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FUTURE

Effects of fully developed vesicular arbuscular mycorrhizal mycelium on seed germination in *Lolium perenne*

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Abstract

Previous studies demonstrate that the inoculation of mycorrhizal spores in seeds can improve speed of germination and establishment in grass seedlings. The universally positive effects of arbuscular mycorrhiza on plants suggest that fully developed mycorrhizal hyphae could potentially induce faster germination in seeds. This study presents data indicating that the inoculation of full grown hyphae in seeds of *Lolium perenne* inhibits speed of germination, independent of level of available soil nutrients. Daily measurements of seedling height between infected and non-infected plants at different starter fertilization rates in a sand profile showed that abuscular mycorhiza influenced seeds emerged later at higher germination success rates. Measurements in shoot biomass height and weight conversely showed that, once emerged, AM plants grew at a faster rate than NM plants. This supports the indication that inoculation of developed AM fungal mycelium, instead of AM spores, cannot further expedite rates of seedling emergence, but could possibly improve rates of seed germination.

Keywords: Lolium perenne; Arbuscular mycorrhiza*;* Germination*;* Abscisic acid*;* Carotenoid biosynthesis*; Glomus*

Introduction

The obligate symbiotic relationship formed by arbuscular mycorrhiza has been associated with numerous benefits to host plants, including, but not limited to, improved plant growth and mineral nutrition (Raju et al. 1990; Marschner and Dell, 1994), improved tolerance of disease (Trotta et al., 1996, Matsubara et al., 2000, 2001) and reduced impact of abiotic stresses like drought (Subramanian and Charest, 1995), chilling (El-Tohamy et al., 1999), and salinity (Ho, 1987). While mycorrhizal associations have been most often noted as positive, there have been instances demonstrating growth retardation (Buwalda & Goh 1982, Kiernan et al. 1983, Modjo & Hendrix 1986, Thompson & Wildermuth 1989, Newsham et al. 1995). Arbuscular mycorrhization has been shown to exist as a general phenomenon that includes the majority of grasses (Newman and Reddel, 1987). Up until recently, the use of arbuscular mycorrhiza in turfgrasses has been seen as less than necessary, as high maintenance sites such as golf courses generally consist of high fertility and pest maintenance programs. U.S. Golf Association (USGA) specifications for the recommended soil profile construction in a putting green (Bengeyfield 1989) do not include the use AM fungi at the time of sowing (Koske et al. 1995). The incorporation of AM fungal spores from the genera *Glomus* and *Gigaspora* in a sand profile can increase leaf matter production during establishment (Gemma et al. 1997). However, populations of fungi are slow to increase within this new green profile (Koske et al. 1997). In turn, the effects of AM fungi on germination and emergence cannot be realized. By supplementing *Lolium perenne* seeds with full grown AM fungal mycelium harvested from established populations, effects on germination and emergence can be observed.

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Abbreviations: AM: arbuscular mycorrhiza; pgm: per growing month; NM: non-mycorrhiza; ABA: abscisic acid; GA: gibberelic acid

Materials and Methods

Plant propagation, inoculation, and fertilization

A 75/25 sand/peat mixture was mixed to fill pots measuring 4-inches in diameter. Mesh was placed in the bottom of each pot to prevent the percolation of the sand through holes in the bottom of each pot. Each pot was spaced approximately 6 inches apart. 6 treatments were administered: *Gigaspora margarita/ Glomus intraradices* inoculum mixture at 1, 0.5, 0.25 lbs of N/m pgm and an uninoculated control at 1, 0.5, 0.25 lbs of N/m pgm. Pots set for infection were inoculated with AM fungal hyphae obtained from cutting of AM infected *Sorghum spp.* roots in calcined clay soil. Uninfected *Sorghum spp.* root cuttings in calcined clay were used as soil amendments for uninfected trials. Root cuttings were evenly spread 1-inch below the soil surface, and top dressed with ½-inches of soil. Seeding rates were issued at 10 lbs/m, and were determined by measuring respective seed weight for the total soil surface area in the 4-inch pots. Germination rates and percentage of inert matter was also calculated into seeding rates. Seeds were randomly dispersed in each pot, and topdressed with 1/8-inches of soil. 18-18-21 water-soluble fertilizer was dissolved in water for application at rates of 20 mL of water for 1 lb of N/m pgm at 3 week intervals. Pots were watered twice daily using 10-minute mist irrigation for the first two weeks. All work was done with uninfected treatments first, as to prevent cross-contamination of the test pots. Trays were not used as to prevent cross contamination through waterborne migration of spores. Pots were arranged in a randomized complete block design, with 7 replications.

Data taken

Differences in color and ground surface coverage between infected an uninfected plants were visually evaluated and noted daily. Shoot height was measured in cm every day for the two weeks following the first sign of germination. Shoot biomass weight was taken from cuttings following initial 2 week growth period.

Results

Emerging shoot growth first appeared on day 2 in NM trials. Numbers of emerged seedlings in NM pots was positively correlated with fertilizer amounts. New shoots in NM trials were not observed after day 3. Plants experienced a constant growth pattern within the first week, slowing around day 10, when daily marginal growth began to decline. Plant height

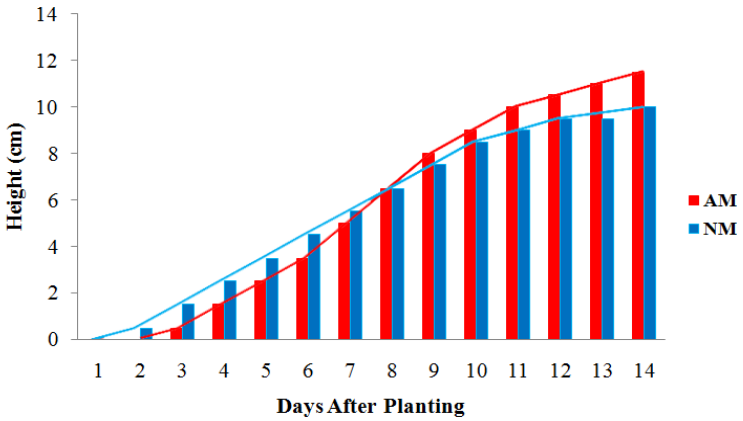

Fig. 1. Growth progression model of shoot heights over initial 2-week observational period. Shoots of AM inhabited seeds exhibited a noticeable reduction in speed of germination. Conversely, AM seeds also experienced a faster rate of growth once germinated, which continued past levels of shoot biomass height of NM plants.

differences between fertilization rates in NM trials was present, but not significant. Numbers of emerged shoots did not change during the initial 2-week observation period.

The first sign of emergence in AM trials occurred on day 3. Numbers of emerged AM shoots was observed to be independent of fertilizer amounts, and were fewer in amount than NM trials at low fertilization rates. AM plants had a slower rate of growth than NM until the end of the first week, when growth rate increased. Shoots continued to emerge in AM trials for the entire 2 week observation period. Shoots emerging beyond the first week were not observed to be propagated from rhizomes. AM and NM plants were similar in height on day 8, and both experienced diminishing marginal growth around day 10. Rate of diminishing marginal growth in AM plants was less than NM plants. AM plant height was independent of fertilization rates for the entire 2 week observation period. AM pot ground cover at the end of two weeks appeared more visually dense than NM trials at high fertilization rates. Shoot biomass cuttings of AM trials weighed more than NM trials at high fertilization rates. No differences in leaf tissue color were observed.

Discussion

While AM inoculated seeds emerged late, they appeared to be more viable through their production of a denser turfgrass stand at the end of the two week period. Emergence of the AM treated seeds appeared to be less synchronized, occurring sporadically for the entire two week period. This can perhaps be explained by the need for C when seeking to establish a

symbiosis. Higher rates of inoculum can cause significant amounts of C to be drained from the developing host plant, causing limited growth (Harley and Smith, 1983). However, when inoculated with varying amounts of AM spores, *Lolium perenne* roots show no significant differences in rates of mycorrhizal root colonization (Pelletier and Dionne, 2004).

More recent studies have suggested that ABA inhibits germination, but not release in seed dormancy in seeds of *Lolium rigida* (Goggin et al., 2009). In plants, ABA is synthesized through the cleavage and oxidation of carotenoids, and is catabolized via hydroxylation or by conjugation to glucose (Nambara and Marion-Poll, 2005). Dormant seeds treated with fluridone (a compound which inhibits carotenoid and, thus, ABA synthesis) often have similar germination characteristics to non-dormant seeds, indicating that the continued synthesis of ABA is required for dormancy maintenance in imbibed seeds of several species (Ali-Rachedi et al., 2004; Kusumoto et al., 2006; Feurtado et al., 2007).

Stimulation of carotenoid biosynthesis pathway occurs in AM infected roots (Fester et al., 2002). In turn, AM colonized roots experience an increase in the production of ABA (Bothe et al., 1994). This is a strong indicator that early AM establishment in seedlings could increase the biosynthesis of precursor carotenoids responsible for the increased production of ABA.

Among the testing of many phytohormones, only ABA is found at a considerably higher level in AM-colonized plants than in controls. Comparatively, the concentration of ABA is about twenty times higher in spores and hyphae of the AM fungus *Glomus* than in roots (Bothe et al., 1994). This suggests that presence of ABA from the introduced AM hyphae could increase the presence of exogeneous ABA.

Increased ABA levels accompany late seed development and discourage premature germination in a range of monocots (King, 1982; Zeevart and Creelman, 1988). ABA is known to limit the production of enzymes such as *alpha*-amylase, which induces the production of gibberellin (e.g. Chrispeels and Varner, 1966; Khan etal., 1973; King, 1976; Stoy and Sundin, 1976). Inhibition of gibberellin-inducible -amylase production is a typical activity of ABA (Chrispeels, 1968). A negative correlation between the effects of increasing ABA and seed sensitivity to GA, could prevent early germination (King et al., 1979).

Trinexapac-ethyl, a PGR that disrupts 3ß-hydroxilation of GA20 to GA1 in late stages of GA biosynthesis (Rademacher, 2000; Tan and Qian, 2003), has been shown to reduce the need for nutrients through limiting plant growth (McCullough, 2004). This occurrence can be viewed as comparable to the possible events surrounding the introduction of ABA to the seed.

The presence of excess N in the plant can reduce plant rooting when carbohydrates stored in the roots are used to support increased shoot growth (McCarty, 2005), a phenomena common during AM colonization, as root carbohydrate exudation patterns may be expected to increase due to the AM fungus acting as a considerable C sink (Douds et al., 2000; Graham, 2000). As a result, shoot growth occurs at the expense of the roots, which may lead to other physiological responses such as cell wall thinning, succulent tissue growth, and, most importantly, reduced root carbohydrate levels (McCarty, 2005). This, along with the increased P status of the plant, can cause a restriction in the colonization of AM, through the reduction of possible fungal metabolites, such as soluble carbohydrates and free amino-nitrogen compounds in roots (Jasper et al., 1979; Same, Robson & Abbott, 1983) and in root exudates (Ratnayake, Leonard & Menge, 1978; Graham, Leonard & Menge, 1981). This supports the idea that, in the seeds of *Lolium perenne*, germination (1) speed could be negatively correlated and (2) rates could be positively correlated with increasing levels of (a) exogenous AM hyphal supplied ABA or (b) AM induced carotenoid biosynthesis stimulation of ABA production, causing limitations on seed responsiveness to GA. In turn, early germination in AM inoculated *Lolium perenne* seeds is prevented and initial growth is stunted so that (i) increased efficiency in nutrient use can improve germination and plant success and (ii) proper AM colonization can occur.

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