Journal of Arid Environments (2002) 51: 449–459
doi:10.1006/jare.2001.0925, available online at http://www.idealibrary.com on IDEAL®

Phosphorus solubilization and uptake by dark septate fungi in fourwing saltbush, *Atriplex canescens* (Pursh) Nutt

J.R. Barrow* & P. Osuna†

**USDA, Agricultural Research Service, Jornada Experimental Range, P.O. Box 30003, MSC 3JER, NMSU, Las Cruces, NM 88003-8003, U.S.A.*
†Department of Biology, NMSU, Las Cruces, NM 88003-8003, U.S.A.

(Received 27 October 2001, accepted 24 September 2001)

Fourwing saltbush, *Atriplex canescens* (Pursh) Nutt., is an ecologically important range plant in arid south-western U.S.A. rangelands. Native populations of this chenopodiacious shrub are more extensively colonized by melanized dark septate fungi (DS) than by conventional mycorrhizal fungi. Seedling radicles of *A. canescens* are colonized at germination by a DS fungus identified as *Aspergillus ustus* that cannot be removed by heat or sterilization. The association of *A. canescens* with *A. ustus* was evaluated by comparing naturally colonized control seedlings receiving no P (0P) or adequate plant available P (AAP) receiving 30 p.p.m. supplied as KH_2PO_4 in the root zone to seedlings whose roots were separated from plant unavailable P (as rock phosphate (RP) or tricalcium phosphate (TCP)) by a barrier that only allowed access by the fungus. *A. ustus* penetrated *A. canescens* roots with hyaline septate hyphae, formed melanized runner hyphae at the root surface, and extended through the root exclusion barrier into RP and TCP. In these treatments *A. ustus* obtained plant carbon, increased shoot and root biomass, and phosphorus use efficiency. *A. ustus* grew well in culture on RP and TCP and internally colonized *in vitro* Ri-T DNA *D. carota* roots. The mutualistic association of DS fungi in arid ecosystems is discussed.

Keywords: mycorrhiza; phosphorus; arid; endophytic fungi

Introduction

Plant–fungal associations are widespread among vascular plants and range from pathogenic to mutually beneficial. With the exception of mycorrhizal and other root-colonizing fungi, the study of plant–fungal relationships has focused primarily on the advantages or disadvantages of the association to the plant with little attention given to the significance of fungal biology (Parbery, 1996).

Mycorrhizal fungi form unique non-pathogenic interfaces with host root cells and extend into the soil expanding the absorptive capacity of the root. Their association

*Corresponding author. Fax: (505) 646-5889. E-mail: jbarrow@nmsu.edu

0140-1963/02/030449 + 11 $35.00/0

with plant roots is considered mutualistic because they enhance plant nutrition in exchange for photosynthetic carbon. They regulate key ecological processes that influence not only the structure and stability of the soil but also plant communities. They benefit host plants by improving water economy during drought (Allen & Boosalis, 1983; Allen, 1996; Duan *et al.*, 1996), protection from pathogens (Johnson *et al.*, 1997) and enhanced nutrient uptake and biomass production in nutrient-deficient ecosystems (Gerdeman, 1964; Chakly & Berthelin, 1982; Harrison, 1997). It is estimated that the external hyphae of arbuscular mycorrhizal (AM) fungi deliver up to 80% of the phosphorus (P) and 25% of a plant's nitrogen requirements. Ectomycorrhizal pine roots supply up to 3·2 times more P and 1·8 times more N than non-mycorrhizal root systems (Marschner & Dell, 1994). Arbuscular mycorrhizal fungi increase P availability by secreting enzymes (phosphatases) that hydrolyse organic P or organic acids that solubilize inorganic P (Bolan, 1991). Extraradicle hyphae take up P, transport it to the root and release it to the plant via unique interfaces (arbuscules) within the root cortex (Smith & Smith, 1990). Other traditional mycorrhizae vary in the way that they interface and exchange resources with their host which has little effect on their physiological function or ecological strategy (Trappe, 1981).

Agronomically important cool season pasture grasses, rye (*Lolium* sp.) and fescue (*Festuca* sp.) are colonized by fungal endophytes that benefit host plants by increasing tolerance to temperature extremes, nutrient deficiency and drought. They produce alkaloids that protect hosts against viruses, insects, nematodes and livestock grazing. In return the plant provides protection, energy and dispersal of the fungus (Belesky & Malinowski, 2000). Soil fungi and other microbes that reside in the energy-rich micro-environment of the rhizosphere at the root surface also enhance mineralization and uptake of nutrients (Bianciotto *et al.*, 1996). Tarafdar & Rao *et al.* (1996) found that among fungi residing in the rhizosphere, species of Aspergillus had the highest phosphatase activity and significantly increased P solubilization and uptake resulting in increased biomass of wheat and chick pea growing in arid soils. *Aspergillus* and *Penecillium* sp. similar to AM fungi secrete organic acids which increase the availability of inorganic P from rock phosphate (RP) and tri-basic calcium phosphate (TCP) (Bolan, 1991). They also produce phosphatase enzymes that hydoliyse organic phosphates resulting in enhanced plant yields (Azcon *et al.*, 1976; Khan & Bhatnagar, 1977; Vassilev *et al.*, 1996; Vassilev *et al.*, 1997*a*,*b*).

Fourwing saltbush, *Atriplex canescens* (Pursh) Nutt. is a dominant, ubiquitous and ecologically important chenopodiacous shrub in arid south-western U.S.A. rangelands. In native populations, its roots were found to be more extensively colonized by melanized, dark septate (DS) endophytic fungi than by arbuscular mycorrhizal fungi (Barrow *et al.*, 1997). Dark septate fungal endophytes are a widely recognized group of undefined fungi that colonize many plant species. Their study and importance have been minimized because they do not conform to traditional mycorrhizal morphology (Trappe, 1981). DS fungal associations have been described as ranging from pathogenic to mutualistic (Jumpponen & Trappe, 1998). They are commonly observed in plant roots as stained or pigmented (melanized) septate hyphae and microsclerotia that grow both inter- and intracellularly within the cortex and at the root surface. Haselwandter & Read (1982) and Newsham (1999) reported that weakly staining hyaline hyphae extended into the cortex from dark septate surface hyphae. Barrow & Aaltonen (2001) stained physiologically active roots of *A. canescens* with sudan IV and analysed them with differential interference contrast microscopy (DIC). They found extensive internal colonization of DS fungi in physiologically active roots that lacked chitin and melanin rendering them invisible to conventional fungus staining methods.

Seed of fourwing saltbush are small (< 2 mm) and develop within a utricle that aids in protection and wind dispersal. Barrow *et al.* (1997) found that emerging radicles of

germinating seedlings are immediately and non-pathogenically colonized by DS fungi. Seedlings germinated from intact utricles were more vigorous than those germinating from excised seeds suggesting that fungi decompose the utricle and supply essential resources for the establishment of seedlings.

The objective of this study was to test the hypothesis that seed-borne DS fungi that naturally colonize *A. canescens* roots are able to enhance nutrition of seedlings by supplying phosphorus from plant unavailable rock and tricalcium phosphate.

Materials and methods

The inability to eliminate DS fungal colonization of seedling radicles by sterilization or heat treatments prevents the comparison of sterile with colonized seedlings. Therefore, plant and fungal responses to different P sources were evaluated using plant containers that separated roots from plant unavailable P by a barrier that only allowed passage by fungi (Fig. 1). A lower fungal chamber was constructed by cutting standard 7·62 cm ID schedule 40, PVC tubing into 7·62 cm lengths. A 2 mm mesh vinyl screen was glued to the bottom of the tube and filter paper was inserted above the screen to retain sand and allow drainage of nutrient solutions. This chamber was filled with acid-washed silica sand (AWS) prepared by soaking a commercial grade of silica blasting sand for 30 min in 0·1 N HCl and copiously rinsed in distilled water to remove all mineral nutrients, particularly P and dried by heating at 110°C for 72 h. The tube was filled and saturated with AWS and packed firmly. The root exclusion barrier was constructed by placing a 10 μm screen over the top of the fungal chamber, a 7·62 mm diameter plastic circle (2 mm thick) designed for needle craft was placed over the screen followed by a second 10 μm screen. A 7·62 cm coupling was lubricated with a light petroleum jelly and driven firmly over the barrier and the tubing of the lower chamber which formed the upper root chamber (8·8 cm diameter × 4·5 cm deep). The barrier separated the root and fungal chambers with a 2 mm air space and prevented roots from entering the lower chamber and the diffusion of P from the lower fungal chamber into the root chamber.

Seeds harvested from a native population of *A. canescens* near the headquarters of the USDA Agricultural Research Service's Jornada Experimental Range in southern

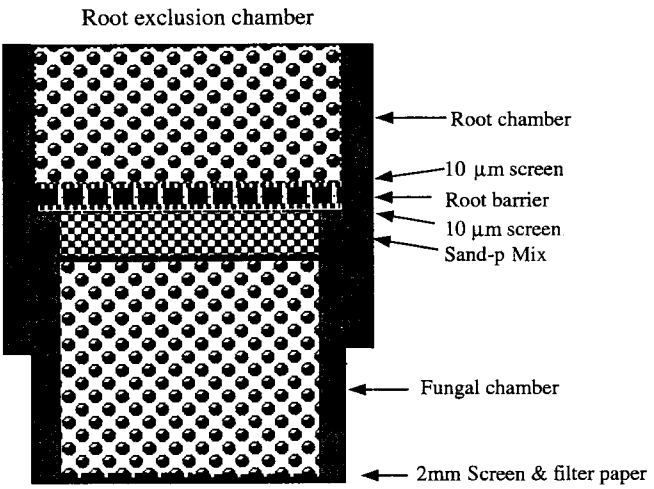

Figure 1. Plant growth tube constructed from 7·62 cm PVC schedule 40 tubing with a root exclusion barrier.

New Mexico were dewinged and germinated on the surface of AWS moistened with deionized water. Three germinants with radicles ~1 cm long, naturally colonized by septate fungi, were transplanted in each tube. Tubes were randomly placed in a growth chamber at 25°C with a 12 h light, 12 h dark regime. Each treatment had 16 replications, 12 of which were used for root and shoot biomass and tissue P analysis at the end of the experiment. Remaining replications were used for microscopic analysis of roots and sub-chamber fungal colonization.

Treatments consisted of two controls, (1) 0P plant roots received all essential nutrients as Hoagland's nutrient solution minus P, and (2) plant roots were supplied a complete Hoagland's nutrient solution containing 30 p.p.m. soluble P as KH_2PO_4. Plants were subjected to two plant unavailable P treatments where in (3) rock phosphate (RP) and in (4) tribasic calcium phosphate (TCP) was mixed as either one part of RP or TCP with 2 parts quartz sand and placed in the top centimeter just below the root barrier. Plants in the 0P, RP, and TCP treatments were watered just prior to wilting (approximately every 72 h) by adding Hoagland's solution with all essential nutrients except P (HS−P) to the root chamber. Nutrient solutions drained quickly to keep sand as dry as possible. Seedlings were watered every third time with deionized water to prevent salt buildup. Nutrient solutions were adjusted to pH 7·5 to prevent solubilization of P from the RP and TCP.

After 2 months, plants were carefully removed from the upper root chamber by submerging and rinsing in water to remove sand. Shoots and roots were separated, dried 3 days at 60°C and weighed. Dried shoots and roots were finely ground and 0·5 g of each tissue sample was analysed for tissue P content using a Jobin Yvon Plus Inductively Coupled Plasma Spectrophotometer. Roots were also harvested from additional replications, cleared and stained using the method of Barrow & Aaltonen (2001) and were analysed with differential interference microscopy to assess internal fungal colonization. Data were analysed with phosphorus as the main effect and dependent variables were shoot, root and plant biomass and P tissue concentrations. Mean differences were compared using the Wilcoxon Signed Rank Test.

Fungal density measurements

The top and bottom screens were cut from four tubes in each treatment and stained with Schiff's reagent, which stained fungal hyphae to a dark red color. Screens were placed in a Petri dish over a grid divided into 1 mm^2 squares. Fungal density was estimated for 50, 1 mm^2 squares for four replications in each treatment with a stereo microscope. Fungal density was scored by estimating the percent of fungus cover of each square: 0, no fungi, 1, <20%, 2, 20–40%, 3, 40–60%, 4, 60–80% and 5, >80%. Data were analysed using a completely randomized one-way ANOVA.

Fungal analysis

Hyphae extending from the root and passing through the barrier into the plant unavailable P sources was isolated and identified as *A. ustus* which was evaluated in two additional experiments. *A. ustus* was cultured on disks 5 mm thick cut from 5 cm diameter PVC tubing. Disks were filled with either AWS, RP, or TCP and sealed top and bottom with10 μm screen and placed in 10 cm Petri dishes which were filled to the upper disk surface with liquid modified White's medium (MWM) (Table 1) supplemented with 30 $g\,l^{-1}$ sucrose l^{-1} and minus P for the 0P, RP, and TCP treatments. For the AAP treatment, the MWP medium was supplemented with 0·048 $g\,l^{-1}$, KH_2PO_4. One millimeter plugs of *A. ustus* were cut from potato dextrose agar (PDA) and placed in the center of disks in all the treatments. Ten replicated Petri dishes comprised each treatment. After 2 weeks, fungal mats from the AAP, and TCP

Table 1. *Modified White's medium for culturing* D. carota *and* A. ustus

Compound	Root stock (Vm) (10 ×) $g l^{-1}$	Symbiosis (root–fungus) ('M') (10 ×) $g l^{-1}$
$MgSO_4 \cdot 7H_20$	7·31	7·31
KNO_3	0·8	0·8
KCl	0·65	0·65
KH_2PO_4	—	0·048
Na_2SO_4	2	—
$NaH_2PO_4 \cdot H_20$	0·190	—
$Ca(NO_3)_2 \cdot 4H_20$	2·88	2·88
NaFe EDTA	1·6	1·6
KI	0·75	0·75
$MnCl_2 \cdot 4H_20$	6	6
$ZnSO_4 \cdot 7H_20$	2·65	2·65
H_3BO_3	1·5	1·5
$CuSO_4 \cdot 5H_2O$	0·13	0·13
$Na_2MoO_4 \cdot 2H_20$	0·0024	0·0024
Glycine	0·3	0·3
Thiamine hydrochloride	0·01	0·01
Pyridoxine hydrochloride	0·01	0·01
Nicotinic acid	0·05	0·05
Myo-inositol	5	5
Sucrose	30	10
Phytagel	8	8

pH adjusted to 5·5 (utilizing HC1 or KOH). Sterilization: 16 min at 121.

treatments were scraped from the upper screen, dried and weighed. Fungal growth was negligible in the 0P and RP treatments at this time and the colony size was measured after 6 weeks.

To demonstrate the potential of *A. ustus* to colonize Ri-T-transformed *Daucus carota* roots, one compartment (root) of a divided Petri dish was filled with MWM containing 30 $g l^{-1}$ sucrose and all required nutrients except phosphorus. The other compartment (fungus) was filled with MWM minus P and sucrose. Disks 5 mm thick were cut from12·7 mm diameter PVC tubing and were filled with TCP and sealed both top and bottom with10 µmm screen and placed in the fungus compartment. Disks were inoculated with 1 mm plugs of *A. ustus*. In 10 Petri dishes, the basal portion of 6 cm segments of Ri-T-transformed *D. carota* roots were placed in the root compartment with sucrose minus P and the meristematic tips were placed adjacent to the TCP disk in the fungal compartment minus sucrose and soluble P. In 10 control Petri dishes roots were not added but the TCP disks were inoculated. After 4 weeks roots were microscopically analysed for fungal colonization and the diameter of fungal colonies were measured.

Results

Table 2 lists the values of shoot and root biomass in the tube experiment. Shoot and root biomass was minimal in the 0P treatment where the only available P was from seed reserves. Maximum shoot biomass was measured in the TCP and AAP treatments but was not significantly different (p=0·587), root biomass in the TCP treatment was significantly greater than in the AAP treatment (p<0·02). Shoot and

Table 2. *Shoot and root dry weight in grams of* Atriplex canescens *seedlings grown with and without soluble phosphorus and treated with rock phosphate (RP) and (TCP)*

Treatments	0P*	AAP	RP	TCP
Shoot	0·089c†	1·149a	0·414b	1·579a
Root	0·078d	0·349b	0·195c	0·725a

*OP=Zero P, AAP=adequate available P, RP= rock phosphate, and TCP= tricalcium phosphate.
†Means within a row followed by different letters are significantly different.
Probabilies of means being different by chance.

Comparison	Shoot	Root
TCP *vs.* AAP	0·587	0·020
AAP *vs.* RP	0·001	0·001
RP *vs.* 0P	0·001	0·001

root biomass in the RP treatment were significantly greater than the 0P and less than the AAP and TCP treatments ($p < 0{\cdot}001$).

Roots did not penetrate the upper 10 μm screen of the root barrier which provided a minimum airspace of 2 mm separating the two chambers. The settling of sand in the lower chamber over the course of the experiment resulted in an additional 1–3 mm airspace. Because of the low solubility of RP and TCP at pH 7·5 and the amount of P accumulated in shoots and roots in these treatments, it was assumed that P was transferred by the fungus and not by diffusion.

Extra-radicle hyphae colonized 30% and 41% of the upper screens of the 0P and AAP treatments, respectively, but did not extend to the lower screens. In the RP and TCP treatments, extra-radicle hyphae more densely colonized the upper screen by 60% and 94% and colonized the lower screen by 29% and 77%, respectively (Table 3).

Roots stained with sudan IV and analysed with DIC microscopy at the end of the experiment revealed inter- and intracellular hyaline septate hyphae within the cortex in all the treatments (Fig. 2). This method improves the visualization of active internal DS structures. Internal fungal structures lacking lipids were not microscopically visible because of the variability in their wall structure making quantitative measurements impossible (Barrow & Aaltonen, 2001). Melanized septate hyphae typical of DS fungi were observed on the root surface and extended into the RP and TCP mixtures below the screen.

Tissue P was 7–8 times greater ($p < 0{\cdot}01$) in the AAP treatment where roots were in contact with soluble P than in the RP and TCP treatments where P was separated from the roots by the barrier (Table 4). No differences in tissue P was measured between the RP and TCP treatments.

The Mean colony weights of *A. ustus* cultured on disks filled with TCP was 20 mg compared to 4 mg for colonies growing on AWS-filled disks supplemented with soluble P. No appreciable growth was observed on either AWS-filled disks in MWM-P or in disks filled with RP. However, *A. ustus* continued to grow slowly on the RP disks supplemented with MWM-P and after 6 weeks, the colony diameter was 17·2 mm where no growth was observed on AWS-filled disks supplemented with MWM-P.

A. ustus grew from the inoculated TCP disk after colonizing the sterile Ri T-DNA-transformed *D. carota* roots, but did not grow in the absence of roots. Hyaline septate hyphae similar to the *A. canescens* roots were observed in the cortex of the *D. carota* roots. Hyphae distinguished only by lipids stained with sudan IV (Fig. 3) were also observed in the cortex. These structures are similar in active internal DS fungal colonization observed in roots of native *A. canescens* populations. Greater hyphal density was observed near the TCP disks in colonized roots compared to inoculated disks without roots.

Table 3. *Percent fungal density on root excluding screens*

Treatment	0P*	AAP	RP	TCP
Top	30%d†	41%c	60%b	94%a
Bottom	0%e	0%e	29%d	77%b

*OP=zero P, AAP=adequate available P, RP= rock phosphate, and TCP= tricalcium phosphate.
†Means within a column followed by different letters are significantly different at $p<0{\cdot}01$.

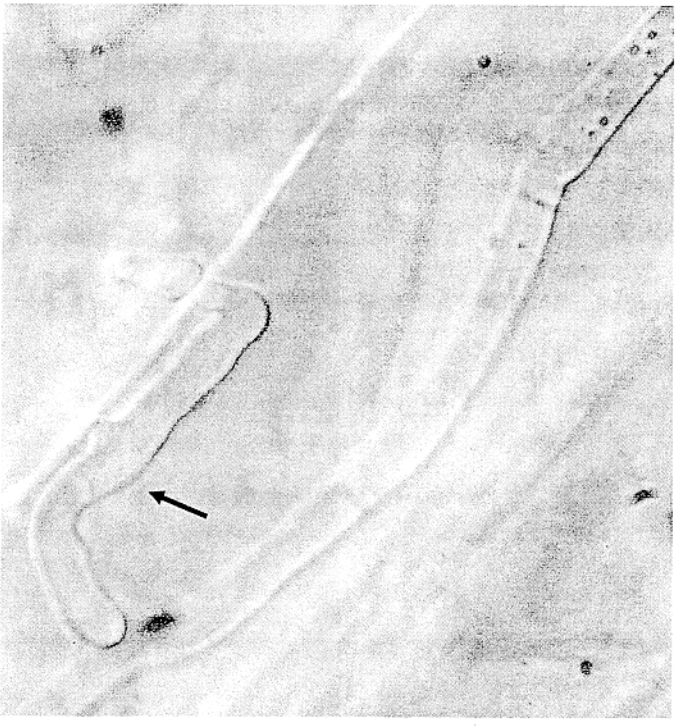

Figure 2. Hyaline septate hyphae of *A. ustus* growing intracellularly in the cortex cells of *Atriplex canescens.*

Discussion

Results of this study indicate a mutualistic association between *A. ustus* and *A. canescens* and are consistent with those found in other symbiotic associations. The equivalent shoot biomass and greater root biomass in the TCP treatment compared to the AAP treatment indicated that plants were receiving P from the plant unavailable TCP below the screen via the fungus. To a lesser extent, shoot and root biomass was greater in RP-treated plants than those receiving no P (0P). Malinowski & Belesky (1999) also found that root dry matter was greater in endophyte-infected tall fescue pastures fertilized with rock phosphate than in pastures fertilized with commercial fertilizer providing plant available P. Microscopic analysis revealed that roots in all treatments were internally colonized by *A. ustus*. Due to the variability in the nature and composition of internal DS fungal structures many of these structures are not microscopically visible making it impossible to accurately quantify their presence (Barrow & Aaltonen, 2001). However, the increased fungal density

Table 4. *Percent tissue phosphorus in roots and shoots*

Treatment	0P*	AAP	RP	TCP
Shoot	nd†	0·712a‡	0·095b	0·080b
Root	nd	0·570a	0·035b	0·054b

*OP=zero P, AAP=adequate available P, RP= rock phosphate, and TCP= tricalcium phosphate.
†nd=not detectable.
‡Means within a column followed by different letters are significantly different at $p < 0{\cdot}01$.

Figure 3. Hyphae of *A. ustus* within the cortex of inoculated *D. carota* roots in culture.

measured on the lower screens in the RP and TCP treatments indicated greater extra-radicle production and suggests greater internal colonization in the RP and TCP treatments and is analogous to the findings of Thomson *et al.* (1992) who observed that AM colonization increased as P availability decreased.

We attribute the increased shoot and root biomass to the solubilization of RP and TCP and uptake and transport of P to the root by *A. ustus*. This suggests that *A. ustus*, like other *Aspergillus* species, *A. niger, A. fumigatus, A. rugulosus*, can solubilize

immobile inorganic phosphates RP and TCP (Tarafdar *et al.*, 1996; Vassilev *et al.*, 1996, 1997*a*,*b*; Khan & Bhatnagar, 1977; Vazquez *et al.*, 2000). Presently, *Aspergillus* species are considered as soil and rhizosphere inhabitants (Tarafdar *et al.*, 1996). Analysis of both *A. canescens* and *D. carota* roots stained with sudan IV with DIC microscopy revealed that *A. ustus* not only grew in the rhizosphere but formed intimate internal associations with cortical cells. These observations indicate that DS fungi non-destructively and internally colonize the root cortex, but differ morphologically from traditional mycorrhizal.

Additionally, it was shown that P use efficiency was greater in plants accessing P from the RP and TCP treatments compared to plants receiving adequate soluble P directly to their roots in the AAP treatments. Malinowski & Belesky (1999) also found that phosphorus use efficiency was 16% greater in endophyte-infected tall fescue plants than in non-infected plants. This suggests that P use efficiency is greater when P is supplied by the fungus. Increased root biomass, external fungal production and P use efficiency suggests a greater energy requirement for plants to take up P by the fungus from recalcitrant sources.

The response of *A. ustus* cultured independently on 0P, AAP, RP and TCP was similar to the tube experiments. It grew well on TCP and more slowly but positively on RP, further demonstrating its ability to solubilize both RP and TCP. Its superior performance of *A. ustus* on TCP suggests its natural adaption and potential of solubilizing Ca–P complexes common to desert soils. The biotrophic nature of *A. ustus* was demonstrated by its growth response after colonizing sterile Ri-T *D.carota* roots in the carbon-free compartment of the divided Petri, where the only available carbon was from the root.

The extensive colonization of *A. canescens* and other dominant plants by DS fungi in arid south-western rangelands suggests that they have a significant ecological role. Staining roots with sudan IV and analysis with DIC microscopy revealed substantial active internal colonization by DS fungi not previously visible using conventional fungus-staining methods (Barrow & Aaltonen, 2001). *A. canescens* produces copious quantities of very small seed (<2 mm) with minimal nutrient reserves. It is suggested that seed-borne *A. ustus*, a DS endophytic fungus, solubilizes and transports P from immobile Ca–P complexes in desert soils to germinating seedlings enhancing their establishment and survival. The ubiquitous presence of DS fungi in dominant plants in arid ecosystems suggests low host specificity. The extension of hyphae into the soil further suggests a substantial hyphal network that could be the primary means of nutrient movement in soils too dry for diffusion or mass flow. Such networks would enhance soil stability and water infiltration and retention through aggregation. DS fungi that differ in strategy and morphology but function similar to conventional mycorrhizal fungi may be best adapted to the harsh conditions of dry soil. There is a critical need to implement innovative microscopy, labeling, molecular and other analytical methods to better understand the unique association and function of DS fungi with their hosts in arid ecosystems.

Thanks are due to Dr Marin Klich, USDA-REE-ARS-MA-SSRC-F&FAR, P.O. Box 19687, New Orleans, LA 70179, for identification of *A. ustus*.

References

Allen, M.F. & Boosalis, M.G. (1983). Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. *New Phytologist*, **93**: 67–76.

Allen, M.F. (1996). The ecology of arbuscular mycorrhizas: a look back into the 20th century and a peek into the 21st. *Mycological Research*, **100**: 769–782.

Azcon, R., Barea, J.M. & Hayman, D.S. (1976). Utilization of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and phosphate-solubilizing bacteria. *Soil Biology and Biochemistry*, **8**: 135–138.

Barrow, J.R. & Aaltonen, R.E. (2001). A method of evaluating internal colonization of *Atriplex canescens* (Pursh) Nutt. roots by dark septate fungi and how they are influenced by physiological activity. *Mycorrhiza*, **11**: 199–205.

Barrow, J.R., Havstad, K.M. & McCaslin, B.D. (1997). Fungal root endophytes in fourwing saltbush, *Atriplex canescens*, on arid rangelands of Southwestern USA. *Arid Soil Research and Rehabilitation*, **11**: 177–185.

Belesky, D.P. & Malinowski, D.P. (2000). Abiotic stresses and morphological plasticity and chemical adaptations of *Neotyphodium*-infected tall fescue plants. In: Bacon, C.W. & White, J.F. (Eds), *Microbial Endophytes*, pp. 455–484. New York: Marcel Dekker Inc.

Bianciotto, V., Minerdi, D., Perotto, S. & Bonfante, P. (1996). Cellular interactions between arbuscular mycorrilizal fungi and rhizosphere bacteria. *Protoplasma*, **193**: 123–131.

Bolan, N.S. (1991). A critical review on the role of mycorrhixzal fungi in the uptake of phosphorus by plants. *Plant and Soil*, **134**: 189–207.

Chakly, M. & Berthelin, J. (1982). Role of an ectomycorrhiza '*Pisolithus tinctorius-Pinus caribaea*' and a rhizosphere bacterium in the mobilization of phosphorus from insoluble mineral or organic phosphates. In: Gianinazzi, S., Gianinazzi-Pearson, V. & Trouvelot, A. (Eds), *Mycorrhizae, an Integral Part of Plants: Biology and Perspectives for Their Use*, Vol. 13, pp. 215–220. Italy: Publication Colloquium INRA.

Duan, X.G., Neuman, D.S., Reiber, J.M., Green, C.D., Saxton, A.M. & Auge, R.M. (1996). Mycorrhizal influence on hydraulic and hormonal factors implicated in the control of stomatal conductance during drought. *Journal of Experimental Botany*, **47**: 1541–1550.

Gerdeman, J.W. (1964). The effect of mycorrhizas on the growth of maize. *Mycologia*, **56**: 342–349.

Harrison, M.J. (1997). The arbuscular mycorrhizal symbiosis: an underground association. *Trends in Plant Science*, **2**: 54–60.

Haselwandter, K. & Read, D.J. (1982). The significance of a root–fungus association in two Carex species of high-alpine plant communities. *Oecologia*, **53**: 352–354.

Johnson, N.C., Graham, I.H. & Smith, F.A. (1997). Functioning of mycorrhizal associations along the mutualism–parasitism. *New Phytologist*, **135**: 575–585.

Jumpponen, A. & Trappe, J.M. (1998). Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytologist*, **140**: 295–310.

Khan, J.A. & Bhatnagar, R.M. (1977). Studies on solubilization of insoluble phosphates by microorganisms. I. Solubilization of indian phosphate rock by *Aspergillus niger* and *Penicillium sp. Fertilizer Technology*, **14**: 329–333.

Marschner, H. & Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil*, **159**: 89–102.

Malinowski, D.P. & Belesky, D.P. (1999). *Neotyphodium coenophialum*-endophyte infection affects the ability of tall fescue to use sparingly available phosphorus. *Journal of Plant Nutrition*, **22**: 835–853.

Newsham, K.K. (1999). *Phialophora graminicola*, a dark septate fungus, is a beneficial associate of the grass *Vulpia ciliata* ssp. *ambigua*. *New Phytologist*, **144**: 517–524.

Parbery, D.G. (1996). Trophism and the ecology of fungi associated with plants. *Biological Review*, **71**: 473–527.

Smith, S.E. & Smith, F.A. (1990). Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. *New Phytologist*, **114**: 1–38.

Thomson, B.D., Robson, A.D. & Abbott, L.K. (1992). The effect of long-term applications of phosphorus fertilizer on populations of vesicular–arbuscular mycorrhizal fungi in pastures. *Australian Journal of Agriculture Research*, **43**: 1131–1142.

Tarafdar, J.C. & Rao, A.V. (1996). Contribution of *Aspergillus* strains to acquisition of phosphorus by wheat (*Triticum aestivum* L) and chick pea (*Cicer arietinum* Linn) grown in a loamy sand soil. *Applied Soil Ecology*, **3**: 109–114.

Trappe, J.M. (1981). Mycorrhizae and productivity of arid and semiarid rangelands. In: Manassah, J.T. & Briskey, E.J. (Eds), *Advances in Food-Producing Systems for Arid and Semiarid Lands Part A*, pp. 581–593. New York: Academic Press.

Vassilev, N., Fenice, M. & Federici, F. (1996). Rock phosphate solubilization with gluconic acid produced by immobilized *Penicillium variable* P16. *Biotechnology Techniques*, **10**: 584–588.

Vassilev, N., Toro, M., Vassileva, M., Azcon, R. & Barea, J.M. (1997*a*). Rock phosphate solubilization by immobilized cells of *Enterobacter* sp. in fermentation and soil conditions. *Bioresource Technology*, **61**: 29–32.

Vassilev, N., Vassileva, M. & Azcon, R. (1997*b*). Solubilization of rock phosphate by immobilized *Aspergillus niger*. *Bioresource Technology*, **59**: 1–4.

Vazquez, P., Holguin, G., Puente, M. E., Lopez-Cortes, A. & and Bashan, Y. (2000). Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils*, **30**: 460–468.