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DIURNAL STOMATAL AND ACIDITY RHYTHMS IN ORCHID LEAVES

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SUMMARY

The stomata of *Arachnis* cv. Maggie Oei, *Aranda* cv. Deborah, *Arundina graminifolia, Bromheadia finlaysoniana, Cattleya bowringiana* × *C. forbesii* and *Spathoglottis plicata* (Orchidaceae) occur only on the lower epidermis of the leaves and are located within hyperstomatic chambers formed by cuticular ledges extending from the guard cells. *Arachnis, Aranda* and *Cattleya* have thick leaves which exhibit Crassulacean acid metabolism, and their stomata open when acidity levels are lowest, or shortly thereafter. *Aranda* and *Arachnis* require higher light intensities for sufficient deacidification to permit stomatal opening than *Cattleya*. Stomata of the thin-leaved *Arundina, Bromheadia* and *Spathoglottis* open during the day. The stomatal rhythms, morphology and distribution, as well as the pathways of carbon fixation and light requirements for deacidification, reflect the natural habitat of each species or the parents of the three hybrids.

INTRODUCTION

Diurnal fluctuations of acidity in orchids were first reported nearly 100 years ago (Warburg, 1886). Since then there have been several reports of increased acidity as well as CO_2 uptake and stomatal opening in the dark in a number of species (Bendrat, 1929; Borriss, 1967; Coutinho, 1964, 1965, 1969, 1970; Coutinho and Schrage, 1970; Khan, 1964; Kristen, 1965; McWilliams, 1970; Milburn, Pearson and Ndegwe, 1968, Nuernbergk, 1963). More recently, dark $^{14}CO_2$ uptake and fixation have been shown to occur in the leaves of *Arachnis* cv. Maggie Oei (*Arachnis hookeriana* × *A. flos-aeris*), *A. hookeriana* var. *luteola, A. flos-aeris, Aeridachnis* cv. Bogor (*Arachnis hookeriana* var. *luteola,* × *Aerides odoratum*), *Aerides odoratum* and *Cattleya* (Borriss, 1967; Knauft and Arditti, 1969; Lee, 1970).

Other findings suggest that certain orchids may fix carbon via the C_4-dicarboxylic acid pathway (C_4-PS). These include mature leaves of *Arachnis* cv. Maggie Oei (Lee, 1970) and possibly *Cattleya* (Knauft and Arditti, 1969). However, this is not the case with *Arundina graminifolia, Bromheadia finlaysoniana, Coelogyne mayeriana, C. rochussenii, Cymbidium sinensis, C.* cv. Cym-doris, and *Eulophia keithii* (Avadhani and Goh, 1974; Hatch, Slack and Johnson, 1967; Wong and Hew, 1973).

Since epiphytic and saxicolic orchids exist under essentially xerophytic conditions, crassulacean acid metabolism (CAM) would hold the same advantages for them as for other xerophytes. Further, because some tropical orchids grow under high light intensities and temperatures, C_4-PS would have the same value for them as for grasses under similar conditions. An obvious question arising from consideration of carbon fixation pathways in

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orchids relates to diurnal acid fluctuations and stomatal rhythms. Studies on these rhythms were therefore made on some of the common orchid species and hybrids in Singapore.

MATERIALS AND METHODS

Plant material

Arachnis cv. Maggie Oei (thick and leathery leaves) and *Aranda* cv. Deborah (*Arachnis hookeriana* × *Vanda lamellata*; thick and rigid leaves) were obtained from the nursery of the Botany Department, University of Singapore, *Cattleya bowringiana* × *C. forbesii* (very thick and very rigid leaves), *Arundina graminifolia* (herbaceous leaves), *Bromheadia finlaysoniana* (leathery, but not very thick leaves) and *Spathoglottis plicata* (thin leaves) were obtained from the Orchid Collection of the Singapore Botanic Gardens. Healthy and uniform mature leaves from the mid-stem region of plants of flower-bearing age were selected for observation.

Plants were brought into a partially shaded courtyard one week in advance to allow for acclimatization. In the case of *Arachnis* and *Aranda*, measurements were also taken on leaves of plants growing in the field. All determinations were made during 2 weeks of successive 24-h periods.

Observation of stomata

Leaf cross-sections and epidermal peels were used to observe stomata. Stomatal density was determined by light microscope in an area measuring 1.6×10^{-2} cm^2. At least six determinations were made and the average expressed as number of stomata/cm^2.

Acidity measurements

Depending on the availability of material, 20 or 40 g fresh weight (FW) of leaves were extracted in boiling distilled water and their acidity determined by titration with 0.01 N NaOH using phenolphthalein as indicator (Osmond and Avadhani, 1970). The results are expressed as μequivalents of acid per g FW of tissue (μequiv./gFW).

Resistance measurements

Stomatal rhythms were investigated by means of a diffusive resistance meter (Lambda Instrument LI-60 with an LI-15S horizontal sensor). In order to eliminate errors due to possible air leakage or changes in leaf physiology, the measurement period was restricted to 75–90 s (meaning that the sensor was on the leaf for about 3–5 min). Thus, the maximum leaf resistance (r_l) that could be measured was about 65 s/cm.

On each of three plants of every orchid, the resistance of three leaves was determined. Measurements were made every 2–3 h and replicated three times. The results are expressed as conductance (C), the reciprocal of leaf resistance ($\times 100\, r_l$).

Light intensity measurements were made with a Weston Illumination Meter Model 756. A bead thermistor attached to the diffusive resistance meter allowed for the simultaneous determination of leaf temperature and resistance. A Casella Recording Thermograph was used to measure air temperatures.

RESULTS

Stomata

All the orchids used in this study have no stomata on the upper epidermis of their

leaves (Table 1). On the lower epidermis, *Arundina graminifolia* and *Bromheadia finlaysoniana* have the highest density, followed by *Spathoglottis plicata* and *Cattleya*; lowest densities are found in *Aranda* cv. Deborah and *Arachnis* cv. Maggie Oei (Table 1).

The guard cells of all six orchids have cuticular ledges which extend over the stomatal pore to form hyperstomatic chambers. Each chamber has a pore which varies in size according to species (Table 1). In *Bromheadia*, the ledges extend upward, whereas, in the other five orchids, they project up and bend towards the centre at a 90° angle. In surface view, the guard cells appear to have the usual kidney shape.

Titratable acidity

The titratable acidity of *Arachnis* cv. Maggie Oei placed in the partially shaded courtyard remained at around 50 μequiv./gFW throughout the 24-h period (Fig. 1e). When the

Table 1. *Stomatal distribution in mature orchid leaves*

Orchid species or hybrid	Leaf thickness (mm)	Stomatal density (number of stomata/cm²) Upper epidermis	Lower epidermis	Size of outer pore of stomata (μm) Width	Length
Arachnis cv. Maggie Oei	1.2	0	4000	12–15	12–15
Aranda cv. Deborah	1.6	0	3000	12–15	15–18
Cattleya bowringiana × *C. forbesii*	1.8	0	11000	9	6–8
Arundina graminifolia	0.3	0	18000	9–11	18–21
Bromheadia finlaysoniana	0.4	0	18000	6–9	6–9
Spathoglottis plicata	0.3	0	14000	4–9	10–15

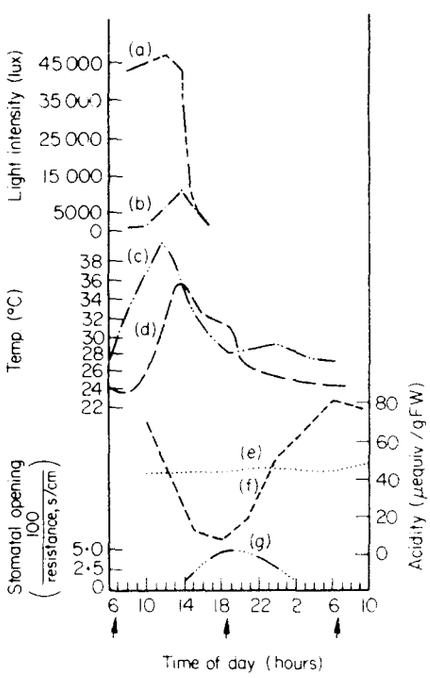


Fig. 1

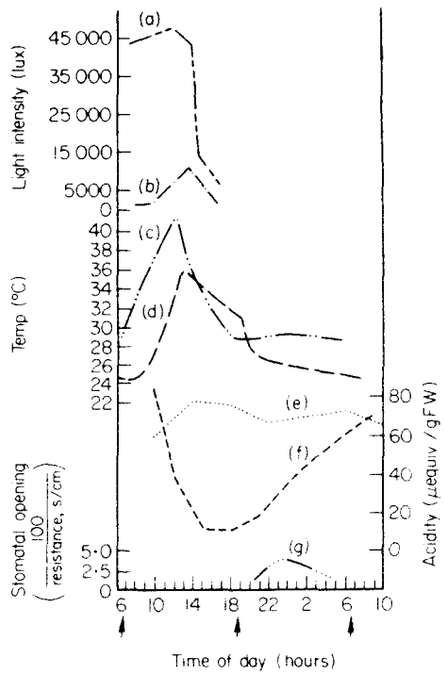


Fig. 2

Fig. 1. Stomatal and acidity rhythms in leaves of *Arachnis* cv. Maggie Oei. (a) Light intensity in open field (full sun); (b) light intensity in partial shade; (c) leaf temperature; (d) air temperature; (e) acidity of leaves in partial shade; (f) acidity of leaves under full sun; (g) stomatal opening as reciprocal of resistance ($100/r_1$). Arrows indicate time of dawn and dusk.

Fig. 2. Stomatal and acidity rhythms in leaves of *Aranda* cv. Deborah. Symbols as in Fig. 1.

plants were moved to the field, deacidification began at 09.00 hours; the titratable acidity reached a minimum at about 18.00 hours and increased thereafter to a maximum at 06.00 hours (Fig. 1f).

The titratable acidity of *Aranda* cv. Deborah placed in courtyard also remained constant, around 70 μequiv./gFW (Fig. 2e). In the field, deacidification began after 10.00 hours; the titratable acidity reached its minimum between 15.00 and 18.00 hours, then increased to a maximum by 10.00 hours (Fig. 2f).

The acidity of *Cattleya* leaves from the shade was high between 09.00 and 10.00 hours, after which it started to drop, reaching a minimum between 22.00 and 01.00 hours. When moved from shade to sun, the acidity dropped from about 175 to 85 μequiv./gFW (Fig. 3e). Deacidification was faster and greater when the plants were maintained under full sinlight in the field; the minimum was reached at 15.00 and the maximum between 06.00 and 10.00 hours (Fig. 3f).

Variations in the acidity of *Arundina graminifolia, Bromheadia finlaysoniana* and *Spathoglottis plicata* were small (Figs. 4, 5, 6).

Stomatal rhythms

The resistance of *Arachnis* cv. Maggie Oei and *Aranda* cv. Deborah leaves (Figs. 1, 2) in the courtyard was always higher than 65 s/cm; therefore, accurate measurements were not possible. However, an increased conductance was suggested in *Arachnis* cv. Maggie Oei between 20.00 and 06.00 hours when the measurement period was prolonged beyond the 90-s limit; a similar increase occurred in *Aranda* cv. Deborah between 23.00 and 07.00 hours.

In the field, under full sun, the diffusive resistance of *Arachnis* cv. Maggie Oei leaves started to drop (i.e., stomata began to open) at about 14.00 hours, reaching a minimum (maximum conductivity *C*) between 18.00 and 20.00 hours, then increasing to above 65 s/cm by 02.00 hours (Fig. 1g). The stomata of *Aranda* cv. Deborah leaves started to open at 20.00 hours, reached a maximum between 21.00 and 24.00 hours, and closed at 04.00 hours (Fig. 2g).

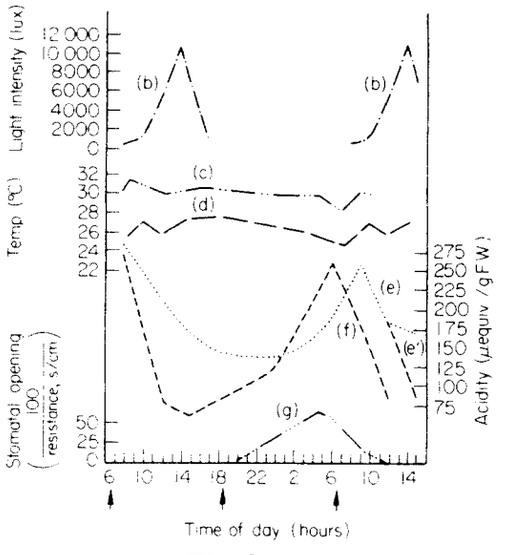


Fig. 3

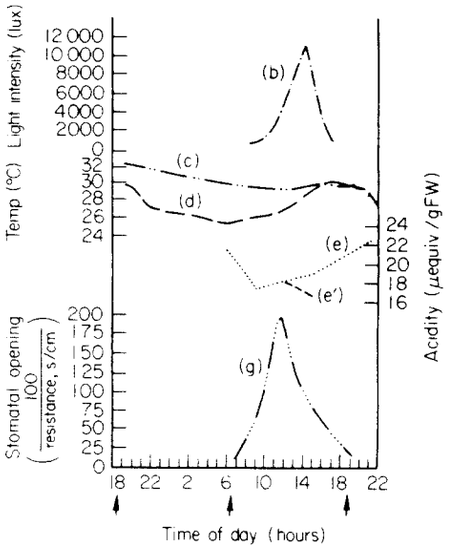


Fig. 4

Fig. 3. Stomatal and acidity rhythms in leaves of *Cattleya bowringiana* × *C. forbesii*. Symbols as in Fig. 1, plus (e') acidity of leaves moved from partial shade to full sun in open.

Fig. 4. Stomatal and acidity rhythms of *Arundina graminifolia*. Symbols as in Fig. 3.

In *Cattleya*, the stomata began to open at 21.00 hours, reaching maximum conductivity between 03.00 and 06.00 hours; by 09.30, the stomata were apparently closed (Fig. 3g). Most leaves showed the same general pattern, but there was very little synchrony among them. At one extreme, the stomata started to open at 21.00 hours, at the other, at 07.00 hours; time of stomatal closure ranged from 07.00 to 09.00 hours. In *Arundina graminifolia, Bromheadia finlaysoniana* and *Spathoglottis plicata*, the stomata remained open during the day (Figs. 4, 5, 6).

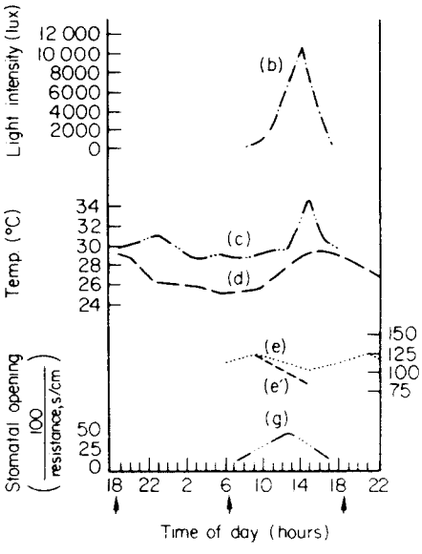


Fig. 5

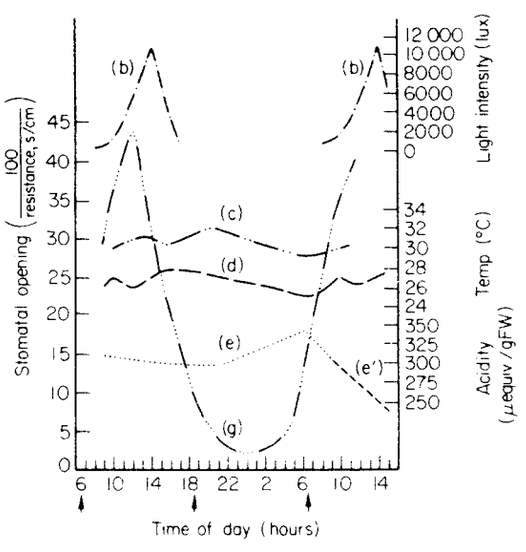


Fig. 6

Fig. 5. Stomatal and acidity rhythms of *Bromheadia finlaysoniana*. Symbols as in Fig. 3.

Fig. 6. Stomatal and acidity rhythms of *Spathoglottis plicata*. Symbols as in Fig. 3.

The stomata of *Arundina graminifolia* opened very quickly and simultaneously in the morning, but their closure in the afternoon was slower and less synchronous (Fig. 4g).

There was little synchrony in stomatal movement in *Bromheadia finlaysoniana* and opening and closing were not as fast as in the other two thin-leaved orchids (Fig. 5g).

Opening and closing of stomata in *Spathoglottis plicata* occurred at similar rates, but with some lack of uniformity within and between plants (Figs. 6g).

The diffusive resistance of the upper surfaces of the leaves of all six orchids was very high (*C* very low) and could not be recorded with the instrument used.

DISCUSSION

Since measurements on the upper epidermal surfaces lacking stomata (Table 1) indicate extremely high resistances, the resistances (r_1) of these orchid leaves reflect largely, if not solely, the stomatal resistances (r_s).

The stomata of the orchids studied vary in size, shape and distribution (Table 1). However, all possess cuticular ledges which cover the stomatal pore, forming a hyperstomatic chamber of the kind observed in *Paphiopedilum* (*Cypripedium*) *venustum* (Haberlandt, 1928), *Vanda tricolor* (Gessner, 1956) and *Cattleya bowringiana* (Borris, 1967). These chambers '... containing stagnant air ...' which '... remains undisturbed, and hence highly charged with water-vapour ...' are '... an arrangement for preventing excessive transpiration' (Haberlandt, 1928). They are common in xerophytes and are found in many orchids. Their presence supports the view that many orchids are xerophytes, a fact not widely appreciated (Gessner,

1956; Knauft and Arditti, 1969; Nuernbergk, 1963). The lack of stomata on the upper surfaces of orchid leaves which has been reported before (Withner, Nelson and Wejksnora, 1974) also contributes to water conservation.

The present findings show that maximum stomatal opening in these orchids can occur very early in the morning (*Cattleya*, Fig. 3g), just before noon (*Arundina*, Fig. 4g), during the early afternoon (*Bromheadia*, Fig. 5g and *Spathoglottis*, Fig. 6g), in the evening (*Arachnis*, Fig. 1g) and at night (*Aranda*, Fig. 2g). In the thick-leaved orchids, maximal stomatal opening coincides with, or shortly follows, the lowest acid content (Fig. 1c). This relationship has also been reported for *Epidendrum* (Coutinho, 1965), *Kalanchoe manurata, K. blossfeldiana* and *Bryophyllum daigremontianum*, but not for *Bryophyllum calycinum* (Nishida, 1963). There are no clear patterns among the thinleaved orchids although in some cases, stomatal opening coincides with lower acidity levels (Fig. 4f).

That stomata close when the CO_2 level increases and open when it decreases is well known. In the thick-leaved orchids which exhibit CAM, stomatal movement appears to be regulated by dark fixation of CO_2 in the mesophyll cells. This lowers the CO_2 level in the internal atmosphere which brings about the opening of stomata. In thin-leaved orchids in which stomatal opening occurs during the day, photosynthetic CO_2 fixation could bring about the same effect.

The stomata of *Arachnis* cv. Maggie Oei and *Aranda* cv. Deborah did not open in the shade of the courtyard during the 24-h period and the acid content of these plants remained high (Fig. 1e, 2e). It is possible, therefore, that no carbon fixation occurred during this time. Since respiration undoubtedly continues, CO_2 probably accumulates in the internal atmosphere of leaves so that the stomata fail to open. In the field, the stomata of *Arachnis* and *Aranda* opened at night (Fig. 1g, 2g); acidification during this period (Fig. 1f, 2f) reduces the internal CO_2 content leading to stomatal opening. This would be especially the case if malate is transported rapidly into the vacuoles, as is considered essential for continued carboxylation in *Bryophyllum calycinum* (Avadhani and Tan, 1974). *Cattleya* behaves in a similar fashion, but its acid content drops even in the shade (Fig. 3e); the stomata open at night when acidification sets in.

The production of malate resulting from dark CO_2 fixation has been observed previously in *Arachnis* (Lee, 1970) *Aranda* and *Cattleya* (Avadhani, unpublished; Knauft and Arditti, 1969). Hence, it is not surprising that stomatal opening and leaf acidity increase in these orchids during periods of dark carbon fixation.

Since the magnitudes of acidification and/or deacidification have been shown to be related to the transport of acid (Avadhani and Tan, 1974), it is reasonable to expect these processes to be influenced by cation fluxes. In this connection, it is interesting to note that stomatal movements have been shown to be associated with potassium fluxes (Dayanandan and Kaufman, 1975).

Of the three thick-leaved orchids studied, only *Cattleya* showed enough deacidification in the shade to allow for stomatal opening whereas, in *Arachnis* and *Aranda*, deacidification occurred only in high light intensities. It is relevant to note that both *Arachnis* cv. Maggie Oei and *Aranda* cv. Deborah are hybrids of species which grow in nature under higher intensities than *Cattleya bowringiana* and *C. forbesii* (Ames and Correll, 1952; Holttum, 1953). Clearly, the light requirements of the three hybrids reflect the ecological habitats of their parents.

Of the three thin leaved orchids studied, *Arundina graminifolia* is always found in open and sunny places in the lowlands and highlands of Malaya, never in the shade; *Bromheadia*

finlaysoniana grows in open scrub and light secondary forest, whereas full sun is the preferred habitat of *Spathoglottis plicata* (Holttum, 1953). All three species fix carbon during the day via the C_3-photosynthetic pathway (C_3-PS) (Avadhani and Goh, 1974; Wong and Hew, 1973). Again, the pathways of carbon fixation and stomatal rhythms of these orchids reflect their natural habitats.

A special and potentially very interesting case is provided by *Arachnis* cv. Maggie Oei. It exhibits dark fixation and low temperature enhancement of acidification typical of CAM plants. In addition, there is also evidence that it may fix carbon via C_4-PS (Lee, 1970). Its stomata start to open early in the day when light intensities are high enough for C_4-PS (Fig. 1). Malate and aspartate, possible intermediates of C_4-PS, have also been found in *Cattleya* (Knauft and Arditti, 1969). It is possible, therefore, that these orchids carry out CAM and C_4-PS. On the basis of fragmentary evidence, it may be speculated that *Arachnis* cv. Maggie Oei may exhibit CAM, C_3-PS and C_4-PS as has been suggested for *Sedum* (Avadhani, Osmond and Tan, 1971). However, much additional work is required if this speculation is to be corroborated.

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