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Influence of Pulse Crops on Abundance of Arbuscular Mycorrhizal Fungi in a Durum-Based Cropping System

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Abstract

Pulses are an important component in crop rotations in southern Saskatchewan. Besides their capability to fix nitrogen, pulse crops establish a strong symbiotic relationship with arbuscular mycorrhizal (AM) fungi, which have been shown to increase nutrient and water uptake through hyphal extensions in the soil. Incorporating strongly mycorrhizal crops in a rotation may increase inoculum levels in the soil and benefit the growth of a subsequent crop. The objective of this study was to determine if AMF colonization of a durum crop is significantly affected by cropping history and to assess the impact of pulses in crop rotations on the abundance of AMF communities in the soil. In 2004 and 2005, soil and root samples were taken on durum with preceding crops of chickpea, pea, lentil, canola, and durum. Arbuscular mycorrhizal colonization was significantly lower in durum roots following canola in both years. Phospholipid fatty acid analysis (PLFA) was completed to analyse the relative abundance of AMF, saprophytic fungi, and bacteria in the soil. These results demonstrated that although previous crop may play a role in microbial community structure, it is not the only influencing factor.

Introduction

Conservation tillage in the brown soil zone of Saskatchewan typically includes a combination of cereals, pulses, and oilseeds in the rotation cycle. Pulse crops form a symbiotic relationship with arbuscular mycorrhizal fungi, helping the plants take up nutrients and water while the plant supplies the fungi with carbon. Arbuscular mycorrhizal fungi can increase uptake through hyphal extensions in the soil. The fungi work by colonizing the root and then extending hyphae out into the soil. Hyphae have the ability to increase soil-root contact, increase exploration in micropores, extract water, and improve water holding capacity (Auge et al., 2003).

The function and abundance of AMF are influenced by a combination of environmental and plant factors. The environmental factors include the soil type, temperature, pH,

moisture, and dissolved nutrients. The plant factors include the species, age, and biomass of the particular plant (Bever et al., 2001). Crop rotation encourages diversity of the AM fungal community (Douds et al., 1993).

Crops that are highly mycorrhizal dependent (i.e. pulses) have shown substantial differences in dry weight of roots and shoots, root length and overall colonization, and P uptake per plot in inoculated plants compared to non-inoculated plants (Shibata and Yano, 2003). Non-host plants (i.e. canola) delay mycorrhizal colonization of subsequent crops in a rotation, presumably a result in the reduction of mycorrhizal propagules in the soil (Gavito 1998).

Pulse crops are important when considering symbiotic plant-microbial interactions because roots are colonized by arbuscular mycorrhizal fungi and nitrogen-fixing rhizobia, both important soil microorganisms (Perotto et al., 1994). There is currently an incomplete understanding of how including pulses in a cropping rotation affects the microbial community. In order to manage AMF in agriculture, an understanding of the interactions existing between crops, AMF, and environmental factors (includes soil factors) must exist.

Arbuscular mycorrhizal fungal abundance may be measured in plant roots and soil. Fatty acid methyl ester (FAME) analysis is one method of examining the size, nature, and physiological state of the soil microbial community. Phospholipid fatty acids (PLFA) are valuable indicators of the active soil microbial community because they are structurally diverse, highly biologically specific, and are able to characterize living biomass and changes in the microbial community (Vestal and White, 1989). Phospholipids are not found in storage products and are rapidly released after cell death and quickly metabolized, resulting in the sole measurement of living or active biomass. Phospholipid fatty acids measurements represent the living or active biomass while PLFA profiles can be used to characterize soil microbial community structure. The marker commonly used as fungal biomass indicator is C18:2ω6, while C16:1ω5 is the AMF indicator.

Materials and Methods

Site Description

The experiment was a two year field plot study (2004 and 2005), conducted at the South Farm of the Semiarid Prairie Agricultural Research Centre, in Swift Current. It was located in the Brown soil zone on a Swinton Silt Loam. Three repetitions of five treatments are compared i.e., the inclusion of *Pisum sativum* L. (pea), *Lens culinaris* Medik (lentil), *Cicer arientinum* L. (chickpea), *Brassica napus* L. (canola) or *Triticum turgidum* L. (durum) at stage II of 3-year rotations with fallow at stage I and durum at stage III (Table 1), in 5 x 24 m plots[1].

Crops were fertilized each year to equalize soil fertility among treatments, as determined by soil tests. For durum plots the total N level is equalized to 65 lbs/ac. This is

[1] Durum following durum was grown in 15 x 24 m plots

calculated as 65 minus amount of N in top 24 inches as determined by fall soil sampling. Phosphorous (11-51-0) is routinely applied with seed at approximately 40lbs/ac.

Table 1. Crop varieties used in experiment were common varieties.

Rotation†	Variety	Inoculant
Fallow-**lentil**-durum	Sovereign	Nitragin C
Fallow-**pea**-durum	Handel	Nitragin C
Fallow-**chickpea**-durum	Myles	Nitragin GC
Fallow-**durum**-durum	Avonlea	n/a
Fallow-**canola**-durum	Argentine	n/a

† Crops in bold were preceding crops of interest and correspond to the indicated variety. All sampling is completed in the durum phase of the rotation.

Soil samples and root samples were taken at intermediate physiological stages (between emergence, five-leaf, flag-leaf, anthesis, and physiological maturity) as predicted using the degree-day model of AC Avonlea crop development (Hong Wong, manuscript in preparation). Soil samples were taken using a hand sampler (r=2.5 cm) to a depth of 7.5 cm. The samples were bulked into one composite sample and put through 2 mm sieves. Part of the soil was placed at -12°C before fatty acid methyl ester (FAME) analysis, and the rest of the soil was air dried and stored at 4°C. Two plants were taken at four locations per plot using a trowel. Roots and shoots were separated.

Root Colonization

Roots were washed thoroughly after sampling to remove any adhering soil, while placed over two mm sieves to minimize fine root loss. The durum roots were then cut into 1-cm fragments and 2 replicates from each plot were placed in plastic cassettes. The roots were then cleared and stained using an ink vinegar solution as described by Vierheilig et al. (1998). The percent root colonization for each plot was determined using the gridline-intersect method from Giovanetti and Mosse (1980).

Fatty Acid Methyl Esters (FAME) Analysis

The soil microbial structures were determined through analyses of phospholipids fatty acids (PFLA). Lipids were extracted from the soil and separated into fractions using silica gel filled columns. The fatty acids of the phospholipids fractions were transmethylated and analyzed by gas chromatography on a VARIAN 3900 GC with the Star Chromatography software using a procedure from Clapperton et al. 2005. The Supelco bacterial mix standards was used as an indicator for the bacterial communities, linoleic acid (C18:2ω6) as indicator for fungi, and C16:1ω5 as indicator for AMF (Balser et al., 2005).

Statistical Analysis

Field plots were replicated three times using a randomized split-plot design where precrop species form the main plots and time, the subplots. The effects of preceding crops on AMF root colonization and soil microbial communities variables were assessed

using ANOVA and least significant difference using JMP v. 3.2.6. All sampling and statistical analysis was completed in 2004 and 2005.

Results and Discussion

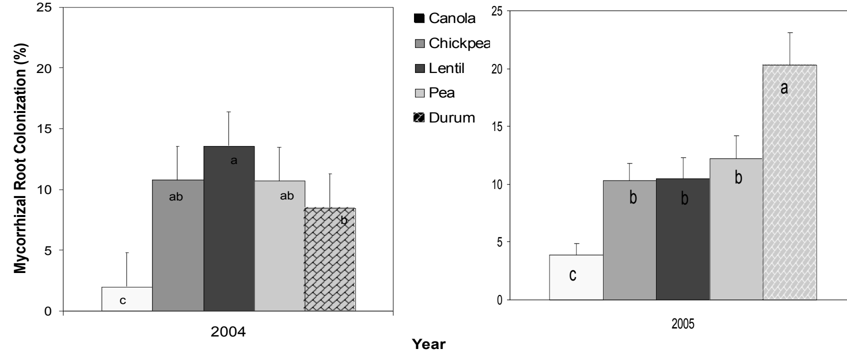

Figure 1. Mean mycorrhizal colonization in durum as influenced by previous crops over the growing seasons of 2004 and 2005. Means with the same letter are not different within each year (LSD *P*=0.05, *N*=60).

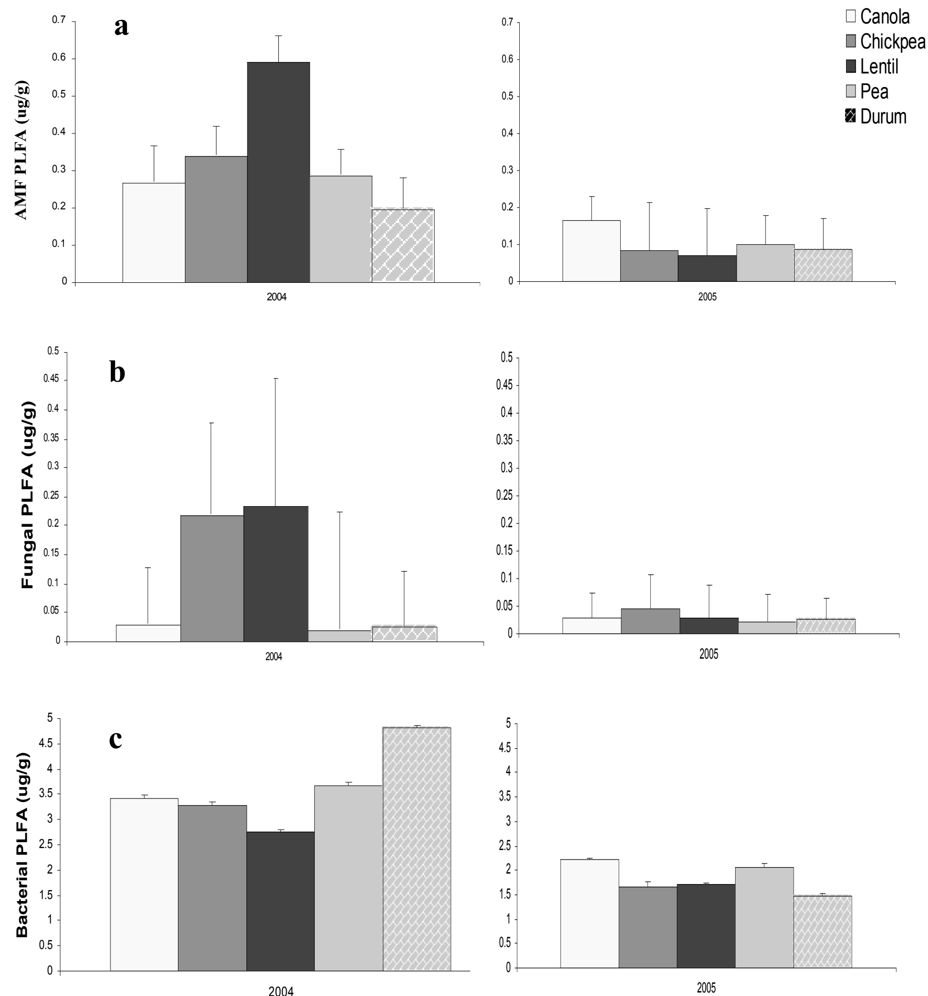

Figure 2. Variation in microbial abundance as evaluated with FAME indicators in the PLFA fractions of soil extracted lipids. Relative abundance of the means of (a) arbuscular mycorrhizal fungi; (b) saprotrophic fungi; and (c) bacteria. Standard errors of means are displayed by error bars (*N*=60).

There was extensive mycorrhizal colonization of durum roots after lentil, pea, chickpea and durum (Figure 1). However after canola, a non-mycorrhizal species, durum root mycorrhizal colonization was delayed. In 2005, AMF colonization on a previous durum crop was significantly higher than all other crops.

In 2004, previous crops of pulses resulted in higher levels of C16:1ω5 as measured by FAME analysis (Figure 2a). In 2005, the highest levels of C16:1ω5 were found in soil cropped to durum following canola. Although previous crop may play a role in soil microbial community structure, it is not the only factor as shown by the differences in PLFA abundance between 2004 and 2005 (Figure 2abc). Durum after non-mycorrhizal canola had the lowest levels of root colonization, but this is not reflected by the soil microbial indicator for mycorrhizal fungi as measured by FAME analysis.

Continuing Research

AMF populations are being investigated to determine if a correlation exists with plant N and P uptake, plant biomass, yield and soil temperature and moisture. Also, DNA analysis using polymerase chain reactions (PCR) and denaturing gradient gel electrophoresis (DGGE) is being used to identify particular species of AMF in the roots and soil.

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