Recovery kinetics of winter stressed conifers: The effects of growth light environment, extent of the season, and species.

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Introduction:

In overwintering evergreen plants, the process of xanthophyll cycle-mediated energy dissipation changes from one that rapidly responds to alterations in excessive absorbed excitation energy in the summer months, to a long-term sustained engagement of energy dissipation that does not respond to a changing light environment during winter (Verhoeven et al. 1998, 1999a; Adams et al. 2001, Öquist and Huner 2003). The winter-induced sustained energy dissipation appears to be critical in maintaining the balance between light absorption and its reduced utilization due to low temperature effects on photosynthetic carbon reduction. This transformation of xanthophyll cycle characteristics appears to involve changes in the composition and characteristics of the photosynthetic apparatus such that there is a functional change from "light harvesting centers" to "dissipating centers" (Öquist and Huner 2003).

Evidence for changes in the composition and characteristics of the photosynthetic apparatus include a seasonal study of *Pinus sylvestris* (Ottander et al. 1995), and a study looking at its acclimation to low temperatures in a growth cabinet (Savitch et al. 2002). Both studies demonstrated that winter acclimation involves decreases in the D1 protein of the PSII reaction center and its light harvesting complexes (LHCs), in addition to increases in the PsbS protein, implicated as a key protein in facilitating xanthophyll cycle-mediated energy dissipation.

In addition to reorganization of the proteins within the photosynthetic apparatus, some studies have suggested that thylakoid protein phosphorylation may be involved in maintaining sustained energy dissipation. Correlations between sustained energy dissipation and dark-sustained phosphorylation of the D1 protein of PSII have been demonstrated in photoinhibited leaves of the shade plant *Monstera deliciosa* (Ebbert et al., 2001), and in the evergreen Douglas fir, measured on subfreezing winter nights (Ebbert et al., 2005).

The goal of this study was to examine seasonal changes in the relative amount of all of the individual light harvesting proteins of both photosystems (and their phosphorylation status) in an overwintering evergreen growing in both low and high light environments, in order to gain further insight into the nature of the dissipating centers. Here we report preliminary data from this ongoing study examining the evergreen Balsam fir (*Abies balsamea* L. Mill.) growing in sun and shade environments in the seasonally very cold climate of Saint Paul, Minnesota.

Methods:

Plant Material and Growth Environment

Four species of conifers (sun and shade needles) were monitored from November of 2007 through February of 2008: eastern white pine (*Pinus strobus* L.), balsam fir [*Abies balsamea* (L.) P. Mill], *Taxus cuspidata* (L.) and blue spruce (*Picea pungens* Engelm.). All plants were growing on the campus of the University of St. Thomas, in Saint Paul, Minnesota (44°59'40"N, 93°05'35"W). For each species, three individual trees were sampled on each date. For pine, only sun needles were sampled, while for the remaining three species both sun and shade needles were sampled.

Monitoring Recovery Kinetics of Winter-Stressed Needles

F_v/F_m was measured on dark acclimated needles in the field. Measurements were done at around 9PM, when all trees/shrubs had been in darkness for at least three hours. For each species, two measurements were conducted on each of three trees/shrubs. After measuring Fv/Fm, the twig on which the measurement was made was cut and placed in a Petri dish. After all samples were measured in the field, the Petri dishes were taken indoors to room temperature and low light (~20°C and light between 5 and 10 μmol photons $m^{-2}s^{-1}$). Once indoors, all needles were organized in Petri dishes with moist paper towels where they were maintained in constant conditions for up to six days. After the needles had warmed for thirty minutes, F_v/F_m was measured again to determine if tehre was any rapid component to the recovery. Needles were subsequently measured after 1.5, 10, 24, 48, 72, 96 and 144 hours of recovery.

Statistical Analysis

In order to determine if there was a rapid component to recovery, paired t-tests were performed on the Fv/Fm values collected in the field compared with the Fv/Fm values measured after 30 minutes warming using Microsoft Excel. In order to determine if there were changes in the kinetics of recovery over the course of the season, if there were differences between sun and shade needles in their recovery kinetics, and if there were differences between species in recovery response, data was analyzed after 24 hours and 100 hours of recovery. For each species, % recovery after 24 hours was calculated as (F_v/F_m at 24 hours - F_v/F_m at time 0)/(0.8 - F_v/F_m at time 0). For shade needles, 0.84 was used instead of 0.8, to indicate the fully recovered value of Fv/Fm. These values were picked based on typical values for unstressed F_v/F_m observed in the field. Recovery after 100 hours was calculated in the same manner. Values for recovery after 24 and 100 hours were analyzed with the software package SAS using a mixed model ANOVA, with date and species as fixed effects. Additional comparisons were done using the Tukey–Kramer HSD comparison.

Results:

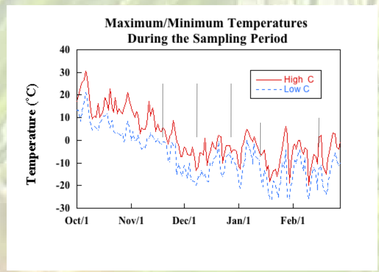
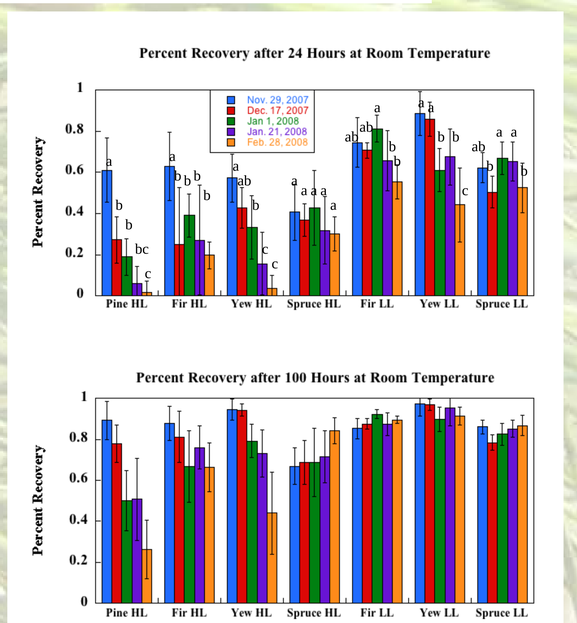


Figure 1. Maximum and minimum temperatures during the sampling period with arrows indicating the approximate dates of sampling.

Table 1. Results of paired t-tests comparing F_v/F_m measured at time 0 in the field with F_v/F_m measured after 30 minutes of warming at room temperature.

		Pine	Fir		Yew		Spruce	
		Pine	Fir		Yew		Spruce	
Temp	Date:	sun	sun	shade	sun	shade	sun	shade
-5	Nov. 29, 2007	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
-6	Dec. 17, 2007	0.092	0.807	**0.001**	0.378	**0.012**	0.527	**0.009**
-16	Jan. 1, 2008	0.99	**0.002**	**0.0001**	0.6	**0.0001**	**0.0013**	**0.00001**
-13	Jan. 21, 2008	0.092	0.807	**0.001**	0.378	**0.012**	0.527	**0.009**
-5	Feb. 28, 2008	0.99	0.52	**0.001**	0.91	0.33	0.59	**0.001**

Figure 2. Percent recovery after 24 (A) and 100 (B) hours of recovery. For sun needles 100% recovery was an Fv/Fm of 0.8, while for shade needles the value was set at 0.84. The letters indicate if there are significant differnces between sampling dates within a species.



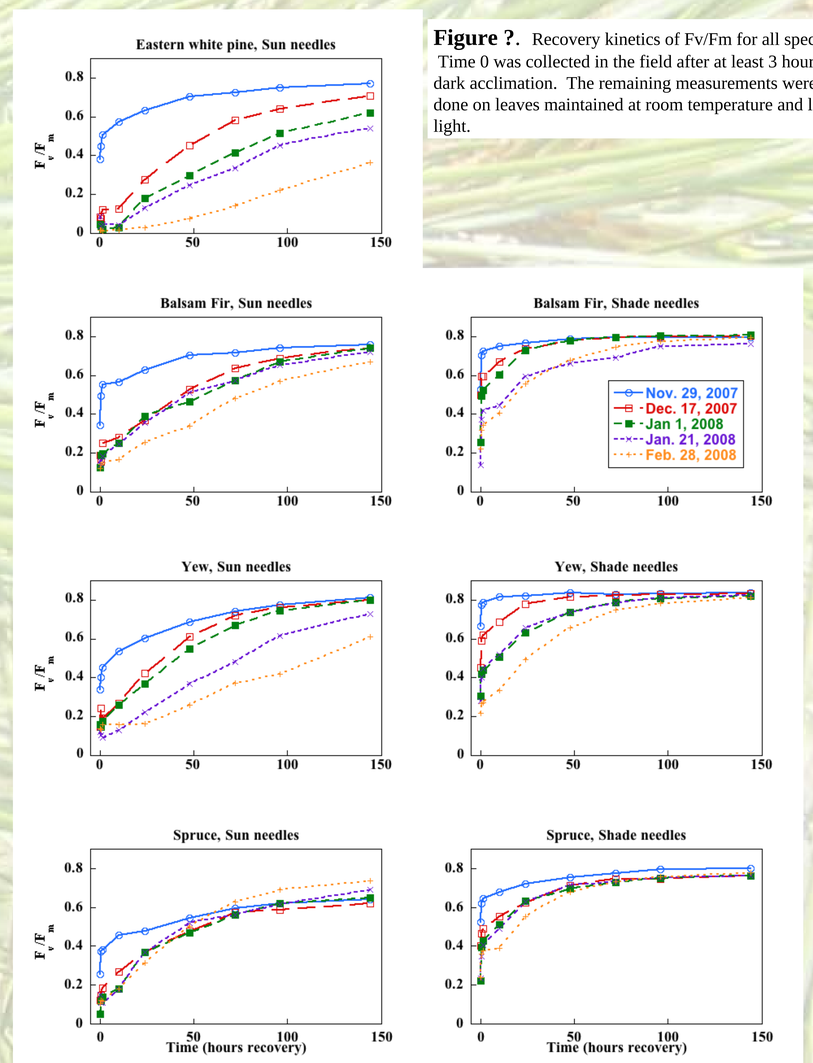


Figure ?. Recovery kinetics of Fv/Fm for all species. Time 0 was collected in the field after at least 3 hours of dark acclimation. The remaining measurements were done on leaves maintained at room temperature and low light.

Discussion/ Conclusions:

- Our preliminary results have demonstrated that there are changes in the relative amounts of individual light harvesting proteins of both photosystem I and photosystem II that occur seasonally.
- Results from a preliminary analysis of the phosphorylation status of the photosynthetic proteins indicate pronounced changes from summer to winter in either the intensity of phosphorylation of a given protein, or in which proteins are phosphorylated. We are in the process of identifying each of the phosphorylated proteins.
- These results are consistent with the hypothesis that there is a structural reorganization occurring in the light harvesting complexes during acclimation to winter stress that accompany the functional change from light harvesting to dissipating centers (Öquist and Huner, 2003). The results are suggestive that proteins that increase during winter (Lhcb2, PsbS) or stay the same or decrease slightly (Lhca2, Lhcb5) may play a role in facilitating the dissipation process relative to light harvesting. Additionally, changes in phosphorylation of the photosynthetic proteins may play a role in this structural reorganization.

Lite
Adams
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vironment during the winter. Photosynth Res 67: 51-62
d thylakoid protein phosphorylation. Photosynth Research 67: 63-78
D1 phosphorylation in zeaxanthin-retaining, photoinhibited needles of overwintering
Jansson S (1994) The light-harvesting chlorophyll *a/b*-binding proteins. Biochim Biophys Acta 1184: 1-19
Öquist G and Huner NPA (2003) Photosynthesis of overwintering evergreen plants. Annu Rev Plant Biol54: 329-55
Ottander C, Campbell D, Öquist G (1995) Seasonal changes in photosystem II organization and pigment composition in *Pinus sylvestris*. Planta 197:176-183
Savitch LV, Leonardos ED, Krol M, Jansson S, Grodzinski B, Huner NPA, Öquist G (2002) Two different strategies for light utilization in photosynthesis in relation to growth and cold acclimation. Plant Cell and Environment 25: 761-771
Verhoeven AS, Adams WWIII, Demmig-Adams B (1998) Photoinhibition during winter stress in sun and shade *Euonymus kiautschovicus* leaves; the xanthophyll cycle and leaf adenylate status. Plant Cell and Environment 21: 893-903
Verhoeven AS, Adams WWIII, Demmig-Adams B (1999a) The xanthophyll cycle and acclimation of *Pinus ponderosa* and *Malva neglecta* to winter stress. Oecologia 118: 277-287

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