How Mycorrhizal Fungal Bio-Fertilizer Impact on Seed Yield of Field Pea and Wheat across Saskatchewan Prairies

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INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are essential component of agricultural ecosystem functioning and sustainability (Gianinazzi et al., 2006). The AMF form symbiotic associations with plant roots, and involved in plant nutrient uptake and growth, and tolerance to biotic and abiotic stress (Smith and Read, 2008). Commercial AMF inoculation (bio-fertilizer) is a potentially emerging technology for crop production in Canada as well as worldwide. The purpose of inoculation is to enhance the soils inoculum potential to enhance plant productivity and sustainability of agricultural ecosystems.

Mycorrhizal fungi are generally considered mutualistic and there has been little concern over potential negative consequences of their introduction in soils. Nevertheless evidence is growing that mycorrhizal function can range from mutualistic to parasitic (Klironomos, 2003) with host plant communities and edaphic conditions. Inoculation by non-native strains of AM fungi as bio-fertilizer is being promoted without clear evidence for symbiotic effectiveness and fungal persistence in field conditions. Justifiable investment for delivering artificially propagated AM fungal inoculants only has potential while it enhances crop yields and the introduced AMF persist among indigenous mycorrhizal fungal field populations without disturbance over a period of time.

In recent years, Saskatchewan pulse growers have been showing interest in applying commercial non-native AM fungal inoculants without knowing the impact of field inoculation on the indigenous AM fungal community composition. Therefore, a three year long field incubation study was undertaken to investigate the impact of commercial AM fungal strain, *Glomus irregulare* on the

diversity, structure and community compositions of indigenous AMF communities under pulse production system of different eco-zones across Saskatchewan prairies. We also hypothesized that commercial AMF inoculant can persist in soil for several years and will promote root colonization and enhance crop yield. In this conference presentation, we focused on the seed yield of pea and wheat (three consecutive years) and most probable number of infective AMF propagules (1st cropping season, 2011) in response to commercial non-native AMF bio-fertilizer strain, *Glomus irregulare* application in different soils and climates of Saskatchewan Prairies.

MATERIALS AND METHODS

Soil core study

The study was conducted using contained soils exposed to ambient outdoor SK conditions. Open ended aluminum cylinders were used to collect soil cores in the May, 2011. The open ended soil cores (37 cm depth, 20 cm diam.) were extracted from four different field sites representing different SK soil zones namely Swift Current (SC) Brown, Scott (ST) Dark Brown, Outlook (OL) Dark Brown and Melfort (MF) Black Chernozem soils. Replicated soil cores from each site were transported to each of the other locations, where cores were reinstalled to the 20 cm depth using a completely randomized design. A commercially available AM fungal inoculant (Myke-Pro GR-containing active propagules of *Glomus irregulare*) was applied to half (16 cores) of the cores at a rate of equivalent to the application rate in the seed-bed. The rest half of the cores were maintained as control (indigenous AMF populations). Field pea (CDC Meadow) was used as host crop. Seed rate was as per growers' manual (3 seeds per soil core). Rhizobia inoculant (N-Prove) was applied as per company manual (3mL per kg seed). The AMF inoculant, *G. irregulare* (MYKE Pro GR ®) having 110 viable spores-propagules g^{-1} of sample (as company manual), was applied at a rate of 2.40 g per soil core (area of soil core 0.03 sq m).Top 5 cm of soils were removed from each core, spreading the inoculants (2.4 g) onto the surface of the soils and then covered with the remaining soils. Rhizobia inoculated seeds were placed at 4 cm depth. Seeds were covered with remaining soils and added 650 mL of water were applied following seeding to the core surface. The inoculation application and seeding operation was done during June 5 to 7, 2011. In 2012 crop season, wheat (var. CDC GO) was seeded in the aluminum cores. Only urea was applied at recommended dose during pea seeded. In 2013 crop season, Pea (CDC Meadow) was seeded as year 2011. The Myke Pro GR® AMF inoculant was applied only in year 2011 and no inoculant was applied in soil cores in subsequent

years (2012 and 2013). The residual effect of first year applied inoculant on seed yield of 2nd (wheat) and 3rd (pea) was examined.

Most probable number (MPN) assay

Most probable number (MPN) assay was conducted using soil samples, collected during first year at field pea crop harvest-August 2011. A method was used to assay the total number of infective propagules (spores, hyphae, arbuscules) in sample soil. Procedures were performed using a modified dilution–series technique (Porter, 1979). Each dilution had five replicated plants. Low P soil (Brown Chernozem soil collected from Swift Current AAFC research field) and sand (1:1w/w) mixture were used as diluent. It involved a series of ten-fold soil dilutions, using test soil and sterilized soil-sand mix. Sterilization was done twice using a two hour liquid autoclave cycle. Seedlings of pea used as trap plants and grown for 6 weeks in growth chamber under ambient day/night temperature of 24 C/18 C a 16 h day length. The growth substrate is supplemented weekly with P-free Hoagland and Arnon (1950) solution. Plant root systems of all samples were washed with deionized H_2O, blotted dry and aseptically cut into small pieces and cleared in 10% KOH for 1h at 80^0C, acidified with 1% HCl for 15 min, and then stained in an ink-vinegar solution (Vierheilig et al., 1998). The stained roots were spread in a petri dish and scored for the presence or absence of AMF colonization. The MPN of AM fungi in the original sample is calculated by reference to an MPN table (Cochran, 1950). Finally, the total AM fungal infective propagules per gram dry soil was estimated.

RESULTS AND DISCUSSION

The most probable number of AMF infective propagules was significantly higher in all treatment combinations (soils and climates) in response to commercial AMF, *G. irregulare* inoculation (Fig 1). While the SC (Brown) and OL (Dark Brown) soil incubated at Scott (ST) site, the number of infective propagules significantly increased both in inoculated and uninoculated soil.

There are three factors (4 Soils x 4 Sites x 2 Inoculations) were considered in analyzing different variables (MPN-2011 and seed yield- 2011, 2012 and 2013). The climates (sites) only had significant influence on MPN-20111 and three years of seed yield. Other single factors (soils and inoculations)

and interaction of factors had variable effect at different significance level ($P \leq 0.01$ & $P \leq 0.05$) on MPN and seed yield of pea and wheat (2011, 2012 and 2013) (Table 1).

Seed yield of pea, 2011 responses were highly variable (Fig 2), and significant yield increases were achieved from SC (Brown Chernozem) and ST soil however SC soil incubated at OL site and OL soil incubated at ST site also significantly increased seed yield of pea. Significantly variable seed yield of wheat, 2012 was observed particularly in two soils (SC and ST). While SC soil incubated at OL site and ST soil incubated in ST site significantly decreased wheat seed yield. Significantly increased wheat seed yield was achieved from SC soil in SC and ST site and ST soil incubated at SC and OL site. The seed yield of wheat, 2012 had no significant variation in OL and MF soils even soils incubated at other sites. The seed yield of pea, 2013 in any soils and sites had no significant response to residual effect of AMF inoculant (applied in 2011).

The number of infective propagules significantly increased both in inoculated and uninoculated soil. Very high and very low soil phosphorus (P) levels may reduce mycorrhizal infection/colonization (Koide, 1991). The SC soil poor in P levels and OL and MF soil rich in P levels showed lower infectivity than ST (Dark Brown) soil, moderate in P levels (Table 2).There is no increasing pattern as MPN was observed in seed yields in response to commercial *G. irregulare* inoculation. Variable seed yield responses to inoculation likely reflect differences in rainfall and temperature across the sites (Figure 5 & 6). Furthermore, successful AMF symbiosis depends on the interactions of soil properties, climatic factors and indigenous AMF community composition (Maherali and Klironomos, 2007; Herrera-Peraza et al. 2011). The year 2011, AMF inoculation had no influence on pea seed yield at Melfort site. This could be happened due to lower rate of rainfall and higher temperature received in Melfort regions compared to other sites (Figure 5 & 6). The variable responses of seed yield to inoculation might be due to inconsistent pattern of rainfall, temperature across the sites. Furthermore, the AMF symbiosis mostly depends on the compatibility of soil properties, climatic factors and indigenous AMF compositions where inoculant introduced (Herrera-Peraza et al. 2011 and Maherali and Klironomos, 2007).

Table 1: Statistical analysis and level of significance for different treatment factors and variables

Factors	MPN 2011	Pea Seed Yield -2011	Wheat Seed Yield -2012	Pea Seed Yield -2013
Inoculation	**<0.0001**	**0.6304**	**0.0224**	**0.5310**
Soil	<0.0001	0.7825	<0.0001	0.0066
Site (Climates)	<0.0001	< 0.0001	< 0.0001	< 0.0001
Soil x Inoculation	**0.3402**	**0.5412**	**0.8250**	**0.6730**
Site x Inoculation	**0.2889**	**0.8471**	**< 0.0001**	**0.0710**
Soil x Site	0.3684	0.0001	< 0.0001	< 0.0001
Soil x Inoculation x Site	**0.4152**	**0.9067**	**0.0279**	**0.6833**

Table 2. Characteristics of Swift Current (Brown), Scott (Dark Brown), Outlook (Dark Brown) and Melfort (Black) soils of Saskatchewan

Field Sites	Soil types	Organic carbon (%)	Organic matter (%)	Total N (%)	Avail-P (mg/kg)	Avail-K (mg/kg)	Avail-S (mg/kg)	Soil pH
Swift Current	Brown	1.9	3.36	0.16	41.1	327	6.7	6.57
Scott	Dark Brown	1.9	3.31	0.15	59.1	708	3.7	7.01
Outlook	Dark Brown	1.7	2.89	0.17	66.8	228	57.8	7.99
Melfort	Black	5.5	9.43	0.5	34.4	371	7.8	5.94

Fig 1. 2011 MPN of AMF infective propagules (colonization) post harvest affected by inoculation, soils and sites (climates)

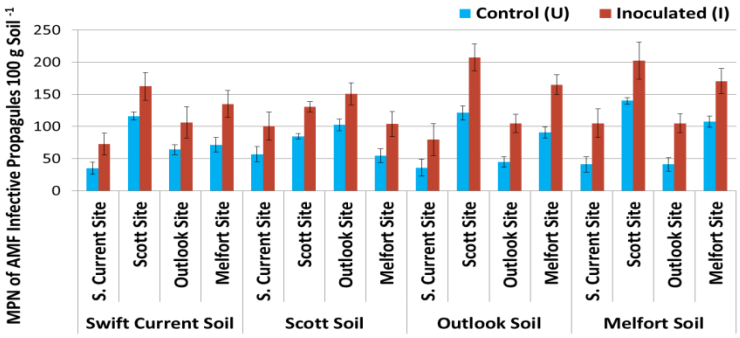


Fig 2. 2011 Pea seed yield affected by inoculation, soils and sites (climates)

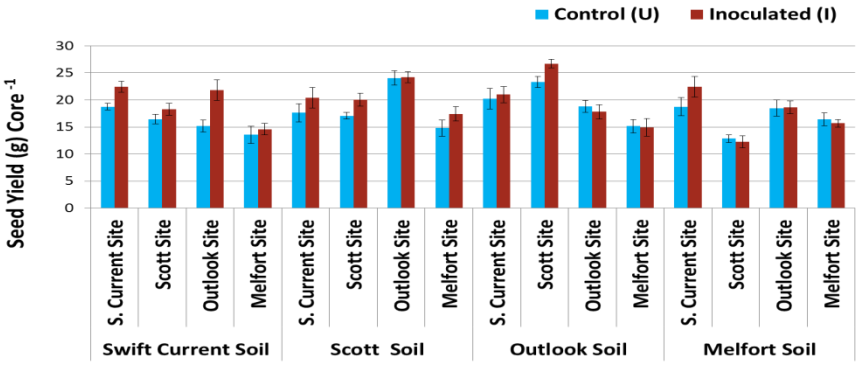


Fig 3. 2012 Wheat seed yield affected by inoculation, soils and sites (climates)

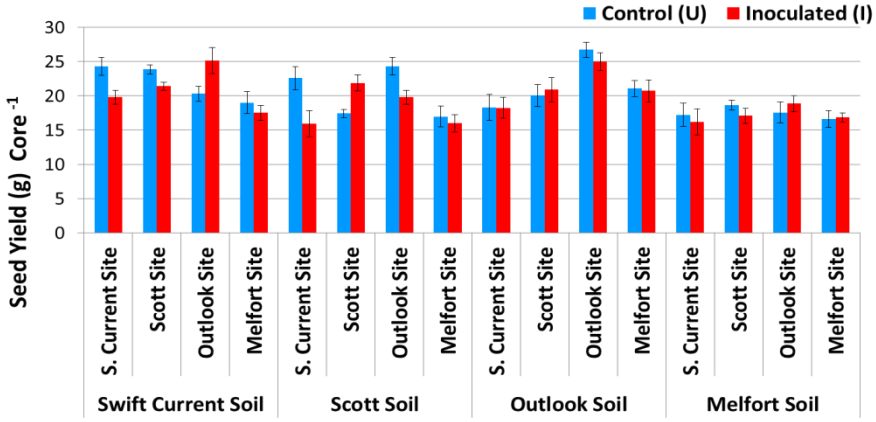
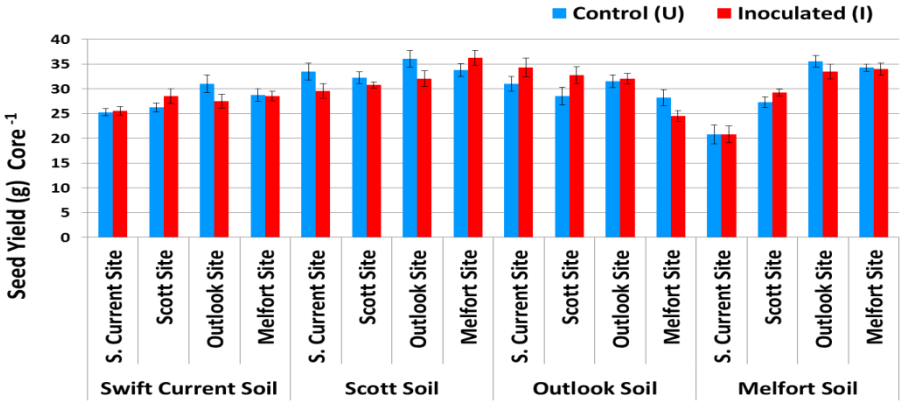
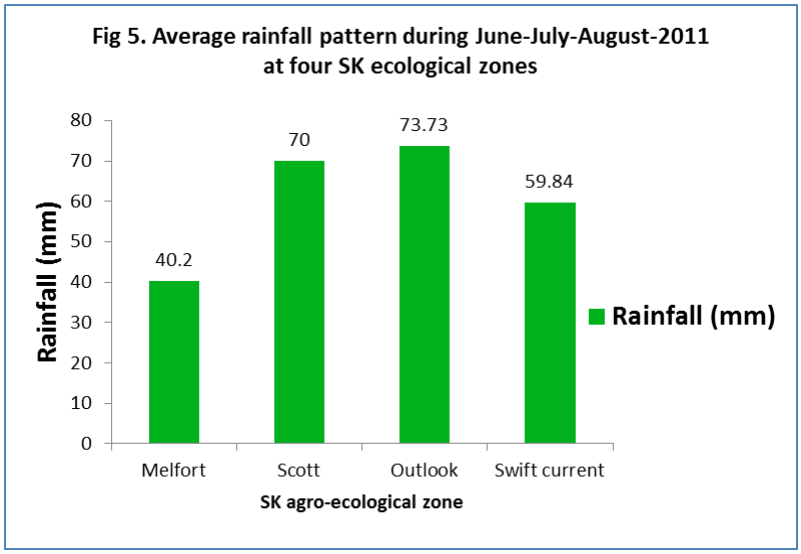
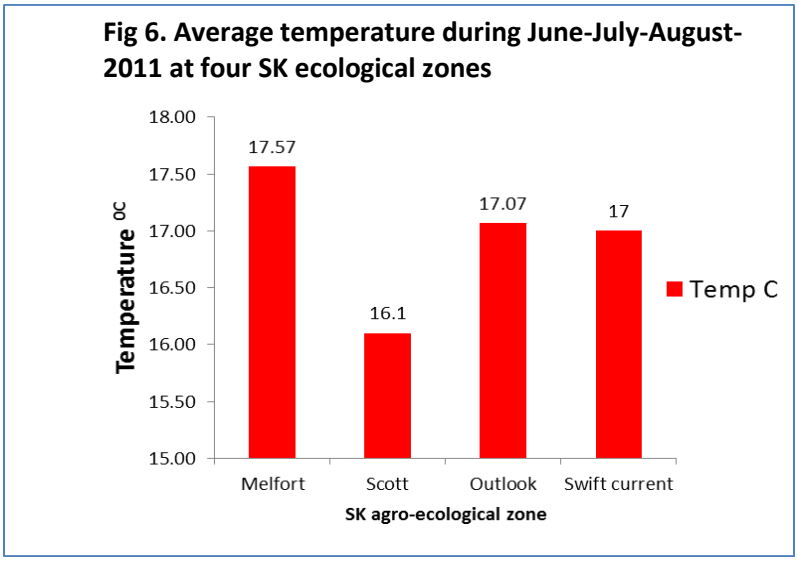


Fig 4. 2013 Pea seed yield affected by inoculation, soils and sites (climate)







CONCLUSION

The commercial AM fungal bio-fertilizer increased number of infective propagules/colonization post-harvest-2011. Yield enhancements at some sites and soils were observed in the first year of the study. Inoculation effects were inconsistent in promoting seed yield in subsequent cropping years. Both climate and soil played important role in yield response. The persistence of the commercial inoculant currently is being investigating using molecular techniques.

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