Leaf canopy display, stomatal conductance, and photosynthesis in seedlings of three tropical pioneer tree species subjected to drought

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Seedlings of *Piper aurtitum*, *Cecropia obtusifolia*, and *Trichospermum mexicanum*, pioneer species of lowland tropical rainforests, were subjected to controlled drought cycles, with stomatal conductance and net photosynthesis monitored to determine species response. The affect of soil moisture on canopy display and the response of stomatal conductance to fluctuations in humidity, light, and temperature were also measured. Stomatal conductance and photosynthesis in *Piper* and *Cecropia* showed no relationship with soil moisture until it declined to approximately 20%. At higher soil moistures, stomatal conductance in *Piper* and *Cecropia* was positively correlated with relative humidity. In contrast, *Trichospermum* stomatal conductance and photosynthesis showed no relationship with relative humidity and declined gradually as soil moisture decreased. Canopy display in all three species showed a sharp decline at the end of the drought cycle. There were marked differences among species in canopy structure. *Piper* and *Cecropia* both have large leaves and a low fibre content in stems, petioles, and leaves, whereas *Trichospermum* has much smaller leaves and a higher fibre content. In addition, *Piper* has a higher leaf to stem ratio than *Trichospermum*. Differences among species in response of stomatal conductance and photosynthesis to environmental factors may reflect the need to maintain a relatively high turgor pressure in *Piper* and *Cecropia* for canopy display.

Key words: canopy architecture, drought stress, leaf size, *Piper auritum*, *Cecropia obtusifolia*, *Trichospermum mexicanum*.

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Les auteurs ont soumis des plantules de *Piper auritum*, *Cecropia abtusifolia* et *Trichospermum mexicanum*, espèces pionnières des basses terres des forêts tropicales ombrophiles, à des cycles de dessèchement contrôlé en mesurant périodiquement la conductance stomatale et la photosynthèse nette pour déterminer la réaction des espèces. Ils ont également mesuré l'influence de l'humidité du sol sur l'étalement de la canopée et sur la réaction de la conductance aux fluctuations de l'humidité, de la lumière et de la température. Chez le *Piper* et chez le *Cecropia*, la conductance stomatale et la photosynthèse ne montrent aucune relation avec l'humidité du sol jusqu'à ce qu'elle diminue à environ 20%. À des humidités plus élevées du sol, la conductance stomatale chez le *Piper* et le *Cecropia* montre une corrélation positive avec l'humidité relative. Au contraire, la conductance stomatale et la photosynthèse du *Trichospermum* ne montrent aucune relation avec l'humidité relative et décroissent graduellement à mesure que l'humidité du sol décroit. Chez les trois espèces, l'étalement de la canopée montre une nette diminution vers la fin du cycle de dessèchement. On note des différences marquées entre les espèces quant à la structure de la canopée. Les *Piper* et les *Cecropia* ont tous deux des feuilles larges et un faible contenu en fibre. De plus, le *Piper* possède un raport feuille à tige plus élevé que le *Trichospermum*. Les différences observées entre les espèces dans la réaction de la conductance stomatale et de la photosynthèse aux facteurs du milieu pourraient refléter le besoin de maintenir une pression de turgescence relativement élevée, pour maintenir l'étalement de la canopée chez le *Piper* et chez le *Cecropia*.

Mots clés : structure de la canopée, stress de la sécheresse, taille des feuilles, *Piper auritum*, *Cecropia obtusifolia*, *Trichospermum mexicanum*.

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Introduction

Tree-fall gaps in wet tropical forests are rapidly colonized by pioneer species that must quickly grow and deploy a canopy to successfully compete for light (Bazzaz 1984; Brokow 1985). These leaves are often large and arranged in a flat crown that maximizes light interception from above (Whitmore 1975; Ashton 1978). The large leaves are supported by sparse branch frameworks, and the few branches they do produce are energetically cheap (Givnish 1978; Oldeman 1978). However, some early successional species have numerous smaller leaves supported by more extensive branching systems (Bazzaz and Pickett 1980). This morphology represents a long-term investment in mechanical support tissue and prolongs canopy durability.

Although lowland tropical forest may receive large amounts of rainfall and are among the wettest habitats, drought periods do occur. Forest seedlings and trees may experience both diurnal and seasonal water deficits (Brunig 1971; Walter 1971; Fetcher 1979; Oberbauer 1982; Chiariello et al. 1987). Large- and small-leaved species can be expected to respond very differently to these water deficits. Large leaves, because of their thick boundary layer, will have reduced sensible-heat transfer (e.g., see Taylor and Sexton 1972). They will have to dissipate large amounts of thermal energy by latent-heat transfer or else become much warmer than the surrounding air. Smaller leaves will be less dependent on transpiration to maintain leaf temperature within physiological limits. Any differences between large- and small-leaved species in leaf energy budgets could in turn lead to differences in the response of photosynthesis to water deficits through their effect on stomatal conductance and leaf temperature. Water stress can also be expected to

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differentially affect canopy display in large- and small-leaved species. Because of a less developed support structure, large-leaved species may be more dependent on maintenance of turgor pressure to optimally display their leaves than do small-leaved species.

Reekie and Bazzaz (1989) found that large-leaved tropical species (*Cecropia obtusifolia, Myriocarpa longipes, Piper auritum*) maintained a substantially higher leaf water potential than small-leaved species (*Senna multijuga, Trichospermum mexicanum*). The present study explores this apparent difference in water relations between large- and small-leaved species by examining the effect of soil water availability on canopy display, stomatal conductance, and photosynthesis in three gap-tree species: two large-leaved species, *C. obtusifolia* Bertol. and *P. auritum* Sieber, and one small-leaved species, *T. mexicanum* Baill.

Materials and methods

Seed was collected at the biological research station of the Instituto de Biologia of the Universidad Nacional Autonoma de Mexico at Los Tuxtlas, Vera Cruz, Mexico. The station is located on the eastern slopes of the Sierra de Los Tuxtlas (95°04′W longitude, 18°34′N latitude), an area of rich soils of recent volcanic origin. The vegetation is high evergreen rainforest (Rzedowski 1978).

Seeds were germinated in moist vermiculite and transplanted to individual pots when plants had at least two true leaves. Pot size was increased as the seedlings grew, with the final pot size being 3.35 L. A 2:1 mix of potting soil and Turface (Plant Products Co. Ltd., Bramalea, Ont.) was used as the growing medium. Plants were placed in a greenhouse where they received unsupplemented natural light. Temperature ranged between 20 and 25°C. Plants were fertilized at monthly intervals by watering with a standard strength Peter's soluble fertilizer (20:20:20). Plants were 12 months old and approximately 1 m tall at the beginning of the experiment.

Prior to the experiment, plants were placed in a greenhouse that provided day−night temperatures of 30 ± 3 and 27 ± 3°C, respectively. They received natural light (maximum light levels approx. $1000-1200 \ \mu mol \cdot m^{-2} \cdot s^{-1}$), and at times when light levels within the 12-h photoperiod dropped below $600 \ \mu mol \cdot m^{-2} \cdot s^{-1}$, natural light was automatically supplemented with illumination from metal halide lamps. The lamps were capable of providing $400 \ \mu mol \cdot m^{-2} \cdot s^{-1}$. Neither relative humidity nor carbon dioxide were controlled, but spot measurements indicated that at midday, relative humidity was approximately 40−60% and CO_2 was between 340 and 370 $\mu L \cdot L^{-1}$. After a 3-week acclimation period, plants were subjected to 5-day drying cycles. Drying cycles were repeated four times with a different set of plants each time. At the start of a cycle, plants were watered to field capacity. Water was then withheld and plants were allowed to dry out in the greenhouse under the above conditions. The following measurements were made.

Soil moisture availability

Soil moisture content was assessed gravimetrically at the time of gas-exchange measurements. Four soil cores were taken from each pot and the samples were bulked to obtain a single composite sample. The cores were 2.5 cm in diameter and were taken to the depth of pot. The samples were weighed, oven-dried at 105°C for 48 h, and then reweighed. Soil moisture content was expressed as a percentage of the oven-dry weight of the soil.

Gas-exchange measurements

Gas-exchange rates of the most recent, fully emerged leaf were measured at various points over the drought cycles. There were a total of 24 measurements per species divided among the four drought cycles. All measurements were made between the hours of 11:00 and 15:00 and were more or less equally distributed across an individual drought cycle. Measurements were made with a LICOR model LI-6200 gas-exchange system (LICOR, Lincoln, Nebr.) with a 1-L

sample chamber (LI-6000-12). The amount of leaf area in the chamber was approximately 20 cm^2 (actual leaf area sampled varied somewhat depending on leaf size). Closed-cell foam-rubber inserts were used to control the amount of leaf area sampled. Photosynthetic rates are expressed on the basis of a single side of the leaf. During measurements the change in CO_2 concentration within the chamber ranged between 5 and 15 $\mu L \cdot L^{-1}$. Changes in humidity were minimized by adjusting the rate of flow through a drying column of magnesium percholate connected in parallel with the plant chamber. Adjustments in the flow rate through the desiccant were made prior to the actual measurement using a leaf of similar age on the same plant. Stomatal conductance was calculated using the change in water-vapour concentration over time and the rate of flow through the desiccant column. Ambient light levels, relative humidity, and temperature within the greenhouse all fluctuated to some degree over the course of the measurements (see above). Stepwise multiple linear regression was used to determine to what extent fluctuations in these variables, along with soil moisture, may have influenced net photosynthesis and stomatal conductance.

Canopy display

To monitor changes in canopy display over the drying period, canopy photographs were taken of each plant at the start of the drying cycle (i.e., when soil was at field capacity) and immediately before measurement of gas-exchange rates. Photographs were taken using Kodacolor 100 print film with the camera fixed at a constant height (2.8 m) directly above the plant. The amount of leaf area displayed perpendicular to the camera was estimated by cutting out and weighing the leaves from a photograph and comparing this weight with that of a circle of known area photographed in the same fashion. Data were expressed as the ratio between leaf area at the time of gas-exchange measurement and that of the same plant at start of the drying cycle. There was little leaf growth over the course of the 5-day drought cycles and therefore a change in leaf area owing to growth is unlikely to be a serious error.

Structural support

Differences among species in the amount of structural support were assessed by calculating the ratio between leaf and stem weight and by determining fibre content of the plant tissues. Leaf to stem ratio was calculated for each plant separately. There were 36 plants (replications) per species. Data were analyzed by one-way analysis of variance. As the F-test for this analysis was significant, paired contrasts were used to compare individual species.

Fibre content was determined separately for leaf blades, petioles, and stems using the neutral detergent method (Van Soest 1967). Plant material was ground (20−30 mesh) and 1 g was placed in a beaker with 100 mL neutral detergent solution, 2 mL decahydronaphthalene, and 0.5 g sodium sulfite. The beaker was heated to boiling and refluxed for 60 min. Contents of the beaker were placed in a Gooch crucible and submitted to vacuum. Contents of the crucible were rinsed twice with hot water and twice with acetone and sucked dry. Crucibles were dried at 100°C for 8 h and weighed. This procedure analyzes for the basic constituents of cell walls (cellulose, hemicellulose, and lignin) and therefore provides an estimate of the structural components of plant tissue. There were six replications for each plant part − species combination. Each sample, regardless of whether it was derived from leaf blades, petioles, or stems, came from a different individual plant. The data were therefore analyzed by standard factorial analysis of variance, with plant part completely crossed with species (i.e., plant part was not nested within individuals). In cases where there was a significant F-test for an effect, means were compared using paired contrasts. The plants used for both the fibre analyses and for determining allocation ratios were those harvested in the experiments described above.

Results

The large- and small-leaved species showed different responses to variation in soil moisture content (Fig. 1). The two large-leaved species (*Piper* and *Cecropia*) exhibited a threshold
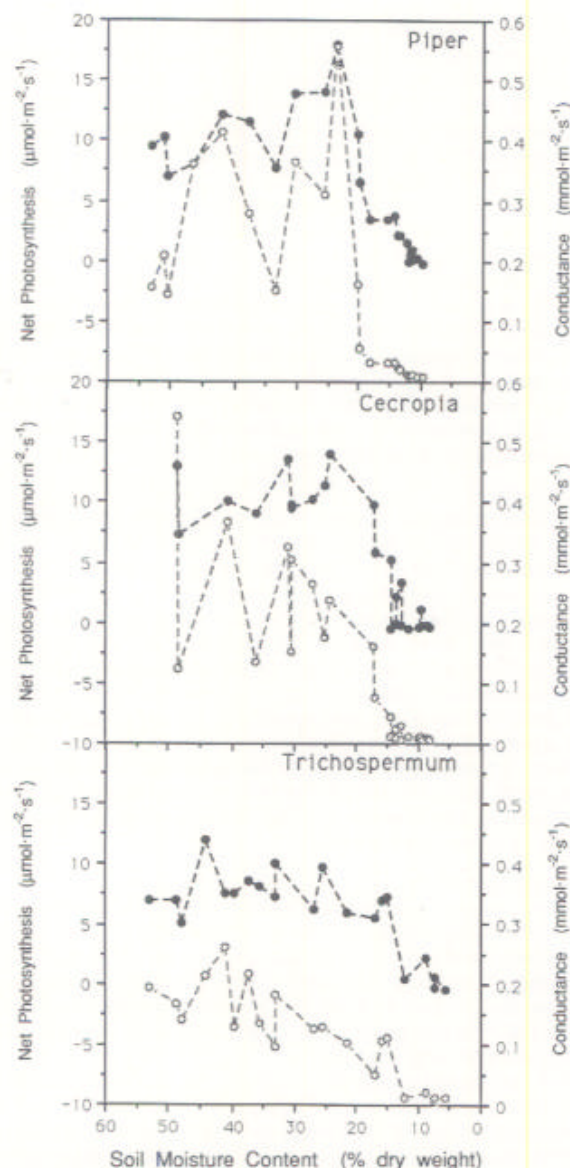
FIG. 1. Relationship between soil moisture content and photosynthesis (○) and stomatal conductance (●) in *Piper*, *Cecropia*, and *Trichospermum*.

TABLE 1. Effect of soil moisture availability (SM), relative humidity (RH), irradiance (I), and air temperature (T) on photosynthesis and conductance in three tropical pioneer species

Species	Dependent variable	Independent variable	Slope estimate	Partial R^2
Piper	Conductance	RH	0.0017	0.56
		T	0.0569	0.20
		SM	0.0011	0.09
	Photosynthesis	SM	0.1957	0.41
		T	1.8181	0.20
		RH	0.2120	0.10
Cecropia	Conductance	SM	0.0073	0.67
		RH	0.0058	0.06
	Photosynthesis	SM	0.3225	0.59
Trichospermum	Conductance	SM	0.0042	0.72
	Photosynthesis	SM	0.1477	0.48
		I	0.0060	0.15

NOTE: Results of stepwise multiple linear regression analysis; independent variables were entered and retained within the model only if they were significant at the 0.05 level.

Photosynthesis varied in soil moisture and irradiance in *Trichospermum*, with soil moisture in *Cecropia*, and with soil moisture, air temperature, and relative humidity in *Piper*.

Leaf display as a proportion of that displayed at field capacity remained close to 1 in both *Piper* and *Cecropia* until soil moisture content dropped to 20%, at which time leaf display declined sharply (Fig. 2). In *Trichospermum*, the proportion of leaf area displayed showed more variability, but there was a drop in leaf display at approximately 20% soil moisture, similar to what occurred in *Piper* and *Cecropia*.

The three species differed in the amount of structural material per unit of leaf. The ratio of leaf to stem weight was higher in *Piper* (3.6) than in either *Cecropia* (2.2) or *Trichospermum* (2.0) ($p < 0.0001$ for the species effect). The species also differed in the fibre content of the various plant parts ($p < 0.0001$ for the main effect of species) (Fig. 3). Fibre content increased from *Piper* to *Cecropia* to *Trichospermum* (differences among all three species significant at the 0.05 level). Differences among species were greater in the plant parts with high fibre content ($p < 0.0001$ for species × plant part interaction). Fibre content increased from leaves to petiole and stem ($p < 0.0001$ for the main effect of plant part).

Discussion

Stomatal conductance and photosynthesis in *Piper* and *Cecropia* were more or less independent of soil moisture content until it had declined to 15−20% (Fig. 1). This is probably a reflection of the ability of these two species to maintain a relatively high plant water potential (Reekie and Bazzaz 1989). A high water potential would allow stomata to remain open longer, prolonging photosynthetic activity. The behaviour of these two large-leaved species contrasts with the constant decline in stomatal conductance and photosynthesis exhibited by *Trichospermum* (Fig. 1). This small-leaved species maintains a much lower water potential than the large-leaved species (Reekie and Bazzaz 1989).

One possible explanation for the dichotomy in the ability of large- versus small-leaved species to maintain shoot water potential concerns the response of stomata to environmental conditions (Table 1). Conductance in *Piper*, and to a lesser extent in *Cecropia*, decreased with relative humidity. The results for

response: photosynthesis and conductance exhibited no relationship with soil moisture content above 20% and declined sharply after this threshold. Photosynthesis and conductance declined more gradually with soil moisture in the small-leaved species (*Trichospermum*).

In *Piper* and *Cecropia*, and to a lesser extent in *Trichospermum*, there was substantial variability in both photosynthesis and conductance that could not be accounted for by variation in soil moisture content. Results of the multiple regression analysis indicated that much of the variability in photosynthesis and conductance was related to fluctuations in relative humidity, light, and temperature (Table 1). However, the species differed in their responses. Stomatal conductance in the small-leaved species, *Trichospermum*, varied only with soil moisture, whereas conductance in the two large-leaved species also varied with humidity, and in the case of *Piper*, with temperature as well.
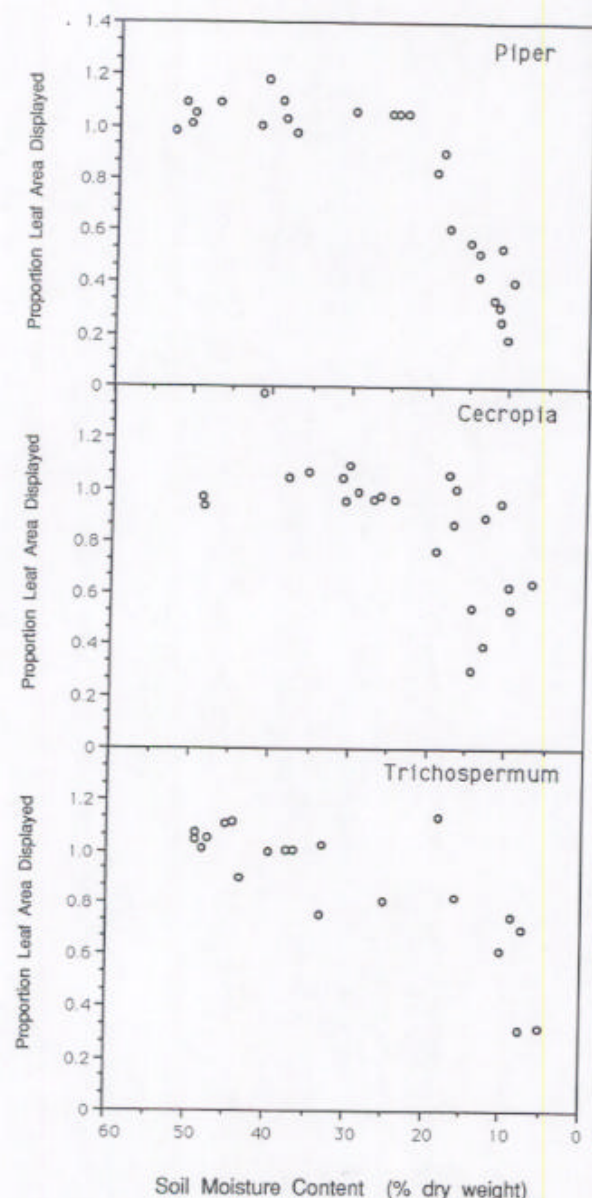
FIG. 2. Relationship between soil moisture content and canopy display in *Piper*, *Cecropia*, and *Trichospermum*. Canopy display is expressed as the ratio of leaf area displayed at time of soil moisture determination to that displayed before the start of the dry-down period when the plants were well watered.
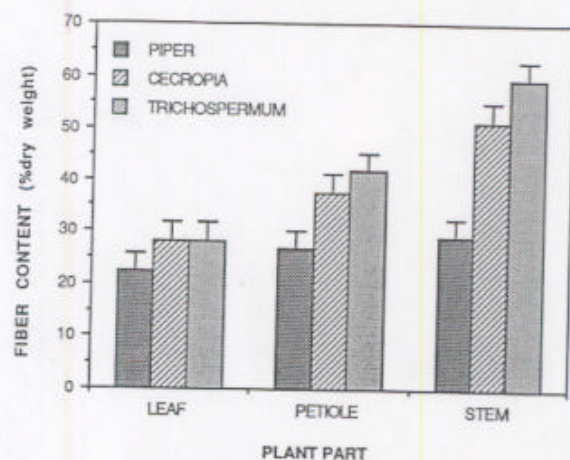

FIG. 3. Fibre content of leaf, petiole, and stem in *Piper*, *Cecropia*, and *Trichospermum*. Error bars represent two standard errors of a single treatment mean.

in the present study. Averaged across plants of different ages grown in a range of environments, both *Piper* (692 cm$^2 \cdot$ g^{-1}) and *Cecropia* (717 cm$^2 \cdot$ g^{-1}) have a lower leaf area to root biomass ratio than does *Trichospermum* (1165 cm$^2 \cdot$ g^{-1}, $p <$ 0.0388 for the difference among species). Therefore, relative to likely demand (i.e., leaf area), the two large-leaved species have a larger water uptake system (i.e., root biomass) than does the small-leaved species. This greater uptake capacity would further facilitate the maintenance of leaf water potential and stomatal conductance.

The different responses to water stress by large- and small-leaved species suggest important relationships exist between seedling water relations and canopy display. A possible explanation for the apparent necessity of maintaining high leaf water potential in *Piper* and *Cecropia* (Reekie and Bazzaz 1989) concerns the role of turgor in canopy display.

Although soil moisture content had more or less the same effect on canopy display in all three species (Fig. 2), the mechanism varied among them. In *Trichospermum*, leaf orientation relative to the light source was controlled by pulvini at the junction between the petiole and leaf blade. As soil moisture decreased, the angle of the leaf blade relative to the petiole decreased from about 180 to 90°, drastically reducing the amount of leaf area perpendicular to an overhead light source. In *Piper*, there are no pulvini controlling leaf orientation. Leaf orientation changed only when there was a generalized wilting of the entire leaf blade, petiole, and young stem. Chiariello et al. (1987) document the importance of this wilting in *Piper* for reducing heat load and maintaining leaf temperature near air temperature. *Cecropia* was intermediate between the above two species. It had pulvini at the junction between the stem and the petiole that, to some degree, controlled the amount of exposed leaf area. However, the blades themselves also wilted at low soil moisture, further reducing leaf display.

In the two large-leaved species, maintenance of a high water potential would be necessary for display of the leaves. In *Trichospermum*, where leaf display is controlled by pulvini, decreases in leaf potential would have little immediate effect on leaf display. Since it is only necessary to maintain turgor within a localized region, the pulvini, decreases in bulk leaf potential have no direct effect on leaf display. Turgor within

Piper are in accord with those of Mooney et al. (1983) who likewise observed a close relationship between conductance and humidity in this species. The direct response of conductance to humidity would minimize water loss under conditions of high evaporative demand (Schulze and Hall 1982). Stomatal conductance of *Trichospermum* did not vary with humidity, and as a result would have a lower water-use efficiency under conditions of fluctuating humidity and therefore a lower water potential.

Other factors are probably also involved in explaining the ability of the large-leaved species to maintain a high water potential, e.g., differences in water uptake capacity. Based on data of Reekie and Bazzaz (1989), it is possible to calculate the ratio between leaf area and root biomass for the three species

the pulvini could be maintained despite decreases in bulk leaf potential by active pumping of ions into the pulvini, lowering osmotic potential relative to the surrounding tissue. In *Piper* and *Cecropia*, where wilting of the entire leaf was responsible for decreases in leaf display, it would be necessary to maintain bulk leaf potential at a high level to ensure sufficient turgor.

The explanation for why *Piper*, and to a lesser extent *Cecropia*, may depend on bulk leaf water potential to display their leaves is probably related to their pattern of growth. The large leaves, high leaf to stem ratio, and lack of structural support within the tissue (Fig. 3) facilitate the rapid growth rates required by these pioneer species. These same characteristics also make it difficult to structurally support the mass that is accumulated. In this situation, supporting the tissue by means of turgor rather than structural material may be a viable solution.

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