Papers in Plant Pathology

Plant Pathology Department

1982

INFLUENCE OF VESICULAR-ARBUSCULAR MYCORRHIZAE ON WATER MOVEMENT THROUGH *BOUTELOUA GRACILIS* (H.B.K.) LAG EX STEUD

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INFLUENCE OF VESICULAR–ARBUSCULAR MYCORRHIZAE ON WATER MOVEMENT THROUGH *BOUTELOUA GRACILIS* (H.B.K.) LAG EX STEUD*

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SUMMARY

Root growth characteristics and water transport were compared between non-mycorrhizal *Bouteloua gracilis* and vesicular–arbuscular mycorrhizal *Bouteloua gracilis* infected with *Glomus fasciculatus*. Mycorrhizal plants did not have significantly different leaf area or root length from non-mycorrhizal plants, but did have significantly fewer and shorter root hairs. Mycorrhizal plants had 50% lower leaf resistance with no change in leaf or root water potentials; thus transpiration was increased 100% with a 50% reduction in whole-plant, soil-to-root and root-to-leaf resistance. Assuming that the difference in water uptake was due to transport via the mycorrhizal hyphae, estimated fungus-to-root transport was 2.8×10^{-5} mg s^{-1} per hyphal entry point which compares favourably with evapotranspiration rates measured in other coenocytic fungi. Thus, one of the major factors causing increased water transport and reduced resistance to water through-flow with mycorrhizal infection may be the increased surface area provided by the hyphae.

INTRODUCTION

Vesicular–arbuscular (VA) mycorrhizae can improve growth and survival of plants in numerous habitats (Hayman, 1980). In mesic to wet habitats, increased phosphate uptake is considered to be the major cause (Sanders *et al.*, 1977), but in the semi-arid to arid regions of the North American Great Plains, water is generally a major factor limiting production (Lauenroth, Dodd and Sims, 1978). If VA mycorrhizae are capable of substantially altering production and survival in these habitats, they must increase plant water uptake and/or improve stress tolerance.

In this paper, I have examined water uptake and movement through *Bouteloua gracilis* (H.B.K.) Lag ex Steud with its symbiont *Glomus fasciculatus* (Thaxter *sensu* Gerdemann) Gerdemann and Trappe to understand further how VA mycorrhizae might alter water transport.

Improved water relations of mycorrhizal versus non-mycorrhizal plants has been noted by several workers (Safir, Boyer and Gerdemann, 1972; Sieverding, 1979; Levy and Krikun, 1980; Allen *et al.*, 1981b; Hardie and Leyton, 1981); however, mechanisms by which mycorrhizal infection might improve water through-flow or increase drought resistance are only beginning to be studied. Safir *et al.* (1972)

reported that mycorrhizae could reduce the resistance to water flow by 41 % in soybeans, apparently due to reduced root resistance; this was attributed to improved phosphate nutrition. Levy and Krikun (1980) found lowered stomatal resistances with no significant differences in estimated plant resistance. They suggested that mycorrhizal infection affected the phytohormone balance which altered stomatal control. Allen *et al.* (1981b) reported that mycorrhizal *Bouteloua gracilis* also had lower stomatal resistance than non-mycorrhizal plants, resulting in increased transpiration rates in saturated and dry soils. Mycorrhizal infection did not change root biomass substantially in these plants but can alter both phosphate transport (Allen *et al.*, 1981a) and phytohormone balances (Allen, 1980; Allen, Moore and Christensen, 1980). Hardie and Leyton (1981) found that the hydraulic conductivities of red clover root systems were higher in mycorrhizal than non-mycorrhizal plants. The higher water flow through the plants could not be accounted for by the increased root lengths in the larger mycorrhizal plants. Thus, they suggested that the difference may be attributable to increased surface area contributed by the fungal hyphae.

MATERIALS AND METHODS

Bouteloua gracilis was grown from seed (Sharp Brothers Seed Company; Healy, Kansas) in 10 cm diameter pots (five seedlings per pot, 15 pots per treatment) in pasteurized greenhouse potting soil (2:2:1, loam:sand:peat). Each pot was inoculated with approximately 600 autoclaved (non-mycorrhizal) or viable (mycorrhizal) *Glomus fasciculatus* spores collected from the Arapahoe Prairie in the Sandhills of central Nebraska. Plants were grown for 2 months in the greenhouse under high intensity incandescent lamps (16 h days, 20 to 30 °C; 8 h nights, 15 to 20 °C; 40 to 50 % r.h, 1150 μE m^{-2} s^{-1}) and were watered regularly throughout the experiment. No fertilizers were added. Following initial sterilization, axenic conditions were not maintained.

After 2 months, five pots (25 plants) of each treatment were harvested. Leaf area was measured on one plant chosen randomly from each pot. Leaf lengths and widths just above the sheath were measured, triangular leaf area computed and area of all leaves summed. Roots were divided into three size classes, and lengths and areas were estimated as described by Fiscus (1977). In addition, density, number, diameter and length of root hairs was recorded.

Water relations measurements were made on plants from five additional pots of each treatment. Stomatal resistance (r_s), leaf temperature, and relative humidity were measured using a steady-state porometer (LI-COR-LI-1600). Leaf water potentials (Ψ_l) were measured using *in situ* leaf hygrometers (Wescor Model L-51A with a Wescor microvolt meter Model HR33T) as described by Campbell and Campbell (1974). Values recorded were similar to earlier measurements of leaf water potentials using the pressure chamber (Allen, 1980). Root water potentials (Ψ_r) were also estimated. Plants were removed from the soil and as much of the soil gently shaken off as possible. Roots were placed inside the hygrometers, sealed in plastic, and placed in foam cups until readings equilibrated (≈ 45 min).

Total diffusive resistance was computed as the parallel sum of the aerodynamic and stomatal resistance for both sides of the leaf and transpiration flux (J_{wv}) estimated using Ohm's Law analogy for water vapour diffusion (Nobel, 1974). Also, whole plant and root-to-leaf resistances were calculated using Ohm's Law analogy (Nye and Tinker, 1977). Because of the small leaf area (LAI = 0·14 to

0·15), total plant water transport could be estimated in two ways; (1) by multiplying the transpiration flux by the leaf area per plant, or (2) estimating canopy resistance and computing transpiration per plant (Campbell, 1977). Water uptake flux could then be estimated assuming negligible water storage, and soil to root resistance using Ohm's analogy equations (Nye and Tinker, 1977; Blizzard and Boyer, 1981).

Following these measurements, the second set of five pots of each treatment was harvested. Roots were washed, stained and the percentage infected root length estimated as described by Allen and Allen (1980). Number of root penetrations by mycorrhizal fungi and hyphal diameter was recorded.

All parameters were compared between mycorrhizal and nonmycorrhizal plants using the Mann–Whitney U-test for non-parametrically distributed data (Zar, 1974).

RESULTS AND DISCUSSION

Mycorrhizal infection altered growth characteristics in *B. gracilis* (Table 1). Mean leaf area was higher in mycorrhizal than non-mycorrhizal plants, but was not statistically significant (Table 1); nor were estimated root length and root areas (Table 2). However, non-mycorrhizal plants had a significantly greater proportion of root length with hairs and the root hairs were longer than in mycorrhizal plants (Table 1). This resulted in a somewhat greater total root surface for non-mycorrhizal than mycorrhizal plants (Table 2). Mycorrhizal fungi occupied 60 % of the root length in infected plants and averaged 10 hyphal entry points per cm of infected root (Table 1).

Mycorrhizal infection increased water movement through *B. gracilis*. Stomatal

Table 1. *Measured growth characteristics of mycorrhizal v. non-mycorrhizal* Bouteloua gracilis (*see Methods*)

Treatment	Leaf area (mm^2)	Root length with hairs (%)	Root hair length (μm)	Root hair width (μm)	No. root hairs $(mm^{-1}$ root length)	Mycorrhizal infection (%)	Mycorrhizal entry $(cm^{-1}$ root length)
Non-mycorrhizal	630	27*	169*	7	132	0*	0*
Mycorrhizal	690	21	135	7	130	60	10

* Indicates significantly different at a confidence level ⩾ 0·95 using the Mann–Whitney U-test.

Table 2. *Calculated growth characteristics of mycorrhizal v. non-mycorrhizal* Bouteloua gracilis (*see Methods*)

Treatment	Root length (mm)	Root area (mm^2)	Root hair length (mm)	Root hair area (mm^2)	Total Root area (mm^2)
Non-mycorrhizal	2880	943	17	391	1330
Mycorrhizal	2650	854	9·8	220	1070

Table 3. *Water relations characteristics of mycorrhizal* v. *non-mycorrhizal* Bouteloua gracilis *(see Methods)*

Treatment	r_s (s m^{-1})	ψ_l (MPa)	ψ_r (MPa)	J_{wv} (mg m^{-2}s^{-1})
Non-mycorrhizal	200*	−1·5	−0·2	59*
Mycorrhizal	100	−1·8	−0·3	119

* Indicates significantly different between treatments as at confidence level $\geqslant 0.95$ using the Mann-Whitney U-test.

Table 4. *Estimated water relations characteristics of mycorrhizal* v. *non-mycorrhizal* Bouteloua gracilis *(see Results and Discussion)*

Treatment	Total plant water transport (μg s^{-1})	Estimated water uptake through roots (mg m^{-2}s^{-1})	Whole plant resistance* (MPa m^{2}s mg^{-1})	Soil-to-root resistance† (MPa m^{2}s mg^{-1})	Root-to-leaf resistance* (MPa m^{2}s mg^{-1})
Non-mycorrhizal	37	28	2.5×10^{-2}	7.1×10^{-3}	2.2×10^{-2}
Mycorrhizal	82	77	1.5×10^{-2}	3.8×10^{-3}	1.3×10^{-2}

* Values are based on J_{wv}.
† Values are based in estimated root water uptake.

resistance was reduced 50 % with infection, but there were no significant changes in plant water potentials (Table 3). This resulted in a 100 % increase in transpiration rates (Table 3). The increased transpiration with no change in Ψ indicates that there was a significant reduction in the whole-plant resistance (Table 4). Assuming negligible water storage in *B. gracilis* the higher transpiration should result in greater water uptake with mycorrhizal infection. The estimated J_{wv} of mycorrhizal plants was always approximately double that of non-mycorrhizal plants and there appeared to be little variation in r_s or leaf temperature. Since a large proportion of the available leaf area was needed for measurement, and the low leaf area index allowed adequate light to permit stomatal opening over most of the plant (Campbell, 1977), the average J_{wv} was used to estimate plant water flow. Estimates based on canopy resistance (Campbell, 1977) were similar to those using an average J_{wv}; non-mycorrhizal water transport was 33 μg s^{-1} v. 37 μg s^{-1} using average J_{wv} and mycorrhizal water transport was 73 μg s^{-1} v. 82 μg s^{-1} using canopy resistance and average J_{wv} respectively.

The whole-plant resistance term can be broken into several resistances: soil, soil-to-root, and root-to-leaf. Soil resistance was considered negligible under saturated conditions (Blizzard and Boyer, 1981). Soil-to-root resistance (based on root water uptake) was reduced 46 % and root-to-leaf resistance (based on J_{wv}) was reduced 41 % (Table 4). Therefore, mycorrhizal infection appeared to decrease the entire resistance pathway through the plants.

Fiscus and Markhardt (1979) and Markhardt *et al.* (1979) demonstrated that hydraulic conductivity (at high flow rates this value approaches resistance^{-1}) is highly dependent on flow rates which are regulated by root area and chemical and

morphological features. Mycorrhizal infection has been shown to improve nutritional status and to alter hormonal balances in *B. gracilis* (Allen, 1980; Allen, Moore and Christensen, 1980; Allen *et al.*, 1981a, b) which may alter water through-flow. However, it has been hypothesized that water may be transported from soil to plant via the fungal hyphae (Hardie and Leyton, 1981). Water moves through fungi in response to changes in water potential similar to that in higher plants (Lucas, 1977; Luard and Griffin, 1981). In the plants measured here, mycorrhizal infection occurred along 60 % of the length with an entry point at approximately every 1 mm. Assuming that the rate of water uptake is similar between mycorrhizal and nonmycorrhizal plants per unit *root* surface area, and ascribing the observed difference to fungal hyphae, the rate of water transport to the roots via fungal hyphae can be estimated as:

$$\text{hyphal transport} = (A_M^{\text{root}} \cdot J_M^{\text{root}}) - (A_M^{\text{root}} \cdot J_{NM}^{\text{root}})$$

where A_M^{root} is the surface area of the mycorrhizal roots, J_M^{root} is the estimated rate of water intake of mycorrhizal roots, and J_{NM}^{root} is the estimated rate of water intake of non-mycorrhizal roots. The difference (hyphal transport) divided by the number of root entry points (≈ 1600 per root system) gives an estimated water transport rate of $2 \cdot 8 \times 10^{-5}$ mg s^{-1} or 100 nl h^{-1} per hyphal entry point. Cowan, Lewis and Thain (1972) estimated that evapotranspiration in the coenocytic fungus *Phycomyces blakesleanus* was as high as 131 nl h^{-1} under a similar vapour pressure deficit.

CONCLUSIONS

VA mycorrhizal infection can improve water uptake in the host, but whether this effect is a direct result of fungal invasion or a secondary response to altered nutrition of phytohormone balance remains unresolved. Safir *et al.* (1972) and Levy and Krikun (1980) considered improved water transport to be a secondary function responding to the altered physiological status of the plant. Regrettably, little information on root system parameters were presented. Hardie and Leyton (1981) reported increased root length and substantially greater water flow rates per unit root length with mycorrhizal infection. They suggested that the external fungal hyphae were required to account for the increased water through-flow. However, altered sizes of root systems alone can change root conduction in a non-linear fashion (Fiscus and Markhardt, 1979). In *B. gracilis*, mycorrhizal infection increased water uptake rates even with similarly sized root systems and the estimated differences were within the range of evapotranspiration in other fungal species. These results tend to support a predominantly direct flow mechanism (Hardie and Leyton, 1981) as the reduced resistances might be attributable to the greater flow rates associated with increased absorptive surface area (Fiscus, 1977).

Mechanisms of mycorrhizal benefits may be a response to one type of stress to which the host is subjected. There appear to be numerous physiological and morphological responses to mycorrhizae which when considered together, can significantly improve adaptations of the host plant. In mesic habitats, where phosphate is one of the major limiting factors, increased phosphate uptake may lead to many of the observed mycorrhizal biomass responses. In semi-arid habitats, increased water uptake and drought tolerance in response to fungal surface area and improved plant physiological status, may be major mechanisms improving survival and production of infected plants.

REFERENCES

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.

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

ALLEN, M. F., SMITH, W. K., MOORE, T. S. JR. & CHRISTENSEN, M. (1981b). Comparative water relations and photosynthesis of mycorrhizal and non-mycorrhizal *Bouteloua gracilis* (H.B.K.) Lag ex Stend. *New Phytologist*, **88**, 683–693.

BLIZZARD, W. E. & BOYER, J. S. (1980). Comparative resistance of the soil and the plant to water transport. *Plant Physiology*, **66**, 809–814.

CAMPBELL, G. S. & CAMPBELL, M. D. (1974). Evaluation of a thermocouple hygrometer for measuring leaf water potential *in situ*. *Agronomy Journal*, **66**, 24–27.

CAMPBELL, G. S. (1977). *An Introduction to Environmental Biophysics*. Springer-Verlag, New York.

COWAN, M. C., LEWIS, B. G. & THAIN, J. F. (1972). Uptake of potassium by the developing sporangiophore of *Phycomyces blakesleanus*. *Transactions of the British Mycological Society*, **58**, 113–126.

FISCUS, E. L. (1977). Determination of hydraulic and osmotic properties of soybean root systems. *Plant Physiology*, **59**, 1013–1020.

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

HARDIE, K. & LEYTON, L. (1981). The influence of VA mycorrhiza on growth and water relations of red clover. I. In phosphate deficient soil. *New Phytologist*, **89**, 599–608.

HAYMAN, D. S. (1980). Mycorrhiza and crop production. *Nature*, **287**, 487–488.

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

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

LUARD, E. J. & GRIFFIN, D. M. (1981). Effect of water potential on fungal growth and turgor. *Transactions of the British Mycological Society*, **76**, 33–40.

LUCAS, R. L. (1977). The movement of nutrients through fungal mycelium. *Transactions of the British Mycological Society*, **69**, 1–9.

MARDHARDT, 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.

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

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

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. & TINKER, P. B. (1973). Phosphate flow into mycorrhizal roots. *Pesticide Science*, **4**, 385–395.

SANDERS, F. E., TINKER, P. B., BLACK, R. L. B. & PALMERLEY, S. M. (1977). The development of endomycorrhizal root systems. I. Spread of infection and growth-promoting effects with four species of vesicular–arbuscular endophyte. *New Phytologist*, **78**, 257–268.

SIEVERDING, E. (1979). Einfluss der Bodenfeuchte auf die Effektivitat der VA-Mykorrhiza. *Angewandte Botanik*, **53**, 91–98.

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