INFLUENCE OF INOCULATION AND HERBICIDE TREATMENT ON CLARK SOYBEAN

by

DAVID WAYNE CUDNEY

B. S., Kansas State University, 1962

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1963

Approved by:

Ernest L. Mader
Major Professor

TABLE OF CONTENTS

INTRODUCTION

In a recent publication by Miss Rachel Carson (6), pp. 53-54, some of the hazards of the use of pesticides as related to soil organisms have been put forth. Miss Carson writes:

> Life not only formed the soil, but other living things of incredible abundance and diversity now exist within it; if this were not so, the soil would be a dead and sterile thing. By their presence and by their activities the myriad of organisms of the soil make it capable of supporting the earth's green mantle.
>
> Bacteria, fungi, and algae are the principal agents of decay, reducing plant and animal residues to their component minerals. The vast cyclic movements of chemical elements, such as carbon and nitrogen, through the soil and air and living tissue would not proceed without these microplants. Without the nitrogen-fixing bacteria, for example, plants would starve for want of nitrogen, though surrounded by a sea of nitrogen-containing air.
>
> The problem that concerns us here is one that has received little consideration: What happens to these incredibly numerous and vitally necessary inhabitants of the soil when poisonous chemicals are carried down into their world.

Although authorities differ on the extent of the pesticide hazard, Miss Carson's publication has stimulated more research in this area and has brought a greater awareness of the danger to the public. In a special report by the President's Science Advisory Committee (15) in May, 1963, the committee concluded that in order to continue the progress made in agriculture, there must be an increase in the use of pesticides. Along with this increase, there must also be an evaluation of the impact on the various segments of biological life.

The lack of information concerning the effect of herbicides on nodulation and the nodulating organism of the soybean stimulated this study.

REVIEW OF LITERATURE

Rhizobium japonicum, the soybean nodulating organism, is a non-spore forming, aerobic, rod-shaped organism 0.5 to 0.9 microns wide and 1.2 to 3.0 microns long. Representatives of the genus are typically motile; however, according to Allen and Allen (2), the type of flagellation can not be used as a criterion for species identification. R. japonicum is typically gram negative; however, positive granules can be identified in the vegetative rods. This well-known unevenness of staining, according to J. M. Vincent (16), appears to be due to large spherical aggregations of polymeric b-hydroxybutyrate. Survival of the organisms in the soil has been noted in some cases up to 13 years where soybeans were not grown during this time. Variation has frequently been encountered in the Rhizobium in respect to cultural characteristics, invasability, effectiveness or symbiosis and other characteristics. Cultural variation is very common; in fact, it is not uncommon, according to Vincent (16), to find an old line showing two or more colony types. Vincent (16) encountered losses of invasiveness in several cultures in the course of working with a large collection of Rhizobium over a period of some 20 years. This constitutes a hazard in the maintenance of stock cultures, especially for commercial inoculates.

The means of differentiating Rhizobium species from related bacteria is highly unsatisfactory (1). This is because the method now in use relies on the ability of the organism to nodulate test plants. Clear differences between strains of root nodule bacteria are apparent, but standard laboratory tests for their differentiation into species are rare. The characteristic upon which classification is based is the capacity of

an isolate to invade roots of a restricted number of plant species in addition to the legume from which the microorganism was obtained. Among the several laboratory reactions which are of diagnostic value is the reaction in litmus milk. It has been noted that R. japonicum does not acidify litmus milk or form a serum zone (1).

The colonies of Rhizobium are distinguishable due to their production of a white sticky gum. While the colonies of the alfalfa organism require only about five days for recognition, the soybean and lupine colonies are slower growing, often requiring 10 to 20 days before colonies are distinguishable (11).

Entrance of the Rhizobium organism into the soybean root and the subsequent production of a nodule is explained very well in Wilson's text (18) on the biochemistry of nitrogen fixation. When a legume seed germinates in a soil containing root nodule bacteria, the bacteria are attracted to the region of the developing root hairs. It has been suggested by Ludwig and Allison (13) that the excretion of a specific growth factor required by the bacteria allows the organism to multiply rapidly in the region of the root hair which favors infection. The increase in the population of organisms is not confined to Rhizobium and leguminous plants but is also common with non-legumes and other microorganisms as well. This area of increased microbiological activity is known as the rhizosphere.

Infection of the root is gained through breaks in the epidermis above and below the region of lateral root emergence and root hairs (18). The process of infection through the root hair begins with the plant's response to the bacteria by causing a curling of the root hair. The

bacteria inside the curl of the root hair grow in a thread toward the cells of the root. During this migration, the Rhizobia are embedded in a gum which they secrete. The cells of the host lay down a sheath about the embedded bacteria to form an infection thread.

The entrance of the infection thread into the proper cell of the host stimulates cell division and the bacteria invade the newly-formed tissue (18). Vincent (16) suggested that the plant roots excrete tryptophane, and the bacteria convert it to indole acetic acid (IAA). He further suggested that a colony of bacteria on the root hair may produce IAA which would make the cell wall plastic and produce osmotic conditions that would cause the root hair cytoplasm to retreat before the colony and so advance the infection thread. Initiation of a nodule in the invaded cortex may result from the combined action of IAA and a sufficiently high level of kinin produced by a desmotic cell.

The mature nodule resulting from this stimulation of cell division consists of a cap of uninfected cortical tissue cells stretched and broken by the development of the nodule. Directly behind this cap is an area of rapidly-dividing cells that are not infected. Behind this meristematic tissue are much larger, infected cells which are filled with bacteria. Vascular bundles connecting with the xylem and phloem elements of the roots develop in the cortex of the nodule. These provide the transport system for bringing food to the bacteria in the nodule and for carrying the fixed nitrogen to the tissues of the host plant (18).

The speed with which nodules are formed on soybeans and become active in nitrogen fixation is impressive. Bergersen (4) observed first nodules on Lincoln soybeans nine days after planting, with nitrogen fixation beginning two weeks following this.

Bond (5) pointed out that once fixation begins in the soybean, from 80% to 90% of the nitrogen fixed is transferred to the plant. He related three stages of fixation:

1. In the early stages a large percent of nitrogen fixed is retained in the nodules.
2. The second stage is a period of plant development. During this stage a fairly constant quantity of the nitrogen fixed is transferred from the nodules to the plant (80 - 90%).
3. In the final stages of growth, blossoming and seed development, heavy taxes are laid on all of the plant's nitrogen store and transfer of nitrogen may reach 90% to a 100% from the nodule.

He also found that efficiency decreases with age, perhaps because the carbohydrates per bacterium decrease as the number of inactive cells increase. A decrease in efficiency does not always occur in soybeans, except in the very last stages of growth.

Strains of R. japonicum vary widely in their ability to benefit the host plant. Many strains found in natural habitats are of ineffective types. Usually nodules formed by poor strains are small, round, and white; whereas effective strains will produce nodules larger in size with a red pigment within the nodules (2). The red pigment in effective nodules is due to the compound leghemoglobin.

Not only are there ineffective strains of Rhizobium, but there are also non-nodulating soybean varieties. These varieties do not develop nodules when inoculated with Rhizobium. This situation is due to a single set of recessive genes (7). These genes have been incorporated into several non-nodulating and nodulating near-isogenic lines and are an

excellent tool in the study of nitrogen fertility problems.

When considering the interaction between microorganisms and herbicides, two effects become of primary significance. The first effect involves the potential inhibition of the herbicide to the microorganisms; the second involves the alteration of the chemicals through the physiological activities of the microorganisms. Whiteside and Alexander (17) listed two simple and rapid techniques designed to test for these interactions. The first was a manometric measurement of the effect of the chemicals on the microorganisms; the second technique involved a spectrophotometric procedure to estimate the influence of the microorganisms on the chemical.

The manometric method, which involes the measurement of gas evolution, has received the widest use as a means of measuring microbiological activities as influenced by herbicides. Most workers have used this method to determine the effect of herbicides, such as 2,4-D (2,4-dichlorophenoxyethyl acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid).

Kratochivil (12) made a comparative study using soils treated in the laboratory and measuring gas evolution over a sixty-hour period. He grouped the herbicides as follows:

1. those causing significant reduction in soil microbiological activities:

   TCA (tricholoracetic acid) at 10 pounds and over per acre

   PCP (pentachlorphenol) at four pounds and over

   IPC (isopropyl-M-penylcarbamate)

2. those having no influence on activity

   2,4-D

2,4,5-T

Maleic hydrazide

In general, he concluded that herbicides have only temporary effects with the possible exceptions of PCP and IPC used at very high rates. Whiteside and Alexander (17), in their experiments with 2,4-D; 2,4,5-T; 2-(2,4-dichlorophenoxy) propionic acid; 4-(2,4,dichlorophenoxy) butyric acid; and amitrole (3-amino-1,2,4-triazole), found no effect on microbiological activity with these chemicals used at normal rates. Chandra, Furtick and Bollen (8) also used the carbon dioxide evolution method of estimating microbiological activity. They studied the effects of 2,3,6-TBA (2,3,6-trichlorobenzoic acid), EPTC (ethyl N,N-di-n-propyl-thiol-carbamate), diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], and simazine [2-chloro-4,6-bis(ethylamino)-s-trazine] on the microbiological activity of nine soils. They discovered that different soils responded differently and there was a decrease in CO_2 evolution for at least 28 days. Their work pointed out the need for further research in this area.

Not only has the effect of herbicides been studied on the microbiological population of the soil as a whole, but some workers have isolated specific organisms and studied the effects of herbicides on them. Chappel and Miller (9) studied the effects of 18 herbicides applied at field rates on six soil-borne plant pathogens. They found the fungi they studied varied in their reaction to the various herbicides, but PCP and DNBP (4,6-dinitro-o-sec-butyl-phenol) at field rates completely inhibited the growth of all six.

Fletcher (10) studied the effects of 2,4-D, MCPA (2-methyl-4-chloro-

phenoxy-acetic acid), 2,4,5-T and 2,4-DB on R. trifolii. The herbicides were incorporated into a yeast mannitol agar. Concentrations of from five to 500 ppm. of herbicide had no effect on the growth of the organism; however, at concentrations above 500 ppm., growth was inhibited by some of the herbicides.

MATERIALS AND METHODS

The purpose of this study was to ascertain the influence of selected herbicides on nodulation of Clark soybean. In order that the subject could be adequately treated, it was approached on three points of view. First, a field test was made to determine the effect of herbicides on the number of nodules formed, nodule weight, weed population, yield of soybean per acre, and protein and oil content of the seed. Second, a similar test was undertaken in the greenhouse. Third, a laboratory test was conducted to determine the effect of herbicides on R. japonicum colonies.

Field Experiments

Field tests were conducted at two locations, the Ashland Agronomy Farm and the Agronomy Farm, during the summer of 1962. The Ashland Farm plots were located on Sarpy fine sandy loam soil, while those on the Agronomy Farm were located on a poorly-drained, silty clay loam, alluvial soil of an unnamed series. Soil test data for the two locations are given in Table 1.

Table 1. Soil test information for the Agronomy and Ashland Farms, 1963.

Location	Soil Type	Organic Matter	pH	Available Phosphorus	Exchangeable Potassium
		%		Lbs/A	Lbs/A
Agronomy Farm	clay loam	3.4	6.5	75	346
Ashland Farm*	sandy loam	0.9	8.2	90	379

* Ashland plots received 50 pounds of nitrogen per acre prior to planting.

The soybean variety Clark was used at each location. Both inoculated and non-inoculated seed treatments were made. Half of the plots were inoculated with the commercial inoculum Nitragin as a wet application immediately prior to planting. The remaining plots were not inoculated. Amiben (3-amino-2,5-dichlorobenzoic acid) and Alanap (N-1-naphthyl phthalamic acid), two soybean pre-emergence herbicides, were used. Each herbicide was applied to both inoculated and non-inoculated plots at three different rates, Amiben at 1½, 3, and 4½ pounds per acre and Alanap at 2, 4, and 6 pounds per acre. A 12-inch band of each herbicide was applied over the row following planting. The experiment was designed to include a no-treatment check plot and an inoculated check plot, along with the inoculated and non-inoculated herbicide-treated plots. Each of the 14 treatments was planted in four-row plots, 16 feet long, and replicated four times in a randomized block design. The two outer rows were used for nodule data, leaving the inner rows for yield information.

The Ashland plots were planted June 7, 1962, and treated with herbicide on June 9 and emerged on June 12. The Agronomy Farm plots were planted on June 12, received herbicide applications on June 15 and emerged on June 17. Both locations received sufficient moisture following planting so that the herbicide treatments were effective. The Agronomy Farm location

received no additional moisture other than that supplied by rainfall; thus, the plants were subjected to considerable moisture stress during the last part of June and the first part of July. However, the Ashland Farm plots received both sprinkler and furrow irrigations during the growing season.

Root samples were taken from both locations, starting 10 days after emergence and proceeding through the end of the blooming stage. The root sampling device designed for the experiment is pictured in Plate I (figures 1 and 2) along with root samples obtained at the first sampling (Plate I, fig. 3). Because most of the microbiological activity is concentrated within the first three inches of the soil and most root nodules develop within this area[1], a core taken 4 inches wide, 6 inches long and 6 inches deep from within the row, including five plants wherever possible, was taken to estimate nodule weights and numbers. The soil-sampling apparatus was driven into the soil in the area to be sampled, the core extracted, and the soil was washed from the roots. The root samples were then placed in quart jars containing water and a mercuric chloride solution for later examination. Later in the laboratory, the nodules were counted, and, in the case of the last sampling, were removed and weighed.

Weed counts were made in each individual plot during mid-season to determine the effectiveness of the herbicides in weed control. Plate II shows the difference in weed control between herbicide and no herbicide application at the Agronomy Farm. The harvesting of the plots was done by mowing each row, bundling it, and threshing each bundle with a small

---

[1]From personal communication with D. J. O. Harris, Department of Bacteriology, Kansas State University, Manhattan, Kansas.

EXPLANATION OF PLATE I

Figure 1. Soil sampling device being driven into the soil.

Figure 2. From left to right, extracted core, soil sampling device, root storage bottle, and wash water container.

Figure 3. Root samples obtained at first sampling.

PLATE I

EXPLANATION OF PLATE II

Figure 1. Note weed-free area within the row for high treatment level of Amiben herbicide.

Figure 2. Note weedy area within the row with no treatment.

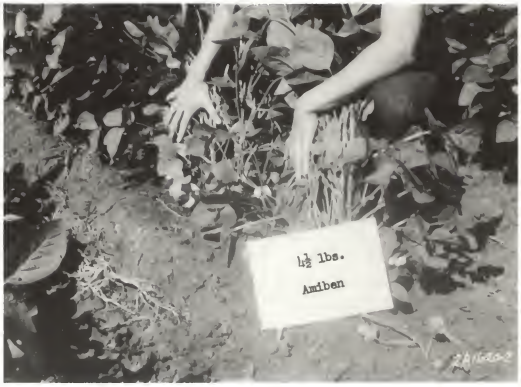

PLATE II

nursery thresher. Protein and oil determinations were made on the harvested seed by the U.S.D.A. Regional Soybean Laboratory at Urbana, Illinois. Approximately 70 grams of seed representing a composite sample from each plot for each treatment was taken for protein and oil content.

Greenhouse Experiment

In order to explain further some of the data obtained in the field experiments, a greenhouse experiment was conducted in February and March of 1963. Herbicides were used at three different levels with both inoculated and non-inoculated seed. In addition to the Amiben and Alanap herbicides, Lorox [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] and Randox (a-chloro-N-N-dially-lacetamid), were incorporated into the experiment. The Lorox- and Randox-treated pots received 2, 4, and 6 pounds per acre equivalent of herbicides. With the inoculated and non-inoculated seed for each herbicide treatment and the checks, a total of 26 pots per replication was needed. A completely randomized block design utilizing four replications was used.

In order to facilitate the removal of the roots from the pots, a mixture of sterile sand and styrofoam was used as a growth medium. Because it was originally anticipated that the plants would be grown in these pots for several weeks, a nutrient solution, as described by Meyer, Anderson, and Bohning (14), was incorporated into the styrofoam-sand mixture. The pots were planted and treated on February 11, 1963. Emergence began on February 18, 1963, and continued through the first of March. The pots were thinned to five plants per pot. Because of the varying dates of emergence and some 2,4-D contamination in the greenhouse, the plants were

not allowed to reach maturity but were harvested for nodule determination on March 21, 1963. Plate III shows the complete tests, along with a comparison of the effect of equivalent rates of the four herbicides.

Microbiological Study

In order to understand more fully the relationship between the soybean nodulating organism and the herbicides under consideration, a test was conducted to determine the effects of various levels of the herbicides on the R. japonicum colonies. Preliminary to the more extensive study, a gradient diffusion plate study was set up to determine the approximate rates at which the herbicides would affect colony growth when incorporated into the growth medium. Taking into account the results obtained in this determination and the work of Fletcher (10), in England, it was decided to use rates of 10, 100, and 1,000 ppm. The four herbicides used in the greenhouse study were again used, with the addition of atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine). Atrazine was included because of its possible carry-over effect as a residue in fields where it has been applied to corn in previous years. The possibility of an undersirable effect of atrazine residue has been suspected.[1]

The cultural medium used, as described by O. N. Allen (3), was an asparagus extract mannitol agar medium. Each herbicide concentration was incorporated into 10 ml. portions of the agar medium per petri dish.

Strain number 311b310, the most competitive strain in use at the U.S.D.A. Laboratory at Beltsville, Maryland, and strain number 61A24,

[1]From personal correspondence with Dr. Herbert Johnson, Research Agronomist, U.S.D.A., Beltsville, Maryland.

EXPLANATION OF PLATE III

Figure 1. Arrangement of pots in complete test.

Figure 2. Effect of the various herbicides on the soybean plants.

PLATE III

an isolate from the commercial product, Nitragin, from the University of Nebraska, Lincoln, Nebraska, were used in the study. With the five herbicides used at three different levels plus the three checks, there were 18 petri dishes per replication. Two replications were made. After the incorporation of the herbicides into the warm agar medium and the solidification of this mixture, both strains were streaked onto the plates in single lines, allowed to grow for four days, and rated on the growth reached at that time (Plates IV and V).

RESULTS

Field Experiments

Measurements pertaining to nodule count at various stages in plant development, nodule weight at the last root sampling, yields, and protein and oil analysis of the seed were compiled and analyzed. These measurements will be discussed separately at each location in an attempt to show the effect of herbicide application on nodulation. Weed counts were also taken and analyzed to show the effectiveness of the herbicides on weed control.

Ashland Farm plots. The first nodule count showed no significant difference at either the 1 or 5% levels due to treatment (Table 2). The last nodule count showed no significant difference among the treatment means (Table 2). With nodule weight, a significant difference was found at the 5% level (Table 2). Statistical analysis by orthogonal comparisons showed this difference was due to the differences in herbicides; that is, the Amiben-treated plots appeared to have greater nodule weight than the Alanap-treated plots.

EXPLANATION OF PLATE IV

Figure 1. Check plate on top followed by paired plates of Alanap, Amiben, and atrazine, with 10 ppm. of herbicide on left and 1,000 ppm. on right.

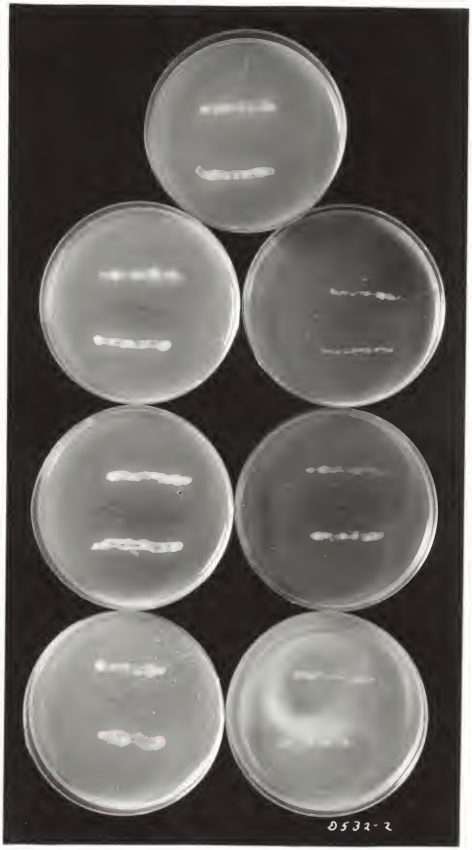

PLATE IV

EXPLANATION OF PLATE V

Figure 1. Check plate on top followed by paired plates of Lorox and Randox, with 10 ppm. of herbicide on left and 1,000 ppm. on right.

PLATE V

Table 2. Effects of inoculation and herbicide treatments on nodule count, nodule weight, and yield of soybeans per acre at the Ashland Farm, 1962.

Treatment	Yield Bu./A	Five Plant Total		
		Nodule Weight Grams	First Nodule Count	Last Nodule Count
	Average of 4 replicates			
Non-inoculated-no treatment	40.6	2.67	76.0	149.3
Inoculated-no treatment	40.5	3.92	72.5	207.5
Non-inoculated 1½ lbs. Amiben/A.	44.7	4.51	88.5	199.0
Inoculated 1½ lbs. Amiben/A.	45.5	2.66	90.3	176.5
Non-inoculated 3 lbs. Amiben/A.	43.1	3.00	82.8	174.0
Inoculated 3 lbs. Amiben/A.	41.2	3.30	71.3	220.2
Non-inoculated 4½ lbs. Amiben/A.	45.1	3.55	94.8	174.0
Inoculated 4½ lbs. Amiben/A.	46.4	3.36	69.3	187.7
Non-inoculated 2 lbs. Alanap/A.	41.3	2.52	74.7	203.3
Inoculated 2 lbs. Alanap/A.	46.0	2.48	56.0	153.5
Non-inoculated 4 lbs. Alanap/A.	45.6	1.95	87.3	158.0
Inoculated 4 lbs. Alanap/A.	43.7	2.33	56.5	177.0
Non-inoculated 6 lbs. Alanap/A.	44.3	2.21	67.3	180.0
Inoculated 6 lbs. Alanap/A.	44.4	2.43	63.8	172.5
Mean	43.7	2.92	75.1	180.9
Significance	n.s.	*	n.s.	n.s.
L.S.D. @ 5% level	n.s.	1.48	n.s.	n.s.
L.S.D. @ 1% level	n.s.	n.s.	n.s.	n.s.
Comparisons				
Alanap vs. Amiben	n.s.	**	n.s.	n.s.
All other comparisons are not significant (See Table 5).				

n.s. - not significant
* - significant at the .05 level
** - significant at the .01 level

The yield data was statistically analyzed; there was no significant difference found among treatments at either the 1 or 5% levels (Table 2). Protein and oil determinations were not analyzed statistically due to the fact that the figures presented are results obtained from the analysis of composited seed samples for the four replications of each treatment (Table 3). Prior studies by the U.S.D.A. Regional Soybean Laboratory at Urbana, Illinois, show that composite samples yield essentially the same information as that obtained from individual plots. With the oil and protein seed analysis, no difference between treatments was illuminated.

The weeds encountered at the Ashland location were, for the most part, Amaranthus species. Upon the statistical analysis of the weed counts, very definite differences, due to treatment, were observed. As may be seen in Table 4, where no herbicide application was made, the weed count within the area not treated by the herbicide reached the highest average levels of 76.0 weeds. A significant reduction in weed count was noted with each herbicide application and the highest level of herbicide application had only 27.0 weeds with Amiben treatment and 15.5 weeds with the Alanap treatment.

Agronomy Farm location. The first nodule count showed significant difference due to treatment at the 1% level (Table 5). Upon analysis of this difference by orthogonal comparisons, it was found that the significance observed was due to the differences between the inoculated and non-inoculated plots and differences between the two herbicides. The non-inoculated plots, contrary to what might be expected, showed a significant increase in nodule number over the inoculated plots. A significant difference at the 5% level was observed between the Amiben and the Alanap

Table 3. Protein and oil seed analysis data from the Ashland Farm, 1962.

Treatment	Percent	
	Protein	Oil
Non-inoculated-no treatment	40.3	22.5
Inoculated-no treatment	41.3	21.5
Non-inoculated 1½ lbs. Amiben/A.	39.8	22.1
Inoculated 1½ lbs. Amiben/A.	40.0	21.9
Non-inoculated 3 lbs. Amiben/A.	40.7	22.6
Inoculated 3 lbs. Amiben/A.	40.2	22.2
Non-inoculated 4½ lbs. Amiben/A.	40.1	22.4
Inoculated 4½ lbs. Amiben/A.	40.0	22.4
Non-inoculated 2 lbs. Alanap/A.	39.8	21.4
Inoculated 2 lbs. Alanap/A.	40.0	21.9
Non-inoculated 4 lbs. Alanap/A.	39.7	21.9
Inoculated 4 lbs. Alanap/A.	40.0	22.2
Non-inoculated 6 lbs. Alanap/A.	40.3	22.5
Inoculated 6 lbs. Alanap/A.	40.4	22.6

Table 4. Weed counts per two rows at the Agronomy and Ashland Farms, 1962.

Treatment	Average of 4 Replications	
	Agronomy Farm	Ashland Farm
No treatment	111.8	76.0
1½ lbs. Amiben/A.	28.8	30.3
3 lbs. Amiben/A.	16.5	27.3
4½ lbs. Amiben/A.	10.5	27.0
2 lbs. Alanap/A.	84.3	25.5
4 lbs. Alanap/A.	74.0	26.8
6 lbs. Alanap/A.	50.0	15.5
Mean	53.7	32.6
Significance	**	**
L.S.D. @ 5% level	12.9	9.3
L.S.D. @ 1% level	17.6	12.7

** - significant at the .01 level

Table 5. Effects of inoculation and herbicide treatments on nodule count, nodule weight, and yield at the Agronomy Farm, 1962.

Treatment	Yield Bu./A	Five Plant Total: Nodule Weight Grams	First Nodule Count	Last Nodule Count
		Average of 4 Replicates		
Non-inoculated-no treatment	38.0	2.59	67.6	198.8
Inoculated-no treatment	34.4	2.36	49.5	139.8
Non-inoculated 1½ lbs. Amiben/A.	41.6	3.87	71.3	224.5
Inoculated 1½ lbs. Amiben/A.	37.0	3.05	54.8	226.3
Non-inoculated 3 lbs. Amiben/A.	44.2	3.60	73.3	242.3
Inoculated 3 lbs. Amiben/A.	38.1	1.49	58.5	176.0
Non-inoculated 4½ lbs. Amiben/A.	45.0	2.84	79.5	204.5
Inoculated 4½ lbs. Amiben/A.	37.2	3.23	62.8	266.7
Non-inoculated 2 lbs. Alanap/A.	43.5	4.39	62.3	218.5
Inoculated 2 lbs. Alanap/A.	36.8	3.46	47.3	258.3
Non-inoculated 4 lbs. Alanap/A.	41.1	2.75	84.5	259.0
Inoculated 4 lbs. Alanap/A.	37.5	3.00	43.8	224.0
Non-inoculated 6 lbs. Alanap/A.	40.7	2.72	56.8	299.0
Inoculated 6 lbs. Alanap/A.	39.5	3.95	41.3	304.3
Mean	39.6	3.09	61.3	231.6
Significance	**	*	**	**
L.S.D. @ 5% level	5.2	1.5	22.0	67.6
L.S.D. @ 1% level	6.9	n.s.	29.4	90.4
Comparisons				
Inoculated vs. non-inoculated	**	n.s.	**	n.s.
Herbicide vs. no herbicide	**	n.s.	n.s.	**
Amiben vs. Alanap	n.s.	n.s.	*	n.s.
Linear relationship - Amiben	n.s.	n.s.	n.s.	n.s.
- Alanap	n.s.	n.s.	n.s.	*

n.s. - not significant
* - significant at the .05 level
** - significant at the .01 level

treatments. The Amiben treatments showed a higher nodule number than the Alanap treatments (Table 5). At the last nodule count, a significant difference was observed at the 1% level. Analysis showed the difference was attributed to the difference between herbicide treatment and no herbicide treatment. The herbicide-treated plots had a much higher number of nodules than the check plots receiving no herbicide. A significant linear relationship at the 5% level also was established with the Alanap-treated plots; the higher level of herbicide treatment had a higher nodule count (Table 5). The nodule weight determinations at the Agronomy Farm, contrary to what might be expected from the significance observed at the last nodule count, showed only a slight difference due to treatment at the 5% level. When this difference was analyzed by orthogonal comparisons, no difference could be found for the comparisons used (Table 5).

The yield measurements showed a significance due to treatment at the 1% level. The difference was attributed to differences between inoculation treatments and herbicide treatments. Inoculation reduced yields. Increased yields were obtained where herbicides were used (Table 5). Oil and protein analysis data showed no difference which could be attributed to treatment (Table 6).

The weeds encountered at the Agronomy Farm location were for the most part Setaria and Amaranthus species. Statistical analysis of the weed counts were made and very definite differences due to treatments were observed (Table 4). The non-treated check plots showed the highest average number of 111.8 weeds, while both herbicides very definitely decreased weed populations (10.5 weeds per plot where Amiben was used and an average of 50.0 weeds per plot for Alanap-treated plots). Amiben was more efficient in the control of Setaria species than Alanap.

Table 6. Protein and oil seed analysis data from the Agronomy Farm, 1962.

Treatment	Percent	
	Protein	Oil
Non-inoculated-no treatment	41.6	20.2
Inoculated-no treatment	39.4	21.8
Non-inoculated 1½ lbs. Amiben/A.	40.9	20.8
Inoculated 1½ lbs. Amiben/A.	40.9	21.0
Non-inoculated 3 lbs. Amiben/A.	41.3	20.9
Inoculated 3 lbs. Amiben/A.	39.9	21.1
Non-inoculated 4½ lbs. Amiben/A.	41.0	20.9
Inoculated 4½ lbs. Amiben/A.	40.4	21.5
Non-inoculated 2 lbs. Alanap/A.	40.6	21.1
Inoculated 2 lbs. Alanap/A.	40.6	20.9
Non-inoculated 4 lbs. Alanap/A.	40.2	21.2
Inoculated 4 lbs. Alanap/A.	40.1	21.2
Non-inoculated 6 lbs. Alanap/A.	40.4	21.5
Inoculated 6 lbs. Alanap/A.	40.7	21.3

Greenhouse Experiment

Because of the coarse-textured nature of the sand-styrofoam medium used in this test, the amount of water which was necessarily applied to the pots due to the high temperature of the greenhouses, and the lack of water-holding capacity exhibited by this mixture, considerable leaching of the herbicide took place. Thus, the soybean plants were subjected to a much greater concentration of herbicides in the confined area of the 4-inch pots than would normally be expected under field conditions, where most of the herbicides would be held in the upper 1/8 to 1/2 inch of the soil surface. Because of these conditions, the soybean plants, particularly those treated with the Lorox, Alanap, and somewhat with the Randox herbicides, were affected. In fact, all the pots treated with Lorox died before March 14, 1963, a week prior to the harvest of the other plants; thus, it was not possible for nodule numbers to be determined for the Lorox treatments. Plants in the pots treated with Alanap were greatly distorted by the herbicide and emergence was very poor at the higher rate. The pots to which Randox was applied showed little plant damage in comparison with the Lorox and Alanap treatments. Plants in pots which received the Amiben treatment showed no stunting effect due to the herbicide (Plate 3, Figure 2).

Because of the variation in emergence and the occurrence of no nodules on the plants of all the herbicide treatments and the checks, statistical analysis of the counts was not made.

Microbiological Study

Both strains of the R. japonicum organisms showed no effect of the herbicide treatments at the 10 ppm. concentration for all herbicides.

The Alanap treatments showed some retardation of growth at 1,000 ppm., a slight effect on strain 311b310 at 100 ppm., and no effect on strain 61A24 at this concentration. With the Amiben-treated plates, a slight effect was noted on strain 311b310 at 1,000 ppm. concentration, with no effect noted on strain 61A24. Neither strain was affected by the 100 ppm. concentration of Amiben. The atrazine-treated plates showed a slight effect due to herbicide treatment with both strains at the 1,000 ppm. concentration; however, neither strain was affected at 100 ppm. The Lorox-treated colonies showed a reduction in growth of both strains at the 1,000 ppm. rate; a slight retardation at the 100 ppm. concentration was noted on both strains. Randox treatment proved to cause the greatest disruption of growth rate at the 1,000 ppm. concentration of both strains, with only a slight effect noted on each strain at the 100 ppm. rate (Table 7).

Table 7. Effects of herbicide treatment on colony growth.*

Herbicide	Concentration					
	10 ppm. Strain		100 ppm. Strain		1000 ppm. Strain	
	311b310	61A24	311b310	61A24	311b310	61A24
Alanap	0	0	1	0	2	2
Amiben	0	0	0	0	1	0
Atrazine	0	0	0	0	1	1
Lorox	0	0	1	1	2	2
Randox	0	0	1	1	3	3
No treatment						

* Growth retardation scale
0 - growth not affected when compared to checks
1 - slight effect on colony growth
2 - colony growth definitely affected
3 - severe effect on colony growth

DISCUSSION

Field Experiments

The figures in Table 2 for the Ashland Farm show inoculation and herbicide treatment had no significant effect on the number of nodules per plant and seed yield. This could be attributed to the fact that 50 pounds of nitrogen per acre was applied prior to planting. Nitrogen application has been shown (7) to reduce nodulation and the effect of inoculation on soybean yields; thus, an effect of treatment on nodule number and seed yield might not be illuminated, particularly, as will be subsequently discussed, if herbicide treatment has no direct effect on nodulation. Table 2 also shows a slightly higher yield for the herbicide-treated plots as opposed to the checks. Although this difference did not show up in the statistical analysis, it parallels the reduction in weeds observed for herbicide-treated plots (Table 4).

Plots which were inoculated in contrast to the plots which were not inoculated on the Agronomy Farm had fewer nodules per plant for both of the sampling dates. It will be noted the differences were significant at the 1% level. Dr. Herbert Johnson, U.S.D.A. of Beltsville, Maryland, and Dr. O. H. Sears[1], formerly in the Department of Agronomy at the University of Illinois, Urbana, Illinois, offered several explanations of this phenomenon. Both Sears and Johnson offered the explanation that the wet treatment given the inoculated seeds perhaps reduced emergence by splitting the seed coat, thus providing a thinner stand. Thin stands

---

[1] Personal correspondences

could conceiveably have influenced seed yields; however, stand counts were not taken; thus no relationship between stand and yields could be established. Nodule numbers were taken from five plants; thus, it was difficult to explain the reduction in nodule number from wet treatment of the seed. Another possible explanation for the smaller number of nodules per plant and lower seed yields resulting from inoculation was that certain strains of nodule bacteria lack invasive ability and some strains may even be parasitic. The possibility of this occurrence stimulated further experiments in this area, which were started in June of 1963. The possibilities of difference in the time of planting and of harvest of the plots was suggested also; but due to the fact that both planting and harvesting took place on the same days, this possibility was discounted. It is the author's opinion that the reduction in yields from the inoculated plots may have been related to the wet treatment given the inoculated seed. The reduction in nodule number may have resulted due to a lack of effectiveness or invasability of the commercial strain used; however, further studies in this area are needed.

Plots which received the Amiben treatments had a greater number of nodules per plant than those receiving Alanap at the Agronomy Farm (Table 5). Where Amiben was used, greater control of weeds, particularly the Setaria species, was noted. A possible explanation for this occurrence was that there may have been less competition between weeds and soybeans, a greater growth of the root system, and more opportunity for nodulation to take place where Amiben was applied.

The larger number of nodules and higher yields obtained from the herbicide-treated plots (Table 5) would indicate that the herbicide used had a beneficial effect on seed yield and nodules per plant. The increase

in yield and number of nodules due to herbicide treatment could be attributed to the reduction in competition between weeds and soybeans (Table 4). The reduced competition allowed the soybeans to develop greater growth, a larger root system, and more opportunity for nodulation to take place.

At neither the Ashland or Agronomy Farm locations was there evidence to suggest that herbicide treatment reduced nodulation. In fact, the last nodule count at the Agronomy Farm location was increased significantly due to herbicide treatment.

Greenhouse Experiment

Although a statistical analysis of the nodule count data obtained from the greenhouse experiment was not made, it is interesting to note that even in the case of extreme distortion of the upper part of the plant and the root system, as was the case with the high level Alanap treatments, nodulation still occurred. It was not possible to note a correlation between the number of nodules produced and the distortion or damage to the soybean plant caused by the various herbicides.

Microbiological Study

The results obtained in this experiment corresponded very closely with those obtained by Fletcher (10), in England, working with R. trifolii and 2,4-D, MCPA, 2,4,5-T, and 2,4-DB. He found no effects of these herbicides on R. trifolii, except at concentrations of 500 ppm. and above. These rates were greater than would be encountered in the field. He maintained the concentrations found in the soil would not exceed 2.0 to 2.5 ppm. per pound of herbicide per acre, assuming complete solution of the herbicide and a 20% water content of the soil.

In the author's study, he found the 100 ppm. concentration of the Alanap, Lorox, and Randox herbicides caused only a slight retardation in colony growth, while the 1,000 ppm. concentration of all herbicides affected colony growth. Randox treatments caused the most severe damage. Using Fletcher's 2.5 ppm. per pound of herbicide per acre, the 100 ppm. would be in excess of the concentrations reached in the field. Unless the concentration of herbicides reached at least 25 pounds per acre, little effect of herbicide treatment would be noticed. Amiben and atrazine could be used at even higher concentrations before colony growth would be affected (Table 7).

SUMMARY

Summarizing the results obtained in these experiments, it was found that:

1. Nodule number, nodule weight, seed yield, protein content, and oil content were not reduced at either location due to herbicide treatment. At the Agronomy Farm, significant increase in nodule numbers was obtained at the last nodule count where herbicides were applied.
2. A significant reduction in seed yield and nodules per plant at the first nodule count was observed due to inoculation at the Agronomy Farm location. Further studies on the problem are needed to explain the reduction in seed yields.
3. In the greenhouse experiment, there was no correlation between the number of distorted soybean plants due to herbicide treatment and the number of nodules produced by the affected plants.
4. Two strains of R. japonicum were used in the microbiological study. Concentrations of 1,000 ppm. for all herbicides inhibited colony

growth. Randox, Lorox, and Alanap inhibited growth somewhat at 100 ppm. while Amiben and atrazine had little effect. Colony growth was not impaired at the concentration below 100 ppm.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Dr. E. L. Mader, his major professor, for his interest, his guidance in these investigations, and his help in the preparation of this manuscript. The author also wishes to express his gratitude to Dr. J. O. Harris, for his counsel in the conduction of the microbiological study. Thanks are also due to Dr. L. F. Marcus, for his aid in the statistical analysis, to Dr. L. E. Anderson, for his suggestions concerning the herbicides used, and to Dr. O. H. Sears and Dr. Herbert Johnson, for their valuable suggestions.

LITERATURE CITED

(1) Alexander, Martin.
Introduction to soil microbiology. New York: John Wiley and Sons, Inc. pp. 227-248, 326-353. 1961.

(2) Allen, Ethel K. and O. N. Allen
Biochemical and symbiotic properties of rhizobia. Bacteriological Reviews. 14(4): 273-330. 1950.

(3) Allen, O. N.
Experiments in soil bacteriology. Minneapolis, Minn.: Burgess Publishing Co. p. 54. 1959.

(4) Bergerson, F. J.
Biochemistry pathways in legume root nodule nitrogen fixation. Bacteriological Review. 24: 246-250. 1960.

(5) Bond, G.
Quantitative observations on the fixations and transfer of nitrogen in the soybean, with especial reference to the mechanism of transfer of fixed nitrogen from bacillus to host. Annals of Botany. 50: 559-578. 1936.

(6) Carson, Rachel.
Silent Spring. Boston: Houghton Mifflin Co. pp. 53-61. 1962.

(7) Cartter, Jackson L. and Edgar E. Hartwig.
The management of soybeans. Advances in Agronomy. 14: 360-408. 1962.

(8) Chandra, Purna, William R. Furtick, and Walter B. Bollen.
The effects of four herbicides on microorganisms in nine Oregon soils. Weeds. 8: 589-598. 1960.

(9) Chappell, W. E. and L. I. Miller.
The effects of certain herbicides on plant pathogens. Plant Disease Reporter. 40: 52-56. 1956.

(10) Fletcher, William W.
Effect of hormone herbicides on the growth of rhizobium trifolii. Nature. 177: 1224. 1956.

(11) Fred, Edwin Broun, Ira Lawrence Baldwin, and Elizabeth McCoy.
Root nodule bacteria and leguminous plants. Madison: The University of Wisconsin, 1932. pp. 44-45.

(12) Kratochivil
Determinations of the effect of several herbicides on soil microorganisms. Weeds. 1: 25-31. 1951.

(13) Ludwig, C. A. and F. E. Allison.
Some factors, affecting nodule formation on seedlings of leguminous plants. Journal of American Society of Agronomy. 27: 895-902. 1935.

(14) Meyer, Bernard S., Donald B. Anderson, and Richard H. Bohning.
Introduction to plant physiology. Princeton, N. J.: D. VanNostrand Co., Inc. pp. 323-324. 1960.

(15) President's Science Advisory Committee
Use of pesticides. U. S. Government Printing Office. pp. 1-2. 1963.

(16) Vincent, J. M.
Presidential address. Australian studies of the root nodule bacteria, a review. Proceedings of the Linnean Society of New South Wales. 87 (Part 1): 8-38. 1962.

(17) Whiteside, Jean S. and M. Alexander
Measurement of microbiological effects of herbicides. Weeds. 8: 204-213. 1960.

(18) Wilson, Perry W.
The biochemistry of symbiotic nitrogen fixation. Madison: The University of Wisconsin Press. 1940.

INFLUENCE OF INOCULATION AND HERBICIDE TREATMENT ON CLARK SOYBEAN

by

DAVID WAYNE CUDNEY

B. S., Kansas State University, 1962

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Agronomy

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1963

To adequately cover the subject, three separate tests were conducted: a field test during the summer of 1962, a greenhouse experiment during February and March of 1963, and a microbiological study during June of 1963.

The field tests were conducted at the Ashland Agronomy Farm and the Agronomy Farm at Manhattan, Kansas, using high, recommended, and low herbicide treatment levels of Alanap (N-1-naphthyl phthalamic acid) and Amiben (3-amino-2,5-dichlorobenzoic acid) on Clark soybean. Herbicide treatments were made on both inoculated and non-inoculated soybean plots. Each plot had four rows, two for root sampling data and two for seed yield, protein content and oil content. The greenhouse experiment was conducted in a manner similar to the field tests with the addition of the Lorox [3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea] and Randox (a-chloro-N-N-diallylacetamid) herbicides. Only nodulation determinations were made. The microbiological study made use of the four previously-mentioned herbicides plus atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-trizaine). In this study three concentrations - 10 ppm., 100 ppm., and 1,000 ppm. - of each herbicide were incorporated into agar plates which were subsequently streaked with strains 311b310 and 61A24 of Rhizobium japonicum.

Nodule number, nodule weight, seed yield, protein content, and oil content were not reduced at either location due to herbicide treatment. At the Agronomy Farm, significant increase in nodule numbers was obtained at the last nodule count where herbicides were applied.

A significant reduction in seed yield and nodules per plant at the first nodule count was observed due to inoculation at the Agronomy Farm location. Further studies on the problem are needed to explain the reduction in seed yields.

In the greenhouse experiment, there was no correlation between the number of distorted soybean plants due to herbicide treatment and the number of nodules produced by the affected plants.

Two strains of R. japonicum were used in the microbiological study. Concentrations of 1,000 ppm. for all herbicides inhibited colony growths. Randox, Lorox, and Alanap inhibited growth somewhat at 100 ppm. while Amiben and atrazine had little effect. Colony growth was not impaired at the concentration below 100 ppm.