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Green light drives photosynthesis in mosses

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Temperate forests are characterised by variable light quality (i.e. spectral composition of light) at or near the forest floor. These understory environments have a high concentration of green light, as red and blue light are preferentially absorbed by upper canopy leaves. Understory species may be well-adapted for using green light to drive photosynthesis. Angiosperms have been shown to use green light for photosynthesis, but this ability has not been demonstrated in shade-dwelling bryophytes. In this study, net photosynthetic rate (P_N) of three temperate understory species of moss (*Dichodontium pellucidum* (Hedw.) Schimp., *Leucobryum albidum* (Brid. *ex* P.Beauv) Lindb. and *Amblystegium serpens* (Hedw.) Schimp.) was measured under green, red + blue, and red + blue + green light to assess green light use efficiency. All three species were capable of photosynthesising beyond their respiratory demands using solely green light, with higher green light use efficiency measured in plants collected from areas with greater canopy cover, suggesting growth in a green light concentrated environment increases green light use efficiency. Each species was also collected from sites differing in their degree of canopy cover and grown under three light treatments (high light, low light, and green light). Photosynthetic efficiency (chlorophyll fluorescence), tissue nitrogen and carbon isotope concentrations were assessed after a short growth period. Growth conditions had little effect on leaf chemistry and monochromatic green light did not significantly degrade photosynthetic efficiency. This study provides the first evidence to date of positive net 'green light photosynthesis' in mosses.

Keywords: Bryophytes, Chlorophyll fluorescence, Green light, Light quality, Photosynthesis

Introduction

Bryophytes occupy a large diversity of niches from pole to pole in environments that vary in light availability, substrate and climate (Gignac, 2001), making them an excellent group to investigate physiological adaptations to variable growth conditions (Miller, 1982). Since bryophytes are the basal taxon of all terrestrial plants, quantifying their physiological adaptations can provide insight into factors that determine the diversity, distribution and relative importance of certain physiological traits across the plant kingdom (Kenrick & Crane, 1997; Qiu *et al.*, 2006; Waite & Sack, 2011). The largest and most diverse group of bryophytes are the mosses, of the division Bryophyta (Goffinet *et al.*, 2001). Mosses lack gametophytic stomata and a vascular tissue system of the form seen in tracheophytes, though (exceptionally) some species contain hydrome and leptome (Garner & Paolillo, 1973). Being small (generally < 100 mm in height) limits the ability of mosses to compete with taller plants in light-limited environments, such as a forest understory. It is well known that heterogeneity in light intensity can drive evolutionary adaptations in plants (Givnish, 1988); however, the impact of light quality (i.e. the spectral composition of light) on plant physiology and its interaction with species adaptations to light-limited conditions is less studied.

The action spectra of isolated chlorophyll, which absorbs predominantly red and blue light, suggests that photosynthesis is driven primarily by red and blue light (Engelmann, 1882; Taiz *et al.*, 2015), however this is a common misconception (Nishio, 2000). The efficient usage of monochromatic green light (520–560 nm) has been demonstrated in a variety of angiosperms and even green algae (Inada, 1976; Lüning & Dring, 1985). Green light drives photosynthesis deep within leaves and can even boost photosynthetic rates and crop yields when used in concert with high intensity blue/red light (McCree, 1972; Sun *et al.*, 1998; Kim *et al.*, 2004; Terashima *et al.*, 2009; Johkan *et al.*, 2012); however,

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the ability of mosses to use solely green light for photosynthesis and the environmental conditions that promote the efficient usage of green light have yet to be studied.

Light quality is reduced as light descends through a forest canopy, as red and blue light are preferentially absorbed by upper canopy leaves and green light is reflected or transmitted to the forest floor (Endler, 1993; Wang *et al.*, 2015). This results in an understory environment highly concentrated with green light, making the forest floor an optimal location to investigate green light use efficiency (Wang & Folta, 2013). Green light has been shown to drive carbon fixation more efficiently than red or blue light within the deepest layers of spinach leaves (Sun *et al.*, 1998). If this phenomenon scales to an entire forest canopy, then leaves and/or plants adapted to growing under a dense canopy may use the available energy of green light more efficiently than leaves exposed to full sunlight.

In this study, we assess photosynthetic responses of temperate moss species to variable light quality (% green light) and quantity (high *vs* low light) and ask the following questions: (1) Can understory mosses use solely green light to drive positive net photosynthesis (P_N), and (2) to what extent is green light use efficiency related to the density of canopy cover? These questions were investigated using three species of ground-dwelling mosses naturally occurring in the understory of a north-eastern US temperate forest with variable canopy cover. We measured photosynthetic responses (P_N and electron transport rate; ETR), carbon-13 discrimination ($\delta^{13}C$) and tissue nitrogen content (%N) of each species of moss in ambient field conditions as well as altered light environments (high light, low light and green light) to test the following hypotheses: (1) green light will drive positive P_N in all species, but more so in species found under more closed canopies, and (2) ETR_{MAX} will be maintained following growth under solely green light.

Methods

Plant material

Three ground dwelling species of moss (*Dichodontium pellucidum* (Hedw.) Schimp., *Leucobryum albidum* (Brid. *ex* P.Beauv) Lindb. and *Amblystegium serpens* (Hedw.) Schimp.) were collected from natural populations growing on a 182 ha forested reserve on Ithaca College's Natural Lands, Ithaca, NY, USA (42° 26′36″N 76°30′0″W). Samples of *A. serpens* and *D. pellucidum* were collected from an area with a closed canopy forest while *L. albidum* was collected from a sparsely forested open canopy clearing. The open canopy species was not found in the closed canopy environment and *vice versa*. To determine the degree of light interception by the forest canopy at each plant collection site, simultaneous measurements of photosynthetically active radiation (PAR) were made at the forest floor from a clearing (open-sky) and at each plant collection site on diffuse days at 10:00 AM using two handheld quantum sensors (LI-COR 210A, LI-COR Biosciences, Lincoln, NE, USA). The fraction of light intercepted by the canopy (LIC) was assessed using the following relationship:

$$LIC = 1 - (LC/LO) \quad (1)$$

where, LC is a measure of PAR under the canopy at each collection site and LO is the simultaneous PAR measurement in a nearby area with open sky (Anderson, 1964; Sack *et al.*, 2006).

Net photosynthesis and respiration

Photosynthesis was measured on gametophyte tissue as the rate of oxygen evolution. Moss samples were collected from the field, placed in sealed bags containing moist paper towels, and immediately brought back to the lab where they were rinsed of any soil or litter. Measurements were made by placing individual gametophytes into a sealed 12 mL- glass cuvette filled with deoxygenated reverse osmosis water at 18.2 mOhms (Milli-Q UFplus SA 67120 Molsheim, France). The cuvette contained 5 mg of $NaHCO_3$ to supply carbon to the tissue during the measurement. The amount of $NaHCO_3$ added did not significantly alter the pH of the solution (monitored using a pH meter).

Prior to taking measurements, the oxygen concentration [O_2] of the solution was lowered by bubbling 100% compressed helium gas (Airgas, Inc.) through the solution for 20 min (this was our baseline O_2 concentration to begin measurements). Oxygen evolution was measured using a micro-oxygen electrode (MI-730 #81950, Microelectrode Inc. Bedford, NH, USA) until a steady state was reached (~10–20 min). Temperature was recorded with a fine-wire copper-constantan thermocouple interfaced to a Campbell Scientific CR10X datalogger (Campbell Scientific, Logan, UT, USA) every second and averaged every 30 s. Percent oxygen evolution was calculated from mV output of the microelectrode by making measurements in a solution saturated with He_2-gas (as the zero O_2 reference point) and the same solution that was vigorously shaken for five minutes in a container that contained ambient outdoor air (~21% O_2). To convert percent O_2 to mol L^{-1} s^{-1} g^{-1}, the solubility (S) of O_2 in mol L^{-1} was calculated using the following relationship obtained from the O_2 sensor calibration manual (Microelectrode Inc.):

$$S = \frac{(a/22.414)(760-p)}{760(r\%/100)} \quad (2)$$

where a is the absorption coefficient of gas at measured temperature, p is the vapour pressure of water at a specific temperature, and r% is the actual reading in percent O_2. In this study, the temperature was maintained between 23.0 to 24.0°C and the corresponding values of a (0.02988 to 0.02881, respectively) were applied during each measurement. The slope of O_2 evolution *vs* time (s) was used to calculate photosynthetic rate. After O_2 evolution reached steady state, samples were placed in a drying oven (Isotemp Oven 630G, Fisher Scientific) set to 72°C for ~48 hours. The data were standardised by dry weight of the sample ($n = 5$ per species) and converted to nmol g^{-1} s^{-1}.

Net photosynthetic rate (P_N) was determined by measuring O_2 evolution in solution (Björkman & Demmig, 1987) under three light conditions with the same incident photon flux density: 100 μmol m^{-2} s^{-1} of green light (max = 528 nm) (G); 100 μmol m^{-2} s^{-1} of red (max = 670 nm) and blue light (max = 465 nm) (R + B); and 100 μmol m^{-2} s^{-1} of red, blue and green light (R + B + G). Light levels were measured with a LI-COR 210A handheld quantum sensor held under water to account for the differential attenuation of wavelengths in water. Two light sources were used to expose samples in the O_2 evolution apparatus to the different light treatments. Green light was supplied from an array of green LED's (Roithner LaserTechnik Vienna, Austria, OVLLx8C7 cylindrical high-intensity LED green diodes with a λ_{max} of 520 nm at 100%, 500–540 nm at 50%, 475–560 nm at 0% relative luminous intensity), while red and blue light were supplied by using a detached LI-COR 6400 02B red/blue light source containing both red and blue diodes. The R + B + G treatment was achieved by combining all three light sources making sure that equal combinations of the three light sources were used (33.33 μmol m^{-2} s^{-1} green light source, 66.67 μmol m^{-2} s^{-1} red + blue light source). Dark respiration rate (R_D) was measured on samples that were dark adapted for 30 min (note: while the units are the same as P_N, R_D is measured as a decline in [O_2] over time).

Growth chamber experiment

Five separate 10 × 10 cm colonies of moss were collected from each field site and grown in a Conviron growth chamber (Model E15, Controlled Environments Ltd., Winnipeg, Manitoba, Canada) set to 20°C, 50% RH, and a 14 h/10 h day/night photoperiod. Plants were grown on absorbent cloths (Chux™) in containers enclosed with clear plastic wrap to prevent desiccation and were misted with chlorine-free water every 2–3 days to ensure that well-watered conditions were maintained throughout the experiments. Gametophyte colonies were exposed to one of the following light treatments: high (100 μmol m^{-2} s^{-1} white light), low (30 μmol m^{-2} s^{-1} white light), and green light (30 μmol m^{-2} s^{-1} of green light) for three weeks ($n = 5$/trt). Green light treated mosses were grown in containers covered with a green Roscogel™ filter (Rosco Inc. Garwood, NJ, USA, No. 273 Medium Green). The filter transmittance, measured with a spectrophotometer (Agilent UV-Vis 8453, Santa Clara, CA, USA), ensured that only green light was being provided to the plants during this time-period (λ_{max} 523 ± 20 nm). Low light treated plants were grown under three layers of Green-Tek 50% white shade cloth and PAR was measured with a LI-COR 250 hand-held quantum sensor. These light levels were chosen based on mean light levels measured at the closed and open canopy field sites (30 and 100 μmol m^{-2} s^{-1}, respectively).

Electron transport rate

Chlorophyll fluorescence was measured on plants using a Technologica™ CF imager (Technologica Ltd, Colchester Essex, UK) following the three-week growth period to test if the treatments impacted electron flow through thylakoid membranes. Gametophytic tissue from each growth condition (green light, high full spectrum light, and low full spectrum light; $n = 5$) was sealed in plastic Ziploc™ bags to prevent desiccation during measurements. The moss samples were dark adapted for 30 min prior to recording optimal quantum efficiency of PSII. Steady-state fluorescence (Fs) was measured continuously while exposing samples to a range of light from 0–300 μmol m^{-2} s^{-1} (CF-imager uses blue light diodes). Saturating pulses of 2750 μmol m^{-2} s^{-1} (blue light) were applied after 10 min of acclimation time when Fs was constant and before each change in light intensity. The saturating pulses were used to measure Fm′ values which were then used to calculate photosystem II efficiency (ΦPSII) from:

$$\Phi\text{PSII} = (\text{Fm}' - \text{Fs})/\text{Fm}' \quad (3)$$

ΦPSII was converted to relative electron transport rate (ETR) from:

$$\text{ETR} = \Phi\text{PSII} \times \text{PAR} \times f \quad (4)$$

where PAR is photosynthetically active radiation, f is a fraction accounting for the distribution of energy between PSII and PSI ($f = 0.5$ was used assuming equal distribution; Maxwell & Johnson, 2000; Stemke & Santiago, 2011). Technical failures limited our measurements of treatment effects on ETR to *D. pellucidum*; however, we did measure light response curves on plant tissue collected from

the field in 2016. This allowed us to assess the light levels at which the thylakoid membranes were light-limited *vs* saturated (Figures S1 and S2).

Tissue biochemistry

Isotopic carbon discrimination ($\delta^{13}C$) and tissue nitrogen (%N) were analyzed on tissues from each species-treatment combination as well as field grown plants. Tissue samples from each clump sample ($n = 5$) were collected from the apices of the gametophyte shoots and oven-dried for 48 hrs. It was not determined if the apices collected were new growth or elongation growth under each treatment. Approximately 3 mg of dried tissue was used for each sample. The samples were analyzed by the Cornell Isotope Laboratory (Cornell University, Ithaca, NY) using a Thermo Delta V isotope ratio mass spectrometer (IRMS) interfaced to a NC2500 elemental analyzer. The average difference in composition of ^{13}C measured as $\delta^{13}C$ against a Vienna Pee Dee Belemnite (VPDB) primary reference scale was assessed to determine the degree of carbon stress seen in each sample (Farquhar *et al.*, 1989). $\delta^{13}C$ was calculated from:

$$[(R_{\text{sample}} - R_{\text{standard}})/(R_{\text{standard}})] \times 1000 = d_{\text{sample-standard}}(\delta) \quad (5)$$

where, R_{sample} is the ratio of the heavy isotope to light isotope in the sample. R_{standard} is the ratio of the heavy isotope to light isotope in the reference gas, and $d_{\text{sample-standard}}$ (δ) is the difference in isotopic composition of the sample relative to that of the reference, expressed in per mil (‰).

More negative values of $\delta^{13}C$ indicate low concentrations ^{13}C in the tissue samples. The percent nitrogen (%N) of each sample was also measured as it is a proxy of tissue protein content (e.g. Rubisco and chlorophyll) and, by association, maximum photosynthetic rate (Evans, 1989).

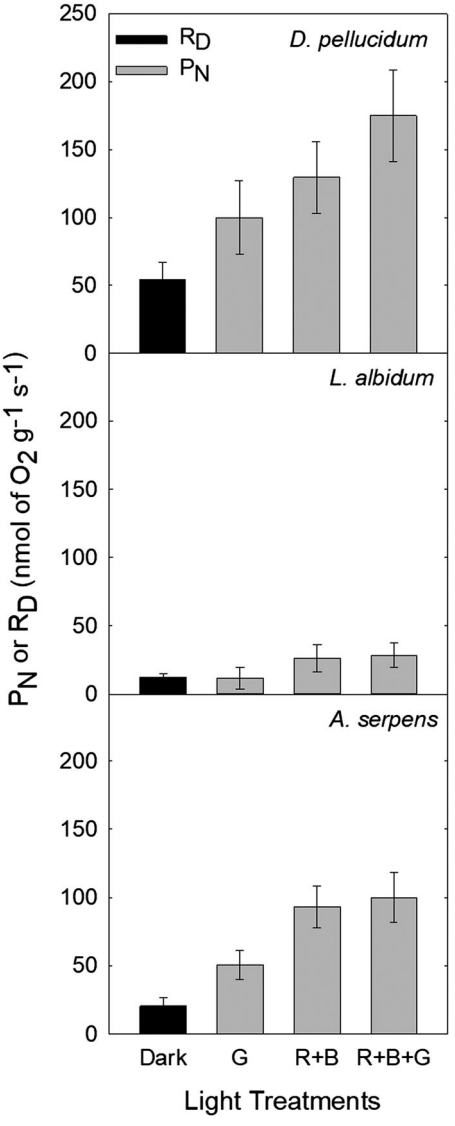

Figure 1 Net photosynthesis (P_N) measured under 100 μmol m^{-2} s^{-1} of green (G), red + blue (RB), and red + blue + green (RBG) light as well as dark respiration (R_D) in three species of moss taken directly from the field. P_N under G light is significantly lower than RB or RBG light (ANOVA; $F = 4.4$, $P = 0.020$), yet is above zero for all species. R_D is significantly lower for *L. albidum* with no difference between other two species (Kruskal-Wallis Test, $H = 12.5$, DF = 2, $P = 0.002$). R_D has negative units (uptake of oxygen), however it is shown with the same absolute units as P_N for ease of visualisation.

Data analysis

All statistical analyses were conducted using Minitab (16). The effect of each light level on P_N was determined using a two-way ANOVA with P_N as the response and 'species' and 'light level' as factors. Similar ANOVAs were run to look for differences in LIC at each collection site as well as growth treatment effects on %N and $\delta^{13}C$ and *t*-tests were used to look for species-specific effects. Significant effects of growth chamber light condition on ETR_{MAX} of *D. pellucidum* were analyzed using a One-Way ANOVA. Univariate linear regressions between %N and $\delta^{13}C$ were run to test whether changes in carbon discrimination were the result of altered protein concentrations.

Results

Canopy cover

The average canopy cover (LIC) for the *D. pellucidum*, *L. albidum*, and *A. serpens* field sites were 0.87 ± 0.06, 0.68 ± 0.03, and 0.81 ± 0.02 (±STDEV), respectively. The site where *L. albidum* was growing had an average of 18% less LIC compared to the site where the other two species were growing indicating higher light transmittance through the canopy in that location (ANOVA; $F = 33.05$, $P < 0.0001$).

Net photosynthesis and respiration

Net photosynthesis (P_N) was positive for all species under green light (Figure 1). Green-light-P_N was significantly lower than P_N measured on plant samples exposed to red + blue and red + blue + green light conditions, with strong species-specific effects (ANOVA; $F = 4.4$, $P = 0.02$). *Dichodontium pellucidum* had the highest rates of P_N in response to green light followed by *A. serpens* and *L. albidum*, respectively (ANOVA; $F = 24.61$, $P < 0.001$). *Dichodontium pellucidum* also had the highest R_D (Tukey adj $P < 0.05$) compared to *A. serpens* and *L. albidum* which had similar R_D (Figure 1; note that R_D reflects reductions in $[O_2]$, whereas P_N reflects oxygen evolution).

As green-light-P_N varied by species, we calculated a relative green light use efficiency index (LUE_G) for each species:

$$LUE_G = \frac{O_2 \text{ evolution under green light}}{O_2 \text{ evolution under red + blue light}} \quad (6)$$

An interspecific comparison of LUE_G can be made here as photosynthesis was measured under light-limiting conditions (100 μmol m^{-2} s^{-1}) (Figures S1 and S2). Not only did *D. pellucidum* have the highest rates of O_2 evolution under green light, it also used green light more efficiently with respect to red/blue light usage ($LUE_G = 0.77$) compared to *A. serpens* or *L. albidum* (LUE_G for these species was 0.55 and 0.44, respectively).

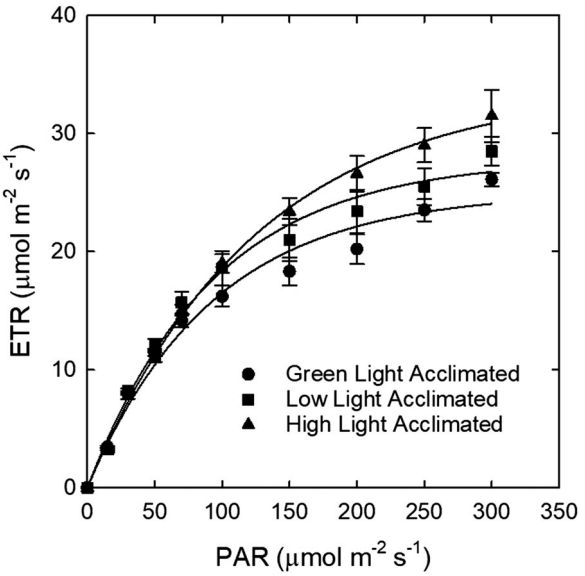

Figure 2 Light response curve for relative electron transport rate (ETR) of *D. pellucidum* acclimated to three light conditions (green light [30 μmol m^{-2} s^{-1}], high full spectrum light [100 μmol m^{-2} s^{-1}], and low full spectrum light [30 μmol m^{-2} s^{-1}]) ($n = 5$/trt). ETR was calculated from chlorophyll fluorescence measurements of PSII efficiency using a Technologica™ CF imager. No significant differences in light response curve parameters (coefficient of nonlinearity or ETR_{MAX}) between treatments were observed.

Electron transport rate

Photosystem II efficiency, expressed as relative electron transport rate (ETR), was measured at varying intensities of blue light using a chlorophyll florescence imaging system (Figure S2). The ETR responses of *D. pellucidum* plants exposed the three light treatments (high light, low light and green light) was assessed after a 3-week growth period. Only small treatment differences at PAR = 300 μmol m^{-2} s^{-1} were observed (Figure 2), and these differences were not significant (ANOVA; $F = 3.19$, $P = 0.077$). To assess maximum photosynthetic efficiency for all species, we measured ETR on field-collected moss samples and found that *D. pellucidum* had the highest photosynthetic capacity compared to *A. serpens* and *L. albidum*, with *A. serpens* and *L. albidum* having similar ETR at varying light intensities (Figure S1); however, all species showed similar light responses at lower light intensities (0–300 μmol m^{-2} s^{-1}) (Figure S1).

Tissue biochemistry

For each species of moss, the high light acclimated tissues had significantly higher (less negative) $\delta^{13}C$ values compared to other treatments (ANOVA; $P < 0.001$; Figure 3), with no significant differences observed among the other three treatments (field, green light, and low light). The $\delta^{13}C$ ratios show a species-specific response with *A. serpens* having a significantly lower $\delta^{13}C$ than *D. pellucidum* (*t*-test; $P = 0.002$) and *L. albidum* (*t*-test, $P = 0.004$), in the high light treatment with no significant differences observed between the latter two species (*t*-test, $P = 0.388$).

Analysis of %N measured from tissue samples of plants grown under each light treatment for 3 weeks varied by species (Table 1). No changes in %N were observed across light treatments for *D. pellucidum* or *A. serpens* ($P = 0.715$; $F = 0.46$ and $P = 0.237$; $F = 1.57$ ANOVA, respectively). However, *L. albidum* grown under green light had significantly higher %N compared to the other three light treatments ($P = 0.032$; $F = 3.76$; Table 1). No significant relationships were observed between %N and $\delta^{13}C$ for all species grown under varied light treatments.

Discussion

Green light photosynthesis

Several studies have shown that supplementing red and/or blue light with green light enhances photosynthesis and plant growth (Inada, 1976; Kim *et al.*, 2004; Terashima *et al.*, 2009). Our findings show that green light alone results in positive net photosynthesis in understory moss species. Intact gametophyte colonies

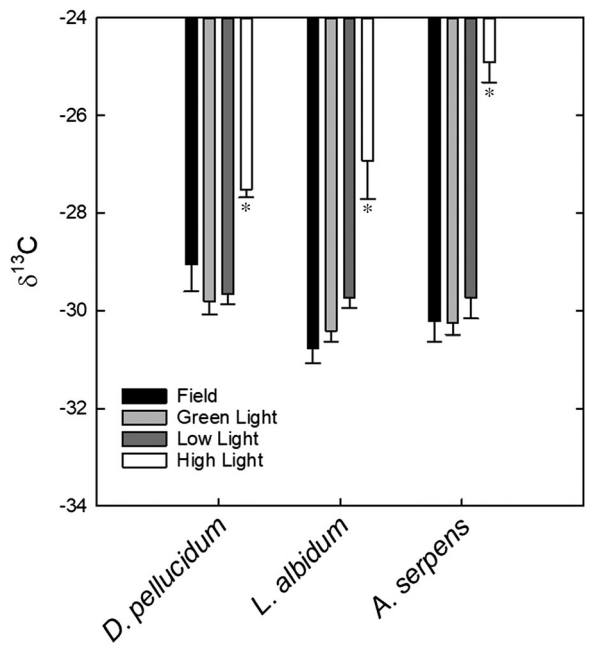

Figure 3 Carbon-13 discrimination ($\delta^{13}C$) for three species of moss grown in green light (30 μmol m^{-2} s^{-1}), high full spectrum light (100 μmol m^{-2} s^{-1}), or low full spectrum light (30 μmol m^{-2} s^{-1}) compared to moss collected from the field. More negative values of $\delta^{13}C$ indicate less carbon discrimination. Asterisks (*) denote significant differences between treatments (ANOVA; $P < 0.01$)

likely have higher green light use efficiency than was measured in this experiment given multiple overlapping layers of tissue which increase light absorption (i.e. the moss canopy); thus, we likely underestimate the ability of these plants to utilise green light for photosynthesis.

Photosynthetic responses varied across the three species of moss studied, with *D. pellucidum,* a shade-dwelling species, having the highest P_N under green light as well as the highest LUE_G compared to *A. serpens* and *L. albidum*, two species that tend to grow in more open canopy environments (Figure 1). These findings support our hypothesis that plants adapted to closed canopy environments have an increased ability to use green light to drive photosynthesis. Species-specific differences in photosynthesis were only significant when normalised by mass (Figure 1) and not by area (Figure S1). Mass-based normalisation of photosynthesis describes the 'return on investment' per gram of biomass produced and may be more useful for comparing photosynthetic abilities of bryophyte species. All P_N measurements were made in aqueous solutions, which should result in similar rates as fully hydrated moss given that the tissue osmolality of fully hydrated moss and submerged moss is nearly the same (Proctor *et al.*, 2007).

We found small differences in ETR_{MAX} for *D. pellucidum* grown under varied light treatments that indicate this trait is plastic, providing *D. pellucidum* with a mechanism to grow in more variable light environments (Figure 2). Under environmentally relevant light levels for this species of moss (<300 μmol m^{-2} s^{-1}), ETR was not significantly different in green light acclimated *vs* full spectrum acclimated plants, which suggests green light did not negatively alter its photosynthetic capacity over the 3-week growth period (Figure 2).

The ability of plants to alter their physiological, anatomical, and morphological traits in response to variations in light intensity has been demonstrated in many species (Lichtenthaler *et al.*, 1981; Givnish, 1988; Sack *et al.*, 2006), including mosses (Hájek & Beckett, 2008). Less studied, however, is the effect of light quality on leaf function (Sun *et al.*, 1998; Kim *et al.*, 2004; Terashima *et al.*, 2009), especially in mosses. In many vascular plants, blue light allows for gas exchange by stimulating a rapid increase in turgor pressure within guard cells causing stomatal opening, a process that can occur rapidly in comparison to photomorphogenic processes (Zeiger & Hepler, 1977). The lack of stomata in moss gametophytes makes blue light unnecessary for gas exchange to occur. With sporophyte stomata aside, all gas exchange in mosses occurs as diffusion across a water film directly between the gametophytic cells and the surrounding boundary layer (Garner & Paolillo, 1973). Thus, gas exchange of mosses may be uninhibited during brief periods of poor light quality, such as the time in between transient sunflecks when red/blue light is absorbed by the upper canopy (Shirley, 1929). Such characteristics of bryophytes likely help them succeed in sub-canopy conditions rich in green light. This is demonstrated by the high LUE_G values observed in species collected from sites with more canopy cover as well as the ability to survive in solely green light conditions.

Table 1 Nitrogen content (%) of gametophytic tissue of three species of moss following 3 weeks of growth in green light (30 μmol m^{-2} s^{-1}), low light (30 μmol m^{-2} s^{-1}), and high light (100 μmol m^{-2} s^{-1}) as well as plant material collected from the field (mean ± SE; $n = 5$).

Species	Field	Green Light	Low Light	High Light
D. pellucidum	1.15 ± 0.039	1.25 ± 0.130	1.28 ± 0.058	1.26 ± 0.057
L. albidum	0.95 ± 0.043	1.19 ± 0.049*	1.03 ± 0.049	0.98 ± 0.048
A. serpens	1.08 ± 0.055	1.18 ± 0.027	1.07 ± 0.045	1.04 ± 0.050

Note: Asterisks (*) denote statistically significant differences between growth conditions (ANOVA; $P < 0.05$).

Understory light environments are also characterised by a high concentration of far-red light, which was not considered in this study. Recent research indicates that green light supplementation can enhance the 'far-red response' by increasing petiole length in *Arabidopsis thaliana* (L.) Heynh., possibly due to a reduction of transcription factors related to shade-response-associated genes (Wang *et al.*, 2015). PhyA and/or PhyB may be required for the green light response (Wang *et al.*, 2015), yet such phytochromes do not exist in mosses. Similar shade responses have been observed in mosses and are possibly regulated by a multitude of other phytochromes present in cryptogams, likely the result of convergent evolution (Possart *et al.*, 2014); thus, the response of moss to combined green light and far-red light, as well as the role of bryophyte-specific phytochromes in shade responses, is worthy of investigation.

There is increasing evidence that a plant 'green light receptor' exists and has yet to be defined (Wang & Folta, 2013). Studies have shown that accessory pigments, such as carotenoids, can transfer energy from the green spectra to Chl-a, but this occurs with low efficiency (McCree, 1972; Inada, 1976; Evans, 1989; Hogewoning *et al.*, 2012). Certain deep-water dwelling green algae, such as *Codium fragile* and *Ulva pertusa*, contain siphonaxanthin, a pigment that absorbs green light and transfers this energy to the photosystems with high efficiency (Kageyama *et al.*, 1977). A similar pigment may be used by bryophytes and perhaps higher plants to efficiently utilise available green light energy.

Leaf chemistry

We found no plastic responses in carbon isotope ratios when comparing field to green-light and low level full-spectrum light. However, we did find that $\delta^{13}C$ levels increased in the high light treatments (Figure 3). Previous studies have shown that bryophytes growing in open canopy environments have higher boundary layer conductance to CO_2 compared to those in closed canopy environments (Johansson & Linder, 1980; Rice, 2000). Our data support these previous findings in that the full spectrum high light treatment increased demand for CO_2 in all species measured in this study compared to the green light or full-spectrum low light treatments (Figure 3). These results may, however, simply reflect light limitation in the low light treatments (both green and full-spectrum) rather than carbon stress. Future studies should measure carbon discrimination under high intensity green and full-spectrum light to elucidate carbon discrimination responses to light quality.

In higher plants, leaf nitrogen content is positively correlated with photosynthetic rate since half of the nitrogen in a plant is allocated towards photosynthetic machinery (Evans, 1989; Durand & Goldstein, 2001; Hikosaka, 2004). Globally, nitrogen addition increases net primary production across ecosystems (LeBauer & Treseder, 2008). In bryophytes, N-addition has little effect on plant growth (Bergamini & Peintinger, 2002), suggesting moss tissue nitrogen is not an important feature for moss photosynthesis; however, synthetic fertiliser has been shown to promote moss photosynthesis (Bigger & Oechel, 1982; Smith, 1993). In our study, the three-week acclimation period did not show a strong effect of light quantity on moss tissue %N. For *L. albidum*, we did observe a slight increase in %N in the green light acclimated plants compared to other treatments ($P < 0.05$, Table 1), which may imply that light quality is a more important driver of tissue nitrogen than light quantity for this species. However, since the acclimation period was only three weeks long, it is not clear if the collected tissue samples were from new growth or simply elongated tissues. Thus, long-term studies are needed to help elucidate the effect of light quality on tissue nitrogen and its ecological significance.

Conclusions

We show that three species of moss can photosynthesise past their respiratory demands using solely green light, a novel finding for this taxon. It is likely that this green light response is even higher in an intact moss colony given that the overlapping tissues will increase light absorption and green light use efficiency. Additionally, the efficiency of green light use may be related to canopy cover in these ground-dwelling species. Lastly, we show that moss can maintain physiological vigour over relatively short periods of time in a monochromatic green light environment. Further research is needed to determine the degree of plasticity in 'green-light photosynthesis' in response to variable growth conditions across plant taxa.

Online Supplementary Material

Supplementary material is available at 10.1080/03736687.2018.1516434.

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