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## Influence of Pulse Crops on 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 symbiotic relationship with arbuscular mycorrhizal fungi, which have been shown to increase nutrient and water uptake through hyphal extensions in the soil. This 2 year study is designed to evaluate the impact of pulses in crop rotations on the biodiversity of AMF communities and their dynamics. Plant N and P uptake and the available soil N and P pools under a durum crop are also measured to assess if there is any correlation with AMF communities. The sampling and analysis is completed on the durum phase of the rotation with preceding crops of pea, lentil, chickpea, canola and durum. The final results will be explained by: (1) the size, nature, and physiological state of the soil microbial community and (2) the nature of the preceding crop residues. Preliminary results from the 2004 season indicate that higher plant P uptake is related to AMF colonization, while no significant change was detected in the soil N and P pools.

### Introduction

Pulses are an important component in crop rotations in southern Saskatchewan. Conservation tillage in the brown soil zone of Saskatchewan typically includes a combination of cereals, pulses, and oilseeds in the cycle. The benefits that pulse crops contribute to a rotation are not completely understood. Previously benefits were mostly attributed to their ability to fix N, but the non-N benefits are not well understood (Stevenson and van Kessel 1996). Besides their capability to fix nitrogen, pulse crops establish a symbiotic relationship with arbuscular mycorrhizal fungi (AMF).

Arbuscular mycorrhizal fungi help the plants take up nutrients and water, while the plant supplies the fungi with carbon derived from photosynthesis. AM 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 extraradical hyphae provide contact between the AM fungi and the host, thereby contributing to nutrient uptake in the soil (Miller and Jastrow 2000). The fungal hyphae (2-5  $\mu$ m diameter) are smaller than root hairs (10-20  $\mu$ m diameter) so have the ability to penetrate soil pores that are inaccessible to non-mycorrhizal roots (Marulanda et al. 2003). Since water, N and P are often limiting factors for plant growth on the Brown Chernozemic soils in the semiarid region of Saskatchewan, increased levels of AMF in the soil may decrease fertilization and disease management costs. Increased water infiltration, decreased soil erosion, and better soil aeration stemming from improved soil aggregation are other AMF-derived benefits.

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 comprise of the species, age, and biomass of the particular plant (Bever et al. 2001). The soil conditions are constantly changing, in addition fluctuations in temperature and plant growth result in changes in the microbial community. Seasonality also plays a large role in determining the microbial community composition.

Crop rotation encourages diversity of the AM fungi community (Douds et al. 1993). Strongly mycorrhizal crops such as pulses with no till cropping systems promote AMF diversity and increased soil inoculum levels. Crops that are highly mycorrhizal dependent (i.e. legumes) 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 (Shibata and Yano 2003). The use of AM field inoculum is not currently practical (Saito and Marumoto 2002) since it has not always been beneficial and predictable (Rayan and Graham 2002). Production of AMF inoculum is complicated because of an inability to grow in a pure culture and its dependency to be multiplied on living roots (Budi et al. 1998). The best option for increasing inoculum levels is by manipulation using plants promoting AMF symbiosis.

The objectives of this study are to: 1) assess the impact of pulses in crop rotations on the biodiversity of AMF communities and their dynamics and 2) measure the plant available N and P pools in soil under a durum crop, and assess any correlation with AMF communities. It is expected that pulse crops will increase the biodiversity and dynamics of AMF communities under a subsequent durum crop and there will be an increase in plant N and P uptake which cannot be explained by changes in the soil N and P pools. The effect of pea, lentil and chickpea on soil biological and biochemical factors will be evaluated through microbial community profiling and examination of nutrient inputs of the preceding crop of pulses, canola, or a subsequent durum wheat crop.

## **Materials and Methods**

The experiment is a two year field plot study (2004 and 2005), conducted at the South Farm of the Semiarid Prairie Agricultural Research Centre, in Swift Current on 5 year old plots. It is located in the Brown soil zone on a Swinton Silt Loam. Three repetitions of

five treatments: pea-durum, lentil-durum, chickpea-durum, canola-durum and durum-durum are being compared (Table 1) in 5 x 24 m plots<sup>1</sup>. The durum monoculture is included in the study since it once represented the best management practice under conventional tillage.

**Table 1.** Crop varieties used in this study are commonly grown in southern Saskatchewan.

<b>Crop</b>	<b>Variety</b>	<b>Inoculant</b>
Durum	cv. Avonlea	n/a
Canola	Argentine	n/a
Lentil	Sovereign	Nitragin C
Pea	Handel	Nitragin C
Chickpea	Myles	Nitragin GC

Crops are 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 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 2.** Variables used as indicators of soil quality in this field experiment and methods used for their evaluation.

<b>Experiment</b>	<b>Sample</b>	<b>Evaluation</b>	<b>Method</b>
Anion Exchange Membranes	Soil	N and P dynamics	Modified from Ziadi et al. 1999
NH <sub>4</sub> /NO <sub>3</sub>	Soil	Point in time available N	KCl extraction (Maynard and Karla, 1993)
Mineralizable N	Soil	N made available over growing season	Incubation extraction (Campbell et al. 1993)
Resin P	Soil	Point in time available P	Hedley extraction procedure (Hedley et al. 1982)
NLFA/PLFA	Soil	Size, nature & physiological state of soil microbial community	Extracting, purifying and analyzing PLFA from soil (Clapperton and Lacey, unpublished)
Biodiversity	Soil	Qualification of AMF population	Polymerase Chain Reaction; Gel Electrophoresis
Inoculum Potential	Soil/Root	AMF potential at beginning of growing season	Infectivity Assay (Brundett 1999)
Root Colonization	Root	Percent of root colonized by AMF	Staining (Vierheilig et al. 1998); Gridline Intersect Method (Giovannetti and Mosse 1980)
Plant N & P	Plant	Nutrient uptake from soil	Digestion of tissue on auto analyzer (Thomas et al. 1967)

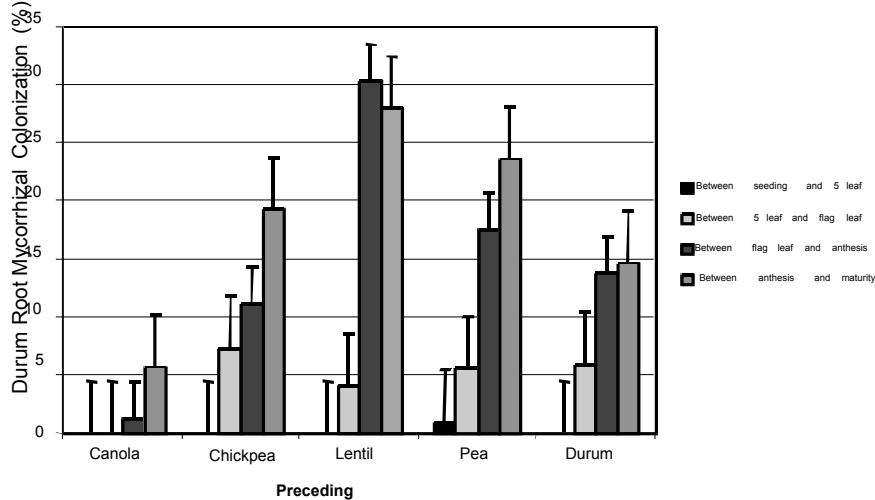
Four soil samples per plot were taken at emergence, five leaf, flag leaf, anthesis, and physiological maturity. The samples were bulked into one composite sample and put through 2 mm sieves before analysis (Table 2). Two plants were taken at four locations per plot and ground before N and P analysis. Soil samples and root samples were also

<sup>1</sup> Durum following durum was grown in 15 x 24 m plots

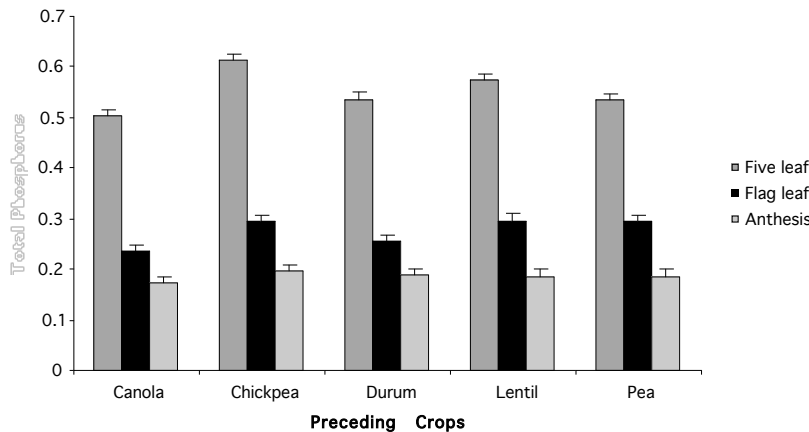
taken at intermediate stages for analysis of phospholipids/neutral lipid fatty acids (PLFA/NLFA), biodiversity, and mycorrhizal root colonization. This sampling scheme will be repeated in 2005.

## Results and Discussion

Soil N and P was not significantly different between the treatments, as determined by  $\text{NH}_4/\text{NO}_3$  and nitrate and phosphate flux measurements (data not shown). The most evident results from the 2004 season were the effect of the treatments on mycorrhizal colonization (Figure 1). Colonization after the pulse crops was high, especially lentil which exhibited the highest colonization between the five leaf and flag leaf stages of development. Using canola as a precrop resulted in no colonization for the first 2 sampling times, with a maximum colonization reaching only 6% between anthesis and maturity. Since canola is a non-mycorrhizal crop, it was expected that AMF development would be delayed in the subsequent durum crop, but the treatment effect did not taper off at the end of the growing season.

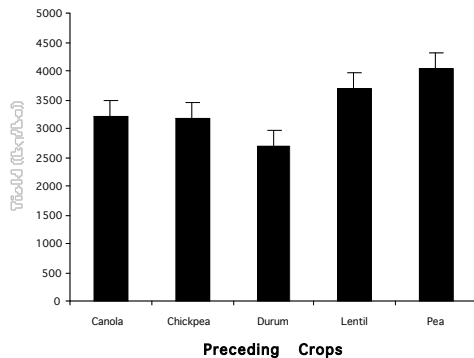


**Figure 1.** Effect of previous crop on mycorrhizal colonization of durum wheat roots. Error bars are standard errors of the means (3 replicates).



**Figure 2.** Effect of previous crop on P concentration of durum wheat. Error bars are standard errors of the means (3 replicates).

The lower colonization levels were also related to lower concentrations of total P in the plants (Figure 2). Durum P uptake was higher after chickpea and lentil and lower after canola and durum. Canola is lower than all others but an interaction ( $P=0.02$ ) showed that differences are greatest at the flag leaf stage and before maturity. The crop preceding durum had no significant difference on plant N uptake (data not shown).



**Figure 3.** Effect of previous crop on grain yield of durum. Error bars are standard errors of the means (3 replicates).

Previous crop was significant ( $P=0.06$ ) after lentil and pea, while the other treatments resulted in lower yields (Figure 3). The 2004 season was unusually wet and cool and the lower durum yields may be partially explained by the large incidence of tan spot resulting from the durum monoculture.

## Conclusions

Increased durum yield following a pulse crop is a result of both the nitrogen and non-N benefits, including AMF symbiosis. The preliminary results of this study indicate that the increased plant P uptake cannot be explained by changes in the soil N and P pools, and are related to increased AMF colonization. The biodiversity of the AMF population and the changes in microbial community structure over the growing season must still be evaluated. The possibility that including pulse crops in a durum-based rotation have a positive influence on the soil microbial populations must still be looked into, as well as repeated sampling and analysis in 2005.

## References

- Auge, R. M., J. L. Moore, K. Cho, J. C. Stutz, D. M. Sylvia, A. K. Al-Agely and A. M. Saxton. 2003. Relating foliar dehydration tolerance of mycorrhizal *Phaseolus vulgaris* to soil and root colonization by hyphae. *Journal of Plant Physiology* 160: 1147-1156.
- Bever, J. D., P. A. Schultz, A. Pringle and J. B. Morton. 2001. Arbuscular Mycorrhizal Fungi: More Diverse than Meets the Eye, and the Ecological Tale of Why. *BioScience* 51(11): 923-932.

- Budi, S. R., J.-P. Caussanel, A. Trouvelot and S. Gianinazzi (1998). The Biotechnology of Mycorrhizas. Microbial Interactions in Agriculture and Forestry. N. S. Subba Rao and Y. R. Dommergues. Enfield, NH, Science Publishers, Inc. 1: 149-160.
- Campbell, C. A., B. H. Ellert and Y. W. Jame (1993). Nitrogen Mineralization Potential in Soils. Soil Sampling and Methods of Analysis. M. R. Carter, Lewis Publishers: 341-349.
- Douds, D. D. J., R. R. Janke and S. E. Peters. 1993. VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. Agriculture, Ecosystems and Environment 43(3-4): 325-335.
- Giovannetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytologist 84: 489-500.
- Hedley, M. J. and J. W. B. Stewart. 1982. Method to measure microbial phosphate in soils. Soil Biology and Biochemistry 14: 377-385.
- Marulanda, A., R. Azcon and J. M. Ruiz-Lozano. 2003. Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. Physiologia Plantarum 119: 526-533.
- Maynard, D.G. and Karla, Y.P. 1993. Nitrate and Exchangeable Ammonium Nitrogen. Soil Sampling and Methods of Analysis – Chapter 4, Canadian Society of Soil Science, Lewis Publishers.
- Miller, R. M. and J. D. Jastrow (2000). Mycorrhizal fungi influence soil structure. Arbuscular Mycorrhizas Physiology and Function. Y. Kapulnik and D. D. Douds. Boston, Kluwer Academic Publishers: 3-18.
- Rayan, M. H. and J. H. Graham. 2002. Is there a role for arbuscular mycorrhizal fungi in production agriculture? Plant and Soil 244: 263-271.
- Saito, M. and T. Marumoto. 2002. Inoculation with arbuscular mycorrhizal fungi: the status quo in Japan and the future prospects. Plant and Soil 244: 273-279.
- Shibata, R. and K. Yano. 2003. Phosphorus acquisition from non-labile sources in peanut and pigeonpea with mycorrhizal interaction. Applied Soil Ecology 24: 133-141.
- Stevenson, F. C. and C. van Kessel. 1996. The nitrogen and non-nitrogen rotation benefits of pea to succeeding crops. Can. J. Plant Science 76: 735-745.
- Thomas, R. L., R. W. Shear and J. R. Moyer. 1967. Comparison of Conventional and Automated Procedures for Nitrogen, Phosphorus, and Potassium Analysis of Plant Material Using a Single Digestion. Agronomy Journal 59: 240-243.
- Vierheilig, H., A. P. Coughlan, U. Wyss and Y. Piche. 1998. Ink and Vinegar, a Simple Staining Technique for Arbuscular-Mycorrhizal Fungi. Applied and Environmental Microbiology 64(12): 5004-5007.
- Ziadi, N., R. R. Simard, G. Allard and J. Lafond. 1999. Field evaluation of anion exchange membranes as a N soil testing method for grasslands. Canadian Journal of Soil Science 79: 281-294.