

*Full Length Research Paper*

# **A comparative ecophysiological study of two forest understory ferns (*Dryopteris marginalis* and *Polystichum acrostichoides*) during summer drought and excessive heat**

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**This is a study of the photophysiology of two understory fern species growing on Torrey Cliff, Palisades, N.Y. during the summer of 2022 when the northeast incurred a significant period of drought and elevated temperatures. During the peak period of stress (July and August), mean photosynthesis rates of both species were lower than during more moderate weather in early summer and in autumn. Leaf fluorescence analyses indicated that the mean  $F_v/F_m$  values, combining the data for both species, were fairly similar across the 5 months suggesting that the quantum yield efficiency of photosystem II may be less susceptible to climate stress. However, there was a significant positive correlation between  $ET_0/RC$  values and net photosynthesis rate ( $r = 0.68$ ,  $n = 10$ ,  $p = 0.03$ ). This indicates that some of the variation in photosynthesis rates between species across the summer weather pattern may be accounted for by the rate of transfer of electrons from PS II through the intermediate quinone to the site of  $CO_2$  fixation as measured by the  $ET_0/RC$  variable. Dark respiration rates were lower for both species during the most severe heat and drought in July and August.**

**Key words:** Carbon exchange balance, climate change, dark respiration, effect of heat and drought, leaf fluorescence analysis, photosynthesis rate.

## **INTRODUCTION**

With increasing evidence of climate change, including rising temperatures and less predictable, but sometimes heavier precipitation in temperate regions; research on the adaptability of plants including ferns has become a topic of interest, especially toward understanding their adaptability and survival in a changing climate. Understory ferns, adapted to more shaded, mesic environments, may

be particularly affected by climate shifts toward elevated temperatures and less predictable precipitation.

Ferns arose historically during the middle Devonian approximately 390 million years ago (mya) becoming a dominant part of the flora in the Carboniferous. They expanded in diversity during the Cenozoic (65 mya), but lost ground when angiosperms were becoming more

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dominant (Schneider et al., 2004; Schuettpelz and Pryer, 2009). However, the understory environments of forests provided hospitable habitats where some fern species diversified and flourished on the forest floor, or through adaptive radiation they became epiphytes on the trunks and limbs of trees (Watkins and Cardelús, 2012). Other fern species have evolved to thrive in diverse environments spanning deserts to marshlands and at varying elevations in mountain ranges (Mehltretter, 1995; Bhattarai et al., 2004; Moran, 2008; Kessler, 2010; Kluge and Kessler, 2011; Anderson, 2021; De Arruda et al., 2021). However, in many temperate locations, ferns are particularly evident in forest understories or other shaded locations.

Considerable research has focused on fern physiological ecology in temperate and montane habitats, and some of these advances have received attention in review articles (Kessler et al., 2016; Zotz, 2005). However, temperate habitats vary substantially in geographic location, elevation, and climate patterns; and as a consequence, the fern flora of a temperate region may vary substantially from one location to another. Nonetheless, ferns in rocky understory locations, and those adapted to grow on human constructed stone structures, may incur particularly challenging habitat demands, including relatively less substrate for anchorage and root development, and more variable moisture; especially at higher elevations where excessive drainage may produce less consistent availability of moisture. Under conditions of climate change and more variable patterns of precipitation, ferns in these locations are likely to incur stress due to insufficient moisture, a situation made more severe if temperatures are higher and evapotranspiration is elevated. Some of these aspects have been addressed more fully in publications that are particularly focused on changing climate and environmental stress (Proctor, 2009; Bystrakova et al., 2014; Rapp and Silman, 2014; Baer et al., 2016; Sharpe, 2019; Testo and Watkins, 2013; Alarcón and Cavieres, 2018; De Gasper et al., 2021; Anderson, 2022; Kessler and Kluge, 2022; Winkel and Wood, 2022). Pittermann et al. (2013) published a review highlighting how functional morphology of the xylem combined with physiological analysis of water relations can account for the capacity of ferns to successfully endure drought-induced stress in the sporophyte stage as well as factors favoring survival of the gametophyte stage. Information of this kind can be useful in planning and interpreting research on fern adaptations and resistance to changing detrimental climate conditions. Increasing attention has also been given to comparative analyses of the physiological mechanisms regulating stomatal conductance under varying environmental conditions, especially comparing ferns to angiosperms. McAdam and Brodribb (2015) examined the effects of transitions in vapor pressure deficit (VPD) and its relationship to foliar concentrations of the plant hormone, abscisic acid (ABA). They reported

that species of conifers and ferns are unable to rapidly increase foliar ABA levels during a VPD transition, which is unlike angiosperm species, suggesting possible differences in mechanisms of stomatal regulation across evolutionary lineages.

In addition to their ecological roles, ferns may serve an important role in the biogeochemical C cycle among other plants on the forest floor. The overall objective of this research was to document the role of ferns in the biogeochemical C cycle at varying locations on Torrey Cliff with emphasis on the balance of carbon gain and loss (assimilation during photosynthesis and loss during dark respiration) in relation to desiccation stress and excessive heat occasioned by seasonal changes in climate. As a consequence, part of the intention of the research was to provide additional evidence of fern resilience during increasingly unfavorable climatic conditions. Two fern species (*Dryopteris marginalis* (L.) A. Gray and *Polystichum acrostichoides* (Michx.) Schott) studied in this research occur on rock ledges and terrestrial shallows in the understory of trees.

The following variables were investigated:

- (1) Net photosynthesis rate ( $\mu\text{mol CO}_2$  assimilated  $\text{m}^{-2} \text{s}^{-1}$ ).
- (2) Leaf chlorophyll content index (CCI), a relative index measuring chlorophyll content per unit leaf area.
- (3) Leaf chlorophyll fluorescence including: (i) evidence of quantum efficiency expressed as ratio of variable fluorescence to maximum fluorescence ( $F_v/F_m$ ) and (ii) electron transfer per reaction center beyond the quinone intermediate ( $Q_A$ ) in the electron-transport chain ( $ET_0/RC$ ).
- (4) Dark respiration rate ( $\mu\text{mol CO}_2$  released  $\text{m}^{-2} \text{s}^{-1}$ ).

## MATERIALS AND METHODS

### Sample site conditions and collection methods

The study site was located at the northern edge of the Lamont-Doherty Earth Observatory campus on Torrey Cliff, Palisades New York ( $41^\circ 00' 26'' \text{ N}$ ,  $73^\circ 54' 27'' \text{ W}$ ; elevation 110 m). The flora of the Hudson River Palisade Cliffs is varied and expansive, with remarkably diverse local ecosystems and biologically rich habitats, spanning ledges on the vertical walls of the cliffs to more substantial stands of trees and understory vegetation on the higher elevations of the ridge overlooking the Hudson River (Airola and Buchholz, 1984). This is a study of the photophysiology of 2 forest understory ferns: *D. marginalis* (Figure 1A) and *P. acrostichoides* (Figure 1B) during 5 months (June to October) including the period of excessive heat and limited precipitation during the summer of 2022. All sites occurred beneath the canopy of overlying deciduous trees with typical light intensities of approximately  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  (LI-COR solar monitor, Li-1776, LiCor Biosciences, Lincoln Nebraska).

Fern leaf samples were collected from three different patches of growth for each of the 2 species (*D. marginalis* and *P. acrostichoides*) and immediately taken to the laboratory for analysis. The number of leaves collected for each species varied during the period of 5 months based on the climatic conditions. In June (2022) when the weather was fairly typical for early summer, 4



**Figure 1.** *Dryopteris marginalis* (A) and *Polystichum acrostichoides* (B) growing on rock ledges beneath a forest canopy on Torrey Cliffs, Palisades, New York. Scale bar = 10 cm.

leaves were collected from each species to provide baseline data. In July, 4 leaves were collected from the *P. acrostichoides*, but six were collected from the *D. marginalis* to more carefully document its response because it exhibited more severe signs of desiccation stress. In August, 5 leaf samples were collected from each species to more fully represent their physiological status in response to the continuing heat and drought. In September, when the temperature returned toward normal and precipitation increased, the 2 fern species showed evidence of recovery with less apparent desiccation stress. Three leaf samples were collected in September and October from each fern species when the weather conditions were more amenable for growth. Each sample of fern leaves was collected in the morning, and the base of the dissected petiole was immediately inserted in 50 ml of distilled water in the bottom of a plastic zip-lock bag containing a pad of absorbent paper moistened with deionized water to provide humidity. The leaf sample was immediately taken to the laboratory located on the campus for analysis.

The sampling dates each month in 2022 as follows: June 3 and 4; July 8, 9, 13 and 16; August 8-15; September 19-22; and October 14-18. The range of dates corresponds to the days when leaves were collected from the sampling sites. It was not possible to complete all laboratory analyses during one day, given that there were 2 species of ferns to be analyzed, and there were several laboratory assessments of variables, including replications to be made for the several leaves collected from each fern. Therefore, several days of sampling were necessary. During the peak period of excessive heat in July and early August, daytime air temperatures reached maximum values approaching 35°C, with uncommonly warm nights. Table 1 contains a report of the minimum, average and maximum temperatures for each of the months from June through October.

There was minimal precipitation in the months preceding June, although temperatures were not extreme, incurring a deficit of precipitation at 50 to 75% of the norm. Therefore, the precursor of severe drought stress had already been initiated prior to the onset of the severe heat wave in July. During this study, the total

precipitation (cm) for each month was as follows: June (6.7), July (0.2), August (1.6), September (2.13), and October (1.0). The precipitation for June and September constituted reasonably moderate values compared to the exceedingly low precipitation amounts during the peak of the heat and drought in July and August. Photographic images of the ferns (Figure 1) were obtained using a Canon PowerShot digital camera (Canon Inc., Ōta, Tokyo).

### Physiological analyses

The net photosynthesis rate of the leaves (expressed as  $\mu\text{mol CO}_2$  assimilated  $\text{m}^{-2} \text{s}^{-1}$ ) was assessed using an infra-red gas analyzer (IRGA) system (model BTA, Vernier, Beaverton Oregon), with an optically clear, 163  $\text{cm}^3$  assay chamber and illuminated with a Light Emitting Diode (LED) source at 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD (LiCor Biosciences, Lincoln, Nebraska), equivalent to the ambient PAR during mid-day at the sampling site, and at a temperature of 25°C. Each sample leaf in the zip-lock bag was removed, the end of the petiole was further cut close to the base of the leaf lamina and immediately immersed in deionized water and remained immersed in deionized water during the photosynthesis and respiration measurements. Relative humidity in the sample cuvette ranged from 85–90% to reduce excessive vapor pressure deficit. The  $\text{CO}_2$  concentration in the assay cuvette was at ambient atmospheric concentration (417 ppm). The time for measurement of each leaf was c. 10 min. Mean respiration rate (expressed as  $\mu\text{mol CO}_2$  released  $\text{m}^{-2} \text{s}^{-1}$ ) was determined at 25°C using the same apparatus with a completely darkened assay chamber. The leaf sample was maintained in the dark condition until the reaction centers of the photosystems of the leaves came to equilibrium with the darkened state (c. 2 min.), and measurements were begun when there was a steady state respiration rate.

The chlorophyll concentration index (CCI) for each leaf sample was obtained using a CCM-300 chlorophyll content meter (Opti-Sciences, Inc., Hudson, New Hampshire). At least 20 measurements were made for each leaf sampled from each of the fern species

**Table 1.** Minimum, average and maximum temperatures (°C) for each of the 5 sampling monthly intervals June to October (2022) at Palisades N. Y.<sup>a</sup>

Sampling date	Minimum temperature	Average temperature	Maximum temperature
June	16.1	21.1	25.0
July	19.1	28.3	29.4
August	23.9	30.6	32.2
September	15.9	23.9	24.4
October	7.6	18.3	19.9

<sup>a</sup>Sparkhill Creek weather station, Palisades, New York.

collected on a specified collection date. Each of these mean values, obtained from the 20 measurements, was used to calculate the overall mean CCI value for the replicates of a given fern species on the specified date (Table 2). An OS-30p+ Chlorophyll Fluorometer (Opti-Sciences, Inc., Hudson, New Hampshire) was used to obtain the leaf fluorescence data. This included leaf quantum yield efficiency expressed as variable fluorescence/maximum fluorescence ( $F_v/F_m$ ), and evidence of electron transport per reaction center ( $ET_0/RC$ ) from photosystem II (PS II) to the quinone intermediate ( $Q_A$ ) and beyond in the electron-transport chain as based on the JIP test application in the OS-30p+ instrument. Leaf samples were dark adapted for 20 to 30 min before the measurements were made to ensure that the reaction centers (RC) had come to equilibrium with the darkened state. All results of the assays are presented as the mean  $\pm$  standard error ( $M \pm SE$ ) calculated using an Excel spreadsheet (Microsoft, Inc. Redmond, WA, USA). Pearson product moment correlation coefficient calculations were obtained using an Excel spreadsheet. Linear regression estimates relating photosynthesis rate ( $A_c$ ) to environmental temperature in °C ( $T$ ) were obtained using the Vassarstats online calculator (<http://vassarstats.net/vscor.html>). A Kolmogorov-Smirnov test was used to verify that the data was sufficiently normally distributed to perform the linear regression analysis.

## RESULTS

The results for the leaf photophysiology and dark respiration measurements are presented in Table 2. In general, the mean rate of photosynthesis for the two species was higher during early summer (June) and early autumn (September into October) than during the peak of the summer drought and heat stress (July and August). During July and August, the overall mean respiration rates ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) were *D. marginalis* ( $0.36 \pm 0.05$ ), and *P. acrostichoides* ( $0.24 \pm 0.04$ ). In general, the mean photosynthesis and dark respiration rates for *D. marginalis* tended to be higher than for *P. acrostichoides*. With respect to the C balance (C lost by respiration/C gained by photosynthesis) the mean respiration rate as a % of photosynthesis  $\pm$  SEM is as follows: *D. marginalis* ( $31\% \pm 3.5$ ) and *P. acrostichoides* ( $23\% \pm 4.7$ ). It is interesting to note that the overall mean leaf chlorophyll concentration index (CCI) was consistently higher for *P. acrostichoides* ( $16.6 \pm 0.30$ ) than for *D. marginalis* ( $5.5 \pm$

$0.53$ ). The leaves of *P. acrostichoides* are deep green in color and relatively thick, suggesting that they may contain more chlorophyll per unit leaf area. The overall mean  $F_v/F_m$  values  $\pm$  SEM for the 2 species during the 5 months was fairly similar: *D. marginalis* ( $0.75 \pm 0.01$ ) and *P. acrostichoides* ( $0.78 \pm 0.11$ ). The mean  $F_v/F_m$  values, combining the June and autumn data for both species were fundamentally equivalent ( $0.76 \pm 0.01$ ). There was no significant correlation between  $F_v/F_m$  and photosynthesis rate ( $r = -0.4$ ,  $n = 10$ ,  $p = 0.15$ ).

The mean  $ET_0/RC$  values  $\pm$  SEM for the 2 species during the 5 months were *D. marginalis*  $1.14 \pm 0.04$  and *P. acrostichoides*  $1.01 \pm 0.01$ . The mean  $ET_0/RC$  value combining the data for the two species for the mild June and autumn time period was  $1.12 \pm 0.04$ , and for the stressful July and August data was  $1.01 \pm 0.02$ ; the means are not significantly different ( $t = 1.91$ ,  $df = 8$ ,  $p = 0.09$ ). However, there was a significant positive correlation between  $ET_0/RC$  values and net photosynthesis rate ( $r = 0.68$ ,  $n = 10$ ,  $p = 0.03$ ). This indicates that some of the variation in photosynthesis rates between species and across the summer weather pattern may be accounted for by the rate of transfer of electrons from PS II through the intermediate quinone to the site of  $\text{CO}_2$  fixation and its reduction to produce the first carbohydrate product as measured by the  $ET_0/RC$  variable.

Overall, there is no statistically significant difference in the mean value of the photosynthesis rate for *D. marginalis* ( $1.75 \pm 0.49$ ) and *P. acrostichoides* ( $1.37 \pm 0.24$ ); although, given the limited sample size ( $N = 10$ ), this may contribute to a lack of significance. The ratio of mean  $\text{CO}_2$  fixation rate ( $A_c$ ) during the most stressful months (July and August) to the mean rate in less stressful months (June, September and October) can provide evidence of resiliency. Combining data for both species, the ratio in July and August (during the greatest climate stress) compared to the 3 months with more moderate climate (June, September and October) was 0.36. That is, the  $\text{CO}_2$  fixation rate for the stressful months was c. 40% of the fixation rate in the less stressful months. This indicates that there may be a substantial decrease in atmospheric C assimilation during

**Table 2.** Means and standard errors of the mean (parentheses) for chlorophyll concentration index (CCI), photosystem II quantum yield efficiency ( $F_v/F_m$ ), electron transfer per reaction center ( $ET_0/RC$ ), photosynthesis  $CO_2$  assimilation rate ( $A_c$ ) and dark respiration rate ( $R_D$ )<sup>a</sup>.

Sampling date	CCI	$F_v/F_m$	$ET_0/RC$	$A_c$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )	$R_D$ ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )
<b>June 2022</b>					
<i>Dryopteris marginalis</i>	4.13 (0.60)	0.72 (0.01)	1.25 (0.03)	3.67 (0.71)	1.49 (0.35)
<i>Polystichum acrostichoides</i>	10.39 (1.10)	0.79 (0.01)	0.98 (0.05)	1.60 (0.12)	0.38 (0.02)
<b>July 2022</b>					
<i>Dryopteris marginalis</i>	4.69 (0.20)	0.73 (0.04)	1.03 (0.07)	1.32 (0.44)	0.44 (0.07)
<i>Polystichum acrostichoides</i>	19.73 (3.85)	0.78 (0.01)	0.96 (0.04)	1.24 (0.42)	0.26 (0.04)
<b>August 2022</b>					
<i>Dryopteris marginalis</i>	5.44 (0.69)	0.76 (0.005)	1.08 (0.01)	1.01 (0.19)	0.27 (0.07)
<i>Polystichum acrostichoides</i>	16.4 (2.85)	0.76 (0.01)	0.96 (0.03)	0.52 (0.22)	0.22 (0.06)
<b>September 2022</b>					
<i>Dryopteris marginalis</i>	6.07 (1.42)	0.78(0.001)	1.12(0.04)	1.27(0.26)	0.45(0.17)
<i>Polystichum acrostichoides</i>	12.98 (2.81)	0.78 (0.004)	1.03 (0.03)	1.54 (0.34)	0.23 (0.06)
<b>October 2022</b>					
<i>Dryopteris marginalis</i>	7.13 (0.54)	0.76 (0.002)	1.22 (0.04)	1.49 (0.10)	0.31 (0.04)
<i>Polystichum acrostichoides</i>	23.53 (3.41)	0.77 (0.004)	1.11 (0.03)	1.95 (0.29)	0.31 (0.02)

<sup>a</sup>Data are means and standard error of the means in parentheses.

the summer stressful period. Consequently, increasing climate stress due to global warming may result in considerable reduction in mean  $CO_2$  fixation by ferns and other herbaceous understory plants, potentially contributing to an increased “greenhouse effect” and greater global warming.

Some of the decreased  $CO_2$  uptake during the climate stressful months may be explained by reduced stomatal opening and less conductance of  $CO_2$  into the leaf. This is particularly likely because the fluorescence evidence of leaf physiological functions (e.g.,  $F_v/F_m$  and  $ET_0/RC$ ) are not substantially different between normal and stressful months. Stomatal closure is a physiological response to conserve leaf hydrature by reducing loss of water by leaf evapotranspiration, but concurrently it also limits diffusion of  $CO_2$  into the leaves.

As shown in Table 1, mean dark respiration ( $R_D$ ) declined considerably during the five-month study from a high value in June of 1.49 for *D. marginalis* and 0.38 for *P. acrostichoides* to 0.31 for both species in October. Some of the decline in the later months may be due to increasing leaf senescence preceding desiccation in the autumn. The mean  $R_D$  for *P. acrostichoides* was consistently lower than the mean  $R_D$  for *D. marginalis*, particularly for June through September. However, with only 4 measurements, the degrees of freedom are too small to likely find a statistically significant difference.

The ratio of dark respiration to photosynthesis rate

( $R_D/A_c$ ) provides an estimate of balance of C exchange by the fern with the environment (that is,  $CO_2$  release/ $CO_2$  assimilation). In June, the ratio was 0.41 for *D. marginalis* and 0.24 for *P. acrostichoides*. During the more stressful months, the ratios for *D. marginalis* in July and August were lower (0.33 and 0.27, respectively). Likewise, the values for *P. acrostichoides* in July and August were 0.21 and 0.42. The somewhat higher value of 0.42 in August is explained by a relatively larger decline in the photosynthesis rate during August compared to the respiration rate. The mean ratios during the subsequent milder period (September and October) were also low: 0.31 for *D. marginalis* and 0.18 for *P. acrostichoides*. At this time most of the leaves were becoming senescent and the relatively low values may be explained by decreasing physiological vigor.

### Linear regression analysis

A linear regression analysis of the relationship between photosynthesis rate ( $A_c$ ) and temperature (T) was made for *D. marginalis* and *P. acrostichoides*. The resulting equation for *D. marginalis* ( $A_c = -0.02 T + 2.27$ ) was not statistically significant ( $t = -0.34$ ,  $df = 19$ ,  $p = 0.74$ ), though the slope of the relationship was negative indicating decreasing photosynthesis rate with increasing environmental temperature. However, the equation for *P.*

*acrostichoides* ( $A_c = -0.09 T + 3.49$ ) was statistically significant ( $t = -2.92$ ,  $df = 18$ ,  $p = 0.009$ ). Differences in the physiological ecology of the 2 ferns may account partially for the differences in the regression results. As explained subsequently, *P. acrostichoides* is typically a more cool-temperature species, surviving winter as a wintergreen fern and exhibiting substantial photosynthesis activity during winter and cool weather of early spring (Noodén and Wagner, 1997). This cooler temperature niche of *P. acrostichoides* may explain the statistically significant negative effect of increasing summer temperatures on its photosynthesis rate as expressed in the linear regression equation.

## DISCUSSION

The drought and heat of summer 2022 produced substantial stress for plants growing on Torrey Cliff, particularly ferns and other herbaceous plants located at more elevated portions of the terrain, leading to severe wilting and in some cases evidence of complete desiccation and death (Anderson, 2022). Evidence presented here, particularly documents the status of 2 commonly occurring temperate fern species on Torrey Cliff during the summer of 2022: (i) the milder early summer (June), (ii) the period of most heat and desiccation stress (July and August) and (iii) autumn (September and October), when more moderate temperatures and precipitation prevailed. Overall, the 2 ferns in this location survived the stressful summer; although during peak stress there was considerable evidence of wilting in some cases, and the physiological evidence indicates substantial decreases in photosynthesis. As of July (2023) when this report was being written, both of the ferns exhibited robust growth further suggesting that they were capable of surviving at least one very serious climate stress and resuming substantial growth in the succeeding year.

*D. marginalis* and *P. acrostichoides* are commonly observed growing as understory terrestrials in occasional patches, typically in depressions near rock outcrops, or other locations where moisture is more prevalent and persistent. One of the *D. marginalis* ferns in this study was growing from a cleft in a north-facing, large rock outcrop beneath a shrub and tree canopy, while all others were growing on rock ledges with relatively thin soil substratum and overlying shrub and tree growth (Figure 1A). *P. acrostichoides* (Figure 1B) known informally as the “Christmas fern” is decidedly wintergreen, and its thick, leathery leaves persist even beneath winter snow. The rachis of the leaf is hinged or softened near the base, thus allowing at least some of the leaves to decline and lie prostrate on the substratum throughout the winter months (Noodén and Wagner, 1997). The position of the leaves (more upright in summer and prostrate in winter) may have important positive adaptive value as shown by

experimental physiological research in field settings (Forget et al., 2018). In some temperate, North American locations, *P. acrostichoides* is a dominant member of the pteridophyte community, occurring in 92% of the plots and constituting 52.4% of the relative importance (Greer et al., 1997).

To place the findings of this laboratory-based study in a broader context, the photosynthesis results reported here are compared to prior published findings for *D. marginalis* and *P. acrostichoides*. During the peak of the summer stress in July and early August, the fern leaves showed signs of wilting, and there was minimal new growth. Nonetheless, after intermittent rain events, the leaves recovered exhibiting normal turgidity. However, the *D. marginalis* mean photosynthesis rates in July through October (in a range of 1.01 to 1.49  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) were lower than the mean in June (3.67) during more favorable growth conditions. The latter rate of 3.67 is comparable to a rate of ca. 3.5  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1}$  reported by Sessa and Givinish (2014) at a light intensity of 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  as interpreted from their graphical data for *D. marginalis* growing in eastern North America.

In the current study, *P. acrostichoides*, growing in shallow soil on the rocky ledges, exhibited major declines in photosynthesis during the period of summer stress reaching a low mean value of 0.52  $\mu\text{mol CO}_2$  assimilated  $\text{m}^{-2} \text{s}^{-1}$  in August, compared to 1.60 in June. The higher June value of 1.60 is comparable to a value of 1.40 reported by Prats and Brodman (2020) for photosynthesis measurements at similar light intensities used in this laboratory research (100  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). In the results reported here, a peak photosynthesis rate of 1.95 was reached when temperatures were more moderate and soil moisture increased in late October. Minoletti and Boerner (1993) reported comparable mean photosynthetic values of 1.96 to 2.14  $\mu\text{mol CO}_2$  assimilated  $\text{m}^{-2} \text{s}^{-1}$  for *P. acrostichoides* growing within ambient  $\text{CO}_2$  levels in forests of Neotoma valley, Ohio during mild winter days. Values of 1.81 to 2.24  $\mu\text{mol CO}_2$  assimilated  $\text{m}^{-2} \text{s}^{-1}$  were published by Noodén and Wagner (1997) for *P. acrostichoides* in early spring while growing in a woodlot near Ann Arbor, Michigan. A value of 1.95 reported here for *P. acrostichoides* during October is within the range reported by Noodén and Wagner. The mean  $F_v/F_m$  values found here, in the range of 0.76 to 0.79 for *P. acrostichoides*, are in agreement with values of approximately 0.79 reported by Reudink et al. (2005) for *P. acrostichoides* growing in southeastern Pennsylvania.

With respect to the biogeochemical C cycle, evidence presented in Table 2, and data from the linear regression analyses, indicate that increasing temperature tends to reduce photosynthesis fixation of  $\text{CO}_2$ , and thus may contribute to relatively higher loss of  $\text{CO}_2$  to the atmosphere through respiration. That is, increasing temperatures tend to shift the balance of  $\text{CO}_2$  exchange toward more  $\text{CO}_2$  loss by the plants. Furthermore, the mean ratio of respiration to net photosynthesis ( $R_D/A_C$ ) for

the 2 species was c. 30% and may account for an appreciable remobilization and release of CO<sub>2</sub> into the atmosphere. Additionally, the lack of new foliage production, particularly during the stressful peak growing season, further limits the potential for drawing down atmospheric CO<sub>2</sub> through photosynthesis fixation due to less physiological activity of senescent leaves (Thomas and Stoddart, 1980).

Overall, the results of this study suggest that these 2 fern species exhibit substantial resilience in the face of increasing evidence of climate change stress, particularly increased mean summer temperatures and reduced precipitation, at least within the weather pattern incurred in summer 2022. However, longer term effects of repeated cycles of exposure to climate change stress over several annual cycles need to be examined to determine if there are accumulated detrimental effects, or if the results reported here within a 5-months period are more indicative of stress resilience across multiple years of climate change. Further research combining laboratory studies such as reported here with field-based research is needed to more fully document the response of ferns in the natural environment to changing climate patterns. Additional research is also needed to assess changing climatic effects on a broader range of temperate fern species, including those in different geographic locales and among more diversified habitats, spanning open locations to more deeply shaded woodlots and forests.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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