

Contrasting leaf phenotypes control seasonal variation in water loss in a tropical forest shrub

(*Psychotria marginata*/phenotypic plasticity/moist tropical forest/water-use efficiency)

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ABSTRACT *Psychotria marginata*, a shrub common to humid tropical forests in Central America, produces leaves in two seasonal flushes with contrasting morphology and physiology. Leaf production is bimodal with a major peak at the beginning of the annual wet season and a secondary peak at the end of the wet season. Together these peaks account for 75–87% of annual leaf production. Leaves produced just prior to the dry season have higher specific mass and, during drought, have lower stomatal conductances and higher water-use efficiencies. Plants irrigated during two consecutive dry seasons continued to produce leaves with these morphological and physiological differences, indicating that this feature has been strongly canalized by some factor other than water availability in this highly predictable yet seasonal habitat. The bimodal leaf production results in acclimation to changing conditions through co-occurring leaves that lack the physiological plasticity usually associated with acclimation. Heterophylly of this form suggests that the moderate drought of the dry season has been a significant selective factor for understory plants.

Leaves with differing resistance to drought produced during periods of contrasting water availability are known from desert and mediterranean habitats (1–5). For shrubs in these habitats, leaf size, shape, and reflectivity vary in ways that may improve function during drought. Possibly the most unlikely place to find drought-related heterophylly is in the humid understory of a tropical rain forest. Despite relatively constant temperatures and high annual rainfall, plants in most tropical forests experience predictable and sometimes large variation in water availability (6). However, compared to habitats where drought-resistant leaves are common, water is relatively abundant in soil and air in most tropical forests even during the dry season (7). Here we report on an understory shrub common to Central America that produces leaves with contrasting drought-resistance characteristics in two seasonal flushes. Thus leaf-type variation is a means of whole-plant response to drought in a tropical forest species.

Acclimation to maintain photosynthesis and minimize water loss during drought may be accomplished through physiological changes within existing leaves (8) or through the production of new leaves with different physiological characteristics (1, 9–11). Deciduousness provides an escape from drought stress and, as the severity and duration of the annual dry season increase, the proportion of species exhibiting leaf loss increases (6). However, in regions with intermediate levels of drought, most species retain their leaves throughout the year, raising the question of how these evergreen leaves respond to a fluctuating environment. Studies of plant responses to seasonal drought suggest that individual leaves are

often capable of physiological adjustments to declining soil moisture and atmospheric humidity, resulting in greater photosynthetic carbon gain and lower transpirational water loss than otherwise would be possible (1, 2, 10–14). Understory tropical plants show strong diurnal variation in stomatal conductance during periods of high humidity and abundant soil water (8), suggesting that controls of water loss may be important even when transpirational demand is low.

An alternative response to drought would be to produce different wet-season and dry-season leaf types. Such leaf types would benefit the plant if they permitted continued photosynthesis and reduced transpiration during drought. This possibility has not been addressed for tropical forest plants, and simultaneous data on photosynthesis and transpiration have been unavailable for heterophyllic plants from other habitats. Here we report on *Psychotria marginata* (Rubiaceae), an understory perennial shrub that shows seasonally adaptive variation in leaf type (7) with two leaf production peaks showing contrasting physiological and morphological characteristics in a seasonally dry Neotropical forest. Leaves produced just prior to the dry season showed the ability to minimize transpiration and maintain photosynthetic assimilation during drought, whereas leaves produced during the early rainy season did not adjust to dry season conditions. Drought-resistant leaves studied did not develop in response to drought stress because they were produced during periods of high water availability and maintained drought-resistant characteristics even when grown under irrigation.

MATERIALS AND METHODS

Naturally occurring *P. marginata* plants were studied in four 2.25-ha plots (1 ha = 1×10^4 m²) in the understory of a 500-year-old lowland moist Neotropical forest at Barro Colorado Island, Panama (15, 16). Mean annual rainfall at Barro Colorado Island is 2600 mm, with the dry season extending from mid-December through April (15). Total rainfall for the first 13 weeks of 1988 was 88 mm, and humidity was always >65% (17). Two plots were irrigated for 1.5 h between 1030 and 1400 h for 5 days each week, whereas two plots experienced normal dry season moisture regimes. Irrigation was accomplished with sprinklers arranged in a hexagonal array, each mounted 1.8 m above the ground. During a typical week, each manipulated plot received 675 metric tons of water, which maintained soil water near field capacity. Soil water was measured weekly at 25 cm at eight sites in each plot with a neutron hydroprobe and converted to water potentials by regression analysis using values derived from psychrometers (18). Soil water potentials averaged -0.04 MPa or higher in irrigated plots and fell to as low as -1.00 MPa in control plots during the study period.

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Gas-exchange studies were conducted with an LCA-2 gas analyzer (Analytical Development, Hoddesdon, Kent, U.K.) on leaves of plants growing in sunfleeked shaded understory in control and irrigated plots during February 1988. Leaves were brought to photosynthetic light saturation and steady-state rates of carbon assimilation and water loss were calculated from equations modified for this equipment (19). To gain some measure of diurnal variation (20), gas exchange was measured between 0900 and 1000 h and again between 1400 and 1500 h. Transpiration values were recalculated relative to a standard cuvette humidity (30 mbar; 1 bar = 100 kPa), allowing the expression of water-use efficiency ($WUE = A/E_{std}$, where A is assimilation and E_{std} is standardized evapotranspiration) in units that are comparable to published values. Leaf temperatures and leaf-to-air humidity deficits were the same for each leaf category but differed between treatments. To determine whether leaf-type differences persisted into the next dry season, we took repeated measures of stomatal conductance with a diffusion porometer (20) (nulled to 65% relative humidity; Li-Cor, Lincoln, NB) throughout the day for 6 days in February 1989 using leaves produced during 1987. Each plant was measured in ambient low light in shaded understory on the abaxial (bottom) leaf surface, which is the only surface containing stomata in this species. There was no pattern of leaf age with respect to treatment for either gas exchange or porometry, and all leaves were mature and nonsenescent at the time of measurement. Analysis of gas-exchange and porometry data was accomplished with repeated-measures analysis of variance (21). Stomatal density was determined from cellulose acetate impressions of the bottom leaf surface. Areas of newly collected leaves were measured and mass was determined after drying at 60°C for at least 72 h.

Biophysical measurements were made on *P. marginata* leaves from 12 to 18 January 1989. Measurements of leaf temperature, air temperature, wind speed, and relative humidity were taken on different days for different plants in irrigated and control plots, using for each plant one pair of previously marked wet-season and dry-season leaves. Leaf temperature was measured using copper-constantan thermocouples (probe diameter, 0.2 mm) in conjunction with a digital thermometer (model 450, Omega, Stamford, CT). Three thermocouples were used per leaf, and a mean leaf temperature was determined; no consistent variation in temperature with position was observed. Ambient air temperature and relative humidity were determined using a digital

hygrometer/thermometer (model RH21, Omega). Measurements of ambient air speed were made with an omnidirectional anemometer (model 1650, TSI, St. Paul, MN) positioned 5 cm from the leaf pairs. Biophysical measurements were averaged over 5-min intervals from 0915 to 1445 h. Convective heat flux was estimated using standard equations (22). Data were pooled by leaf type and treatment and a two-factor analysis of variance was used to evaluate leaf type and treatment differences in leaf temperature differential ($T_{leaf} - T_{ambient}$) and convective heat flux.

RESULTS

In Central Panama, *P. marginata* produces leaves throughout the year, but with two major peaks, one beginning in May during the early wet season (referred to here as wet-season leaves) and the other beginning in December just prior to and during the initial weeks of the dry season (dry-season leaves; Fig. 1). Wet-season leaves studied were produced during the early wet season of 1987, and dry-season leaves were produced during December 1987 and early January 1988. Leaves generally persist on the plant for at least 2 years. From 100 censuses, we found that leaves produced during the early wet season accounted for 43.0–67.5% of annual leaf production, while leaves produced during the late wet season accounted for 19.5–32.0% of annual leaf production from January 1985 through December 1988 (7).

Dry-season leaves had lower transpiration and higher water-use efficiency than leaves produced during the early wet season. This difference occurred under both irrigated and control conditions (Fig. 2 and Table 1). Assimilation (A_{max}) at light saturation did not differ significantly between treatment or between leaf types, whereas afternoon values were always lower than morning values ($F = 45.77$; $df = 1, 17$, $P < 0.0001$). Evapotranspiration measured concurrently and standardized to a leaf chamber humidity of 30 mbar (E_{std}) showed a strong effect of leaf type and weak effect of treatment (Table 1), which is significant given the a priori prediction of lower transpiration in the control treatment. Afternoon values of E_{std} tended to be lower than morning values ($F = 3.67$; $df = 1, 17$; $P < 0.08$). Atmospheric humidity was markedly lower and leaf temperatures were 0.8–0.9°C higher in control sites at 1200 h. Calculated values of water-use efficiency show a strong effect only for leaf type and there was no treatment-induced leaf type effect (Table 1). The ratio of mesophyll to

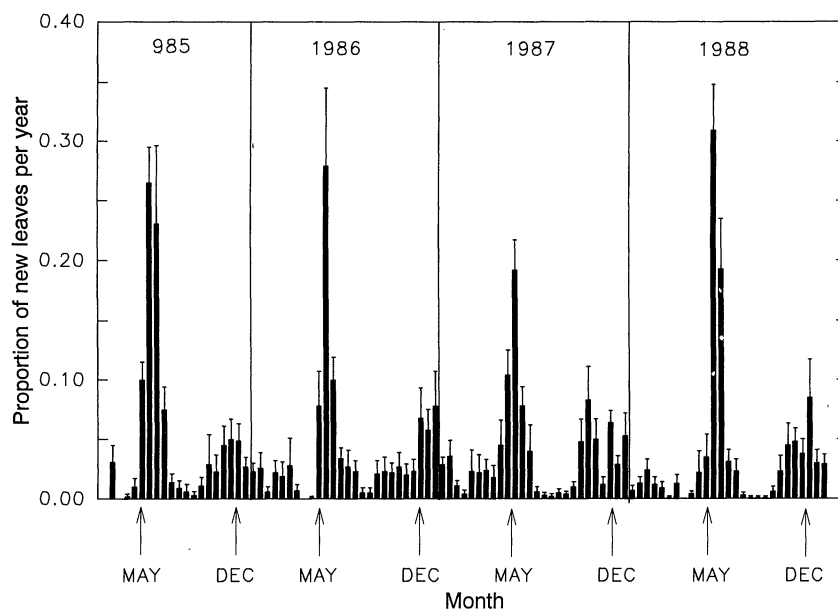


FIG. 1. Yearly production of new *P. marginata* leaves plotted against census period from 1985 through 1989. Censuses were conducted every 2–3 weeks. Values are the proportion of the leaf production in a given year (mean \pm SEM). The highest period of productivity for each year coincides with the months of May and June at the beginning of each wet season, and a secondary peak occurs at the end of the rainy season in December and January.

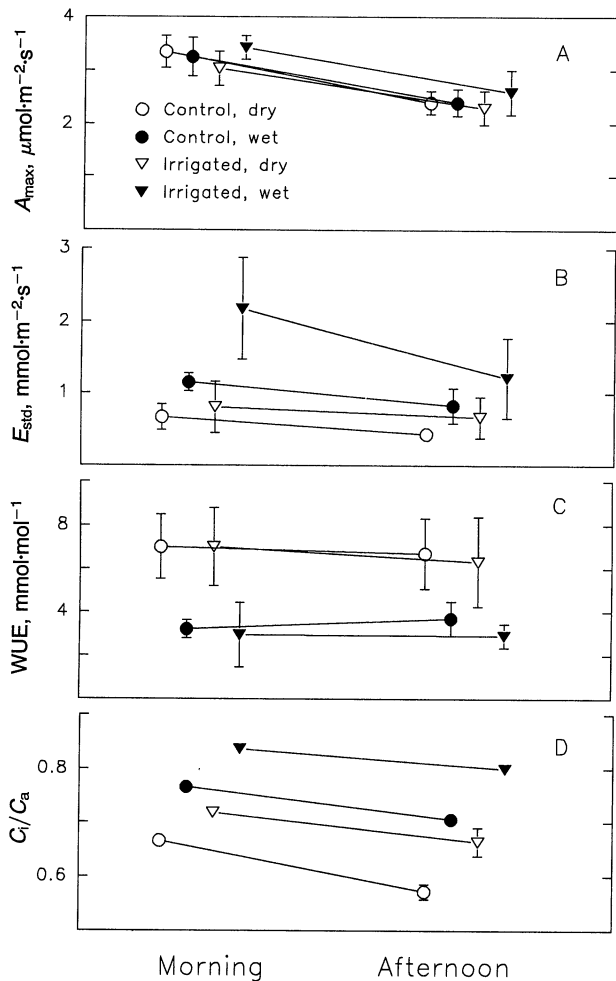


FIG. 2. (A) Profile diagram of assimilation (A_{max} , mean \pm SEM), (B) Evapotranspiration standardized to 30 mbar (E_{std}). (C) Water-use efficiency (WUE). (D) C_i/C_a at light saturation in the morning and afternoon in control and irrigated *P. marginata* during the 1988 dry season. Wet, wet season; dry, dry season. Statistics are given in text and Table 1. The absence of error bars indicates the error is smaller than the symbol.

atmospheric CO_2 (C_i/C_a) was consistently higher in wet-season leaves irrespective of treatment (Fig. 2 and Table 1).

Repeated measures analysis of stomatal conductance to water vapor (g_{wv}) measured under diffuse low-light conditions (Fig. 3) showed that stomatal control of water loss in 1987 dry-season leaves persisted until the 1989 dry season. During morning hours, wet-season leaves had significantly higher rates of conductance in both treatments and this difference was maintained throughout the day ($F = 4.83$; $df = 1,70$; $P < 0.05$). Although an effect of treatment was apparent later in the day ($F = 12.2$; $df = 1,70$; $P < 0.01$), there was no treatment-induced leaf type effect ($P > 0.25$).

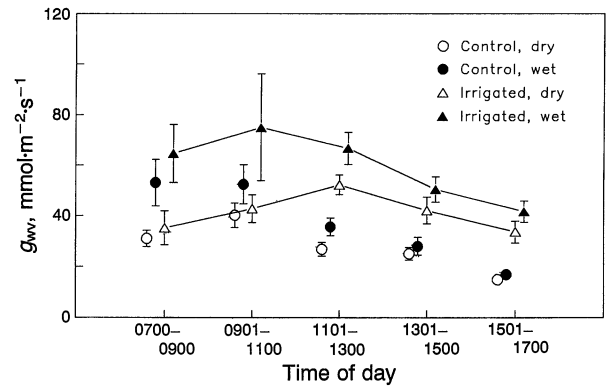


FIG. 3. Profile diagram of stomatal conductance in irrigated and control *P. marginata* plants measured in the 1989 dry season (mean \pm SEM). Leaves are of the same 1987 cohorts as those in Fig. 2. Symbols are as in Fig. 2. Statistics are given in text. The absence of error bars indicate the error is smaller than the symbol. The time of day (eastern standard time) is shown.

Dry-season leaves of *P. marginata* had more dry mass per unit surface area than did wet-season leaves ($F = 7.84$; $P < 0.01$; sample sizes in Table 2), but there was no treatment-induced leaf type effect ($F = 0.39$; $P < 0.6$). There were no differences in stomatal density (Table 2), leaf length, width, or total area with respect to leaf type.

Compared to control plots, irrigated plots experienced slightly lower ambient wind speeds (a reduction of $0.04 \text{ m}\cdot\text{s}^{-1}$; $F = 7.3$; $P < 0.01$), were significantly cooler (0.2°C ; $F = 34.4$; $P < 0.001$), and were significantly more humid (4% relative humidity; $F = 88.6$; $P < 0.001$). Consistent with the direct measurements of leaf conductance, wet-season leaves displayed significantly more negative leaf temperature differentials ($F = 65.6$; $P < 0.001$) and greater leaf-to-air convective heat flux ($F = 65.6$; $P < 0.001$) compared to dry season leaves (Table 2). Leaf temperature differential was frequently positive in control plots and typically negative in irrigated treatments ($F = 10.6$; $P < 0.001$), supporting the suggestion (above) of higher transpiration in irrigated plots. A treatment-induced leaf type effect was only weakly apparent ($P < 0.06$).

DISCUSSION

Dry-season leaves clearly control water loss, while maintaining assimilation under conditions of drought (Figs. 2 and 3 and Table 1). Although stomatal closure produced higher temperatures in dry-season leaves (Table 2), within each treatment these leaves maintain lower transpiration for a given leaf-to-air humidity difference (Fig. 2). High water-use efficiency is usually correlated with leaf specific mass because of greater surface area of mesophyll per leaf surface (23). In such leaves assimilation may not be greatly affected even when stomata are partially closed (as in dry-season leaves, Fig. 2). Thus we expect higher water-use efficiency in

Table 1. Repeated measures of analysis of variance for *P. marginata* gas exchange data

Parameter	Error SS	Source of variation								
		Treatment			Leaf type			Treatment \times leaf type		
		SS	F	P	SS	F	P	SS	F	P
Assimilation	1.8656	0.0004	0.00	<0.95	0.0333	0.30	<0.59	0.0511	0.47	<0.50
Evapotranspiration	10.057	2.2309	3.77	<0.07	4.6222	7.81	<0.02	0.8009	1.35	<0.27
Water-use efficiency	12.5881	0.2829	0.39	<0.55	5.5881	7.64	<0.02	0.0125	0.02	<0.90
C_i/C_a	0.29222	0.0824	4.79	<0.05	0.1868	10.87	<0.005	0.0012	0.07	<0.80

Only between-subjects effects are shown; within-subjects effects are discussed in the text. Assimilation and evapotranspiration values were \log_e -transformed, and water-use efficiency and C_i/C_a were transformed by the square root and arcsin. $df = 1,17$. Evapotranspiration is calculated relative to a standard humidity of 30 mbar. SS, Sum of squares; F, Fisher statistic.

Table 2. Abaxial stomatal density, leaf specific mass, and biophysical measures of *P. marginata* leaf phenotypes taken from irrigated and control plants

Parameter	Control		Irrigated	
	Wet	Dry	Wet	Dry
Stomatal density, mm ⁻²	88.0 ± 6.2 (6)	91.4 ± 12.5 (3)	91.0 ± 3.1 (7)	89.7 ± 2.3 (6)
Specific mass, g/m ² *	54.4 ± 1.7 (6)	59.4 ± 2.4 (5)	49.9 ± 2.1 (7)	57.0 ± 2.5 (6)
Temperature differential, °C*	-0.17 ± 0.04 (59)	0.06 ± 0.05 (59)	-0.44 ± 0.05 (59)	-0.38 ± 0.04 (55)
Convective heat flux, W·m ² *	-4.4 ± 1.2 (59)	-2.2 ± 1.1 (59)	-15.0 ± 1.5 (59)	-12.6 ± 1.5 (55)

Values are the mean ± SEM. Sample sizes are indicated in parentheses. An asterisk indicates a significant leaf-type effect ($P < 0.05$). Statistics are given in text.

dry-season leaves that have high specific mass but no other apparent morphological differences. High specific mass could also be due to a heavy layer of cutin that would tend to reduce cuticular water loss, but this would not function to maintain assimilation. If some water remains in the soil as drought progresses, then the presence of dry-season leaves may be crucial for continued carbon gain and survival until the beginning of the rains. This may be especially true during years when the dry season is extended because of the southern oscillation ("El Niño") (17). In addition, water-use-efficient gas exchange may help maintain higher xylem water potentials and thus contribute to maturation of fleshy fruits, which occurs during the beginning of the dry season.

Both leaf types are present at the same time on *P. marginata* and each type may serve to improve plant performance at different times of the year. Although less apparent, the advantage of producing a wet-season leaf phenotype is likely a function of their ability to have higher stomatal conductance during periods when water is abundant (Fig. 3 and Table 2). During the dry season, these leaves would lose more water than dry-season leaves, but they would have a 2-fold advantage in the shaded understory during the rainy season due to higher mesophyll CO₂ concentrations. Higher C_i/C_a (Fig. 2) helps to offset photorespiration in C₃ plants, and thus wet-season leaves would maintain higher quantum yield in low light (24). Higher mesophyll CO₂ permits greater carbon uptake during brief periods of direct high light (sunflecks) that typically occur in the shaded understory (25).

Acclimation responses are usually quantitatively related to the degree of environmental change (25, 26), whereas true polymorphisms occur as contrasting alternative forms (27). The seasonal changes in leaf phenotypes reported here occur several times during the lifespan of a given individual plant, and they continue to occur even when the associated environmental variation has been removed. These leaf types may not represent a polymorphism because a few leaves are produced at all times of the year and these leaves may possess intermediate characteristics. Nevertheless, this heterophyllic habit is remarkable in that it assists in acclimation to seasonal variation in water availability but individual leaves do not exhibit physiological plasticity normally associated with acclimation. The seasonal leaf types of this species are not a developmental response governed by water availability at the time of leaf flush because (i) the dry-season leaves are produced largely prior to the dry season, (ii) the two leaf types are produced even under long-term irrigation, and (iii) the treatment-induced leaf type effect was weak or absent for morphological and physiological characteristics. The fact that the phenotypic differences persisted in leaves produced in 1987 for two dry seasons makes it unlikely that the effect observed in 1988 was due to dry-season leaves being younger than wet-season leaves.

Heterophyly in *P. marginata* suggests that water availability has been an important selective factor even in a moist humid tropical forest where dry season conditions in the understory are quite moderate (7). Leaf production November through January may be under strong genetic control such that

leaf characteristics respond weakly or not at all to conditions at the time of development. Alternatively, contrasting phenotypes may be cued by an environment that consistently induces or selects for specific character states under specific conditions (26). Wright (7) has shown that some *Psychotria* species slowly lose synchrony of leaf flush when under irrigation for several years. Thus the mechanism of seasonal synchrony is subject to environmental influence, but the nature of the cue is as yet unknown. The lack of a rapid facultative response to irrigation in *P. marginata* in this study indicates that the annual dry season has been an ecologically important and predictable environmental factor, canalizing the production of these leaf types over ecological time or selecting for plants with this ability over evolutionary time.

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