SHADE PROMOTES THORN DEVELOPMENT IN A TROPICAL LIANA, *ARTABOTRYS HEXAPETALUS* (ANNONACEAE)

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The liana *Artabotrys hexapetalus* (L.f.) Bhand., which is widely planted in the Tropics and native to African rain forests, produced new reiterations (new leader shoots) normally and after damage induced by Hurricane Andrew (August 24, 1992). In each new orthotropic shoot, there is a gradient in lateral branch structures from basal thorns, to vegetative leafy branches, to distal leafy flowering branches. We noted that reiterations developing in shade had more thorns than similar reiterations developing in full sun. Tents with clear (66% photosynthetically active radiation [PAR]) and shaded plastic film (12%–14% PAR) were placed over nodes when the axillary buds began to expand to produce reiteration shoots. After 2 mo of growth inside the tents and in the open, the types of lateral outgrowths (thorn vs. branch) were recorded. Shoots in spectrally neutral shade (red to far red of full sun) and spectrally altered shade (red to far red of canopy shade) produced significantly more thorns at the lower nodes of the shoots as compared to those in full sun. Shoots in control clear plastic tents were the same as those in full sun. We conclude that the fate of lateral bud development is controlled by irradiance (light level) but not by light quality. Increased thorn production in shade could be advantageous to plants growing in the deep shade of rain forests. Thorns in the self-shaded regions of the plant, and well below the forest canopy, could aid in protection from herbivory and in climbing by acting as hooks.

Keywords: architecture, *Artabotrys*, branch development, liana, thorn, red to far-red ratio, shade effects.

Introduction

Plants undergo morphological changes as they grow from seedling to adult. In most plants, the changes are subtle, involving internode lengths and leaf shape. In understory woody plants and particularly lianas (woody climbers), the morphological changes are striking, involving dramatic changes in leaf anatomy, morphology and size, internode length, and phyllotaxis (Lee and Richards 1991). Such change in morphology with development has been termed "heteroblasty" (Goebel 1900) or, rather, changes in leaf form along a plant axis from the juvenile plant. Heteroblastic changes in development are particularly marked in root climbers, where different lateral branches are produced, as in *Hedera helix* (Wareing and Frydman 1976). Changes in leaf form are correlated with shifts in microclimate, particularly irradiance, as lianas grow into the forest canopy. Heteroblastic development is presumably under genetic control but probably modified by environmental signals, including gravity and irradiance. Such influences have been demonstrated in *Ipomoea* (Njoku 1956; Peñalosa 1983), but these putative environmental controls need more research. Here, we report on the influence of irradiance and spectral quality on lateral shoot development in *Artabotrys hexapetalus* (L.f.) Bhand.

Artabotrys (in the Annonaceae) is a genus of 100 or more species of lianas that grow in the Old World Tropics (Mabberley 1997). The lianas climb by means of thorns at the base of the shoot and by hooks borne on distal lateral branches (fig. 1). The hooks form in series and can become swollen and woody after becoming attached to an object. In some woody plants, suckers or water sprouts (basal reiterations) may display juvenile morphology, e.g., branch thorns in *Citrus* (Spiegel-Roy and Goldschmidt 1996). In *Artabotrys*, the opposite is true. Juvenile shoots (seedlings) initially lack thorns. However, the basal regions of new shoots (reiterations) produce thorns, while higher regions produce flowering foliage branches.

In saplings and older plants, two to six serial buds occur on the stem directly above the leaf insertion (Posluszny and Fisher 2000). The uppermost one or two buds are the largest and produce one or two branches if they develop without a resting stage. Such immediate bud outgrowth is called sylleptic branching (Hallé et al. 1978). The lower buds remain and always form orthotropic shoots if they develop after a period of rest. Such outgrowth from resting renewal buds is called proleptic branching (Hallé et al. 1978). Without fail, the proleptic buds on leader shoots produce only vertical shoots (reiterations or new leader shoots), and sylleptic buds produce either horizontal leafy branches or horizontal thorns. Thus, new orthotropic (vertical) axes or leader shoots develop either from proleptic buds in distal nodes of the leader after its terminal bud is damaged or stops growing or from proleptic buds in proximal nodes of older shoots or leaders.

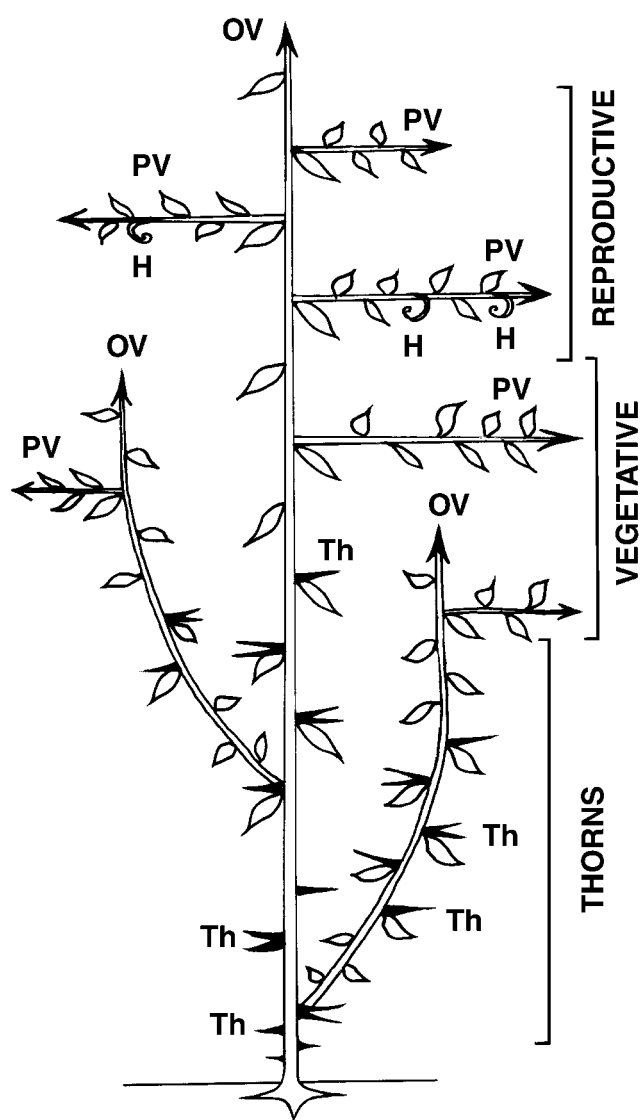[1] Author for correspondence; e-mail jfisher@fairchildgarden.org.

Fig. 1 Diagram of the architecture of a mature plant of *Artabotrys hexapetalus* with three main architectural regions in the main orthotropic axis and two reiterations. Serial axillary buds can grow out as thorns, vegetative plagiotropic branches, and reproductive plagiotropic branches with hooks. H = hook; OV = orthotropic leader; PV = plagiotropic branch; Th = thorn (from Posluszny and Fisher 2000).

Each orthotropic leader, whether it is the original leader or a reiteration, has a similar structure and gradient in morphology from the proximal to distal nodes (fig. 1). The lowest few nodes lack axillary appendages and have only buds. A series of nodes usually has two thorns of slightly unequal size on either side of the serial bud complex (fig. 2A) or a single thorn (fig. 2B). Thorns often bear one or two foliage leaves and one or more scale leaves, with the larger thorns having more appendages. At higher nodes, the thorns take on a transitional form with a swollen thornlike base, continued terminal meristem activity, and production of more foliage leaves with the eventual abortion of the thorn apex. Initially, two and then one branch (fig. 2C) develops at each node higher on the leader.

Lateral vegetative branches tend to grow without hooks at more proximal nodes and when growing in shade. More distal branches and those in full sunlight form hooks. They produce an inflorescence within two to 10 branch nodes from the orthotropic stem. This inflorescence bears one to several flowers and bracts and becomes recurved as a hook, terminating the growth of the branch unit (Posluszny and Fisher 2000).

In older horizontal (plagiotropic) branches, especially those in full sun, one of the several axillary buds is released to form second-order branches in the horizontal plane. The overall architecture of a large plant is based on repeated reiteration of the sapling architectural model with release of proleptic buds to form new orthotropic leaders. All leaders produce plagiotropic branches and thorns. Cultivated plants grow up into neighboring tree crowns to a height of 10–15 m.

Two established plants were severely damaged by winds and the falling of supporting tree limbs caused by Hurricane Andrew (August 24, 1992). After 6 mo, there was much new growth. New orthotropic shoots arose from lateral buds on the nodes of old leaders, old lateral branches, or old thorns near the base of the plant. In some plants, leaders growing in unshaded sites produced only plagiotropic vegetative branches and few, if any, thorns. Such full-sun-lit reiterations showed seedling morphology with juvenile features, such as lack of both thorns and inflorescence hooks. Thorns were more common at the base of those leaders growing in dense shade. To document these observations and to test the hypothesis of light control of thorn development, we devised shade experiments using the mature cultivated plants.

Material and Methods

Material

Two established plants of *Artabotrys hexapetalus* (L.f.) Bhand. growing for more than 25 yr at Fairchild Tropical Garden, Miami, Florida, were examined. This species (syn. *Artabotrys odoratus* R. Br.; *Artabotrys uncinatus* [Lam.] Merrill) is known as "climbing ylang-ylang" (Liberty Hyde Bailey Hortorium 1976). New natural basal sprouts, or water shoots, and those induced by decapitated old stems were observed.

Experimental Design

Wire frames were constructed at the bases of old stems whose damaged crowns were cut off. Their axillary buds were swollen, indicating that they were released from apical dominance and were about to grow out as reiterations. Each frame enclosed one or more old nodes with one or more swollen buds. The frames formed cylinders that were 50–70 × 22–45 cm in height and diameter, respectively. A bag of clear plastic or shade film was placed over and taped to the frame to form a rigid tent around and above the bud. The top was constructed with vents that permitted air to circulate into the open bottom and out through the top but blocked direct rays of sunlight. This venting allowed the shade and clear tents to have only slightly elevated internal temperatures (1°–3°C) compared to ambient temperature outside. New shoots were collected and scored after 2 mo. The experiment was repeated by setting up tents twice, in April–May and August–September, 1993.

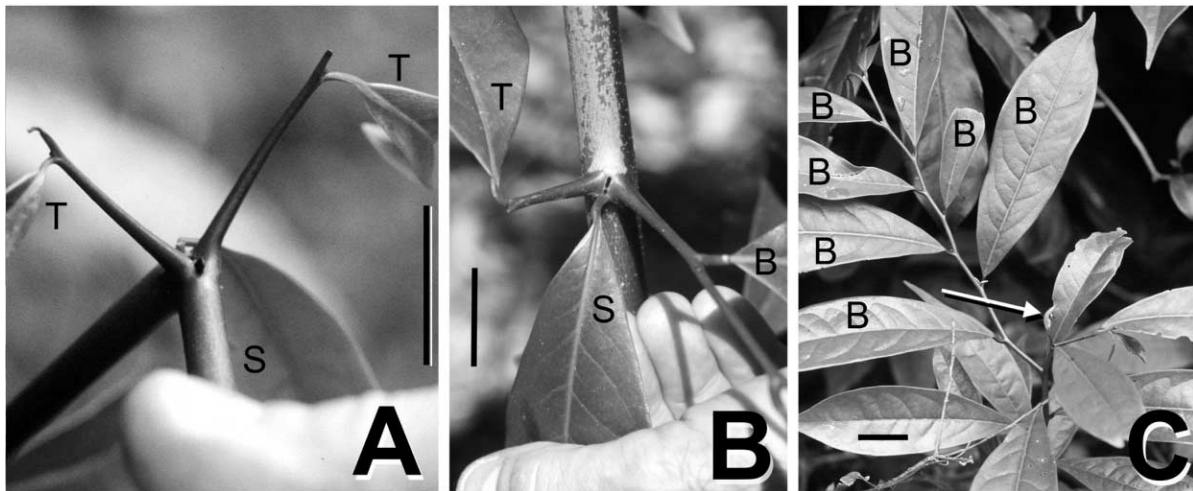For the clear plastic control treatment, we used clear 3-mil

Fig. 2 *Artabotrys* thorns and foliage branches. *A*, Node with two thorns on either side of serial axillary bud complex viewed from above the node; the main leader axis was bent so that the subtending leaf is behind the thorns. *B*, Node with one thorn and one foliage branch viewed from the side with subtending leaf below serial axillary bud complex. *C*, Node with one foliage branch viewed from above with subtending leaf not visible; arrow denotes apex of leader shoot. *B* = branch leaf on foliage branch; *S* = subtending leaf of node; *T* = thorn leaf on thorn. Scale bar = 2 cm.

polyvinyl construction film. For the neutral-shade and reduced red to far-red ratio (R : FR) shade treatments, we used two films manufactured by 3M Corporation, St. Paul, Minnesota: a metal-coated film that reduced photon flux density (PFD) but was spectrally neutral and did not alter the R : FR of sunlight, RE20, and a dye-impregnated film that reduced PFD and R : FR, NR20 (Lee et al. 1996). We documented the temperature and radiation environments in all treatments near solar maximum on two sunny days in April (table 1). We measured temperatures ($N = 6$ for each treatment) with a thermistor probe on a model 43TA telethermometer (YSI, Yellow Springs, Ohio). We measured irradiance ($N = 10$ for each treatment) as photosynthetic photon flux density (400–700 nm in μmol m^{-2} s^{-1}) and spectral quality as the quantum ratio of R : FR (Smith 1995). These values were calculated from 400–800-nm scans with a Li-1800 spectroradiometer with fiber-optic probe and remote cosine-corrected sensor (Li-Cor Instruments, Lincoln, Nebr.).

As the new reiterations grew, care was taken to adjust the tent to prevent restriction of shoot growth. The enclosed shoots were harvested when they filled the tent. We recorded the morphological nature of their lateral organs starting from the basal node position of the reiteration shoot.

Data Analysis

All shoots within one treatment were pooled. Nodes were counted successively with the basal (first) node equal to 1. At each node position, we determined the total number of branches and thorns and the number with mixed branches and thorns, aborted leafy branches, and no axillary structures. A few outgrowths that were too immature or intermediate between branch and thorn were not scored, i.e., they were counted as no structure. Our null hypothesis was that the tented treatments would be the same as the full-sun control with respect to the production of thorns. To test this hypoth-

esis, we compared the control with each treatment separately and at each node position as a contingency table using the *G*-test of independence, model 1 (Sokal and Rohlf 1981*a*). Only the number of thorns versus the number of branches were used in two-way tables. The χ^2 value was used at 1 df. To account for the multiple comparisons at each node (three treatments compared to control), we also applied a more conservative level of significance that was three times that of the usual χ^2 value. When this Bonferroni correction or "unplanned comparisons" described by Sokal and Rohlf (1981*a*, 1981*b*, table 15) was different from the usual χ^2 test, the result was noted within brackets in table 2.

Results

The vigor of reiteration shoots, from the one to several nodes enclosed in the experimental tent, was variable. Some elongated rapidly while others grew slowly or stopped growth as if they were under apical dominance. Consequently, when the space was filled with growth and the plastic tent was removed, the several enclosed reiterations had variable numbers of nodes. The most vigorous shoots had eight or more nodes with mature leaves and lateral bud outgrowths. Smaller reiterations had few matured nodes. When the experiment was repeated (experiment 2), the tents were larger; therefore, more elongation was possible, and more mature nodes were measured.

In both experiments, the first node always lacked bud outgrowth, and the second node rarely had an outgrowth of either thorn or branch. There were no significant differences among the three treatments and the control at nodes 1 and 2. Therefore, results are only indicated for nodes 3–7 (experiment 1) and 3–8 (experiment 2), for which the distalmost nodes had enough replicates (node position 7 had six or more measurable nodes in experiment 1, and node position 8 had 19 or more measurable nodes in experiment 2). A typical sequence of ax-

Table 1

Light and Temperature of Treatments

Treatment description	PFD radiation (μmol m^{-2} s^{-1})	Quality (R : FR ratio)	Temperature (°C)	No. replicates in two experiments	
				Tents	Shoots
Full-sun control (no tent)	1558 ± 15 (100)	1.22	29.6 ± 0.4	7, 3[a]	15, 20
Clear plastic (tented)	1024 ± 67 (66)	1.22	32.1 ± 0.4	3, 2	20, 20
Neutral shade (tented)	190 ± 18 (12)	1.22	32.4 ± 0.5	2, 2	30, 25
Altered shade (tented)	220 ± 27 (14)	0.24	32.5 ± 0.5	2, 2	22, 22

Note.   Midday readings on a sunny day were taken at level of shoots. Summaries are given as means ± SEs. Values in parentheses are percentages of full-sun photon flux density (PFD).

[a] Nodes since tents were not constructed for these controls.

illary structures on a reiterated shoot in full sun (control without tent) and in neutral shade (neutral-shade tent) in experiment 2 is shown in table 3.

As an example of the pooled data, the node position 5 of 14 shoots growing in full sun (experiment 1) had the following distribution of axillary structures: 20 branches and no thorns. For node 5 of 20 shoots growing in altered shade, there were 17 branches and 10 thorns. Ten thorns were observed, but zero thorns were expected. Thus, there was a significant difference in the frequency of thorns between the full-sun and altered-shade treatments at node 5 in experiment 1 (table 2).

There were significantly more thorns produced in neutral and altered shade in experiments 1 and 2 variously at node positions 3–6 (table 2). For both treatments, the G-test of independence between neutral and altered-shade treatments was not significant at all nodes. This indicates that the two shade treatments do not differ from one another in thorn number, although they each differed from the full-sun control in thorn number at nodes 4 and 6 (table 2).

The few values in brackets in table 2 indicate those comparisons in which conservative χ^2 values gave a different outcome from the more usual values used for single comparisons. Nevertheless, the null hypothesis that thorn number in shade equals that in full sun is rejected, at least for node positions 3–6.

The total number of bud outgrowths in both experiments were not significantly different at all node positions. Therefore, the treatments did not affect the number of axillary buds released. Only the type of outgrowth (thorn vs. branch) was affected by irradiance.

Discussion

Thorns and leafy branches arise from equivalent buds of the leader shoot. The distal one or two buds of the series of axillary buds in *Artabotrys* were the largest and grew out first, similar to the behavior of serial buds of *Bougainvillea* (Hackett and Sachs 1968), some Rhamnaceae (Tourn et al. 1989; Tortosa et al. 1996), and other species with serial buds (Troll 1937). In *Artabotrys*, these nonresting, sylleptic buds developed into either thorns or leafy branches. Thorn buds varied in the number of leaves produced before the apical meristem shrank in size and finally matured (Posluzsny and Fisher 2000). Leaf number and size on thorns varied similarly in *Ulex* (Bieniek and Millington 1967). They found that the apical meristem of a vegetative axillary bud was converted into a thorn by cessation of both meristem activity and leaf initiation, as was described for the apices of branched thorns of *Gleditsia* (Blaser 1956).

What factors control the divergent development of these buds? Thorns were most frequent at the base of an orthotropic leader shoot with a transition to foliage branches at higher nodes. Intermediate elongated thorns with foliage leaves occurred at nodes between the extremes of short thorns and long foliage

Table 2

Differences in Thorn Production (Number per Node Position) between Full-Sun Control and Treated Shoots of *Artabotrys*

Treatment	Node 3	Node 4	Node 5	Node 6	Node 7	Node 8
Experiment 1:						
Clear plastic	NS	NS	NS	NS	NS	...
Neutral shade	NS	NS	*	**	NS	...
Altered shade	NS	*[NS]	**	**	NS	...
Experiment 2:						
Clear plastic	NS	NS	NS	NS	NS	NS
Neutral shade	**[*]	*	*[NS]	**	NS	NS
Altered shade	**	**[*]	*[NS]	NS	NS	NS

Note.   Experiment 1 conducted April–May; experiment 2 conducted August–September. Separate comparisons are made for each node position numbered from the base using the G-statistic. Bracketed values indicate a different outcome when multiple comparisons are taken into account by using a more conservative χ^2 that has three times the level of significance (table 15 in Sokal and Rohlf 1981*b*). Significant χ^2 values based on single comparisons; NS = not significant.

* Significant at the 0.05 level.

** Significant at the 0.01 level.

Table 3

Typical Sequence of Axillary Structures on a Reiterated Shoot in Experiment 2

Node	Full sun (control without tent)	Neutral shade (neutral-shade tent)
1	None	None
2	None	None
3	One branch + one aborted branch	One thorn
4	Two branches	One thorn
5	Two branches	One thorn + one branch
6	One branch	Two thorns
7	Two branches	One thorn + one branch
8	Two branches	Two thorns

branches. Thorns were correlated with shaded low-irradiance conditions as well as basal positions on a leader shoot. Conversely, foliage and later hook inflorescence branches were correlated with high-irradiance conditions and distal node positions. Our findings indicated that low irradiance promoted thorn development from the same meristems that produced leafy branches in full sun. Light quality (R : FR) did not significantly affect bud development because we found no differences in thorn production between neutral and altered-shade treatments. Low R : FR has been shown to suppress lateral bud initiation in various rain forest trees (Lee et al. 1996), but no such effect was seen in these experiments with *Artabotrys*. In simple experiments with seedlings of *Ulex europaeus* (Fabaceae), Bieniek and Millington (1968) found that low irradiance delayed formation of thorns but concluded that this was due to reduced growth that simply delayed the onset of the adult phase. This was similar to their finding that short and long day length affected the transition from juvenile to adult phases, but day length had no effect on thorn development once the plants reached their adult phase. In *Artabotrys*, irradiance affected thorn development in newly induced leader shoots. However, we did not carry out the reciprocal experiment in which branch-bearing leader shoots growing in high irradiance were shaded to see whether their distal nodes would then produce thorns.

We can speculate on the possible adaptive significance of these morphological gradients in a liana growing in the rain forest. New orthotropic leader shoots arise near the forest floor from either seedlings or from the base of mature shoots as reiterations. In both cases, these new shoots face the task of climbing above the understory and reaching the upper level of the forest canopy. The understory of a rain forest has 1%–10% of the irradiance found at the upper surface of the forest canopy (Chazdon et al. 1996; Lee et al. 1997). Thus, it appears that the seedling grows under a low-irradiance environment but produces no thorns. The juvenile tolerates deep shade as a suppressed sapling that does not climb. As an adult (like the plants that we experimented on), it forms new leaders that produce thorns under the same deep-shade conditions.

The thick woody thorns may aid in climbing by hooking onto supporting branches and preventing slippage. We assume that thorns are found on the first-formed climbing shoots of a sapling, but we have no supporting observations. If this is true, then thorn production can be seen as an adult characteristic found only in the climbing leader shoot, which itself is a feature of the adult plant. Shade-induced thorn production would then also be an adult characteristic.

Thorns may also help to defend the base of the leader from herbivores. Although the thorns are large and neither particularly sharp nor spiny, they may help to protect the stem from large herbivores in the native African habitat. However, we feel it is premature to dwell on the adaptive significance of these thorns. Grubb (1992) discussed the complexities and difficulties of offering an ecological interpretation for plants being armed. Very different kinds of plants, in terms of succession, growth rate, or water availability, display similar protection by spines. His ideas might be applicable here, but we cannot speculate further because we lack basic information about the native habitat of *Artabotrys*, its seedling establishment, successional status, or herbivore pressures.

Acknowledgments

Literature Cited

Bieniek ME, WF Millington 1967 Differentiation of lateral shoots as thorns in *Ulex europaeus*. Am J Bot 54:61–70.

——— 1968 Thorn formation in *Ulex europaeus* in relation to environmental and endogenous factors. Bot Gaz 129:145–150.

Blaser HW 1956 Morphology of determinate thorn-shoots of *Gleditsia*. Am J Bot 43:22–28.

Chazdon RL, R Pearcy, D Lee, N Fetcher 1996 Photosynthetic responses of tropical forest plants to contrasting light environments. Pages 5–55 *in* SS Mulkey, RL Chazdon, AP Smith, eds. Tropical forest plant ecophysiology. Chapman & Hall, New York.

Goebel K 1900 Organography of plants. Pt 1. General organography. Oxford University Press, Oxford.

Grubb PJ 1992 A positive distrust in simplicity—lessons from plant defences and from competition among plants and animals. J Ecol 80:585–610.

Hackett WP, RM Sachs 1968 Experimental separation of inflorescence development from initiation in *Bougainvillea*. Proc Am Soc Hortic Sci 92:615–621.

Hallé F, RAA Oldeman, PB Tomlinson 1978 Tropical trees and forests: an architectural analysis. Springer, Berlin.

Lee DW, K Baskaran, M Mansor, H Mohamad, SK Yap 1996 Irradiance and spectral quality affect Asian tropical rain forest tree seedling development. Ecology 77:568–580.

Lee DW, S Oberbauer, K Baskaran, M Mansor, H Mohamad, SK Yap 1997 Effects of irradiance and spectral quality on the development of two *Hopea* species. Oecologia 110:1–9.

Lee DW, JH Richards 1991 Heteroblastic development in vines. Pages

205–243 *in* FE Putz, HA Mooney, eds. Biology of vines. Cambridge University Press, New York.

Liberty Hyde Bailey Hortorium 1976 Hortus third: a concise dictionary of plants cultivated in the United States and Canada. Macmillan, New York.

Mabberley DJ 1997 The plant book. 2d ed. Cambridge University Press, Cambridge.

Njoku E 1956 Studies in the morphogenesis of leaves. XI. The effect of light intensity on leaf shape in *Ipomoea caerulea*. New Phytol 55:91–110.

Peñalosa J 1983 Shoot dynamics and adaptive morphology of *Ipomoea phillomega* (Vell.) House (Convolvulaceae), a tropical rainforest liana. Ann Bot 52:737–754.

Posluszny U, JB Fisher 2000 Thorn and hook ontogeny in *Artabotrys hexapetalus* (Annonaceae). Am J Bot 87:1561–1570.

Smith H 1995 Sensing the light environment: the functions of the phytochrome family. Pages 187–217 *in* RE Kendrick, GHM Kronenberg, eds. Photomorphogenesis in plants. 2d ed. Kluwer Academic, Dordrecht.

Sokal RS, FJ Rohlf 1981*a* Biometry. 2d ed. WH Freeman, New York.

——— 1981*b* Statistical tables. 2d ed. WH Freeman, New York.

Spiegel-Roy P, EE Goldschmidt 1996 Biology of *Citrus*. Cambridge University Press, Cambridge.

Tortosa RD, L Aagesen, GM Tourn 1996 Morphological studies in the tribe Colletieae (Rhamnaceae): analysis of architecture and inflorescences. Bot J Linn Soc 122:353–367.

Tourn GD, D Medan, RD Tortosa 1989 Yemas multiples en Ramnaceas: organización del complejo axilar con especial referencia a *Ziziphus* y *Paliurus*. Kurtiana 20:101–111.

Troll W 1937 Vergleichende Morphologie der höheren Pflanzen. Band 1, teil 1. Borntraeger, Berlin.

Wareing PF, VM Frydman 1976 General aspects of phase change, with special reference to *Hedera helix*. Acta Hortic 56:57–69.