

Carbon Transport by Symbiotic Fungi in Fourwing Saltbush, *Atriplex canescens* (Pursh) Nutt.

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Abstract—Mycorrhizal fungi enhance the nutrition and survival of host plants in native ecosystems. Arid rangelands severely challenge plants because of chronic nutrient and water stress. Fourwing saltbush, *Atriplex canescens* (Pursh) Nutt., a dominant and important shrub of western arid rangelands, generally considered to be non-mycorrhizal, is more extensively colonized by dark septate (DS) fungal endophytes than by traditional mycorrhizal fungi. Roots of fourwing saltbush colonized by DS fungi were stained with sudan IV and analyzed with differential interference microscopy that revealed extensive internal colonization by vacuolated hyaline hyphae that is not evident using conventional fungus staining methods. Fungal vacuoles accumulated substantial quantities of lipids in the sieve elements and cortex when roots were physiologically active. The widespread colonization of fourwing saltbush by DS fungi and their extensive accumulation of lipids suggests that these fungi transport and manage carbon in arid ecosystems. Their potential role in ecosystems stability is discussed.

Mycorrhizal fungi form specific internal interfaces by which they exchange resources with their host. Their hyphae extend from the root into soil enhancing the uptake and transport of mineral nutrients. They perform varied and significant ecological functions in native ecosystems such as enhanced nutrition, water relations and survival of host plants. They regulate key processes that impact soil, vegetation structure, and stability. Recent evidence suggests that external hyphae also link plants together within a community, forming a common mycorrhizal network by which plants may share carbon and mineral nutrients. The transport and sharing of essential resources such as carbon and mineral nutrients could significantly affect the structure and stability of native plant communities (Ayling and others 1997; Brandes and others 1998; Duan and others 1996; Graves and others 1997; Simard and others 1997).

The mycorrhizal status of desert plants is not clearly defined, but their survival in water and nutrient stressed arid ecosystems is likely enhanced by mycorrhizal or other symbiotic fungi. Chenopodiaceous shrubs, common in many arid ecosystems, are generally considered to be non-mycorrhizal (Aguilera and others 1998). Fourwing saltbush, *Atriplex canescens* (Pursh) Nutt., is an ubiquitous and ecologically important shrub in arid southwestern U.S.A. rangelands. Its roots are more extensively colonized by dark

septate endophytic fungi (DS) than by traditional mycorrhizal fungi (Barrow and others 1997). DS fungi are widespread root colonizers in many stressed ecosystems. These fungi form nondestructive interfaces with roots and species include recognized pathogens, saprophytes, soil and rhizosphere inhabiting fungi (Jumpponen and Trappe 1998). It has been suggested that they may establish beneficial associations with specific plants under stressed conditions that are unfavorable for traditional mycorrhizal fungi (Kohn and Stasovski 1990). DS fungi are typically recognized as stained or pigmented (melanized) hyphae and microsclerotia in roots prepared and stained with conventional fungal staining and microscopic methods. Their ubiquitous presence in stressed ecosystems strongly suggests an important but presently unresolved ecological function (Jumpponen and Trappe 1998).

Fungal associations with plants range from parasitic to mutually beneficial. This relationship is based upon the costs expended or benefits received by each partner (Smith and Smith 1996). Mycorrhizal fungi form nondestructive interfaces with plant roots and supply mineral nutrients in exchange for organic carbon constituting a mutually beneficial relationship. Mycorrhizal fungi vary in their strategy for accessing and utilizing carbon (Smith and Read 1998). The internal mycelia of obligate arbuscular mycorrhizae (AM) fungi can only assimilate photosynthetically derived glucose from the host, from which they synthesize lipids, and transfer them to the external mycelia. The external mycelia are incapable of assimilating glucose or other forms of organic carbon (Sachar-Hill and others 1995; Pfeffer and others 1999). The external hyphae of ectomycorrhizal fungi are able to hydrolyze and utilize complex external polysaccharides (Smith and Read 1998). Ericoid mycorrhizal fungi degrade cellulose, pectin, and lignin (Cairney and Burke 1998) and wood rotting orchid mycorrhizal fungi can degrade lignin and other complex soil organic matter and transfer simple sugars to the heterotrophic protocorm for growth and differentiation of a shoot and root system (Peterson and others 1998).

Barrow and Aaltonen (2001) analyzed roots of *A. canescens* colonized by DS fungi with differential interference microscopy (DIC). They observed extensive nondestructive colonization of the cortex and vascular cylinder with hyaline hyphae that are not visible with conventional staining and microscopy. Staining of physiologically active roots with sudan IV revealed substantial accumulation of lipids in fungal vacuoles that further enhanced fungal visibility and suggested that carbon management and transport may be a significant ecological function of DS fungi. The objective of this study was to analyze *A. canescens* roots colonized by DS fungi at different levels of physiological activity and to

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determine if lipid accumulation is affected by seasonal influence.

Materials and Methods

Roots were sampled from a native population of *A. canescens* near the headquarters of the USDA Agricultural Research Service's Jornada Experimental Range in southern New Mexico from winter, early to late spring, summer, until late fall of 1999. They were prepared and cleared using modified methods developed by Bevege (1969), Brundrett and others (1983), Kormanik and others (1980), and Phillips and Hayman (1970) for *A. canescens* roots. They were stained with sudan IV and analyzed with DIC microscopy using the method developed by Barrow and Aaltonen (2001).

Results

The expression of DS fungal colonization in *A. canescens* roots was highly variable and was affected by the conditions of the plant or soil at the time of sampling. Typical DS fungal structures, pigmented (melanized) septate hyphae, and microsclerotia, were most frequently observed within the cortex of physiologically inactive roots sampled during the winter or during extended drought. In the spring, fall, and following precipitation events when roots were physiologically active, melanized fungal structures decreased and a corresponding increase in unique vacuolated hyaline hyphae were observed. Hyaline hyphae were more frequently associated with metabolically active roots and were considered active stages of DS fungi. Hyaline hyphae form an extensive inter- and intracellular network within the cortex and sieve elements. In the cortex, hyphae were larger, 2 to 5 μm diameter, and had distinctly visible walls. Within the sieve elements, they were smaller in diameter, 1 to 2 μm , and their walls were less visible. Staining with sudan IV positively stained lipids in fungal vacuoles further enhancing the visibility of internal colonization of DS fungi.

Lipid accumulation in fungal vacuoles was highly variable and was also influenced by physiological activity of the roots. Lipids began to accumulate in spherical fungal vacuoles in hyphae growing in the cortex and in the vascular cylinder in early spring before any growth response was observed and continued until new leaves were established, at which time lipids were not observed in fungal vacuoles. Lipid accumulation was again observed in fungal vacuoles in response to intense, short duration precipitation events that increased soil moisture in late summer then they decreased as soil moisture decreased. In late fall, as mean temperatures dropped, fungal vacuoles again accumulated substantial lipids in the cortex. The vacuoles were elongated and more abundant than the smaller, more spherical vacuoles observed in the spring.

Discussion

Dark septate fungal endophytes are not only the dominant colonizers of native plant roots in arid southwestern rangelands (Barrow and others 1997), but they are wide spread in other stressed ecosystems (Jumpponen and Trappe

1998). The staining of colonized roots with sudan IV and analysis with DIC (Barrow and Aaltonen 2001) revealed extensive internal colonization by hyaline hyphae that are not evident using conventional fungus staining methods. Dark septate fungi are highly polymorphic and produce hyaline internal structures from external pigmented septate hyphae (Barrow and Aaltonen 2001; Haselwandter and Read 1982; Newsham 1999). They form unique nondestructive internal interfaces with cortical cells and sieve elements. Their cumulation of substantial quantities of lipids in response to physiological activity suggests that they have a significant ecological role in the transport and management of carbon.

The small diameter hyaline hyphae that nondestructively colonize the sieve elements are virtually indistinguishable with DIC microscopy because they lack chitin and melanin and are very thin walled. These characteristics would increase their permeability and facilitate resource exchange with the host (Barrow and Aaltonen 2001; Cousin 1996; Money and others 1998). The accumulation of lipids in fungal vacuoles within the sieve elements (carbon transport cells) suggests that the fungus is accessing host photosynthate and converting it to fungal lipids. The amount of lipids within hyaline hyphae in both the vascular cylinder and the cortex suggests that they are being transported by the fungus, but the direction of carbon transport is not clear. Both pathogenic and symbiotic fungi access photosynthetic carbon from their hosts. DS fungi accumulate substantial quantities of lipids without any deleterious effects on the host suggesting that they manage carbon for the benefit of the host.

Mycorrhizal fungi not only enhance nutrient and water uptake in exchange for carbon, but they also contribute other significant ecological factors that enhance the stability and survival of native plant communities. They increase total chlorophyll and carotenoid content of host plants, accelerate net photosynthesis, increase total carbohydrate accumulation in the roots, and carbon flow into the soil contents (Graves and others 1997). Soil organic carbon and nitrogen pools were found to be 13 percent greater under tall fescue pastures with high fungal endophyte colonization compared with soil pools under low colonized pastures (Franzluebbers and others 1999). Plants likewise exude substantial quantities of carbon as simple sugars, organic acids, and polysaccharides in the rhizosphere (Isobe and Tsuboki 1998) into the soil that favorably modifies the microenvironment of the rhizosphere, improving soil properties and enhancing plant stability (Grayston and others 1997; Perry and others 1992). Mucigels or polysaccharides have high water binding capacity (Chenu and Roberson 1996), maintain hydraulic continuity between the root and soil (Read and others 1999), and enhance nutrient diffusion and uptake in dry soils (Sutherland 1998). Organic acids change pH and enhance mineralization and nutrient uptake (Jones 1998). Soil polysaccharides are polymers of simple sugars, which are released by hydrolysis and provide an energy source for soil microflora involved in mineralization (Bianciotto and others 1996). Soil carbon enhances soil aggregation and stability, water-holding capacity, and serves as an energy reserve for plants and microbes.

It is proposed that DS fungi stimulate photosynthesis, convert host photosynthate to lipids, transport it to the soil

where it insulates plant roots allowing them to survive and function in harsh dry soil. The dynamic and variable response of lipid accumulation by DS fungi as it was influenced by physiological activity of the host suggested the potential of bidirectional transport of carbon between the host and the soil. In the spring, before any new growth was evident and when photosynthetic activity was low, substantial lipid transport and accumulation was observed in hyphae in the cortical cells and in the sieve elements. It is suggested that the fungi were accessing carbon from soil reserves and supplying the plant energy rich carbon for the synthesis of new leaves, similar to the mechanism used by orchid mycorrhizal fungi, which degrade complex external carbohydrates and supply the host with simple sugars for the production of roots and shoots (Peterson and others 1998). After new leaves were established and the host was presumed to be photosynthetically active, lipid accumulation ceased. A pathogenic association would suggest that the fungi would extract more carbon from the host when it was photosynthetically active. It is suggested that DS fungi receive carbon from the host to meet its physiological and growth requirements and transport substantial quantities to the soil to recharge soil supplies essential for water retention, mineralization, and nutrient uptake. During periods of stress, they may reverse the flow of carbon to supply the host sufficient energy rich carbon for growth and survival.

The widespread colonization of native grasses and shrubs by DS fungi in arid rangelands, their nondestructive interface with sieve elements, and accumulation of substantial quantities of lipids during periods of peak physiological activity in the host suggest that these fungi manage and recycle carbon for the survival and stability of plant communities. Future studies must focus on the direction of carbon flow, the nature of carbon profiles that are established in the soil, and how they relate to ecosystem stability.

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