A Comparison of Physiological and Morphological Properties of Deciduous and Wintergreen Ferns in Southeastern Pennsylvania

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ABSTRACT.—Physiological and morphological properties of a deciduous, perennial fern (*Onoclea sensibilis*) and three wintergreen, perennial ferns (*Polystichum acrostichoides*, *Polypodium virginianum*, and *Dryopteris intermedia*) were examined using leaf fluorescence, chlorophyll a:b ratios, total chlorophyll content, water potential, and leaf edge to surface area ratios. *Onoclea sensibilis* differed significantly from the wintergreen ferns in morphology and physiology for almost every parameter measured. Interspecific differences were also observed within the wintergreen group. *Dryopteris intermedia* differed most within the wintergreen group and showed more similarity in physiology to *O. sensibilis*. *Dryopteris intermedia* was found occupying the same high-light, higher soil moisture habitat as *O. sensibilis*, which may indicate that inherent leaf morphology, physiological characteristics, and a wintergreen or perennial life cycle, play important roles in determining habitat preference.

Eastern hardwood forests are host to a plethora of understory plants, including 66 species of pteridophytes (Rhoads et al., 2000). In southeastern Pennsylvania, both deciduous perennial and wintergreen perennial ferns can be found in sympatry. Unlike deciduous perennial ferns, whose fronds undergo senescence during the fall and early winter, wintergreen perennial ferns maintain their fronds throughout the winter and do not begin to senesce until spring, when new fronds begin to unfold (Tessier, 2001).

Abiotic factors such as sunlight, available water, and substrate conditions can dramatically alter plant life history, distribution, growth, physiology and overall morphology (Brach et al., 1993; Smith et al., 1997). Greer et al. (1997) found in a southeastern Ohio hardwood forest that the distribution of pteridophytes was significantly influenced by moisture and soil nitrates. They also observed *Onoclea sensibilis* L., the sensitive fern, only in sunny and disturbed habitats, such as by riverbanks or streams, and almost never in deeply forested areas, whereas *Dryopteris intermedia* (Muhl. ex Wild.) A. Gray, the intermediate shield-fern, was often found at the base of rock outcrops near streambanks. *Polystichum acrostichoides* (Michx.) Schott, the Christmas fern, is more abundant and more variable in its distribution than the other two ferns, but is often found on the forest floor in damp, shady regions (Greer et al., 1997; Minoletti and Boerner, 1993). *Polypodium virginianum* L., the rock-cap fern, is unique in that it primarily grows on boulders and large rocks (Foster, 1984).

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Light is a particularly important factor influencing plant morphology and physiology. Brach et al. (1993) notes that shade leaves are thinner with more leaf surface area, have higher chlorophyll contents and less dry mass per unit leaf area than sun leaves. In order to examine the effects of shade on plant photosynthesis, Hill (1972) studied three species of fern, two from open habitats and one from a shaded habitat. He found that species from open, sunny habitats had higher light compensation points, light saturation points, and maximum photosynthesis rates than the shade species. A study by Poole and Conover (1973) showed that in Polystichum adiantiforme (Forst.) J. Sm., elemental composition of individual plants differed with respect to light conditions. In 80% shade-grown plants, levels of K, Zn, and Mn were elevated and Ca, Cu, Fe, and Mg were lower compared to those plants grown in 60% shade.

In southeastern Pennsylvania, ferns occur throughout hardwood forests; however, the micro-distribution and morphological characteristics of ferns may be influenced by the amount of available light, moisture, soil properties, and habitat conditions. We examined three species of wintergreen perennial ferns (P. acrostichoides, P. virginianum and D. intermedia) and one species of deciduous perennial fern (O. sensibilis). This study investigates leaf fluorescence, chlorophyll content, chlorophyll a:b ratios, water potential, and the ratio of leaf edge to leaf surface area to test the hypothesis that deciduous perennial and wintergreen perennial ferns differ in their physiological and morphological characteristics, and that these characters influence habitat selection.

We predicted that those species living along a riverbank in a damp, sunny environment (O. sensibilis, D. intermedia, and sun-grown P. acrostichoides) should have a higher leaf variable to maximum fluorescence (Fv/Fm) ratio than those species living on a deep-forest hilltop with less available sunlight and water (P. virginianum and shade-grown P. acrostichoides). Onoclea sensibilis should have higher chlorophyll a:b ratios and more chlorophyll than the three wintergreen species, based on local light conditions and habitat preference. The ferns exhibiting the lowest chlorophyll a:b ratios and chlorophyll contents should be those species growing in the deep forest, low light conditions (P. virginianum and deep forest P. acrostichoides). We also hypothesize that all ferns growing at the riverbank site (O. sensibilis, D. intermedia, and riverbank P. acrostichoides) would have less negative water potential than those plants growing at the deep forest site (P. virginianum and deep forest P. acrostichoides). Polypodium virginianum was predicted to exhibit the most negative water potential, as it is found only growing on boulders and should have the least amount of available water. We further hypothesized that there would be intraspecific differences in P. acrostichoides between the two sites, with plants growing at the deep forest site exhibiting a more negative water potential. Plants exposed to higher amounts of sunlight (O. sensibilis, D. intermedia, and sun-grown P. acrostichoides) should have leaves with lower leaf edge to surface area ratios than those plants growing in low sunlight conditions (P. virginianum and shade-grown P. acrostichoides).
Materials and Methods

Study site.—Ridley Creek State Park is located in Delaware County, southeastern Pennsylvania and encompasses 2,606 acres of multi-use forest. It is a typical, second-growth hardwood forest dominated by Quercus alba L., Q. velutina Lam., and Q. prinus L., Liriodendron tulipifera L., Carya spp., and several Acer species. The park is largely contiguous, but is punctuated by rivers, streams, trails, and roads, creating canopy gaps of various sizes. Onoclea sensibilis, D. intermedia, and P. acrostichoides are found growing together on streams and riverbanks. Polystichum acrostichoides has a more widespread distribution than any of the other species in this study, a finding consistent with pteridophyte distribution in southeastern Ohio (Greer et al., 1997), and is also found in deep forest, shady habitats. Polypodium virginianum was not observed along riverbanks, but was only found growing on boulders in deep forest, high shade habitat.

Data collection took place in the fall of 2003. All fern data were collected from two sites at Ridley Creek State Park. The riverbank site was located stream-side with a partly open canopy and contained O. sensibilis, D. intermedia and P. acrostichoides. The deep forest site was located on the top of a hill with a nearly closed canopy and consisted of P. acrostichoides and P. virginianum, the latter of which was only found growing on boulders. At the riverbank site, 3 microsites were used for P. acrostichoides, 2 microsites were used for D. intermedia, and 3 microsites were used for O. sensibilis. At the deep forest site, 2 microsites were used for P. acrostichoides and 1 microsite was used for P. virginianum. Because sampling was destructive and was conducted repeatedly, not all fern species could have equal numbers of microsites due to variation in the species abundance.

The average ambient temperature and humidity were recorded at three locations within each study site on three separate occasions using a sling psychrometer. The average light intensity for the riverbank site and deep forest site was obtained using an International Light Inc. radiometer/photometer (Newburyport, MA 01950 US) and taking three measurements within each microsite at mid-frond level.

Soil samples.—At each microsite, 25 ml of soil were collected in 50 mL disposable centrifuge tubes and taken back to the lab for further analysis. Soil samples were weighed, and then distilled water was added to each sample to bring the total of mixed water and soil up to 40 ml. Next, the samples were shaken and allowed to settle for approximately 1 hour. The free water was poured off the top and the soil was weighed to determine saturated weight. Samples were then dried in an oven at 60°C for 48 hours, after which the samples were weighed to determine the dry weight, bulk density (saturated weight/saturated volume) and particle density (saturated weight – water weight/saturated volume – water volume).

Chlorophyll fluorescence.—Leaf fluorescence was obtained by first dark adapting pinnae for at least 5 minutes (N = 6 leaves/microsite) with plastic cuvettes and then exposing the dark adapted part of the pinnae to high
frequency light for one second. By using a modulated fluorometer (Optiscience OS1-FL, Tyngsboro, MA 01879 USA), the maximum efficiency of photosystem II was measured by recording the Fv/Fm ratios – the ratio between the variable fluorescence and the maximum fluorescence.

Chlorophyll a:b ratios.—Approximately 0.25–0.5g of fresh leaf material were collected (n = 2 pinnae/microsite) and brought back to the laboratory for processing. Leaves were soaked in 15 ml of 80% acetone and left for seven days at 4°C in the dark. The acetone (with chlorophyll) was then diluted 1:1 with 80% acetone (total dilution 1:30) and absorbance was measured at two wavelengths (663 nm and 645 nm). Chlorophyll a was recorded by using the equation (Arnon, 1949):

\[
[(12.7(A663) - 2.69(A645))(\text{amount diluted})]/(\text{sample weight})
\]

Chlorophyll b was recorded using the equation (Arnon, 1949):

\[
((22.9(A645) - 4.68(A663))(\text{amount diluted})]/(\text{sample weight})
\]

Total chlorophyll was recorded using the equation (Arnon, 1949):

\[
((8.02(A663) + 20.2(A645))(\text{amount diluted})]/(\text{sample weight})
\]

The chlorophyll a:b ratio was calculated by dividing the value for chlorophyll a (in µg/ml) by the value obtained for chlorophyll b.

Water potential.—Water potential measurements were obtained using a PMS model 1003 plant pressure chamber (PMS Instrument, Corvallis, Oregon, USA). Two fronds were taken from each microsite and analyzed.

Leaf edge to surface area.—Counting from the tip of the frond, pinnae (or lobes in the case of P. virginianum) 3, 6, and 9 were removed from the left, right, and left sides respectively. Eight fronds were analyzed using 3 pinnae or lobes per frond per microsite. Pinna or lobe 1 was determined to be the first pinna or lobe that was distinguishable as being separate from the previous pinna or lobe (the tips of the fronds are often comprised of small, webbed immature pinnae). The pinnae or lobes were then scanned into Image J (NIH shareware) for analyses via a Canon 900 scanner. Images were converted to binary code and scored as either leaf material or empty space based upon a preset threshold value. Based on the number of pixels per cm, a scale was calibrated from a known distance scanned with each image. Image J then was used to analyze each pinna or lobe measuring both edge and surface area.

Data analysis.—For each morphological and physiological factor studied, measurements from different weeks were pooled together for each individual species and then compared to each of the other species one at a time using t-tests. Since Polystichum acrostichoides occurred both at the riverbank site and the deep forest site, individuals from the deep forest site were also compared with those inhabiting the riverbank site. If results between the two populations were significantly different, they were treated as separate populations in the statistical tests. If significant differences were not found, individuals from deep forest and riverbank sites were pooled.
Table 1. Environmental conditions monitored at the deep forest site and the riverbank site. Soil measurements were done once at the beginning of the experiment.

<table>
<thead>
<tr>
<th>Environmental conditions</th>
<th>Average temperature (F)</th>
<th>Average relative humidity</th>
<th>Average light intensity (Em⁻²s⁻¹)</th>
<th>Soil bulk density (gm⁻³)</th>
<th>Soil particle density (gm⁻³)</th>
<th>Soil moisture %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep Forest Site</td>
<td>53.8</td>
<td>63</td>
<td>105.1</td>
<td>1.27</td>
<td>1.38</td>
<td>65.5</td>
</tr>
<tr>
<td>Riverbank Site</td>
<td>52.1</td>
<td>70</td>
<td>165.2</td>
<td>1.34</td>
<td>1.5</td>
<td>76.7</td>
</tr>
</tbody>
</table>

Results

Study site observations.—Polystichum acrostichoides was widespread in its distribution, found on both riverbanks, as well as in shaded, deep forest habitat. Onoclea sensibilis and Dryopteris intermedia were only found on or near the riverbanks, and Polypodium virginianum was found growing only on boulders in the deep forest. Typically (across its natural range), P. acrostichoides grows in shade but can also be found in sun. Onoclea sensibilis is most frequently found in the sun but is also occasionally found under forest canopy. Interestingly, Dryopteris intermedia is usually found in the shade, but is also found in the sun, while P. virginianum is most frequently found in the shade, but can also occur in canopy gaps (Foster, 1984; Jones, 1987).

The riverbank site had higher light intensity, relative humidity, soil moisture, and lower ambient temperature, than the deep forest site, and also exhibited higher soil bulk density and particle density (Table 1).

Polystichum acrostichoides from the deep forest site did not differ from individuals from the riverbank site in chlorophyll florescence (p = 0.406, t = 0.832, df = 228; Fig. 1A), chlorophyll a:b ratio (p = 0.873, t = -0.160, df = 56; Fig. 1B), total chlorophyll (p = 0.090, t = -1.726, df = 56; Fig. 2A), and leaf edge to surface area ratio (p = 0.790, t = -0.273, df = 10; Fig. 2B). Therefore, data from both deep forest and riverbank sites were pooled and treated as one species in comparisons with other species. However, P. acrostichoides from the deep forest site did differ from individuals from the riverbank site in leaf water potential (p = 0.041, t = -2.139, df = 28) and so were treated as separate populations (Fig. 3).

Deciduous versus Wintergreen Ferns.—Overall, the deciduous perennial fern, O. sensibilis, differed from all three wintergreen perennial species in chlorophyll florescence, chlorophyll a:b ratio and total chlorophyll (Figs. 1–3). Among the four species, chlorophyll florescence of O. sensibilis was higher than D. intermedia (p = 0.003, t = -3.088, df = 100) but lower than P. acrostichoides (p = 0.002, t = 3.143, df = 264) and P. virginianum (p = 0.004, t = 2.965, df = 74; Fig. 1A). Onoclea sensibilis had the highest chlorophyll a:b ratio and total chlorophyll (Figs. 2 & 3). Leaf edge to surface area ratio of O. sensibilis was significantly higher than P. acrostichoides (p < 0.001, t = -6.622, df = 19) and lower than D. intermedia (p < 0.001, t = 8.997, df = 10), but was not different from that of P. virginianum (p = 0.375, t = -0.928, df = 10; Fig. 2B).
Fig. 1. Chlorophyll comparisons among species; data for Polystichum acrostichoides and Onoclea sensibilis were pooled from deep forest and riverbank sites. A. Mean chlorophyll fluorescence readings of four species. B. Mean chlorophyll a:b ratios of four species. Letters (a–c) indicate significant differences between species (P < 0.05).
Fig. 2. Chlorophyll content and morphological comparisons among species. A. Mean total chlorophyll readings of four species. Data for *Polystichum acrostichoides* and *Onoclea sensibilis* were pooled from deep forest and riverbank sites. Letters (a–c) indicate significant differences between species (P < 0.01). B. Mean edge to surface area ratios of four species. Data for *Polystichum acrostichoides* were pooled from deep forest and riverbank sites. Letters (a–c) indicate significant differences between species (P < 0.05).
HI.20

Mean water potential readings of four species. Note that *Polystichum acrostichoides* from deep forest and riverbank sites were treated as two populations. *Onoclea sensibilis* was not included due to lack of data. Letters (a–c) indicate significant differences between species (*P* < 0.05).

Water potential data were not available for *O. sensibilis* because most fronds had already senesced at the time of second sample collection.

**Wintergreen Ferns.—Chlorophyll fluorescence.**—Chlorophyll fluorescence of *D. intermedia* (mean = 0.712 μEm²s⁻¹) was significantly lower than that of both *P. acrostichoides* (mean = 0.785 μEm²s⁻¹; *p* < 0.001, *t* = 8.838, df = 294) and *P. virginianum* (mean = 0.786 μEm²s⁻¹; *p* < 0.001, *t* = -4.955, df = 104). However, there was no significant difference between *P. acrostichoides* and *P. virginianum* (*p* = 0.842, *t* = -0.2, df = 268; Fig. 1A).

Chlorophyll a:b ratio.—Chlorophyll a:b ratios did not differ among *P. acrostichoides*, *D. intermedia*, and *P. virginianum*. Among these three species, *Polystichum acrostichoides* had the highest and *Dryopteris intermedia* had the lowest readings (Figure 2).

Total chlorophyll.—Total chlorophyll was significantly higher in *D. intermedia* than for both *P acrostichoides* (*p* < 0.001, *t* = -7.583, df = 82) and *P. virginianum* (*p* < 0.001, *t* = 4.142, df = 36). *Polystichum acrostichoides* and *P. virginianum* were not significantly different from each other (*p* = 0.348, *t* = 0.946, df = 68; Fig. 2A).

Leaf edge to surface area ratio.—*Dryopteris intermedia* and *P. acrostichoides* had the highest and lowest edge to surface area ratio respectively among all four species, including *O. sensibilis*. All species differed significantly from
each other except *O. sensibilis* and *P. virginianum*, which, as mentioned before, did not show significant differences in leaf edge to surface area ratio (\(p = 0.375, t = -0.928, \text{df} = 10\); Fig. 2B).

*Water potential.* — *Polystichum acrostichoides* from the deep forest site and the riverbank site were treated as separate populations because there was a significant difference in the preliminary test. Deep forest *P. acrostichoides* had a significantly more negative water potential than riverbank *P. acrostichoides* (\(p = 0.041, t = -2.139, \text{df} = 28\), *D. intermedia* (\(p < 0.001, t = -5.22, \text{df} = 23\)), and *P. virginianum* (\(p = 0.008, t = -3.044, \text{df} = 16\)). Water potential of riverbank *P. acrostichoides* was significantly more negative than that of *D. intermedia* (\(p = 0.006, t = -2.941, \text{df} = 29\)), but was not significantly different from *P. virginianum* (\(p = 0.643, t = -0.469, \text{df} = 22\)). Although *P. virginianum* had a more negative mean water potential measurement than *D. intermedia*, there was no statistically significant difference between these two species (\(p = 0.089, t = 1.806, \text{df} = 17\); Fig. 3).

**Discussion**

*Onoclea sensibilis* was found to be significantly different from the three wintergreen species for each morphological and physiological parameter measured in the study, with the exception of leaf edge to surface area ratios. *Onoclea sensibilis* and *D. intermedia* were found in areas with open canopies (i.e. along riverbanks and trails), whereas *P. acrostichoides* was found both under full canopy and in higher light conditions. *Polypodium virginianum* was found only in small patches growing on the surface of boulders in deep forest habitat.

Chlorophyll fluorescence was highest for *P. acrostichoides* and *P. virginianum*, with no significant differences detected between the two species. The lowest values were for *D. intermedia* (Fig. 1A). Readings for all species were indicative of healthy plants (\(Fv/Fm = 0.6-0.8\); Maxwell and Johnson, 2000). Another factor that must be taken into consideration is that the readings were taken in late autumn. *Polystichum acrostichoides* and *P. virginianum*, with the highest readings, are both wintergreen species and the process of senescence does not start until spring. *Onoclea sensibilis*, with lower readings, is a species that has deciduous fronds that senesce in the autumn of each year. In order to ensure that lower Fv/Fm values are not indicative of senescence, a series of measurements would need to be taken throughout the growing season. While *D. intermedia* appeared to be healthy, it had the lowest Fv/Fm value. As this species does not undergo senescence in autumn it is possible that the low Fv/Fm value was due to water stress as it had the highest edge to surface area ratio (Fig. 2B) and the most negative water potential (Fig 3). As *D. intermedia* is typically found in shaded, moist areas, this suggests that in our study site soil moisture was a more important parameter than light intensity for habitat selection. Further, this species apparently was under the most stress of the four species examined, suggesting limitations on the ability to acclimate to higher light intensities.
The three wintergreen species had significantly lower chlorophyll a:b ratios than *O. sensibilis*, but there was no significant difference among wintergreen species (Fig. 1B). The higher chlorophyll a:b ratio in *O. sensibilis* may be indicative of a smaller light harvesting system (less stacking of thylakoid membranes) and is expected for plants found in higher light environments (Ludlow and Wolf, 1975). A future study of the anatomy of chloroplasts in deciduous and wintergreen species could shed light on the mechanisms employed by ferns to deal with two different life history strategies.

*Onoclea sensibilis* had twice the total chlorophyll of *D. intermedia* and was five-fold higher than *P. acrostichoides* and *P. virginianum* (Fig. 2A). These results are surprising, yet consistent, as both *O. sensibilis* and *D. intermedia* were restricted to the same habitat (i.e. riverbanks with higher light intensities, higher soil moisture, and presumably a wider range of both diurnal and seasonal temperature fluctuations). Additionally, higher edge to surface area ratios may increase transpirational pull, thus increasing the strain on roots to uptake water. It would be interesting to further investigate the correlation between high total chlorophyll measurements and the occurrence of compound leaves in ferns of the eastern hardwood forest.

The ability to dissipate heat is important in protecting photosystem II and optimizing overall photosynthesis. The ratio of edge to surface area is of interest because a high edge to surface area ratio allows the plant to dissipate heat more efficiently (Givnish and Vermeij, 1976). Plants found in high light conditions with ample water are expected to have high edge to surface areas. Analysis of edge to surface area ratios revealed that *D. intermedia* had the highest edge to surface area and that *O. sensibilis* had the second highest edge to surface ratio. Both of these plants are found in open, sunny, high moisture areas. This may also indicate that both *Dryopteris intermedia* and *Onoclea sensibilis* are more capable of dissipating heat that can disrupt photosynthesis in high light conditions, as has been observed in many plant species (Givnish and Vermeij, 1976) and may also have a higher capacity for transpiration. However, as mentioned above, this also increases transpirational strain through water loss from the leaves and may also contribute to the observation that highly dissected ferns are often found in deeply shaded habitats (Bannister and Wildish, 1982).

Water plays an important role in the overall health of all plant species (Raven *et al.*, 1999). Water stress causes the pressure in the xylem to become more negative, making it increasingly difficult for the plant to take up water. Therefore, by measuring water potential we are able to discern the amount of water stress a plant is under. Not surprisingly, *D. intermedia*, the fern with the lowest Fv/Fm ratio, also had the most negative water potential, indicating a higher amount of stress due to a lack of available water, or a condition whereby the rate of transpiration exceeds water uptake by the root system. *Polypodium virginianum* had the next lowest value. As this species is typically found growing on boulders with minimal amounts of soil (Foster 1984) it is reasonable to hypothesize that it will exhibit low water potential values. *Polystichum acrostichoides* found at the riverbank site had more negative
water potential values than the *P. acrostichoides* found at the deep forest site. This suggests that the higher light intensities at the riverbank site had a greater impact on water potential than the measured differences in soil moisture. The lower light intensity at the deep forest site might result in lower transpiration rates for plants when compared to the riverbank site and would therefore result in less strain given similar root water uptake capacities for the two populations.

In conclusion, the deciduous, perennial fern *O. sensibilis* was found to be significantly different when compared to the three wintergreen perennial fern species for almost all morphological and physiological parameters studied. The wintergreen fern, *D. intermedia* shared characteristics with the *O. sensibilis* as well as with its wintergreen counterparts, and physiological measurements suggest that it was experiencing the most stress of the three species found at the sunny, higher soil moisture (riverbank) site. We suggest that leaf morphology may play a significant role in the ability of ferns in the NE USA to establish in habitats of differing light intensities, temperature fluctuations, and soil moisture and that physiological acclimation to light intensity (photosynthesis and transpiration) may be not only secondary mechanisms to cope with changing light quality over time in wintergreen fern species, but also useful indicators of species fitness for differing microsites within a habitat.

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**LITERATURE CITED**


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