Original article

Chlorophyll fluorescence characteristics, performance and survival of freshly lifted and cold stored Douglas fir seedlings

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Abstract – The physiological condition of three-year-old Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] was periodically assessed from October to May 1998/1999 during propagation of seedlings in an Irish nursery. Seedling physiological status was evaluated in situ using determinations of chlorophyll fluorescence and plant water status. Pre- and post-cold stored (either –2 °C or +0.5 °C) plant vitality was examined using determinations of root growth potential (RGP), root electrolyte leakage (REL), shoot water content and chlorophyll fluorescence. Data obtained from freshly lifted and cold stored stock showed that chlorophyll fluorescence provides a reliable (non-destructive) method of evaluating a seedlings potential field performance in both pre-lift (direct planting) and post-storage (outplanting) situations. Variations in survival and an index of plant health were paralleled by inverse changes in the effective quantum yield of photosystem II (Φ PSII) from November to April. Significant positive non-linear relationships were found between RGP and Φ PSII, though they were of limited predictive ability in terms of outplanting performance. Post-storage fluorescence assessments indicated down-regulation and/or damage of the plants photosynthetic light-harvesting complex, which depended on storage temperature and duration.

chlorophyll fluorescence / cold storage / plant quality / photosystem II / *Pseudotsuga menziesii*

Résumé – Caractéristiques de la fluorescence chlorophyllienne, performances et survie de plants de Douglas vert récemment arrachés et stockés au froid. La condition physiologique Douglas [*Pseudotsuga menziesii* (Mirb.) Franco] âgés de trois ans a été évaluée périodiquement de octobre à mai 1998/1999 pendant la dans une pépinière irlandaise. Le statut physiologique des semis a été évalué in situ par la détermination de la fluorescence chlorophyllienne et de l'état hydrique des plants. La viabilité des plants avant et après stockage au froid (–2 °C ou +0,5 °C) fut examinée par la détermination du potentiel de croissance racinaire (RGP), du relargage d'électrolytes par les racines (REL), de l'humidité des bourgeons et de la fluorescence chlorophyllienne. Les données obtenues sur des plants récemment arrachés et conservés au froid montrent que la mesure de la fluorescence chlorophyllienne est une méthode fiable (non destructive) pour évaluer la performance potentielle au champ des germinations dans les situations de plants avant levage (plantation directe) et de plants stockée. Les variations de survie et index de vitalité du plant étaient inversement proportionnelles aux variations du rendement effectif du photosystème II (ΦPSII) de novembre à avril. Des relations positives significatives non-linéaires entre RGP et ΦPSII furent trouvées, bien qu'elles aient une capacité limitée à prédire la performance des plants transplantés. Après stockage, les mesures de fluorescence montrèrent l'inhibition et/ou la détérioration de l'efficacité photochimique du photosystème II qui dépendait de la température et de la durée du stockage.

fluorescence chorophyllienne / stockage au froid / qualité des plants / photosystème II / *Pseudotsuga menziesii*

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1. INTRODUCTION

Predicting conifer seedling performance in the field, prior to outplanting, is a major goal in many forest seedling development programmes [28]. These assessments are based on two main criteria: material attributes, which can be measured directly, and performance attributes, which measure aspects of seedling physiological response under specific test conditions [37, 38]. Although relationships between morphological characteristics and seedling tolerance to cold storage have been reported, they have been of limited value in predicting field performance because of variation within morphological grades [12, 21, 44]. Consequently, several physiological indices have been used in an attempt to provide a more rapid and predictive test of seedling vitality [28, 29, 39].

Root growth potential (RGP) is one of the most commonly used seedling quality tests and is defined as the ability of seedlings to grow new roots when placed in a favourable environment. This test has been shown to be an accurate predictor of seedling quality at the time of lifting and often, but not always [42], correlates well with survival [36, 37, 38]. Several less time-consuming tests have also been investigated and, of these, the root electrolyte leakage (REL) technique has become the most widely employed [13]. The REL technique has been used as a performance indicator for several conifers following cold storage in the UK, including Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] [24, 27] and has been found to be correlated with survival [24, 25].

Assessments of photosynthetic performance may also be of value in scoring plant responses to environmental conditions, since photosynthesis is sensitive to changes in temperature, water availability and light [23, 30]. One increasingly used method for assessing the "integrity" of a trees photosynthetic apparatus is via the use of chlorophyll fluorescence signals [40, 41]. Although chlorophyll fluorescence assessments originally focused on measurements made on initially dark-adapted samples, there is now increasing evidence that these may not always give an accurate assessment of a plants actual photosynthetic status (under constant illuminated conditions), due to differences between light- and dark- adapted quenching processes [22]. The development of portable, pulse-modulated instruments (e.g. [1]) can circumvent this problem, and enable routine measurements to be made in the field under ambient light or illuminated conditions. Chlorophyll fluorescence measurements of stock quality have included the study of winter dormancy induction, cold hardiness development and photosynthetic reactivation in the spring, following winter dormancy, or after cold storage [4, 5, 6, 16, 47].

In Ireland, direct planting of freshly lifted bare-root seedlings onto forest sites is standard practice, but low survival and/or poor growth of Douglas fir is common [32]. Transplantation shock has also resulted in highly variable survival under the climatic conditions that prevail in both Britain [26] and France [14]. This has been attributed to shoot desiccation and frost damage [26] or poor plant/soil water status [14]. In Douglas fir, new adventitious root production is required for water uptake and this is known to require current photosynthate [45]. However, both photosynthesis and root growth processes are limited under conditions of low temperature [8, 9, 11, 34]. Therefore, outplanting in mid-winter may be less favourable than cold storage, even under Irish conditions.

Cold storage is becoming a more prevalent practice in Ireland (10–20% of seedlings are now cold stored) and has managerial and practical benefits [24, 26]. The typically mild and moist climatic conditions found in Ireland may result in Douglas fir failing to develop full winter dormancy and stock may show a reduced tolerance to storage [32]. Also cold storage in Ireland is carried out at sub-zero temperatures (–2 °C); such storage temperatures have been shown to have detrimental effects upon Douglas fir seedling quality in the UK [e.g. 26]. Therefore, predictions of ideal cold storage "windows" requires assessment of the interactions between lift date, cold store temperature and duration of cold storage.

In this study, the physiological status of three-year-old Douglas fir seedlings was assessed over the normal operational lifting period, as well as during cold storage. The primary aim was to assess the utility of the pulse-modulated chlorophyll fluorescence technology, alongside the more common tests of dark-adapted fluorescence, RGP and REL, as a screening tool for assessing the development of cold hardiness and the ideal period for lifting and cold storage. A field trial was also established to determine if the physiological measures employed could be used to predict variations in performance and survival success.

2. MATERIALS AND METHODS

2.1. Plant material

Three-year-old Douglas fir transplants (1 $\frac{1}{2}$ + 1 $\frac{1}{2}$) (Seedlot [797]241: Elma, Washington, 350 m elevation) were grown in Ballintemple Nursery, Co. Carlow, Ireland (lat. 52°44' N, long. 6°42' W, 100 m elevation). Seedlings were lined out into fumigated beds in July 1997, once shoot elongation had ceased. This is common nursery practice under Irish conditions, and promotes dormancy development in the stock. Seedlings used in

this study were systematically sampled from the normal operational nursery stock. Soil at the nursery site is a sandy loam (pH 5.7, organic content 6–8%, and sand, silt and clay fractions 66, 19 and 15% respectively). Plants received monthly additions of nitrogen at 14 kg N ha^{-1} from April to July, with top dressings in July of K and Mg.

2.2. Treatments

Plants were lifted on six dates from October 1998 to May 1999, at four to six week intervals, and packaged into light-tight co-extruded seedling bags, each containing 100 morphologically (i.e. visually) graded seedlings. Some material was then directly planted at a farm-field site [32] soon after lifting (see 2.6. Outplanting performance). Additional stock ($n = 100$) was lifted and cold stored, in sealed bags, at one of two temperatures (sub-zero: –2 °C or above-zero: +0.5 °C, respectively) in dark, controlled temperature storage units. A further subset ($n = 15$) of the freshly lifted stock underwent physiological assessments in the laboratory.

All cold-stored plants, irrespective of lift date, were removed from storage, assessed using chlorophyll fluorescence, and then planted in mid-May, 1999. A subset of the cold-stored plants was also assessed under controlled environment conditions using RGP and fluorescence methodologies, after removal from storage ($n = 7$), for each cold storage temperature × lift date combination. The relatively small sample size was due to space limitations.

2.3. Pre-lift assessments

At each lift date, pulse modulated chlorophyll fluorescence measurements of dark adapted and light exposed (both ambient and controlled light conditions) tissue were made at two hours intervals on previous year's needles of first-order lateral shoots ($n = 15$), from pre-dawn to dusk. Pre-dawn shoot water potential (Ψ_{SHOOT}) was also assessed ($n = 5$). For each plant sample two Ψ_{SHOOT} measurements were taken using a Scholander pressure bomb (Model 1400, Skye Instruments Ltd., Llandrindod Wells, UK), on 2-year-old shoots. To minimise water loss during measurements, the samples were immediately placed in sealed polythene bags containing moist paper towels. Measurements were completed within 20 min of sample collection. Direct determinations of relative water content in current and previous year's shoots ($n = 10$) were also taken on pre-dawn collected material. The needles were immediately removed and samples wrapped in Nescofilm^{TM} (Nescofilm, Nippon

Shoji, Osaka, Japan) to prevent sample desiccation. The relative water content was obtained using the method of Sobrado et al. [43] and sample volume and density estimates followed the protocol of Borghetti et al. [2]. Nursery soil temperatures at 5 cm depth, were logged continually (hourly averages) with Tinytag^{TM} dataloggers (Gemini Data Loggers Ltd, Chichester, UK).

2.4. Chlorophyll fluorescence protocols

The fluorescence equipment (FMS 2, Hansatech Instruments Ltd, Kings Lynn, UK) was first parameterised for use with Douglas fir tissue, to ensure saturating light pulses were sufficient to close all reaction centres. The potential quantum efficiency of phostosystem II (Fv / Fm), in the dark-adapted state, was assessed pre-dawn, in the field, and also under growth chamber (for RGP assessment) and cold storage conditions (at either –2 °C or +0.5 °C temperatures), after 30 min dark-adaptation, using a leaf clip. All laboratory based measurements were taken at 20 °C. Further estimates of fluorescence parameters (the photochemical efficiency of open reaction centres measured under steady state, light adapted, conditions), Fv' / Fm', and the quantum efficiency of photosystem II, ΦPSII, were derived and calculated from data obtained under ambient temperatures and controlled light conditions (using a "background" illumination source integral to the FMS2 modulated equipment). For controlled illumination studies the actinic (background) light induction level was 600 μmol m^{-2} s^{-1} which was maintained for 5 min before estimates were obtained. This duration was sufficient for all seedlings, irrespective of storage conditions, to reach steady-state fluorescence. The calculation of parameters measured under illuminated conditions followed the nomenclature of van Kooten and Snel [46], where ΦPSII = Fv' / Fm' × q_p.

2.5. Post-lift assessments

2.5.1. Physiological assessments

Measurements of REL were determined on excised fine roots (<2 mm diam., fresh mass 100–500 mg) of 15 plant replicates from each lift date. The relative conductivity method of Wilner [48] was used to determine REL, following the modifications of McKay [24].

Measurements of post-lift fluorescence were determined on 15 plant replicates from each lift date. Samples were dark adapted for 30 min prior to measurement of Fv / Fm, and then exposed to saturating pulses with

background illumination, to allow calculation of Fv' / Fm' and ΦPSII, under steady state conditions.

2.5.2. Post storage assessments of root growth potential (RGP) and fluorescence

The assessment of RGP was made after growth of seedlings for 14 days under controlled environment conditions: 20 °C, 75% RH (relative humidity) and an irradiance of 300 µmol m^{-2} s^{-1}, with a 16 h photoperiod [8, 24]. Measurements of RGP were made at the end of cold storage on 7 median-size replicate plants, per lift date × storage treatment. Root growth was assessed as the number of new white roots (>10 mm in length) produced at the end of the 14 day trial. On the first day of RGP assessment fluorescence parameters were also measured, for each plant, on one-year-old needle clusters on the first whorl ($n = 3$, per plant).

2.6. Outplanting performance (field trial)

At each sampling lift date at Ballintemple Nursery, 80 seedlings were transplanted to the Coillte Teoranta Tree Improvement Centre, Kilmacurra, Co. Wicklow (lat. 52°56' N, long. 6°49' W, 120 m elevation). Soil characteristics at the outplanting site are: pH 5.7, 7% organic matter content and sand, silt and clay fractions of 40, 32 and 27%, respectively. Additionally, 80 cold stored seedlings from each of the lift dates (excluding October lift as all plants were dead on removal from storage) and for each of the two cold storage temperatures (–2 °C or +0.5 °C) were sent to Kilmacurra in May, 1999, and planted in a split-plot randomised block design, alongside freshly lifted stock, to allow a comparative assessment of post-storage field survival and growth. Storage (freshly lifted or cold stored at –2 °C or +0.5 °C) was the main plot and lifting date the subplot. Each of the four blocks contained one replicate of each lift date × "storage" combination as a row of 20 seedlings. Spacing was 30 cm intra-row and 50 cm inter-row. Data analyses were made using block means.

Survival was scored in September, 1999, after bud set, for both cold stored and freshly lifted plants. Total survival was assessed and a scalar plant health index (PHI) was constructed, which transformed a single visual score assessment of needle browning [10, 32], for the whole plant, to a continuous linear scoring scale (value range: 0 = brown, *presumably dead* to 1 = completely green, *presumably healthy*). PHI has an advantage over simple measurements of survival as it incorporates a "score" related to the health of the plant. PHI was calculated for

each lift date/storage combination, and means were grouped by planting block, as:

$$1 - \left[\frac{\Sigma\, individual\, visual\, score\, assessments}{maximum\, sum\, total\, score\, of\, n\, individuals} \right].$$

2.7. Statistical analyses

Pre-lift field measured variables were tested for significance between lift dates using a repeated measures ANOVA. Post cold storage, where no clear interaction was observed between lift date and storage temperature, a one way ANOVA, with multiple pairwise analysis, was performed using Dunn's test. Outplanting survival and plant health data were analysed by ANOVA, to test separately for the effects of block and lift date for each storage treatment. A factorial split-plot analysis was not attempted as data sets were not balanced due to some loss of stock from cold store. Means for each lift date (within treatment) and differences between storage regimes (by lifting date), for fluorescence and REL measurements, were analysed further using *t*-tests, after arcsine transformations. Data were analysed using SAS (SAS Institute Inc., Cary, NC), except regression analyses. Regressions of RGP against fluorescence, measured post cold storage, were fitted using a non-linear "Hill" function. Linear regressions were used to assess the relationship between light adapted fluorescence signals (Fv' / Fm' and ΦPSII), and either survival or plant health. All regression functions were fitted to sample means using Sigmaplot^{TM}.

3. RESULTS

3.1. Physiological assessments of freshly lifted stock

Soil temperatures, at 5 cm depth, declined to a minimum (0 to 4.5 °C) in January-February (*figure 1A*), and this decline was broadly correlated with changes in measured physiological variables. Shoot relative water content (RWC) increased significantly in current year's (1 y.o.) shoots after November 1998 ($p < 0.05$) (*figure 1B*). Pre-dawn shoot water potential (Ψ_{SHOOT}) did not vary significantly from October to May, with the exception of the measurement made in March ($p < 0.05$) (*figure 1C*), which coincided with a period in which there was sustained rainfall. The potential maximum quantum efficiency of PS II photochemistry in the dark-adapted state (Fv / Fm) declined to a minimum in January ($p < 0.05$) with the highest values during October-November and March-April (*figure 1D*). Little change in the efficiency
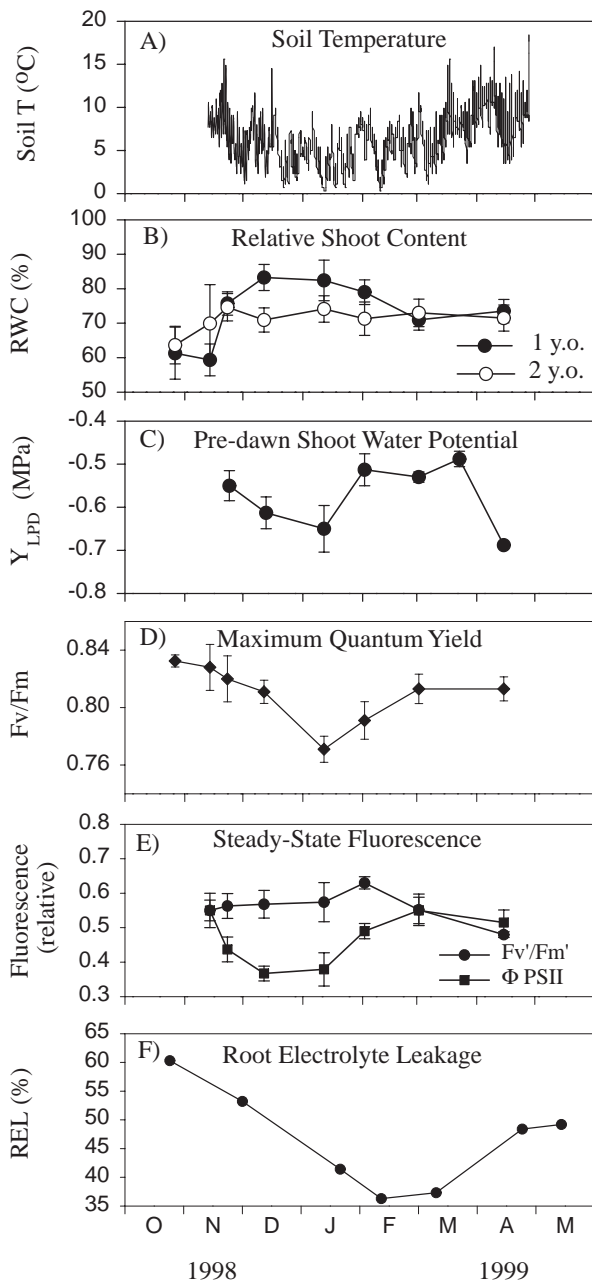
Figure 1. Variation in (A) soil temperature (°C), (B) shoot relative water content (RWC) ($n = 10$; y.o. means year old), (C) pre-dawn shoot water potential (Ψ_{SHOOT}) ($n = 2$), (D) the potential quantum efficiency of phostosystem II (Fv / Fm) ($n = 15$), (E) the quantum efficiency of open PSII reaction centres (Fv' / Fm') ($n = 15$), and quantum efficiency of photosystem II photochemistry (ΦPSII) ($n = 15$), and (F) root electrolyte leakage (REL) ($n = 15$), of Douglas fir grown at Ballintemple Nursery during October-May 1998/1999. Vertical bars represent 1 standard error of the mean (where error bars are not shown they are smaller than symbols used).

of open PSII reaction centres (Fv' / Fm') measured under steady state, light adapted, conditions was found, although there was a significant decline in the overall quantum efficiency of PSII (ΦPSII), which reached a minimum in December-January (*figure 1E*). The decline in ΦPSII preceded the decline observed for Fv / Fm. Subsequently, there was a recovery of ΦPSII by March to values comparable to those found at the beginning of the measurements. Root vitality, measured as root electrolyte leakage (REL), declined to a minimum in February/March (*figure 1F*), somewhat later than the decline in Fv / Fm and ΦPSII (*figure 1D,E*). Increases in Fv / Fm and, particularly ΦPSII, were observed prior to increases in REL. The REL estimates were significantly lower in January, February and March than on other lift dates ($p < 0.05$), and were similar to those previously reported under Irish conditions [32].

3.2. Fluorescence characteristics and RGP of cold stored stock

The RGP of cold stored plants, assessed in May after various storage durations, reached a maximum in plants lifted to store in February (*figure 2A*). Post cold storage these higher values, in February, were consistent with higher values for Fv / Fm (*figure 2B*), Fv' / Fm' (*figure 2C*) and ΦPSII (*figure 2D*), measured on the first day of RGP assessment. For other lift (to storage) dates low RGP's, post storage, were not linearly related with reductions in fluorescence parameters.

Fluorescence parameters measured on material removed from cold store to control conditions ranged from 0.5 to 0.82 for Fv / Fm (*figure 2B*), 0.3 to 0.7 for Fv' / Fm' (*figure 2C*), and 0.4 to 0.78 for ΦPSII (*figure 2D*). Regression analysis, using a sigmoidal function (for grouped dates) gave a significant correlation between RGP and both light and dark-adapted fluorescence measurements ($p < 0.05$) for cold stored seedlings (*figures 3A,B,C*). Overall, the best correlations were found between RGP and Fv / Fm ($R^2 = 0.97$) or ΦPSII ($R^2 = 0.94$) (*figures 3A,C* respectively).

3.3. Outplanting survival

Survival, measured in September 1999, of directly planted stock was poor (<70 %) for seedlings lifted in October 1998 and then remained above 87% up to April (*figure 4*). Survival was significantly lower for stock lifted in November to the cold store, and outplanted in May (i.e. 24 weeks storage) ($p < 0.05$) (*figure 4*). For stock stored in April, survival at –2 °C was significantly lower than those stored at +0.5 °C ($p < 0.05$). None of the stock
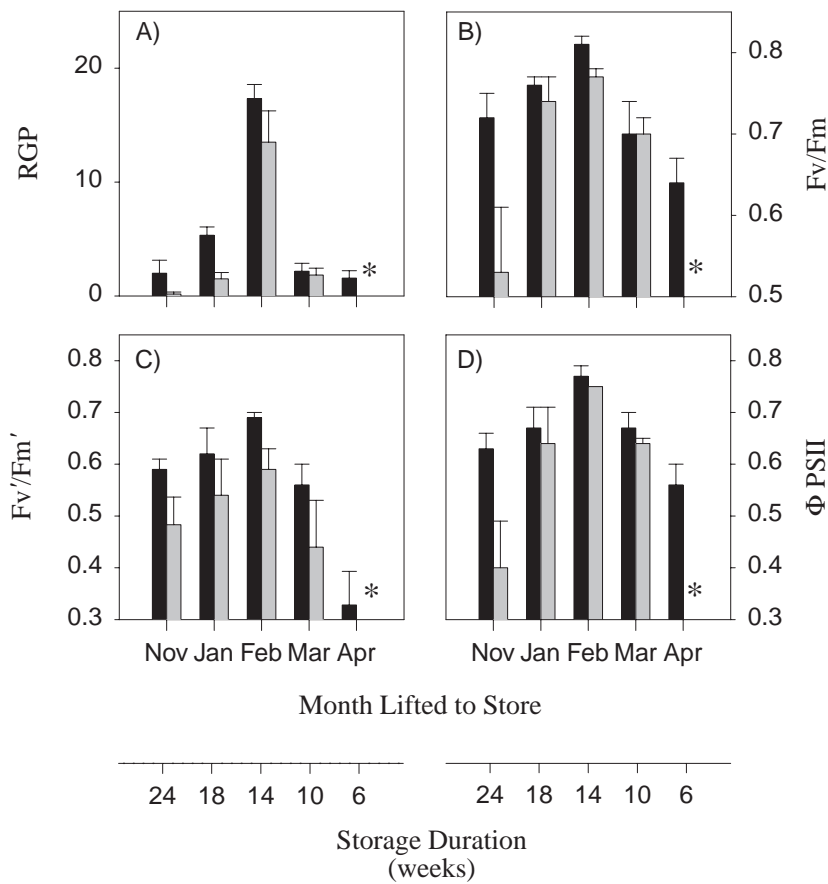
Figure 2. Effects of duration of cold storage (dependent on date lifted to store) on (A) root growth potential (RGP) (*n* = 7), and corresponding fluorescence values (*n* = 3 *per plant*) of (B) Fv / Fm, (C) Fv' / Fm' and (D) ΦPSII, of Douglas fir lifted at Ballintemple Nursery. Plants were assessed during May, 1999, immediately prior to planting in the field. Cold storage temperatures were +0.5 °C (filled bars) and –2 °C (shaded bars). Vertical bars represent 1 standard error of the mean, * denotes not determined.
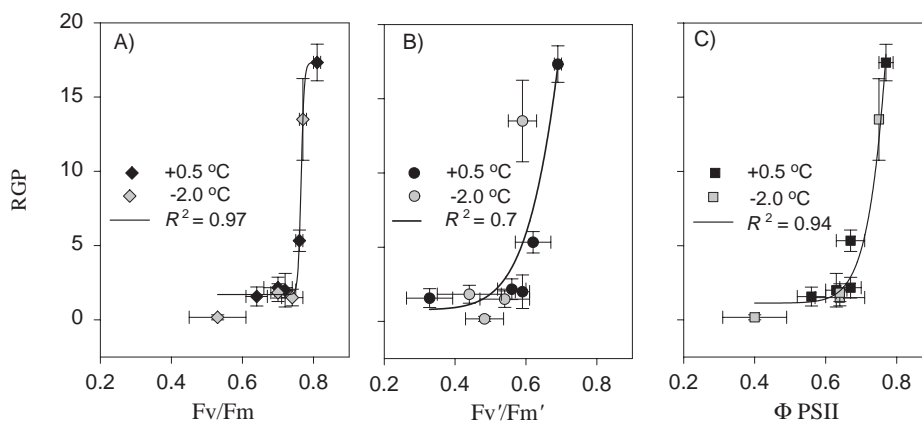

Figure 3. Relationship between root growth potential (RGP) (*n* = 7) and corresponding fluorescence values (*n* = 3 *per plant*) of (A) Fv / Fm, (B) Fv' / Fm' or (C) ΦPSII for cold stored Douglas fir. Cold storage temperatures were +0.5 °C (filled symbols) and at –2 °C (shaded symbols). Vertical and horizontal bars indicate 1 standard error of the mean.

stored in October was of sufficient quality to warrant planting to the field.

3.4. Comparisons of outplanted stock health or survival with fluorescence

Fluorescence measurements of ΦPSII on freshly-lifted plants, from October to April, were high when survival was poor (compare *figure 1E* and *figure 4*). Plants measured immediately prior to planting, after cold storage (+0.5 °C or –2 °C), showed increases in ΦPSII from October to November, which mirrored increases in plant health, as measured at the end of the season, in September 1999. An inverse relationship was then evident between ΦPSII and plant health index for measurements made between November to April (*figures 5G,H*). In contrast, no clear relationships were found between Fv / Fm or Fv' / Fm' and PHI of cold-stored material (*figures 5C–F*).

Measurements of ΦPSII were negatively correlated with survival in the field (*figure 6A*). Although the correlation for freshly lifted stock was poor, this was due to an anomalous (high) value in April which reduced the significance of the relationship (*figure 6A*). This anomaly was less evident when comparisons were made with plant health index scores (*figure 6B*). Linear regression analysis showed an excellent fit between measurements of post cold storage ΦPSII and both survival (–2 °C, $R^2 = 0.93$; +0.5 °C, $R^2 = 0.83$) and plant health (–2 °C,

$R^2 = 0.88$; +0.5 °C, $R^2 = 0.62$) with similar slopes, for each "storage" condition.

4. DISCUSSION

This study has shown over winter reductions in REL, Fv / Fm and the overall quantum efficiency of photosystem II (ΦPSII), for Douglas fir measured at the time of lifting. Dark-adapted values of Fv / Fm showed significant declines during the winter, which were concomitant with a decline in soil temperature. This contrasts with the findings of Fisker et al. [12] who showed no variation in Fv / Fm under comparable mild-winter conditions for both a provenance selected from a coastal, low elevation site and for the same provenance used in the present study. However, the values reported by Fisker et al. are far lower than those found in this study, possibly indicating either poor instrument resolution or additional limitations to plant performance. The observed reduction in Fv / Fm noted in this study was not, however, accompanied by a change in shoot water status. Other studies have also shown a reduction in Fv / Fm during the coldest winter period that are thought to indicate winter photosynthetic "inactivation" [20], with reduced light-saturated rates of net photosynthesis and electron transport [30, 31]. Despite the mild field temperatures experienced by seedlings in this study, declines, particularly in ΦPSII, were still evident indicating that either the plants are susceptible to relatively small temperature changes or other environmental factors are involved.
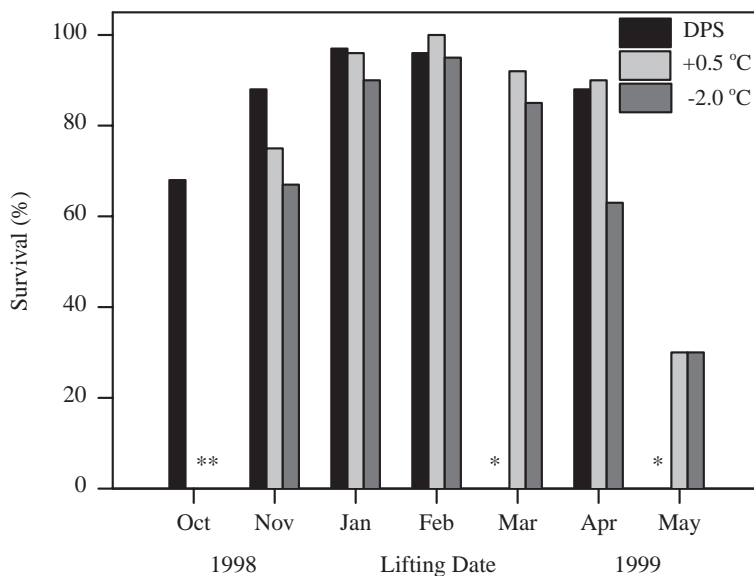

Figure 4. Seedling survival assessed in the field (Kilmacurra) at the end of one growing season in September 1999 for directly planted stock (DPS), and plants stored at either +0.5 °C or –2 °C. Results are mean values ($n = 80$), * denotes not determined except for plants cold stored in October which were dead on removal from store, in May, 2000.
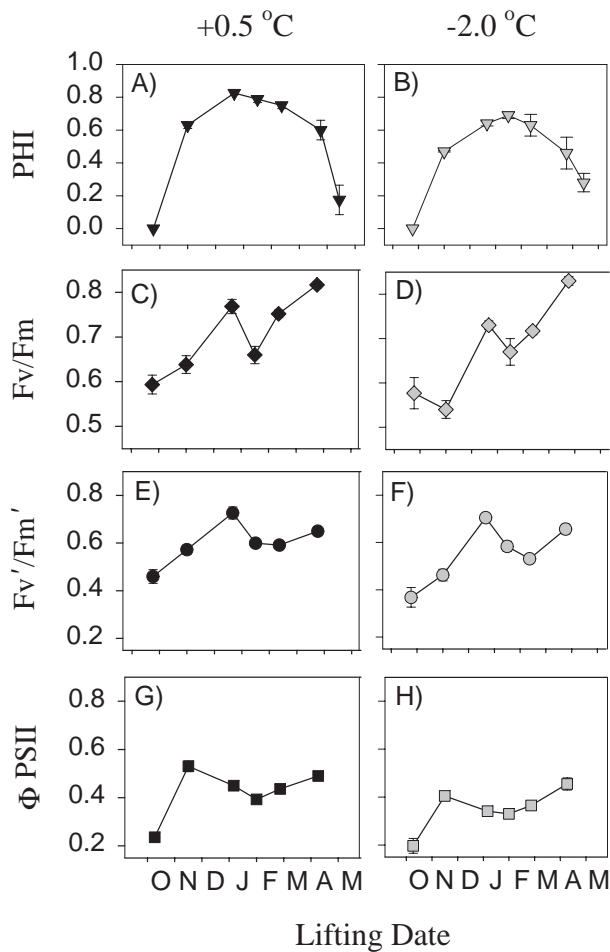
Figure 5. Comparisons between (A,B) plant health index (PHI) of seedlings assessed in the field (Kilmacurra) at the end of one growing season (September 1999) and fluorescence parameters (C,D) Fv / Fm, (E,F) Fv' / Fm' and (G,H) ΦPSII, determined immediately prior to removal from cold storage, at either +0.5 or –2 °C. Points are mean values: for PHI ($n = 80$), and for fluorescence parameters ($n = 15$). Vertical bars indicate 1 standard error of the mean.

Declines in ΦPSII, with little or no alteration in Fv' / Fm', indicates that these changes were largely associated with variations in the proportion of open PSII reaction centres (q_p, or photochemical efficiency), rather than a decline in the photochemical efficiency of open reaction centres under steady state, light adapted, conditions (Fv' / Fm'), as ΦPSII = Fv' / Fm'·q_p [15]. A similar conclusion has been suggested for changes in ΦPSII associated with the effects of low temperature, CO_2 enrichment or

restricted "sink" activity [19, 22]. This suggests that changes in field performance are linked to processes downstream of PSII that increasingly constrain light-driven electron flow. The more rapid decline observed in ΦPSII could, therefore, reflect a decrease in net assimilation rate and, compared with the decrease in (pre-dawn) Fv / Fm, suggests that these constraints are evident earlier in illuminated material. Furthermore, our data suggest that variable fluorescence may possibly detect declines, not only in cold stored stock, but also in stock that has not received substantial environmental perturbations.

In cold stored material subsequently exposed to "ideal" growth conditions there were reductions in Fv' / Fm' (*figures 2C and 3B*), which were not attributable to changes in photochemical quenching. This suggests that continued exposure to low temperatures does cause a down-regulation in the efficiency of electron transfer to the reaction centres.

For REL, the minimum values obtained lagged behind the minimum soil temperatures. This suggests that root hardiness developed in response to the accumulated chilling sum, rather than the instantaneous temperature. As spring (March 1999) approached, Fv / Fm and ΦPSII increased (*figure 1*), but this was not reflected, initially, in alterations in baseline REL values. Similarly, the initial decline in Fv / Fm and ΦPSII occurred earlier than the reduction in REL. This suggests that REL, whilst an excellent indicator of root vitality [25, 27], may not give an accurate assessment of current, whole-plant performance during hardening/de-hardening processes. In support of this it has been suggested that REL can be used as a measure of seedling quality only when the dormancy status is known [13]. It also suggests that the fluorescence parameters used, rather than REL, are more sensitive predictors of variations in plant vitality occurring in the field, during the hardening and overwintering phases.

Variable fluorescence measurements taken on plants "in cold store" were found to be negatively correlated with post-planting survival and plant health (*figure 6*). Transplantation shock is thought to be due to plants being subjected to water deficits [7, 17, 18], this can be largely overcome through increased water uptake by new root production [8]. In Douglas fir, current photosynthate is thought to be the primary carbon source for new root growth, as little carbon is available from storage tissues [34, 45]. Therefore, we suggest that measures of ΦPSII "in cold store" can be used to predict post planting vitality of cold stored stock, once inter-seasonal variations in threshold values are known. This is due to the fact that a low ΦPSII under cold storage conditions appears to correlate with the ability for rapid photosynthetic
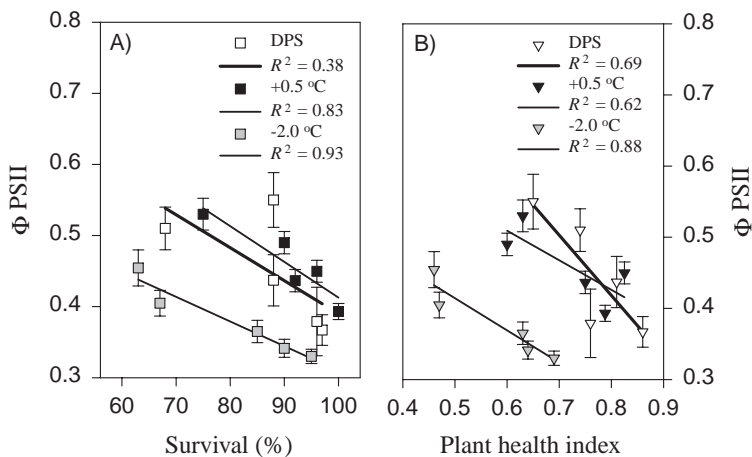
Figure 6. Relationships between ΦPSII ($n = 15$) measured at the end of cold storage and (A) survival ($n = 80$), or (B) plant health index ($n = 80$) of Douglas fir. Data is grouped according to treatments prior to planting: directly planted stock (DPS), or plants stored at +0.5 °C or −2 °C. Vertical bars indicate 1 standard error of the mean.

reactivation (in the field) and, ultimately, to the plant's potential to establish successfully.

For freshly lifted stock, however, REL was a sensitive indicator of post planting survival. Due to the lower soil temperatures experienced by winter transplants, active root growth may not occur [11] and water uptake will be largely reliant upon the existence of a viable, cold-hardened root system. In Ireland, establishment of directly planted Douglas fir is most successful for early plantings, when RGP is high and soils are warm enough to realise this potential [32]. Stock planted in winter often performs poorly due to low soil temperatures, irrespective of RGP and, in spring, declining RGP also results in poor performance. Therefore, despite an ability for continued photosynthesis in spring, as indicated by the high values for ΦPSII, the plants may still be limited by postplanting conditions. The present study suggests that measures of root growth potential (RGP) can be pre-empted by using assessments of shoot photosynthetic processes, under ideal conditions. The sigmoidal nature of the relationship between RGP and ΦPSII assessed post storage is due, in part, to the fact that the maximum, fully relaxed, levels of photosystem II photochemistry give a peak value of ≈ 0.83 [15]. The positive relationship found suggests that photosynthetic reactivation is rapid after removal from cold storage to conditions "ideal" for growth, and this result may be of particular relevance in post-planting assessments, that are used for prediction of survival. The findings also suggests that RGP may not necessarily predict future performance and field survival, as poor root growth was evident for plants which established successfully [cf. 42]. This reinforces the notion that RGP provides unreliable estimates of the quality of cold stored stock [24, 35] and should not be used as a stand-alone test.

Whilst good relationships were found between fluorescence signals (particularly ΦPSII) measured on cold stored stock immediately prior to planting, and survival their use in predicting field performance is probably best utilised in a threshold manner. The threshold fluorescence values for survival and root growth also highlight potential limitations in the use of fluorescence assessments, particularly a requirement for seasonal variability to be taken into account. Regression analysis of cold hardiness LT_{50} values, obtained for each lift date as part of the overall study (data not shown), with pre-lift ΦPSII measurements from 1998–1999 gave a significant positive relationship (r^2 of 0.65). A rough estimate of the inter-seasonal utility of measurements of ΦPSII was obtained by regression analyses using further cold hardiness LT_{50} values, obtained during the years 1993–1994 and 1994–1995 (data taken from [32]). These gave r^2 values of 0.69 and 0.45 respectively, with all points falling within the 95% confidence limits.

Values for Fv / Fm indicated a recovery from cold inhibition of photosynthesis in the field [33] but were, in general, positively correlated with cold storage duration (*figures 5C,D*). Therefore, this parameter appears not to be appropriate in predicting field survival of cold stored stock *after* the resumption of photosynthetic activity in spring. They could, however, be used in the assessment of storage duration-induced declines in plant quality for cold stored stock lifted *prior* to the resumption of photosynthesis in the field.

In conclusion, variations in photosynthetic efficiency (the fluorescence parameter ΦPSII) are shown to be a useful predictor of plant vitality and post-planting establishment, both for stock that had been directly planted and for those subjected to cold storage. The relationship between measures of RGP and PSII photochemistry

offers the potential for a significant reduction in the time required to predict the ability of the plant to produce new roots, under favourable conditions [cf. 16], but the utility of such measurements in predicting survival appears limited. Our investigations suggest that modulated fluorescence (related to steady state photosynthetic performance) provides a more physiologically useful measurement than dark-adapted fluorescence alone [cf. 3]. Thus, modulated fluorescence measurements have the potential to provide an "instantaneous" measure that, with further parameterisation to take into account seasonal variability, could be used to identify and predict the vitality of stock, particularly that previously subjected to cold storage.

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