

Carbon Fixation by *Paphiopedilum insigne* and *Paphiopedilum parishii* (Orchidaceae)*†

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ABSTRACT

Measurements of stomatal conductance, $^{14}\text{CO}_2$ fixation and acidity levels indicate that *Paphiopedilum insigne* (Walhch) and *P parishii* (Rchb. f) Stein, Orchidaceae, do not fix carbon via the Crassulacean acid metabolism pathway.

Key words: Acidity, C_3 pathway, CAM, stomatal conductance

Thin-leaved orchids fix carbon via the C_3 pathway, thick-leaved ones do so through Crassulacean acid metabolism (CAM) There is no firm evidence regarding the existence of the C_4 pathway in orchids (Withner, 1959, 1974, Nuernbergk, 1963, Szarek and Ting, 1977, Kluge and Ting, 1978, Arditti, 1979, Avadham and Arditti, 1981, Avadhani *et al*, 1982) The available information about *Paphiopedilum* Phtz is inconclusive According to one report *Paphiopedilum* cf. Mildred Hunter (a very complex hybrid) is a CAM plant (Rubenstein *et al*, 1976), but nocturnal acidification does not occur in *P villosum* (Lindl.) Stein (Bendrat, 1929) or *P venustum* (Walhch) Pfitz. McWilhams, 1970), which has been described as a C_3 plant (Thorpe, 1980) like an unidentified *Paphiopedilum* Nuernbergk, 1963) It is possible that conclusive evidence regarding carbon fixation by *Paphiopedilum* is not available partly because the water stress of plants used in previous experiments is not clear.

P insigne (Walhch) Pfitz (subgenus *Paphiopedilum* Bneger) is a terrestrial species from Assam and Nepal, with thin leaves (0.65-0.8 mm, mesophyll 0.5 mm) and therefore probably not a CAM plant *P. parishii* (Rchb. f) Stein (subgenus *Brachypetalum* Hall) is an epiphyte from Burma, Thailand and Yunnan, China (Grant, 1895, Graham and Roy, 1983) Because of its thick, almost fleshy leaves (2.0-2.1 mm, mesophyll 0.83 mm) it is the most likely species of *Paphiopedilum* to fix carbon via CAM These species were selected as models for the genus because they represent two extremes in leaf thickness (Karasawa and Saito, 1982)

The plants were watered well (i.e. they were not under water stress) and maintained in a greenhouse under a night temperature of $18-5\pm 1.5^\circ\text{C}$, a day temperature of $25-5\pm 2.5^\circ\text{C}$, with a 12 h photoperiod, and high solar irradiance Gas-exchange parameters were measured with a dual-isotope porometer that feeds simultaneously ^3HOH and $^{14}\text{CO}_2$ to the underside of a leaf Stomatal conductance and carbon dioxide fixation rates were calculated using isotope uptake (Johnson, Rowland and Ting, 1979) Acidity was determined by titration of leaf samples to pH 7.0 endpoint using an automatic titrator and 0.01 N NaOH (Goh *et al*, 1971) Replicate samples (three per species during two 24 h periods) were taken every 3 h starting at 09:00 hours.

* The nomenclature adopted in this paper is for the most part that of Cribb (1983)

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Donovan, Arditti and Ting—Carbon Fixation by Paphiopedilum

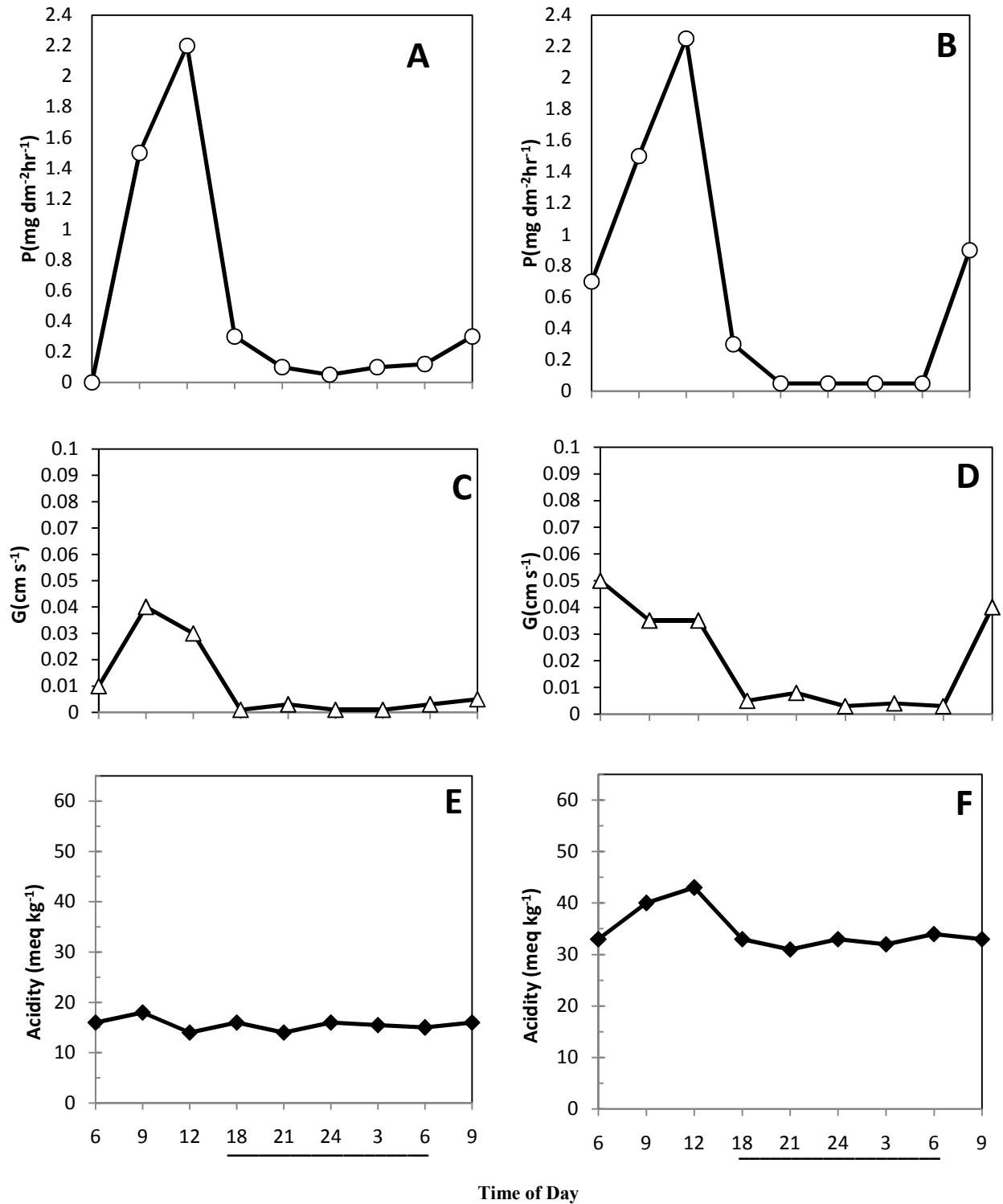


Fig 1. Carbon fixation (*P*) and stomatal conductance (*G*) as measured in a greenhouse with dual-isotope porometer and acidity A, C, E, in *Paphiopedilum parishii* (Rchb f) Stein, B, D, F, in *P. insigne* (Wallich) Pfitz Numbers on the horizontal axis are the time of day, with night hours underlined Acidity is given in milliequivalents per kilogram fresh weight

Donovan, Arditti and Ting—Carbon Fixation by *Paphiopedilum*

Virtually all carbon fixation by both species occurred during daylight hours. Only a small amount was fixed during the beginning and towards the end of the dark period by *P parishii* (Fig 1. C, D) The stomata of *P. insigne* opened earlier in the morning than those of *P. parishii*. All gas exchange data are typical of C₃ plants and show no indication of any CAM activity Titratable acidity over a 24 h period was different for each species and somewhat high (Fig 1. E, F) Such high acidity levels are unexpected for C₃ plants, but not indicative of CAM due to the lack of any discernible diurnal fluctuation.

Neither *P. insigne* nor *P. parishii* exhibits physiological characteristics of CAM orchids. Therefore, our findings are consistent with previous reports regarding carbon fixation by an unspecified plant of *Paphiopedilum* (Nuernbergk, 1973), *P. villosum* (Bendrat, 1929), and *P. venustum* (McWilliams, 1970; Thorp, 1980) The question of the 'C₃-CAM' *Paphiopedilum* cv. Mildred Hunter (Rubenstein *et al.*, 1976) remains unsolved.

All *Paphiopedilum* species studied to date, including the thick-leaved *P. parishii*, are apparently C₃ plants and do not show evidence of CAM. Thus, contrary to previous speculations (Kluge and Ting, 1978), leaf thickness may not be a reliable indicator for the presence of CAM in orchids Other species of *Paphiopedilum* in the subgenus *Brachypetalum* [e.g. *P. bellatum*, *P. niveum* (Richb f) Stein and *P. concolor* (Batem) Plitz, as well as *P. godefroyae* (Godefr Lebeuf) Stein, which may be a variety of *P. bellatum*], with leaves as thick as those of *P. parishii*, have yet to be examined Several species of the Aizoaceae and Portulacaceae shift from C₃ photosynthesis to CAM during drought (Kluge and Ting, 1978) Furthermore, some species of *Peperomia* Ruiz & Pav (Piperaceae) when water-stressed shift from apparent C₃ photosynthesis to a condition in which stomata are closed both day and night, but organic acids tend to fluctuate diurnally just as in CAM (Kluge and Ting, 1978, Ting, 1982). Evidently, respiratory CO₂ is recycled through the CAM pathway which is induced by drought. These species, when not performing CAM, have high levels of organic acids similar to *P. parishii* and *P. insigne* (Fig 1 E, F) Therefore the potential for CAM may be present in some *Paphiopedilum* species, but we do not know how these plants respond to drought However, it is important to bear in mind that under water stress 'CO₂ assimilation [by other orchids] was greatly reduced and exclusively nocturnal' (Fu and Hew, 1982)

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