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The impact of arbuscular mycorrhizal fungi on strawberry tolerance to root damage and drought stress

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ABSTRACT

Individually, arbuscular mycorrhizal fungi (AMF), drought stress, and root damage can alter terrestrial plant performance but the joint effects of these three factors have not been explored. Because AMF can improve water relations, colonization by these root symbionts may increase the host's tolerance of drought especially when roots have been compromised by herbivory. This full factorial study examined effects of AMF, water deficit, and artificial root herbivory in three genotypes of wild strawberry, *Fragaria virginiana* Duchesne that originated from the same restored tallgrass prairie as the AMF inoculum. Drought stress and root damage altered allocation to roots vs. shoots but the effects were not additive and the interaction did not depend on AMF treatment. Effects of AMF were absent with one exception: root damage significantly reduced belowground mass only in plants inoculated with AMF. Although drought stress did not interact with the AMF treatment, both drought stress and root damage reduced the abundance of arbuscules, and especially vesicles, and colonization varied among genotypes. Failure to detect strong effects of AMF on host growth could be due to variable responses of individual AMF species summing to no net effects. Functionally, AMF were primarily commensals of strawberry in this study.

Introduction

Arbuscular mycorrhizal fungi (AMF) and terrestrial plants form one of the most ubiquitous symbioses in terrestrial plant communities. Extramatrical hyphae of the fungal partner function like an extended root system because they grow extensively from the root, increasing the distance and volume of soil explored by the fungus–host symbiosis (Rhodes and Gerdemann, 1975; Augé et al., 2003). As obligate symbionts, AMF benefit from the interaction. However, the direction of the partnership's effects on the host is conditional. In nutrient-poor or dry soil, AMF can facilitate P uptake and improve water relations (de Silva et al., 1996; Dell'Amico et al., 2002; Augé, 2004) as well as increase root longevity (Eissenstat et al., 2000). Better growing conditions where these services of AMF are not needed may select for symbionts that function as commensals or even parasites (Johnson, 1993; Johnson et al., 1997).

While a number of studies have examined the responses of the AMF–plant partnership to nutrient (e.g., de Silva et al., 1996; Charron et al., 2001) or drought stress (e.g., Hernández-Sebastià et al., 2000; Dell'Amico et al., 2002; Borkowska, 2002; Augé, 2004), effects of AMF on plant tolerance to root herbivory have received scant attention. In experiments that examined belowground herbivory, AMF reduced or eliminated growth deficits from root-feeding beetles (Gange et al., 1994; Gange, 1996, 2001). Root herbivory reduces surface area for water and mineral absorption while leaving the photosynthetic area of the shoot intact and therefore alters the root:shoot ratio. Mycorrhizal hyphae extending into the soil may effectively replace some of the root functions that are lost to herbivory and help bring the root and shoot functions into better balance.

Association with AMF demands a significant portion of energy fixed by the host (Jakobsen et al., 2002). Net gain from the investment may be realized chiefly during transient periods of heightened physiological demand (Dunne and Fitter, 1989) or during episodes of diminished root function. AMF's role in mineral and water balance may be especially valuable to a root-damaged host challenged by poor soil conditions.

Plants experience multiple sources of stress simultaneously but generally, effects of mycorrhizae on host plant growth are examined under two or more levels of a single form of stress, such as drought. This study examined the individual and combined effects of AMF, drought stress, and root damage on growth of wild strawberry (*Fragaria virginiana* Duchesne). Plants and inoculum originated from a common field site, a restored tallgrass prairie in central Illinois, USA. It was hypothesized that the effects of AMF on host growth are context dependent and predicted that beneficial effects of AMF on host growth would be most evident when host plants experienced the dual stresses of drought and root damage. Studies exploring compatibility of AMF–plant

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interactions have demonstrated that pairings of host and AMF may vary in degree or even direction of the effect on host performance (Klironomos, 2003; Gustafson and Casper, 2006; Bennett and Bever, 2007). Although compatibility was not the focus, this study also examined whether the effects of colonization by a natural, mixed community of AMF from a restored prairie varied among genotypes of strawberry plants originating from the same field site.

Materials and methods

Genotypes and growing conditions

F. virginiana plants spaced >10 m apart were collected from John English Prairie, Comlara Park, McLean Co., Illinois, USA, 1 yr before this experiment was conducted and cloned in the greenhouse. The 3.6 ha site is a nutrient-poor, reasonably diverse restored tallgrass prairie originally converted from farmland more than 30 yr ago (Hedberg et al., 2005). Based on distinctiveness of phenotypes after propagation in the greenhouse, three presumed genotypes (here referred to as A, B, C) were chosen from among the larger group of plants. In mid-June the three genotypes were propagated by pegging stolons into 38 ml cups containing autoclaved medium consisting of 4:2:1 sand:topsoil:peat. After 2 weeks the young plants were transplanted to 0.5 L pots containing a 7:1 mix of autoclaved medium and autoclaved soil collected from John English Prairie. Eight treatments that were combinations of the presence or absence of AMF inoculum, drought stress, and root damage were randomly assigned to plants of each genotype. There were three or four replicates for each treatment-genotype combination (9 to 12 replicates summed across genotypes) for a total of 89 plants.

Pots were randomly assigned a position on the greenhouse bench and grown under unsupplemented light that diminished in day length from 15 h of sunlight at propagation to 12.25 h at harvest. To reduce extremely rapid drying of the well-drained medium on sunny days, shade cloth on the greenhouse extended when light at the rooftop sensor exceeded 90% of maximum. 3 weeks after transplanting, each pot received 0.5 g of Osmocote® (Scotts Co., Maryville, OH, USA) fertilizer (19-6-12), or approximately 0.075 g of slow release P per liter of soil.

Spidermites appeared during the pre-manipulation growth phase. Twice during the first 5 weeks of growth all leaf surfaces were wiped with a cotton ball soaked in Bon-Neem™ (Bionide Products, Inc., Oriskany, NY, USA). Prior to the start of drought stress and root damage the extent of infestation was rated visually from 0 (no mites) to 6 (heavy infestation and visible damage) for each young, fully expanded leaf and the mean for each plant was calculated. Then all plants were sprayed with insecticidal soap and rinsed with water under high pressure. The water rinse was repeated on two additional days. Upon reappearance of mites 2 weeks later each plant was treated daily by inverting it and thoroughly rinsing the shoot with water from a hose, taking care to avoid the entry of water to the pot. Mites were virtually absent with this treatment.

AMF treatment

At transplanting, each plant was inoculated with either 30 ml of freshly collected prairie soil and a small amount of strawberry and grass roots (+AMF) or with 30 ml of autoclaved prairie soil and autoclaved roots (−AMF). Inoculum was placed directly under the plant and in contact with the roots. Several liters of DI water were mixed with 1700 ml prairie soil, passed twice through a 20 μm sieve, and distributed among −AMF pots to add bacteria to the sterilized soil. +AMF plants were given an equal volume of DI water. At planting all developing stolons were removed from the plants, most of which had two or three small leaves. 1 week before other treatments commenced, AMF colonization in +AMF plants and absence of colonization in −AMF treatments were confirmed by staining and examining roots in extra replicates for each genotype.

Drought stress

In mid-August all plants were watered to excess 1 day before root damage commenced and weighed after water stopped dripping from the pot to determine weight at field capacity. Thereafter, drought-stressed plants were weighed daily and then watered to bring them up to 85% of this weight. After 1 week, this maximum level of hydration was increased to 87% due to severe wilting by some plants within 24 h of watering. Several plants continued to wilt on sunny days and these were given an additional 5 ml daily. Nonstressed plants were watered daily just until water appeared below the pot to avoid excessive drainage. A tensiometer reading, which measures the soil matric potential, was taken on selected plants prior to daily watering to confirm drought stress. Values depend on soil characteristics but in this experiment they were typically 28–40 kPa for stressed plants and <12 kPa for well-watered plants on a scale from 0 kPa (saturated soil) to 40 kPa. On the final day before harvesting began, the 47 drought-stressed plants averaged 82.2% ($\pm 0.9\%$ sd) of weight at field capacity 24 h after last watering, whereas measurements taken on a subset of nonstressed plants averaged 89.8% ($\pm 2.1\%$ sd, $n = 15$) of field capacity.

Root damage

Root damage was intended to simulate increasing damage by a growing root herbivore. Artificial herbivory may not mimic natural damage faithfully and consequently may fail to induce biochemical responses or capture complex biotic interactions (see Hjältén, 2004). Nonetheless artificial damage and natural herbivory produced similar effects on plant growth in many experiments (Lehtilä and Boalt, 2004) and artificial damage has the advantage of greater control of type, timing, and intensity of damage with fewer confounding effects (Hjältén, 2004). Given the number of factors in this study, artificial herbivory presented a straight-forward method for examining effects on plant growth, including accurate estimation of investment in roots because severed roots could be recovered. Damage was first imposed 6 weeks after transplanting by inserting a #8 cork borer (1.2 cm diameter) through the soil to the bottom of the pot and returning the soil to the hole. Single cores were taken on days 0, 7, 13, 18, 21, 23, and 24, rotating positions clockwise around the pot. Two cores were taken on day 25 in the positions initially cored on days 0 and 7. This damage severed approximately 30% of root mass whereas roots of undamaged plants were essentially intact at harvest. Control pots were lifted and handled but roots were not damaged.

Harvest

At harvest each plant was separated into stolons, leaves, and roots+crown. Stolon length and dry mass of stolons were recorded. Severed roots were washed, dried, and weighed. Freshly harvested, fine, attached roots were weighed and then cut into 1 cm pieces. The pieces were thoroughly mixed and then approximately 1 g was removed for evaluation of AMF colonization. Remaining fine roots