Effect of Chemical Treatment of Pea Seed on Nodulation by Rhizobium leguminosarum

By K. J. Kadow
L. E. Allison
H. W. Anderson

UNIVERSITY OF ILLINOIS
AGRICULTURAL EXPERIMENT STATION

Bulletin 433

CONTENTS

PAGE

THE PROBLEM 4

DATA FROM EXPERIMENTS 6

Greenhouse Studies, 1934 and 1935 6

Field Studies, 1935 and 1936 7

Greenhouse Studies, 1936 and 1937 7

DISCUSSION AND CONCLUSIONS 10

LITERATURE CITED 12

Urbana, Illinois May, 1937

Publications in the Bulletin series report the results of investigations made by or sponsored by the Experiment Station

EFFECT OF CHEMICAL TREATMENT OF PEA SEED ON NODULATION BY RHIZOBIUM LEGUMINOSARUM

By K. J. Kadow, L. E. Allison, and H. W. Anderson[a]

THE VALUE of chemical treatment of seed in improving the stands of certain varieties of peas has been recognized for some time by commercial growers tho the treatments have only recently come into general use. In repeated tests under Illinois field and greenhouse conditions stands of Surprise, Wisconsin Early Sweet, and Perfection have been more than doubled by the chemical treatment of the seed. Not only have the stands been improved markedly, but the plants from the treated seed have usually been much larger and more vigorous. Alaska variety, on the other hand, has shown very little need of chemical seed treatment; and accordingly the benefits from treatment have been small.

The improvement in stands and in plant vigor from chemical treatment (Fig. 1) comes about by preventing the premature rotting of the cotyledons and the activity of certain undesirable microorganisms such as the "damping-off" fungi. When the seed is untreated, the cotyledons usually rot away soon after the young seedlings are above ground, if not before, and the seedlings are thus deprived of part of their food supply. Also the rotting often affords a point of infection from which soil-inhabiting organisms invade the seedlings and subsequently cause rots. But when the seeds are treated with either Semesan or cuprous oxid (Cuprocide or Metrox), the cotyledons do not rot, but shrivel gradually as their food reserves are used by the young growing plants (Fig. 2).

Considerable data on the control of rots of seed and seedlings have been gathered at the Illinois Station and are being published in a forthcoming bulletin. Some of the results with cuprous oxids were

[a]K. J. Kadow, Associate Pathologist in Horticulture; L. E. Allison, formerly Assistant in Soil Biology; and H. W. Anderson, Chief in Pomological Pathology.

The authors wish to acknowledge the advice given upon the conducting of this study by R. H. Bray, Assistant Chief in Soil Survey Analysis, and O. H. Sears, Associate Chief in Soil Biology; and the assistance in taking technical records given by the following students: S. L. Hopperstead, R. B. McKenzie, C. H. Ackerman, and M. D. Appleman.

FIG. 1.—EFFECT OF CUPROUS OXID SEED TREATMENT ON STAND OF PEAS

One hundred seeds of Surprise variety were planted in each pot of garden soil. Those in the left-hand pot were untreated, and 23 seedlings emerged; the seed in the right-hand pot were treated with cuprous oxid (Metrox), and 94 seedlings emerged. Except for the chemical treatment, the peas in the two pots were planted and grown under the same conditions. Note the apparent difference in vigor as well as in number. (The Metrox used in this study was supplied by the Metals Refining Company, Hammond, Indiana.)

published in an article in *Phytopathology*.[2*] The general trend of the data is indicated in the limited material on this problem presented in this bulletin in Table 2.

THE PROBLEM

Inasmuch as the chief function of seed treatment is to inhibit the activity of certain undesirable microorganisms such as the "damping-off" fungi, the question naturally arises as to the effect of this treatment on the bacteria applied to the seed for the purpose of facilitating nitrogen fixation. The inoculation of peas grown for canning purposes has been practiced for many years, and its importance is widely recognized. A well-nodulated pea crop is adequately supplied with available nitrogen,[8*] which fact not only assures a high quality product but also lengthens the period of time over which the crop may be harvested. Because quality of peas is so closely associated with nitrogen supply, canners insist that the seed be inoculated with a high-grade inoculant even tho the land may have been occupied previously

*These numbers refer to literature citations on page 12.

FIG. 2.—EFFECT OF CUPROUS OXID SEED TREATMENT ON COTYLEDONS AND GROWTH AND VIGOR OF PEAS

The seedlings on the left were taken from the left pot shown in Fig. 1. The cotyledons are badly rotted and in some cases the stem shows disease invasion at the point of attachment. The seedlings on the right, from the right-hand pot in Fig. 1, were from seed treated with cuprous oxid, and aside from the cotyledons being shriveled are in perfect condition. Note difference in size of these seedlings.

by inoculated peas. In response to the many inquiries from growers concerning the effect of the chemical treatments on the nodule-producing bacterium, the studies reported in this bulletin were begun.

The scientific literature on this subject indicates that the organic mercuries were the first products in general use for the treatment of pea seeds. In connection with their use Müller and Stapp[6*] found that they did not influence nodulation of peas when the nodule-producing organism was present in the soil. Sayre[7*] and Jones[5*] stated, but offered no substantiating data, that the treatment of pea seed with organic mercuries kills the bacteria previously added to the seed.

In 1934 Horsfall *et al*[4*] suggested the use of cuprous oxid as a treatment for pea seed. According to experiments with cuprous oxid and Semesan at Urbana, the data from which have not yet been published, cuprous oxid, tho not always superior to Semesan (organic mercury), is a much more reliable treatment under a large variety of field conditions.

DATA FROM EXPERIMENTS

Greenhouse Studies, 1934 and 1935

In the fall of 1934 and again in the fall of 1935, preliminary studies on the effect of Semesan and Cuprocide on the nodulation of peas were conducted under greenhouse conditions. The soil was a dark-colored silt loam with a pH of 6.5. Manure with a high straw content was heavily applied, and the soil was then sterilized and kept fairly wet until plantings were made about six weeks later. Pure cultures of *Rhizobium leguminosarum* were used thruout these studies, and were added to the seed in water suspension. As soon as the seed dried the various chemicals were added in recommended concentrations. The seeds were planted immediately thereafter.

From the results which were obtained (Table 1), it is evident that nitrates were not present in sufficient amounts[1*] to hinder nodulation. The application of the straw to the soil was effective in holding the nitrate nitrogen at a low level because the straw stimulated nitrate-assimilating bacteria to utilize the nitrates about as rapidly as they were produced. Records were taken six to eight weeks after the planting dates.

Nodulation was excellent on the inoculated controls altho actual

TABLE 1.—EFFECT OF SEMESAN AND CUPROUS OXID ON THE NODULATION OF PEAS[a]
(Greenhouse studies)

Seed treatments, listed in order applied, and year	Moisture content of soil	Nodulation
No bacteria or chemical treatment added		
1934	Optimum	No nodules present
1934	Suboptimum	No nodules present
1935	Optimum	No nodules present
Bacteria added, no chemical treatment		
1934	Optimum	Heavy nodulation
1934	Suboptimum	Nodulation about 20 percent less than in moist soil
1935	Optimum	Heavy nodulation
Bacteria added, also cuprous oxid[b]		
1934	Optimum	Few nodules evident
1934	Suboptimum	Nodulation 20 to 40 percent of that of checks
1935	Optimum	Few nodules evident
Bacteria added, also Semesan[c]		
1934	Optimum	Few nodules evident
1934	Suboptimum	Nodulation 20 to 40 percent of that of checks
1935	Optimum	Few nodules evident

[a]One hundred peas of Alaska variety were used in each test. Soil, pH 6.5; temperature, about 60° F.
[b]The cuprons oxid used was Cuprocide, furnished by Röhm and Haas, 222 West Washington Square, Philadelphia, Pennsylvania.
[c]Semesan was supplied for these experiments by DuPont, Wilmington, Delaware.

counts of nodules were not made. The suboptimum moisture content of the soil, altho significantly lower than the optimum content, only slightly reduced the nodulation and was not low enough to restrict plant growth.

Where Semesan or cuprous oxid was applied to the seed subsequent to inoculation, the number of nodules per plant was greatly reduced. In the soil with optimum moisture the chemicals practically prevented nodulation, while in the soil with a suboptimum water content nodulation was not reduced so severely.

Field Studies, 1935 and 1936

In the springs of 1935 and 1936 attempts were made to secure experimental data on the effect of chemical seed treatments on nodulation under field conditions, but the various soils in which the peas were planted contained the nodule-forming bacteria and nodulation consequently occurred on all plots whether or not the seed was inoculated. This is, however, a very significant point, for no peas had been grown for from six to ten years on the soil used in these field studies. Thus where peas are grown in a three- to five-year rotation on fertile soils that are not too acid, it should not be necessary to inoculate the seed of later crops if earlier crops have been well nodulated, for nodulation will result from the bacteria already present in the soil.

These field studies showed conclusively that the treatment of pea seed with Semesan, cuprous oxid, or zinc oxid does not influence nodulation if the nodule-producing bacteria are already present in the soil. The value of treatments in controlling seed and seedling rots (Table 2) also was demonstrated.

Greenhouse Studies, 1936 and 1937

The greenhouse studies in 1936 failed because the available nitrates in the soil were so high that nodulation did not occur.

The most conclusive and thoro series of all the experiments was conducted in the spring of 1937 under greenhouse conditions. The soil in this study was a silt loam taken from a well-drained bank about 5 feet below the soil surface. The purpose in obtaining soil from such a location was to obtain soil with a low organic-matter content and therefore to avoid, in so far as possible, the interference of a high nitrate level. The soil was steamed in the greenhouse, and after partial sterilization it was recontaminated with damping-off species of Pythium and Rhizoctonia and several other soil-inhabiting fungi.

After two months the plantings were made with the nitrate level at

TABLE 2.—EFFECT OF CHEMICAL SEED TREATMENTS ON STAND AND NODULATION OF PEAS: SURPRISE VARIETY

(Field and greenhouse studies)

Seed treatments, listed in order applied, and year	Number of replications	Final stand[a]	Average number of nodules per plant
No bacteria or chemical treatment added		*perct.*	
1936, field	5	22	No record[b]
1937, greenhouse	2	60	.5
Bacteria added, no chemical treatment			
1936, field	5	25	No record
1937, greenhouse	2	47	75.0
Bacteria added, also cuprous oxid[c]			
1936, field	5	71	No record
1937, greenhouse	2	99	4.0
Bacteria added, also Semesan (organic mercury)			
1936, field	5	74	No record
1937, greenhouse	2	99	3.0
Bacteria added, also Vasco 4[d] (zinc oxid)			
1936, field	5	49	No record
1937, greenhouse	2	63	12.0
Cuprous oxid added, bacteria added to soil, not to seed			
1936, field	1	79	No record
1937, greenhouse	1	99	96.0
Bacteria added, then cuprous oxid, then graphite[d]			
1937, greenhouse	2	99	4.0
Bacteria added, then graphite, then cuprous oxid			
1937, greenhouse	2	94	17.0

[a]221 ± 2 seeds were included in each greenhouse planting; 228 ± 2 were included in each field planting.
[b]"No record" indicates that no records were taken because the soil contained the nodule-producing bacterium, and consequently all plantings had nodules.
[c]Cuprocide.
[d]Vasco 4 was supplied for this study by the Virginia Smelting Company, Norfolk, Virginia; and graphite by the Joseph Dixon Crucible Company, Jersey City, New Jersey. (See footnotes to Table 1 for sources of other materials.)

2 pounds per acre. During the course of the study the nitrate nitrogen increased slowly, and after five weeks it had reached 15 pounds per acre, a level which was not sufficient to inhibit nodulation. At this time the records were taken.

In this series Vasco 4 (principally zinc oxid) was included along with Semesan and cuprous oxid, inasmuch as it was thought possible that the zinc might have some application as a pea-seed treatment in those sections of the country which have soils with a low soluble-zinc content.[a] Likewise graphite, which is recommended by Arnold and Horsfall[3]* to prevent drill clogging and cracking of pea seed treated with cuprous oxid, was included. It was determined in the laboratory

[a]In Illinois zinc oxid has no practical value as a pea-seed treatment.

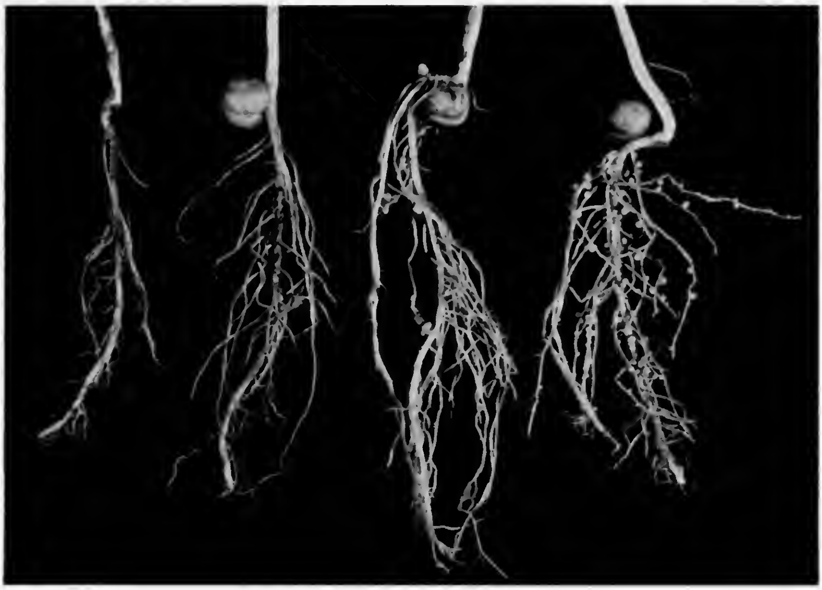
that graphite was not toxic to the nodule producing bacterium. Consequently it seemed important to learn if graphite could be added to the seed in such a manner as to prevent the harmful effects of chemicals on nodulation. In each of the treatments, many of which were duplicated, 221 ± 4 seeds were planted. In laboratory tests the seed germinated 100 percent.

Records were taken of the final stand (Table 2), altho seed and seedling rots were not nearly so severe as under field conditions. The plants were washed free of the soil and the nodules counted. The outstanding results are shown in Figs. 3 and 4. Table 2 includes, in addition to the results of this study, stand records secured from field studies conducted during the growing season of 1936. No records on nodulation were secured in the field study of 1936 because the soil contained the pea nodule bacteria and all plantings, whether treated or not, showed nodulation.

(1) (2) (3) (4)

FIG. 3.—EFFECT OF DIFFERENT CHEMICAL SEED TREATMENTS ON NODULATION OF PEAS

(1) Seed not inoculated nor treated; note lesion on stem. (2) Seed inoculated but not treated; nodules averaged 80 per plant. (3) Seed inoculated and treated with cuprous oxid; nodules averaged 4 per plant. (4) Seed inoculated and treated with zinc oxid; nodules averaged 12 per plant. Seed treated with Semesan (not shown) averaged 3 nodules per plant.



(1) (2) (3) (4)

FIG. 4.—RELATION OF GRAPHITE TO NODULATION WHEN GRAPHITE WAS APPLIED BEFORE OR AFTER SEED TREATMENT—ALSO EFFECT OF SEED TREATMENT WHEN BACTERIA ARE IN THE SOIL

(1) Seed not inoculated nor treated. (2) Seed inoculated and treated with cuprous oxid and then with graphite; nodules averaged 4 per plant. (3) Seed inoculated and treated with graphite and then with cuprous oxid. Nodules averaged 17 per plant and were larger than in No. 4. (4) Seed not inoculated but treated with cuprous oxid, and a water suspension of Rhizobia added to the soil at planting time; nodules averaged 96 per plant.

DISCUSSION AND CONCLUSIONS

The nodulation of peas induced by inoculation of the seed with *Rhizobium leguminosarum* is practically prevented by treating the inoculated seed with Semesan (organic mercury), cuprous oxid, or Vasco 4 (principally zinc oxid), according to the results of the experiments described in the foregoing sections. But these chemicals have no noticeable effect on nodulation by bacteria present in the soil.

In many instances the reduction in the actual number of nodules as a result of seed treatment appeared to be partially offset by a considerable increase in the size of the individual nodules. While this increase in size of nodules often offsets reductions as large as 50 percent in numbers of nodules, it does not seem to do so in the general run of cases involving greater reductions in numbers.

It seems worth while to mention that in spite of the steadily accumulating evidence of the harmful effects of seed treatment on nodulation, a few instances have been observed under commercial growing conditions where cuprous oxid treatment of inoculated seed did not materially reduce nodulation. These cases occurred when the peas were growing on rather dry soil, a condition which seems to be directly in line with the limited data presented on this point in Table 1. No explanation of this observation is offered as yet. In the Illinois experiments cuprous oxid has been found to be the most consistently satisfactory chemical for the treatment of pea seeds under field conditions.

Not all varieties of peas show marked benefits from chemical seed treatment; accordingly only those should be treated which are found to be benefited. Varieties which do not need treatment might better be inoculated and not treated, so that the bacteria may thus be established in the soil.

Since seed treatment of such varieties as Surprise, Wisconsin Early Sweet, and Perfection increases the stand and vigor of the seedlings, and since inoculation of the seed is the most practical means of securing nodulation in soils which do not contain the bacteria, further research appears necessary as a means of rendering the two practices compatible. Direct application of the pea Rhizobia to the soil at time of planting would involve considerable technical difficulty as well as considerable added expense, altho if properly done it would undoubtedly be effective. The addition of the bacteria to the soil would in all probability require much larger quantities of Rhizobia than would be needed to accomplish the same results from seed inoculation.

Whether or not the addition to the seed before chemical treatment of certain nontoxic materials, such as graphite, offers a practical method of ameliorating the harmful effect of chemical seed treatment on nodulation, the authors are not yet prepared to say. It does seem, however, that if the benefits of both inoculation and seed treatment are to be had, some such procedure offers a likely means of solving the problem. From very limited data and experience it appears that graphite added to inoculated seed before a chemical treatment is applied permits about one-fourth normal nodulation to occur, and does not materially influence the effect of the treatment on the stand and vigor of the seedlings. This indication is in keeping with the findings of L. T. Leonard,[a] bacteriologist of the U. S. Department of Agriculture, who found that cuprous oxid prevented nodulation when pure·

[a]Correspondence between L. T. Leonard and L. E. Allison, November 24, 1936.

cultures of Rhizobia were used, but that when the bacteria were added to the seed in charcoal or peat some nodulation resulted.

Another likely method of securing the benefits of both seed treatment and inoculation would be to establish the bacteria in the soil by planting inoculated seed of varieties which do not need seed treatment. Once the bacteria become established in the soil, varieties which should be chemically treated may be treated and grown without interfering with nodulation, providing the soil is maintained in good cultural condition and is not allowed to become too acid.

LITERATURE CITED

1. ALLISON, F. E., and LUDWIG, C. A. The cause of decreased nodule formation on legumes supplied with abundant combined nitrogen. Soil Sci. **37**, 431. 1934.
2. ANDERSON, H. W., KADOW, K. J., and HOPPERSTEAD, S. L. The evaluation of some cuprous oxides recommended as seed-treatment products for the control of damping-off. Phytopathology **27**, 575-87. 1937.
3. ARNOLD, E. L., and HORSFALL, J. G. Use of graphite to prevent clogging of drills when sowing dusted pea seed. N. Y. (Geneva) Agr. Exp. Sta. Bul. 660. 1936.
4. HORSFALL, J. G., NEWHALL, A. G., and GUTERMAN, C. E. F. Dusting miscellaneous seeds with red copper oxide to combat damping-off. N. Y. (Geneva) Agr. Exp. Sta. Bul. 643. 1934.
5. JONES, L. K. The treatment of pea seed with chemical materials. N. Y. (Geneva) Agr. Exp. Sta. Circ. 118. 1934.
6. MÜLLER, A., and STAPP, C. Beiträge zur Biologie der Leguminosenknöllchenbakterien mit besonderer Berücksichtigung ihrer Artverschiedenheit. Arb. Biol. Reichanst. Land. u. Forstw. **14**, 455-554 (see especially pp. 534-36). 1926.
7. SAYRE, C. B. Better methods of canning crop production. N. Y. (Geneva) Agr. Exp. Sta. Bul. 553. 1928.
8. WHITING, A. L., FRED, E. B., and STEVENS, J. W. Inoculation increases yield and quality of peas for canning. Wis. Agr. Exp. Sta. Bul. 372. 1925.

5,050—5-37—11974