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Full Length Research Paper

An ecophysiological study of the fern *Onoclea sensibilis* growing in three environmental locations varying in incident light intensity

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Onoclea sensibilis **L. plants growing in three locations with varying light intensities (dense shade, partial shade, and open sun) at a temperate site in Palisades, New York, were studied to assess net photosynthesis rate, dark respiration rate, leaf micromorphology, and leaf fluorescence in relation to these light conditions. The mean maximum net photosynthesis rate (3.6 µmoles CO_2 m^{-2} s^{-1}) was highest for leaves sampled from the open sun location, compared to lower rates for leaves from partial and dense shade (3.0 and 2.5 µmol CO_2 m^{-2} s^{-1}, respectively). Mean stomatal densities (stomata cm^{-2}) were higher in leaves from the open sun and partial shade locations (5,661 and 5,252, respectively) compared to leaves from the dense shade location (2,777). Mean stomatal size was approximately the same for O.** ***sensibilis*** **leaves across the three locations, measuring around 40 to 45 µm in length and 24 to 26 µm in width. A specific leaf area (SLA) was highest in leaves from dense shade (641) and lower in leaves from partial shade and open sun locations (429 and 354, respectively). Overall, the data indicate that** ***O. sensibilis*** **can survive across a broad range of light intensities in this temperate location.**

Key words: Chlorophyll concentration, dark respiration, leaf micromorphology, photosynthesis rate, specific leaf area, varying light intensities.

INTRODUCTION

Onoclea sensibilis L. is a temperate, perennial fern widely distributed in the northern hemisphere, with a native range extending from central and eastern Canada to north-central and eastern U.S.A., and from southeastern Siberia to Japan and China in Asia (Govaerts, 2024). Commonly known as the "sensitive fern" due to its susceptibility to frost damage late in the growing season, it is also referred to as the "bead fern" for its clusters of bead-like fertile fronds. This species has a well-documented fossil record spanning the major forested regions of the northern hemisphere, with occurrences in western Canada and Alaska from the late Cretaceous and Tertiary periods. It has also been reported from interior North America (Colorado and New Mexico) during the Cretaceous, from eastern North America (including the Dakotas) from the Cretaceous onward, and from northwest Europe and Japan in the Eocene (Lloyd, 1971). Barrington (1993) attributed the present, somewhat disjunct distribution to vicariance, contrasting with the broader northern distribution suggested by the fossil

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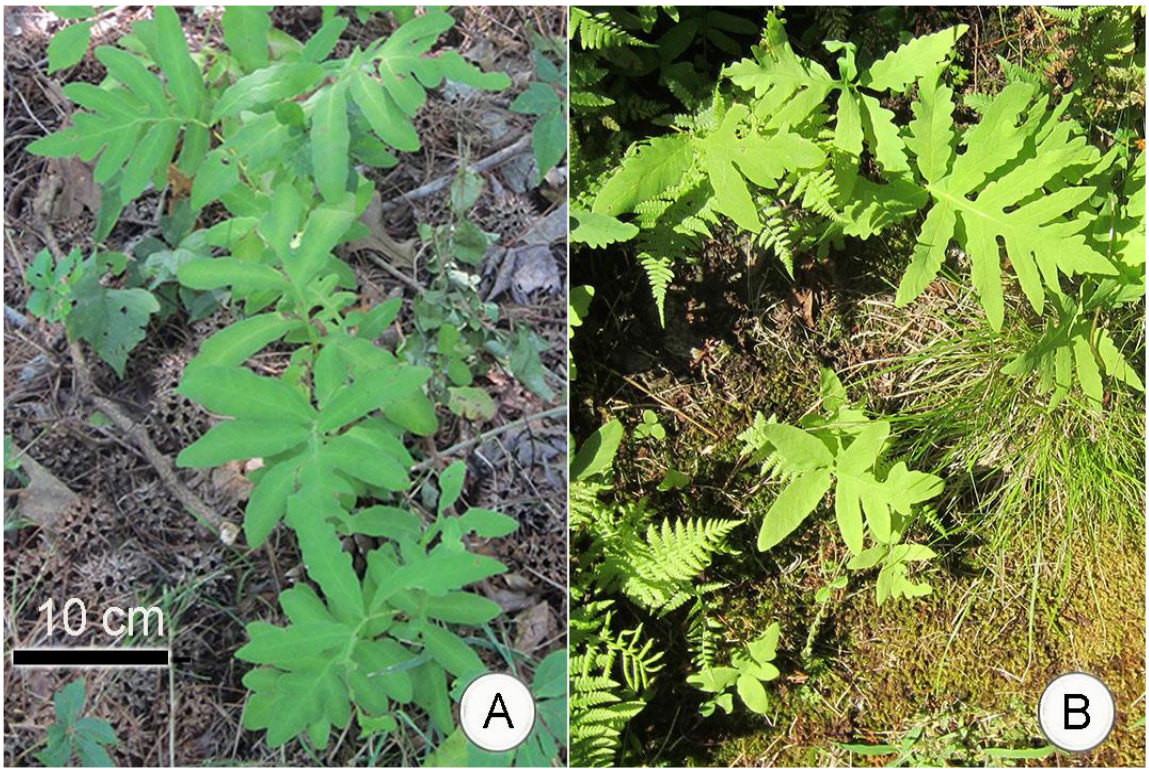


Figure 1. Photographs of *O. sensibilis* plants from the sampling locations for dense shade in the understory of a stand of deciduous trees (A) and open sun on a north-facing slope (B). Marker = 10 cm.

record.

In terms of habitat, Greer et al. (1997) observed that *O. sensibilis* in a southeastern Ohio hardwood forest was found only in sunny, disturbed areas, such as along riverbanks or streams, and almost never in deeply forested zones.

Contrastingly, Khrapko and Tsarenko (2015) reported that in Russia's Far East, *O. sensibilis* was found in forests, clearings, forest edges, and even on bog outskirts, often subdominant in the herbaceous layer or scattered within plant communities, occasionally forming small clumps. While *O. sensibilis* is considered well adapted to stable, mesophytic environments, some evidence suggests moderate resistance to summer heat and drought (Anderson, 2022).

In an ecophysiological comparison of *O. sensibilis*, a deciduous-leaf fern, with three wintergreen fern species (*Dryopteris intermedia, Polypodium virginianum*, and *Polystichum acrostichoides*), Reudnik et al. (2005) found significant differences in morphology and physiology, including total chlorophyll content, chlorophyll a ratio, chlorophyll fluorescence, and water potential. Additional studies by Nobel et al. (1984) on photosynthetic active radiation (PAR), temperature, and water vapor effects on gas exchange in *O. sensibilis* and three other ferns (*Asplenium nidus*, *Polypodium aureum*, and *Pteridium aquilinum*) cultivated in greenhouse pots showed that *P. aquilinum* had the highest photosynthesis rate, with *O. sensibilis* ranked second. Maximum photosynthetic CO_2 assimilation in *O. sensibilis* occurred at PAR levels of 400 to 600 µmol m^{-2} s^{-1}.

Given evidence that *O. sensibilis* grows in a broad range of environments with varying light intensities—from forest understories to open areas such as clearings and bog outskirts—further research is warranted to document its physiological responses to variations in light intensity. This research study investigates the ecophysiology of *O. sensibilis* growing in three locations within the same geographic area, each with different light intensities ranging from dense shade to open sunlight. Images of *O. sensibilis* in dense shade are as shown in Figure 1A, and in open sun in Figure 1B. The physiological variables analyzed include: 1) Net photosynthesis rate as a function of light intensity, measured as photosynthetic photon flux density (PPFD); 2) Stomatal size and density; 3) Leaf chlorophyll content index (CCI), a relative index of chlorophyll content per unit leaf area; 4) Specific leaf area (SLA); 5) Leaf chlorophyll fluorescence, including: (i) quantum efficiency, expressed as the ratio of variable fluorescence to maximum fluorescence (Fv/Fm), (ii) electron transport per reaction center beyond the quinone intermediate (Q_A) in the electron transport chain (ET_0/RC), (iii) probability of electron transfer to the quinone and beyond in the electron transport chain (Ψ_o);

Table 1. Leaf micromorphology and SLA for leaf samples from three locations (dense shade, partial shade, and open sun) expressed as means ± SEM.

Location	Stomatal density[a]	Stomatal length[b]	Stomatal width[b]	Specific leaf area (SLA)[c]
Dense shade	2,777 ± 9	44 ± 1.67	24 ± 0.88	641 ± 8
Partial shade	5,661 ± 159	40 ± 4.04	24 ± 1.00	429 ± 36
Open sun	5,252 ± 322	45 ± 1.33	24 ± 1.00	354 ± 12

[a]Number per cm^2, [b]Dimensions (µm), [c]Area (cm^2) per dry weight (g).

and 6) Dark respiration rate.

MATERIALS AND METHODS

Study location

The research was conducted on the campus of the Lamont-Doherty Earth Observatory (Columbia University) in Palisades, New York, U.S.A., situated on cliffs overlooking the western bank of the Hudson River (41°00'14.93" N, 73°54'32.52" W) at an elevation of 108 m. The three sampling sites included: 1) a densely shaded area within the understory of a broad-leaf tree stand (primarily mature oak and maple) with a light intensity (PPFD) of 30 to 40 µmol m^{-2} s^{-1}; 2) a partially shaded area at the crest of a north-facing slope, protected from direct sunlight by surrounding trees, with a light intensity of approximately 100 µmol m^{-2} s^{-1}; and 3) an open sunlight area near the base of the slope, fully exposed and reaching a PPFD of about 1,700 µmol m^{-2} s^{-1} from early morning to mid-afternoon on cloudless days. Light intensity measurements were taken in July and August 2024.

Leaf sample collection

Leaves were collected from mid-morning to early afternoon at each of the three sites during July and August 2024. Samples were enclosed in a zip-lock plastic bag containing a moistened absorbent pad with distilled water and promptly transported to a nearby laboratory for analysis under controlled conditions. For chlorophyll concentration estimates, fluorescence analysis, stomatal density and size measurements, and photosynthesis and respiration analyses, at least three leaves from three representative *O. sensibilis* plants were collected. Four additional leaves, minus the petioles, were collected for SLA estimates.

Methods of analysis

The net photosynthesis rate of the leaves (expressed as µmol CO_2 m^{-2} s^{-1}) was measured using an infrared gas analyzer (IRGA) system (model BTA, Vernier, Beaverton, Oregon, U.S.A.) with an optically clear assay chamber (163 cm^3 volume, corrected for the leaf sample volume) at a temperature of 25°C. Leaves were illuminated with an LED light at six different light intensities, ranging from 10 to 800 µmol m^{-2} s^{-1} PPFD, to simulate semi-shade natural conditions or possible cultivation settings, measured with a LiCor solar monitor 1775 (Biosciences, Lincoln, Nebraska, USA). The net photosynthesis rate was also calculated relative to leaf dry weight (gDW). The relative humidity in the cuvette was maintained at 85 to 90%, with a vapor pressure deficit (VPD) of 0.48-0.32 kPa to limit excessive transpiration. CO_2 concentration was held at ambient atmospheric levels (417 ppm). Each leaf measurement took approximately 10 min.

The mean respiration rate (µmol CO_2 released m^{-2} s^{-1}) was determined at 25°C using the same apparatus with the assay chamber completely darkened. The leaf sample remained in the dark until the reaction centers of the photosystems reached equilibrium with the darkened state (about 2 min), and measurements began once a steady-state respiration rate was achieved.

Leaf area, expressed in cm^2, was measured with a Leaf Area Meter (Model AM-350, Opti-Sciences, Inc., Hudson, New Hampshire, USA).

Leaf fresh weight and dry weight (after drying overnight at 60°C in a laboratory oven) were measured using a Sartorius digital balance (Göttingen, Germany). These data were also used to calculate the mean specific leaf area (SLA), expressed as leaf area in cm^2 per dry weight in g (Wolf et al., 1972). The mean chlorophyll concentration index (CCI) for each leaf sample was measured with a CCM-300 chlorophyll content meter (Opti-Sciences, Inc., Hudson, New Hampshire, USA). Leaf fluorescence data were collected using an OS-30p+ Chlorophyll Fluorometer (Opti-Sciences, Inc., Hudson, New Hampshire, USA). This included quantum yield efficiency (Fv/Fm), electron transport per reaction center (ET_0/RC) from photosystem II (PS II) to the quinone intermediate (QA) and beyond in the electron-transport chain, and Ψ_o, the probability that an electron produced by photon excitation in PS II will pass to the intermediate quinone and beyond in the electron-transport chain. These measurements were based on the JIP test application within the OS-30p+ instrument. Leaf samples were dark-adapted for 20 to 30 min before measurements to ensure that the reaction centers (RC) were in an open state.

The mean stomatal density (number cm^{-2}) on the abaxial surface of the leaves from the three collection sites was estimated using a leaf peel method, in which acetate varnish was applied to the abaxial surface, and a replica of the leaf epidermis was created by gently peeling away the thin, hardened varnish layer. A wet mount slide preparation with deionized water was made, and the acetate peel was examined under 400X magnification using a Nikon phase-contrast compound light microscope (Nikon Instruments, Melville, NY, USA). All assay results are presented as the mean ± standard error of the mean (SEM), calculated using an Excel spreadsheet (Microsoft, Inc., Redmond, Washington, USA).

RESULTS

Leaf micromorphology and specific leaf area (SLA)

The results for leaf micromorphology (stomatal size and density, stomatal density) and SLA are presented in Table 1. Images of stomata on the abaxial epidermis of leaves from shade, partial-shade and sun samples of *O. sensibilis* are presented in Figure 2.

The mean stomatal densities for *O. sensibilis* leaves

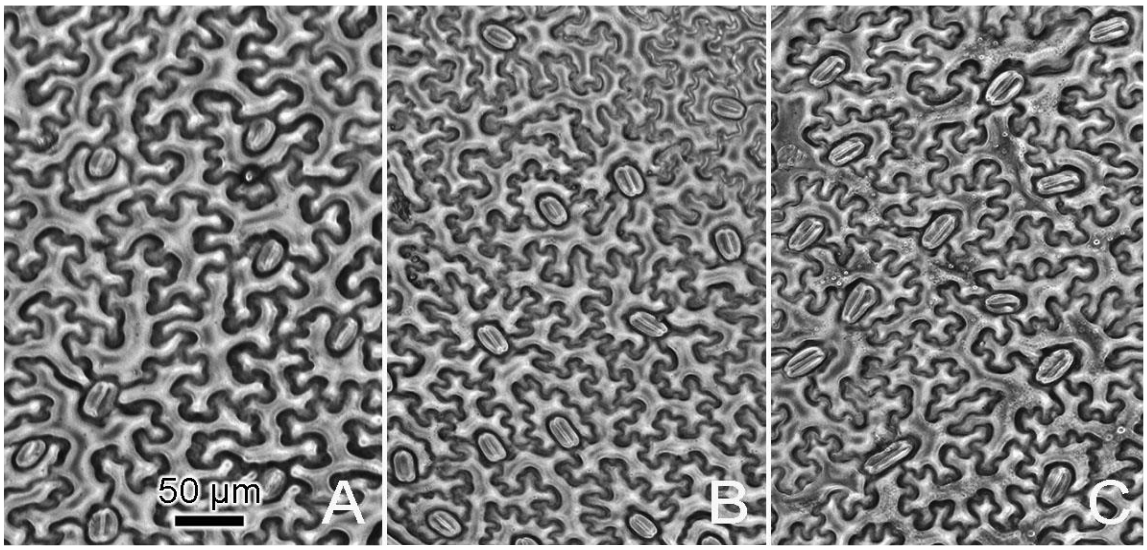


Figure 2. Replica of the abaxial leaf epidermis showing comparative images of stomata for leaf samples from plants in dense shade (A), partial shade (B) and open sun (C). Marker = 50 µm.

Table 2. Leaf fluorescence data for leaf samples from three locations (dense shade, partial shade, and open sun) including means ± SEM[a].

Location	F_v/F_m	ET_0/RC	Ψ_o	CCI
Dense shade	0.74 ± 0.02	1.26 ± 0.06	0.71 ± 0.01	8.03 ± 0.67
Partial shade	0.78 ± 0.01	1.08 ± 0.14	0.72 ± 0.01	8.11 ± 0.58
Sun	0.78 ± 0.01	1.13 ± 0.06	0.69 ± 0.01	8.27 ± 0.30

[a]Based on mean values from three leaves from three different plants per each location.

sampled from the open sun location (5,252 ± 322 cm^{-2}) and partial shade (5,661 ± 159 cm^{-2}) are significantly higher than those for leaves sampled from *O. sensibilis* growing in dense shade (2,777 ± 9 cm^{-2}). However, mean stomatal length and width does not differ substantially among the leaves sampled from the three locations. Notably, the mean SLA for leaves from dense shade is the highest, followed by leaves grown in partial shade, and is lowest in leaves grown in open sun. As shown in Figure 2, although stomatal sizes are comparable across the three leaf samples (dense shade, partial shade, and open sun), the dense-shade sample has fewer stomata per area, consistent with the results reported in Table 1. Additionally, the epidermal cells appear larger in the dense-shade-adapted leaves (Figure 2A) compared to those in leaves from *O. sensibilis* grown in partial shade and open sun (Figure 2B and C).

Leaf fluorescence data

Mean data for the results of fluorescence analyses of *O. sensibilis* leaves sampled from the three light intensity sampling sites are presented in Table 2.

Overall, the mean leaf fluorescence data is appreciably similar for leaves sampled from the three sampling sites, with perhaps a somewhat higher mean ET_0/RC value obtained for leaves sampled from the dense shade location compared to the values obtained for samples from the other two locations.

Photosynthesis and dark respiration data

The light saturation curves, illustrating the mean photosynthesis rate in relation to light intensity as photosynthetic photon flux density (PPFD), are shown in Figure 3. The mean maximum net photosynthesis rates for each of the three sampling sites, expressed per unit area and per unit leaf dry weight, are presented in Table 3. These data, also shown in Figure 3, indicate that the highest rate per unit area is found in samples from the open sun location, with rates decreasing progressively for samples from the partial shade and dense shade locations, respectively. While the mean maximum photosynthesis rate per unit leaf area varies across the three sampling locations—lowest in dense shade and highest in open sun—the results expressed per unit leaf

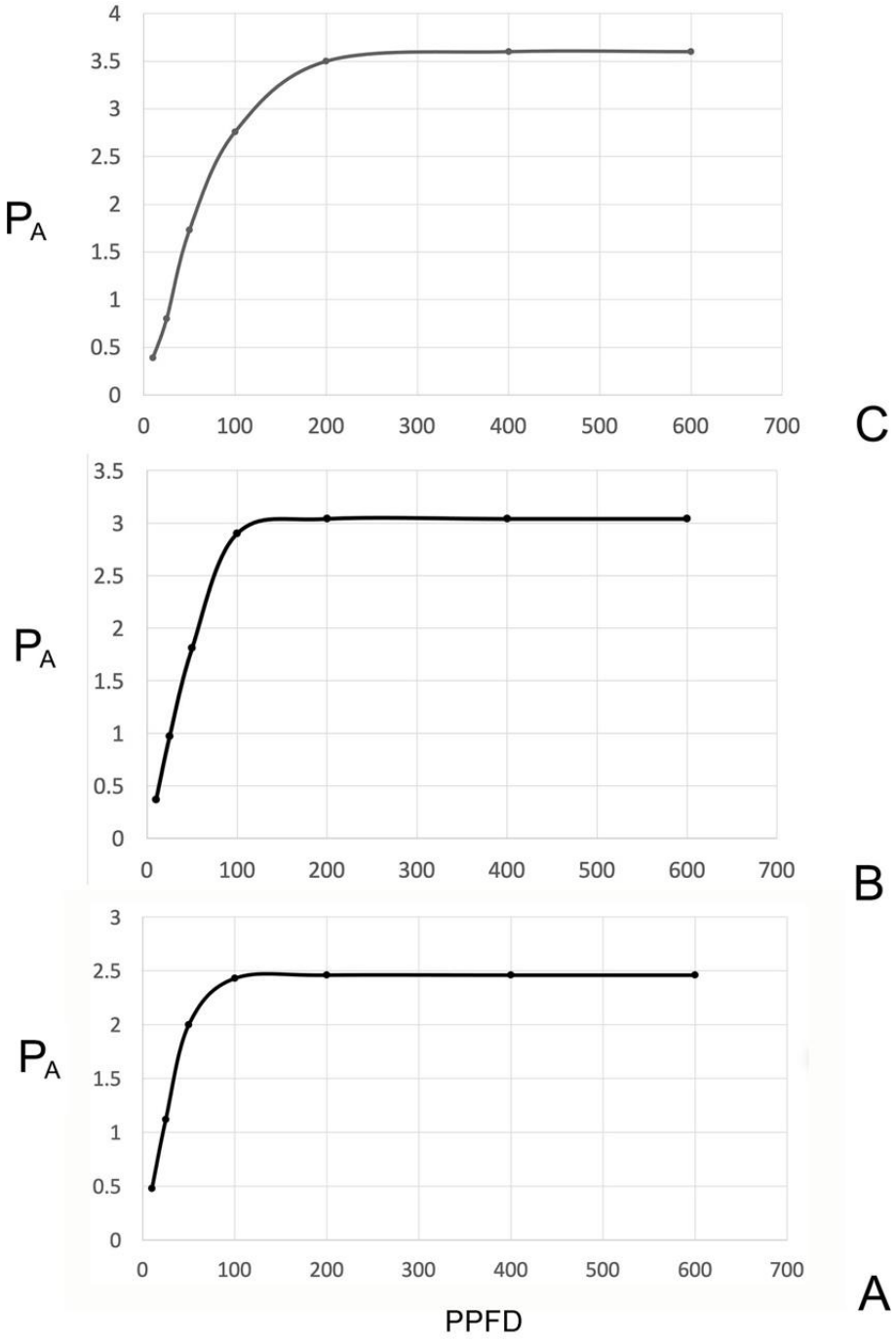


Figure 3. Photosynthesis light saturation curves for leaf samples from dense shade (A), partial shade (B), and open sun (C) locations. PPFD = photosynthesis photon flux densities (µmol photons m^{-2} s^{-1}). P_A = Mean net photosynthesis CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1}).

dry weight show less variation. The mean values for leaves from both the open sun and partial shade locations are comparable and higher than those from the dense shade location.

The mean ± SEM dark respiration rates for leaves from the three sampling sites expressed as µmol CO_2 released per m^{-2} s^{-1} are as follows: dense shade (0.20 ± 0.04), partial shade (0.31 ± 0.05) and open sun (0.63 ± 0.14). As may be expected, the mean rates for the shade-adapted leaf samples are lower than for the mean

Table 3. Mean ± SEM net maximum net photosynthesis rate of *O. sensibilis* leaves growing in three locations of varying light intensity (open sun, partial shade and dense shade) expressed per unit leaf area and per unit leaf dry weight.

Location	Mean maximum net photosynthesis rate[a]	
	Per leaf area (μmol CO_2 m^{-2} s^{-1})	Per leaf dry weight (μmol CO_2 g^{-1} min^{-1})
Open sun	3.6 ± 0.12	8.4 ± 0.80
Partial shade	3.0 ± 0.40	8.6 ± 1.50
Dense shade	2.5 ± 0.02	6.3 ± 0.70

[a]Mean data based on leaf samples from three different plants per each location.

obtained for the sun-adapted leaves.

DISCUSSION

The results presented for the three sampling locations—dense shade, partial shade, and open sun—provide comparative data on photosynthesis rate, respiration rate, and leaf morphology in relation to previously published data for these variables in habitats where *O. sensibilis* has been observed, including forest understories, open clearings, and bog peripheries (Greer et al., 1997; Khrapko and Tsarenko, 2015). Overall, the current data indicate that even in the most shaded location within the understory of a deciduous forest (dense shade), the potential maximum photosynthesis rate was appreciable at 2.5 μmol m^{-2} s^{-1}. Moreover, based on the saturation curve data (Figure 3A), the mean photosynthesis rate at a PPFD of 50 μmol m^{-2} s^{-1} (similar to the ambient light intensity in the densely shaded location) was approximately 2.0 μmol m^{-2} s^{-1}. Notably, this rate is nearly identical to the rate at a PPFD of 50 μmol m^{-2} s^{-1} for samples from the other two locations with higher light intensities. However, as shown in Figure 3, the photosynthesis curves saturated at a PPFD of about 100 μmol m^{-2} s^{-1} for samples from the dense shade and partial shade sites, whereas saturation for leaves sampled from the open sun location—with an overall higher maximum photosynthesis rate—occurred at a PPFD of approximately 200 μmol m^{-2} s^{-1}. Mean photosynthesis rates expressed per unit dry weight were also higher for leaves from the open sun and partial shade locations than for dense shade leaves of *O. sensibilis* (Table 3). These differences may reflect variations in leaf thickness and biomass allocation between plants growing in higher light intensities versus those in dense shade. Generally, sun leaves compared to shade leaves exhibit higher stomatal density, increased CO_2-fixation rates, and higher light saturation of photosynthesis (Lichtenthaler et al., 1981; Mathur et al., 2018), features that align with the findings reported here for the sun and shade leaves of *O. sensibilis* (Tables 1 and 3). The mean stomatal dimensions of *O. sensibilis* in Table 1 (approximately 40 to 45 μm in length and 24 μm in width) are consistent with previously published values (39.4 μm in length and 26.6 μm in width) by Khrapko and Tsarenko (2015) for *O. sensibilis* in the southern Russian Far East. Their line drawings of the micromorphology of the abaxial epidermis of *O. sensibilis* are comparable to the features reported here (Figure 2), showing epidermal cells with undulating, interdigitated margins and individual stomata positioned roughly at the intersection of three adjacent epidermal cells.

In prior research, sun leaves have also demonstrated higher quantum efficiency of photosystem II compared to shade leaves, as indicated by greater Fv/Fm values (Mathur et al., 2018). However, in this study, the Fv/Fm values for sun leaves and partial shade leaves (0.78) were only modestly higher than those for shade leaves (0.74), shown in Table 2. In general, fern leaves tend to have lower Fv/Fm values than angiosperm leaves (Björkman and Demmig, 1987; Dobrikova et al., 2022), which may partly explain the smaller differences in Fv/Fm observed between high-light and shade leaves of *O. sensibilis* in this study. Much more research is needed to clarify the effects of variations in light intensity and other environmental factors on leaf fluorescence in ferns and other taxa. Shade leaves, being typically thinner than sun leaves, tend to have a higher SLA (Mather and Jazoo, 2018), a trend that was also found for *O. sensibilis* in this study (Table 1).

In conclusion, the data presented here indicate that *O. sensibilis* can grow across a wide range of light intensities, from dense shade (ca. 20 to 40 μmol m^{-2} s^{-1}) to moderately open sunlight (ca. 1,700 μmol m^{-2} s^{-1}), which is within the range reported for full sunlight (2,000 μmol m^{-2} s^{-1}). Additional research is needed to understand the basis for this adaptability, including potential genetic and/or phenotypic variations among *O. sensibilis* plants growing in different environments. The mean net maximum photosynthesis rates across the three sampling sites at low to moderate light intensities (ca. 50 to 100 μmol m^{-2} s^{-1}) were similar in magnitude, as shown in Figure 3, though the maximum photosynthesis rates varied more significantly. For instance, the maximum photosynthesis rate per unit leaf area across all sites ranged from 2.5 to 3.6 μmol CO_2 m^{-2} s^{-1}, while the rate per unit dry weight ranged from 6.3 to 8.4 μmol

CO_2 g^{-1} min^{-1}. Mean stomatal densities on the abaxial epidermis of open sun and partial shade leaves were consistently higher than those from dense shade, corresponding with the higher mean maximum net photosynthesis rates of sun and partial-shade samples. Mean dark respiration rates were lower for leaves from dense and partial shade sites compared to those from high-light locations, contributing to greater net photosynthesis efficiency in carbon fixation under low-light conditions. Further research is required to examine the interaction of other environmental factors (e.g., soil moisture, temperature, humidity, and soil nutrients) with light intensity to more fully explain the ecophysiology of *O. sensibilis* across different climatic, edaphic, and altitudinal environments.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

REFERENCES

Anderson OR (2022). Photophysiology and respiration of the temperate fern *Onoclea sensibilis* in spring and during summer stress in a changing climate. International Journal of Biodiversity and Conservation 14(3):158-164.

Barrington DS (1993). Ecological and historical factors in fern biogeography. Journal of Biogeography 20:275-279.

Björkman O, Demmig B (1987). Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170:489-504.

Dobrikova AE, Apostolova I, Adamakis S, Hanc A, Sperdouli I, Moustakas M (2022). Combined impact of excess zinc and cadmium on elemental uptake, leaf anatomy and pigments, antioxidant capacity, and function of photosynthetic apparatus in clary sage (*Salvia sclarea* L.). Plants 11:2407.

Govaerts R (2024). WCVP: World Checklist of Vascular Plants. Facilitated by the Royal Botanic Gardens, Kew. https://doi.org/10.15468/6h8ucr

Greer GK, Lloyd RM, McCarthy BC (1997). Factors influencing the distribution of pteridophytes in a southeastern Ohio hardwood forest. Journal of the Torrey Botanical Society 124:11-21.

Khrapko OV, Tsarenko NA (2015). Adaptive strategies of two species from the family Onocleaceae. Contemporary Problems of Ecology 8:148-154.

Lichtenthaler HK, Buschmann C, Döll M, Fietz HJ, Bach T, Kozel U, Meier D, Rahmsdorf U (1981). Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. Photosynthesis Research 2:115-141.

Lloyd RM (1971). Systematics of the onocleoid ferns. University California Publications in Botany 61:1-87.

Mathur S, Jain L, Jajoo A (2018). Photosynthetic efficiency in sun and shade plants. Photosynthetica 56:354-365.

Nobel PS, Calkin HW, Gibson AC (1984). Influences of PAR, temperature and water vapor concentration on gas exchange by ferns. Physiologia Plantarum 62:527-534.

Reudnik MW, Snyder JP, Xu B, Cunkelman A, Balsamo RA (2005). A comparison of physiological and morphological properties of deciduous and wintergreen ferns in southeastern Pennsylvania. American Fern Journal 95(2):45-56.

Wolf DD, Carson EW, Brown RH (1972). Leaf area index and specific leaf area determinations. Journal of Agronomy Education 1:24-27.