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GROWTH AND PHOTOSYNTHESIS OF THREE AZOLLA SPECIES IN RESPONSE TO IRRADIANCE

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DANIEL J. N. and BARTHOLOMEW D. P. *Growth and photosynthesis of three Azolla species in response to irradiance*. BIOTRONICS 22, 1-14, 1993. Physiological studies of *Azolla* (azolla) species in relation to comparative responses to environment are limited. In this study, growth, carbon dioxide exchange rate (*CER*) and evapotranspiration (*ET*) were measured in *Azolla caroliniana*, *A. microphylla* and *A. pinnata* mats grown in a greenhouse at 100, 65, and 35% of ambient light levels. Although *A. caroliniana* had higher frond area index (*FAI*) and specific frond area, its *CER* per unit frond weight was significantly lower than *A. microphylla* and *A. pinnata* at a photosynthetic photon flux density (*PPFD*) of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ probably due to mutual shading; at 1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, however, the differences in *CERs* among the species were not significant. At any given *PPFD*, *CER* per unit weight decreased in the order *A. caroliniana* > *A. microphylla* > *A. pinnata*, and with increasing biomass per unit area from 500 to 1500 g m^{-2} . *ET* of the azolla canopy was more closely related to *FAI* than to *CER*. The results indicate that *A. caroliniana*, which has a lower optimum *FAI* than *A. microphylla*, needs to be more frequently harvested to maximise biomass production. Measurements on biomass mats of azolla provided realistic estimates of *CER*, but in *in-situ* measurements of *ET*, evaporation appeared to be a significant component at low growth light levels.

Key words: *Azolla* species, photosynthesis, light level, biomass, frond area, evapotranspiration.

INTRODUCTION

The *Azolla*-*Anabaena* symbiotic system has been a subject of research because of its value as a nitrogen-fixing green manure crop for paddy crops (10, 17, 22, 29). Agronomic aspects of *Azolla* (azolla) species have been investigated for the past several decades by researchers in China (13), Vietnam (28) and the United States (27). However, studies on the basic physiology of azolla are recent and limited. Photosynthesis and nitrogen fixation occur only during the vegetative phase which is represented by the sporophyte of azolla. The cavity within the dorsal lobe of the azolla leaf contains the nitrogen fixing blue-green algae *Anabaena azollae*. Thus, unlike legumes, there is no spatial separation of

carbon and nitrogen fixation sites in azolla.

The photosynthetic rates obtained in different experiments vary widely, probably due to differences in species or strain, biomass per unit area, and other conditions of experimentation. Reported photosynthetic rates for *A. caroliniana* ranged from 40 to 123 $\mu\text{moles CO}_2 \text{ mg}^{-1} \text{ chlorophyll hr}^{-1}$ (20, 23, 24). Net photosynthesis of *A. pinnata* varied from 2.0 to 12 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (1). No studies of assimilation rates of other species of azolla were found.

Carbon dioxide exchange rate (*CER*) and transpiration rate provide a means of explaining plant responses to environmental variation and can help characterize the potential productivity of a species. *CER* of single leaves is usually expressed as the moles of CO_2 assimilated per unit time and area of photosynthetic surface. Accurate measurement of the photosynthetic surface of azolla is difficult because the individual leaves are small and arranged compactly on the fronds. Moreover, the fronds become inter-twined to form a dense mat. Therefore, *CER* typically has been expressed on a unit weight or chlorophyll basis. If CO_2 exchange rates are to reflect typical field responses, *CER* of azolla should be measured on mats that represent the type of biomass accumulation found in the field.

In a mixed cropping system with rice, azolla is subjected to changes in irradiance as the rice leaf canopy develops. Since productivity can only be predicted if the interactions between a plant and its environment are characterized, the effects of irradiance on growth and photosynthesis of azolla need to be studied under conditions that simulate natural conditions.

Measurements of *CER* and evapotranspiration (*ET*) for three species of azolla grown at different irradiances using minimally disturbed mats is reported in this paper. The effects of frond biomass per unit surface area on *CER* was also examined and differences in *CER* were related to foliar characteristics such as frond area and thickness.

MATERIALS AND METHODS

Azolla material and nutrient solution

Accessions of *Azolla caroliniana*, *A. microphylla* and *A. pinnata* used in this study were obtained from a collection maintained at the University of Hawaii, Honolulu, U.S.A. (Table 1). The collection and the experimental cultures were grown in nitrogen-free nutrient solution (4) with a sodium supply for the blue-green algae (19). The concentrations of nutrients in the solution in ppm are as follows: P-5; K-6.25; Mg-7.5; S-8.5; Na-2.5; Ca-7.5; Fe-1.24; Cu, B, Mo-0.05; Mn, Zn-0.5; and Co-0.005. The experimental cultures were raised in cylindrical containers of 1.0 l volume and 85 cm^2 surface area.

Establishment of experiments

The effect of irradiance during growth on the CO_2 exchange rate (*CER*) of the three species of azolla was studied by growing cultures in the greenhouse in full sun and under black polypropylene shade cloth to provide 100, 65, and 35% of

Table 1. Species, subgenus, site of collection, and natural distribution of three *Azolla* species.

Species	Subgenus	Site of collection	Natural distribution
<i>A. caroliniana</i> Willdenow	Euazolla	Ohio, U.S.A.	America, Carribean
<i>A. microphylla</i> Kaulfuss	Euazolla	Galapagos Islands	Central America
<i>A. pinnata</i> R. Brown	Rhizosperma	Bogor, Indonesia	Asia, Africa, Oceania

ambient light levels. The average mid-day photosynthetic photon flux density (*PPFD*) in the greenhouse as measured with a LI-COR Instruments Inc. LI-190 SB quantum sensor was $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$. Shade cloth such as that used in this study does not significantly alter light quality (12, 26).

Containers were inoculated with 5.0 g fresh weight of the appropriate azolla species (equivalent to 588 g m^{-2}). The light treatments were imposed and the cultures were grown for one week. Azolla biomass was then reduced to 4.0 g per container (470 g m^{-2}) on the "day of planting". Because measurements could only be made on six containers per day, treatments (*Azolla* species, light level) were selected at random and assigned a planting date. Planting dates were staggered so that six azolla containers were available for gas exchange measurement on any given day. The containers were brought to the laboratory six days after planting and *CER* was measured at *PPFDs* of 300, 800 and $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$. A randomized complete block design with factorial arrangement of treatments with three replicates was used and treatment effects were evaluated by analysis of variance.

Due to the differential growth rates during the growing period, the weights of azolla in different treatments were not equal at the time of *CER* measurement. In addition to weight, other attributes influencing *CER*, such as chlorophyll content and photosynthetic area (length and number of leaves), were also not uniform among the treatments. These confounding effects cannot be eliminated in studies with plant material like azolla biomass mats.

In a subsequent study, the effect of azolla biomass on *CER* of the three species was measured. Plant material for that study was multiplied in the greenhouse for 10 days in containers having a volume of 15 l. On the day *CER* was measured, weighed quantities of azolla were transferred to 1.0 l containers filled with culture solution and the fronds were arranged to construct a biomass mat. Weights were adjusted to produce mats having a biomass of 500, 1000 and 1500 g m^{-2} . *CERs* were measured on three replicate samples under *PPFDs* of 500, 1000, 1500, 2000 and $2500 \mu\text{mol m}^{-2} \text{s}^{-1}$. The experimental design and analysis of variance procedure were as described above.

Gas exchange measurements

CER was measured in a semi-closed gas exchange system similar to that described previously (5). The assimilation chamber was formed by cementing an acrylic plastic cover to a sleeve having the same taper as the culture containers. The assembly was inserted into the 1.0 l culture container and secured by bolts to form a gas-tight seal (Fig. 1). The volume of the head space was approximately 0.15 l. The temperature of the cultures was maintained at 25°C during measurement of the *CER* by immersing the 1.0 l container in a temperature-controlled water bath.

Air was circulated between a Beckman Instruments Inc. IR-215 infrared gas analyzer (IRGA) and the assimilation chamber with a diaphragm pump at 1.0 l min⁻¹. A second diaphragm pump, connected only to the assimilation chamber, circulated air between the pump and the chamber at 8.6 l min⁻¹ (about 57

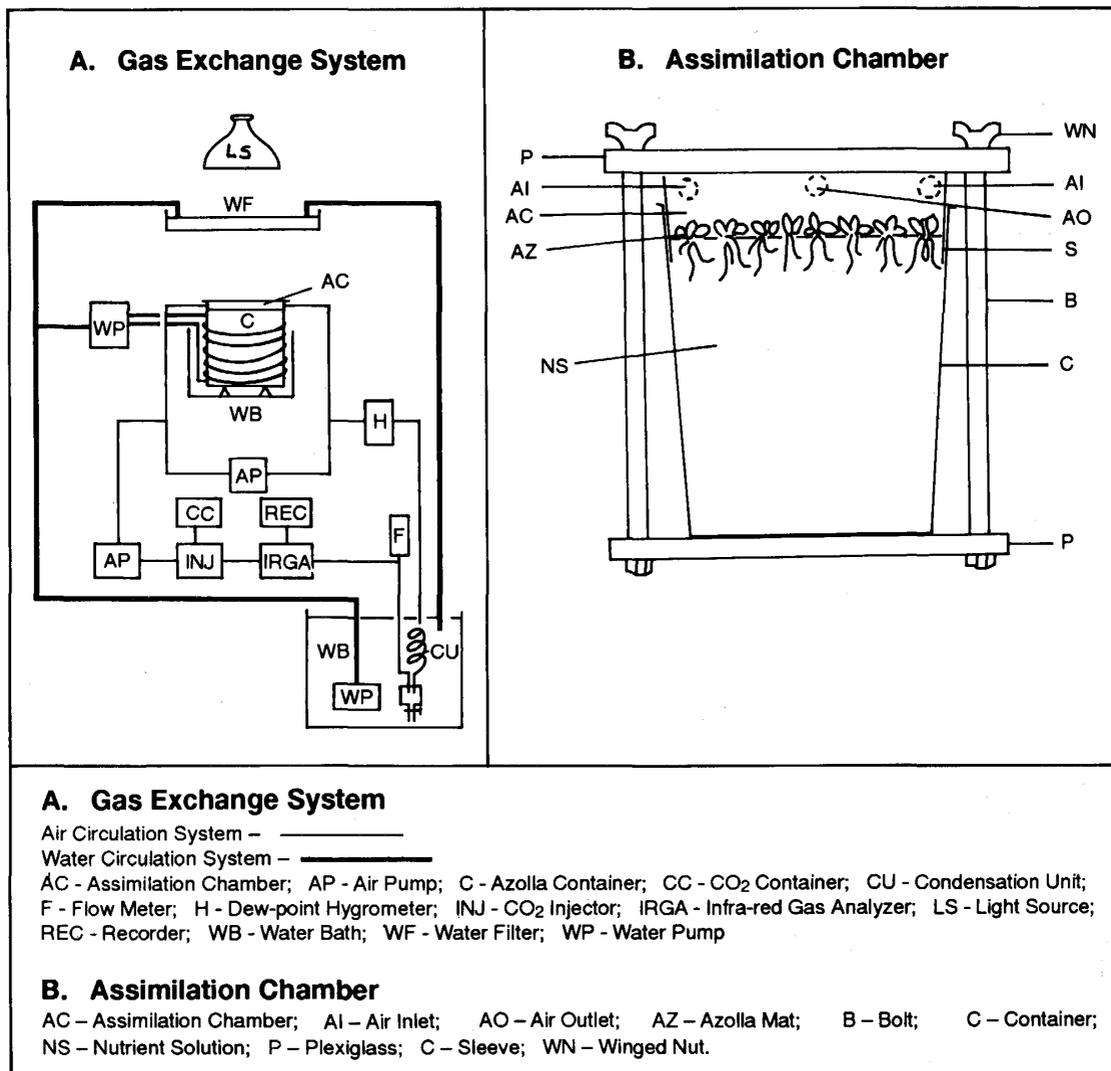


Figure 1. Diagram of assimilation chamber.

changes per minute) to maximize boundary layer conductance. The IRGA and associated electronics injected 84 μl of pure CO_2 into the assimilation chamber when the CO_2 concentration dropped to approximately 275 vppm. Every injection of CO_2 increased the CO_2 concentration to 350 vppm, resulting in an average concentration of 312 vppm. The IRGA was calibrated daily with 99.99% N_2 and an upscale standard containing 301 vppm CO_2 . Assimilation rates were calculated from the mass of CO_2 injected into the chamber over a known period of time.

The dew point temperature of air entering the assimilation chamber was maintained at $15.0 \pm 1.0^\circ\text{C}$ by passing the air stream through a copper coil immersed in a temperature-controlled water bath. Condensate formed in the coil drained into a trap. The dew point temperature of the air exiting the assimilation chamber was measured with a General Eastern Corp. dewpoint hygrometer. Evapotranspiration (*ET*) was calculated from the average difference in dew point temperature of the air entering and exiting the assimilation chamber during the time *CER* was being measured.

The light source used in the photosynthesis studies was a 1,000 watt high pressure sodium (lucalox) lamp. A 3.0 cm circulating water filter was placed between the lamp and the assimilation chamber to reduce thermal radiation. The *PPFD* in the assimilation chamber was measured with a LI-COR. LI-190 SB quantum sensor and was varied either by changing the distance between the assimilation chamber and the lamp or by placing shade cloth inside the water filter. Before collecting data on *CER* and *ET*, the cultures were equilibrated, usually for about 15 minutes, under a given *PPFD* until the dew point temperature of the air exiting the assimilation chamber remained constant.

Measurement of biomass and photosynthetic structures

Azolla fresh weight, chlorophyll content, and frond area were determined after gas exchange measurements were completed. Fresh weight was determined after blotting off the water on the surface of fronds and roots. Since azolla fronds are nonfibrous, whole fronds were liquified with alcohol in a blender, and the chlorophyll content was determined colorimetrically (2). Frond area was obtained by measuring the area of a 1.0 g sub-sample with a LI-COR. LI 3100 area meter and multiplying that value by total fresh weight. The length of individual leaves and the number of leaves per mm of frond length were measured with an optical micrometer.

Derived parameters

The following parameters were derived from the basic data on frond biomass, frond area and chlorophyll content:

1. Relative growth rate (*RGR*) = $(\ln w_2 - \ln w_1) / (t_2 - t_1)$ where w_1 and w_2 are fresh weights at times t_1 and t_2 ;
2. Frond area index (*FAI*) = frond area/unit container surface area;
3. Specific frond area (*SFA*) = frond area/unit frond fresh weight;
4. Specific chlorophyll content (*SCC*) = chlorophyll content/unit frond area

where *SFA* and *SCC* are expressed in $\text{cm}^2 \text{g}^{-1}$ fresh weight and μg chlorophyll cm^{-2} frond area, respectively.

RESULTS AND DISCUSSION

In the studies reported here, there were significant differences between the main effects (species and light level) but no significant interactions. Therefore, only the main effects of the treatments are presented.

Effect of species and irradiance on growth

The highest *RGR* was obtained for *A. pinnata* though it was not significantly greater than that for *A. microphylla* (Table 2). Biomass of the three species increased linearly with increasing growth light level (Figure 2) with small, but non-significant differences between the species. *RGR* also increased linearly with increasing light (Table 2). However, in 100% light, values were about half the maximum values reported previously for these species (16). The lower values obtained here probably were due mainly to the higher initial inoculum level used in this study (470 vs the 160 g m^{-2} in the previous study) which would

Table 2. Relative growth rate (*RGR*), frond area index (*FAI*), and specific frond area (*SFA*) as influenced by *Azolla* species and growth light level.

Treatment	<i>RGR</i>	<i>FAI</i>	<i>SFA</i>
	— $\text{g kg}^{-1} \text{day}^{-1}$ —		— $\text{cm}^2 \text{g}^{-1}$ —
a. <i>Azolla</i> species (averaged over growth light level)			
<i>A. caroliniana</i>	78b ⁺	2.93a	38.44a
<i>A. microphylla</i>	84ab	2.14c	26.96c
<i>A. pinnata</i>	94a	2.46b	29.33b
b. Growth light level (averaged over species)			
35%	40c	2.03c	34.11a
65%	92b	2.55b	31.25b
100%	127a	2.95b	29.38c
SE (16)*	6.5	0.12	0.80

⁺ Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

* Standard error of the mean with error degrees of freedom in parentheses.

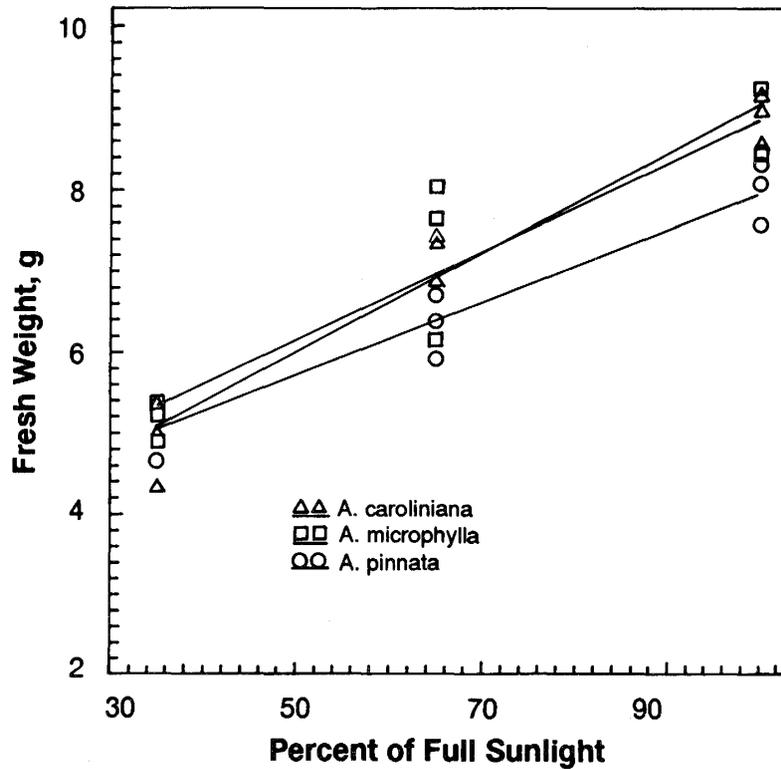


Figure 2. Effect of growth light level on fresh weight accumulation of three *Azolla* species. Equations for the lines are *A. caroliniana*, $Y=2.944+0.0612x$, $r^2=0.946$; *A. microphylla*, $Y=3.435+0.0542x$, $r^2=0.863$; and *A. pinnata*, $Y=3.482+0.0448x$, $r^2=0.934$.

have increased the competition for light.

FronD area indices (*FAI*) for the species were significantly different (Table 2) with the highest value approaching 3.0. *FAI* is comparable to leaf area index and a value of 3.0 is typical of a fully developed leaf canopy. The azolla mats were only 1.0 to 2.0 cm thick, indicating that an azolla canopy is essentially planar. Consequently, it would have a light extinction coefficient (14) approaching 1.0, and light attenuation in such a canopy would be very rapid.

FAI increased and *SFA* decreased as light available for growth increased (Table 2). This increase in *FAI*, despite a decrease in *SFA*, was due to a greater number of fronds per unit surface area. The inverse relationship between *SFA* and light available during growth is a typical response to light. Leaf thickness increases with increasing light level, resulting in a corresponding decline in specific leaf area (3, 6). Growth and yield, or both, of other crops is linearly related to intercepted light (25) and highly correlated with available light (11). While no measurements of intercepted light were made in this study, light interception was assumed to be high because azolla almost completely covered the surface of the container at planting and *FAI* was greater than 2.0 (Table 2) for all treatments six days later.

SCCs of *A. caroliniana* and *A. pinnata* were significantly greater than that of

Table 3. Effects of *Azolla* species and light level during growth on specific chlorophyll content (SCC), leaf length, and number of leaves per unit of frond length.

Treatment	SCC — $\mu\text{g cm}^{-2}$ —	Leaf length ⁺ — mm —	Leaves ⁺ — no. mm ⁻¹ —
a. <i>Azolla</i> species (averaged over growth light level)			
<i>A. caroliniana</i>	8.98a ⁺	1.1	3.28
<i>A. microphylla</i>	7.55b	1.3	2.27
<i>A. pinnata</i>	9.07a	1.6	2.22
b. Growth light level (averaged over species)			
35%	8.53a	1.3	2.68
65%	8.68a	1.4	2.63
100%	8.38a	1.4	2.59
SE (16)*	0.34	—	—

⁺ Mean of 15 to 20 observations.

* Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

Standard error of mean with error degrees of freedom in parentheses.

A. microphylla, but differences generally were small (Table 3): *A. microphylla* characteristically has a light green color and this appears to be due to its significantly lower SCC. There were significant differences in SCC between light levels but the cause is not obvious. The lack of a significant effect of light level on plant morphology suggests that the 35% level was not low enough to inhibit chlorophyll formation nor 100% light high enough to cause chlorophyll bleaching.

A. pinnata had the longest leaves and *A. caroliniana* the shortest; the reverse was true in the case of number of leaves per mm of frond length (Table 3). Light level during growth had little effect on leaf length or number of leaves per mm of frond (Table 3). The latter parameter might be expected to be inversely related to light because internode length usually increases as available light decreases (6). *Azolla* is a fairly shade-tolerant plant that is believed to light saturate at 50% of full sunlight or less (17). The lack of an effect of shade on morphology may be because even the lowest light level was adequate for normal growth.

Effect of species and light level on CER

CERs for the three species (Table 4) were much lower than is typical for other C₃ plants (7), but are comparable to rates reported for ferns; the maximal photosynthetic rates of ferns are generally around 2.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (18). The rates for *azolla* ranged from 2.77 to 5.46 $\mu\text{mol m}^{-2}$ frond area s^{-1} in the present

Table 4. Effects of *Azolla* species, light level during growth, and photosynthetic photon flux density (PPFD) on the carbon dioxide exchange rate (CER) per unit frond area.

Treatment	PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
	300	800	1300
— $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ —			
a. <i>Azolla</i> species (averaged over growth light level)			
<i>A. caroliniana</i>	2.66b ⁺	4.07a	4.85a
<i>A. microphylla</i>	3.08a	4.41a	4.82a
<i>A. pinnata</i>	3.18a	4.62a	4.82a
b. Growth light level (averaged over species)			
35%	2.93b	3.90a	4.05b
65%	3.22a	4.58a	5.46a
100%	2.77b	4.62a	5.00a
SE (16)*	0.15	0.43	0.42

⁺ Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

* Standard error of mean with error degrees of freedom in parentheses.

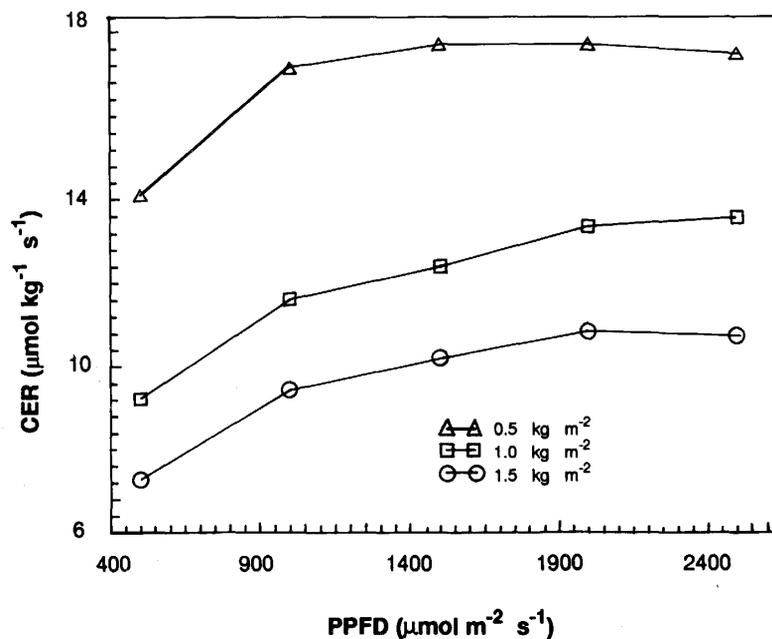


Figure 3. Effect of photosynthetic photon flux density (PPFD) on the CO₂ exchange rate (CER) per kg fresh weight of three *Azolla* species.

study. Rates for *A. pinnata* and *A. microphylla* were about 20% greater than for *A. caroliniana* at a *PPFD* of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 4). Values for all three species increased with increasing *PPFD* but the increase for *A. caroliniana* was relatively greater than for the other two species. *A. caroliniana* had a higher *FAI* than the other two species so it also required more light to saturate the canopy than did the other two species (Figure 3).

Expressed on the basis of chlorophyll content (data not shown), the *CERs* ranged from 94 (*A. caroliniana* grown at 100% light and measured at a *PPFD* of $1300 \mu\text{mol m}^{-2} \text{s}^{-1}$) to $262 \mu\text{mol CO}_2 \text{ mg}^{-1} \text{ chlorophyll hr}^{-1}$ (*A. microphylla* grown at 65% light and measured at a *PPFD* of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$). The range of values reported by others for *A. caroliniana* were 40 (20) to $123 \mu\text{mol CO}_2 \text{ mg}^{-1} \text{ chlorophyll hr}^{-1}$ (23).

At each growth light level, *CER* increased with increasing *PPFD* but the increase was smaller (about 35%) for plants grown at 35% light than it was for those grown at 65 and 100% light (about 70%) (Table 4). The small increase in *CER* of azolla grown at 35% light with increasing *PPFD* is a typical response of shade-grown plants to increasing light. The response of azolla grown at higher light levels was not completely typical. Plants grown at high light often have greater *CERs* in high light than do plants grown at lower light levels (6), but that was not the case in this study. The most likely explanation is that azolla is better adapted to low to moderate levels of sunlight than to full sunlight (17).

Effect of PPFD and frond biomass on CER

In the experiment where biomass per unit area was varied, *CER* is expressed as $\mu\text{mol g}^{-1} \text{ fresh weight s}^{-1}$ because frond area was not measured. The photosynthetic response of *Azolla* species, averaged over biomass, to increasing *PPFD* (Figure 3) was typical of results presented previously (Table 4). The rates, expressed per gram fresh weight, were highest for *A. caroliniana* followed by *A. microphylla* and *A. pinnata* at all *PPFDs*. The lower *CER* per unit frond area of *A. caroliniana* (Table 4) was more than compensated for by a higher *FAI* and *SFA* (Table 2), resulting in greater *CER* per kg fresh weight than the other two species. The greater *CER* of *A. caroliniana* did not result in higher growth rates (Table 2). Many studies have shown that *CER* measured over a short time period is not a good indicator of dry matter production (8). Light saturation of *A. caroliniana* mats occurred at about $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ whereas the other two species saturated at slightly lower *PPFDs* (Figure 3). Previous studies showed that light saturation of *A. caroliniana* occurred at a *PPFD* of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (24) and at 8.0 klux (21). The lack of agreement between the results reported here and the data of others appears to be due to differences in frond biomass per unit area. As was noted previously, the higher *FAI* of *A. caroliniana* likely accounts for the higher *PPFD* required to saturate photosynthesis of the mat.

The *PPFDs* required to light saturate photosynthesis of the azolla canopies in this study also were higher than levels required to light saturate the *CER* of other fern species. Of the published studies found, all utilized only a single frond. Single fronds of *Botrychium virginianum* and *Pellaea atropurpurea* light

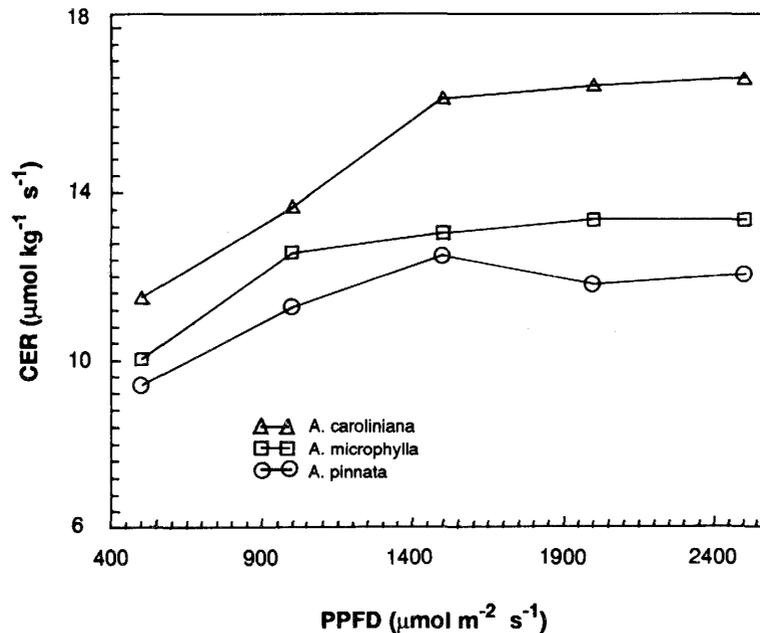


Figure 4. Effect of photosynthetic photon flux density (*PPFD*) and azolla biomass per unit area on the CO_2 exchange rate (*CER*) per kg fresh weight of *Azolla* species.

saturated at 800 and 2500 ft. candles, respectively (15) while saturation of *Pteris cretica* was at about 25 W m^{-2} (9). Mats having a *FAI* greater than one would be expected to saturate at higher light levels than single fronds.

Increasing biomass per unit area resulted in a corresponding decrease in the *CER* per unit weight of azolla (Figure 4). This is to be expected as competition for light would be more acute as mat biomass increased. Further evidence for this is the fact that mats having a biomass of 500 g m^{-2} saturated at a *PPFD* of $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ while mats with a biomass of $1,000 \text{ g m}^{-2}$ required about $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ to light saturate.

Effect of species and light level on ET

A. microphylla had the highest *ET* rate followed by *A. pinnata* and *A. caroliniana* (Table 5). The significant differences among the species in *ET* rates (Table 5) for almost equal *CER*s (Table 4) suggest that evaporation was a sizeable component of *ET*. The vertical growth of fronds in *A. microphylla* resulted in a more open canopy while *A. caroliniana* had a compact canopy and more closely spaced leaves (Table 3). The result of these morphological differences would be an increased area of water surface exposed to evaporation for *A. microphylla* and a reduced area for *A. caroliniana*. Thus, *ET* of *Azolla* species was inversely related to *FAI* and *SFA* (Table 2). However, *ET* increased with growth light level (Table 5) despite a corresponding increase in *FAI* (Table 2). The reason for this may be the increase in transpiration as a result of increased *CER* at higher growth light level (Table 4).

Table 5. Effects of photosynthetic photon flux density (*PPFD*) on evapotranspiration rates (*ET*) of *Azolla* species grown at different light levels.

Treatment	<i>PPFD</i> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
	300	800	1300
	— $\text{g m}^{-2} \text{s}^{-1}$ —		
a. <i>Azolla</i> species (averaged over growth light level)			
<i>A. caroliniana</i>	59.42b ⁺	67.14c	69.26c
<i>A. microphylla</i>	85.25a	95.59a	95.86a
<i>A. pinnata</i>	78.83a	79.86b	83.55b
b. Growth light level (averaged over species)			
35%	69.25b	46.20b	72.96c
65%	79.04a	73.26b	81.96b
100%	75.21ab	93.12a	93.75a
SE (16)*	4.19	3.85	3.76

⁺ Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

* Standard error of mean with error degrees of freedom in parentheses.

There was little stomatal control over *ET* as indicated by the increase in *CER* by approximately 63% when *PPFD* increased from 300 to 1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 4) while *ET* increased by only about 12% (Table 5). *Azolla* leaves contain stomates (17), but no data were collected or found in literature on the functionality of *azolla* stomates or on stomatal number per unit leaf.

General discussion

The present study has shown that there are distinct morphological differences among the *Azolla* species and these differences influenced rates of photosynthesis and growth. *A. caroliniana*. has better light-harvesting attributes in having a higher *SFA*, more leaves per unit length of frond and a higher *SCC* than *A. microphylla*. However, fronds of *A. caroliniana*. are prostrate and form a very dense mat that occupies a relatively small volume above the growth medium. As a result of the high *SFA* and low volume occupied by the mat, *A. caroliniana* appeared to have a lower optimum *FAI*, and photosynthesis was lower than other species at low light. Moreover, *A. caroliniana* light saturated at a higher *PPFD* compared to other species. Therefore, when *A. caroliniana* is grown as a green manure, the quantity of inoculum should be smaller and harvesting or incorporation should be done at shorter intervals to sustain high growth rates. Conversely, *A. microphylla* which has a lower *SFA*, fewer leaves

per unit length of frond, a lower SCC, and an upright growth habit would require more initial biomass and harvesting at longer intervals.

This study further demonstrated the dependence of *CER* on the biomass per unit of culture area. The data demonstrate the need to report *CER* data for free-floating aquatic species that form biomass mats on the basis of frond weight per unit area or *FAI* so that comparisons among different studies can be made. Similarly, nitrogen fixation being a light-dependent process in azolla, assays must be carried out on undisturbed mats and the rates be expressed on a per unit of frond weight or area basis. Caution is also necessary in relating in-situ *ET* measurements to dry matter production because transpiration can be a sizeable fraction of *ET*, particularly at low growth light levels.

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