

Responses of photosynthesis to irradiance in bryophytes of the Azores laurel forest

¹ROSALINA GABRIEL and ²JEFFREY W. BATES

¹Universidade dos Açores, Terceira, Portugal and ²Imperial College London, Ascot, U.K.

SUMMARY

Photosynthetic responses to light intensity were studied under laboratory conditions in seven bryophyte species from evergreen laurel forest, a threatened habitat, on Terceira island in the Azores. Four mosses (*Andoa berthelotiana*, *Echinodium prolixum*, *Fissidens serrulatus*, *Myurium hochstetteri*) and three liverworts (*Bazzania azorica*, *Frullania tamarisci*, *Lepidozia cupressina*) were selected to encompass a range of potential responses to variations in the forest light environment. Carbon dioxide exchange measurements were made, using an infra-red gas-analyser, at photosynthetic photon flux densities (PPFD) of 0–900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a mean temperature of 21°C in fully hydrated shoots. Most species achieved light saturation of photosynthesis below 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$, the lowest value being for *A. berthelotiana* (20 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and the highest for *M. hochstetteri* (68 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The liverwort *F. tamarisci* had the highest maximum photosynthetic rate (P_{max} , 23 $\mu\text{mol CO}_2 \text{g}^{-1} \text{h}^{-1}$) whereas P_{max} was lowest in the mosses *E. prolixum* and *M. hochstetteri* (10 $\mu\text{mol CO}_2 \text{g}^{-1} \text{h}^{-1}$). Dark respiration rate, a critical factor in toleration of shade by forest floor plants, was highest in the species with the highest values for P_{max} . Compensation point was extremely low (7 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in *Fissidens serrulatus*, a species found in the deep shade of forest ravines and caves, and highest in *M. hochstetteri* a moss restricted to better illuminated habitats within and outside the forest. No photoinhibition was detected during the relatively short exposures to high irradiances. Comparison of these responses with data on the forest light environment indicates that, despite the possession of considerable shade adaptations, during winter in the evergreen laurel forest, low light levels may often limit photosynthetic rates of the bryophytes.

KEYWORDS: Azores, laurel forest, bryophytes, photosynthesis-illumination curves, compensation point, photoinhibition.

INTRODUCTION

The natural evergreen forests of the Azores island group support a species-rich and luxuriant bryophyte flora including both archipelago and Macaronesian endemics (Sjögren, 1978; Sérgio, 1984; Bates & Gabriel, 1997; Gabriel, 2000). Regrettably, the extent of these forests continues to diminish owing to felling to provide land for agriculture and commercial forestry, and several of their distinctive bryophytes are becoming threatened (ECCB, 1995). The original richness of the bryophyte flora is linked to a favourable combination of climatic and microclimatic factors. These include the relative warmth and extremely high rainfall owing to the mid-Atlantic position, the mountainous character of the larger islands stimulating rainfall and mist formation, and shading and trapping of humidity inside the forest by the dense canopy (Sjögren, 1978). Under the evergreen canopy

of the predominant trees and shrubs the RH is *ca* 94% in August and light levels are only 5–10 % of those of the exterior (Gabriel, 2000). Removal of the forest can be expected to be damaging to its bryophytes by greatly increasing their exposure to light and desiccation as well as by removal of specific habitats (Gradstein, 1992). This study is part of a wider investigation of the physiological and ecological features of some of the characteristic oceanic bryophytes of the native forests of the Azores undertaken as an aid to their effective conservation (Gabriel, 2000). Although oceanic bryophytes have received some attention from an ecological point of view (e.g. Ratcliffe, 1968; Sjögren, 1978; Edwards, 1986), there have been very few studies of their physiological limitations. In this paper we report on the photosynthetic responses to light intensity in a range of Azorean forest bryophytes.

MATERIALS AND METHODS

Plant material and cultivation conditions

Seven bryophyte species, four mosses (*Andoa berthelotiana* (Montin) Ochyra, *Echinodium prolixum* (Mitt.) Broth., *Fissidens serrulatus* Brid. and *Myurium hochstetteri* (Schimp.) Kindb.) and three liverworts (*Bazzania azorica* Buch & Perss., *Frullania tamarisci* (L.) Dum. and *Lepidozia cupressina* (Sw.) Lindenb.) were chosen for study. They were selected to encompass a range of tolerances to drought and to low and high light levels inferred from field observations. It was hypothesized that obligate forest species (*Bazzania azorica*, *Lepidozia cupressina*) would be the most sensitive to high light and low water availability. Other species (*Andoa berthelotiana*, *Echinodium prolixum*, *Myurium hochstetteri*), although frequent in the forest, may occasionally be found outside and are presumably less demanding of extreme shelter and shade. *Fissidens serrulatus* is a common species in the Azores wherever shaded conditions are present but is not restricted to forest. In comparison with these, *Frullania tamarisci* is a generalist. It is found in exposed habitats and on all kinds of substrata within a wide altitudinal range in the Azores, and also has a wide distribution in Europe. Healthy material of these species was collected from extensive colonies in natural laurel forest habitats at Pico Alto (UTM 1-km grid square 8289; altitude 600 m) in the centre of Terceira. *A. berthelotiana*, *E. prolixum*, *F. tamarisci*, and *M. hochstetteri* were collected from the bark of *Laurus azorica* (Seub.) Franco, *L. cupressina* from bark of *Juniperus brevifolia* (Seub.) Antoine, *B. azorica* was sampled from lava walls of a ravine with some soil, and *F. serrulatus* was taken from basaltic rocks. Three species bore sporophytes: *A. berthelotiana*, *F. tamarisci*, and *M. hochstetteri*.

The plants were transported to the laboratory at Imperial College in a fully hydrated condition within one week in July 1996. In the laboratory, the shoots were spread on horticultural capillary matting that had been saturated with distilled water within seed trays (60 × 30 cm) covered with transparent plastic propagator lids. They were regularly watered with distilled water but care was taken to avoid flooding. The closed trays were placed in a constant temperature (CT) room at 15°C, 80% RH and 16 h photoperiod at 40 μmol photon m⁻² s⁻¹ for about one month prior to use. This period was considered desirable to avoid effects caused by any desiccation or by high or low temperature stresses suffered during transportation.

Gas exchange measurements

Net photosynthesis and dark respiration were measured with an infrared gas analyser (IRGA, Series 225, Analytical Development Co. Ltd. England), using a method similar to that of Larson & Kershaw (1975). Thirty or more fully hydrated shoots (10–20 mm fully green apical segments depending on species) were inserted at their bases into a piece (5 mm height) of saturated florist's 'oasis'. The shoots were arranged evenly to avoid self-shading. Each such unit

was placed in a cylindrical perspex assimilation chamber (volumes 98–117 cm³) with removable lid and with the block in contact with free distilled water to help maintain hydration of the shoots during the incubation. Some evidence was obtained that blotting of surface water from the shoots with absorbent paper is damaging (lowered photosynthetic rates), therefore the gentler expedient of shaking off the excess water prior to planting in 'oasis' was adopted.

The assimilation chambers were immersed in a shallow water bath maintained at 21°C (± 2°C). Photosynthetically active radiation (PPFD) was provided by four 100 W tungsten spot lamps in a reflector hood placed above the waterbath. A range of PPFD was achieved by switching on different combinations of the four lamps, and by placing layers of white and black cheesecloth over the chambers. Ten points were below 100 μmol photon m⁻² s⁻¹ and eight approximately evenly distributed upwards to 900 μmol m⁻² s⁻¹. Each chamber was incubated in the water bath for 20 min at each PPFD, and also for 10 min in the dark (chamber covered with aluminium foil) to determine respiration rate. Immediately prior to each incubation the CO₂ concentration was brought to a known value (usually 350 v.p.m.) by flushing for 1 min at a flow rate of 1200 cm³ min⁻¹ and sealing at ambient air pressure. At the end of the incubation a 5 cm³ sample of the chamber air was withdrawn by hypodermic syringe and injected into the CO₂-free airstream leading to the sample cell of the IRGA. Peak height was recorded on a chart-recorder. The bryophyte shoots were freeze-dried to obtain dry weight. Net photosynthesis and dark respiration rates were expressed as moles of carbon dioxide absorbed or released per gram dry weight per hour (μmol CO₂ g⁻¹ h⁻¹).

Curve fitting

Photosynthesis-illumination curves were fitted using the equations of Baly (1935) modified by Titus & Wagner (1984). This function is a two-parameter hyperbola, bounded by zero and one.

$$P_{gn} = (P_{max} * b * L) / (P_{max} + b * L),$$

where, P_{gn} is the normalized rate of photosynthesis (Titus & Wagner, 1984; Alpert & Oechel, 1987), P_{max} is the maximum photosynthetic rate, b is the slope of the initial proportional increase and is a constant, and L is the photon flux density. P_{gn} accounts for the respiration and transforms the data so they fall between zero and one,

$$P_{gn} = (\text{given rate of net photosynthesis} - R) / (P_{max} - R),$$

where, R is the respiration rate. The coefficient b was estimated by non-linear regression in SYSTAT 8.0. Light saturation point (I_s) was considered to have been achieved when net photosynthesis was at least 90% of the maximum value of photosynthesis for the species. Light compensation point (I_c) was estimated using the linear part of the relationship between photon flux density and net photosynthesis. The averages of three replicate datasets were used to fit the curve for each species.

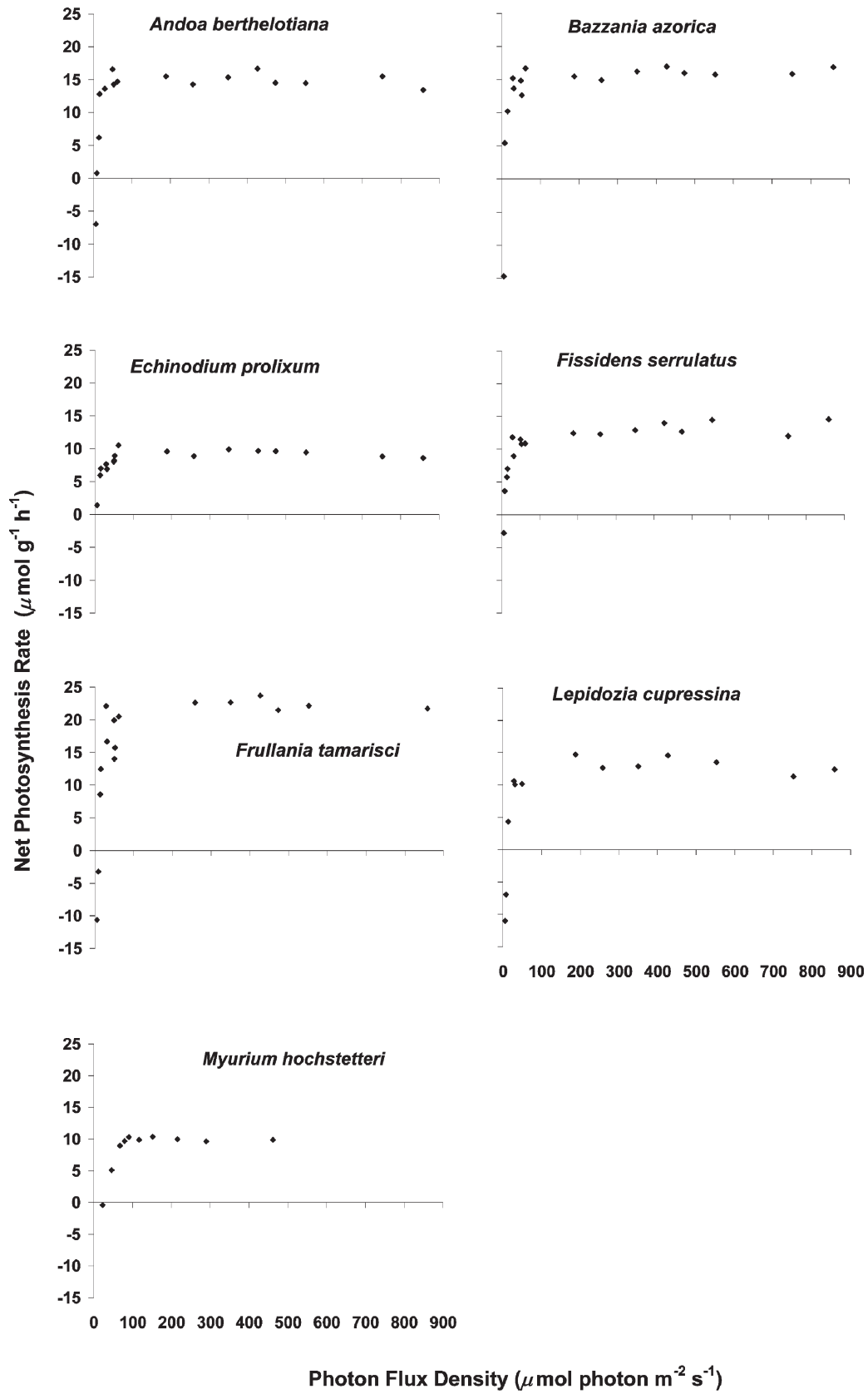


Figure 1. Dependence of net photosynthesis on photosynthetically active radiation at 20°C for: *Andoa berthelotiana*; *Bazzania azorica*; *Echinodium prolixum*; *Fissidens serrulatus*; *Frullania tamarisci*; *Lepidozia cupressina*; *Myurium hochstetteri*.

RESULTS

The species investigated responded differently to increasing PPFD in their CO₂ assimilation rates (I_C, I_S, P_{max}), photosynthetic efficiencies (b, slope of the curve), and dark respiration rates (Fig. 1 Table 1). *Fissidens serrulatus* exhibited the lowest compensation point values (7 μmol photon m⁻² s⁻¹), while other species presented intermediate figures (8 to 12 μmol m⁻² s⁻¹) and *Myurium hochstetteri* the highest (31 μmol m⁻² s⁻¹). Light saturation (I_S) was generally achieved below 30 μmol m⁻² s⁻¹, the lowest value being for *Andoa berthelotiana* (20 μmol m⁻² s⁻¹) and the highest for *Myurium hochstetteri* (68 μmol m⁻² s⁻¹). The highest values of net P_{max} were obtained for *Frullania tamarisci* (23 μmol CO₂ g⁻¹ h⁻¹) among the liverworts and for *Andoa berthelotiana* (15 μmol g⁻¹ h⁻¹) among the mosses. The species with the lowest values of P_{max} were the mosses *Myurium hochstetteri* and *Echinodium prolixum* (10 μmol g⁻¹ h⁻¹). There was no inhibition of photosynthetic rates at the highest PPFD provided (900 μmol m⁻² s⁻¹). Dark respiration rates were highest for the species that yielded the highest photosynthetic rates, *Andoa berthelotiana* (28 μmol CO₂ g⁻¹ h⁻¹), *Frullania tamarisci* (25 μmol g⁻¹ h⁻¹), and *Fissidens serrulatus* (23 μmol g⁻¹ h⁻¹). Respiration rates were lowest for *Echinodium prolixum* and *Myurium hochstetteri* (15 and 16 μmol g⁻¹ h⁻¹, respectively). The slopes of the photosynthesis-illumination curves for each species (estimated parameter b) and their associated standard errors are given in Table 1. Two groups of values were observed, those around 0.10 (*F. tamarisci*, *L. cupressina*, and *M. hochstetteri*) and those higher than 0.22 (*A. berthelotiana*, *E. prolixum*, and *F. serrulatus*). The proportions of the variation that was explained by the fitted curves were high, r² varying from 0.986 in *Bazzania azorica* and *Frullania tamarisci* to 0.999 in *Fissidens serrulatus*.

DISCUSSION

Two aspects of the responses to light that may be especially important to forest bryophytes are the ability to maintain a positive carbon balance under deep shade, and susceptibility to photoinhibition at high irradiance (Seel, Hendry & Lee, 1992), e.g. through removal of the forest canopy. The species investigated appear well adapted to low light levels, exhibiting low compensation points and low saturation

points (Table 1). *Fissidens serrulatus* exhibited a remarkably low compensation point (7 μmol photon m⁻² s⁻¹) comparable to those reported in *Marchantia foliacea* and *Monoclea forsteri* (Green & Snelgar, 1982). *F. serrulatus* is normally found in deep shade in ravines, cave entrances, and on other shaded rocks and soil. *Myurium hochstetteri* had the highest compensation value (31 μmol photon m⁻² s⁻¹) which matches its prevalence in well-illuminated habitats, especially as an epiphyte on *Laurus azorica*, (generally above 60 cm height), or on rocks in clearings or even outside the forest. The other species have very low compensation points (8 to 12 μmol photon m⁻² s⁻¹) which may permit net photosynthetic gains in shaded forest habitats (Kershaw & Webber, 1986). Values of light saturation for the species studied here lay between 20 and 69 μmol photon m⁻² s⁻¹, with *Andoa berthelotiana* and *Fissidens serrulatus* showing the lowest values, and *Myurium hochstetteri* the highest. Comparable light saturation levels have been found in other forest bryophytes (Green & Snelgar, 1982; Bakken, 1995), whereas non-forest bryophytes usually exhibit much higher values (300–600 μmol photon m⁻² s⁻¹; Larcher, 1995). Somewhat higher light saturation values have been reported on the basis of chlorophyll fluorescence estimates of relative electron flow rate in two pendulous epiphytes of tropical African forests and in a range of British woodland bryophytes (Proctor, 2002). Comparison of our I_C and I_S results with the actual irradiances experienced in native evergreen forest (Gabriel, 2000) indicates that sufficient irradiance is available for some bryophyte growth in all months. However, from November until March, plants may often photosynthesize under light levels that are below those needed to saturate the photosystems.

High respiration rates are characteristic of heliophytes and rapidly growing species. Mosses, herbaceous plants, deciduous trees, and evergreen trees form a series of increasing respiration rates (Larcher, 1995). Respiration of mosses proceeds at a low rate, from 1–4 mg CO₂ g⁻¹ h⁻¹ (Skre & Oechel, 1981; Sveinbjörnsson & Oechel, 1992). The highest dark respiration rates observed in this investigation were achieved by *Andoa berthelotiana* (28 μmol g⁻¹ h⁻¹ or, in units allowing comparison with the figures above, 1.2 mg CO₂ g⁻¹ h⁻¹) and *Frullania tamarisci* (25 μmol CO₂ g⁻¹ h⁻¹ or 1.1 mg CO₂ g⁻¹ h⁻¹), and most of the other species exhibited similar values. *Echinodium prolixum* and *Myurium hochstetteri*, however, exhibited lower respiration values (16 and 15 μmol

Table 1. Comparison of species photosynthetic attributes based on the data in Fig. 1. See text and Fig. 1 for explanation. Figures are means of three replicates and their standard errors.

Species	Compensation Point, I _C		Saturation Point, I _S		Net P _{max}		Respiration rate		b	r ²	
	(μmol photon m ⁻² s ⁻¹)		(μmol photon m ⁻² s ⁻¹)		(μmol CO ₂ g ⁻¹ dw h ⁻¹)		(μmol CO ₂ g ⁻¹ dw h ⁻¹)				
<i>Andoa berthelotiana</i>	8	±0.2	20	±4.7	15	±0.5	28	±3.0	0.22	±0.03	0.996
<i>Bazzania azorica</i>	9	±0.5	29	±0.0	16	±0.3	19	±3.7	0.14	±0.03	0.986
<i>Echinodium prolixum</i>	9	±1.1	27	±12.5	10	±0.2	15	±1.7	0.28	±0.04	0.997
<i>Fissidens serrulatus</i>	7	±0.5	24	±4.7	12	±0.3	23	±3.8	0.24	±0.02	0.999
<i>Frullania tamarisci</i>	10	±0.7	36	±6.4	23	±0.6	25	±2.6	0.09	±0.01	0.986
<i>Lepidozia cupressina</i>	12	±0.7	30	±0.7	13	±0.5	19	±3.3	0.10	±0.02	0.991
<i>Myurium hochstetteri</i>	31	±2.8	68	±0.0	10	±0.3	16	±3.3	0.10	±0.02	0.996

$\text{g}^{-1} \text{h}^{-1}$ or $0.7 \text{ mg g}^{-1} \text{h}^{-1}$). The two species with the highest values also produced the highest relative growth rates in laboratory growth experiments (Gabriel, 2000).

The photosynthesis-illumination curves revealed a wide range of P_{max} for the species studied (Fig. 1, Table 1). *Frullania tamarisci* had the highest photosynthetic rate among the liverworts ($P_{\text{max}} 23 \mu\text{mol CO}_2 \text{ g}^{-1} \text{h}^{-1}$) and *Andoa berthelotiana* presented the highest photosynthetic rate for the mosses ($15 \mu\text{mol g}^{-1} \text{h}^{-1}$). Their comparably high concentrations of total chlorophyll are probably connected with this performance (Gabriel, 2000). *Myurium hochstetteri* and *Echinodium prolixum* exhibited low maximal photosynthetic values. The dry weight of the samples is the sum of photosynthetic and non-photosynthetic tissues, and plants without supporting tissues (e.g. aquatics and seaweeds) normally exhibit higher productivity on a dry weight scale (McIntire *et al.*, 1996). The low P_{max} values of the two mosses may be related to morphological features such as the presence of relatively dense structural tissues composed of non-chlorophyllose cells. To avoid this complication, photosynthesis may be expressed in terms of the weight of chlorophyll present (Martin & Adamson, 2001), but this measure is, arguably, mainly of physiological interest and less relevant to ecological performance (Davey & Rothery, 1997), and thus not used here.

The range of PFD used to obtain the photosynthesis-illumination curves was extended towards much higher values (up to $900 \mu\text{mol m}^{-2} \text{s}^{-1}$) than the species experience in the Azores forests to investigate depression of photosynthesis at high irradiance. However, photo-inhibition was not detected. This may be related to the short time of incubation (20 min) in comparison with the 12-hour treatments applied by Seel *et al.* (1992). Effects of longer exposures are considered by Gabriel (2000) and will be explored in a later communication.

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ROSALINA GABRIEL, Departamento de Ciências Agrárias. Universidade dos Açores, 9700-851 Angra do Heroísmo, Portugal. E-mail: rgabriel@angra.uac.pt

JEFFREY W. BATES, Department of Biological Sciences, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY, U.K.

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