Antioxidant Activity in Summer Versus Winter in Three Species of Conifers

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Abstract:

Evergreen plants that grow in seasonally cold climates deal with an imbalance of light absorption and utilization because much less photosynthetic activity occurs at low temperatures. In these conditions photoprotective mechanisms, such as the antioxidant systems in plants, are crucial. Previous work has shown that glutathione and the enzyme glutathione reductase (GR), components of the ascorbate-glutathione cycle, increased in winter stress in Taxus cuspidata needles. Other components of the ascorbate-glutathione cycle did not increase. This led to the hypothesis that GR and glutathione have a unique role in winter stress that is separate from their role in the ascorbate-glutathione cycle. The present study aims to determine whether this change in antioxidant response during winter is common for various plant species or a species-specific trend. We examined the level of glutathione and the activity of GR and of the enzyme ascorbate peroxidase (APX) in winter and summer conditions in three conifer species: eastern white pine (*Pinus strobus L.*), balsam fir [*Abies balsamea (L.) P. Mill*] and white cedar (*Thuga occidentalis L.*). The enzyme results indicate that GR increased significantly in all three species during winter, while APX increased in pine and cedar but not in fir. Results from glutathione show significant increases in pine, while results from the other species are pending. The data suggest that different species have distinct strategies for antioxidant acclimation to cold stress: pine and cedar apparently up-regulate their ascorbate-glutathione cycle, while fir up-regulates GR but not APX, as seen in *Taxus cuspidata*.

Introduction:

At the low temperatures characteristic of a Minnesota winter, plants absorb a lot more light than they use to power photosynthesis because photosynthetic rates are reduced in cold stress. This imbalance causes stress that exacerbates the production of reactive oxygen species that are harmful to the cells. The cells mitigate these harmful effects by using antioxidant systems. The ascorbate-glutathione cycle is one such antioxidant pathway that reduces superoxide to water over a series of chemical reactions. This is illustrated in Figure 1 (Asada, 1999). Previous work has shown that two components of this cycle, glutathione and glutathione reductase (GR) increased in winter stress in *Taxus cuspidata* needles, but other components of the ascorbate-glutathione cycle did not increase. (Verhoeven, 2004) This led to the hypothesis that GR and glutathione have a unique role in plant adaptations to winter stress that is different from their role in the ascorbate-glutathione cycle. The present study aims to determine whether this observed increase in GR and glutathione in winter, but not the other components of the ascorbate-glutathione cycle, is a response of conifers in general or if there are species-specific effects.

Methods:

Plant material

White Pine (*Pinus strobus L.*) was collected at Cedar Creek Ecological Station in summer (7/31/2009) and winter (01/12/2010). White cedar (*Thuga occidentalis L.*) was collected at the University of St. Thomas Saint Paul Campus in summer (08/12/2009) and winter (01/08/2010). Balsam Fir [*Abies balsamea (L.) P. Mill*] was collected at St. Croix Bluffs Regional Park in summer (09/21/09) and winter (01/28/2010). Light, temperature and the chlorophyll fluorescence parameter ΦPSII from each tree was recorded at the time of collection. (Table 1). All measurements were taken between 11AM and 1PM on days that were mostly sunny.

Glutathione

Plant material was weighed and ground using a mortar and pestle in 7% sulflosalicylic acid with polyvinylpyrrolidone (PVP:40) in the acid solution. Plant extracts were centrifuged and then neutralized using Triethanolamine (2,2',2''-Nitrilotriethanol) immediately before being assayed using a spectrophotometer at absorbance 412 as described in Griffith, 1999. We were unable to obtain results for cedar using this protocol, likely due to interference from secondary metabolites.

Glutathione Reductase (GR) & Ascorbate Peroxidase (APX)

Plant material was ground in the following extraction buffer: 50mM KH_2PO_4, 0.1 mM EDTA, 0.3% (w/v) Triton X-100, 4% (w/v) soluble PVP-10, 1.3 mM ascorbic acid, pH 7.6. Plant extract samples were assayed using a spectrophotometer according to Grace and Logan, 1996.

Ascorbate

Plant material was ground in 6% perchloric acid with PVP:40, partially neutralized to pH 1-2 with sodium carbonate, and assayed using a spectrophotometer at absorbance 265 according to Grace and Logan, 1996.

Statistics: A t-test was run (using Microsoft Excel) to find statistically significant differences between summer and winter.

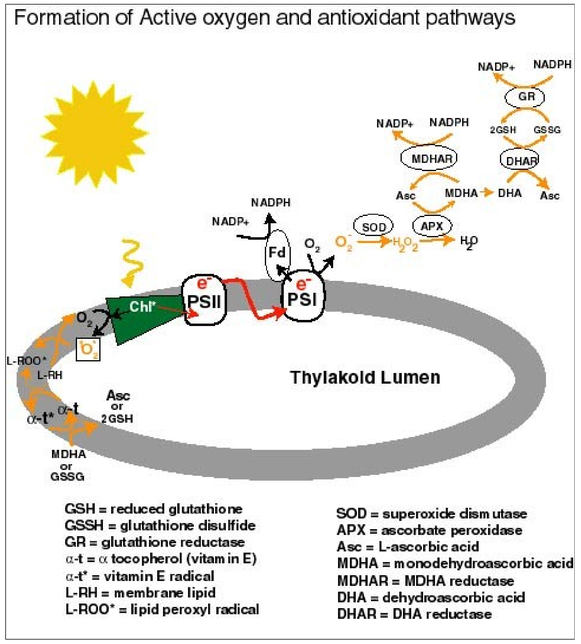

Figure 1. Simplified diagram of antioxidant pathway with ascorbate-glutathione cycle. Adapted from Asada 1999.

	Summer			Winter		
	Light	Temp (˚C)	ΦPSII	Light	Temp (˚C)	ΦPSII
Pine	1743 ±136	27±1.8	0.321±0.045	820±360	-8±1.2	0.009±0.013
Cedar	1606±250	30±2.2	0.311±0.094	1070±520	-12±2.2	0.012±0.012
Fir	320±190	20±1	0.685±0.156	1025±290	-16±1	0.002±0.004

Table 1. Light, temperature, and the chlorophyll fluorescence parameter ΦPSII for all species in both summer and winter.. Plant material was collected between 11 AM and 1 PM. Data are averages ± standard deviation, n=6-10.

Results and Conclusions:

In all species we found significant increases in the activity of GR in winter compared to summer (Figure 3A). Additionally, in the two species for which we could measure glutathione, we found significant winter increases in total glutathione (Figure 3C). Significantly higher APX activity in winter versus summer was found in pine and cedar but not fir (Figure 3B). Significantly higher ascorbate levels in winter versus summer were only found in pine (Figure 3D).

The differences shown between the antioxidant response in summer versus winter of the three species in the present study and a previous study examining *Taxus cuspidata* (Verhoeven 2005), suggest that there are species specific patterns of regulation of the antioxidant system to deal with cold winter stress. While all species examined seem to up-regulate glutathione and the enzyme GR in winter, up-regulation of ascorbate and APX were variable depending on the species. This indicates, in fir and *Taxus*, a possible role of glutathione which is independent of the ascorbate-glutathione cycle.

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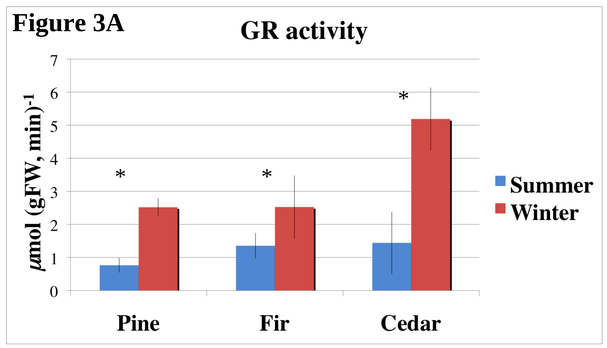
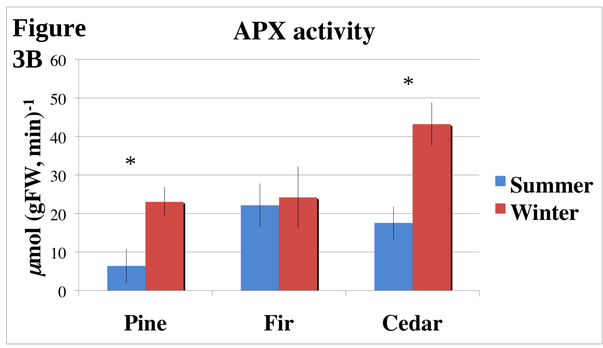
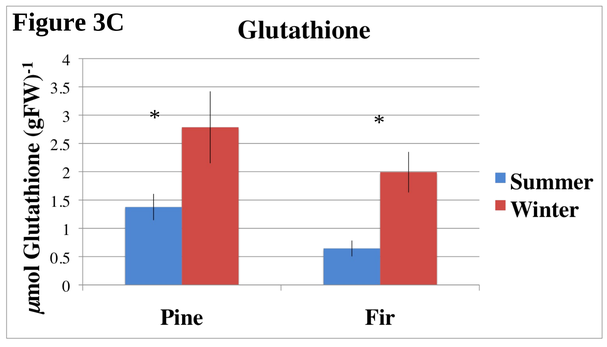
Figure 2. From top to bottom: Picture of pine, fir, and cedar.

Results:







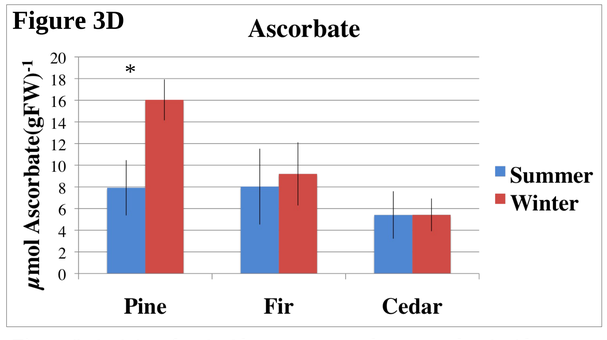


Figure 3. Activity of antioxidant enzymes and amount of antioxidant metabolites expressed as a function of leaf fresh weight in summer compared to winter. Error bars represent the standard deviation from the mean for that sample group (n=6-10). An asterisk indicates a significant difference between summer and winter.