Seed-Borne Fungal Endophytes on Fourwing Saltbush, *Atriplex canescens*

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Fourwing saltbush, Atriplex canescens *(Pursh) Nutt., produces copious quantities of small seed, with minimal nutrient reserves, protected in a hard porous capsule (utricle) that aids in dispersal. At germination, saprophytic fungi simultaneously colonize the utricle, testa, and root cortex cells of the emerging radicles. Seedling vigor was determined by measuring hypocotyl and radicle lengths after germination on minimal carbon or cellulose-supplemented medium. Comparisons were made between seedlings from utricle-excised and intact seed. Comparisons were also made between surface-sterilized and nonsterile excised seed. Minimal growth responses were observed in germinating seedlings from excised seeds on minimal carbon medium. Fungi, utricles, and cellulose supplementation positively affected seedling vigor. The results support a hypothesis that septate fungi recycle utricles and access organic carbon for seedling establishment.*

Keywords arid ecology, endophytic fungi, fertility, germination, land restoration, mycorrhizae, mutualism, native plants, plant nutrition, saprophytic fungi, symbiosis

Symbiotic fungal associations are common among plants, and their benefits to plant growth and survival are numerous (Clay, 1990; Harley & Smith, 1983). Fungal endophytes protect plants from herbivory (Gehring & Whitham, 1994; Strahan et al., 1987). Mycorrhizal fungi improve nutrient uptake and water relationships under stress (Allen, 1991; Miller, 1987). Plant roots and external vesicular-arbuscular mycorrhizal hyphae aggregate soil particles, which improves soil structure, stability, and retention of soil resource (Jastrow et al., 1987). Read (1991) stated that over 100 years of mycorrhizal research has demonstrated a need for better understanding of the full range of mycorrhizal functions in order to comprehend ecosystem dynamics.

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Atriplex canescens (Pursh) Nutt., a common native shrub on western North American rangelands (McArthur & Sanderson, 1984), is valued as a soil stabilizer, a palatable and nutritious forage for many herbivores, and an important cover species for wildlife habitat (Geirst & Edgerton, 1984; Aldon, 1972; McArthur et al., 1984). A small seed with minimal reserves forms within a fibrous utricle with four subtending bracts that facilitates wind dispersal. Germination is characteristically low, difficult, and highly variable (Springfield, 1970, Young et al., 1984).

In our studies of autecological characteristics of *A. canescens*, utricles and seed coats were consistently colonized by septate fungi. At germination these fungi penetrated emerging radicles and formed nondestructive interfaces with the cortex cells. Isolated fungi grew readily on potato dextrose agar (PDA) and other media with simple or complex carbohydrates and were capable of surviving outside the host. Several fungal species are likely involved, and one species was identified by Craig Liddell, New Mexico State University, Las Cruces, New Mexico, USA, as *Alternaria alternata*, an ubiquitous saprophytic fungus.

The objective of this study was to determine if these fungi positively influence germination, nutrition, and establishment of *Atriplex canescens* seedlings.

Materials and Methods

Atriplex canescens seed was collected from native plants in south central New Mexico, USA, in the fall of 1990. Seed was cleaned by removing bracts in a hammer mill at 750 rpm with a 2.5-cm screen, and stored in paper bags at room temperature for further use.

Seeds were carefully excised from the utricle with a surgical knife or left intact. Intact and undamaged excised seeds were germinated and compared for vigor. Excised seeds were surface sterilized by sequentially soaking them in distilled water with one drop of commercial detergent for 3–4 min, in 95% ethanol for 2 min, in 50% solution of 5.25% Na-hypochlorite for 15 min, and thoroughly rinsing them 4 times in sterile distilled water. Surface sterilization removed external saprophytic fungi that grew on PDA agar.

In experiment 1, vigor was compared between seedlings of nonsterilized intact and excised seeds germinated in sterile silica sand and watered with distilled water. Six PVC tubes (7.6 × 15.2 cm) were filled with sterile, washed silica sand, and each tube was planted with either 20 intact or 20 excised seeds and placed in a growth chamber at 25°C with a 12-h photoperiod. After 2 weeks, the numbers of seedlings were recorded and the stem heights were measured from the base to the terminal bud.

A minimal carbon germination medium (1/10 MWR–C) was prepared using 1/10 strength mineral salts of modified White's root medium (MWR) excluding sucrose and vitamins (Table 1; Chabot et al., 1992). A cellulose medium (1/10 MWR+C) was prepared by adding 0.03 g L^{-1} pure cellulose to the above medium. Media pH were adjusted to 5.5 and media were autoclaved at 121°C for 20 min.

We could not effectively germinate or remove external fungi from surface sterilized intact seeds because of the fibrous nature of the utricle. This made direct comparisons with nonsterile intact seed impossible. Therefore, in experiment 2, seedling response was compared between 500 surface-sterilized (SS) and 500 non-surface-sterilized (NSS) excised seeds germinated on either 1/10 MWR–C or 1/10 MWR+C (2000 seeds) at 25°C and a 12-h photoperiod for 7 days. Dependent variables were lengths and diameters of hypocotyls and radicles of seedlings measured after 8 days. Measurements were made using a stereomicroscope with an ocular micrometer. Data were analyzed as a 2 × 2 factorial in a completely randomized design. Means were separated using least significant difference (lsd) when significant F values ($p < .01$) were detected for a treatment.

Table 1
Composition of modified White's medium (MWR) used to culture roots and isolated fungi[a]

Chemical compound	mg L^{-1}
$MgSO_4 \cdot 7H_2O$	731.00
$Na_2SO_4 \cdot 10H_2O$	453.00
KNO_3	80.00
KCl	65.00
$NaH_2PO_4 \cdot 2H_2O$	21.50
$Ca(NO_3)_2 \cdot 4H_2O$	288.00
Iron: NaFeEDTA	8.00
Trace Minerals	
KI	0.79
$MnCl_2 \cdot 4H_2O$	6.00
$ZnSO_4 \cdot 7H_2O$	2.65
H_2BO_3	1.50
$CuSO_4 \cdot 5H_2O$	0.13
$Na_2MoO_4 \cdot 2H_2O$	0.0023
Vitamins	
Glycine	3.00
Thiamine	0.10
Pyridoxine	0.10
Nicotinic acid	0.50
Myo-Inosotol	50.00

[a]After Chabot et al. (1992).

In experiment 3, seedlings from NSS excised seeds were compared with nonsterile intact seeds germinated on either 1/10 MWR–C or 1/10 MWR+C. To obtain sufficient seedling numbers, 5000 intact seeds were soaked in distilled water for 2 h, plated on sterile silica sand in 1 × 10 cm petri dishes, and incubated simultaneously with the excised seed to obtain 250 germinating seeds (radicles 1–3 mm) for each treatment. Likewise, 250 seedlings from nonsterile excised seed were plated to each medium. Seedlings were measured and analyzed as already described.

After measurement, radicles were cut into 2–4 mm pieces, fixed, rinsed, postfixed, and dehydrated following procedures outlined by Spurr (1969). Material was embedded in resin, polymerized, sectioned (1–5 μm) with an ultramicrotome, mounted on slides, and stained with 0.1% toluidine blue to microscopically determine the nature of fungal colonization.

Results

Experiment 1

Approximately 16% of the intact seeds germinated after 2 weeks in silica sand. Seedlings averaged 47 mm in height. Only 2.5% of the excised seeds germinated, with a mean seedling height of 17 mm. The differences in germination and height were attributed to enhanced seedling nutrition by fungal decomposition of the utricle.

Table 2
Experiment 2: Hypocotyl (H) and radicle (R) length (L, mm) and diameter (D, mm) of fourwing saltbush seedlings from excised surface-sterilized (S) and non-surface-sterilized (NS) seeds germinated on either carbon-free (–C) or cellulose-supplemented (+C) medium

Surface treatment	Germination medium	n[a]	HL	HD	RL	RD
NS	+C	38	20.0a[b]	0.59a	10.3a	0.47a
S	+C	26	15.9b	0.58a	9.4a	0.50ab
NS	–C	26	9.7c	0.58a	3.8b	0.47a
S	–C	27	8.8c	0.60a	4.6b	0.63b

[a]*n* is the number of seedlings measured in each treatment.

[b]Means within a column followed by a different letter differ ($p < .01$).

Experiment 2

Table 2 shows increased hypocotyl and radicle lengths of seedlings germinated on 1/10 MWR+C medium from SS and NSS excised seeds ($p > .01$) compared to these germinating on 1/10 MWR–C. Seedlings from excised NSS seeds were more vigorous than those from excised SS seeds ($p < 0.01$). Microscopic examination of seedling radicles from SS seed revealed internal colonization by other septate fungi not eliminated by surface sterilization. Increased seedling height and radicle lengths were attributed to sugars released by fungal hydrolysis of cellulose. Radicle diameters were greater in seedlings from excised SS seed germinated on 1/10 MWR–C medium ($p < .01$) than those from NSS seeds, which were longer.

Experiment 3

Table 3 illustrates differences ($p > .01$) in hypocotyl and radicle lengths of germinating seedlings in response to different substrates. Seedlings from excised seed germinated on cellulose-supplemented medium were larger than those without cellulose. A greater

Table 3
Hypocotyl (H) and radicle (R) length (L, mm) and diameter (D, mm) of fourwing saltbush seedlings from non-surface-sterilized excised (–utricle) and intact (+utricle) seeds germinated on either carbon-free (–C) or cellulose-supplemented (+C) medium

Seed treatment	Germination medium	*n*[a]	HL	HD	RL	RD
+Utricle	+C	43	29.8a[b]	0.62a	28.5a	0.54a
+Utricle	–C	49	25.5b	0.59b	21.0b	0.51b
–Utricle	+C	36	18.3c	0.50c	13.2c	0.41c
–Utricle	–C	63	15.7d	0.48c	9.6d	0.33d

[a]*n* is the number of seedlings measured in each treatment.

[b]Means within a column followed by a different letter differ ($p < .01$).

response was observed in seedlings from intact seeds germinating on 1/10 MWR–C than in those from excised seeds germinating on 1/10 MWR–C. The most vigorous seedlings were from intact seeds germinated on 1/10 MWR+C medium.

Seed-borne fungi nonpathogenically colonized seedling root cells (Figure 1), forming a plant–fungal interface for nutrient exchange. These interfaces were similar to those observed in the roots of native grasses and shrubs under field conditions throughout the southwestern United States (Barrow et al., 1997). In both cases considerable external hyphae were observed.

Discussion

Seed-borne septate fungi formed nondestructive, inter- and intracellular associations with the seedling root (Figure 1), similar to colonization observed in native *Atriplex canescens* populations (Barrow et al., 1997). From the data, we hypothesize that dark septate fungi decompose utricles, cellulose, and complex organic substrates and utilize resulting metabolites for seedling nutrition. Orchid mycorrhizal fungi, identified as saprophytes, parasites, and wood-rotters, colonize roots of achlorophyllous seedlings, hydrolyze external organic polymers, and transport soluble carbohydrates for plant development and survival. They differ from other mycorrhizae that supply nutrients to the host in exchange for carbon (Harley & Smith, 1983; Warcup, 1981). Dighton (1991) reported that some ectomycorrhizal fungi acquire carbon either from the host or from organic matter. Fungi were more efficient decomposers when associated with the plant.

Boursnell (1950) found a septate, seed-borne fungus that was essential for normal development of *Helianthemum chamaecystus* Mill. seedlings. In our study, seedlings from

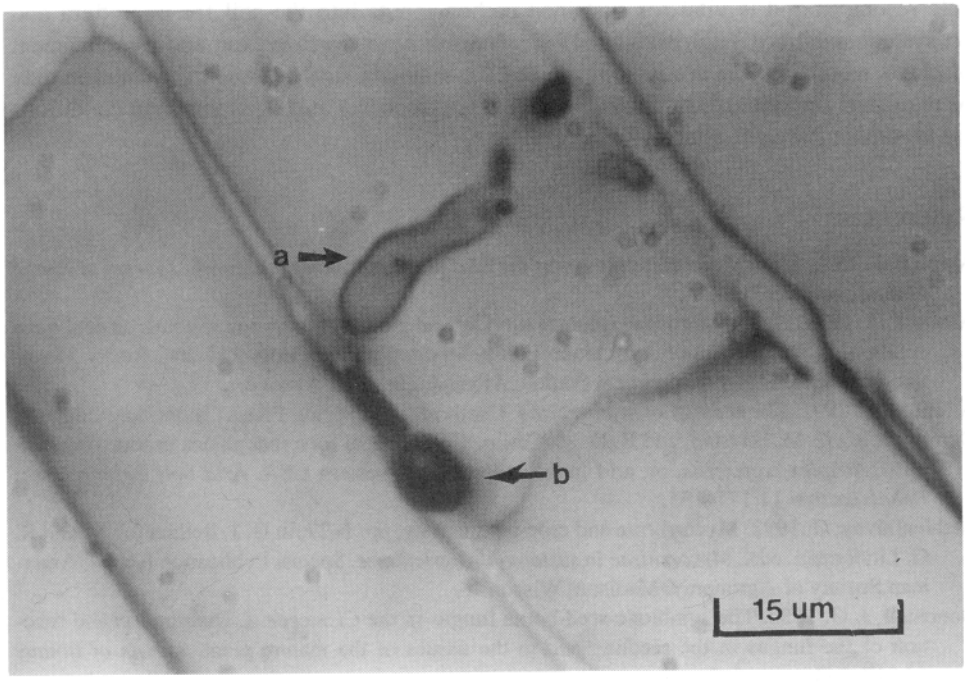

Figure 1. (a) Inter- and (b) intracellular hyphae colonizing cortex cells of germinating seedlings of fourwing saltbush (400×).

SS seeds were more distorted than those from NS seeds, indicating a fungal influence for normal development. Garvin et al., (1996) summarized reports that *Alternaria* and other fungi improved germination of several *Atriplex* species by decomposing bracts, utricles, and possibly inhibitors. Aldon (1975) improved seedling growth and survival of *A. canescens* on mine spoil sites by inoculating with endomycorrhizal fungi.

Endophytic and mycorrhizal fungi significantly enhance host plant nutrition and survived in major ecosystems. They interface with root cells and form an extensive absorptive hyphal networks in the soil. Networks serve as a metabolic bridge where carbon, nutrients, and water flux between interspecific plant communities, soil, and other microflora (Bethlenfalvay, 1992; Read, 1992). Fungi are particularly adapted to arid environments because they can remain active in absorbing and transporting resources at low soil water potentials (Griffin, 1979).

Saprophytic fungi, similar to those we observed, have extensive symptomless, endophytic phases in plants that are not apparent nor understood by casual observers (Parbery, 1996). Others demonstrated their mycorrhizal function and proposed ecological significance (Harley & Smith, 1983; Hall, 1976; Haselwandter & Read, 1982; Odell et al., 1993; Sengupta et al., 1989).

Atriplex canescens has a functional relationship with endophytic fungi that efficiently manages limited vital resources for establishment and survival. Resources are used for utricle and bract formation, in lieu of seed reserves, and are recycled at germination. Subsequent linkage to the absorptive network is essential for establishment and survival.

The distribution and frequency of mature plants is relatively stable in native *A. canescens* populations. Annual seed production is enormous, yet new seedlings are rare. However, plants removed by disturbance are often replaced in the original distribution and frequency (J. R. Barrow, unpublished). This suggests that plant cover is regulated by available resources and is vital for sustained carbon inputs into the soil for microflora and ecosystem stability (Bethlenfalvay, 1992). Indiscriminate germination and establishment of plants would deplete available resources. To maintain stability, seed germination may be regulated and initiated by fungal stimuli when resources and environmental conditions are favorable for plant establishment.

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