Lindleyana 4(2): 53-60. 1989.

WHY DO NONFOLIAR GREEN ORGANS OF LEAFY ORCHIDS FAIL TO EXHIBIT NET PHOTOSYNTHESIS?

D. H. Benzing and W. T. Pockman

Department of Biology, Oberlin College, Oberlin, Ohio 44074

ABSTRACT

Nonfoliar organs of numerous epiphytic orchids, including fruit of *Encyclia tampensis*, are green. Regenerative rather than net photosynthesis is characteristic of these structures except when well-developed leaves are absent. The uneven distribution of photosynthetic capacity among the body parts of these plants can be explained by the relative cost effectiveness of investing scarce resources (in particular, N) for autotrophy. Historical change in vegetative form and accompanying shifts in function during transitions become clearer when plant economics are considered.

ALLUSIONS to chlorophyll in plant organs that are primarily concerned with nontrophic functions abound in the orchid literature, but seldom do even those few studies that report photosynthesis at these sites (Avadhani et al., 1982) address its full implication. Crop scientists (e.g., Flinn, Atkins, and Pate, 1977; Pate, Sharkey, and Atkins, 1977) and others (e.g., Bazzaz, Carlson, and Harper, 1979) have demonstrated that carbon/energy inputs from what appear to be relatively trivial sources, together with associated influences on water economy, can be significant to plant performance. Emphasis was placed on the importance of nonfoliar supplements to yield — particularly seed production — but there are also potential consequences for historic change in plant form.

Because they so often grow in strong light and on impenetrable substrata, orchids can utilize an exceptional variety of organs for energy harvest. Indeed, *Encyclia tampensis*, like thousands of its relatives, conducts photosynthesis in stems, roots, and fruit as well as in leaves, but not with equal intensity. Results of previous studies of this epiphyte (Benzing and Ott, 1981; Benzing et al., 1982; Benzing et al., 1983) and data provided below are used here to develop an economic perspective on why nonfoliar organs of so many leafy orchids are green yet possess insufficient photosynthetic capacity to exhibit net CO_2 consumption. This study also illustrates how a comprehensive cost/benefit analysis will help explain why such an extraordinary variety of body plans exists among the more advanced autotrophic orchids.

MATERIALS AND METHODS—Plants were collected in April, 1987 from a variety of phorophytes in the mixed cypress/hardwood forest that occupies much of the Fahkahatchee Slough in Collier County, Florida. Growth continued on lath strips in the Oberlin College greenhouse until flowering took place 3-5 months later. Following manual cross-pollination (2-6 flowers/inflorescence), plants were moved into a growth chamber where fruit developed under 14 hr of light (ca. 170 µmol $m^{-2}s^{-1}$) and day/night temperatures of about 25 C and 20 C.

Subsets of a population of about 200 capsules produced by approximately 50 plants were examined at five stages during the 8-10 months required to reach dehiscence (Fig. 2). Stages 1 through 3 were defined by capsule width/length ratios of: less than 0.09/0.12; 0.15/0.18; and 0.23/0.26. Stages 4 and 5 were defined by time elapsed in the 6 to 15 weeks after the end of Stage 3.

Fruit and leaves were collected at 7 a.m. (at the end of the dark period) and at 7 p.m. They were weighed immediately, sectioned, and ground in 40 ml distilled H_20. Acidity was measured by titrating the extract to pH 7.5 with 0.01 M NaOH. Chlorophyll content was determined spectrophotometrically according to Arnon (1949). A third set of oven-dried samples were wet-digested in H_2S0_4 using metallic selenium as a catalyst. Following neutralization with 30% NaOH, digests were steam-distilled and the distillate titrated with 0.01 HCl to determine total (Kjeldahl) nitrogen content. Seeds that initially represented little of the total mass could not be separated from Stage 1 and 2 fruit; thereafter, capsule values are for wall tissue only.

Gas exchange (CO_2 and H_2O) in detached fruit placed in a 250-ml cuvette was measured in a closed system using a LI-COR LI-6200 portable infrared gas analyzer fitted with a hygrometer. Data were collected between noon and 2 p.m. and between midnight and 2 a.m. Measurements were made in the growth chamber in order to avoid abrupt changes in conditions, particularly humidity, that might affect stomatal conductance. Several Stage

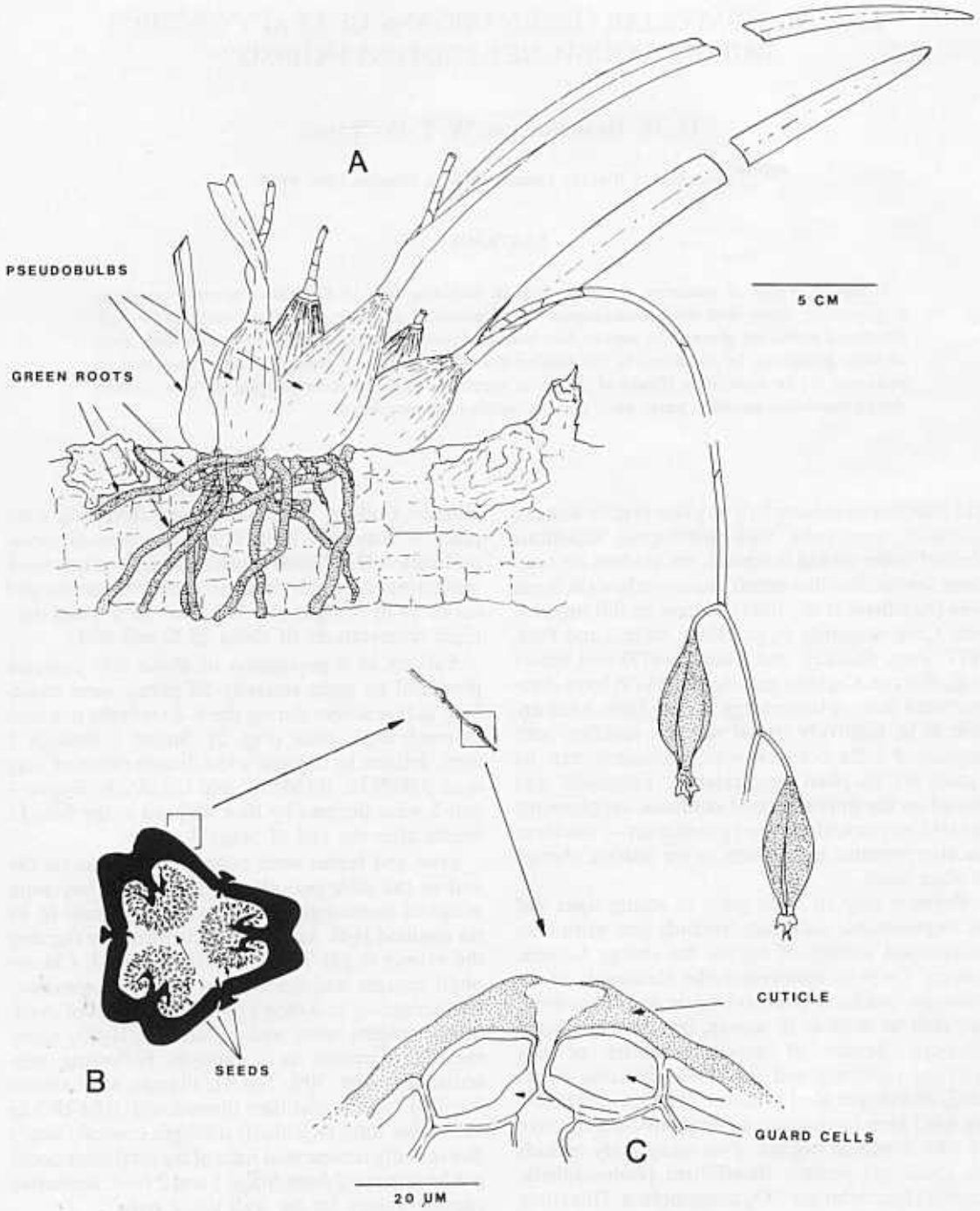


Fig. 1. Habit and morphology of *Encyclia tampensis*. **A.** Whole fruiting specimen. **B.** Fruit cross-section. **C.** A sectioned stoma.

1 and 2 capsules were used for each determination; larger, older fruit was assayed singly. Occasionally, as few as three replicates were run, but usually n=8-10. Capsules from Stages 3, 4, and 5 were first analyzed intact and then removed from the cuvette and bisected with a razor blade. The initial burst

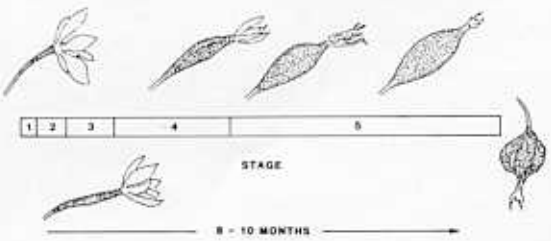


Fig. 2. Fruit development illustrating capsule morphology and the duration of the five growth stages.

of CO_2 evolution (presumably representing escape of trapped gas) was measured; fifteen min later, fruit was again monitored for steady state CO_2 efflux. Three capsules from Stages 2-5 were maintained over $CaCl_2$ for 72 hr to quantify further rates of water loss.

Leaf and fruit sections fixed in FAA were postfixed in 2% OsO_4 for 24 hr and infiltrated with acetone. After critical-point drying and sputter-coating with gold, specimens were examined with a Hitachi S-405A SEM operating at 15-25 Kv. Additional materials were embedded in Paraplast, sectioned, and stained with toluidine blue for light microscopy.

RESULTS—Stages 1 and 2 capsules exhibited high and statistically identical acid content; acidity subsequently dropped with concurrent development of a stronger diurnal CAM rhythm (Fig. 3) which continued through Stage 5. Leaves showed more pronounced acid fluctuation throughout the study.

Carbon dioxide exchange, although negative at night during fruit development, diminished between Stages 2 and 3 as CAM intensified (Fig. 4). Net losses were also detected at midday from young fruit (data not shown), but at rates below those recorded at night. By Stage 5, CO_2 evolution was about the same day and night. Leaves exchanged little or no CO_2 at midday, but always engaged in nocturnal uptake. Stages 3-5 capsules liberated bursts of CO_2 at night that were well above the steady-state evolution demonstrated 15 min later.

Fruit became increasingly watertight with age and by Stage 5 were losing only about 8% as much moisture at night as had Stage 1 samples (Fig. 5). Foliar transpiration at night was relatively high and statistically indistinguishable from that of Stage 1 and 2 capsules on a fresh weight basis. Nocturnal and diurnal transpiration by fruit (data not shown) was statistically undifferentiated. Intact Stage 2 specimens maintained over desiccant for 72 hr lost 17.6% of their initial weight while percentages for Stages 3, 4, and 5 were 4.7, 3.1, and 2.7, respectively.

Chlorophyll and total N concentrations in fruit walls decreased initially and leveled off after Stage 2 at about half to one-third that in leaves (Fig. 6).

The most conspicuous epidermal feature distinguishing fruit from foliage was stomatal location and density (Fig. 7, 8). Occurrence on leaves (abaxial side only; Fig. 8) averaged 46 mm^{-2} while numbers on capsules were much lower — 2 mm^{-2} near the three ribs (Fig. 1) and about 3 mm^{-2} elsewhere. Stomata were recessed in fruit, one at the summit of each small papilla. Full-sized capsules were bounded by a thick-walled epidermis with a stout cuticle; sizable locular cavities had developed by Stage 3 (Fig. 1).

DISCUSSION—Perspectives on performance of, and broader implications of green tissue in, capsules of *Encyclia tampensis* and many additional orchids (and also in their stems and roots) are improved through comparison with nonorchids. Developing pods of some Leguminosae and a variety of other green fruits exhibit substantial surface-to-volume ratios and fix modest amounts of atmospheric CO_2 as well as much respired carbon. Enough photosynthesis, including net CO_2 uptake during early development, occurs in *Pisum sativum* fruit to reduce appreciably the amount of imported photosynthate needed to produce seeds (Flinn et al., 1977). Specifically, capsules import 17% less dry matter because they are green, certainly a benefit for a plant destined to allocate one-third or more of its biomass to a single reproductive effort at the end of a short life. In contrast, capsules of perennial, slow-growing, xerophytic *Encyclia tampensis* continuously leak some CO_2 that foliage must replace. In effect, similarity in overall carbon balance between fruit and leaves of *Pisum sativum* and its kind exceed that of *E. tampensis* except when drought induces the orchid's foliage to CAM-idle, as described below.

The fruit of C_3 *P. sativum* is not particularly watertight, although transpiration occurs at a lower rate than does that from leaves. If the water balances of the two species considered here are contrasted, pods of *E. tampensis* are the more resistant to desiccation relative to foliage—just how much more is impossible to determine without additional data. Nevertheless, at least quantitative differences in photosynthetic performance and water use (fruit versus leaf) exist, and they, along with the distinct environmental conditions prevailing in native habitats, prompt us to suggest that peculiarities of pea and *E. tampensis* capsules vis-à-vis water and carbon economy influence plant survival according to the following hypothesis.

Photosynthesis by fruit can yield several advantages that vary in relative importance, depending on the nature of that organ, the life style of the parent plant, and growing conditions. Green tissue

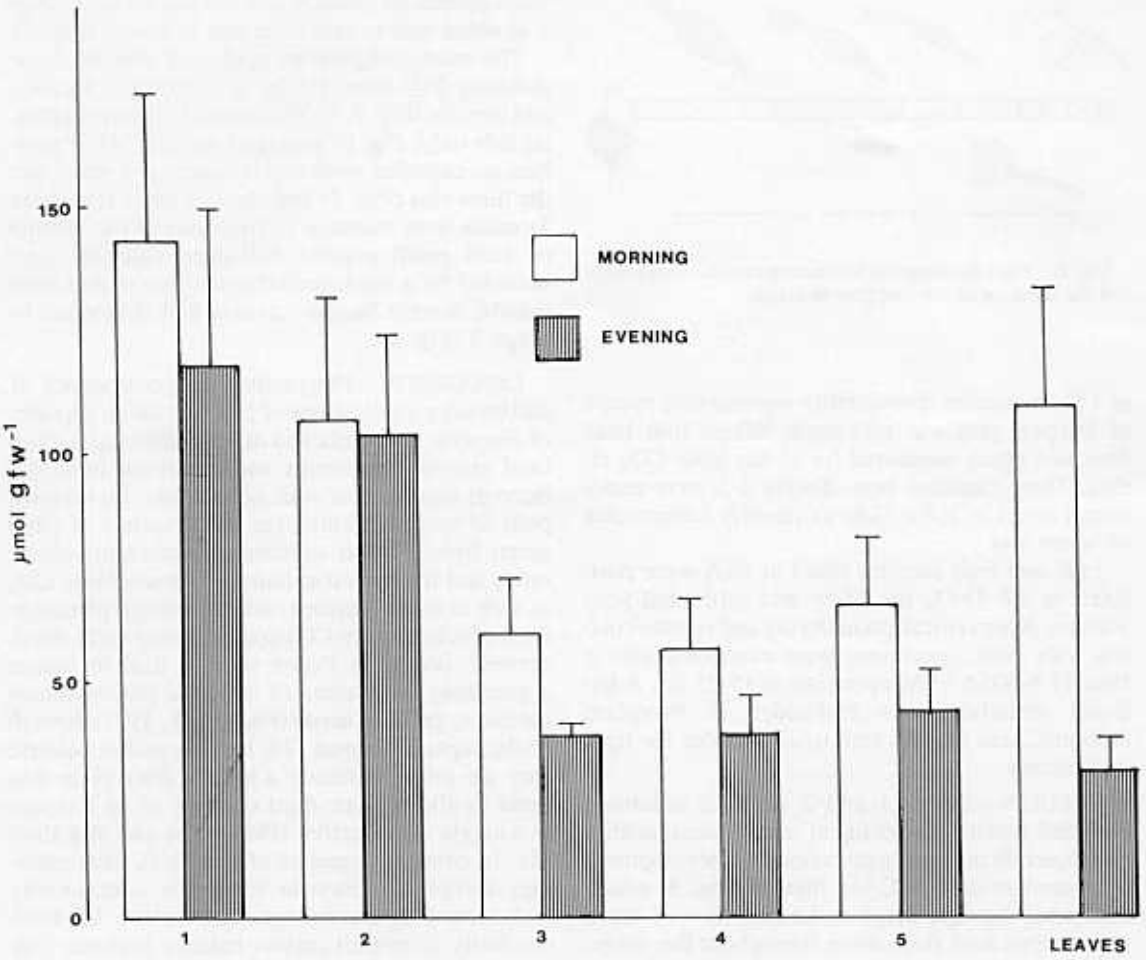


Fig. 3. Mean titratable acidity, including standard deviation (SD), at 7 a.m. and 7 p.m. in capsules and leaves. Differences between readings for Stages 1 and 2 are not statistically distinguishable (one-tailed Mann-Whitney U). Values for Stages 3-5 and foliage are significant ($P<0.001$).

in fruit of slow-growing *Encyclia tampensis* increases fitness through its positive effects on stress tolerance. In contrast, enhanced vigor is the principal advantage of capsule photosynthesis for *Pisum sativum* and for similar species in equable habitats where rapid maturation more than pronounced drought tolerance favors survival. Our argument, that benefits from green fruit tissue reflect plant life history and conditions in home ranges, is inspired by successful interpretation of leaf form and function (Chabot and Hicks, 1982) using tools borrowed from economists (Bloom et al., 1985). Other green organs presumably operate under some of the same rules, hence are equally amenable to cost/benefit analysis using the same currencies (i.e., carbon, water, and nitrogen).

Plant economics dictate that photosynthetic performance is determined by constraints related to organ (or whole canopy, etc.) architecture, cost and longevity (Table 1), and by ambient aridity and nutrient availability. The efficacy of the *E. tampensis* capsule for net photosynthesis should be below that of *Pisum sativum*; benefit from fruit-enhanced conservation and efficient use of scarce resources (particularly water) should, on the other hand, be paramount for this and other similarly constructed xerophytes. In effect, the advantages of net versus strictly regenerative photosynthesis (a continuous process that is otherwise similar to the transitory, stress-induced, CAM-idling mechanism of CAM xerophytes) are determined by the way carbon and growth-limiting supplies of water, nitrogen, and possibly other mineral nutrients are deployed in fruit compared to the way they are used in leaves on the same plant.

Substrates consumed for energy by developing

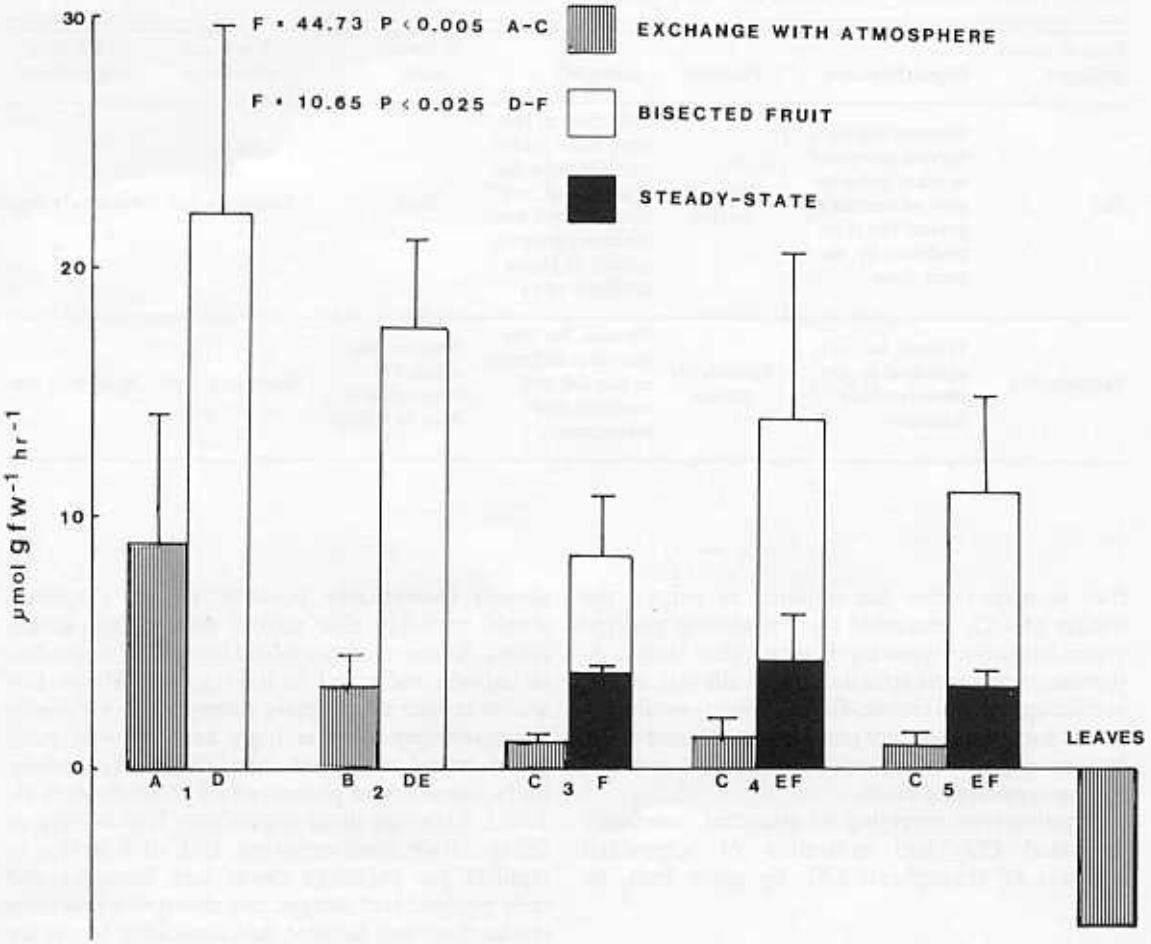


Fig. 4. Mean nocturnal CO_2 evolution rates ± SD by intact capsules and leaves and the initial burst of CO_2 from sectioned fruit and steady-state CO_2 15 min later. The negative value for leaves represents net CO_2 consumption. Values of F and P (Kruskal-Wallis ANOVA, or "K-W A") are indicated to the left of the key. Columns have been lettered so that those with the same letter are not significant (Student-Newman-Kuels, or "S-N-K"; $P<0.01$).

seed and associated heterotrophic tissue must be replaced at greater cost if, instead of being refixed, respired CO_2 is lost through fruit walls. Carbon recycling, including CAM-idling, unlike net photosynthesis, incurs no transpiration nor energy inputs for phloem import, but these two advantages alone do not determine how green tissue in fruit can most effectively promote plant success. Neither does capsule shape (which is rather poor for efficient light harvest and gas exchange in *E. tampensis*) fully explain why this epiphyte's fruit never exhibits net CO_2 uptake. Other species possessing thicker organs (e.g., cactus cladodes) that are even less suitable for harvesting CO_2 and photons, occasionally provide most or all required photosynthate, but only for species that possess no other type of organ capable of delivering equal or greater return on plant investment.

Two constraints based on form and time, plus a third due to peculiarity of the growing capsule, militate against plant investments that would permit *E. tampensis* fruit to perform net photosynthesis. First, there is foliage which, by its planar structure, is better suited for carbon gain than is any other part of the plant. Second, capsules live less than a year; foliage survives about three to four times longer, i.e., a year-old leaf has two or three more seasons to repay construction and maintenance costs and then turn an energetic profit. Finally, walls of ripening capsules tap an enriched internal CO_2 supply and fix it day and night with less protein (N) than leaves require to capture carbon at the same rate from the atmosphere—a more dilute and hence less accessible source in the sense of enzyme kinetics. Buildup and partial leakage of CO_2 indeed suggests that carboxylation capacity in *E. tampensis*

TABLE 1. *Additional features that distinguish organs characterized by net or regenerative photosynthesis.*

Type of photosynthesis	Organ Structure	Function	Longevity	N investment	Water use efficiency	Diffusive conductance
Net	Superior for light harvest compared to other green organs of similar or greater life span produced by the same plant	Primarily trophic	Sufficient to produce more photosynthate than that required for construction and maintenance (inversely related to photosynthetic rate)	High	Relatively low	Relatively high
Regenerative	Various, but subordinated to nonphotosynthetic functions	Secondarily trophic	Various, but less than that sufficient to pay for construction and maintenance	Various, but often less concentrated than in foliage	Relatively high	Relatively low

fruit is even below that required to recycle the stream of CO_2 generated by a relatively gastight organ containing growing heterotrophic tissue. Astomatous or more expensive fruit walls (i.e., richer in chlorophyll and carbon-fixing protein) would further reduce this loss, but perhaps not without creating unacceptable complications (anoxia?) or incurring unsupportable costs.

Simultaneous recycling of abundant, internally generated CO_2 and utilization of substantial amounts of atmospheric CO_2 by green fruit, although theoretically possible for *E. tampensis*, would probably also require unprofitable investments. Nitrogen inputs would have to be equivalent in capsule walls and in foliage, a condition that would reduce whole-plant nitrogen-use efficiency for autotrophy. Similar logic explains why most green orchid roots and stems, including pseudobulbs, show no net photosynthesis (Avadhani et al., 1982). Although these organs may live as long as foliage, their stout structure, lack of a device to regulate gas exchange (roots lack stomata), and their predominant storage and absorptive functions render them less suitable than associated leaves for vigorous photoassimilation (Benzing and Ott, 1981; Benzing et al., 1983). A conservation system similar to that in *E. tampensis* capsules probably exists in many orchid roots and stems, although almost certainly carbon turnover there is slower than in fruit. Foliage temporarily falls into this same category when drought-imposed CAM-idling slows photosynthesis and continuous stomatal closure eliminates access to atmospheric CO_2. This is also the period during which similarities in carbon and water balance (but not in budgets) between fruit and leaves of *E. tampensis* and those of *Pisum sativum* are greatest.

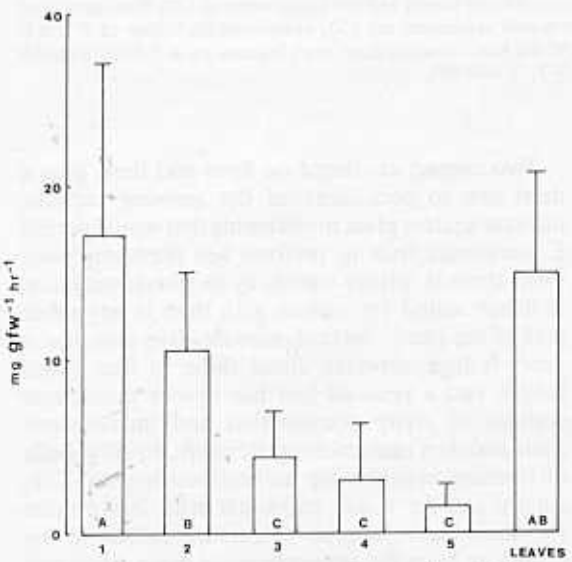


Fig. 5. Mean rates of nocturnal water loss ± SD from intact fruit and leaves. Differences between columns with the same letter are not significant (K-W A, F=21.96; P<0.001; S-N-K, P<0.01).

A few orchids lack developed foliage and instead photosynthesize via relatively N-rich roots (e.g., essentially shootless *Polyradicion*; Benzing and Ott, 1981; Benzing et al., 1983) or stems (e.g., leafless *Vanilla*). These exceptional taxa raise important questions about plant resource economy and unusual tradeoffs during angiosperm evolution. They also remind us that some of the greatest challenges facing evolutionary botanists today concern the reasons why organs that appear to be much better designed for one particular service are occasionally

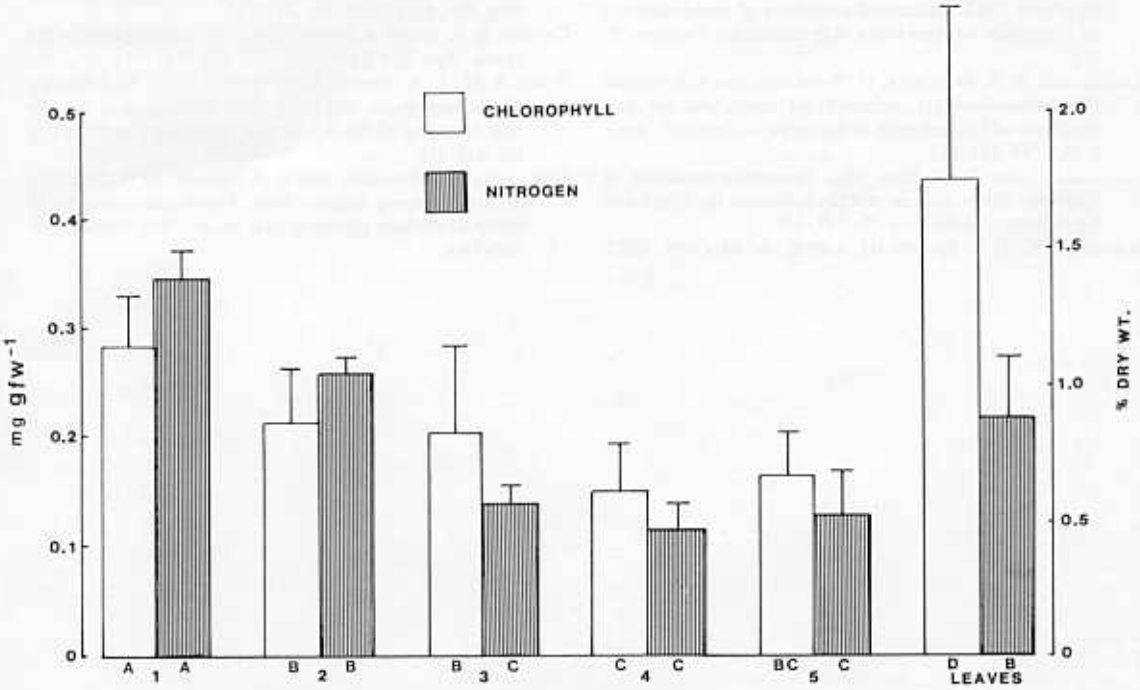


Fig. 6. Mean concentrations of chlorophyll and total N in fruits and leaves. Differences between columns with the same letter are insignificant. Chlorophyll: K-W A, F=18.90, P<0.001; S-N-K, P<0.001. Total N: K-W A, F=66.62, P<0.001; S-N-K, P<0.01.

co-opted for another wholly different function. Apparently, advantages powerful enough to override the biomechanical (Table 1) and environmental constraints that underlie the ancient division of labor that still describes tens of thousands of leafy relatives have affected several orchid lineages. The identity of these selective forces as well as important details of the shifts in form and function that represent responses to them remain largely unknown.

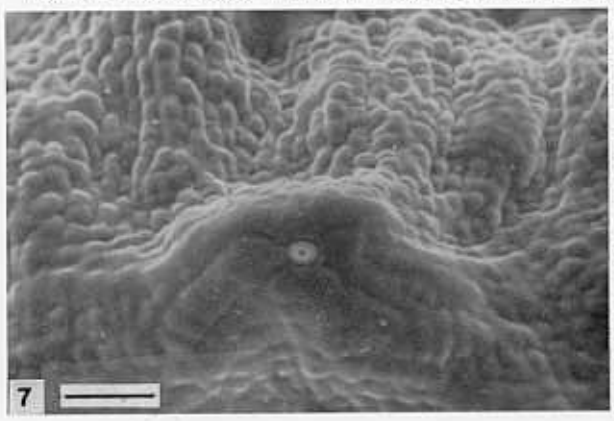
LITERATURE CITED

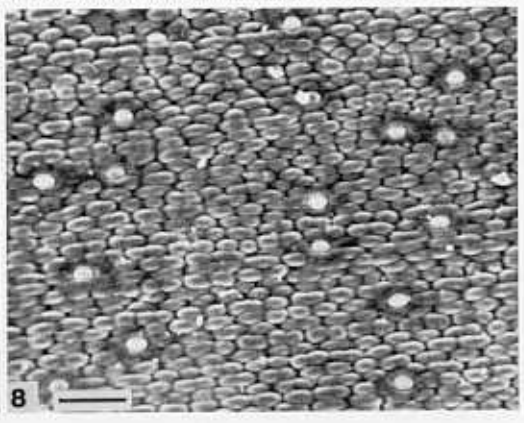
ARNON, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24: 1-15.

AVADHANI, P. N., C. J. GOH, A. N. RAE, AND J. ARDITTI. 1982. Carbon fixation in orchids. Pages 173-193 *in* J. Arditti [ed.], *Orchid biology: reviews and perspectives, II*. Cornell University Press, Ithaca, New York.

BAZZAZ, F. A., R. W. CARLSON, AND J. L. HARPER. 1979. Contribution to reproductive effort by photosynthesis of flowers and fruits. *Nature* 279: 554-555.

Fig. 7, 8. **7.** Capsule stoma at the summit of a papilla (bar=0.3 mm). **8.** Stomata on the abaxial leaf surface (bar=0.3 mm).





BENZING, D. H., A. BENT, D. MOSCOW, G. PETERSON, AND A. RENFROW. 1982. Functional correlates of deciduousness in *Catasetum integerrimum* (Orchidaceae). *Selbyana* 7: 1-9.

_____, W. E. FRIEDMAN, G. PETERSON, AND A. RENFROW. 1983. Shootlessness, velamentous roots, and the preeminence of Orchidaceae in the epiphytic biotope. *Amer. J. Bot.* 70: 121-133.

_____, AND D. W. OTT. 1981. Vegetative reduction in epiphytic Bromeliaceae and Orchidaceae: its origin and significance. *Biotropica* 13: 131-140.

BLOOM, A. J., F. S. CHAPIN III, AND H. A. MOONEY. 1985. Resource limitation in plants—an economic analogy. *Ann. Rev. Ecol. Syst.* 16: 363-392.

CHABOT, B. F., AND D. J. HICKS. 1982. The ecology of leaf life spans. *Ann. Rev. Ecol. Syst.* 13: 229-259.

FLINN, A. M., C. A. ATKINS, AND J. S. PATE. 1977. Significance of photosynthetic and respiratory exchanges in the carbon economy of the developing pea fruit. *Plant Physiol.* 60: 412-418.

PATE, J. S., P. J. SHARKEY, AND C. A. ATKINS. 1977. Nutrition of a developing legume fruit: Functional economy in terms of carbon, nitrogen, and water. *Plant Physiol.* 59: 506-510.