# Photosynthetic Capacity and Leaf Reorientation in Two Wintergreen Ferns, Polystichum acrostichoides and Dryopteris intermedia

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ABSTRACT.—Some ferns growing in areas with severe winters maintain green leaves through the winter; however, very little is known about the physiology of their remarkably hardy leaves, and the function of the wintergreen trait has not been completely established. Both *Polystichum acrostichoides* and *Dryopteris intermedia* retain high concentrations of chlorophyll (2.4 mg/g fresh weight) and a high quantum efficiency (0.6–0.8) in photosystem II. Even after a winter, the *P. acrostichoides* leaves show a significant photosynthetic rate (ca. 2  $\mu$ moles CO<sub>2</sub>/m<sup>2</sup>/sec). During fall, a 1 to 2-cm zone in the lower part of the stipe senesces and softens forming a hinge that allows the leaves to sink to the ground. This prostration does not cut off their xylem flow, and it apparently minimizes their exposure to cold and desiccation.

A number of ferns growing in temperate climates with a cold winter are wintergreen (Wherry, 1961), and this appears to represent an advantageous adaptation. Wintergreen refers to plants that maintain green leaves through a single winter, after which they die as they are replaced by a new set of leaves (Wagner, 1996). In Michigan, 24 (31%) species of ferns have fully wintergreen leaves, 4 (5%) are partially wintergreen, and 49 (64%) have leaves that die before winter each year (W. H. Wagner, unpublished data).

One obvious possible value of the wintergreen trait is extension of the photosynthetic period, but this trait may also enable the plants to retain limiting nutrients and to redistribute them from the old leaves to the new leaves more efficiently (Moore, 1984; Van Buskirk and Edwards, 1995). The importance of wintergreen leaves as photosynthetic organs in climates with severe winters has been questioned (Moore, 1984). Nonetheless, the value of the wintergreen leaves is clearly demonstrated in *Dryopteris intermedia* (Muhl.) A. Gray where removal of the old leaves in the early spring (April 1) before the new growing season greatly suppresses (60% reduction) the new growth (Van Buskirk and Edwards, 1995). Exactly what these leaves contribute after April 1 and their role in general remains to be established.

Not only is the wintergreen phenomenon an interesting aspect of natural history that needs further study, but the wintergreen leaves appear to be resistant to some extreme environmental stresses, and understanding the nature of this resistance may be of practical value in offering ways to engineer crop plants to increase stress resistance.

Both the Christmas fern, *Polystichum acrostichoides* (Michx.) Schott, and the "evergreen" or glandular wood fern, *Dryopteris intermedia* (Muhl. ex Willd.) A Gray, remain green and fresh in appearance through the winter in northeastern U.S.A.; however, only very limited data are available on their photosynthetic activity during this time (Minoletti and Boerner, 1993). This paper examines in a preliminary way their winter photosynthesis and some related phenomena. We also address some of the remarkable mechanical adaptations that these ferns appear to use to protect their leaves and their photosynthetic machinery from damage by the cold and desiccation that occur during winter.

# METHODS

Whole *Polystichum acrostichoides* plants or leaves alone were collected from one isolated clump of plants in an oak forest near Ann Arbor. *Dryopteris intermedia* leaves were collected from a rich, mixed woodlot near Ann Arbor. The plants or whole detached leaves were stored in darkness at 5°C in a plastic bag with a moist (not wet) paper towel until they were analyzed within 48 hr.

Chlorophyll was extracted by placing the leaflets in dimethylformamide and quantified spectrophotometrically as described by Canfield et al. (1995). Photosynthesis was measured as net  $CO_2$  uptake at 20°C, 20% relative humidity, and 600 mol/m<sup>2</sup>/sec red light (ca. 670 nm) with an infrared gas analyzer, a LI-COR LI 6400 portable photosynthesis meter (LI-COR, Inc., Lincoln, NB) as described by Guiamét et al. (1990). The quantum efficiency (Fv/Fm) of photosystem II was measured at 25°C with an OS-500 Modulated Fluorimeter (PP Systems, Haverhill, MA 01830).

To study xylem flux and the integrity of the xylem system, leaves were cut at their base, below the trophopod (the swollen basal region in the leaf axis [cf. Wagner and Johnson, 1983 and Fig. 1B]), and they were placed in a solution of the vital dye trypan blue (0.4% w/v in water [Artis et al., 1985]). The leaves were supported vertically in an indoor laboratory at 25°C, 25% relative humidity and 11 mol/m<sup>2</sup>/sec from cool white fluorescent lights.

## RESULTS

During the fall (November in the Ann Arbor, Michigan area), the leaves of *P. acrostichoides* and *D. intermedia* decline from an upright to a prostrate posture (Fig. 1A). This change is caused by softening of a 1–2 cm zone in the lower stipe above the trophopod (Fig. 1B), and, thereafter the stipe no longer supports the weight of the leaf, forming a hinge and bending at the softened zone. In contrast to the rest of the stipe, this softened zone can be compressed easily by squeezing it between the fingers. The surface of this hinge zone is brown-colored, whereas the neighboring stipe surface is green. Except for the vascular sheath, the cells in the hinge are dead, and they are friable, i.e., loose-ly connected.

If dye (trypan blue) was supplied through the base of the stipe cut off below this soft zone, the dye was pulled up into the leaf blades by transpiration. When the stipe was viewed in cross section, it could be seen that the dye was restricted to the xylem, i.e., it did not leak out into the surrounding tissues

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FIG. 1. *Polystichum acrostichoides* leaves. A) Prostration of the leaves; B) Location of the hinge (soft zone) that causes the prostration; C) Cross sections of the stipe showing the vascular bundles (circles) and dye location marked by Xs inside the circles. In order to simplify and clarify these diagrams (A), only the major axes are shown; the leaflets have been omitted.

(Fig. 1C). Unlike the other stipe tissues, most of the tissue in this elbow was dead and quite friable. As would be expected from the permeability results, the sheath around the vascular tissue appeared to be alive.

The chlorophyll concentrations in these leaves in late winter (Feb. 12, 1996) following some periods of relatively low temperature (ca.  $-10^{\circ}$ C) without snow cover remained high, on par with soybean, *Glycine max* (L.) Merr. (Table 1) during its peak photosynthesis. Soybean was selected for comparison here, because of its high photosynthetic activity. The chlorophyll a/b ratios were 2.35 and 2.03 for *P. acrostichoides* and *D. intermedia* respectively compared with 2.95 for *G. max*. At this time, *P. acrostichoides* and *D. intermedia* showed PSII quantum yield of 0.6–0.8, which is similar to 0.8 for *G. max* (Table 2).

Later, in early spring (Apr. 5), most of the leaves of *P. acrostichoides* were still fresh and green in appearance, and they carried on net  $CO_2$  fixation at a rate of about 2 mol/m<sup>2</sup>/sec at 20°C compared with about 20 mol/m<sup>2</sup>/sec for *G. max* at 25°C (Table 3). Interestingly, discolored (darkened) *P. acrostichoides* leaves still had substantial amounts of chlorophyll (50–75%), and the chlorophyll a/b ratios were similar to the normal, green leaves, yet they were essentially inactive in terms of  $CO_2$  uptake or release (Table 3).

During late winter through mid spring, i.e., Feb. 12 through Apr. 27, the leaves of *P. acrostichoides* not only failed to increase their dry weight (biomass), but they actually lost some dry matter (Table 4).

company they there be	mg Chl/g fresh weight	g Chl/cm <sup>2</sup>	Chl a/b
Polystichum acrostichoides	$2.4 \pm 0.2$	44.3 ± 2.7	$2.35 \pm 0.04$
Dryopteris intermedia	$2.3 \pm 0.05$	N.D.	$2.03 \pm 0.06$
Glycine max	N.D.	50 <sup>a</sup>	2.95 <sup>a</sup>

TABLE 1. Chlorophyll concentrations in the leaves of two stay-green ferns in late winter (Feb. 12, 1996) compared to soybean. <sup>a</sup>Data from Guiamét et al. (1991).

## DISCUSSION

Those wintergreen ferns that grow in climates having a cold winter are particularly impressive among wintergreen species. They seem to be delicate, yet they are able to endure very harsh winter conditions, i.e., cold and desiccation. One factor in their durability is their ability to reposition their leaves to a less exposed posture. During fall, *P. acrostichoides* and *D. intermedia* leaves change their posture from upright to prostrate (Fig. 1A). Although this change in the orientation of certain fern leaves has been noted incidentally (Parsons, 1899), it seems not to have been studied in any detail. This reorientation undoubtedly reduces their exposure to cold, drying winds (Givnish, 1982); however, these prostrate leaves could still carry on photosynthesis, at least while uncovered on warm, sunny days.

To produce this reorientation, a 1–2 cm zone in the stipe, near the ground but above the trophopod (Fig. 1B), softens and is unable to bear the weight of the leaf. This soft zone becomes a hinge causing the leaf to lie flat on the ground. Even though most of the supporting tissue in this hinge has died and collapsed, dye movement indicates that the vascular strands themselves are not blocked and remain functional. Moreover, because the dye used, trypan blue, is an indicator of cell integrity (Artis et al., 1985), we were also able to determine that the xylem symplastic domain retains its integrity, that is the solutes do not leak out. The hinge zone seems to form through a localized senescence or apoptosis.

Two quite different explanations have been proposed for the function/benefit of the wintergreen character (Moore, 1984; Van Buskirk and Edwards, 1995). First, the wintergreen leaves may extend the period of photosynthesis for these plants. It may be a considerable advantage for these understory species to carry on photosynthesis when the leaves of the forest canopy are absent; however, the plants may have to pay a "cost" for developing cold resistant leaves and for maintaining these leaves during the winter. A second idea relates to storage and transfer mechanisms. Basically, it may cost less for these organisms to retain limiting nutrients in their old leaves and redistribute them directly to new leaves after the winter (one transfer) than to transfer them first to specialized storage tissues in the rhizome and/or trophopod and then to new leaves in the spring (2 transfers). Each transfer step presumably has a cost. Removal of the foliage from *D. intermedia* during the winter shows that the old foliage makes an important contribution to the growth of the new leaves

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F <sub>v</sub> /F <sub>m</sub>	
0.55-0.71	
0.59-0.76	
0.78–0.81 <sup>a</sup>	

TABLE 2. Light-harvesting capacity (quantum yield of PSII) in the leaves of two stay-green ferns in late winter (Feb. 12, 1996) compared to soybean. <sup>a</sup>Unpublished data from Guiamét et al. (1995).

(Van Buskirk and Edwards, 1995), but this could involve either or both of the two mechanisms cited.

Determination of the chlorophyll concentrations shows that the wintergreen leaves of *P. acrostichoides* and *D. intermedia* maintain a high concentration of chlorophyll even into the spring. The chlorophyll a/b ratio in fern leaves is similar to but slightly lower than the ratio for *G. max* leaves at their photosynthetic peak. The lower chlorophyll a/b in ferns indicates that they have a slightly larger light-harvesting or antenna system which would be advantageous in shady conditions (Guiamét et al., 1991; Scheer, 1991; Sundqvist and Ryberg, 1993). The high quantum efficiency of photosystem II in these leaves indicates that these chlorophylls are not only retained, but they function efficiently (Scheer, 1991; Sundqvist and Ryberg, 1993; Bolhàr-Nordenkampf and Öquist, 1993). Clearly, the cold caused no significant damage to PSII, which is a particularly sensitive indicator of cold stress.

We and Minoletti and Boerner (1993) find that the leaves of *P. acrostichoides* also retain their capacity for  $CO_2$  fixation through the winter. The  $CO_2$  fixation rates we observed were only 10% of those measured for soybean leaves (Guiamét et al., 1990), and they were also less than other understory herbs (Minoletti and Boerner, 1993); however, our rates were similar to those reported by Minoletti and Boerner (1993) for *P. acrostichoides*. The fact that PSII and net  $CO_2$  fixation are active during late winter and early spring certainly supports the idea that wintergreen leaves could make an important photosynthetic contribution to the plant and to new growth in the spring. Nonetheless, the fact that these leaves fail to accumulate dry matter does raise some questions about what this photosynthesis contributes. If the production of new carbohydrate by photosynthesis exceeds the consumption of carbohydrate for self maintenance, then photosynthate must accumulate outside the leaf blades, e.g., in the rhizome and/or the trophopod.

These data do not exclude the second function of wintergreen leaves, i.e., temporary storage of mineral nutrients. Usually, but not always, leaf senes-

TABLE 3.	Photosynthetic rates and chlorophyll contents in the leaves of the stay-green fern (Polystichu	n
acrosticho	des) in early spring (April 5, 1996).	

ante musicory bor test	moles/m <sup>2</sup> /sec	g Chl/cm <sup>2</sup>	Chl a/b
Fresh green leaves Darkened (discolored)	1.81–2.24 0.06	39–59 29	2.20–2.35 2.21

Date	Dry weight (mg)/leaflet	Dry weight (mg)/cm <sup>2</sup>
Feb. 12, 1996	35 ± 3	$7.1 \pm 0.1$
Apr. 27, 1996	$18 \pm 2$	$5.1 \pm 0.3$

TABLE 4. Changes in the dry weight (biomass) of the leaves of the stay-green fern (*Polystichum acrostichoides*) during the late winter through early spring.

cence is accompanied by the removal/release of stored nutrients such as nitrogen and phosphorous (P) from the senescing leaves (Noodén, 1988a,b). A seasonal profile of phosphorous content as g P/kg dry matter in leaves has been reported for *P. acrostichoides* (Minoletti and Boerner, 1993), but their data do not permit clear conclusions about redistribution from the leaves. A measure of P/leaf or P/unit of leaf area would be better. Because the dry weight content of the leaves decreases, at least under our conditions, the small decreases reported for g P/kg dry matter could represent some redistribution of P within the plant; however, this decrease is small and is not reciprocally related to the growth of the new leaves. Thus, the mineral nutrient distribution in these plants needs further analysis, and the trophopods and rhizomes will have to be included. In addition, why these leaves survive the winter only to die after the weather becomes favorable is also an open question.

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