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Photochemical efficiency of adult and young leaves of the neotropical understory shrub *Psychotria limonensis* (Rubiaceae) in response to changes in the light environment

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Abstract: We explored the short-term adjustment in photochemical efficiency (F_v/F_m) in adult and young leaves of the understory neotropical shrub *Psychotria limonensis* Krause (Rubiaceae) in response to rapid changes in the light environment. Leaves were collected from 20 individual plants growing under sun and shade conditions on Gigante Peninsula, Barro Colorado Natural Monument (Republic of Panama), during the wet season of 1996. Leaves were distributed in four sequences of light treatments (AB leaves were expanded under sun and were transferred to shade, BA leaves experienced the opposite transfer, and the controls AA and BB leaves that were expanded and maintained under sun or shade conditions). Adult and young leaves did not differ in overall photochemical efficiency. Instead, differences were found among light environments, for which leaves transferred from shade to sun showed the lowest F_v/F_m ratios. There was no relationship between photochemical efficiency and leaf temperature. In *P. limonensis*, understory plants are susceptible of photoinhibition independently of the leaf ontogenetic stage. The approach utilized in this experiment allowed the rapid exploration of this capacity, and could be applied to poorly studied understory species. Rev. Biol. Trop. 52(4): 000-000. Epub 2005 Jun 24.

Key words: chlorophyll fluorescence, photoinhibition, photosynthesis, *Psychotria limonensis*, tropical forest, Panama.

Light conditions of tropical forests are highly heterogeneous across successional environments (Chazdon and Fetcher 1984, Fetcher *et al.* 1994, Chazdon *et al.* 1996) and seasons (Wright 1996, Barone 1998). Individual leaves are exposed to transient and drastic changes in the light environment during development, and must adjust their physiology accordingly (Avalos and Mulkey 1999). Plants must adjust as well to extreme differences in light between wet and dry seasons (Kitajima *et al.* 1997). The degree of adjustment should match the temporal and spatial heterogeneity of the habitat where the species occurs, with pioneer species expected to show a wider range of physiological plasticity rela-

tive to shade-tolerants (Bazzaz and Carlson 1982, Strauss-De Benedetti and Bazzaz 1996). Since basic information on light acclimation through structural and functional adjustment of whole, mature individuals is lacking for most species, metabolic responses are studied at the leaf level assuming that leaves largely regulate whole-plant responses to light changes through the control of shoot and root growth and carbon allocation (Dickson and Isebrands 1991, Field 1991). It is at the leaf level where the most critical selective pressures affecting the whole individual converge. Therefore, leaf structure and function should reflect the selective pressures acting upon the whole plant (Field 1991).

In tropical forests, understory plants are exposed to sudden changes in forest vertical structure associated with gap apertures, or continuous shading due to canopy closure. High light levels often lead to photoinhibition. Photoinhibition consists in the reduction of the maximum quantum yield of photosynthesis and the decrease in the convexity of the photosynthetic light response curve as reflected in the pattern of variable to maximum chlorophyll fluorescence emissions (F_v/F_m ratio, Demmig and Björkman 1987, Long *et al.* 1994). The capacity to respond to such light changes at the leaf level represents a critical adaptive trait that is likely to influence the distribution and diversity of tropical species (Grubb 1977, Denslow 1980, Bazzaz and Carlson 1982, Denslow 1987, Clark and Clark 1987, Fetcher *et al.* 1994). The adjustment capacity depends on leaf age and structure, plant ontogeny, and the characteristics of the light environment where the leaf developed before the light change (i.e., Bauer and Thöni 1988).

Although the mechanism of photoinhibition has been largely investigated under controlled conditions (i.e., Gouallec *et al.* 1990, 1991, Mulkey and Pearcy 1992), there remains to be shown whether it represents a common response of understory plants to increased light levels. The current data suggest little evidence for strong and permanent damage to the photosynthetic apparatus during highlight exposure in field conditions for tropical species (Fernández and Fetcher 1991, Castro *et al.* 1995, Chazdon *et al.* 1996). Although moderate photoinhibition does occur, species differ in their susceptibility to become photoinhibited (Araus and Hogan 1994, Lovelock *et al.* 1994).

In this study we compared the short-term adjustment in photochemical efficiency of adult and young leaves expanded under sun and shade conditions to different light sequences in the neotropical understory shrub *Psychotria limonensis* Krause (Rubiaceae). Since fluorescence emission can be affected by ambient temperature, we also explored the relationship between leaf temperature and photochemical efficiency.

The evergreen shrub *P. limonensis* is abundant in the understory of moist forests, from southern Mexico to northern Colombia (Croft 1978). Variation in photochemical efficiency was assessed as the time-dependent change in the ratio of variable to maximum chlorophyll fluorescence F_v/F_m , where F_v is the difference between the maximum (F_m) and the minimal (F_0) fluorescence emissions. Variation in the F_v/F_m ratio provides a non-intrusive parameter to assess the capacity of photosystem II to use light in photosynthesis (Long *et al.* 1994), and constitutes a good predictor of performance under changing light conditions. Photochemical efficiency (F_v/F_m) has a high value of 0.83 in unstressed leaves (Demmig and Björkman 1987).

The study was done during the first week of August of 1996 (mid-rainy season) on Gigante Peninsula, Barro Colorado Natural Monument, in the Republic of Panama. At this site, *P. limonensis* is mainly associated with shaded environments, such as the understory under deep canopy cover, but it can persist under relatively high light environments, such as gap edges. The F_v/F_m ratio was measured with a portable fluorometer (Modulated Fluorometer OS-500, Opti-Sciences, Tyngsboro MA). Adult and young leaves were collected before sunrise from seven plants established under sun-exposed conditions (middle of a young gap with no canopy influence) and seven plants growing under typical shade conditions (understory under deep canopy cover). Young leaves of similar size were collected from the terminal node of top branches, whereas adult, fully expanded leaves, were collected from the third node at the apex. Leaf petioles were immediately submerged and maintained under water using 250 ml plastic cups. Leaves were arranged in the following sequences of light treatments: AA (leaves collected from sun plants and maintained under sun conditions), AB (leaves collected from sun plants but moved to shade conditions), BA (leaves collected from shade plants but moved to sun conditions), and BB (leaves collected and maintained under shade

conditions). Measurements were taken on five leaves per light sequence. Leaves were dark-acclimated for 5 min by positioning a leaf-clip with an aperture of 0.5 cm in diameter onto the leaf. The aperture was sufficiently large to allow the fiber optic probe to cover the dark-acclimated section. We used a red light pulse to completely saturate all electron acceptors. Measurements were taken every hour from 8 am to 12 m under sunny and warm conditions (27-32°C). Since measurements were done on the same individual leaves through time we used a Repeated Measures ANOVA to correct F values and account for reduced degrees of freedom due to the temporal autocorrelation of the response. Since F_v/F_m ratios were truncated between zero and one, values were arcsine transformed prior to analyses. Leaf temperatures were measured simultaneously relative to F_v/F_m by clipping the OS-500 leaf chamber thermocouple onto the leaf.

We found no differences among young and adult leaves relative to photochemical efficiency (Hotelling-Lawley Trace = 0.006, $P= 0.72$). Young leaves were expected to have lower F_v/F_m due to incomplete leaf expansion and photochemical capacity necessary to utilize most of the incident radiation. The extent of differences in overall F_v/F_m values for young and adult leaves were minimal (Fig. 1). Reduction in F_v/F_m ratio was not severe across sequences, and may reflect increased levels of photoprotection, although young BA leaves showed the lowest and most variable F_v/F_m ratios, whereas leaves in the BB and AB sequences showed the highest. In this case, levels of reduction of electron carriers were low, indicating an increased opportunity to convey all the radiation during saturation pulses. The high F_v/F_m values of leaves in the AB sequence could be due to extra photochemical capacity developed within the former high light environment. Both adult and young leaves followed a similar pattern of temporal variation in F_v/F_m , and significant differences were found only among light sequences (Hotelling-Lawley Trace = 9.84, $P< 0.05$), specially for BA leaves (Fig. 2).

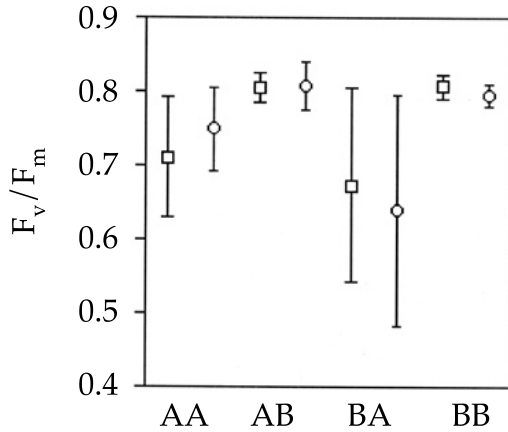


Fig. 1. Overall differences in the F_v/F_m ratio of adults -squares- and young leaves -circles- of *P. limonensis* under different sequences of light environments, Gigante Peninsula, August 1996. Values are means (\pm S.D.) of 20 leaves per sequences and ontogenetic stage.

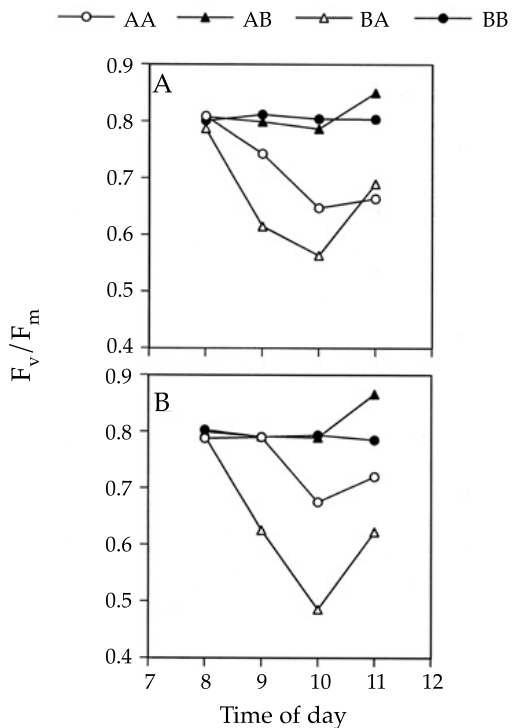


Fig. 2. Temporal variation in F_v/F_m from early morning to noon in adult (A) and young leaves (B) of *P. limonensis* under different sequences of light environments, Gigante Peninsula, August 1996. The symbols in the legend are explained in the text.

Variation in leaf temperature during the morning was minimal for adult (mean = 28.46°C, sd = 1.18) and young leaves (mean = 29.06°C, sd = 1.55). There were no significant differences in temperature among stages (Hotelling-Lawley = 2.56, P = 0.13), or sequences of light treatments (Hotelling-Lawley = 0.56, P = 0.58), although there was a small but significant interaction between sequence and life stage (Hotelling-Lawley = 0.53, P = 0.04) reflecting the effect of time on temperature increase throughout the morning. We could expect an increase in leaf temperature to cause a decrease in F_v/F_m due to the synergistic effect of increasing temperature and photorespiration. In this case, photorespiration might act as a photoprotective mechanism, providing an alternate pathway for electron flow (Osmond 1981). However, the low extent of differences in temperature among treatments, and the magnitude of the temperature itself, made the influence of photorespiration minimal in this case.

The results show that shade leaves of *P. limonensis* are prone to short-term decreases in photochemical efficiency, and are likely to suffer photoinhibition and stress due to high light, independently of their ontogenetic stage. This contrasts with data reported by Krause *et al.* (1995), who found higher photoinhibition in young canopy leaves. In the canopy, leaves are exposed to higher radiation levels for longer periods. Cycles of photoinhibition and recovery may reflect the role of photoinhibition as protective rather than a damaging mechanism (Krause 1988) under these circumstances. However, our data suggest a minimal influence of photoinhibition in canopy leaves, even during the dry season (Avalos and Mulkey 1999, Avalos 1999). In the understory, leaves experience lower light levels and it is likely that leaf expansion occurs mostly under shade conditions. Here, exposure to high light is more likely to lead to long term damage than in the case of canopy leaves, specially if high light exposure is combined with drought (see Lovelock *et al.* 1994). However, *P. limonensis* seems to be well buffered against this condition

due to its deep root system and the ability to maintain low stomatic conductance during the dry season (Wright *et al.* 1992).

This study suggests the utility of collecting and analyzing leaves *in situ* by maintaining their petioles submerged in water, a technique that could be useful to perform rapid surveys of photochemical efficiency in response to rapid changes in light in poorly explored tropical species. A more detailed study, coupled with the measurement of photosynthetic capacity, is needed to fully characterize the response of *P. limonensis* to light changes that may take place during longer periods.

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RESUMEN

Se exploró el ajuste a corto plazo en la eficiencia fotosintética (F_v/F_m) en hojas jóvenes y adultas del matorral del sotobosque neotropical *Psychotria limonensis* Krause (Rubiaceae) en respuesta a cambios rápidos de luz ambiental. Las hojas fueron recolectadas de 20 plantas individuales bajo condiciones de sol y sombra en Península Gigante, Monumento Natural Barro Colorado (Panamá), durante la estación lluviosa de 1996. Las hojas fueron distribuidas en una secuencia cuatro tratamientos de luz (AB las hojas fueron extendidas bajo el sol y fueron transferidas a la sombra, BA las hojas experimentaron la transferencia contraria, y las hojas controles AA y BB que fueron extendidas y mantenidas bajo condiciones de sol o de sombra). Las hojas adultas o jóvenes no difieren en la eficiencia fotoquímica general. Por el contrario, se encontró diferencias entre los ambientes de luz (iluminados), para los cuales las hojas transferidas de la sombra al sol mostraron las menores tasas F_v/F_m . No hubo relación entre la eficiencia fotoquímica y la temperatura de las hojas. En *P. limonensis*, las plantas son susceptibles a la foto

- inhibición independientemente del estado ontogenético de la hoja. El enfoque utilizado en este experimento permitió la rápida exploración de esta capacidad y demostró que puede ser utilizado a otras especies poco estudiadas del sotobosque.

Palabras clave: fluorescencia, clorofila, fotoinhibición, fotosíntesis, *Psychotria limonensis*, bosque tropical, Panama.

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