Photosynthetic Responses Of *Microstegium vimineum* (Trin.) A. Camus, A Shade Tolerant, C4 Grass, To Variable Light Environments

By: J.L. Horton and **H.S. Neufeld**

Abstract

Microstegium vimineum (Trin.) A. Camus, a shade-tolerant C4 grass, has spread throughout the eastern United States since its introduction in 1919. This species invades disturbed understory habitats along streambanks and surrounding mesic forests, and has become a major pest in areas such as Great Smoky Mountains National Park. The focus of this study was to characterize the photosynthetic induction responses of M. vimineum, specifically its ability to utilize low light and sunflecks, two factors that may be critical to invasive abilities and survival in the understory. In addition, we were curious about the ability of a grass with the C4 photosynthetic pathway to respond to sunflecks. Plants were grown under 25% and 50% ambient sunlight, and photosynthetic responses to both steady-state and variable light were determined. Plants grown in both 25% and 50% ambient sun became 90% light saturated between 750–850 μmol m−2 s−1; however, plants grown in 50% ambient sun had significantly higher maximum steady-state photosynthetic rates (16.09 ± 1.37 μmol m−2 s−1 vs. 12.71 ± 1.18 μmol m−2 s−1). Both groups of plants induced to 50% of the steady-state rate in 3–5 min, while it took 10–13 min to reach 90% of maximum rates, under both flashing and steady-state light. For both groups of plants, stomatal conductance during induction reached maximum rates in 6–7 min, after which rates decreased slightly. Upon return to low light, rates of induction loss and stomatal closure were very rapid in both groups of plants, but were more rapid in those grown in high light. Rapid induction and the ability to induce under flashing light may enable this species to invade and dominate mesic understory habitats, while rapid induction loss due to stomatal closure may prevent excess water loss when low light constrains photosynthesis. The C4 pathway itself does not appear to present an insurmountable barrier to the ability of this grass species to respond to sunflecks in an understory environment.

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Key words *Microstegium vimineum* · Sunflecks · Photosynthesis · Induction · C_4 pathway

Introduction

Plants in understory habitats are subject to highly variable light environments. Long periods of low light are frequently punctuated by relatively short duration sunflecks of high intensity via direct beam penetration through holes in the canopy. Sunflecks can contribute 50–80% of the daily total photosynthetic photon flux density (PPFD) in tropical understories (Pearcy 1983; Chazdon and Fetcher 1984; Chazdon 1988) and 50% of total daily PPFD in temperate deciduous forests (Hutchinson and Matt 1977). Carbon gain during these transient flecks may account for 30–60% of the total daily carbon gain (Bjorkman et al. 1972; Pearcy and Calkin 1983; Pearcy 1987). Therefore, it is important to understand how understory plants are able to utilize this variable resource.

Osterhaut and Haas (1919) first showed that there is a lag time before a leaf reaches its maximum photosynthetic rate when exposed to high light after being in low light for a long time. This lag, or induction period, is often biphasic (Kobza and Edwards 1987; Pearcy 1988), with a fast initial increase in assimilation rate in the first few minutes and a second slower increase to steady-state rates that can last up to an hour (Pearcy et al. 1985). Biochemical limitations, such as the light activation of necessary enzymes and the autocatalytic build-up of Calvin cycle metabolites, are thought to be primarily responsible for the induction response. Although stomatal limitations may also be present (Chazdon and Pearcy 1986a; Kobza and Edwards 1987), the role of

stomatal conductance (g_s) during induction may be dependent on initial g_s, with greater stomatal limitations when initial g_s is low (Kirschbaum and Pearcy 1988b). However, stomatal limitations are thought to be insignificant during induction of C_4 plants, because internal CO_2 concentrations remain above saturating levels (Usada and Edwards 1984; Furbank and Walker 1985). Induction can also occur under flashing or transient light (Pearcy et al. 1985) and usually follows the same time course as induction under steady-state light (Chazdon and Pearcy 1986a).

Some fully induced plants exposed to sunflecks actually achieve light fleck use efficiencies greater than those predicted from steady-state rates (Chazdon and Pearcy 1986b). These greater efficiencies under transient light may be explained by post-illumination carbon fixation (PICF). During transient light, rates of non-cyclic electron transport are higher than necessary for the concurrent rates of carbon assimilation (Kirschbaum and Pearcy 1988a). The energy captured by this excess electron transport may be used to form a capacitance of assimilatory charge in the form of high-energy metabolites (Sharkey et al. 1986), and carbon fixation may continue into the following dark period until this metabolite pool is depleted (Pearcy 1990).

A fully induced leaf returned to low light conditions will undergo a period of induction loss. For many plants the response to decreases in light is slower than that to increases in light (Chazdon and Pearcy 1986a; Poorter and Oberbauer 1993), allowing plants to maintain high induction states during low-light periods between sunflecks. Thus, how a plant responds to sunflecks will depend on the timing of the sunfleck relative to its current induction state, and on the ability of the plant to perform PICF.

C_4 plants are expected to have more complex induction responses due to the spatial separation of the initial carbon fixation reactions and the Calvin cycle. C_4 plants must not only build up metabolite pools for the Calvin cycle but must also create separate pools for the initial fixation reactions and metabolites that shuttle between the mesophyll and bundle sheath cells (Furbank and Walker 1985). Induction in NADP-ME type C_4 plants may be further complicated because the build-up of assimilatory charge occurs in the mesophyll whereas its utilization occurs in the bundle sheath cells (Krall and Pearcy 1993). However, there are few studies of the responses of C_4 plants to variable light and/or sunflecks. Krall and Pearcy (1993) found that corn (*Zea mays* L.), a shade-intolerant C_4 plant, was less efficient at utilizing short-duration (< 1 min) lightflecks than some C_3 species that have been studied. Pearcy et al. (1985) found that *Euphorbia forbesii*, a shade-tolerant Hawaiian rainforest understory tree that possesses the C_4 pathway, induced quickly under both steady-state and flashing light. *E. forbesii* was shown to have higher rates of photosynthesis and faster stomatal responses to sunflecks than a sympatric C_3 species, *Claoxylon sandwicense*. And, finally, there have been a few studies on long-term light responses by shade-tolerant C_4 grasses in the genera *Muhlenbergia* and *Microstegium* (Winter et al. 1982; Smith and Martin 1987a, b, c; Smith and Wu 1994). However, to our knowledge, no studies have addressed the responses of shade-tolerant C_4 grass species to "transient," i.e. sunfleck, light conditions.

Microstegium vimineum is an exotic, shade-tolerant C_4 grass (NADP-ME subtype) that has become a noxious weed in the eastern United States since its introduction in 1919 (Fairbrothers and Gray 1972). *M. vimineum* occurs in a variety of habitats from open moist fields, to roadsides and forest edges, to deep-understory sites (Redman 1995). This species has the ability to invade disturbed, moist understory habitats and can easily establish a monoculture (Barden 1987), crowding out much of the native vegetation. It is considered to be a major plant pest in such places as Great Smoky Mountains National Park (GSMNP). The purpose of this study was to assess the induction and induction-loss responses of *M. vimineum* to various light regimes when grown under different light conditions typical of those experienced in the field. In particular, we were interested in comparing induction responses of this C_4 shade-tolerant grass to those reported for other C_4 shade-tolerant plants, such as *E. forbesii* (Pearcy et al. 1985) and the C_4 shade-intolerant grass, *Z. mays* (Krall and Pearcy 1993).

Materials and methods

Field light environment

To characterize the heterogeneity of the light environment of *M. vimineum* over a range of habitats, measurements of PPFD in the field were made at three sites in Cades Cove, GSMNP. One site was under a partially closed canopy of tulip poplar (*Liriodendron tulipifera* L.) and white oak (*Quercus alba* L.) on a dry, west-facing slope (hereafter referred to as PCD, for partially closed, dry site). The second site was under a partially closed canopy of American beech (*Fagus grandifolia* L.) and yellow birch (*Betula lutea* L.) in a moist depression (PCM, for partially closed, moist site). The third site was in a moist open site (GAP, for gap, moist site) along a slightly sloping drainage between stands of white pine (*Pinus strobus* L.). This site received direct sunlight for 2–3 h during the middle of the day, whereas the other two sites did not receive as much direct radiation because of their more closed canopies.

To characterize the understory light environment at these three sites, light measurements were made for a full day once a month from April through August using a 1-m^2 light sensor array equipped with nine evenly spaced fast-response GaAsP photodiodes (G-1118 Hamamatsu, Bridgewater, NJ). Sensors were calibrated against a LI-185B quantum sensor (Li-Cor, Lincoln, NB), which was also placed in the field. The light sensor array was leveled to ensure that all sensors were horizontal. Data from all ten sensors were pooled when assessing the light environment of each site.

Past researchers have characterized temporal variability in understory light environments by measuring light intensity every 5 or 10 s (Pearcy 1983; Chazdon et al. 1988; Roden and Pearcy 1993), with some authors averaging individual measurements over short time scales of 5–10 min (Chazdon and Fetcher 1984; Oberbauer et al. 1988; Messier and Puttonen 1995). In this study, light intensity was measured every second; the mean, maximum, and minimum values were then calculated for each 10-s interval. Data were stored on a Campbell 21X datalogger with a SM192 storage module (Campbell Scientific, Logan, UT) until downloaded to a

portable computer. High-light events were designated as PPFD > 100 μmol m^{-2} s^{-1} (Weber et al. 1985). Data for each site were used to construct histograms of high-light-event duration and intensity and contribution to total daily PPFD by high-light events of different lengths.

Plant material

Seeds of *M. vimineum* were collected in topsoil near the Primitive Baptist Church (elevation 450 m) in Cades Cove, GSMNP, and were germinated in a laboratory at Appalachian State University (ASU) under a fluorescent light bank providing 100 μmol photons m^{-2} s^{-1} PPFD on a 16:8 h light:dark cycle. After 1 month, seedlings were transplanted and moved to the ASU Biology Department greenhouse, where they were randomly assigned to one of two light treatments, either 25% or 50% of ambient sunlight (75% or 50% shade, respectively). These treatments are representative of light environments in the range of habitats in which *M. vimineum* occurs in the field (Redman 1995). Plants were allowed to grow in their assigned light regime for at least 26 days before gas exchange measurements were taken. This was enough time for new leaves to develop under these growth conditions.

Gas exchange

For all experiments, gas exchange measurements were made on the newest fully expanded leaf on a randomly chosen shoot and replicated three to four times on different plants using a Li-Cor 6200 Portable Photosynthesis System with a 0.25-l chamber. In all experiments, light was provided by a 1000-W metalarc lamp (GTE Products, Manchester, NH), with a 10-cm-deep water bath placed between the lamp and the cuvette to absorb heat from the lamp. Neutral density filters were used to provide various light levels. Air used to flush the cuvette between measurements was scrubbed of both CO_2 and H_2O before entering the system. CO_2 was then added back to the airstream from a tank containing 2% CO_2 in air at a rate to maintain the CO_2 concentration of the airstream entering the chamber between 350–375 ppm. Part of the airstream was bubbled through a water bath to maintain the relative humidity in the chamber between 60 and 70%.

Steady-state light response curves

Steady-state light response curves were constructed for both groups of plants. In each trial, a leaf was placed in the chamber and allowed to acclimate stepwise to a maximum PPFD of 1875 μmol photons m^{-2} s^{-1}. Then, gas exchange was measured as PPFD was decreased stepwise to 0 μmol photons m^{-2} s^{-1} (achieved by wrapping the cuvette in aluminum foil). Leaves were allowed to equilibrate to each light level for 20–30 min before gas exchange measurements were taken. At each light level five measurements were taken 1–2 min apart, with the median value used to calculate means of the three to four replicates. Between each measurement, the system was opened and the cuvette flushed with the control air described above.

For each replicate, the apparent quantum yield (slope) and light compensation point (x-axis intercept) were calculated from a linear regression equation, using the first three data points above 0 (60–330 μmol photons m^{-2} s^{-1}) of the response curve. These values were compared between both groups of plants (25% and 50% of ambient sun) using analysis of variance (ANOVA). Entire light response curves were fitted using the Von Bertalanffy equation:

$$p = a + b(1 - \exp^{-k*\mathrm{PPFD}})$$

where p = observed photosynthetic rate, a = dark respiration rate, b = maximum photosynthetic rate, and k = apparent quantum yield.

Induction responses

Plants used for induction measurements were kept at low light (< 10 μmol photons m^{-2} s^{-1}) for at least 2 h before gas exchange measurements were taken (as per Pearcy et al. 1985; Poorter and Oberbauer 1993). Leaves were placed in the cuvette and allowed to equilibrate to cuvette conditions and control air (as described above) and remained in the chamber throughout the duration of the trial. Once leaves were equilibrated to low light they were exposed to one of four light regimes: constant light at either 550 or 1100 μmol photons m^{-2} s^{-1} and flashing light with 1 min of high light at either 550 or 1100 μmol photons m^{-2} s^{-1} with intervening 1-min low-light periods of ≈10 μmol photons m^{-2} s^{-1}. Low light during the flashing experiments was achieved manually by placing a piece of cardboard between the light source and the plant. Measurements were taken every minute starting at 5 min before high-light exposure and continuing for 30 min thereafter. For each measurement, the system was closed and allowed to equilibrate to a steady rate of CO_2 depletion (10–15 s) before measuring gas exchange rates, in order to decrease measurement error caused by the lag in the system in detecting changes in CO_2 concentration (Knapp 1992; Fay and Knapp 1993). Preliminary experiments with such short equilibration times using an empty chamber resulted in photosynthetic rates of less than 0.7 μmol m^{-2} s^{-1}. Results from empty-chamber experiments with longer equilibration times of 1 min were not significantly different from those with 15-s equilibration times. Therefore, the results presented here represent actual biological results and not results from system lags or errors.

The rate of CO_2 depletion was measured for 15 s which was timed to coincide with the last 15 s of the light treatment (flash or shade). After each 15-s measurement, the system was opened and the cuvette flushed with control air for 30–40 s before the next measurement cycle. This maintained cuvette relative humidity between 60–70% and the CO_2 concentration between 350–370 ppm during the entire trial. An analysis of the potential errors induced by non-linearity in rates of gas exchange showed that such errors in rate calculations never exceeded 3.5% of those expected if rates were linear with time.

Maximum photosynthetic rates were calculated for each trial by taking the mean of the last 10 min of high-light measurements (n = 10 for steady light and n = 5 for flashing light). The times to 50% (T_{50}) and 90% (T_{90}) of maximum rate were calculated. All responses were compared among treatments using ANOVA.

Induction loss

Fully induced leaves were exposed to periods of low light (≈10 μmol photons m^{-2} s^{-1}) of 1, 2, 5, 10, 15, 20, or 30 min in length, after which they were exposed to a 30-s lightfleck of 1175 μmol photons m^{-2} s^{-1}, an intensity often encountered in the field (Horton 1996). A short light fleck of 30s was used to reduce the amount of induction incurred during the fleck in order to better assess photosynthetic induction state attained after the low-light period. Gas exchange rates measured over the last 15 s of the fleck were used to calculate the induction state of the leaf using the following equation from Chazdon and Pearcy (1986a):

$$\text{induction state (\%)} = (P_{\mathrm{LF}} - P_{\mathrm{L}})/(P_{\mathrm{H}} - P_{\mathrm{L}}) * 100$$

where P_{LF} is the rate of CO_2 assimilation at the end of the lightfleck, P_{L} is the steady-state CO_2 assimilation rate at low light, and P_{H} is the steady-state CO_2 assimilation rate at high light. An analogous equation was used to calculate conductance state during induction loss.

Induction and conductance loss were modeled using a two-parameter exponential decay function (Ratkowsky 1990):

$$y = 100 * \exp^{[(-C/D)+(C/(D+x))]}$$

where y equals induction or conductance state, x is the length of the dark period, and C and D are parameters to be estimated. We then reparameterized the model, following the procedures described by

Lee et al. (1990) in order to directly calculate the time to reach either 50% or 80% induction or conductance loss. To do this we created a new variable:

$$X_p = -D^2 * \ln(1-p)/[D * \ln(1-p) + C]$$

where X_p is the time to either 50% or 80% loss of initial induction or conductance, p is the relative amount of induction loss, and $1-p$ the relative induction loss state. We then expressed C as a function of D and X_p as follows:

$$C = -D * \ln(1-p) * (D + X_p)/X_p$$

Substituting for C in the above model, we obtain the revised two-parameter model:

$$y = 100 * \exp^{\ln(1-p)*(D+Xp)*[Xp*(D+Xp)]}$$

We used non-linear regression in SAS (SAS Institute, Cary, NC) to obtain parameter estimates of D and X_p, and then used a standard t-test to look for growth treatment effects in the estimates of times to 50% and 80% losses of induction and conductance states.

Results

Field light environment

Figure 1 shows the diurnal light regime from one representative sensor at each of three sites in Cades Cove in July 1995. Sampling took place over 3 warm, partly cloudy days, typical of summer in GSMNP. The PCD site had fewer fleck events and most flecks were of low intensity. The PCM site had more fleck events and many of the flecks were of high intensity. The GAP site received an order of magnitude greater daily photon flux than the partially closed canopy sites (12.6 ± 2.0 mol m^{-2} versus 1.0 ± 0.2 mol m^{-2} at PCD, and 1.3 ± 0.3 mol m^{-2} at PCM), much of this coming during the middle of the day as high-intensity direct sunlight.

The majority of fleck events in the closed-canopy sites were less than 1 or 2 min long, while the gap site had a large proportion of fleck events less than 5 min, but also several high-light events longer than 20 min (Fig. 2A). Very short (< 1 min) flecks contributed 80% of the total daily photon flux at the PCD site and > 50% at the PCM site. The PCM had a more equitable contribution from longer flecks. The GAP site received > 80% of the total daily photon flux in high-light events lasting more than 20 min, due to a large opening in the canopy (Fig. 2B).

Figure 2C shows the distribution of maximum intensities achieved during sunflecks. In all sites > 60% of

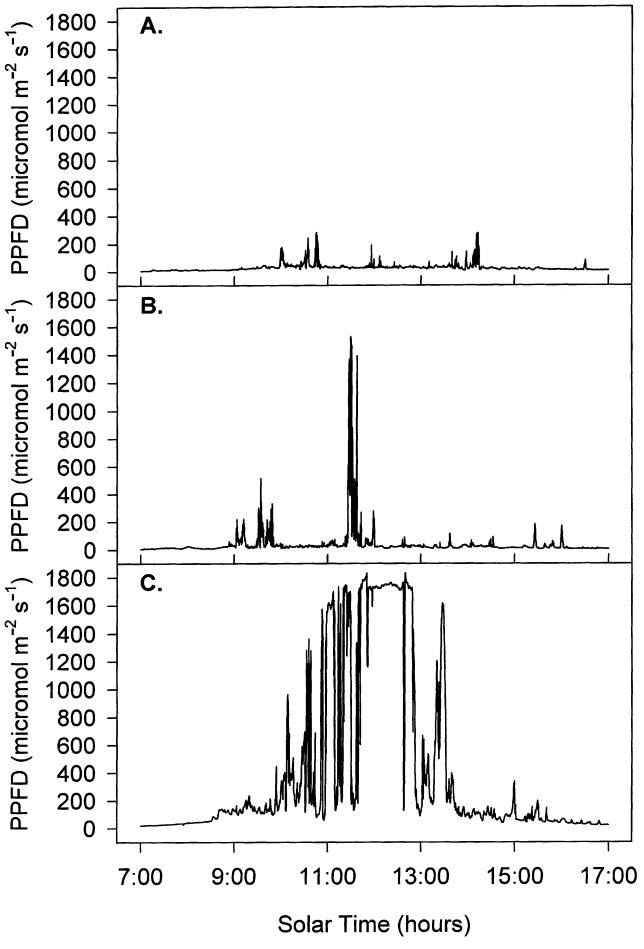

Fig. 1 Diurnal course of photosynthetic photon flux density (*PPFD*) from one representative sensor at each of three sites in Cades Cove, Great Smoky Mountains National Park: a partially closed canopy dry site (**A**), a partially closed canopy moist site (**B**), and an open-canopy, gap site (**C**). Data were collected on three consecutive days in July 1995

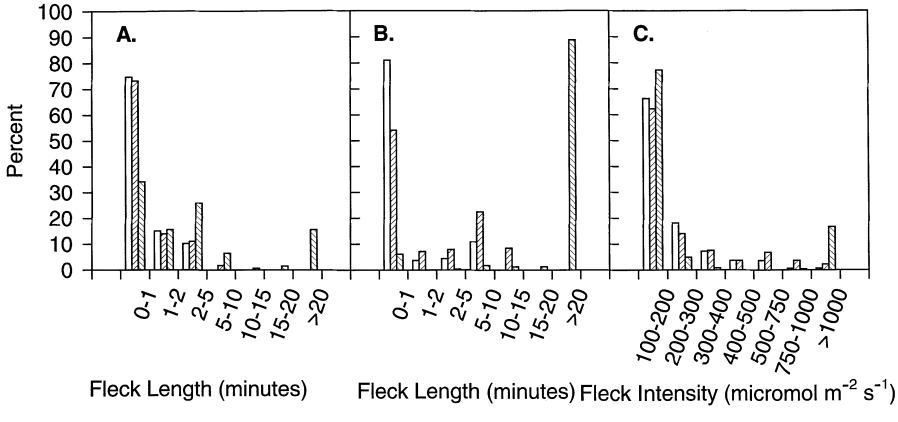

Fig. 2 Frequency histograms for sunfleck duration (**A**), the percent of total daily PPFD contributed by flecks of specific duration classes (**B**), and fleck maximum intensity (**C**). Three sites were sampled over three consecutive days in July 1995, a partially closed canopy dry site (□), a partially closed canopy moist site (▨), and an open-canopy, gap site (▧). Histograms represent combined data from ten sensors at each site

the fleck events were between 100 and 200 μmol m^{-2} s^{-1}. However, all sites received some higher-intensity flecks, often ranging up to >1000 μmol m^{-2} s^{-1}.

Steady-state light curves

Comparisons of photosynthetic characteristics were made on *M. vimineum* grown under either 25% or 50% of ambient sunlight by constructing steady-state light response curves (Fig. 3). Plants grown under 25% ambient sun achieved maximum steady-state CO_2 assimilation rates of 12.71 ± 1.18 μmol m^{-2} s^{-1} and became 90% light saturated at 750 ± 100 μmol m^{-2} s^{-1}, while those grown at 50% full sun had significantly ($P < 0.001$) higher maximum CO_2 assimilation rates of 16.09 ± 1.37 μmol m^{-2} s^{-1}, but a similar ($P = 0.81$) 90% light saturation point of 815 ± 240 μmol m^{-2} s^{-1}. Both the 25% and 50% full sun plants had similar light compensation points (13.2 ± 10.0 and 12.8 ± 0.3 μmol m^{-2} s^{-1}, respectively, $P = 0.94$) and low dark respiration rates (0.63 ± 0.11 and 0.67 ± 0.36 μmol m^{-2} s^{-1}, respectively, $P = 0.85$), which are typical of shade-adapted plants (Boardman 1977).

Induction responses

Induction under steady light was very rapid, but did not show the biphasic nature seen in other induction studies (Figs. 4A, D), possibly because of our inability to make measurements rapidly enough to detect such a pattern. Plants grown in 50% ambient sun induced to significantly higher rates of CO_2 assimilation than those grown in 25% ambient sun (Table 1). In all experiments, the time required to reach 50% of steady-state rates (T_{50}) was 3.5–5 min. On the whole, both groups of plants required 8–14 min to reach 90% of steady-state rates (T_{90}). However, 25% ambient sun plants induced at low levels of flashing light required longer to reach T_{90} than both 50% ambient sun plants under the same induction regime and 25% ambient sun plants induced under higher-intensity flashing light (Table 1).

Stomata were essentially closed during the low-PPFD pretreatment, but showed a rapid response to increasing PPFD (Fig. 4B, E). Conductance increased quickly to high rates but then showed a decline before stabilizing at an intermediate value. Intercellular CO_2 concentration (C_i) showed an initial drop when PPFD was increased (Fig. 4C, F), followed by an increase as stomata opened, and then a decline to a slightly lower stable level. When plants from both growth treatments were tested for their induction responses under flashing light, each successive lightfleck produced higher rates of CO_2 assimilation until steady-state rates were achieved (Fig. 5A, D). Plants grown in 50% ambient sun had significantly ($P < 0.001$) higher maximum rates of CO_2 assimilation under flashing light when induced at 1175 μmol m^{-2} s^{-1} than those grown at 25% full sun. However, the 50% ambient sun plants induced under low-intensity flashing light achieved similar rates as those of the 25% ambient sun plants (Table 1).

The stomata of both plant groups responded very rapidly to changes in PPFD (Fig. 5B, E). The overall induction pattern was similar to that seen under steady-state light, with a fast initial peak followed by a decline to steady rates, although the stomata tracked changes in PPFD very closely. Internal CO_2 concentration closely followed changes in stomatal conductance, dropping very low during lightflecks, especially later in the in-

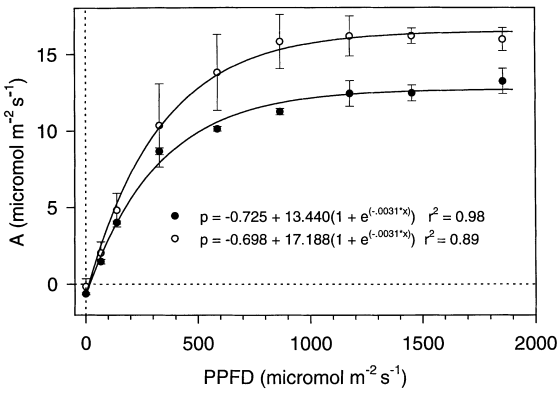

Fig. 3 Fitted steady-state photosynthetic responses to varying light levels in *Microsteguim vimineum* grown under 25% (*filled circles*) and 50% (*open circles*) of ambient sun. Curves were fitted using the Von Bertalanffy equation: $p = a + b(1 - \exp^{-k*\text{light}})$ where p = observed photosynthetic rate, a = respiration rate, b = maximum photosynthetic rate, and k = apparent quantum yield. Each point represents the mean ± SE of three replicates

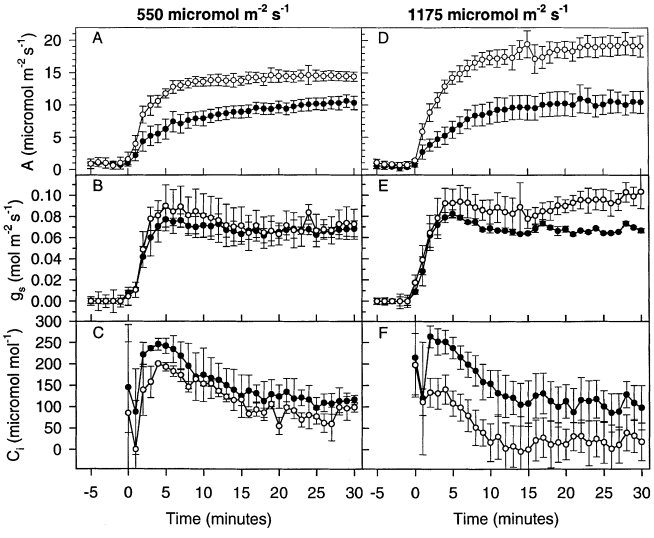

Fig. 4 Responses of CO_2 assimilation (**A, D**), stomatal conductance (**B, E**), and internal CO_2 concentration (**C, F**) when exposed to steady-state light of 550 or 1175 μmol m^{-2} s^{-1} after a 2-h low-light (10 μmol m^{-2} s^{-1}) period, for plants grown under 25% ambient sun (*closed circles*) and 50% ambient sun (*open circles*). Due to the inaccuracy of internal CO_2 calculations when stomata are closed, values during the low-light pretreatment were omitted. Each point represents the mean ± SE of three replicates

Table 1 Maximum steady-state rates of CO_2 assimilation achieved during the induction period, and the time required to reach 50% (T_{50}) and 90% (T_{90}) of those steady-state rates. Data shown are the mean ± SE ($n = 3$). *Lower-case letters* show significant differences ($P < 0.075$) for comparisons within treatment across growth levels; *upper case letters* show significant differences ($P < 0.075$) for comparisons within plant group across induction light treatments (steady or flash), and unmarked means are not significantly different across all comparisons (*PPFD* photosynthetic photon flux density)

Plant group/treatment	Steady-state rate (μmol CO_2 m^{-2} s^{-1})	T_{50} (min)	T_{90} (min)
25% ambient sun			
550 μmol m^{-2} s^{-1} PPFD steady	9.74 ± 1.40^{a}	3.3 ± 2.3	13.3 ± 6.4^{a}
1175 μmol m^{-2} s^{-1} PPFD steady	10.40 ± 3.01^{a}	4.3 ± 1.3	12.7 ± 3.8^{a}
550 μmol m^{-2} s^{-1} PPFD flash	9.18 ± 2.37^{a}	5.3 ± 2.3	20.0 ± 2.0^{bA}
1175 μmol m^{-2} s^{-1} PPFD flash	8.75 ± 1.60^{a}	4.0 ± 3.5	8.7 ± 4.6^{aB}
50% ambient sun			
550 μmol m^{-2} s^{-1} PPFD steady	14.44 ± 1.36^{bA}	2.0 ± 0.0	7.7 ± 2.1^{a}
1175 μmol m^{-2} s^{-1} PPFD steady	19.13 ± 2.62^{bB}	3.0 ± 1.0	11.0 ± 4.4^{a}
550 μmol m^{-2} s^{-1} PPFD flash	12.13 ± 4.94^{a}	4.0 ± 2.0	10.7 ± 6.1^{a}
1175 μmol m^{-2} s^{-1} PPFD flash	14.20 ± 3.52^{b}	4.0 ± 0.0	14.0 ± 2.0^{a}

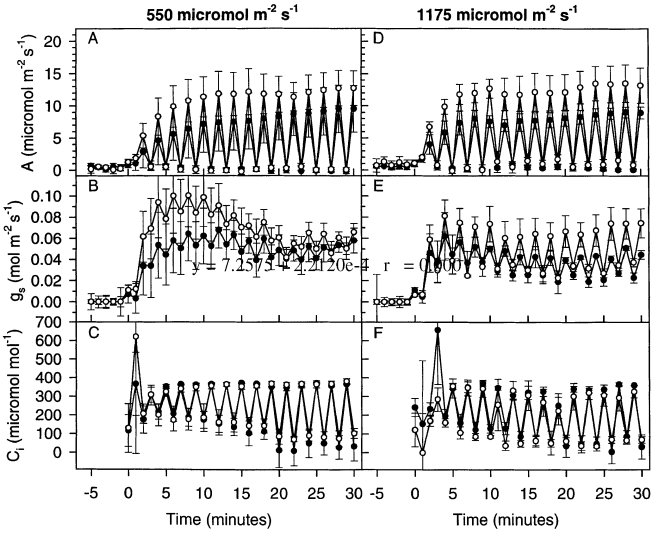

Fig. 5 Responses of CO_2 assimilation (**A, D**), stomatal conductance (**B, E**), and internal CO_2 concentration (**C, F**) when exposed to flashing light (1 min 550 or 1175 μmol m^{-2} s^{-1}/1 min 10 μmol m^{-2} s^{-1}) after a 2-h low-light (10 μmol m^{-2} s^{-1}) period, for plants grown under 25% ambient sun (*closed circles*) and 50% ambient sun (*open circles*). Due to the inaccuracy of internal CO_2 calculations when stomata are closed, values during the low-light pretreatment were omitted. Each point represents the mean ± SE of three replicates

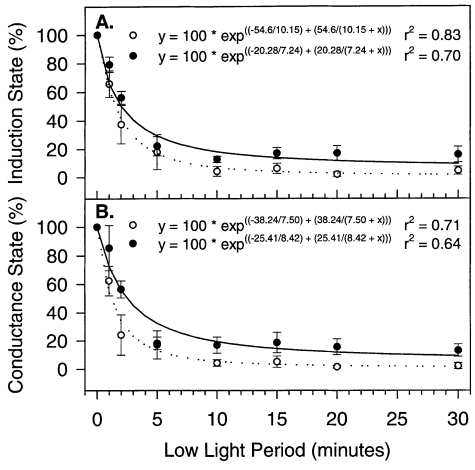

Fig. 6 Time course of induction loss of *M. vimineum* grown under 25% (*filled circles*) and 50% (*open circles*) full sun following different low-light exposure periods. Each point represents the mean ± SE of four replicates. Curves were fitted using a two-parameter exponential decay function ($y = 100 * \exp^{-C/D+[C/(D+x)]}$)

duction period (Fig. 5C, F). Although these low levels of C_i may not be limiting for photosynthesis, they come very close (Usada and Edwards 1984; Furbank and Walker 1985).

Induction loss

Induction loss in *M. vimineum* followed a negative exponential decay function and was very rapid in both groups of plants (Fig. 6A). However, the rate of induction loss was significantly faster in the plants grown under 50% full sunlight than in the 25% full sun plants (Table 2). After 30 min of low light, the 25% full sun plants maintained a somewhat higher induction state than the 50% full sun plants, although the difference was not very significant statistically ($P = 0.11$). The stomatal conductance state followed a decay pattern similar to that of the induction state (Fig. 6B). Both groups of plants lost conductance state quickly but, the decline was more rapid in the 50% full sun plants (Table 2).

Discussion

M. vimineum grows in highly variable light environments with long periods of low-intensity diffuse light punctuated by short periods of high-intensity sunflecks. It possesses low dark respiration rates and low light compensation points characteristic of shade-tolerant plants

Table 2 Time required for fully induced leaves of *Microstegium vimineum* grown in two light environments to reduce to 50% and 80% losses of initial induction and conductance states. Values were calculated from a two-parameter negative exponential function (see Fig. 6) fitted to mean induction states and conductances after each low-light period. *t*-tests were used to compare times to 50% and 80% induction and conductance loss between the two groups of plants ($n = 4$)

	Plants grown in 25% ambient sun (min)	Plants grown in 50% ambient sun (min)	*P*
Induction loss			
50%	2.4	1.5	0.056
80%	9.7	4.3	0.015
Conductance loss			
50%	2.5	1.2	0.015
80%	9.6	3.4	0.012

(Boardman 1977), allowing it to maintain a small but positive carbon gain during the long periods of low light in the understory. Plants grown in high light show some ability for photosynthetic acclimation as evidenced by the higher light-saturated rates of CO_2 assimilation. However, these plants also retain their shade-tolerant attributes of low dark respiration rates and light compensation points.

Induction under both steady-state and flashing light was very rapid in both 25% and 50% ambient sun plants. Stomatal response was also very rapid and was probably not limiting during the induction phase. Limitations in the initial stages of induction are probably due to light activation of enzymes such as Rubisco, which has been shown to require up to 4 min to reach maximum activation (Kobza and Edwards 1987). The build-up of Calvin cycle metabolites and a metabolite gradient between the mesophyll and bundle sheath cells may account for the later stages of induction (Krall and Pearcy 1993). Plants grown under 25% ambient sunlight required more time to reach 90% of their steady-state rates when induced under low flashing light. Perhaps it takes longer under this light regime to acquire adequate energy to build up the metabolite pools needed to drive the Calvin cycle and/or transport carbon from the mesophyll to the bundle sheath cells. This trend was not seen under flashing light of greater intensity. To fully assess this phenomenon, metabolite pool sizes and turnover rates during induction would need to be measured.

Stomatal conductance also responded rapidly to both increases and decreases in PPFD. Stomatal tracking in C_4 prairie grasses has been suggested to conserve water and increase water use efficiency in areas with high but variable light (Knapp 1993). These rapid responses may not be as adaptive in understory environments (Knapp 1993) because low conductances at the beginning of subsequent flecks may limit CO_2 assimilation (Pearcy and Calkin 1983; Kirschbaum and Pearcy 1988b). Brown (1977) suggests that the C_4 mechanism in understory plants may represent reverse evolution from high to low light environments. Thus, stomatal tracking in *M. vimineum* may be a relic from when the species occurred more frequently in high light environments.

Maintaining a high induction state during periods of low light will enable an understory plant to more effectively utilize a subsequent sunfleck. However, induction loss was far more rapid in *M. vimineum* than was expected for an understory plant. This rapid loss could limit CO_2 assimilation during sunflecks following brief (2–5 min) low-light periods. *Alocasia macrorrhiza*, an Australian rainforest herb, was shown to require more than an hour to fully lose induction and was able to maintain higher induction states than a gap species (Chazdon and Pearcy 1986a). Loss of induction state results from the deactivation of enzymes in the Calvin cycle, stomatal closing, and depletion of high-energy metabolite pools needed for the Calvin cycle and transfer of carbon from the mesophyll to the bundle sheath cells. Deactivation of Rubisco and other Calvin cycle enzymes is slower than activation (Chazdon and Pearcy 1986a) and therefore appears not to explain the rapid induction loss seen in *M. vimineum*. Reduction in stomatal conductance state during low-light periods followed a similar pattern as observed for the induction state and could account for much of the rapid induction loss. Furthermore, a rapid depletion of high-energy metabolites in bundle sheath cells during a low-light period may also account for the rapid induction loss seen in *M. vimineum*. C_4 plants require energy in the form of ATP to shuttle metabolites from the mesophyll to the bundle sheath cells. In NADP-ME-type C_4 plants, which have little or no photosystem II reaction centers in bundle sheath cells, PGA must be shuttled from the bundle sheath cells to the mesophyll cells, where it is reduced to Triose-P and returned to the bundle sheath cells where it may enter the Calvin cycle (Edwards and Walker 1983). In low light, NADP-ME enzyme C_4 plants may quickly run out of reducing potential in the bundle sheath cells and be unable to shuttle metabolites to the mesophyll for reduction. Plants grown in 25% full sun maintained higher induction and conductance states than those grown in 50% full sunlight, again suggesting that acclimation to high light environments limits the ability to utilize sunflecks as effectively. Sharkey et al. (1986) found that C_3 plants grown under low light were able to generate greater pools of Triose-P than those grown under higher light, and the same may hold true for C_4 plants. Thus *M. vimineum* plants grown in 25% full sun may be able to maintain their induction state longer than those grown in 50% full sun because they are able to create and maintain higher levels of Triose-P under high light. Biochemical analyses of metabolite pool sizes are needed to fully understand the mechanisms of induction and induction loss in *M. vimineum*.

In conclusion, *M. vimineum* is a shade-tolerant C_4 grass, and may be an example of reverse evolution of a C_4 species from a high to a low light environment. It has been suggested that C_4 photosynthesis may be competitively advantageous in very hot understory environ-

ments, where photorespiration becomes limiting for C_3 plants (Ehleringer and Bjorkman 1977). But the C_4 pathway may not confer any advantage in cool, temperate understories. Pearcy (1983) suggested that *E. forbesii*, a C_4 tree in the Hawaiian rainforest understory, may have evolved from an ancestor in high light environments. It maintained C_4 photosynthesis because it was adaptively neutral in the rainforest understory, yet advantageous if the tree entered the canopy. *M. vimineum* possesses many characteristics that enable it to tolerate the low light typical of understory habitats, yet it still maintains characteristics of plants adapted to high light environments, such as rapid stomatal movements in variable light, rapid induction loss, and a high-light requirement for successful seed production (Barden 1987). So what is its competitive advantage over native C_3 understory species? Rapid induction may allow for greater carbon gain during sunflecks than that possible for native species, resulting in greater growth. Comparisons of carbon gain of *M. vimineum* and native C_3 understory plants in the field need to be performed to better understand the competitive advantage, if any, that the C_4 pathway provides for *M. vimineum* in temperate understory habitats.

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