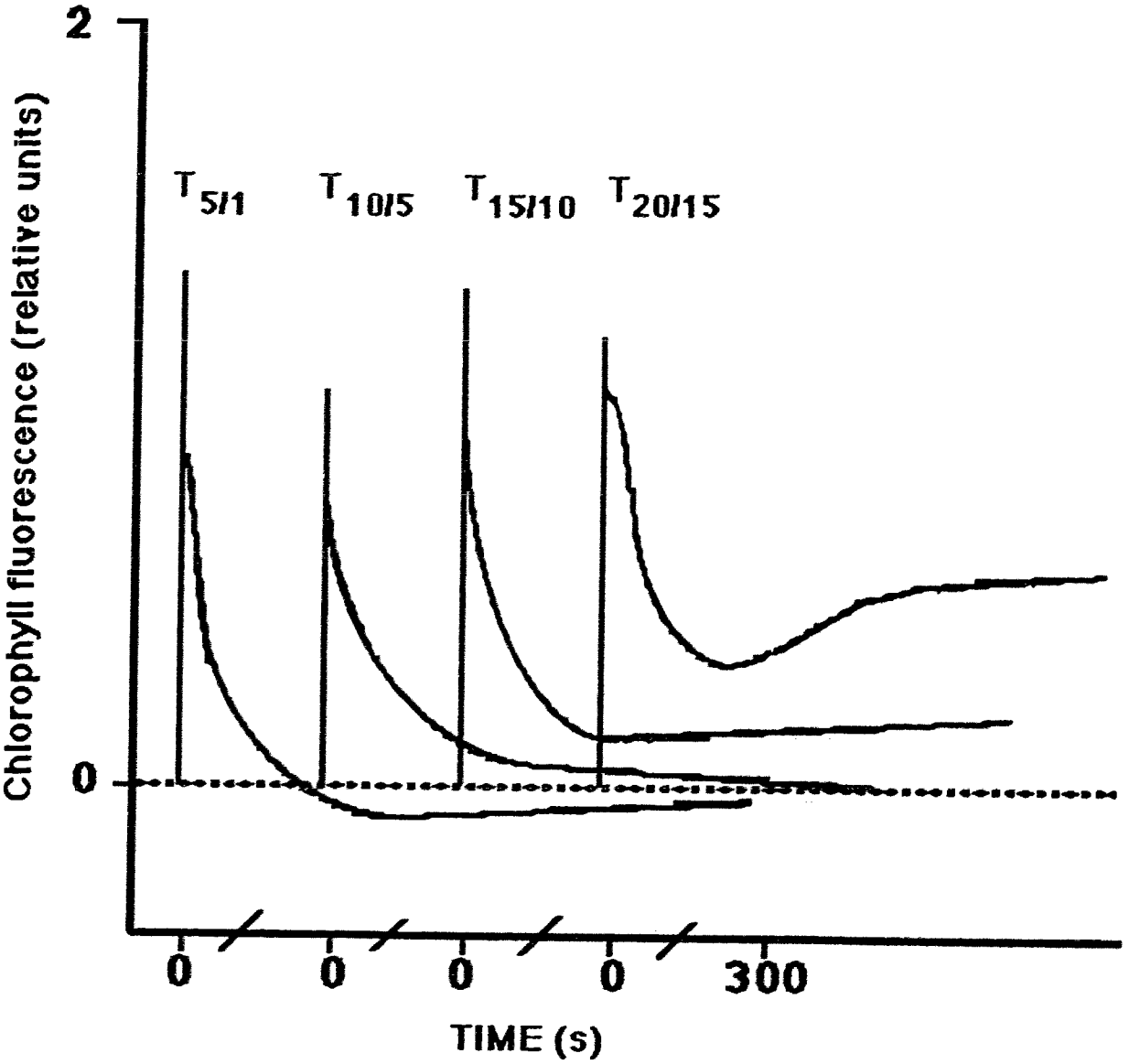

Figure 11. Normalized chlorophyll fluorescence induction curves of white spruce seedlings after 48 hour temperature regimes. Each curve represents the average of ten seedlings. Day/night temperature treatments were 5/1, 10/5, 15/10, and 20/15°C.

Table 8. Physiological parameters measured after 48 hour temperature regimes. Day/night temperature treatments were 5/1, 10/5, 15/10, and 20/15°C. Mean ± SE (n=45).

Species	pretreatment	$T_{5/1}$	$T_{10/5}$	$T_{15/10}$	$T_{20/1°C}$
F_T (relative units)					
White spruce	-.08±.01	.04±.01[a]	.02±.01[a]	.13±.02	.40±.02
BC Douglas-fir	.12±.04	.08±.02	.18±.03	.40±.04	.62±.08
Oregon D-f	.22±.09	.08±.04[a]	.08±.05[a]	.10±.04[a]	.22±.05
Fv/Fm (relative units)					
White spruce	.43±.05	.42±.05	.41±.06	.47±.05	.45±.06ns
BC Douglas-fir	.46±.07	.46±.06	.47±.07	.46±.07	.46±.05ns
Oregon D-f	.54±.07	.67±.07	.57±.06	.66±.07	.66±.08ns
P_N [$\mu mol \cdot m^{-2} \cdot s^{-1}$]					
White spruce	1.07±.12	1.02±.1[a]	1.06±.1[a]	1.46±.12	1.85±.1
BC Douglas-fir	2.39±.2	0.92±.12	1.37±.1	2.12±.1[a]	2.0±.2[a]
Oregon D-f	3.39±.48	1.92±.1[a]	2.37±.06	2.12±.06	2.0±.1[a]
Cold Hardiness (LT_{50})					
White spruce	-32±1	-31±1[a]	-28±1[a]	-24±1[b]	-24±1[b]
BC Douglas-fir	-23±1	-22±1[a]	-22±1[a]	-20±1[ab]	-18±1[b]
Oregon D-f	-18±1	-18±1[a]	-17±1[a]	-15±1[ab]	-14±1[b]

[a,b] same letter signifies results are not significant between columns at p=.01 (Bonferroni test).
ns not significant
D-f - Douglas-fir

Post-storage

The pre-storage temperature treatments had a significant effect on the post-storage recovery of all seedlings (Table 9). There was a strong negative relationship between pre-storage temperature and the number of white roots; white spruce (r^2=-.65), B.C. coastal Douglas-fir (r^2=-.83) and Oregon Douglas-fir (r^2=-.93). The lowest temperature treatment ($T_{5/1°C}$) seedlings had the best root growth for all species.

Vigour, as measured by the rapidity of budbreak, decreased with increased treatment temperature (Table 9). Temperature treatment also had a significant effect (p=.001) on the length and dry weight of the new flushed leaders (Table 9). The lowest temperature treatments yielded the longest new leaders with the highest dry weight.

Net photosynthesis, P_N, measured over the 28 day recovery period, increased significantly for most treatment groups; a strong species*treatment interaction was also found (Figure 12). Net photosynthesis varied significantly with treatment temperature for each of the species tested, with P_N being lowest in the $T_{15/10}$ and $T_{20/15}$ treatment groups, in particular on day 28. A relationship was found between P_N and root growth for both species (r^2=.87), where seedlings with the highest P_N had the greater number of roots.

Steady state fluorescence, F_T, increased significantly (p=.001) over the 28 days for all seedlings, but varied widely with treatment temperature for each species and provenance

(Figure 13). Steady state fluorescence, F_T, measured on day 28 correlated with root growth (r^2=.86) and P_N (r^2=.89).

There was a significant (p=.001) treatment effect on Fv/Fm over time in the B.C. coastal Douglas-fir seedlings (Figure 14). Seedlings from the $T_{5/1}$ treatment group had the highest Fv/Fm on day 1 for all seedlings, and Fv/Fm on day 1 correlated with root growth (r^2=.73). The Fv/Fm ratio was significantly (p=.01) lower in the $T_{15/10}$ and $T_{20/15}$ treatment groups for all seedlings.

Steady state fluorescence, F_T, measured after temperature treatment prior to cold storage was linearly correlated to the treatment temperature (Figure 15A), as well as post-storage root growth (Figure 15B).

Table 9. Morphological parameters of seedlings determined after 15 weeks cold storage (-2±1°C) and 28 days in growth chamber. Pre-storage day/night temperature treatments were 5/1, 10/5, 15/10, and 20/15°C. Mean ± SE (n=45).

Species	$T_{5/1}$	$T_{10/5}$	$T_{15/10}$	$T_{20/15°C}$
Root growth (number of white roots>1 cm length/seedling)				
White spruce	18.8±.5	11.1±.5	7.2±.5[a]	7.1±.4[a]
B.C. Douglas-fir	34.6±.6[a]	28.3±.6[ab]	21.7±.6[bc]	16.2±.5[c]
Oregon D-fir	36.3±.6[a]	31.6±.7[a]	20.8±.5	4.2±.4
New leader length (cm)				
White spruce	6.1±.4[a]	5.8±.5[ab]	4.8±.4[b]	5.0±.6[b]
B.C. Douglas-fir	5.2±.6[a]	5.0±.5[ab]	4.6±.5[bc]	4.2±.3[c]
Oregon D-fir	8.6±.5	6.4±.6	4.3±.4	NF
New leader Dry weight (g)				
White spruce	0.64±.08[a]	0.63±.07[a]	0.42±.05	0.50±.07
B.C. Douglas-fir	0.54±.07[a]	0.52±.06[a]	0.43±.07[b]	0.39±.06[b]
Oregon D-fir	0.69±.09[a]	0.61±.08[a]	0.41±.07	NF
Days to bud break				
White spruce	7	8	9	9
B.C. Douglas-fir	6	7	9	10
Oregon D-fir	17	18	19	NF

[a,b,c] same letter signifies results are not significant between columns at p=.01
NF apical buds did not flush
D-fir Douglas-fir

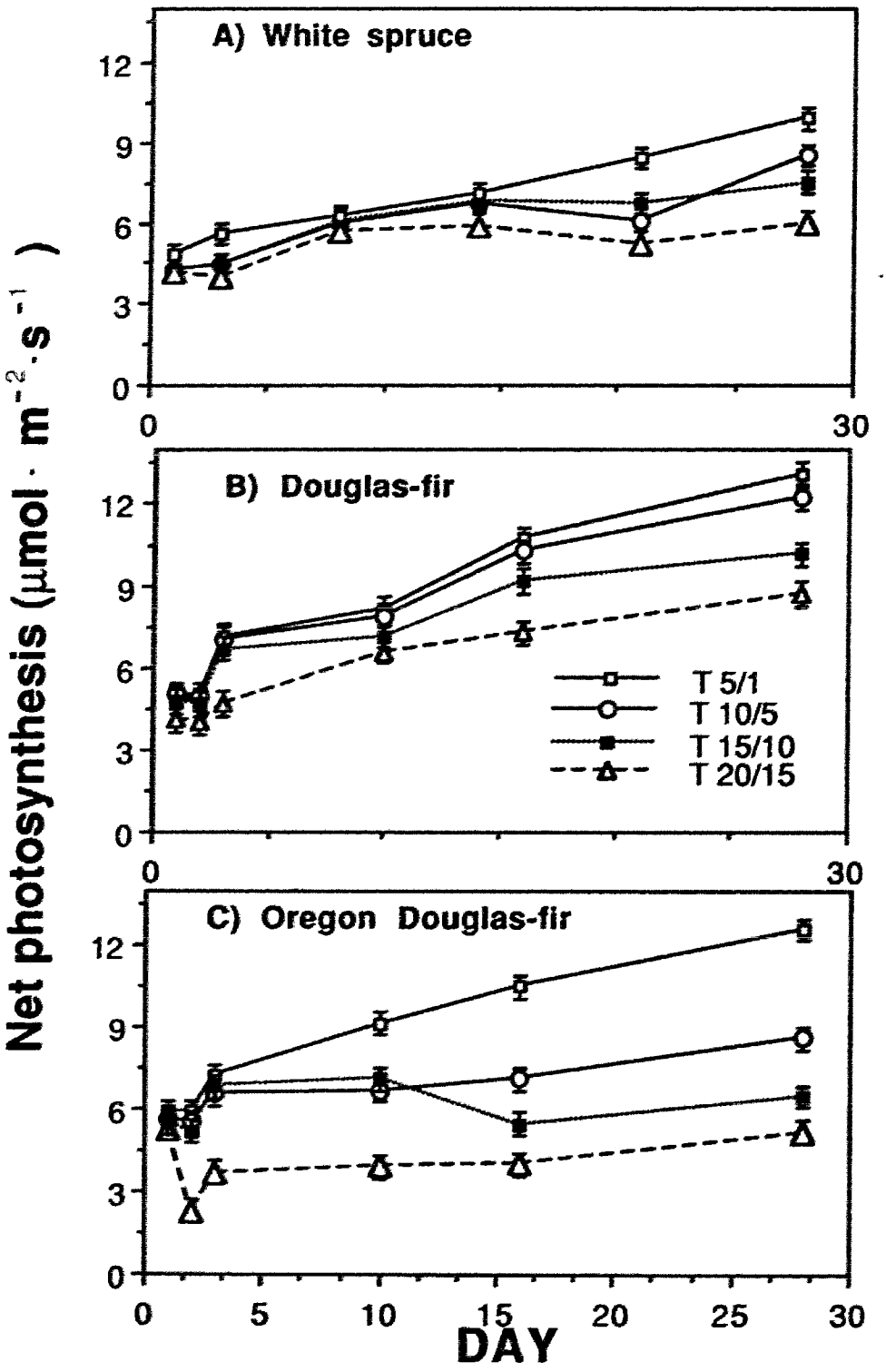

Figure 12. Net photosynthesis, P_N, of seedlings determined during 28 days in growth chamber. Seedlings were cold-stored for 15 weeks (-2±1°C) after pre-storage day/night temperature treatments 5/1, 10/5, 15/10, and 20/15°C.

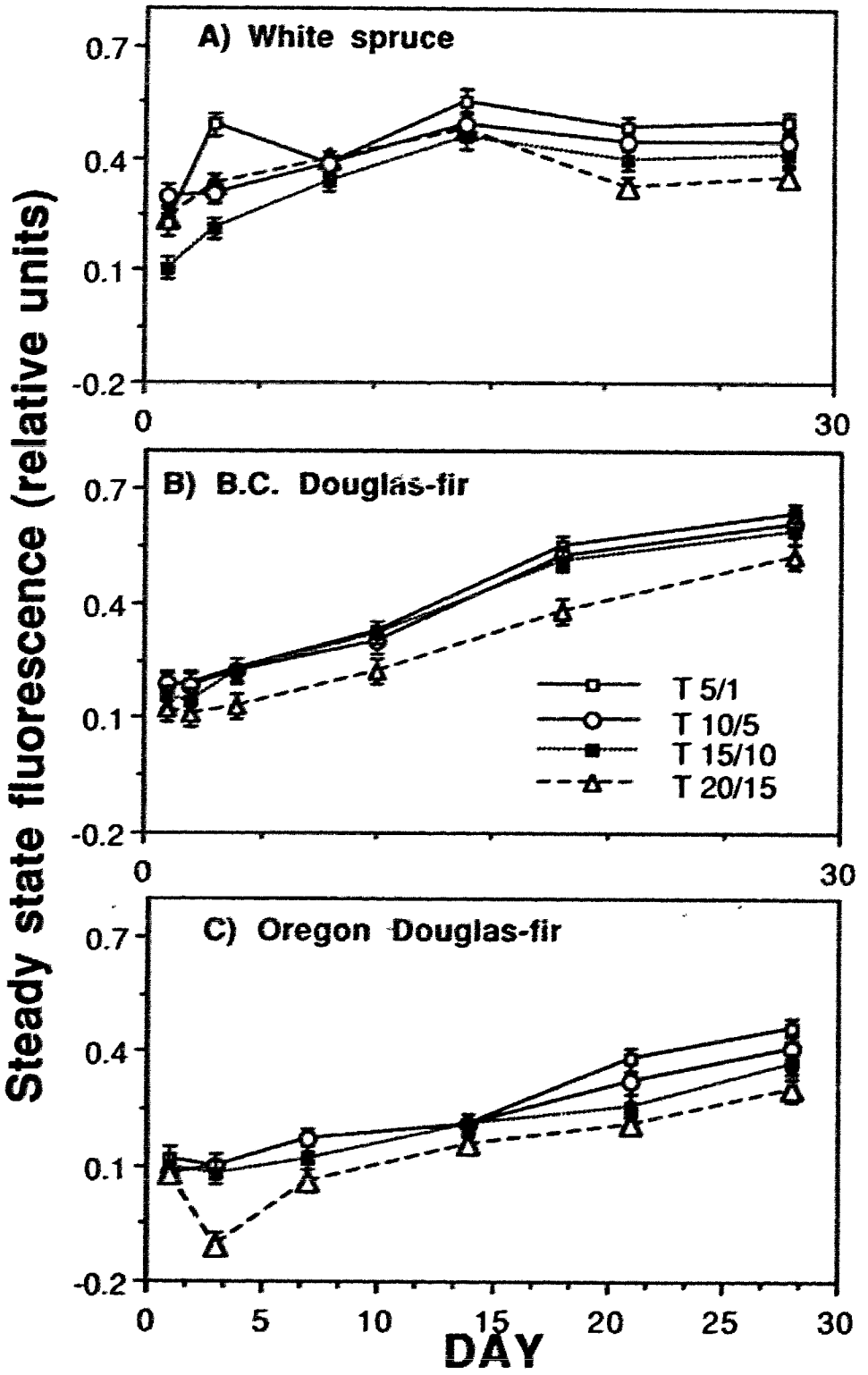

Figure 13. Steady state fluorescence, F_T, of seedlings determined during 28 days in growth chamber. Seedlings were cold-stored for 15 weeks (-2±1°C) after pre-storage day/night temperature treatments 5/1, 10/5, 15/10, and 20/15°C.

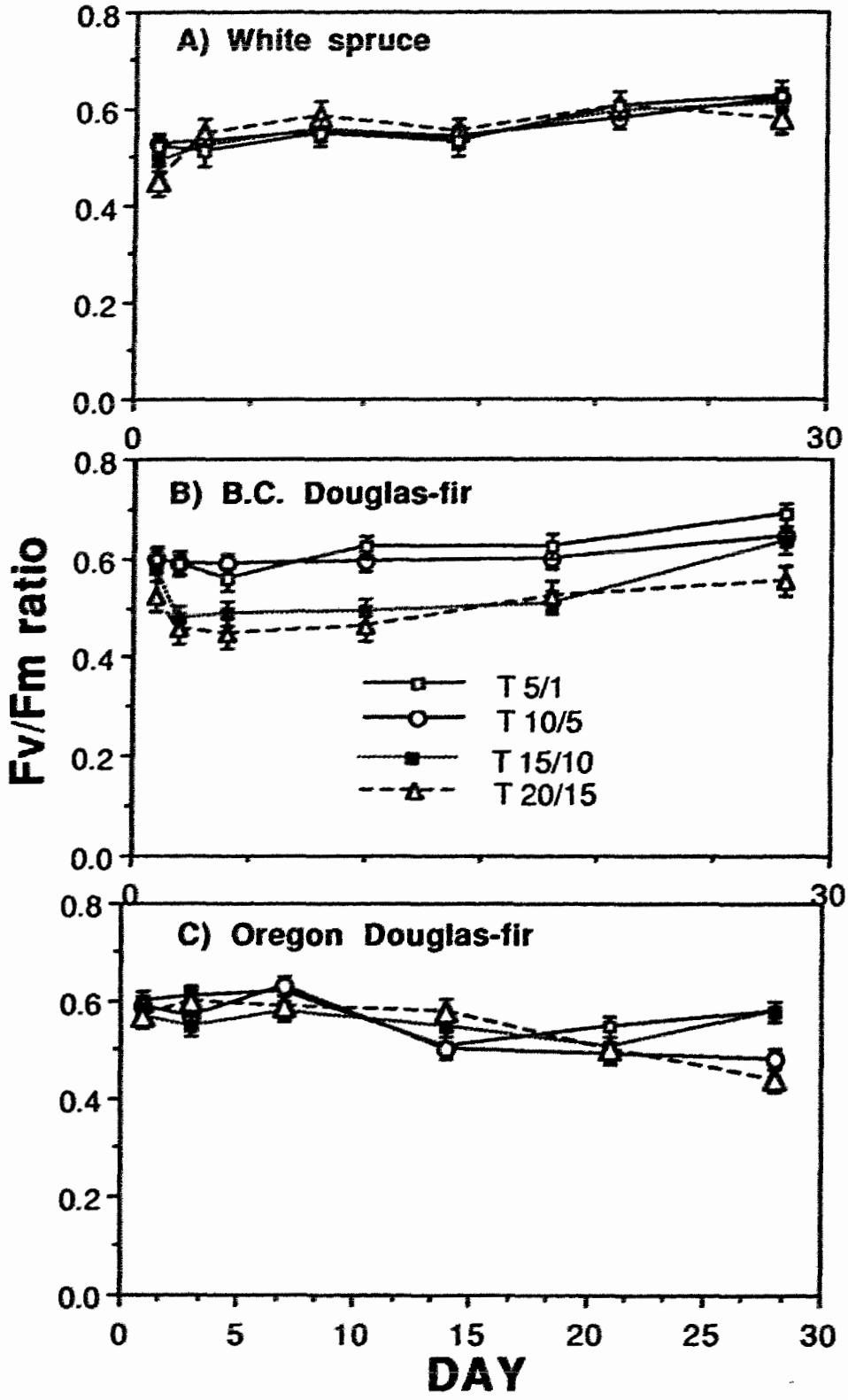

Figure 14. Ratio of variable fluorescence to maximum fluorescence, Fv/Fm, of seedlings determined during 28 days in growth chamber. Seedlings were cold-stored for 15 weeks (-2±1°C) after pre-storage day/night temperature treatments 5/1, 10/5, 15/10, and 20/15°C.

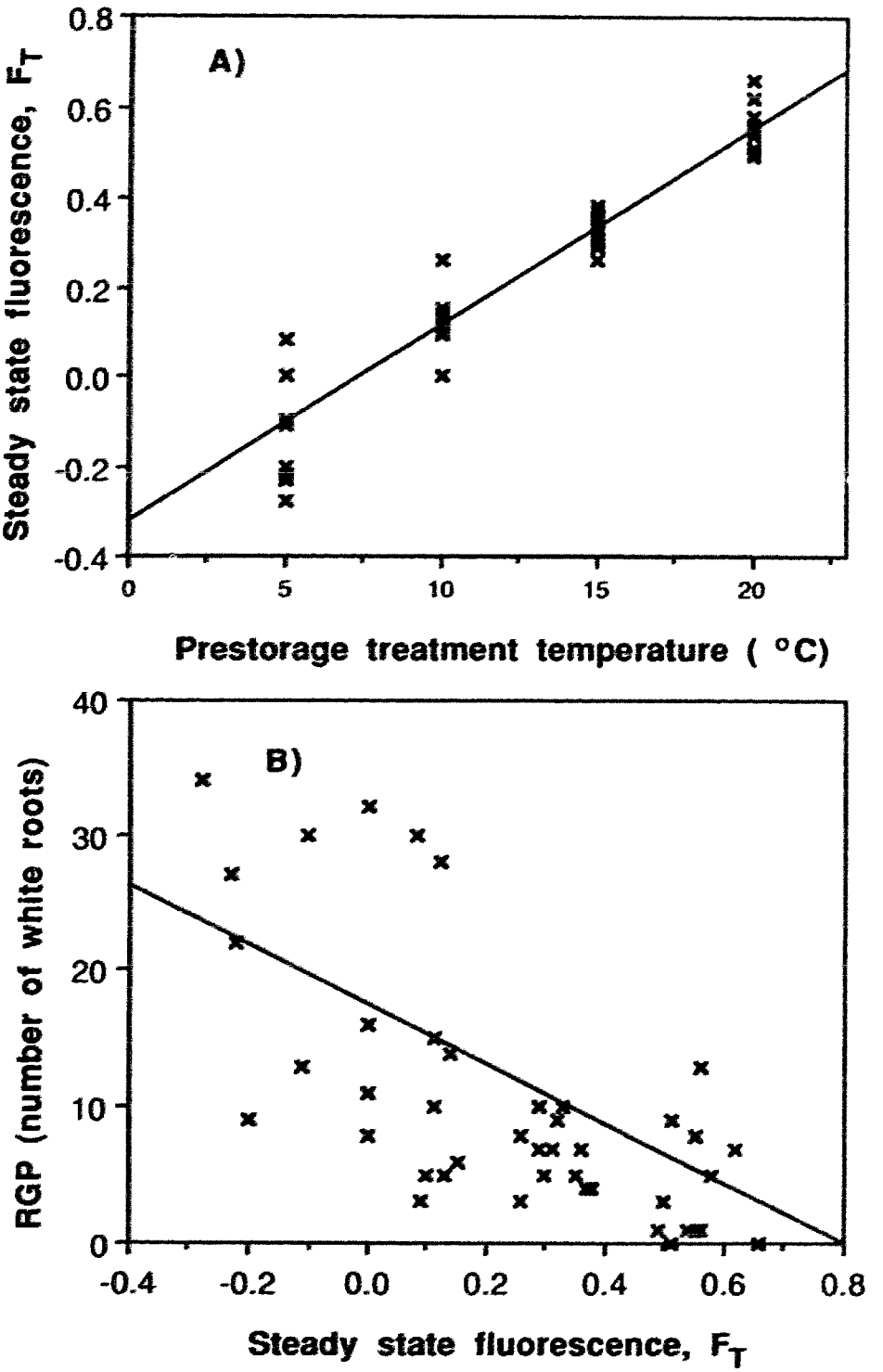

Figure 15. A) Prestorage steady state fluorescence, F_T, related to prestorage treatment temperature. Equation for the line is $y = -0.322 + 0.044\ x$, $r^2=.92$. B) number of white roots after 28 days in growth chamber related to prestorage steady state fluorescence, F_T, measured after temperature treatment. Equation for the line is $y = 16.33 - 25.85x$, $r^2=.52$.

DISCUSSION

Similar to results found by other researchers (Martin et al. 1978, Hawkins & Lister 1985, Strand & Öquist 1988 and Vidaver et al. 1989a) seedlings under natural winter conditions had low variable chlorophyll fluorescence or Fv/Fm prior to temperature treatments in December and January. Öquist and Malmberg (1989) found that frost hardened pine remained susceptible to short term (2 hour) photoinhibition, as seen by a decline in Fv/Fm, and suggests the decline reflects a protective function. In contrast, this study found low temperatures with moderate light levels (350 $\mu mol \cdot m^{-2} \cdot s^{-1}$) in the growth chamber caused a decline in steady state fluorescence, F_T, in frost hardened conifers; but there was no significant change in Fv/Fm. The already low Fv/Fm levels of the seedlings prior to treatment may have contributed to this insignificance.

There was a decline in F_T below apparent F_O levels in the $T_{5/1}$ group of the white spruce seedlings, which Bilger & Schreiber (1986) have termed F_O-quenching, and is likely caused by structural changes in the pigment bed. This may serve a protective function by allowing excess excitation energy to be released as heat.

Forty-eight hours dehardening treatment, $T_{15/10}$ and $T_{20/15°C}$, was sufficient for seedlings to lose some cold hardiness. Photosynthetic activity also generally increased as temperature treatment increased. These seedlings, although considered dormant, were quite capable of responding rapidly

to increases in temperature. The increased physiological activity seen in the seedlings placed in warm temperatures appears to make them less suitable for cold storage. The capacity of the these seedlings to rapidly increase their photosynthetic activity in response to increased temperatures suggests that the term physiological quiescence should be used, similar to the terminology used for bud dormancy. In this state, the physiological activity of the seedlings is maintained at a low level by environmental conditions, not necessarily by internal controls.

A decrease in suitability for cold storage was the most evident in the Oregon Douglas-fir seedlings, where dehardening temperatures resulted in poor survival after storage. Although the treatment had the least effect on the pre-storage photosynthetic gas exchange of the Oregon Douglas-fir, F_T increased significantly with increasing temperature regime, indicating altered physiological activity.

The British Columbia coastal Douglas-fir was also strongly affected by the treatment temperature. Douglas-fir from the Pacific Northwest has been found to be photosynthetically active in the fall and winter, depending upon the ambient temperatures (Waring & Franklin 1979). Dehardening in the growth chamber for 48 hours appeared to reactivate the photosynthetic and metabolic activities quicker in the Douglas-fir than for a boreal species such as white spruce.

Resistance to the effects of short-term temperature increases may therefore relate to the provenance origin. The most southerly Oregon Douglas-fir seedlings are adapted to a mild winter, as are the B.C. coastal Douglas-fir seedlings, though to a lesser extent. The capacity to be photosynthetically active in the fall and winter may be an advantage to these seedlings. Typically these seedlings endure dry summers, which limits photosynthesis. Photosynthetic activity during mild fall and winter periods, when water is plentiful, potentially accounts for up to 40% of the total accumulated photosynthate each year for these seedlings (Waring & Franklin 1979). In contrast, white spruce is a boreal species subject to harsh winter conditions, a short-term warming in the fall or winter can be followed by sudden temperature declines, rapid reactivation of photosynthesis would be a disadvantage to such a species, leaving them susceptible to freezing damage.

There was a strong inverse relationship between the quality of the seedlings after storage and temperature treatment prior to storage. The recovery of photosynthetic activity after cold storage was significantly affected by the pre-storage treatment in all species; the lower the temperature the higher the P_N and F_T after storage. Van den Driessche (1987) has suggested that new photosynthate is required for new root growth in conifer seedlings; therefore the higher photosynthetic gas exchange rates in the $T_{5/1}$ and

$T_{10/5}$ treatment groups could be responsible for the higher root growth and greater vigour seen in these groups.

The importance of assessing root growth in conifer seedlings has been investigated by Burdett (1979, 1987). Seedlings with poor root growth usually have poor field survival, whereas seedlings with good root growth and high vigour, as measured by rapid budburst, have the greatest field survival and height growth (McCreary & Duryea 1987). The decreased root growth associated with increased temperature treatment prior to cold storage seen in this experiment could have a significant effect on the survival of the seedlings in the field.

Vidaver et al. (1988) suggest that, in the event of a warming trend in the fall or winter, chlorophyll fluorescence could be used to assess physiological reactivation of seedlings to prevent losses in seedling quality during cold storage. Decreases in chlorophyll fluorescence during frost hardening or dormancy have been found for several conifer species (Martin et al. 1978, Hawkins & Lister 1985, Strand & Öquist 1988, Bolhar-Nordenkampf & Lechner and Vidaver et al. 1989a) and has been used as a predictor of cold hardiness in Scots pine (Sundblad et al. 1990), Douglas-fir (Hawkins & Lister 1985) and white spruce (Vidaver et al. 1989a).

Cold hardiness, CO_2 gas exchange and chlorophyll fluorescence are capable of measuring dynamic and rapid responses in the plant to changing environmental conditions; however, mitotic index is much slower to respond to such

changes. This study indicates pre-storage measurements of chlorophyll fluorescence, CO_2 gas exchange and cold hardiness might be good indicators of post-storage quality, although a more rapid technique for measuring cold hardiness would be of greater value in assessing seedlings immediately prior to cold storage. Ambient temperatures have a significant effect on the quality of seedlings, suggesting that, when possible, nursery personnel attempt to lift seedlings during periods of cold (≤5°C) temperatures to help maximize seedling quality.

It should be noted there were difficulties in storing the Douglas-fir seedlings from British Columbia. The experiment had to be repeated several times for this group in order to obtain complete data sets. Losses in storage due to grey mold (Botryitis sp.) especially in $T_{15/10}$ and $T_{20/15}$ seedlings were prevalent. Further research is indicated to see if pre-storage temperature is a factor in the development of grey mold during cold storage.

Natural chilling is necessary for seedlings prior to cold storage (Carlson 1985). Cold temperature with low light levels or just cold nights may be adequate to induce reductions of chlorophyll fluorescence and CO_2 gas exchange (Strand & Öquist 1988, Strand & Lundmark 1987); however, high light levels in combination with low temperature have been found to cause a much larger decline of variable chlorophyll fluorescence (Krause & Somersalo 1989). The relationship between temperature and light levels needs to be investigated

to assess whether they have a synergistic effect on the storability of conifer seedlings.

In this study, the physiological activity of the seedlings was maintained at a low level by environmental conditions, and not necessarily by internal controls; however, previous studies have indicated that photochemical regulation of the photosynthetic apparatus is important in the induction of dormancy in conifers (Vidaver et al. 1989a). Section 3 addresses the effects of both photoperiod and chilling temperatures as dormancy induction treatments to determine if photochemical regulation is initiated by either treatment.

III. The influence of photoperiod and temperature on photochemical regulation of white spruce and Douglas-fir seedlings

INTRODUCTION

A decline in the variable chlorophyll fluorescence induction curve or the ratio of variable fluorescence to maximal fluorescence, Fv/Fm, indicates decreased photochemical efficiency, and is commonly seen in conifers under winter conditions (Bolhar-Nordenkampf & Lechner 1988, Hawkins & Lister 1985, Vidaver et al. 1989a). This decline of Fv/Fm has been attributed to photoinhibition (for a review see Krause 1988) and results in a decrease of excitation energy transfer from the light harvesting antenna complex to the reaction centers (Öquist & Malmberg 1989). Vidaver et al. (1989a) have suggested an alternative explanation for white spruce seedlings, where photosynthetic regulation, as seen as a decline in Fv/Fm and photosynthetic gas exchange, occurs either under conditions of environmental stress or is initiated by decreased day length. Day length mediated photochemical regulation of the photosynthetic apparatus would be highly adaptive for boreal species such as white spruce, where the onset of freezing temperatures is rapid in the fall. Under freezing conditions biochemical reactions are inhibited but photochemical reactions are not, resulting in the production of toxic-oxygen species within the chloroplasts (Salin 1987). To prevent cell damage, production of toxic-oxygen species can be reduced by inactivating PSII (Gillies & Vidaver, 1990). Reversible inactivation of photosynthetic photochemistry within the

chloroplasts would decrease photodamage under conditions of low temperatures (Wise & Naylor 1987), water stress (Sibbald & Vidaver, 1987, Toivonen & Vidaver, 1988), and when CO_2 assimilation is reduced (Maenpaa et al. 1988).

Previous studies by Vidaver et al. (1989a) suggested that shortened day length initiated the decline of photosynthetic activity in the fall prior to chilling temperatures. If this is the case, seedlings placed under a short day length with warm temperatures would be expected to exhibit photochemical regulation, resulting in photosynthetic inactivation: declines in net photosynthesis and chlorophyll fluorescence. Other researchers suggest temperature as an important factor in the decline of net photosynthesis and chlorophyll fluorescence (Strand & Öquist 1988, Öquist & Malmberg 1989). If so, seedlings under chilling temperatures with a long photoperiod might also exhibit photosynthetic inactivation due to chilling initiated photochemical regulation.

This study examined the relationship between photoperiod and temperature on the morphology and photosynthetic activity of white spruce and Douglas-fir seedlings, in particular, evidence of photochemical regulation of the photosynthetic apparatus. The second objective of this experiment was to provide seedlings for cold storage after different dormancy induction treatments.

MATERIALS AND METHODS

Approximately 1100 one year old white spruce and 1100 one year old Douglas-fir seedlings were used for study in 1990, another 1100 one year old seedlings of each species were used in 1991. Seedlings used in this experiment are described in General Materials and Methods. In the first week of August of each year, seedlings were randomly divided into a control and two treatment groups.

Control seedlings (n=360) were left in styroblock containers under ambient natural conditions (AMB) in an unheated, well ventilated glass greenhouse. Temperature was monitored continuously in the greenhouse (Figure 16).

Long day length, chilling temperature conditions (LD-C) seedlings (n=360) were placed in styroblock containers in a growth chamber (Conviron) with a 16 hour photoperiod, a PPF of 375 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 70-85% RH and day/night temperature of 23/18°C. The temperature was lowered at the rate of 2°C every three days until it reached 10/5°C day/night, then maintained at this setting for the remainder of the study.

Short day length, warm temperature treatment (SD-W) seedlings (n=360) were placed in styrobock containers in a growth chamber (Conviron) for one month with a 12 hour photoperiod, 25/20°C day/night temperature, PPF of 375 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 70-85% RH. On September 19, seedlings were moved to a temperature controlled glass greenhouse: day/night temperature was 25/20°C, with 70-85% RH and a natural photoperiod (<12 hour).

Due to practical considerations the other potential experimental conditions were not used in this study: short day length, chilling temperatures; and long day length, warm temperatures.

Mitotic index, cold hardiness, chlorophyll fluorescence and net photosynthesis (P_N) were measured (Figure 17) as per General Materials and Methods.

Root/shoot ratio was the ratio of the oven dry weight of the root divided by the oven dry weight of the entire shoot (stem and needles). It was calculated by washing all material from roots and separating roots and shoots at the root collar. Roots and shoots were oven dried at 70°C for 48 hours then cooled in a desiccator and weighed.

Statistical analysis was performed using SAS/STATS (1988) on the main frame computer at SFU. Two-way ANOVA on one factor, with multiple comparisons contrasted for both time and treatment, and the Bonferroni test of differences were used to evaluate significance of treatment effects (Winer 1977). Simple linear correlations were used to determine relationships between factors where neither factor is assumed to be functionally dependent upon the other. For these cases, the coefficient of determination, r^2, is reported as the measure of the strength of the straight-line relationship, no dependent and independent variable is implied (Zar 1985). Due to differences in climatic conditions between the two years, data for each year is dealt with separately.

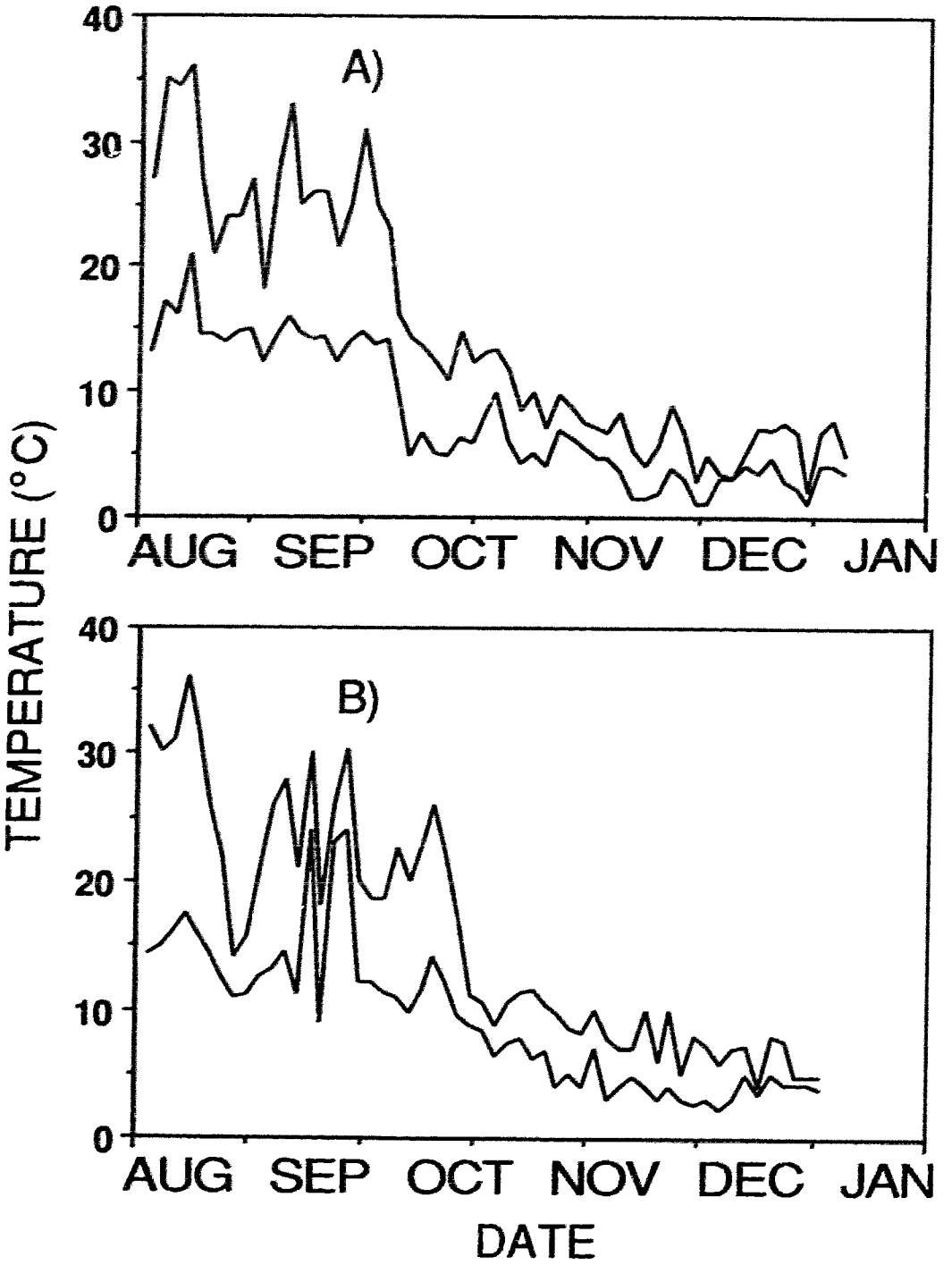

Figure 16. Three day average minimum and maximum temperatures measured in unheated greenhouse at Simon Fraser University. a) 1990-1991, b) 1991-1992.

Table 10. Environmental conditions for white spruce and Douglas-fir seedlings. Day length is the period from sun rise to sun set for Vancouver, the average air temperature is reported for the three days prior to testing. Dormancy induction treatments groups were: ambient (AMB), short-day, warm temperature (SD-W) and long day, chilling temperature (LD-C).

GROUP	DATE						
	Aug 8	Aug 27	Sep 11	Oct 24	Nov 15	Dec 20	Jan 4
Day length (hours)							
AMB	14.75	13.75	12.75	10.9	9.0	8.2	8.5
LD-C	16.0	16.0	16.0	16.0	16.0	16.0	16.0
SD-W	12.0	12.0	12.0	10.9	9.0	8.2	8.5
Three day average temperature (°C)							
AMB 1990	22.8	17.0	18.9	10.2	5.9	4.4	4.7
AMB 1991	21.2	15.6	15.7	7.7	6.6	5.9	5.3
LD-C	6.6	6.6	6.6	6.6	6.6	6.6	6.6
SD-W	22.5	22.5	22.5	22.5	22.5	22.5	22.5

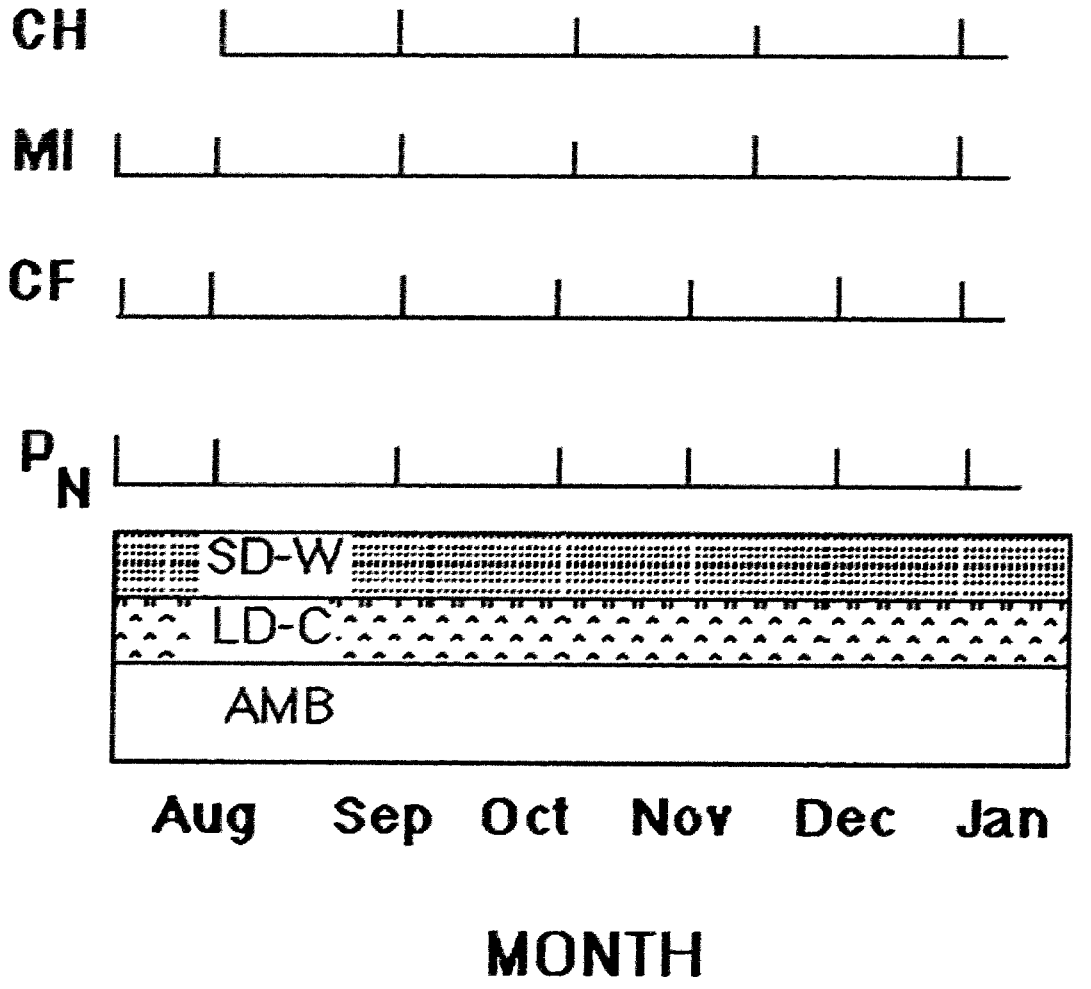

Figure 17. Schedule of experiment. Bars indicate when measurements were taken: CH - cold hardiness, MI - mitotic index, CF - chlorophyll fluorescence, P_N - net photosynthesis. Treatment groups were AMB - ambient temperatures and day length, SD-W - ≤12 h photoperiod, 25/20°C, LD-C - 16 h photoperiod, 10/5°C.

RESULTS

White Spruce

Similar results were found for both 1990 and 1991. Seedlings had all set bud prior to the start of the study; however, apical buds were still mitotically active in August. The mitotic index declined to 0% by the end of September for all groups (Table 11).

There was no significant morphological difference between the three groups, with the exception of root/shoot ratio. Mean height for all groups was 16.4 cm in 1990, 15.7 cm in 1991, mean stem diameter was 3.2 cm in 1990, 3.3 cm in 1991. The root/shoot ratio of the AMB seedlings increased significantly in September and remained at this level until December, when it then decreased (Table 11). The LD-C group had a continuous increase in root/shoot ratio from August to December, attributable to somewhat more rapid growth of roots than shoots. The root/shoot ratio of the SD-W seedlings increased in late August and September, then decreased in November and December; the ratio was significantly lower than for the other two groups from October to December, and may be because root growth ceased.

Cold hardiness increased during the fall and winter in 1990 and 1991 (Table 11) for the AMB group, and correlated with photoperiod ($r^2=0.91$, $r^2=0.88$) and average air temperature ($r^2=0.97$, $r^2=0.92$). There was no significant change in the cold hardiness of the SD-W group after the treatment began in August; seedlings were significantly less

Table 11. Parameters of white spruce seedlings, 1990. AMB seedlings were under natural conditions; SD-W - ≤12 h photoperiod, 25/20°C; LD-C - 16 h photoperiod, 10/5°C.

Group	Aug 8	Aug 27	Sep 11	Oct 4	Oct 24	Nov 15	Dec 20	Jan 4
Days	1	19	34	58	78	99	134	149
	Root/shoot ratio (n=10)							
AMB	0.43±0.1[a]	0.44±0.1[a]	0.96±0.3[b]	1.0±0.3[b]	0.99±0.3[b]	0.93±0.3[b]	0.64±0.2	-
LD-C	-	0.57±0.1	0.68±0.2[c]	0.71±0.2c	0.75±0.1[cd]	0.79±0.2[d]	-	-
SD-W	-	0.69±0.2[c]	0.72±0.2[c]	0.44±0.1[a]	0.46±0.2[a]	0.50±0.2[a]	-	-
	Cold Hardiness (LT_{50})							
AMB	-10±1	-14±2	-20±2	-22±2	-25±2	-27±2	-28±2	<-35±2*
LD-C	-	-10±2	-10±2	-16±2	-21±2	-26±2	-10±2	- 9±2
SD-W	-	-12±2	-14±2	-13±2	-13±2	-12±2	-14±2	-14±2
	Mitotic Index (%) (n=10)							
AMB	2.7	1.91	0.80	0	0	0	0	-
LD-C	-	1.62	0.96	0	0	1.7	5.1	-
SD-W	-	0.95	0.55	0	0	0	0	-

* Note minimmum temperature of freezer was -35°C.
a,b,c, same letter signifies no significant difference between columns and rows.
- no measurements taken on that date

cold hardy than the other two groups. The cold hardiness of the LD-C group increased until the end of November; seedlings began to lose hardiness when bud swell started and the mitotic index increased (Table 11). Cold hardiness was inversely correlated with mitotic index for the LD-C seedlings (r^2=-0.54).

The AMB group had a average of 7.9 white roots per seedling on each sampling date after mid-September in 1990, 8.3 in 1991, the LD-C group had an average of 12.2 white roots per seedling in 1990, 12.6 in 1991; the SD-W seedlings had no white roots on any sampling date.

Multivariate analysis of net photosynthesis and Fv/Fm ratios from all three treatment groups indicate that both photoperiod and air temperature are significant factors (p=0.001). Net phototsynthesis in white spruce was related to photoperiod (p), (P_N = 0.470p, r^2=0.81) and air temperature (t) (P_N = 0.353t, r^2=0.86) and both together (P_N= 0.213p + 0.222t, r^2=0.91). Similarly the Fv/Fm ratio was related to photoperiod (p), (Fv/Fm = 0.038p, r^2=0.98) and air temperature (t), (Fv/Fm = 0.025t, r^2=0.78) and both together (Fv/Fm = 0.03 p + 0.004t, r^2=0.98).

Figure 18 shows typical fluorescence induction curves obtained at room temperature with dark-pretreated white spruce seedlings sampled in August, September, October, November, December and January (1990/91). The changes in fluorescence yield at P were mainly attributable to changes

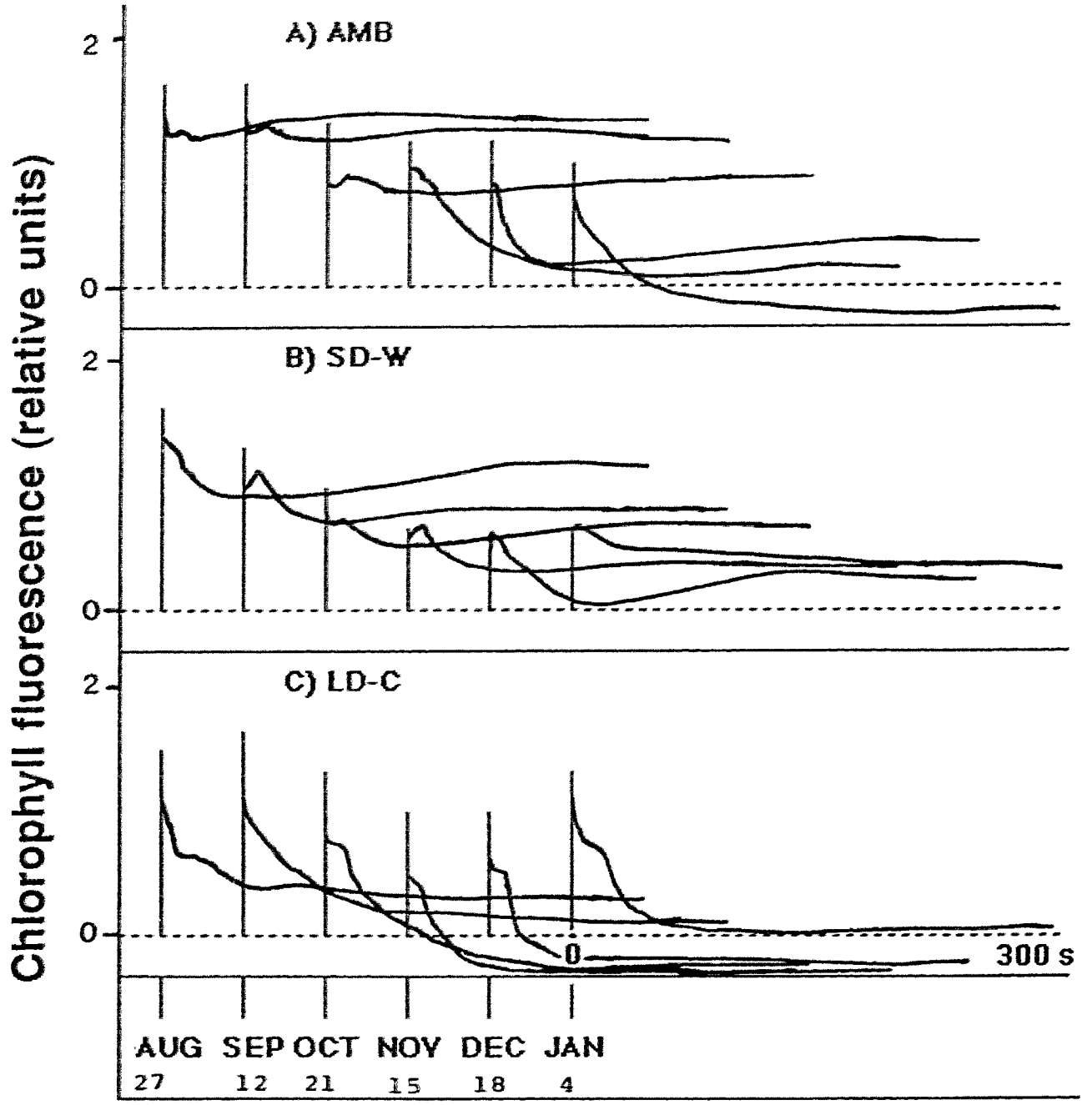

Figure 18. Chlorophyll fluorescence induction curves of white spruce seedlings measured at room temperature in 1990. Each curve represents the average of 10 seedlings. AMB seedlings were under natural conditions, SD-W was ≤12 h photoperiod, 25/20°C, LD-C was 16 h photoperiod, 10/5°C.

in variable fluorescence, rather than changes in F_O, which remained relatively stable throughout the period of study. As can be seen in Figure 18, the kinetics of the chlorophyll fluorescence induction curves changed over the fall for all three treatment groups, and appeared to be different for each group. A decline in all portions of the chlorophyll fluorescence induction curve was observed in the AMB and SD-W seedlings throughout the fall. All portions of the chlorophyll fluorescence induction curves of the LD-C declined from August to December, some recovery was seen in January.

The chlorophyll fluorescence ratio, Fv/Fm, (Figure 19) and steady state fluorescence, F_T, (Figure 20) of the AMB seedlings declined over time in both 1990 and 1991, and correlated with photoperiod ($r^2=0.89$, $r^2=0.93$) as well as with average air temperature ($r^2=0.79$, $r^2=0.97$). The Fv/Fm ratio and F_T of the SD-W treatment group also declined over time in 1990 and 1991 and correlated with photoperiod ($r^2=0.94$, $r^2=0.98$). The LD-C treatment group showed a significant ($p=0.01$) change in Fv/Fm over time; the Fv/Fm declined until December 15, then it began to increase, coinciding with flushing of the buds. Steady state fluorescence, F_T, of the LD-C seedlings decreased in October 1990 and in November of 1991 to levels below apparent F_O, then increased significantly ($p=0.01$) in January with the appearance of newly flushed foliage.

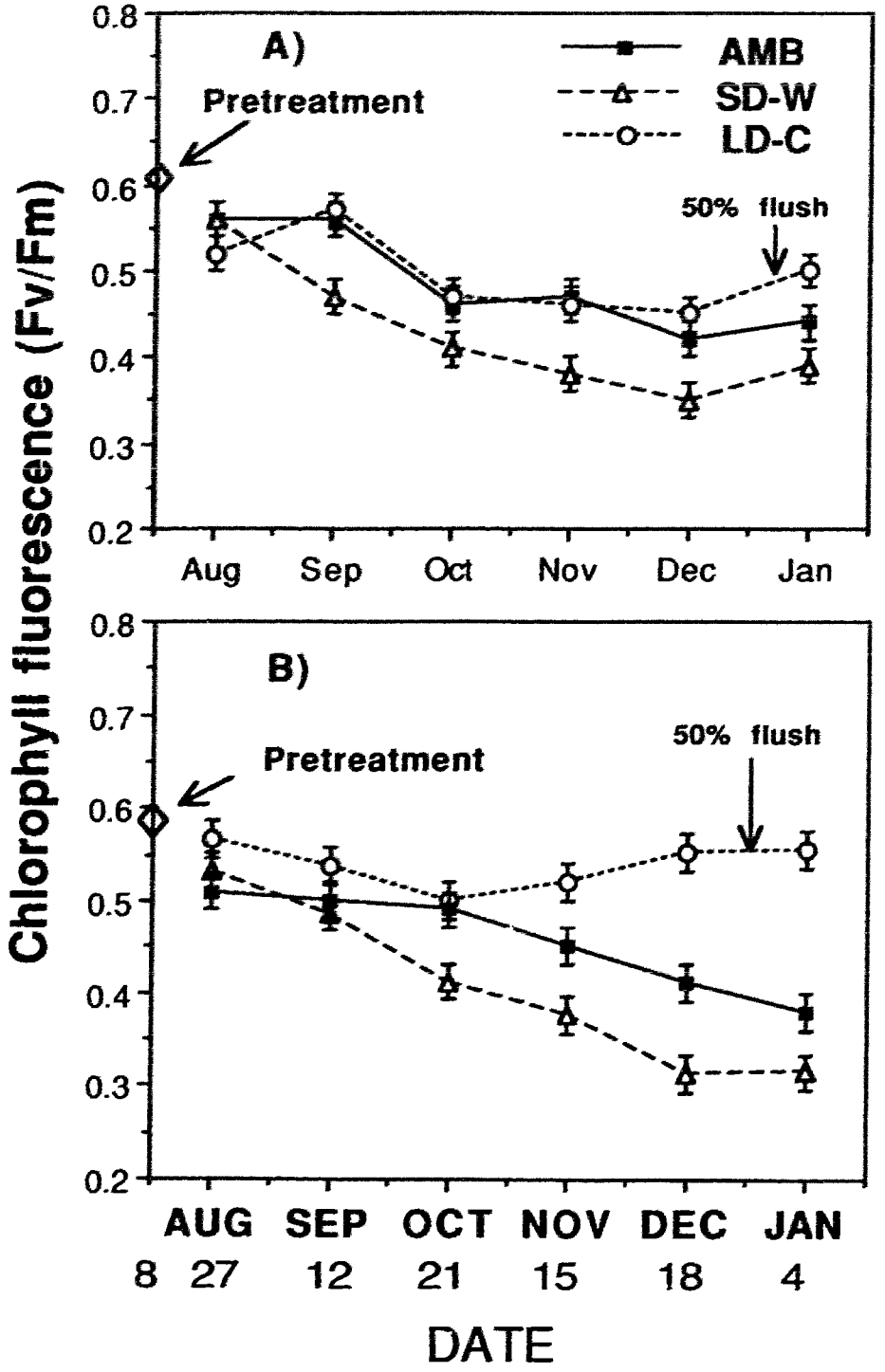

Figure 19. Ratio of variable fluorescence to maximal fluorescence, Fv/Fm, of white spruce seedlings, A) 1990 and B) 1991. AMB seedlings were under natural conditions, SD-W was ≤12 h photoperiod, 25/20°C, LD-C was 16 h photoperiod, 10/5°C.

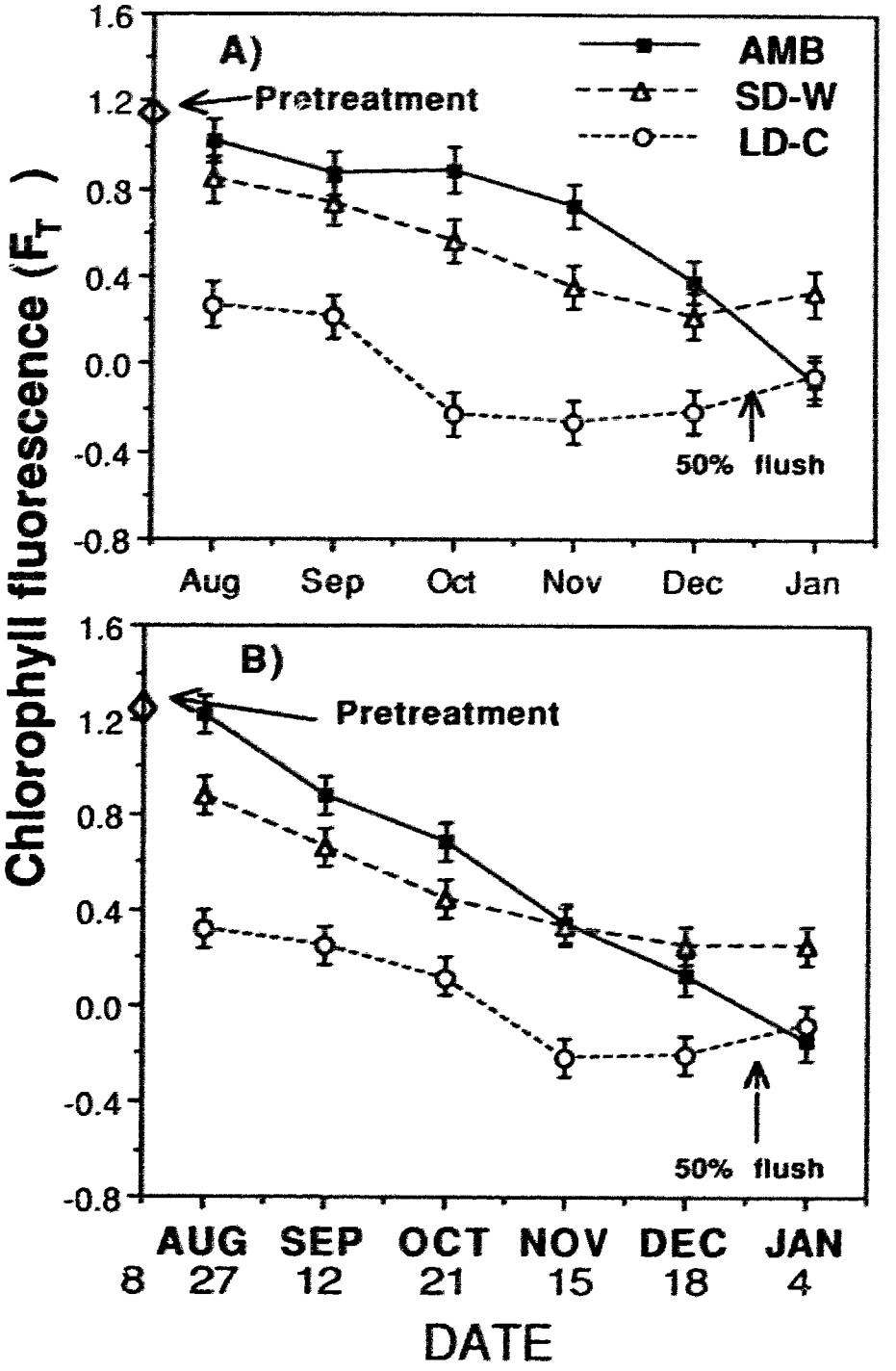

Figure 20. Steady state fluorescence, F_T, of white spruce seedlings. A) 1990 and B) 1991. AMB seedlings were under natural conditions, SD-W was ≤12 h photoperiod, 25/20°C, LD-C was 16 h photoperiod, 10/5°C.

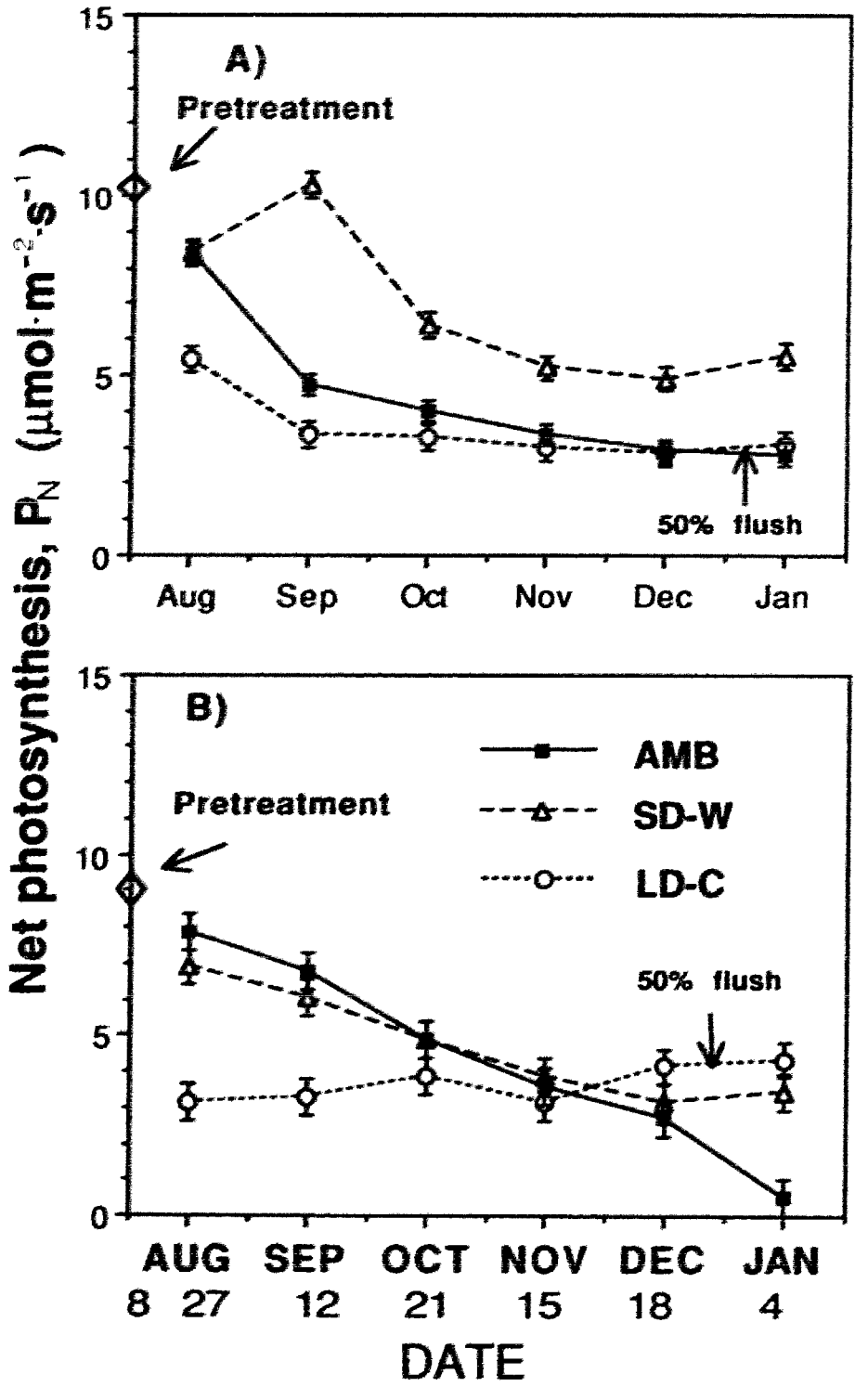

Figure 21. Net photosynthesis (P_N) of white spruce seedlings, A) 1990 and B) 1991. AMB seedlings were under natural conditions, SD-W was ≤12 h photoperiod, 25/20°C, LD-C was 16 h photoperiod, 10/5°C.

In both 1990 and 1991, net photosynthesis decreased from a maximum in August to a minimum in January in the control AMB seedlings, correlating with average air temperature (r^2=0.88, r^2=0.85) and photoperiod (r^2=0.95, r^2=0.91) (Figure 21). A similar decrease was seen in the SD-W seedlings in 1990 and 1991, but a correlation was found only between P_N and photoperiod (r^2=0.86, r^2=0.89). The P_N rates of the LD-C seedlings decreased during the first month in the growth chamber in 1990 and 1991, and was linearly correlated with growth chamber temperature (r^2=0.81, r^2=0.78). After the chamber reached 10/5°C, P_N remained at a low level until new foliage flushed.

Douglas-fir

Similar results were found in both 1990 and 1991. The SD-W group set bud rapidly after treatment began, mitotic index decreased to zero by the end of September (Table 12). Bud set was not complete in the AMB group until January, the LD-C group had not yet completed bud set by the end of the study.

The root/shoot ratio of the SD-W seedlings increased to a stable level in September (Table 12). The root/shoot ratio of the AMB group increased to a maximum in October, then decreased. There was no significant change in the LD-C seedlings.

Height increased in the AMB group to an average of 23.0 cm in early December 1990, 21.9 cm in 1990, when buds set. Height growth ceased in the SD-W group when buds set, and

Table 12. Parameters of Douglas-fir seedlings, 1990. AMB seedlings were under natural condition; SD-W - ≤12 h photoperiod, 25/20°C; LD-C - 16 h photoperiod, 10/5°C.

Group	Aug 8	Aug 27	Sep 11	Oct 24	Nov 15	Dec 20	Jan 4
Days	1	19	34	78	99	134	149
	Root/shoot ratio (n=10)						
AMB	0.29±0.1[a]	0.30±0.1[a]	0.32±0.2[a]	0.77±0.3[b]	0.69±0.3[c]	0.52±0.1	-
LD-C	-	0.31±0.2[a]	0.41±0.1[d]	0.42±0.1[d]	0.44±0.1[d]	0.42±0.1[d]	-
SD-W	-	0.89±0.4	0.69±0.2[c]	0.78±0.2[b]	0.79±2[b]	0.81±0.2[b]	-
	Cold Hardiness (LT_{50})						
AMB	0	0±1	0±1	-2.5±1	-5.5±1	-9.5±1	-17±1
LD-C	-	0±1	0±1	0±1	-2.2±1	-5.4±1	-5.7±1
SD-W	-	-6.5±1	-7.5±1	-10±1	-7.4±1	-6.2±1	-7.1±1
	Mitotic Index (%) (n=10)						
AMB	13.7	11.2	11.9	11.1	7.9	6.1	0.2
LD-C	-	14.1	12.8	11.9	10.2	10.7	8.1
SD-W	-	6.5	4.0	0	0	0	0

a,b,c, same letter signifies no significant difference between columns and rows.
- no measurements taken on that date

seedlings were much shorter than the other two groups, with an average height of 15.36 cm in 1990, 15.07 cm in 1991. Height growth was slow in the LD-C seedlings, increasing to 19.2 cm in December 1990, 18.23 cm in 1991.

Cold hardiness (Table 12) of the AMB seedlings correlated to photoperiod (r^2=0.81, r^2=0.77), average air temperature (r^2=0.86, r^2=82) and bud set (r^2=-0.69, r^2=-0.73) in both 1990 and 1991. There was no significant change in the cold hardiness of the SD-W seedlings after bud set was complete. The LD-C group increased cold hardiness much slower than the AMB group, and were significantly less hardy; cold hardiness was correlated to bud set (r^2=-0.91) in both 1990 and 1991.

In November of both years, lammas growth of individual buds was present in 10% of the SD-W seedlings, increasing to 16% in December. No lammas growth of buds was seen for either of the two other groups.

The LD-C seedlings had significantly more white roots present at each sampling date than the other two groups with 14.7 roots per seedling in 1990, 13.9 in 1991. The SD-W seedlings had no white roots visible on any sampling date.

Multivariate analysis of net photosynthesis and Fv/Fm ratios from all three treatment groups indicate that both photoperiod and air temperature are significant factors (p=0.001). Net phototsynthesis in Douglas-fir was related to photoperiod (p), (P_N = 0.42p, r^2=0.66), and average air temperature (t) (P_N = 0.38t, r^2=0.97). When net

photosynthesis is related to both photoperiod and air temperature together, (P_N = 0.40t - 0.044p, r^2=0.97), the r^2 does not increase above the value for air temperature alone.

The Fv/Fm ratio pooled from all treatment groups is related to photoperiod (p), (Fv/Fm = 0.042p, r^2=0.95), and average air temperature (t), (Fv/Fm = 0.027t, r^2=0.72). When Fv/Fm is related to both photoperiod and air temperature together, (Fv/Fm = 0.002t + 0.40p, r^2=0.96), the r^2 does not significantly increase above the value for photoperiod alone.

Figure 22 shows the typical chlorophyll fluorescence induction curves obtained for the Douglas-fir seedlings in August, September, October, November, December and January (1990/91). As can be seen in Figure 22, the chlorophyll fluorescence induction curves changed over the fall for each of the three treatment groups.

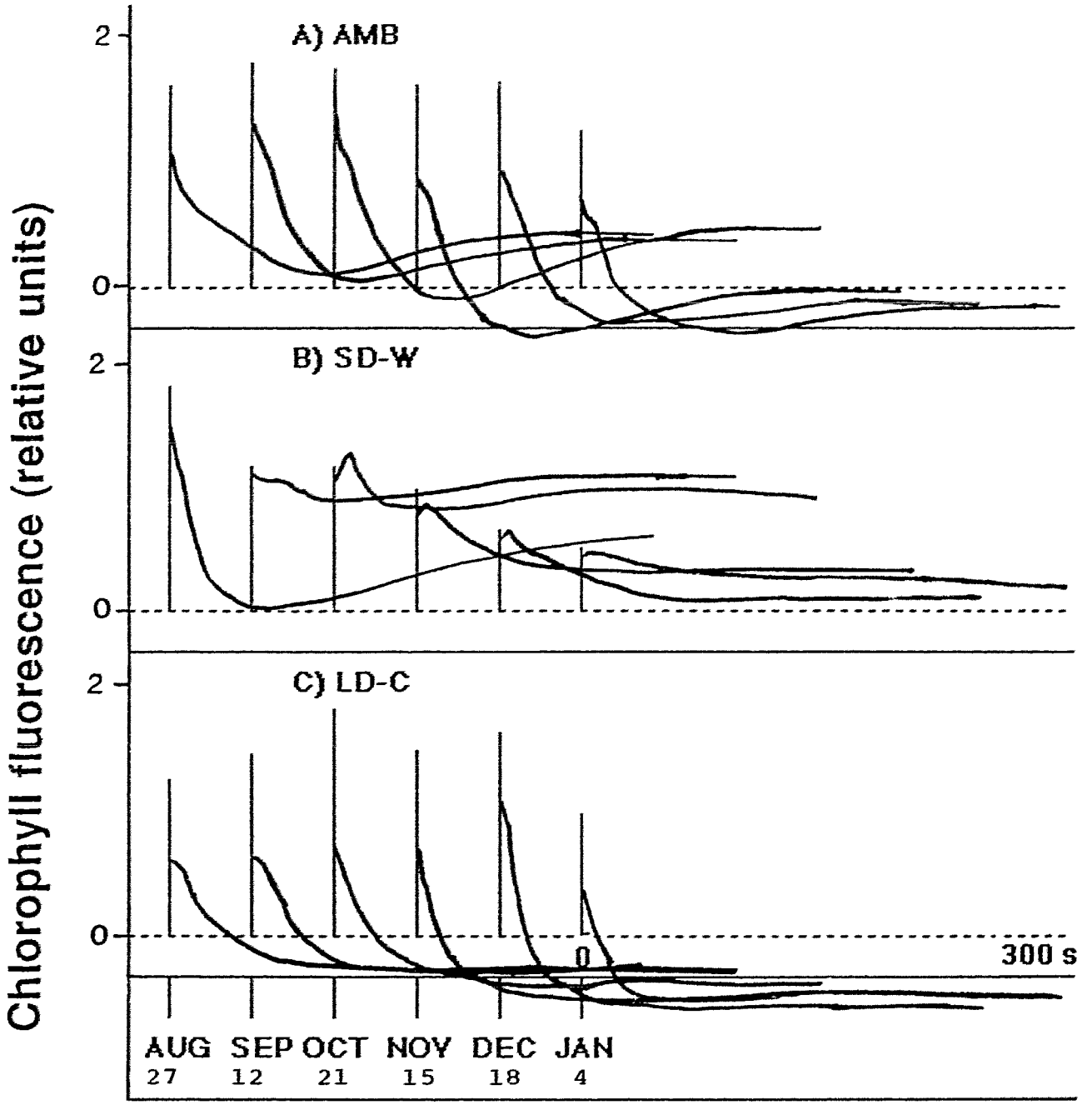

Figure 22. Chlorophyll fluorescence induction curves of Douglas-fir seedlings measured at room temperature. Each curve is an average of 10 seedlings. AMB seedlings were under natural conditions, SD-W was ≤12 h photoperiod, 25/20°C, LD-C was 16 h photoperiod, 10/5°C.

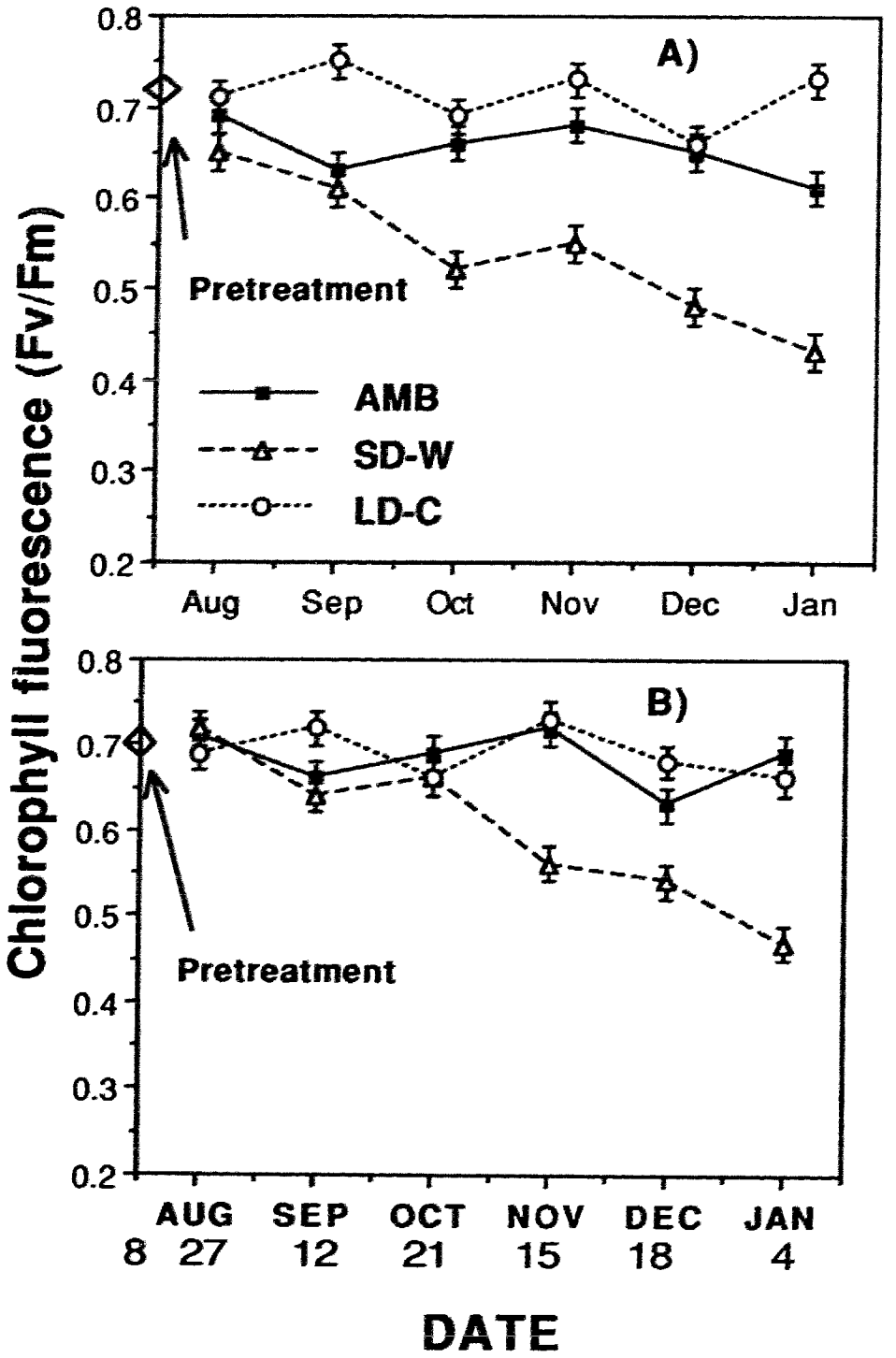

Figure 23. Ratio of variable fluorescence to maximal fluorescence, Fv/Fm, of Douglas-fir seedlings, A) 1990 and B) 1991. AMB seedlings were under natural conditions, SD-W was ≤12 h photoperiod, 25/20°C, LD-C was 16 h photoperiod, 10/5°C.

There was no significant change in the Fv/Fm ratio (Figure 23) of the AMB and LD-C groups from August through January, although F_O did vary from August to December in both 1990 and 1991. This change in F_O did not appear to be due to damage to the reaction centers, rather, it appeared to be related to seedling size. As seedlings increased in size, Fo also increased, and was linearly correlated to dry weight (r^2=0.56). The SD-W white spruce showed no significant change in F_O from August through January, consistent with the lack of growth seen in these seedlings. A significant increase in F_O was found in the LD-C seedlings, although F_O remained lower than for AMB seedlings. The growth of the these seedlings was also much slower than the AMB seedlings.

Steady state fluorescence (Figure 24) decreased significantly in the AMB seedlings over time in 1990 and 1991 (p=0.001), and was correlated to both photoperiod (r^2=0.71, r^2=0.81) and average air temperature (r^2=0.77, r^2=0.79). A decline in F_T was seen in the SD-W seedlings from October to January, which correlated to photoperiod (r^2=0.57, r^2=0.59) in 1990 and 1991.

Net photosynthesis decreased significantly in the AMB seedlings from August to September in 1990 and 1991 (Figure 25), and correlated to photoperiod (r^2=0.82, r^2=0.86) and average air temperature (r^2=0.89, r^2=0.92). There was no significant change in P_N for LD-C seedlings in 1990 and 1991 once the growth chamber temperature reached 10/5°C. There was also no significant change in P_N for SD-W seedlings in

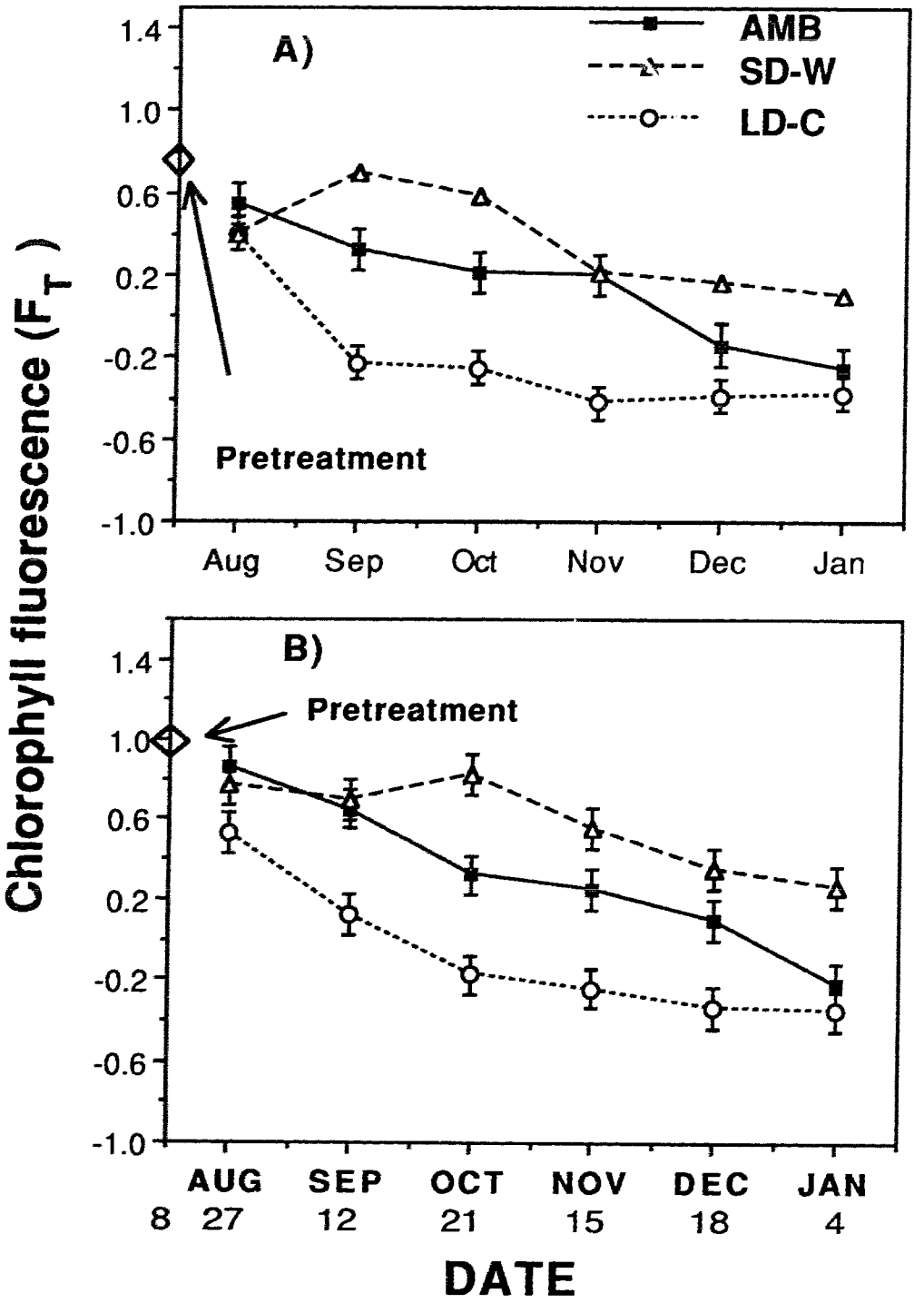

Figure 24. Steady state fluorescence, F_T, of Douglas-fir seedlings, A) 1990 and B) 1991. AMB seedlings were under natural conditions, SD-W was ≤12 h photoperiod, 25/20°C, LD-C was 16 h photoperiod, 10/5°C.

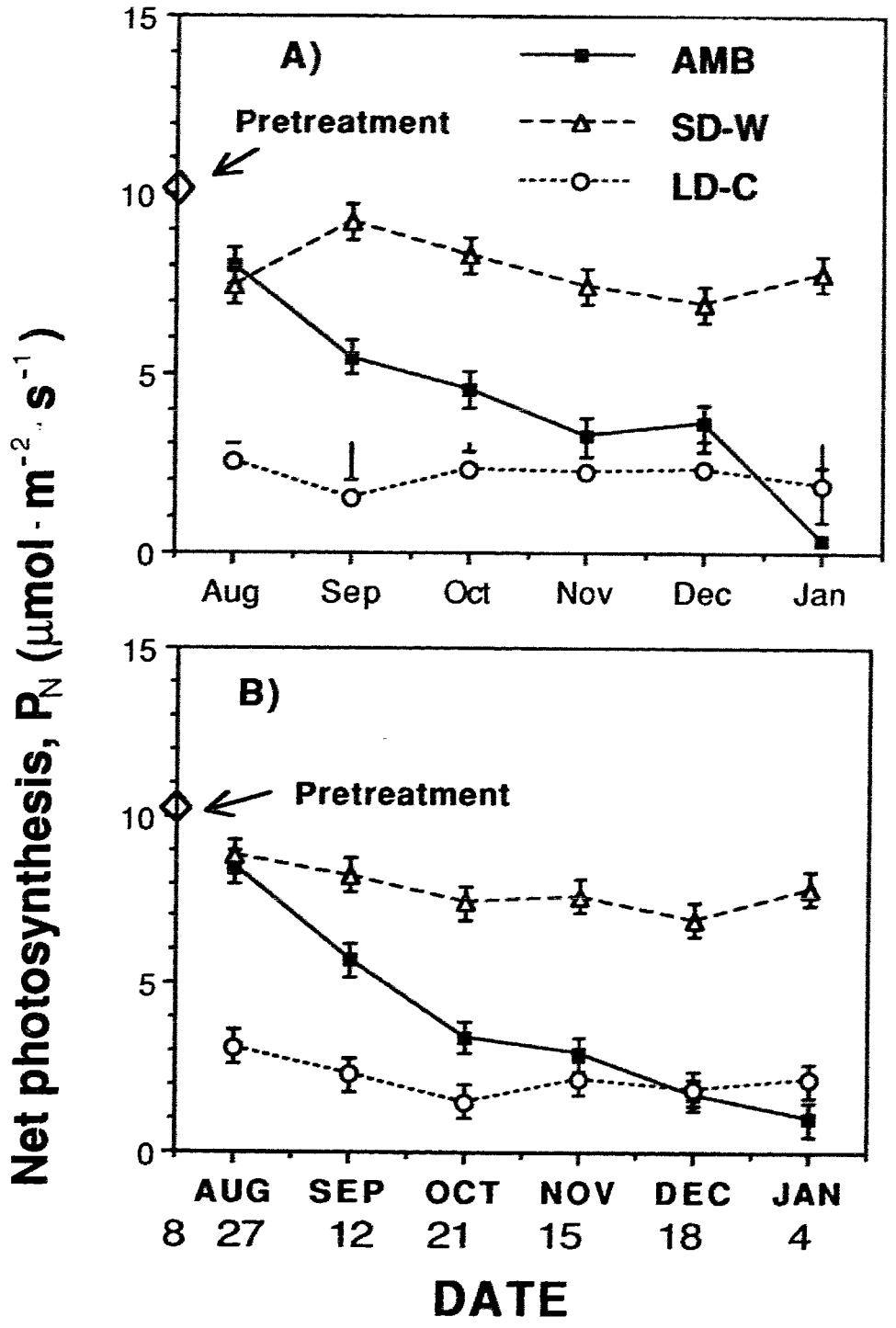

Figure 25. Net photosynthesis (P_N) of Douglas-fir seedlings, A) 1990 and B) 1991. AMB seedlings were under natural conditions, SD-W was ≤12 h photoperiod, 25/20°C, LD-C was 16 h photoperiod, 10/5°C.

1990 and 1991, P_N was not correlated to day length.

DISCUSSION

White Spruce

Evidence was found for day length initiated photochemical regulation of photosynthesis in white spruce seedlings.

The ratio of variable fluorescence to maximal fluorescence, Fv/Fm, is used as an indicator of PSII photochemical efficiency, and relates to the probability of a photon absorbed by the chlorophyll matrices being utilized to drive PSII photochemistry (Baker 1978). Decreased PSII activity, as seen by reduced Fv/Fm ratios, was evident during the fall in all three treatment groups. This decline of Fv/Fm was not due to increases in F_O, indicating no damage was evident in the PSII reaction centers (Briantais, et al. 1986). Reduced Fv/Fm ratios have been observed during the fall and winter in many conifer species, and has previously been related to both cold hardiness and photoinhibition.

Changes in Fv/Fm have been used to predict cold hardiness in conifers (Sunblad et al. 1990). In this study, the Fv/Fm ratio of the AMB and LD-C seedlings did decrease as cold hardiness increased; however, this was not the case for the SD-W seedlings, where Fv/Fm decreased but cold hardiness did not increase. Short day length did not cause an increase in cold hardiness in white spruce seedlings in this study, similar to results found by Burr et al. (1989). Therefore,

the decrease in PSII electron transport can not be explained by changes in cold hardiness only.

Reductions in Fv/Fm ratios have also been attributed to photoinhibition in many chilling sensitive and resistant plants (Strand & Lundmark 1987, Smillie et al. 1988, Hetherington et al. 1989, Krause & Somersalo 1989, Öquist & Malmberg 1989), including conifers (Öquist & Huner 1991). Photoinhibition results in a loss of both electron transport capacity and variable chlorophyll fluorescence, and is characterized by light-induced degradation of the D1-protein of PSII (Richter et al. 1990).

Northern species, such as white spruce, are subject to sudden frosts in late summer. White spruce is protected by setting bud in mid-summer in response to declining day length. It would also be adaptive for physiological changes associated with dormancy, including the reduction of photosynthetic activity, to begin early in the fall. A reduction of photosynthetic electron flow would prevent over excitation of the photosynthetic apparatus and protect chloroplasts from photodamage during periods of high light intensity and reduced CO_2 uptake (Gillies & Vidaver 1990). Therefore, the capacity to reduce electron flow prior to potentially damaging conditions, observed in conifers would be highly adaptive, enabling them to survive harsh conditions.

Ottander & Öquist (1990) have suggested that photoinhibition, measured as a decrease in Fv/Fm ratio, may

serve to regulate the rate of photochemistry to match reduced rates of CO_2 fixation, thereby balancing the energy flows in Scots pine at chilling temperatures. Under such conditions, excess energy may be dissipated as heat by the transformation of active reaction centers to photochemically inactive fluorescence quenchers, perhaps without degradation and reassembly of the reaction centers (Krause et al. 1990), making this a rapidly reversible reaction. Öquist et al. (1992) suggests that photoinhibition may also represent a mechanism for the long term regulation of PSII, especially in the fall and winter. In this study, such regulation appeared to be day length dependent, not stress (high light and chilling temperature) induced.

Seedlings in the SD-W treatment were not exposed to environmental stresses, therefore CO_2 fixation should have continued unabated; but even though the photosynthetic rates were higher than for the other two treatment groups, variable chlorophyll fluorescence and net photosynthesis did decline. The decrease in the Fv/Fm ratio and net photosynthesis observed in the SD-W seedlings was correlated to day length, suggesting photoperiod induced photochemical regulation of PSII in white spruce. This is consistent with the results of Vidaver et al. (1989a) who found photosynthetic regulation, decline in chlorophyll fluorescence and CO_2 gas exchange of white spruce seedlings began in mid-August in the absence of low temperatures, and approached completion in late October, suggesting a short day length dependent mechanism. This

decline in photosynthetic activity has also been found to be provenance dependent: even when seedlings were grown in the same nursery, more northern seedlots showed decreased chlorophyll fluorescence and CO_2 gas exchange earlier than more southern seedlots (Vidaver et al. 1988, Vidaver et al. 1989a, Vidaver et al. 1989b).

Dormancy induction had already been triggered in the LD-C seedlings, as indicated by the initiation of bud set prior to the beginning of the study. Therefore, results were not conclusive; the decline of net photosynthesis and chlorophyll fluorescence seen in these seedlings after placement in LD-C conditions could have been the result of depressed enzymatic activity brought on by chilling temperatures and photoinhibition, or possibly, was the result of photochemical regulation associated with dormancy.

After dormancy induction is initiated, and bud set nearly complete, reactivation of growth in white spruce requires chilling (Lavender 1985). The 16 hour day length within the growth chamber appeared to have less effect on the physiological activity of these seedlings than the chilling temperature. The growth chamber conditions permitted seedlings to acquire sufficient chilling hours for the release of bud dormancy, and seedlings flushed in December. Net photosynthesis and variable chlorophyll fluorescence increased once the newly flushed needles were present, indicating physiological reactivation, similar to that commonly seen in spring under natural conditions. It would

appear that the growth chamber conditions met the seedling's chilling requirements, as well as being conducive to flushing. Essentially, the growth chamber conditions reduced the period of time these seedlings might otherwise have remained dormant. Had this study begun prior to bud set, day length may have been a more important environmental signal for these seedlings.

Similar to the results found in Section I, F_O quenching was observed, steady state fluorescence, F_T, declined below apparent F_O level in the AMB seedlings when the temperature declined, and was consistently below apparent F_O in the LD-C treatment seedlings. The LD-C seedlings showed F_O quenching at much warmer temperatures than the AMB seedlings. Seedlings under LD-C conditions may have become shade adapted due to lower light levels in the growth chamber, rendering them more sensitive to F_O quenching than the control AMB seedlings.

It has been suggested that there are two mechanisms involved in the protection of the photosynthetic apparatus (Demmig-Adams 1990). This study also found evidence for two mechanisms; one which involves F_O quenching in the pigment bed (Bilger & Schreiber 1986), and may be zeaxanthin mediated (Demmig et al. 1987), the other causes a decline in electron flow, is seen as a decline in Fv/Fm and appears to be triggered by photoperiod in white spruce. Both mechanisms could function to protect the photosynthetic apparatus from damage during periods when excess energy must be removed.

Öquist & Huner (1991) proposed that photoinhibition of PSII might be an efficient way of controlling the dissipation of excessive excitation as heat in evergreens during prolonged winter conditions; however, this study found evidence of day length controlled decline of PSII activity, indicating photochemical regulation might be a more appropriate term than photoinhibition for white spruce.

Douglas-fir

Day length mediated photochemical regulation of photosynthesis in white spruce is characterized by both a reduction of net photosynthesis and the chlorophyll fluorescence ratio Fv/Fm. No evidence was found for day length initiated photochemical regulation of photosynthesis in coastal Douglas-fir. The seasonal decline of Fv/Fm ratios observed in the SD-W Douglas-fir seedlings were not accompanied by an associated decrease in net photosynthesis, indicating they had only lost excess electron carrying capacity; photosynthesis did not appear to be limited. This is similar to results of Öquist & Malmberg (1989), who found that pine has considerable surplus capacity for electron transport versus capacity for carbon metabolism. Declines in Fv/Fm did not relate to decreases in photosynthesis and may not be indicative of day length mediated photochemical regulation in Douglas-fir.

Net photosynthesis declined over the fall in the AMB seedlings, yet no decline in the Fv/Fm ratio was observed in

either the AMB or LD-C seedlings. This is in contrast to the results reported in Section I and by Hawkins & Lister (1985) for Douglas-fir where, in winter under freezing conditions, a decline of peak fluorescence, P, occurred in conjunction with a decline in net photosynthesis.

The difference between the results reported here and those of Section I may be attributed to the level of development of the seedlings at the beginning of each study. In Section I, seedlings had been subjected to 2 weeks short day length in the summer to induce bud set. The seedlings had well developed buds, the needles were mature and dark green by August. In this study, bud set as measured by mitotic index was not complete until the end of December for AMB seedlings, similar to the observations of Fielder & Owens (1988) and the study ended prior to completion of bud set for LD-C seedlings; the needles remained light green and succulent, and acquired little cold hardiness during the fall.

These results indicate a significant difference in the response of seedlings at different levels of development. The reversible decline of Fv/Fm, as well as an increase in cold hardiness, was observed only in the fall and winter in seedlings with resting buds and mature needles. There was no decline of Fv/Fm or increase in cold hardiness observed in seedlings without buds and with immature needles under fall and winter conditions. A decrease of Fv/Fm ratio in

immature, unhardened needles might be indicative of damage to rather than regulation of PSII.

Because AMB seedlings experienced both decreasing day length and temperature, it is not possible to determine which factor had more influence on the net photosynthetic rate; however, SD-W seedlings experienced decreasing day length only, and seedlings had high photosynthetic rates throughout the fall. Conversely, the long day length (16 hours) given to LD-C seedlings did not result in high photosynthetic rates. Temperature appeared to be an important factor influencing net photosynthesis in all treatment groups.

Comparison of white spruce and Douglas-fir

The different responses of these two species can be partly explained by the disparate climatic regimes they are adapted to. White spruce is a boreal species that experiences the sudden onset of freezing temperatures in late summer and early fall; without the initiation of dormancy in mid-summer, seedling mortality in the early fall could be very high. In contrast, coastal Douglas-fir is from a temperate region, and is an opportunistic species that can be photosynthetically active in the fall and winter (Waring & Franklin 1979).

Both AMB and LD-C seedlings had white roots when sampled throughout the fall, indicating ongoing root growth. The occurrence of large numbers of white roots in the fall seen in this study is similar to the results seen by Johnson-

Flanagan & Owens (1985). These observations suggest root growth in white spruce and Douglas-fir may be temperature related; cool temperatures appeared to induce root growth. The LD-C seedlings at 10/5°C had the greatest number of white roots, and the peak root growth of the AMB seedlings occurred when the ambient temperature averaged between 5-10°C.

In this study, temperature was the most important factor in the development of cold hardiness in the white spruce seedlings; bud set was the most important for the Douglas-fir seedlings. White spruce seedlings appeared to require chilling to increase cold hardiness, short day length alone was not sufficient. If this study had begun prior to bud set in the white spruce seedlings, mitotic index might have been a more important factor in the development of cold hardiness in the AMB seedlings, as it was in the Douglas-fir seedlings.

The photosynthetic activity of these two species were found to response to both temperature and photoperiod. Both temperature and photoperiod were equally important factors affecting the net photosynthesis of white spruce, but photoperiod was a more significant factor affecting the Fv/Fm ratio. Air temperature was a more significant factor affecting the net photosynthesis of Douglas-fir than photoperiod, but photoperiod was a more signficant factor affecting the Fv/Fm ratio.

There was a significant difference between the two species at the beginning of the study: the white spruce seedlings had set bud prior to the onset of treatment, the

Douglas-fir seedlings had not. The effect of the presence or absence of buds on the physiological responses of seedlings to dormancy induction treatments should be explored. Alternative photoperiod and temperature regimes would also provide more information on the control of dormancy, cold hardiness and photochemical regulation of photosynthetic activity.

IV. Physiological differences between cold hardened, dehardened and newly flushed white spruce needles

INTRODUCTION

There is general agreement that photoinhibition occurs under conditions when photon flux energy exceeds the plant's capacity to dissipate it through heat production, fluorescence and use in photosynthesis (Powles 1984). Photoinhibition is based primarily on an inactivation of the electron transport system in the thylakoids (for a review see Krause 1988) and has been at least partly attributed to the degradation of the D1-protein (Richter et al. 1990).

Photoinhibition is characterized by the quenching of variable chlorophyll fluorescence, causing a decrease in the ratio of variable fluorescence to maximum fluorescence, Fv/Fm (Richter et al. 1990). An initial increase in the variable chlorophyll fluorescence may occur prior to this decrease (personal communication Vidaver, W.). The quenching of Fv/Fm results predominantly from a decrease in variable fluorescence, Fv, and not from a decrease in F_O (Krause 1988). Chlorophyll fluorescence has therefore been used extensively to investigate photoinhibition (Strand & Lundmark 1987, Smillie et al. 1988, Bolhar-Nordenkampf et al. 1991, Ögren 1991).

Low temperatures can increase the susceptibility to photoinhibition in both chilling sensitive and resistant plants (Hetherington & Smillie 1989, Krause & Somerasalo 1989) as well as cold tolerant plants such as white spruce (Strand & Öquist 1985, Öquist & Malmberg 1989, During et al. 1990).

Plants chilled in the light show greater injury than plants chilled in darkness (Wise & Naylor 1987), and even cold hardened conifers appear to be susceptible to photoinhibition (Strand & Öquist 1988, Bolhar-Nordenkampf & Lechner 1988).

Because photoinhibition is rapidly reversible, taking from minutes to hours, it can be viewed as a controlled protective mechanism that serves to dissipate excess energy (Krause 1988) under conditions of low temperatures and high photon flux (Krause & Somersalo 1989).

Physiological differences were observed between newly flushed needles and dehardened one year old needles in Section 1. This study looks at the differences in the susceptibility of cold hardened, dehardened one year old and newly flushed white spruce needles to sub-zero temperatures in light and darkness.

MATERIALS & METHODS

White spruce seedlings used in this study were supplied as per General Materials and Methods. Seedlings were brought to SFU in November 1991 and placed in an unheated glass greenhouse.

Cold hardiness testing was conducted in January 1992, as per General Materials and Methods. Seedlings were considered hardened when cold hardiness LT_{50} was >-25°C. In February, approximately 190 seedlings were placed in a styroblock container in a growth chamber at 24/18°C day/night temperature, 16 hour photoperiod, PPF of 375 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 70-

85% RH. Seedlings were well watered and maintained under these conditions for 35 days, at which time they were considered dehardened. All new foliage was removed except the leader; needles remained tightly bunched and had not fully expanded at this time.

Carbon dioxide gas exchange and needle water content were measured on randomly selected hardened seedlings in January, sub-zero temperature treatments were also done at this time. This was repeated in March for dehardened one year old and newly flushed needles after the 35 days of dehardening in the growth chamber.

The CO_2 gas exchange was measured at three O_2 concentrations (percent O_2 = mole fraction X 100%): 2.1%, 21% and 51.3%. Gas for low and high O_2 concentrations was supplied by Linde (Vancouver). Room air was used for 21% O_2. Gas exchange was measured on three seedlings, each measurement was repeated twice at each oxygen concentration.

All CO_2 gas exchange measurements were taken between 310 and 370 $\mu L \cdot L^{-1}$ CO_2 using the system described in the General Materials and Methods. The PPF for light saturation curves used a range from 2350 to 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$, measurements were taken from highest PPF to lowest. Seedlings were acclimated for a minimum of 30 minutes at 2350 $\mu mol \cdot m^{-2} \cdot s^{-1}$ prior to measurements.

The CO_2 gas exchange was measured in the following order of O_2 concentration for each individual seedling: 21%, 2.1% then 51.3% O_2. The CO_2 gas exchange was measured twice at 21%

O_2, then the system was flushed with 2.1% O_2 for 3 minutes. CO_2 gas exchange was measured twice at 2.1% O_2, the system was then flushed with room air for 5 minutes. The system was then flushed with 51.3% O_2 for 3 minutes before CO_2 gas exchange at 51.3% O_2 was measured twice.

Rates of photorespiration, R_p, ($\mu mol \cdot m^{-2} \cdot s^{-1}$), were estimated as the difference between photosynthesis at 2.1% and 21% O_2 (Mebrahtu et al. 1991). Rates of high O_2 concentration photorespiration, Rp*, were estimated as the difference between photosynthesis at 2.1% and 51.3% O_2.

Needle water content was estimated by weighing needle fresh and dry weights. Plants were well watered prior to weighing. Water content was calculated as 100 minus the percentage of dry weight to fresh weight using the following formula:

Water Content = $[(FWT - DWT)FWT^{-1} \times 100\%)]$.

Chlorophyll fluorescence followed the procedures of General Materials and Methods. To measure chlorophyll fluorescence of the old and new foliage separately in the integrating sphere, foliage not measured was either left external to the sphere or covered by crushed foil, the foil had no effect on chlorophyll fluorescence measurements (data not presented).

Chlorophyll fluorescence was also measured using the SF-30 (Brancker, Ottawa), 10 needles were removed from each sample and lined up on black masking tape after dark pretreatment. The probe was placed over needle samples and

chlorophyll fluorescence measured. This was repeated three times for each sample.

Randomly selected seedlings from each group (hardened needles, newly flushed needles and dehardened one year old needles) were given sub-zero temperature treatments in both light and darkness. Sub-zero temperature treatments were performed in a modified deep freeze (Figure 26). The freezer lid was removed and replaced with an insulating foam lid with a 40 X 30 cm double pane glass window. A Plexiglass water bath filled with 9 cm of water was placed over the window, the external light source was located above the water bath. Light was supplied by a high pressure sodium lamp (400 watt Poot Elektra, Type PC 1078/4 lamp, with a GE Lucalox LU400/40 bulb) and the PPF at foliage height was 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as measured by a LI-COR 185A radiometer with a quantum sensor (Lincoln, NE). To ensure the maximum amount of foliage received a high photon flux density, samples were placed in a horizontal position in the freezer (Figure 26). All seedlings experienced light for the last 4 hours they were in the freezer, regardless of the minimum temperature. Seedlings receiving sub-zero temperature treatments in darkness were enclosed in a light-proof black box and placed in the freezer at the same time as light-treatment seedlings. Temperature was monitored in both the black box and the freezer using thermistors.

Initial freezer temperature was 15°C, temperature was then lowered at $6°C \cdot h^{-1}$, and maintained at the desired

temperature for 1 hour. To ensure uniform air temperature, air circulation within the freezer was provided by two fans in the bottom of the freezer.

Six sub-zero temperatures were used, ranging from -2°C to -22.5°C; the minimal temperature attainable in the freezer with the light and fans on. Five samples were used at each temperature, each temperature treatment was repeated three times.

After sub-zero temperature treatment was complete, the light was turned off and both light and dark-treatment seedlings were warmed at a rate of $6°C \cdot h^{-1}$ to room temperature in the dark. After 16 hours recovery in darkness, seedlings were then placed under a PPF of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 2 hours at room temperature to slowly acclimate them to light. Chlorophyll fluorescence was then measured as per General Materials and Methods, and repeated again after 3 days.

Control seedlings were placed under identical conditions in the freezer, but experienced a temperature of 25±2°C. All seedlings were then placed in an incubator with a PPF of 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 23±2°C and 75-85% RH for two weeks.

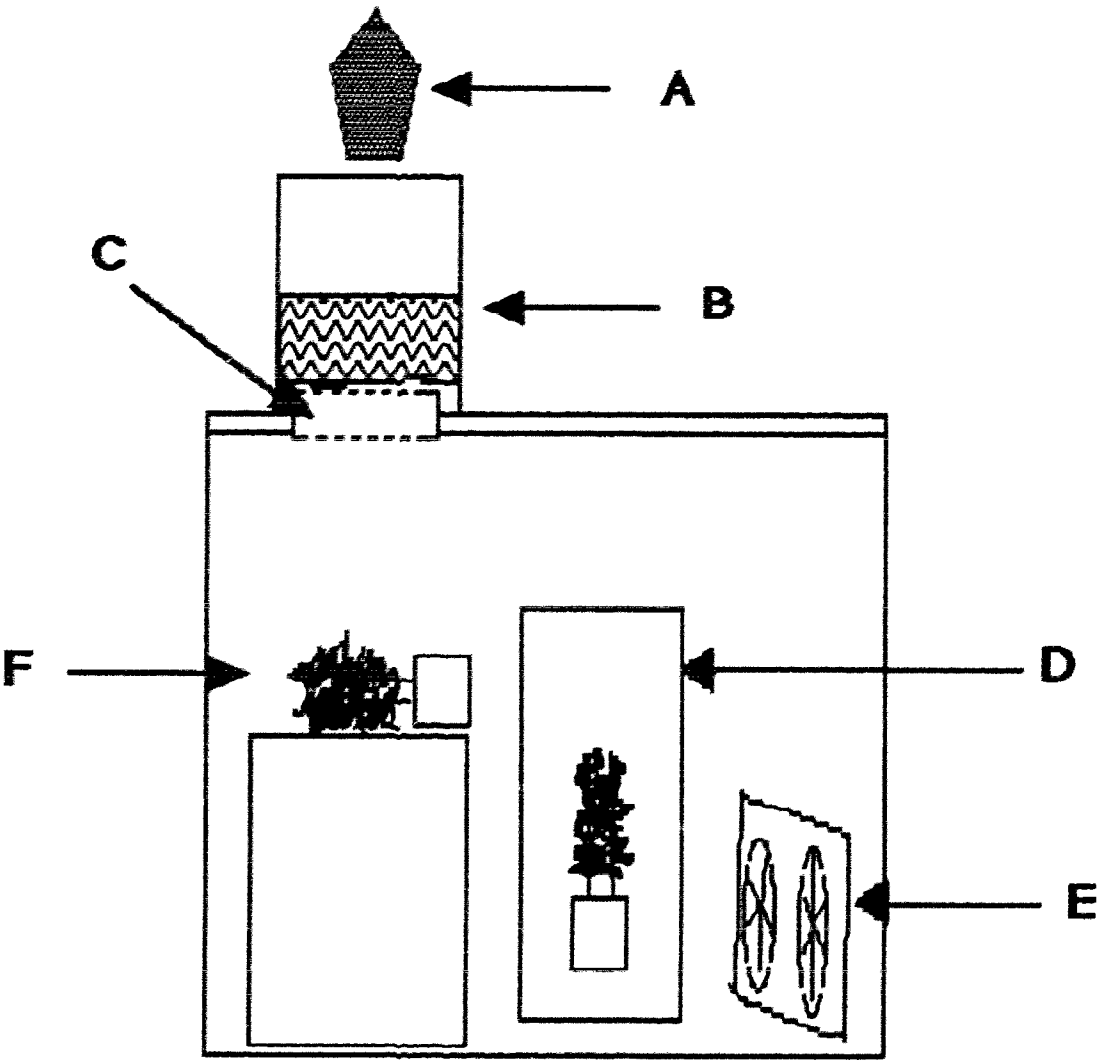

Figure 26. Modified deep freeze used for sub-zero temperature treatments. A - light source, B - water bath, C- double pane window, D - black box containing seedlings given dark-treatment, E - fan, F - seedlings given light-treatment.

The procedure for measuring electrolyte leakage was modified from Colombo et al. (1984). Needles were removed from seedlings for electrolyte leakage determination after sub-zero temperature treatments. Electro-conductivity was measured using a Digital Conductivity Meter Model 1481-90,82 (Cole-Parmer Inst. Co., Chicago IL).

Damage to foliage was visually assessed after two weeks recovery in the incubator as per General Materials and Methods.

Statistical analysis was performed using SAS/STAT (1988). Analysis of covariance, using needle status (hardened, dehardened, newly flushed) as the covariant and temperature and light as factors, was used to analyze electrolyte leakage, chlorophyll fluorescence and damage data, a sample analysis of co-variance table is shown in Table 13. Bonferroni tests of differences was used to determine significance between individual means (Winer 1971).

Table 13. Analysis of Co-Variance table for the ratio of variable fluorescence to maximum fluorescence (Fv/Fm) of dehardened seedlings after various sub-zero temperature treatments in the light (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or darkness. Needle status - hardened, dehardened, or newly flushed - was used as the co-variant.

Analysis of Co-Variance Table

Source	Sum-of-squares	DF	Mean-Square	F-ratio	P
Temp	10.078	6	1.680	123.654	0.000
Light	0.160	1	0.160	11.802	0.001
Status	0.108	1	0.108	7.986	0.005

RESULTS

Carbon dioxide gas exchange

Dehardened one year old needles had a significantly higher light saturation ceiling at 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than hardened and newly flushed needles (Figure 27). The O_2 concentration had a significant effect (p=.001) on photosynthetic rate for hardened, dehardened and new needles. At the high O_2 concentration, photosynthesis was inhibited.

There was no significant difference in the Rp of hardened needles, dehardened one year old needles and newly flushed needles (Figure 28). There was a significant difference in Rp* between hardened one year old needles and newly flushed needles (Figure 28). At 51.3% O_2, newly flushed needles were strongly inhibited and the photosynthetic rate was 30% lower than the gross photosynthetic rate at 2.1%, whereas the dehardened needles were reduced by 43% of the Pg rate.

Water content

Newly flushed needles had the highest water content at 80.3%, dehardened one year old needles had 63.5%, hardened needles had the least water content at 41.2%.

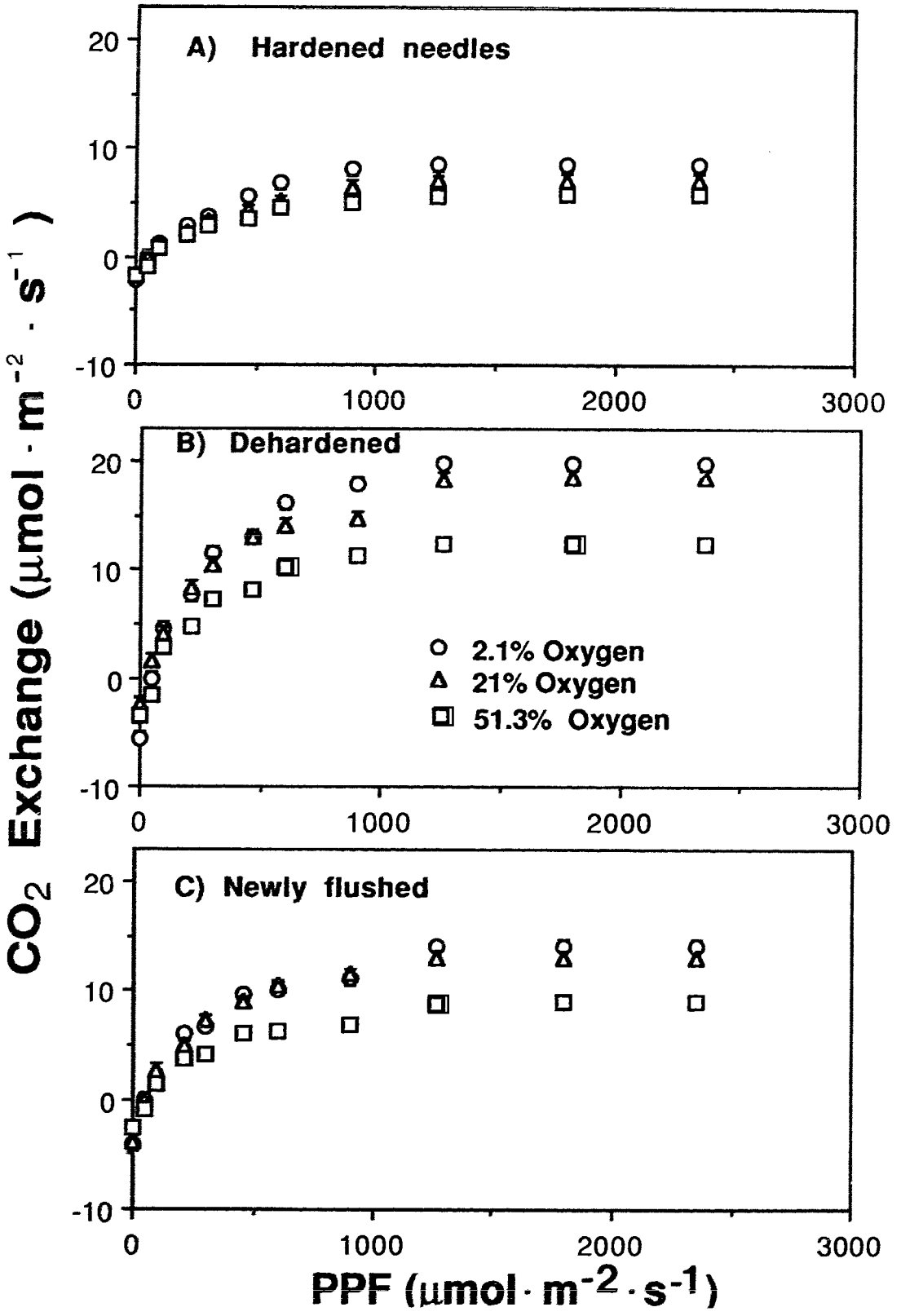

Figure 27. Light saturation CO_2 gas exchange curves at three O_2 concentrations for white spruce. A) hardened needles, B) dehardened one year old needles, and C) newly flushed needles.

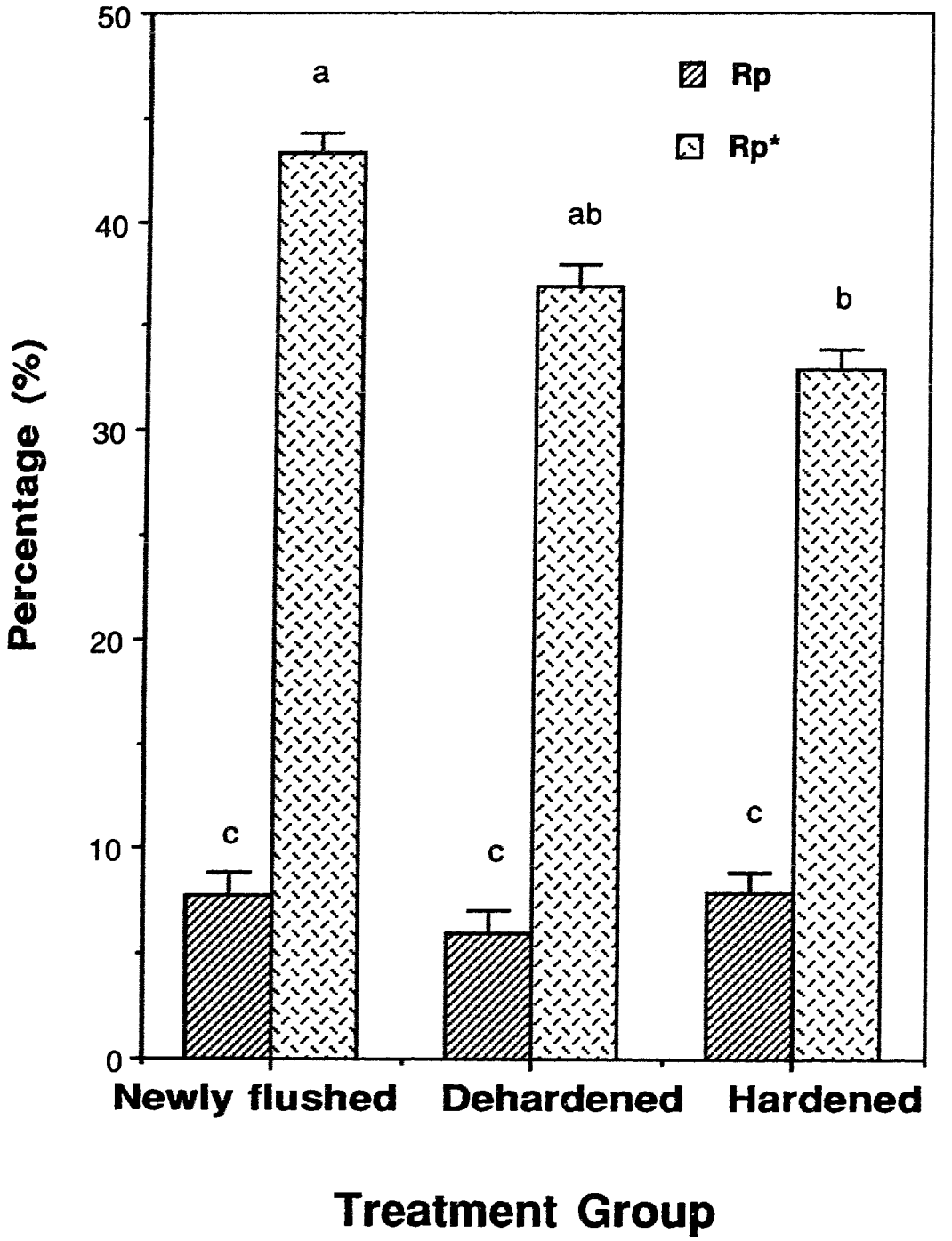

Figure 28. Photorespiration (Rp) at 21% O_2, and photorespiration* (Rp*) at 51.3% O_2 calculated as a percentage of photosynthesis measured at 2.1% O_2. For hardened needles, dehardened one year old needles, and newly flushed needles at a PPF of 2350 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

a,b,c - same letter signifies no significant differences between columns

Sub-zero temperature treatments

Hardened Needles

There was no significant difference between the Fv/Fm ratio of control seedlings (25°C) or seedlings given sub-zero temperatures in both the light and dark (Figure 29A & B). For all temperatures, Fv/Fm recovered fully when measured after three days. There was no visible damage to foliage after two weeks recovery (Figure 32A) and electrolyte leakage was not significantly affected by the sub-zero treatments in either the light or the dark (Figure 32B).

Dehardened Needles

Dehardened needles were significantly affected by the sub-zero temperature treatment ($p=.001$), and were more susceptible in the light than in the dark ($p=.005$). Sub-zero temperatures had a significant effect ($p=.001$) on all measured parameters, Fv/Fm, visible damage and electrolyte leakage.

There was no significant change in Fv/Fm for dehardened one year old needles at temperatures above -10°C (Figure 30A); however, the Fv/Fm ratio was significantly lower at temperatures below -10°C, and after 3 days Fv/Fm did not recover (Figure 30). There was a positive correlation between Fv/Fm and sub-zero temperature ($r^2=.79$); Fv/Fm declined as temperature declined. At temperatures above -10°C, Fv/Fm recovered after 3 days. There was no 3 day recovery of Fv/Fm for temperatures of -10°C and lower (Figure 30).

For seedlings given sub-zero temperatures in the light there was a positive correlation between the sub-zero

temperature and Fv/Fm (r^2=.79). Sub-zero temperature treatments in the dark did not show the same characteristic decline, Fv/Fm did not decrease as temperature decreased, instead Fv/Fm declined only at temperatures which also appeared to cause tissue damage.

The percent visible damage to needles after two weeks (Figure 32A) showed that damage occurred at a higher temperature in the light than darkness. There were strong linear correlations between percent visible damage and electrolyte leakage (r^2=.88) and percent visible damage and Fv/Fm measured immediately after treatments (r^2=.91) (Figure 33). The LT_{50} calculated using data from all three methods were very similar. From visible damage data, dehardened one year old needles had an LT_{50} of -10.5°C in the light and -12.4°C in the dark.

Newly Flushed Needles

Similar to the results seen in dehardened needles, there was a positive correlation (r^2=.82) between sub-zero temperature and Fv/Fm in the light. For seedlings given sub-zero temperature treatments in the light, the Fv/Fm ratio declined as temperature treatment declined, at temperatures above -4°C, Fv/Fm recovered after three days, at -6°C there was no recovery of Fv/Fm after 3 days (Figure 31).

For dark-treated seedlings, Fv/Fm did not decrease as temperature decreased, instead Fv/Fm declined only at temperatures which also appeared to cause tissue damage. There was no significant change in the Fv/Fm ratio for dark-

treated newly flushed needles seedlings given sub-zero temperatures above -6°C, at -6.2°C Fv/Fm decreased and did not recover after 3 days (Figure 31).

Damage occurred at a higher temperature in the light than darkness (Figure 32B).

Using visible damage data, the newly flushed needles had an LT_{50} of -4.8 when light-treated, and when treated in the dark had an LT_{50} of -6.2°C (Figure 32A).

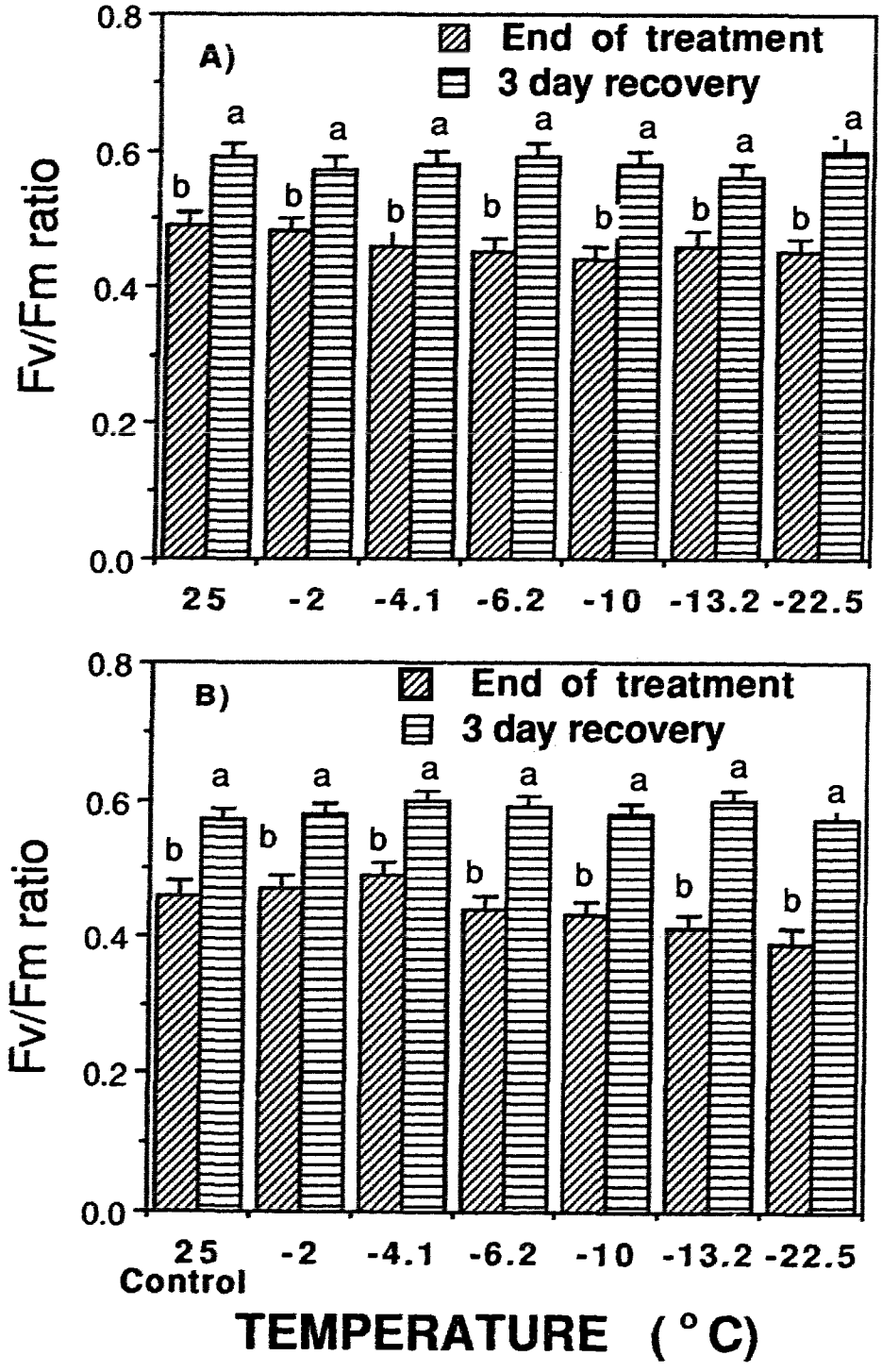

Figure 29. The variable fluorescence to maximum fluorescence ratio (Fv/Fm) of hardened white spruce seedlings after sub-zero temperature treatment, and after three days recovery in low light 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. A) dark-treated and B) light-treated (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (mean ± SE).

a,b same letter signifies no significant difference between means.

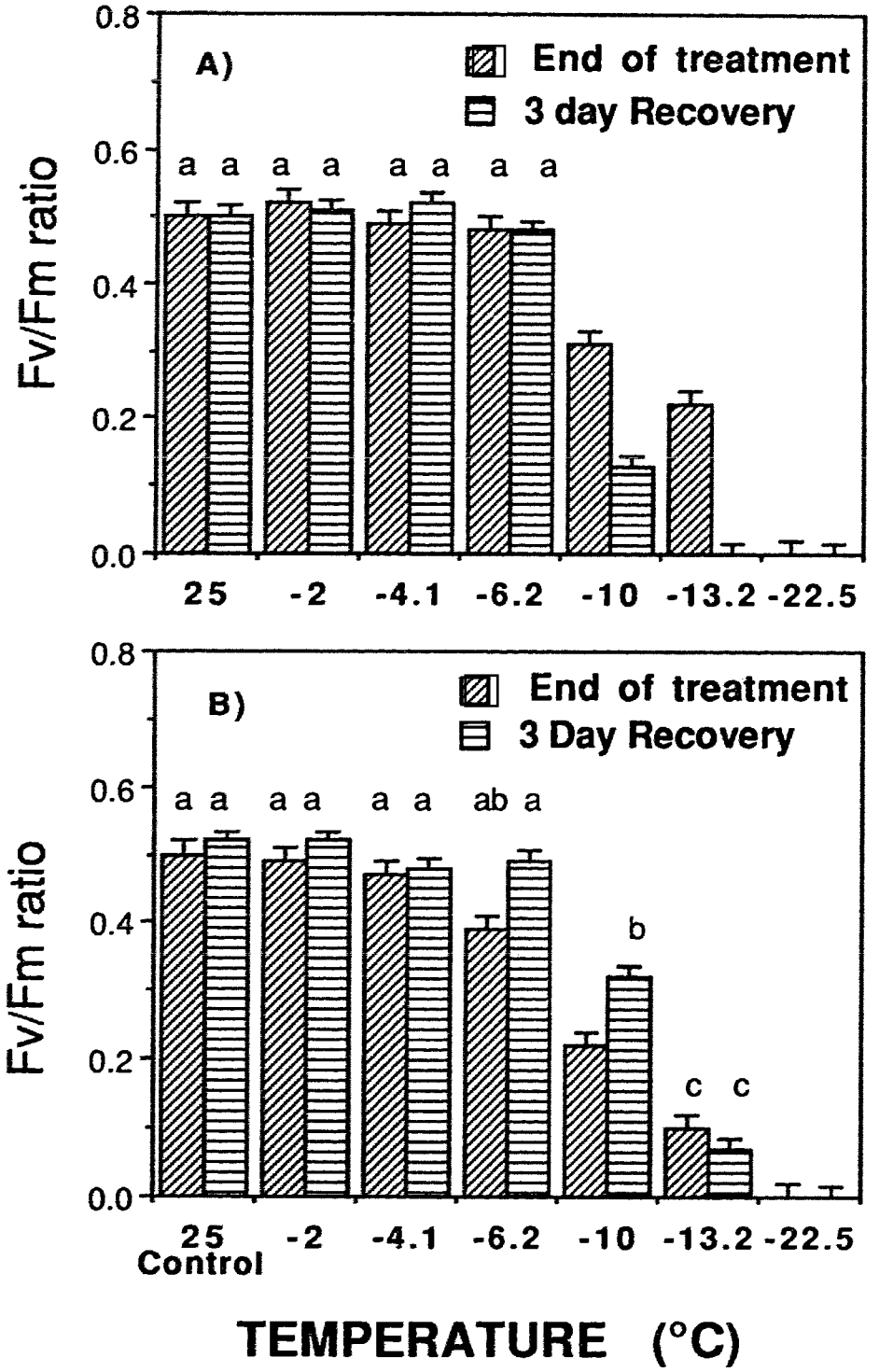

Figure 30. The variable fluorescence to maximum fluorescence ratio (Fv/Fm) of dehardened white spruce seedlings after sub-zero temperature treatments and after three days recovery in low light 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. A) dark-treated, and B) light-treated (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (mean ± SE).

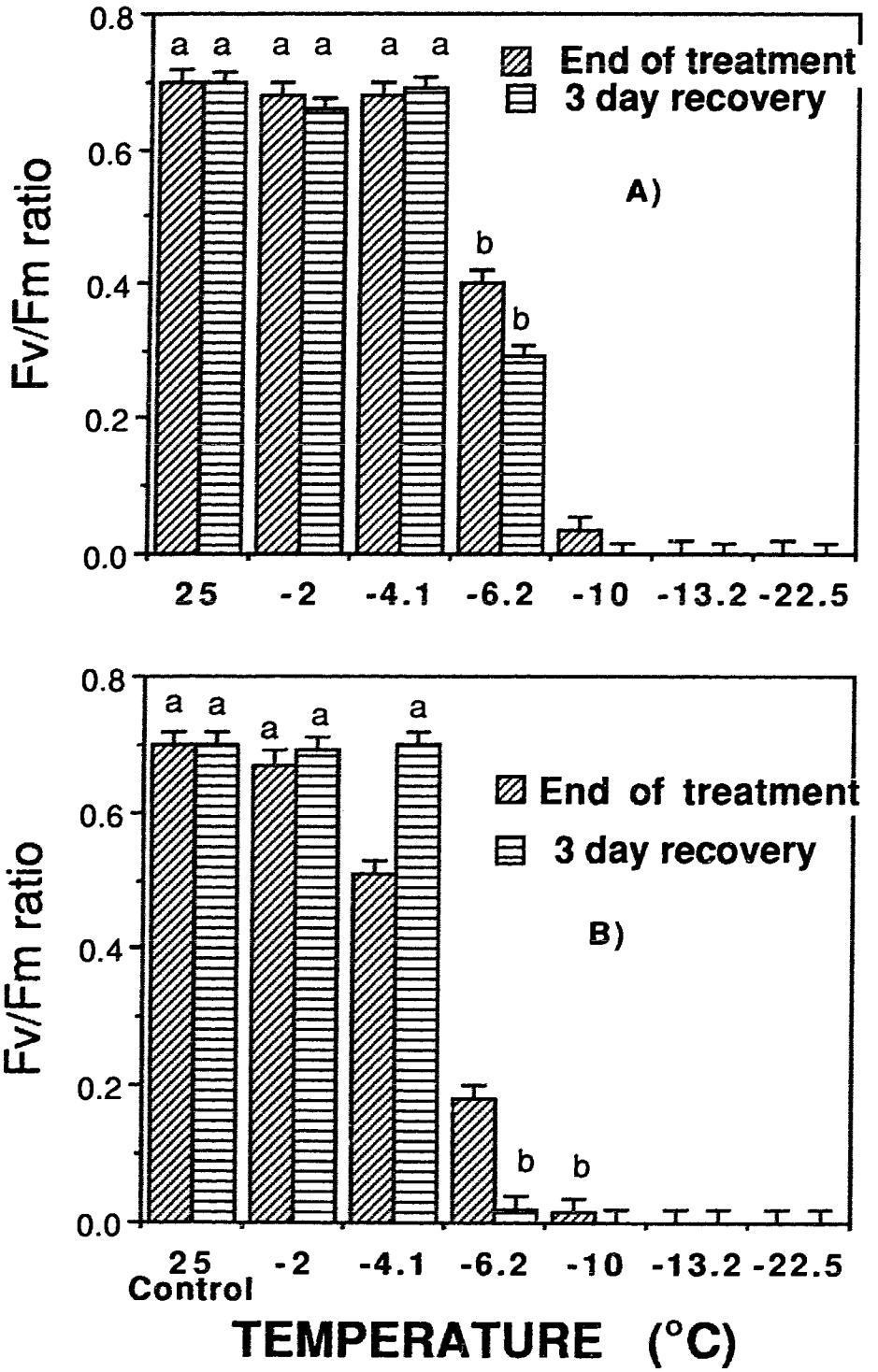

Figure 31. The variable fluorescence to maximum fluorescence ratio (Fv/Fm) of newly flushed white spruce needles after sub-zero temperature treatments, and after three days recovery in low light 120 $\mu mol \cdot m^{-2} \cdot s^{-1}$. A) dark-treated, and B) light-treated (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (mean ± SE).

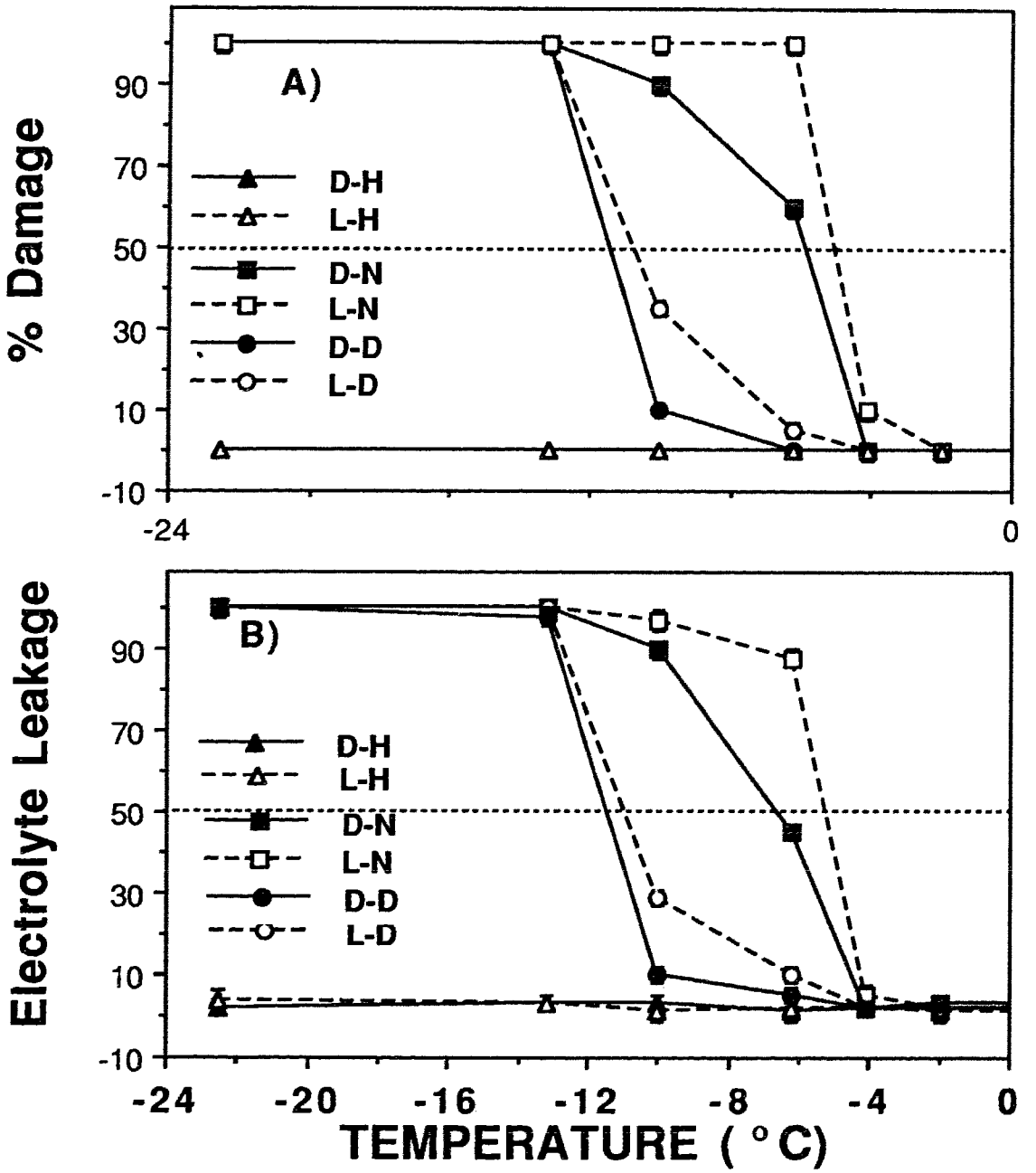

Figure 32. A) The percent of visible foliar damage of white spruce seedlings after two weeks recovery from sub-zero temperature treatments in the light and the dark, and
B) Electrolyte leakage of the needles of white spruce seedlings after sub-zero temperature treatments in the light and the dark.
D-H- dark-treated hardened, L-H- light-treated hardened, D-N- dark-treated newly flushed, L-N- light-treated newly flushed, D-D- dark-treated dehardened, L-D- light-treated dehardened needles.

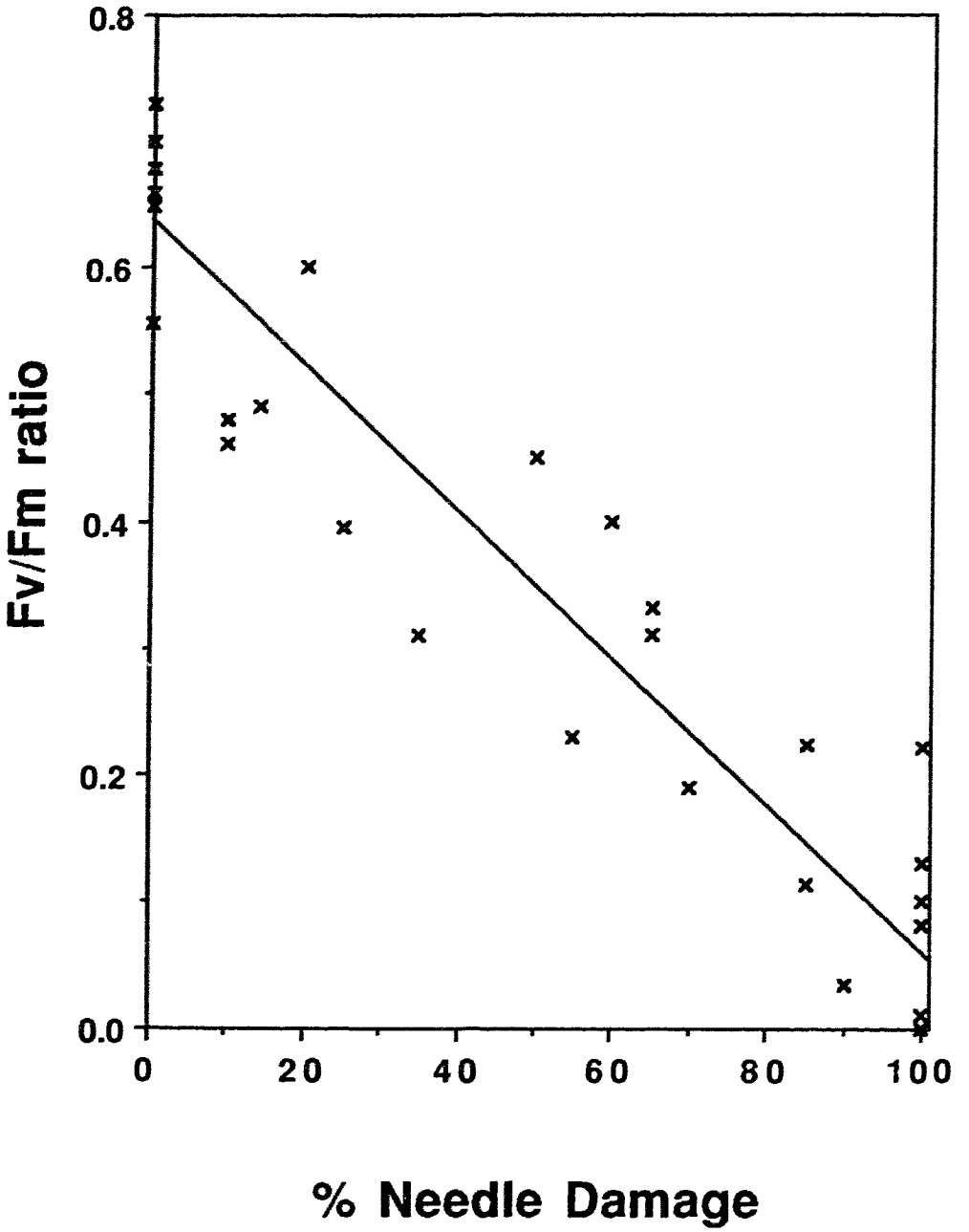

Figure 33. The variable fluorescence to maximum fluorescence ratio (Fv/Fm) related to needle damage. Dehardend and newly flushed needles were given sub-zero temperature treatments in the light (400 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Each point represents the mean of 5 replicates. Equation for the line is $y = 0.638 - 0.0058x$ ($r^2 = -.91$).

DISCUSSION

Needle maturity and hardening were important factors in understanding the photosynthetic rates observed in the three treatment groups. The low photosynthetic rates of hardened needles, as seen in this study, are frequently observed in hardened conifers in the fall and winter (Strand & Oquist 1988, Vidaver et al. 1989a); however, the low photosynthetic rates observed in newly flushed needles, which were tightly bunched rather than fully expanded, may have been the result of self-shading.

Oxygen concentration had a significant affect on the photosynthetic rate; at ambient (21%) and high O_2 concentrations (51.3%), photorespiration, as well as the Mehler reaction, decreased photosynthesis in all seedlings. Photorespiration was most pronounced in the new needles, they demonstrated greater photosynthetic inhibition under high oxygen concentrations than either hardened or dehardened one year old needles.

Photoinhibition, seen as a reversible decline of Fv/Fm, was observed in all light-treated seedlings after sub-zero temperatures, but not in dark-treated. Newly flushed needles showed photoinhibition at warmer temperatures than dehardened or hardened needles. Recovery of Fv/Fm occurred after three days only if there was no damage to tissue. This agrees with the results of Öquist & Malmberg (1989), and indicates photoinhibition, acting as a protective mechanism, functions in the light for these seedlings.

Tissue damage in seedlings occurred at warmer temperatures in the light than in darkness. In both the light and dark, substantial and irreversible decreases in Fv/Fm were indicative of tissue damage. The combination of low temperatures and high light can result in photodamage, caused by photooxidation - light and oxygen dependent bleaching - by the production of strong oxidants (Wise & Naylor 1987). Irreversible decreases in Fv/Fm after sub-zero temperature treatments in the light indicate a lowering of the photochemical efficiency of PSII; D1-protein has been proposed as the site of damage (Strand & Öquist 1988).

Damage to tissue given sub-zero temperature treatments in darkness cannot be explained by light dependent photodamage. In the dark, damage to needles would likely be due to freezing injury and appeared to be related to the water content of the needles. Sub-zero temperatures generally cause needle water to freeze at about the -4°C range (Pisek 1973, Bauer et al. 1975), which can disrupt the plasma membrane, the primary cause of freezing injury in plants (Steponkus & Lynch 1989). The increased electrolyte leakage seen these seedlings would indicate that membranes had indeed been damaged.

In this study, hardened needles had the lowest water content; newly flushed needles had the highest. At -6.2°C it is possible water in the needles would freeze and damage membranes through water crystal formation and dehydration. The hardened needles with low water content did not seem to suffer from freezing damage, whereas freezing damage was

observed in the newly flushed needles after sub-zero temperature treatments of -6.2°C in the dark.

Reversible decreases in Fv/Fm were the result of decreases in Fv and not increases in F_O, and are attributable to photoinhibition as a form of photochemical regulation. Irreversible decreases in Fv/Fm; however, were the result of decreases in Fv and large increases in F_O, and are attributable to photodamage and freezing damage in light-treated seedlings, and membrane damage caused by freezing in dark-treated seedlings.

Susceptibility to photoinhibition and photodamage appeared to depend upon the developmental stage of the needles. Newly flushed needles were the most affected by high O_2 concentrations, having the highest photorespiration rates. High photorespiration rates may be indicative of the increased sensitivity to photodamage caused by strong oxidants observed in the newly flushed needles. Needle water content appeared to be an important factor in susceptibility to freezing damage in the dark, newly flushed needles with the highest water content suffered damage at the warmest temperature, hardened needles with the lowest water content showed no damage.

V. Evaluating the efficacy of seedling quality assessment methods

INTRODUCTION

Effectively assessing seedling quality has long been crucial in the forest and conifer nursery industries. In the nursery, morphological tests are frequently used to select and grade seedlings, they are easy and rapid to perform (Thompson 1985), and result in a relatively morphologically uniform crop. Problems arise because there can be a large variation in physiological quality of apparently uniform seedlings, resulting in little relationship between morphological parameters and the survival and growth of seedlings in the field.

Information on the physiological status of seedlings, in combination with morphological tests and standards, would provide siliviculturalists with greater information for making decisions. In any reforestation programme, planting seedlings in the field incurs the largest expense. If seedlings are of poor quality, it is more cost effective to not plant these seedlings, plant them in a low stress location or to increase the planting density.

Physiological tests are already used in two areas of seedling production: determining lifting dates for storage and determining seedling quality after cold storage. Current methods for assessing seedlings' physiological status rely on single tests: cold hardiness for determination of suitability for cold storage, and root growth potential to measure seedling quality in the spring prior to planting. Some physiological tests may also have the potential to be

prescriptive during the growing season, facilitating any necessary alteration of cultural treatments.

Many different physiological tests have been suggested, and some techniques have proved to be of value in evaluating seedling quality at different times in the production cycle. In this study, several morphological parameters were measured (height, stem diameter, number of white roots visible, root/shoot ratio and bud set) to ensure seedlings met the B.C. Ministry of Forests standards for each species. Physiological assessment methods used prior to lifting for cold storage include CO_2 gas exchange, chlorophyll fluorescence, mitotic index and cold hardiness testing. After storage, CO_2 gas exchange, chlorophyll fluorescence, root growth potential, timing of bud flush and leader growth are used as measures of seedling quality in the spring. Root growth potential is used as the standard to assess the other methods because it is the standard used by the B.C. Ministry of Forests.

Zaerr (1985) proposed that an ideal test of plant vigour should have several characteristics:

a) rapid, yielding final results immediately
b) simple to understand and use at all levels of operation
c) inexpensive, accessible to all potential users
d) reliable, the test works everytime
e) non-destructive, enabling test plants to be outplanted

f) quantitative and non-subjective, allowing probability values to be assigned, rather than qualitative, and

g) diagnostic, so that the cause of seedling damage could be indicated.

Puttonen (1989) suggests adding several other criteria, four of which will be used in this study:

h) basis of assessment known: Is what you are measuring understood or empirical?

i) able to accommodate changes in seedlings; for example, seasonal changes

j) prediction span: How long is the information useful, and does it actually predict future performance?

k) applicability for quality control in the nursery.

In assessing the methods used in these studies, emphasis was placed on the prediction span, how long was the information useful, and did it actually predict future performance? If the parameter or test was not useful in predicting the future performance of the seedlings, or the information was only useful for a very short period of time then the test would be of little value in determining lifting dates and subsequent seedling quality. The objective of this study was to assess the efficacy of various parameters and physiological tests in determining lifting dates and seedling quality.

MATERIALS & METHODS

In order to determine the effectiveness of the physiological assessment methods, a large range of seedlings varying in quality was required. Seedlings used in this study were therefore provided from the dormancy induction study conducted in 1990 and described in Section III.

From August 1990 to January 1991, on a regular basis, 10 randomly selected seedlings of each species from each of the three treatment groups (AMB, LD-C and SD-W) were lifted and placed in cold storage (-2±1°C). Seedlings were cold-stored for 15 weeks, then potted and placed in a growth chamber for 28 days as per General Materials and Methods.

There are difficulties involved in estimating the PSA (projected surface area) of seedlings with newly flushed, tightly bunched needles, as well as the associated problem of determining a satisfactory basis on which to express CO_2 gas exchange measurements. For needles that are not fully expanded and still tightly bunched, expressing CO_2 gas exchange on either a dry weight or PSA basis over-estimates the effective photosynthetic area because of shelf-shading. Toivonen (1985) and Murphy (1990) have suggested initial fluorescence, F_O, can provide a estimate of seedling size as a basis for CO_2 gas exchange calculations because it proportional to the size of the active photosynthetic pigment bed (Krause & Weis 1984).

A linear correlation was found between P_N calculated per PSA (m^2) and P_N calculated per F_O unit, using data from both

white spruce and Douglas-fir seedlings (r^2=.69). To keep the range of P_N calculated per F_O unit within a similar scale as P_N values calculated per surface area, net photosynthesis was calculated F_O/500 as an estimate of seedling size; where 500 is the slope of the linear correlation.

Statistical analysis was performed using SAS/STATS (1988). Simple and multiple linear correlations were used to determine the strength of the straight line relationship between two or more independent variables; the coefficient of determination, r^2, is reported (Zar 1984). Analysis of variance and the Bonferroni test of differences were used to evaluate significance of treatment effects (Winer 1977).

RESULTS

Dormancy induction treatments produced seedlings with a large range of quality in the spring after cold storage. Pre-storage parameters for white spruce seedlings are summarized in Tables 14A and B, Douglas-fir in Tables 15A and B. Post-storage parameters for white spruce seedlings are summarized in Tables 16A and B, Douglas-fir in Tables 17A and B.

Table 14. Parameters for white spruce seedlings prior to cold storage. A) Cold hardiness, mitotic index, average number of white roots per seedlings, and number of chilling hours. Treatment groups: AMB - ambient seedlings under natural conditions; LD-C - 16 hour day length, 10/5 day/night temperature; SD-W, ≤12 hour day length, 25/20°C day/night temperature.

DATE	28/8	15/9	21/10	15/11	28/11	12/12	19/1
LIFT	1	2	3	4	5	6	7
Treatment							
Cold hardiness LT_{50} (°C)							
AMB	-14.0±1[a]	-20.5±1[b]	-24.8±1[bc]	-25.6±1[bc]	-27.8±1[c]	-28.6±1[c]	<-35
LD-C	-10.3±1[a]	-10.7±1[a]	-15.0±1[a]	-23.0±1[b]	-26.2±1[c]	- 9.6±1[a]	NA
SD-W	-12.1±1[a]	-14.5±1[a]	-12.9±1[a]	-13.1±1[a]	-14.4±1[a]	-14.2±1[a]	NA
Mitotic Index (%)							
AMB	1.9±0.5	0.8±0.5	0	0	0	0	0
LD-C	1.6±0.5	0.9±0.5	0	0	1.6±0.5	5.4±0.5	NA
SD-W	0.9±0.5	0	0	0	0	0	NA
Average number of white roots per seedling							
AMB	2.9±0.5[a]	6.6±0.5[b]	7.2±0.4[b]	8.2±0.4[b]	7.6±0.5[b]	6.9±0.3[b]	6.7±0.5[b]
LD-C	4.6±0.4[a]	12.7±0.5[c]	13.1±0.5[c]	11.6±0.6[c]	14.2±0.4[c]	12.9±0.4[c]	NA
SD-W	0	0	0	0	0	0	NA
Number of chilling hours (≤5°C)							
AMB	0	0	27.7	154.2	178.2	673.4	1221
LD-C	0	120	360.0	608.0	712.0	840.0	NA
SD-W	0	0	0	0	0	0	NA

Table 14. Parameters for white spruce seedlings prior to cold storage continued. B) Fv/Fm ratio at lift, F_T at lift, net photosynthesis at lift, and maximum/minimum air tempreature day before lift.

DATE	28/8	15/9	21/10	15/11	28/11	12/12	19/1
LIFT	1	2	3	4	5	6	7
Treatment							
	Fv/Fm ratio at lift						
AMB	0.56 ± 0.03^{a}	0.56 ± 0.04^{a}	0.46 ± 0.04^{b}	0.46 ± 0.03^{b}	0.47 ± 0.03^{b}	0.42 ± 0.02^{bc}	0.44 ± 0.03^{bc}
LD-C	0.52 ± 0.04^{ab}	0.57 ± 0.03^{a}	0.47 ± 0.03^{b}	0.47 ± 0.04^{b}	0.46 ± 0.03^{b}	0.45 ± 0.03^{b}	NA
SD-W	0.56 ± 0.03^{a}	0.47 ± 0.03^{b}	0.41 ± 0.03^{c}	0.38 ± 0.03^{c}	0.38 ± 0.03^{c}	0.35 ± 0.03^{c}	NA
	F_T at lift						
AMB	1.02 ± 0.03	0.89 ± 0.03^{a}	0.78 ± 0.03^{b}	0.51 ± 0.03^{c}	0.42 ± 0.03^{d}	0.38 ± 0.03^{d}	$-.08\pm0.03$
LD-C	0.27 ± 0.03^{e}	0.21 ± 0.03^{e}	$-.23\pm0.03^{f}$	$-.24\pm0.03^{f}$	$-.27\pm0.03^{f}$	$-.22\pm0.03^{f}$	NA
SD-W	0.84 ± 0.03^{a}	0.73 ± 0.03^{b}	0.56 ± 0.03^{c}	0.48 ± 0.03^{cd}	0.35 ± 0.03^{d}	0.22 ± 0.03^{e}	NA
	Net photosynthesis ($\mu mol \cdot m^{-2} \cdot s^{-1}$) at lift						
AMB	8.4 ± 0.1	4.7 ± 0.1	3.9 ± 0.1^{a}	3.3 ± 0.1^{ab}	2.9 ± 0.1^{b}	2.9 ± 0.1^{b}	2.8 ± 0.1^{b}
LD-C	5.5 ± 0.1	3.4 ± 0.1	2.7 ± 0.1^{b}	2.9 ± 0.1^{b}	2.8 ± 0.1^{b}	2.8 ± 0.1^{b}	NA
SD-W	8.4 ± 0.1	10.3 ± 0.1	6.4 ± 0.1	5.2 ± 0.1^{c}	5.0 ± 0.1^{c}	4.9 ± 0.1^{c}	NA
	Maximum/minimum air temperature day before lift (°C)						
AMB	22/14	23/16	8/3.5	8/2.9	6.8/-.09	4/2.5	3/-1.2
LD-C	10/15	10/15	10/15	10/15	10/15	10/15	NA
SD-W	25/20	25/20	25/20	25/20	25/20	25/20	NA

NA test not conducted
^{a,b,c,d,e,f} same letter signifies no significant difference between rows and columns for each parameter

Table 15. Parameters for Douglas-fir seedlings prior to cold storage. A) Cold hardiness, mitotic index, average number of white roots per seedlings, and number of chilling hours. Treatment groups: AMB - ambient seedlings under natural conditions; LD-C - 16 hour day length, 10/5 day/night temperature; SD-W, ≤12 hour day length, 25/20°C day/night temperature.

DATE	28/8	15/9	21/10	15/11	28/11	12/12	19/1
LIFT	1	2	3	4	5	6	7
Treatment							
Cold hardiness LT_{50} (°C)							
AMB	0±1[a]	0±1[a]	-2.5±1[a]	-5.5±1[b]	-9.5±1[c]	- 17±1[d]	-19.9±1[d]
LD-C	0±1[a]	0±1[a]	0±1[a]	-2.2±1[a]	-5.4±1[b]	-5.7±1[b]	- 5.6±1[b]
SD-W	-6.5±1[b]	-7.5±1[b]	-10±1[c]	-7.4±1[b]	-6.2±1[b]	-7.1±1[b]	NA
Mitotic Index (%)							
AMB	11.2±0.4[a]	11.9±0.6[a]	11.1±0.5[a]	7.9±0.5[b]	7.9±0.7[b]	6.1±0.4[b]	0.16±0.09
LD-C	11.9±0.5[a]	12.8±0.5[a]	11.9±0.6[a]	10.2±0.4[a]	10.7±0.6[a]	9.6±0.5[a]	6.2±0.4
SD-W	1.2±0.5	0	0	0	0	0	NA
Average number of white roots per seedling							
AMB	4.7±0.5[a]	5.6±0.5[a]	6.8±0.4[a]	8.1±0.4[b]	8.4±0.5[b]	7.2±0.3[a]	4.5±0.5[a]
LD-C	NA	10.7±0.5	13.4±0.5[c]	12.6±0.6[c]	13.2±0.4[c]	13.9±0.4[c]	13.8±0.5[c]
SD-W	0	0	0	0	0	0	NA
Number of chilling hours (≤5°C)							
AMB	0	0	27.7	154.2	178.2	673.4	1221
LD-C	0	120	360.0	608.0	712.0	840.0	980
SD-W	0	0	0	0	0	0	NA

Table 15. Parameters for Douglas-fir seedlings prior to cold storage continued. B) Fv/Fm ratio at lift, F_T at lift, net photosynthesis at lift, and maximum/minimum air temperature day before lift.

DATE	28/8	15/9	21/10	15/11	28/11	12/12	19/1
LIFT	1	2	3	4	5	6	7
Treatment							
	Fv/Fm ratio at lift						
AMB	0.61±0.03[a]	0.60±0.04[a]	0.58±0.04[a]	0.50±0.03	0.57±0.03[a]	0.47±0.02[b]	0.49±0.03[b]
LD-C	NA	0.66±0.03	0.48±0.03[b]	0.48±0.04[b]	0.46±0.03[b]	0.38±0.03	0.32±0.03
SD-W	0.56±0.03[a]	0.56±0.03[a]	0.58±0.03[a]	0.50±0.03[b]	0.57±0.03[a]	0.48±0.03[b]	NA
	F_T at lift						
AMB	0.42±0.03[a]	0.32±0.03[a]	0.23±0.03[a]	0.21±0.03[b]	0.22±0.03[bc]	-.18±0.03[c]	-.17±0.03
LD-C	NA	0.26±0.03[c]	-.22±0.03[d]	-.24±0.03[d]	-.36±0.03[d]	-.32±0.03[d]	-.32±0.03[d]
SD-W	0.44±0.03[a]	0.69±0.03[a]	0.82±0.03	0.82±0.03	0.27±0.03[c]	0.18±0.03[c]	NA
	Net photosynthesis ($\mu mol \cdot m^{-2} \cdot s^{-1}$) at lift						
AMB	8.7±0.1	6.0±0.1	3.8±0.1	2.9±0.1[a]	2.9±0.1[a]	2.0±0.1[b]	1.0±0.1
LD-C	NA	2.5±0.1[a]	1.8±0.1[b]	2.4±0.1[a]	2.4±0.1[a]	2.5±0.1[a]	2.3±0.1[a]
SD-W	8.4±0.1	6.2±0.1	9.3±0.1	8.4±0.1	7.5±0.1[c]	7.9±0.1[c]	NA
	Maximum/minimum air temperature day before lift (°C)						
AMB	22/14	23/16	8/3.5	8/2.9	6.8/-.09	4/2.5	3/-1.2
LD-C	10/5	10/5	10/5	10/5	10/5	10/5	10/5
SD-W	25/20	25/20	25/20	25/20	25/20	25/20	NA

NA test not conducted

[a,b,c,d,e,f] same letter signifies no significant difference between rows and columns for each parameter

Table 16. Parameters for white spruce seedlings after cold storage (-2±1°C). A) Root growth potential, days to terminal bud flush, and new leader length. Treatment groups: AMB - ambient seedlings under natural conditions; LD-C - 16 hour day length, 10/5 day/night temperature; SD-W, ≤12 hour day length, 25/20°C day/night temperature.

DATE	28/8	15/9	21/10	15/11	28/11	12/12	19/1
LIFT	1	2	3	4	5	6	7
Treatment							
	Root growth potential						
AMB	0.9±0.6	5.2±3.2	8.8±3.2	18.0±2.8	9.3±2.2	8.9±2.6	8.7±2.2
LD-C	NA	17.1±1.7	20.3±2.9	28.9±2.7	13.8±3.1	NA	NA
SD-W	0.0	0.9±1.1	0.5±1.2	0.4±0.8	0.0	NA	NA
	Days to terminal bud flush						
AMB	>28	28	20	16	15	16	11
LD-C	NA	14	15	15	13	NA	NA
SD-W	NF	NF	NF	NF	NF	NA	NA
	New leader length (cm)						
AMB	0	1.91±0.04	4.82±0.03[a]	5.68±0.04[a]	5.38±0.02[a]	5.98±0.04[a]	6.85±0.04[b]
LD-C	NA	6.51±0.04[b]	4.87±0.04[a]	4.68±0.03[a]	6.01±0.04[b]	NA	NA
SD-W	0	0	0	0	0	NA	NA

note: NF = no flush

Table 16. Parameters for white spruce seedlings after cold storage continued. B) Fv/Fm ratio 5 hours after removal form cold storage, net photosynthesis 5 after removal from cold storage and damage to foliage after 28 days.

DATE	28/8	15/9	21/10	15/11	28/11	12/12	19/1
LIFT	1	2	3	4	5	6	7
Treatment							
	Fv/Fm ratio 5 hours after removal from cold storage						
AMB	0.27±0.02^{a}	0.26±0.02^{a}	0.51±0.03^{b}	0.48±0.02^{b}	0.48±0.02^{b}	0.38±0.02^{c}	0.39±0.02^{c}
LD-C	NA	0.43±0.03^{bc}	0.35±0.02^{c}	0.31±0.02^{c}	0.32±0.02^{c}	NA	NA
SD-W	0.23±0.02^{a}	0.25±0.02^{a}	0.26±0.02^{a}	0.22±0.01^{a}	0.21±0.01^{a}	NA	NA
	Net photosynthesis 5 hours after removal from cold storage ($\mu mol \cdot m^{-2} \cdot s^{-1}$)						
AMB	0.06±0.11	2.39±0.54^{a}	2.04±0.25^{a}	2.96±0.34^{a}	3.74±0.42	1.24±0.22^{b}	1.16±0.27^{b}
LD-C	NA	2.12±0.16^{a}	1.08±0.09^{b}	0.91±0.33^{b}	2.19±0.26^{a}	NA	NA
SD-W	0.67±0.15	1.14±0.22^{b}	1.83±0.39^{ab}	0.02±0.05	0.10±0.12	NA	NA
	Damage to foliage after 28 days (%)						
AMB	64	15	0	0	0	0	0
LD-C	NA	0	0	0	0	NA	NA
SD-W	90	36	41	46	78	NA	NA

NA test not conducted
^{a,b,c,d,e,f} same letter signifies no significant difference between rows and columns for each parameter

Table 17. Parameters for Douglas-fir seedlings after cold storage (-2±1°C). A) Root growth potential, days to terminal bud flush, and new leader length. Treatment groups: AMB - ambient seedlings under natural conditions; LD-C - 16 hour day length, 10/5 day/night temperature; SD-W, ≤12 hour day length, 25/20°C day/night temperature.

DATE	28/8	15/9	21/10	15/11	28/11	12/12	19/1
LIFT	1	2	3	4	5	6	7
Treatment							
	Root growth potential						
AMB	0.0	0.9±2.6	21.9±3.1	26.3±3.9	27.6±2.2	20.6±2.7	33.1±3.5
LD-C	NA	0.0	33.2±2.8	44.7±3.1	37.8±2.9	27.2±2.8	24.1±2.2
SD-W	0.0	0.9±1.3	0.0	0.0	0.5±2.3	0.4±0.9	NA
	Days to terminal bud flush						
AMB	>28	26	22	18	16	14	13
LD-C	NA	>28	>28	>28	>28	28	25
SD-W	NF	NF	NF	NF	NF	NF	NA
	New leader Length (cm)						
AMB	0	1.91±0.04[a]	4.82±0.03[b]	5.68±0.04[bc]	5.38±0.02[b]	5.98±0.04[c]	6.85±0.04
LD-C	NA	0	0	0.58±0.43	1.21±0.35[a]	1.09±0.46[a]	1.43±0.43[a]
SD-W	0	0	0	0	0	0	NA

Note: NF = no flush

Table 17. Parameters for Douglas-fir seedlings after cold storage continued. B) Fv/Fm ratio 5 hours after removal from cold, storage, net photosynthesis 5 hours after removal from cold storage, and damage to foliage after 28 days.

DATE	28/8	15/9	21/10	15/11	28/11	12/12	19/1
LIFT	1	2	3	4	5	6	7
Treatment							
	Fv/Fm ratio 5 hours after removal from cold storage						
AMB	0.43±0.02^{a}	0.66±0.05^{b}	0.65±0.03^{b}	0.65±0.04^{b}	0.55±0.02^{b}	0.59±0.03^{b}	0.39±0.02^{ac}
LD-C	NA	0.46±0.03^{a}	0.43±0.02^{a}	0.59±0.04^{b}	0.45±0.05^{a}	0.47±0.05^{a}	0.49±0.04^{a}
SD-W	0.31±0.09^{c}	0.30±0.08^{c}	0.41±0.07^{ac}	0.40±0.09^{ac}	0.31±0.07^{c}	0.38±0.10^{c}	NA
	Net photosynthesis 5 hours after removal from cold storage ($\mu mol \cdot m^{-2} \cdot s^{-1}$)						
AMB	0.06±0.11	2.39±0.54^{a}	2.04±0.25^{a}	2.96±0.34^{a}	3.74±0.42	1.24±0.22^{b}	1.16±0.27^{b}
LD-C	NA	0.29±0.12^{c}	0.67±0.07^{d}	0.91±0.21^{bd}	1.12±0.27^{b}	0.87±0.11^{d}	1.23±0.14^{b}
SD-W	-2.3±0.15	-.24±0.22	0.20±0.14^{c}	0.09±0.05^{c}	0.05±0.10^{c}	0.09±0.07^{c}	NA
	Damage to foliage after 28 days (%)						
AMB	64	15	0	0	0	0	0
LD-C	NA	100	21	8	5	4	0
SD-W	94	91	43	56	79	84	NA

NA test not conducted.
^{a,b,c,d,e,f} same letter signifies no significant difference between rows and columns for each parameter

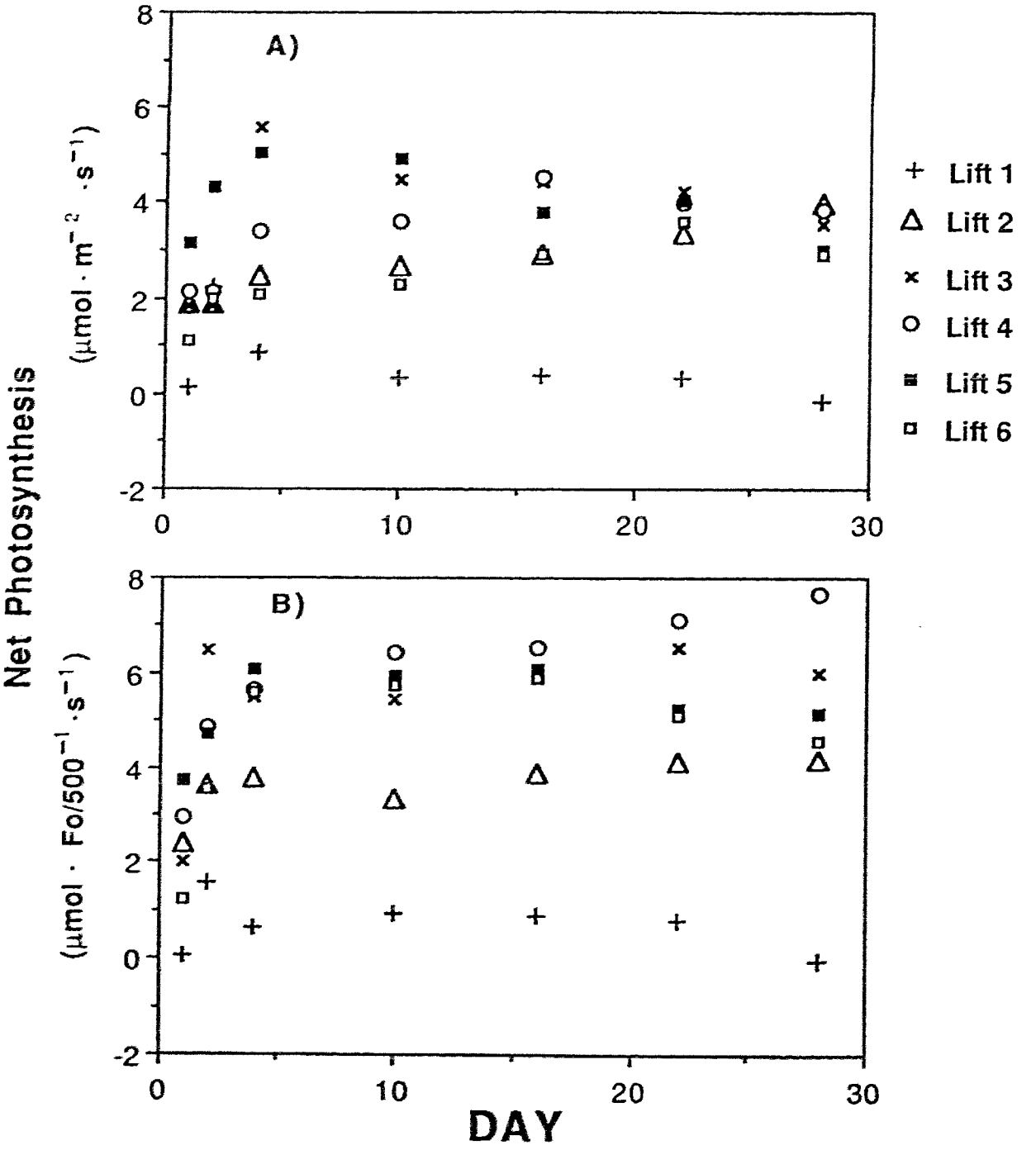

Figure 34. Net photosynthesis of AMB white spruce seedlings measured after cold-storage during the 28 days' recovery in growth chamber, A) calculated per projected surface area (m^2) and B) calculated per area estimated by $F_O/500$.

Fall lifting assessment: was the test or parameter capable of predicting seedling quality (RGP) after cold storage? Figures 35 and 36 summarize the relationships between post-storage RGP and prestorage parameters for white spruce and Douglas-fir seedlings.

Cold hardiness was predictive of RGP in the spring for both AMB white spruce and Douglas-fir. For all treatment groups of both white spruce and Douglas-fir, seedlings with little cold hardiness had poor storability.

Mitotic index was only predictive of RGP in the spring for AMB white spruce and Douglas-fir, but not when all treatment groups are lumped together. Seedlings with low or 0% mitotic indices did not necessarily store well, particularly seedlings given SD-W dormancy induction treatment. Conversely, Douglas-fir seedlings with high mitotic indices did store well.

The presence of white roots at the time of lifting seedlings in the fall appeared to be predictive of good recovery after storage for both white spruce and Douglas-fir, in particular the relationship was strongest when data from all treatment groups was used.

Net photosynthesis, measured using CO_2 gas exchange, decreased over the fall for all dormancy induction treatment groups, but was somewhat predictive of RGP in the spring. When the AMB group is considered independently, it was more predictive for the Douglas-fir seedlings, where as net photosynthesis decreased, storability did increase.

Chlorophyll fluorescence decreased over the fall for all dormancy induction treatment groups; however, there were characteristic differences in the curves for each group (see Figures 18 and 22, Section 3). For white spruce seedlings Fv/Fm decreased as storability increased for AMB seedlings and was predictive of RGP after storage; however, when all groups are taken together Fv/Fm was not predictive. Steady state fluorescence was somewhat predictive for white spruce, and in particular AMB Douglas-fir seedlings. The lack of budset in the Douglas-fir seedlings may have been confounding the results, since in Section I, Fv/Fm was found to decline in the fall and relate to storability.

Temperature measured the day before lifting was predictive of RGP after storage for both white spruce and Douglas-fir seedlings. Seedlings lifted when the temperature was low had higher RGP values after storage.

Chilling hours, the number of accumulated chilling hours at ≤5°C, was somewhat predictive of RGP after storage, although the regression was not significant (r^2=.00). Seedlings without any accumulated chilling hours stored poorly for all treatment groups for both white spruce and Douglas-fir.

Several of these tests: mitotic index, net photosynthesis, and chlorophyll fluorescence Fv/Fm ratio, were not capable of distinguishing between white spruce seedlings given SD-W treatment (≤12 hour day length, 25/20°C day/night temperature) which did not store well, and AMB

(seedlings under ambient conditions) which generally did store well, although it is unlikely that seedlings would be lifted prior to any chilling. There is no test which directly measures 'storability', the capacity of a seedling to survive winter cold storage without the loss of quality, in this case measured as RGP. Many of these tests measure different morphological or physiological phenomena related to fall and winter dormancy in these seedlings, how they relate to storability is still questionable.

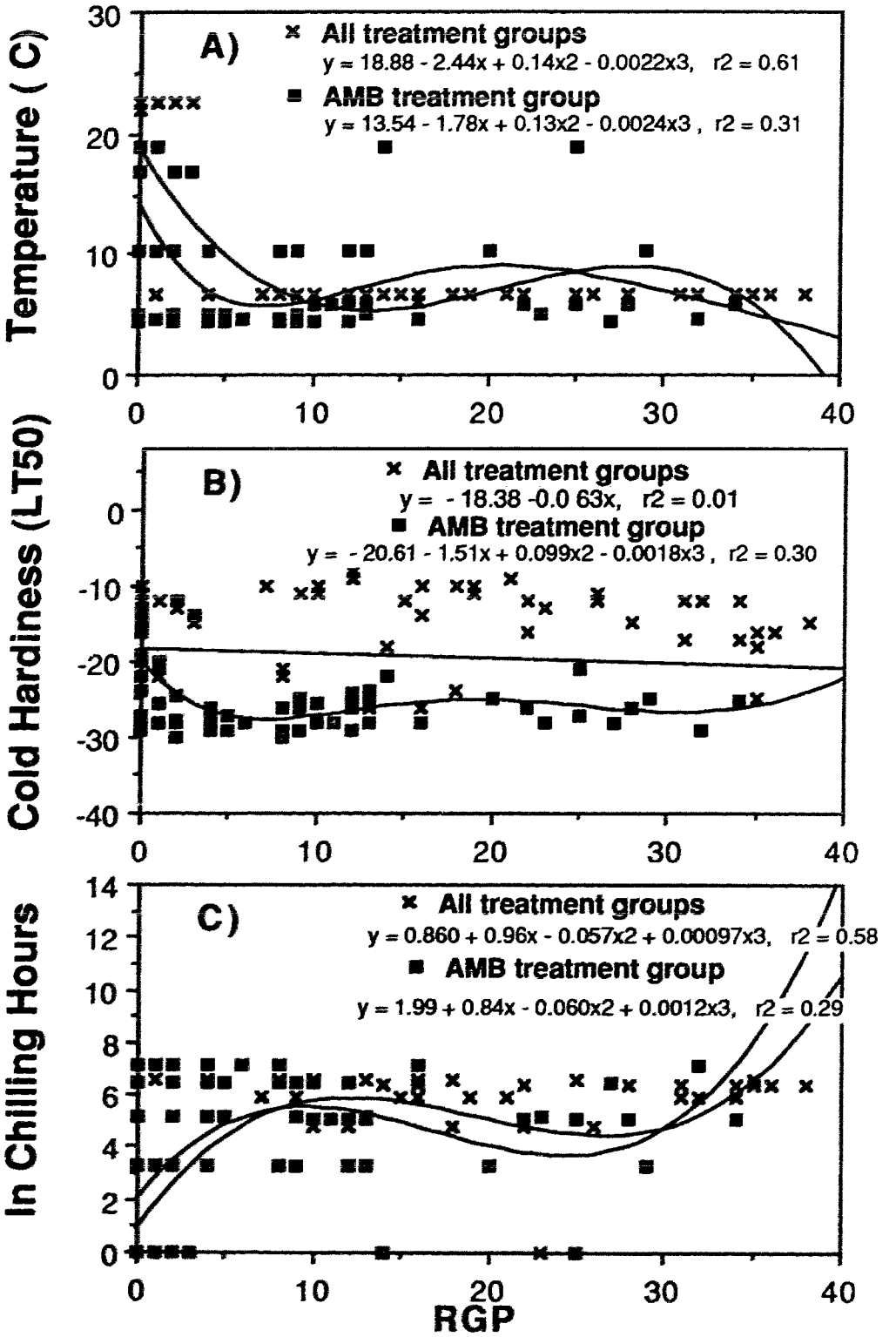

Figure 35. Relationship between post-storage root growth potential (RGP) and prestorage parameters for white spruce seedlings. A) RGP related to air temperature. B) RGP related to cold hardiness. C) RGP related to ln chilling hours.

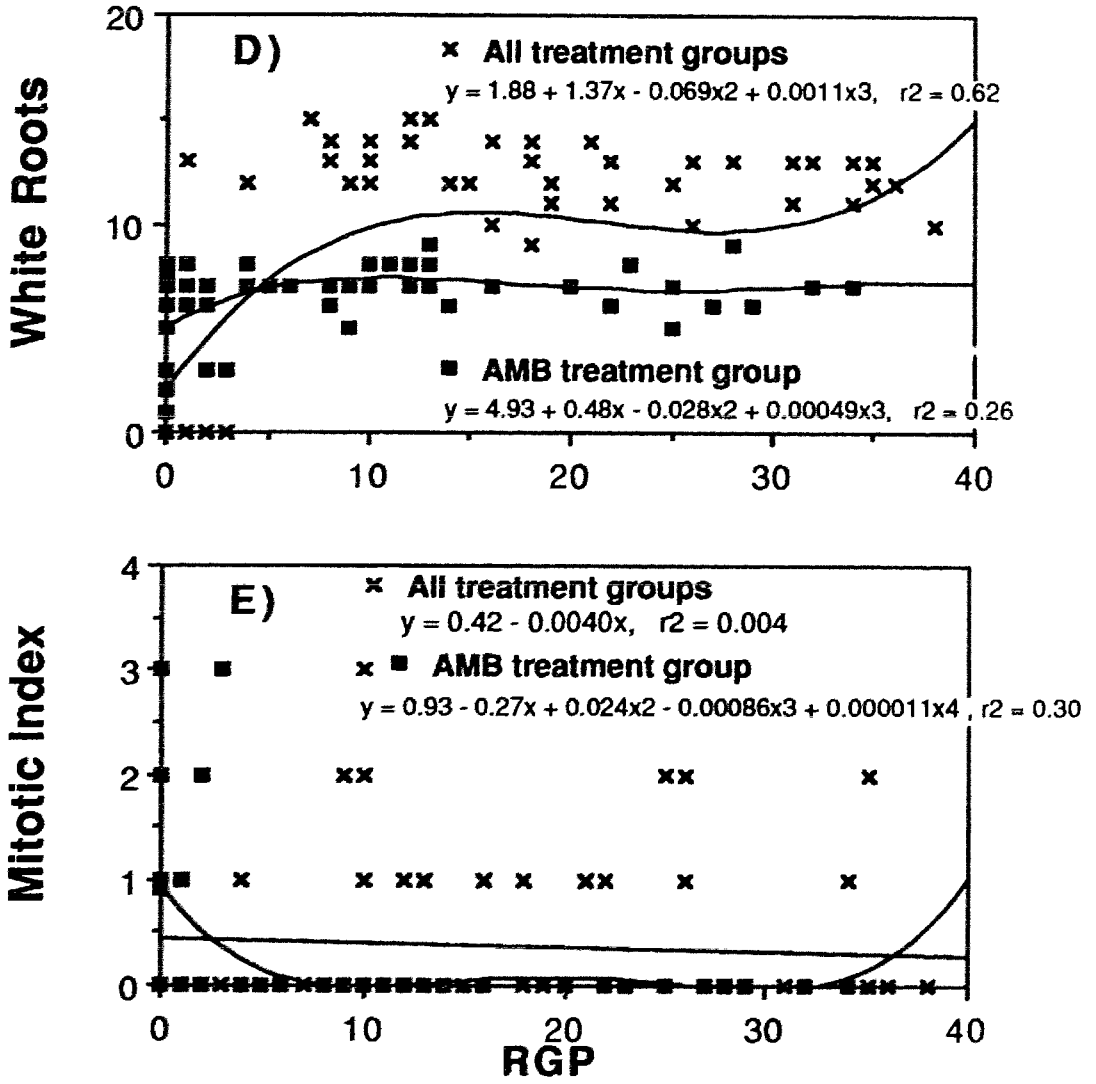

Figure 35. Relationship between post-storage root growth potential (RGP) and prestorage parameters for white spruce seedlings. D) RGP related to the number of white roots at time of lift. E) RGP related to mitotic index.

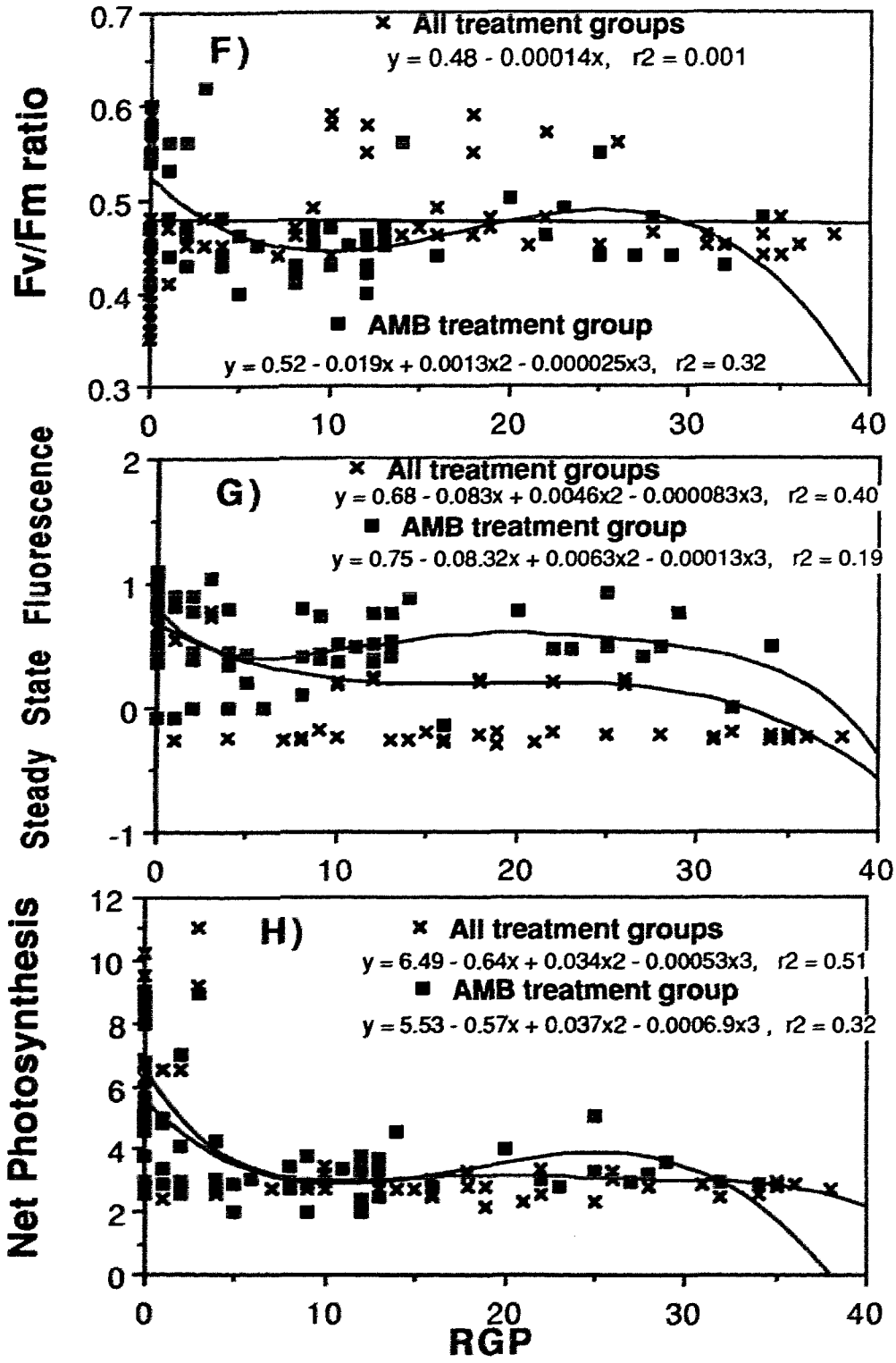

Figure 35. Relationship between post-storage root growth potential (RGP) and prestorage parameters for white spruce seedlings. F) RGP related to Fv/Fm ratio. G) RGP related to steady state fluorescence, F_T, and H) RGP related to net photosynthesis.

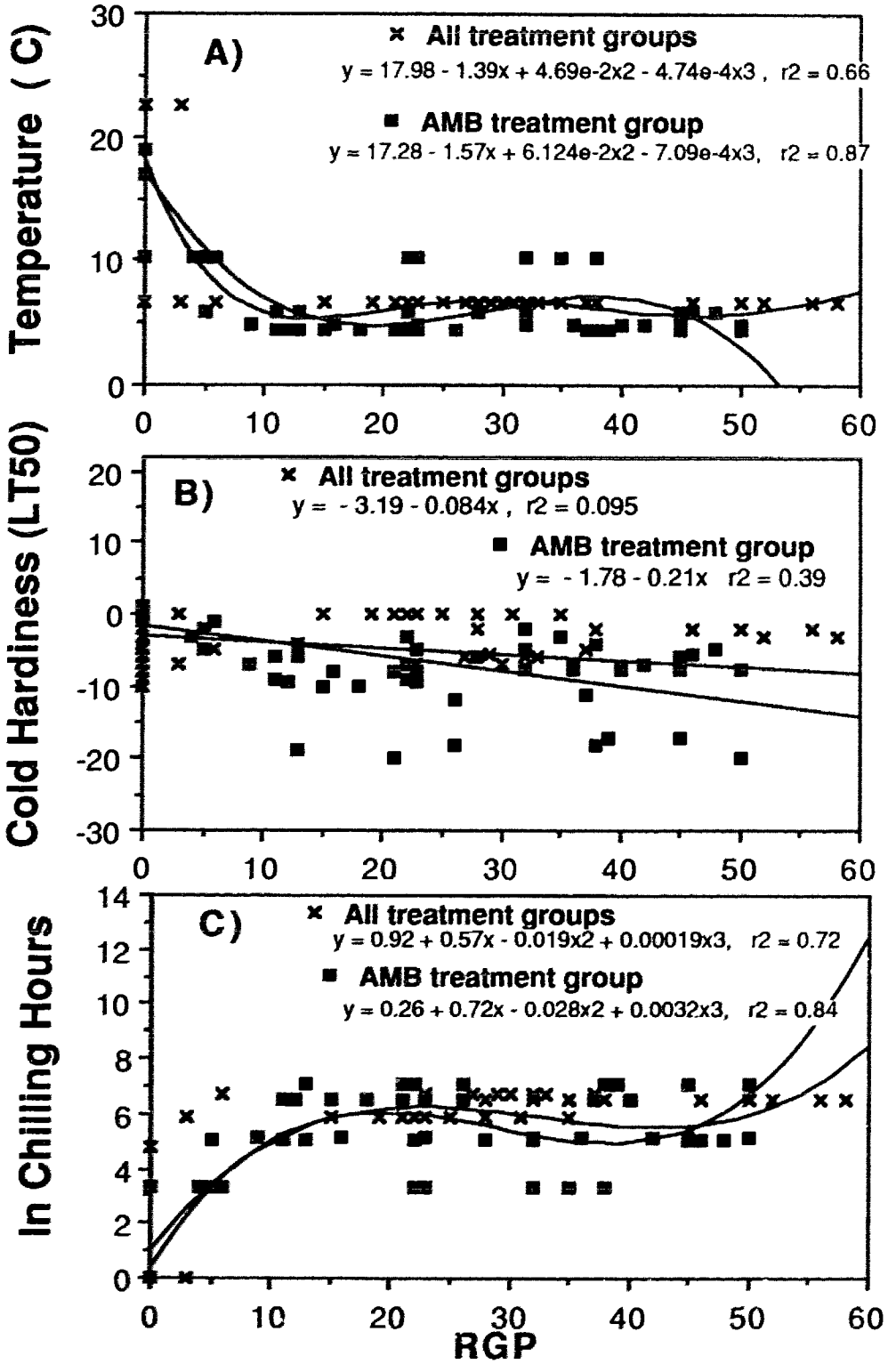

Figure 36. Relationship between post-storage root growth potential (RGP) and prestorage parameters for Douglas-fir seedlings. A) RGP related to air temperature. B) RGP related to cold hardiness. C) RGP related to ln chilling hours.

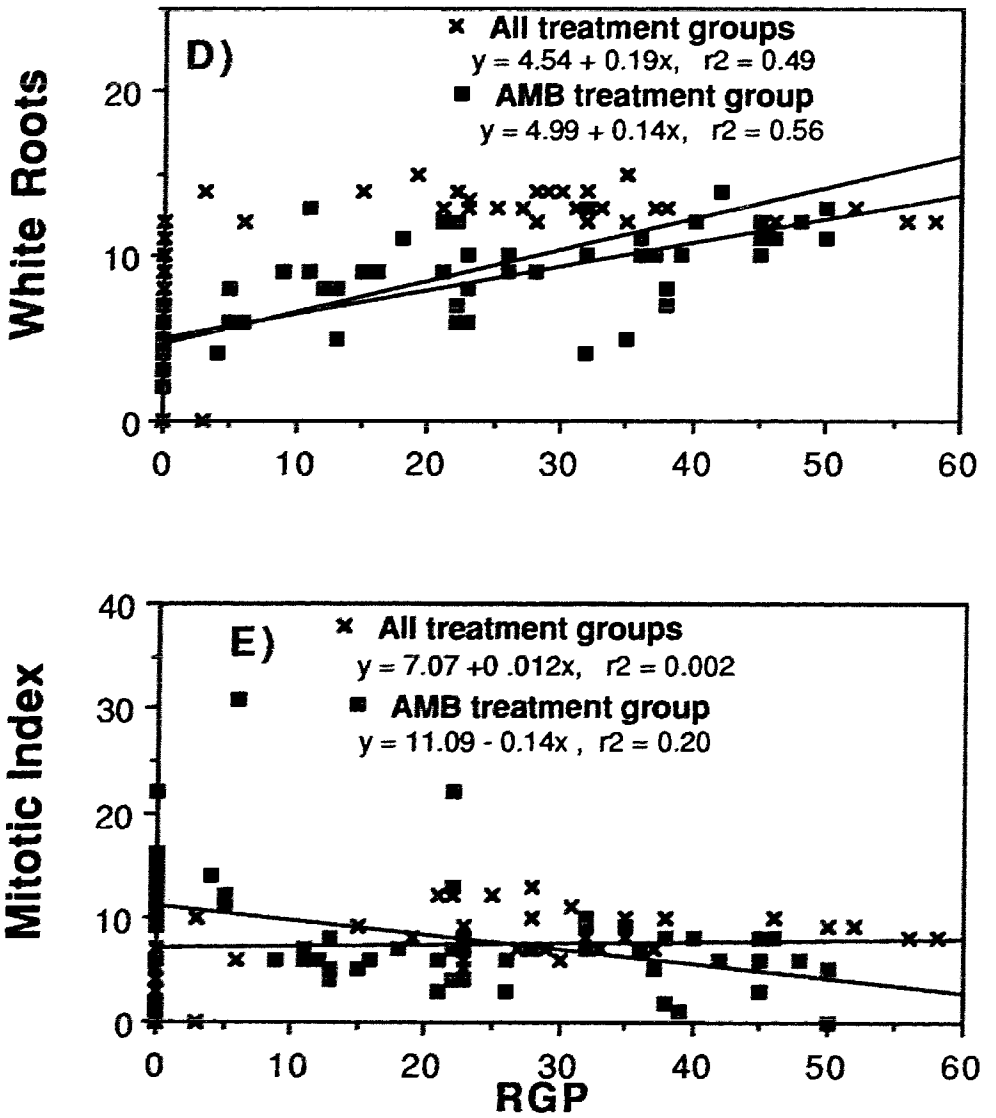

Figure 36. Relationship between post-storage root growth potential (RGP) and prestorage parameters for Douglas-fir seedlings. D) RGP related to the number of white roots at time of lift. E) RGP related to mitotic index. .

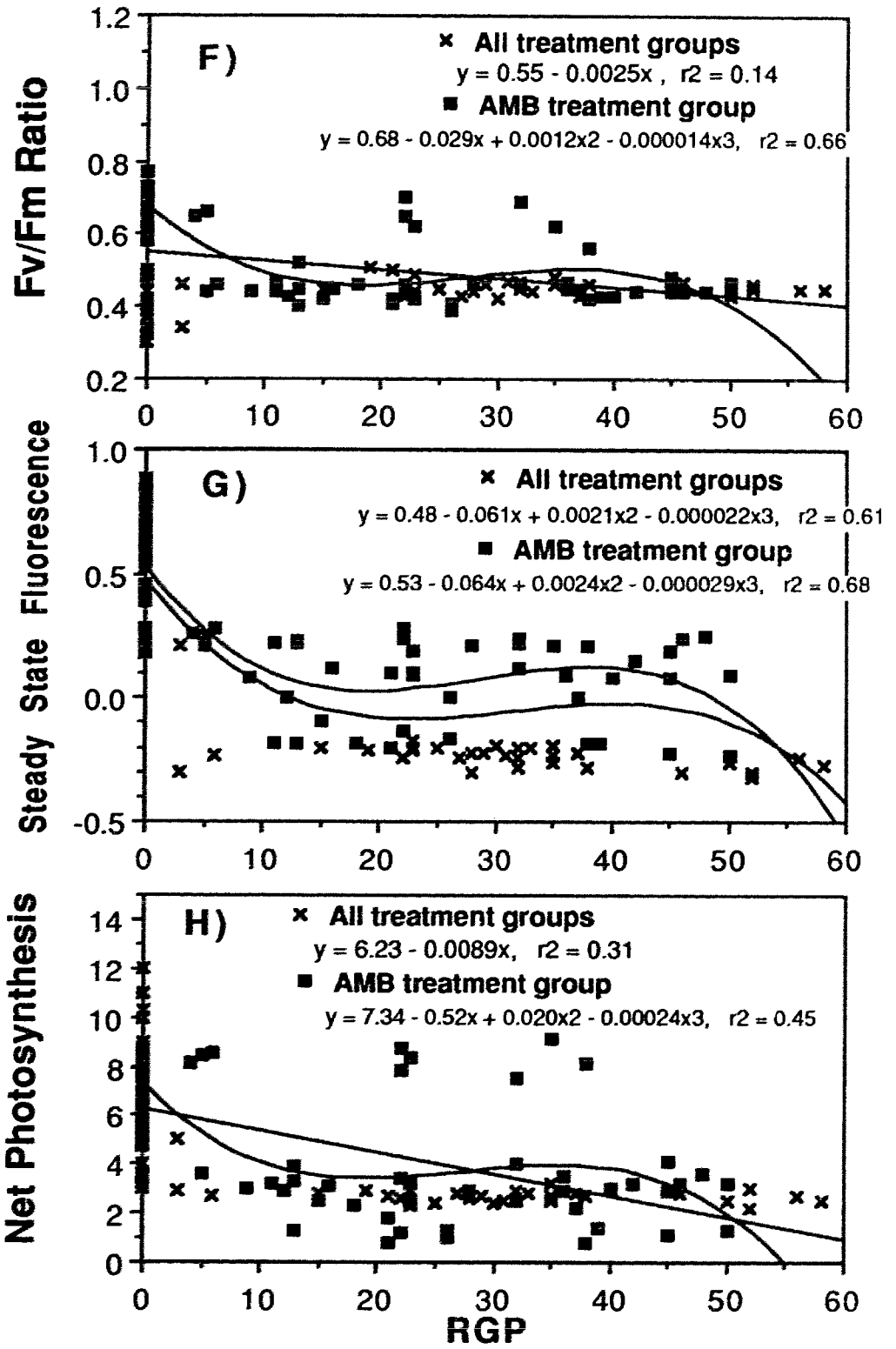

Figure 36. Relationship between post-storage root growth potential (RGP) and prestorage parameters for Douglas-fir seedlings. F) RGP related to Fv/Fm ratio. G) RGP related to steady state fluorescence, F_T, and H) RGP related to net photosynthesis.

Spring quality assessment: How well did the test or parameter compare to RGP? Figures 37 and 38 summarize the relationships between post-storage RGP, measured as the number of white roots greater than 1 cm in length after 28 days in the growth chamber, and post-storage parameters for white spruce and Douglas-fir seedlings.

Chlorophyll fluorescence Fv/Fm ratio was predictive of RGP measured after 28 days for white spruce. There was a linear correlation between Fv/Fm five hours after removal from cold storage and the RGP on day 28 for AMB white spruce seedlings (r^2=.76) (not shown). When SD-W and LD-C data are also considered, the linear correlation was reduced (Figure 37A). The different dormancy induction treatments resulted in variation in the response of the Fv/Fm after cold storage; in particular, the LD-C seedlings had high RGP and lower Fv/Fm than the AMB seedlings.

For the AMB Douglas-fir seedlings there was also a linear correlation between Fv/Fm measured five hours after removal from cold storage and RGP calculated after 28 days (r^2=.66) (not shown). The linear correlation between Fv/Fm measured five hours after removal from cold storage and RGP was also less pronounced when the LD-C and SD-W groups are also considered (Figure 38A).

Both AMB and LD-C Douglas-fir seedlings came out of storage with high F_O levels, indicating damage to PSII reaction centers (Briantais et al. 1986); there was no relationship between F_O and needle dry weight. Initial

fluorescence, F_O, decreased significantly over the 28 days in the growth chamber and coincided with the senescence of apical needles. The increased F_O levels would result in erroneous Fv/Fm values.

Net photosynthesis, CO_2 gas exchange measured five hours after seedlings were removed from cold storage, was also predictive of RGP measured after 28 days, in particular for AMB seedlings (not shown). For both white spruce and Douglas-fir, net photosynthesis measured five hours after removal from storage was linearly correlated to RGP (Figures 37B, 38B) for all treatment groups. In the spring, net photosynthesis calculated on a needle dry weight (data not shown) or PSA (projected surface area) basis increased rapidly to a maximum on day 4, then decreased throughout the rest of the 28 days in the growth chamber (Figure 34A). When the P_N was calculated per $F_O/500$ it continued to increase over the 28 days for most lifts (Figure 34B). A linear correlation was found between P_N calculated per $F_O/500$, rates and the number of roots produced ($r^2=.78$), but not when P_N was calculated per unit surface area or unit dry weight.

Bud flush generally occurred sooner in seedlings with high RGP values and new leader length was generally longer. Although new leader length was related to RGP it was not predictive because it was measured at the same time as RGP.

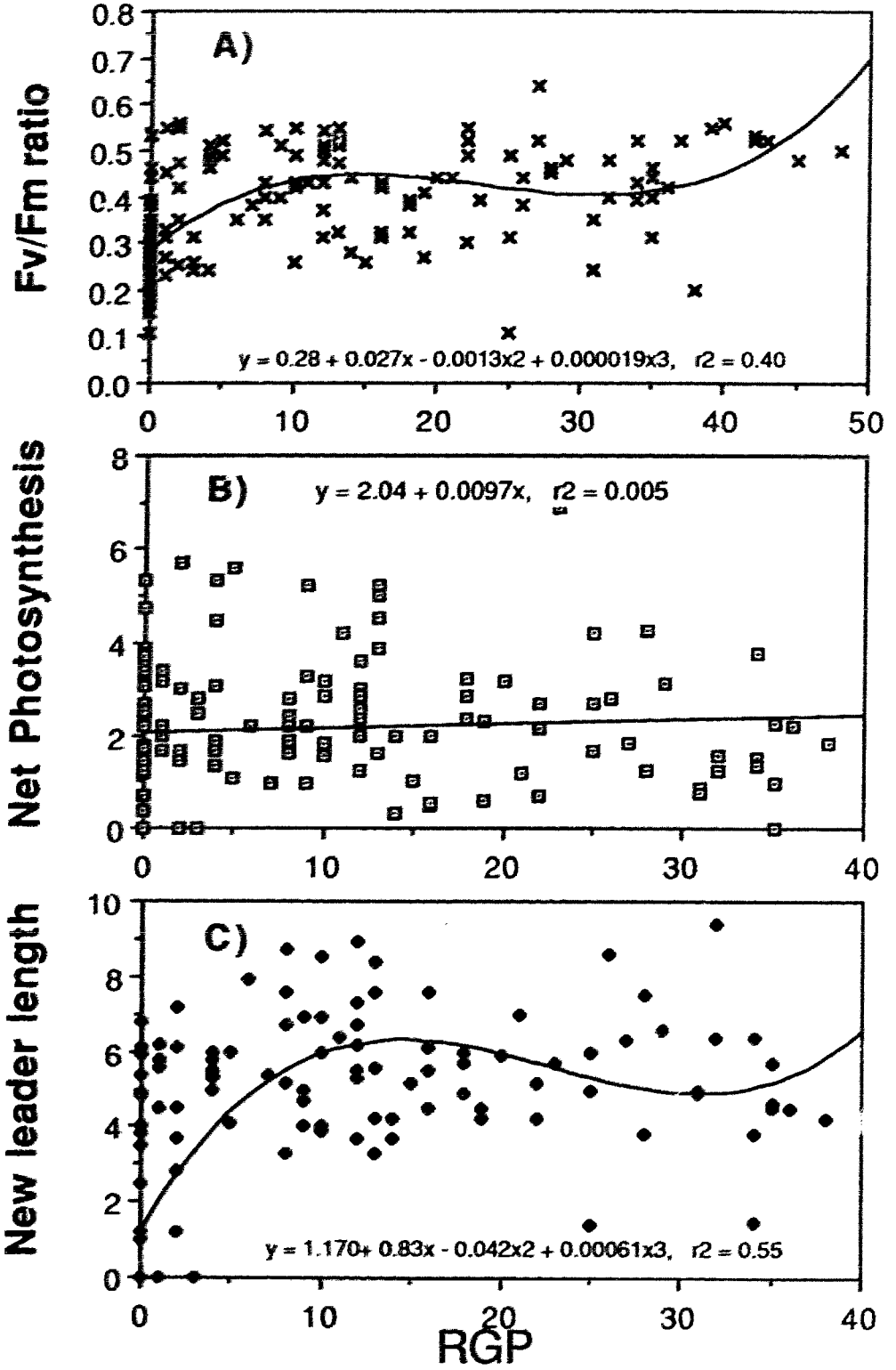

Figure 37. Relationship between post-storage root growth potential (RGP) and post-storage parameters for white spruce seedlings. A) RGP related to Fv/Fm ratio 5 hours after removal from storage. B) RGP related to net photosynthesis 5 hours after removal from storage. C) RGP related to new leader length after 28 days.

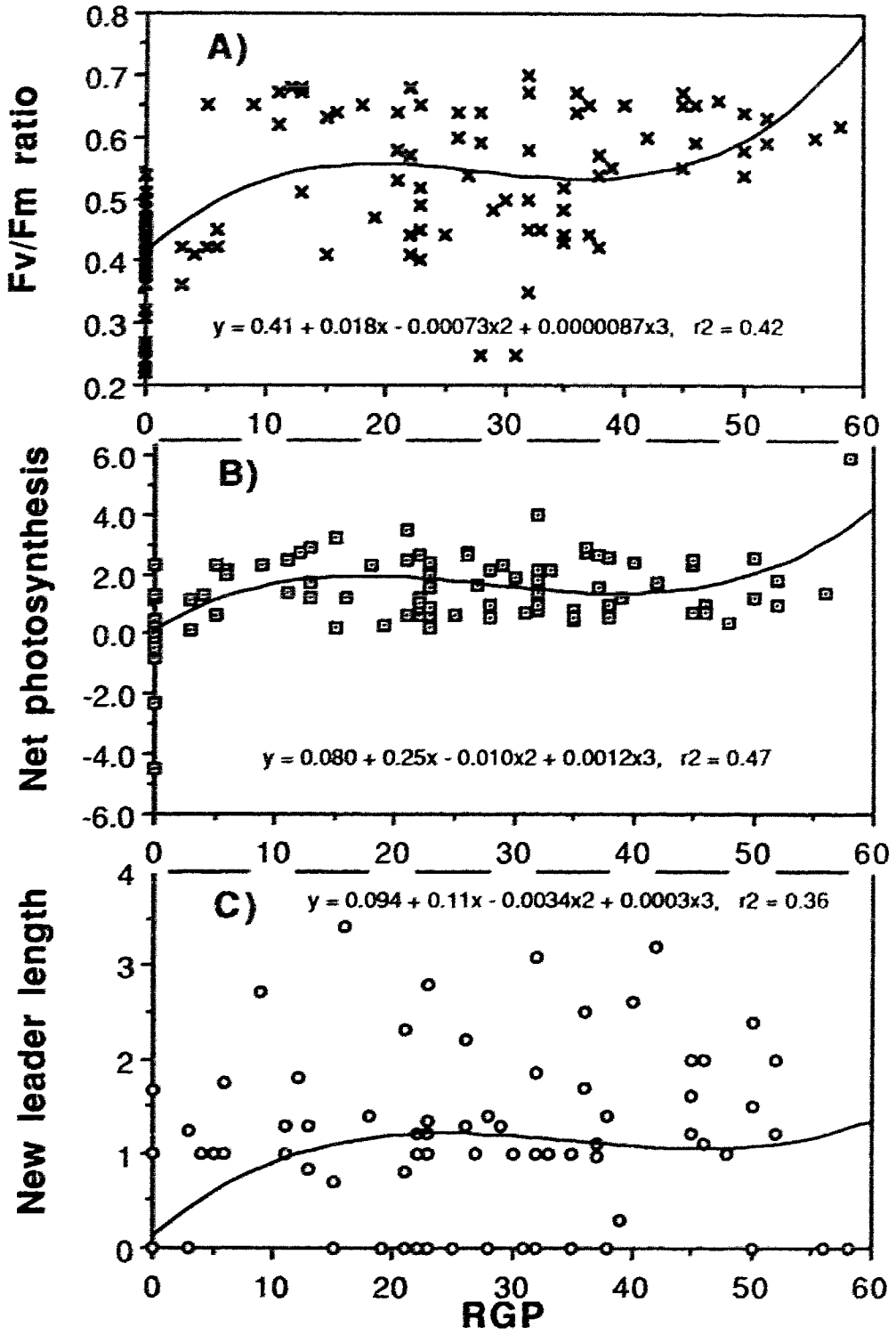

Figure 38. Relationship between post-storage root growth potential (RGP) and post-storage parameters for Douglas-fir seedlings. A) RGP related to Fv/Fm ratio 5 hours after removal from storage. B) RGP related to net photosynthesis 5 hours after removal from storage. C) RGP related to new leader length after 28 days.

Table 18 lists the eleven criteria used to evaluate the assessment methods, ranking scores were assigned to each criteria similar to those used by Hawkins & Binder (1991). Table 19 summarizes the relative ranking of each of the nine different seedling quality assessment methods or measured parameters used in these studies.

Table 18. Criteria used to assess tests.

Characteristic	Ranking	Criteria
Rapid:	3	≤ 24 hours
	2	1-3 days
	1	> 3 days
Simple:	3	anyone can use
	2	requires technician
	1	researcher use only
Cost:	3	< $100
	2	$100-1000
	1	>$1000
Reliable:	2	very reliable
	1	reliable
	0	not reliable
Nondestructive:	2	no damage
	1	partial damage
	0	seedling can not be planted
Quantitative:	2	quanitative, results can be statistically analyzed
	1	qualitative
Diagnostic:	2	able to determine cause of problem
	1	not able to determine cause of problem
Basis:	2	known physiological basis
	1	basis unknown
	0	non-physiological
Adaptability:	3	more than one function and season
	2	more than one function
	1	useful at more than one time
	0	useful at one time in growing season
Prediction:	3	highly predictable of future quality and long prediction span after testing
	2	highly predictable
	1	somewhat predictive
	0	not predictive of future performance
Control:	3	highly useful for quality control
	2	moderately useful
	1	slightly useful
	0	not useful for quality control

Table 19. Sum ranking of various seedling quality assessment methods. Based on criteria and rating scale outlined in Table 17 (adapted from Hawkins & Binder 1991).

TEST	RAPD	SIMP	COST	RELI	NOND	QUAN	DIAG	BASI	ADPT	PRSP	CONT	SUM
MORPH[a]	3	3	3	2	2	2	1	0	1	0	1	18
AIR T	3	3	3	1	2	2	1	0	0	1	1	17
CHILL H	3	3	3	1	2	2	1	0	0	1	1	17
WATER P	3	3	2	2	1	2	1	1	2	1	1	19
GAS EX	3	1	1	2	2	2	1	2	3	2	2	21
COLD H	1[b]	1	2	1	0	2	1	1	0	1	1	11
MI	2	2	2	1	1	1	1	1	0	1	1	13
RGP	1	3	2	2	0	2	1	1	2	2	0	16
CHL F	3	1	1	2	2	2	2	2	3	1	3	22

[a], Abbreviations: MORPH, morphology; AIR T, air temperature; CHILL H, chilling hours; WATER P, water potential; GAS EX, gas exchange; COLD H, cold hardiness; MI, mitotic index; RGP, root growth potential; CHL F, chlorophyll fluorescence.

[b], Test normally takes 2 weeks using visible assessment of foliar damage, can be 1 week if electroconductivity is measured, or 24 hours if chlorophyll fluorescence is used, in which case can be viewed as a 2 giving a sum of 12.

DISCUSSION

None of the physiological tests met all the criteria of an ideal test of plant vigour. The prediction span of all these tests is likely to be very short; seedling physiology can change very rapidly. Therefore, repeated testing would be required to monitor any changes in seedling physiology. For several of the tests, frequent testing would be time consuming and labour intensive, or results would be acquired too late to be useful, therefore the ability to get rapid results becomes a primary prerequisite. Tests, such as RGP and cold hardiness, which take up to a week to perform may be of less value during critical periods when decisions must be made immediately.

Several of the tests are suitable for use only during one portion of the growth (production) cycle. For example, measuring mitotic indices and cold hardiness testing are of value only in the fall.

Tracking environmental conditions is simple, rapid and inexpensive for a nursery; however, neither average air temperature nor the number of acquired chilling hours have any physiological basis and is not suitable for other times in the growing season.

The average air temperature measured the day before lifting for storage appeared to be predictive of subsequent seedling quality after cold storage. Lifting seedlings under low temperature conditions produced higher quality seedlings, in agreement with the results presented in Section 2.

Chilling hours also appeared to have a strong affect on the storability of white spruce seedlings; after seedlings had acquired even as few as 27.7 chilling hours, a significant increase in storability was seen. Any increase in chilling hours beyond this time did not have a major affect on the post-storage vigour of seedlings. The AMB white spruce seedlings stored well after acquiring 27.7 chilling hours; however, the acquisition of chilling hours does not appear to be independent of other factors, as shown with lift 2 of the LD-C white spruce seedlings, which did not survive storage even after acquiring 120 chilling hours. The mitotic index of the LD-C seedlings was higher at lift 2 than at the other lifts, and may have been a factor.

Monitoring morphological parameters is rapid, simple and inexpensive. Generally, seedlings are required to meet morphological criteria set by the Ministry of Forests, therefore, morphological parameters are routinely measured by a nursery. There is no physiological basis for this test and there may be little relationship between morphological measurements and future performance after planting; however, a nursery can alter cultural conditions to produce seedlings of specified morphological parameters. The effect of morphological parameters, such as height and stem diameter, on spring RGP has been well researched and will not be discussed here. The number of white roots greater than 1 cm in length per seedling at the time of lifting for storage was found to be related to subsequent spring RGP. Seedlings with

white roots present at the time of lifting had the best RGP values in the spring.

Root growth potential is the standard physiological test used by the B.C. Ministry of Forests, and other tests were judged against this standard. This technique did not score highly. It is slow, taking from 7 to 14 days to complete, and is labour intensive. It is destructive, seedlings are not suitable for planting after testing, and results are not diagnostic. It is therefore unlikely that RGP would be useful for quality control within the nursery; however it is a good predictor of seedling quality after cold storage.

The mitotic index of the apical bud met few of the criteria of an ideal test. The technique requires a technician to perform and is slow, taking three days to get results. Determination of the mitotic stage of the cells can be subjective, and vary with individuals. The technique is destructive, leaving seedlings in a condition not suitable for planting. Information obtained using this technique is nondiagnostic and not suitable for quality control in the nursery.

Mitotic index measured in the fall was not predictive of subsequent root growth of the seedlings in the spring. Seedlings given SD-W treatment in the fall had mitotic indices of 0% for all lifts, yet seedlings were uniformly of poor quality after storage. Conversely, AMB and LD-C Douglas-fir seedlings had high mitotic indices in the fall and terminal buds had not completed budset, yet these

seedlings had high RGP measurements in the spring after storage; however, especially for early lifted material, nonhardened needles and apices were unable to survive freezing temperatures in storage. The loss of the apices in storage may have resulted in new photosynthate going to root growth rather than apical growth, resulting in higher RGP values than may have been observed if apices were not damaged.

After September, the mitotic index of all white spruce treatment groups declined to 0%. After this time, mitotic index could give not further information that would assist in the selection of lifting periods.

Cold hardiness testing also met few of the criteria of an ideal test. Depending upon the method used, it can be very slow, taking from 3 to 7 days for results. The amount of training required to perform cold hardiness testing also depends upon the technique used. Assessment of visible damage can be very simple to perform and requires little training, but measuring electrolyte leakage requires a trained researcher. Cold hardiness testing is destructive, seedlings can not be planted after testing.

Cold hardiness testing was of little predictive value for both white spruce and Douglas-fir, although seedlings stored better once some degree of cold hardiness had developed. Seedlings with poor cold hardiness, in particular, AMB seedlings lifted in August and September and the SD-W seedlings, did not store well.

In Section 2, a loss of cold hardiness was associated with a decline in storability. Optimal use of cold hardiness testing as a predictor of seedling quality after storage would require frequent testing in the fall and winter prior to lifting, requiring labour intensive, destructive sampling.

Needle water potential (using a Leaf Plant Press) was used in Section I to assess seedling recovery after cold storage, and was found to be predictive of good recovery. Although this method was not used during other times in the growing season it is possible that it may be useful in the summer to indicate drought, and in the fall, decreased needle water potential might also be an indicator of suitability for storage, particularly when used in conjunction with another method. This technique is rapid and simple to operate.

Chlorophyll fluorescence ranked the highest of all the assessment methods utilized. It was rapid, reliable, non-destructive, quantitative and diagnostic. The basis of assessment is becoming clearer and many reviews have been published on using this technique for determining the stress of plants (Lichtenthaler 1990). This method has the greatest potential for its applicability for quality control in seedling nurseries, as well as in the field. Changes in the variable chlorophyll fluorescence induction curves can indicate when seedlings are under environmental (Krause & Somersalo 1989, Lichtenthaler 1990) or nutritional stress (Boddi et al. 1985, Conroy et al. 1986, Rao et al. 1986), or exposed to toxins or herbicides (Schrieber & Bilger 1985)

during the growing season, permitting remedial action to be taken by nursery personnel.

Chlorophyll fluorescence prior to storage indicated increasing photosynthetic inactivation during the fall and winter. In the fall, a decrease of Fv/Fm was indicative of photochemical inactivation of photosynthesis, which correlated with storability in AMB white spruce seedlings and Douglas-fir seedlings under fall natural conditions in Section I. The different results seen in this study and in Section I were because seedlings in Section I had already set bud prior to lifting for storage. The effect of storing Douglas-fir seedlings prior to completion of budset confounded the data seen in this section and may not be indicative of what might actually occur in a nursery situation, since seedlings would not be placed in storage prior to budset.

Steady state fluorescence, F_T, also declined as storability increased. An analysis of the whole curve (Sundblad et al. 1990) may give more information than selection of a few features, Fv/Fm ratio and F_T. Analysis of the whole curve might have provided sufficient information to successfully differentiate between the dormancy induction treatments and be predictive in selecting lifting dates for high quality seedlings in the spring after storage.

One area of concern was noted. For both white spruce and Douglas-fir, the linear correlation between the Fv/Fm ratio measured five hours after removal from cold storage and

RGP is reduced when the LD-C and SD-W treatment groups are considered. This brings into question the robustness of the Fv/Fm ratio as a potential measurement of seedling quality after cold storage, although it is unlikely that seedlings would be lifted prior to budset or the acquisition of any chilling hours.

To address this potential problem, chlorophyll fluorescence should be used in combination with other parameters: cold hardiness, mitotic index and environmental conditions, or the number of acquired chilling hours. By doing this, ideal lifting windows for cold storage may be determined.

Because of the rapidity of this technique, seedlings can be monitored frequently in the fall. As shown in Section 2, photosynthetic reactivation caused by warming trends prior to lifting resulted in increases in steady state fluorescence, F_T. Therefore, frequent testing could help avoid any loss of storability.

Chlorophyll fluorescence can also provide valuable information to the nursery at other times in the growing season. After cold storage, seedling quality can be rapidly assessed using this technique. This study found that five hours after removal from storage, photosynthetic reactivation, seen as an increase in Fv/Fm ratio, indicative of PSII activity, was predictive of seedling quality for white spruce seedlings. Damage to the reaction centers also resulted in high Fv/Fm ratios immediately after removal from

storage for many of the Douglas-fir seedlings, decreasing the predictability; however, in Section I when Douglas-fir seedlings were stored after budset, a good relationship was found between Fv/Fm ratio after removal from storage and subsequent RGP.

Initial fluorescence, F_O, may also be of value in assessing damage to foliage after cold storage or exposure to sub-zero temperatures in the field. High F_O values are indicative of damage to the reaction centers (Briantais et al. 1986). The AMB and LD-C Douglas-fir seedlings came out of storage with very high F_O values, which then declined over the 28 days in the growth chamber, coinciding with the senescence of damaged tissue.

Still to be addressed using this system is the potential for F_O quenching within the pigment bed, this should be accounted for since it decreases the slow kinetics part of the induction curve and can lead to improper interpretation of curves.

Chlorophyll fluorescence has the potential to increase the speed of cold hardiness testing as well. As shown in Section IV, 24 hours after seedlings are tested for cold hardiness, damage to the photosynthetic apparatus can be rapidly assessed using chlorophyll fluorescence. The results correlated with both membrane leakage and visible damage assessed after two weeks. Conventional cold hardiness testing is slow, requiring one to two weeks for completion, measuring membrane leakage is labour intensive, and requires

three working days to complete. Results can be available within 24 hours after removal from the freezer using chlorophyll fluorescence.

Carbon dioxide gas exchange also ranked high. It is rapid and results are quantitative. It was of moderate predictive value, capable of measuring photosynthetic inactivation in the winter, and when used in conjunction with environmental conditions or other parameters, results were similar to those of chlorophyll fluorescence. This technique does not appear to have the potential to be diagnostic, because it is not possible to determine the cause of seedling damage from the data, it does appear to be useful at many times during the growing season.

The use of at least two parameters appeared to be a better predictor of seedling quality after storage than a single method. Chlorophyll fluorescence used in conjunction with the number of chilling hours, average air temperature or seedling cold hardiness was a more reliable predictor of seedling quality than either parameter alone. Chlorophyll fluorescence with mitotic index was not predictive, as is evident with the SD-W seedlings, which had both low chlorophyll induction curves and mitotic indices.

Many of these techniques require expensive equipment: fluorometers, IRGAs, microscopes, growth chambers or temperature controlled greenhouses. The cost of the fluorometer; however, could be considered to be low when its flexibility is taken into account.

The ability to monitor the physiological status of a crop of seedlings may become as integral a part of nursery protocol as monitoring the morphological characteristics. By altering cultural treatments, nurseries may be able to produce seedlings which meet a physiological criteria similar to the current morphological one used. In the quest for an ideal test of seedling physiological status, many potential techniques have been offered (for a review see Hawkins & Binder 1991); however, no test has been granted this status yet. Of all the techniques used in this study, chlorophyll fluorescence was found to have the greatest potential, although the ideal test of seedling physiological status may prove to be a combination of several tests.

D. General Summary and Conclusions

Some results seen in these studies appear to be related to inherent differences between white spruce and Douglas-fir. White spruce is a boreal species adapted to a harsh climate. It can survive winter temperatures below -60°C. The white spruce seed source used in these studies was from the Prince George area, where the sudden onset of sub-zero temperatures is common in late summer and early fall. To prevent frost damage from such abrupt drops in temperature, white spruce has adapted by initiating bud dormancy in mid-summer and decreasing its physiological activity in the early fall. In the dormant state, seedlings are the most resistant to environmental stresses (Levitt 1980).

White spruce was found to have low sensitivity to short-term dehardening temperatures in the winter (48 hours at 15/10 and 20/15°C), indicating that once seedlings become dormant in the fall and winter, they are likely to have delayed or minimal responses to sudden temperature increases, that would otherwise lead to rapid physiological reactivation and leave these seedlings susceptible to damage.

In contrast, coastal Douglas-fir is adapted to a mild maritime climate. Photosynthesis may be limited by lack of water during the summer, and up to 40% of the total carbon can be assimilated in the fall and winter (Waring & Franklin 1978). Douglas-fir has adapted by becoming opportunistic, exhibiting good photosynthetic reactivation in response to short-term temperature increases in the winter.

Short-term temperature treatments (48 hours at 5/1, 10/5, 15/10 and 20/15 °C day/night temperatures) in the winter affected the storability of all seedlings, the lower the temperature at lifting, the better the seedlings stored. The degree of reactivation and the rapidity of the response may be related to provenance; the more southern coastal Douglas-fir from Oregon were most affected by dehardening temperatures.

Bud dormancy is triggered by day length in both of these species, with white spruce also displaying photochemical inactivation of photosynthesis in response to decreasing day length under warm temperatures. Photochemical inactivation was characterized by decreased PSII activity, seen as a decrease in Fv/Fm ratio, and a parallel decline in net photosynthesis.

Net photosynthesis did not decline with day length in Douglas-fir under warm temperatures, as would be expected in such an opportunistic species. Low temperatures did appear to inhibit photosynthesis in this species, consistent with the results of Hawkins & Lister (1985).

Photoinhibition, rapidly reversible declines of Fv/Fm, has been proposed as a protective mechanism to prevent damage in the light (Öquist & Malmberg 1989). Photoinhibition was observed in white spruce needles after sub-zero temperature treatments in light, but not in darkness. Damage occurring at sub-zero temperatures in the light was attributable to photodamage, and was characterized by irreversible declines of Fv/Fm. Photodamage is primarily induced by light and oxygen

dependent photooxidation (Wise & Naylor 1987). Damage at sub-zero temperatures in darkness was attributable to freezing of needle water, resulting in laceration of membranes by ice crystals and dehydration (Steponkus & Lynch 1989), and was related to needle water content.

Susceptibility of white spruce needles to damage from sub-zero temperatures in light and darkness was dependent upon needle maturity and degree of hardiness. Newly flushed needles were highly susceptible to photoinhibition, photodamage and freezing damage. They also had the highest needle water content and photorespiration rates. High photorespiration rates under 51.3% oxygen may indicate the production of high levels of toxic oxygen species, making them more susceptible to photodamage.

Chlorophyll fluorescence was found to be the most efficacious method for determining seedling quality. It was useful for multiple purposes throughout the growing season; when measured prior to lifting and five hours after removal from cold-storage it was predictive of seedling quality as ascertained by root growth potential. The prediction span was very short for all tests because seedling physiology can change rapidly, therefore testing should be done frequently. The rapidity and nondestructive nature of chlorophyll fluorescence gave it a clear advantage over the other seedling quality assessment methods.

Several topics for future research emerge from these studies: (i) the biochemistry and physiology of cold-stored

versus naturally over-wintered seedlings, to be studied with respect to changes occurring during cold storage; (ii) the importance of morphological and physiological differences between cold-stored and naturally over-wintered seedlings, in particular average root length and photochemical reactivation; (iii) the presence or absence of day length mediated photochemical regulation of photosynthesis in other conifers and evergreen plants; (iv) the potential role of chlorophyll fluorescence in forestry.

In conclusion, determination of suitable lifting times for placement of seedlings in cold storage was essential in producing quality seedlings. From a practical perspective, chlorophyll fluorescence can easily be used to quickly and nondestructively determine suitability for lifting. If seedlings are then lifted when the air temperature is low, chlorophyll fluorescence becomes a good predictor of post-storage seedling quality.

Improvement and assessment of seedling quality is critical to successful reforestation in British Columbia. Planting poor quality seedlings or failing to understand the eco-physiological needs of the outplanted seedlings can result in expensive plantation failures. Costs of reforestation may be reduced if seedling quality can be easily and effectively assessed. Plantation success depends not only on the production of quality seedlings, but also on site conditions. Eco-physiological studies in the field should be the next target for seedling quality assessment.

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