EFFECT OF SELECTED POSTEMERGENCE HERBICIDES ON GROWTH, NODULATION, AND NITROGEN FIXATION OF SOYBEANS (*GLYCINE MAX*)

by

OZAIR AHMAD CHAUDHRY

B.Sc., Punjab University, 1973

M.Sc., (Biology), University of Islamabad, Pakistan, 1976

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Approved by:

Loren J. Moshier

Major Professor

To my Parents

WITH LOVE

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INTRODUCTION

Soybean [*Glycine max* (L. Merr.)] is a very important crop in the United States. In the past five years, U.S. soybean production has been 60 to 65% of the world soybean production (1). 51.6 million metric tons of soybeans were grown on 27 million hectares in 1981 (2).

More than 60 weed species infest soybean fields in the United States. Major losses are caused by annual broadleaf and grassy weeds in all regions (24). Use of herbicides is a very important component in weed control systems for soybeans. Herbicides applied postemergent (after soybean and weeds emerge) are now commonly used to control weeds that escape herbicides applied at planting time and/or to replace cultivation operations.

Leguminous plants such as soybeans contain higher concentrations of nitrogen as protein and other nitrogenous compounds than crops of other families. Each kilogram of soybean seed contains 60 to 70 grams of nitrogen; the associated vegetative growth contains an additional 30 to 35 grams. Approximately 600 kilograms nitrogen are required for seed yields of 6000 kg/ha. These nitrogen inputs may be provided by soil/fertilizer nitrogen and/or symbiotically fixed nitrogen (17). Due to limited energy sources available for the manufacture of nitrogen fertilizer, there is at present considerable interest in fixation of atmospheric nitrogen within legumes (7).

The U.S. Environmental Protection Agency (EPA) guidelines to pesticide manufacturers include studies on nitrogen fixation in herbicide-treated soybeans and other leguminous crops (16). Relatively few studies have been reported regarding effects of herbicides on a) soybean-*Rhizobium* symbiosis and b) nitrogen content in seed.

We therefore felt studies were necessary to determine effects of selected herbicides on growth, nodulation, and nitrogen fixation capability in soybeans.

Herbicides selected for these studies were acifluorfen [5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate], bentazon [3-isopropyl-1-H-2,1,3-benzothiadiazin-4-(3H)-one2,2-dioxide], chloramben [3-amino-2,5-dichlorobenzoic acid)], and 2,4-DB [4-(2,4-dichlorophenoxy)butryic acid]. These herbicides are currently registered in the U.S. to be applied post-emergent to soybeans to control certain broadleaf weeds (20).

REVIEW OF LITERATURE

Payne and Fults (28) observed that 2,4-D (2,4-dichlorophenoxyacetic acid) applied at 0.08 kg/ha prevented nodulation in beans (<u>Phaseolus</u> spp.) in a greenhouse study. Carlyl and Thorpe (8) reported that 2,4-D at a concentration of 0.5 ppm in sand reduced nodulation and growth of alfalfa (<u>Medicago sativa</u> L.), beans, peas (<u>Pisum sativum</u> L.), and red clover (<u>Trifolium pratense</u> L.). Elfadi and Fahmy (13), however, reported that 2,4-D or MCPA [(4-chloro-<u>o</u>-tolyl)oxy]acetic acid] applied at rates up to 3.0 kg/ha had no effect on nodulation of inoculated cow peas (<u>Vigna sinensis</u> Savi.)

Kust and Struckmeyer (22) observed that trifluralin [α-α,α-trifluoro-2, 6-dinitro-<u>N</u>-<u>N</u>-dipropyl-<u>p</u>-toluidine] applied at 0.4 to 0.7 kg/ha to soil prior to planting and incorporated reduced nodulation in soybeans. Plant growth and nitrogen fixation were reduced in chick pea (<u>Cicer arientinum</u> L.) grown in soil treated with trifluralin (25). Trifluralin treatments also reduced nodulation and growth of clovers (<u>Trifolium</u> spp.) (5).

Avrov (3) reported a reduction in nodule number and size in peas treated with prometryne [2,4-bis(isopropylamino)-6-(methylthio)-<u>s</u>-triazine]. Formation of pseudonodules was observed in alfalfa treated with amitrole (3-amino-<u>s</u>-triazole) or dalapon (2,2-dichloropropionic acid) (23) and in several leguminous crops treated with 2-bromo-3,5-dichloro-benzoic acid (4).

Herbicides may reduce nodulation as a result of reducing growth in treated plants. Reduction in nodulation in alfalfa and red clover grown in soil treated with EPTC (<u>s</u>-ethyl dipropylthiocarbamate), benefin (<u>N</u>-butyl-<u>N</u>-ethyl-α,α,α-trifluoro-2,6-dinitro-<u>p</u>-toluidine) or foliarly treated with dichlofop [2-[4-(2,4-dichlorophenoxy)phenoxy]proponate] or 2,4-DB was usually associated with reduction in top growth (29). Trifluralin treatments were observed to reduce growth and nodulation in several *Trifolium* spp. without reducing *Rhizobia* populations in the soil (5). Paraquat (1,1'-dimethyl-4, 4'-bipyridillium ion), which causes immediate cessation of growth in treated plants, decreased nitrogenase activity on both per plant and per nodule weight basis in white clover (*Trifolium repens* L.) within 24 hours after treatment (31).

Dunigan et al., (11) however, reported no detrimental effects in greenhouse or field-grown soybeans treated with alachlor [(2-chloro-2',6'-diethyl-*N*-(methoxymethyl)acetanilide], chloramben, linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea), DCPA (dimethyl tetrachloroterephthalate), or prometryne. These investigators also observed that dalapon treatments did not affect nodule number or nodule weight in birdsfoot trefoil (*Lotus corniculatus* L.). Other studies revealed that nodule formation was enhanced in lupine (*Lupinus spp*.) treated with simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] (3), in beans treated with prometryne (31), and in red clover treated with dalapon (27).

MATERIALS AND METHODS

Greenhouse Studies.

A soil sample was collected in February 1982 from the Kansas State University Agronomy Farm located 7.2 km southwest of Manhattan from a site with Eudora silt loam (Fluventic Hapludoll, coarse-silty, mixed, mesic), with 26 percent sand, 65 percent silt, and 9 percent clay, with pH 6.3, and 1.4 percent organic matter. Soybeans were grown on this site the previous two years. The soil was removed at random from the upper 30 cm of the profile, sieved with 4-mm screen to remove plant debris, and uniformly mixed. The soil then was stored outdoors in galvanized cans lined with polyethylene bags and covered to prevent drying.

A soil-sand mixture prepared by adding three parts soil to one part sand was placed in 946-ml paper pots to a depth of 12.5 cm. Sand was added to improve aeration, reduce crusting, and facilitate root harvesting. Soybean (var. 'Williams') seeds that were treated with the fungicide thiram [bis(dimethylthio-carbamoyl)disulfide] at a rate of 2 g/kg seed (normal use rate) were planted five per pot 2.5 cm deep. Thiram has been reported to have no effect on symbiotic nitrogen fixation in soybean when applied at normal use rates (9).

Plants were thinned to three per pot one week after emergence. Plants were watered as necessary, fertilized weekly with a modified Hoagland's nutrient solution (18) diluted to half strength and without nitrogen, and grown without supplemental lighting in a day/night temperature regime of 32/18 ±2C. Pots were rotated on greenhouse bench every several days to ensure uniform growth.

Sodium salt of acifluorfen at 0.6 kg active ingredient (ai)/ha, sodium salt of bentazon at 1.1 kg ai/ha, ammonium salt of chloramben at 2.8 kg acid (ae) equivalent/ha, and dimethylamine salt of 2,4-DB at 0.13 ae/ha were applied when plants were at V2 stage or when trifoliate leaf at second node was fully developed (15). Two-way combinations of these herbicides were also applied with each component applied at the same rate as when applied alone. Herbicides were applied with water as diluent at 234 L/ha with a flat fan nozzle spraying at 1.3 kg/cm^2 and attached to a stationary boom mounted over a moving table. Pots were watered carefully during the initial 24 hours after treatment so that water did not come in contact with leaves.

Plants were harvested two weeks later after removing paper from the root-soil continuum, soaking the roots and soil in water for approximately 20 minutes, and then placing plants and soil on a 2-mm sieve and washing soil from roots with dispersed tap water. Plants were harvested from 1300 to 1500 when nitrogen fixation would be expected to proceed at a rapid rate (17). Roots were immediately surface-dried by placing them in contact with water-absorbent paper and then severed from shoots. Both shoots and roots were immediately weighed.

Roots from plants in each pot were placed in 250-ml glass bottles and bottles were then sealed with rubber septa. Twenty-five ml of air in each bottle were removed by syringe and replaced with acetylene. Roots were incubated at 30 ± 1C for one hour to allow the nitrogenase enzyme to convert acetylene to ethylene. Amount of ethylene in a 0.5-ml sample was then analyzed by a gas-liquid chromatograph equipped with a column packed with Porapak-R[1] in an oven maintained at 45 C and a flame ionization detector operating

[1]Waters associates, Framingham, MA.

at 140 C. Immediately after acetylene reduction assay was completed, root nodules were counted and weighed. Ethylene produced was calculated on both per plant and per nodule fresh weight base and standaridized on a per unit time basis (19).

Treatments were replicated four times. Analysis of variance was performed on all data based on a completely randomized design except for ethylene produced per plant. This parameter was statistically analyzed based on a randomized complete block design since all plants from a replicate were harvested together and incubation times and temperatures were kept constant for that replicate. Means were separated statistically by a Duncan's multiple range test. Data presented are means of three experiments.

In the second greenhouse study, 2,4-DB rate was reduced to 0.04 kg ae/ha and chloramben at 5.6 kg ae/ha and chloramben plus 2,4-DB at 2.8 plus 0.04 kg/ha were applied at additional treatments. Plants were grown under supplemental fluorescent lighting with a photoperiod of 15 hours to prevent early flowering and treated at V3 stage. Soil was removed from roots by gently shaking plants. This method allowed faster removal of soil from roots than washing them and reduced likelihood of diffusion of oxygen into nodules, which inactivates the nitrogenase enzyme.

Data presented for this study are means of two experiments. Otherwise, experimental and statistical procedures were similar to those described for the first study.

Field study.

A site on the Kansas State University Agronomy Farm was selected with a soil as previously described. Trifluralin at 0.8 kg/ha was applied and incorporated 7.5 cm deep with two discing operations on 15 April 1982 to control grassy weeds. Trifluralin applied at 0.8, 1.1, 1.4 and 2.2 kg/ha did not affect nitrogen fixation, nodulation and growth in greenhouse-grown soybeans in a preliminary study (Table I, appendix). On 1 June, the plot area was tilled with a power driven rotary action cultivator and then planted to soybeans (var. 'Williams') 3 cm deep and spaced 5.0 cm apart in the row with a 76-cm row spacing. Plots consisted of four rows 9 m long which were kept weed free throughout the growing season and were not irrigated.

Plants at the V3 stage were treated on 26 June with herbicides at the rates reported in the first greenhouse study with water as diluent at 187 L/ha. A tractor-mounted sprayer equipped with flat fan nozzles operating at 1.1 kg/cm^2 pressure was used to apply herbicides. Two weeks after treatment, three neighboring plants from one of two middle rows in each plot were selected at random and removed by excavating roots and soil up to 15 cm from plants and to a 25-cm depth. Careful removal of soil from selected plants revealed that essentially all the nodules were on the primary root or on lateral roots close to the primary root. Plants were excavated from 1000 to 1300 and soil was removed from roots by washing. Roots were surface-dried, separated from shoots, and both shoots and roots were weighed. Acetylene reduction assay to measure nitrogen fixing capability of nodules was then performed as described in previous studies. Nodules were also counted and weighed.

On 2 August, plants were at the R1 stage (beginning bloom) and again excavated from 1300 to 1500 as previously described except to 30 cm depth rather than 25 cm depth. Soil was removed from roots by gently shaking plants and roots then placed in 2.5-L plastic containers. Containers were immediately sealed and 100 ml of acetylene was injected into each container after volume of air was withdrawn by syringe. Containers were kept in shade in the field for one hour and 1-ml sample was then withdrawn with syringe which was subsequently inserted into a rubber stopper until gas-liquid chromtographic analysis could be performed.

Soybeans were manually harvested on 3 October by removing 4.5 m of the plants in the two middle rows of each plot and then threshing with a nursery combine. Percent nitrogen in the harvested seed was later analyzed by micro-kjeldahl method.

Treatments were replicated four times in a randomized complete block design. Data were subjected to an analysis of variance. Means were separated statistically by Duncan's multiple range test.

RESULTS

Greenhouse Studies. None of the postemergence herbicides applied in the first greenhouse study significantly reduced shoot weight, root weight, nodule weight, nodule number, or nitrogen-fixing capability as measured by ethylene production (or acetylene reduction) assay (Table 1). The low nodule weights and low amount of ethylene production in nontreated plants and significant increase in nodule weight and nodule number due to bentazon plus 2,4-DB and acifluorfen plus 2,4-DB, respectively, in this study cannot be explained.

All herbicide treatments except bentazon alone significantly reduced shoot weight of soybeans in the second greenhouse study (Table 2). Root weight also was reduced when plants were treated with the bentazon plus 2,4-DB combination. Nodule weight was significantly reduced by all except three treatments. Chloramben at 5.6 kg/ha, acifluorfen plus bentazon, acifluorfen plus chloramben, and bentazon plus 2,4-DB significantly reduced both nodule weight and nodule number. All treatments except bentazon alone significantly reduced ethylene production per plant. Five of these treatments also significantly reduced ethylene production on unit nodule weight basis.

Field Studies. Acifluorfen alone, chloramben alone, three combinations with either acifluorfen or chloramben, and bentazon plus 2,4-DB significantly reduced root growth but not shoot growth of soybeans during the two-week interval after treatment (Table 3). Bentazon or 2,4-DB did not reduce shoot or root growth. Shoot and root weight of plants treated with herbicide combinations was seldom less than weights of plants treated with herbicides alone.

Table 1. Effect of postemergence herbicides on growth, nodulation, and nitrogen fixation capability of greenhouse-grown soybeans (Study 1).

Herbicide	Rate	Shoot weight	Root weight	Nodule weight	Nodule frequency	Ethylene produced per plant	Ethylene produced per nodule weight
	(kg/ha)	(g/plant)[a]	(g/plant)[a]	(g/plant)[a]	(no/plant)[a]	(μmoles/hr)[a]	(μmoles/g/hr)[a]
Acifluorfen	0.6	3.92 a	4.03 abc	0.35 ab	35.6 b	4.2 ab	10.7 ab
Bentazon	1.1	3.63 abc	3.88 bc	0.32 ab	37.4 b	4.5 a	12.0 ab
Chloramben	2.8	3.60 abc	3.98 abc	0.34 ab	36.2 b	4.2 ab	12.6 a
2,4-DB	0.14	3.75 abc	6.56 a	0.36 ab	36.0 b	4.2 ab	11.7 ab
Acifluorfen + bentazon	0.6 + 1.1	3.84 ab	3.50 c	0.32 b	36.4 b	7.7 a	14.9 a
Acifluorfen + chloramben	0.6 + 2.8	3.57 abc	3.75 c	0.35 ab	31.4 b	4.9 a	13.2 a
Acifluorfen + 2,4-DB	0.6 + 0.14	3.37 c	4.03 abc	0.36 ab	44.4 a	4.3 ab	10.8 ab
Bentazon + chloramben	1.1 + 2.8	3.63 abc	3.70 c	0.32 b	32.2 b	4.1 ab	12.7 a
Bentazon + 2,4-DB	1.1 + 0.14	3.50 ac	4.38 ab	0.37 a	37.3 b	5.3 a	13.8 a
None	---	3.66 abc	3.98 abc	0.32 b	31.9 b	2.8 b	7.9 b

[a]Means within columns followed by common letters are not significantly different according to Duncan's multiple range test at 5% level.

Table 2. Effect of postemergence herbicides on growth, nodulation, and nitrogen fixation capability of greenhouse-grown soybeans (Study 2).

Herbicide	Rate	Shoot weight	Root weight	Nodule weight	Nodule frequency	Ethylene Produced per plant	Ethylene Produced per nodule weight
	(kg/ha)	(g/plant)[a]			(no/plant)[a]	(μmoles/hr)[a]	(μmoles/g/hr)[a]
Acifluorfen	0.6	5.90 c	2.68 ab	0.75 b	35.1 ab	7.4 cd	10.0 c
Bentazon	1.1	6.83 ab	2.83 ab	0.87 a	39.5 ab	11.4 ab	12.8 abc
Chloramben	2.8	6.51 bc	2.84 ab	0.73 bc	35.6 ab	7.9 cd	10.5 bc
Chloramben	5.6	5.98 c	2.72 ab	0.61 c	32.4 b	7.5 cd	12.2 abc
2,4-DB	0.04	6.40 c	2.88 ab	0.78 ab	35.9 ab	10.1 bcd	13.1 abc
Acifluorfen + bentazon	0.6 + 1.1	6.19 bc	2.66 ab	0.75 b	31.6 b	9.4 bcd	12.1 abc
Acifluorfen + chloramben	0.5 + 2.8	5.81 c	2.89 ab	0.71 bc	31.7 b	7.8 cd	11.0 bc
Acifluorfen + 2,4-DB	0.6 + 0.04	6.08 bc	2.57 ab	0.73 bc	35.2 ab	9.9 bcd	14.1 abc
Bentazon + chloramben	1.1 + 2.8	6.27 bc	2.68 ab	0.81 ab	36.8 ab	8.6 bcd	10.7 bc
Bentazon + 2,4-DB	1.1 + 0.04	6.36 bc	2.48 b	0.72 bc	31.6 b	10.5 bc	14.4 ab
Chloramben + 2,4-DB	2.8 + 0.04	6.53 bc	2.54 ab	0.71 bc	33.8 ab	7.2 d	10.4 bc
None	---	7.40 a	3.06 a	0.89 a	41.4 a	13.5 a	15.9 a

[a]Means within columns followed by common letters are not significantly different according to Duncan's multiple range test at 5% level.

Table 3. Effect of postemergence herbicidea on growth, nodulation, nitrogen fixation capability, yield, and percent nitrogen in seed of field grown soybeans in 1982.

		2 weeka after V2 stage						R1 Stage		
						Ethylene produced		Ethylene produced		Nitrogen
Herbicide	Rate	Shoot weight	Root weight	Nodule weight	Nodule frequency	per plant	per nodule weight	per plant	Yield	Concentration in seed
	(kg/ha)	(g/plant)[a]			(no/plant)[a]	(μmoles/hr)[a]	(μmoles/g/hr)[a]	(μmoles/hr)[a]	(kg/ha)[a]	(%)[a]
Acifluorfen	0.6	11.1 ab	2.5 b	0.21 b	28.2 a	1.8 c	8.1 bc	9.3 a	2350 a	8.8 a
Bentazon	1.1	18.6 a	3.8 ab	0.43 ab	34.2 a	2.7 abc	6.7 bc	6.2 a	2170 a	9.0 a
Chloramben	2.8	12.7 ab	2.9 b	0.36 ab	34.8 a	2.6 abc	7.9 bc	9.7 a	2210 a	8.2 a
2,4-DB	0.14	15.1 ab	3.6 ab	0.39 ab	40.6 a	2.0 bc	5.4 c	6.6 a	2140 a	9.0 a
Acifluorfen + bentazon	0.6 + 1.1	11.7 ab	2.7 b	0.28 ab	32.7 a	4.2 ab	14.1 a	7.3 a	2380 a	9.4 a
Acifluorfen + chloramben	0.6 + 2.8	10.61 b	2.8 b	0.25 ab	29.7 a	3.0 abc	11.7 ab	6.5 a	2440 a	9.7 a
Acifluorfen + 2,4-DB	0.6 + 0.14	11.4 ab	2.8 b	0.23 b	28.8 a	2.5 abc	10.1 ab	7.3 a	2020 a	9.7 a
Bentazon + chloramben	1.1 + 2.8	15.2 ab	3.2 ab	0.36 ab	34.3 a	2.1 bc	6.4 bc	11.6 a	2490 a	9.0 a
Bentazon + 2,4-DB	1.1 + 0.14	10.8 b	2.7 b	0.28 ab	29.2 a	2.2 abc	7.4 bc	7.7 a	2280 a	9.8 a
None	---	17.9 ab	4.4 a	0.48 a	45.0 a	4.5 a	9.6 abc	7.5 a	2430 a	10.0 a

[a]Means within columns followed by common lettera are not aignificantly different according to Duncan'a multiple range test at 5% level.

Acifluorfen alone and acifluorfen plus 2,4-DB significantly reduced nodule weight. Nodule number was not significantly affected by any treatment. Amount ethylene produced per plant was significantly reduced by acifluorfen, 2,4-DB, and bentazon plus chloramben. Ethylene production on unit nodule weight basis was not significantly affected by any treatment.

None of the herbicides or herbicide combinations significantly reduced ethylene production by nodules of plants at early flowering stage. Yield and nitrogen content in seed also was not significantly affected by any of the treatments.

DISCUSSION

Coefficients of variation were 40 and 28 percent for ethylene production measurements on a per plant basis and 41 and 30 percent for ethylene production measurements on a per nodule weight basis for the first and second greenhouse studies, respectively (Tables IV and V, appendix). Coefficients of variation were 15 and 14 percent for nodule weight measurements for the first and second greenhouse studies, respectively. Johnen et al (21) also reported large variation associated with measurements of nitrogenase activity.

In our greenhouse studies, several sources of variation were present. Soybeans were planted at different times of the year and therefore grown under different light intensity regimes since no supplemental lighting was used in first greenhouse study and only low intensity lighting was used in second greenhouse study to extend photoperiod to 15h. Variation in nitrogenase activity would therefore occur among experiments in each study since nitrogenase activity increases as light intensity increases (16).

Washing roots with water to remove soil in first greenhouse study may explain larger coefficients of variations observed in nitrogenase activity. Oxygen diffusion into nodules during washing process would have cause inactivation of nitrogenase enzyme which is very sensitive to oxygen (12,14). Length of time that roots were soaking in water and subsequently washed varied 20 minutes between experimental units.

Effects of herbicide applications on nodule weight depended on the specific experiment within both studies and effects on nitrogen fixation capability depended on the specific experiment within the first greenhouse study. Factors previously discussed may have possibly contributed to experiment by treatment interactions for nodule weight and ethylene production and also contributed to large experimental error that we encountered in ethylene production measurements. Also the time of day and stage of growth varied some between experiments within greenhouse studies. Nodule weight and nitrogenase activity are influenced by stage of growth (16); nitrogenase activity also is affected by time of day (17). Thus effects of herbicides on these parameters may have varied between experiments depending on nodule growth patterns and nitrogenase activity within plants at time of treatment.

All treatments except bentazon reduced nodule weight in the second greenhouse study. Therefore magnitude of ethylene production values calculated on per nodule weight basis were much closer to values for untreated plants than when calculated on per nodule weight basis. Data from the field study, collected when soybeans were harvested two weeks after treatment, revealed a similar pattern. Data from these studies indicate that ethylene production on per plant basis rather than per nodule weight basis should be considered when evaluating effects of herbicides on nitrogen fixation.

Acifluorfen, 2,4-DB and their metabolites are essentially immobile in leguminous plants (10,32). It therefore is unlikely that sufficient amount of these herbicides or their metabolites would translocate into the nodules of treated soybean plants and directly interfere with the nitrogen fixation process. Reduction in nitrogen fixation ability would appear to be a secondary rather than primary response to these herbicide treatments. Bentazon which is also essentially immobile in treated soybean plants (26) did not reduce nitrogen fixing ability. Literature pertaining to chloramben translocation in plants when foliarly applied was not found.

In field-grown soybeans, two herbicide treatments (acifluorfen and bentazon plus 2,4-DB) reduced nitrogen fixing ability two weeks after treatment but had no effect on seed yield. Bello et al (6) observed that significant differences existed in total nitrogen fixed per hectare in three different soybean varieties but not in seed yield. Apparently there was sufficient available soil N to meet the plants' demands for nitrogen in their study and therefore allow soybeans to balance supply of nitrogen from soil nitrogen plus nitrogen-fixation to demand. Our data would indicate that plants treated with postemergence herbicides that reduce nitrogen fixation are also able to compensate by removing more soil nitrogen but in consequence, may deplete soil nitrogen to a greater extent in fertile soils.

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APPENDIX

Effect of Trifluralin on Plant Growth, Nodulation and Nitrogen Fixation in Soybeans

MATERIALS AND METHODS

Soil was sampled in February, 1982 from the site used in the field study as previously described, sieved to remove plant debris and treated with trifluralin at 0.8, 1.1, 1.4 and 2.2 kg/ha. The soil was immediately mixed uniformly to distribute the herbicide and then placed in 946-ml paper pots. Soil which was not treated was also placed in pots to serve as a control. Spraying, planting, culturing, harvesting, and data collecting procedures were identical to those in other greenhouse studies as previously described. Data presented are means of one experiment.

RESULTS AND DISCUSSION

No significant reduction occurred in shoot weight, root weight, nodule weight, nodule number, or ethylene production in plants grown in soils treated with trifluralin at various rates. Trifluralin applied at 0.8 kg/ha in the field six weeks prior to planting should have no effect on growth, nodulation and nitrogen fixation in soybeans.

Table 1. Effect of trifluralin application on growth, nodulation and nitrogen fixation of soybean grown in the greenhouse.

Trifluralin	Shoot weight	Root weight	Nodule weight	Nodule Frequency	Ethylene produced per plant	Ethylene produced per nodule weight
(kg/ha)	(g/plant)[a]			(no/plant)[a]	(μmoles/hr)[a]	(μmoles/g/hr)[a]
0	4.13 a	3.79 a	0.38 a	37.25 a	4.35 a	11.3 a
0.8	4.98 a	4.66 a	0.43 a	32.10 a	4.98 a	11.7 a
1.1	4.01 a	4.78 a	0.41 a	28.10 a	4.83 a	11.7 a
1.4	4.36 a	5.11 a	0.44 a	37.58 a	4.94 a	11.6 a
2.2	3.79 a	3.19 a	0.36 a	32.25 a	4.14 a	11.7 a

[a]Means within columns followed by common letters are not significantly different according to Duncan's multiple range test at the 5% level.

Effect of Selected Postemergence Herbicides on Soybean Growth and Nodulation

MATERIAL AND METHODS

Effects of six herbicides, each applied postemergent at three stages, on soybean growth and nodulation were evaluated in the greenhouse. A soil mix consisting of one part Muir silt loam soil and one part decomposed leaf litter was placed in 2.5 L pots. Soybean plants (var 'Williams') were thinned to three per pot after emergence. Six postemergence herbicides that control grassy weeds were applied to soybeans at V1 to V2 stage (15). Common and chemical names for these herbicides are shown in Table II. Herbicides were applied using a moving belt sprayer with a single flat fan nozzle, attached to a fixed boom. The sprayer was calibrated to deliver 187 L/ha with a pressure of 1.45 kg/cm^2. The nozzle was placed 45 cm above the soybeans. Fresh weight of shoot, root, and nodules were measured and number of nodules were counted two weeks after treatment.

A completely randomized design for 19 treatments with three replications was used. An analysis of variance and a Duncan's Multiple range test was performed on data. Data presented are means of two experiments.

RESULTS AND DISCUSSION

Mefluidide applied at 0.28 and 0.42 kg/ha significantly reduced shoot fresh weight (Table III). Reduction due to mefluidide treatments was 16 and 22% at 0.28 and 0.42 kg/ha rates, respectively. Other treatments did not significantly reduce shoot weight.

None of the treatments significantly reduced root fresh weight. Nodule weight but not nodule number was reduced by mefluidide applied at

Table II. Names of selected post emergence herbicides used in greenhouse study.

Common name or Code designation	Chemical Name
Sethoxydim	2-[1-(ethoxyimino)-butyl]-5-[2-(ethylthio)-propyl]-3-hydroxy-2-cyclohexene-1-one
Fluazifop	(±)-butyl 2-[4-[(5-(trifluoromethyl)-2-pyridinyl)oxy]phenoxy]propanoate
Haloxyfop	Methyl 2-(4-((3-chloro-5-(trifluoromethyl)-2-pyridinyl)oxy)phenoxy)propanoate
HOE-33171	Not available
CGA-82725	2 propynl 2-[4-(3,5-dichloro-2-pyridyloxy)-phenoxy]-propanoate]
Mefluidide	N-[2,4-dimethyl-5-[(trifluoromethyl)sulfonyl]amino]phenyl]acetamide

Table III. Effect of different rates of selected postemergence herbicides on soybean growth and nodulation in greenhouse.

Treatment	Rate	Shoot weight[a]	Root weight	Nodule weight	Nodule number
	(kg ai/ha)	(g/plant)			(no/plant)
Sethoxydim[b]	0.28	23.5 a	15.8 a	1.64 a	99.8 ab
	0.56	22.0 a	10.2 bc	1.43 abc	98.4 ab
	1.12	21.7 a	11.6 bc	1.50 abc	93.6 abc
Fluazifop[b]	0.28	23.6 a	11.1 bc	1.53 ab	90.7 abc
	0.56	21.2 a	10.9 bc	1.25 bc	84.5 abc
	1.12	22.6 a	12.6 b	1.45 abc	90.0 abc
Haloxyfop[c]	0.07	24.0 a	11.5 bc	1.37 abc	96.3 ab
	0.14	24.4 a	11.9 bc	1.42 abc	90.0 abc
	0.28	21.3 a	9.9 bc	1.31 abc	89.3 abc
HOE 00581	0.17	22.7 a	11.8 bc	1.43 abc	85.9 abc
	0.34	22.1 a	11.5 bc	1.47 abc	91.6 abc
	0.68	22.4 a	11.4 bc	1.42 abc	93.3 abc
CGA-82725[b]	0.28	22.5 a	11.7 bc	1.40 abc	101.5 a
	0.56	23.0 a	10.8 bc	1.43 abc	89.9 abc
	1.12	21.0 a	11.7 bc	1.27 bc	89.1 abc
Mefluidide	0.14	21.1 a	10.7 bc	1.35 abc	101.8 a
	0.28	17.4 b	9.0 c	0.90 d	74.5 c
	0.42	16.6 b	10.2 bc	1.18 cd	90.5 abc
No Treatment	----	21.6 a	11.5 bc	1.33 abc	79.1 c

[a] Soybeans were grown, treated, and harvested for shoot fresh weight measurements by Mr. Felix Ayala as a portion of his Master of Science thesis research.

[b] Crop oil concentrated was used at the rate of 2.3 L/ha.

[c] Surfactant was used at the rate of 0.94 L/ha.

Means within the columns with the same letters are not significantly different according to Duncan's multiple range test at 5% level.

0.28 and 0.42 kg/ha. In this study, treatments that reduced shoot weight also reduced nodule size in soybeans. The low number of nodules found on untreated plants cannot be explained.

Table IV. Analysis of variance and coefficients of variation of the effects of selected postemergence herbicides on growth, nodulation and nitrogen fixation of soybeans grown in the greenhouse (study 1).

Source of variation	df[b]	Mean Square				Ethylene Produced	
		Shoot weight	Root weight	Nodule weight	Nodule number	per plant	per nodule weight
		g/plant				(μ moles/hr)	(μmoles/g/hr)
Experiment	2	9.14[a]	4.96[a]	0.21[a]	4433.43[a]	587.19[a]	5083.49[a]
Replication	3	----	----	----	-------	52.74[a]	410.88[a]
Treatment	9	0.30	1.18[a]	0.004	170.57	5.08[a]	45.08
Exp. x Treatment	18	0.31	0.73	0.006[a]	83.95	5.18[a]	42.74[a]
Error	81	1.18	0.44	0.003	66.62	2.95	24.03
Coefficient of variation		12	17	15	23	40	41

[a]Denotes that these are significant differences at 0.05 level.

[b]Degrees of freedom for error mean square for ethylene produced is 78.

Table V. Analysis of variance and coefficients of variation of the effects of selected postemergence herbicides on growth, nodulation and nitrogen fixation of soybean growth in the greenhouse (study 2).

Source of variation	df[b]	Mean Square: Shoot weight	Mean Square: Root weight	Mean Square: Nodule weight	Mean Square: Nodule numbers	Ethylene Produced: per plant	Ethylene Produced: per nodule weight
		(g/plant)	(g/plant)	(g/plant)	(No/Plant)	(μmoles/hr)	(μmoles/g/hr)
Experiment	1	140.14[a]	19.76 a	1.82[a]	6215.2[a]	115.75	36.02
Replications	3	---	---	---	---	51.94[a]	82.35[a]
Treatment	11	1.55[a]	0.23	0.04[a]	78.23	29.03[a]	26.06[a]
Experiment x Treatment	11	0.77	0.56[a]	0.04[a]	73.60	7.32	6.67
Error	66	0.50	0.22	0.01	48.97	6.78	13.46
Coefficient of Variation	--	11	17	14	20	28	30

[a]Significant differences for this parameter do exist at 0.05 level.

[b]Degrees of freedom for error mean square for ethylene produced is 78.

Table VI. Analysis of variance and coefficients of variation of the effects of selected postemergence herbicides on growth, nodulation and nitrogen fixation of soybeans (Field Study, two weeks after V3-stage).

Source of variation	df	Mean Square				Ethylene Produced	
		Shoot weight	Root weight	Nodule weight	Nodule number	per plant	per nodule weight
		(g/plant)				(μmoles/hr)	(μmoles/g/hr)
Replication	3	13.71	0.77	0.0019	24.70	6.83[a]	67.07[a]
Treatment	9	35.59	1.53[a]	0.0328	119.60	3.39	28.14[a]
Error	27	20.42	0.59	0.021	115	1.93	10.13
Coeffieent of variation	--	33	24	44	32	50	36

[a]Denotes that there are significant differences at 0.05 level.

Table VII. Analysis of variance and coefficients of variation of the effects of selected postemergence herbicides on nitrogen fixation at flowering stage yield and seed nitrogen content of soybeans. (Field study, R1-Stage and when harvested).

Source of Variation	df	Mean Square[a]		
		Ethylene Produced	Yield	Grain Nitrogen Concentration
		(μmoles/plant/hr)	(kg/ha)	(%)
Replication	3	4.63	107753.33	1.36
Treatment	9	11.68	93686.61	1.26
Error	22	29.27	145072.79	2.78
Coefficient of variation	--	62	17	18

[a]Denotes that these are not significant differences at 0.05 level.

Table VIII. Analysis of variance and coefficients of variation of the effect of trifluralin treatment on growth, nodulation and nitrogen fixation in soybeans.

Source of Variation	df	Mean Sqaure				Ethylene Produced	
		Shoot weight	Root weight	Nodule weight	Nodule number	(per plant)	(per nodule weight)
		(g/plant)			No/plant	(μmoles/hr)	(μmoles/g/hr)
Replication	3	----	----	----	----	3.11[a]	12.75[a]
Treatment	4	0.30	0.99	0.007	62.16	0.57	0.09
Error	15[b]	0.56	0.36	0.004	48.70	0.83	3.6
Coefficient of variation	--	18	13	21	21	20	16

[a]Denotes that there are significant differences at 0.05 level.

[b]Degrees of freedom for error source of variation is 12 for ethylene production.

Table IX. Analysis of variance and coefficients of variation of the effect of selected post emergence herbicide treatment on growth and nodulation of soybean growth in the greenhouse.

Source of variation	df	Mean Square			
		Shoot weight	Root weight	Nodule weight	Nodule Number
		(g/plant)			(No/Plant)
Experiment	1	1932.8[a]	1168.9[a]	1.02[a]	1454.8[a]
Treatment	18	23.15[a]	10.89[a]	0.15[a]	285.8
Exp. x Treat.	18	2.76	7.56[a]	0.06	200.2
Error	70	5.61	5.00	0.06	229
Coefficient of variation	--	11	20	18	17

[a]Denotes that there are significant differences at 0.05 level.

EFFECT OF SELECTED POSTEMERGENCE HERBICIDES ON GROWTH, NODULATION, AND NITROGEN FIXATION OF SOYBEANS (*GLYCINE MAX*)

by

OZAIR AHMAD CHAUDHRY

B.Sc., Punjab University, 1973

M.Sc., (Biology), University of Islamabad, Pakistan, 1976

AN ABSTRACT OF A MASTER'S THESIS

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Acifluorfen [5-[2-chloro-4(trifluoromethyl)phenoxy]-2-nitrobenzoate], bentazon [3-isopropyl-1-H-2,1,3-benzothiadiazin-4-(3H)-one2,2,dioxide], chloramben [3-amino-2,5-dichlorobenzoic acid], 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid], and two way combinations of these herbicides were foliarly applied to soybeans [Glycine max (L.) Merr.] at V2 to V3 stage under both greenhouse and field conditions. None of the herbicide treatments significantly reduced shoot weight, root weight, nodule weight, nodule number or nitrogen fixation ability in soybeans harvested two weeks after treatment in one greenhouse study. In a second greenhouse study, all treatments except bentazon, 2,4-DB, and bentazon plus chloramben reduced nodule weight. Four of eleven treatments significantly reduced both nodule weight and nodule number. Ethylene production measurements after nodules were exposed to acetylene to determine nitrogen fixation ability were reduced by all treatments except bentazon. Five of these treatments reduced ethylene production significantly when converted to a per unit nodule weight basis. None of the treatments reduced shoot growth but six of nine treatments including acifluorfen and chloramben alone reduced root growth in field grown soybeans treated at V3 stage and harvested two weeks later. Acifluorfen also reduced nodule weight. Ethylene production per plant but not per nodule weight was adversely affected by acifluorfen, 2,4-DB, and bentazon plus chloramben treatments. Herbicide treatments did not reduce ethylene production when plants were harvested at R1 stage and also did not adversely affect yield or nitrogen content in seed.