HERBICIDE EFFECTS ON PULSE CROP NODULATION AND NITROGEN FIXATION

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Introduction

High protein pulse crops such as field pea and lentil have high nitrogen requirements that typically are met through inoculation with effective nitrogen-fixing rhizobia. The amount of nitrogen supplied by fixation depends not only on the ability of the inoculant rhizobia to fix nitrogen, but also on the ability of the plant to provide energy to the rhizobia in the nodules. Thus, any factor or factors that influence either the rhizobia directly or the ability of the plant to send energy to the nodules, may have a negative impact on nitrogen fixation and ultimately, crop yield. Herbicide application, both in-crop and soil applied, is known to affect plant growth and microbial activity and thus it is possible that some herbicides may influence nitrogen fixation. Our current understanding of the nature and the magnitude of these effects, however, is incomplete and in some instances research results are somewhat contradictory. The uncertainty regarding herbicide-inoculant interactions largely is due to the seemingly inexhaustible combinations of herbicides, crops, crop varieties, *Rhizobium* species and strains, and environments - and the interactions amongst all of these factors. Thus research conducted in different environments might not be directly applicable to conditions encountered in Western Canada. Irrespective of the complexity, current research and literature suggests that we must be aware of the potential for herbicide-inoculant interactions to occur, and this awareness should guide our decisions regarding appropriate inoculant strategies to maximize benefits of the legume-*Rhizobium* association.

Herbicide-Rhizobia Interactions

Although various research studies have demonstrated that some herbicides (at some rates) can influence nitrogen fixation, it is not always clear if the herbicide directly affects rhizobial survival and functioning, or if observed effects are somewhat indirect, i.e., due largely to the affect of the herbicide on general plant growth and development. A number of possibilities exist and have been investigated by researchers worldwide (Table 1) but separating direct and indirect effects of herbicides remains a challenge.

Table 1. Examples of studies examining possible direct and indirect herbicide effects on pulse crop nodulation and nitrogen fixation. In some instances, the studies concluded that there was no impact of the herbicide on the proposed mechanism.

Possible herbicide effects	Reference
Rhizobial growth	Moorman et al. 1992; Anderson et al. 2004
Rhizobial survival	Singh and Wright 1999, 2002
Rhizobial recognition of host plant	Fox et al. 2001; 2004
Nodule formation/root hair deformation	Mårtensson 1992; Musarrat and Haseeb 2000; Singh and Wright 1999, 2002
Nitrogenase (nitrogen fixation) activity	Mårtensson 1992; Anderson et al. 2004
Reduced plant growth and photosynthate supply to nodules; reduced root biomass and thus reduced infection sites	Rennie and Dubetz 1984; Bertholet and Clark 1985; Sprout et al. 1992, Vidal et al. 1992; Abd-Alla et al. 2000; Singh and Wright 1999, 2002; Gupta et al. 2002

For example, Singh and Wright (1999, 2002) examined the impact of terbutryn/terbuthylazine, trietazene/simazine, prometryn (pre-emergence) and bentazon (post-emergence) on growth, nodulation and nitrogen fixation of pea in a growth chamber study. They reported that all herbicides decreased nodulation, and total root and shoot production. Moreover, herbicide application reduced leaf area and thus total photosynthate supply to the nodules, ultimately reducing total nitrogen fixed. Interestingly, however, the activity of a critical enzyme in the root nodules, called "nitrogenase", remained unaffected by herbicide application. These results suggested that the reduction in fixed nitrogen was not related to the direct impact on critical biochemical processes within the nodules - rather, it was suggested that the herbicide effect was due simply to reduced plant growth. When they examined the impact of the various herbicides on rhizobial growth under controlled laboratory conditions, they concluded that the herbicides "did not adversely affect the growth of rhizobia at the concentrations expected to be normally experienced by the rhizobia under field conditions" (Singh and Wright 2002). Moreover "…decreased growth of herbicide treated plants was due to direct effects of herbicide on peas and not due to indirect effects of herbicides on rhizobia".

Although many studies similarly have concluded that herbicides affect nitrogen fixation largely via indirect affects on plant growth and consequent availability of photosynthate to the root nodules (e.g., Rennie and Dubetz, 1984; Bertholet and Clark 1985; Sprout et al. 1992; Vidal et al. 1992; Abd-Alla et al. 2000), there is evidence that some pesticides might impair the ability of the rhizobia to recognize appropriate host plants. For example, Fox et al. (2004) reported that some pesticides can mimic naturally occurring biochemicals and thereby interfere with various biochemical signaling processes between rhizobia and appropriate host plants. As a consequence, early nodulation events can be disrupted. However, according to their research, not all pesticides had a negative impact on nodulation and the degree to which nodulation was inhibited was dependant on pesticide concentrations.

In some instances, results from various studies have been contradictory. For example, when examining the effects of chlorsulfuron under laboratory conditions, Anderson et al. (2004) observed that even at rates equivalent to two times field rates, chlorsulfuron did not influence rhizobial growth. However, although rhizobial growth was not influenced, the subsequent ability of these rhizobia to form nodules was reduced. Thus, they reported that when rhizobia were pre-exposed to relatively high levels of chlorsulfuron, subsequent nodule size and total nitrogen fixation was reduced. In contrast, Mårtensson (1992) reported that nodulation ability was unaffected by previous exposure to chlorsulfuron. These contrasting results suggest that the impact of various herbicides on specific nodulation events may be highly dependent on specific environmental conditions, including different soil characteristics (i.e., pH, organic matter, moisture, etc.) and weather conditions.

Mårtensson (1992) examined the impact of various herbicides on root hair formation. Rhizobia infect plant roots through root hairs and thus it was hypothesized that herbicides affecting root hair development might interfere with nodulation. He reported that some herbicides , including glyphosate, caused root hair deformations that apparently resulted in fewer nodules being formed. It is important to note, however, that this was a laboratory study and consequently the herbicide rates used in these experiments were not necessarily similar to rates that would be encountered in soils under field conditions. Thus, although the research demonstrates the possibility for herbicides to affect nodulation via root hair deformations, it is not known if this phenomenon occurs under field conditions.

Working in Australia, Gupta et al. (2002) reported that application of flumetsulam caused shoot yellowing and reduced plant growth. They also observed a reduction in effective nodule numbers on six-week pea roots when evaluated 10-d after herbicide application. Furthermore, they observed a partial recovery in the appearance of the plants four to five weeks after herbicide application, apparently because new nodules formed on new roots. Their observations led them to conclude that herbicide application can result in substantial loss of nodules from the roots, likely due to the herbicide-induced stress on the plant-Rhizobium symbiosis. These results are consistent with the idea that any stress, whether induced by chemical applications or by natural causes such as drought, intense heat, flooding, hail or insect damage, can have a negative impact on nitrogen fixation by limiting the ability of the plant to support a healthy relationship with the rhizobia housed in root nodules. Thus, when assessing the potential for any chemical treatment to have an impact on nitrogen fixation, our first question likely should be "Is this having a negative effect on the plant?" Because nodules depend on the host plant for an energy supply (in the form of plant photosynthates), striving to maintain healthy, actively growing plants is the first step in achieving optimal levels of nitrogen fixation.

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