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Mycorrhizal associations in *Opuntia humifusa* in southern Illinois

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by
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INTRODUCTION

A mycorrhiza is a mutualistic association between a fungus and the roots of a plant. Mycorrhizae are known to occur in most plant families and in 96% of plant species examined (Dhillion and Friese 1994). These associations are essential to the success of many plants in natural environments (Allen 1991). In fact, Pirozynski and Malloch (1975) have hypothesized that the colonization of land by plants and their subsequent evolution was possible only because of a symbiotic relationship between a semi-aquatic ancestral alga and an aquatic fungus-like organism (an oomycete). Mycorrhizae are evident in even the earliest known fossilized soil-absorbing organs of plants--the rhizomes of lycopods from 370 million years ago (Nicolson 1975).

Mycorrhizae have since evolved into at least three major types: ectomycorrhizae, ectendomycorrhizae, and arbuscular mycorrhizae (AM). Ecto- and ectendomycorrhizae are generally developed by Basidiomycetes and Ascomycetes, while arbuscular mycorrhizae are developed by Zygomycetes. Ectomycorrhizal fungi penetrate the spaces between the host plant's root cells but do not enter the cells themselves. Ectendo- and arbuscular mycorrhizal fungi, however, do penetrate the cortical cells and form specialized structures within the cells (Allen 1991). This research focuses only on arbuscular mycorrhizae, as this is the only type associated with cacti (Trappe 1981).

Like other typical Zygomycetes, AM fungi form aseptate hyphae. With these hyphae, AM fungi penetrate the plant host's root cell walls, but not the cell membranes. These intraradical hyphae may then produce structures called vesicles and arbuscules inside the cell lumens (Allen 1991). Johnson et al. (1999) describe vesicles as more or less spherical sacs that form within or between cortical cells and probably function as

storage units (Figure 1). They describe arbuscules as highly branched masses of hyphae that exchange nutrients and water between the fungus and the plant (Figure 2). The fungus also forms extraradical hyphae that act as "extended roots" and may expand to form asexual spores, either singly (Figure 3) or in clusters (Figure 4). AM fungal spores and external hyphae are involved in asexual reproduction of the fungal partner and in colonization of new sites.

The mutualistic association between the AM fungus and the plant provides several benefits to both symbionts. One of the main benefits to the plant is improved nutrient uptake. The fungus extends its external hyphae beyond the phosphate-depletion zone around the roots to capture more soluble phosphates, which are transferred to the plant roots (Hayman 1983). AM fungi also produce exogenous enzymes, such as phosphatases, phytases, and nitrate reductase, which improve the uptake and metabolism of nutrients (Trappe 1981). In some plants, mycorrhizal associations have also been shown to increase levels of Zn, Cu, and N (Hayman 1983). Cui and Nobel (1992) found that CO_2 uptake and water-use efficiency were higher in specimens of *Agave deserti* Engelm. inoculated with AM fungi than in uninoculated ones. Other benefits conferred on plants by AM mycorrhizal symbiosis include increased plant hormone levels, chlorophyll content, and decreased leakage of electrolytes from diseased plant cells (Hayman 1983).

The fungal symbiont benefits by having an abundant supply of carbohydrate supplied by the host plant. The fungus is also provided with a protective niche where it is relatively safe from antagonistic soil microbes (Hayman 1983).

According to Morton and Benny (1990), arbuscular mycorrhizal fungi are

Figure 1. Vesicle formed by AM fungus in root of *Opuntia humifusa* from Cove Hollow, Jackson County, Illinois. Bar = 20 μm

Figure 2. Arbuscule (indicated by arrow) formed by AM fungus in root of *O. humifusa* from Cove Hollow, Jackson County, Illinois. Bar = 20 μm

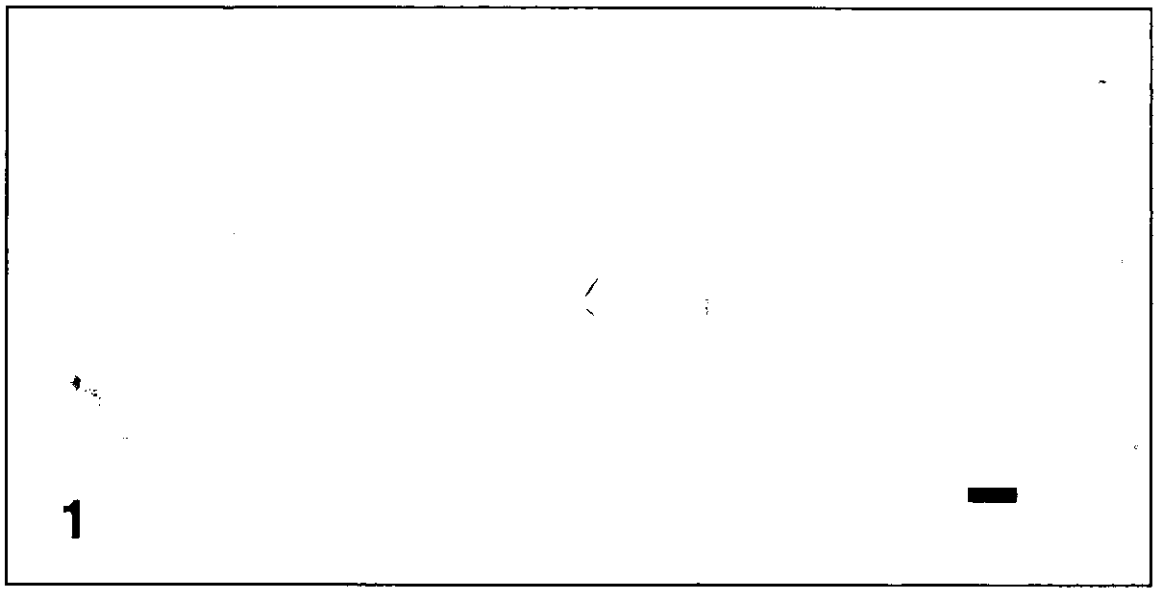
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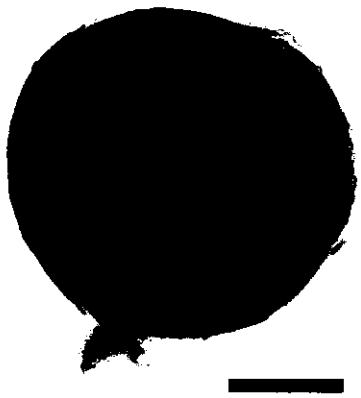
Figure 3. AM fungal spore extracted from soil around roots of *Opuntia humifusa* at Jackson Hole Natural Area, Pope County, Illinois. Bar = 50 μm

Figure 4. AM fungal spores formed at tip of dichotomously branched hypha. Extracted from soil around roots of *O. humifusa* at Jackson Hole Natural Area, Pope County, Illinois. Bar = 50 μm



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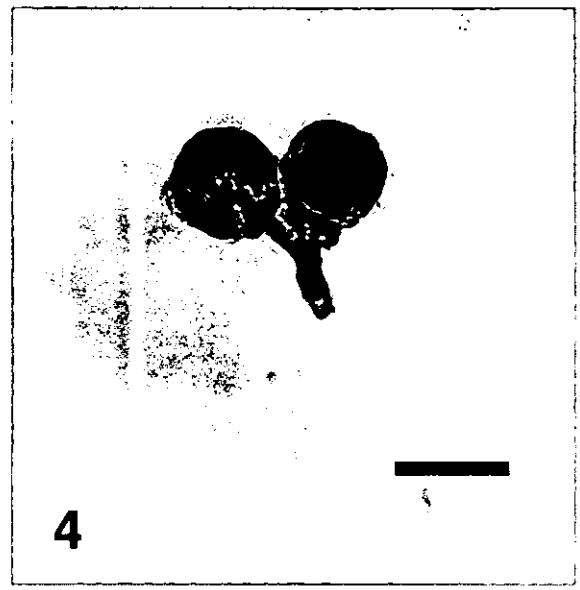

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Zygomycetes in the order Glomales. The six currently recognized genera in the order are divided among three families: Glomaceae, Acaulosporaceae, and Gigasporaceae. Genera and species can be distinguished by spore wall characteristics, presence or absence of sporocarps, germination method, and a few other characters. Identification and classification of taxa can be very difficult because of the limited number of morphological characters.

Some authors have attempted to classify plant families and genera as mycorrhizal or non-mycorrhizal based on the extent of mycotrophy among members of the taxa in question (e.g., Gerdemann 1968). This is probably an over-simplification, however. The presence or absence of mycotrophy apparently has more to do with ecological adaptations than to taxonomic categories (Allen 1991). Newman and Reddell (1987) reviewed the literature on the mycorrhizal status of 25 plant families and found that none were consistently non-mycorrhizal, even those families such as Chenopodiaceae and Brassicaceae, which have traditionally been considered non-mycorrhizal. Similarly, Trappe (1981) found several genera, and even species, in the literature that are sometimes mycorrhizal and sometimes are not. Species that are often non-mycorrhizal tend to be early successional annuals such as *Chenopodium* and *Amaranthus* species (Trappe 1981).

The Cactaceae is a family that has been categorized as typically non-mycorrhizal (Dhillion and Friese 1994). Dhillion and Zak (1993) reviewed the literature on mycorrhizae in arid and semi-arid regions and found that of nine cactus species examined, seven were mycorrhizal and two species were not. One objective of this study was to perform a literature search and review to further develop what is known about the mycorrhizal status of species of the Cactaceae. The results of this search are summarized

in the Literature Review section below, with nomenclature according to The Plant Names Project (1999).

A second objective of this study was to expand the knowledge of the mycorrhizal status of a prickly pear cactus, *Opuntia humifusa* (Raf.) Raf., which is native to southern Illinois (Figure 3). Dhillion and Friese (1994) reported that *O. humifusa* plants from two sand prairie sites in west central Illinois had "moderate" (15-40%) mycorrhizal colonization in the roots. Dhillion, et al. (1988) found spores of several AM fungal species near the root zones of *O. humifusa* plants on burned and unburned sites in the Sand Prairie Scrub Oak Nature Preserve in Mason County, Illinois. Given that some cactus species have been reported as mycorrhizal in some areas but not in others due to environmental differences (Staffeldt and Vogt 1975), I was interested in determining whether the mycorrhizal colonization level of *O. humifusa* and spore densities of associated soils in southern Illinois are similar to those in plants from other areas of the state.

LITERATURE REVIEW

Literature reviews by other authors, based on a limited number of reported species, have concluded that the Cactaceae are typically non-mycorrhizal or sometimes non-mycorrhizal (Trappe 1981; Dhillion and Friese 1994). A thorough literature search performed by us has revealed that of the 25 cactus species reported on in the literature, only three were always or sometimes non-mycorrhizal (Table 1).

To my knowledge, Staffeldt and Vogt (1975) made the first reference to mycorrhizal associations in cacti in a study of mycorrhizae in desert plants of several families. They found that *Opuntia engelmannii* Salm-Dyck was mycorrhizal in one area, but non-mycorrhizal in another. They also found that *O. leptocaulis* DC was non-mycorrhizal.

Based on plants sampled from a shortgrass prairie in northeastern Colorado, Davidson and Christensen (1977) found that *Opuntia polyacantha* Haw. had mycorrhizal fungi associated with its roots. Miller (1979) studied the occurrence of arbuscular mycorrhizae in revegetated mine spoils and undisturbed areas of the Red Desert in Wyoming. In the undisturbed community, he found a sample of *O. polyacantha* that was mycorrhizal. Reeves et al. (1979) studied disturbed and undisturbed areas in a mid-elevation sage community in Colorado. They also found mycorrhizal *O. polyacantha* in the undisturbed area.

Rose (1981) sampled endemic plants of the Sonoran Desert of Baja California for mycorrhizal associations. Of the ten species sampled, three were cacti. An unidentified *Opuntia* species contained AM fungal hyphae and arbuscules in cortical cells, but no fungal spores were recovered from surrounding soil. *Machaerocereus gummosus* Britt.

Table 1. Mycorrhizal status of cactus species

Plant species[a]	Mycorrhizal status[b]
Carnegiea gigantea	+
Echinocactus acanthodes	+
E. engelmannii	+
Ferocactus acanthodes	+
F. wislizeni	+
Machaerocereus gummosus	-
Opuntia sp.	+
Opuntia sp.	+
Opuntia acanthocarpa	+
O. basilaris	+
O. bigelovii	+
O. coccinellifera	+
O. cylindrica	+
O. echinocarpa	+
O. engelmannii	+/-
O. ficus-indica	+
O. fulgida	+
O. humifusa	+
O. leptocaulis	-
O. phaeacantha	+
O. polyacantha	+
O. santa-rita	+
O. spinosior	+
O. vulgaris	+
Pachycereus pringlei	+

[a] Nomenclature according to The Plant Names Project (1999).

[b] Mycorrhizal status is noted as follows:

+ indicates presence of mycorrhizal fungi;

- indicates absence of mycorrhizal fungi;

+/- indicates presence in some samples, absence in others

and Rose was completely non-mycorrhizal, and no soil spores were recovered. *Pachycereus pringlei* Britt. and Rose roots contained AM fungal hyphae but no functional arbuscules. No spores were recovered from the soil.

Bethlenfalvay et al. (1984) sampled plants of 19 families for colonization by AM fungi at four sites in a southern California desert. At one site in Anza-Borrego Desert State Park, they found four species of cactus, *Echinocactus acanthodes* Lem., *Echinocereus engelmannii* (Parry ex Engelm.) Rümpler in C. F. Forst., *Opuntia acanthocarpa* Engelm. and Bigel., *O. bigelovii* Engelm. All had mycorrhizal fungi present in greater than half of the roots by length. They found three cactus species at another site in the State Park, *E. acanthodes*, *O. basilaris* Engelm. and Bigel., and *O. echinocarpa* Engelm. and Bigel. All of these were mycorrhizal as well.

Bloss (1985) sampled soils from the Sonoran Desert in Arizona to isolate AM fungal spores for identification and culture. While there were several cactus species in the sampling areas, no list of mycorrhizal species is provided. However, mycorrhizal roots of *Opuntia fulgida* Engelm. and *O. phaeacantha* Engelm. are depicted in the figures. Bloss and Walker (1987) reported on the mycorrhizal status of plants of the Santa Catalina Mountains in Arizona. Roots from five cactus species were sampled: *Ferocactus wislizeni* Britt. and Rose, *O. phaeacantha*, *O. spinosior* Toumey, *O. fulgida*, and *Carnegiea gigantea* Britt. and Rose. All of the samples were mycorrhizal.

Mathew et al. (1991) examined the mycorrhizal status of introduced spineless cacti used as cattle fodder in the desert of Rajasthan, India. Six *Opuntia* species were sampled and their roots were examined and scored for percent colonization of mycorrhizal fungi by length. *O. ficus-indica* Mill. had a 16.0% colonization level, *O.*

santa-rita Rose had 22.0% colonization, *O. vulgaris* Mill. had 17.0% colonization, *O. coccinellifera* Steud. was 35.6% colonized, *O. cylindrica* DC had 32.0% colonization, and an unidentified *Opuntia* species was 12.0% colonized by AM fungi.

Cui and Nobel (1992) determined the percent colonization levels of *Ferocactus acanthodes* Britt. and Rose from a desert in southern California. The roots of this species had a mycorrhizal colonization level of between 6.1% (±2.2%) and 7.2% (±1.8%). They also examined the roots of greenhouse-grown *Opuntia ficus-indica* and *F. acanthodes*, and found that they had a maximal colonization level of 9% and 12%, respectively.

Dhillion and Zak (1993) sampled *Opuntia humifusa* plants on two sand prairie sites in west central Illinois. The plants sampled were mycorrhizal. Similarly, Dhillion and Friese (1994), in a study on mycorrhizae in prairie plants, reported that *O. humifusa* from a sand prairie was moderately colonized (15-40%) by AM fungi.

MATERIALS AND METHODS

Study sites

Specimens of *Opuntia humifusa* (Figure 5) were sampled between 13 July and 12 October 1999 from six different sites in southern Illinois: Little Grand Canyon, Jackson Hole, Stone Face, Cove Hollow, Lusk Creek, and Devil's Backbone.

Little Grand Canyon National Natural Landmark is northwest of Pomona in Jackson County. The plants were found on a steep, dry northwest-facing ridgetop overlooking the Big Muddy River in an area with Neotoma series soils. Neotoma soils are characterized by a stony loam surface layer, moderate permeability, and rapid runoff. The soils have low organic matter, moderate water holding capacity, and an extremely acid to somewhat acid reaction (USDA 1979). The site was sampled 13 July 1999.

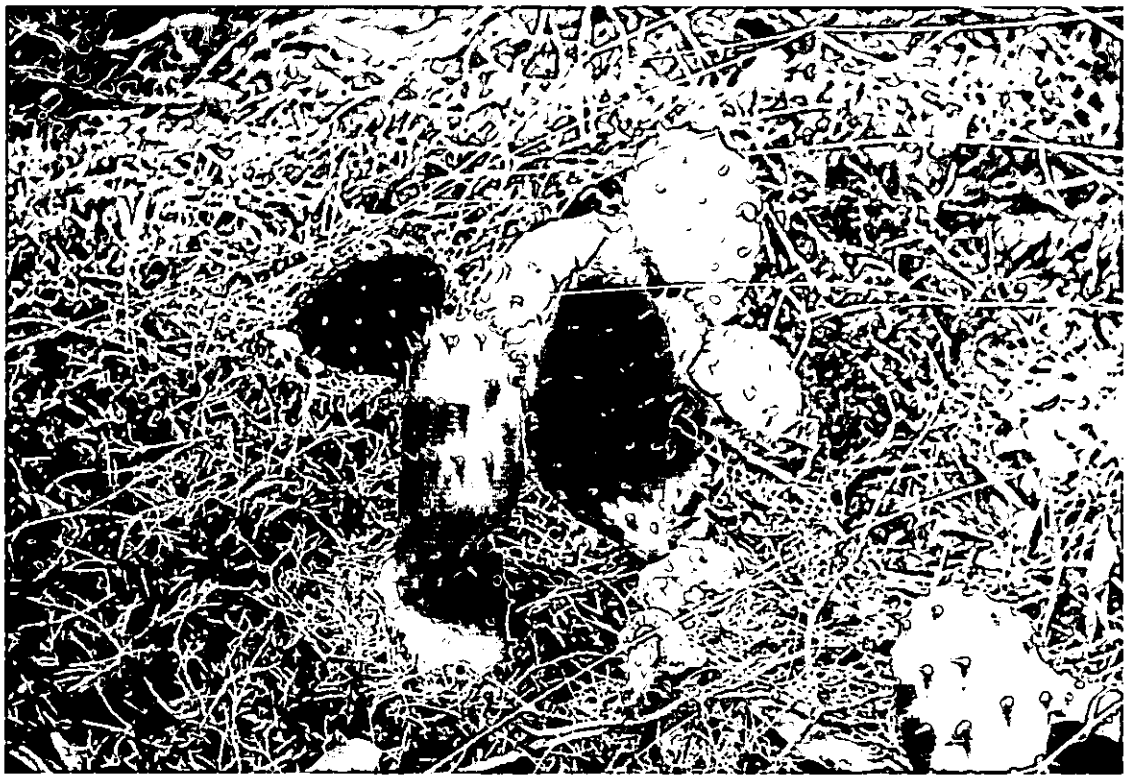
Jackson Hole Natural Area is in Pope County, west of Eddyville. The collection site is a dry ridgetop with Wellston series soils. Wellston soil is formed from weathered sandstone and loess. It has low organic matter content, moderate permeability, and high acidity (USDA 1975). The plants at this site were collected on 17 July 1999.

Stone Face Natural Area was sampled on 25 July 1999. Stone Face is located south of Harrisburg in Saline County. The site was a west-facing ridge with Wellston soils (USDA 1978).

The Cove Hollow plants were collected on 22 August 1999 on a south-facing ridgetop. Cove Hollow is east of Pomona in Jackson County, on Cedar Lake. The area contains Alford silt loam soil, which is formed from loess and weathered bedrock, has moderate permeability, high acidity, and low organic matter (USDA 1979).

Lusk Creek Wilderness is east of Eddyville in Pope County. The site was sampled on 2 October 1999. The collection site was a southwest-facing ridge with Wellston soils

Figure 5. *Opuntia humifusa* population at Jackson Hole Natural Area, Pope County, Illinois. Picture taken 17 July 1999



(USDA 1975).

Devil's Backbone State Park is north of Grand Tower in Jackson County. The plants were found on a southwest-facing bluff overlooking the Mississippi River. The area contains well-drained Alford soils (USDA 1979).

Field Procedures

The plants were excavated and the root systems were carefully removed on site. The roots were then stored in plastic bags with a moist paper towel at room temperature for up to 5 days. Plant rhizosphere soil was collected, air dried, and stored at room temperature for further processing. At least two cladophylls were removed at each site to be used as voucher specimens. Vouchers are housed in the Southern Illinois University Herbarium in Carbondale, Illinois (SIU).

Assessment of mycorrhizal colonization

Roots were rinsed in distilled water to remove soil and fixed in glass vials of FAA (formalin:glacial acetic acid:ethyl alcohol, 1:1:18 by volume). The fixed roots were processed according to Dhillion et al.'s (1988) modification of Kormanik and McGraw's (1982) clearing and staining procedure. Roots were cleared in 10% KOH at room temperature for 3-6 days, rinsed in distilled water, and bleached in alkaline H_2O_2 for 20 min. They were then acidified in 1% HCl for 3 min and placed in acid fuchsin/lactic acid/glycerin stain for 5 days. Excess stain was removed by placing roots in a lactic acid/glycerin mixture.

Roots were scored for percent colonization according to a method described by Giovannetti and Mosse (1980). Ten stained 1-cm root segments were mounted on a glass slide in lactic acid/glycerin and examined under a compound microscope. Five slides were prepared in this manner for each of the six root samples, for a total of 50 1-cm segments per site. A segment was considered mycorrhizal if it contained vesicles,

arbuscules, or both. The total number of mycorrhizal segments in each sample was multiplied by two in order to determine the percentage of fungal colonization per site.

Spore counts

The soil samples were processed for spore extraction using a method similar to the wet-sieving and decanting technique described by Walker et al. (1982). A 100-g subsample of each soil sample was thoroughly mixed and all large peds were broken by hand. The soil was placed in a plastic bucket and stirred with an excess of water. The heavier particles were allowed to settle for a few seconds and the water was decanted onto a 270-µm sieve. The sieve was washed and scanned for the presence of large spores and sporocarps. None were detected in any of the subsamples, so the sievings were discarded. The mixture from this sieving was stirred vigorously and allowed to settle for a few seconds, then decanted through a 180-µm sieve nested on a 53-µm sieve. This captures all but the smallest fungal spores. The sievings from the 180-µm and 53-µm sieves were washed into funnels lined with coarse filter paper, and the filter paper was air dried and stored in a Petri dish at room temperature.

These sievings were later combined and rinsed into 50-ml centrifuge tubes with about 40 ml of water. The tubes were centrifuged at about 1500 rpm for 3 min in a swinging bucket centrifuge with a 41-cm rotor diameter (about 850-900×*g*). The supernatant was decanted onto a 53-µm sieve and scanned for spores. Spores were present in some samples, so the sievings were collected in a beaker. Thirty to 40 ml of 2 mol/l sucrose was added to each tube and they were centrifuged at about 1500 rpm for 1.5 min. The supernatant was poured onto a 53-µm sieve and the spores were quickly rinsed with water. The spores were combined with the sievings from the water centrifugation.

The spores were poured onto coarse filter paper in a vacuum filtration apparatus and vacuum filtered. The filter paper was air dried and stored in a Petri dish at room

temperature. The spores were later scraped and rinsed into a plastic Petri dish divided into quarters by scoring the underside with a dissecting needle. Enough water was added to the dish to suspend the spores. The spores were stirred to evenly distribute them over the plate and a one-quarter section of the plate was chosen at random for counting. All spores in the section were counted, and the number was multiplied by four to obtain a total spore count for the sample. This was divided by the volume of the soil subsample and the mass of the subsample in order to determine the number of spores per cm^3 of soil and the number of spores per g of soil, respectively. No attempt was made to identify taxa by spore types.

RESULTS

Mycorrhizal colonization

Plants from all sites were mycorrhizal. Colonization levels varied from 38% in the plants from Devil's Backbone to 74% in the Cove Hollow plants (Table 2). Most plants had intraradical hyphae, arbuscules, and vesicles present in the roots. However, no vesicles were seen in the sample from Devil's Backbone; only arbuscules and intraradical hyphae were present. One segment from the Cove Hollow roots also contained what appeared to be clusters of AM fungal spores inside the cortical cells. Some samples were infected by Basidiomycetes or Ascomycetes as well.

Spore counts

Soil from each of the sites contained AM fungal spores. Spore counts ranged from 7.26 spores/cm^3 in the Jackson Hole soil to 93.56 spores/cm^3 in the Stone Face soil. Spore counts for all sites are reported in Table 2. Results are reported as spores per cm^3 of soil and spores per g of soil.

Table 2. Root colonization levels and spore counts of AM fungi associated with *Opuntia humifusa* in southern Illinois

Site	Root Colonization (percent)	Spore count (per g soil)	Spore count (per cm^3 soil)
Little Grand Canyon	44	47.8	47.8
Jackson Hole	56	27.5	7.3
Stone Face	52	93.6	93.6
Cove Hollow	74	59.1	35.5
Lusk Creek	58	266.0	76.0
Devil's Backbone	38	9.9	8.6

DISCUSSION

The results of this study show that *Opuntia humifusa* populations in southern Illinois are highly colonized by arbuscular mycorrhizal fungi. The 38-74% colonization of the plants in this study is higher overall than the 15-40% reported by Dhillion and Friese (1994) for *O. humifusa* from two sand prairie sites in west central Illinois.

Though the sites studied were not uniform, root colonization appeared to increase through the summer, peak in August, and decline in the fall. Similarly, Dhillion and Anderson (1993) found significant seasonal variation in root colonization of *Schizachyrium scoparium* (Michx.) Nash. However, differences between our sites (soil texture, moisture, nutrient levels, etc.), and the fact that each site was sampled only once, make such comparisons difficult at best. Sampling each site in this study several times over the growing season should help more effectively elucidate the seasonal dynamics of root colonization in *Opuntia humifusa*.

Wide variations in spore densities were detected at the six sites. This agrees with those who have found that spore numbers vary widely among sites with different disturbance levels, moisture inputs, soil textures, and other complex factors, and when sampled in different seasons (Ianson and Allen 1986; Dhillion, et al. 1988; Jacobson 1997). In addition to the seasonal variation, soil texture and density play an important role in spore formation and mechanical retrieval. Dense clay soils have smaller spaces for percolation, air circulation, and growth, which may inhibit external hypha and spore production. According to Ianson and Allen (1986), clay soils also tend to hold spores more readily due to electrostatic attraction, while spores are more easily extracted from loam soils. This makes the wet-sieve and decant method less effective for soils high in

clay. In agreement with this observation, the soil samples with high clay content used in this study had lower spore counts than those that were higher in organic matter.

Further work should incorporate different spore extraction techniques to obtain the most accurate spore counts. Because of differences in soil densities at the different sites, spore counts reported per unit volume of soil are comparatively more meaningful than counts reported per unit mass of soil.

CONCLUSIONS

Based on the results of the literature search, it appears that species in the Cactaceae are usually mycorrhizal. Only three of the 25 examined are non-mycorrhizal. This differs from earlier views that cacti are usually non-mycorrhizal or only sometimes mycorrhizal. The mycorrhizal status of some species is different in plants collected from different areas. Therefore, it is probably more useful to classify plants' mycorrhizal status based on the ecological conditions in which they're found, rather than taxonomically.

Opuntia humifusa plants in southern Illinois are highly colonized by arbuscular mycorrhizal fungi. Colonization levels are higher than those reported for *O. humifusa* plants from other areas of the state. However, many environmental factors affect percent colonization. Further work should be done to get a better picture of the mycorrhizal associations of this species.

Arbuscular mycorrhizal fungal spore densities from *Opuntia humifusa* root zones vary widely among southern Illinois sites sampled at different times. Further studies should sample individual sites at different seasons to better understand the seasonal dynamics of fungal spore formation. Spores could also be identified to species to compare fungal diversity at the sites.

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