Effect of Trifluralin on Growth, Nodulation, and Nitrogen Fixation of Peas

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INTRODUCTION

A variety of herbicides are used in Saskatchewan in order to protect crops and increase yields. This is of particular importance when herbicides are used with legume crops because these chemicals may not only control plant growth, but may also affect the Rhizobium-legume interaction or the population of rhizobia in the soil. The consequences of herbicides affecting such non-target systems might result in a decrease in nitrogen fixation and thus affect soil fertility. Soil-incorporated herbicides such as trifluralin should be of special concern as they contact the root system where nodulation and nitrogen fixation processes occur.

Our previous studies (Germida et al., 1983; Olson et al., in press) have shown that the soil incorporated herbicide trifluralin does not significantly affect soil microorganisms or decomposition processes in situ in soil; however, excessive rates (i.e., > 25,000 ppm) did affect the growth of soil microorganisms, including rhizobia, as pure cultures on laboratory media. Investigations by other workers (Brock, 1972; Dunigan et al., 1972; Kurst and Struckmeyer, 1971; DeRosa et al., 1978) have shown that trifluralin causes a reduction in the nodulation of legumes such as soybean and clover. A limited study by Harvey and Gritton (1977) has examined the effects of this herbicide on the growth of two pea cultivars. With this in mind, the objective of the present study was to investigate the effects of trifluralin on the growth, nodulation, and nitrogen fixation of pea plants grown in a greenhouse or in the field.

MATERIALS AND METHODS

Growth Chamber Study

Two separate experiments were conducted using a Dark Brown Chernozemic soil (Bradwell loam). Basically, pea seeds were subjected to three types of inoculation (see below) and then seeded into soil treated with four rates of trifluralin (see below). Each experiment consisted of 4 replicates per treatment in a completely randomized block design.

The characteristics of the Bradwell loam have been described previously (Germida et al., 1983). For herbicide incorporation, 1560 g of air-dried, sieved (2 mm) soil received 140 ml of distilled water containing a commercial preparation of trifluralin (Treflan EC) sufficient to provide the appropriate soil concentration (0, 1 ppm, 2 ppm, 3 ppm), 100 ml of a nutrient stock solution (0.0121 M K_2HPO_4 and 0.0047 M K_2SO_4) and then mixed in a large polyethylene bag. The control soil was treated with the appropriate amount of nutrient solution and distilled water. All treated soils were allowed to equilibrate for 24 h before potting in 6" plastic pots.

Pisum sativum L. (var. Homesteader) seeds were surface sterilized in a 20% javex bleach solution for 5 min and then rinsed in sterile distilled water 7 times. One set of seeds was treated with a commercial inoculum (Nitragin Co.) according to the instructions. Another set was inoculated (0.5 ml/seed) with an unwashed 72 h broth culture of Rhizobium leguminosarum strain 128C52. This rhizobium strain was grown in 50 ml of YEM broth (Vincent, 1970) on a gyrotory shaker at 27C. Control seeds were surface sterilized but received no inoculum. Initially, ten seeds were planted in each pot (3/4" depth) and the soil covered with styrofoam beads (4 mm dia.) to protect against water loss; after germination the number of seedlings was thinned to three. All pots were placed in a growth chamber with controlled temperature, lighting, and humidity as described previously (Germida et al., 1983). All pots were weighed and the soil maintained at field moisture-holding capacity (about 20% moisture) during the 5-week growth period.

At harvest, the plants and soil were dumped from the pot and the bulk soil carefully removed to prevent excessive root loss. The shoot and root systems were separated. Shoot material was oven dried (65 C), weighed, and analyzed for total nitrogen by the Kjeldahl procedure. Root systems and nodules were subjected to the nitrogenase assay in order to measure their nitrogen fixation potential. For this assay, three unwashed root segments (and clinging soil) were immediately placed in 455 ml glass mason jars with lids fitted with rubber septums. Twenty-five cc of the atmosphere was replaced with C_2H_2, and duplicate samples of the head space were collected after 30 min for determination of C_2H_4 as described previously (Nelson and Child, 1981). Samples (0.2 cc) were analyzed on a Hewlett Packard 5730A gas chromatograph fitted with a 1.5 m x 3 mm column of Poropak R (column temp was 90 C) using N_2 carrier gas and a H_2 flame ionization detector at 200 C. Peak heights were measured using a Hewlett-Packard 3373B integrator. After analysis, roots were recovered, washed, number of nodules counted and root fresh weight and tap root measurements taken.

Field Study

In order to assess the effects of trifluralin on peas grown under field conditions, a preliminary experiment was set up on a Dark Brown Chernozemic soil (Sutherland clay loam) at the University of Saskatchewan Kernen Farm (for soil characteristics see Germida et al., 1983). This study consisted of a complete randomized block with split plot design on a 10 M x 45 M field plot. For herbicide incorporation, one pass of an 80 inch swath (4 nozzles set at 18 inches above the ground at 3 mph) for the recommended field rate (1.1 kg/ha) and 2 passes for 2 x recommended rate was made. Pisum sativum L. (var. Trapper) seeds (4.6 seeds/ft or 125 kg/ha) were sown at a depth of 2 1/2" using a Press drill (6" spacing). Seeds were either inoculated with a commercial inoculum of R. leguminosarum or else un-inoculated. No fertilizer was applied to the field.

Plots were examined at various time intervals and observations made on the growth of pea plants and nodulation of representative plants from all plots. No attempt was made to recover root systems and count nodules or determine nitrogenase activity; however, after

plant maturity, seeds were collected and analyzed for total nitrogen content as described above.

RESULTS AND DISCUSSION

Growth Chamber Study

Effect of Herbicide on Pea Plant Growth. In general, there was no effect of trifluralin on the germination of pea seeds in the Bradwell loam soil. Other studies (not reported) have shown some toxicity of trifluralin on seed germination in sandy (Asquith) soil. Plants in trifluralin treated soil (Bradwell loam) exhibited slower growth than plants in non-treated soil, and the shoot growth of plants in soil receiving 3 ppm of trifluralin showed visible signs of damage (e.g., curled leaves and leaf-tip browning). These results are consistent with previously reported studies on various legumes (Brock, 1972; Dunigan et al., 1972; Harvey and Gritton, 1977).

The effects of trifluralin on the growth of pea plants is shown in Table 1. There was significant reduction of plant root fresh weight and shoot dry weight due to 3 ppm trifluralin. This was the case regardless of whether the plants had been nodulated by rhizobia. Lower concentrations of trifluralin caused significant but less severe effects. There was, however, no significant effect or clear-cut trend on tap root length, or on shoot nitrogen content by any of the trifluralin concentrations tested. Visual observations indicated that lateral root development was extensive on all plants, although it was somewhat stunted on plants in soil containing 3 ppm trifluralin. Similar effects; i.e., reduction in shoot and root weight due to trifluralin, have been reported for several Trifolium (clover) species

Table 1. Effect of trifluralin on the growth of pea plants after 5 weeks in a growth chamber.

Inoculum	Trifluralin concentration	Root fresh wt per pot (g)	Tap root length per plant (cm)	Shoot dry wt per plant (g)	Shoot-N per plant (%)
None	Control	13.55a*	26a	1.17a	3.15a
None	1 ppm	10.55bc	26a	1.20a	3.20a
None	2 ppm	11.75ab	29a	0.88b	2.89a
None	3 ppm	8.97c	32a	0.58c	3.30a
Commercial	Control	12.02a	29a	1.08a	3.14a
Commercial	1 ppm	10.39a	26a	1.09a	3.03a
Commercial	2 ppm	10.84a	24a	0.88b	3.05a
Commercial	3 ppm	6.45b	33a	0.45c	3.58b
Strain C52	Control	11.22a	26a	1.10a	3.46a
Strain C52	1 ppm	7.62b	20a	0.88b	3.41a
Strain C52	2 ppm	9.37ab	26a	0.92ab	3.09a
Strain C52	3 ppm	4.18c	26a	0.36c	3.67a

* Means of four replicates; values followed by the same letter within each column for each inoculum do not differ at the 95% level as determined by Duncan's multiple range test.

(Brock, 1972) and for Glycine max (soybeans) (Dunigan et al., 1972). Harvey and Gritton (1977) found that trifluralin at a rate of 1.68 kg/ha (about 0.8 ppm) caused a reduction in root fresh weight of greenhouse grown "Alaska" and "Perfection" pea cultivars.

Effect of Herbicide on Nodulation of Plants. Pea plants grown in this Bradwell soil were not nodulated unless they had been inoculated with an appropriate rhizobium strain. The effect of trifluralin on nodulation and nitrogenase activity of inoculated pea plants is shown in Table 2. There was a highly significant correlation ($r = .95$) between a decrease in root nodulation or nitrogenase activity, with increasing herbicide concentration. Note that 2 ppm and 3 ppm trifluralin caused a significant reduction in the number of nodules per g of root fresh weight and in the nitrogenase activity of these roots. These results were essentially the same regardless of the rhizobium inoculum used. Harvey and Gritton (1977) did not examine the effect of trifluralin on nodulation and nitrogenase activity of their pea cultivars. Other investigators, however, have shown that trifluralin (at rates comparable to those in the present study) causes a reduction in the number and size of nodules on various legumes (Brock, 1972; Dunigan et al., 1972; Kurst and Struckmeyer, 1971). Because there is a direct relationship between the number of nodules and nitrogenase activity (Fig. 1), it would appear that the main effect of trifluralin was a reduction in nodulation which then resulted in a corresponding decrease in nitrogenase activity. It has been suggested that the reason for reduced nodulation of legumes in trifluralin treated soils is

Table 2. Effect of trifluralin on the number of nodules and nitrogenase activity of pea plants after 5 weeks in a growth chamber

Inoculum	Trifluralin concentration	No nodules (per g root fresh wt)	Nitrogenase activity (umoles C_2H_2 per g per h)
None	Control	0	0
None	1 ppm	0	0
None	2 ppm	0	0
None	3 ppm	0	0
Commercial	Control	7.9a*	0.154a
Commercial	1 ppm	7.1ab	0.123ab
Commercial	2 ppm	3.8bc	0.035bc
Commercial	3 ppm	1.0c	0.002c
Strain C52	Control	9.3a	0.161a
Strain C52	1 ppm	5.5ab	0.062ab
Strain C52	2 ppm	3.0bc	0.049bc
Strain C52	3 ppm	0.5bc	0.005bc

* Means of four replicates; values followed by the same letter within each column for each inoculum do not differ at the 95% level as determined by Duncan's multiple range test.

due to the abnormal root growth caused by this herbicide (Brock, 1972). We also detected abnormal root growth and our results would seem to support this hypothesis.

The results obtained were similar for both of the growth chamber studies.

Field Study

General Observations. The field study plots were seeded on June 2, 1983 and were damaged approximately 3 weeks later due to a heavy rain. At 1 month of age, the pea plants on trifluralin treated plots, especially the 2 x field rate plot, were visibly stunted compared to the plants on the control plots. After 5 weeks of growth, plants were selected at random from all plots and examined for nodulation. All plants examined, even control uninoculated plants, exhibited good to heavy nodulation. By harvest, all plants appeared to have recovered from the earlier effects of trifluralin. Based on an analysis of the pea seeds from plants on the various treated plots, there was no effect of trifluralin on the nitrogen content of seeds.

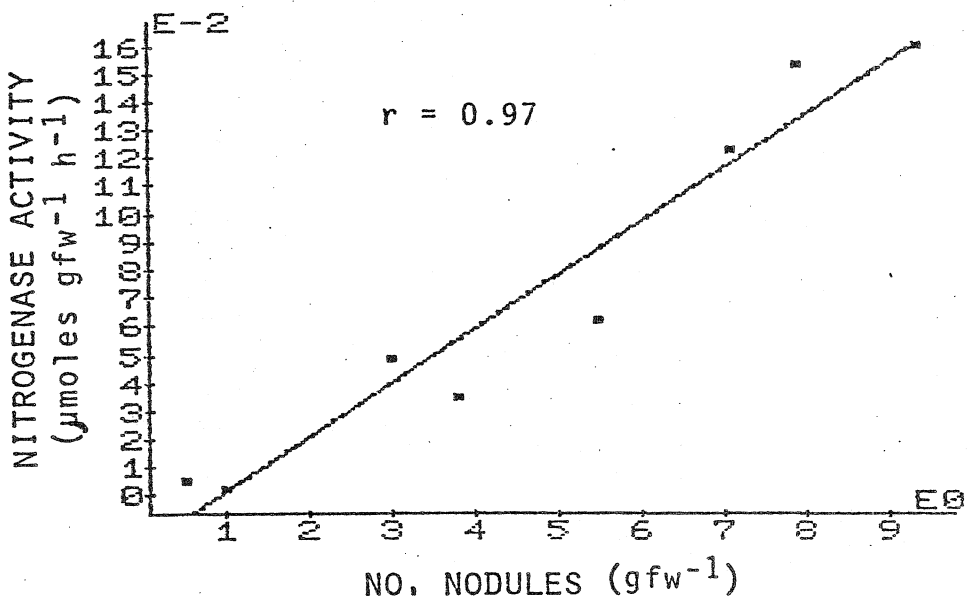

Fig. 2 Relationship between nodulation and nitrogenase activity of pea plants; note y-axis scale E - 2 = 10^{-2}

SUMMARY

In general, trifluralin reduced pea plant shoot and root growth, and caused a reduction in plant nodulation under growth chamber conditions. Under field conditions, trifluralin did not appear to cause major effects on pea plant growth and concentrations up to 2 x field rate were not a problem. On the basis of this short-term study, it

would appear that recommended rates of trifluralin would not impair legume nodulation and growth in the field. Nevertheless, based on the growth chamber results, caution should be exercised when using trifluralin with legumes, as carelessness and accidental spills may have significant effects on legume growth and nodulation. The consequence of less effective nitrogen-fixing legumes could affect soil nitrogen fertility.

ACKNOWLEDGEMENTS

We extend thanks to B. Drew and L. Mills of the U. of S. Crop Development Center for setting up the field plots, and also to L. Nelson and F. Chalifour of the Plant Biotechnology Institute for advice and gas chromatography facilities for the nitrogenase assay.

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