# Chlorophyll Fluorescence as a Tool in Propagation

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#### INTRODUCTION

Failures in the rooting of cuttings can often be attributed to unhealthy or stressed stock plants. Since stress levels detrimental to plant health may be present before they are visible to the naked eye, a method of measuring this stress would be useful in stockplant selection, as well as in monitoring cuttings throughout the propagation process.

Chlorophyll fluorescence is a technique increasingly used to measure plant photosynthetic health. When chlorophyll molecules absorb light in the processes of photosynthesis, a small portion of that light is re-emitted, or fluoresced. Measurement of this fluorescence provides an estimation of photosynthetic efficiency, which is an indirect measure of plant stress (Adams et al., 1990; Genty et al., 1989). Variable fluorescence over maximum fluorescence ( $F_{\downarrow}/F_{m}$ ), the measurement used in these studies, has been used extensively in environmental stress studies, including dormancy assessments in douglas fir (*Pseudotsuga menziesii*) (Hawkins and Lister, 1985), Scotch pine (*Pinus sylvestris*), lodgepole pine (*P. contorta*) (Lindgren and Hallgren, 1993), and Norway spruce (*Picea abies*) (Westin et al., 1995) and as an estimate of freeze damage and seedling survival in douglas fir (Fisker et al., 1995). In addition, it has been used to examine light levels in cold storage of white spruce (*P. glauca*) and jack pine (*P. banksiana*) (Camm and Lavender, 1993).

The objective of these studies was to examine chlorophyll fluorescence ( $F_v/F_m$ ) as a potential tool for stockplant selection, assessment of storage conditions, and measurement of stress over the course of propagation in Taxus.

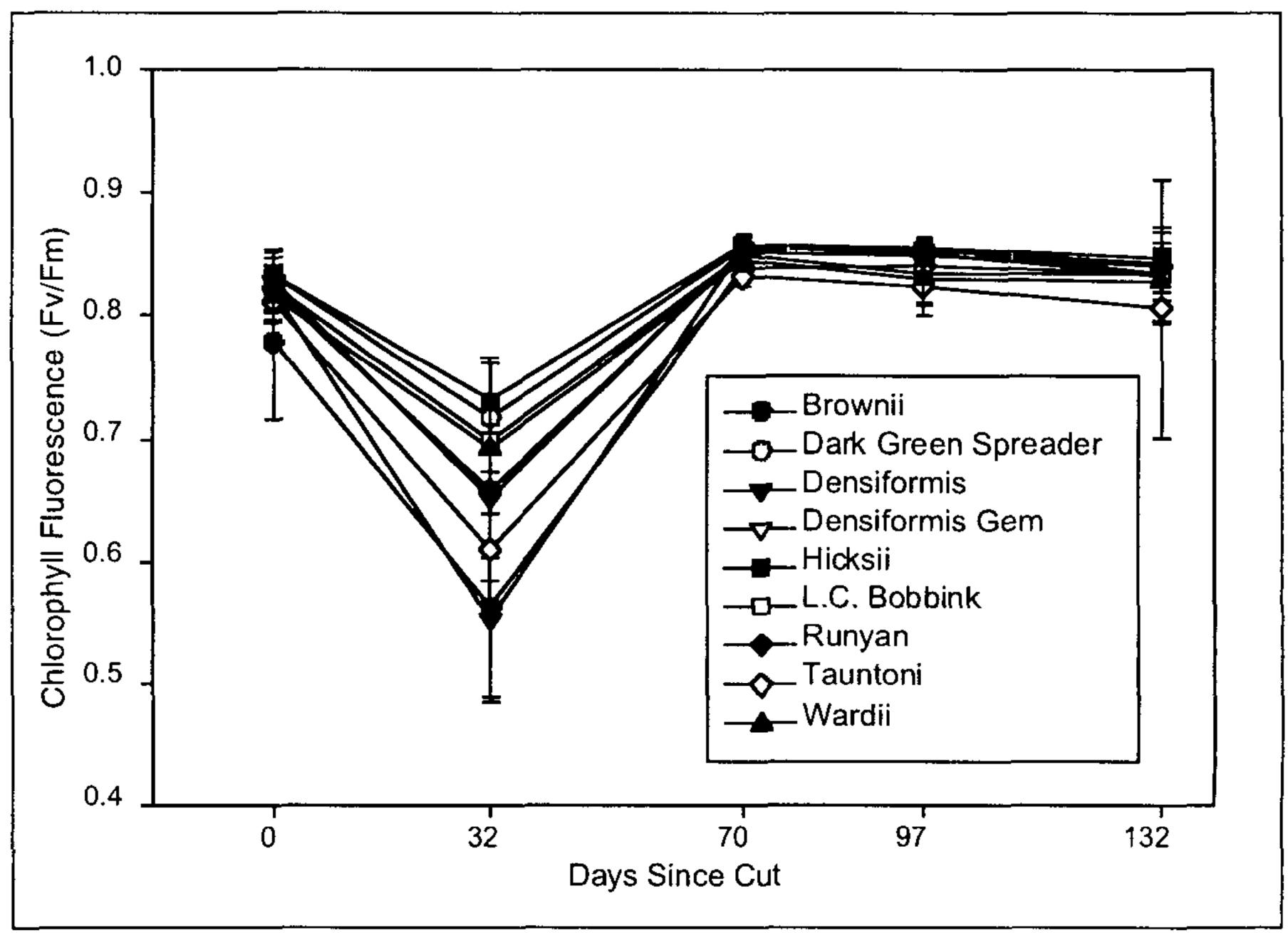
## **MATERIALS AND METHODS**

Nine cultivars of  $T.\times media$  (Brownii, Dark Green Spreader, Densiformis, Densiformis Gem, Hicksii, Bobbink, Runyan, Taunton, and Wardii) were selected to examine cultivar differences, changes in  $F_v/F_m$  over the course of propagation, and to compare initial  $F_v/F_m$  values with final rooting data. Cuttings were taken in late October from field grown plants at Zelenka Nursery, Grand Haven, Michigan. Each cutting was measured for chlorophyll fluorescence levels, placed in cold storage at 5°C for 32 days, and then stuck at Zelenka (re-cut to 4.5 inches, dipped in Woods Rooting Hormone [IAA 1.03%; NAA 0.66%] at 2800 ppm, and placed in perlite medium, air temperature 18°C, bottom heat 21°C). Cuttings were harvested after 100 days in the propagation bed and rooting percentage, root number, root length, and root dry weight measured. Chlorophyll fluorescence levels were measured monthly.

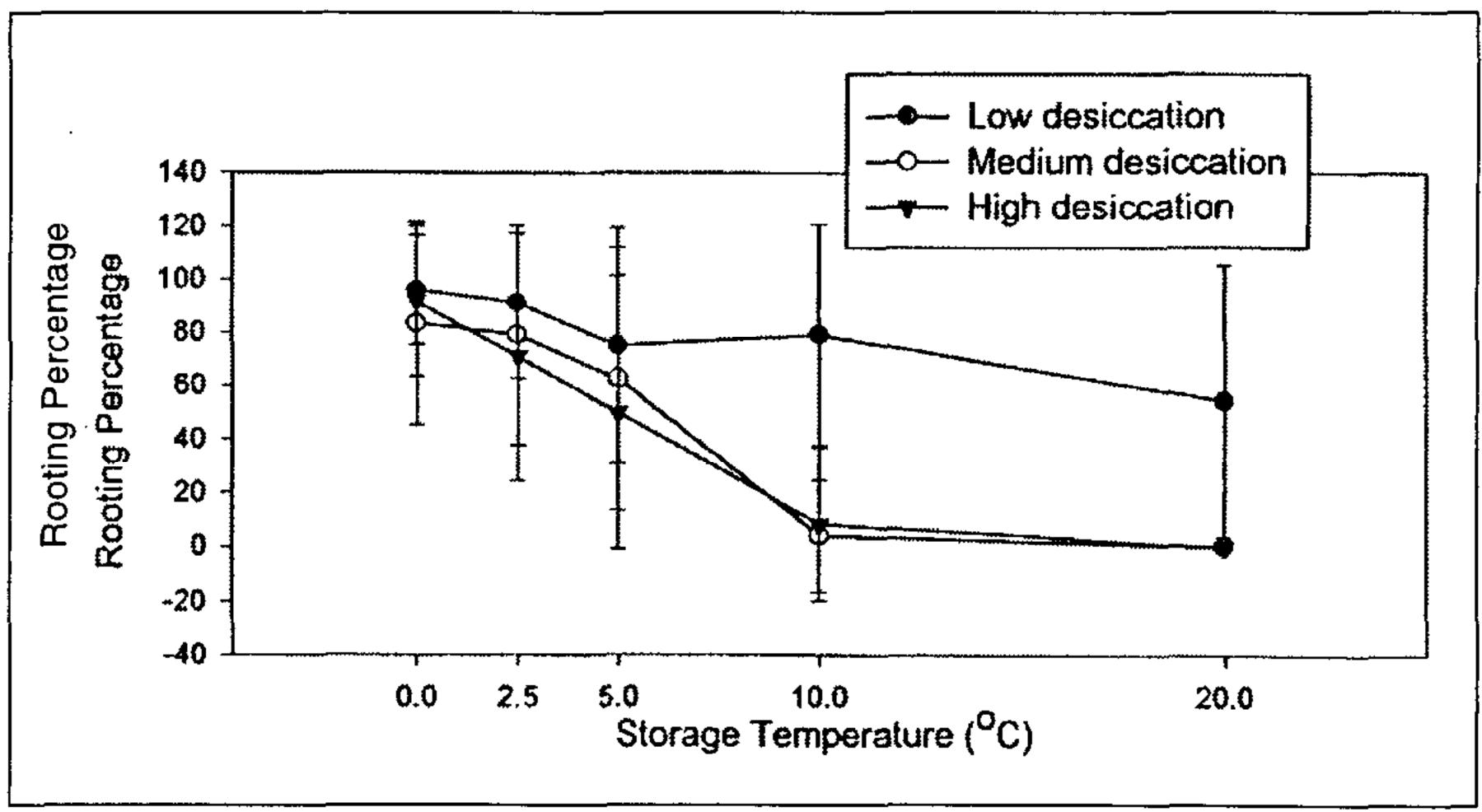
Four cultivars of *T.* ×*media* (Brownii, Dark Green Spreader, Hicksii, and Wardii) were selected to study storage conditions, as measured by chlorophyll fluorescence. Storage condition treatments consisted of desiccation (low, sealed plastic bag; medium, perforated plastic bag; and high, open plastic bag), duration (34, 70, and 107 days), and temperature (-2.5, 0, 2.5, 5, 10, and 20°C). Propagation was performed as previously described, except for the various storage treatments. Chlorophyll fluorescence was measured at cutting collection and sticking of each duration. Data was collected over two propagation seasons.

## **RESULTS AND DISCUSSION**

Initial chlorophyll fluorescence values ( $F_v/F_m$ ) ranged from a high 0.833 (relative units) in 'Dark Green Spreader' to a low 0.778 in 'Brownii' (Table 1). Significant differences existed between some cultivars, however, there was much overlapping between them. Fluorescence levels dropped dramatically during cold storage,



**Figure 1.** Chlorophyll flourescence in *Taxus* over the course of propagation at Zelenka Nursery 1998-1999 Season.



**Figure 2.** Effect of storage temperature and desiccation on rooting in Taxus 'Hicksii' 1997-1998. Storage duration = 34 days

**TABLE 1.** 1998-1999 Initial chlorophyll fluorescence ( $F_v/F_m$ ) of 10 cultivars of  $Taxus \times media$  from stock plant material at Zelenka Nursery.

Cultivar	$F_{v}/F_{m}$								
Dark Green Spreader	0.833	a	b	c	d				
Hicksii	0.832	a	b	$\mathbf{c}$	d	e			
Densiformis Gem	0.828	a	b	$\mathbf{c}$	d	e			
L.C. Bobbink	0.825	a	b	$\boldsymbol{c}$	d	$\mathbf{e}$	$\mathbf{f}$		
Runyan	0.822	a	b	c	$\mathbf{d}$	e	f	g	
Densiformis	0.821		b	$\mathbf{c}$	d	e	$\mathbf{f}$	g	
Wardii	0.814			$\mathbf{c}$	d	e	f	g	
Tauntoni	0.811				d	e	f	g	
Brownii	0.778								h

Mean separation among cultivars by LSD, P 0.05.

Means with the same letter are not significantly different

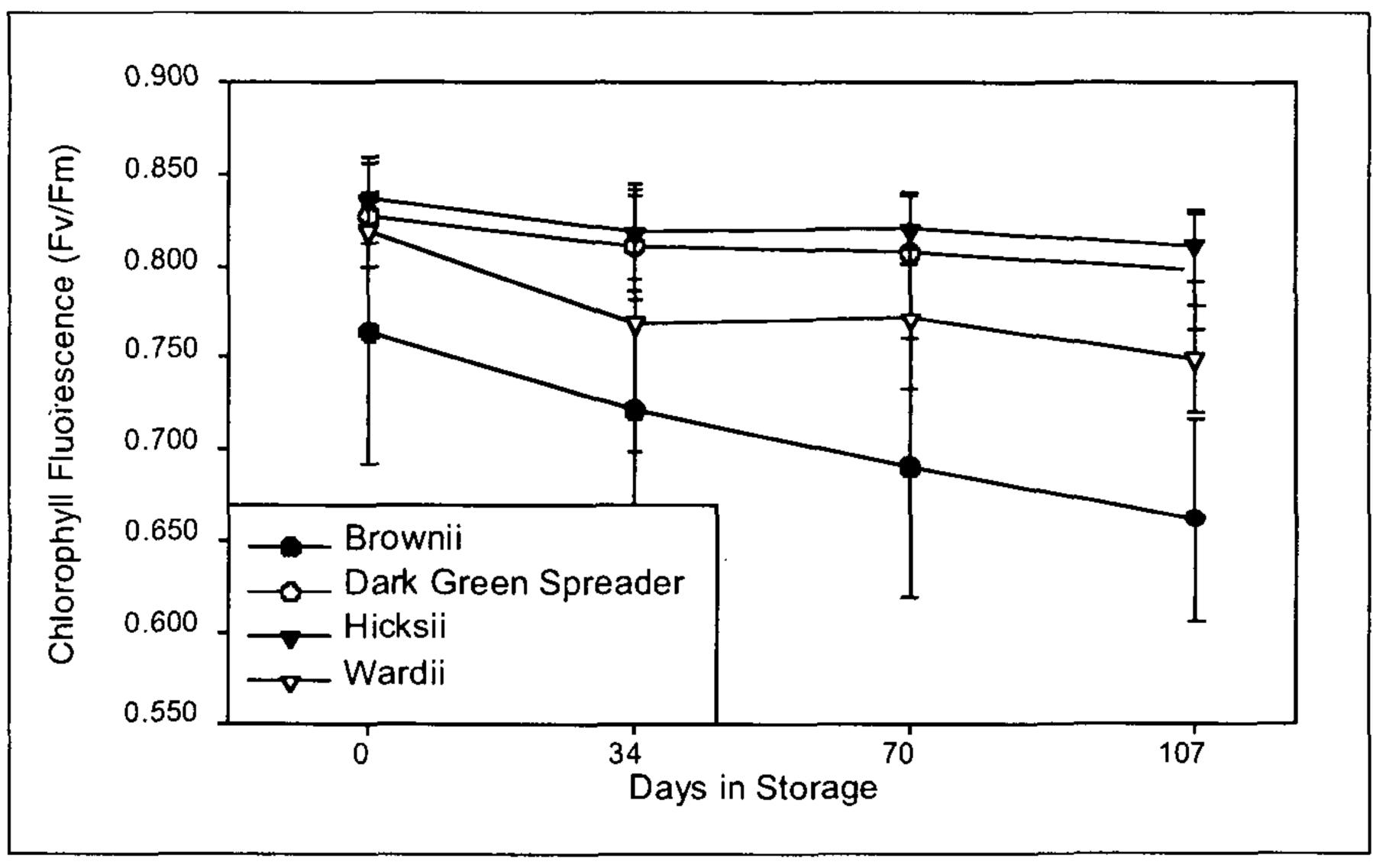
**TABLE 2.** 1998 -1999 Rooting percentage of ten cultivars of  $Taxus \times media$  at Zelenka Nursery after 32 days cold storage at 5°C and 100 days in the propagation bed.

Cultivar	Rooting %							
Brownii	96.6	a	b	c				
L.C. Bobbink	95.0	a	b	c	d			
Hicksii	86.7	a	b	c	d	e		
Densiformis Gem	83.3		b	$\mathbf{c}$	d	е	f	
Tauntoni	75.0			$\mathbf{c}$	$\mathbf{d}$	e	f	
Wardii	71.7				$\mathbf{d}$	e	f	
Dark Green Spreader	48.5							g
Runyan	46.7							g
Densiformis	45.0							g

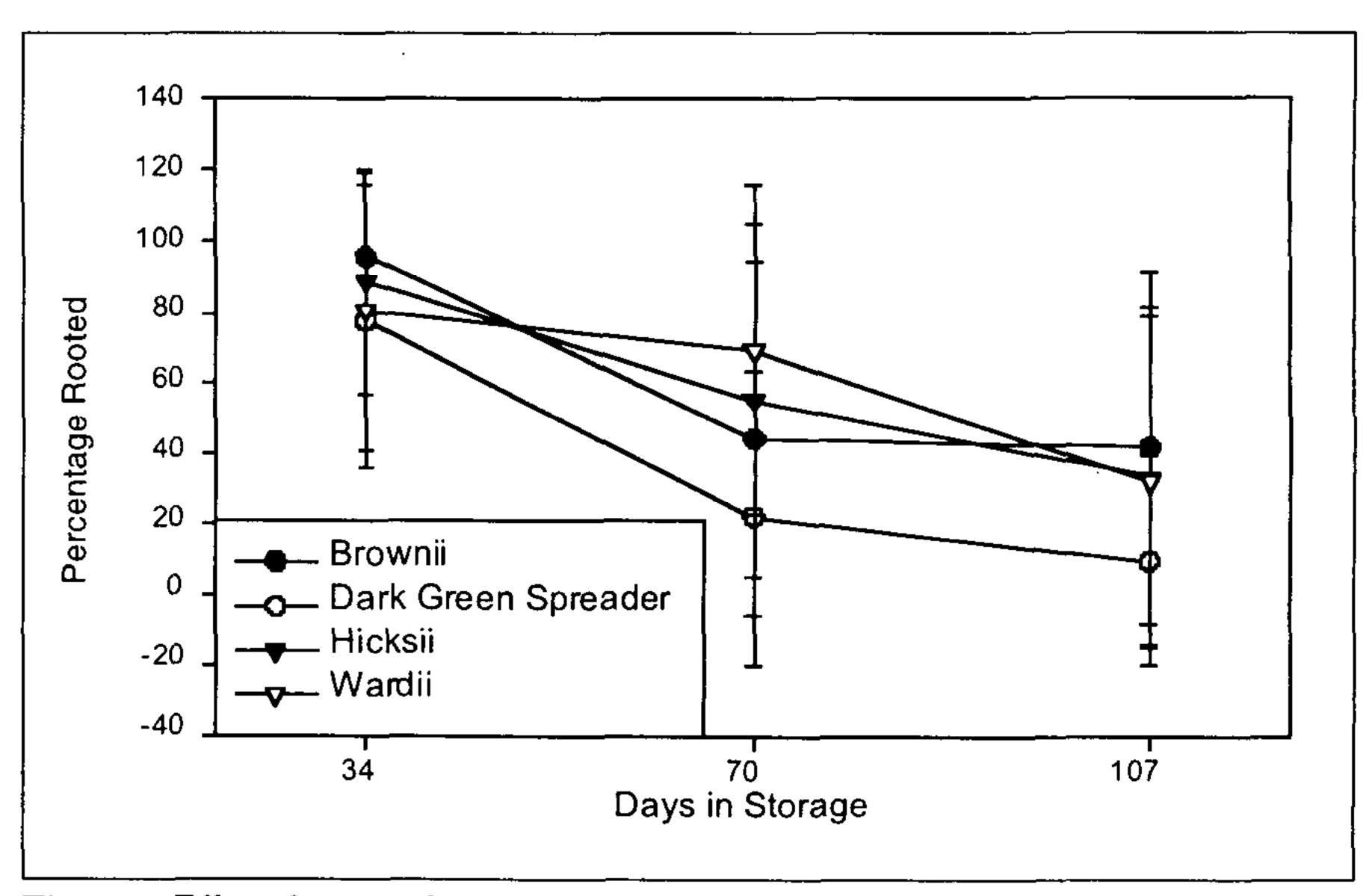
Mean separation among cultivars by LSD, P 0.05.

Means with the same letter are not significantly different

demonstrating dormancy effects, and then rose quickly to pre cold storage levels within a month after sticking (Fig. 1) and remained high until harvest. Rooting percentages ranged from 96.6% ('Brownii') to 45% ('Densiformis') (Table 2). No strong correlations were found between initial  $F_{\rm v}/F_{\rm m}$  and rooting percentage, root number, root length, or root dry weight.



**Figure 3.** Effect of storage duration on chlorophyll fluorescence (Fv/Fm) of four cultivars of Taxus. 1998-1999 Season, storage temperature = -2.5 - 2.5 °C



**Figure 4.** Effect of storage duration on rooting of four cultivars of Taxus 1998-1999 Season. Storage temerature = -2.5 - 2.5 °C. Rooting percentages measured 96-99 days after sticking.

High desiccation levels and high temperatures during storage inhibited rooting, however, at low desiccation relatively high rooting occurred at 10°C (Fig. 2). Temperature treatments of 0, 2.5, and 5°C did not result in differing rooting responses, and desiccation did not affect these cooler temperatures. Chlorophyll fluorescence read-

ings were reduced in 20°C treatments but remained indistinguishable for 0 to 10°C treatments. Similar results were obtained for all four cultivars, so only data for 'Hicksii' is presented.

To test effects of storage duration, cuttings were stored for 34, 70, and 170 days at temperatures of -2.5, 0, and 2.5°C. There were no significant differences among temperatures, so data was combined across temperatures. Chlorophyll fluorescence levels did not decline appreciably with longer durations in storage (Fig. 3). Longer storage durations did lead to decreases in rooting percentage (Fig. 4), root number, root length, and root dry weight. Rooting percentages were often halved with a doubling of the storage duration (34 to 70 days). 'Wardii' showed the most initial resistance to extended storage (at 70 days), however, with the longest duration (107 days), it also showed significantly diminished rooting.

#### CONCLUSIONS

Correlations were not found between initial  $F_v/F_m$  and rooting percentage, root number, root length, or root dry weight, indicating that  $F_v/F_m$  is not a reliable indicator of stockplant propagation potential. Short storage durations at temperatures ranging from -2.5 to 2.5°C were found to be ideal. Higher rooting could be maintained at 5°C and possibly 10°C when combined with low desiccation. Longer storage durations led to significant decreases in rooting and root quality.  $F_v/F_m$  could detect substandard storage conditions only at temperature and desiccation extremes.

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#### LITERATURE CITED

- Adams, W.W. III, B. Demmig-Adams, K. Winter, and U. Schreiber. 1990. The ratio of variable to maximum fluorescence from photosystem II, measured in leaves at ambient temperature and at 77K, as an indicator of the photon yield of photosynthesis. Planta 180:166-174.
- Camm, E.L. and D.P. Lavender. 1993. Photosynthetic apparatus in cold-stored conifer seedlings is affected by nursery and storage photoperiod. Forest Sci. 39(3):546-560.
- **Fisker, S., R. Rose,** and **D.L. Haase.** 1995. Chlorophyll fluorescence as a measure of cold hardiness and freezing stress in 1+1 Douglas-Fir seedlings. Forest Sci. 41(3):564-575.
- Genty, B., J.M. Briantais, and N.R. Baker. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophy. Acta 990:87-92.
- **Hawkins, C.D.B.** and **G.R. Lister.** 1985. In vitro chlorophyll fluorescence as a possible indicator of the dormancy state in Douglas-fir seedlings. Can. J. For. Res. 15:607-612.
- **Lindgren, K.** and **J.E. Hallgren.** 1993. Cold acclimation of *Pinus contorta* and *Pinus sylvestris* assessed by chlorophyll fluorescence. Tree Physiol. 13:97-106.
- Westin, J., L. G. Sundblad, and J. E. Hallgren. 1995. Seasonal variation in photochemical activity and hardiness in clones of Norway spruce (*Picea abies*). Tree Physiol. 15(10):685-689.