Chapter 3

PHOTOSYNTHETIC LIGHT RESPONSES OF FERN SPECIES ADAPTED TO DIFFERENT LIGHT REGIMES

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ABSTRACT

Sunlight is the energy source for plant photosynthesis and one of the major environmental factors influencing the growth and distribution of plant species. Most ferns live in moist and shady environments; however, some are able to recruit energy under high-irradiance conditions.

Here, we tested 4 fern species and found that fern species adapted to high light always had a high photosynthetic rate. Shade-adapted ferns showed a low photosynthetic rate but high stomatal conductance at the initiation of light induction and required a short time to reach a maximal photosynthetic rate. In addition, when leaves were illuminated with 50 to 2000 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) for 30 min, the degree of photoinhibition increased with increasing PPFD. Despite the different light-adaptation capabilities of the four species, they could be divided into 2 groups by degree of photoinhibition under the same PPFD.

Pyrrosia lingus, a slight-shade–adapted fern, could use more sunlight with high photosynthetic capacity, whereas *Asplenium antiquum*, a slight- to medium-shade fern, and *Diplazium donianum*, a medium- to heavy-shade fern, could dissipate excess light energy via xanthophyll-cycle–dependent energy quenching (qE).

Therefore, these 3 species could maintain low photoinhibition. These characteristics are probably related to their adaptation to direct sunlight (*P. lingus*) and sunflecks (*As. antiquum* and *D. donianum*). In contrast, *Archangiopteris somai,* a heavy-shade–adapted fern, showed both low photosynthetic rate and qE and therefore high excess energy and low photoprotection, for high photoinhibition.

INTRODUCTION

Sunlight is the energy source for plant photosynthesis and one of the major environmental factors influencing the growth and distribution of plant species (Boardman 1977, Lambers et al. 1998). Studying photosynthetic light response is fundamental to plant eco-physiological research and rare species management because it can be used to assess the light-utilizing ability and optimal light conditions of plants (Bazzaz and Carlson 1982, Lüttge 1997, Aleric and Kirkman 2005). Photosynthesis is a combination of light and carbon reactions. The former converts light energy into ATP and NADPH, which are consumed by the carbon reaction (e.g., Taiz and Zeiger 2006). Under insufficient light energy, plants may reduce net carbon assimilation and growth. In contrast, under high irradiance, the light reaction may absorb more photons than the carbon reaction can use, which often leads to photoinhibition (Demmig-Adams et al. 1996, Kato et al. 2003, Morosinotto et al. 2003). This is the damages of photosystem, especially photosystem II (PSII), caused by the formation of toxic photoproducts, such as O_2^-, H_2O_2, and OH^- (Demmig-Adams et al. 1996, Kato et al. 2003, Adams et al. 2004). To avoid the damage caused by excess absorbed energy, plants have developed several strategies to balance the captured and consumed photon energy, such as the enhanced photosynthetic rate (*P*) and downregulated photosystem efficiency (Demmig-Adams et al. 2006). The latter mechanism, mainly via xanthophyll-cycle–dependent non-photochemical quenching (NPQ) dissipates the excess energy as heat, and plays an important role in protecting the photosystem (Demmig-Adams and Adams 1996, Li et al. 2000, Adams et al. 2004). Plant species adapted to different light regimes show differential photosynthetic characteristics. High-light adapted species tend to have higher *P* and/or NPQ

than shade-adapted species and thus could utilize and/or dissipate more light energy (Scholes et al. 1997, Demmig-Adams et al. 2006, Golan et al. 2006).

At a lower layer of the canopy and understory, leaves commonly receive a very low level of diffuse light and highly variable high light because of frequent exposure to bright sunflecks. In these layers, intense sunflecks might range from seconds to minutes or even longer (e.g., Chazdon 1988, Pearcy 1990). Photosynthetic adaptation or acclimation to light has been studied both for responses to steady light and to dynamic changes in light (e.g., Schulte et al. 2003, Bai et al. 2008, Chen et al. 2011). When leaves are exposed to a sudden increase in irradiance, some understory plants and late-successional species have lower photosynthetic capacity and faster induction than do gap- or open-grown plants and early successional pioneer species (e.g., Han et al. 1999, Portes et al. 2008, Chen et al. 2011). Demmig-Adams et al. (2006) suggested that shade-grown *Monstera deliciosa*, a shade-tolerant perennial monocot species, transferred to high light still showed a low photosynthetic rate but enhanced xanthophyll-cycle–dependent NPQ to avoid photoinhibition.

NPQ is a heterogeneous process. According to formation and dark-relaxation kinetics, NPQ can be divided into at least 3 different components, namely, energy-dependent quenching (qE), transition-dependent quenching (qT) and photoinhibitory quenching (qI) (Müller et al. 2001). qE is the fastest, with induction and relaxation within seconds to minutes. It is related to xanthophyll-cycle–dependent energy quenching. qT, which forms and relaxes in tens of minutes, is state-transition quenching. qI develops when leaves are under prolonged exposure to highly excessive light and was originally ascribed to the photoinhibition of PSII. It needs more time to relax (Müller et al. 2001, Morosinotto et al. 2003, Finazzi et al. 2004, Kalituho et al. 2007, Zulfugarov et al. 2007). High-light–grown *Arabidopsis thaliana* showed high qE and low qI indicates increased dissipation of excess light energy and diminished photoinhibition, whereas low-light–grown *A. thaliana* had the opposite results with more photodamage (Ballottari et al. 2007). Also, we previously found that vegetable cultivars with low photosynthetic capacity had higher qI or lower qE, or both, than the cultivars with high photosynthetic capacity among the same species (Weng et al. 2011).

Stomatal conductance (g_s) is another limiting factor for photosynthesis. Stomata prevent water loss and facilitate CO_2 diffusion to mesophyll cells. Stomatal guard cells may respond to intercellular CO_2 concentration, determined by atmospheric CO_2 and by the mesophyll assimilation rate, to maintain water economy during photosynthesis (Vavasseur and Raghavendra, 2005). For this reason, there is a tight coupling between P and g_s under many

conditions, involving at different light intensity (Yu et al. 2004, Huang et al. 2007) and during photosynthetic light induction (Schulte et al. 2003, Bai et al. 2008, Chen et al. 2011). Leaves with low g_s at the initiation of sunflecks, tend to require a relatively long time to reach maximal P (Han et al. 1999, Bai et al. 2008). Also, the response to sunfleck by stomata is faster for some understory plants than sun-adapted species (Valladares et al. 1997, Han et al. 1999). However, the P–g_s relationship may vary by species (Bai et al. 2008, Portes et al. 2008).

Most ferns live in moist and shady environments; however, some species are able to grow under high irradiance; light-adaptation capabilities vary greatly among species (Saldaña et al. 2010, Wong et al. 2012a). Moreover, stomata of ferns have low ability to detect and respond to increases in CO_2 concentration, which leads to a lower P/g_s ratio as compared with angiosperms (Brodribb et al. 2005, Haworth et al. 2011). However, the light responses of P, g_s and energy quenching of fern species adapted to different light regimes have not been studied in detail, let alone compared with that in angiosperms.

In this study, we used 4 fern and 3 woody species with different light-adaptation capabilities to elucidate their light responses of P and g_s.

In addition, chlorophyll fluorescence analysis is a fast, simple, non-invasive, and reliable method to assess changes in the function of PSII and the fraction of NPQ (Demmig-Adams et al. 1996, Roháček and Barták 1999, Maxwell and Johnson 2000, Müller et al. 2001).

Thus, we used a same equipment to combine measurements of light responses of P, g_s and chlorophyll fluorescence.

MATERIALS AND METHODS

Plant Materials

We used 4 ferns with different light-adaptation capabilities (ranked from high to low, *Pyrrosia lingus*, *Asplenium antiquum*, *Diplazium donianum*, Archangiopteris *somai*); 1 broad-leaved pioneer tree, *Alnus formosana*; and 2 broad-leaved understory shrubs, *Ardisia crenata* and *Ardisia cornudentata*. One- to 2-year-old *A. formosana* seedlings about 30–50 cm tall and adult plants of the other 6 species, about 30–60 cm tall, were collected from central Taiwan (23°49'–24°05'N, 120°54'–121°01'E, 560–800 m a.s.l.), then transplanted to pots (16-cm diameter, 12-cm depth, 1 plant per pot for *As. antiquum* and 3 woody species, and 1 rhizome with 3–4 leaves per pot for the

other 3 ferns) filled with organic soil and maintained outdoors in the nursery at the Endemic Species Research Institute, Chichi Township, Nantou County, Taiwan (23°49'N, 120°48'E, 250 m a.s.l.).

Materials were regularly watered and fertilized (half-strength Hoagland's nutrient solution per month) and received up to 1 to 3 light intensities according to the light condition of their habitat: (1) complete sunshine, 100% sunlight; (2) filtered 50% sunshine beneath a shade cloth, 50% sunlight; and (3) filtered 90% sunshine beneath a shade cloth, 10% sunlight.

The 2 slight- to medium-shade ferns, *P. lingus* and *As. antiquum*, received 10%, 50% and 100% sunlight. One medium-to-heavy shade fern, *D. donianum,* and 2 broad-leaved understory shrubs received both 10% and 50% sunlight; one heavy-shade fern, *Arc. somai*, received 10% sunlight, and the broad-leaved pioneer tree, *A. formosana*, received 100% sunlight.

Measurements were made at least 6 months after transplanting.

Measurements

1. Light Responses of P, g_s and Electron Transport Rate (ETR)

At dawn of the measurement day, fully expanded younger leaves were selected and dark-adapted by covering with aluminum film and kept under the eaves of a cottage at the Endemic Species Research Institute until measurement. From 9:30 to 15:00 h, photosynthesis, g_s and chlorophyll fluorescence were measured simultaneously by use of a portable, open-flow gas exchange system (LI-6400, LI-COR, Lincoln, NE, US) with an integrated fluorescence chamber head (LI-6400-40) stepwise from low to high levels of photosynthetic photon flux density (PPFD) (i.e., 0, 100, 200, 400, 800, 1200 and 2000 µmol m^{-2} s^{-1}).

Throughout the measurements, the leaf temperature was kept at 25°C and CO_2 concentration and relative humidity in the chamber were kept at 350−400 µmol mol^{-1} (no control) and 75% (air entering the chamber controlled by passing temperature-controlled water), respectively. The values of CO_2 exchange, g_s and chlorophyll fluorescence were recorded every 2 min until CO_2 exchange was stable (about 4 min in the dark and 8−16 min under each level of illumination).

For the chlorophyll fluorescence measured in darkness, F_o and F_m, the minimal and maximal fluorescence, respectively, were determined by applying a weak pulse of light (0.1 µmol quanta m^{-2} s^{-1}) and a 0.8-s pulse of saturating flashes of approximately 6000 µmol m^{-2} s^{-1}, respectively. For leaves under

each level of illumination, F was determined for each PPFD level, and F_m' was determined by the same process as for F_m. F and F_m' are the actual and maximal levels, respectively, of fluorescence during illumination.

2. Light Induction of P, g_s and ETR

Overnight dark-adapted fully expanded younger leaves of 100% and 10% sunlight-acclimated plants were selected for measurements with the same instrument and under the same leaf temperature, CO_2 concentration and relative humidity as mentioned above. First, CO_2 exchange and chlorophyll fluorescence of dark-adapted leaves were measured simultaneously.

Then the leaves were exposed to 500 or 2000 $\mu mol\ m^{-2}\ s^{-1}$ PPFD by the light source of the fluorescent chamber head for 30 min, and the values of CO_2 exchange, g_s and chlorophyll fluorescence were recorded simultaneously every 2 min.

3. Energy Quenching and Photoinhibition during Light Induction

Overnight dark-adapted potted materials were placed in a growth chamber (F-360DN) at 25°C. Chlorophyll fluorescence was measured under darkness. Then the leaves were exposed to luminescent lamps (halogen lamp and optical fiber) stepwise from low to high PPFD (i.e., 50, 100, 300, 500, 1000 and 2000 $\mu mol\ m^{-2}\ s^{-1}$) for 30 min. Chlorophyll fluorescence was recorded every 2 min during illumination. After illumination, leaves were dark-adapted for 30 min, and chlorophyll fluorescence was recorded every 2 min for 10 min, then every 5 min for 20 min. Chlorophyll fluorescence was measured by use of a portable chlorophyll fluorometer (PAM-2100, Walz, Germany), and variables were taken under the same conditions as mentioned above.

Calculation and Statistical Analysis

Gross photosynthetic rate (P_g) was calculated as net photosynthetic rate (P_n) + dark respiration rate. P_n is the stable CO_2 exchange rate under each level of PPFD, and dark respiration rate is the CO_2 exchange rate measured in the dark before illumination. The potential quantum efficiency of PSII (F_v/F_m) of dark-adapted leaves was calculated as $(F_m–F_0)/F_m$. For leaves under each level of illumination, the actual PSII efficiency ($\Delta F/F_m$') was calculated as $(F_m'–F)/F_m$'. ETR was calculated as $\Delta F/F_m' \times PPFD \times 0.5 \times \alpha$ (Maxwell and Johnson 2000). We used the mean value of leaf absorption (α) for green leaves of 0.84 (Björkman and Demmig 1987). qE and qI were calculated as (F_{mD2} −

F_m') / F_m' and (F_m − F_{mD30}) / F_m', respectively. F_{mD2} and F_{mD30} is F_m with dark adaptation for 2 and 30 min after illumination, respectively (Bilger and Björkman 1990, Demming-Adams et al. 1996, Maxwell and Johnson 2000, Müller et al. 2001).

Data are mean ± SE for 4 to 6 leaves from 4 plants for each species grown under each light condition. Each leaf was considered one replicate for statistical analyses. Statistical analyses involved the use of Sigma Plot v10.0 (Systat Software, Point Richmond, CA, US).

RESULTS AND DISCUSSION

1. The Light Responses of *P, g_s* and ETR

Similar to other results (e.g. Lambers et al. 1998, Taiz and Zeiger 2006), the P_n of all materials increased with increasing PPFD in a curve-linear fashion (Figure 1 and Wong et al. 2012*a*). The relation of P_n and PPFD for Arc. *somai* could be best fitted by the equation $y = a (1 − e^{−bx})$ and the equation for the others, including 3 ferns and 3 woody species, could be best fitted by $y = ax/(b + x)$ ($y = P_n$, x = PPFD (Wong et al. 2012*a*). Plants adapted or acclimated to high light always had a higher light saturation point and maximal P_n (Figures 1–2 and Wong et al. 2012*a*). For 4 fern species with different light adaptation capabilities, maximal P_n ranged from ~2 (Arc. *somai*, a heavy-shade–adaptable fern) to ~9 (*P. lingus*, a slight-shade–adaptable fern) μmol (CO_2) m$^{−2}$ s$^{−1}$. This range was similar to that observed for 3 fern species of the genus *Blechnum* of contrasting ecological breadth in a forest light gradient (Saldaña et al. 2010). Yet, the highest maximal P_n we obtained reached about 80% of that for fern species with recorded high photosynthetic capacity, ~11 μmol (CO_2) m$^{−2}$ s$^{−1}$ (Durand and Goldstein 2001, Hunt et al. 2002). However, the maximal P_n of *P. lingus* was only about 50% of that of the pioneer tree, *A. formosana*, when acclimated to 100% sunlight; and was near to that of 2 understory shrubs, when they were acclimated to 50% sunlight, despite the fact that there was no significant difference in between P_n acclimated under 100% and 50% sunlight for *P. lingus* (Figure 2). In contrast, the lowest maximal P_n we obtained in the present study, ~2 μmol (CO_2) m$^{−2}$ s$^{−1}$, was similar to that of many fern species (Brodribb et al. 2007, Funk and Amatangelo 2013).

Photosynthesis is limited by both stomatal and non-stomatal factors. The former is associated with decreased leaf *C*i caused by stomata closure and the

latter with decreased photochemical efficiency and CO_2 fixation (Berry and Downton 1982, Brodribb 1996).

Some reports noted that the light-response curve of g_s was close to that of P (Yu et al. 2004, Huang et al. 2007), and late successional species or shade-grown plants showed low P and g_s (Hölscher et al. 2006, Huang et al. 2007).
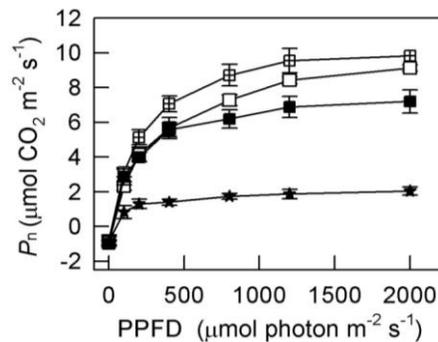

Data are mean ± SE (n = 4–6).

Figure 1. The responses of the net photosynthetic rate (P_n) to photosynthetic photon flux density (PPFD) for *Pyrrosia lingus* (squares, a slight-shade adaptable fern) and *Archangiopteris somai* (stars, a heavy-shade adaptable fern) cultivated under 100% (open symbols), 50% (within the cross symbols) and 10% (closed symbols) sunlight. Variables were measured at 25°C.
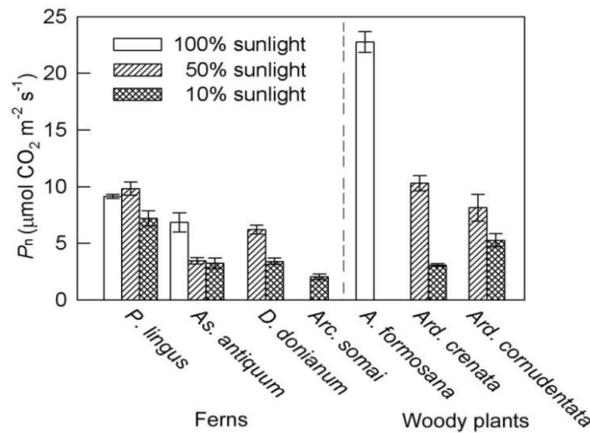
Data are mean ± SE (n = 4–6).

Figure 2. Maximal net photosynthetic rate (P_n) for 4 fern species and 3 woody species cultivated under 100%, 50% and/or 10% sunlight. Variables were measured at 25°C and 0 to 2000 μmol m^{-2} s^{-1} photosynthetic photon flux density.

a   b
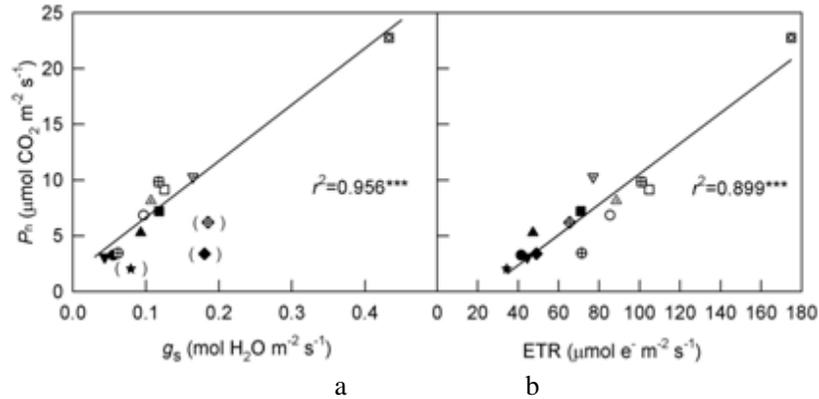
Each datapoint represents the mean of 4–6 leaves. ***: $P < 0.001$ [Panel A excludes data for *D. donianum* (diamonds), *Arc. somai* (star)].

Figure 3. The relationships between net photosynthetic rate (P_n) and stomatal conductance (g_s), and between P_n and electron transport rate (ETR) for *Alnus formosana* (⊡), *Pyrrosia lingus* (squares), *Asplenium antiquum* (circles), *Diplazium donianum* (diamonds), *Archangiopteris somai* (stars), *Ardisia cornudentata* (upward triangles) and *Ardisia crenata* (downward triangles) cultivated under 100% (open symbols), 50% (within the cross symbols) and 10% (closed symbols) sunlight. Variables were measured at 25°C and 2000 μmol m^{-2} s^{-1} photosynthetic photon flux density.

Our materials with high P_n under high PPFD always showed high g_s (Figure 3A), which agree with previous findings.

However, the P_n–g_s relationship may vary by species.

Ferns have a low ability for detecting and responding to an increase in CO_2 concentration, which leads to lower P/g_s ratio than for angiosperms (Brodribb et al. 2005, Haworth et al. 2011). We found the same tendency only for *Arc. somai* and *D. donianum*, heavy- and medium- to heavy-shade–adaptable ferns (Figure 3A). In addition, with PPFD increased from 0 to ~400 μmol m^{-2} s^{-1}, the increased rate of g_s was higher than that of P_n (Wong et al. 2012*a*). Thus, in the well water condition of the present study, g_s might not be a main limiting factor for P_n, when combining all obtained data.

Conversely, at 25°C and 2000 μmol m^{-2} s^{-1} PPFD, ETR was strongly correlated with P_n, when merging data from all species for analysis (Figure 3B). This observation, caused by high light, often leads to absorption of more photons than can be consumed (Stuhlfauth et al. 1990, Valladares et al. 1997).

To avoid the damage caused by excessive absorbed energy, plants often downregulate the efficiency of the photosystem, especially PSII (Demmig-

Adams et al. 1996, Kato et al. 2003, Adams et al. 2004). In addition, we found the ratio of ETR to P_g similar for the 4 fern species at 25°C and from low to high PPFD (Wong et al. 2012a). However, except for CO_2 fixation, electrons from PSII have several energy sinks, such as photorespiration (Peterson 1994), water–water cycle (Asada 1999) and cyclic electron flow within PSII (Miyake and Okamura 2003), as well as nitrogen assimilation (Robinson 1990).

In C_3 plants, both CO_2 fixation and photorespiration are major sinks for electrons from PSII; therefore, the ETR/P_g ratio may increase with increasing temperature because of the increase in photorespiration (Oberhuber and Edwards 1993). We found that the relation of the ETR/P_g ratio to temperature could best be fitted by first- or second-order equations, depending on plant species (Wong et al. 2012a).

These equations contain only the 2 easily obtained dynamic indicators, ETR and leaf temperature, and thus could be used to simulate dynamic variation in a leaf-scale photosynthetic rate under different temperature and PPFD conditions (Wong et al. 2012a).


2. Light Induction of P, g_s and ETR

When dark-adapted leaves were suddenly exposed to light, P_g increased hyperbolically (Figure 4).

However, photosynthetic induction varied by species and light intensity of material growth and measurement. For all tested materials, the time required to reach 90% of maximum P_g ($T90_{Pg}$) was 7.3–23.0 and 5.3–21.1 min when measured at 500 and 2000 µmol m^{-2} s^{-1} PPFD, respectively. The light induction of P_g was faster under 2000 than 500 µmol m^{-2} s^{-1} PPFD, and plants adapted or acclimated to low light tended to require a short time to reach $T90_{Pg}$ (Figure 5A,B).

During photosynthetic light induction, the time-course variation in ETR hyperbolically increases for all materials (Wong et al. 2012b).

P was previously found to be related to ETR in dynamic photosynthetic induction of woody plants (Han et al. 1999, Bai et al. 2008). Our ferns and woody plants showed great differences in light adaptation.

Moreover, materials cultivated under two levels of light intensity showed a broad range of P_g and ETR. Even though CO_2 assimilation may be influenced by stomatal, biochemical and photoinhibitory limitations, P_g was previously found positively correlated with ETR for all materials under the same PPFD and induction time (Wong et al. 2012b).
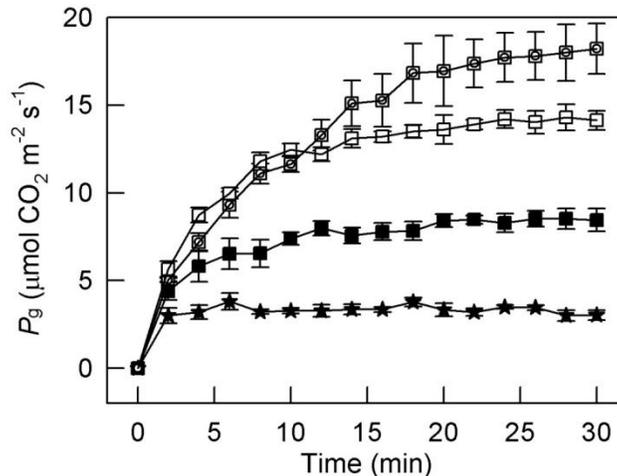
Data are mean ± SE (n = 4–6).

Figure 4. Time course variation in gross photosynthetic rate (P_g) for *Alnus formosana* (⊙), *Pyrrosia lingus* (squares) and *Archangiopteris somai* (stars) cultivated under 100% (open symbols) and 10% (closed symbols) sunlight. Variables were measured at 25°C and 2000 μmol m^{-2} s^{-1} photosynthetic photon flux density.

Yet, light induction of ETR is more rapid than that of CO_2 assimilation (Han et al. 1999, Bai et al. 2008, Wong et al. 2012*b*); thus, the slope of the regression equation between P_g and ETR increases almost with increasing induction time (Wong et al. 2012*b*). In addition to CO_2 fixation, electrons from PSII have several alternative energy sinks (Robinson 1990, Peterson 1994, Asada 1999, Miyake and Okamura 2003). In C_3 plants, both CO_2 fixation and photorespiration are major sinks for electrons from the photosystem and may be influenced by temperature (Oberhuber and Edwards 1993) as well as CO_2 and O_2 concentrations (Krall and Edwards 1990, Cornic and Briantais 1991, Ripley et al. 2007). Thus, our finding of a close relation between P_g and ETR may have been due to (1) measurements made under a constant temperature and a small variation in CO_2 and O_2 concentrations; (2) both CO_2 exchange and ETR, measured simultaneously by an equipment under constant temperature and PPFD conditions, and environmental and physiological factors not differing during measurement; and (3) materials with close proportions in allocating between CO_2 fixation and alternative electronic sinks (Wong et al. 2012*b*). ETR is a readily obtainable dynamic parameter and may be a good indicator for estimating the light induction of the *P* of species across a wide range of light adaptation capability.
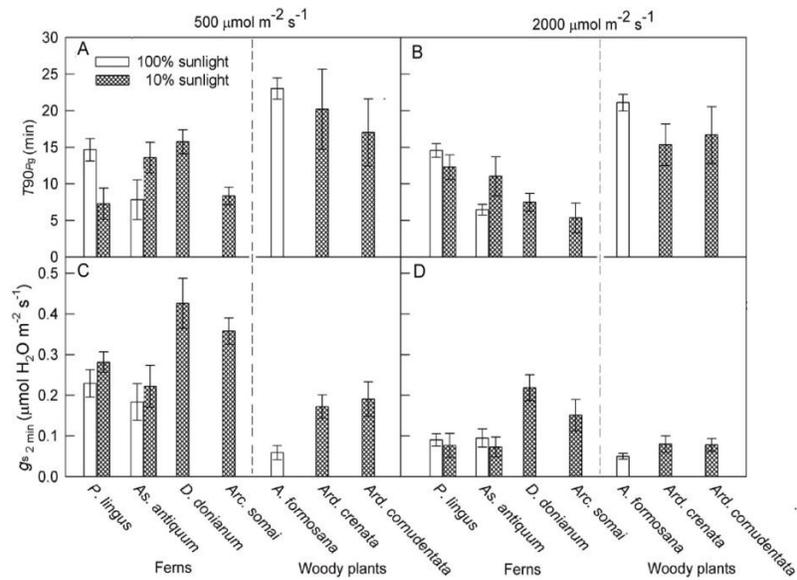
Data are mean ± SE (n = 4–6).

Figure 5. Time required to reach 90% maximal gross photosynthetic rate ($T90_{Pg}$), and stomatal conductance (g_s) obtained at 2 min after the start of light induction for 4 fern species and 3 woody species cultivated under 100% and 10% sunlight. Variables were measured at 25°C and 500 and 2000 μmol m^{-2} s^{-1} photosynthetic photon flux density.

Stomatal conductance is an important limiting factor for photosynthesis. Leaves with low g_s at the initiation of sunflecks tend to require a relatively long time to reach maximal P (Han et al. 1999, Bai et al. 2008).

As well, the response to sunflecks is faster for stomata of some understory plants than those of sun-adapted species (Valladares et al. 1997, Han et al. 1999). We showed that material with high g_s at the initiation of illumination always had short $T90_{Pg}$ than material with low g_s at both 500 and 2000 μmol m^{-2} s^{-1} PPFD (Figure 5 and Wong et al. 2012*b*), which agrees with previous results (Han et al. 1999, Cai et al. 2005, Bai et al. 2008).

As in previous reports (Brodribb et al. 2005, Haworth et al. 2011), we found g_s higher for all 4 of our tested fern species, especially heavy-shade adapted ferns, than the pioneer tree species at the initial stage of light induction, with a short time to reach maximal P (Figure 5).

3. Energy Quenching and Photoinhibition during Light Induction

When leaves were suddenly exposed to 50 to 2000 μmol m^{-2} s^{-1} PPFD, $\Delta F/F_m$' decreased rapidly during the first 2 min of illumination, then increased with increasing illumination time almost to a stable level within ~5 to 20 min of illumination (Figure 6). The transient decrease in $\Delta F/F_m$' at the initial stage of light induction was mainly due to the downregulation of PSII efficiency (Finazzi et al. 2004, Kalituho et al. 2007, Zulfugarov et al. 2007). Because light induction is more rapid for PSII efficiency than CO_2 assimilation (Han et al. 1999, Bai et al. 2008, Wong et al. 2012*b*), the energy may be absorbed more at the early stage of photosynthetic induction. To avoid the damage caused by excess absorbed energy, PSII efficiency was sharply downregulated to a low level, with greater downregulation under higher than lower light because of greater excess absorbed energy (Thiele et al. 1998, Tausz et al. 2005, Bai et al. 2008).

The excess absorbed energy may decrease with increasing photosynthetic rate (Müller et al. 2001, Finazzi et al. 2004, Wong et al. 2014), which led to an increase in $\Delta F/F_m$' with increasing illumination time and P_g (Figure 4) to a stable level (Figure 6).

a   b
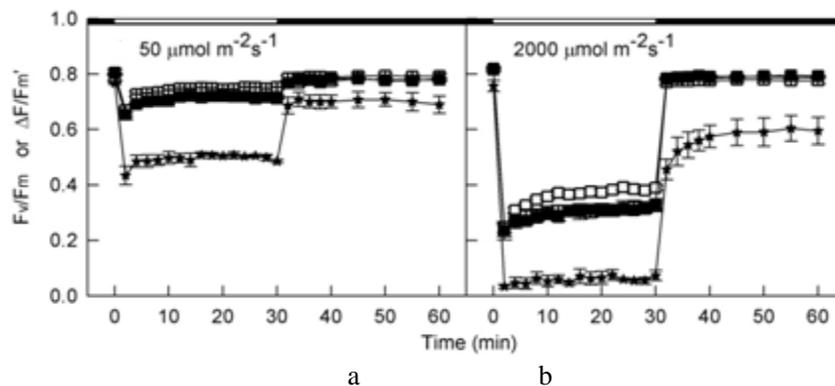
Data are mean ± SE (n= 4). ■: darkness; □: illumination.

Figure 6. Time course of illumination and darkness and variation in photosystem II efficiency (F_v/F_m under darkness and $\Delta F/F_m$' under illumination) for *Pyrrosia lingus* (squares) and *Archangiopteris somai* (stars) cultivated under 100% (open symbols), 50% (within the cross) and 10% (closed symbols) sunlight. Variables were measured under 50 and 2000 μmol m^{-2} s^{-1} photosynthetic photon flux density.

The steady value of $\Delta F/F_m'$ decreased with increasing light intensity and explains why the excess absorption of light energy often leads to decreased PSII function (Demmig-Adams et al. 1996, Kato et al. 2003, Morosinotto et al. 2003).

After the light was turned off, F_v/F_m increased sharply within 2 min, then slowly reached a steady state within 2 to 15 min. When illuminated leaves were dark-adapted for 30 min, the relative value of F_v/F_m ($F_v/F_{mD30}\%$, F_v/F_m value for each treatment before illumination set to 100%) was negatively correlated with PPFD for the 4 species.

Therefore, the degree of photoinhibition increased with increasing light intensity. However, even though the 4 tested fern species had different light-adaptation capability, the descending slope of $F_v/F_{mD30}\%$ with increasing light intensity led to the classification of only 2 groups. This slope was lower for *P. lingus*, *As. antiquum* and *D. donianum* than *Arc. somai* (0.003–0.004 vs. 0.006), so *Arc. somai* was more vulnerable to photoinhibition than the other 3 species at the same PPFD (Figure 7A–D). These species-specific differences in photoinhibition were closely related to the species' energy utilization and quenching with adaptation to different light regimes.
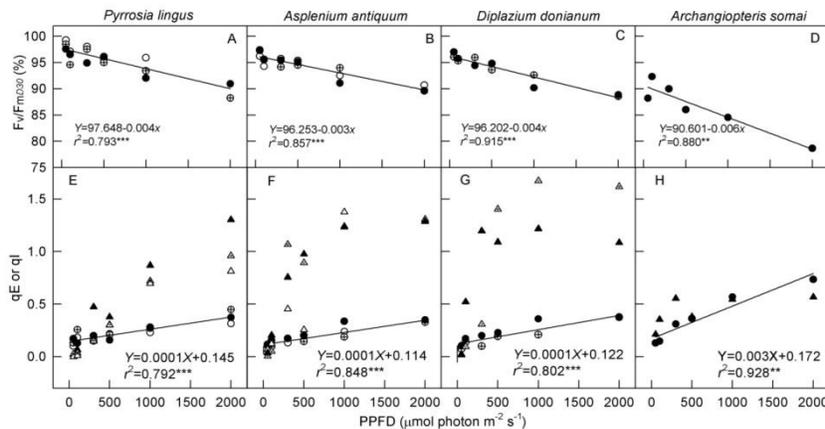
** and ***: $P < 0.01$ and $P < 0.001$.

Figure 7. The relative values of photosystem II efficiency ($F_v/F_{mD30}\%$, F_v/F_m value for each treatment before illumination set to 100%), energy-dependent quenching (qE, triangles) and photoinhibitory quenching (qI, circles) in leaves of 4 fern species under 50, 100, 300, 500, 1000 and 2000 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux density (PPFD) for 30 min. Materials were cultivated under 100% (open symbols), 50% (within the cross) and 10% (closed symbols) sunlight for at least 6 months.

When leaves were suddenly exposed to 50–2000 μmol m^{-2} s^{-1} PPFD for 30 min, the qE with all treatments increased near-linearly (*P. lingus*) or asymptotely (the other 3 fern species) with increasing PPFD (Figure 7E–H). The order for qE was *D. donianum > As. antiquum > P. lingus > Arc. somai* with PPFD > 300 μmol m^{-2} s^{-1}. In addition, qI increased linearly with increasing PPFD for all species, and the increase of the slope was greater for *Arc. somai* than the other 3 species (0.003 vs. 0.001).

The photosynthetic rate of tested species was in the order of *P. lingus > As. antiquum > D. donianum > Arc. Somai* (Figure 2). qE related to xanthophylls-cycle–dependent energy quenching could downregulate the efficiency of the photosystem and represents the major portion of photoprotection, whereas qI is related to photoinhibition and represents the major portion of damage to PSII (Müller et al. 2001, Morosinotto et al. 2003, Kalituho et al. 2007). Thus, *P. lingus,* a slight-shade–adapted fern, could use more sunlight with high photosynthetic capacity and did not need high qE to dissipate the excess light energy. This characteristic was probably related to the species' adaptation to direct sunlight. In contrast, even *As. antiquum*, a slight- to medium-shade–adapted fern, and *D. donianum*, a medium- to heavy-shade–adapted fern, showed lower photosynthesis ability than *P. lingus* but could dissipate more excess light energy via qE. Thus, these 2 species could maintain a similar level of qI and photoinhibition as *P. lingus* under the same level of PPFD probably because of their adaptation to sunflecks. *Arc. somai* showed a low photosynthetic rate and qE but high qI. The species might retain more excess light energy, which leads to greater photoinhibition. This characteristic probably relates to the species' adaptation to heavy shade.

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