EFFECTS PESTICIDES POSE UPON NITROGEN FIXATION AND NODULATION BY DRY BEAN (PHASEOLUS VULGARIS L. 'BONUS')

by

MICHAEL ALLEN SCHNELLE

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LITERATURE REVIEW

INTRODUCTION

Nitrogen is often the most limiting nutrient in crop production. Some organisms are able to supply their needs and those of associated plants via fixation of atmospheric nitrogen. Global biological nitrogen fixation results in approximately 122×10^6 metric tons fixed nitrogen per year (Muller and Newton, 1983).

This phenomena is restricted to free-living organismisms such as some actinomycetes, blue-green algae and bacteria or symbioses between higher plants and bacteria as in the legume Rhizobium association. The relationship of nitrogen-fixing bacteria with roots of higher plants has long been an intricate part of agriculture.

Pesticides are widely used by producers to obtain maximum quality and yield. All classes of these chemicals contain compounds which have reduced nodulation and/or nitrogen fixation in free-living organisms (Moiroud and Faure-Raynaus, 1983) and in symbionts.

Alaa-Eldin (1981) found the herbicide nitralin to be inhibitory to nodulation of soybean (Glycine max

(L.) Merr.). Trifluralin inhibited soybean nodulation and nitrogen fixation when applied at planting and was detrimental when applied five days prior to seeding. There were no effects, however, when this chemical was applied ten days before planting (Balatazar and Brontonegro, 1979). Trifluralin, as well as 2,4-DB, alachlor, glyphosate and metribuzin all adversely affected nodulation and nitrogen fixation in G. max at five and ten times normal rates (Mallik and Tesfai, 1985).

A marked reduction in nodulation and leghemoglobin formation of chickpea (Cicer arietinum L.) resulted from simazine application. An increase in nitrogen fixation, however, was observed in C. arietinum exposed to prometryne (Kumar et al., 1981).

Nodulation and nitrogen fixation by broad bean (Vicia faba L.) were adversely affected by linuron and nitralin, while dinoseb, terbucarb and chlorthaldimethyl had no effect (Ibrahim, et al., 1975). Foliar and soil treatments of bentazon resulted in temporary reduction of nitrogen fixation by P. vulgaris (Bethlenfalvay, et al., 1978).

EPTC, 2,4-DB, benefin, butralin and diclofop- caused some reduction in nodulation of alfalfa Medicago sativa L.) and red clover (Trifolium pratense L.

This inhibition, however, corresponded with plant growth reductions resulting from herbicide applications (Peters and Zbida, 1979).

Several insecticides have been injurious to legume physiological processes. Oftanol inhibited nodulation by red clover (Melilotus rubra L.), sweet clover (Melilotus alba L.) and M. sativa (Smith et al., 1978). Nodulation by cowpea (Vigna unguiculata (L.) Walp.) was reduced by aldicarb and fensulfothion (Sekar and Balasbramanian, 1978). Nitrogen fixation in peanut (Arachis hypogea L.) was significantly depressed when exposed to carbofuran (Mundade et al., 1980).

Additionally, nitrogen fixation by soybean was reduced by carbaryl and malathion at 10x label rates and by 5 and 10x applications of acephate, diazinon and toxaphene (Mallik and Tesfai, 1985). Dieldrin and lindane suppressed nodulation by Vicia faba at 20x and recommended rates (Selim, et al., 1970). Witty et al., (1980), however, found that nitrogenase activity of V. faba was increased by application of aldicarb.

Fungicides have both suppressed and enhanced legume-bacteria associations. Nodulation and nitrogen fixation in G. max were depressed when subjected to carboxin and carboxin plus captan at 10 times the

recommended level (Mallik and Tesfai, 1985) and by thiram (Tu, 1981).

Curley and Burton, (1975) found that thiram and carboxin at label rates were compatible with inoculum for G. max, but PCNB and captan reduced taproot nodulation and Rhizobium survival.

Additionally, nodulation and nitrogen fixation have been reduced in peanut by fungicides. Methoxyethyl-mercury chloride and thiram were inhibitory, but benomyl, aureofungin, carboxin, copper oxychloride and zineb were stimulatory (Mundade, et al., 1980).

Nitrogenase activity and nodule dry weight were increased in birdsfoot trefoil (Lotus corniculatas L.) when exposed to carbofuran, a nematicide (Belanger, et al., 1985).

Mallik and Tesfai, (1983) found captan, thiram, mancozeb and carboxin reduced viability in some Rhizobium cultures, however, PCNB and fenaminsulf proved non-toxic even at the highest concentrations. Herbicides such as chlorthal, terbutol, nitralin and linuron have also inhibited growth of Rhizobium strains (Ibrahim et al., 1977).

CHAPTER II

PESTICIDAL EFFECTS ON NITROGEN FIXATION AND NODULATION BY PHASEOLUS VULGARIS L. 'BONUS'

INTRODUCTION

Biological processes for fixing atmospheric nitrogen hold the key to a long-term world food supply. This nutrient is frequently limiting to crop production, and plants which are able to utilize atmospheric nitrogen are not dependent on synthetic sources. The Haber-Bosch process and other methods of synthesizing ammonia for agricultural use are energy expensive and require enormous capital expenditures for new production facilities (Muller and Newton, 1983). This often limits availability in developing regions.

Use of legume culture has long been an important fertility strategy for worldwide agriculture. Nitrogen fixing legumes supply their own nitrogen requirements and also reduce nitrogen requirements for non-leguminous crops grown next season. As a result, manufactured fertilizers can be avoided or greatly reduced, decreasing capital cost input.

Dry bean (Phaseolus vulgaris L.) is one of many widely grown legumes in the United States and abroad. 1,500,000 acres were planted in the United States in

1986 with over 17,000 acres in Kansas (Kansas Crop and Livestock Reporting Service, 1986). This legume was was chosen as the test species, due to its widespread adaptation and acreage.

Prior research has shown pesticides to be inhibitory to nitrogen fixation and nodulation in dry bean as well as other agriculturally important legumes. Interruption of the symbiosis, from environmental or other factors, can create a nitrogen deficiency resulting in lowered crop yields and quality. Improved understanding of apparent sensitivities to numerous pesticides, would allow for better production decisions and maximum yield.

The objective of these studies was to determine effects of twenty labelled pesticides on nitrogen fixation and nodulation by P. vulgaris.

MATERIALS AND METHODS

Nitrogen Fixation Study

Seeds of dry beans (P. vulgaris 'Bonus')[1] were inoculated with commercially prepared Rhizobium phaseoli[2] prior to planting. Seeds were germinated and

[1] Phaseolus vulgaris 'Bonus' was supplied by Roger's Brothers Seed Company, Twin Falls, ID.

[2] Rhizobium phaseoli peat-based inoculum was supplied by Nitragin Company, Milwaukee, WS.

plants grown for the duration of the experiment in (14.5 x 18.0 cm) cell packs. Seedlings, thinned to one plant per cell, were grown under greenhouse conditions in a sand/loam media (5:1, v/v) and periodically fertilized with a nitrogen-free Hoagland solution (Hoagland and Arnon, 1938).

Plants were grown four weeks before exposure to postemergent fungicides, herbicides or insecticides. All pesticides were applied at triple the manufacturer's recommended rate (Table II-1). A chlorofluorocarbon aerosol propellent was utilized to apply all fungicides and insecticides to the point of run-off, whereas postemergent herbicides were applied over the top of plants in measured amounts of distilled, deionized water. Nodules were harvested from treated and control plants, two and six days after application. Preemergent herbicides were applied at the day of planting and nodules harvested after four weeks growth.

Nitrogen fixation rates of excised nodules of treated and control plants, were determined using modified acetylene reduction techniques described by Hensley and Carpenter (1979). Excised nodules were placed in 18 ml stoppered culture tubes and one cc of air replaced with acetylene. Nodules were incubated

Table II-1. Pesticides and rates (3x manufacturer's recommended rate) evaluated for their influence on nitrogen fixation and nodulation by Phaseolus vulgaris.

Common Name	Trade Name	Rate
FUNGICIDES		
benomyl	Benlate	5.0 g/L
captan	Captan	13.7 kg/ha
chlorothalonil	Daconil 2787	5.0 kg/ha
copper hydroxide	Kocide 101	1.6 kg/ha
maneb	Maneb	6.3 g/L
PCNB	Terrachlor	120.0 kg/ha
thiabendazole	Thiabendazole	13.0 g/L
HERBICIDES		
alachlor	Lasso	13.3 kg/ha
bentazon	Basagran	6.7 kg/ha
chloramben	Amiben	13.3 kg/ha
chlorthal dimethyl	Dacthal	35.0 kg/ha
dinoseb	Premerge 3	12.0 kg/ha
EPTC	Eptam	25.5 kg/ha
sethoxydim	Poast	0.3 kg/ha
trifluralin	Treflan	6.7 kg/ha
INSECTICIDES		
carbaryl	Sevin	5.5 g/L
diazinon	Diazinon	7.5 g/L
dicofol	Kelthane	3.7 g/L
malathion	Malathion	6.3 g/L
fenvalerate	Pydrin	0.7 kg/ha
endosulfan	Thiodan	1.8 g/L

at 26 C for one hour. Samples for analysis were withdrawn using one cc disposable syringes. A Varian 6000 automated gas chromatograph, with a stainless steel Porapak R column was utilized for all assays. Column, injector and ionization temperatures were 50 C, 90 C and 105 C respectively. Nodules were dried for 24 hours at 80 C before weighing.

Acetylene reduction data were expressed in nmoles ethylene per nodule dry weight per hour. One way analysis of variance was performed on data after log transformation (Snedecor and Cochran, 1983).

All treatments and controls were replicated five times and each study repeated. Any pesticide which showed an effect was analyzed at label rate in a separate evaluation, as described earlier.

Nodulation Study

Seeds of P. vulgaris were inoculated, sown and plants cultivated as in the previous nitrogen fixation study. Pesticides and means of application were the same as the prior study, except that postemergent chemicals were applied when seedlings had one set of true leaves. Nodules were excised, counted and weighed four weeks after treatment. All treatments and controls were replicated five times and each study

repeated. Data were statistically analyzed by means of one way analysis of variance.

RESULTS AND DISCUSSION

Nitrogen Fixation Study

No insecticide (Table II-2), fungicide (Table II-3) or herbicide, except bentazon (Table II-4) consistently reduced nitrogen fixation. Bentazon, a postmergent herbicide at 3x label rate, repeatedly depressed nitrogenase rates within 48 hours of application. Nitrogen fixation activity, however, was comparable to controls by the six day evaluation. Bethlenfalvay et al., (1978) found similar results with P. vulgaris, that nitrogen fixation rates were depressed within two days of exposure to bentazon, but recovered by one week. In a separate study bentazon was tested at label rate and proved not to be depressive to nitrogenase activity within two days after exposure (Table II-4).

The temporary recession in acetylene reduction values, in bentazon treated plants, may result from several factors. Bethlenfalvay et al., (1978) reported bentazon application probably reduced translocation of carbohydrates to the nodules. The material may also affect leghemoglobin formation, act on

TABLE II-2. Influence of insecticides (3x rate) on nitrogen fixation (acetylene reduction) by Phaseolus vulgaris two and six days application.

INSECTICIDES	NITROGEN FIXATION[Z] (nmoles C_2H_4/g/hr)
Carbaryl 2 days	24166
Control	19841
Carbaryl 6 days	23326
Control	21107
Diazinon 2 days	3649
Control	2734
Diazinon 6 days	10758
Control	8400
Dicofol 2 days	15444
Control	12249
Dicofol 6 days	13889
Control	12607
Malathion 2 days	43765
Control	36154
Malathion 6 days	18036
Control	16161
Fenvalerate 2 days	1862
Control	1885
Fenvalerate 6 days	989
Control	959
Endosulfan 2 days	3835
Control	4841
Endosulfan 6 days	1703
Control	1295

[Z]There were no statistical differences (F test, 0.05) when treatments were compared to appropriate controls.

TABLE II-3. Influence of fungicides on nitrogen fixation (acetylene reduction) by Phaseolus vulgaris two and six days after application.

FUNGICIDES	NITROGEN FIXATION[Z] (nmoles C_2H_4/g/hr)
Benomyl 2 days	3078
Control	2195
Benomyl 6 days	3054
Control	2172
Captan 2 days	4532
Control	4471
Captan 6 days	2900
Control	4388
Chlorothalonil 2 days	5910
Control	5869
Chlorothalonil 6 days	3542
Control	4659
Copper hydroxide 2 days	2543
Control	2815
Copper hydroxide 6 days	4069
Control	2969
Maneb 2 days	7184
Control	9395
Maneb 6 days	6818
Control	5927
PCNB 2 days	6543
Control	6851
PCNB 6 days	1964
Control	1405
Thiabendazole 2 days	9308
Control	9177
Thiabendazole 6 days	6699
Control	6829

[Z]There were no statistical differences (F test, 0.05) when treatments were compared to appropriate controls.

Table II-4. Influence of herbicides on nitrogen fixation (acetylene reduction) by Phaseolus vulgaris two and six days after application of postemergent herbicides, and after four weeks of growth following application of preemergent materials.

HERBICIDES	NITROGEN FIXATION (nmoles C_2H_4/g/hr)
Preemergent	
Alachlor	7702
Control	6804
Chlorthal dimethyl	7868
Control	6122
Dinoseb	5491
Control	4547
EPTC	13655
Control	11177
Trifluralin	5995
Control	5923
Postemergent	
Bentazon 2 days (3x rate)	756*
Control	2817
Bentazon 6 days (3x rate)	795
Control	1336
Bentazon 2 days (1x rate)	1891
Control	2703
Bentazon 6 days (1x rate)	2554
Control	2771
Sethoxydim 2 days	7260
Control	5643
Sethoxydim 6 days	8667
Control	4766

*Treatment means were significantly different (F test, 0.05) when compared to appropriate controls.

nitrogenase or affect the process in in other ways.

Although most pesticides in this study proved harmless to nitrogen fixation, this was not observed during some earlier investigations. Mallik and Tesfai (1985) found carbaryl and malathion did not alter nitrogen fixation in soybean at recommended rates, but noted a depression at a 10x level. Besides insecticides, alachlor and trifluralin both depressed nitrogenase activity at 5x rate as well as recommended levels. Though this present work showed no effect from these materials at 3x rate, application of much higher rates (5 and 10x) may indeed have damaged nitrogen fixation potential. These extreme rates, however, are unrealistic in a production situation and thus were not tested.

Trifluralin, alachlor (Mallik and Tesfai, 1985) and captan (Rennie et al., 1985) all were found to depress nitrogenase activity in soybean at label rate. However, species differences as well as experimental conditions may explain this discrepancy.

Although nitrogen fixation capacity in P. vulgaris was not harmed in this study, plants were nevertheless affected. All plantings treated at elevated rates of herbicides showed marginal to severe chlorosis and necrosis.

Nodulation Study

No differences were found in nodule dry weight or number with insecticides (Table II-5), fungicides (Table II-6) or herbicides (Table II-7) when compared against appropriate controls.

Adverse effects have been noted on nodule weight and number by other investigators. Carbaryl and malathion at 10x rate showed no depression on nodulation, but alachlor at 5 and 10x rates did depress nodule number and weight in soybean (Mallik and Tesfai, 1985). Again such extreme rates may result in various abnormalities.

Necrosis and chlorosis also resulted from application of herbicides at 3x label rate in this study. Although nodule dry weight and number were not affected by tested pesticides at elevated (3x) rate, this may not have held true had experimental conditions changed or different species been tested.

Summary

Chlorosis and necrosis to plants' foliage resulted from some pesticides applied at elevated (3x) rates, but nitrogen fixation and nodulation were unaffected. Bentazon was the exception. Nitrogenase activity in P. vulgaris was repeatedly depressed within 48 hours after application of this herbicide at

Table II-5. Influence of insecticides (3x rate) on nodulation by Phaseolus vulgaris four weeks after application.

	NODULATION[Z]	
	avg. no. nod./ plant	avg. dry nod. wt./plant (g)
INSECTICIDES		
Carbaryl	12	.0177
Control	15	.0109
Diazinon	21	.0155
Control	18	.0144
Dicofol	16	.0199
Control	18	.0129
Malathion	14	.0175
Control	17	.0190
Fenvalerate	23	.0200
Control	25	.0215
Endosulfan	19	.0167
Control	21	.0199

[Z]There were no statistical differences (F test, 0.05) when treatments were compared to appropriate controls.

Table II-6. Influence of fungicides on nodulation by *Phaseolus vulgaris* four weeks after application.

FUNGICIDES	NODULATION[Z] avg. no. nod./ plant	avg. dry nod. wt./plant (g)
Benomyl	18	.0118
Control	13	.0106
Captan	19	.0180
Control	20	.0117
Chlorothalonil	16	.0109
Control	12	.0104
Copper hydroxide	16	.0162
Control	13	.0134
Maneb	19	.0156
Control	23	.0194
PCNB	15	.0115
Control	18	.0118
Thiabendazole	19	.0200
Control	24	.0211

[Z]There were no statistical differences (F test, 0.05) when treatments were compared to appropriate controls.

Table II-7. Influence of herbicides on nodulation by Phaseolus vulgaris four weeks after application of preemergent chemicals and 6 days after application of postemergent materials.

HERBICIDES	NODULATION[Z] avg. no. nod./ plant	avg. dry nod. wt./plant (g)
Alachlor	18	.0112
Control	21	.0115
Chlorthal dimethyl	16	.0120
Control	14	.0115
Dinoseb	14	.0113
Control	17	.0156
EPTC	19	.0189
Control	15	.0161
Trifluralin	15	.0156
Control	18	.0117
Bentazon	28	.0207
Control	27	.0189

[Z]There were no statistical differences (F test, 0.05) when treatments were compared to appropriate controls.

3x label rate, but returned to levels comparable to controls six days after application. Bentazon applied at label rate, however, showed no effect on the symbiosis within 48 hours or six days. This suggests the herbicide is safe to use when applied as labelled for P. vulgaris.

CHAPTER III

MODE OF ACTION OF BENTAZON IN DEPRESSING NITROGEN FIXATION BY PHASEOLUS VULGARIS

INTRODUCTION

Bentazon, which inhibits the Hill reaction in photosynthesis (Akihiko and Matsunaka, 1975), is widely used for postemergent weed control in legume production. Prior work in this study has shown bentazon, at 3x label rate, to depress nitrogenase activity in Phaseolus vulgaris 48 hours after application. These results are consistent with work by Bethlenfalvay, et al., (1978) who found nitrogen fixation rates to be rapidly depressed in P. vulgaris after treatment with bentazon. The herbicide may disrupt one or more physiological activities in the symbiosis.

LEGHEMOGLOBIN

In 1939, pink coloration in legume nodules was shown to be due to hemoglobin (Kubo, 1939). This protein, referred to as leghemoglobin, can only be found in active root nodules of legumes. The heme group is of bacterial origin and genetic determinants for the apoprotein are contained in the plant (Verma et al., 1974).

Leghemoglobin is associated with oxygen transport necessary for successful symbiotic nitrogen fixation. Bergersen et al., (1979), found when leghemoglobin was introduced to bacteroid suspensions, acetylene reduction was stimulated. These researchers also found detached nodules lost nitrogenase activity when exposed to carbon monoxide.

A study was designed, therefore, to determine if leghemoglobin concentration in nodules of P. vulgaris, was affected by application of bentazon.

CARBOHYDRATES

Several studies have indicated the importance of carbohydrates to the symbiosis. Nitrogenase, which catalyzes the reduction of dinitrogen by bacteroids, depends on host photosynthates to provide substrate (Dilworth, 1974). Further work has shown disruption of nitrogenase activity in the Rhizobium-legume symbiosis may be due to limited photosynthate availability (Hardy and Havelka, 1976).

Schweitzer and Harper, (1980) examined effects of various lengths of darkness on nodulated soybeans. Nitrogen fixation by nodules of plants exposed to continuous darkness at 27 C decreased within three days and ceased by seven days. Bethlenfalvay, et al., (1978) showed nitrogen fixation by P. vulgaris 'Blue

Lake' dependent upon recently translocated photosynthates. There was a strong correlation between a depressed carbon dioxide exchange rate and reduced nitrogen fixation rates in plants treated with bentazon. A study was initiated to determine carbohydrate content in nodules of P. vulgaris after exposure to bentazon.

MATERIALS AND METHODS

NITROGENASE STUDY

Seeds of P. vulgaris were inoculated, sown and seedlings grown as described earlier. Nodules of P. vulgaris, previously untreated, were detached from four-week-old plants and placed in 100 ml flasks containing 0, 1, 3 and 5x label rates of bentazon (0.0, 12.5, 37.5 and 62.5ml/L respectively). This was conducted to determine direct effects of bentazon on nitrogenase. Nodules were vacuum infiltrated by faucet aspiration for one hour to allow for penetration of the herbicide. Upon infiltration, nodules were removed from flasks, blotted dry and placed in 18 ml culture tubes. One cc of air was replaced with acetylene and nodules allowed to incubate one, two and three hours before samples were withdrawn for gas chromatography. Five replications were used and the

study repeated. Data were analyzed by use of two and one way analyses of variance.

LEGHEMOGLOBIN STUDY

Seeds of P. vulgaris were inoculated, sown and plants allowed to grow four weeks as before. Plants were treated prior to harvest with a 3x rate of bentazon as described earlier. Nodules were harvested two and six days after exposure for determination of leghemoglobin concentration.

Concentration of this hemoprotein was determined by methods described by Appleby and Bergersen, (1980). Nodules were detached from root systems and placed in 0 C 0.1M sodium phosphate buffer at pH 7.4, then mixed with 1-3 volumes of air equilibrated 0.1M sodium phosphate buffer containing 1.0mM EDTA at pH 7.4. At this point, nodules were macerated and the resulting brei strained through a triple layer of cheesecloth, whereby nodule debris was discarded.

Centrifugation at 10,000g for 30 minutes was used to clarify the filtrate. The supernatant was brought to 55% saturation with ammonium sulfate and centrifugation was again conducted as before. The mixture was then brought to 80% saturation, again by use of ammonium sulfate, and centrifugation performed as above.

After three centrifugations, leghemoglobin precipitate was dissolved in 50mM ammonium sulfate buffer, again containing 1mM EDTA with pH 7.4. The solution now placed in dialysis tubes, were sealed and dialyzed against the same buffer for four hours in refrigeration (0 C).

Leghemoglobin concentrations were determined by use of pyridine reagent assays. Using alkaline pyridine reagent (4.2M pyridine in 0.2 M NaOH) to which an equivalent volume of leghemoglobin was added, the mixture was divided between two cuvettes. One cuvette was reduced and the other oxidized by a few crystals of sodium dithionite and potassium hexacyanoferrate respectively.

Values A_{556nm} minus A_{539nm} were recorded using a Beckman 25 spectrophotometer. Spectral differences were measured, compensating for a crude leghemoglobin extract obtained. Three replications were used and the study repeated. Data were analyzed by one way analysis of variance.

CARBOHYDRATE STUDY

Seeds of dry bean were inoculated, sown and seedlings grown as described earlier. In a preliminary study, plants were treated with bentazon at 3x rate at

four weeks of age and placed in a darkened growth chamber at 27 C. Acetylene reduction rates were monitored daily with activity ceasing within six days. This is presumably attributable to lack of photosynthate for bacteroid nourishment (Wheeler, 1971).

Upon this six day period, plants were removed for examination of their nitrogen fixation potential. Nodules were then excised, and vacuum infiltrated one hour with 0.0, 0.5, 1.0 and 2.0M sucrose. Acetylene reduction assays were performed at one, two and three hour intervals, to determine if nitrogen fixation could be restored in plants treated with bentazon. Treatments were replicated five times, and data analyzed by three and one way analyses of variance.

In a separate study plants treated with bentazon were again placed in a darkened growth chamber as described earlier. However, plants were not treated with bentazon until four days after their placement in the growth chamber. Plants remained in darkness an additional two days and as before nodules were excised, vacuum infiltrated and subjected to sucrose concentrations. Nodules were then assayed for nitrogenase activity as before.

In yet another study, plants were grown in the

greenhouse as described earlier and treated with bentazon. Two days after exposure, nodules were infiltrated with sucrose and assayed for nitrogenase activity as described above.

RESULTS/DISCUSSION

NITROGENASE STUDY

Two way analysis of variance showed significant differences among treatment and time main effects, but no significant interactions (Table III-1)(Appendix 1). Acetylene reduction by nodules imbibed with the highest herbicide rate (5x) was significantly less than all other treatments (Table III-1). There were, however, no significant differences between other herbicide rates. Nitrogen fixation rates were significantly greater after three hours than one and two hours.

Concentrations of herbicide within the nodules are likely many times greater than what would occur from foliar application and absorption.

Even at these amplified levels, there were no short-term effects on nitrogenase detected, except the highest concentration (5x). This indicates acetylene reduction depression within 48 hours after application may be due to factors other than disruption

TABLE III-1. Acetylene reduction (nmoles C_2H_4/g/hr.) by nodules of *P. vulgaris* vacuum infiltrated with 0.0, 12.5, 37.5 and 62.5 ml/L rates of bentazon.

Treatment	nmoles C_2H_4 g/dry nod. wt.
	Treatment Main Effects
Control	11722a[Z]
Bentazon 1x	10880a
Bentazon 3x	12427a
Bentazon 5x	9100b
Exposure Time (hours)	Time Main Effects
1	9535a
2	10912a
3	12648b

[Z]Mean separation utilizing Newman-Keul, 5% level. Means within each main effect column followed by the same letter are not significantly different.

of the enzyme itself. The enzyme functioned in the presence of the herbicide for only a short period. However, longer exposure may have resulted in disruption of present enzyme or interruption in synthesis of replacement nitrogenase.

LEGHEMOGLOBIN STUDY

Foliar application of an elevated (3x) rate of bentazon did not affect leghemoglobin concentrations in nodules of P. vulgaris after two or six days exposure (Table III-2). Apparently, oxygen transport or other systems involved are not interrupted by the presence of this herbicide. Kulkarni, et al., (1974) found foliar application of carbofuran, thimet, dasanite and heptachlor did not significantly affect leghemoglobin content in peanut.

CARBOHYDRATE STUDY

Results of the preliminary study indicate acetylene reduction in nodules of P. vulgaris is unaffected after six days exposure to bentazon, supporting earlier work (Table III-3). However, a significant interaction occurred between sucrose and herbicide concentrations (Table III-4) (Appendix 2). This study, however, did not address effects on nitrogenase 48 hours after exposure when differences have

Table III-2. Leghemoglobin concentration (nmoles lb/g nod.) of nodules of P. vulgaris, two and six days after foliar application of bentazon at 37.5 ml/L.

HERBICIDE TREATMENT	LEGHEMOGLOBIN CONCENTRATION
Study 1	nmoles lb/g nodule
Bentazon 2 days	.0709[Z]
Control	.0883
Bentazon 6 days	.0712
Control	.0621
Study 2	
Bentazon 2 days	.0840[Z]
Control	.0695
Bentazon 6 days	.0792
Control	.0806

[Z]There were no statistical differences (F test, 0.05) when treatments were compared to appropriate controls.

Table III-3. Influence of sucrose concentrations (0.0 0.5, 1.0 and 2.0M) and bentazon at 37.5 ml/L on nitrogen fixation by P. vulgaris.

Treatment	nmoles C_2H_4 g/dry nod. wt.
	Treatment Main Effects
Herbicide	960a[Z]
Control	936a
Concentration	Sucrose Main Effects
0.0M	13a
0.5M	729b
1.0M	910c
2.0M	1208d
	$y = (543.1)x + 239.8$
	$r^2 = 0.91$
Exposure Time	Time Main Effects
1.0	881a
2.0	945a
3.0	1020b
	$y = (69.5)x + 809.6$
	$r^2 = 0.99$

[Z]Mean separation utilizing Newman-Keul, 5% level. Means within each main effect column followed by the same letter are not significantly different.

Table III-4. Influence of bentazon (37.5 ml/L) and sucrose concentrations (0.0, 0.5, 1.0 and 2.0M) six days after herbicide application.

	nmoles C_2H_2/g/hr.			
	0.0M	.5M	1.0M	2.0M
Herbicide	20.1a[Z]	805c	910c	1166d
Control	5.9a	652b	908c	1249d

[Z]Mean separation utilizing Newman-Keul, 5% level. Means followed by the same letter are not significantly different.

been shown to occur. This was due to unanticipated carbohydrate storage of nodules.

Additional studies, described earlier, allowed for examination of carbohydrate depleted nodules two days after exposure to bentazon at 3x label rate. Significant differences occurred between sucrose concentrations when nodules were examined from plants grown in the dark (Table III-5) (Appendix 3). Also, nitrogen fixation activity in bentazon treated nodules was consistently lower than in nodules from plants vacuum infiltrated with distillied water. A companion study conducted with light-grown plants showed no significant differences among sucrose concentrations at any time period (Table III-6) (Appendix 4). Although sucrose was being utilized in control as well as bentazon treated nodules, nitrogenase activity was still significantly depressed in nodules from plants exposed to bentazon at 3x label rate 48 hours earlier. This suggests that carbohydrate defiency may not be a factor in the depression noted earlier in the symbiosis under these conditions. No significant interactions occurred between treatments and sucrose concentrations in either dark or light studies.

Table III-5. Effect of bentazon at 3x label rate 48 hours after exposure to nodules of P. vulgaris grown in the dark and infiltrated with sucrose at 0.0, 1.0 and 2.0 M concentrations.

Sucrose	Control	Bentazon	Mean
0.0	159	71	115 A[Z]
0.5	342	109	225 AB
1.0	533	237	385 B
2.0	553	411	482 C
Mean	397 A[Y]	207 B	

[Y]Means in row followed by different letters are significantly different (F test, 0.05).

[Z]Means followed by different letters are significantly different (Newman-Keul, 0.05).

Table III-6. Effects of bentazon at 3x label rate 48 hours after exposure to nodules of P. vulgaris grown in the light and infiltrated with sucrose at 0.0, 1.0 and 2.0 M concentrations.

Sucrose	Control	Bentazon	Mean
0.0	11871	6818	9344 NS[Z]
0.5	14368	6959	10663 NS
1.0	10890	7337	9113 NS
2.0	12956	10436	11696 NS
Mean	12521A[Y]	7887B	

[Y]Means followed by different letters are significantly different using F test, 0.05.

[Z]NS indicates no significant differences among means (Newman-Keul, 0.05).

SUMMARY

No short-term effects on nitrogenase were evident, from directly exposing nodules to bentazon, except where the herbicide was studied at a 5x rate. Even so, this high concentration of herbicide would probably not be found in nodules during normal field applications.

Leghemoglobin concentration or activity in nodules exposed to bentazon two and six days earlier, was apparently not affected. This suggests this hemoprotein functions normally in the presence of bentazon, and does not contribute to the depression in nitrogenase activity.

No effects were evident on carbohydrate supply when bentazon was applied at 3x rate to P. vulgaris, after six days exposure.

Further work demonstrated that regardless of sucrose supply, treated plants could not regain nitrogenase activity comparable to plants treated with distilled water. This suggests that carbohydrate supply may not be a factor in the depression noted earlier.

However, work by Bethlenfalvay et al.,(1978) showed a positive correlation in inhibition of carbon dioxide exchange rate and nitrogen fixation rate in

dry bean. They concluded, therefore, bentazon probably does not affect root nodules directly, but rather restricts the availability of photosynthates. This is reasonable since bentazon is known to disrupt the Hill reaction possibly creating a reduction in carbohydrates moving to the nodules.

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Appendix 1. Anova table for nitrogenase study.

Source	DF	Sum of Sq.	Mean Sq.	F	P>F
Treat	3	9.264826E+07	3.088276E+07	6.75	0.00
Time	2	9.72248E+07	4.86124E+07	10.63	0.00
TT	6	4002278	667046.3	0.15	0.99
Model	11	1.938753E+08	1.762503E+07	3.85	0.00
Error	48	2.195564E+08	4574091		
Adj Tot	59	4.134317E+08	7007317		

Appendix 2. Anova table for preliminary carbohydrate study.

Sour.	DF	Sum-Sq.	MEAN-Squares	F	P > F
Treat.	1	12721.1	12721.1	.45	.500
Sucrose	3	3505663	1168871	62.08	0.000
Time	2	288320.5	144160.3	5.11	.009
TrS	2	214195.7	107097.8	3.79	.026
TrT	2	10623.83	5311.916	.19	.818
ST	4	22857.78	5714.444	.2	.934
TrST	4	54434.3	13608.57	.48	.752
ERROR	72	2032834	228233.8		
MEANS					

Appendix 3. Anova table for carbohydrate study conducted in a darkened growth chamber.

Source	DF	Sum of Suares	Mean Square	F	P > F
Treat.	1	359671	359671	12.83	0.00
Sucrose	3	801407	267135	9.53	0.00
TS	3	64795	21598	0.77	0.50
Model	7	1225874	175124	6.25	0.00
Error	32	896973	28030		
Adj. Tot.	39	2122848	54432		

Appendix 4. Anova table for carbohydrate study conducted in light.

Source	D.F.	Sum of Squares	Mean Square	F	P > F
Treat.	1	2.1473+08	2.1473+08	24.30	0.00
Sucrose	3	4.3654+07	1.4552+07	1.65	0.20
TS	3	3.3784+07	1.1261+07	1.27	0.30
Model	7	2.9217+08	4.1739+07	4.72	0.00
Error	32	2.8281+08	8838040		
Adj. Tot.	39	5.7499+08	1.4743+07		

EFFECTS OF PESTICIDES ON NITROGEN FIXATION AND NODULATION BY DRY BEAN (*PHASEOLUS VULGARIS* L. 'BONUS')

by

MICHAEL ALLEN SCHNELLE

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Effects of seven fungicides, seven herbicides and six insecticides on nitrogen fixation and nodulation by dry bean Phaseolus vulgaris L. 'Bonus') were investigated. All pesticides examined were found inocuous to nitrogen fixation (acetylene reduction) except bentazon, a postemergent herbicide. There were no differences in nodulation determined from any pesticides applied.

Bentazon at (37.5 ml/L) 3x label rate depressed nitrogen fixation rates within forty-eight hours after application, however, rates recovered and were comparable to control plants after 6 days. No effects were observed on nitrogen fixation when bentazon was applied at 12.5 ml/L (label rate).

Many of the herbicides, though not depressing or inhibiting nitrogen fixation or nodulation, did stunt plants and create necrotic lesions on the plants' canopy when applied at the higher (3x) rate.

Bentazon's possible mode of action on nitrogen fixation in P. vulgaris was investigated, by examining direct effect of the herbicide on nitrogenase activity, on leghemoglobin concentration and on carbohydrate supply to plants' nodules.

Bentazon applied to nodules at 0.0, 12.5, 37.5 and 62.5 ml/L by vacuum infiltration, was conducted to

observe direct effects of this herbicide on nitrogenase in P. vulgaris. Upon infiltration of the herbicide for one hour, acetylene reduction was conducted one, two and three hours later. No effects were observed, except with bentazon at 5x (62.5 ml/L) rate on acetylene reduction rates which increased linearly over time as expected.

Leghemoglobin concentration in nodules of P. vulgaris was investigated by use of spectrophotometry. Leghemoglobin concentration, of nodules examined two and six days after exposure to bentazon at 3x rate, repeatedly proved comparable to appropriate controls. This study indicates no contribution of leghemoglobin malfunction or synthesis inhibition in reduced nitrogen fixation rates observed earlier.

Another study was undertaken to examine carbohydrate levels in P. vulgaris exposed to bentazon (3x). Treated plants were grown in a darkened growth chamber for six days. Upon this time, nodules were assayed for nitrogenase activity with and without the addition of sucrose at 0.0, 0.5, 1.0 and 2.0M concentrations. Sucrose levels were found to significantly differ, with 2.0M concentration providing the greatest recovery in nitrogenase activity in carbohydrate depleted nodules.

Further work was conducted to examine carbohydrate depleted nodules exposed to bentazon at 3x label rate 48 hours earlier. Again nodules were infiltrated with sucrose concentrations as described earlier in studies with plants grown in darkness as before and in light. In both studies, nodules from bentazon treated plants proved significantly lower in nitrogenase activity than in control plants. Sucrose concentrations differed significantly in terms of their effect on nitrogen fixation activity in the dark study, but these concentrations were statistically equivalent in the light study. No significant interactions occurred between sucrose levels and treatments in either light or dark studies.

Although these studies do not indicate potential modes of action in bentazon depressing nitrogen fixation in P. vulgaris, other work has suggested the herbicide restricts photosynthate availability to legume nodules.