A STUDY OF STEWART'S DISEASE OF SWEET CORN CAUSED BY PHYTOMONAS STEWARTI

BY

C. W. FRUTCHEY

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Table of contents

Introduction

In the summer of 1932 there was a serious outbreak in Michigan of bacterial wilt commonly known as Stewart's disease of sweet corn. This disease is caused by the bacterial organism Phytomonas stewarti (Smith) com. S. A. B. According to The Plant Disease Survey (31) it also was prevalent in high percentages throughout the middle western and eastern Atlantic states as well as being reported in Ontario, Canada that year. In the central and southern counties of Michigan sweet corn fields with 100 percent infection were not uncommon. Although the severity of the disease is influenced by climatic conditions, it has been present each year since 1932 in smaller quantities with regional and individual field outbreaks from time to time. Prior to 1932 Stewart's disease had not caused any serious trouble in Michigan although it had been found as early as 1898 by Erwin F. Smith in Berrien county in the southwestern portion of the state. Since that date a few plants affected with bacterial wilt have been found from year to year within the state.

The general epiphytotic occurring in 1932 raised the question how it was possible for such large amounts of natural inoculum to have accumulated either within the soil, plant debris, seed, or insects and to have caused such high percentages of infection that year without having caused greater losses prior to this.

Studies of Stewart's disease of sweet corn were therefore begun in the summer of 1932. The various phases of the disease and its control studied were seed treatment practices, transmission of the disease by insect vectors, methods of artificial inoculation and varietal resistance, the soil as a source of inoculum, the role of infected seed in the transmission of the disease, and wilt caused by P. stewarti and associated organisms.

Literature Review

Bacterial wilt of sweet corn was first discovered in New York by Stewart (28) in 1894. Stewart described the disease symptoms and was able to isolate and culture the bacterial organism but was unsuccessful in attempts at artificial inoculation. The organism was briefly described and its presence in great abundance demonstrated in the vascular tissue although its pathogenicity was not established.

In 1897 Stewart sent a culture to Erwin F. Smith requesting him to name the organism. After a series of cultural tests he described the bacterium as a medium sized rod, rounded at the ends, with one polar flagellum, and gave it the name Pseudomonas stewarti in honor of the discoverer. In the same publication Smith reported seeing the disease in the southwestern portion of Michigan in 1898.

Halsted (2) mentions finding the bacterial wilt in New Jersey in 1899 but states that only one variety, First-of-all showed symptoms of the disease.

In 1901 Smith (21) published a more complete account of the cultural characteristics of the organism and in 1903 (22) was able to complete proof that Pseudomonas stewarti was the causal agent of bacterial wilt. Inoculating five hundered sweet corn plants by spraying a bacterial suspension on the leaves and by placing drops of bacteria in the fluid oozing from the water pores at the tips of the leaves, typical infections were obtained. By both methods of inoculation a total of more than three hundred plants showed wilt symptoms. In a publication six years later he (23) concluded that infected seed was the main source of dissemination of the disease and that treatment of the seed with mercuric chloride 1 : 1000 for 15 minutes would control it.

Between 1909 and 1914 Smith (24) in a section in Bacteria in Relation to Plant Diseases and in his book (25) Bacterial Diseases of Plants, gave a comprehensive report of five years data, describing all phases of the disease in great detail. The organism was described and demonstrated in all of the vascular tissue of the plant including the base of the kernel. The symptoms were described and infected seed as a means of dissemination was stressed with a statement that treatment with mercuric chloride 1 : 1000 for 20 minutes was not an

adequate control.

McCulloch (11) in 1918 after making fourteen isolations of the bacterial wilt organism on corn from several states was unable to find motile forms and could not demonstrate flagella by the use of special stains. Two types of agar colonies were obtained, alike in every respect except the surface view on agar, one having a smooth flat surface, the other a central depression. The organism was renamed Aplanobacter stewarti (Smith) because of the absence of motility.

Rand and Cash (14) reported in 1921 that the early varieties of sweet corn were more susceptible than the later ones with only a trace of wilt in field corn. They were able to produce no infection from infested soil nor old infected corn stalks but isolated the wilt bacterium from the base of the kernel and from parts of the endosperm, finding no trace of it in the embryo. They concluded that abundant soil moisture and high temperature were principal factors influencing the development of Stewart's disease although they believed that injury caused by insect larvae was closely correlated with its appearance. Later (15) they discovered that the adults of the brassy flea beetle Chaetocnema pulicaria Horn., the toothed flea beetle Chaetocnema denticulata Horn., and the twelve-spotted cucumber

beetle Diabrotica duodecimpunctata Oliv. were direct agents of transmission of bacterial wilt and found larval injury of an unknown origin associated with the disease plants found in the controls. Their previous findings were verified (16) in 1933 and they suggest that much of the late season infection is due to insect transmission. They stated that infected seed probably plays its major role in the transportation of the disease from one region to another. Very low percentages of wilt were found in plants grown in the greenhouse where insects were controlled.

Reddy (17), experimenting with resistance and susceptibility of dent, flint, and sweet corn found that none of them showed any marked resistance. There was no evidence of infection from soil previously exposed to bacterial infestation and it was concluded that seed disinfection was ineffective in control.

Reddy and Holbert (18) tested bacterial wilt resistance of various inbred and hybrid lines of dent corn. The wilt organism was isolated from wilted plants of the Golden Bantam variety, and inoculations were made by means of hypodermic injection into the stalk of the partially grown seedings. No apparent correlation between resistance and vegetative vigor was found.

Thomas (29) reported Stewart's disease prevalent in

Ohio in 1924. The disease was briefly described and the suggestion was made that the main source of the inoculum is in the infected seed. Some of his results indicated that the organism might cause infection from infested soil. Later (30) Thomas gave a report on a study of bacteriophage associated with P. stewarti. The bacteriophage could be isolated from roots and lower nodes of diseased plants and from infected seed. Soaking naturally infected seed in bacteriophage cultures of relatively high titer, the percentage of wilted plants were greatly decreased in comparison to the controls. All plants were subjected to the presence of the twelve-spotted cucumber beetles, flea beetles, and chinch bugs.

As a result of the epiphytotic of bacterial wilt of corn occurring in 1932, investigations were initiated at various institutions and several publications concerning Stewart's disease have been released since that time. Noteworthy among these is the work of Ivanoff of Wisconsin, and that of Elliott and co-workers of the United States Department of Agriculture.

Ivanoff (4) reports that the western corn root worm, the larval stage of Diabrotica longicornis Say., transmits bacterial wilt from diseased to healthy plants. Several bacterial organisms were found in wilted plants which on

inoculation into corn plants caused symptoms such as striping, yellowing, and wilting. These organisms differed from Phytomonas stewarti both in the symptoms they caused and in their physiology. In a later publication (5) on pathogenesis a selective medium was developed for culturing P. stewarti. It was demonstrated that the organism would not infect corn plants without the presence of a wound; the organism was found to be present in the kernel but did not produce infection to any great extent when insects were absence. Still later (6) he learned that Phytomonas vascularum an organism causing a vascular disease in sugar cane, when inoculated into sweet corn produced symptoms similar to those caused by P. stewarti. When inoculated into sorghum Holcus sorghum, Sudan grass H. sundanensis, and yellow fox-tail Setaria glauca, P. stewarti produced symptoms similar to those which it causes on sweet corn.

Ivanoff and Riker (8) tested resistance against P. stewarti by means of natural and artificial inoculations into a large number of corn plants. Resistance was found in some of their inbred and hybrid strains and they conclude that it is due to inherent vigor, time of maturation, and true resistance. Resistance seemed to show as a dominant inherited factor.

Ivanoff, Riker, and Dickson (9) state that Diabrotica

longicornis Say. transmitted the disease from wilted to healthy plants. They also found isolates of Stewart's organism which differed somewhat in pathogenicity and physiology.

Holbert, Elliott, and Koehler (3) working in Illinois found bacterial wilt to be prevalent with abundant local lesions on the leaves. The bacteria were not present in the leaf veins below the lesions on the leaf blade nor were they present in the sheath nor in the bundles of the stalk below the infected leaf. They noted different strains of the pathogen and a marked resistance to bacterial wilt in certain inbred strains of corn and their crosses.

Poss and Elliott (12) isolated Phytomonas stewarti from overwintered adults of the flea beetle, Chaetocnema pulicaria Horn. Of 40 species of insects from which isolations were made only C. pulicaria yielded the wilt organism. P. stewarti was found in about 75 percent of the cases of this insect collected before any corn plants were present. In their work there was no evidence that infection might occur from soil infested with the bacterial organism.

Clinton and Singleton (1) made a large number of crosses producing inbred and hybrid strains of Whipple some of which they report to be quite resistant to bacterial wilt.

Smith (26) reports a hybrid variety of sweet corn

Golden Cross Bantam which stood up very well in resistance to bacterial wilt in his field trials.

Mahoney, Muncie, and Marston (10) found the early varieties to be the most susceptible to bacterial wilt. Golden Cross and Top Cross Bantam showed some infection in the field but slight loss in marketable ears.

Economic Importance.

Bacterial wilt on the more susceptible sweet corn varieties and depending on the intensity of infection may cause 100 percent loss in the field. In the Summer of 1932 in Michigan sweet corn fields were in many instances a total loss. One field of 55 acres of sweet corn near Morcenci was completely ruined by general infection of bacterial wilt. Another crop near Lansing was so severely attacked that the grower was unable to sell more than $105 worth from a forty acre field. Infection followed by necrosis was exceedingly high in all of the College plots and small garden plots as well as commercial fields suffered greatly from this disease. Since 1932 Stewart's disease in Michigan has been present but not nearly so intense as it was that year.

Similar conditions have been reported from the middle western and eastern Atlantic states. The Plant Disease Survey (31) reports the losses caused by Stewart's disease for 1931, 1932, and 1933. In 1931 only three states showed as much as three percent reduction in yield due to wilt. Losses in West

Virginia were estimated to be ten percent, Ohio and New Jersey each three percent. In 1932 seven states reported losses of five or more percent due to bacterial wilt. Pennsylvania had 45 percent, Indiana 20 percent, Michigan 15 percent, West Virginia, Ohio, and New York each 10 percent, Iowa five percent, and Connecticut four and one-half percent. In 1933 the intensity of the disease decreased somewhat in comparison to that in 1932. Nine states showed a loss of five or more percent. Maine, Connecticut, and Iowa each record a loss of five percent, Massachusetts and Indiana each ten percent, New York 11 percent, Michigan and Ohio six and seven percent respectively, and Pennsylvania 25 percent. Illinois reports heavy losses for 1932 and 1933 (32) but there is no recorded estimate of percentage loss. The disease decreased with nearly the same sharp break in 1934 as it arose in 1932.

Symptoms of the Disease

In the light of recent knowledge it has been found that the same or very similar symptoms heretofore attributed exclusively to infection by Phytomonas stewarti may be caused by certain other organisms as well. Ivanoff (4), (6) mentions other organisms which are able to infect corn and which cause symptoms similar to those produced by P. stewarti and made some distinction between these symptoms. Nevertheless the general symptoms attributable to infection by P. stewarti are

widely known, and the wilt disease can be identified by these for all particable purposes. If the plant is in the seedling stage when general infection sets in, the leaves wilt, showing a water-soaked bluish cast at first. The plants become a lighter or yellowish color later turning brown with a shrivelled dried out appearance. Dwarfing shrinking and finally dying results. This type of wilting is always accompanied by a discoloration of the interior of the nodes three or more above the adventitious roots and a general basal rotting, usually if not always, associated with other bacterial and fungus organisms. The plant may not be completely infected, but the disease is often manifested by local leaf lesions which occur on the veins, at the tips, or at the margins. These local lesions are quite similar in color to those resulting from severe infection. When infected, the leaf margin becomes water soaked and darkened, gradually shrinking and rolling upward so that the edges turn in toward the midrib. The water-soaked, discolored streaks may appear along the veins from a few millimeters in length to that of the entire leaf, and from one or two millimeters in width to one-half inch or more. These areas shrink and sink below the level of the uninfected epidermis. The infected portion may dry and shrink until it pulls away from the live tissue in which case it falls out leaving a hole through the leaf. The tips of the leaves may show lesions which often becoming discolored and dried,

roll up and break off leaving the green lower portion of the leaf with a blunt end. If the plant is in the tassel stage when infection sets in, the tassels become dwarfed and bleached in appearance, and the leaves may partly or entirely wilt. The leaves may stick together above the enclosed tassel due to exuding organisms when they are in a water soaked condition. This prevents emergence of the tassels and causes the stalk to bend in the form of a loop due to later elongation. It is not the intention to give the impression that all of these conditions appear on any one plant nor even in one field where wilt is prevalent, but to present a composite of symptoms which may be found. Pathogenic strains of the bacterial wilt organism have been isolated from plants showing the various individual symptoms described.

Symptoms that may closely resemble those of Stewart's disease occur both in sweet and field corn. The writer has found a species of Fusarium causing a foot rot and wilting which is similar in appearance to the seedling stage of Stewart's disease. A bacterial organism producing white colonies on potato dextrose agar has also been recovered which if not identical is closely related physiologically to Phytomonas dissolvens Rosen. This organism causes basal rotting and leaf wilting very similar to the symptoms of bacterial wilt caused by P. stewarti and which may be readily

mistaken unless proved otherwise by isolations. Drought alone, if severe, may produce a type of wilting and yellowing of corn plants somewhat similar to those of the systemic type of Stewart's disease. Late spring frosts and mechanical injury at the base of the plant can likewise give similar results.

Methods of Inoculation and Varietal Resistance

After a number of isolations were made and cultures of P. stewarti obtained, various methods of inoculation were tried in order to cause wilt artificially. A suspension of the bacteria was sprayed on the leaves and stems, the seeds were soaked in a broth culture of the organism, and hypodermic injections were made into the stems of plants a foot or more in height. All of these methods gave more or less negative results. The hypodermic injections into the stems gave local lesions but not the typical wilting occurring in the field at that time. From these preliminary trials it was found that a heavy hypodermic injection of a broth culture of the bacterial wilt organism at the crown of the plant was the most effective way of producing bacterial wilt artificially. Ivanoff (7) recently reported a modification of this method.

In order to determine if there was any difference in resistance inoculations were made using a large number of hybrid and inbred strains of corn. The included lots of seed

received by Dr. C. H. Mahoney of the Department of Horticulture from Dr. E. S. Haber of Iowa State College and from Mr. Glen M. Smith of the United States Department of Agriculture as well as some commercial varieties of corn. Two plots of seed of these varieties were planted in the greenhouse at different times. In each plot 10 lots of 20 seed or a total of 400 seeds of each variety were planted in the two plots. The corn was planted in rows and inoculated when about three inches tall. The first ten plants in each lot were inoculated and the second ten left as controls, there being ten replications of inoculated and control plants of each variety in each plot. All of these inoculations were made with a hypodermic needle at the crown of the plant and observations were made to determine the length of time necessary for symptoms to develop. The incubation period not only in these tests but in others was found to vary from two to ten days with symptoms becoming noticeable usually in about four or five days, and the most severely infected plants being completely dead in about three weeks. The results of these inoculation tests are recorded in Table I.

Table I

The number of plants, the percentages of wilt developing in the inoculated and control plants, and the average percent of wilt for both of these groups.

Table I (continued)

Variety used	Plot I number plants	Plot I percent wilt	Plot II number plants	Plot II percent wilt	average percent wilt
Iowa hybrids					
B--23 inoculated	96	66.6	97	85.5	76.2
" uninoculated	92	0	96	1	0.5
B--64 inoculated	85	82.3	98	67.3	74.3
" uninoculated	89	22.5	97	11.3	16.6
B--122 inoculated	91	87.6	87	81.6	84.8
" uninoculated	83	0	86	0	0
B--34 inoculated	97	84.5	89	75.3	80.1
" uninoculated	84	25	99	10.1	16.8
B--69 inoculated	93	78.5	83	100	88.6
" uninoculated	91	0	94	0	0
B--10 inoculated	87	100	83	100	100
" uninoculated	86	0	73	0	0
B--50 inoculated	72	83.3	96	80.2	81.5
" uninoculated	69	30	86	19.8	24.5
B--30 inoculated	87	70.1	88	75	72.6
" uninoculated	83	15.7	87	16.1	15.9
B--38 inoculated	65	77	87	81.6	79.6
" uninoculated	67	0	89	0	0
B--19 inoculated	73	100	--	--	100 *
" uninoculated	75	28	--	--	28 *
B--21 inoculated	94	54.2	--	---	54.2*
" uninoculated	81	0	--	--	0 *
C--108 inoculated	84	100	--	--	100 *
" uninoculated	88	0	--	--	0 *
C--118 inoculated	90	11.1	94	50	31
" uninoculated	92	33.7	98	19.4	26.3
C--20 inoculated	100	23	79	31.7	26.8
" uninoculated	94	11.7	87	11.5	11.6
C--34 inoculated	77	79.2	--	--	79.2*
" uninoculated	71	0	--	--	0 *
C--117 inoculated	87	37.9	86	53.5	45.7
" uninoculated	82	37.8	89	10.1	23.4
C--125 inoculated	100	40	100	39	39.5
" uninoculated	91	0	100	1	1.5
C--23 inoculated	93	10.7	85	38.8	24.1
" uninoculated	99	0	84	0	0
C--31 inoculated	87	60.9	91	75.8	68.5
" uninoculated	86	17.4	85	14.1	15.2
C--113 inoculated	93	34.4	90	51.1	42.6
" uninoculated	84	0	93	2.1	1.1
C--115 inoculated	91	100	92	93.5	96.7
" uninoculated	87	57.5	91	12.1	26.9

Table I (continued)

Variety used	Plot I number plants	Plot I percent wilt	Plot II number plants	Plot II percent wilt	average percent wilt
Iowa hybrids					
C--22 inoculated	100	50	93	51.6	50.8
" uninoculated	92	44.5	88	22.8	33.9
126 inoculated	100	40	100	54	47
" uninoculated	100	0	93	2.1	1
Iowa selfed					
1857 inoculated	100	90	90	66.6	78.9
" uninoculated	91	11	78	1.3	6.5
1876 inoculated	90	100	94	85.1	92.4
" uninoculated	90	0	99	11.1	5.8
1804 inoculated	94	88.3	89	97.8	92.8
" uninoculated	89	0	93	4.3	2.2
Golden Cross Bantam					
inoculated	100	91	95	85.3	88.2
uninoculated	90	0	98	0	0
Purdue Bantam					
inoculated	99	81.8	90	94.4	87.8
uninoculated	98	21.4	91	11	16.4
Yellow Bantam Maine					
inoculated	70	57.1	93	83.9	72.4
uninoculated	70	42.8	96	19.8	29.5

* Note In plot II these records were lost and there was not enough seed left to repeat the trials.

These results indicate that immunity to the disease in a corn plant would be relatively difficult to obtain, but that there may be a difference in resistance between varieties. Clinton and Singleton (1) found that several of their Whipple stains of sweet corn were quite resistant under field conditions, depending upon natural inoculation for infection. Golden Cross Bantam has been grown very successfully in Indiana, Ohio, Pennsylvania, and New York with little loss from wilt. However, both of these varieties are susceptible when inoculated artificially in the above manner, and in 1934 Golden Cross Bantam

showed 100 percent in the field in three different plots at the Michigan Station and was the most susceptible of any variety grown. Ivanoff and Riker (8) report hybrids and inbreds which stood up well in the field when subjected to this method of inoculation. This method of inoculation is very severe and puts a variety to the most vigorous test of resistance. Immunity may not be needed for practical purposes, and there may be varieties that will stand up well in the field where conditions for wilt development are very favorable. Such varieties, however, might not be resistant enough to withstand this method of inoculation, using a virulent culture of P. stewarti.

It will be noted in Table I that high percentages of wilted plants were found in some of the uninoculated varieties. Early workers carrying on investigations of Stewart's disease believed infected seed to be the main source of inoculum and the chief agent of dissemination. The results of later tests, however, cast some doubt upon this hypothesis due to the fact that bacterial wilt did not always develop from seed obtained from plants showing high percentages of infection. Rand and Cash (16) found only about two percent wilt resulting from infected seed where other sources of inoculum were negligible, and Ivanoff (5) reports similar results.

The percentages of wilt as shown in the uninoculated

controls in Table I do not harmonize with these later discoveries. As the plants were grown in the greenhouse, there were only three sources of inoculum which presented themselves, namely, infected seed, infested soil, and insects. Experiments were conducted, therefore, to determine from which source or sources the inoculum could have arisen that caused the infection in the controls of Table I.

Infested Soil a Source of Inoculum

Smith (20) believed the organism causing bacterial wilt of corn was disseminated from plant to plant through the soil by cultivation. The results of Thomas' (29) experiments indicated that infection might occur from infested soil. Reddy (17) states that there was no evidence that infection occurred from soil previously exposed to bacterial infestation. Rand and Cash (16) were not able to obtain any infection from infested soil nor from old infected corn stalks. Roos and Elliott (12) found no evidence to show that infection of plants ever occurred by means of soil infested with the bacterial wilt organism. Ivanoff (5) found no evidence of soil infestation by p. stewarti causing the disease without other agencies present but was able with his selective medium to isolate the organism from the soil and from infected plant debris.

The writer has never been able to isolate the organism from the soil. It can be isolated from diseased corn stalks

for a limited length of time, but the results obtained in these experiments show no indications that the bacterial organism is able to winter over in soil or plant debris. During the winters of 1932 and 1933 and 1934 both of which were extremely cold for this section, flasks of sterilized and unsterilized soil heavily inoculated with a culture of P. stewarti were left out of doors. In the spring after they had thawed plantings were made from the soil both of Ivanoff's selective medium and on potato dextrose agar. The bacterial wilt organism was not recovered. The same is true of soil in the field. However, there is a possibility that this condition may vary in different sections of the country and there may be some soils which would harbor the organism for longer periods of time.

From the above results it can be concluded that soil infestation did not cause the wilt that occurred in the uninoculated controls as shown in Table I. With this factor eliminated it was necessary therefore to determine what role infected seed and insect transmission played in the production of Stewart's disease.

Infected Seed as a Source of Inoculum

As the control plants shown in Table I were not uniformly free from the disease, and as there was some question regarding the ability of the seed to carry the bacterial organism

from generation to generation, it became necessary to determine the actual percentage of wilt that would be due only to infection from the seed. Plants were grown both in the greenhouse and the field. The inbred and hybrid strains shown in Table I in addition to some others were grown as one group. These were grown in the greenhouse only, due to a limited amount of seed; the soil was sterilized and the greenhouse fumigated with nicotine sulfate as often as was necessary to keep down the insect population. Another group or lot of plants from seed of diseased Golden Bantam corn was grown both in the greenhouse and the field. The only source of inoculum that was eliminated in this instance in the greenhouse was insects because the soil was not sterilized but fumigation was carried out regularly thus eliminating to a great extent insect agents of transmission.

The fact that the same varieties of plants grown in the field had a higher percentage of wilt than did those which grew in the greenhouse indicated a correlation between insect injury and the amount of wilt produced. The results of this experiment are tabulated in Table II.

Table II

Greenhouse plants grown in pots of sterilized soil with the number healthy, the number wilted, and the average percent wilted.

Table II (continued)

Variety used	Lot I number wilted	Lot I number clean	Lot II number wilted	Lot II number clean	Lot III number wilted	Lot III number clean	Average percent wilt
Iowa hybrids							
B--23	1	14	1	34	2	44	4.2
B--64	1	33	2	13	3	26	7.7
B--122	5	21	0	15	1	46	6.8
B--34	1	51	1	11	4	40	5.6
B--69	1	39	1	41	-	--	2.4
B--10	1	31	0	11	1	29	2.7
B--50	1	49	1	29	-	--	2.5
B--30	0	41	1	24	2	21	3.4
B--38	0	60	2	43	-	--	1.9
B--19	0	49	1	11	0	19	1.2
B--21	0	41	0	42	0	14	0
C--108	0	41	0	16	1	29	1.1
C--118	1	21	1	63	2	13	3.9
C--20	0	51	1	41	4	12	4.6
C--34	0	31	1	15	0	43	1.1
C--117	0	24	2	39	0	14	2.5
C--125	1	51	0	27	1	12	2.2
C--23	1	60	1	10	1	34	2.8
C--31	1	51	0	47	-	--	1.0
C--113	0	61	1	10	0	20	1.1
C--115	1	26	2	47	0	25	2.9
C--22	1	36	1	31	2	25	4.2
126	0	35	0	16	0	37	0.0
69	0	31	1	36	0	16	1.2
Iowa selfed							
1857	0	34	3	70	-	--	2.8
1876	2	49	1	32	-	--	3.6
1804	2	44	2	36	0	24	3.7
917	0	16	1	42	2	29	3.3
13	1	43	1	33	0	15	2.1
69	0	31	1	36	0	16	1.2
42	0	41	1	41	1	19	1.9
9	0	35	2	22	0	22	2.4
Purdue hybrids							
Golden Cross Bantam	1	41	1	48	-	--	2.2
Purdue 51	0	26	3	64	-	--	3.2
1403	0	27	3	41	1	14	4.7
1310	0	24	0	19	3	39	3.5
1303	0	41	1	31	-	--	1.3
1413	0	23	6	41	3	31	8.6

Table II (continued)

Variety used	Lot I number wilted	Lot I number clean	Lot II number wilted	Lot II number clean	Lot III number wilted	Lot III number clean	percent wilt
Hybrid Golden Cross Bantam	0	17	2	43	0	19	2.4
Commercials							
Pur. Bantam	0	41	1	40	2	17	3.0
Pur. Bantam(b)	0	35	0	29	-	--	0
Yellow Bantam Maine	0	29	1	37	0	26	1.1
Quaker Hill Golden Cross Bantam	2	35	3	32	2	21	6.4
Associated Seed Growers Golden Cross Bantam	0	44	0	13	1	35	1.1
Sheeley Golden Cross Bantam	1	24	2	42	1	23	4.3
Barron Sunshine	0	31	0	32	1	31	1.1
Average of all Varieties	1.3	36.5	1.6	32.5	1.9	25	3.0

Total for all varieties, clean = 4075; wilted = 95; percent wilt=2.6

The seed of the above strains of sweet corn came from plants showing a high percentage of wilt in 1932. These strains were all more or less susceptible to the attack of P. stewarti as can be concluded from the results of inoculations presented in Table I, yet none of them produced any large amount of wilt when grown in the greenhouse in sterilized soil in the absence of insects. Golden Cross Bantam had 8.6 percent wilted plants which was the highest reading of any strain, and in many there was not a single plant which showed the disease. It is also

true that in many cases the uninoculated controls in Table I showed a much higher wilt reading than occurred in any of the same strains in Table II. It is therefore quite evident that either insect agencies or soil infested with the bacterial wilt organism had some influencing effect upon the production of wilt in the controls of Table I. It would also indicate that infected seed has a small role in the production of the disease even when plants are grown in the greenhouse with other sources of infection eliminated.

It was therefore decided to eliminate only one of the above mentioned infection possibilities when a duplication of plants were grown in the field. In the summer of 1934 seed from diseased Golden Bantam plants was planted both in a greenhouse which was kept fumigated frequently and in the field. The soil was not sterilized in either case but the greenhouse plants were kept free from insects. The results are shown in Table III and IV.

Table III

Plants grown in a fumigated greenhouse in unsterilized soil, showing the relation of insect injury to the number of wilt, and the percentage of wilt.

plantings	number plants	number healthy	number wilted	number wilted with insect injury	percent wilt
1.	334	324	6	4	3.0
2.	184	173	8	3	6.0
3.	291	286	5	0	1.7
4.	212	203	5	4	4.2
5.	534	526	8	0	1.5
6.	182	167	10	5	8.2
7.	107	103	4	0	3.7

Table IV

Two plots of plants grown in the field not excluding insects.

Readings taken	number plants	number healthy	number wilted	number wilted with insect injury	percent wilt
		Plot I			
July 1, 1934	2331	2312	19	19	0.8
Aug. 6, 1934	2327	2286	41	36	1.7
Sept. 1,1934	2322	1509	813	711	35.0
		Plot II			
July 1, 1934	1101	1090	11	8	1.0
Aug. 6, 1934	1096	1081	15	15	1.5
Sept.1, 1934	1090	733	357	297	32.8

The most striking feature of table IV is that very little wilt showed up until August. It will likewise be noticed that insect injury of diseased plants increased proportionally with the increase in wilt. The insect injury was largely the work of larvae feeding around the base of the plants. At no time could definite bacterial wilt leaf lesions be attributed to inoculation by leaf feeding insects. It is doubtful in this case that the late infection was due to diseased seed as the kernels were rotted away and no longer in evidence before the wilt became noticeable to any great extent. The only means by which infected seed could have played a part would have been for the organism to have gained entrance into the young seedling from the seed and there remained dormant until August when the plants were practically mature. Delayed appearance of the symptoms after infection was thought by early workers to occur. However, in the light of present

day knowledge of the appearance of the symptoms after artificially inoculation, it is very doubtful if such a dormant period exists. Due to the fact that the same lots of seed produced very little wilt in the greenhouse where conditions, other than insect infectation, were very favorable for the appearance of the disease, it is reasonable to conclude in this experiment that larvae of insects played a major role in infection in the field rather than infested soil or infected seed.

The results in Table II show clearly that not all of the wilt in the control plants in Table I could be due to infected seed. Varieties C--22, C--115, C--118, and Maine Yellow Bantam for examples had 33.9, 26.9, 26.3, and 29.5 percent wilt respectively. These varieties when grown in sterilized soil in the greenhouse where other sources of inoculum were eliminated showed only 4.2, 2.9, 3.9, and 1.1 percent wilted plants respectively. Soil infested with the bacterial wilt organism and insect transmission of the disease were the only sources of infection eliminated in these experiments. It was found that isolations from suspected infected seed yielded P. stewarti in approximately 12 percent of the trials. This as can be seen is a higher percentage than there were infected plants when factors other than infected seed were eliminated. As this fact indicates that not all of the infected seed will give rise to wilted plants, studies of this nature were made

and are given in the next phase of the work.

The role of infected seed in the transmission of the disease

Results of previous experiments had shown that the organism was carried in the seed, and that it was possible to isolate an organism from the interior of the seed and to produce bacterial wilt with the organism obtained. It was also found by isolations that the percentages of seed infected with the organism were greater in all cases than were the percentages of wilted plants occuring when this seed was planted and the insects agents were controlled. Various workers have shown by isolations from and sectioning of the diseased kernel that the organism was present in the old vascular tissue of the chalazal region of the endosperm. Rand and Cash (16) isolated P. stewarti from the endosperm, but there is no record of the organism being recovered from the embryo region. The question at once was raised as to why greenhouse plants grown in sterilized soil from seed known to be infected did not show a greater percentage of bacterial wilt. In addition isolations of the pathogen could be readily made from kernels one year old. This condition showed that the bacterium was still alive and did not die out before the next year's planting. Also seed one year old would produce just as much wilt as seed only a month or two old. Experiments were therefore inaugurated to determine what connection there might be between inoculum within the seed and the occurence of the disease.

In order to be certain that infection came from the interior of the seed, surface sterilized kernels were germinated and grown on sterile agar slats in large test tubes until the young seedlings were about four inches in length. Altogether 500 young seedlings were grown in this manner from seed collected from Golden Bantam plants definitely affected with bacterial wilt. Only 53 or 10.6 percent of these plants showed symptoms of the disease. Plantings of bits of the infected leaf and stem tissue were made on potato dextrose agar plates and P. stewarti and a white bacterial organism were isolated from each of the diseased plants.

Experiments also were carried on to verify the results of Ivanoff's (5) work showing that infection with P. stewarti takes place only through wounds. In these tests 500 kernels of clean seed were germinated and grown on agar in petri dishes. After the kernels had germinated 0.1 c.c of a virulent broth culture of P. stewarti was dropped upon the kernel at the point of emergence of the epicotyl. No wilt was produced even when the organism was growing on the agar around the tip of the shoot and root and over the kernel. This experiment as well as those of Ivanoff show that a wound is necessary for P. stewarti to gain entrance into the plant tissue. From these facts it seemed reasonable to

assume that the organism within the vascular tissue at the base of the kernel was fixed and not able to invade the embryo at the time of germination, unless some injury occurred which would afford an avenue of entrance. Experiments were therefore carried on to determine if possible the soundness of the above hypothesis.

Several hundred sweet corn seeds were surface sterilized by repeated rinsing in alcohol, mercuric chloride 1:1000, and sterile water. By this method any organisms within the seed were not affected. These seeds were then germinated by the "rag doll" method and after the epicotyl and hypocotyl had emerged the kernels were punctured with a fine needle through the chalazal region into the embryo to a point where the young root and shoot separate. Two punctures were made in each seed, each time passing the needle through some of the vascular tissue that had connected the kernel to the cob. Controls were treated in a like manner in every way except puncturing. These plants were grown in the greenhouse in sterilized soil and for the most part under a large muslin covered insect proof cage. The greenhouse was fumigated each time a new lot of seedlings was planted. The results of the experiments expressed as percentages of wilted plants in the punctured seedlings and the controls are shown in Table V. The percentages of infected seed as determined by isolations are given in the text. These isolations were made

from corresponding lots of seed as those used for the puncture experiments in order to know approximately the percentages of inoculum present.

Table V

Number of wilted plants and the percentages of wilt as found in the punctured seedlings and controls.

Punctured seedlings

Plantings	Variety used	number plants	number healthy	number wilted	percent wilt
1.	Golden Bantam	68	43	25	37
2.	" "	53	34	19	36
3.	" "	75	50	25	33.3
4.	Golden Nugget	186	153	33	18
5.	Golden Bantam	191	165	26	13.6
6.	" "	214	174	40	18.7
7.	Extra Early Golden Bantam	374	295	79	21.1
8.	" "	232	191	41	17.1

Controls

1.	Golden Bantam	94	67	7	9.5
2.	" "	48	44	4	8.3
3.	" "	207	196	11	5.3
4.	Golden Nugget	161	145	16	10
5.	Golden Bantam	263	261	2	0.76
6.	" "	211	194	17	8.1
7.	Extra Early Golden Bantam	241	226	15	6.2
8.	" "	197	179	18	9.1

The results of this experiment show very clearly that injury through the chalazal region of the corn kernel, after germination, into the base of the young shoot caused the disease to be expressed in much greater percentages than when injury had not taken place. The percentages of wilted plants were much higher in the injured plants than in the controls.

It is also clearly shown in Table V that in the controls there were more kernels with bacterial infection than there were wilt infected seedlings when the seed was planted. It may then be concluded that the greater part of the bacterial wilt inoculum is unable to gain entrance into the young seedling at the time of germination unless a path of entrance was made by some agent other than P.stewarti. If there were no agent to create the avenue of entrance and P. stewarti was not able to gain entrance in the absence of wounds as proved, the controls in Table V should have been completely free of wilt. As this was not found to be the case and as isolations from corresponding lots of seed showed on the average 18.7 percent of the kernels to be infected with P. stewarti and 7.2 percent of the kernels to contain a bacterial organism which produced white colonies on potato dextrose agar, studies were made of insect transmission of the disease, and the role played by organisms associated with P. stewarti.

The fact that wilted plants were more prevalent when the tissues of the seed were punctured through the chalazal region into the young seedling correlates very well with other phases of our knowledge of Stewart's disease. There are known to be a number of insect larvae that feed within the kernel shortly after germination and if they penetrate farther, as some of them do, they could infect the young plant from its own seed

merely as a contact agent of dissemination. Examples of some of these insects are the seed-corn maggot and several small maggots of this group, as the cabbage and the onion maggots. Figures 1, 2, and 3 show the relation between the chalazal region and the embryo.

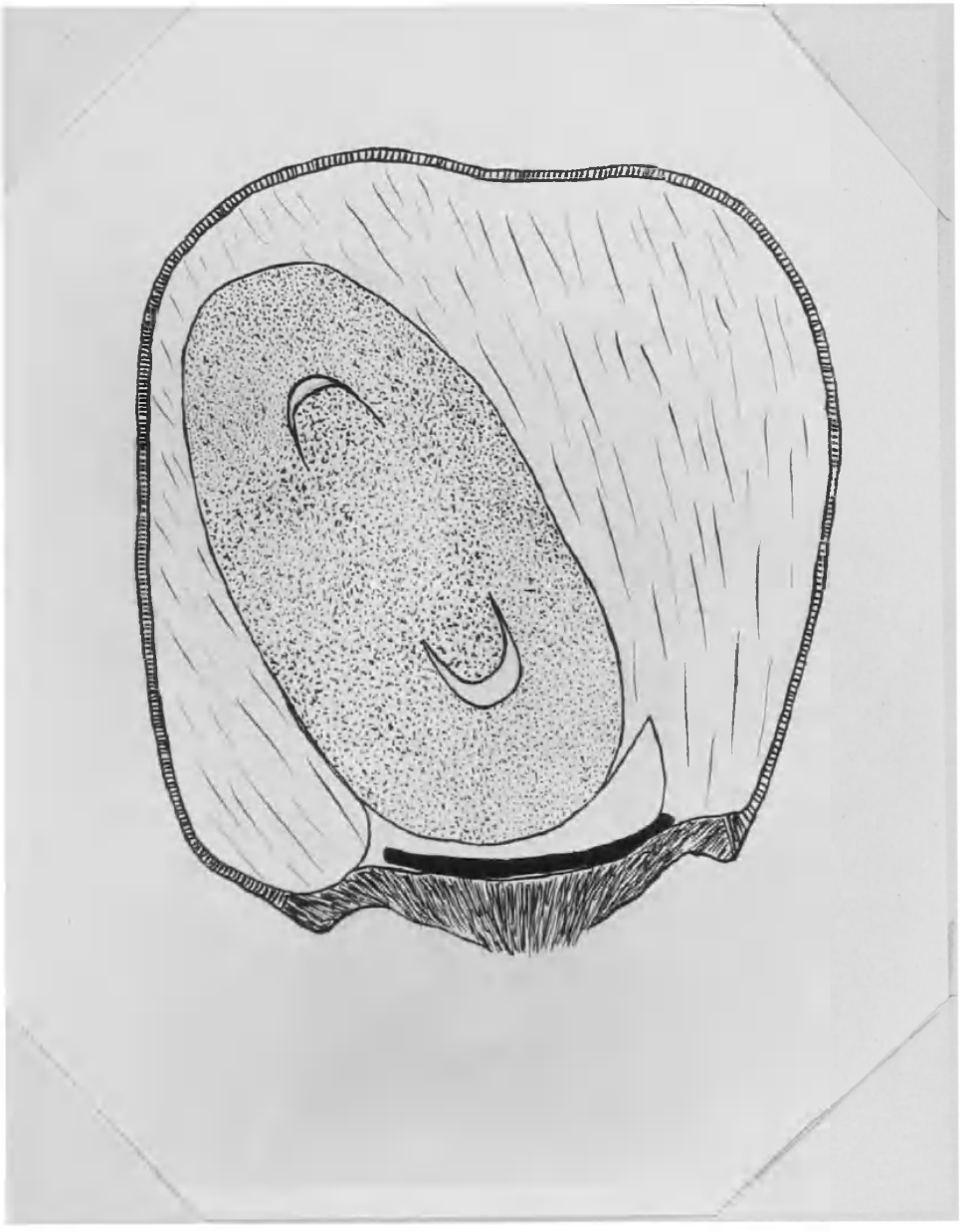

Figure I

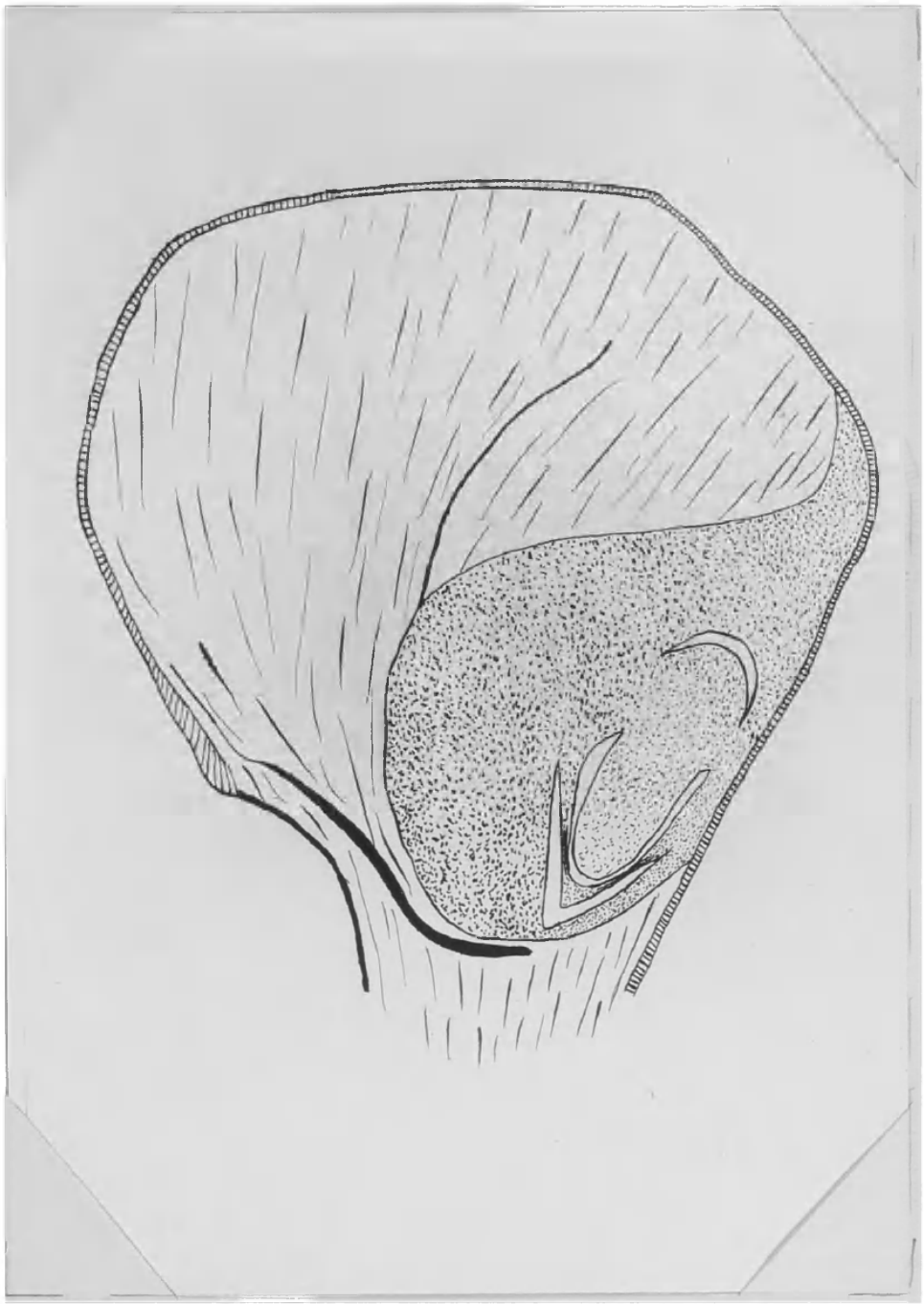

Figure II

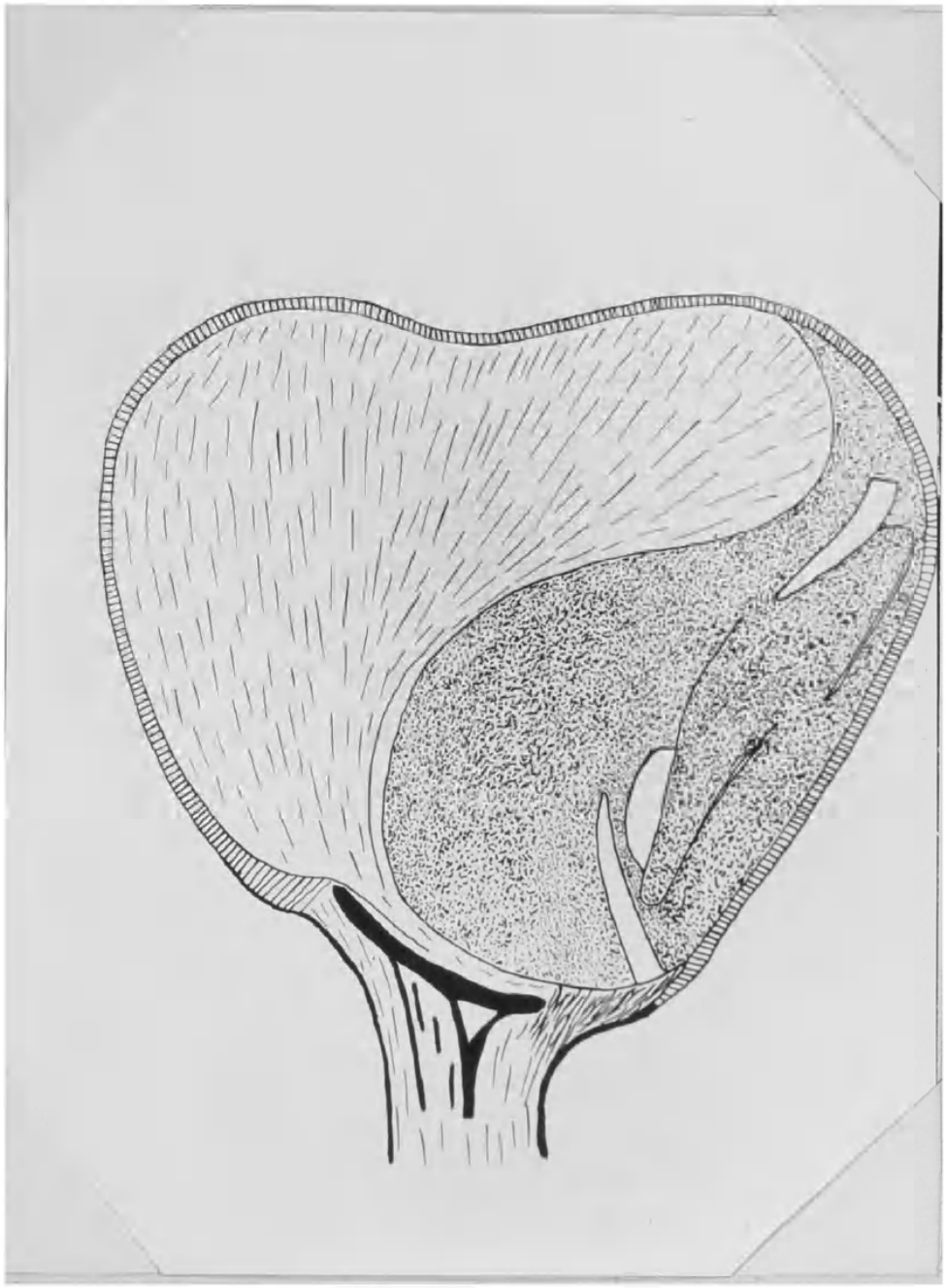

Figure III

Insects in relation to disease transmission

During the severe epiphytotic of Stewart's disease in 1932 many specimens were sent in to the plant pathological laboratory by growers. These specimens varied in height from six to eighteen inches and nearly all of them were infected systemically with P. stewarti. Most of the plants showed the wilting and necrotic conditions typical of the disease, accompanied by a soft rot at the base of the stalk. In many of these plants small larvae were found feeding in the base of the stem and often two or three inches above the crown. It was assumed at first that these larvae were those which commonly follow the soft rot condition in plant tissue. Later they were identified by the Entomology Department as larvae of the seed-corn maggot, Hylemyia cilicrura Meigen. Isolations were made both from the exterior surface by placing the maggot in a tube of broth and plating from this medium, and from the alimentary tract after disinfecting the surface of the maggot and dissecting. Cultures of P. stewarti were readily recovered from both regions but especially from the latter. In 1933 only a few of these maggots were found and again isolations made from the alimentary tract yielded P. stewarti. In 1934 some of the adults of Hylemyia cilicrura were collected and placed upon plants growing under muslin cages in the field. A close watch was kept but none of the plants showed any symptoms until three weeks after introduction of the insects, when symptoms began to

appear. A short time later when about three-fourths of the plants had become diseased, they were pulled and examined. Every wilted plant showed larval injury, some still having the larvae within the plant tissue, although most of them had gone into the ground to pupate before they could be recovered. Of the 93 plants exposed to these insects 67 plants or 72 percent showed typical wilt while the controls showed only an occasional wilted plant. These maggots were not recovered from the soil and as they were rather scarce during 1934 probably due to the preceding cold winter, the experiment had to be discontinued for the time being. It is hoped that more larvae of the seed-corn maggot can be secured in 1935 and more data obtained regarding their activity as an agent in dissemination. The fact that the maggot does attack corn plants and also carries P. stewarti in a virulent condition internally is conclusive evidence of its role in wilt dissemination. The length of time P. stewarti is carried within the insect and how long the organism remains pathogenic there, is however, still a question.

Another type of insect found to carry P. stewarti from diseased to healthy plants was the common wheat wireworm, Agriotes mancus Cand . These insects were found in wilt infected field corn. The field from which the specimens were taken had been in sod for a number of years and consequently was well infested with wireworms. On cutting across the stem

of corn plants injured by wireworms bacteria oozed out in a slimy yellow mass from the vascular bundles, while only a few uninjured plants showed bacterial wilt. Some of these worms were collected, brought into the laboratory and placed on agar plates of Ivanoff's (5) selective medium where they were allowed to crawl about for a few minutes. In a few days colonies of P. stewarti were in evidence and proved to be pathogenic when inoculated into corn plants. Of the inoculated plants 78 percent showed wilting while the controls showed only one or two percent of wilt. Isolations were made from the alimentary tract of a few of the wireworms but in no case could P. stewarti be recovered. After allowing the wireworms to crawl about on the agar plates, they were removed and allowed to feed upon corn plants in pots of sterilized soil. Wilting of the plants followed soon after injury by feeding of the wireworms. Isolations made from the wilted plants showed the presence of the bacterial wilt organism. Every plant that was injured by the wireworms wilted and wilting always followed injury as long as they were present. When the wireworms were left in pots for two weeks in the absence of corn plants, the wilt organism could no longer be isolated from them. In this case they probably acted merely as mechanical carriers of inoculum. Wireworms are of minor importance in the field because they are never prevalent in large enough numbers to cause severe loss unless the soil is soddy. Because they do not

harbor the causal bacterium for any length of time, the inoculum would have to be present in an infected plant before wireworms could act as agents of dissemination.

Rand and Cash (15) were the first workers to definitely prove that insects were associated with the transmission of bacterial wilt. They were able to transmit the disease from diseased to healthy plants by transferring two species of flea beetle, Chaetocnema pulicaria Horn. and C. denticulata Horn., and the twelve-spotted cucumber beetle, Diabrotica duodecimpunctata Oliv. to leaves of the corn plant. Poos and Elliott (12) isolated cultures of P. stewarti from the flea beetle Chaetocnema pulicaria Horn. early in the spring before there were any corn plants that could have furnished the inoculum. This is the only proven case of an insect carrying the organism over winter, although there may be other similar insects which harbor P. stewarti. The infected flea beetles produced the disease when allowed to feed on leaves of healthy plants. There is little evidence that any great amount of inoculum can live over winter in the soil, and it is almost certain that the organism would not live in the soil for as long as two or more years.

From evidence at hand and from that of the above workers it can be concluded that insects play an important role in the dissemination of the disease. However, there is also evidence that the wilt producing organism is carried in the

seed and in the absence of both insects and soil infestation will cause a small amount of wilt. Smith (24), Rand and Cash (16), and Ivanoff (5) were able to isolate the organism from the seed, and it also has been repeatedly done by the writer at this station.

Wilt produced by organisms associated with P. stewarti

During the progress of the various experiments there were found two parasitic organisms that are sometimes associated with P. stewarti. One of them is a species of Fusarium and the other is a white bacterial organism producing glistening white colonies on potato agar and believed to be closely related physiologically to Phytomonas dissolvens Rosen. Both of these organisms were found to be parasitic and to play a part in the appearance of Stewart's disease under some conditions.

The species of Fusarium causing a foot rot of corn may be but is not necessarily associated with P. stewarti. This Fusarium in the wilt disease is probably not an important factor for the reason that it is so virulently parasitic, that the effects of the wilt bacterium are overshadowed by those of the fungus. Many of the plants attacked by this Fusarium were killed before they reached the soil surface. Those that were attacked after they were several inches tall wilted with somewhat different symptoms than those of Stewart's disease. The plants became pale yellow in color with the absence of a water

soaked appearance. The fungus could be isolated from wilted plants along with Stewart's organism, and the two together in culture would often be closely associated, the bacterium growing along the fungus hyphae. However, but very little loss from bacterial wilt was caused by this combination as the Fusarium is a more virulent pathogen than P. stewarti. In none of the specimens could the wilting which somewhat resembled Stewart's disease be attributed to the bacterial wilt organism. In this case P. stewarti seemed to follow rather than to be the direct causal agent in wilting.

It was found in a previous phase of the work that both P. stewarti and this white bacterium could be isolated from infected kernels. They could also be isolated from diseased plants. This was particularly true in the controls of Table V all of which yielded both organisms upon isolation. It is especially to be noted that none of the wilted plants in the controls contain P. stewarti alone, and that many of the wilted plants in the punctured seedlings contained P. stewarti alone. In Table VI is recorded the results of isolations from corresonding lots of seed as used in Table V.

Table VI

Organisms present as determined by isolation in lots of seed corresponding to those of Table V.

Isolations	number of kernels	number with P. stewarti	number with white organism	percent P. stewarti	percent white organism
1.	220	57	24	25.9	10.9
2.	180	41	13	22.9	7.2
3.	272	63	15	23.2	5.5
4.	260	31	23	11.9	8.8
5.	204	24	4	11.8	2.0
6.	215	33	19	15.3	8.8
7.	271	62	20	22.9	7.4
8.	231	37	17	16.0	7.3

The white bacterial organism plays a very important role in the production of the disease from infected seed when other factors are absent. It is capable of causing a rotting of the seed and the basal portions of the plant and producing wilt symptoms in the leaves. This organism alone is not highly pathogenic but causes a basal rotting with some leaf symptoms as shown in plate III. After the adventitious roots are formed the infected plants seem to take on new vigor and resume growth, although the organism is in the plant tissue as determined by isolations. This organism is often found associated with P. stewarti in the seed and plant tissue although this is not necessarily the case. When making isolations the two organisms may be recoveredin a mixed culture and are sometimes difficult to separate on plating. Stanley and Orton (27) reported the presence of a white organism when isolations were made for P. stewarti. It is possible that the organism reported by them

and identified as P. dissolvens Rosen may be the same bacterium as the one found in these investigations.

Inoculations were made with pure cultures of the white organism by hypodermic injections into the base of seedlings grown in sterilized soil in the greenhouse, and it was re-isolated both from the leaves and stems of the infected plants. The organism travels up the vascular bundles as is the case with P. stewarti and the symptoms are manifested within a few days. This pathogen seems to be more destructive in cloudy weather at relatively high temperatures. This is probably due to the slower growth of the plant in cloudy weather and the rapid growth of the organism at relatively high temperatures, 25 to 30 degree Centigrade. The peculiar leaf rolling and water soaking of the central leaves as shown in Plate III are characteristic symptoms produced by this organism, although P. stewarti may also be present in plants showing these symptoms. P. stewarti alone may produce symptoms somewhat similar to these.

There was no attempt made to identify or classify this organism other than to note the symptoms it produced on corn and to determine some of its physiological properties on culture media. It grows as a white, glistening, round colony on potato dextrose agar. In this medium the colonies are raised, smooth, convex, and opaque. Under oil immersion in a

hanging drop there appeared to be both motile and non-motile forms. The organism is a short rod often occurring in chains; it forms acid and gas in dextrose, galactose, mannit, sucrose, maltose, lactose, and raffinose; the bacterium made only a fair growth in gelatin and caused only slight liquefaction. Growth is cloudy in potato dextrose broth producing a scummy pellicle around the top of the tube. It precipitates slowly and stains readily with carbol fuchsin.

It will be noted that the percentage of kernels showing the white bacterial organism in Table VI corresponds closely to the percentages of wilted plants which appeared in the control plants as shown in Table V. The question arose as to the part played by this organism in the wilted plants of the controls. The answers that presented themselves were two, (1) that this organism could induce a type of wilt similar to that caused by P. stewarti, and (2) that this organism was held within the seed with P. stewarti and at the time of germination was able to cause a rotting of the embryo tissue allowing P. stewarti to gain entrance into the young plant. Results of inoculation tests as indicated above proved the organism to be parasitic, producing on the corn plant symptoms similar to those induced by P. stewarti.

To determine the effect of this white bacterium when associated with P. stewarti, pathogenicity tests were made of the organism alone and in combination. Apparently clean corn

seed that had been soaked in water was divided into two lots and treated as follows: (1) the seed coats were broken in several places by scratching with a needle on the opposite side from the embryo, and (2) the seed coats were left unbroken. Each lot of seed was separated into three groups of 200 kernels each. One group was soaked in a broth culture of the white organism, another group was soaked in a broth culture of P. stewarti, and the third group was soaked in a mixture of broth cultures of the two organisms. Both lots received the same treatment. The results are striking. In only two groups were there any wilted plants, both of which occurred in groups where the seed coat had been broken. One had been soaked in a broth culture of the white organism alone, and the other had been soaked in a mixture of broth cultures of the white organism and P. stewarti. No wilted plants resulted in those groups in which the seed coat was left unbroken and P. stewarti was not able to infect after the seed coat had been broken. The results are given in Table VII.

Table VII

Results of inoculations with P. stewarti, the white bacterium associated with it, and a mixture of the two organisms into which corn kernels with broken and unbroken seed coats were soaked.

Inoculum	Kernels with broken seed coats		Kernels with unbroken seeds coats	
	plants wilted		plants wilted	
	number	percent	number	percent
White organism	121	60.5	0	0
P. stewarti	0	0	0	0
Both organisms	167	83.5	0	0

Neither of the organisms were able to penetrate through the seed coat either alone or together. The white bacterium was able to penetrate the tissues of the young seedling at germination and produce wilt after the seed coat was broken, but P. stewarti alone was not able to penetrate even after the seed coat was broken. Both organisms could be isolated from the wilted plants which resulted after the seed with broken coats had been soaked in a mixture of broth cultures of the two organisms. This is evidence that P. stewarti does not distintegrate living tissue to any extent and is not able to migrate from the chalazal region into the embryo and vascular tissue of the seedings. The white organism apparently is able to play a double role. It can infect the plant alone if present in the seed causing wilting and in some cases death, and it can attack and rot away the tissue allowing P. stewarti to gain entrance into the vascular tissue where the wilt organism causes more necrotic and systemic conditions than does the white bacterium. Plates I, II, and III show different types of wilting as caused by these two organisms.

Anatomy of corn in relation to wilt.

From observation in the field and from published results of other workers some varieties are more resistant directly or indirectly than others, and the suggestion was made that the number of vascular bundles might be an influenceing factor in resistance. Experiments were therefore conducted to determine

if possible the relation between the number of vascular bundles of corn plants and the amount of wilt found in these varieties.

Both healthy mature plants and those showing symptoms of Stewart's disease of five different varieties were collected and the bundles counted at the second internode above the crown. Free hand sections were made of the entire cross section. To more clearly differentiate the vascular bundles, the sections were treated with phloroglucinol and a drop of 25 percent hydrochloric acid which gave them a pink color that showed very well under the binocular microscope. The cross sections were suspended from a slide which was mounted on another with small strips of wood separating the two. This allowed enough space between the slides for the sections which were about one-half millimeter in thickness. The moisture on the sections caused them to adhere to the top slide which was charted in squares about two millimeters square with a diamond point pencil. The five varieties of corn selected were extreme in that some showed great susceptibility and others showed high resistance. The number of bundles, area in square millimeters, and average number of bundles per square millimeter of cross section are given in Table VIII.

Table VIII

The number of bundles, the area in square millimeters, the average number of bundles per square millimeter for both healthy and wilted corn plants of five different varieties.

	Healthy			Wilted		
Variety used	number bundles	area in sq. mm.	ave. number bundles per sq. mm	number bundles	area in sq. mm.	ave. number bundles per sq. mm.
1593	472	346.37	1.37	365	78.54	4.65
"	462	298.64	1.55	416	148.25	2.8
"	566	330.05	1.71	414	113.09	3.66
"	507	346.36	1.46	506	275.8	1.83
"	408	247.18	1.65			
"	458	290.72	1.58			
"	376	220.07	1.71			
"	405	201.06	2.01			
"	396	201.06	1.97			
"	430	258.43	1.66			
Average of all	448	277.99	1.61	425.25	153.92	2.76
1588	410	254.47	1.61	523	320.73	1.63
"	454	247.14	1.84	508	261.28	1.94
"	435	240.52	1.81	489	268.79	1.82
"	434	247.14	1.75	479	247.14	1.94
"	406	207.13	1.96	493	240.52	2.05
"	521	261.28	1.99	441	153.93	2.86
"	441	220.1	2.0	496	298.64	1.66
"	476	336.53	1.41	469	240.52	1.95
"				405	233.42	1.75
"				472	296.78	1.59
Average of all	447.12	256.18	1.77	477.5	251.79	1.86
51xG.B.	491	233.42	2.1	536	254.47	2.1
"	510	330.05	1.54	490	254.47	1.92
"	598	283.53	2.11	554	298.64	1.86
"	564	272.88	2.06	503	213.82	2.35
"	559	293.77	1.90	570	317.3	1.79
"	499	251.64	1.98	457	254.47	1.79
"	509	287.71	1.77	600	309.13	1.94
"	553	286.51	1.93	394	265.91	1.48
"	478	306.02	1.56	610	151.74	4.02
"	494	324.93	1.52	507	208.66	2.43
Average of all	475.5	287.04	1.65	522.1	252.86	2.06

Table VIII (continued)

	Healthy			Wilted		
Varity used	number bundles	area in sq. mm.	ave. number bundles per sq. mm	number bundles	area in sq. mm.	area. number bundles per sq. mm.
1594	519	337.81	1.53	489	261.28	1.87
"	631	388.46	1.62	504	268.79	1.88
"	540	306.02	1.76	454	298.64	1.52
"	566	268.79	2.11	460	240.52	1.91
"	476	314.16	1.52	408	240.52	1.69
"	531	298.64	1.78	512	330.05	1.55
"	410	275.8	1.48	421	128.68	3.27
"	437	314.16	1.39			
"	514	420.53	1.22			
"	618	419.09	1.47			
Average of all	524.2	334.34	1.57	464	252.64	1.84
2x1339	540	406.11	1.33	573	170.62	3.35
"	566	380.13	1.49	555	233.42	2.38
"	528	314.16	1.68	637	226.98	2.80
"	565	388.45	1.45	541	283.53	1.91
"	587	380.13	1.54	508	226.98	2.24
"	560	367.78	1.52			
"	466	311.01	1.49			
"	555	433.73	1.28			
"	494	448.62	1.10			
"	573	347.68	1.64			
Average of all	543.4	377.78	1.44	562.8	228.31	2.46

Too little research has been done on the anatomy of the corn plant to substantiate any hypothesis relating the number of vascular bundles to resistance. It is known that the number of vascular bundles in a corn plant besides being dependent on hereditary factors are also dependent on the number of leaves that develop. The number of leaves in turn are likewise dependent on inherent factors, two facts which

mean that the total number of bundles within a corn stem at any one place is quite variable. It will be noted in Table VIII that the number of bundles per square millimeter in the wilted stems is greater than that in the healthy stems, but it will also be noted that the total cross section area is smaller in the wilted plant stems than in the healthy stems. This probably means that the total size of the stem was stunted due to the disease, and the number of vascular bundles being more or less fixed developed the same as in the healthy stem, thus crowding the same number of bundles together in a smaller stem.

There is one possibility that the number of vascular bundles might influence the resistance which a variety might exert. The number of bundles in a corn stem is dependent upon the number of leaves and the number of leaves is dependent upon the time of tassel formation. There are no more leaves formed after the tassel begins to develop and as the total number of vascular bundles depends upon the number of leaves, the date of development of the tassel would influence the total number of vascular bundles. However, there is so little known about this subject that definite conclusions cannot be drawn.

There is a large field open in which to work on the subject of resistance in corn to Stewart's disease. It is of course difficult to determine the factor in the host that makes it resistant unless it is known definitely just what

factors, if any, other than the pathogen influence the development of the disease. If insects are the main disseminating agents, then a variety would undoubtedly have to be resistant to their attack as well as the action of the parasite itself. For practical purposes though the common methods of plants breeding are the quickest for yielding a remedy.

Control

There are no known methods of controlling this disease other than the use of resistant varieties. Smith's (26) Golden Cross Bantam has proven resistant in the field in other states. Clinton and Singleton (1) found strains of Whipple to be quite resistant. And Ivanoff and Riker (8) report several strains of sweet corn that were resistant to both artificial and natural inoculation in both the field and greenhouse. The use of these varieties has probably saved considerable loss in some sections.

Control by seed treatment has been found to be ineffective. As early as 1909 Smith (23) reported results of experiments in which mercuric chloride seed treatment seemed to control bacterial wilt to some extent. Later (24) he found the bacterial organism within the kernel and expressed doubt as to the effectiveness of this treatment for the internal disinfection of the kernel. At the Michigan Station, the

writer in cooperation with Dr. J. H. Muncie tried out under field conditions in 1932 and 1933 various seed treatments none of which proved effective. These treatments were mercuric chloride 1:1000, Semesan jr. as a dust treatment, several phenol compounds, Hexylresorcinol of varying concentrations, a chlorine dust compound, and dry heat at 60° C. for one hour. The results were based on percentage of wilted plants in the treated plots in comparison to those found in the controls.

In the winter of 1934 and 1935, a series of isolations was made from the interior of kernels from wilted plants. The results showed that the organism was recovered in fewer cases and that the percentage of infected kernels decreased in the same lot of corn when 95 percent alcohol was used to remove the air before the kernels were surfaced sterilized with mercuric chloride. Accordingly, four replications of seed treatments were carried on (1) using 95 percent alcohol soak for 15 minutes followed by a 15 minute soak in 1:1000 mercuric chloride, (2) mercuric chloride 1:1000 alone as a soak for 15 minutes, and (3) untreated controls. It is obvious that for the controls it was impossible to have seed surface sterilized without using some disinfectant. This was accomplished by washing for long periods of time in sterile water and by repeated rinsing for short periods of not more than 30 seconds in alcohol and repeated washing in

sterile water. The number and percent of kernels infected with P. stewarti and an associated white bacterial organism are shown in Table IX.

Table IX

Total number of kernels treated and those from which P. stewarti and the associated bacterium could be isolated after treatment with alcohol, mercuric chloride, and from those untreated.

Treatment used		number treated	number healthy	number with P. stewarti	number with white organism	percent P. stewarti	percent white organism
Trial treated							
I	(a)	204	204	0	0	0	0
	(b)	220	218	1	1	0.5	0.5
	(c)	136	121	6	3	4.4	2.2
II	(a)	228	204	5	2	2.2	0.9
	(b)	216	188	15	9	6.9	4.2
	(c)	332	200	81	31	24.4	9.4
III	(a)	360	327	12	21	3.3	5.8
	(b)	340	277	39	24	11.5	7.0
	(c)	300	184	69	47	23	15.6
IV	(a)	280	260	13	7	4.6	2.5
	(b)	312	273	25	14	8	4.5
	(c)	380	250	91	39	23.9	12.6
V	(a)	268	248.7	7.5	7.5	2.52	2.3
	(b)	272	239	20	12	6.72	4.05
	(c)	287	188.7	61.7	30	18.95	9.95

V. represents an average of all.
(a) represents ~~that~~ treatment of 95 percent alcohol followed by mercuric chloride 1:1000.
(b) represents treatment with mercuric chloride alone.
(c) represents untreated controls.

It will be noted that the first lot of seed treated showed but small percentages of wilt even in the controls. This was a Golden Bantam variety which had shown very little wilt in the field and came from relatively clean plants.

The evidence at hand and that by other workers, of insect dissemination of Stewart's disease, and the evidence that infected seed alone as an agent of dissemination does not cause a high percentage of wilted plants, indicates that seed disinfection although completely effective would not control the disease under most conditions. However, the fact that the organism is carried within the seed and, therefore affords a source of inoculum to insect agents, makes desirable some seed treatment that will sterilize the interior of the seed without injuring the embryo. The results of seed disinfection in the beginning of these investigations are probably in error due to the fact that the insect agents of dissemination at that time were not considered nor controlled.

The results show that the treatments employed are not effective in disinfecting the interior of the kernel although the alcohol and the mercuric chloride treatment reduced the number of infected seeds somewhat. P. stewarti was recovered from a rather high percentage of the kernels in the controls as shown in Table IX. It is possible by isolations to determine that the percentage of infected kernels within a variety is higher than the percentage of wilted plants that will be produced when the same kernels are planted.

Discussion

Stewart's disease caused by P. stewarti (Smith) com.

S. A. B. is a disease of corn plants and in general causes a wilting of the entire plant at any time from the seedling stage until the plants are nearly mature. The bacterial organism is a vascular parasite and in systemic cases of wilt plugs up the xylem vessels from the base of the plant causing a deficiency of water and subsequent wilting. Depending on the region of the plant inoculated either naturally or artificially, local or systemic conditions may result. If a young susceptible plant is inoculated at the crown in such a manner that a large number of bundles become infected with a virulent culture of P. stewarti, complete wilting will occur in a very short time, However, if only a small portion of the bundles is infected, local lesions and wilting will occur and the plant may survive and produce more or less normal ears. Sometimes the ears and kernels are small and deformed with the ear poorly filled on a plant that shows quite general systemic cymptoms but that has survived the disease until it reached maturity.

The conditions and factors influencing outbreaks of Stewart's disease are undoubtedly many, some of which are quite well known. It is believed by most investigators that high temperature, abundant soil moisture, and high humidity favor the development of wilt. The conditions that influence the development of the disease other than weather are: (1) the presence of the pathogen, (2) the resistance of the host to

the pathogen, (3) infected seed, (4) insect carriers, and (5) other organisms associated with the pathogen.

The pathogen causing Stewart's disease is present in, and can be isolated from, kernels produced on diseased plants. According to Ivanoff (5) it can be isolated from old infected corn stalks and from the soil. Poos and Elliott (12) found it present in the alimentary tract of the flea beetle Chaetocnema pulicaria Horn., and the writer was able to isolate it from the alimentary tract of the seed-corn maggot Hylemyia cilicrura Meigen. So the sources of inoculum are numerous enough to provide infection when conditions are favorable.

There have been a number of varieties of corn produced which have shown resistance to bacterial wilt. Smith (26) produced the variety Golden Cross Bantam which has stood up well in the field in Indiana, New York, and other states. This variety is very susceptible to artificial inoculation and has shown little promise of resistance in Michigan. In the summer of 1934 Golden Cross Bantam was the only variety in any of the College plots that showed 100 percent wilt. The Whipple variety developed by Clinton and Singleton (1) seems to be quite tolerant to the disease in Michigan but not highly resistant to artificial inoculation. Ivanoff (8) has produced some inbreds and hybrids which stood up well under artificial inoculations both in the field and greenhouse. These strains have not been tested in Michigan.

Phytomonas stewarti has been found in the seed of wilted plants since the discovery of the disease, Smith (24) and Ivanoff (5) were able to clearly demonstrate the presence of the bacterium in the chalazal region. These findings have also been corroborated by the writer. The pathogen is easily isolated from infected kernels and some lots of seed have shown as high as 25 percent infection. The organism is carried over winter in this manner and affords a source of inoculum when the seeds are germinated. In no case when insects were controlled was the percentage of wilt caused by infected seed high. It was found that puncturing through the chalazal region into the base of the epicotyl at germination would greatly increase wilt if the organism was present in the seed. The percent of wilt in these punctured seedlings was much greater than in the controls. This indicates that the organism is fixed within the endosperm of the seed and is freed upon injury as might occur by the larvae of insects feeding within the kernel.

As there is no evidence that the pathogen is harbored in the soil for any length of time, it must be present from some other source to cause infection. The two known sources of the organism are the seed and insects. Poos and Elliott(12) isolated the organism from the adults of the flea beetle. Chaetocnema pulicaria Horn. early in the spring before these

insects could have obtained the parasite by feeding in infected host plants. This is definite proof that the wilt bacterium may be carried over winter by insects. There is some doubt as to the amount of wilt infection produced by leaf and stem feeding insects. Using a pathogen that reacted very virulently when inoculated into the crown of corn plants, the leaves were inoculated by spraying on a broth culture followed by pin pricks and by direct injection into the mid-rib with a fine pointed hypodermic needle. In none of the plants was there a single case of systemic wilt. Local lesions varying in size would form but always toward the tip of the leaf from the point of inoculation. It was impossible to isolate the organism from a part of the leaf below the point of inoculation and it was quite easy to isolate from the region above or toward the tip from this point. This would indicate that the pathogen will travel up the vascular tissue but is not able to pass downward. This point requires further study. In all probability the larva of these insects play a more important role as an agent of inoculum inoculation than do the adults as they feed near the base of the plant and would account for more plugging of the vascular tissue by the bacterial wilt organism in this manner. There is evidence that several other species of insects transmit the disease from plant to plant in the field. Rand and Cash (16) found the adult of the two flea beetles, Chaetocnema pulicaria and C.

denticulata, and the larval stage of the twelve-spotted cucumber beetle, Diabrotica duodecimpunctata Oliv. to disseminate wilt in the field. Ivanoff (4) transmitted the disease from wilted to healthy plants with the western corn root worm, larval stage of Diabrotica longicornis Say., and states that the wilt bacterium may gain entrance into the roots of corn plants through wounds made by the white grub, Phyllphaga sp. In this work the larval stage of the seed-corn maggot, Hylemyia cilicrura Meign. was found associated with wilted plants and cultures of P. stewarti could be isolated from the alimentary tract. The wheat wireworm, larval stage of Agriotes mancus Cand. was also found to harbor the wilt organism on its surface for a short period of time. Both of these insects were capable of transmitting wilt, the wireworm mechanically, and the seed-corn maggot by carrying the organism internally.

Two other organisms found associated with P. stewarti were parasitic on corn plants. A species of Fusarium causing a foot rot of corn was accompanied by P. stewarti and the two grew together in culture. A white bacterial organism was found associated with P. stewarti in the seed and in wilted plants. Its presence was essential for Stewart's organism to gain entrance into the plant from infected seed, and it was able, in pure culture to cause wilt symptoms similar to those caused by P. stewarti. This bacterium is not highly parasitic and does not as a rule cause necrotic conditions.

Ivanoff (4) also mentions organisms other than P. stewarti that would produce symptoms in corn plants similar to those of bacterial wilt.

Summary and Conclusions

1. Inoculation tests were made on a large number of inbred, hybrid, and commercial varieties of sweet corn. None of these strains showed marked resistance. Hypodermic injections of a broth culture of the pathogen at the base of the plant was found to be the best method of producing wilt artificially. Strains of corn immune to Stewart's disease will be relatively difficult to obtain.

2. Leaf inoculation by hypodermic injections and the needle prick methods failed to develop systemic infection of bacterial wilt.

3. P. stewarti was found not to be carried in the soil for any great length of time.

4. Infected seed grown in sterilized soil in the absence of insects produced very little wilt. Alone in infected seed P. stewarti will not cause the disease to develop unless other agencies are present which will carry the inoculum into the young plant.

5. P. stewarti was found to be fixed within the endosperm of the kernel and could not penetrate into the seedling at germination unless aided by injury. Insects and associated organisms will produce an avenue of entrance through

the embryo and into the vascular tissue of the young seedling.

6. The larvae of the seed-corn maggot Hylemyia cilicrura Meigen. carries P. stewarti in its alimentary tract. The wheat wireworm, larval stage of Agriotes mancus Cand. transmitted the disease from one plant to another. Insects were the chief agents of dissemination of the disease in the field.

7. Organisms associated with P. stewarti were studied. One, a species of Fusarium was the leading parasite with P. stewarti secondary. The other a white bacterium was secondary in parasitic activity but its presence was essential before P. stewarti could gain entrance into the seedling from infected seed.

8. Alcohol as a pre-soak for mercuric chloride helped reduce the percentage on internally infected seed. Seed treatment practices at present are ineffective as control measures.

9. Certain varieties of sweet corn showing field resistance in many localities showed a high percentage of bacterial wilt under Michigan conditions. Some of these varieties have not been tested at this Station. However, the main methods of control at the present time are through the use of resistant varieties.

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Explanation of plates

Plate I --- Plants showing systemic infection after inoculation with a pure culture of *P. stewarti*.

Plate II --- Plant showing the leaves sticking together due to bacteria oozing from the leaf surface. *P. stewarti* and the white bacterium are often found together in plants showing symptoms of this type.

Plate III --- Healthy plants inoculated with a pure culture of the white bacterium. Note the center leaf rolled up tightly. This is a very characteristic symptom produced by this organism.

Plate IV --- Plants grown in sterilized soil and in the absence of insects. Wilt produced by puncturing through the chalazal region at the base of the kernel with a fine needle.

Plate I

Plate II

Plate III

Plate IV