1981

COMPARATIVE WATER RELATIONS AND PHOTOSYNTHESIS OF MYCORRHIZAL AND NON-MYCORRHIZAL *BOUTELOUA GRACILIS* H.B.K. LAG EX STEUD.

Michael F. Allen
University of Nebraska-Lincoln

William K. Smith
University of Wyoming

Thomas S. Moore Jr.
University of Wyoming

Martha Christensen
University of Wyoming

COMPARATIVE WATER RELATIONS AND PHOTOSYNTHESIS OF MYCORRHIZAL AND NON-MYCORRHIZAL *BOUTELOUA GRACILIS* H.B.K. LAG EX STEUD.

By MICHAEL F. ALLEN*, WILLIAM K. SMITH, THOMAS S. MOORE, Jr and MARTHA CHRISTENSEN

Department of Botany, University of Wyoming, Laramie, Wyoming 82071, U.S.A.

(*Accepted 16 December 1980*)

Summary

The rangeland grass, *Bouteloua gracilis* was inoculated with its mycorrhizal symbiont, *Glomus fasciculatus*, to determine the influence of vesicular–arbuscular mycorrhizae on water status, stomatal behaviour and photosynthesis as well as gross plant morphology, biomass and phosphorus content. Mycorrhizal infection increased transpiration rates by over 100 % with 50 to 70 % lower leaf resistances to water vapour diffusion. Leaf xylem pressure was not different between mycorrhizal and non-mycorrhizal plants indicating that whole-plant resistance to water transport was reduced by more than 50 %. Photosynthetic rates under saturating light conditions increased 68 % with infection as a consequence of a 33 % reduction in stomatal resistance and a 67 % reduction in mesophyll resistance to CO_2 uptake. Mycorrhizal infection did not affect biomass or gross plant morphology after 30 weeks of growth, but increased chlorophyll and phosphate concentrations by 28 % and 70 % respectively. These physiological changes indicate that mycorrhizae may substantially alter survival ability of *Bouteloua gracilis*.

Introduction

Vesicular–arbuscular (VA) mycorrhizae improve survival and growth of many plants, especially those living in marginal habitats (Aldon, 1975; Kahn, 1975; Ruehle and Marx, 1979). Direct transport of several nutrients from soil to plant by mycorrhizal fungi has been demonstrated (Hattingh, Gray and Gerdemann, 1973; Rhodes and Gerdemann, 1978, 1979) suggesting that the increased absorption provided by hyphae increased plant growth (Sanders and Tinker, 1971). However, the effects of mycorrhizal infection on other physiological processes that influence growth, such as water relations, stomatal behaviour, and CO_2 uptake, are relatively unknown.

In many arid and semiarid regions, the major factor limiting plant production and nutrient uptake may be water availability (Harner and Harper, 1973; Fischer and Turner, 1978; Lauenroth, Dodd and Sims, 1978). Safir, Boyer and Gerdemann (1971, 1972) demonstrated that, under mesic soil water conditions, VA mycorrhizal infection lowered resistance to water uptake by 40 % in soybean and Mosse and Hayman (1971) reported that non-mycorrhizal onions wilted when transplanted, whereas mycorrhizal plants did not. Aldon (1975) noted that survival of *Altriplex canescens* on coal mine spoils in New Mexico increased with VA mycorrhizal infection and he hypothesized that increased water uptake was a contributing

* Present address: Institute of Agriculture and Natural Resources, Plant Pathology Department, University of Nebraska-Lincoln, Lincoln, Nebraska 68583, U.S.A.

0028-646X/81/080683+11 $02.00/0

factor. More recently, Levy and Krikun (1980) found that VA mycorrhizal infection increased transpiration and $^{14}CO_2$ uptake in citrus upon recovery from drought.

We evaluated the influence of VA mycorrhizae on the water and photosynthetic relations of a common North American rangeland grass, *Bouteloua gracilis*, using its native mycorrhiza symbiont, *Glomus fasciculatus* (Thaxter sensu Gerdemann) Gerdemann and Trappe. *B. gracilis* shows typical Kranz anatomy and the C_4 photosynthetic pathway (Gutierrez, Gracen and Edwards, 1974).

MATERIALS AND METHODS

B. gracilis was grown from seed (Sharp Brothers Seed Company, Healy, Kansas) in 10 cm diameter pots (five seedlings per pot, 15 pots per treatment) containing steam-sterilized grassland sandy loam (Olney soil series) collected from the Pawnee grassland IBP site near Ft. Collins, Colorado, U.S.A. Each pot was inoculated with approximately 150 sterile (non-mycorrhizal treatment) and viable (mycorrhizal treatment) *G. fasciculatus* spores collected from clones of *B. gracilis* in the Pawnee grasslands by a sucrose flotation method (Allen *et al.*, 1979). The greenhouse was adjusted to approximate a typical growing season for a shortgrass steppe region of the north-central United States (16 h day, 30 to 35 °C, 8 h night, 15 to 20 °C, 13 to 23 % relative humidity) (Lauenroth and Sims, 1976). Plants were watered regularly until experiments began. No fertilizers were added throughout the study. Lighting was supplemented with high-intensity incandescent lamps. Following inoculation, axenic conditions were not maintained.

Plant growth and nutrient status

After 5 months growth, infection frequency was determined as described by Allen and Allen (1980) and roots and shoots were washed and dried at 55 °C for 48 h for dry wt determination. It was impossible to separate all soil particles from the roots; therefore, following dry wt determinations, the roots were ashed at 550 °C for 6 h and the ash weight was subtracted from the dry wt. Leaf size was estimated on one plant randomly selected from each pot; leaf length and width were measured just above the sheath and triangular leaf area computed. From the total number of leaves per plant the leaf area per plant was determined. Stomatal characteristics were determined at midday by spreading a thin layer of Duco^R cement (Du Pont Co.) over the surface of the flag leaf, near the sheath. After drying, the cement was carefully stripped off and stomatal length, width and density measured microscopically. Other leaves were frozen and hand-sectioned just above the sheath to determine mesophyll cell size, bundle sheath cell size and number and leaf thickness.

Root and shoot phosphate concentrations were analyzed as described by Dittmer and Wells (1969). Leaf concentrations of total chlorophyll, chlorophyll *a* and *b* were measured spectrophotometrically and chlorophyll *a*/*b* ratios calculated (Ross, 1974).

Water relations

Pots of mycorrhizal and non-mycorrhizal plants were allowed to dry out and soil water potential (ψ_s), air temperature and absolute air humidity monitored until ψ_s reached the lowest measurable limit of the soil thermocouple psychrometers

(minus 5 to minus 6 MPa). Measurements of leaf temperature, stomatal resistance to water vapour diffusion (R^s_{wv}) and leaf xylem pressure (ψ_x), were made on one plant from each pot at midday daily throughout the drying period. Aerodynamic resistance was estimated to be approximately 10 s m^{-1} for both treatments (Nobel, 1974; Taylor, 1975). Total diffusion resistance (R_{wv}) was then computed as the parallel sum of the aerodynamic and stomatal resistance for both sides of the leaf (Nobel, 1974).

Transpiration rates ($\mathcal{J}_{wv}$) for mycorrhizal and non-mycorrhizal plants were computed using the Ohm's Law analogy for water vapour diffusion (Nobel, 1974) and resistance to water flux from soil to leaves calculated as described by Nye and Tinker (1977). Leaf temperatures were measured with a Barnes Instatherm (Model 14-220-4) and air temperature and humidity using a ventilated thermocouple psychrometer in order to calculate the difference in water vapour concentration between leaf interior and surrounding air (Nobel, 1974). Stomatal resistance to water vapour diffusion was measured with a diffusion resistance porometer (Lambda Inst. Corp., Model HAW-103), leaf xylem pressure with a Scholander-type pressure bomb (PMS model 1000) and soil water potential with thermocouple psychrometers (Wescor Inc. model PT51-10) and a Dewpoint microvoltmeter (Wescor Inc. model HR-33T).

Stomatal behaviour and light

Mycorrhizal and non-mycorrhizal plants in saturated soils ($\psi_s \sim 0{\cdot}01$ MPa, $n = 15$) were taken from the greenhouse at midday when the stomata were fully open and placed in a ventilated dark growth chamber at 30 °C (20 % relative humidity). Changes in R^s_{wv} for leaves of three individual plants (alternating within a pot) were measured each minute for the first 10 min and every 2 min thereafter for 30 min using the diffusion resistance porometer. Two additional sets of plants ($n = 15$) were kept in the dark for 24 h at 30 °C and were then placed in a growth chamber at 30 °C using supplemental incandescent and fluorescent lighting at an illumination of 400 μE m^{-2} s^{-1}. Stomatal resistance was monitored and leaf resistance calculated approximately every minute for the first 6 min and every 2 min thereafter for 30 min as above.

Photosynthesis

CO_2 uptake by mycorrhizal and non-mycorrhizal plants was monitored using a flow-through infrared gas exchange system incorporating a Beckman model 865 CO_2 analyzer under near saturating light conditions (1600 μE m^{-2} s^{-1}). Individual plants (8 to 10 blades) were sealed inside a Plexiglass cuvette and blade temperatures were monitored with fine-wire thermocouples (36 gauge). Temperature was controlled ($\pm 1{\cdot}6$ °C) by circulating cold water through a false-bottom in the cuvette and by filtering the light source through 10 cm of water. The CO_2 concentration entering the cuvette was maintained at approximately 320 μl l^{-1} in N_2. The influence of mycorrhizae on the CO_2 diffusion pathway from the ambient air to the carboxylation sites in the chloroplasts was quantified using electrical circuit analogies (Nobel, 1974). The linearity of the equations when the internal concentration of CO_2 was assumed negligible was confirmed by plotting CO_2 flux ($\mathcal{J}_{CO_2}$) against ambient CO_2 concentration over the range 90 to 400 μl l^{-1} of CO_2 in N_2.

Statistics

Standard data transformation and appropriate parametric statistical techniques were used as described by Zar (1974) and Neter and Wasserman (1974). Student's *t*-test was applied to biomass, nutrient content, morphological features, R_p and CO_2 uptake, also to the linear regressions for water status of mycorrhizal and non-mycorrhizal plants. Values of R_{wv} for leaf resistance changes with light were transformed logarithmically and comparisons based on linear regressions.

Table 1. *Plant biomass, morphology and anatomy of VA mycorrhizal and non-mycorrhizal* Bouteloua gracilis *expressed as mean ± s.e. mean* (n)

Parameter	Treatment	
	Non-mycorrhizal	Mycorrhizal
Infection frequency (%)	0 ± 0 (5)	48 ± 5 (15)
Root dry wt (mg per plant)	189 ± 21 (15)	229 ± 19 (15)
Shoot dry wt (mg per plant)	160 ± 19 (15)	197 ± 14 (15)
Total dry wt (mg per plant)	350 ± 28 (15)	425 ± 31 (15)
Average leaf width at base (mm)	0·79 ± 0·02 (10)	0·79 ± 0·02 (10)
Average leaf length (mm)	56·1 ± 2·5 (10)	59·6 ± 2·3 (10)
Average leaf thickness (μm)	124 ± 13 (10)	136 ± 4 (10)
Leaf area (mm^2 per plant)	1059 ± 137 (10)	1223 ± 150 (10)
Average no. of leaves per plant	20·6 ± 1·8 (10)	24·4 ± 1·9 (10)
Stomatal density (number mm^{-2})	205 ± 7 (10)	227 ± 14 (10)
Stomatal length (μm)	20·2 ± 1·9 (10)	22·4 ± 1·7 (10)
Stomatal width (μm)	13·4 ± 1·5 (10)	11·8 ± 2·2 (10)
No. cells per bundle sheath	9 ± 1 (10)	9 ± 0 (10)
Cell diameter (μm)	20 ± 3 (10)	20 ± 1 (10)
Mesophyll cell width (μm)	2 ± 0 (10)	2 ± 0 (10)
Mesophyll cell length (μm)	13 ± 1 (10)	13 ± 3 (10)

Table 2. *Chlorophyll and phosphate concentrations of VA mycorrhizal and non-mycorrhizal* Bouteloua gracilis

Treatment	Leaf chlorophyll (mg kg^{-1} fresh wt)				Phosphate (mmol kg^{-1} fresh wt)	
	a	*b*	Total	*a*/*b*	Leaves	Roots
Non-mycorrhizal	619 ± 50	624 ± 78	1243 ± 121	1·04 ± 0·08	26·4	7·8
Mycorrhizal	867 ± 98*	961 ± 53	1597 ± 111*	1·26 ± 0·05	49·8†	11·5†

* Denotes significant differences at confidence level ⩾ 0·95.

† Significant differences at confidence level ⩾ 0·95 by Mann-Whitney U-test.

Leaf chlorophyll was measured on seven composite samples of four plants [mean ± s.e. (mean)], phosphate on three composite samples of four plants (mean values only).

RESULTS

Plant growth, anatomy and nutrient status

After 4 to 5 months growth, no significant differences in biomass or gross plant morphology were apparent between mycorrhizal and non-mycorrhizal plants (Table 1); the same applied to mesophyll cell size, bundle sheath cell size and number and stomatal size and density (Table 1). Mycorrhizal infection increased

leaf chlorophyll concentrations by 28 %, did not affect chlorophyll *a*/*b* ratios and increased P concentration by 89 and 47 % in leaves and roots respectively (Table 2).

Water relations

Although no significant differences in leaf xylem pressure potentials were noted between mycorrhizal and non-mycorrhizal plants [Fig. 1(a)], R_{wv} was significantly lower in mycorrhizal plants throughout the soil drying period,

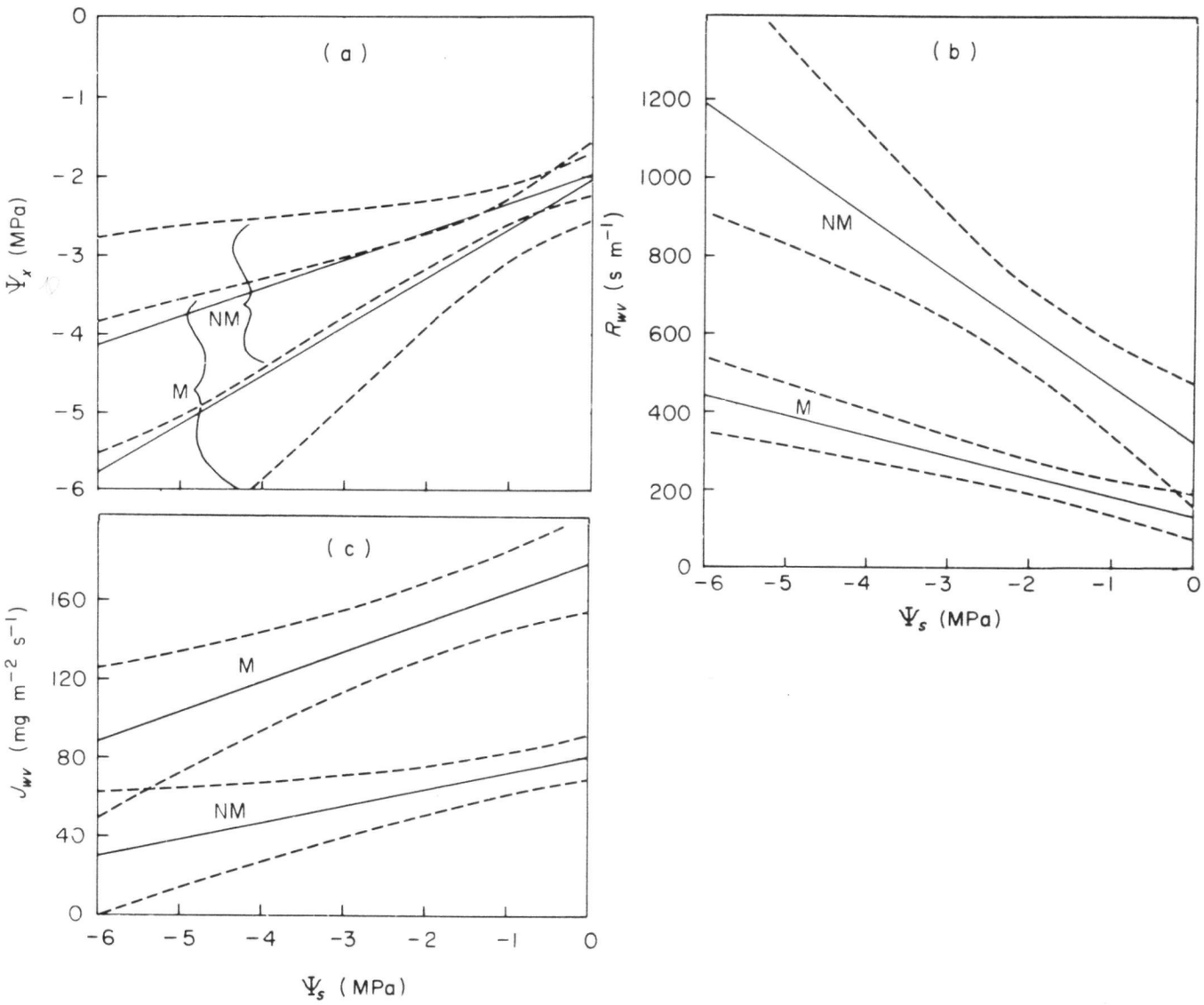

Fig. 1. Linear regressions of leaf water potential (a) (ψ_x), resistance to water vapour diffusion (b) (R_{wv}) and transpiration rates (c) ($\mathcal{J}_{wv}$) against soil water potential (ψ_s) for VA mycorrhizal (M) and non-mycorrhizal (NM) *Bouteloua gracilis*. ——, Linear regressions; – – – –, 95 % confidence levels.

ranging from 50 % less than control plants in saturated soil to 70 % less in extremely dry soils [Fig. 1(b)]. Transpiration fluxes for mycorrhizal plants were more than twice those for non-mycorrhizal plants throughout the range of ψ_s [Fig. 1(c)]. Stomatal resistances of mycorrhizal plants remained low down to the lowest measurable ψ_s (approximately minus 6 MPa), whereas those of many non-mycorrhizal plants increased until no measurable transpiration occurred at ψ_s values near minus 5·5 MPa. However, no significant differences in ψ_s with drying could be detected between pots containing mycorrhizal and non-mycorrhizal plants (Table 3).

Mycorrhizae also appeared to affect water transport from soil to leaves. At high ψ_s (0 to minus 0·5 MPa) there was a 50% reduction in R_p (Table 4). Similar differences between mycorrhizal and non-mycorrhizal plants ocurred in moderately dry soils (minus 0·6 to minus 1·5 MPa), although the variability among individual plants was much greater. In dry soils (less than minus 1·5 MPa), the variability in R_p within treatments was greater than differences between mycorrhizal and non-mycorrhizal treatments.

Table 3. *Water potentials (ψ_s) of drying soil containing mycorrhizal and non-mycorrhizal* Bouteloua gracilis *expressed as mean ± s.e. (mean)* (n = *five per day per treatment*)

Days of drying	ψ_s (MPa)	
	Non-mycorrhizal	Mycorrhizal
0	0·01 ± 0	−0·01 ± 0
2	−0·01 ± 0	−0·01 ± 0
4	−0·1 ± 0·0	−0·1 ± 0·1
6	−1·1 ± 1·0	−1·6 ± 1·4
7	−1·6 ± 0·4	−2·6 ± 0·8

Table 4. *Whole plant resistance to water flux* (R_p) *of VA mycorrhizal and non-mycorrhizal* Bouteloua gracilis *expressed as mean ± s.e. (mean)* (n)

ψ_s range (MPa)	R_p (MPa m² s mg⁻¹ × 10⁻³)		Percentage reduction with infection
	Non-mycorrhizal	Mycorrhizal	
0 to −0·5	20 ± 2 (15)	10 ± 1 (12)*	50
−0·6 to −1·5	15 ± 3 (10)	7 ± 2 (10)†	53
< −1·5	9 ± 10 (9)	1 ± 1 (8)	89

* Significant difference between treatments at confidence level ⩾ 0·99.
† Significant difference between treatments at confidence level ⩾ 0·90.

Stomatal behaviour and light

Although there was little R_{wv} response to decreases in leaf xylem pressure potentials during the day, there were significant differences in response to light–dark transitions between mycorrhizal and non-mycorrhizal plants (Fig. 2). Leaf diffusion resistance of mycorrhizal plants remained approximately 50% lower than in non-mycorrhizal plants during a 30 min exposure to light [Fig. 2(a)], but became much greater during darkness [Fig. 2(b)].

Photosynthesis

VA mycorrhizal infection significantly increased CO_2 uptake by 68% as compared with non-mycorrhizal plants (Table 5). One-third of the increase was due to a 51% reduction in the combined gas-phase resistance ($R^{gas}_{CO_2}$) and two-thirds to a 77% reduction in the liquid-phase resistance of the mesophyll cells ($R^{liq}_{CO_2}$).

DISCUSSION

Bouteloua gracilis is a dominant grass in western North America and has VA mycorrhizal infection frequencies of 88 to 100% in the field (Davidson and

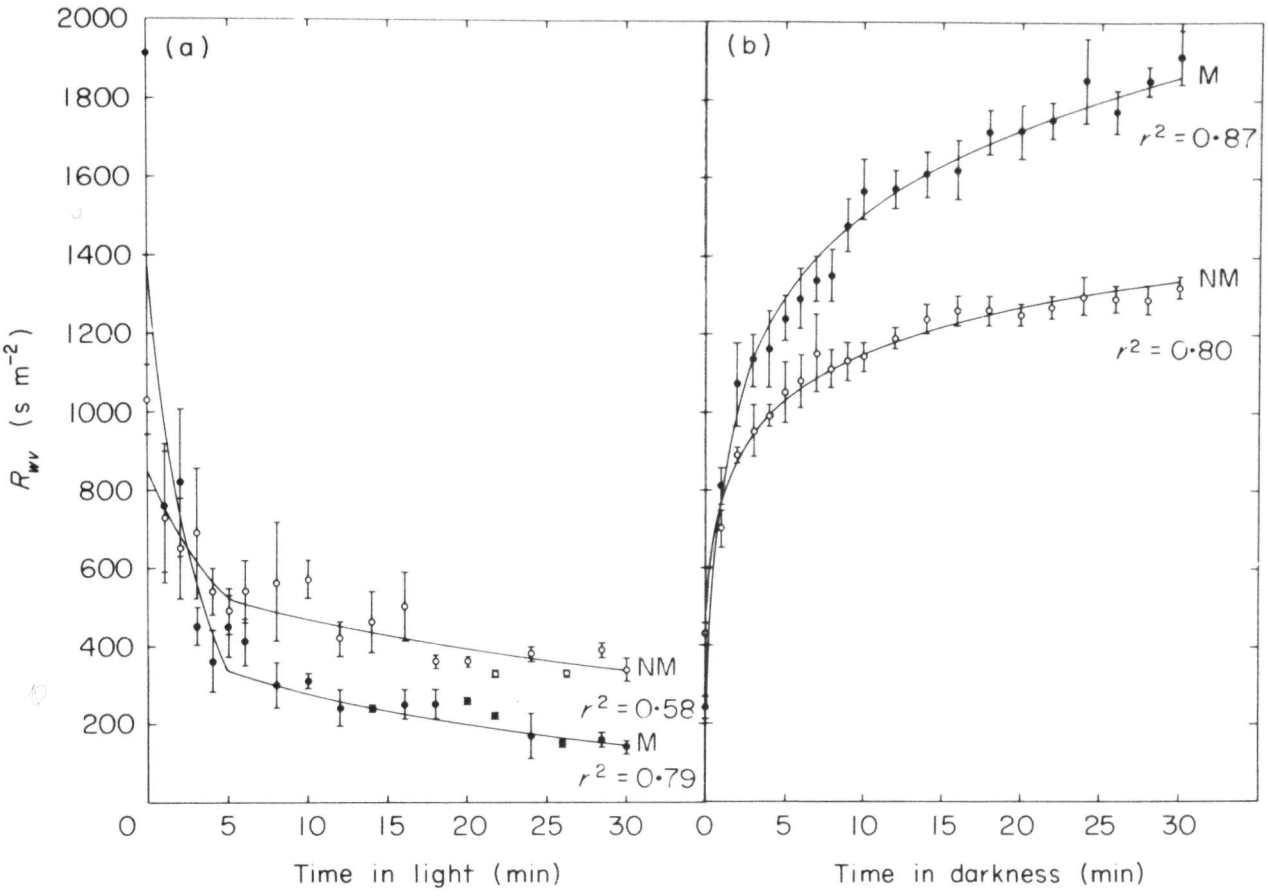

Fig. 2. Diffusion resistance of opening (a) and closing (b) stomata of VA mycorrhizal (●) and non-mycorrhizal (○) *Bouteloua gracilis* in light and dark. Mean values for $R_{wv} \pm$ s.e. (mean).

Table 5. *Gas exchange of VA mycorrhizal and non-mycorrhizal* Bouteloua gracilis *expressed as mean ± s.e. (mean)*

Treatment	No. of plants	R_{wv} (s m^{-1})	$R^{gas}_{CO_2}$ (s m^{-1})	$R^{liq}_{CO_2}$ (s m^{-1})	$\mathcal{J}_{CO_2}$ (mmol s^{-1} m^{-2})	Percentage increase due to $R^{gas}_{CO_2}$	Percentage increase due to $R^{liq}_{CO_2}$
Non-mycorrhizal	11	840 ± 311*	1310 ± 430*	2050 ± 780*	3·8*	—	—
Mycorrhizal	13	560 ± 200	870 ± 310	1160 ± 560	6·4†	33	67

* All measured and calculated values are significantly different between treatments at confidence level ⩾ 0·95.

† Indicates a 68 % increase in photosynthesis in mycorrhizal as compared with non-mycorrhizal plants.

Christensen, 1977; Allen, personal observations). Infection with VA mycorrhizae has been shown to enhance water status in soybean (Safir, Boyer and Gerdemann, 1972), onion (Mosse and Hayman, 1971), citrus (Levy and Krikun, 1980) and to improve survival of *Atriplex canescens* in arid mine spoils (Aldon, 1975). However, only a few investigators have considered possible physiological mechanisms which could contribute to these effects. Daft and Okusamya (1973) reported an increased number of vascular bundles in maize following mycorrhizal infection which could reduce resistance to water transport. Safir *et al.* (1972) estimated that root resistance to water uptake was reduced 40 % by mycorrhizal infection in soybean. They also noted that phosphate fertilizers reduced root resistance and attributed the improved water transport to improved nutritional status, especially phosphorus.

However, Levy and Krikun (1980) suggested that in similarly sized plants, mycorrhizae only increase stomatal conductance and do not alter root resistance.

Transpiration rates were significantly higher in mycorrhizal than in non-mycorrhizal plants with no changes in xylem pressure potentials (Fig. 1). These results were similar to those reported for citrus (Levy and Krikun, 1980). Under high transpirational demands such as in western grasslands (Lauenroth and Sims, 1976) and in our greenhouse (less than 25 % humidity and higher than 30 °C air temperature), xylem pressure potentials closely approached leaf water potentials (Weatherly, 1975). Thus, whole-plant resistance (R_p) was reduced by mycorrhizal infection. Assuming that R_p approaches the inverse of the hydraulic conductivity at high transpiration rates (Fiscus and Markhardt, 1979), *B. gracilis* had fairly high whole-plant resistances when compared with crop plants (Boyer, 1971; Safir *et al.*, 1972; Fiscus and Markhardt, 1979). However, as reported previously by Safir *et al.* (1972), whole-plant resistances dropped sharply with mycorrhizal infection.

Whole-plant resistance is a composite term which incorporates many possible factors including root surface area and permeability (Nye and Tinker, 1977). Although root biomass for *B. gracilis* was not altered significantly here, increased root branching (Allen *et al.*, 1981) could lead to substantial increases in root surface area without changes in total biomass. The absorbing area for water uptake also could be increased in mycorrhizal plants by the fungal hyphae, especially by reducing or eliminating 'dry zones' which might surround the slower growing roots during low soil moisture periods (Nye and Tinker, 1977). Finally, changes in hydraulic conductivity have been observed following changes in phytohormone levels (Markhardt *et al.*, 1979; Tal *et al.*, 1979) such as have been attributed to mycorrhizal infection (Allen, Moore and Christensen, 1980; Allen, 1980).

Leaf resistance to water vapour diffusion in mycorrhizal plants was lower than in non-mycorrhizal plants throughout the drying period [Fig. 1(b)] and in response to illumination (Fig. 2), but became considerably higher in response to darkness (Fig. 2). The reduced resistance could have resulted from enhanced water uptake and thus, higher leaf water potentials or possibly increased photosynthesis (Meidner and Mansfield, 1968). Additionally, leaves from VA mycorrhizal *B. gracilis* grown in axenic culture can have elevated cytokinin levels (Allen *et al.*, 1980) which may stimulate stomatal opening (Incoll and Whitelam, 1977) and transpiration (Cooper, Dibgy and Cooper, 1972). The increased R_{wv} for mycorrhizal plants during darkness (Fig. 2) suggests either incomplete stomatal closure in non-mycorrhizal plants (stomatal sizes and densities were not different between mycorrhizal and non-mycorrhizal plants, Table 1) or a higher cuticular resistance to water vapour diffusion in mycorrhizal plants. To our knowledge, the possibility of a better developed cuticle or other more impermeable epidermal covering following mycorrhizal infection has not been investigated.

The increase in photosynthesis by *B. gracilis* infected with VA mycorrhizae was due to a 51 % reduction in the gas-phase and a 77 % reduction in the liquid-phase resistance (Table 5). The reason for the reduction in gas-phase resistance is probably the same as for the observed reduction in stomatal resistance to water vapour transport (see also Levy and Krikun, 1980). We found higher phosphate levels in mycorrhizal than in non-mycorrhizal plants which might reduce the liquid-phase resistance to CO_2 uptake, but would not be expected to alter the gas-phase resistance (Longstreth and Nobel, 1980). The liquid-phase resistance also incorporates resistance to CO_2 uptake associated with changes in photophosphorylation (Nobel, 1974). The increased amounts of leaf chlorophyll without

significant differences in leaf thickness or chlorophyll *a*/*b* ratios, may indicate an increase in the number of photosynthetic units (Alberte, Fiscus and Naylor, 1975) contributing to the lower resistance.

Despite increased photosynthesis, we found no significant increase in biomass of mycorrhizal plants. We are not sure why this was so. Possibly there was no major phosphate response as has been reported elsewhere (Safir *et al.*, 1972) since phosphate is not as limiting, generally, as water and nitrogen in the Pawnee grassland soils (Lauenroth *et al.*, 1978). Furthermore, *B. gracilis* is a slow growing perennial and may require much longer establishment periods for significant growth enhancements to be observed. According to Tranquillini (1964), as much as 38 % of the CO_2 fixed by *Pinus cembra* may be incorporated in its ectomycorrhizae. Thus, the possibility exists that a substantial portion of the increased photosynthate in our mycorrhizal plants may have been incorporated into the fungus (see Sanders, Mosse and Tinker, 1975). Further research is needed to clarify the role of carbon allocation in the symbiotic relationships of VA mycorrhizal fungi and their hosts.

B. gracilis is one of the few C_4 perennial plants predominating in western North American high plains. In the field, CO_2 fixation was still substantial even at soil water potentials below minus 3 MPa (Brown and Trlica, 1977). Leaf potentials as low as minus 6 to minus 7 MPa have been measured in the field in areas where soil water potentials commonly fall to minus 6 or minus 8 MPa during the growing season (Hutcheson, 1972; Allen, 1979). Therefore, *B. gracilis* must be able to conduct water vapour and CO_2 at extremely low soil water potentials. We noted that conductance of water vapour, and therefore CO_2, was greater in mycorrhizal than in non-mycorrhizal plants down to the lowest measurable soil and leaf water potentials. Furthermore, even though midday transpiration rates were higher in mycorrhizal than in non-mycorrhizal plants, the rate of soil water depletion was not significantly different (Table 3). This suggests that mycorrhizal plants are more efficient in obtaining the available moisture and that a mycorrhizal relationship might be an essential adaptation in these habitats.

The enhanced water and nutrient uptake and photosynthetic rates of *B. gracilis* with mycorrhizal infection indicates an important role for this association in plant survival. As marginal habitats are utilized increasingly for agriculture and energy development, the successful re-establishment of desirable native plant species may require the restoration of VA mycorrhizal associations.

Acknowledgements

We thank Dr Edith Allen, Peter Stahl and James Sexton for helpful discussions and laboratory assistance. This research was supported by National Science Foundation Grant no. DEB-7825222 and a grant from the Industrial Fund of the Rocky Mountain Institute of Energy and Environment at the University of Wyoming.

References

Alberte, R. S., Fiscus, E. L. & Naylor, A. W. (1975). The effects of water stress on the development of the photosynthetic apparatus in greening leaves. *Plant Physiology*, **5**, 317–321.

Aldon, E. F. (1975). *Endomycorrhizae Enhance Survival and Growth of Four-wing Saltbush on Coal Mines Spoils*. USDA Forest Service Research Note RM-294.

ALLEN, E. (1979). *The competitive relationships of introduced annual weeds on some native and reclamation species in the Powder River Basin, Wyoming*. Ph.D. Dissertation. University of Wyoming, Laramie, WY. U.S.A.

ALLEN, E. B. & ALLEN, M. F. (1980). Natural re-establishment of vesicular–arbuscular mycorrhizae following stripmine reclamation in Wyoming. *Journal of Applied Ecology*, **17**, 139–147.

ALLEN, M. F. (1980). *Physiological alterations associated with vesicular–arbuscular mycorrhizal infection in* Bouteloua gracilis. Ph.D. Dissertation, University of Wyoming, Laramie, WY, U.S.A.

ALLEN, M. F., MOORE, T. S., JR. & CHRISTENSEN, M. (1980). Phytohormone changes in *Bouteloua gracilis* infected by vesicular–arbuscular mycorrhizae. I. Cytokinin increases in the host plant. *Canadian Journal of Botany*, **58**, 371–374.

ALLEN, M. F., MOORE, T. S., JR, CHRISTENSEN, M. & STANTON, N. (1979). Growth of vesicular–arbuscular mycorrhizal and non-mycorrhizal *Bouteloua gracilis* in a defined medium. *Mycologia*, **71**, 666–669.

ALLEN, M. F., SEXTON, J. C., MOORE, T. S., JR. & CHRISTENSEN, M. (1981). Influence of phosphate source on vesicular–arbuscular mycorrhizae of *Bouteloua gracilis*. *New Phytologist*, **87**, 687–694.

BOYER, J. S. (1971). Resistances to water transport in soybean, bean and sunflower. *Crop Science*, **11**, 406–407.

BROWN, L. F. & TRLICA, M. J. (1977). Interacting effects of soil water, temperature and irradiance on CO_2 exchange rates of two dominant grasses of the shortgrass prairie. *Journal of Applied Ecology*, **14**, 197–204.

COOPER, M. J., DIBGY, J. & COOPER, P. S. (1972). Effects of plant hormones on the stomata of barley: a study of the interaction between abscisic acid and kinetin. *Planta*, **105**, 43–49.

DAFT, M. J. & OKUSAMYA, B. O. (1973). Effect of *Endogone* on plant growth. *New Phytologist*, **65**, 343–350.

DAVIDSON, D. E. & CHRISTENSEN, M. (1977). Root–microfungal associations in a shortgrass prairie. In: *The Belowground Ecosystem : A Synthesis of Plant-associated Processes* (Ed. by J. K. Marshall), pp. 279–287. Colorado State University, Ft. Collins, Colorado.

DITTMER, J. C. & WELLS, M. A. (1969). Quantitative and qualitative analysis of lipid and lipid components. *Methods in Enzymology*, **14**, 482–530.

FISCHER, R. A. & TURNER, N. C. (1978). Plant productivity in arid and semiarid zones. *Annual Review of Plant Physiology*, **29**, 277–317.

FISCUS, E. L. & MARKHARDT, A. H., III. (1979). Relationships between root system water transport properties and plant size in *Phaseolus* and *Glycine*. *Plant Physiology*, **64**, 770–773.

GUITERREZ, M., GRACEN, V. E. & EDWARDS, G. E. (1974). Biochemical and cytological relationships in C_4 plants. *Planta*, **119**, 279–300.

HARNER, R. F. & HARPER, K. T. (1973). Mineral composition of grassland species of the eastern Great Basin in relation to stand productivity. *Canadian Journal of Botany*, **51**, 2037–2046.

HATTINGH, M. J., GRAY, L. E. & GERDEMANN, J. W. (1973). Uptake and translocation of ^{32}P-labelled phosphate to onion roots by endomycorrhizal fungi. *Soil Science*, **116**, 383–387.

HUTCHESON, J. L. (1972). *Response patterns of* Bouteloua gracilis *leaf water potential*. M.S. Thesis. University of Wyoming, Laramie, Wyoming.

INCOLL, L. D. & WHITELAM, G. C. (1977). The effect of kinetin on stomata of the grass *Anthephora pubescens* Nees. *Planta*, **137**, 243–245.

KHAN, A. G. (1975). Growth effects of vesicular–arbuscular mycorrhiza on crops in the field. In: *Endomycorrhizas* (Ed. by F. E. Sanders, B. Mosse & P. B. Tinker), pp. 419–436. Academic Press, New York.

LAUENROTH, W. K., DODD, J. L. & SIMS, P. L. (1978). The effects of water- and nitrogen-induced stresses on plant community structure in a semiarid grassland. *Oecologia* (Berlin), **36**, 211–222.

LAUENROTH, W. K. & SIMS, P. L. (1976). Evapotranspiration from a short-grass prairie subjected to water and nitrogen treatments. *Water Resources Research*, **12**, 437–442.

LEVY, I. & KRIKUN, J. (1980). Effect of vesicular–arbuscular mycorrhiza in *Citrus jambhiri* water relations. *New Phytologist*, **85**, 25–32.

LONGSTRETH, D. J. & NOBEL, P. S. (1980). Nutrient influences on leaf photosynthesis, effects of nitrogen, phosphorus, and potassium for *Gossypium hirsutum* L. *Plant Physiology*, **65**, 541–543.

MEIDNER, H. & MANSFIELD, T. A. (1968). *Physiology of Stomata*. McGraw-Hill, New York.

MARKHARDT, A. J., III, FISCUS, E. L., NAYLOR, A. W. & KRAMER, P. J. (1979). Effect of abscisic acid on root hydraulic conductivity. *Plant Physiology*, **64**, 611–614.

MOSSE, B. & HAYMAN, O. J. (1971). Plant growth responses to vesicular arbuscular mycorrhiza. II. In unsterilized field soils. *New Phytologist*, **70**, 29–34.

NETER, J. & WASSERMANN, W. (1974). *Applied Linear Statistical Models : Regression, Analysis of Variance, and Experimental Designs*. Richard D. Irwin, Inc., Homewood, IL.

NOBEL, P. S. (1974). *Introduction to Biophysical Plant Physiology*. W. H. Freeman and Co., San Francisco.

NYE, P. H. & TINKER, P. B. (1977). *Solute Movement in the Soil–root System*. University of California Press, Berkeley.

RHODES, L. H. & GERDEMANN, J. W. (1978). Hyphal translocation and uptake of sulfur by vesicular–arbuscular mycorrhizae of onion. *Soil Biology and Biochemistry*, **10**, 355–360.

RHODES, L. H. & GERDEMANN, J. W. (1979). Translocation of calcium and phosphate by external hyphae of vesicular–arbuscular mycorrhizae. *Soil Science*, **126**, 125–216.

Ross, C. W. (1974). *Plant Physiology Laboratory Manual*. Wadsworth Publishing Company, Inc., Belmont, California.

Ruehle, J. W. & Marx, D. H. (1979). Fiber, food fuel, and fungal symbionts. *Science*, **206**, 219–422.

Safir, G. R., Boyer, J. S. & Gerdemann, J. W. (1971). Mycorrhizal enhancement of water in soybeans. *Science*, **172**, 581–583.

Safir, G. R., Boyer, J. S. & Gerdemann, J. W. (1972). Nutrient status and mycorrhizal enhancement of water transport in soybeans. *Plant Physiology*, **49**, 700–703.

Sanders, F. E., Mosse, B. & Tinker, P. B. (1975). *Endomycorrhizas*. Academic Press, New York.

Sanders, F. E. & Tinker, P. B. (1971). Mechanism of absorption of phosphate from soil by *Endogone* mycorrhizas. *Nature*, **233**, 278–279.

Tal, M., Imber, D., Erez, A. & Epstein, E. (1979). Abnormal stomatal behavior and hormonal imbalance in *Flacca*, a wilty mutant of Tomato. V. Effect of abscisic acid on indoleacetic acid metabolism and ethylene evolution. *Plant Physiology*, **63**, 1044–1048.

Taylor, S. E. (1975). Optimal leaf form. In: *Perspectives of Biophysical Ecology*, *Ecological Studies*, vol. 12 (Ed. by D. M. Gates & R. B. Schmerl), pp. 73–86. Springer, Verlag, New York.

Tranquillini, W. (1964). Photosynthesis and dry matter production of trees at high altitudes. In: *The Formation of Wood in Forest Trees* (Ed. by M. H. Zimmerman), pp. 606–618. Maria Moors Cabot Foundation for Botanical Research, New York.

Weatherly, P. E. (1975). Water relations of the root system. In: *The Development and Function of Roots* (Ed. by J. G. Torey & D. T. Clarkson), pp. 397–413. Academic Press, New York.

Zar, J. H. (1974). *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, New Jersey.