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Seed Treatment for the Control of Bean Blight.

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SEED TREATMENT FOR THE CONTROL OF BEAN BLIGHT

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ABSTRACT

In different ways during the past forty years a great variety of materials have been tried as seed-treating agents for the control of bacterial bean blight. As most of these treatments were unsatisfactory, no seed treatment has been recommended for practical control for a considerable period. Since the bacteria may be situated deep within the seed, an effective treating agent must be capable of penetrating that portion of the seed in which bacteria may be found or carried at germination, and there causing the destruction of the bacteria without appreciably injuring the seed itself. The fact that aqueous solutions cause slipping of the bean seed coat in 12-15 minutes necessitates the use of a solution which will either penetrate the seed and kill the bacteria within this time limit, or will prevent the seed coat from slipping so that the treatment may be carried out over a longer period of time. With this in mind, Person and Edgerton reported in 1939 a fairly effective seed treatment using a solution of 1:500 mercury bichloride in 70 per cent ethyl alcohol and 3 per cent acetic acid for 12-15 minutes. This work has been continued with a view to improving effectiveness and eliminating the disadvantages of injury to germination and slipping of the seed coat.

A number of materials were given preliminary laboratory tests to determine the optimum concentration and maximum time over which the treatment could be safely utilized without causing appreciable injury to the bean seed. Laboratory tests were also run to determine

the bactericidal effectiveness of the solutions by treating diseased seed lots and transferring the treated seed aseptically to tubes of sterile dextrose broth. The number of tubes showing bacterial growth after a week's incubation period was used as the criterion for determining the success or failure of a solution as a seed-treating agent. Bactericidal tests were also run on cultures of the bean blight organisms to determine the minimum concentration at which a particular disinfectant killed the bacteria. On the basis of numerous laboratory tests several treatments were selected for field trials, using several lots of known diseased seed. The treatments had the following composition:

(1) 1:500 mercury bichloride in di-ethyl ether.
(2) 1:20,000 brilliant green in 50% ethyl alcohol plus 3% acetic acid.
(3) 1:500 mercury bichloride in 70% ethyl alcohol plus 3% acetic acid.
(4) 1:20,000 gentian violet in 50% ethyl alcohol plus 3% acetic acid.

Seed was treated by immersing it in the treating solution for definite time intervals. The solution was then poured off and the seed spread out to dry for a short while. The treated seed was then taken to the field and planted in measured plots, using the same amount of seed for each plot. Check plots were isolated from the treated plots by several barrier rows in order to reduce the spread of bacteria from the check plants to those developing from the treated seed insofar as possible.

Blight counts were made on plants after the simple leaves had fully emerged but before any trifoliate leaves had formed. At this stage the plants were well separated and could be examined individually.

All primary infections on the simple leaves were recorded. Striking differences in blight percentages were noted between the plants developing from treated and untreated seed. A fraction of 1 per cent was the usual amount of blight initially developing in the plants grown from treated seed, while the untreated seed gave rise to plants which contained as much as 60 per cent blight.

Periodic examination of the plots revealed that the treatment increased the vigor of the plants and that these plants were not as severely injured by secondary spread of the bacteria as were those in the check plots. Plants grown from untreated seed were stunted and yellowed, and a great many were killed before they had a chance to blossom. With this in mind, yields were taken on the "treated" and "untreated" plots by picking the center two or three rows of each plot and weighing the green beans when they were at their best market quality. The data collected revealed that the "treated" plots outyielded the "untreated" plot from 2-7 times. The quality of green beans obtained was correspondingly higher in the treated plots. Pods showed less spotting and were not shriveled or deformed as were the beans in the "untreated" plots. The treatments gave rise to as high and satisfactory yields as would have been expected in fields planted with healthy seed.

Samples of treated bean seed were stored for varying time intervals in order to determine the effect of treatment on storage of the seed. Periodic germination tests were made on these samples and it

was found that no injury to germination had occurred, even after a seven months storage period. Thus the tests indicated that bacterial bean blight might be effectively controlled by seed treatments that had no injurious effect on seed stored over a fairly long period of time.

INTRODUCTION AND HISTORICAL

Bacterial bean blight has been known for a number of years. It was first reported in this country by Halsted (8), from New Jersey, and Beach (1), from New York, in 1892. According to Halsted (8), the disease had probably been noted as early as 1886 in certain bean fields of western United States. The earliest probable European account of the disease was that by Delacroix (6), who reported the disease as occurring on beans at the outskirts of Paris in 1899. Since that time bacterial bean blight due to one or more organisms has been reported from a number of countries. It is now considered to be practically world-wide in its distribution.

According to Burkholder (2), a total of six bacterial diseases of the common bean, Phaseolus vulgaris L., have been described, but only three of these are considered to be common and therefore of great importance. Common blight, caused by Phytomonas phaseoli and first described by Smith (23), was formerly thought to be the sole organism associated with bean blight. Since that time, halo blight, caused by Phytomonas medicaginis var. phaseolicola has been described by Burkholder (3) and has been reported as causing serious losses of beans. Bacterial wilt, caused by Phytomonas flaccumfaciens and first described by Hedges (10), is the third bacterial disease of major importance on beans.

No distinction has been made in this work between cultural or field characteristics of the various organisms, although isolations

from leaves, stems, and pods of diseased plants have indicated that common blight and the halo blight are of greatest importance to the bean crop of Louisiana. Plate I, figures 1 and 2, and Plate II, figure 1, show typical symptoms of blight infections on leaves and pods. These three organisms are seed-borne. Diseased seed of certain varieties may show shriveling and yellowing, particularly in the white-seeded beans, as described by Hedges (11) and (12). No outward differences could be detected in certain dark-seeded varieties such as Black Valentine, which was extensively used in this research. Zaumeyer (27, 29) has shown histologically that Phytomonas phaseoli may enter the seed either through the micropyle, if the pod cavity is invaded, or through the vascular system from prior infection of the dorsal suture of the pod. Seedlings arising from such severely infected seed are naturally weakened and may be stunted as shown in Plate II, figure 2. These stunted and severely infected plants act as initial infection centers from which the bacteria spread to neighboring plants. Such dissemination may cause losses of entire fields, as shown in Plate III, figures 1 and 2, in which a healthy and a diseased field of beans are contrasted.

According to Burkholder (4), infected seed may give rise to the so-called "snake-heads" in which the growing tip of the seedling has been destroyed by the bacteria and only the cotyledons remain. A number of such severely infected seed may fail to push their way through the ground and this failure may result in poor stands. Harter (9) believed an undue emphasis had been placed on blight as the causal

agent of such failures. He demonstrated that thresher injury might cause the same condition and believed this factor might frequently account for this condition.

Soil transmission of bacterial bean blight due to Phytomonas phaseoli was claimed by Muncie (17) for Michigan conditions. Patel (18) showed that Phytomonas phaseoli, along with several other pathogens, was capable of living over winter in sterilized and non-sterilized soil. Wager (26), working in Transvaal on bacterial wilt and blight of French beans, claimed that infection arose from the seed, with no root infection taking place from infected soil. Skoric (22), working on bacterial blight of peas, found no evidence that the organism causing this disease was capable of overwintering in the soil. Zaumeyer (28), on the other hand, collected data showing that beans planted in a field having had a severely infected crop the previous year became blighted with B. phaseoli. No experimental data on overwintering of blight bacteria under Louisiana conditions were collected. Observations, however, indicate that the high soil temperatures reached during the summer and the dry fall weather possibly eliminated the organisms from the soil. Since blight was not usually important in all plantings of Louisiana beans, due to the hot dry weather at this time of the year, there was little inoculum to be carried in the soil over the relatively mild winter period. Soil transmission of bacterial bean blight in Louisiana is therefore probably of minor consequence.

The emergence of a few seed-infected plants from the soil gives a primary source of inoculum from which secondary spread of bacteria to surrounding plants may take place. Treatment of the primary infected plants was the basis of this work, since there is little that can be done after infection has gained a foothold in the seedling plants. Early workers believed that spraying infected plants with Bordeaux or other fungicides and insecticides might control the disease directly or indirectly through control of insects which might act as transmission agents. Rapp (21), Muncie (17), and Townsend (25) have stated that spraying met with little or no success and was not to be recommended. Roguing of diseased plants might be practical if the disease was recognized by the individual. On the other hand, this practice might be a means of spreading infection through the handling of both diseased and healthy plants. On a commercial basis this method did not appear to be feasible and other methods were sought.

The use of resistant varieties has long been anticipated as a possible solution of the problem and breeding work has been carried on intensively at various stations over a period of years.

Rands and Brotherton (20) ran a four-year test on bean varieties resistant to anthracnose and bacterial blight. They found that no variety tested showed any high degree of resistance to blight. Rapp (21) tested 43 varieties of beans and found a great deal of variation in susceptibility to infection. No variety, however, showed any great resistance to blight. Zaumeyer (28) found a few varieties of the Refugee type which showed some resistance to blight infection but none

showed complete resistance. At present there is still no commercial variety of beans giving a high degree of resistance to bacterial blight.

One of the most logical but also most difficult procedures concerns seed treatment for control of the blight. Since the organisms are located deep within the seed, a treatment must be used which is capable of penetrating the seed and killing the bacteria wherever they are located without causing injury to seed germination or slipping of the seed coat*. Edgerton and Moreland (7) found that seed coats of beans treated in aqueous solutions slipped after 18-20 minutes treatment. Some seed coats slipped earlier than this and the beans in such cases were considered to be poor seed that probably would not have germinated anyway. Some experiments of this research, involving rigidly selected seed, indicated that those seeds showing no visible cracks or fissures in the seed coat were less likely to slip after a 15-minute immersion in an aqueous solution than seeds which had minute cracks and fissures. As many as 50 per cent of some lots of seed examined were found to have defective seed coats, yet such defects had no appreciable influence upon germination. Edgerton and Moreland (7) treated bean seed in hot water at 50° C. for eight minutes and found that this treatment greatly increased the blight infection, due possibly to decreased seedling vitality or to the probability that the water acted as a carrier for the bacteria. Leonard (14) and (15) showed that bean wilt might be greatly increased by inoculating seed with a liquid culture of legume bacteria. He explained this as being due to stimulation of the wilt organisms inside

*Used throughout this work as a collective term for both inner and outer seed coats.

the seeds when water was absorbed. In experiments in which diseased seeds were soaked in water while other seeds from the same lot were planted dry, the seeds soaked in water prior to planting emerged with a great deal more blight than seeds planted dry.

A great variety of disinfectant materials has been used by investigators over a forty-year period. Early work was concerned primarily with hot water, mercury bichloride, or formalin as seed-treating agents. Edgerton and Moreland (7) treated respective lots of bean seeds for 18-30 minutes in each of the following solutions: 1:1,000 mercury bichloride, 1:50 benetol, 1:100 formalin, or 1:1,000 mercury bichloride in a 1:50 solution of glycerine. None of the above treatments caused a great reduction in germination and the benetol and mercury bichloride treatments were found to be somewhat effective in reducing blight. Kapr (21) treated bean seeds for various lengths of time in solutions of formaldehyde, mercury bichloride, sulfuric acid, hot water, and with dry heat. All of these treatments weakened germination considerably and gave no adequate blight control. Muncie (17) used sodium nitrate in combination with mercury bichloride solutions to prevent slipping of the seed coat and found that this treatment was unsuccessful for blight control. He obtained best results by soaking seeds for thirty minutes in a 35 per cent solution of bleaching powder or by sprinkling the seed with one pint of formaldehyde to 30 gallons of water. None of the treatments, however, gave complete control. His treatments and results are fairly representative of the work carried out over a number of

years. Recently, Person and Edgerton (19) reported a fairly effective treatment using a solution of 1:500 mercury bichloride in 70 per cent ethyl alcohol plus 3 per cent acetic acid. This treatment was used for 12-15 minutes and caused neither appreciable decrease in germination nor slipping of the seed coat.

The major work reported in this thesis has involved the use and possible improvement of the Edgerton-Person treatment for the control of bean blight. This treatment gave indications of a fairly effective control but it could not be used longer than 12-15 minutes without causing injury to germination and slipping of the seed coat. The restricted use of ethyl alcohol and the necessity of preparing correct relative proportions of materials might easily impair the effectiveness of the treatment if not handled properly. The loss or dilution of toxic ingredients after continued use over a period of time was also considered as a possible factor in determining the effectiveness of the treatment. The above limitations on the treatment have made recommendations for its use to farmers and seedsmen impractical and further study has been carried out in an attempt to solve some of the attendant difficulties and at the same time to improve, if possible, the effectiveness of the treatment. Experiments were conducted to determine the feasibility of substituting the cheaper methyl alcohol for ethyl alcohol in the treatment. Work was also carried out in which non-aqueous solutions were used as carriers for the toxic materials in an effort to prolong the time over which seed treatment could be carried out without causing slipping of the bean seed coat. All of the above procedures

showed some sort of promise and tended to converge the various materials and methods toward a common point from which an attempt has been made to evolve a fairly effective and practical seed treatment.

Another line of attack used in the present research was that of treating seeds with certain dyes which had been shown to exhibit marked bacteriostatic action. Churchman (5) showed that the acridine and triphenyl methane group of dyes were highly effective in killing certain bacteria. He also demonstrated that a number of dyes had inhibited bacterial movement and multiplication. Tilley (24) demonstrated the bactericidal effectiveness of a number of dyes. He found that basic dyes were usually more effective toward gram-positive organisms than toward gram-negative ones. The addition of phenol or orthocresol increased the efficiency of triphenyl methane dyes against gram-negative bacteria. The addition of phenol to brilliant green or malachite green did not, however, increase their bactericidal efficiency. Kobs and Robbins (13) claimed that increase in H-ions, in general, increased the toxicity of dyes except where solubility was concerned. Monteith (16) used malachite green and gentian violet as a means of controlling brown patch of grass caused by Rhizoctonia solani.

MATERIALS AND METHODS

The work of Person and Edgerton had previously shown that a 1:500 concentration of mercury bichloride in 70 per cent ethyl alcohol plus 3 per cent acetic acid by volume was capable of controlling bacterial bean blight to some extent. The treatment, however, could not be prolonged beyond 15 minutes without causing serious injury to the seed through slipping of the seed coat. The acetic acid in the solution presumably acted as a penetrating agent carrying the mercury bichloride to the site of bacterial infection, but it also had a somewhat detrimental effect on seed germination, particularly under prolonged treatment. With these things in mind, a number of materials were used in preliminary laboratory tests to determine the effect of various chemicals as toxic agents in bean blight control and as agents possibly influencing seed germination. Since aqueous solutions caused slipping of the bean seed coat in 12-15 minutes, a solution was required which was capable of penetrating the seed within this short time period, killing the bacteria within the seed and at the same time causing a minimum amount of injury to the seed both in relation to germination and to subsequent vigor of the young seedling. Another possibility considered was that of using some material which would prevent the slipping of the seed coats and at the same time would act as a suitable vehicle to carry the toxic materials to the places in which the bacteria were located and there cause their destruction. At the same time, it was recognized that this substance must be one which would

not cause appreciable decrease in germination even though the treatment was carried out over a relatively long period of time.

Numerous difficulties were encountered in attempting to find reliable toxicity tests. Early in the work diseased seeds were treated with the particular disinfectant on which data was desired and they were then transferred aseptically to tubes of Difco dextrose broth. Ten tubes were usually used for each disinfectant to be tested and from one to four treated bean seeds were dropped into each tube. The tubes were then incubated for one week with frequent examinations each day. Any series of tubes showing little or no bacterial growth after one week were considered to have been effectively treated with bactericidal agents. The disinfectant was then deemed desirable for further laboratory tests and possible improvement or for field trials. The fact that no accurate knowledge as to the amount of infection in diseased seed lots could be obtained without first running field trials made this type of test rather inaccurate, since one would not know whether the seeds were diseased or not. The growers' or seedsman's statement was not scientifically reliable. Further, a large number of tubes had to be used in order to detect the diseased seeds, and this involved excessive time and labor, and since the organisms developing in the tubes had to be isolated and compared as to cultural characteristics with Phytomonas medicaginis var. phaseolicola and Phytomonas phaseoli. This procedure was used during most of the earlier studies but was later discarded in favor of a more efficient method.

Preliminary tests of disinfectants carried out according to accepted bacteriological procedures using pure cultures of the desired organism gave indications of being satisfactory, and gave reliable information regarding the toxicity of various materials. The following method was therefore used to test the toxicity of a number of substances to the bean blight bacteria.

Five cubic centimeters of sterile Difco dextrose broth were added aseptically to each of a series of 10 sterile culture tubes by means of a sterile pipette. Ten cc. of the particular disinfectant solution to be used were then pipetted into the first tube and mixed well with the broth. Ten cc. of the broth-disinfectant mixture from tube number one were then pipetted to tube number two and mixed well in the broth in that tube, leaving 5 cc. of broth-disinfectant mixture in tube number one. Ten cc. of this mixture in tube number two were then pipetted into tube number three and the process repeated through each of the ten dilutions, thus leaving 5 cc. of broth-disinfectant mixture in each preceding tube. The excess 10 cc. in the last dilution tube, or tube No. 10, were discarded and all tubes then had equivalent amounts of materials in a uniformly decreasing series. Five cc. of a broth culture containing a mixture of strains of Phytomonas phaseoli at least 72 hours old were then pipetted aseptically into each of the disinfectant dilution tubes at 30-second intervals. This left a 10 cc. volume of broth-disinfectant-culture mixture in each tube.

Since ten dilutions were ordinarily used, two disinfectant tests were run simultaneously in series by adding the 5 cc. of broth culture at 30-second intervals to each of the twenty tubes, ten in each series.

After completing the twentieth tube the bacterial culture in the first tube had been exposed to the action of the disinfectant for 10 minutes and subculturing was then carried out. This was done by transferring a loopful of material from each of the disinfectant dilution tubes to a tube of sterile dextrose broth. These tubes were then incubated at 20-25° C. and readings made on the subculture tubes at 24, 48, and 72-hour intervals. The dilution at which growth ultimately occurred in the subculture tube was used as the criterion of how toxic any given material was to the bacteria. After the final reading the broth tubes from the check, the greatest dilution used, and the greatest concentration of disinfectant showing growth were plated as dilution series in Difco dextrose agar to check the organisms surviving as being the same as those introduced into the tubes.

Disinfectant materials were usually given preliminary trials from a 1:100 stock solution. The dilution proportions and milligrams per 100 cc. of disinfectant materials at the various dilutions are shown in Table 1. Stock solutions of some disinfectants were made up in 1:500 and 1:1,000 dilutions and the proportions and milligram per 100 cc. calculations are shown in Tables 2 and 3.

A number of materials, including sulfanilamide and some of its derivatives were tried out on cultures of the blight organism to determine whether or not they might also exhibit marked toxicity toward plant pathogenic bacteria. As may be seen from Table 4, sulfanilamide in ethylene glycol had very little if any effect on the bacteria used

Table 1. Dilutions of materials using a 1:100 stock solution.

Tube No.	Final dilution after adding bacteria	Mg/100 cc. 1:100 stock solution
1	1:300	333.33
2	1:450	222.22
3	1:675	148.13
4	1:1012	98.75
5	1:1518	65.83
6	1:2278	43.89
7	1:3417	29.26
8	1:5125	19.51
9	1:7688	13.00
10	1:11532	8.66

Table 2. Dilutions of materials using a 1:500 stock solution.

Tube No.	Final dilution after adding bacteria	Mg/100 cc. 1:500 stock solution
1	1:1500	66.6
2	1:2250	44.4
3	1:3375	29.6
4	1:5062	19.73
5	1:7593	13.15
6	1:11390	8.77
7	1:17085	5.84
8	1:25628	3.89
9	1:38443	2.59
10	1:57665	1.73

Table 3. Dilutions of materials using a 1:1.000 stock solution.

Tube No.	Final dilution after adding bacteria	Mg/100 cc. 1:1,000 stock solution
1	1:3000	33.3
2	1:4500	22.2
3	1:6750	14.80
4	1:10125	9.96
5	1:15187	6.57
6	1:22781	4.38
7	1:34171	2.92
8	1:51207	1.94
9	1:76810	1.29
10	1:115215	.86

Table 4. Toxicity limits of various disinfectants.

Disinfectant	Dilutions		Mg/100 cc.	
	Lower	Higher	Lower	Higher
8-hydroxyquinoline sulfate in H_2O (1:100)	1:675	1:1012	148.13	98.75
Sulfanilamide in ethylene glycol (1:100)	1:300*	1:300	333.33	333.33
Brilliant green in H_2O (1:100)	1:3417	1:5125	39.26	19.51
Auramine O in H_2O (1:100)	1:300*	1:300	333.33	333.33
Sulfapyridine in 95% methyl alcohol (1:100)	1:450	1:675	222.22	148.13
Gentian violet in H_2O (1:100)	1:1012	1:1518	98.75	65.83
95% methyl alcohol 1:500 $HgCl_2$ in 95% ethyl alcohol	1:11390	1:17085	8.77	5.84
95% ethyl alcohol 1:500 $HgCl_2$ in H_2O	1:25628	1:38443	3.89	2.59
1:500 $HgCl_2$ in 95% methyl alcohol	1:11390	1:17085	8.77	5.84
Sulfamethylthiazole in 95% methyl alcohol (1:100)	1:450	1:675	222.22	148.13
1:1000 brilliant green in 1/2 H_2O, 1/2 glycerine	1:6750	1:10125	14.80	9.86
1:1000 brilliant green in 1/2 95% methyl alcohol, 1/2 glycerine	1:6750	1:10125	14.80	9.86
1:1000 brilliant green in 1/2 95% methyl alcohol, 1/2 glycerine, 2% HCl	1:15187	1:22781	6.57	4.38
1:1000 $HgCl_2$ in 1/2 95% methyl alcohol, 1/2 glycerine	1:15187	1:22781	6.57	4.38

*Growth in greatest concentration used.

in the toxicity tests. Likewise, sulfamethylthiazole[1], as a 1:100 dilution in 95 per cent methyl alcohol, was but slightly toxic in the tests in which it was tried. Another sulfanilamide derivative, sulfapyridine[2], at 1:100 dilution in 95 per cent methyl alcohol, was found to have but slight toxicity in the laboratory tests.

Laboratory tests in the early stages of this research consisted primarily of trying various dilutions of ethyl alcohol and organic acids in combination and at varying concentrations in order to determine the effect on seed germination and the toxicity to bacteria in supposedly infected bean seed. Acetic, propionic, and benzoic acids were tried in 2, 4, and 6 per cent concentrations in combination with ethyl alcohol. Acetic and propionic acids were found to be readily miscible with ethyl alcohol and mercury bichloride. Some difficulty was experienced in attempting to dissolve benzoic acid at the lower dilutions. A saturated solution of the acid was therefore made up in 95 per cent ethyl alcohol and this was then used for additions at various concentrations to the mercury bichloride - alcohol mixture. Clouding took place if 6 per cent benzoic acid was added to 15 per cent alcohol, however, and this dilution was therefore discarded from subsequent tests.

The fact that various dyes had been shows to exhibit bacteriostatic action led to the trial of brilliant green, ventian violet, and auramine O.[3]

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[1] 2-sulfanilamidomethylthiazole or M. T. 3920 was kindly supplied by the Winthrop Chemical Co., New York, N. Y.

[2] Kindly supplied by the Lederle Laboratories, Inc., Pearl River, N. Y.

[3] Purchased from Eimer and Amend Co., New York, as commercial, uncertified dye.

The ready solubility of brilliant green and gentian violet in water, ethyl alcohol, methyl alcohol, and toluene led to extensive laboratory and field tests of these dyes in various solvents. The results of these tests have been brought together in Table 4. As may be seen, brilliant green exhibited greater toxicity than gentian violet in the laboratory tests and this activity was further confirmed under field tests. Acidification of the brilliant green - alcohol solution seemed to increase its toxicity under laboratory conditions.

The restrictions and expense connected with the use of ethyl alcohol led to trials with methyl alcohol as a carrier for the toxic chemical agents. The results of laboratory tests given in Table 4 indicate that methyl alcohol gave as good results as ethyl alcohol not only as a solvent but also as a toxic agent. Methyl alcohol, however, did not appear to retain mercury bichloride in solution as well as did the ethyl alcohol, particularly when stored for a considerable length of time in the laboratory. Brilliant green and other materials seemed to be as readily soluble in methyl alcohol as they were in ethyl alcohol or water.

Since bean seed could not be treated longer than 12-15 minutes in any of the solutions previously tried without causing serious slipping of the seed coat, laboratory trials were made with a number of non-aqueous solutions. Among such solvents tried and considered worthy of further tests were petroleum ether, di-ethyl ether, chloroform, and toluene. Mercury bichloride was found to be readily soluble in only two of these, di-ethyl ether and toluene. The ready combination

of di-ethyl ether or toluene with ethyl alcohol appeared to afford an excellent means of preventing the slipping of the seed coat while still using alcohol as a toxic agent, and laboratory experiments along this line justified this belief. A fairly low concentration of toluene or di-ethyl ether in alcohol readily prevented slipping of the bean seed coat, even when the seeds were treated over a period of several hours. The highly inflammable properties of ether, however, somewhat restricted its use as a seed disinfectant or carrier for toxic materials unless handled with extreme care. Further laboratory investigations involved the use of glycerin to prevent the slipping of the seed coat. Glycerin was found to mix well with other solvents such as ethyl and methyl alcohol and at the same time it retained materials such as brilliant green and mercury bichloride in solution.

LABORATORY GERMINATION TESTS

Germination tests were run on treated bean seeds in connection with laboratory tests on the toxicity of various materials to the bean blight bacteria. In these tests, usually 50 to 100 commercial bean seeds of the varieties Bountiful or Black Valentine were treated for a definite length of time and then placed directly on germination blotters. The germination blotters were made by placing a layer of absorbent cotton between double sheets of 9 1/2 x 11 1/2 paper toweling. Treating was done by placing the counted seeds in a 1 x 4 1/2 inch glass centrifuge tube which had been perforated several times through the bottom with a red-hot steel needle. A loop of fine but strong piano wire around the lip of the tube formed a convenient handle by means of which the tube could be easily manipulated in or out of the bottle containing the treating solution. A wide-mouth bottle of several hundred cc. capacity was used to hold the treating solution so that when several tubes of beans were lowered into the bottle enough liquid was present to sufficiently cover the beans being treated, the solution gaining entrance to the tube through the perforated bottom. At specified time intervals a tube was lifted from the bottle without disturbing the remaining tubes and the excess treating solution was allowed to drain through the bottom of the tube. The treated seeds were then placed directly on the previously moistened germination blotters and spread out evenly before rolling the blotter into a compact bundle. The rolled-up germination blotter was then labeled with a tag and all the excess water lightly squeezed from it before placing on end in the

germination incubator. The temperature in the incubator remained at 20-25° C. and the blotters were examined daily and moistened when necessary. At the end of 72 hours the germinated seeds were examined and counts were made. Any seeds showing no emergence of the hypocotyl were considered as being non-germinated, even though swelling of the entire seed had taken place. In this manner a large number of germination tests were run on materials believed desirable as seed-treating agents for bacterial bean blight.

The success of Person and Edgerton in treating bean seeds infected with bacterial bean blight by using a solution of 1:500 mercury bichloride in 70 per cent ethyl alcohol plus 3 per cent acetic acid suggested the problem of varying the concentration of some of the constituents and determining the effect on germination. Table 5 shows the results of germination tests following the use of 15, 30, 50, 70, and 95 per cent ethyl alcohol containing 1:500 mercury bichloride and 1, 4, or 6 per cent acetic acid by volume. As may be seen from the table, germination was not greatly injured by the treatments until the higher acid and alcohol concentrations were reached. No slipping of the seed coats took place unless the treatment was prolonged beyond 15 minutes, while the control seeds treated with tap water slipped after a 10-minute treatment.

These results suggested further work to determine the possible effect of other organic acids as penetrants in combination with alcohol and mercury bichloride. Table 6 shows the results obtained when

Table 5. Per cent germination of bean seeds treated with various materials at timed intervals.

Treatment		3 min.	6 min.	12 min.	18 min.
15% Ethyl alcohol, 1:500 $HgCl_2$	1% acetic acid	92	96	90	84
30% " " " "		88	94	96	94
50% " " " "		72	84	82	80
70% " " " "		95	88	84	70
95% " " " "		91	70	88	82
H_2O - Check		94	92	100	96
15% Ethyl alcohol, 1:500 $HgCl_2$	4% acetic acid	92	90	86	88
30% " " " "		88	98	79	86
50% " " " "		88	82	90	66
70% " " " "		82	76	74	70
95% " " " "		86	56	64	74
H_2O - Check		96	85	92	94
15% Ethyl alcohol, 1:500 $HgCl_2$	6% acetic acid	84	84	76	78
30% " " " "		72	76	88	75
50% " " " "		88	74	68	42
70% " " " "		82	88	56	64
95% " " " "		72	72	74	70
H_2O - Check		92	90	86	88

Table 6. Per cent germination of bean seeds treated with various materials at timed intervals.

Treatment		3 min.	6 min.	12 min.	18 min.
15% Ethyl alcohol, 1:500 $HgCl_2$	2% propionic acid	100	96	98	86
30% " " " "		90	90	96	86
50% " " " "		86	90	92	59
70% " " " "		80	84	80	60
95% " " " "		86	82	64	68
H_2O - Check		92	96	90	96
15% Ethyl alcohol, 1:500 $HgCl_2$	4% propionic acid	88	90	92	68
30% " " " "		84	96	92	64
50% " " " "		92	80	78	46
70% " " " "		72	76	82	63
95% " " " "		80	70	82	72
H_2O - Check		86	98	96	98
15% Ethyl alcohol, 1:500 $HgCl_2$	6% propionic acid	68	64	50	70
30% " " " "		78	64	76	72
50% " " " "		72	66	54	42
70% " " " "		62	66	56	44
95% " " " "		68	66	54	46
H_2O - Check		96	90	86	90

propionic acid was substituted for acetic acid in the above treating solution. The higher acid concentrations and prolonged exposure time here, as in the acetic acid treatments, were found to have a harmful effect on seed germination.

Table 7 shows the results of germination tests involving the use of benzoic acid in the alcohol - mercury bichloride mixture. Some difficulty was experienced in preventing the acid from precipitating and clouding the solutions, particularly in the lower alcoholic dilutions. Since benzoic acid was insoluble in cold water, a saturated solution was made by dissolving the acid crystals in 95% ethyl alcohol and using this as a stock solution from which to make dilutions. Six per cent benzoic acid was found to cloud badly in 15 per cent ethyl alcohol and this dilution series was therefore eliminated from the germination trials.

The marked bacteriostatic action of brilliant green toward blight bacteria suggested the use of this material in various alcohol and acid concentrations. A 1:20,000 dilution was selected on the basis of the results of previous toxicity tests on pure cultures of the blight organism and was used during part of the germination tests in connection with laboratory tests on the dye. As may be seen from Table 8, the brilliant green treatment had little harmful effect on germination of the treated seed. The 95 per cent ethyl alcohol treatment was eliminated from these tests, due to its obvious injurious effect on germination in the previous trials. Very little slipping of the seed coat was observed in any of the treatments over a 15-minute treatment period.

Table 7. Per cent germination of bean seeds treated with various materials at timed intervals.

Treatment		3 min.	6 min.	12 min.	18 min.
15% Ethyl alcohol, 1:500 $HgCl_2$	2% benzoic acid	100	86	80	88
30% " " " "		88	84	88	42
50% " " " "		93	74	60	64
70% " " " "		66	78	40	52
95% " " " "		71	78	56	28
H_2O - Check		84	96	98	96
15% Ethyl alcohol, 1:500 $HgCl_2$	4% benzoic acid	90	92	84	80
30% " " " "		82	78	84	84
50% " " " "		74	43	34	54
70% " " " "		76	66	66	30
95% " " " "		80	50	46	38
H_2O - Check		96	90	100	90
15% Ethyl alcohol, 1:500 $HgCl_2$	6% benzoic acid	*			
30% " " " "		76	78	68	62
50% " " " "		88	50	60	24
70% " " " "		78	68	68	66
95% " " " "		84	48	45	66
H_2O - Check		96	82	88	87

*Benzoic acid precipitated.

Table 8. Per cent germination of bean seeds treated with various materials at timed intervals.

Treatment		3 min.	6 min.	12 min.	18 min.
15% Ethyl alcohol, 1:20,000 br. green	2% acetic	88	92	96	90
30% " " " " "		82	84	78	86
50% " " " " "		82	78	60	54
70% " " " " "		74	80	76	46
H_2O - Check		96	92	86	82
15% Ethyl alcohol, 1:20,000 br. green	4% acetic	98	98	90	78
30% " " " " "		88	72	82	78
50% " " " " "		88	78	84	67
70% " " " " "		84	83	76	70
H_2O - Check		98	98	94	100
15% Ethyl alcohol, 1:20,000 br. green	6% acetic	88	90	90	78
30% " " " " "		88	78	82	76
50% " " " " "		90	82	62	70
70% " " " " "		80	66	86	74
H_2O - Check		96	98	98	96

Since ethyl alcohol was found to give excellent results in connection with mercury bichloride, it was deemed desirable to determine whether or not methyl alcohol might give equally good results as a carrier for the mercury bichloride or brilliant green. The cheapness and non-restricted use of this solvent would be an obvious advantage under commercial treatment practices. Bean seeds were therefore treated in varying concentrations of methyl alcohol containing mercury bichloride and acetic acid. As is shown in Table 9, no great decrease in germination took place until the longer treatment periods were reached. The 6 per cent acetic acid treatment was eliminated, due to

Table 9. Per cent germination of bean seeds treated with various materials at timed intervals.

Treatment		3 min.	6 min.	12 min.	18 min.
15% Methyl alcohol, 1:500 $HgCl_2$	2% acetic acid	96	100	94	94
30% " " " "		92	94	94	94
50% " " " "		80	94	88	92
70% " " " "		90	94	92	88
95% " " " "		80	88	63	68
H_2O - Check		96	96	89	100
15% Methyl alcohol, 1:500 $HgCl_2$	4% acetic acid	92	86	90	78
30% " " " "		88	96	89	90
50% " " " "		98	94	96	88
70% " " " "		96	72	88	64
95% " " " "		74	61	64	62
H_2O - Check		98	96	96	60

the injurious effects of acid concentrations greater than 4 per cent. Some difficulty was encountered with mercury bichloride which tended to settle out of methyl alcohol solutions after standing for some time.

The fact that most of the treatments thus far reported could not be used more than 15 minutes without causing serious slipping of the seed coat led to an investigation of beans for defects in the seed coat. A number of bean seeds were examined closely and all those showing defects such as cracks or fissures were separated from those showing no visible defects. The two sets of seeds were then treated separately in varying concentrations of ethyl alcohol, brilliant green, and acetic acid for different periods of time.

The results of these treatments have been brought together in Table 10. The selected seed showed a remarkable ability to withstand slipping in comparison with ordinary run seed which exhibited defects in the seed coat. No great decrease in germination, however, could be found between the two sets of seed. It was therefore concluded that the slipping of the bean seed coats was due to certain defects in the seed coat itself and might possibly be remedied by using various non-aqueous solutions as carriers for the toxic materials. Preliminary germination tests were tried, using solutions of petroleum ether, di-ethyl ether, and chloroform. The results obtained have been brought together in Table 11.

Table 10. Per cent of seeds showing slipping of seed coats between selected and non-selected bean seeds.

Treatment		3 min.		6 min.		12 min.		18 min.	
		Selected	Non-sel.	Selected	Non-sel.	Selected	Non-sel.	Selected	Non-sel.
15% Ethyl alcohol, 1:20,000 brilliant green	2% acetic acid	10	24	10	30	0	26	0	36
30% " " " " "		0	24	0	32	10	38	10	34
50% " " " " "		0	32	10	42	10	36	10	46
70% " " " " "		0	6	0	34	10	34	0	46
H_2O - Check		10	24	10	36	10	34	10	50
15% Ethyl alcohol, 1:20,000 brilliant green	4% acetic acid	0	24	0	35	0	28	0	38
30% " " " " "		10	27	0	32	10	21	0	37
50% " " " " "		0	36	10	42	0	31	30	30
70% " " " " "		0	21	0	34	0	27	0	33
H_2O - Check		0	22	0	40	0	74	10	32
15% Ethyl alcohol, 1:20,000 brilliant green	6% acetic acid	10	25	0	35	0	28	0	26
30% " " " " "		10	27	0	32	0	34	0	46
50% " " " " "		10	36	0	42	20	31	10	30
70% " " " " "		0	21	0	35	0	27	0	36
H_2O - Check		0	22	0	20	0	37	0	32

Table 11. Per cent germination of bean seeds treated with various materials at timed intervals.

Treatment	3 min.	6 min.	12 min.	18 min.
25% Ethyl alcohol. 1:10,000 brilliant green	94	91	88	84
50% Ethyl alcohol. 1:10,000 brilliant green	92	80	70	64
Petroleum ether	94	97	98	92
Ethyl ether	98	96	96	96
Chloroform	92	88	74	82
H_2O - Check	96 Per cent			

These results show that very little decrease in germination took place with any of the above solutions and no slipping of the seed coat took place after an 18 minute exposure to the solutions. Further work revealed that bean seeds could be treated in any of the three solutions for as long as 24 hours without causing the seed coat to slip. Furthermore, no great decrease in germination took place even after such prolonged treatment. The solubility of mercury bichloride was then tried in the three solutions in a 1:500 concentration and some variation was noted. Mercury bichloride was found to be very soluble in di-ethyl ether, insoluble in petroleum ether, and only slightly soluble in chloroform. On the basis of these data, chloroform and petroleum ether were eliminated from consideration as carriers of mercury bichloride.

Various combinations of ethyl alcohol, ether, acetic acid, and mercury bichloride were tried on seed germination. The results are brought together in Table 12.

Table 12. Per cent germination of bean seeds treated with various materials at timed intervals.

Treatment	5 min.	10 min.	15 min.	30 min.	60 min.	24 hrs.
15% ethyl alcohol, 1:500 $HgCl_2$ 90% di-ethyl ether, 3% acetic acid	92	84	82	54	76	86
30% ethyl alcohol, 1:500 $HgCl_2$ 70% di-ethyl ether, 3% acetic acid	94	96	66	88	84	71
50% ethyl alcohol, 1:500 $HgCl_2$ 50% di-ethyl ether, 3% acetic acid	86	90	84	78	92	61
70% ethyl alcohol, 1:500 $HgCl_2$ 30% di-ethyl ether, 3% acetic acid	78	73	66	67	80	67
H_2O - Checks	94 Per cent					

A 24-hour treatment with any solution using di-ethyl ether gave surprisingly good germination, considering the length of time of treatment. The ether effectively eliminated all slipping of the seed coat and was relatively non-toxic except when combined with high alcohol percentages.

The highly inflammable nature of the ether and chloroform broadened the field of search somewhat for a good substitute solvent not quite so precarious to handle under average conditions. After several trials with some materials of varying degrees of inflammability, toluene was selected as exhibiting the most desirable properties. Prolonged exposures of bean seeds to toluene alone did not cause a great reduction in germination or cause slipping of the seed coat. Table 13 gives germination percentages obtained in tests using toluene in various combinations with ethyl alcohol, brilliant green, gentian violet, and mercury bichloride.

Table 13. Per cent germination of bean seeds treated with various materials at timed intervals.

Treatment	5 min.	15 min.	30 min.	60 min.
1:5,000 Gentian violet, 1/4 ethyl alcohol, 3/4 toluene	76	52	52	42
1:5,000 Brilliant green, 1/4 ethyl alcohol, 3/4 toluene	74	68	74	82
1:500 $HgCl_2$, 15% ethyl alcohol, 85% toluene	66	74	66	50
H_2O - Check		90 Per cent		
1:500 $HgCl_2$, di-ethyl ether		96	96	90
Chloroform		95	95	94
H_2O - Check		96 Per cent		

The low germination percentages are possibly due to the low viability seed lot used in this test, since the checks themselves only germinated 90 per cent.

Further experimentation using toluene to eliminate seed coat slipping was carried out after laboratory tests revealed that a 1:1000 concentration of brilliant green might be more effective toward the blight bacteria. The results of these trials are given in Table 14.

The germination percentages shown in Table 14 substantiate the contention that toluene is not overly injurious to germination of bean seeds. In these tests methyl alcohol was substituted for ethyl alcohol and a new constituent, glycerine, was introduced. Glycerine was found to eliminate seed coat slipping just as well as some of the non-aqueous solutions used and also readily formed combinations with materials used

Table 14. Per cent germination of bean seeds treated with various materials at timed intervals.

Treatment	15 min.	30 min.	60 min.
1:1,000 Brilliant green, 1/2 methyl alcohol, 1/2 toluene	86	87	80
1:1,000 Brilliant green, 1/2 H_2O, 1/2 glycerine	94	92	94
1:1,000 Brilliant green, 1/2 glycerine, 1/2 methyl alcohol	92	98	96
1:1,000 $HgCl_2$, 2% HCl, 1/2 methyl alcohol, 1/2 glycerine	90	94	96
1:1,000 Brilliant green, 2% HCl, 1/2 methyl alcohol, 1/2 glycerine	92	90	98
1:1,000 $HgCl_2$, 1/2 glycerine, 1/2 H_2O	98	97	94
1:1,000 $HgCl_2$, 1/2 metnyl alcohol, 1/2 toluene	94	90	78
1:1,000 $HgCl_2$, 1/2 methyl alcohol, 1/2 glycerine	100	98	98
Check	96 per cent		

previously in the seed treatments. As seen in Table 14, none of the combinations of materials used in these tests caused great decrease in germination or slipping of the seed coat. Hydrochloric acid was substituted for the acetic acid, since it was thought that the more highly ionized acid might be a factor in making some of the constituents more toxic.

Since most of the treatments given laboratory germination tests gave no immediate decrease in germination, it was deemed desirable to

find out what effect a prolonged storage period might have on treated bean seeds. Three treatments were accordingly selected early in the experimental work, made up of the following constituents:

(1) 1:500 mercury bichloride in 50% ethyl alcohol plus 4% acetic acid.

(2) 1:500 mercury bichloride in 50% ethyl alcohol plus 4% propionic acid.

(3) 1:20,000 brilliant green in 50% ethyl alcohol plus 4% acetic acid.

Checks were composed of seeds soaked in tap water. All of the above treatments were for 15 minutes. For each treatment 325 grams of Bountiful bean seed were weighed and covered with 200 cc. of solution. After 15 minutes the solutions were drained off and the seeds spread out to dry on paper toweling. A germination test was run immediately after treatment on 100 seeds. The remainder were placed in quart jars with loosely-capped tops and stored at room temperature. As shown in Table 15, occasional germination tests were run over a period of seven months.

From the data obtained, it would appear that no appreciable decrease in germination took place after treating bean seeds and the seeds could be safely kept in storage over a considerable time period after treating.

Table 15. Per cent germination of bean seeds over a prolonged storage period.

Treatments	Date of Examination				
	2/18/39	2/26/39	3/12/39	5/10/39	9/4/39
1:500 $HgCl_2$. 50% ethyl alcohol. 4% acetic acid	73	83	88	75	80
1:500 $HgCl_2$. 50% ethyl alcohol. 4% propionic acid	81	73	80	80	74
1:20,000 Brilliant green 50% ethyl alcohol 4% acetic acid	85	80	88	87	80
Check	99	96	96	90	90

FIELD TRIALS

Laboratory tests revealed that certain disinfectant materials might be worthy of field trials and these were tried during the spring and fall of 1939. A total of seven plantings was made during the spring at approximately ten-day intervals. Five fall plantings were also made the same year. Disinfectant solutions were used at various concentrations and time intervals. Diseased seed lots were obtained from several commercial seed companies which had received blighted seed from their own or contracted fields in some of the western seed-growing areas. The following seed lots were used at some time or other in the field trials.

Lot No. 1. Full Measure Stringless, "Heavy blight" according to the grower, but found to contain only moderate blight under the field conditions of these experiments.

Lot No. 2. Asgrow Black Valentine, "Heavy blight" according to the grower and substantiated by field trials.

Lot No. 3. Asgrow Black Valentine, "Heavy blight" according to the grower and checked as such in field trials.

Lot No. 4. Round Pod Kidney Wax, "Heavy blight" according to the grower and found in field trials to be fairly heavily infected.

Lot No. 5. Asgrow Black Valentine, "Heavy blight," according to the grower. Seeds arrived in the spring too late for an accurate field check of blight prevalence.

Equal quantities of weighed seeds were used for each treatment plot in each planting. The weighed seed portions for each field treatment plot were treated in the laboratory prior to being taken to the field for planting. The seeds were placed in wide-mouth jars and

covered with the treating solution for the desired length of time. After the desired treatment time had elapsed, the treating solutions were poured from the seed through a screen strainer and the seed spread out on paper toweling to dry before taking to the field in labeled paper bags.

All planting was done by hand, dropping the beans at approximately 1 to 1 1/2 inch intervals in the row. The treatment plots for any particular planting were grouped together but the groups were separated from each other by barrier rows of some other crop such as potatoes or peas. Check plots were usually placed adjacent to the treated plots but separated from them by barrier rows. This arrangement placed all plots under more or less similar environmental conditions and at the same time prevented too great spread of blight during the early stages of plant development when notes on primary infection were taken. Blight spread due to secondary infection from diseased plots when the plants were maturing and beginning to touch each other could not be adequately controlled and no great amount of importance was attached to this type of infection, since control was based on eliminating the primary infection as a means of preventing secondary spread.

Bacterial bean blight counts were made after the seedlings had emerged and were about six inches tall. At this stage only the first two simple leaves had emerged and were fully developed. No trifoliate leaves had opened. All plants were closely examined at this stage of

development and any infection found on the leaves, either upper or lower side, was considered sufficient evidence that the plant had arisen from diseased seed and that, in consequence, the infection should be designated as primary. It was considered unlikely that much secondary spread and development of the bacteria would have taken place at this early stage of leaf development. Examinations of treated plots were made first and the checks or blighted plots last in order to eliminate spreading the bacteria from diseased to healthy plants by handling.

After counting the number of blighted plants in each row, stand counts were made on the entire plot and the percentage of infection calculated for each treatment and check plot. Periodic examinations were made on the various plots in order to note the development made by plants treated with the various materials and also the incidence of possible secondary spread. In general, plots showing marked differences in infection also showed striking differences in plant vigor and consequently in set of fruit. Yield data were later taken from several of these plantings by picking the snap beans at their best market stage of development.

Late plantings of beans, particularly after the middle of April, gave poor sets of fruit as well as low blight infections, due to the onset of hot, dry weather. Fall plantings likewise gave very low blight infection in heavily-diseased lots, probably due to the high fall temperatures and the extremely dry weather of this season. The

data on control of blight with various seed treatments is taken up for individual plantings in the pages that follow.

Planting No. 1

On March 1, 1939, a planting was made using Full Measure Lot No. 1 seed only. Four treatments and a dry and wet check were used. The treating agents were made up as follows in 500 cc. portions.

(1) 1:500 mercury bichloride in 70% ethyl alcohol plus 3% acetic acid.

(2) 1:500 mercury bichloride in 70% ethyl alcohol plus 3% propionic acid.

(3) 1:20,000 brilliant green in 50% ethyl alcohol plus 3% acetic acid.

(4) 1:20,000 gentian violet in 50% ethyl alcohol plus 3% acetic acid

For each treatment 685 grams of seeds were used and allowed to remain in the treating solution for 12 minutes. A wet check was soaked in tap water for 12 minutes while a dry check was left untreated. After treatment each seed portion was spread out on paper toweling and allowed to dry before taking to the field plots. Each plot was composed of five rows 35 feet long. The plots were laid out end to end with a three-foot space between the row ends of one plot and those of the next. Check plots were placed adjacent to the treated plots but separated from them by five barrier rows of Irish potatoes. Previous to planting, 100 seeds of each treatment were retained for laboratory germination tests. The results of these tests were as follows:

Treatment No.	1 — 92%	germination
"	2 — 94%	"
"	3 — 93%	"
"	4 — 96%	"
Wet Check	— 95%	"
Dry Check	- 100%	"

Blight counts were made on March 25 and stand counts on March 30. Table 16 indicates the number of plants in each row giving typical blight lesions.

Table 16. Initial infection occurring in seed treatments for Planting No. 1

Treatment	Total number plants	Blighted plants
1:500 $HgCl_2$ in 70% ethyl alcohol + 3% acetic acid	1410	4
1:500 $HgCl_2$ in 70% ethyl alcohol + 3% propionic acid	1599	8
1:20,000 brilliant green in 50% ethyl alcohol + 3% acetic acid	1298	6
1:20,000 gentian violet in 50% ethyl alcohol + 3% acetic acid	1332	3
Wet Check	1041	48
Dry Check	1656	24

Since the infection was so low in the above seed lot, no infection percentage was calculated, since it would have been so slight as to have had little significance. As the season advanced, notice-

able differences became evident between the treated and check plots. These may have been due to latent infections becoming expressed under more favorable environmental conditions or to secondary spread from the primary infected plants. The former explanation would seem to be more logical in that secondary spread would tend to distribute the blight more or less uniformly over the plots rather than just in the check plots. These differences became more pronounced as the season advanced, and as a result it was decided to take yield tests from the plots and determine whether the treated plots might not be more productive than the untreated. Two of the five rows of each plot were picked clean of all marketable snap beans and the weights taken for each plot. The picking was made on May 11 and the weights in pounds of green beans are shown in Table 17.

Table 17. Yields of green beans for Planting No. 1.

Treatment	Yield in lbs.
1:500 $HgCl_2$ in 70% ethyl alcohol + 3% acetic acid	29.8
1:500 $HgCl_2$ in 70% ethyl alcohol + 3% propionic acid	18.0
1:20,000 brilliant green in 50% ethyl alcohol + 3% acetic acid	26.5
1:20,000 gentian violet in 50% ethyl alcohol + 3% acetic acid	24.2
Wet Check	15.9
Dry Check	15.7

Planting No. 2

Four seed lots were used in a planting made on March 9, 1939. The varieties for each lot were as follows:

Lot No. 1 - Full Measure

Lot No. 2 - Asgrow Black Valentine

Lot No. 3 - Asgrow Black Valentine

Lot No. 4 - Round Pod Kidney Wax

Five treatments and a wet check for each seed lot were used in this planting. The treatments were all in one block with the exception of the treatment using 1:500 mercury bichloride in 70 per cent ethyl alcohol plus 3 per cent acetic acid. This treatment was isolated from the other treatments and placed in a different part of the field in order to test it more thoroughly and also to remove possible chances of spread of bacteria from the new and untried treatments.

The four seed treatments tested together in the field were as follows:

(1) 1:500 mercury bichloride in 70% ethyl alcohol plus 3% propionic acid.

(2) 1:20,000 brilliant green in 50% ethyl alcohol plus 3% acetic acid.

(3) 1:20,000 gentian violet in 50% ethyl alcohol plus 3% acetic acid.

(4) 1:500 mercury bichloride in di-ethyl ether.

The ether treatment was added because laboratory tests had previously shown that non-aqueous solutions such as ether kept the seed coat from slipping, even if the seeds were soaked in such a solu-

tion for an indefinite period. Ether also had the property of readily dissolving mercury bichloride and not causing any appreciable reduction in germination over a fairly long treatment period. The highly inflammable nature of ether placed some restriction on its use unless it was handled in the absence of a flame and with proper aeration.

A 375-gram sample of each of the four seed lots was weighed out for each of the four treatments and checks planted together. Each treatment was for 12 minutes, including that of the wet check which was soaked in tap water for the required time. After the seeds had been dried on paper toweling, they were taken to the field and planted in plots of six 16-foot rows with a 3-foot space between the ends of adjoining plots. The treated and check plots were separated longitudinally from each other by means of several barrier rows. The isolated plot was planted with 625 grams of each of the four seed lots treated 12 minutes with 1:500 mercury bichloride in 70 per cent ethyl alcohol plus 3 per cent acetic acid. The same amount of seed was used for each of the check lots which were separated from the treated plots by several barrier rows. Each plot for treated and check alike was composed of five 35-foot rows with a 3-foot space between the ends of adjoining plots.

Blight counts on all treatments were made March 25 and stand counts on March 30. Tables 18 and 19 give the relative blight percentages for the treated and check plots used in this planting. The excellent control obtained is shown in the photographs of "treated" and "untreated" rows shown in Plate IV, figures 1 to 4.

Table 18. Initial infection occurring in seed treatments for Planting No. 2.

Lot 1			Lot 2			Lot 3			Lot 4			Treatment
Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	
1002	0	.00	901	0	.00	1055	0	.00	780	0	.00	12 min. in 1:500 $HgCl_2$ in 70% ethyl alcohol + 3% propionic acid
1051	0	.00	935	0	.00	1029	2	.10	837	0	.00	12 min. in 1:20,000 brilliant green in 50% ethyl alcohol + 3% acetic acid.
1015	0	.00	928	1	.10	1075	1	.09	814	0	.00	12 min. in 1:20,000 gentian violet in 50% ethyl alcohol + 3% acetic acid
1134	0	.00	929	0	.00	1016	1	.09	928	0	.00	12 min. in 1:500 $HgCl_2$ in di-ethyl ether
1117	42	3.76	917	608	66.3	1097	325	29.6	891	235	26.4	Check

Table 19. Initial infection occurring in isolated acetic acid treatment. Planting No. 2.

	Lot 1		Lot 2		Lot 3		Lot 4	
	Total	Blight	Total	Blight	Total	Blight	Total	Blight
Total plants	1498	0	1508	0	1757	0	1223	0
Per cent blight	.00		.00		.00		.00	
	C h e c k s							
Total plants	1668	15	1505	254	1790	215	1439	79
Per cent blight	.89		16.8		12.01		5.48	

The striking control of primary blight infection in this planting by the various treatments led to the belief that there might also be great differences in the yield of green beans between treated and untreated plots. Random rows were therefore picked in the treated and check plots from May 9 to May 11 and the weights recorded in pounds as shown in Table 20.

Table 20. Yields in pounds of green beans for Planting No. 2.

Lot 1		Lot 2		Lot 3		Lot 4	
Tr. 3	Tr. 4	Tr. 3	Tr. 4	Tr. 3	Tr. 4	Tr. 3	Tr. 3
19.0	25.2	21.7	26.5	24.0	29.5	39.8	44.5
Tr. 1	Tr. 2	Tr. 1	Tr. 2	Tr. 1	Tr. 2	Tr. 1	Tr. 2
21.3	22.2	20.5	26.0	22.5	27.8	32.7	35.4

Check	Check	Check	Check
14.0	3.7	7.7	10.9

Acetic acid treatment

Lot 1	Lot 2	Lot 3	Lot 4
18.0	17.9	20.4	23.7
Check	Check	Check	Check
9.8	6.3	5.9	11.4

Planting No. 3

Each of the four previous seed lots were used in a planting made on March 23. 1939. Eight treatments of the following composition

were used, the treatment time being given for each treatment.

(1) 12 minutes in 1:500 mercury bichloride in 70% ethyl alcohol plus 3% acetic acid.

(2) 12 minutes in 1:20,000 gentian violet in 50% ethyl alcohol plus 3% acetic acid.

(3) 12 minutes in 1:20,000 brilliant green in 50% ethyl alcohol plus 3% acetic acid.

(4) 10 minutes in 1:500 mercury bichloride in equal portions of a 95% ethyl alcohol - di-ethyl ether mixture.

(5) 60 minutes in 1:500 mercury bichloride in equal portions of a 95% ethyl alcohol - di-ethyl ether mixture.

(6) 10 minutes in 1:500 mercury bichloride in equal portions of a 95% ethyl alcohol - di-ethyl ether mixture plux 1% acetic acid.

(7) 60 minutes in 1:500 mercury bichloride in equal portions of a 95% ethyl alcohol - di-ethyl ether mixture plux 1% acetic acid.

(8) 12 minutes in 1:500 mercury bichloride in a mixture of 70% ethyl alcohol - 30% di-ethyl ether.

Checks of each seed lot were soaked in tap water for 12 minutes. For each treatment, 218 grams of each seed lot were used. The seeds were planted in plots of four rows 18 feet long with 1 1/2 foot spacing between the ends of the plots. A 325-gram sample of seed was used for each of the check plots, which were made up of three 36-foot rows with 3 1/2 foot spacing between the ends of the plots. Thus the 36-foot check plots were laid out end to end the length of the field. Since the treated plots were one-half the length of the check plots, two treated plots for each seed lot could be laid to the length of one check plot. Thus the treated plots were laid in pairs the width of the field for each seed lot. Several barrier rows were left between

the check plots and the nearest treated plots, with one barrier row between treated plots.

Blight counts were made on this planting on April 7. As will be seen from the results given in Table 21, some reduction in blight took place due to extremely cool weather during the emergence period of the seedlings. Heavy rains just prior to making blight counts also caused dirt to spatter up on the leaves and obscure isolated and small infection areas. Later field observations revealed heavy general infection spread through the check plots with only minor and sporadic infection centers in the treated plots.

Yield tests of green beans were made on the plots by picking the two inside rows of each treated plot and two of the three rows in the check plots. As may be seen from Table 22, appreciable differences in yield were obtained.

Table 21. Initial infection occurring in seed treatments for planting No. 3.

Lot 1			Lot 2			Lot 3			Lot 4			Treatment
Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	
579	0	0.0	570	0	0.0	641	1	0.1	485	0	0.0	12 min. in 1:500 $HgCl_2$ 70% ethyl alcohol + 3% acetic acid
651	0	0.0	563	0	0.0	651	2	0.3	476	0	0.0	12 min. in 1:20,000 gentian violet, 50% alcohol + 3% acetic acid
611	0	0.1	563	0	0.0	628	2	0.3	503	0	0.0	12 min. in 1:20,000 brilliant green, 50% ethyl alcohol + 3% acetic acid
664	0	0.0	542	1	0.1	656	0	0.0	479	0	0.0	10 min. in 1:500 $HgCl_2$ 1/2 di-ethyl ether, 1/2 ethyl alcohol
625	0	0.0	549	0	0.0	584	0	0.0	471	1	0.2	60 min. in 1:500 $HgCl_2$ in 1/2 di-ethyl ether, 1/2 ethyl alcohol
654	0	0.0	577	0	0.0	633	1	0.1	468	0	0.0	10 min. in 1:500 $HgCl_2$ in 1/2 di-ethyl ether, 1/2 ethyl alcohol + 1% acetic acid
623	0	0.0	536	0	0.0	613	0	0.0	466	0	0.0	60 min. in 1:500 $HgCl_2$ in 1/2 di-ethyl ether, 1/2 ethyl alcohol + 1% acetic acid
647	2	0.3	566	0	0.0	650	1	0.1	531	1	0.1	12 min. in 1:500 $HgCl_2$ in 70% ethyl alcohol 30% ether
956	5	0.5	861	43	4.9	937	83	8.8	774	42	5.4	Check

Table 22. Yields in pounds of green beans for Planting No. 3.

Lot 1	Lot 2	Lot 3	Lot 4	Treatment
9.2	11.0	13.2	15.3	12 min. in 1:500 $HgCl_2$ in 70% ethyl alcohol + 3% acetic acid
12.2	11.8	11.8	19.0	12 min. in 1:20,000 gentian violet in 50% ethyl alcohol + 3% acetic acid
13.5	15.0	12.0	20.2	12 min. in 1:20,000 brilliant green in 50% ethyl alcohol + 3% acetic acid
15.0	18.0	14.5	12.0	10 min. in 1:500 $HgCl_2$ in 1/2 95% ethyl alcohol, 1/2 di-ethyl ether
11.5	9.5	9.5	15.3	60 min. in 1:500 $HgCl_2$ in 1/2 95% ethyl alcohol, 1/2 di-ethyl ether
13.0	11.4	13.0	9.2	10 min. in 1:500 $HgCl_2$ in 1/2 95% ethyl alcohol, 1/2 di-ethyl ether + 1% acetic acid
11.6	8.8	10.0	10.3	60 min. in 1:500 $HgCl_2$ in 1/2 di-ethyl ether + 1% acetic acid
13.5	9.2	10.0	10.3	12 min. in 1:500 $HgCl_2$ in 70% ethyl alcohol and 30% di-ethyl ether
8.8	5.8	6.9	6.9	Check

Planting No. 4

In this planting seed lots 2, 3, and 4 were used, since Lot No. 1 from previous tests was found to be only lightly infected. Four treatments were used in a planting made on April 6, 1939. Two series of tests were run, one treating each of the three seed lots for 12 minutes, and the other treating Lot No. 4 only with the four treating solutions but for varying time intervals. The following solutions were used:

(1) 1:500 mercury bichloride in 70% ethyl alcohol plus 3% acetic acid.
(2) 1:20,000 gentian violet in 50% ethyl alcohol plus 3% acetic acid.
(3) 1:20,000 brilliant green in 50% ethyl alcohol plus 3% acetic acid.
(4) 1:500 mercury bichloride in di-ethyl ether.

For one series, 350 grams of each of the three seed lots were used in each treatment. All seeds were treated as previously, at 12 minutes, including a wet check soaked in tap water. A dry check of each seed lot was also used. The treated seeds were planted in plots of six 20-foot rows such that plots one and two were end to end, with a six-foot space between the ends of the plots. Plots one and two were adjacent to plots three and four which were likewise placed end to end in a manner similar to plots one and two. Thus each seed lot was represented by a square block of four treatments. An eight-foot space separated the plots of one seed lot and those of the next seed lot. Wet and dry checks for each seed lot were planted in plots of four 20-foot rows adjacent to the treated plots for that

particular seed lot but separated from the treated plots by several barrier rows. The dry and wet check plots for each seed lot were placed end to end but separated from each other by a six-foot space.

The time treatments were run using the same solutions but varying the time of each treatment. Each treatment and time test required a 175-gram sample of Lot No. 4 seed. Each treatment was run at a 4, 8, or 12-minute period. After treating, the seeds were taken to the field and planted in plots of three 20-foot rows in a manner similar to the preceding tests, such that each set of four treatments at a certain time interval was arranged in a square with two sets of treatments end to end and adjacent to the next set. The ends of two treated plots were separated by a six-foot space. Each complete set of treatments was separated from the next by an eight-foot space. The same set of checks was used in this and the preceding test.

Blight counts on the treatments were made on April 28. As shown in Tables 23 and 24, considerable blight developed in the checks while the treated plots remained relatively free of disease. The tables give the counts based on primary infected plants.

Since considerable difference was evident between the treated and check plots, not only in blight infection but also in subsequent vigor of plants, yield tests were made by picking the green beans at their stage of highest marketable value. Green beans were picked only from the treated plots of the three seed lots and four treatments, as is shown in Table 25. The four inside rows of the six rows in each plot were picked and the weight in green beans taken. The two inside

Table 23. Initial infection occurring in timed treatments for Planting No. 4.

4 minutes			8 minutes			12 minutes			Treatment
Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	
378	0	.00	339	0	.00	338	0	.00	1:500 $HgCl_2$ in 70% ethyl alcohol + 3% acetic acid
360	5	1.38	308	0	.00	359	3	.83	1:20,000 gentian violet in 50% ethyl alcohol + 3% acetic acid
353	0	.00	377	0	.00	370	0	.00	1:20,000 brilliant green in 50% ethyl alcohol + 3% acetic acid
394	0	.00	429	1	.23	371	1	.26	1:500 $HgCl_2$ in di-ethyl ether

Table 24. Initial infection occurring in seed treatments for Planting No. 4.

Lot 2			Lot 3			Lot 4			Treatment
Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	
892	0	.00	1056	3	.28	746	0	.00	12 min. in 1:500 $HgCl_2$ in 70% ethyl alcohol + 3% acetic acid
926	6	.64	1041	12	1.15	819	1	.12	12 min. in 1:20,000 gentian violet in 50% ethyl alcohol + 3% acetic acid
896	0	.00	1003	9	.89	809	2	.24	12 min. in 1:20,000 brilliant green in 50% ethyl alcohol + 3% acetic acid
966	0	.00	1025	4	.39	893	2	.22	12 min. in 1:500 $HgCl_2$ in di-ethyl ether
931	316	33.9	1018	446	43.8	808	446	55.19	Wet Check
943	4	.42	1029	125	12.1	858	21	2.44	Dry Check

Table 25. Yields in pounds of green beans for Planting No. 4.

Lot 2	Lot 3	Lot 4	Treatment
35.4	30.6	21.9	12 min. in 1:500 $HgCl_2$ in 70% ethyl alcohol + 3% acetic acid
31.8	23.2	19.0	12 min. in 1:20,000 gentian violet in 50% ethyl alcohol + 3% acetic acid
34.7	34.3	24.8	12 min. in 1:20,000 brilliant green in 50% ethyl alcohol + 3% acetic acid
36.3	24.0	23.8	12 min. in 1:500 $HgCl_2$ in di-ethyl ether
15.0	8.7	6.1	Wet Check
8.3	8.2	9.0	Dry Check

rows of the four check rows were likewise picked and weighed. Since the same amount of seed was used in the four check rows as in the six treated rows, the yields had to be balanced accordingly. The weights in pounds of the checks were therefore multiplied by 2/3 in order to make the weights of green beans in the checks comparable with that of the treated plots. The quality of beans in the treated plots was much better than that in the checks.

Planting No. 5

Lots No. 2 and 4 were used in a planting made on April 13, 1939. Sixteen treatments were used, mostly of individual materials to determine the effectiveness of the separate materials used in the previous treatments as toxic agents in the control of bacterial bean blight. For each treatment 175 grams of seed were used, all treatments being carried out for 12 minutes with the exception of the wet check which was treated for only 10 minutes. The treatments used were as follows:

(1) 70% ethyl alcohol alone.

(2) 70% ethyl alcohol plus 3% acetic acid.

(3) 1:500 mercury bichloride in 70% ethyl alcohol.

(4) 1:500 mercury bichloride in 70% ethyl alcohol plus 3% acetic acid.

(5) 3% acetic acid in water.

(6) 1:500 mercury bichloride in water.

(7) Chloroform alone.

(8) Di-ethyl ether alone.

(9) Petroleum ether alone.

(10) 1:20,000 gentian violet in water.

(11) 1:20,000 brilliant green in water.

(12) 3% propionic acid in water.

(13) 50% ethyl alcohol alone.

(14) 1:500 mercury bichloride in chloroform.

(15) 1:20,000 brilliant green in 50% ethyl alcohol.

(16) 1:20,000 brilliant green in 50% ethyl alcohol plus 3% acetic acid.

There was also a dry and wet check for each seed lot.

After treating and drying on paper toweling, the seeds were taken to the field and planted in plots of four rows 18 feet long, with a one-foot space between the ends of rows separating plots. Each seed lot was represented by a series of four treatment plots placed end to end and in a lateral series of four plots so that each seed lot was made up of a block of 16-treatment plots. The check plots were composed of two 18-foot rows with the plots laid out end to end. A four-foot space separated the ends of adjoining plots. Several barrier rows were used to separate the "treated" and "untreated" plots.

Blight and stand counts were made from May 1 to May 4. Very low infection was recorded in the plots, probably due to one or more of several factors. The lateness in the season and the hot weather undoubtedly inhibited the development of the blight organism, as well as the best development of the bean plants. The seed was planted at a time when the soil was extremely dry and no rain fell for a considerable length of time thereafter. These circumstances have been shown previously to be unfavorable for the development of bean blight.

No data were compiled for this or the remaining two plantings, which also developed very little blight and therefore gave no index as to the control being effected on blighted seed. These observations substantiate previous investigations as to the necessity of soil moisture and high humidity for the best development of the bean

blight organism. These results were further borne out in the fall plantings of the same year.

Fall Plantings, 1939

Laboratory tests carried out after the results of the spring plantings of beans suggested that toluene might be a good non-aqueous carrier for the brilliant green or mercury bichloride. The following six treatments were therefore used during the fall plantings.

(1) 1:500 mercury bichloride in di-ethyl ether.

(2) 1:500 mercury bichloride in a mixture of equal portions of 95% ethyl alcohol and toluene.

(3) 1:20,000 brilliant green in a mixture of equal portions of 95% ethyl alcohol and toluene.

(4) 1:500 mercury bichloride in a mixture of equal portions of 95% ethyl alcohol and di-ethyl ether.

(5) 1:500 mercuric cyanide in a mixture of equal portions of 95% ethyl alcohol and toluene.

(6) 1:500 mercury bichloride in 70% ethyl alcohol plus 3% acetic acid.

All of the above treatments except the latter were carried out for 30 minutes. The acetic acid treatment was carried out for 12 minutes. Five plantings were made during the fall of 1939 on the following dates: September 8, 16, 24, 29, and October 13. The same procedure was used in these treatments and plantings as in the spring series. Disappointing results were obtained as far as any blight development was concerned. The hot, dry weather of the fall season

in Louisiana was extremely unfavorable for the development of blight and the observations made on these plantings amply corroborated other work, particularly that of Edgerton, in showing that fall-grown beans usually escape blight infection in Louisiana. No accurate conclusions or results could therefore be given on any of the fall plantings, since in most cases the heavily-infected seed lots in the spring plantings showed very little or no blight infection.

Spring Plantings, 1940

Several plantings were made during the spring of 1940 in which some new seed lots and treatments were tested. The seed lots used were as follows:

Lot No. 5 - Asgrow Black Valentine, western grown, found to be lightly infected in field trials.

Lot No. 6 - Round Pod Kidney Wax, western grown, found to be heavily infected in field trials.

Lot No. 7 - Asgrow Black Valentine, western grown, found to be heavily infected in field trials.

Lot No. 8 - Bountiful, purchased on the open market, source unknown, found to be very lightly infected in field trials.

Lot No. 9 - Black Valentine, purchased on the open market, source unknown, found to be very lightly infected in field trials.

The seed treatments used during 1939 were revised somewhat for the 1940 plantings in an attempt to increase toxicity and length of treatment time. Glycerine was introduced as a carrier for the toxic materials since laboratory tests showed that a 50 per cent solution of

glycerine was capable of preventing seed coat slipping over a period of at least one hour. The concentration of brilliant green was increased to 1:1,000 since it was assumed that the more concentrated solution should be more effective and less liable to dilution within the seed. Methyl alcohol was introduced as a substitute solvent to replace ethyl alcohol in combinations with toluene and glycerine. All solutions were made up in liter portions.

The first planting was made on March 2, 1940, using seed lots 5, 6, 7, 8, and 9. The following solutions were used as treating agents.

(1) 1:1,000 brilliant green in a mixture of equal portions of 95% methyl alcohol and toluene.

(2) 1:1,000 brilliant green in a mixture of equal portions of glycerine and water.

(3) 1:1,000 brilliant green in a mixture of equal portions of 95% methyl alcohol and glycerine.

(4) 1:1,000 mercury bichloride in a mixture of equal portions of 95% methyl alcohol and glycerine plus 2% hydrochloric acid.

(5) 1:1,000 brilliant green in a mixture of equal portions of 95% methyl alcohol and glycerine plus 2% hydrochloric acid.

(6) 1:1,000 mercury bichloride in a mixture of equal portions of glycerine and water.

(7) 1:1,000 mercury bichloride in a mixture of equal portions of 95% methyl alcohol and toluene.

(8) 1:1,000 mercury bichloride in a mixture of equal portions of 95% methyl alcohol and glycerine.

(9) 1:20,000 brilliant green in 50% ethyl alcohol plus 3% acetic acid.

(10) 1:500 mercury bichloride in 70% ethyl alcohol plus 3% acetic acid.

A sample of each seed lot weighing 147 grams was used for each of the 10 treatments in this planting. All seeds were treated for 30 minutes in the laboratory before planting. A wet check of each seed lot obtained by treating the seed in tap water for 10 minutes and a dry check without any treatment were included in the test. Each seed lot was represented by 10 "treated" plots and a dry and wet check plot. Individual plots were made up of four 15-foot rows. The "treated" series of plots were separated from the check plots by three barrier rows.

Blight counts were made on March 27. A heavy rain at the time the seedlings were emerging packed the soil into a crust so that many of the plants were broken in attempting to force their way through the hard ground. A poor stand resulted and may account in some measure for the low blight counts since the severely blighted plants would have had difficulty in emerging, due to their weakened condition. Blight counts are brought together in Table 26. No stand counts were made, due to the poor distribution of plants in the plots.

Table 26. Numbers of blighted plants in various treatments, Planting No. 1, Spring 1940.

Lot 5	Lot 6	Lot 7	Lot 8	Lot 9	Treatment
2	0	6	0	0	1:1,000 brilliant green in 1/2 methyl alcohol, 1/2 toluene
2	0	1	0	0	1:1,000 brilliant green in 1/2 glycerine, 1/2 water
0	0	7	0	0	1:1,000 brilliant green in 1/2 methyl alcohol, 1/2 glycerine
0	1	2	5	14	1:1,000 mercury bichloride in 1/2 methyl alcohol, 1/2 glycerine, plus 2% HCl
0	1	6	0	0	1:1,000 brilliant green in 1/2 methyl alcohol, 1/2 glycerine, plus 2% HCl
5	0	1	0	0	1:1,000 mercury bichloride in 1/2 glycerine, 1/2 water
0	0	0	0	1	1:1,000 mercury bichloride in 1/2 methyl alcohol, 1/2 toluene
0	4	3	0	0	1:1,000 mercury bichloride in 1/2 methyl alcohol, 1/2 glycerine
4	0	3	2	2	1:20,000 brilliant green in 50% ethyl alcohol plus 3% acetic acid
0	0	15	0	1	1:500 mercury bichloride in 70% ethyl alcohol plus 3% acetic acid
13	16	12	0	1	Dry Check
32	71	29	2	5	Wet Check

A second planting was made on March 18, 1940. The same treating solutions were used as in the previous planting but were first filtered free of sediment. Seed lots No. 5, 6, 7, 8, and 9 were used in 146-gram portions and treated for 15 minutes instead of 30 minutes as in the previous planting. Wet checks of each seed lot were given a 10-minute treatment period in water. The field plots were of the same size and order as those of the previous planting, including a dry and wet check and 10 "treated" plots for each seed lot. Blight and stand counts were made from April 9 to April 11. A heavy rainstorm with a wind of near gale proportions occurred on April 6 and undoubtedly caused a great deal of spread of bacteria from the check plots to the "treated" plots. These results are brought together in Table 27. Considerable blight developed in the check plots of lots No. 6 and 7. These plots could easily have acted as initial infection centers from which the bacteria spread to the "treated" plots over the few intervening barrier rows. Several of the treatments gave fairly good control of blight but the table indicates that most treatments containing glycerine were ineffective in penetrating the seed or carrying the toxic materials to the regions where bacteria were located.

Table 27. Initial infection occurring in seed treatments, planting No. 2, Spring 1940

Lot 5			Lot 6			Lot 7			Lot 8			Lot 9			Treatment
Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	
404	1	.24	229	1	.43	438	10	2.28	263	0	.00	406	0	.00	1:1000 brilliant green in 1/2 methyl alcohol, 1/2 toluene
463	0	.00	327	0	.00	450	11	2.44	360	0	.00	387	4	1.03	1:1000 brilliant green in 1/2 glycerine, 1/2 water
464	1	.21	285	13	4.56	455	16	3.51	351	0	.00	416	0	.00	1:1000 brilliant green in 1/2 methyl alcohol, 1/2 glycerine
433	1	.23	278	0	.00	475	18	3.78	313	0	.00	438	1	.22	1:1000 $HgCl_2$ in 1/2 methyl alcohol, 1/2 glycerine + 2% HCl
421	0	.00	281	11	3.91	468	27	5.89	321	0	.00	291	3	.76	1:1000 brilliant green in 1/2 methyl alcohol, 1/2 glycerine + 2% HCl
471	0	.00	332	2	.60	483	1	.20	332	0	.00	392	0	.00	1:1000 $HgCl_2$ in 1/2 glycerine, 1/2 water
434	0	.00	230	0	.00	421	3	.71	292	0	.00	379	2	.52	1:1000 $HgCl_2$ in 1/2 methyl alcohol, 1/2 toluene

Table 27 (continued)

Lot 5			Lot 6			Lot 7			Lot 8			Lot 9			
Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	Total	Dis.	%	Treatment
457	2	.43	315	4	1.26	424	19	4.48	319	0	.00	395	0	.00	1:1000 $HgCl_2$ in 1/2 methyl alcohol, 1/2 glycerine
343	0	.00	194	6	3.09	405	6	1.48	226	0	.00	322	0	.00	1:20,000 brilliant green in 50% ethyl alcohol + 3% acetic acid
396	1	.25	93	1	1.07	404	11	2.72	230	0	.00	298	1	.33	1:500 mercury bichloride in 70% ethyl alcohol + 3% acetic acid
473	5	1.05	327	103	31.49	407	17	4.17				404	0	.00	Dry Check
444	4	.90	278	51	18.34	419	183	43.67	348	0	.00	430	0	.00	Wet Check

DISCUSSION

Seed treatment for the control of bean blight has involved numerous difficulties. The toxic agent must penetrate the seed without injuring germination appreciably and at the same time it must be toxic to the bacteria located deep within the seed. Complications arise in attempting to treat seed in aqueous solutions for longer than 12-15 minutes since the bean seed coat tends to absorb water rapidly, after which it wrinkles and slips. Cracks and fissures in the seed coat apparently are involved in early slipping, particularly those which occur during a treatment of less than 15-minute duration. Slipping of the seed coat was partially overcome by using certain non-aqueous solutions, such as di-ethyl ether and toluene, as carriers for the toxic materials. Glycerine was used in the spring plantings of 1940 but was not overly successful since considerable blight developed in some of the treatments used. The viscous character of the glycerine apparently prevented it from penetrating the seed and carrying the toxic agent to the site of the bacterial infection. Since laboratory experiments indicated that mercury bichloride and brilliant green were highly toxic to bacteria, these materials were used throughout this study as the principal ingredients of toxic mixtures. No great difficulty was experienced in maintaining the solubility of these compounds in most of the carriers tried. Methyl alcohol was used in the spring plantings to overcome the expense and restrictions associated with the use of ethyl alcohol. The methyl alcohol did not appear to be quite

as effective as the ethyl alcohol under the experimental conditions tried. The addition of acetic acid to the extent of 3 per cent improved the effectiveness of the treatments somewhat. The acid apparently aided in penetration of the toxic materials. Blight failed to develop under dry conditions and also when the temperatures were rather high. No single treatment as yet gave complete control of blight but the data presented indicated a high degree of success for several treatments. Other combinations of some of these materials might eventually be successful in evolving a treatment giving control not only of bean blight but also of other seed-borne bacterial and fungus diseases.

SUMMARY

A number of materials were examined in the laboratory to determine their relative toxicity toward cultures of bean blight bacteria and toward bean seeds.

The bean seed coat was found to slip after a 12-15 minute treatment in an aqueous solution. To avoid this slipping, non-aqueous solutions such as di-ethyl ether or toluene were used as carriers for the toxic materials.

The best control of initial infection was obtained by soaking bean seeds for 12-15 minutes in the following solutions.

(1) 1:500 mercury bichloride in di-ethyl ether.

(2) 1:20,000 brilliant green in 50% ethyl alcohol plus 3% acetic acid.

(3) 1:500 mercury bichloride in 70% ethyl alcohol plus 3% acetic acid.

(4) 1:20,000 gentian violet in 50% ethyl alcohol plus 3% acetic acid.

Control of initial infection by seed treatment resulted in increased yields of green beans with a corresponding improvement in the quality.

Storage after treatment apparently had little effect on the viability of the treated seed.

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BIOGRAPHY

Kermit William Kreitlow was born in Minneapolis, Minnesota, July 24, 1913. He was graduated from Theodore Roosevelt High School of Minneapolis in June, 1931, and entered the University of Minnesota the following fall. In June, 1936, he received the Bachelor of Science degree from the College of Agriculture. He entered the Graduate School of the University of Minnesota in the winter of 1937 and continued his study at Louisiana State University, where he received the Master of Science degree in May, 1938. He has continued his study at Louisiana State University and is a candidate for the degree of Doctor of Philosophy in June, 1940.

LEGEND FOR PLATES

PLATE I

Figure 1. Leaflets of Asgrow Black Valentine beans showing the marked chlorosis and extensive halos produced with severe blight infection. A healthy leaflet is shown in the lower right hand corner.

Figure 2. Mature, heavily infected bean leaves showing numerous blight spots with surrounding halos.

PLATE II

Figure 1. Mature pods of variety Full Measure showing blight spots and early necrosis.

Figure 2. Seedlings of Round Pod Kidney Wax beans showing stunting and chlorosis of severely infected plants. A healthy plant of the same age is at the right.

PLATE III

Figure 1. A healthy commercial field of beans containing vigorous plants of uniform growth.

Figure 2. Portion of a severely diseased bean field showing gaps in the stand and stunted growth of diseased plants.

PLATE IV

Figure 1. Portion of a check row of Asgrow Black Valentine Beans.

Figure 2. Portion of a row of Asgrow Black Valentine beans treated with 1:20,000 brilliant green in 50% ethyl alcohol plus 3 per cent acetic acid.

Figure 3. Portion of a check row of Asgrow Black Valentine beans.

PLATE I

Figure 1

Figure 2

PLATE II

Figure 1

Figure 2

PLATE III

Figure 1

Figure 2

PLATE IV

Figure 1

Figure 2

Figure 3

Figure 4

EXAMINATION AND THESIS REPORT

Candidate: KERMIT W. KREITLOW

Major Field: BOTANY, BACTERIOLOGY, AND PLANT PATHOLOGY

Title of Thesis: SEED TREATMENT FOR THE CONTROL OF BEAN BLIGHT

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

May 6, 1940