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Photophysiology, dark respiration and leaf desiccation resilience of the fern *Adiantum capillus-veneris* L.

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Adiantum capillus-veneris **is a cosmopolitan, globally distributed terrestrial fern in warm temperate to tropical regions. It is also widely cultivated in indoor and outdoor settings. The natural habitat of *A. capillus-veneris* includes limestone rocky places with consistent sources of water, including shady alkaline, moist cliffs and on vertical surfaces in moist forested locations, preferably with calcareous soils; however, it is also found on sandstone. There is increasing evidence that it is relatively tolerant of desiccation stress, under appropriate environmental circumstances. This is a report of a laboratory-based study of the photophysiology of *A. capillus-veneris,* including an analysis of the effects of leaf excision and varied degrees of desiccation on leaf resilience and physiological properties, with comparisons to *Nephrolepis exaltata* (L.) Schott and *Adiantum tibeticum* Ching. Evidence is also presented on the rate of excised leaf desiccation in relation to the role of humidity in mitigating stress due to limited access to moisture. The ecophysiologial variables examined include: Variations in photosynthesis rate relative to light intensity, dark respiration rate, leaf parameters including chlorophyll concentration index (CCI), specific leaf area (SLA), and leaf fluorescence evidence for functioning of photosystem II (PS II).**

Key words: Adaptation, climate change, comparative physiology, desiccation stress, environmental research, functional anatomy, leaf fluorescence analysis, physiological ecology.

INTRODUCTION

Adiantum capillus-veneris L. (known as the maidenhair fern) is a cosmopolitan terrestrial fern species widely distributed globally in warm temperate to tropical regions. It is commonly grown as a cultivated plant (Jones, 1987). The delicate, thin leaves (triangular to ovate-deltate in outline) arise as an erect or arching array from a brown-scaly, creeping rhizome. The leaf lamina is bi- or tri-pinnate, but typically pinnate near the apex. Pinnules are wedge-shaped with straight sides, entire to deeply incised on the outer margin into narrow lobes, with outer margin minutely toothed (Figure 1). Further details of the species can be found in Jones (1987) or Khullar (2000) for formal botanical information. The habitat of *A. capillus-veneris* includes limestone rocky places with consistent sources of water, including shady alkaline, moist cliffs and on vertical surfaces in moist forested locations, preferably with calcareous soils; however, it is also found on sandstone (Furnald, 1950). Occasionally, it is found growing in acidic to neutral soils. In some montane locations (e.g., Himalaya) it is found in a variety

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Figure 1. Foliage of *A. capillus-veneris* in laboratory cultivation. Scale bar = 4 cm.
Source: Author

of locations, but also grows effectively as a lithophyte on moist or wet rocks (Punetha et al., 2013). Fossil evidence indicates the presence of *Adiantum* spp. in the Miocene, ca. 23 to 5 million years ago, at geographic locations where paleontological evidence suggests the climate was warm and humid (Yao et al., 2011).

As is characteristic of the genus (*Adiantum*), the thin pinnae have an inner chlorophyll-bearing mesophyll that is one- to two-cells thick, bounded by an upper and lower epidermis with a substantial hydrophobic cuticle (Wiley, 1948) that provides some protection from desiccation of the thin leaf, by impeding water diffusion through the epidermis during desiccation stress.

Although the leaf is delicate, current evidence indicates that *A. capillus-veneris* is relatively resistant to desiccation stress. Wu et al. (2013) reported that *A. capillus-veneris* was the most drought resistant among six temperate ferns they studied in north China. In a study of 43 fern species, Kessler and Siork (2007) reported that the desiccation tolerance of excised *A. capillus-veneris* leaves was intermediate between poikilohydric and mesomorphic species, with the capacity to survive desiccation loss up to 81% of the leaf water content. However, the relative rate of water loss by the thin leaves of *A. capillus-veneris* may be more rapid than thicker leaves of other ferns, and additional research on this aspect is needed as addressed more fully in Objective 4 of this research study.

In general, a substantial amount of research has been done on comparative morphology and physiology, systematics, biogeography, distribution of fern species, ecology and medicinal uses, including *A. capillus-veneris* (Li et al., 2013; Al-Snafi, 2015; Liao et al., 2017; Oloyede et al., 2017; Deans et al., 2019). However, there is increasing interest in their physiological ecology (Mehltreter et al., 2010; Anderson, 2021). Much of the basic research on functional morphology and physiology of *A. capillus-veneris* was done several decades ago as summarized earlier; and less research has been devoted to photophysiology. Therefore, additional research is needed using modern instrumentation and research methods as reported here. This is a report of a laboratory-based study of the photophysiology of *A. capillus-veneris,* including an analysis of the effects of leaf excision and varied degrees of desiccation on leaf resilience and physiological properties. More specifically, the objectives of this study are as follows:

(1) Assess the photosynthesis rate expressed relative to: i) total leaf surface area, ii) leaf fresh weight, and iii) leaf dry weight.
(2) Determine leaf physiological variables: i) chlorophyll concentration index (CCI), ii) specific leaf area (SLA), iii) the quantum yield efficiency (Fv/Fm) of the leaves, iv) electron-transport per reaction center (ET_0/RC) from Photosystem II (PS II) through the quinone intermediate Q_A and beyond in the electron-transfer chain, and v) probability of electron transfer from PSII to the intermediate quinone (Q_A) and beyond in the electron-transport pathway (ψ_0).

(3) Assess dark respiration rate expressed relative to: i) total leaf surface area, ii) leaf fresh weight, and iii) leaf dry weight.
(4) Examine the effects of leaf excision, curtailing hydraulic conductance, on leaf percent water content, photosynthesis rate, and leaf physiological variables as listed in Objective 2.

MATERIALS AND METHODS

Laboratory maintenance of fern plants

A. capillus-veneris plants were purchased as potted plants from a commercial plant vendor (Hirt's Gardens, Medina, Ohio, geographic coordinates: 41.14°N, 81.73°W) and maintained in an environmentally controlled culture room (24°C, relative humidity at 62%, and light intensity of 100 µmol photons m^{-2} s^{-1} provided by a LED fluorescent source, with a light:dark cycle of 14:10). Three plants were used in the experimental studies, and all measurements were replicated either three or eight times as explained subsequence.

Photosynthesis and respiration measurements

The net photosynthesis rate of the fern leaf sample was assessed using an infra-red gas analyzer (IRGA) system, with an optically clear, 163-cm^3 cuvette (CO_2 sensor model BTA, Vernier, Beaverton, Oregon), and illuminated with a LED light source at intensities of 10, 25, 50 and 100 µmol m^{-2} s^{-1} (verified using a Li-Cor 1776 Solar Monitor; LiCor Biosciences, Lincoln, NE) and a temperature of 25°C. Relative humidity in the sample cuvette ranged from 85 to 88%. Dark respiration was measured by enclosing the IRGA cuvette containing the leaf sample in an opaque enclosure to determine the rate of CO_2 production in complete darkness at 25°C. The fern leaf sample was maintained in the dark condition until the reaction centers of the photosystems of the leaf came to equilibrium with the darkened state, and measurements were begun when there was a steady state respiration rate, achieved at approximately 1 min after inception of the measurement. The CO_2 concentration in the assay chamber was at ambient atmospheric concentration (417 ppm). All measurements were replicated for eight leaf samples.

Following the assay for photosynthesis rate and dark respiration, the area of the leaf expressed as cm^2 was assessed using a leaf area meter (Model AM-350, Opti-Sciences, Inc., Hudson, NH). The fresh weight of the leaf and the dry weight, after drying overnight at 60°C in a laboratory oven, were determined using a Sartorius digital balance (Model: GD-503-NTEP, Sartorius AG, Göttingen, DEU). Using this data, mean specific leaf area (leaf area in cm^2/ dry weight in g) was also calculated using eight replicates.

The mean photosynthesis rate ± standard error of the mean (SEM) for the analyzed leaves was determined based on the quantity of CO_2 assimilated for each of the following conditions: i) per leaf area (cm^{-2}), ii) per leaf fresh weight (g_{FW}^{-1}), and iii) per leaf dry weight (g_{DW}^{-1}). Additionally, the mean dark respiration rate ± SEM for each leaf sample was assessed based on the quantity of CO_2 released relative to the same three leaf parameters: i) per leaf area (cm^{-2}), ii) per leaf fresh weight (g_{FW}^{-1}), and iii) per leaf dry weight (g_{DW}^{-1}).

Leaf physiological parameters

The mean ± SEM for the chlorophyll content index (CCI) was obtained for each leaf sample using a Chlorophyll Content Meter (Model CCM-200+GPS; Opti-Sciences, Inc., Hudson, NH) based on at least 10 measurements per leaf sample and using three leaves for a total of 30 measurements. The percent water content (H_2O %) of the leaf sample was obtained using the fresh leaf weight and dry leaf weight (Equation 1).

$$H_2O\ \% = [(\text{fresh leaf wt.} - \text{dry leaf wt.})/\text{fresh leaf wt.}] \times 100 \quad (1)$$

Specific leaf area (SLA) was also calculated (Equation 2).

$$SLA = \text{Leaf area } (cm^2)/\ \text{Leaf dry wt. (g)} \quad (2)$$

Eight leaves were measured and weighed using a Sartorius digital balance (Model: GD-503-NTEP, Sartorius AG, Göttingen, DEU). Also, for comparative purposes, the SLA of the fern *Nephrolepis exaltata* (L.) Schott (commonly known as the Boston fern) was also calculated, using eight samples. The percent water content (H_2O %) of the *N. exaltata* leaf was also calculated for each of the leaf samples by using Equation 1.

An OS-30p+ Chlorophyll Fluorometer (Opti-Sciences, Inc., Hudson, NH) was used to obtain the quantum yield efficiency based on the ratio of variable fluorescence/maximum fluorescence of the leaf (F_v/F_m), electron transport per reaction center (ET_0/RC), and probability of electron transfer from PSII to the quinone and beyond (ψ_0) based on the JIP test application in the OS-30p+ instrument. Each measurement was replicated eight times. Leaf samples were dark adapted for 20 min before the F_v/F_m measurements were made.

Leaf desiccation resilience evidence

To assess the rate of percent water loss by leaves of *A. capillus-veneris* when subjected to loss of xylem stream hydraulic conductance, freshly excised leaves were analyzed experimentally as explained subsequently.

Percent water loss during desiccation

A freshly excised leaf from the laboratory-grown *A. capillus-veneris* was placed on an 8-cm^2 sheet of weighing paper in the Sartorius digital balance situated in a temperature and climate controlled laboratory room (25°C) with relative humidity (RH) = 15-20%, and the weight was monitored for 350 min with at least 20 continuous weight measurements. The decrease in percent water content was plotted as a function of time (Figure 2). The weighing was stopped when the decrease in leaf weight approached a steady lowest level and the leaves were severely dehydrated. The analysis was replicated three times to confirm the negative curvilinear slope of the plot as shown in Figure 2.

Resilience as related to humidity

Based on the aforementioned evidence of decline in water content at moderately low humidity, additional evidence was obtained regarding the resilience of excised leaves when maintained at higher humidity (ca. 90%). The humidity treatment was replicated three times. Photosynthesis rate and dark respiration were measured as explained earlier at two times: (1) prior to maintenance in elevated humidity for 7 days, and (2) after humidity treatment for 7 days. The treatment was replicated three times. After the photosynthesis and dark respiration measurements at Step 1, each leaf was immediately placed in a transparent, plastic 'zip-lock' bag containing three Whatman No. 1 discs of filter paper thoroughly moistened with distilled water. A plastic disc (ca. 5 cm dia., × 3 cm height) was enclosed to elevate the plastic bag above

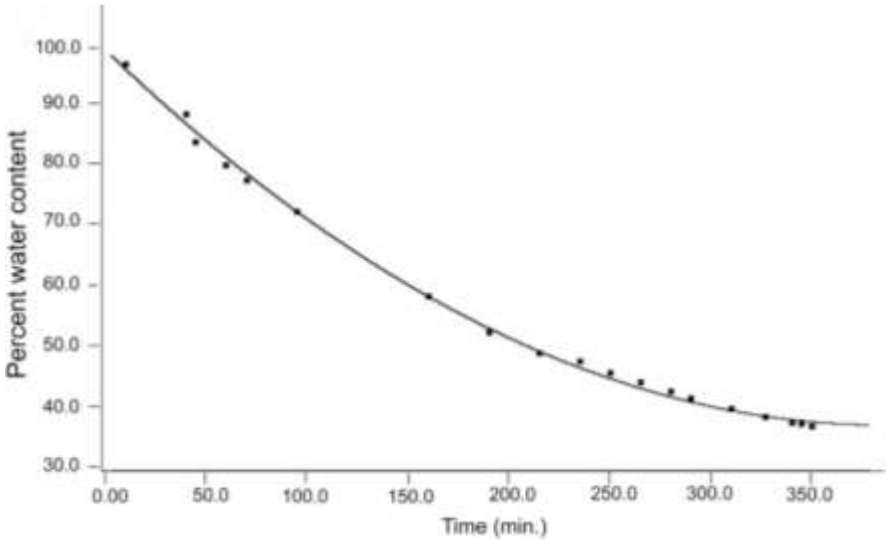

Figure 2. Desiccation-decay curve showing percent change in water content based on change in fresh weight of *A. capillus -veneris* leaf as a function of time in minutes (min) while drying at 25°C and relative humidity of ca. 20%.
Source: Author

the *A. capillus-veneris* leaf to provide adequate space for gas exchange with the leaf while in the higher humidity treatment. The internal humidity was determined by inserting the probe of a Fisher Scientific, digital thermo-hygrograph into the partially opened zip lock enclosure while sealing the plastic closure around the casing of the probe.

The leaves within the zip-lock bags were placed in the controlled climate room under the same culture conditions, including illumination, used for the *A. capillus-veneris* plants as described earlier. After seven days (Step 2), each leaf was retrieved from the 'zip-lock' bag and immediately analyzed for photosynthesis and dark respiration rates as described earlier in the subsection on "Photosynthesis and Respiration measurement." After these measurements, leaf physiological measurements were made as described earlier in the subsection on "Leaf physiological parameters". These measurements were compared with data previously gathered on untreated leaves at the beginning of the seven-day experiment. Leaf fresh weight and dry weight (after drying overnight in an aluminium foil planchet at 60°C in a laboratory oven) were obtained using the Sartorius balance.

Statistical analyses

Each result is reported as a mean ± standard error of the mean (SEM). An unpaired t-test (GraphPad, Dotmatics.COM, Boston, MA) was used to assess mean differences between data from *A. capillus-veneris* and *N. exaltata* as specified in the results. A Kolmogorov-Smirnov test was used to determine that the data were sufficiently normally distributed to perform the parametric t-Test.

RESULTS

The results of the assays for photosynthesis rates are presented in Table 1.

There is an approximate linear increase in photosynthesis rate up to a PPFD of 50 µmol photons m^{-2} s^{-1}, for all three column categories; but thereafter, the slope becomes increasingly less as is characteristic at higher light intensities. The maximum PPFD (100 µmol photons m^{-2} s^{-1}) is consistent with the higher light intensities in forest understories with relatively open canopies and at northern facing locations under a cloudless sky at temperate locations.

The mean ± SEM for dark respiration rate based on leaf area was 0.33 ± 0.06 (µmol CO_2 released m^{-2}, s^{-1}), based on leaf fresh weight was 289.97 ± 64.41 (nmol CO_2 released g_{FW}^{-1} min^{-1}), and on a dry weight basis was 1174.21 ± 281.42 (nmol CO_2 released g_{DW}^{-1} min^{-1}). Data for leaf physiological variables are presented in Table 2.

Leaf desiccation resilience

When an excised leaf was weighed continuously during a period of ca. 350 min with relative humidity of ca. 20%, the percent water content decreased continuously with a negative curvilinear slope (Figure 2), reaching a near steady state minimum of 35% of water content relative to the initial fresh weight) and the leaves were decidedly dehydrated. When oven dried, the mean percent dry mass ± SEM of *A. capillus-veneris* leaves relative to the fresh weight was ca. 25% ± 1.8.

For purposes of comparison, the leaves of *Nephrolepis exaltata* (L.) Schott (Boston fern) were also analyzed for rate of water loss, including three replicates, using a similar desiccation experiment as was used for *A. capillus-veneris*. *N. exaltata* leaves are decidedly more robust and thicker. They lost relatively less water water content (12%) during the drying experiment, compared to

Table 1. Mean ± SEM net photosynthesis rate for *A. capillus-veneris* under controlled climate cultivation at 25°C at light intensities expressed as photosynthetic photon flux density (PPFD) of 10, 25, 50 and 100 µmol photons m^{-2} s^{-1}.

PPFD	Photosynthesis rate[a]		
	µmol CO_2 m^{-2} s^{-1}	nmol CO_2 g_{FW}^{-1} min^{-1}	nmol CO_2 g_{DW}^{-1} min^{-1}
10	0.31 ± 0.07	303 ± 81.80	1257 ± 361.54
25	0.67 ± 0.10	621 ± 135.62	2617 ± 566.85
50	1.07 ± 0.11	979 ± 138.86	4026 ± 669.71
100	1.38 ± 0.18	1238 ± 190.21	5081 ± 894.61

[a]Mean data are based on eight measurements for each parameter.
Source: Author

Table 2. Mean ± SEM leaf physiological parameters for *A. capillus-veneris*[a].

CCI	SLA	H_2O %	F_v/F_m	ET_0/RC	ψ_0
5.73 ± 0.19	615 ± 47.82	75 ± 1.04	0.76 ± 0.013	1.03 ± 0.03	0.67 ± 0.01

[a]CCI = Chlorophyll Content Index, SLA = specific leaf area , H_2O % = leaf percent water content, F_v/F_m = variable fluorescence/maximum fluorescence, ET_0/RC = electron transport in PS II per reaction center; and ψ_0 = probability that an electron, produced by photon excitation in PS II, will pass to the intermediate quinone and beyond in the electron-transport chain. Mean data are based on eight measurements.
Source: Author

65% for *A. capillus-veneris* as reported earlier, where the graph declined from 100% to a residual concentration of 35%. There are many differences in leaf morphology of *N. exaltata* compared to *A. capilllus-veneris*. The thicker mesophyll layer and overall robustness of *N. exaltata* leaves may have contributed to its greater resistance to evapotranspiration loss of water from the excised leaves. However, this comparative data between the two species illustrates the relative difference in water loss of *A. capillus-veneris* under desiccation stress compared to that of *N. exaltata*.

N. exaltata was chosen as an interesting comparative fern species because it occurs in a variety of habitats (pantropical), at low to middle elevations (sea level to 1,170 m). In addition to the desiccation data, the specific leaf area (SLA) of *A. capillus-veneris* and *N. exaltata* were analyzed. The SLA (mean ± SEM) of *A. capillus-veneris* (647 ± 35.7) was higher than that of *N. exaltata* (527 ± 18.34). The differences are statistically significant ($t = 2.82$, $p < 0.01$, $df = 22$). This partially reflects the differences in morphology of the two leaves. The relatively thin leaves of *A. capillus-veneris* have a much higher surface to volume ratio than the leaves of *N. exaltata*; thus, contributing to a large surface: dry weight ratio, and contributing to a larger SLA. Moreover, the SLA of each species has both positive and negative adaptive values as explained more fully, hereafter.

A larger SLA (larger surface to weight ratio) as reported for *A. capillus-veneris* favors enhanced reception of light photons, while reducing leaf construction costs by developing less mesophyll biomass. However, with a thin mesophyll, there is likely greater water vapor conductivity in the mesophyll tissue free space, permitting more diffusion of water toward and through the epidermis by evapotranspiration, thus leading comparatively to greater percent water loss. On the other hand, the smaller SLA (smaller surface to weight ratio) of *N. exaltata*, favors less loss of water by evapotranspiration, assuming the thicker mesophyll likely impedes water vapor diffusion within the mesophyll tissue interstices; and thus reduces diffusion toward the epidermis and eventual loss to the atmosphere. However, there is less surface area to absorb incident photons, and there is relatively increased construction cost to produce the more massive mesophyll internal layer.

Overall, the results of this phase of the study suggest that under adverse dry conditions, with reduced hydraulic conductance, *A. capillus-veneris* plants in environments with relatively moderate humidity in the range of 20 to 30% R.H. may incur serious water loss within a period of hours due to evapotranspiration; at least within the limitations of this experimental evidence, as discussed subsequently.

To more fully explore these implications experimentally, excised leaves of *A. capillus-veneris* were placed in elevated humidity within zip-lock bags as explained earlier. The purpose was to determine the possible ameliorative effects of higher humidity on the leaf resilience to water loss. The mean weight of the leaves prior to treatment was 0.17 ± 0.05 g and seven days afterwards was 0.16 ± 0.04 g, indicating relatively little loss of water content. Moreover, the net photosynthesis

Table 3. Means ± SEM: net photosynthesis rate for excised leaves of *A. capillus-veneris* prior to and after seven days treatment in elevated humidity[a].

Treatment	µmol m^{-2} s^{-1}	nmol g_{FW}^{-1} min^{-1}	nmol g_{DW}^{-1} min^{-1}
Pre-treatment	1.33 ± 0.16	968.65 ± 164.42	3,323.08 ± 347.84
Post-treatment	1.26 ± 0.24	888.98 ± 64.06	3,045.56 ± 42.78

[a]Mean data are based on three measurements.
Source: Author

Table 4. Means ± SEM: dark respiration rate for excised leaves prior to and post seven days treatment in elevated humidity[a].

Treatment	µmol m^{-2} s^{-1}	nmol g_{FW}^{-1} min^{-1}	nmol g_{DW}^{-1} min^{-1}
Pre-treatment	0.27 ± 0.07	164.52 ± 19.57	606.33 ± 50.34
Post-treatment	0.20 ± 0.06	134.70 ± 18.64	467.25 ± 71.93

[a]Means are based on three measurements.
Source: Author

rates before the seven-day humidity treatment and afterwards also are comparable, but slightly lower after the seven-days of treatment (Table 3).

Overall, after the seven days in the illuminated and elevated humidity treatment, there was a reduction in net photosynthesis rate of 5% based on leaf area, 8.2% based on leaf fresh weight and 8.4% based on leaf dry weight. This indicates that leaves of *A. capillus-veneris* in environments with substantially higher humidity may incur less leaf stress and remain more physiologically viable under conditions of limited available water and reduced hydraulic conductivity, compared to environments with lower humidity, where evapotranspiration rates would be expected to be higher (Figure 2).

The mean dark respiration rates were more markedly reduced for excised leaves maintained in the high humidity, illuminated environment shown in Table 4. Overall, there was a reduction in dark respiration rate of 27% based on leaf area, 18% based on leaf fresh weight and 23% based on leaf dry weight after seven days of treatment in the high humidity, illuminated environment.

It is not entirely clear why the dark respiration rates were more markedly reduced after the seven-day treatment compared to net photosynthesis rates. However, there may have been down-regulation of dark respiration to conserve starch and other reserve organic substances and/or there may have been a reduction of available reserve organic storage compounds as a result of the seven-day high humidity treatment, thus providing less carbon compounds to be metabolized. This is an aspect that requires additional experimental analysis.

Leaf fluorescence analyses indicated that the major markers for Photosystem II function in the leaves maintained in the higher humidity conditions were comparable to mean values reported in Table 2 for untreated leaves; that is, $F_v/F_m = 0.75 \pm 0.003$, $ET_0/RC = 0.98 \pm 0.06$, and $\psi_0 = 0.70 \pm 0.01$.

DISCUSSION

A. capillus-veneris, a cosmopolitan plant found in relatively diverse geographic and environmental locations, is an attractive species for ecological and ecophysiological studies; particularly, research to better document its response to varying environmental and climatic variables toward an improved understanding of how such widely-distributed ferns adapt to and survive in different ecosystems, where they may be an important component of local plant communities (Sharpe et al., 2010).

Recently, there has been increasing interest in the dynamics of fern interactions with their environment, particularly where there are diverse edaphic and climatic variables. This is partly due to increasing evidence of climate change and the need to determine to what extent, if any, climate change may have on fern adaptability, distribution, and survival (Anderson, in press; Mehltreter and McAdam, 2022; Sharpe, 2019). Given the changing characteristics of the environment, there is also interest in better understanding the role of ferns, and other plant groups, as environmental indicator species (Carignan and Villard, 2002; Marimuthu et al., 2022); that is, evidence of how the particular adaptive qualities of a fern species, that make it successful in a particular environment, may be used to characterize or categorize different environments where the fern species are growing. For example, Higa et al. (2013) examined the predictive capacity of three fern species: *Matteucia struthiopteris* (L.) Tod., *Pteridium aquilinum* (L.) Kuhn, and *Osmunda japonica* Thunb. in relation to 16 other angiosperm species growing in varying environmental

settings. Increasing evidence from ecological and physiological research on ferns in varied geographic locales and habitats may contribute to more successful application of these bioindicator methods.

Based on a search of the literature, there is limited research on the physiological ecology of *A. capillus-veneris*, especially fundamental aspects such as primary productivity and adaptations to environmental stressors, including variations in light intensity, available moisture, temperature and interactions with other biota. The research reported here particularly focused on responses of *A. capillus-veneris* to variations in light intensity (PPFD) and some aspects of reduced hydrature based on leaf excision experiments.

Overall, the results of the data on photosynthesis for *A. capillus-veneris* (Table 1) are consistent with general expectations for plants adapted to survive in environments with low to moderate photosynthetic active radiation (PAR). This is to be expected for representatives of a plant lineage that has evolved in a world increasingly dominated by angiosperms, especially forest-producing angiosperms, where ferns became increasingly established as forest understory flora in the Cenozoic, ca. 65 million years ago (Watkins and Cardelús, 2012). There is appreciable net photosynthesis at the lowest PPFD used in this study (10 µmol photons m^{-2} s^{-1}); namely: 0.31 ± 0.07 µmol m^{-2} s^{-1} expressed on a leaf area basis. The highest rate at PPFD of 100 µmol photons m^{-2} s^{-1} was 1.38 ± 0.18 µmol CO_2 m^{-2} s^{-1}.

Furthermore, in a prior laboratory-based photophysiological study of *Adiantum tibeticum* Ching (a high-altitude, Himalayan species found in semi-open areas, among rocks and low bushes) a photosynthesis rate of 1.08 ± 0.05 µmol CO_2 m^{-2} s^{-1} at a PPFD of 100 µmol m^{-2} s^{-} was reported (Anderson, in press). This is somewhat lower than the value of 1.38 ± 0.18 CO_2 µmol m^{-2} s^{-} reported earlier for *A. capillus-veneris* at a PPFD of 100. However, *A. tibeticum* is a much smaller plant with a creeping rhizome, low spreading growth habit, and leaves approximately 30 cm in length.

The values for leaf physiological parameters of *A. capillus-veneris* are fairly representative of ferns. For example, the F_v/F_m mean value of 0.76 is in the range typical of ferns. Ferns generally have lower F_v/F_m mean values than angiosperms; where values as high as 0.83 are recorded as an optimum (Björkman and Demmig, 1987; Dobrikova et al., 2022). Moreover, the mean F_v/F_m value of 0.76 for *A. capillus-veneris* is comparable to the value reported for *A. tibeticum* (0.75).

The mean leaf SLA for *A. capillus-veneris* (615) is relatively large, compared to other broad-leaf plants, and may be partially explained by the thin leaf mesophyll with comparatively low dry mass. Overall, the photosynthesis data suggest that *A. capillus-veneris* could adapt to environments of varying PPFD, depending on suitable temperature ranges, edaphic variables and available humidity or other sources of moisture.

In this study, excised leaves of *A. capillus-veneris* were used to examine evapotranspiration loss of water and the effects of elevated humidity on leaf resilience against desiccation. This is a technique that has been used in prior studies (Kessler and Siork, 2007). There are advantages and limitations to this approach. Using freshly excised leaves allows for carefully controlled measurements of leaf weight loss during evapotranspiration under controlled conditions of low available water and limited hydraulic conductance. However, excision also incurs some additional stress factors, including loss of transport of nutrients and hormones carried by xylem flow from the root to the leaves. Some of these factors are likely not so significant for short-term experiments over several hours. For longer-term experiments, it may be more significant. However, in the seven-day elevated humidity experiment in this study, the examined physiological variables (Table 4) showed minimal evidence of major changes after 7 days, suggesting that there was less likely adverse effects, overall, of the excision.

There is fairly good evidence to suggest that excised leaves, or even excised discs of leaf tissue, are sufficiently normal physiologically to permit valid, relatively short-term laboratory experimental studies (Bartos et al., 1960); although, in any laboratory excision preparation, care must be taken in the experimental arrangements to prevent blockage of leaf surfaces that may limit diffusive uptake of atmospheric CO_2, etc (Kato et al., 2002). However, longer term studies may require more careful monitoring to account for possible adverse effects of excision.

With respect to the effects of available moisture on desiccation tolerance as examined with the excised leaves in this study, the results reported here are consistent with those of other studies indicating that *A. capillus-veneris* is capable of adjusting to, or recovering from, limited environmental available moisture (Kessler and Siork, 2007; Wu et al., 2013). However, these findings must be tempered by the added caveat that such resilience may only be substantial assuming there is sufficient relative humidity, or other atmospheric sources of moisture such as fog or water spray, to prevent excessive rapid leaf transpiration and drying.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

REFERENCES

Al-Snafi AE (2015). The chemical constituents and pharmacological effects of *Adiantum capillus-veneris* – A Review. Asian Journal of Pharmaceutical Science Technology 5:106-111.

Anderson OR (2021). Physiological ecology of ferns: Biodiversity and conservation perspectives. International Journal of Biodiversity and Conservation 13(2):49-63.

Anderson OR (In press). Comparative photophysiology of two ferns with contrasting morphology and physiological ecology: *Adiantum tibeticum* Ching and *Dryopteris lepidopoda* Hayata. Indian Fern Journal.

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.

Bartos J, Kubin S, Setlík I (1960). Dry weight increase of leaf disks as a measure of photosynthesis. Biologia Plantarum (Praha) 2:201-215.

Carignan V, Villard MA (2002). Selecting indicator species to monitor ecological integrity: a review. Environmental Monitoring and Assessment 78:45-61.

Deans RM, Brodribb TJ, Busch FA, Farquhar GD (2019). Plant water-use strategy mediates stomatal effects on the light induction of photosynthesis. New Phytologist 222:382-395.

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.

Furnald ML (1950). *Adiantum Capillus-veneris* in the United States. Rhodora 52:201-208.

Higa M, Nakao K, Tsuyama I, Nakazonjo E, Yasuda M, Matsui T, Tanaka N (2013). Indicator plant species selection for monitoring the impact of climate change based on prediction uncertainty. Ecological Indicators 29:307-315.

Jones DL (1987). Encyclopedia of Ferns. Timber Press, Portland OR P 214.

Kato MC, Hikosaka K, Hirose T (2002). Leaf discs floated on water are different from intact leaves in photosynthesis and photoinhibition. Photosynthesis Research 72:65-70.

Kessler M, Siork Y (2007). Desiccation and rehydration experiments on leaves of 43 pteridophyte species. American Fern Journal 97:175-185.

Khullar SP (2000). An Illustrated Fern Flora of the West Himalaya (Vol. I). International Book Distributors, Dehra Dun, India.

Li X, Fang Y-H, Yang J, Bai S-N, Rao G-Y (2013). Overview of the morphology, anatomy and ontogeny of *Adiantum capillus-veneris*: An experimental system to study the development of ferns. Journal of Systematics and Evolution 51(5):499-510.

Liao JX, Zhang H, Mo L, Huang YQ, Sun YJ, Li YQ (2017). Differences in growth and biomass allocation of *Adiantum flabellulatum* and *A. capillus-veneris* as a result of light and water availability. Botany Letters 164(4):393-400.

Marimuthu J, Fernández H, Kumar A, Thangaiah S (Eds.) (2022). Ferns: Biotechnology, Propagation, Medicinal Uses and Environmental Regulation. Springer Nature, Singapore pp. 587-713.

Mehltreter K, McAdam SAM (2022). Ecology and ecophysiology of ferns and lycophytes in a changing climate: A special issue of the American Fern Journal. American Fern Journal 112(4):227-232.

Mehltreter K, Walker LR, Sharpe JM (Eds.) (2010). Fern Ecology. Cambridge University Press, Cambridge, UK.

Oloyede FA, Olatunji TY, Ogbimi ER (2017). Spore germination, gametophyte development and studies of the growth rate of *Adiantum capillus-veneris* L. Notulae Scientia Biologicae 9(4):557-562.

Punetha R, Bhakuni K, Kolia BA (2013). Lithophytic ferns and fern allies of Kumaon. In: Singh, RA (Ed.), Landslides and Environmental Degradation. Gyanodaya Prakashan, Nainital, India pp. 239-352.

Sharpe JM (2019). Fern ecology and climate change. Indian Fern Journal 36:179-199.

Sharpe JM, Mehltretter K, Walker LR (2010). Ecological importance of ferns. In: Mehltreter K, Walker LR, Sharpe JM (Eds.), Fern Ecology. Cambridge University Press, Cambridge, UK. pp. 1-21.

Watkins JE Jr., Cardelús CL (2012). Ferns in an angiosperm world: Cretaceous radiation into the epiphytic niche and diversification on the forest floor. International Journal of Plant Science 173(6):695-710.

Wiley RB (1948). The dominant role of the epidermis in leaves of Adiantum. American Journal of Botany 35:465-473.

Wu F, Wang MM, Xi Z, Zhao SW, Xing S (2013). Study on drought stress of six common ferns in North China. Acta Horticulturae 1035:113-124.

Yao Y-F, Bruch AA, Mosbrugger V, Li CS (2011). Quantitative reconstruction of Miocene climate patterns and evolution in Southern China based on plant fossils. Paleogeography, Palaeoclimatology, Paleoecology 304:291-307.