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Short Communication

Gas Exchange in *Paphiopedilum*[1]

LACK OF CHLOROPLASTS IN GUARD CELLS CORRELATES WITH LOW STOMATAL CONDUCTANCE

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ABSTRACT

Net photosynthesis and stomatal conductance were measured in attached leaves of *Paphiopedilum insigne*. At 20°C and a vapor-pressure deficit of 0.5 kilopascal, both net photosynthesis and stomatal conductance were light-saturated below 0.2 millimole per square meter per second, a response typical of shade plants. The absolute values of photosynthetic rate and conductance however were remarkably low, presumably reflecting an adaptation to the low-light, limited-nutrient habitat characteristic of these orchids. The leaves also showed a vapor-pressure deficit response, with net photosynthesis and conductance varying over a 2-fold range between 0.3 and 1.6 kilopascals.

These results confirm that *Paphiopedilum* stomata are functional. The correlation between achlorophyllous guard cells and low conductance rates, however, singles them out as an exceptional biological system, exhibiting basic differences from typical stomata in higher plants. Available evidence showing that guard-cell chloroplasts are needed to sustain high conductance rates at moderate to high irradiances indicates that the genetic changes leading to the loss of chloroplast differentiation in *Paphiopedilum* guard cells were not deleterious because of the low conductance rates characteristic of this genus.

The genus *Paphiopedilum*, Orchidaceae, has attracted considerable attention from stomatal physiologists because of the lack of chloroplasts in its guard cells (12, 17, 19, 21). With the exception of *Paphiopedilum* chloroplasts are remarkably conserved in guard cells of virtually all species (9, 23). This structural invariance argues for a central role of guard-cell chloroplasts in stomatal function, although its precise nature remains to be elucidated. Recent findings demonstrating a capacity of guard-cell chloroplasts to photophosphorylate (10, 13, 18), and the characterization of a photon efficiency of stomatal conductance under blue and red light (24) indicate that guard-cell chloroplasts function to sustain high conductance rates under moderate to high irradiances (22). The apparent functional normalcy of stomata from *Paphiopedilum* (8, 12), especially their reported ability to respond to blue and red light (12), appears to contradict these findings and deserves investigation.

Here we report on gas-exchange analysis of attached leaves of *P. insigne*, in which stomatal conductance and net photosynthesis were simultaneously measured. Both photosynthesis and stomatal conductance changed with light and VPD[3] but the absolute values of these two leaf responses were low. These low prevailing rates of net photosynthesis and stomatal conductances can provide an explanation for the apparent functional normalcy of *Paphiopedilum* stomata despite their lack of chloroplasts.

MATERIALS AND METHODS

Plants of *Paphiopedilum insigne* were purchased from a local nursery and grown in a greenhouse at 20° ± 5°C, with prevailing RH around 50%. The plants were shaded to avoid high irradiances, which were observed to cause leaf damage. Gas exchange in attached leaves was measured in a differential system (2, 11) at an ambient CO_2 concentration of 340 μl/l, a leaf temperature of 25°C, and a VPD of 0.5 kPa. In experiments measuring VPD responses, the water-vapor concentration of the air supplied to the leaf chamber was changed while keeping the chamber at constant temperature.

RESULTS

Net photosynthesis and stomatal conductance in an attached leaf of *P. insigne*, as a function of increasing irradiance is shown in Figure 1. Both leaf functions saturated at irradiances below 0.2 mmol m^{-2} s^{-1}, or about 10% of full sunlight, a typical saturation value for a shade plant (1). The absolute values of the responses, however, were unusually low (1, 5); in comparison, leaves from *Viola sempervirens*, a species native to the understory of a redwood forest, where it grows in extreme shade, had saturating rates of photosynthesis and conductance of 6 μmol CO_2 m^{-2} s^{-1} and 5.5 mm s^{-1}, respectively, at an irradiance of 0.2 mmol m^{-2} s^{-1} and an ambient CO_2 concentration of 340 μl/l (Castellanos and Zeiger, unpublished). Extensive measurements of stomatal conductance in *P. insigne* and *P. harrissianum* leaves kept in a greenhouse, obtained with a LiCor steady-state porometer, were usually less than 1 mm s^{-1} (data not shown). As discussed below, *Paphiopedilum* leaves are very sensitive to VPD, suggesting that the lower conductance values measured with the porometer in the greenhouse were most likely a result of higher prevailing VPD (see Fig. 2). Both sets of data, however, showed that leaves from *Paphiopedilum* have very low rates of stomatal conductance, which appear associated with the slow growth typical of these orchids.

The decreases in net photosynthesis and conductance seen at higher irradiances (Fig. 1) are indicative of photoinhibition. The extent and threshold levels of this inhibition, however, varied

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[3] Abbreviation, VPD, vapor pressure deficit.

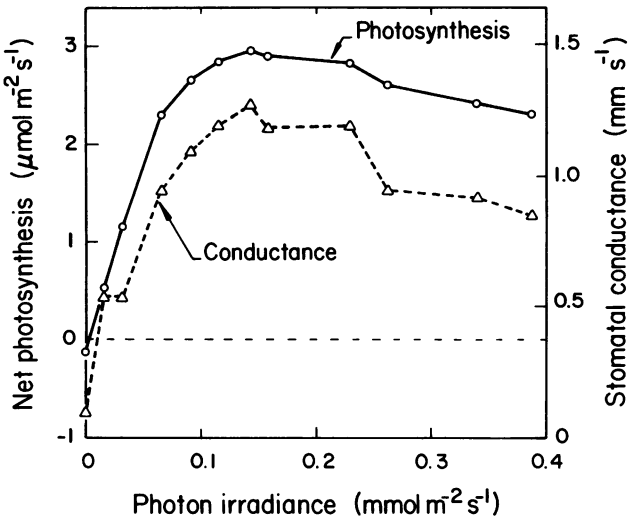

FIG. 1. Net photosynthesis (O——O) and stomatal conductance (Δ- - -Δ) in an attached leaf of *P. insigne* as a function of increasing intensities of white light. Leaf temperature was 20°C and the VPD was 0.5 kPa.

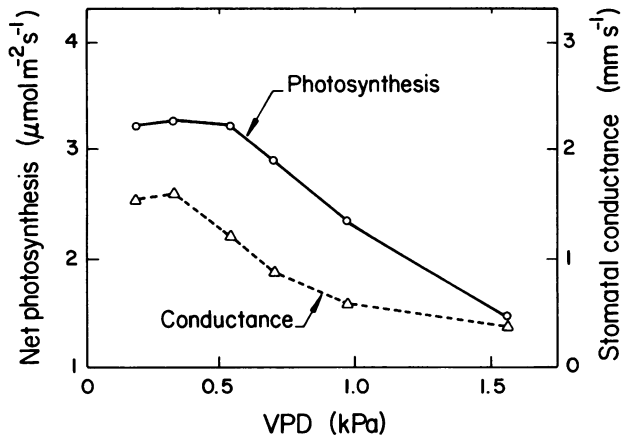

FIG. 2. VPD response of an attached leaf of *P. insigne*, at 20°C. Light intensity was 0.13 mmol m^{-2} s^{-1}. (O——O), Net photosynthesis; (Δ- - -Δ), stomatal conductance.

markedly between leaves.

A typical VPD response of these leaves is shown in Figure 1. Both stomatal conductance and net photosynthesis decreased sharply with increasing VPD. While low intercellular CO_2 concentrations most likely limit photosynthesis at the higher VPD values, the steep reductions in net photosynthesis observed in the low range of the VPD curve could be indicative of a direct inhibition of photosynthesis by the increasing VPD (3).

DISCUSSION

The kinetics of net photosynthesis and stomatal conductance in intact leaves of *P. insigne* in response to light and VPD are similar to those observed in other species having chloroplasts in their guard cells (4). This confirms previous reports indicating that stomata from *Paphiopedilum* are functional (8, 12). On the other hand, the observed maximum rates of net photosynthesis and stomatal conductance were remarkably low, even for shade plants (1, 5). The low conductance values were not restricted to *Paphiopedilum*; we measured comparable rates in two other genera of this family, *Cymbidium* and *Phragmipedium* (Zeiger, Grivet, and Williams, unpublished). These low photosynthetic rates probably constitute an adaptation to the low-light, limited-nutrient habitat of these Orchidaceae, and their conductance rates exhibit concomitantly low values because of the characteristic coupling of the two leaf functions (3, 20, 24).

These prevailing low rates of stomatal conductance could provide an explanation for the apparent functional normalcy of *Paphiopedilum* stomata, despite the absence of chloroplasts in their guard cells. Guard-cell chloroplasts appear crucial as an energy source sustaining high conductance rates at moderate to high irradiances (10, 13, 24). The genetic changes causing the loss of chloroplast differentiation in *Paphiopedilum* would have been deleterious in species requiring high conductances, but appear to have been harmless in *Paphiopedilum* because of the low prevailing conductance rates. Whether this loss provides any intrinsic advantage under those growing conditions, for example by preventing high stomatal conductances under high irradiances, with net photosynthesis having saturated at low irradiances, remains to be determined.

If our interpretation is correct, the normal functioning of *Paphiopedilum* stomata does not provide evidence for the lack of a role of guard-cell chloroplasts in stomatal movements (6, 7, 12, 14–16); it rather constitutes an exceptional biological system whose stomatal light responses depend solely on the blue light photosystem of the guard cells (22; Zeiger, Assmann, and Meidner, unpublished), with oxidative phosphorylation of guard-cell mitochondria providing an additional energy source for ion transport (22; Assmann and Zeiger, unpublished). Comparative studies of *Paphiopedilum* and related genera of the Orchidaceae having guard-cell chloroplasts should be useful for further investigations on the role of these organelles in stomatal function.

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