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Increased abundance of arbuscular mycorrhizal fungi in soil coincides with the reproductive stages of maize

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Abstract

Arbuscular mycorrhizal (AM) fungi are recognized for their positive effects on plant growth, playing an important role in plant P nutrition. We used C16:1*cis*11 and C18:1*cis*11 fatty acid methyl ester (FAME) biomarkers to monitor the dynamics of AM fungi during the reproductive stages of maize (*Zea mays* L.) grown at high yield in Nebraska, USA. Two fields with four different levels of P availability were sampled throughout the reproductive stages. Chambers, made of PVC enclosed mesh fabric to allow passage of roots and hyphae (+R) or hyphae alone (−R) and amended with either KH_2PO_4 (+P) or distilled water (−P), were installed in the field at tasselling and removed after three, six and nine weeks. Our objectives were (i) to provide evidence for C allocation to AM fungi during the reproductive stages of high productivity maize and (ii) to link AM fungal growth dynamics with changes in soil P availability. We observed that initial AM FAME concentration was lower at sites with a high availability of P. During the reproductive growth of maize, AM biomarkers increased inside the chambers and were consistent with the biomarker increase observed in adjacent field soil. This confirms that there is C allocation from the plant to the symbiont during the reproductive stages of maize. We also observed a reduction in available P in +R and −R chambers. This observation implies that hyphae were as efficient as roots and hyphae in reducing the P concentration in chambers. These results demonstrate that AM fungi are active during the reproductive growth stages of maize and may benefit high productivity maize crops by facilitating P uptake.

Keywords: AM fungi; FAMEs; Bray P; Maize (*Zea mays* L.)

1. Introduction

Arbuscular mycorrhizal (AM) fungi form symbiotic relationships with up to 80% of land plants and are also recognized for their positive effects on plant growth and soil quality (Smith and Read, 1997). The extramatrical growth of the mycelium is a key factor in nutrient acquisition by the mycorrhizal symbiont (Olsson et al., 1997). Phosphorous is an essential plant nutrient and, following N, is the second most common fertilizer nutrient applied in crop production. Although P supply during early development has an effect on crop yield potential (Grant et al., 2001), there may also be a requirement for additional P later in crop growth. In maize (*Zea mays* L.), P accumulates steadily until maturity, with a high proportion (approximately 60%) being absorbed during the reproductive period (Karlen et al., 1988). Phosphate transport across the root is usually faster than diffusive transport in soil. This lowers the concentration of phosphate in the soil solution surrounding the root forming a P depletion zone (Barber, 1977). This gap may be bridged by AM fungi.

Several studies document reduced mycorrhizal colonization of plants with increased P availability (Olsson et al., 1997); however, plant P concentration (Koide and Li, 1990) and N supply (Liu et al., 2000) may be more important. Recently, it was shown that AM fungi contribute significantly to P uptake by wheat, even in the presence of added P (Li et al., 2006). In maize, Liu et al. (2000) found a positive correlation between the shoot/root ratio and the degree of AM colonization of roots. The amount of dry matter in

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maize roots remains almost constant shortly after reproductive growth begins at R1 (Ritchie et al., 1997). The below ground dry matter/above ground dry matter ratio is maximum at V10 (61%) and decreases with time, being 25–34% 7 days before R1, 17–20% at R1, and 10–13% at R5 (Plenet, 1995). Thus, maize grown at high yield may be more dependent on AM fungi for nutrient acquisition to support increased above ground biomass.

The uniqueness and utility of fatty acid methyl ester (FAME) biomarkers for AM fungi has been demonstrated by several authors. Larsen et al. (1998) used fatty acid signatures to study mycelial interactions between AM fungi and saprotrophic fungi in root-free soil. The dominant fatty acid for saprotrophic fungi was C18:2*cis*9,12 while it was negligible in mycelium of *Glomus intraradices*. The fatty acids C16:1*cis*11, C20:4, and C20:5 were found in this AM fungus, but not in the saprotrophic ones. FAME analysis performed on the spores of four AM fungi (*G. coronatum*, *G. mosseae*, *Gigaspora margarita* and *Scutellospora calospora*) showed C16:1*cis*11 to be the dominant fatty acid present (Madan et al., 2002). Furthermore, Olsson and Johansen (2000) found that the amount of phospholipid fatty acid (PLFA) C16:1*cis*11 per unit biomass of two AM species (*G. intraradices* and *G. clarideum*) remained rather constant as the mycelium aged, and its distribution between spores and hyphae was highly consistent making it a suitable biomass indicator. Gryndler et al. (2006) reported significant positive correlations between whole cell extracted C16:1*cis*11 and AM hyphal lengths in field soils. van Aarle and Olsson (2003) observed that NLFA and PLFA were similarly correlated with AM % root colonization of *Plantago lanceolata* L in monoxenic cultures. In soils, NLFA C16:1*cis*11 may be a more sensitive indicator of AM fungal biomass due to high background concentrations of PLFA C16:1*cis*11 (Olsson, 1999).

The relative amount of C16:1*cis*11 in roots may provide an index of the amount of C allocated for fungal growth and lipid storage in the root during the colonization process (Graham et al., 1995). We used this approach to understand the dynamics of mycorrhizal fungi during the reproductive stages of high productivity maize. Management systems that enhance natural mechanisms for P acquisition will help to optimize the use of P fertilizer resources. We hypothesize that AM fungi are essential to meet the demand for P during the reproductive stages of maize managed for high yield. Our objectives were (i) to provide evidence for C allocation to AM fungi during the reproductive stages of high productivity maize and (ii) to link AM fungal growth dynamics with changes in soil P availability.

2. Materials and Methods

2.1. Study area

The field study was conducted during 2004 near Shelton NE (40°45′01″N, 98°46′01″W) and Lincoln, NE (40°49′12″N, 95°39′W). Soils at Shelton are Hord silt loam (Fine-silty, mixed mesic Pachic Argiustoll, 0–1% slope) and Blendon loam (Coarse-loamy, mixed mesic Pachic Haplustoll, 0–1% slope). Continuous maize has been cropped since 1990 under conventional disk tillage with furrow irrigation, and since 1996 under reduced tillage (disk and field cultivator) with sprinkler irrigation. Prior to planting, 168 kg N ha^{-1} as urea ammonium nitrate solution was incorporated with a field cultivator. Maize (Pioneer 33B51) was planted on 2 May, 2004 with 19.5 kg P ha^{-1}, and 13.1 kg N ha^{-1} as diammonium phosphate applied adjacent to the seed. Stand density was 71,600 plants ha^{-1} and field average yield was 12.2 Mg ha^{-1} at 155 g kg^{-1} moisture content.

In May 2003, apparent electrical conductivity (ECa) was measured with an EM 38 dual dipole conductance meter (Geonics Ltd., Mississauga, Ont., Canada) pulled behind an all terrain vehicle (Grigera et al., 2006). The data collected were processed using ERDAS Imagine (ERDAS Inc., Atlanta, GA) to create four ECa classes based on ranges of ECa measurements using unsupervised classification (ERDAS, 1997) (Johnson et al., 2001). Six sites in ECa classes II and IV were randomly selected for soil sampling and to study mycorrhizal dynamics.

Soil at Lincoln is a deep Kennebec silty clay loam (Fine-silty, mixed, superactive, mesic Cumulic Hapludolls 0–1% slope). Continuous maize has been cropped since 1999 under conventional tillage with sprinkler irrigation. The field was plowed in the fall using a Salford conservation tillage plow (Salford Farm Machinery Limited, Salford, ON, Canada) (about 25–30 cm deep), and field cultivated before planting. The experiment was arranged in a randomized complete block design with four replicates. Two levels of fertilizer-nutrient management were applied: recommended (M1) and intensive (M2). For M1, a total of 200 kg N ha^{-1} as ammonium nitrate was applied: 100 kg N ha^{-1} incorporated with a field cultivator prior to planting, and 100 kg N ha^{-1} at V6. For M2, a total of 280 kg ha^{-1} of N was applied as follows: 50 kg N ha^{-1} in October 2003 as UAN on crop residue (before plowing), 80 kg ha^{-1} as ammonium nitrate prior to planting, 60, 50 and 40 kg N ha^{-1} as ammonium nitrate at V6, V10 and V14, respectively. In addition, 45 kg ha^{-1} P as single super phosphate and 85 kg ha^{-1} K as KCl were broadcasted before planting and incorporated. Maize (Pioneer 31N28) was planted on 12 May, 2004. Stand density was 74 000 plants ha^{-1}, and field average yield for M1 was 15.5 Mg ha^{-1} and for M2 was 15.6 Mg ha^{-1} at 155 g kg^{-1} moisture content.

2.2. Preparation and installation of soil chambers

Bulk soil to fill the chambers was collected from the 0- to 15-cm depth on 22 June from two sites each in ECa II and ECa IV at Shelton and M1 and M2 at Lincoln. Field moist soil was passed through a 6 mm mesh sieve to remove plant residues and stored at 4 °C until chambers were prepared.