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EFFECTS OF TWO SPECIES OF VA MYCORRHIZAL FUNGI ON DROUGHT TOLERANCE OF WINTER WHEAT*

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SUMMARY

Roots and soils from western Nebraska fields of native and planted grasslands, and winter wheat of varied fallow-wheat cultivation duration, were evaluated for vesicular–arbuscular (VA) mycorrhizal root infection and spore numbers and types. Increased cultivation decreased percentage mycorrhizal infection in wheat and reduced spore numbers of *Glomus fasciculatus*, the dominant VA mycorrhizal fungus in these soils. Spore numbers of other VA mycorrhizal fungi did not change significantly with cultivation although mean numbers of *G. mosseae* increased with continued wheat production. Water relations and growth were determined for greenhouse-grown non-mycorrhizal, *G. fasciculatus*-infected, and *G. mosseae*-infected wheat in wet and dry soils. Stomatal conductances were higher in mycorrhizal than in non-mycorrhizal plants in both wet and dry treatments. Stomatal closure in mycorrhizal plants occurred at lower leaf water potentials (ψ_1) and after greater desiccation than in non-mycorrhizal plants, but some leaves of *G. mosseae*-infected plants showed no stomatal response to drought and continued to transpire at ψ_1 as low as $-4{\cdot}1$ MPa. Leaf osmotic adjustment was greatest for *G. fasciculatus*-infected plants. Non-mycorrhizal and *G. fasciculatus*-infected plants had equal dry wts in both wet and dry conditions. Infection by *G. fasciculatus* appeared to increase wheat drought tolerance while infection by *G. mosseae* did not.

INTRODUCTION

In recent years there has been a renewed interest in converting range lands of the western Great Plains of North America to cereal crops. Wheat yields are high following breakup of the grassland sod, but tend to decline with continued cropping. Although precise reasons for these declines are not known, deficiencies of water, nitrogen and phosphorus may be involved.

Vesicular–arbuscular (VA) mycorrhizae commonly occur in these habitats (Davidson and Christensen, 1977; Allen and Allen, 1980) and improve drought tolerance and nutrition of the native grasses (Allen, Allen and Boosalis, 1981; Allen *et al.*, 1981). However, tillage, pesticides and fertilizers can eliminate mycorrhizal fungi or shift abundances of dominant species (Hayman, 1980; Schenck and Kinlock, 1980; Nemec *et al.*, 1981). Thus, productivity could be enhanced or reduced with cropping in response to interactions between different host and fungal species (Carling and Brown, 1980; Azcón and Ocampo, 1981).

As an initial step in understanding the role of mycorrhizae in dryland wheat production in western Nebraska, we surveyed adjacent different-aged wheat fields

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and grasslands for VA mycorrhizal fungi and have investigated the influence of two dominant endophytes, *Glomus fasciculatus* (Thaxter sensu Gerdemann) Gerdemann and Trappe and *Glomus mosseae* (Nicol. and Gerd.) Gerdemann and Trappe, on growth and drought tolerance of young winter wheat plants, *Triticum aestivum* L. 'Centurk'.

MATERIALS AND METHODS

Field observations

A farm 20 km west of Scottsbluff, Nebraska, contained adjacent native grasslands, planted grasslands and wheat-fallow rotations. Six fields were sampled in June 1980 for VA mycorrhizal fungal spores: site *a*, the native grassland (dominated by *Stipa comata* Trin. & Rupr., needle and thread grass); site *b*, the planted grassland (dominated by *Agropyron desertorum* (Fisch.) Schult., crested wheatgrass); site *c*, a fallow field newly broken from crested wheatgrass; site *d*, a wheat field following the first year of fallowing after crested wheatgrass; site *e*, a field of wheat which had undergone wheat-fallow rotation for > 20 years and site *f*, a fallow field which had undergone wheat-fallow rotation for > 20 years.

Samples were collected from the top 20 cm and spores extracted and counted as described by Allen *et al.* (1979). Individuals of dominant grass from each of sites *a*, *b*, *d* and *e* were sampled for VA mycorrhizal infection. Roots of plants in the top 20 cm were collected, washed, stained (Kormanick, Bryan and Schultz, 1980), and percentage infection estimated (Allen and Allen, 1980). All soils were sandy-loams (Keith soil series). Samples from sites *c* and *f* were analysed by the University of Nebraska Soil Testing Laboratory.

Greenhouse experiments

Wheat plants were grown from seed in plastic pots, 11 cm diameter, containing 800 g pasteurized soil (2:2:1, loam:sand:peat, Table 1). A 3 × 2 factorial design was used consisting of three biotic treatments, non-mycorrhizal, *G. fasciculatus*-

Table 1. *Status of greenhouse potting soil used for experiments*

pH	Nitrogen (NO_3 residual) (p.p.m.)	Phosphorus (Bray1 extraction) (p.p.m.)	Potassium (p.p.m.)	Organic matter (%)
5·6	30·0	42·0	219·0	1·6

infected and *G. mosseae*-infected, and two abiotic treatments, wet versus dry. Spores of *G. fasciculatus* and *G. mosseae* collected from site *c* soil by wet-sieving and decanting (Gerdemann and Nicolson, 1963) were used for the first replication. In subsequent replications we used spores collected from pots of the first replication. Seedlings were inoculated 2 to 3 days after germination with 500 to 700 spores of the appropriate fungus in Ringer's solution pipetted onto the soil surface at the base of each plant. All plants were watered regularly for 2 weeks to permit mycorrhizal establishment. Wet and dry treatments were then created by saturating half of the pots daily; the other half were watered only when the plants began to wilt. Plants were grown in the greenhouse under high intensity

incandescent lamps (16 h day, 20 to 30 °C, 1150 μE m^{-2} s^{-1}, 15 to 20 °C night, 40 to 50% r.h.). No fertilizers were added and axenic conditions were not maintained. The experiments were replicated three times.

After 2·5 months, five pots of each treatment were harvested and leaves and roots separated. Dry wts were determined after drying at 60 °C for 48 h.

The rest of the plants were used for water relations measurements. Because lighting was not constant in the greenhouse (clouds, overhead beams), at the end of 2 months, plants from all treatments were placed in a growth chamber (16 h day, 25 °C, 500 μE m^{-2} s^{-1}, 15 °C night, 35 to 45% r.h.). After 1 week of equilibration soils of all treatments were saturated. Stomatal resistances and leaf water potentials of plants from the wet treatments were determined at mid-day following saturation; those of plants from the dry treatments were determined as the pots dried out with daily readings after plants approached steady-state conditions. Leaf resistances were measured on both sides of the leaf using a LI-COR steady-state porometer (model LI-1600) and stomatal resistance (R_s) was estimated using electrical circuit analogy equations (Nobel, 1974). Boundary layer resistance was calculated to be 30 to 50 S/m (Nobel, 1974). Cuticular resistance (R_c) was assumed to equal pre-dawn stomatal resistance. Leaf water potentials (ψ_l) were measured using a pressure chamber (PMS model 1000). At the end of the drying period, leaves were detached, allowed to wilt, sealed in foil and plastic tape, placed in plastic bags, and frozen at −10 °C. After 24 h, the leaves were thawed and osmotic potentials (ψ_π) measured (Slavik, 1974) using leaf psychrometers (Wescor Model L-51A with a Wescor microvolt meter model HRT33T). Roots were harvested, stained (Kormanick *et al.*, 1980), and mycorrhizal infection estimated (Allen and Allen, 1980).

Statistics

Field infection frequencies and spore counts were compared using the Kruskal-Wallis test followed by the Mann-Whitney U-test for non-parametrically distributed data (Zar, 1974). Values for greenhouse material were compared using Analysis of Variance followed by *t*-tests using pooled variances (Zar, 1974). Stomatal resistances versus ψ_l values were combined for all replications and compared using piecewise regressions (Neter and Wasserman, 1974) followed by *t*-test comparisons (Zar, 1974).

Results

Field observations

Plants from the different fields had different mycorrhizal infections (Table 2). Total infection was highest in needle and thread grass followed by crested wheatgrass, wheat (site *d*) and wheat (site *e*), respectively. Arbuscule formation was high in the wheat (site *d*) and low in other plants. Vesicle formation was high in needle and thread grass and crested wheatgrass, but low in wheat from both sites.

Spore counts were dependent on the degree of soil disturbance (Table 3). Total spore numbers were highest in the grassland and newly disturbed sites and decreased with continued wheat production. The decrease was due to reduced numbers of *G. fasciculatus* spores as there were no significant changes in other species. Mean numbers of *G. mosseae* spores tended to increase with the transition from the grassland to wheat production.

The decline in VA mycorrhizae with age of wheat fields did not appear to be

Table 2. *Mycorrhizal infection in field (see Methods)*

Plants	Site	Percentage infection			
		Arbuscules	Vesicles		Total
			Internal	External	
Needle and thread grass	*a*	17^{b*}	49^{a}	73^{a}	79^{a}
Crested wheatgrass	*b*	14^{b}	43^{a}	18^{b}	68^{b}
Wheat	*d*	33^{a}	6^{b}	3^{c}	46^{c}
Wheat	*e*	16^{b}	1^{b}	0^{c}	23^{d}

* Letters represent significant differences at a confidence level $\geqslant 0{\cdot}95$ using the Kruskal-Wallis test followed by the Mann-Whitney U-test for non-parametrically distributed data.

Table 3. *Influence of land use on spores of VA mycorrhizal fungi (see Methods)*

Site	No. spores g^{-1} soil					
	Glomus				*Gigaspora* spp.	Total
	fasciculatus	*macrocarpus*	*mosseae*	Other spp.		
a	16^{a*}	$0{\cdot}7^{a}$	$0{\cdot}2^{a}$	0^{a}	$0{\cdot}1^{a}$	18^{a}
b	11^{a}	$0{\cdot}6^{a}$	$0{\cdot}2^{a}$	0^{a}	0^{a}	11^{ab}
c	11^{ab}	$0{\cdot}3^{a}$	$0{\cdot}3^{a}$	$0{\cdot}1^{a}$	0^{a}	12^{ab}
d	$5{\cdot}6^{b}$	$0{\cdot}6^{a}$	$0{\cdot}6^{a}$	$0{\cdot}1^{a}$	0^{a}	7^{b}
e	$5{\cdot}8^{b}$	$1{\cdot}0^{a}$	$0{\cdot}7^{a}$	$0{\cdot}1^{a}$	$0{\cdot}1^{a}$	8^{b}
f	$1{\cdot}6^{c}$	$0{\cdot}2^{a}$	$0{\cdot}1^{a}$	0^{a}	0^{a}	2^{c}

* Letters represent significant differences among sites at a confidence level $\geqslant 0{\cdot}95$ using the Kruskal-Wallis test followed by the Mann-Whitney U-test for non-parametrically distributed data.

Table 4. *Selective soil characteristics of newly broken sod and long-term wheat fallow known to influence mycorrhizae*

	Fallow treatment	
	Site *f*	Site *c*
Soil pH	7·4	7·3
Total nitrogen (p.p.m.)	13·0	14·0
NH_4-N	5·9	4·1
Potassium (p.p.m.)	1·6	1·6
Phosphorus ($NaHCO_3$ extractable) (p.p.m.)	7·4	8·8
Phosphorus (Brayl extraction) (p.p.m.)	18·0	19·0
CEC	14·9	15·4
Organic matter (%)	1·6	1·6

a nutrient response (Table 4) as there was little difference between soils of newly tilled fields and those in long-term production. However, both fields could be characterized as nutritionally deficient. Yield was 17% higher in site *d* than site *e*, but two different varieties (Scout and Centurk) had been planted (Mr Goodell, pers. comm.).

Greenhouse experiments

Plants infected with *G. fasciculatus* had dry wts similar to non-mycorrhizal plants; infection with *G. mosseae* significantly reduced growth of wheat under both wet and dry conditions (Table 5). Dry wts of both non-mycorrhizal and *G. fasciculatus*-infected plants were significantly reduced (27 and 33%, respectively) by drought. Leaf dry wts were 34% lower in *G. mosseae*-infected plants with dry versus wet treatments, but there was no difference in root dry wts.

Table 5. *Dry wts of VA mycorrhizal and non-mycorrhizal wheat grown in wet and dry soils* ($\bar{\chi} \pm C.I._{0.95}$)

Treatment	Plant organ	Dry wts (mg per plant)		
		Non-mycorrhizal	*G. mosseae*-infected	*G. fasciculatus*-infected
Wet	Leaves	$162 \pm 14^{a*}$	$121 \pm 14^{b*}$	$163 \pm 14^{a*}$
	Roots	$342 \pm 23^{a*}$	132 ± 23^{b}	$325 \pm 23^{a*}$
Dry	Leaves	131 ± 14^{a}	80 ± 14^{b}	117 ± 14^{a}
	Roots	236 ± 28^{a}	129 ± 28^{b}	228 ± 28^{a}

Different letters represent significant differences among mycorrhizal treatments at a confidence level $\geq 0{\cdot}95$.

* Represents significant differences between wet and dry treatments at a confidence level $\geq 0{\cdot}95$.

Table 6. *VA mycorrhizal infection of roots of wheat grown under wet and dry conditions*

Treatment	Infection type	Mean percentage infection	Mean percentage segments with vesicles
Wet	*G. mosseae*	43^{a}	17^{ab}
	G. fasciculatus	59^{b}	20^{b}
Dry	*G. mosseae*	46^{a}	9^{a}
	G. fasciculatus	66^{b}	9^{a}
Pooled confidence interval$_{(0.95)}$		12	11

Different letters represent significant differences among treatments at a confidence level $\geq 0{\cdot}95$.

Mycorrhizal infection frequency did not differ between wet and dry treatments for either fungus, although *G. fasciculatus* colonized a slightly higher proportion of the root than did *G. mosseae* (Table 6). The frequency of segments containing vesicles was not significantly different between *G. mosseae*- and *G. fasciculatus*-infected plants, but was higher in wet than in dry treatments.

Plants grown under wet conditions showed no differences in leaf water potentials between non-mycorrhizal and mycorrhizal treatments (Table 7). Stomatal resist-

Table 7. *Water relations of VA mycorrhizal and non-mycorrhizal wheat (see Methods)*

	Abiotic treatment			
	Wet soils		Dry soils	
Infection type	R_s s/m	ψ_l MPa	R_c S/m	ψ_π MPa
Non-mycorrhizal	146[a]	−1·6[a]	370[a]	−2·1[a]
G. mosseae-infected	65[ab]	−1·6[a]	320[a]	−1·9[a]
G. fasciculatus-infected	56[b]	−1·7[a]	380[a]	−2·9[b]
Pooled confidence interval (0·95)	84	0·2	230	0·5

Different letters represent significant differences among treatments at a confidence level ⩾ 0·95.

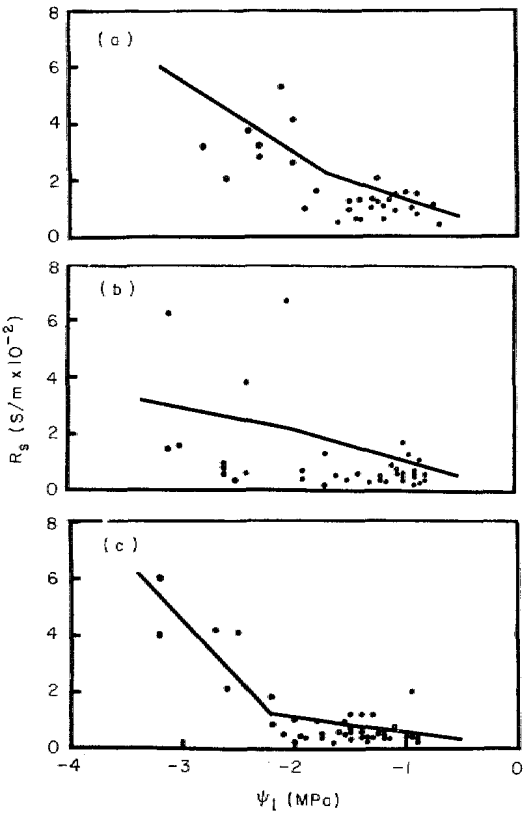

Fig. 1. Stomatal resistance (R_s) versus leaf water potentials (ψ_l) for (a) non-mycorrhizal, (b) *G. mosseae*-infected, and (c) *G. fasciculatus*-infected 2-month-old wheat plants during droughting. Values are combined from all experiments. *F*-test values and R^2 are significant ($\alpha < 0{\cdot}01$) in all cases. (a) $r^2 = 0{\cdot}62$, $F = 26$; (b) $r^2 = 0{\cdot}38$, $F = 13{\cdot}4$; (c) $r^2 = 0{\cdot}76$, $F = 67$.

ance was significantly reduced by infection with *G. fasciculatus* compared with non-mycorrhizal, whilst *G. mosseae*-infected plants were intermediate (Table 7).

Stomatal resistances of plants grown in the dry soils were different between all three treatments (Fig. 1). Non-mycorrhizal plants had higher resistance when soils were saturated and closed stomata earlier with drought stress than either of the mycorrhizal plants. Stomatal resistance of leaves of *G. mosseae*-infected plants fell more or less uniformly with increasing drought stress and some leaves had low resistances with ψ_l as low as $-4{\cdot}1$ MPa. Leaves of *G. fasciculatus*-infected plants had resistances similar to those of *G. mosseae*-infected plants, but stomata closed between $-2{\cdot}2$ and $-2{\cdot}7$ MPa. Stomata of non-mycorrhizal plants began to close 4 days after saturation whereas the mycorrhizal plants continued to transpire for 6 to 7 days (Fig. 2).

Cuticular resistance did not differ between treatments (Table 7, Fig. 2). However, *G. fasciculatus*-infected wheat plants had significantly lower ψ_π than both non-mycorrhizal and *G. mosseae*-infected plants.

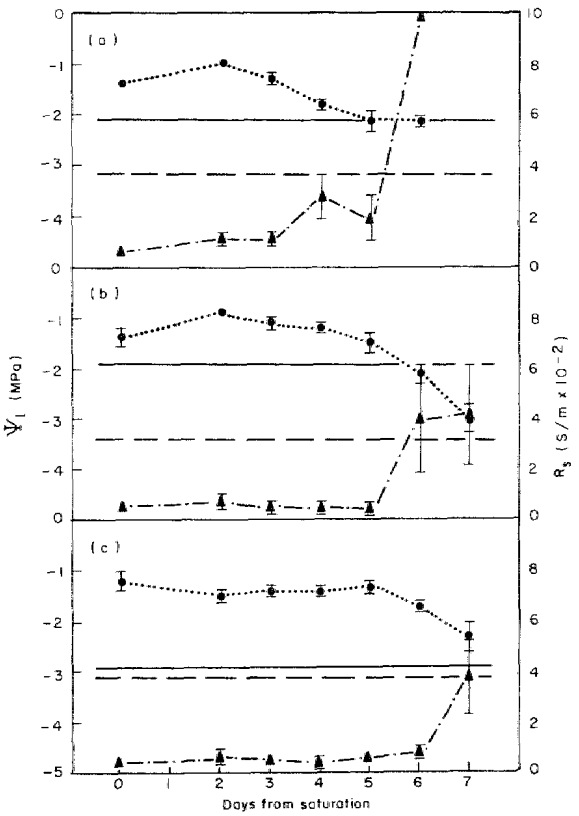

Fig. 2. Leaf water potentials (ψ_l) and stomatal resistances (R_s) from saturation of soil at day 0 to stomatal closure. Values shown are mean ± s.e.m. from (a) non-mycorrhizal, (b) *G. mosseae*-infected and (c) *G. fasciculatus*-infected wheat plants. (——) Leaf osmotic potential, (– – –) cuticular resistance are also shown. (——) Leaf osmotic potential (ψ_π), (●—●) leaf water potential (ψ_l), (– – –) cuticular resistance (R_c), (▲—▲) stomatal resistance (R_s).

DISCUSSION

Field observations

The results suggest that continued wheat-fallow rotations can reduce the incidence of VA mycorrhiza and change dominance of the fungi. The high level of infection in the grasses indicates good mycorrhizal development. Tillage itself apparently does not immediately affect spore numbers significantly, but by the time the wheat is growing (1 year later), spore numbers were lower than in the grasslands. Wheat was probably favoured by the high mycorrhizal populations following break-up of crested wheatgrass sod as the percentage root length supporting arbuscules was higher in site *d* than in *e*. However, there appeared to be low mycorrhizal production as indicated by both the low vesicle frequency and spore counts compared with the grasslands.

There was a slightly higher wheat grain yield from site *d* than *e*, but we cannot determine whether this was due to mycorrhizae, variety, or tillage. Soil nutrient status alone probably did not reduce mycorrhizal infection, but the low infection of wheat under long-term production coupled with low available phosphorus could be responsible for the observed yield reductions (e.g. Hayman, 1980; Hardie and Leyton, 1981).

Greenhouse experiments

The results substantiate the hypotheses that physiological differences among endophytes from a single field can exist and that not all associations improve plant growth (Carling and Brown, 1980; Hayman, 1980; Azcón and Ocampo, 1981). With both endophytes mycorrhizal infection decreased stomatal resistance and hence CO_2 diffusion resistance. It has been shown that infection by mycorrhizal fungi can increase CO_2 fixation (Levy and Krikun, 1980; Allen *et al.*, 1981; Wallace, 1981), but at the same time, the fungus requires carbon from the host (Ho and Trappe, 1973) and can increase below-ground respiration (Pang and Paul, 1980). Thus, in this experiment, the carbon balance of the association resulted in a similar biomass between non-mycorrhizal plants and those infected with *G. fasciculatus*, but was detrimental when plants were infected by *G. mosseae* (see also Buwalda and Gol, 1982). Hayman (1970) concluded that mycorrhizae have little effect on wheat growth before flowering. The lack of biomass response between non-mycorrhizal plants and those infected by *G. fasciculatus* in the dry treatments, despite the reduction in stomatal resistance, may also be inherent in experiments designed to keep plants alive for measurement. Stomata of non-mycorrhizal plants began to close and leaves to wilt after 4 days of drought, whereas *G. fasciculatus*-infected plants continued transpiring for 7 days.

Altered plant water relations have important consequences in long-term drought tolerance. Infection reduced R_s and lowered the ψ_1 at which stomata closed. However, some *G. mosseae*-infected plants appeared to have lost their ability to regulate stomatal closure. We are not sure why stomata did not close as ψ_1 approached ψ_π; it may have been simply sample variation. However, a low root resistance (Hardie and Leyton, 1981; Allen, 1982) combined with an altered hormone balance (Allen, Moore and Christensen, 1980, 1982) could result in wilt without stomatal closure (Tal *et al.*, 1979). *G. fasciculatus*-infected plants also maintained open stomata at low ψ_1 but showed significantly greater osmo-regulation than the other treatments, resulting in an improved ability to maintain positive turgor (Ackerson, Kreig and Sung, 1980; Sionit, Teare and Kramer, 1980).

Conclusions

In arid agro-ecosystems promotion of mycorrhizae is important for improving wheat production with minimum cost inputs. However, control of endophyte species surviving land preparations and continued cropping may be of equal or greater significance. Our results suggest that traditional tillage might reduce populations of an endophyte that promotes drought resistance and increase that of one less adapted to wheat production. *G. fasciculatus* is a dominant VA mycorrhizal fungus in several nutrient- and drought-stressed habitats (Williams and Aldon, 1976; Nicolson and Johnston, 1979; Allen and Allen, 1980). In contrast, *G. mosseae* may be more adapted to other crop species in more mesic sites or microhabitats (Gerdemann and Trappe, 1974) where it can facilitate water transport (Safir, Boyer and Gerdemann, 1972) and thereby increase CO_2 conduction. Therefore, knowledge of the effects of various alternative agricultural practices, especially minimum-tillage, on mycorrhizal infection and on the different endophytes will be essential for promoting optimal productivity.

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