

Alteration of leaf movement of *Abutilon theophrasti* (Malvaceae) by mycorrhizal infection

R. T. KOIDE* and R. P. SCHREINER†

Graduate Program in Plant Physiology, The Pennsylvania State University, University Park, Pennsylvania 16802, USA

Summary

1. *Abutilon theophrasti* (Malvaceae) plants orient their leaves in a downward folded position at night but display them more horizontally during the light period.
2. The absence of vesicular–arbuscular mycorrhizal infection of *Abutilon* resulted in an alteration in the range of leaf movement. Mycorrhizal plants held their leaves nearly horizontal during the middle portion of the day. Uninfected plants, however, reoriented their leaves less during the night/day transition and held them several degrees below horizontal during midday.
3. This modification of leaf angle caused by the absence of mycorrhizal infection was associated with lower leaf P concentration, lower stomatal conductance and higher leaf temperature.

Key-words: Leaf movement, phosphorus, photosynthesis, vesicular–arbuscular mycorrhiza

Functional Ecology (1994) **8**, 384–388

Introduction

Abutilon theophrasti Medic., an early successional annual member of the Malvaceae, displays both nyctinastic (Fuhrman & Koukkari 1981) and heliotropic (personal observations) leaf movements. Nyctinastic movements are exhibited by several members of the Fabaceae and Malvaceae (Darwin 1880, in Barrett & Freeman 1989). They consist of the rhythmic folding (near dusk) and unfolding (near dawn) of leaves and/or leaflets. Little is known about the ecological significance of nyctinasty, but other kinds of leaf movements such as heliotropic movements may have a profound influence on plant carbon gain (Mooney & Ehleringer 1978; Ehleringer & Werk 1984; Gamon & Pearcy 1989).

The infection of roots by mycorrhizal fungi is common in most terrestrial ecosystems. Many researchers have shown that the absence of vesicular–arbuscular mycorrhizal infections can depress plant growth and/or reproduction (reviewed by Koide 1991). This most often occurs when phosphorus (P) is a limiting plant nutrient. Huang, Smith & Yost (1985) recently showed that non-mycorrhizal *Leucaena leucocephala* (Fabaceae) had significantly lower shoot P concentrations, lower stomatal conductances, higher leaf temperatures and increased paraheliotropic (sun-

avoiding) behaviour of leaves compared to normal mycorrhizal plants. By causing the leaves to avoid direct sunlight, paraheliotropic movements can reduce the likelihood of damage by excess light and heat (Ludlow & Björkman 1984; Forseth 1990). In several of our experiments with *Abutilon* grown in the field and in the greenhouse we have noticed that leaf movements (in particular, nyctinastic movements) were substantially altered by the absence of vesicular–arbuscular mycorrhizal infections. Here we report on the effect of mycorrhizal infection on nyctinastic leaf movements in *A. theophrasti*.

Materials and methods

Seeds of *A. theophrasti* were treated in concentrated H₂SO₄ for 7 min followed by a 10-min rinse in distilled water. On 14 December 1989, four seeds were placed into each of 128 pots (11.4 cm on each side) filled with approximately 0.560 dm³ of soil mix. This consisted of equal volumes of a low P soil (Hagerstown silty-clay loam, bicarbonate-extractable P concentration 2.9 µg P g⁻¹) and sand. Half the pots (64) were inoculated with the mycorrhizal fungus *Glomus etunicatum* Becker & Gerd (Native Plants, Inc., Salt Lake City, Utah, USA). Approximately 3000 spores were placed into each of these pots 2 cm below the seeds. The other half were inoculated with washings from the fungal spores to provide comparable non-mycorrhizal microbial inputs (Koide & Li 1989).

All pots were placed in a greenhouse receiving 14 h of supplemental illumination each day provided by

* Present address and address for reprint requests: Dr R. T. Koide, Department of Horticulture, The Pennsylvania State University, University Park, PA 16802, USA.

† Present address: Dr R. P. Schreiner, USDA/ARS, Horticulture Crops Research Laboratory, 3420 N.W. Orchard Ave., Corvallis, Oregon 97330, USA.

two high-pressure sodium lamps operating from 06.00 to 20.00 h. Sixty-two days after sowing (on 14 February) the photoperiod was altered to extend from 06.00 to 18.00 h in order to induce flowering. Starting 14 days after sowing all pots were fertilized five times weekly with 0.050 dm³ of one-fifth strength Hoagland nutrient solution (one-tenth strength iron) lacking micronutrients and phosphate (Machlis & Torrey 1956). Supplemental irrigation was provided by an automatic drip irrigation system. All pots were thinned to a single seedling 26 days after sowing. Remaining seedlings were selected on the basis of uniformity. From 39 days after sowing onwards (corresponding to the exponential growth phase), one-half strength Hoagland solution (one-tenth strength iron) lacking micronutrients and phosphate was used.

DOCUMENTATION OF NYCTINASTY

Thirty-five days following sowing, 16 plants from each treatment [mycorrhizal (M) and non-mycorrhizal (NM)] were randomly selected from the pool of 128 plants in the greenhouse and placed in a growth chamber providing more controlled environmental conditions. There, illumination of the plants was from directly above. In this way we eliminated the movement of the sun as a factor. The plants received approximately 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR, 400–700 nm) at the top of the plant canopy. The photoperiod extended from 06.00 to 20.00 h, the air temperatures were 25 °C (day) and 20 °C (night) and relative humidities were constant at 50%. The angles of the second and fourth leaves (counting from the oldest leaf) on each plant were monitored every 1.5 h from approximately 05.00 to 20.00 h. This was done using a protractor with a plumb line attached to the midpoint of the flat side. The flat side was held against the abaxial surface of the lamina at the midrib and the angle made by the plumb line in relation to the protractor was noted. The angle β (degrees below horizontal) was then calculated from the protractor angle. $\cos \beta$ was used as a measure of leaf angle so that fully horizontal leaves (with the greatest exposure to light directly overhead) had a value of 1 while leaves held straight down (with minimal exposure to overhead light) had values of 0. Negative values of $\cos \beta$ indicated that the leaves were tucked in, that is, they rotated past the vertical and could actually receive overhead light on the abaxial surface.

PHOTOSYNTHESIS UNDER AMBIENT CONDITIONS

Forty-nine and 50 days after sowing, photosynthesis measurements were made in the growth chamber on the 128 plants (64 per treatment). Thirty-two plants from each treatment were measured on each of the 2 days. Plants were placed in the growth chamber and

allowed to equilibrate for a full photoperiod prior to measurement. Just prior to placing the leaves into the cuvette, the angle of the leaf was measured using the protractor device previously described.

All leaves were held horizontally during the measurement regardless of the leaf angle prior to measurement. Photosynthesis was measured between 10.00 and 15.00 h using a Li-Cor model 6200 portable photosynthesis system (Li-Cor Inc., Lincoln, Nebraska, USA) equipped with the standard 4 dm³ leaf cuvette. The most recently fully expanded leaf fully exposed to the light was measured on each plant. Carbon dioxide concentrations in the growth chamber ranged from 400 to 450 μmol^{-1} during the measurement periods. The mean PAR was 545 $\mu\text{mol mol}^{-2} \text{s}^{-1}$ at the level of the measured leaf. All measured leaves were held horizontally the same distance from the bank of lights. On average, r.h. changed less than 10% during the measurements on each leaf. Leaf temperatures generally did not vary more than 1 °C during the entire period within the cuvette. Variation in leaf temperature was minimized by using a fan to blow air across the closed cuvette during measurements. The CO₂ concentration within the cuvette was monitored to ensure a steady rate of assimilation prior to taking a measurement. Measurements were based on 5 $\mu\text{mol mol}^{-1}$ CO₂ depletions which took approximately 20 s.

After the measurement of photosynthesis, leaves were removed from the cuvette and leaf length and width were used to estimate the leaf area ($\text{area} = l \times w \times 0.84$) in order to compute the photosynthetic rate per leaf area. Photosynthesis, stomatal conductance, leaf temperature and leaf angle were recorded for each plant. Subsequently the measured leaves were removed from 25 plants per treatment, dried, weighed, digested in concentrated sulphuric acid/hydrogen peroxide, and colorimetrically analysed for total N and P concentrations using procedures as described in Koide & Li (1989).

FINAL HARVEST

On 19 April (126 days after sowing) all plants were harvested. Shoots were dried at 70 °C and weighed. Root systems were sampled from each pot using a soil core. The degree of mycorrhizal infection was assessed using a line intersect technique as described in Koide & Mooney (1987).

STATISTICAL METHODS

The significance of treatment (M vs NM) to the several measured and calculated variables (dry weight, photosynthetic rate, P concentration, etc.) was assessed using a single-factor analysis of variance (STSC 1991).

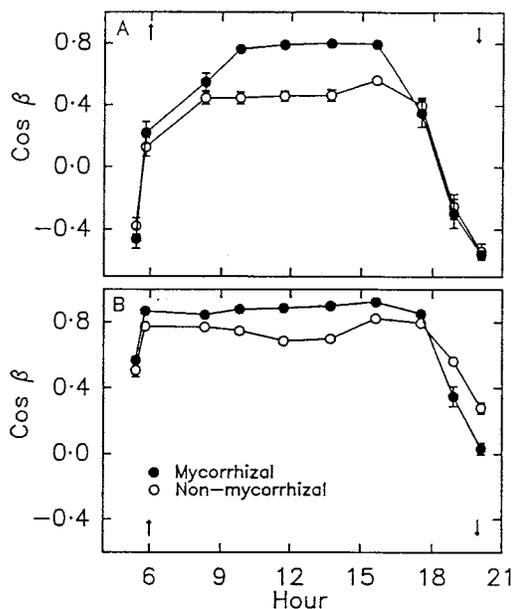


Fig. 1. (A) Mean $\cos \beta$ (degrees below horizontal) over time for the second leaf from each M and NM plant ($n=16$). (†) lights on in growth chamber; (‡) lights off in growth chamber. Error bars are ± 1 SE. Where not shown, error bars were too small to project beyond the symbol. (B) Same for fourth leaf.

Results

When measured 35 days after sowing, the angles of the leaves from M and NM plants differed substantially over the course of one photoperiod, particularly during midday. The leaves of the M plants were held nearly horizontal during the middle portion of the day. While leaves of NM plants rotated up with much the same timing as the leaves of M plants, they did not attain the nearly horizontal position of the leaves of M

plants (Fig. 1a,b). Both time and treatment (M vs NM) contributed significantly to variation in leaf angle for both leaf 2 and leaf 4 and the very small standard errors made further statistical testing unwarranted.

These nyctinastic leaf movements were apparently endogenously controlled. This is most obvious for the folding movement of leaves back towards the vertical in the late afternoon. For leaf 2, this movement was initiated near the start of the natural dark period, 2 or 3 h prior to the start of the dark period in the growth chamber (Fig. 1a). Indeed, the initiation of leaf folding corresponded to neither growth chamber photoperiod nor artificial greenhouse light photoperiod, both of which extended from 06.00 to 20.00 h during the period when leaf angles were monitored.

Associated with more horizontal leaves of the M plants was a greater rate of photosynthesis on a leaf area basis (Table 1). When the leaves were held horizontally within the cuvette, the temperature of the leaves of NM plants was significantly higher than that of the leaves of M plants (Table 1). Higher leaf temperatures for the NM plants were associated with lower stomatal conductances (Table 1).

At the time photosynthesis was measured (49 and 50 days after sowing), M plants had leaves with significantly greater P concentrations but significantly lower N concentrations compared to those from NM plants (Table 2). At the final harvest (126 days after sowing), M plants had acquired significantly more shoot dry weight and were highly infected by mycorrhizal fungi while the NM plants were essentially non-mycorrhizal (Table 3).

Discussion

The ecological consequences of nyctinastic leaf movements have not been well studied. Forseth

Table 1. Means \pm SE of several variables measured on the most recently fully expanded leaves of individual M or NM *Abutilon theophrasti* plants. Measurements were made 49–50 days after sowing ($n=62$)

	Leaf angle*	Photosynthesis ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	Leaf temperature ($^{\circ}\text{C}$)	Stomatal conductance ($\text{mol m}^{-2}\text{s}^{-1}$)
M	$46.1 \pm 1.1^{\text{a}\dagger}$	$13.7 \pm 0.1^{\text{b}}$	$27.4 \pm 0.1^{\text{a}}$	$0.228 \pm 0.006^{\text{b}}$
NM	$69.0 \pm 1.2^{\text{b}}$	$10.6 \pm 0.2^{\text{a}}$	$28.3 \pm 0.1^{\text{b}}$	$0.121 \pm 0.004^{\text{a}}$

* Leaf angle is degrees below horizontal prior to placement in cuvette.

† Different superscript letters indicate a significant ($P < 0.05$) difference between M and NM treatments according to the single-factor analysis of variance.

Table 2. Means \pm SE of nutrients of most recently fully expanded leaves of individual M or NM *Abutilon theophrasti* plants. Leaves were sampled 49–50 days after planting ($n=25$)

	N (%)	P (%)	N ($\mu\text{g cm}^{-2}$)	P ($\mu\text{g cm}^{-2}$)
M	$3.14 \pm 0.10^{\text{a}*}$	$0.305 \pm 0.006^{\text{b}}$	$90.6 \pm 1.5^{\text{a}}$	$8.87 \pm 0.16^{\text{b}}$
NM	$3.49 \pm 0.08^{\text{b}}$	$0.144 \pm 0.004^{\text{a}}$	$121.7 \pm 3.5^{\text{b}}$	$5.05 \pm 0.17^{\text{a}}$

* Different superscript letters indicate a significant ($P < 0.05$) difference between M and NM treatments according to the single-factor analysis of variance.

Table 3. Final harvest variables measured 126 days after sowing ($n=63$). Means \pm SE

	Shoot dry weight (g)	Mycorrhizal infection (% of root length)
M	3.87 \pm 0.16 ^{b*}	74.4 \pm 1.7 ^b
NM	1.84 \pm 0.07 ^a	3.2 \pm 1.1 ^a

* Different superscript letters indicate a significant ($P < 0.05$) difference between M and NM treatments according to the single-factor analysis of variance.

(1990) has outlined several possible consequences that could have selected for nyctinasty. These include reduced radiant heat loss by leaves and buds at night, and reduced leaf wetting by dew formation. Our studies indicated that M and NM plants folded their leaves at night to about the same extent. Consequently, both types of plants would have received the same benefit (whatever it might be) from leaf folding.

We showed that in the growth chamber M and NM plants unfolded their leaves to different extents during the night/day transition. M plants held their leaves more nearly horizontal during the light period than did NM plants. The ecological significance of this alteration in the range of leaf movement is unclear. However, it could substantially alter the reception of light in the field. We therefore feel that the phenomenon warrants further study.

In this and some other studies, the range or nature of leaf movements have been shown to be correlated with plant P status. In soybean, increased paraheliotropism was associated with P deficiency (Lauer *et al.* 1989). In *Leucaena leucocephala*, Huang *et al.* (1985) showed that M plants with a higher P status exhibited less paraheliotropism than NM plants with a lower P status. In the current study, M infection was associated with increased leaf P concentrations and decreased leaf N concentrations. Therefore, the effect of M infection on stomatal conductance and leaf angle could not have been due to an improved N status.

We showed that stomatal conductances of M plants were significantly greater than those of NM plants and that horizontal leaves of M plants were about 1 °C cooler than those of NM plants. Several other studies performed with P-deficient soil have also shown that M plants have higher stomatal conductances than NM plants (Allen *et al.* 1981; Johnson *et al.* 1982; Johnson 1984; Koide 1985b; Fitter 1988). Koide (1985b) showed that the effect of mycorrhizal infection on stomatal conductance can be linked to its effect on leaf P status.

Reduced stomatal conductances and lower transpirational cooling in the NM plants would be consistent with leaves held less than horizontal at midday if regulation of leaf temperature within narrow limits were

important. We stress here that we do not know if temperature regulation by such a mechanism is important in the field. Nevertheless, a possible set of links between plant mycorrhizal status and leaf angle may involve the fact that, independent of mycorrhizal infection, P deficiency can reduce root hydraulic conductivity (Graham & Syvertsen 1984). Thus the roots of P-deficient NM plants may have a reduced capacity to supply leaves with water. This would require either producing less leaf area or reducing stomatal conductance, or both (as seen in this study) in order to match the water supply to the water demand. Reduced stomatal conductances would result in higher leaf temperatures unless the reception of radiant energy were reduced such as by the downward folding of leaves.

While the above hypothesis may suggest a possible selective advantage for the alteration of leaf angle in NM plants, it does not itself provide a mechanism for such. One hypothesis of mechanism is that a low P status is associated with a reduced physiological or anatomical capacity to hold leaves horizontally. Unlike many species which are capable of leaf movements, *A. theophrasti* does not possess distinct pulvini. The mechanism responsible for leaf movement, however, may be similar to that operating in a pulvinus (Wetherell 1990). If, for example, membrane transport of ions necessary to generate turgor in motor cells was limited by P deficiency, NM plants might be expected to have a reduced capacity to hold leaves horizontally. Indeed, P deficiency has been shown to be associated with membrane leakiness (Graham, Leonard & Menge 1981).

In summary, although neither the selective advantage for altered leaf movement in NM plants nor the mechanism to account for the alteration have been made clear, our findings suggest that the absence of symbiosis may reduce carbon gain by altering the display of leaves towards the light source in addition to previously described negative effects on leaf expansion (Koide 1985a; Fredeen & Terry 1988) and leaf photosynthetic capacity (Johnson *et al.* 1982). Whether the phenomenon described in this study is attributable solely to an alteration in P nutrition is not shown here, but preliminary observations suggest that it is related to P nutrition as high-P NM plants do not show a reduced range of leaf movement.

Acknowledgements

We thank the A.W. Mellon Foundation and the National Science Foundation for financial support of this research. We also thank Dr D. Shumway for help with the analysis of the data, X. Lu for laboratory assistance and A. Omeis for assistance in the greenhouse. Drs D. Shumway and I. Sanders and an anonymous reviewer kindly provided helpful editorial comments.

References

- Allen, M.F., Smith, W.K., Moore, T.S., Jr & Christensen, M. (1981) Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis* H.B.K. lag ex Steud. *New Phytologist*, **88**, 683–693.
- Darwin, C. (1880) *The Power of Movement in Plants*, vol. 27, *The Works of Charles Darwin* (1989) (eds P. H. Barrett & R. B. Freeman). William Pickering, London.
- Ehleringer, J.R. & Werk, K.S. (1984) Modifications of solar radiation absorption patterns and implications for carbon gain at the leaf level. *On the Economy of Plant Form and Function* (ed. T. J. Givnish), pp. 57–82. Cambridge University Press, Cambridge.
- Fitter, A.H. (1988) Water relations of Red Clover *Trifolium pratense* L. as affected by VA mycorrhizal infection and phosphorus supply before and during drought. *Journal of Experimental Botany*, **39**, 595–603.
- Forsyth, I.N. (1990) Function of leaf movements. *The Pulvinus: Motor Organ for Leaf Movement* (eds R. L. Satter, H. L. Gorton & T. C. Vogelmann), pp. 238–261. American Society of Plant Physiologists, Rockville, Maryland.
- Fredeen, A.L. & Terry, N. (1988) Influence of vesicular-arbuscular mycorrhizal infection and soil phosphorus level on growth and carbon metabolism of soybean. *Canadian Journal of Botany*, **66**, 2311–2316.
- Fuhrman, M.H. & Koukkari, W.L. (1981) Anatomical and physiological characteristics of the petiole of *Abutilon theophrasti* in relation to circadian leaf movements. *Physiologia Plantarum*, **51**, 309–313.
- Gamon, J.A. & Pearcy, R.W. (1989) Leaf movement, stress avoidance and photosynthesis in *Vitis californica*. *Oecologia*, **79**, 475–481.
- Graham, J.H. & Syvertsen, J.P. (1984) Influence of vesicular-arbuscular mycorrhiza on the hydraulic conductivity of roots of two citrus rootstocks. *New Phytologist*, **97**, 277–284.
- Graham, J.H., Leonard, R.T. & Menge, J.A. (1981) Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhizal formation. *Plant Physiology*, **68**, 548–552.
- Huang, R.-S., Smith, W.K. & Yost, R.S. (1985) Influence of vesicular-arbuscular mycorrhiza on growth, water relations, and leaf orientation in *Leucaena leucocephala* (Lam.) De Wit. *New Phytologist*, **99**, 229–243.
- Johnson, C.R. (1984) Phosphorus nutrition on mycorrhizal colonization, photosynthesis, growth and nutrient composition of *Citrus aurantium*. *Plant and Soil*, **80**, 35–42.
- Johnson, C.R., Menge, J.A., Schwab, S. & Ting, I.P. (1982) Interaction of photoperiod and vesicular-arbuscular mycorrhizae on growth and metabolism of sweet orange. *New Phytologist*, **90**, 665–669.
- Koide, R.T. (1985a) The nature of growth depressions in sunflower caused by vesicular-arbuscular mycorrhizal infection. *New Phytologist*, **99**, 449–462.
- Koide, R.T. (1985b) The effect of VA mycorrhizal infection and phosphorus status on sunflower hydraulic and stomatal properties. *Journal of Experimental Botany*, **36**, 1087–1098.
- Koide, R.T. (1991) Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist*, **117**, 365–386.
- Koide, R.T. & Li, M. (1989) Appropriate controls for vesicular-arbuscular mycorrhizal research. *New Phytologist*, **111**, 35–44.
- Koide, R.T. & Mooney, H.A. (1987) Spatial variation in inoculum potential of vesicular-arbuscular mycorrhizal fungi caused by formation of gopher mounds. *New Phytologist*, **107**, 173–182.
- Lauer, M.J., Pallardy, S.G., Blevins, D.G. & Randall, D.D. (1989) Whole leaf carbon exchange characteristics of phosphate deficient soybeans (*Glycine max* L.). *Plant Physiology*, **91**, 848–854.
- Ludlow, M.M. & Björkman, O. (1984) Paraheliotropic leaf movement in *Siratro* as a protective mechanism against drought-induced damage to primary photosynthetic reactions: damage by excessive light and heat. *Planta*, **161**, 505–518.
- Machlis, L. & Torrey, J.G. (1956) *Plants in Action*. Freeman, San Francisco.
- Mooney, H.A. & Ehleringer, J.R. (1978) The carbon gain benefits of solar tracking in a desert annual. *Plant, Cell and Environment*, **1**, 307–311.
- STSC (1991) *Statgraphics Statistical Graphics System, version 5.0*. STSC, Inc., Rockville, Maryland.
- Wetherell, D.F. (1990) Leaf movement in plants without pulvini. *The Pulvinus: Motor Organ for Leaf Movement* (eds R. L. Satter, H. L. Gorton & T. C. Vogelmann), pp. 72–78. American Society of Plant Physiologists, Rockville, Maryland.

Received 4 August 1992; revised 13 April 1993; accepted 29 April 1993