

# Stable Isotopes and Plant Carbon-Water Relations

Edited by

**James R. Ehleringer**

Stable Isotope Ratio Facility  
for Environmental Research  
Department of Biology  
University of Utah  
Salt Lake City, Utah

**Anthony E. Hall**

Department of Botany and Plant Science  
University of California  
Riverside, California

**Graham D. Farquhar**

Plant Environmental Biology Group  
Research School of Biological Sciences  
Australian National University  
Canberra, Australia

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## Environmental and Developmental Effects on Carbon Isotope Discrimination by Two Species of *Phaseolus*

**Qingnong A. Fu, Thomas W. Boutton, James R. Ehleringer, and  
Richard B. Flagler**



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### I. Introduction

Isotopic discrimination ( $\Delta$ ) against  $^{13}\text{CO}_2$  during photosynthesis in  $\text{C}_3$  plants is positively correlated with the long-term, integrated ratio of intercellular to atmospheric  $\text{CO}_2$  concentration ( $c_i/c_a$ ), a parameter which reflects the balance between consumption of  $\text{CO}_2$  by photosynthetic activity and supply of  $\text{CO}_2$  through stomatal diffusion (Farquhar *et al.*, 1982, 1989). This relationship has been shown to fit the equation

$$\Delta = a + (b - a)(c_i/c_a), \quad (1)$$

where  $a$  is the isotopic fractionation due to the slower diffusion of  $^{13}\text{CO}_2$  versus  $^{12}\text{CO}_2$  in air (4.4‰), and  $b$  is the net isotopic fractionation associated with carboxylation activities (~27‰) (Farquhar *et al.*, 1989). As a consequence of an independent relationship between  $c_i/c_a$  and water-use efficiency (the ratio of photosynthetic carbon gain ( $A$ ) to transpirational water loss ( $E$ )),  $\Delta$  is negatively correlated with  $A/E$  (Farquhar and Richards, 1984; Farquhar *et al.*, 1989). This relationship between  $\Delta$  and  $A/E$  has been verified under controlled laboratory conditions and in the field (Farquhar and Richards, 1984; Hubick *et al.*, 1986; Farquhar *et al.*, 1989) and has been used extensively to estimate  $W$ , long-term  $A/E$  in crops and native plants. Because  $c_i/c_a$  in  $\text{C}_3$  plants is responsive to environmental conditions (e.g., irradiance, soil moisture, soil nutrient status, salinity, gaseous air pollutants),  $\Delta$  can function as a useful integrator of plant physiological response to environment.

In this study, we have investigated the effects of environment and plant developmental status on carbon isotope discrimination by two species of *Phaseolus*. *Phaseolus vulgaris* L. (common bean) is the most important of the five cultivated species in the genus *Phaseolus*. However, this species is very drought-sensitive, and yields are often reduced by even mild water stress (Haterlein, 1983; Castonguay and Markhart, 1991). Common bean follows the theoretically expected relationship between  $\Delta$  and  $c_i/c_a$  (Seeman and Critchley, 1985; Ehleringer *et al.*, 1991), and there is considerable genetic variability with respect to  $\Delta$  within this species (Ehleringer, 1990; Ehleringer *et al.*, 1990, 1991; White *et al.*, 1990).

By contrast, *P. acutifolius* A. Gray (tepary bean) is native to the southwestern United States and northern Mexico, is adapted to heat and drought stress, and outyields *P. vulgaris* under hot, dry conditions (Petersen and Davis, 1982; Thomas *et al.*, 1983; Pratt and Nabhan, 1988). Although cultivation of tepary bean is presently limited to subsistence farming in the southwestern United States and northern Mexico, this species may represent an important source of variability for genetic improvement of common bean (Schinkel and Gepts, 1989); furthermore, tepary bean could play a role in the development of low-input, sustainable agricultural systems in regions affected by drought and/or salinity.

In order to better understand the ecophysiological characteristics of these two species, we compared carbon isotope discrimination by *P. vulgaris* and *P. acutifolius* in response to environmental variation, plant developmental status, and their interactions.

## II. Methods and Materials

*P. vulgaris* L. var. "Cahone" and *P. acutifolius* A. Gray var. "Sonora" were grown in the Biology Experimental Garden on the University of Utah campus in Salt Lake City during the summer of 1989. Soil in the experimental garden belonged to the Parleys Series (fine-silty, mixed, mesic Calcic Argixeroll). Seeds of both species were germinated in vermiculite in small pots outdoors. Half of the seeds from each species were germinated approximately 5 weeks earlier (Group 1) than those of the other group (Group 2) in order to have plants at two different stages of development. Seedlings were transplanted into the field at the primary leaf stage, at a row spacing of 100 cm and a distance between plants within rows of 50 cm. All plants were watered daily with a drip irrigation system.

Four days after transferring Group 2 seedlings to the field, all plants from both groups were each fertilized with 5 g of multipurpose N:P:K fertilizer (16:16:8). At late seedling stage, half of the plants in Groups 1 and 2 were assigned to a high nitrogen (HN) treatment and each plant received an additional 10 g of nitrogen (46:0:0) fertilizer, while the remainder of the plants were assigned to a low nitrogen (LN) treatment and were not fertilized for the remainder of the study. One week after in-

stallation of the nitrogen treatments, water treatments were initiated. Half of the plants in each nitrogen treatment continued to receive daily drip irrigation as before (HW), while the other half of the plants (LW) received no further irrigation for the remainder of the study. There was no rainfall during the study period. Each of the above treatment combinations was replicated twice.

Ten days after initiating the water treatments, leaves were collected for stable carbon isotope analysis from plants of both species in Group 1 (fruiting stage) and Group 2 (vegetative stage). From two plants in each replicate, two terminal leaflets from each of three leaf age classes were sampled randomly from the tops of the plant canopies. The leaf age classes were (1) newly emerged leaves (main vein length less than half the length of the main vein in a fully mature leaf); (2) expanding leaves (main vein length greater than half the length of main vein in fully mature leaf); and (3) mature leaves.

Leaves were dried at 75°C, ground to a fine powder, and combusted to CO<sub>2</sub> using a sealed-tube technique (Boutton, 1991). The CO<sub>2</sub> was isolated and purified cryogenically, and its isotopic composition determined relative to the international PDB standard on a dual-inlet, triple collector gas isotope ratio mass spectrometer (VG Micromass 903; VG Isogas, Middlewich, UK).  $\delta^{13}\text{C}_{\text{PDB}}$  values were determined with an overall precision (machine error plus sample preparation error) of <0.15‰ ( $\pm 1 \text{ SD}$ ).  $\Delta$  values were calculated according to the equation

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p}, \quad (2)$$

where  $\delta_a$  is the  $\delta^{13}\text{C}_{\text{PDB}}$  value of atmospheric CO<sub>2</sub> (-8‰; Mook *et al.*, 1983), and  $\delta_p$  is the  $\delta^{13}\text{C}_{\text{PDB}}$  value of the plant sample.

Each treatment combination was replicated twice, and each replicate comprised measurements made on two different plants. Data were analyzed by analysis of variance to test for differences due to the main effects of water, nitrogen, species, developmental stage, and leaf age. In addition, all two-, three-, and four-way interactions between treatment effects were evaluated.

### III. Results and Discussion

#### A. Response of $\Delta$ to Environmental and Developmental Variation

Mean  $\Delta$  values for *P. vulgaris* had a range of 3.7‰ (16.8 to 20.5‰) across all treatment combinations (Table I), equivalent to an overall difference in  $c_i$  among treatments of approximately 56  $\mu\text{l liter}^{-1}$ . A comparable range of  $\Delta$  values was found in *P. vulgaris* plants exposed to salinity (Seeman and Critchley, 1985). Mean  $\Delta$  values of *P. acutifolius* had a range of 2.7‰ (16.3 to 19.0‰) across all treatment combinations (Table I), indicating a differ-

**Table I** Mean Foliar  $\Delta$  Values (%) for Both *Phaseolus vulgaris* and *Phaseolus acutifolius*<sup>a</sup>

	Low water				High water			
	Low nitrogen		High nitrogen		Low nitrogen		High nitrogen	
	Vegetative	Fruiting	Vegetative	Fruiting	Vegetative	Fruiting	Vegetative	Fruiting
<b>Leaf age-class 1</b>								
P. vulgaris	19.3(0.8)	17.5(0.1)	17.7(0.0)	16.8(0.5)	19.9(0.1)	19.3(0.2)	19.6(0.1)	18.8(0.4)
P. acutifolius	17.2(0.1)	16.6(0.4)	16.3(0.3)	16.5(0.1)	18.5(0.3)	17.6(0.5)	18.7(0.0)	18.2(0.5)
<b>Leaf age-class 2</b>								
P. vulgaris	20.1(0.8)	19.0(0.5)	19.2(0.0)	18.0(0.2)	19.7(0.3)	19.3(0.0)	19.8(0.2)	18.9(0.2)
P. acutifolius	17.3(0.2)	16.9(0.3)	17.3(0.3)	16.7(0.1)	18.4(0.9)	18.7(0.3)	18.5(0.2)	17.8(0.8)
<b>Leaf age-class 3</b>								
P. vulgaris	20.4(0.5)	20.5(0.1)	19.1(0.2)	18.9(0.5)	19.9(0.0)	19.9(0.1)	19.7(0.0)	18.7(0.1)
P. acutifolius	18.4(0.2)	18.4(0.5)	17.8(0.1)	18.0(0.3)	19.0(0.0)	18.6(0.9)	18.9(0.0)	18.1(0.8)

<sup>a</sup> Parentheses indicate SEM.

ence in  $c_i$  among treatments of approximately  $38 \mu\text{l liter}^{-1}$ . These data demonstrate that both species have a high degree of plasticity in terms of the response of  $c_i/c_a$  to soil water and nitrogen status during different stages of plant development.

### B. Interspecific Variation in $\Delta$

Leaf  $\Delta$  values were significantly lower ( $P < 0.001$ ) for *P. acutifolius* ( $17.8 \pm 0.1\%$ ; mean  $\pm$  SE) than for *P. vulgaris* ( $19.2 \pm 0.1\%$ ) in all experimental treatments (Tables I and II, Fig. 1). The average difference between species across all treatments was  $1.3\%$ , corresponding to a difference in  $c_i$  of  $22 \mu\text{l liter}^{-1}$ . These data indicate greater long-term  $A/E$  for *P. acutifolius* relative to *P. vulgaris*, suggesting that high  $A/E$  may be one mechanism underlying the drought resistance of this species. Since comparisons between these species were made in identical environmental and developmental circumstances, differences in  $\Delta$  values should be a consequence of genetic variation.

### C. Effect of Soil Moisture Availability

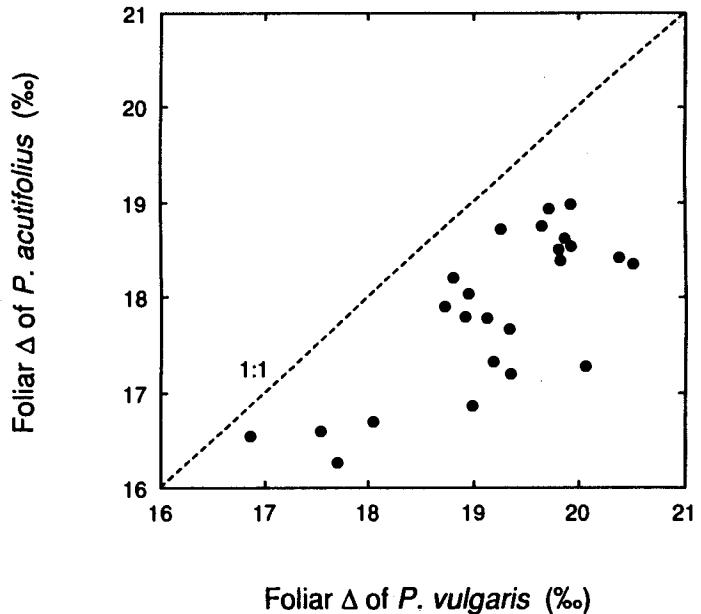
Plants from both species grown at low water availability had significantly lower  $\Delta$  values ( $P < 0.01$ ) than those grown at high water availability (Tables I and II, Fig. 2), indicating higher  $A/E$  for the low water plants. Similarly, White *et al.* (1990) found that 10 genotypes of *P. vulgaris* grown under rain-fed conditions had lower  $\Delta$  values than the same genotypes grown under irrigation. Many additional studies on both native and crop species have documented lower carbon isotope discrimination by plants growing under conditions of low soil water availability (Winter, 1981; Farquhar and Richards, 1984; Hubick and Farquhar, 1987; Ehleringer and Cooper, 1988).

A significant water  $\times$  species interaction ( $P < 0.05$ ; Table II) indicated that the difference in  $\Delta$  values between high and low water treatments was greater in *P. acutifolius* than in *P. vulgaris*. Mean  $\Delta$  values of *P. vulgaris* in

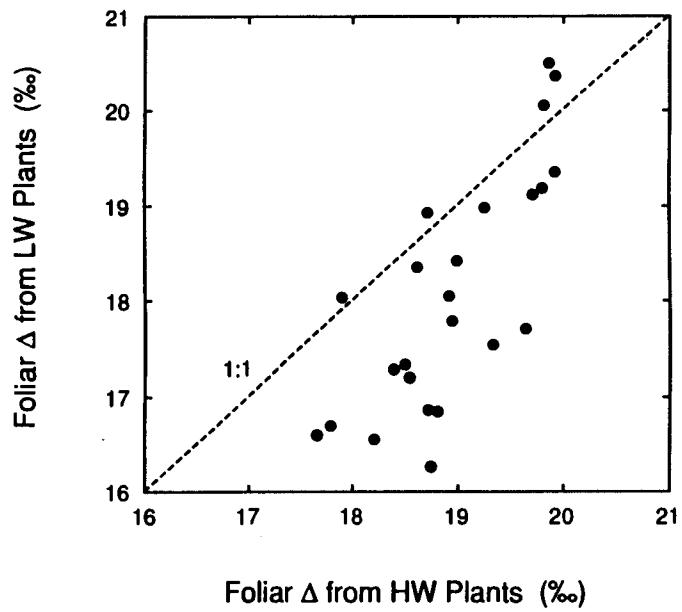
**Table II** Results of Analysis of Variance (ANOVA)\*

Source of variation	Degrees of freedom	Mean square	F value	Probability
Nitrogen	1	6.0	17.8	0.05
H <sub>2</sub> O	1	18.2	90.7	0.01
Species	1	41.7	196.3	0.001
Developmental stage	1	6.9	21.1	0.001
Leaf age	2	7.5	22.9	0.001
H <sub>2</sub> O $\times$ species	1	1.9	8.7	0.05
Nitrogen $\times$ species	1	1.9	8.9	0.05
H <sub>2</sub> O $\times$ leaf age	2	4.1	12.4	0.001

\* Only significant interactions are given.



**Figure 1.** Relationship between foliar  $\Delta$  values of *Phaseolus vulgaris* and *Phaseolus acutifolius* grown in the field under similar treatments. Values represent the means of each treatment combination. The dashed line represents 1:1.



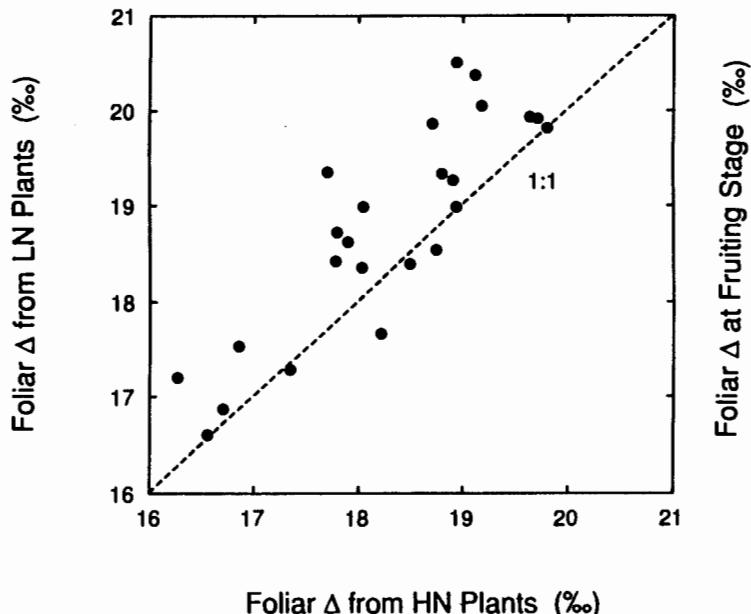
**Figure 2.** Relationship between foliar  $\Delta$  values of *Phaseolus vulgaris* and *Phaseolus acutifolius* from high water (HW) and low water (LW) treatments. Values represent the means of each treatment combination. The dashed line represents 1:1.

the high water treatment were  $19.5 \pm 0.1\%$ , while those in the low water treatment were  $18.9 \pm 0.2\%$ . By contrast, *P. acutifolius* plants in the high water treatment had mean  $\Delta$  values of  $18.4 \pm 0.1\%$ , and those in the low water treatment were  $17.3 \pm 0.2\%$ . These data indicate that *P. acutifolius* operates at lower  $c_i$  than *P. vulgaris* under both well-watered and water-limited conditions and that *P. acutifolius* can greatly reduce  $c_i$  and increase  $A/E$  in response to water limitation.

Similarly, Markhart (1985) found that stomatal conductances of *P. acutifolius* decreased earlier and to a greater extent than those of *P. vulgaris* during the imposition of water stress. While *P. vulgaris* maintained relatively high stomatal conductance at leaf-water potentials as low as  $-1.8$  MPa, stomata of *P. acutifolius* were effectively closed at leaf-water potentials of  $-1.0$  MPa (Markhart, 1985). As result, *P. acutifolius* is able to maintain relatively high leaf-water potentials under conditions of soil water limitation, thereby postponing dehydration and maintaining cell volume more effectively than *P. vulgaris* (Markhart, 1985; Castonguay and Markhart, 1991).

#### D. Effect of Soil Nitrogen Status

Leaf  $\Delta$  values were significantly higher ( $P < 0.05$ ) for plants grown at low soil nitrogen levels ( $18.8 \pm 0.2\%$ ) than for those grown at high soil nitrogen levels ( $18.3 \pm 0.2\%$ ) (Tables I and II, Fig. 3). These results suggest lower  $c_i$



**Figure 3.** Relationship between foliar  $\Delta$  values of *Phaseolus vulgaris* and *Phaseolus acutifolius* from high nitrogen (HN) and low nitrogen (LN) treatments. Values represent the means of each treatment combination. The dashed line represents 1:1.

and higher  $A/E$  for plants grown at high soil nitrogen availability. A significant nitrogen  $\times$  species interaction ( $P < 0.05$ , Table II) revealed that  $\Delta$  values of *P. acutifolius* were relatively unresponsive to nitrogen treatment; however, *P. vulgaris* plants in the low nitrogen treatment had  $\Delta$  values ( $19.6 \pm 0.2\%$ ) that averaged 0.8% higher than those of plants in the high nitrogen treatment ( $18.8 \pm 0.2\%$ ).

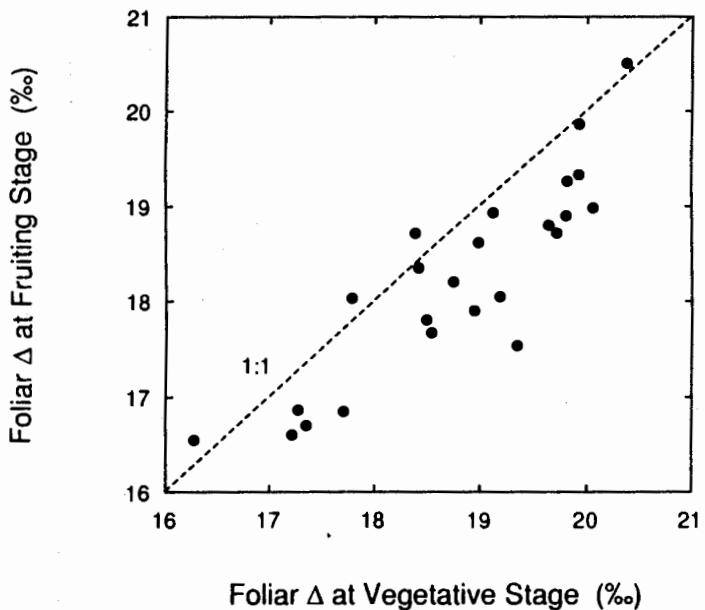
Data evaluating potential relationships between  $\Delta$  and mineral nutrition are limited. White *et al.* (1990) found no effect of leaf nitrogen concentration on  $\Delta$  in 10 genotypes of *P. vulgaris* growing under rain-fed and irrigated conditions in two locations in Columbia. Similarly, Hubick (1990) reported that nitrogen treatments had no effect on  $\Delta$  in genotypes of peanut (*Arachis hypogaea* L.). Results on *P. acutifolius* in the present study conform to this pattern of no effect of nitrogen on  $\Delta$ .

However, some studies demonstrate that mineral nutrition can in fact influence  $\Delta$ . For example, Bender and Berge (1979) found that *Phleum pratense* L. plants grown at optimum temperature and fertilized with nitrogen and potassium generally had lower  $\Delta$  values than unfertilized plants. Fu and Ehleringer (1992) demonstrated that for both container- and field-grown *P. vulgaris*, plants in high fertilizer treatments had significantly lower  $\Delta$  values than those grown at low fertilizer levels, in agreement with results from the present study. Additional supporting evidence comes from gas exchange studies on *Helianthus annuus* (Fredeen *et al.*, 1991) and *Larrea tridentata* (Lajtha and Whitford, 1989), which both demonstrated higher  $A/E$  at higher concentrations of leaf nitrogen.

In the absence of gas exchange data, it is difficult to explain the mechanism by which nitrogen enhancement has resulted in higher  $A/E$  in this study. However, there is a well-documented positive correlation between photosynthetic capacity and leaf nitrogen concentration (Field and Mooney, 1986; Evans, 1989). For any given value of stomatal conductance, leaves with high nitrogen concentrations and high photosynthetic capacities should have lower  $c_i$  values and higher  $A/E$  than leaves with lower nitrogen concentrations and photosynthetic capacities. Therefore, the lower  $\Delta$  values (higher  $A/E$ 's) observed in the high nitrogen plants in this study may be a consequence of a relatively high ratio of photosynthesis to leaf conductance.

#### E. Effect of Plant Developmental Stage

For both species, leaf  $\Delta$  values were significantly higher ( $P < 0.001$ ; Table II) for plants in the vegetative stage than for those in the fruiting stage (Table I, Fig. 4), suggesting higher  $A/E$  during fruiting. This change in  $A/E$  during development may be a consequence of paraheliotropic leaf movements, which result in a higher photosynthetic photon flux density (PPFD) incident on leaves during the vegetative stage. Leaves at the tops of the canopies of both species have been shown to orient more obliquely toward direct solar radiation during the fruiting stage than during the vegetative stage (Fu and Ehleringer, 1991). The consequence of this reduction in

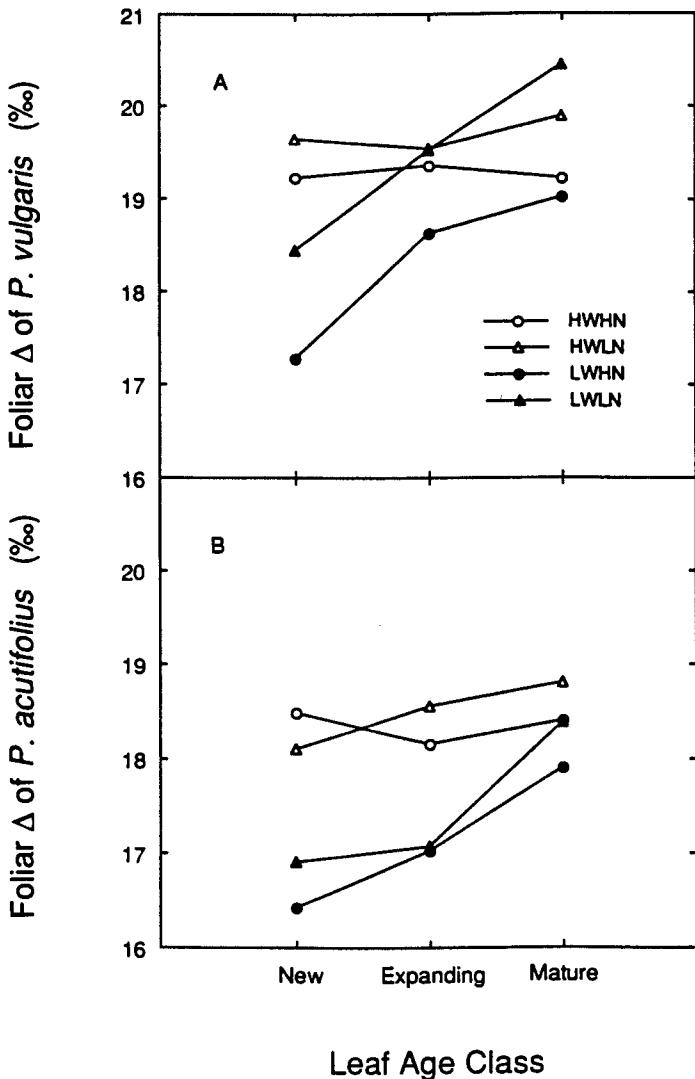


**Figure 4.** Relationship between foliar  $\Delta$  values of *Phaseolus vulgaris* and *Phaseolus acutifolius* from vegetative and fruiting stages of development. Values represent the means of each treatment combination. The dashed line represents 1:1.

PPFD incident on leaves during the fruiting stage may be a decrease in stomatal conductance. Because of the higher resistance to  $\text{CO}_2$  diffusion relative to water diffusion in leaves (Nobel, 1991), stomatal closure results in a proportionally greater decrease in transpiration than in  $\text{CO}_2$  fixation (Raschke, 1979, Cowan, 1982) and results in higher  $A/E$ . Therefore, leaves receiving less PPFD as a result of paraheliotropic leaf movement during the fruiting stage may have higher  $A/E$  than leaves during the vegetative stage. Conversely, higher PPFD incident on leaves during the vegetative growth stage should result in higher stomatal conductances and higher  $c_i$ , thereby maximizing carbon gain and allowing young plants to grow and establish rapidly.

#### F. Effect of Leaf Age

Leaf  $\Delta$  values increased significantly ( $P < 0.001$ , Table II) as leaf age increased in both species, especially for the low water treatment (Table I, Fig. 5). Within treatments, variation in  $\Delta$  due to leaf age was substantial. For example, in the low water-low nitrogen treatment,  $\Delta$  values for *P. vulgaris* ranged from approximately 18.4% in the new leaves to 20.4% in mature leaves (Fig. 5). The large difference in  $\Delta$  values between leaves of different ages within treatments emphasizes the importance of sampling leaves of similar developmental status when conducting comparative ecophysiological studies using  $\Delta$  as an index.



**Figure 5.** Relationship between foliar  $\Delta$  values and leaf age-class for *Phaseolus vulgaris* (A) and *Phaseolus acutifolius* (B). LWLN, low water, low nitrogen; LWHN, low water, high nitrogen; HWLN, high water, low nitrogen; HWHN, high water, high nitrogen.

Comparable data have not been published for any crop species. However, Ducatti *et al.* (1991) documented that older leaves from several Amazonian tree species had  $\Delta$  values that were approximately 0.7‰ higher than those of younger leaves. Similarly, we found that 1-year-old live oak (*Quercus virginiana*) leaves differed from those that were only 2–3 months old by approximately 3‰ (Boutton *et al.*, unpublished data). These changes in  $\Delta$  may reflect changes in stomatal function and/or photosynthetic capacity associated with leaf aging. Alternatively, environmental conditions prevail-

ing during growth and development of "old" leaves may differ significantly from those prevailing during growth and development of "new" leaves, resulting in different  $c_i/c_a$  and  $\Delta$  values.

Because the effect of leaf age on  $\Delta$  was more pronounced in plants grown at low water availability relative to those grown at high water availability (Fig. 5), the water  $\times$  leaf-age interaction was significant ( $P < 0.001$ , Table II). Leaves of different ages from plants in the well-watered treatment would have all experienced optimum soil water status during growth and development. By contrast, in the low water treatment, older leaves developed prior to or soon after the cessation of irrigation, while new leaves developed later during a period of reduced water availability. Thus, lower  $\Delta$  values in the younger leaves may reflect an increase in  $A/E$  in response to a decrease in soil moisture status as the growing season progressed. The near constancy of  $\Delta$  values with respect to leaf age in the well-watered treatments supports an environmental rather than developmental explanation for the decrease in  $\Delta$  in newer leaves in the low water treatments.

#### IV. Summary

*P. vulgaris* L. and *P. acutifolius* Gray were grown in the field at two levels of soil moisture and two levels of soil nitrogen to evaluate environmental and developmental effects on carbon isotope discrimination ( $\Delta$ ) and water-use efficiency ( $A/E$ ). For both species,  $\Delta$  was measured on three age-classes of leaves sampled from each of two developmental stages (vegetative and fruiting) growing simultaneously. Leaf  $\Delta$  values were significantly lower for *P. acutifolius* than for *P. vulgaris* in all experimental treatment combinations. This suggests greater long-term  $A/E$  in *P. acutifolius*, which is adapted to warmer, drier environments. Since comparisons between these species were made under identical environmental and developmental circumstances, differences in  $\Delta$  values should be a consequence of genetic variation. Plants from both species grown at low water availability had significantly lower  $\Delta$  values than those grown at high water availability. A significant water  $\times$  species interaction occurred because the difference in  $\Delta$  values between high and low water treatments was greater in *P. acutifolius* than in *P. vulgaris*. Leaves from plants of both species grown at low soil nitrogen levels had significantly higher  $\Delta$  values than those grown at high soil nitrogen levels, suggesting that plants in high nitrogen treatments had higher  $A/E$ . This result agrees with the positive correlation between leaf nitrogen content and  $A/E$  demonstrated previously by others. A significant nitrogen  $\times$  species interaction revealed that *P. vulgaris* was more responsive to soil nitrogen status than *P. acutifolius*. For both species, leaf  $\Delta$  values were significantly higher from plants in the vegetative stage than from those in the fruiting stage, suggesting higher  $A/E$  during fruiting. This change in  $A/E$  during development may be a consequence of stronger

paraheliotropic leaf movements, which result in higher photon flux density incident on leaves during the vegetative stage. Leaf  $\Delta$  values increased significantly as leaf age increased in both species. This effect was more pronounced in plants grown at low water availability compared to those grown at high water availability, indicating that it was probably due to the changing environment and not to effects of leaf development.

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