Control of Paraheliotropism in Two *Phaseolus* Species[1]

Fei Yu[2] and Virginia S. Berg*

Department of Biology 0421, University of Northern Iowa, Cedar Falls, Iowa 50614

Paraheliotropic (light-avoiding) leaf movements have been associated with high light intensity, high temperature, and drought. We investigated leaf elevation for intact plants, pulvinus bending for excised motor organs, and size change for protoplasts from motor tissue for two *Phaseolus* species: *Phaseolus acutifolius* A. Gray, native to hot, arid regions, and *Phaseolus vulgaris* L., the common bean. Leaf angles above horizontal were measured for central trifoliolate leaflets of intact plants at 24, 27, and 30°C at 500 and 750 μmol photons (400–700 nm) m^{-2} s^{-1} over a range of water potentials; equivalent angles were determined for excised motor organs under similar conditions. Diameters were measured for protoplasts from abaxial and adaxial motor tissue over a range of photon flux density values, temperatures, and water potentials. In general, higher photon flux density and temperature resulted in elevation of leaves, bending of excised pulvini, and equivalent changes in protoplast volume (swelling of abaxial protoplasts and shrinking of adaxial protoplasts). In intact plants, lower water potentials yielded greater paraheliotropism; abaxial protoplasts increased in size, whereas adaxial ones did not change. *P. acutifolius* typically exhibited greater paraheliotropism than did *P. vulgaris* under the same conditions, a set of physiological responses likely to be highly adaptive in its native arid habitat.

Many plants experience water deficits on a daily or seasonal basis or between natural or artificial irrigation events. Faced with the prospect of injuriously low water potentials, plants must regulate water use, usually by stomatal closure. For many plants, including a substantial number of legumes, light-avoiding leaf movements (paraheliotropism) also reduce water loss (Darwin, 1881; Dubetz, 1969; Mooney and Ehleringer, 1978). In the field, as paraheliotropism increases, light interception by leaves decreases, reducing leaf temperatures to below those of static, horizontal leaves (Gates, 1980). This reduces transpirational water loss (Forseth and Ehleringer, 1980, 1982), keeps leaf temperatures nearer photosynthetic optimum (Fu and Ehleringer, 1989), and lessens the potential for photoinhibition (Ludlow and Björkman, 1984; Gamon and Pearcy, 1989).

Leaves incline upward through changes in cell size in the motor tissue of the pulvinus at the base of each leaf or leaflet: abaxial motor cells swell, and adaxial motor cells shrink (Satter and Galston, 1981). (For clarity, no distinction will be made between the pulvinus at the base of leaves and the pulvinule at the base of leaflets.) Ion flux through cell membranes causes an osmotically induced water flux, changing cell volume (Pfeffer, 1881; Satter et al., 1974; Lee, 1990). Paraheliotropic leaf movements in intact plants are promoted by conditions associated with current and future low water potential: high temperatures (Fu and Ehleringer, 1989), high PFD (Berg and Heuchelin, 1990), and low water potential (Shackel and Hall, 1979; Oosterhuis et al., 1985; Berg and Hsiao, 1986; Kao and Forseth, 1991). Because these factors often occur together in the field, and even in the laboratory, it has been difficult to establish the independence and interaction of the effects.

Darwin (1881) observed that many species in arid environments exhibited paraheliotropism and discussed the potential advantages of such responses in such habitats. Because plant species and varieties vary in their leaf movements (Wofford and Allen, 1982; Travis and Reed, 1983), closely related taxa from contrasting environments may exhibit differences in paraheliotropic responses that would be beneficial in their respective habitats. The two species examined here are closely related members of the genus *Phaseolus*: *Phaseolus vulgaris*, common bean, a highly productive species from mesic habitats, and *Phaseolus acutifolius*, tepary bean, a less productive species typically found in arid habitats. The objectives of this study were to characterize the independent and interactive effects of PFD, water potential, and temperature in whole plants of two contrasting species, to examine the responses of excised pulvini of these species, and to determine whether protoplasts act in a manner consistent with that of intact plants and excised organs. By examining the responses of excised organs and protoplasts, investigators can determine whether these units can act autonomously, without direct input from the rest of the plant, and ascertain whether at least some of the differences between species are located in the motor organ. In addition, differences between the responses of adaxial and abaxial tissue can be detected.

MATERIALS AND METHODS

Plant Material and Growing Conditions

Phaseolus acutifolius var *latifolius* G. Freeman and *Phaseolus vulgaris* cv Red Cloud Bean were grown in 15-cm pots in a well-fertilized 2:2:2:1 mixture of soil:perlite:peat moss:sand. The seeds were germinated at 26°C and transferred to a greenhouse at the University of Northern Iowa; all plants were grown during the summer, when typical midday temperatures were near 30°C and PFD values were near 1400

[2] Present address: Botany Department, Bessey Hall, Iowa State University, Ames, IA 50011.

* Corresponding author; fax 1-319-273-2893.

Abbreviation: PFD, photon flux density.

μmol m^{-2} s^{-1}. Each pot was placed in a shallow plastic box to which 100 to 200 mL of water were added daily, the amount depending on transpirational demand. Plants were fertilized with a 200 ppm 20-20-20 (N-P-K) solution every other week. Insecticides were applied as needed to control white flies.

Measurements on Intact Plants

Two days before measurement, plants of similar developmental stage were transferred to the growth chamber. Plants in the controlled environment growth chamber (Conviron E-15; Controlled Environments, Winnipeg, Canada) were subjected to 16 h of light and 8 h of dark daily. PFD (400-700 nm) was 500 μmol m^{-2} s^{-1} (quantum sensor and light meter; Li-Cor, Lincoln, NE). Light was provided by an overhead bank of fluorescent lamps (160-W VHO; Sylvania, Danvers, MA) supplemented by incandescent bulbs (150-W Excel, Sylvania). A 500-nm cutoff filter (Optical Coding Lab, Inc., Santa Rosa, CA) and the quantum sensor (above) were used to determine that 21% of this light was between 400 and 500 nm, the range associated with paraheliotropic leaf movement. Temperatures (day/night) were 24/18°C, 27/20°C, or 30/20°C. Light and temperature increased gradually from 0700 to 0900 h, remained constant through the next 12 h, and then declined gradually from 2100 to 2300 h. For each treatment, 10 to 14 plants were supplied with different amounts of water to establish a range of water potentials.

Measurements were taken on the terminal leaflet of the first trifoliolate leaf when the latter was just fully expanded, about 30 d after germination. For each plant, the angle between the leaf lamina and horizontal was measured with an inclinometer (Angle Finder; Dasco Pro, Rockford, IL). Since the light source was overhead, all leaf angles above horizontal represent paraheliotropism; negative angles indicate leaves below horizontal. Leaf conductance was measured with a steady-state porometer (Li-Cor 1600); water potential was measured using a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA). Plants were measured at approximately midday in the growth chamber at the temperature under which they were grown in the chamber. At 27°C, plants were measured at a PFD of 500 or 750 μmol m^{-2} s^{-1} to determine the effect of PFD over a range of water potentials. At a PFD of 500 μmol m^{-2} s^{-1}, plants were measured at 24, 27, or 30°C. At each PFD or temperature, two groups of five (P. acutifolius) or seven (P. vulgaris) plants were measured. At 27°C and 750 μmol m^{-2} s^{-1}, two additional groups of plants were measured.

Measurements of Excised Pulvini

Measurements were taken when the first trifoliolate leaf was just fully expanded. For 3 d before the pulvini were excised, all plants were placed in the growth chamber at 27/20°C (day/night) and 500 μmol m^{-2} s^{-1} and subjected to mild drought for 2 d (by withholding water) to stimulate paraheliotropism. Plants were well watered for 1 d before the pulvini were excised; no paraheliotropism was evident at the beginning of the measurement period. Terminal leaflets were excised 1 cm below the pulvinus, and leaf lamina were re-moved, leaving a 1-cm length of midvein to facilitate measurement of the angle between the petiolule and the midvein with a protractor. Pulvini were excised at approximately 12 PM to minimize interaction with circadian rhythms. The petiolules of the excised pulvini were held horizontal, with the end of the petiolule in distilled water. The pulvinus was placed in the growth chamber, and then angle measurements were recorded initially and at intervals throughout the trial (approximately 2 h total).

The angles of three sets of excised pulvini were measured at 27°C at 500 or 1000 μmol m^{-2} s^{-1}, using five pulvini each time for each PFD level. Similar measurements were obtained for three sets of pulvini at 1000 μmol m^{-2} s^{-1} for six pulvini each at 18, 21, 24, 27, 30, and 33°C. The maximum angle from each pulvinus was used for comparisons among temperatures and species.

Measurements of Motor Tissue Protoplasts

Adaxial and abaxial tissues were dissected from motor organs of both species and treated separately to isolate protoplasts. For P. vulgaris, the sides of the motor organ were first sliced off; for both species, the adaxial and abaxial epidermal tissue were then sliced off, leaving the vascular cylinder with adaxial and abaxial motor tissue attached. To avoid differences due to endogenous rhythmic effects, all pulvini were isolated at 8 AM. Motor tissue from approximately 30 pulvini of P. acutifolius or 20 pulvini of P. vulgaris was weighed, chopped finely in 1 mL of plasmolysis solution (described below), then added to 4 mL of the same solution and allowed to plasmolyze for 30 min. The plasmolyzed tissue was then incubated with 3 mL of enzyme solution (described below) for 2 h, after which it was filtered through 221-μm nylon mesh, followed by a wash of 3 mL more plasmolysis solution. The filtrate was then passed through 62-μm nylon mesh, which was washed with 3 mL of additional plasmolysis solution. The resultant filtrate was centrifuged at low speed (25g) for 10 min at room temperature, and the protoplasts in the pellet were then suspended in sorbitol solution (-1.0 to -2.0 MPa, depending on subsequent measurements) supplemented with 20 mM KCl and 1 mM CaCl$_2$. Yield was typically 1.7×10^6 to 6.2×10^6 protoplasts/g tissue, estimated by counting samples of protoplast suspension.

Solutions were as follows. Plasmolysis solution: 0.6 M sorbitol, 1 mM CaCl$_2$, 5 mM Mes (Sigma), adjusted to pH 5.6. Enzyme solution: plasmolysis solution supplemented (w/v) with 0.5% macerozyme R-10, 2% cellulase "Onozuka" R-10 (both Yakult Honsha, Tokyo, Japan, from Ganematsu-Gosha, Los Angeles, CA), 0.15% pectolyase Y-23 (Seishin Pharmaceutical, Tokyo, Japan, from Ganematsu-Gosha), 0.5% BSA (Sigma), adjusted to pH 5.6.

The diameters of three independent samples of 50 to 100 protoplasts were measured for each treatment (given below), tissue type (adaxial and abaxial), and species, using a microscope with an ocular micrometer. Protoplast suspensions were placed in a temperature-controlled glass-bottomed aluminum cuvette and measured after approximately 10 min, because Kim et al. (1992) showed that ion flux from motor cell protoplasts occurred immediately after changes in con-

ditions. Illumination was maintained at 100 or 1000 μmol m^{-2} s^{-1}; 8 and 13%, respectively, of the light was between 400 and 500 nm. Measurements were made between 1100 and 1400 h on the day of isolation to avoid interaction with rhythmic responses.

The effect of PFD was tested by measuring the size of protoplasts from adaxial and abaxial tissues at 100 and 1000 μmol m^{-2} s^{-1}, each at 27 and 30°C, using −1.0 MPa (0.4 molal) sorbitol solution as a bathing medium. The effect of temperature was tested by measuring protoplast diameter at 24, 27, 30, and 33°C at 100 μmol m^{-2} s^{-1} PFD in −1.0 MPa bathing solution. (The 27 and 30°C treatments were the ones used to test PFD effect.) The effect of water potential was determined by maintaining protoplasts at 24°C and 100 μmol m^{-2} s^{-1} PFD with sorbitol bathing solutions at different water potentials (−2.0, −1.5, or −1.0 MPa); sorbitol solutions were supplemented with 20 mM KCl and 1 mM CaCl$_2$.

Statistical Analysis

All statistical analyses were performed using Crunch software (version 3; Crunch, Oakland, CA). Analysis of variance was used unless otherwise specified. Repeated-measures-design analysis of variance was used whenever comparisons dealt with a series of sequential measurements of the same plants or tissue.

RESULTS

Measurements on Intact Plants

For both species, leaf angle and leaf conductance were affected by leaf water potential, by PFD, or by both (Fig. 1). Leaf angles of *P. acutifolius* were significantly correlated with water potential at both 500 and 750 μmol m^{-2} s^{-1} (P = 0.004 and 0.002, respectively). There was no significant difference between the two regressions. Leaf conductance was significantly correlated with water potential at both PFD values (P = 0.003 and 0.001 for 500 and 750 μmol m^{-2} s^{-1}, respectively). The regressions were significantly different at P = 0.01. Leaf angles of *P. vulgaris* were significantly correlated

with water potential at 750 μmol m^{-2} s^{-1} (P = 0.008) but not at 500 μmol m^{-2} s^{-1} (P = 0.067). Leaf conductance was significantly correlated with water potential at both PFD values (P = 0.001 for both). The regressions were significantly different at P = 0.01.

For *P. acutifolius*, leaf angle and leaf conductance were affected by leaf water potential and by temperature (Fig. 2). Leaf angles of *P. acutifolius* were significantly correlated with water potential at temperatures of 24, 27, and 30°C (P < 0.001 for all). Leaf conductance was significantly correlated with water potential at 24, 27, and 30°C (P < 0.001 for all). Leaf angles of *P. vulgaris* were not significantly correlated with water potential at 24°C (P = 0.887). The relationship was nearly statistically significant at 27°C (P = 0.067) and was significant at 30°C (P = 0.014). There was a significant difference between the two regressions against water potential for 27 and 30°C (P < 0.050). Leaf conductance was not significantly correlated with water potential at 24°C, but the relationship was significant at 27 and 30°C (P < 0.001 for both).

Measurements of Excised Pulvini

For both species, pulvinar angles increased to maximum values in approximately 20 to 30 min and then declined, attaining near steady values by 45 to 60 min; this value was maintained until measurements ended after 2 h (Fig. 3). Pulvini of *P. acutifolius* had higher angles than those of *P. vulgaris* at equivalent times; there was a significant difference between species (P < 0.001). For each species, PFD had a significant effect on pulvinar angle (P = 0.001 and <0.001 for *P. acutifolius* and *P. vulgaris*, respectively).

There was an increase in pulvinar angle associated with increases in temperature for both species (Fig. 4). Both species and temperature had significant effects on pulvinar angle (P < 0.001 for both). There was no significant difference between species at 18 or 21°C, but they were significantly different at higher temperatures (P < 0.03 for all).
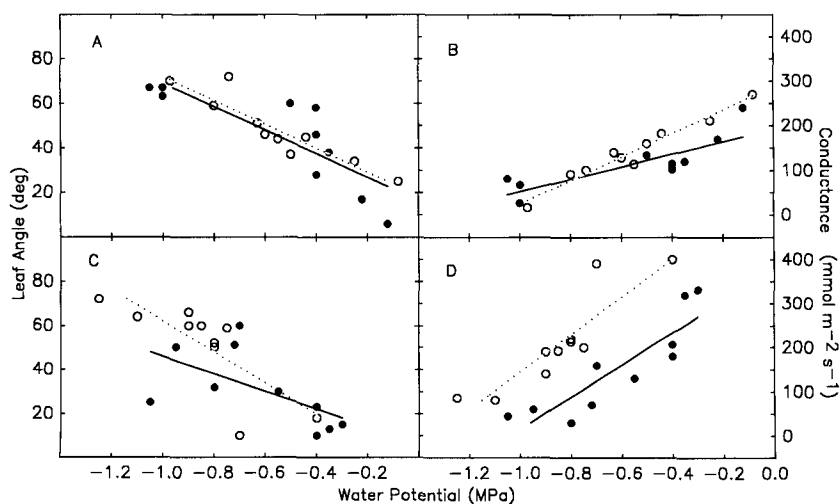

Figure 1. Effect of water potential and PFD on leaf angle and leaf conductance of *P. acutifolius* and *P. vulgaris*. A and B illustrate effects on *P. acutifolius*; C and D illustrate effects on *P. vulgaris*. Filled and open circles represent data from trials at 27°C (both) with PFD values of 500 and 750 μmol m^{-2} s^{-1}, respectively. Solid and dotted lines represent regressions for trials at 500 and 750 μmol m^{-2} s^{-1}, respectively.

Figure 2. Effect of water potential and temperature on leaf angle and leaf conductance of *P. acutifolius* and *P. vulgaris*. A and B illustrate effects on *P. acutifolius*; C and D illustrate effects on *P. vulgaris*. Filled circles, open circles, and squares represent conditions in which the temperatures were 24, 27, and 30°C, respectively. Solid, dotted, and dashed lines represent corresponding regressions.
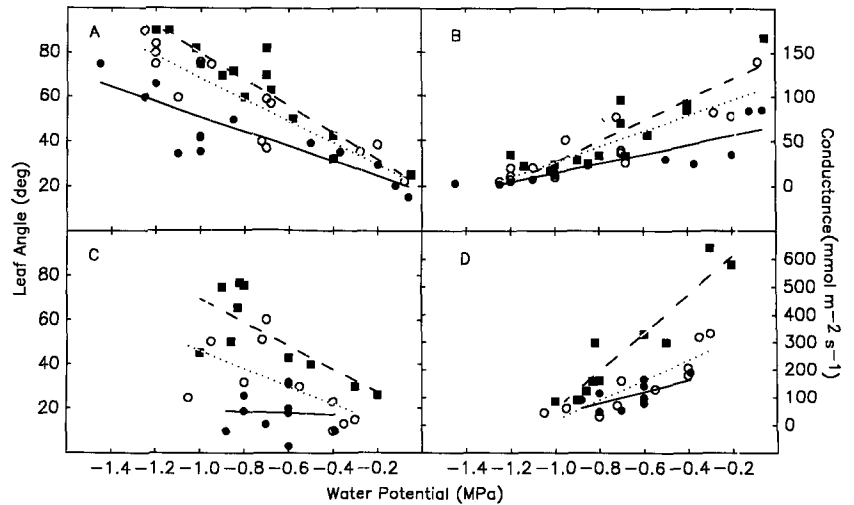
Measurements of Motor Tissue Protoplasts

Mean diameters of protoplasts isolated from adaxial tissue were greater at 100 μmol m^{-2} s^{-1} than at 1000 μmol m^{-2} s^{-1} at both temperatures tested and for both species (Fig. 5). Mean diameters of protoplasts from abaxial tissue were smaller at 100 μmol m^{-2} s^{-1} than at 1000 μmol m^{-2} s^{-1} for both temperatures and both species. The effect of PFD was significant for adaxial and abaxial tissues for both species (Fig. 5; $P < 0.001$ for protoplasts from both adaxial and abaxial tissue of *P. acutifolius*; $P = 0.001$ and 0.005 for corresponding protoplasts of *P. vulgaris*). Temperature had a significant effect for protoplasts from adaxial tissue of both species (Fig. 5; $P < 0.001$ for both *P. acutifolius* and *P. vulgaris*), but protoplasts from abaxial tissue did not have

significantly different sizes when measured at 27 and 30°C (Fig. 5).

Mean diameters of protoplasts from adaxial tissues decreased significantly as temperature increased over the range 24 to 33°C, and those of protoplasts from abaxial species increased significantly as temperature increased for *P. acutifolius* (Fig. 6; $P < 0.001$ for both). For *P. vulgaris*, mean diameters of protoplasts from adaxial tissues decreased significantly with increasing temperature (Fig. 6; $P = 0.004$), whereas temperature had no significant effect on the size of protoplasts from abaxial tissue. Increased water potential was associated with a significant increase in mean diameter of protoplasts from adaxial tissues of both species (Fig. 7; $P < 0.001$ for both). There was no significant effect of water potential on mean diameter of protoplasts from abaxial tissues from either species (Fig. 7).
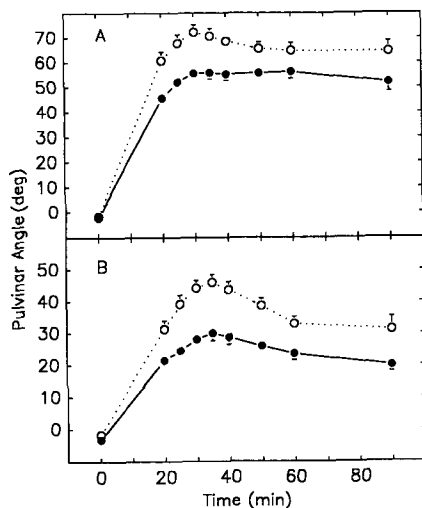
DISCUSSION

Paraheliotropism and associated cell size changes were controlled by PFD, temperature, and water potential. Responses were consistent at the whole plant, motor organ, and cell levels.
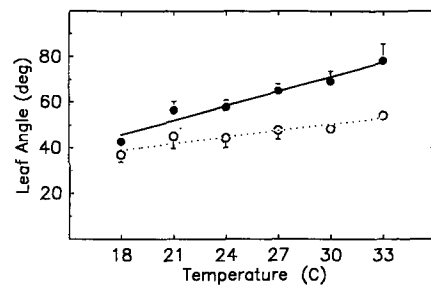

Figure 3. Effect of PFD on pulvinar angle of excised pulvini of *P. acutifolius* and *P. vulgaris*. Pulvini were measured at an air temperature of 27°C. A, Data from *P. acutifolius*; B, data from *P. vulgaris*. Filled circles and solid lines indicate pulvini at 500 μmol m^{-2} s^{-1}; open circles and dotted lines indicate pulvini at 1000 μmol m^{-2} s^{-1}. Error bars indicate SE.

Figure 4. Effect of temperature on pulvinar angle of excised pulvini of *P. acutifolius* and *P. vulgaris*. Measurements were made at 1000 μmol m^{-2} s^{-1} PFD. Filled circles and solid line represent symbols and regression from *P. acutifolius*; open circles and dotted line represent symbols and regression from *P. vulgaris*. Error bars indicate SE.
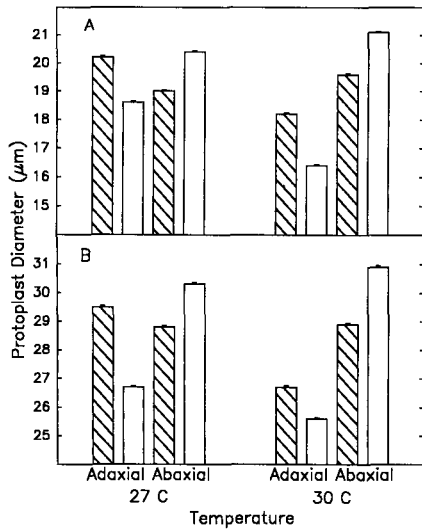
Figure 5. Effect of PFD on diameter of isolated motor tissue protoplasts at two temperatures. A and B illustrate effects on *P. acutifolius* and *P. vulgaris*, respectively. Shaded and open bars represent PFDs of 100 and 1000 μmol m^{-2} s^{-1}, respectively. Error bars represent SE.

Protoplasts of both species responded to changes in temperature and water potential at the lowest PFD level used, 100 μmol m^{-2} s^{-1} (Figs. 5–7). Erath et al. (1988) similarly demonstrated protoplast swelling at low PFD levels. Activity at low PFD is to be expected, because the PFD within abaxial pulvinar tissues is low (Donahue et al., 1990). Initial angles of excised pulvini of both species (start of trials, Fig. 3) indicate the lack of paraheliotropism at very low PFD (room lighting, approximately 10 μmol m^{-2} s^{-1}), but subsequent
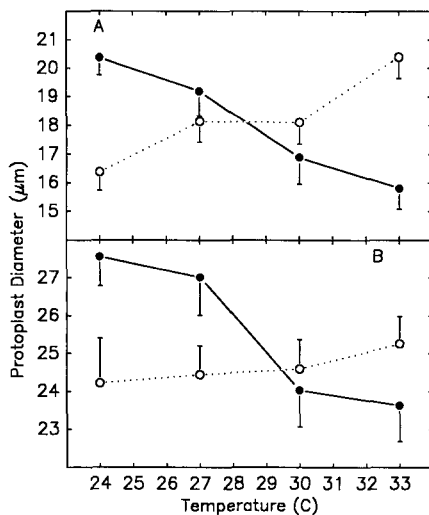

Figure 6. Effect of temperature on diameter of isolated motor tissue protoplasts. A and B illustrate effects on *P. acutifolius* and *P. vulgaris*, respectively. Filled circles represent protoplasts from adaxial motor tissue; open circles represent those from abaxial motor tissue. Data given are from a single trial. Error bars represent SE.
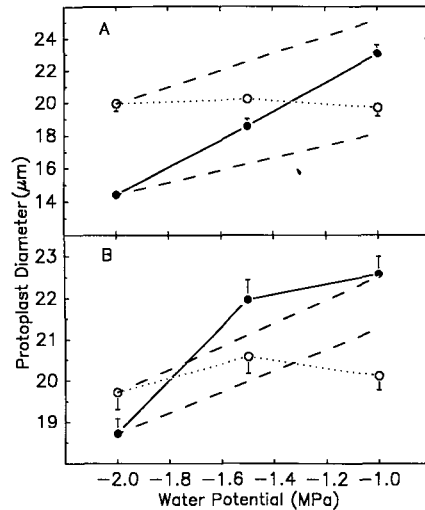

Figure 7. Effect of water potential on diameter of isolated motor tissue protoplasts. A and B illustrate effects on *P. acutifolius* and *P. vulgaris*, respectively. Filled circles represent protoplasts from adaxial motor tissue; open circles represent those from abaxial motor tissue. Dashed lines indicate the change in diameter based on osmotic effects alone for protoplasts of each tissue if taken from −2.0 to −1.0 MPa. Data given are from a single trial. Error bars represent SE.

changes in angle demonstrate a response at 500 μmol m^{-2} s^{-1} (Fig. 3). Although intact plants did not exhibit significant paraheliotropism at 500 μmol m^{-2} s^{-1} when cool and at high water potential (Fig. 2), both *P. acutifolius* and *P. vulgaris* exhibited a paraheliotropic response to a PFD of 500 μmol m^{-2} s^{-1}, when plants were under some water stress and at elevated temperature (Figs. 1 and 2). Although several field and laboratory studies have shown that many species exhibit little paraheliotropism at low PFD (Darwin, 1881; Forseth and Ehleringer, 1982; Berg and Hsiao, 1986; Fu and Ehleringer, 1989), low PFD conditions were typically accompanied by high water potential and relatively low temperature, the usual combination in the field.

For protoplasts, the pattern of swelling (abaxial protoplasts) and shrinking (adaxial protoplasts) with increased PFD (Fig. 5) corresponds well with the response of excised pulvini, where organs at 1000 μmol m^{-2} s^{-1} exhibited greater paraheliotropism than those at 500 μmol m^{-2} s^{-1} (Fig. 3). The fact that no difference was observed between intact plants at 500 μmol m^{-2} s^{-1} and those at 750 μmol m^{-2} s^{-1} was probably due to the small difference in PFD. Previous research has showed that PFD has a significant effect on paraheliotropism of *P. vulgaris* (Sato and Gotoh, 1983) and *Glycine max* (Berg and Heuchelin, 1990), but for those studies the PFD differences were greater. In all of these studies of intact plants, high irradiance may have increased the pulvinus temperature, adding a compounding temperature effect. In the present study, pulvinus temperatures were 4 to 5°C above air temperature, with no significant differences in temperature elevation among species, air temperatures, or PFD levels (F. Yu and V.S. Berg, unpublished data).

Both *P. acutifolius* and *P. vulgaris* exhibited increased re-

sponses as temperature increased, a situation consistent with the findings of Fu and Ehleringer (1989) for leaf movements of well-watered (but not necessarily high water potential) plants of *P. vulgaris*. In the present study, protoplasts responded to changes in temperature, i.e. increasing temperature led to larger abaxial protoplasts for *P. acutifolius* (+93% volume change) and smaller adaxial protoplasts for *P. acutifolius* and *P. vulgaris* (−53 and −37% volume changes, respectively; Fig. 6), again consistent with the elevation of leaves. Similar results were obtained for excised pulvini (Fig. 6) and for intact plants (Fig. 2). Elevated temperature also alters rhythmic leaf movements of *Samanea*, enhancing leaf opening but slowing leaf closure (Satter and Galston, 1981); related patterns have been observed for *Samanea* protoplasts (Satter et al., 1987; Lee and Satter, 1988).

Paraheliotropism in response to lowered water potential has been observed in the field and in the laboratory (Forseth and Ehleringer, 1980; Oosterhuis et al., 1985; Berg and Hsiao, 1986; Berg and Heuchelin, 1990). In the present study, plants of both *P. acutifolius* and *P. vulgaris* responded to water potential, although the responses of the two species appear to have different thresholds of temperature and PFD (Figs. 1 and 2). At the lowest temperature and PFD level tested, 24°C and 500 μmol m^{-2} s^{-1}, *P. acutifolius* exhibited a significant paraheliotropic response to changes in water potential (Figs. 1 and 2). For *P. vulgaris*, no paraheliotropic response to water potential was evident under these conditions; either a higher temperature (30°C at 500 μmol m^{-2} s^{-1}; Fig. 2) or a higher light level and temperature (750 μmol m^{-2} s^{-1} at 27°C; Fig. 1) was necessary to elicit a significant response. It generally appears that changes in temperature multiply the effect of water potential in stimulating paraheliotropism by whole plants (Fig. 2). The effect of water potential on the response of excised pulvini was not tested because of difficulty in getting uptake of osmoticum into excised organs; therefore, no conclusions can be drawn from these data regarding the interaction between water potential and temperature or PFD for excised motor organs.

Changes in leaf angle arise from changes in the turgor and size of motor cells in the pulvinus, with opposite responses expected from cells from abaxial and adaxial tissue. Protoplasts, not constrained by cell walls, should exhibit corresponding volume changes (Nobel, 1991). When the water potential of the bathing solution was lowered from −1.0 to −2.0 MPa, the volume of protoplasts would be expected to decrease 50% because of osmotic effects alone. Deviations from the expected change indicate active responses of the cells to the external conditions, as water flux is driven by the osmotic response to ion flux. Protoplasts from adaxial motor tissue decreased in volume more than would be expected from osmotic changes alone (Fig. 7; 76 and 63% for *P. acutifolius* and *P. vulgaris*, respectively), indicating additional ion flux from the protoplasts. In contrast, the volume of protoplasts from abaxial tissue decreased less than expected (Fig. 7; 0 and −10% volume change for *P. acutifolius* and *P. vulgaris*, respectively), indicating additional ion flux into the protoplasts. Protoplasts from abaxial motor tissues changed relatively little in size between −2.0 and −1.0 MPa bathing solutions, indicating that active ion flux essentially just countered the osmotic effects. A much greater change in diameter

was observed for protoplasts from adaxial tissue, where ion flux added to osmotic effects. This size change is consistent with the response of whole plants. In the case of *Samanea*, both sides of the pulvinus are under compression, so changes in leaf angle are associated with changes in the turgor pressure difference between abaxial and adaxial tissue (Gorton, 1987a, 1987b). Ion flux associated with volume change in protoplasts would result in a change in the turgor of walled cells.

Although several investigators have shown the functioning of protoplasts in a manner associated with rhythmic movement (Iglesias and Satter, 1983; Lee and Satter, 1987, 1988), this is the first report of changes in cells consistent with paraheliotropic leaf movements. This indicates that each motor cell contains all of the components necessary for this response, including the receptors that detect blue light (Donahue and Berg, 1990), the membrane-based pumps and ion channels (Gorton, 1990), and the metabolic power supply. However, this does not preclude interaction with the rest of the plant through hormones and through factors influencing water potential, such as leaf and root area, rooting patterns, and stomatal conductance.

Species differences were apparent at all levels. Given the same conditions of water potential, temperature, and PFD, *P. acutifolius* exhibited a stronger response than did *P. vulgaris*, whether measurements were of leaves of intact plants (change in orientation, Figs. 1 and 2), of excised pulvini (change in shape, Figs. 3 and 4), or of protoplasts (change in volume, Figs. 5–7). Furthermore, *P. acutifolius* was active even at low temperatures and PFD levels. The more sensitive paraheliotropic response acts to reduce water loss whenever water is in short supply, as indicated by low plant water potential. In addition, the stomatal conductance of *P. acutifolius* was characteristically lower than that of *P. vulgaris* under the same conditions, further conserving water. Paraheliotropism and stomatal conductance were closely coordinated in *P. acutifolius* ($r > 0.7$ and P < 0.004 for all temperatures at 500 μmol m^{-2} s^{-1}), giving this species two parallel, additive mechanisms for conserving water. Paraheliotropism in *P. vulgaris* occurs only at the higher temperatures and PFD levels and is less pronounced. Under the same conditions, leaves of *P. vulgaris* would therefore be exposed to higher temperatures, higher water loss, and higher PFD levels; negative consequences may include excess water loss, leaf temperatures above the optimum for photosynthesis (Fu and Ehleringer, 1989), and photoinhibition associated with high PFD and temperature and low water potential and stomatal opening (Ludlow and Björkman, 1984; Gamon and Pearcy, 1989).

The fact that *P. acutifolius* increases paraheliotropism in response to high PFD and temperature, as well as to low water potential, means that this species can act as a more effective drought postponer than can *P. vulgaris* under the very conditions under which low plant water potential is likely to develop and persist. The degree and sensitivity of the paraheliotropic responses of these two species fit well with the environments in which the plants are normally found. The paraheliotropic response of *P. acutifolius*, the species found in hot, sunny, arid habitats, conserves water in response to low water potential; it also responds to high

PFD and temperature, conditions likely to lead to water deficits. Unlike developmentally controlled responses to environmental stress, leaf movements are fast and reversible. Combined with small leaves and leaf area, a greater root to shoot ratio, and tolerance of high temperature (Markhart, 1985; Sanders and Markhart, 1992), paraheliotropism adds to the capacity of this species to survive in extreme conditions. In contrast, *P. vulgaris* exhibits characteristics that help increase yield in more favorable conditions: large leaf area, open stomates (Markhart, 1985), and leaves with greater light interception through reduced paraheliotropism. Thus, paraheliotropism adds to other characteristics that distinguish between the two species in terms of adaptation to and interaction with their usual habitats.

ACKNOWLEDGMENTS

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