

AGR1006
Assessment of Arbuscular Mycorrhizal Fungal Inoculants for Pulse Crop Production Systems
INVESTIGATORS
Principal Investigator: Fran Walley, University of Saskatchewan Co-Investigator(s): Jim Germida, University of Saskatchewan
STUDY SPONSORS
Saskatchewan Pulse Growers, NSERC
TYPE OF STUDY
AGRONOMY
OBJECTIVES
<p>This project examined the impact of a commercially available AMF inoculant in pulse crop production systems. Specifically, the objectives of this project were to:</p> <ol style="list-style-type: none"> 1) Assess the growth promotion characteristics (including synergies with rhizobial inoculants and biological nitrogen fixation) of commercially available AMF inoculants as compared to local inoculum sources (i.e., AMF isolated from Saskatchewan soils) 2) Assess the impact of AMF inoculation on populations and colonization success by indigenous AMF species 3) Examine the impact of soil and climate on the persistence of introduced commercial AMF isolates 4) Examine the influence of AMF inoculant rate on growth promotion of pulse crops, and competition with other indigenous AMF, and determine the economic viability of using an AMF inoculant
WHY STUDY NEEDED
<p>Commercially available inoculants containing arbuscular mycorrhizal fungi (AMF) were recently introduced in western Canada. The benefits of AMF are well established; AMF are known to enhance uptake of relatively immobile nutrients, such as phosphorus (P) and are also implicated in improved drought resistance, disease tolerance and nitrogen (N) uptake. Little is known, however, regarding the impact of introduced AMF on the indigenous AMF community, or the efficacy of introduced AMF in terms of enhancing pulse crop yields in field soils with indigenous AMF communities.</p>
HYPOTHESIS

STUDY DESIGN

Growth chamber and field experiments were conducted to determine the efficacy and persistence of an introduced AMF inoculant in pulse crop production, and to examine the impact on the indigenous AMF community. Specifically, inoculation with spores from a commercially available AMF (*Rhizophagus irregularis*, also known as *Glomus intraradices*) and four native AMF isolates, *G. claroideum*, *G. irregularis*, *G. monosporum*, and *G. sp.* (MixG), were compared in terms of plant growth promotion of pea, lentil and chickpea, in both sterile and non-sterile soils. Early experiments also served to develop and assess appropriate molecular technique protocols for use in the studies. Crop response to AMF was assessed and molecular techniques for assessing AMF colonization were refined. Additionally, a growth chamber experiment was conducted to determine the impact of AMF inoculant rate on consequent plant growth promotion of pea. A comparison of growth promotion of pea by AMF versus *P. bilaiae* was conducted in a growth chamber experiment.

Field studies examined the impact of a commercial AMF inoculant on growth promotion characteristics of lentil (two field sites) and field pea (three field sites). Experimental plots were established in spring 2012 and 2013 in commercial farm fields at five different locations in Saskatchewan, including Kelvington and Stewart Valley in 2012; and Stewart Valley, Outlook and Pampbrun in 2013. Sites were under cereal cultivation in the previous years at all locations and seeded to field pea (CDC Meadow) and lentil (CDC Impress). Treatments included an AMF commercial inoculant MYKE®PRO GR (Premier Tech Ltd.) applied at three rates (0, 7.5 and 15 kg/ha) alone and in combination with 16.8 kg P₂O₅/ha as mono ammonium phosphate (11-52-0). Nodulator® (BASF Canada) peat Rhizobium inoculant for pea and lentil was seed placed across all the treatments at the recommended field rate (5.6 kg/ha). Canola (*Brassica napus* cv. Clearfield) was seeded perpendicularly to the experimental plots in both the pea and lentil and served as the non-N fixing reference crop. Plants were harvested by hand at mid-pod fill stage from a 1 m-row length for above-ground biomass and root sampling. At physiological maturity, plants were harvested from three 1m long rows for determination of harvest index. Plots were combined with a small-plot combine for final seed yield. A composite soil sample for each treatment was also collected and stored at 4°C for soil AMF infective propagule assay.

A three-year field incubation study was initiated in 2011 to determine if an introduced AMF inoculant (i.e., non-native) would persist in SK soils and to determine the impact of non-native AMF on the indigenous AMF population. The study was established at four AAFC research stations in Saskatchewan (Scott, Swift Current, Melfort and the Saskatchewan Irrigation Centre at Outlook) in the spring of 2011. The sites represented soils from an environmental gradient, extending from the Brown soil zone, through to the Black soil zone. Intact cores were collected from each site and a subset of each set of cores were transported to all other sites where cores were reinstalled using a completely randomized design stations. Following installation of the cores in 2011, the cores were seeded to pea (CDC Meadow) either treated with a commercial AMF inoculant containing *Rhizophagus irregularis* at the recommended field rate or left untreated. Peas were grown to maturity and aboveground biomass was harvested.

FINDINGS

Experiments revealed that application of an introduced AMF inoculant altered the community composition in colonized roots and rhizosphere soils, indicating that introduced inoculants

can compete effectively with indigenous AMF. The results also indicated that inoculation at rates equivalent to current commercial inoculant recommendations are adequate to ensure successful colonization, even in soils with rich and varied indigenous AMF communities. The impact of AMF on plant growth characteristics was variable, and responses to inoculation generally varied between the host plant, soil, and environment.

Results from growth chamber experiments suggested that dependency on mycorrhiza for growth promotion varied between crops as follows: pea > chickpea > lentil, whereas field experiments failed to detect any seed yield responses in lentil or field pea. Researchers concluded that if the indigenous AMF population is able to meet all plant needs, the addition of AMF spores conferred no further benefits.

Results from growth chamber experiments also showed that a mixed taxa AMF inoculant was superior to single taxon AMF inoculant for promoting field pea growth, N and P uptake, and biological N fixation. These results suggest that development and use of mixed taxa inoculants might be desirable both from ecological and agronomic perspectives, although further research is required.

Field experiments conducted in 2012 and 2013 with field pea and lentil as test crops revealed significant treatment effects and interactions between AMF, rhizobial inoculation and phosphorus fertilizer that resulted in various plant growth benefits. In many instances there was a positive impact of AMF inoculation on nodulation and, in some instances, on N fixation, suggesting a synergy between AMF and rhizobial inoculants. These observations suggest that AMF may enhance both P acquisition and N fixation in pulse crop systems. Although varied plant growth benefits were detected during the growing season, these benefits did not translate to enhanced seed yield for either field pea or lentil, in either year of the field study. When applied at higher than recommended rates, AMF inoculation either did not confer additional benefits, or was associated with negative plant growth responses, suggesting that current application rates are sufficient.

However, it is important to recognize that there are situations where native AMF levels could be low. For example, we know that the number of infective propagules (including spores and hyphal strands) can decline unless a suitable host crop is grown. Consequently, including summer fallow or a non-host crop such as canola or mustard in a rotation could result in lower populations of indigenous AMF. Equally, it might be possible that extended flooding could reduce AMF populations. In these situations in particular, inoculation of AMF could be beneficial. This possibility warrants further investigation.

In conclusion, seed yield responses to AMF inoculation are likely to be variable at best, and dependent on a variety of factors, and the interactions between these factors. It remains clear that P nutrition is important for maximizing pulse crop production goals, although responses to P fertilizer application and biofertilizers intended to enhance P nutrition are variable. Although AMF clearly aid in P uptake, and uptake of P from fertilizer may be enhanced by AMF inoculation, introduced AMF must confer benefits above those provided by the indigenous AMF populations to achieve further benefits in terms of seed yield.

Finally, the persistence of AMF inoculant was examined in a three-year incubation study. Introduced AMF inoculants may persist in soils, although data suggest that persistence of any AMF taxa is controlled by both biotic and abiotic factors. Specifically, although differences in community composition were retained in some soils for as long as three years, AMF communities tended to converge in uninoculated and inoculated soils after three years.

Identifying and understanding the impact of these factors is likely to assist in the development of management strategies to enhance the benefits conferred by introduced and indigenous AMF.

SIGNIFICANCE OF STUDY

The introduction of AMF commercial inoculants provides another tool for producers in nutrient management for pulse crop production. Undoubtedly, AMF are an important group of soil microorganisms that play a critical role in maximizing crop yields. In pulse crop production, the study results suggest that AMF also play a role in enhancing nodulation. In Saskatchewan, soils already contain an abundance of a variety of AMF taxa.

PUBLICATIONS, PRESENTATIONS, EDUCATIONAL MATERIALS PRODUCED

Jin, H., J.J. Germida and F.L. Walley. 2013. Impact of arbuscular mycorrhizal fungal inoculants on subsequent arbuscular mycorrhizal fungi colonization in pot-cultured field pea (*Pisum sativum* L.). *Mycorrhiza* 23:45-59 (DOI 10.1007/s00572-012-0448-9).

Results of this work have been presented (or will be presented) as follows: Biswaray, A., J. Germida, and F. Walley. 2012. The impact of microbial interactions on the success of the tripartite association between pulse crops, rhizobia, and arbuscular mycorrhizal fungi. Ninth Canadian Pulse Researchers Workshop, November 6-8, Niagara Falls, Ont.

Biswaray, A., J. Germida, and F. Walley. 2013. The impact of microbial interactions on the success of the tripartite association between pulse crops, rhizobia, and arbuscular mycorrhizal fungi. Soils and Crops Workshop, March 5-6, Saskatoon, SK.

Islam, Nazrul, J. Germida and F. Walley. 2013. Impact of inoculation with *Glomus irregulare* on colonization and phosphorus uptake by pea (*Pisum sativum*). Canadian Society of Soil Science Annual Meeting, July 23-25, Winnipeg, MB.

Islam N., J. Germida and F. Walley. 2013. Impact of inoculation with *Glomus irregulare* on colonization and seed yield of field pea (*Pisum sativum* L.) as affected by soil and climate. Canadian Soil Science Society (CSSS) Conference: July 22-25, 2013, Front Garry Hotel, Winnipeg, Manitoba, Canada.

Biswaray, A., J. Germida, and F. Walley. 2014. Do interactions between arbuscular mycorrhizal and rhizobial inoculants affect growth, nutrient uptake and yield of peas and lentils? Soils and Crops, March 11-12, Saskatoon. SK.

Biswaray, A., Germida, and Walley, F. 2014. Do interactions between arbuscular mycorrhizal and rhizobial inoculants affect growth, nutrient uptake and yield of peas and lentils? Canadian Society of Soil Science Annual Meeting, May 4-6, Banff, AB.

Islam N., J. Germida and F. Walley. 2014. Commercial mycorrhizal fungi impact yield of pea. Soils and Crops Conference, March 11-12, 2014, Saskatoon, Canada.

Islam N., J. Germida and F. Walley. 2014. Impact of commercial arbuscular mycorrhizal fungi (AMF), *Rhizophagus irregularis* on the diversity, structure and community composition of indigenous AMF across Saskatchewan Prairies. International Union of Microbiological Societies Congresses, July 27 to August 1:2014, Montréal Canada.

Walley, F.L. 2014. Assessment of arbuscular mycorrhizal fungal inoculants for pulse production systems. IFLRC VI & ICLGG VII, July 7-11, Saskatoon, SK.

Wijesinghe, M.A.K., F.L. Walley and J.J. Germida. 2014. Commercial arbuscular mycorrhizal (AM) inoculants change the diversity of AM communities associated with roots and rhizosphere of field pea. IFLRC VI & ICLGG VII, July 7-11, Saskatoon, SK.

Wijesinghe, M. A. K., J. Germida, and F. Walley, 2015. Plant-associated bacterial communities vary with host crop, habitat and soil characteristics, but not with AM inoculation. 19th Annual meeting, Canadian Society for Ecology and Evolution. May 22-25, University of Saskatchewan, SK.

VALUE TO PRODUCERS

This research project generated knowledge that will support further AMF inoculant development. With increasing interest in 'biofertilizers' the knowledge generated in this study will be of interest to pulse crop producers, as well as the AMF industry, which continue to develop new products.