EFFECTS OF TREFLAN AND THIRAM AND THEIR INTERACTION ON NODULATION, NITROGEN FIXATION, AND YIELD OF INOCULATED AND UNINOCULATED GARDEN BEANS (*PHASEOLUS VULGARIS* L.)

by

SRINIVAS KOMANDURI
Bachelor of Science (Agriculture) 1977
A.P. Agricultural University, Hyderabad, INDIA

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1979

Major Professor.

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To my Parents

WITH LOVE

ACKNOWLEDGMENTS

I would like to express my sincere appreciation to Dr.Carl.D. Clayberg, Professor of Horticulture for his guidance and encouragement as Chairman of my graduate committee.

A special thanks is due to Dr. Charles W. Marr, Extension Horticulturist, for his generous contribution of time and photographic skills and equipment and for his additional assistance while serving on my committee.

Sincere appreciation is also extended to Dr. Peter Wong for his advice as a member of my committee and to Dr. George A. Milliken for his assistance in statistical analysis of the data, and to all others who have helped me in this research at one time or another.

Finally I am forever indebted and grateful to my parents and to my brother whose encouragement and sacrifice made my graduate study possible.

CHEMICAL NAMES OF PESTICIDES USED IN THE LITERATURE REVIEW

Chemical names of pesticides used in the literature review.

FUNGICIDES :

Trade Name or Common Name	Chemical Name
1. Thiram	Tetramethylthiuram disulfide
2. Semesan	Hydroxymercurichlorophenol
3. Bavistan (Carbendazin)	2-(Methoxycarbonylamino)-benzimidazole
4. Captan	cis N((Trichloromethyl)thio)-4-cyclohexene-1, 2-dicarboximide
5. Dithane M-45	Zinc + 80% Manganese ethylenebisdithiocarbamate
6. Ceresan	Ethylmercuric chloride
7. Spergon	2,3,5,6-Tetrachloro-1,4-benzoquinone
8. Phygon	2,3,-Dichloro-1,4-napthoquinone
9. Rhizoctol	Methylarsinic sulfide
10.Vitavax	2,3-Dihydro-5-carboxyanilide-6-methyl-1, 4-oxathiin
11.Terracoat L 205	Terrazole + Terraclor (PCNB)
12.Terrazole	5-Ethoxy-3-trichloromethyl-1,2,4-thiadiazole
13.T.C.M.T.B.	2-(thiocyanomethylthia)benzothiazole
14.Benlate (Benomyl)	Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate
15.Dexon	Sodium (4-(dimethylamino)phenyl) diazene sulfonate
16.Manzate	Manganese ethylene bis dithiocarbamate
17.Saadtan	N-(trichloromethylthio)cyclohex 4-ene dicarboximide + phenyl mercury acetate
18.Brassicol (PCNB)	Pentachloronitrobenzene

Trade Name or Common Name	Chemical Name
19. Oxycarboxin	5,6,-Dihydro-2-methyl-1,4-oxathin-3-carbox-anilide-4,4-dioxide
20. Ethylan	1,1-Dichloro-2,2-bis (4-ethylphenyl)ethane
21. Dodine	n-Dodecylguanidine acetate
22. H.P.M.T.S.	2-hydroxypropyl methanethio sulfonate
23. Ethirimol	5-Butyl-2-ethylamino-4-hydroxy-6-methylpyrimidin
24. Tridemorph (Calixin)	N-Tridecyl-2,6-dimethylmorpholine
25. Triforine	N,N-(1,4-Piperazinediylbis(2,2,2-Trichloro-ethylidene))-bis-(Formamide)
26. Thiophanate Methyl	Dimethyl ((1,2-phenylene)bis-(iminocarbonothioyl)) bis(carbamate)
27. Botran (DCNA)	2,6-Dichloro-4-nitroaniline
28. Difolatan	cis-N-((1,1,2,2-Tetrachloroethyl) thio)4-cyclo-hexene-1,2-dicarboximide
29. Milcurb (Dlmethirimol)	5-n-Butyl-2-dimethylamino-4-hydroxy-6-methyl-pyrimidine

HERBICIDES

30. Simazine	2-chloro-4,6-bis(ethylamino)-s-triazine
31. Atrazine	2-Chrolo-4-ethylamino-6-isopropylamino-s-triazine
32. Aretit	0-acetyl-2-sec-butyl-4,6-dinitrophenol
33. 2,4-D	2,4,-Dichlorophenoxy acetic acid
34. MCPA	((4-chloro-o-tolyl)oxy) acetic acid
35. 2,4-D_B	4-(2,4-dichlorophenoxy)butryic acid
36. Dalapon	2,2-dichloropropionic acid

Trade Name or Common Name	Chemical Name
37. Treflan	α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine
38. Nitralin	4(Methylsulfonyl)-2,6-dinitro-N,N-dipropyl-aniline
39. Alachlor (Lasso)	2-chloro-2',-6'-diethyl-N-(methoxymethyl) acetanilide
40. Chloramben (Amiben)	3-Amino-2,5-dichlorobenzoic acid
41. DCPA (Dacthal)	Dimethyl tetrachloroterepthalate
42. Dinoseb	2-sec-Butyl-4,6-dinitrophenol
43. Chloroxuron	3-(p-(p-chlorophenoxy)phenyl)-1, 1-dimethylurea
44. Afalaon (Linuron)	3-(3,4-Dichlorophenyl)-1-methoxyl-1-methylurea
45. Troptox (MCPB)	4-((4-Chloro-o-tolyl)Oxy)butryic acid
46. Aresin	N-(4-Chloro-phenyl-N'-methyl)-N'-methoxy-N' -methylurea
47. Cotoran	1,1-Dimethyl-3-(α,α,α-trifluoro-m-tolyl)urea
48. Paraquat	1,1-'Dimethyl-4,4'-bipyridinium ion
49. Bentazon	3-Isopropyl-1 H-2,1.3-benzothiadiazin-(4) -3 H one-2,2-dioxide
50. Amitrole	3-Amino-1,2,4-triazole
51. 2,4,5T	2,4,5-Trichlorophenoxy acetic acid
52. Caparol (Prometryne)	2,4-bis(isopropylamino)-6-(methylthio)-s -triazine

<u>INSECTICIDES:</u>

53. Lindane	γ isomer of 1,2,3,4,5,6-hexachlorocyclohexane
54. D.D.T.	Dichloro Diphenyl Trichloro Ethane

Trade Name or Common Name	Chemical Name
55. B.H.C.	Benzene Hexachloride
56. Phorate (Thimet)	0,0-diethyl-S-ethylmercaptomethyl dithiophosphate
57. Endrin	Hexachloroepoxyoctahydro-endo,endo-dimethano-nephthalene
58. Chlorazine	2-Chloro-4,6-bis(diethylamino)-s-triazine
59. Temik	2-Methyl-2(methylthio)propionaldehyde)-(methylcarbamoyl)oxime
60. Dursban	0,0-diethyl 0-(3,4,6-trichloro-2-pyridyl)-phosphorothioate
61. Birlane	2-chloro-1-(2,5-dichlorophenyl)-vinyl diethyl phosphate
62. Nemafos	0-0-Diethyl 0-2-pyrazinyl phosphorothioate
63. Terracur	0,0-Diethyl 0-(P-(methylsulfinyl)phenyl) phosphorothioate
64. Bayluscide	5,2'-dichloro-4'-nirtosalicylanilide, ethanolamine salt
65. Nemacur	Ethyl 3-methyl-4-(methylthio)phenyl (1-methylethyl)phosphoramidate

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LITERATURE REVIEW

LITERATURE REVIEW

Several fungicides, herbicides, insecticides, and other pesticides have been studied for their effects on nodulation, nitrogen fixation, yield, and different other parameters of various leguminous crops, and their effect varied with the concentration of the chemical used, the legume crop, environmental conditions and soil.

A: EFFECTS OF FUNGICIDES ON LEGUME NODULATION AND YIELD.

The effects of most of the fungicides studied, indicated an inhibitory action of the chemical on nodulation, nitrogen fixation, yield, and other characters of different legumes. There have been however, a few reports of fungicides being stimulatory, or having no effect on nodulation, nitrogen fixation and yield of legumes.

Puppin and Decosta (31) reported a reduction in nodule number, nitrogen fixation, and yield of inoculated beans (Phaseolus) treated with Semesan and Neantina under greenhouse conditions. They attribute the injury to the mercury content in the chemicals used. Misra and Gaur (23) found Ceresan at normal concentrations significantly reducing nodulation, nitrogen uptake, plant growth, and yield of Cicer arietinum in a greenhouse study. Brinkerhoff et al.,(7) reported Spergon, Phygon, and Arasan tending to reduce or in some instances nearly eliminating nodulation in non-infested soils, when soybeans and cowpeas were grown from treated, inoculated seeds under field conditions. They found no evidence that the addition of nodule inoculam to the seed will decrease the protective ability of the seed treatment fungicides.

Kadow et al.(19) found a reduction in nodulation of English peas treated with semesan. Arasan and Spergon were also reported to be reducing yield of inoculated soybeans under field conditions on a soil having no previous legume bacterium, Sherf and Reddy (36). Afifi et al., found Phygon, Ceresan, TMTD, Orthocide 75, and Rhizoctole harmfull to most of the rhizobium species in culture. They concluded that these fungicides would have deleterious effects on legume nodulation and consequently on nitrogen fixation. Evaluating the toxicity of 13 commonly used seed dressing fungicides on 2 strains of cowpea group bacteria, Staphorst and Strijdom (38) reported fungicides Brassicol, Terracoat L205 and Thiram 50 were least toxic when applied to seed of Vigna anguiculata before inoculation with a peat based inoculant. The other fungicides Benlate, Botran-Difolatan 50:50, Captan, Dexon, Dithane S.P.C., Manzate, Saadtan, T.C.M.T.B.+H.P.M.T.S., and Vitavax either impaired or prevented nodulation with atleast one of the strains tested. They also found that the relative effects of the fungicides tested on nodulation were less marked when uninoculated V.anguiculata seeds were planted in sand containing rhizobia.

Kranth and Vasanthrajan (21) applied Dexon to sunn hemp seeds in pots and found germination, root elongation, and nodulation being adversely affected. However, rhizobium in culture media was found to be resistant to this fungicide. They concluded that the inhibitory effect of Dexon is due to its effect on plant metabolism rather than on rhizobia. In a field study, Nitragin Co.(3) found very little reduction in soybean nodulation between inoculated and uninoculated seeds treated with Arasan.

Fisher (12) found Thiram, Oxycarboxin, and Ethylan C.P., affecting nitrogen fixation of white clover in pots, while Benomyl, Captan, Carbendazin, Carboxin, Dodine, Dimethirimol, Ethirimol, Tridemorph, Triforine or Thiophanate methyl had no effect. None of these fungicides had any effect on leghaemoglobin content, or on rhizobium in vitro. Increase in nodulation of peas treated with Thiram, Captan, Bavistan, Ceresan, or Dithane M-45, at 0.25% concentration was reported by Singh et al.(37), Various parameters associated with nitrogen fixation like nodule dry wt., leghaemoglobin content were significantly influenced by the treatments. Bavistan and Dithane M-45 treatments had a marked increase in nodulation and leghaemoglobin synthesis. Appleman (4) obtained good nodulation with Semesan in English peas and soybeans. Ceresan was found to reduce nodulation in peas but not in soybeans. Soil treatment with Thiram resulted in an increase in bacterial population and decrease in fungal counts, Richardson (33). Vlitos and Preston (43) also found an increase in nodulation of inoculated soybeans and cowpeas treated with Spergon, Phygon, and Arasan in greenhouse conditions.

B: EFFECTS OF HERBICIDES ON LEGUME NODULATION AND YIELD.

Several herbicides were found to be inhibitory to legume nodulation at various concentrations.

Payne and Fults (30) found 2,4-D preventing nodulation in common beans at a concentration of 0.075lbs/acre in a greenhouse study. Salem et al.(34) reported Cottoran to be harmfull to both the inoculated broad bean plants and to the bacterium alone under greenhouse conditions. This was felt due to the high concentration of the chemical used. Misra and Guar (23) reported a drastic reduction in nodulation, plant growth, and nitrogen fixation of Cicer, when Treflan was applied as a pre-plant herbicide at normal concentrations in a greenhouse study.

Carlyle and Thorpe (8) found a concentration of 0.5 ppm of 2,4-D in buildders sandy soil seriously affecting nodulation and growth of some legumes studied, causing partial inhibition of germination of red clover and alfalfa and delayed germination of beans and peas. Avrov (5) reported a reduction in nodule number and size of pea nodules due to Prometryne, Simazine was however found to be stimulatory to lupine nodule bacteria.

Dunigan et al.(10) reported no detrimental affects of Alachlor, Treflan, Linuron, DCPA, Prometryne, and Chloramben in field and greenhose studies on soybeans. Dalapon was also found to have had no affect on nodule number and nodule weight of birds foot trefoil (Lotus corniculatus), Garcia and Jordan (13). Hauke (17) found Afalon, Aretit, Chwastox, and Embutox reducing yield of peas and serradellas under greenhouse conditions. very little residues of Simazine was found to be harmfull to nodulation and nitrogen fixation of peas.

Elfadi and Fahmy (11) found no significant effect of 2,4-D, or MCPA on nodulation of inoculated cowpeas until the concentration reached to 3 lbs/feddan (about an acre) in greenhouse studies. Kaszubiak (20) observed considerable diffrenece in the suceptability of the slow and fast growing strains of rhizobium to 17 herbicides.

Szabo (40) found a reduction in number and size of pea nodules by Aretit and A-114. Formation of Pseudonodules on roots of lucerne by Dalapon and Amitrole was observed by Lakshmikumari et al. (22) in a greenhouse study. Allen et al. (2) also observed pseudonodules on several leguminous crops tretaed with 2 bromo-3,5-dichlorobenzoic acid.

Increase in nodule number of beans by Prometryne in a field study was reported by Rankov et al. (32). Nutman (26) also reported enhanced rate of nodulation by Dalapon in inoculated red clover.

Overall the effects of the herbicides were found to be mixed, being inhibitory in most cases, or having no effect on nodulation, nitrogen fixation, or yield in others. However, since most herbicides studied have been replaced by new herbicides which are more toxic chemically, and are hence being more widely used, there is a necessicity to study the effects of these new chemicals on nodulation, nitrogen fixation, and yield of legumes.

C : EFFECTS OF INSECTICIDES AND OTHER PESTICIDES ON LEGUME NODULATION AND YIELD.

Very few studies have been reported on the effects of insecticides and other pesticides on nodulation, nitrogen fixation or yield of legumes in comparison with either fungicides or herbicides, and most of the reports indicated either a stimulatory or no effect of the chemicals on legume nodulation and yield.

Misra and Gaur (23) found Lindane reducing nodulation, nitrogen fixation, and yield of Cicer arietinum in greenhouse studies. Salem et al. (34) found Birlane, Dursban, Temik, and Thimet analog affecting the formation of effective nodules on common beans (vicia faba) in different concentrations,under greenhouse conditions, nodulation was very much affected at higher concentrations, due to the accumilation of the chemical in the soil. Nematicides, Nemafos and Terracur, and molacide, Bayluscide were also found to have the same effect as the insecticides. In another study Salem et al. (35) and Taha et al. (41) found an increase in nodulation and nitrogen fixation of white clover, lucerne, broad beans and lentil plants with Lindane, Dyfonate, Endrin, and Dipterex.

Magu et al. (24) reported no toxic effect of Lindane at normal rates on nodulation, and plant growth of Cicer arietinum in a pot culture experiment. At high rates, they found an in inhibition in nodulation, plant growth, and seed weight. DDT at normal and 10X normal dosage was found to have no effect on nodulation, leghaenoglobin content nitrogen uptake, and yield of Phaseolus aureus in greenhouse studies, Pareek and Gaur (28).

Puppin and dacosta (31) found no effect of Gesarol 33, Carunchol 50, Malagran and Phostoxin on rhizobium or nitrogen fixation of beans in a greenhouse study. Payne and Fults (30) reported DDT reducing root nodulation of common beans, in a greenhouse study, they found Colorado 9, an insecticide similar to DDT not having any affect on nodulation, evan at higher concentrations.

D : EFFECTS OF RHIZOBIAL INOCULATION AND NITROGENOUS FERTILIZERS ON LEGUME NODULATION AND YIELD.

Inoculation of legumes before sowing was found to be enhancing yields in some crops, but application of nitrogenous fertilizers to legumes did not result in any increase in yields.

Sundara rao (39) reported an increase in yield of peas and Cicer on inoculation with a general rhizobial strain. Increase of yield varied with the cultivar, the bacterial strain and the location. Bajpai et - al.(6) also found inoculation increasing yield of berseem (Trifolium), cowpea and peanuts to the extent of 74, 46, and 21% respectively. Yield was also high in inoculated soybeans and Cicer. Inoculation of peanuts with a general strain did not have any affect on yield, Gaur et al. (14). Nagaraj rao (25) working with four strains of peanut rhizobium, found no significant increase with any strain in pod yield under greenhouse conditions, while there was a significant increase in pod yield and nitrogen uptake under field conditions with one of the four strains. This was concluded to be due to the compatability of the strain with the host. Variable increase in yield of haricot beans on inoculation with a local rhizobial strain was reported by Habish and Ishaq (16).

Guss & Dobereiner(15)found inoculation having no affect on nodulation and plant growth of Phaseolus. Application of nitrogenous fertilizer also did not have any influence on nodulation or plant growth. Inoculation was found to be increasing yield and protein content of one soybean variety while having no affect on another variety,in a greenhouse study. Ammonium sulfate and amm.nitrate increased yield of one -

soybean variety, Puppin and dacosta (31). Hardaker and Hardwick (18) found inoculation giving inconsistant results. Reasons advanced for this, include invasion of the plant by indeginous but ineffective bacteria or the failure of the inoculation process in Phaseolus vulgaris.

Das and Bhaduri (9) working with four strains of Rhizobium phaseoli on five cultivars of Phaseolus vulgaris found competetion between strains for nodule sites. They concluded that specific host preferentiality towards different strains of bacteria constitute a primary limiting factor in its inter strain competetion for the sites in different cultivars of P.vulgaris. Variable increase in nodulation and yield of soybeans with different strains of Rhizobium was reported by Patil et al.,(29). Poor nodulation with one bacterial strain was thought to be due to absence of viable cells in the inoculant which was stored for 2 months. They stressed the need for the selection of an effective strain to match different cvrs. of soybeans and other legumes, and to different agroclimatic conditions.

Olsen et al.(27) found no increase in yield of soybeans due to application of ammonium nitrate in field conditions, higher rates of mmonium nitrate resulted in a reduction in soybean nodulation and plant height.

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MANUSCRIPT

MANUSCRIPT

This manuscript is written in the style of and for publication in HortScience.

Effects of Treflan and Thiram and their interaction on Nodulation, Nitrogen Fixation, and Yield of Inoculated and Uninoculated Garden Beans[1]

S.Komanduri, C.D.Clayberg, and C.W.Marr[2]

Department of Horticulture
Kansas State University
Manhattan, KS 66502.

Additional index words : *Phaseolus vulgaris*, tetramethylthiuram - disulfide, α,α,α-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toludine.

Abstract : Treflan, a pre-emergence herbicide at 1.41 l/ha, inoculation with *Rhizobium phaseoli*, and application of ammonium nitrate at 33.65 kg/ha did not have any effect on nodulation, nitrogen fixation, or yield of garden beans (*Phaseolus vulgaris* L.) under field conditions. Thiram, used as a seed treatment fungicide at 1.26 g/kg seed reduced early nodulation but had no effect on dry seed yield or nitrogen fixation. Two interesting interaction effects were observed, i) Interaction between Treflan and Thiram resulted in a significant increase in dry seed yield due to Thiram treatment, only in absence of Treflan, ii) Interaction between Treflan and *Rhizobium* also resulted in a significant increase in root nodule volume, 64 days after planting in treatments with *Rhizobium* and Treflan and *Rhizobium* inoculation alone.

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[1]Received for publication Contribution No. Kansas Agricultural Experimental Station, Kansas State University, Manhattan, KS 66506.

[2]Graduate Student and Research Horticulturists.

Many legumes produce root nodules that are efficient in fixing atmospheric nitrogen through symbiosis with Rhizobium. This is not true of *Phaseolus vulgaris* L., for which application of nitrogenous fertilizer at low rates is commonly recommended. No particular reason has been advanced for this poor nitrogen fixing ability. However it is possible that agricultural practices, particularly the use of various pesticides in a crop protection scheme are toxic to Rhizobium bacteria, thus reducing the nitrogen fixing capability.

It has been reported that Treflan, a commonly used herbicide on beans, suppressed nodulation, nitrogen fixation, plant growth, and yield in *Cicer arietinum* (20). Several other herbicides also reduced nodulation, nitrogen fixation and yield of beans, peas, soybeans, and other legumes (9,11,20, 24,29). Seed treatment of some legumes including beans, with fungicides is commonly practiced to prevent pre-emergence rots. Several researchers have reported an inhibitory action of these chemicals on nodule bacteria, leading to suppression of nodulation, nitrogen fixation, and yield of various legumes, including beans (1,6,20,25,31,32).

Pesticides in certain concentrations were also found to stimulate legume nodulation and yield (2,22,26). Rhizobium strains that were resistant to certain pesticides in cultures, or in sterile soil suspensions did not retain this resistance under greenhouse or field conditions (17 ,18) Staphorst and Strijdom (32) found Thiram, a seed treatment fungicide, was toxic to Rhizobium strains in agar media, but proved harmless in greenhouse tests. Even independent field study reports of pesticidal effects on soybeans were found to be contradictory (5,30). All these indicate that soil, environmental, and other factors might also influence the overall nitrogen fixing ability of a legume.

One area that has been poorly studied is that of possible interaction effects on soil bacteria of two different types of pesticides, commonly used during a crop season. Consequently, this study was initiated to evaluate the effects of a pre-emergence herbicide and a seed treatment fungicide and their interaction on nodulation, nitrogen fixation, and yield of snap beans under field conditions with and without inoculation with a strain-specific Rhizobium.

In a preliminary study, ten snap bean and dry bean cultivars inoculated with a commercial mixture of Rhizobium bacteria were evaluated for nodulation in pots under greenhouse conditions. A snap bean cultivar, Tendercrop (Stokes Seeds, Inc., Buffalo, N.Y.), which produced more and larger, pinker nodules, and had a shallow root system, was chosen for the field study. The study was conducted at the Kansas State University horticultural research farm, on a Heyne fine sandy loam soil.

Eight treatments in all possible combinations were made with and without Thiram seed treatment, Rhizobium inoculation, and plot treatment with Treflan herbicide. Thiram 50 Red (tetramethylthiuram disulfide) applied as a slurry at 1.26 g/kg seed, was used in preference to Captan, the commonly used commercial seed treatment fungicide, as it was reported to have caused less effective nodulation in beans than Thiram in greenhouse studies (unpubl. Nitragin Sales Corp.,).

Treflan 7E (a,a,a-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) was sprayed with a boom sprayer at a normal rate of 1.41 l/hectare and immediately incorporated into the soil.

Seeds were inoculated on the day of planting with *Rhizobium phaseoli* (strain 127K47) using a sticking agent, Nitra-coat, (both supplied by Nitragin Sales Corp.) in a slurry form. Two additional treatments involving post-planting (23 days after planting) application of ammonium nitrate at 33.65 kg/ha with and without Treflan, were made to compare the effectiveness of inoculation. The experimental design was a randomized split plot of ten treatments and three replications.

Nodulation counts were made on 5 consecutive plants from a randomly selected portion of each plot, starting on the 46th and 64th day after planting. Plants were dug with about .028 mt^3 of soil, and roots were washed through a 16 mesh sieve; and nodule number, and size diameter to the nearest millimeter were recorded for each plant. Total nodule volume per plant was estimated from the diameter classes, based on assuming a spherical nodule shape.

Effectiveness of nodulation was determined by assaying the amount of nitrogen fixed by the acetylene reduction method 51 days after planting (full bloom stage) on 2 plants per treatment in only 2 replications, as there was some plant loss in the third replication due to *Pythium* root rot.

Dry seed yield per plant was obtained 101 days after planting from consecutive plants and data was analysed by Analysis of variance using Duncan's multiple range test to separate means. LSD values were calculated to compare a herbicide and no herbicide treatments and treatment combinations, because of the unequal sample sizes, and as some of the comparisons were made between whole plot treatments (7).

Significant differences were encountered for some of the treatments as well as for some interaction combinations on snapbean nodulation and yield (Table 1). Thiram without Treflan or Rhizobium inoculation significantly decreased nodule number (Table 2) 46 days after planting, but this combination had no effect on yield. Staphorst and Strijdom (32) reported no significant difference in nodule dry mass between Thiram treated and untreated peanuts in a greenhouse study. Affifi et al. (1), however, reported that Thiram inhibited several strains of bacteria including some of Rhizobium phaseoli in agar media. Increased nodulation following fungicide application has been reported for inoculated peas (27,31), and inoculated alfalfa and cowpeas (33). Brinkerhoff et al. (6) observed that Thiram decreased nodulation in inoculated cowpeas, and Fischer (12) found Thiram decreased nitrogen fixation in white clover.

Inoculation with Rhizobium significantly increased nodule number and nodule volume (Table 3) 64 days after planting, but there was no effect of inoculation on dry seed yield. Field trials with Rhizobium inoculation of Phaseolus have given inconsistent results (10,13,14). Reasons given for this were invasion of the plant by indigenous but ineffective strains of Rhizobium or failure of the inoculation process (16).

Application of fertilizer at 33.65 kg/ha did not have any effect on dry seed yield. Guss and Döbereiner (15), reported no effect of mineral nitrogen applied to Phaseolus vulgaris under greenhouse conditions on nodulation and plant growth. Similar results for soybeans were reported by Olson et al. (21). At higher fertilization rates, they found nitrogen decreased yields.

The report that Treflan drastically reduced nodulation and yield of Cicer arietinum (20) led us to consider that this herbicide might act similarly on garden beans. However, our data showed no effect of Treflan on bean nodulation, nitrogen fixation, or yield. Dunigen et al. (9) likewise reported no effect of Treflan on soybean nodulation and yield. Most of the work described to date with pesticides has been done with compounds that are not in current use; thus, it would be beneficial to test the effect of modern pesticides on nodulation and yield of beans and other legumes.

A significant herbicide and fungicide interaction effect was noticed on dry seed yield as seen in Table 4. Two observations can be made on the interaction effect, about which no information has previously been reported. First, in the absence of herbicide, fungicide treatments increased dry seed yield. Second, in the presence of herbicide no significant differences in yield were found between fungicide and no fungicide treatments. Singh et al. (31) found an increase in nodulation, nodule dry weight, nitrogen fixation, and leghemoglobin content in inoculated peas treated with Thiram. Rewari et al., (27) found an increase in grain yield of inoculated cowpeas, treated with Thiram, over inoculation alone, but there have been no studies reported on the interaction effect of a herbicide and fungicide, or any two pesticides, on nodulation, nitrogen fixation, or yield of beans or any other legume. Richardson (28) has reported that Thiram treatment of soil resulted in an increase in bacteria and decrease in fungal populations. This is exactly what we observed in Table 4 for Thiram and no Thiram treatment in the absence of Treflan, which resulted in an increase in seed yield. Presence of Treflan along with Thiram might have inhibited the increase in soil bacterial population, as such no effect on yield was observed. Since fungicide increased dry seed yield only in the absence of herbicide, and no significant effect on yield was observed for any of the treatments individually, it appears that herbicide effect though not detrimental, is more pronounced on bacteria in soil than effect of the fungicide. In the absence of the fungicide, herbicide increased yield, but not significantly.

The herbicide, treflan, also interacted significantly with Rhizobium on root nodule volume 64 days after planting (Table 5). No significant difference in nodule number was observed. Root nodule volume was estimated, because we noticed plants with few nodules often had larger nodules than those with many nodules. Herbicide with Rhizobium inoculant gave a significant increase in root nodule volume over herbicide alone. In the absence of the herbicide, inoculation increased root nodule volume, but insignificantly.

Our attempts to correlate nodulation (46th and 64th day counts) with yield, or nitrogen fixation (assayed by acetylene reduction method 51 days after planting) with yield did not give any evidence of a positive correlation. One reason that may be involved is, that formation of efficient nodules might have started late in the crop season and thus had little impact on yield of a short-season crop like the bush bean cultivar that we used.

In our experiment we did not observe large, pink nodules of the type usually considered to be efficient in nitrogen fixation until 85% of the pods were set. This suggests that one or both of the pesticides may be delaying efficient nodule formation, or causing the formation of pseudonodules that have no capability to fix nitrogen and hence have no impact on yield. Formation of pseudonodules on the roots of some legumes treated with different herbicides has been reported by Lakshmikumari et al., (19), Allen et al., (14). Acetylene reduction analysis for total nitrogen fixed indicated no significant difference. This may be due to small number and size of the samples analysed, or it may mean that the nodules analysed were immature.

Overall our data indicate that neither Treflan nor Thiram at normal rates will have any effect on yield of snapbeans, even when used together, as is commonly practiced in commercial bean cultivation. One interesting observation made is the increase in seed yield due to fungicide treatment, but in absence of the herbicide, in the interaction effect. It would be desirable to see whether a reduction in concentration of Treflan will result in an increase in yield, when used with Thiram, without any appreciable reduction in weed control. Though inoculation with a strain specific Rhizobium did not have any influence on yield, we feel that further trials should be made using a different strain, specific to Phaseolus to determine whether inoculation can substitute for nitrogenous fertilizer application. There have been reports about the host-specific strains performing differently, according to their degree of compatibility with the host, on soybeans and gardenbeans. (8,18). In our case, may be the strain used was not sufficiently compatible with the cultivar, or with the environmental conditions.

Table 1. Analysis of variance F values for herbicide, fungicide, rhizobium inoculation, and fertilizer application treatments, measured for yield, nodule number (46 and 64 days after planting), root nodule volume (46 and 64 days after planting), and nitrogen fixed in snap beans. (Entries corresponding to Error A and Error B are Error mean squares).

Source	D.F.	Yield	Nod. 46	Nod. 64	Vol. 46	Vol 64	Nitr. fix.
Herbicide	1	0.05	0.15	0.24	0.16	0.46	1.03
Error A (Rep * H)	2	21.96	21.96	118.72	33968.79	622008.96	1230.60[1]
Fungicide	1	0.58	7.81[a]	4.00	2.18	1.22	3.08
Rhizobium	1	0.01	1.40	22.22[B]	0.20	14.02[B]	1.82
Fertilizer	1	0.43	2.37	0.37	3.91	0.85	0.00
H * F	1	5.94[a]	1.80	0.03	1.13	0.13	0.09
H * R	1	0.35	0.61	3.36	0.99	6.83[a]	0.27
H * Fe	1	0.84	3.44	0.00	2.72	0.42	0.34
F * R	1	0.31	1.88	1.71	1.79	0.00	3.59
H * R * F	1	5.77[a]	0.04	0.14	0.65	0.05	0.58
Error B	16	5.70	387.92	26.23	8847.89	156314.34	2723.37[2]

[a] Means significant at 5% level.
[B] Means significant at 1% level.

[1] Error (A) has 1 DF as it has only 2 reps.
[2] Error (B) has 8 DF as it has only 2 reps.

Table 2. Effect of Thiram on nodulation of Garden beans, 46 days after planting (mean of three replications).[z]

TREATMENT	NODULES / PLANT
THIRAM	18.83
NO THIRAM	32.04

[z] Mean separation in columns by LSD, 5 % level

Table 3. Effect of Rhizobium inoculation on nodule number/plant, and on nodule volume/plant, 64 days after planting of Garden beans (mean of three replications).[z]

TREATMENT	NODULE NUMBER	NODULE VOLUME
RHIZOBIUM	15.68	1009.81
NO RHIZOBIUM	5.47	365.63

[z] Mean separation in columns by LSD, 5 % level

Table 4. Effect of Treflan, Thiram interaction on dry seed yield (gms/plant) of Garden beans (least squares means of three replications).[z]

	THIRAM	NO THIRAM
TREFLAN	16.80	18.43
NO TREFLAN	18.60	15.48

[z] Mean separation in columns or rows by LSD, 5 % level. Between rows within column comparisons (between whole plot) for Thiram means were made with LSD = 4.01, and for no Thiram means with LSD = 3.27. Between column within row comparisons (within whole plot) were made with LSD = 2.66.

Table 5. Effect of Treflan, Rhizobium interaction on root nodule volume, 64 days after planting of Garden beans (least squares means of three replications).[z]

	RHIZOBIUM	NO RHIZOBIUM
TREFLAN	1270.10	243.81
NO TREFLAN	538.47	355.99

[z] Mean separation in columns or rows by LSD,5 % level. Between rows within column comparisons (between whole plot) for Rhizobium means were made with LSD = 672.30, and no Rhizobium means with LSD = 548.93. Between columns within row comparisons (within whole plot) were made with LSD = 441.76.

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APPENDIX

Table 1 : Greenhouse trial for identification of a good nodulating bean cultivar for field study (mean of two plants)

	PERFORMANCE RANKING	
CULTIVAR	NODULATION[a]	ROOTING[b]
Jacobs Cattle	7	9
Green Crop	8	6
Star	5	1
UI 111	4	2
Gold Crop	3	10
Royal Burgandy	10	7
Tendercrop	1	8
BBL 290	9	5
Provider	2	4
Taylors Hort.	6	3

[a]largest root system (size and number)
[b]largest root system
*cultivar selected for field study

EXPERIMENTAL DESIGN

DESIGN:	Randomized Split Plot
TREATMENTS:	10
REPLICATIONS:	3
PLOT SIZE:	350' x 20'
SPACING:	Between rows 3'
	Within rows 1'

TREATMENTS:

1 + H +R +F	6 -H +R +F	
2 + H +R -F	7 -H +R -F	H = Herbicide
3 + H -R +F	8 -H -R +F	R = *Rhizobium phaseoli*
4 + H -R -F	9 -H -R -F	F = Fungicide
5 + H -R -F +FE	10 -H -R -F +FE	FE = Fertilizer

RANDOMIZATION OF TREATMENTS

REP I		REP II		REP III	
4	10	7	4	2	6
2	8	6	1	4	10
3	7	8	2	5	8
5	6	9	5	3	7
1	9	10	3	1	9

Table 2 : Effect of Thiram, Treflan, and Rhizobium phaseoli on Nodulation (46 and 64 days after planting and yield of Garden beans (least squares means of three replications)

		THIRAM		NO THIRAM	
		INNOCULATED	UNINNOCULATED	INNOCULATED	UNINNOCULATED
NODULATION 46 (NO./PLANT)	TREFLAN	21.73	18.67	46.13	17.60
	NO TREFLAN	-3.10	3.03	39.50	26.96
NODULATION 64 (NO./PLANT)	TREFLAN	14.21	4.04	22.29	5.09
	NO TREFLAN	8.45	4.36	14.18	6.22
YIELD (GMS/PLANT)	TREFLAN	16.15	17.45	19.58	17.28
	NO TREFLAN	19.72	17.48	13.72	17.25

Table 3 : Effect of Thiram, Treflan and *Rhizobium phaseoli* on root nodule volume and N-fixation of Garden beans (least square means of three replications)

		THIRAM		NO THIRAM	
		INNOCULATED	UNINNOCULATED	INNOCULATED	UNINNOCULATED
NODULE VOLUME (46)	TREFLAN	62.78	124.01	161.02	57.46
	NO TREFLAN	-27.68	47.95	90.30	125.10
NODULE VOLUME (64)	TREFLAN	1195.35	199.54	1344.85	288.09
	NO TREFLAN	439.91	216.95	637.03	495.03
N-FIXATION	TREFLAN	-8.22	-0.18	61.00	9.92
	NO TREFLAN	14.96	35.46	136.23	18.12

EFFECTS OF TREFLAN AND THIRAM AND THEIR INTERACTION ON NODULATION, NITROGEN FIXATION, AND YIELD OF INOCULATED AND UNINOCULATED GARDEN BEANS (*PHASEOLUS VULGARIS* L.)

by

SRINIVAS KOMANDURI
Bachelor of Science (Agriculture) 1977,
A.P. Agricultural University, Hyderabad, INDIA.

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Horticulture

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1979

Effects of Treflan and Thiram and their interaction on nodulation, nitrogen fixation, and yield of inoculated and uninoculated Garden Beans.[1]

S.Komanduri, C.D.Clayberg, and C.W.Marr[2]

Department of Horticulture

Kansas State University, Manhattan, KS 66502.

Additional index words : *Phaseolus vulgaris*, Tetramethylthiuram-disulfide, a,a,a-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toludine.

Abstract : Treflan, a commonly used pre-emergence herbicide did not have any affect on nodulation, nitrogen fixation, or yield of garden beans under field conditions. At 1.41 l/ha. it gave an excellent weed control. Thiram, used a seed treatment fungicide reduced early nodulation, but had no affect on dry seed yield. Inoculation with a strain specific *Rhizobium* did not have any influence on yield, nor did application of ammonium nitrate at 33.65 Kg/ha. Two interesting interaction effects were observed, 1) Interaction between Treflan and Thiram, 2) Interaction between Treflan and *Rhizobium*.

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[1]Received for publication Contribution No.
Kansas Agricultural Experimental Station

[2]Graduate student and Research Horticulturists.