Impact of In-crop and Soil Residual Herbicides on Effective Nitrogen Fixation in Field Pea (*Pisum sativum* L.) and Chickpea (*Cicer arietinum* L.)

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Abstract

A three-year project was initiated in 2004 to examine the effects of residual herbicides and registered “in-crop” herbicides, both soil and foliar applied, on N fixation and consequent yield of field peas and chickpeas. Inoculation strategies were examined to determine if inoculant formulation (i.e., peat powder versus granular inoculant) influences the degree to which herbicides can affect N fixation. This research is on-going and thus all results are considered preliminary. Preliminary results in field pea, suggest that where herbicides had a negative effect on N fixation, the effects occurred at relatively early growth stages (i.e., soon after herbicide application) and were typically overcome at later growth stages. In addition, granular inoculants were associated with increased N fixation as compared to peat powder inoculants, and may have mitigated any negative herbicide effects. Chickpea incurred damage from the herbicides and all treatments had significantly less N fixation than the control. In general, results suggest that N fixation may be compromised if herbicides cause significant plant damage; however, improved weed control associated with herbicide application may counter the negative impact on early N fixation.

Introduction

Legumes such as field pea and chickpea are high in protein and have high nitrogen (N) requirements that generally are met through a symbiotic relationship with N-fixing rhizobia. However, the amount of N_2 fixed is influenced by several environmental factors that include soil type, nutritional status of soil, crop species and varieties, water availability and temperature as well as soil and crop management (Ledgard and Steele, 1992). In addition, N_2 fixation depends on the ability of the plant to provide photosynthically-fixed carbohydrates to the rhizobial partner (Bergersen, 1982, Mårtensson, 1992; Yoneyama, 2005). Thus, any factor or factors that influence this relationship may negatively impact the N_2-fixing association and consequently the N supply to the plant. For example, the chemicals presently used in agriculture may be upsetting the natural balance in the soil and therefore affecting the symbiotic relationship between N_2-fixers and plants (Flores and Barbachano, 1992).

The common use of herbicides in agriculture may negatively impact N fixation, by either directly affecting rhizobia (Mallik and Tesfai, 1985; Anderson et al., 2004) or indirectly by reducing photosynthate allocation to N_2 fixation (Sprout et al., 1992; Eberbach, 1993; Koopman et al., 1995) or by restricting root growth and hence, the number of root sites available for infection (Eberbach and Douglas, 1991). As well, there is the possibility that herbicides that are persistent in the soil may have a longer lasting impact on rhizobial survival and function (Eberbach and Douglas, 1989; Mårtensson and Nilsson, 1989; Koopman et al., 1995; Eliason et al., 2004).

While considerable research has examined the impact of herbicides in pulse crop production, few studies have been conducted in western Canada using herbicides common on the Canadian prairies. By scrutinizing the impact of herbicides on N_2 fixation, the consequent yield, and the mechanisms by which herbicides may impact nodulation and subsequent nodule occupancy, we can begin to develop effective strategies to minimize the impact of herbicides on the N_2-fixing association.
The objective of this project is to:

1. Assess the impact of in-crop and soil residual herbicides commonly used in pulse crop rotations on nodulation and subsequent N_2 fixation in field pea and chickpea.
2. To compare the use of inoculation types (i.e., granular soil implant versus peat applied) on N_2 fixation by pea subject to herbicide stress and to determine if inoculation type influences the impact of herbicides on N_2 fixation.

Materials and Methods

Site Description for Field Trials

In 2004, two experimental locations were established at Saskatoon (U of Saskatchewan Goodale Research Farm) and at Beaverlodge, Alberta at the Agriculture and Agri-food Research Station. At each location, experiments were established to investigate the impact of herbicides on N_2 fixation by pulse crops. The experiments included: (1) 'residual' soil applied herbicide applications (Table 1) and (2) 'in-crop' herbicide applications (Tables 2 and 3). The 'residual' herbicide experiments received a suite of herbicide treatments applied to wheat (Table 1) in 2004. These plots were then seeded to field peas (cv. Mozart) inoculated with Nitragin™ inoculant in 2005. The 'in-crop' experiments similarly included a variety of herbicides (Table 2) applied to either pea or chickpea (Table 3). In 2005, in addition to these field sites, another field location was added at Clavet, Saskatchewan. As well, the experiment at Beaverlodge also included two types of inoculants, granular and peat powder for in-crop treatments.

Standard field-plot (small plot) experiment techniques were used and plot size reflected the different seeding equipment for each location. Treatments were replicated four times, except at Clavet where they were replicated six times. Treatments were arranged in a randomized complete block design.

Table 1. Soil residual herbicide treatments in Beaverlodge, Ab and Goodale, SK

Treatment	Chemical Name	Rate (g ai/ha)	Timing	Inoculant
1	Clopyralid + Clodinafop-propargyl +Score	660 56 0.8%V/V	Post-emerg	Peat powder
2	clopyralid Clodinafop- + propargyl +Score	660 56 0.8%V/V	Post-emerg	Granular
3	flucarbazone-sodium + Agral 90	20 0.25%V/V	Post-emerg	Peat powder
4	flucarbazone-sodium + Agral 90	20 0.25%V/V	Post-emerg	Granular
5	flucarbazone-sodium + Agral 90	30 0.25%V/V	Post-emerg	Peat powder
6	flucarbazone-sodium + Agral 90	30 0.25%V/V	Post-emerg	Granular
7	sulfosulfuron + Finnish + Merge	20 0.25%V/V 0.5%V/V	Post-emerg	Peat powder
8	sulfosulfuron + Finnish + merge	20 0.25%V/V 0.5%V/V	Post-emerg	Granular
9	check			Peat powder
10	check			Granular
11	Flax check			Peat powder
12	Flax check			Granular

Table 2. Field pea herbicides used at Beaverlodge, AB and Goodale, SK

Treatment	Chemical Name	Rate (g ai/ha)	Timing
1	amitrole	970	Pre-emerg
2	glyphosate	450	Pre-emerg
3	MCPB + MCPA	1700	Post-emerg
4	MCPA	270	Post-emerg
5	imazamox + imazethapyr + Merge	30 + 0.5%V/V	Post-emerg
6	metribuzin	280	Post-emerg
7	bentazon + Cittowet	1080 0.25%V/V	Post-emerg
8	Check (in Goodale sprayed accidentally with bromoxynil		
9	check	Seeded to flax	

Table 3. In-crop herbicide treatments on chickpea

Treatment	Chemical Name	Rate (g/ai/ha)	Timing
1	Metribuzin	205	Post-emerg
2	Metribuzin	410	Post-emerg
3	Sulfentrazone	280	Pre-emerg
4	Isoflutol	105	Pre-emerg
5	Imazethapyr/glyphosate	16.5/450	Post-emerg
6	Hand weeded check		
7	check	Seeded to flax	

Acetylene Reduction Assays

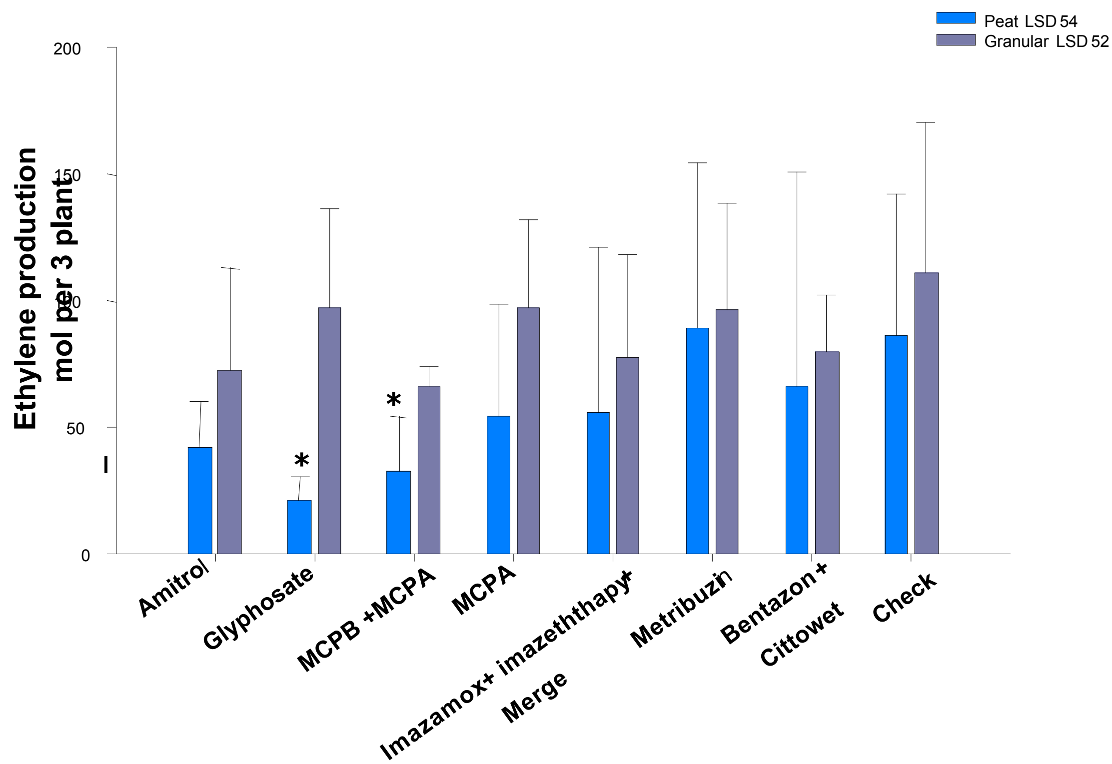
In order to measure the efficacy of the nodules, an acetylene reduction assay (ARA) was conducted (Hardy et al., 1973). For the ARA, three plant samples were removed from the soil and shaken gently. Shoots were cut off and the exposed roots were placed in a 1 L mason jar. One hundred mL of air was removed with a 24 cc syringe and replaced with 100 mL of acetylene (C_2H_2). Jars were buried in the soil and intermittently shaken to maximize nodule exposure to acetylene. Before sampling, the air in the jars was mixed by pumping the syringe 4-5 times to endure a homogeneous mixture of the gases. Ten mL was removed and placed into 12 mL evacuated x-tainers for gas chromatography (GC) analysis. The acetylene reduced to ethylene via the nitrogenase activity was measured using a GC (Hewlett-Packard 5890A). The "in-crop" experiments near Saskatoon were sampled two times throughout the season. The first sampling was done 10 d after herbicide application and the second was taken 20 d after herbicide application.

The second sampling coincided with the flowering. In Beaverlodge, roots were sampled once near the end of July, during flowering and pod-filling.

Biomass and seed yield was determined. Hand-harvested samples were air-dried and threshed and the harvest index was determined. In Beaverlodge and Goodale, the residual plots were harvested using a small plot combine and seed yield was measured and recorded.

Preliminary Results

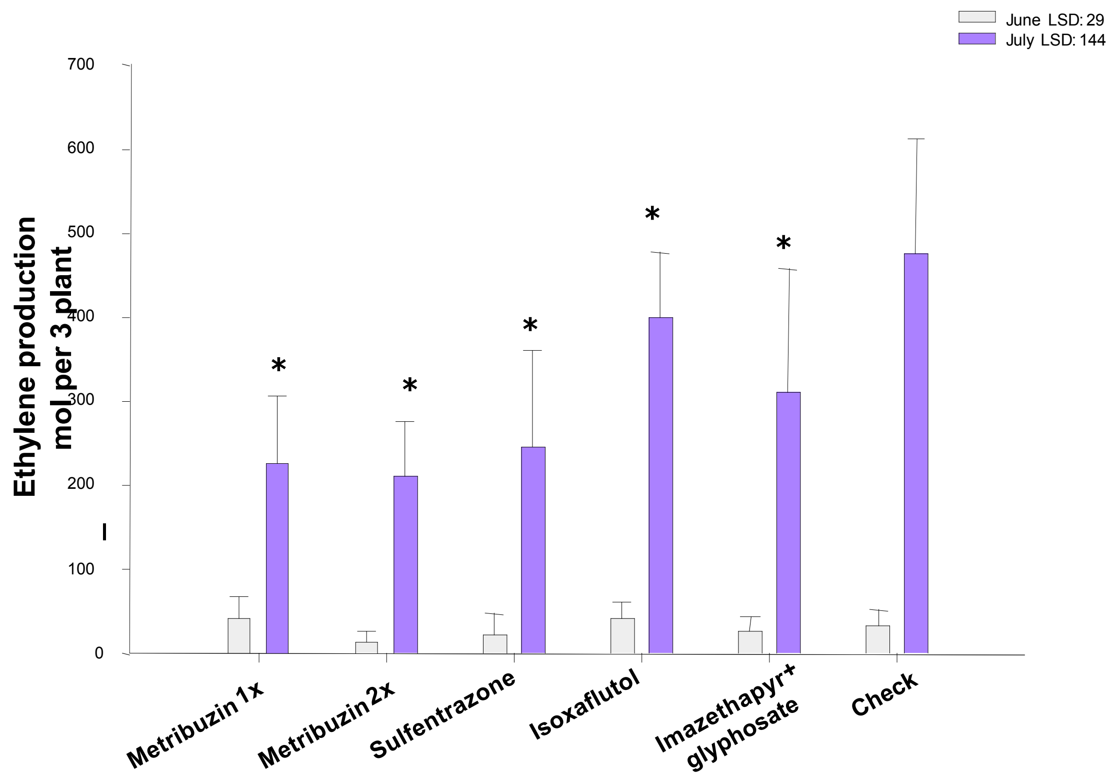
For all "in-crop" field pea trials, any differences in ARA activity that were detected in June were overcome by the July sampling period. Apparently, any damage that the herbicides may have had on the plants, had since been remedied. Typically, the granular treatment was associated with higher nitrogenase activity (Fig. 1).



*denotes a significant difference

Figure 1. Nitrogen fixation activity at Beaverlodge for the "in-crop" herbicide treatments.

For chickpea, only the metribuzin 1x treatment was a registered treatment. The 2x treatment was a “worse case scenario”, while the others were being evaluated for minor use registration. After the initial herbicide treatments, significant herbicide injury was detected (data not shown) at early growth stages which were exacerbated for the metribuzin at later growth stages. Nitrogenase activity, assessed using the ARA, typically reflected overall plant herbicide injury (Figure 2).



*denotes a significant difference

Figure 2. Chickpea ARA assessments

Yield

Few significant differences in final yield were detected (data not shown). These results suggest that although some herbicides may affect N fixation, the benefits of improved weed control may outweigh any negative effects on N fixation. This study is ongoing and further experimentation in 2006 will help clarify the impact of herbicide application on N fixation and yield of field pea and chickpea.

Summary

In field peas, ARA suggest that although there may have been several negative effects on N fixation 10 days after herbicide application, 20 days later, these inhibitions largely were overcome. However, in chickpea, herbicide damage was so severe that by the second sampling period, the plants had not fully recovered and N fixation was significantly less than the check. This included the recommended and registered metribuzin application.

The impact of herbicides on final yield varied between treatments and treatment effects varied between experimental sites. This suggests that the environment may influence herbicide/nitrogen fixation interactions. As well, granular inoculant, which was associated with enhanced fixation, apparently mitigated the impact of the herbicides.

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