Unique Characteristics of a Systemic Fungal Endophyte of Native Grasses in Arid Southwestern Rangelands

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Abstract: Native grasses and shrubs in arid Southwestern rangelands are more extensively colonized by dark septate endophytic fungi than by traditional mycorrhizal fungi. A histochemical method was developed that revealed active internal structures that have not been observed using conventional protocol for fungal analysis. These fungi nonpathogenically and totally colonize all sieve elements, cortical and epidermal cells. They grow intercellularly and intracellularly, forming an intimate, integrated association with all root and leaf cells. Their internal morphology is often atypical of commonly recognized symbiotic or pathogenic fungal colonization. These endophytes accumulate and disperse large quantities of lipids through the root. They form a saturated mucilage layer on both the root and leaf surface, which protects them and maintains hydraulic continuity with dry soil. Our results suggest that these fungi function as carbon managers and enhance nutrient and water uptake in arid ecosystems.

Mycorrhizal fungi profoundly influence plant communities in all ecosystems. The most extensively studied are the arbuscular mycorrhiza (AM) that are associated with at least 85 percent of all vascular plants. These fungi colonize the root cortex, extend into the soil, and transport P to arbuscules within the root cortex where it is released to the plant. The term "mycorrhizae" means fungus root (Smith and Read 1997). These fungi are harmonious components of and are an extension of the root system. Fungi are well adapted for nutrient acquisition; their small size allows exploration of microscopic soil pores, and they are able to function at lower water potentials than other organisms (Griffin 1979). They function as microscopic pipelines where carbon and minerals are actively transported simultaneously to and away from the plant.

Little is known of the mycorrhizal status of desert plants, and studies have focused primarily on the presence of arbuscules in plant roots. Plant roots are also colonized by many other kinds of fungi, including saprophytic or weakly pathogenic fungi that may have symptomless endophytic or biotrophic phases in their life cycles that are not apparent to causal observers (Parbery 1996). Factors that distinguish between beneficial and detrimental plant-fungus relationships are relative to the quantitative reciprocal exchange of photosynthetic carbon for mineral nutrients, water, or protection (Smith and Smith 1996). Nonmycorrhizal plants are frequently colonized by septate fungi that may function like mycorrhizal fungi, but their study and importance have been minimized because they do not conform to established mycorrhizal morphology (Trappe 1981). Barrow and others (1997) have found that dominant shrubs and grasses are more extensively colonized by dark septate fungal endophytes (DSE) than by traditional mycorrhizae. DSE are characterized by stained and melanized septate fungal structures (Jumpponen 2001). In a review by Jumpponen and Trappe (1998), DSE fungi colonize a wide range of plant species in stressed ecosystems. Barrow and Aaltonen (2001) developed dual-staining methodology and high magnification differential interference microscopy that revealed substantially greater incidence of unique, active fungal structures that are not detected using conventional fungus-staining methods and casual low-magnification observations.

Materials and Methods

Material Collection

Roots and leaves were sampled weekly during 2001 from a native population of black grama, *Bouteloua eriopoda* (Torr.) Torr. (BOER), located on the USDA Agricultural Research Service's Jornada Experimental Range in southern New Mexico. Soil was chronically dry, and soil moisture was generally less than 3 percent at most sampling times, except for brief periods following precipitation events when the soil was nearly saturated. Blue grama, *Bouteloua gracilis* (Kunth) Lag. Ex Steud. (BOGR), from various northern and high elevations was also collected periodically. BOGR is adapted to more mesic, higher elevations than BOER on the Jornada Experimental Range. Some samples were taken from actively growing sites that received normal and above precipitation during the growing season. Fungal colonization was determined to be consistent, and expression was uniform in this and previous studies within the population at each sampling period. For this study, roots from two to five plants of each species were collected, bulked, and sealed in a plastic bag and taken to the laboratory for preparation and analysis.

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Tissue Preparation and Clearing

Methods developed by Bevege (1968), Brundrett and others (1983), Kormanik et al. (1980), and Phillips and Hayman (1970) were modified for optimal visualization of fungi in native grass roots. Roots were washed in tap water to remove soil. From each bulked sample, healthy feeder roots of uniform maturity and appearance (approximately 0.250 mm in diameter) were randomly selected and cleared by placing in an autoclave in 2.5 percent KOH. Temperature was increased to 121 °C over 5 minutes, maintained for 3 minutes, and after 8 minutes, samples were removed from the autoclave. Roots were rinsed in tap water, bleached in 10 percent alkaline H_2O_2 for 10 to 45 minutes to remove pigmentation, and placed in 1 percent HCL for 3 minutes. Decolorized roots were rinsed for 3 minutes in dH_2O before staining with either trypan blue (TB) or sudan IV (SIV), or they were dual stained with TB and SIV. To stain with TB, roots were placed in prepared TB stain (0.5 g trypan blue in 500 ml glycerol, 450 ml dH_2O and 50 ml HCL), autoclaved at 121 °C for 3 minutes, and stored in acidic glycerol (500 ml glycerol, 450 ml H_20, and 50 ml HCL). For SIV staining, roots were placed in prepared SIV (3.0 g Sudan IV in 740 ml of 95 percent ETOH plus 240 ml dH_2O), autoclaved at 121 °C for 3 minutes, and stored in acidic glycerol. For dual staining, roots were stained first in TB, autoclaved as above, and destained 24 hours in the acidic glycerol. Roots were then transferred to the SIV stain and autoclaved as above. Dual stained roots were destained in dH_2O for 3 minutes, and roots were stored in acidic glycerol until mounting.

Ten to twelve 2-cm root segments were placed on a microscope slide in several drops of permanent mounting medium. A cover slip was placed over the root sections and pressed firmly to facilitate analysis at high magnification. Analysis was done with a Zeiss Axiophot microscope using both conventional and DIC optics at 1000x. Leaves were prepared similar to the roots.

Results

This method revealed a number of unexpected associations of DSE fungi with the native grama grasses that differ from known pathogenic and symbiotic fungal associations (Barrow 2003). Active fungal structures were dynamic and morphologically different from typical fungal morphology and were influenced by external environmental conditions. The most prevalent structures in physiologically active roots were fungal protoplasts that had no distinguishable walls. As root activity decreased, structures with hyaline, stained, or pigmented walls characteristic of DSE fungi became increasingly evident and were connected with fungal protoplasts. A distinguishing feature of fungal structures was a virtually constant association with lipid bodies of varied size and shape. The fungus was systemic and formed an intimate, integrated interface with all root and leaf cells. The nondestructive colonization of sieve elements of healthy roots was unexpected. Colonization of cortical cells was less evident in physiologically active roots, but as roots became dormant, DSE fungi were observed in all cortical cells. Another unique observation was the colonization of all root meristematic cells. The fungus was observed extending from the root cortex and formed a loosely configured network within a mucilaginous layer on the root surface. Fungi were also observed extending from the root surface into the soil. The same fungal morphology was observed associated with sieve elements and bundle sheath and mesophyll cells of the leaves. Colonization of epidermal cells and the stomatal complex was likewise unique. Lipid bodies within fungal structures that ranged from very small to occupying the entire internal volume of the subsidiary cells were also observed. Lipid-filled fungal protoplasts were also observed extruding from the stomatal apertures.

Disscussion

The novel method used in this study revealed a substantially greater colonization of native grasses by DSE fungi and atypical fungal structures that escape detection by conventional methodology. Systemic colonization is assured because of colonization of meristem cells and distribution of the fungus at cell division to developing roots and shoots. The nature and extent of fungal colonization indicate several potential ecological roles. The colonization of sieve elements is presently relegated to pathogenic fungi and is unique for beneficial or endophytic fungi. This also distinguishes DSE fungi from traditional mycorrhizal fungi that are restricted to the root cortex and surface.

Staining with sudan IV, specific for lipids, revealed many internal fungal structures that are not observed using conventional microscopy. The quantity and size of fungal lipid bodies indicate substantial assimilation of host photosynthate. This would generally be viewed as a parasitic association; however, there is no detrimental affects to the host plants. Therefore, it is concluded that DSE fungi are carbon managers and utilize host carbon for the protection and benefit of the host plant. Evidence for this is the protective mucilaginous layer observed on both root and leaf surfaces. Mucilage is an important organic carbon component of plants and soil that affects water infiltration, retention, and soil stability. This matrix is viewed as a protective, saturated microenvionment that would maintain hydraulic continuity between the root and dry soil (Read and others 1999). Equally important is the colonization of cells of the stomatal complex. Accumulation of lipids in the subsidiary cells could physically influence stomatal closure. Lipid-filled fungal protoplasts occupying stomatal apertures could potentially trap and retain transpired water. Such mechanisms would enhance water use efficiency and regulation and plant survival in drought-stressed arid ecosystems.

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