UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 828

Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER May 10, 1920

BACTERIAL WILT OF CUCURBITS

By

FREDERICK V. RAND, Assistant Pathologist, and ELLA M. A. ENLOWS, formerly Assistant Pathologist
Laboratory of Plant Pathology

CONTENTS

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CONTENTS.

FOREWORD.

The studies of the writers on bacterial wilt of cucurbits (caused by *Bacillus tracheiphilus* Erwin F. Smith), published in 1915 and in the spring of 1916, have been continued during the past three seasons. The previously published work[1] demonstrated that the striped cucumber beetle (*Diabrotica vittata* Fab.) and also the 12-spotted cucumber beetle (*D. duodecimpunctata* Oliv.) are the most active and probably the only summer carriers of this bacterial wilt in the localities investigated. Some evidence was given that the striped cucumber beetle may be also a winter carrier.[2] It was shown that the soil had no relation to the disease either as a source of early spring or of summer infection. Seed from wilted plants in all cases failed to transmit the disease, and all attempts at infection through the stomata gave negative results. A fair degree of control was obtained by early treatments with Bordeaux mixture and lead arsenate combined, and it was shown that this control was related not only to the repellent and insecticidal qualities of the mixture, but also to a direct bactericidal action upon the wilt organism.

The present paper deals with further studies of the relation of soil and insects to the distribution and control of the disease, and includes also a portion of our work with the causal organism itself.

[1] Rand, F. V. Dissemination of bacterial wilt of cucurbits. (Preliminary note.) *In* Jour. Agr. Research, v. 5, no. 6, p. 257-260, pl. 24. 1915.

Rand, F. V., and Enlows, Ella M. A. Transmission and control of bacterial wilt of cucurbits. *In* Jour. Agr. Research, v. 6, no. 11, pp. 417-434, 3 figs., pl. 53-54. 1916.

[2] Other species of Diabrotica have not been tested.

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SUMMER TRANSMISSION OF WILT.

DIRECT INSECT TRANSMISSION EXPERIMENTS.

The earlier experiments on insect transfer of the wilt disease have been repeated many times during the past three years (1916–1918) with results similar to those already published. As an example of the method used the following details may be given:

September 17, 1916. Twelve squash bugs (*Anasa tristis* De G.) were fed four days upon cucumber plants wilted from pure culture inoculation. Two bugs were then caged (Pl. I) with each of six healthy cucumber plants for two days, during which time they were all observed to have fed. The plants at this time had become slightly flabby as a direct result of the insect feeding. However, this condition practically always results from squash-bug injury to young cucumber plants and is in no way connected with bacterial wilt. Cultures and microscopical examination in such cases fail to show bacteria present; the flabbiness often begins to appear within a few hours after the bugs start feeding, while wilt at the very earliest does not appear sooner than three to five days after inoculation; this flabbiness at once affects the plant as a whole, while in the bacterial disease the wilting is progressive from the point of inoculation; and finally, unless too far gone, plants always recover their turgor after the squash bugs are removed, while vigorous young cucumber plants inoculated with virulent strains of bacterial wilt have never been known to recover. In this experiment the squash bugs were all removed after two days and the plants soon regained their turgor. Although under observation until October 13, no wilt developed. This experiment was repeated many times with like results.

The squash lady bird (*Epilachna borealis* Fab.) was tested in 10 or more further sets of experiments, but no wilt ever followed its injuries to healthy cucumber plants, and negative results were obtained by inoculation with intestines of wilt-fed individuals (see p. 24).

The cotton aphis (*Aphis gossypii* Glov.) and the potato flea-beetle (*Epitrix cucumeris* Harr.) were also retested by similar methods and the negative results confirmed by observations in the experimental fields. Furthermore, in the field cage experiments they have always had access through the wire netting to the cucurbit plants within, but have never carried the disease from the numerous wilt cases in the surrounding field. These observations corroborate the results of the direct experiments.

During August, 1917, honeybees (*Apis mellifera* L.) were collected at random from the experimental cucurbit field where wilt was prevalent, and many were taken directly from blossoms of wilting vines. One to several bees were placed in 15 large cages containing cucumber or cantaloupe vines (Pl. II, fig. 1). No wilt followed in any case. It might be noted in passing that the cucumber fruit set very much more freely in these cages than where bees were excluded.

These five different species have all given constantly negative results, while the striped and 12-spotted cucumber beetles tested in the same way have repeatedly given positive results. For example, using the striped beetle in 10 direct experiments (1916 and 1917) similar to the one detailed above for the squash bug, 7 out of the 10 tests gave positive results. In these cases the beetles were fed upon wilted leaves and then caged with cucumber plants for a sufficient length of time to determine the result. These experiments were entirely separate from the successive infection experiments detailed later (cf. p. 21, 22). The 12-spotted cucumber beetles have not been tested so fully, but sufficient work has been done to show that they are also capable of direct wilt transmission.

TRANSMISSION FROM RANDOM COLLECTIONS OF BEETLES.

Collections of striped cucumber beetles and 12-spotted cucumber beetles taken at random in the fields have given widely varying results as to wilt transmission according to time of year, prevalence of wilt, amount of territory devoted to cucurbits, and length of time the beetles had fed since attaining the adult stage. In eastern Long Island from the first collections of early spring only an occasional striped beetle has proved to be a wilt carrier. Later in the season some collections have shown a large percentage capable of spreading the disease. For example, on September 1, 1916, striped beetles were collected at random in a cucurbit field and several put into each of four beetle-proof cages containing healthy cucumber plants. Many of the vines in each of the cages contracted bacterial wilt, so that at least one of the beetles introduced into each cage must have been a wilt carrier. About two weeks previously six beetles collected in the same locality failed to give infection. In this locality late cucumbers constitute one of the main crops.

However, in the trucking sections around the District of Columbia late cucurbits are rather the exception, most of these crops being planted there in early spring. In that locality random collections from the field have given a much smaller percentage of infection. During the summer of 1917, at Tuxedo, Md., from five to seven large insect cages were kept constantly as storage cages for both species of cucumber beetles. Healthy plants grown in the cages and healthy potted cucumber plants brought frequently from the greenhouses at Washington, D. C., were kept in the cages as food for the beetles. Collections of beetles made at frequent intervals from fields where wilt was present were placed in these cages, but throughout the season only two cases of wilt occurred in these cages. In many instances the beetles were taken directly from wilting vines.

Twelve striped beetles, among the first of the season found, were collected May 20, 1918, in an early cymling field at Kenilworth, D. C. Careful search over the field failed to reveal any cases of wilt. These beetles were caged for four days with 10 cucumber seedlings, after which the plants were held for observation in a beetle-free cage. On June 4 two of the ten plants had wilted. Cultures were made and *Bacillus tracheiphilus* was isolated and tested by successful inoculations.

In a large collection of striped beetles made at Norfolk, Va., about October 1, 1916, wilt resulted in one out of several cages among which the beetles were distributed.

In most instances only a very small proportion of the cucumber beetles present in a field actually carry infection.

The truth of this statement is seen at once when one thinks of the large numbers of beetles often present in a field during their maximum prevalence. If any considerable proportion were carriers of wilt not a single plant could escape infection at some time during the season.

WILT CURVES VERSUS BEETLE CURVES.

In a preceding publication [1] by the writers a discussion was given of the records of striped-beetle prevalence versus wilt prevalence (1915) in three experimental fields at East Marion, Long Island (fig. 1, Fields I, II, and III). The graphs here reproduced (fig. 2) show that a definite relation existed between rise and fall in beetle and wilt curves under two sets of conditions in which the beetles, appearing at widely different dates, were the variable environmental factor. No

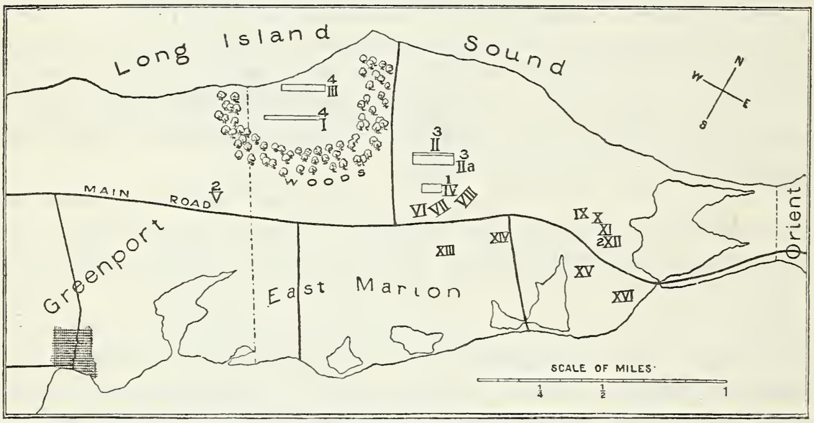

FIG. 1.—Map of northeastern Long Island, showing location of experimental fields I to IV and of wilt sequence records in fields I to XVI. The arabic numerals show the sequence of wilt appearance during the spring of 1916. (Map drawn by Wayland C. Brown, formerly of the Bureau of Plant Industry.)

direct relation to meteorological conditions could be found. In all three fields about one month intervened between the maximum prevalence of striped cucumber beetles and of bacterial wilt.

The wilt records of 1915 were made by counting at each date of observation all living cucumber plants infected with wilt and including not only all new cases but also any wilted plants that might have lived over from the time of the previous count. It was thought possible that by this method some cases might have been counted more than once, thus moving the maximum of the wilt curve forward too far. Therefore, during the following season (1916) in the same locality much more careful records were kept of both beetle and wilt prevalence in Fields II and IIa (figs. 1 and 3). At each date of beetle observation an area one-tenth the size of the whole field was measured off, and so far as possible all striped cucumber beetles in this area were

[1] Rand, F. V., and Enlows, Ella M. A., 1916. Op. cit., p. 420–423.

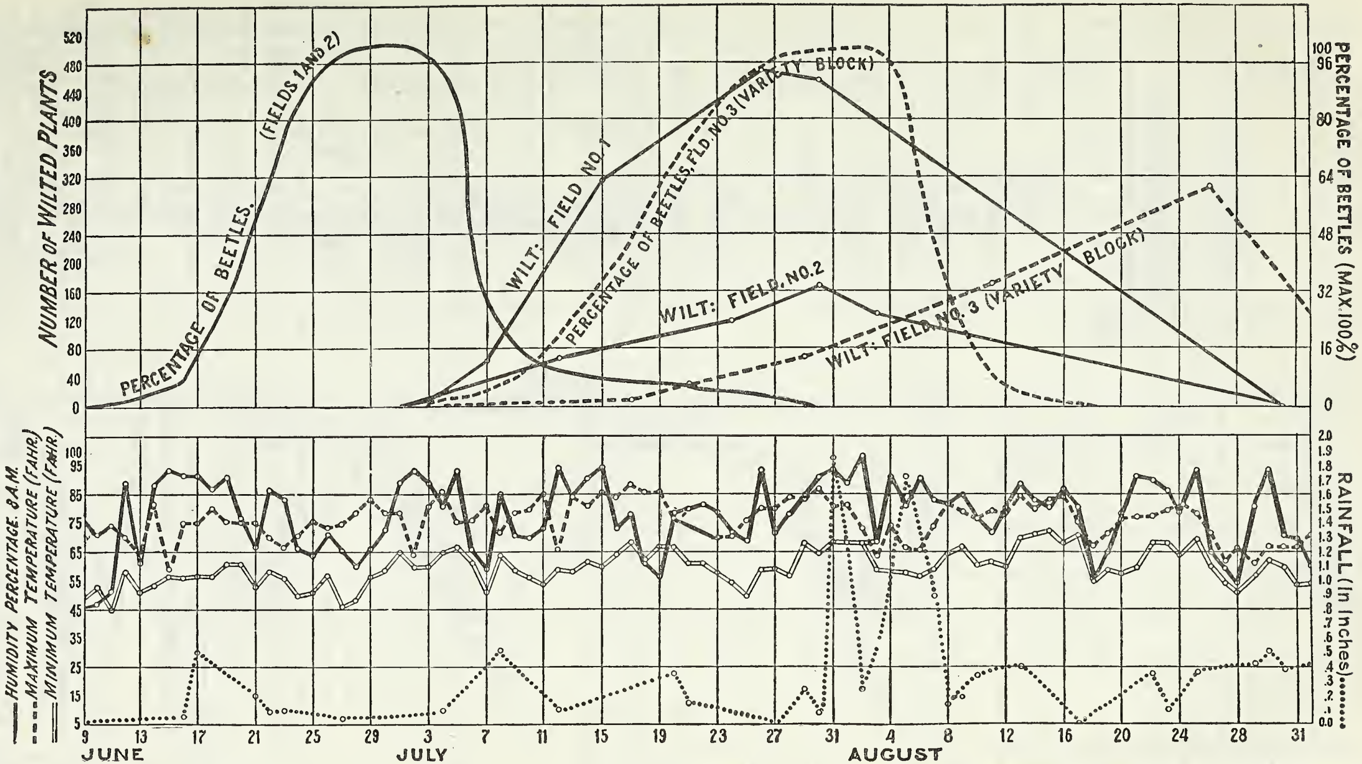

FIG. 2.—Comparison of the amount of wilt with the prevalence of the striped cucumber beetle and with meteorological phenomena in Fields I, II, and III, East Marion, Long Island, N. Y., season of 1915. (For the beetle curves, maximum prevalence is indicated at 100 per cent, regardless of the actual numbers present.)

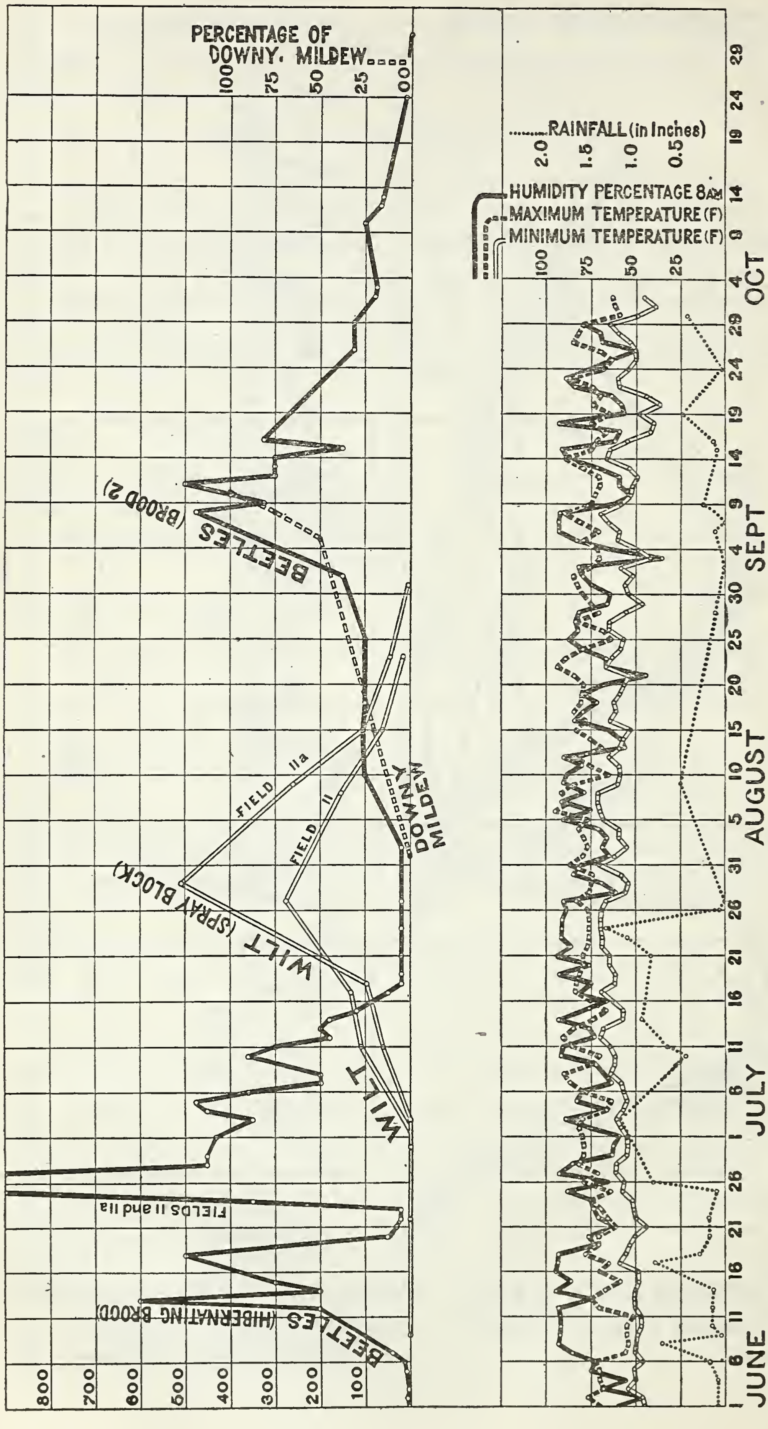

FIG. 3.—Comparison of the prevalence of wilt with that of striped cucumber beetles and with meteorological phenomena in Fields II and IIa, East Marion, Long Island, N. Y., season of 1916. (The downy-mildew curve is also shown. The beetle and wilt curves are based on actual numbers of beetles and cases of wilt present.)

counted. These figures were in each case multiplied by 10 to give the assumed actual number of beetles in the whole field at each date, and these totals were used in plotting the curves. Obviously it would be impossible to make these figures absolutely accurate, but the counts were carefully made, always by the same person, and were checked up by general observations over the field; and there is no doubt that the figures are comparable for purposes of plotting the curve.

In the case of the wilt records, each plant showing wilt was marked by a stake dated when the disease was first observed, and careful records were kept by row, hill, and plant, so that a wilted plant was counted once and only once. These records were as accurate as it was possible to make them.

During the summer of 1917, similar careful records were kept in the experimental field near Tuxedo, Md. The cucumber and cantaloupe spray blocks and the cucurbit variety block were all contained in one rectangular field of 1½ acres, and the beetle and wilt records cover this field as a whole. In the graphs (fig. 4) made from these figures the wilt is expressed in actual number of new cases at each date of observation, and the beetle prevalence is given in percentages, 100 per cent representing the maximum number of beetles for each brood.

Similar records also were kept (1916) of beetle and wilt prevalence in two experimental fields at Giesboro Point, D. C. (fig. 5; XI, XVI), with records over a part of the season in several other fields in the same locality; and records for two other fields at East Marion, Long Island, were made.

A detailed discussion of the beetle and wilt records follows:

In Fields I and II (figs. 1 and 2), East Marion, 1915, the striped-beetle curve shows that the maximum covers several days during the last of June and first of July, while the maxima of the corresponding wilt curves cover the last few days of July, almost one month later. In Field III, though planted only three days after Field I, the striped beetles were much later in appearing. Here the maximum of the beetle curve is about the first of August and the maximum of the wilt curve the last few days of August, almost a month later. Fields I and III were less than a quarter of a mile apart; in fact, both were a portion of one larger field, so that the meteorological conditions were similar, yet the wilt curves in the two cases were approximately a month apart. In Fields I and II the maxima of the wilt curves came just before the greatest rainfall of the season, while in Field III the reverse was true. No definite relation between the wilt and the temperature or rainfall could be detected in any case. Thus in these three fields wilt prevalence bore a clear and definite relation to striped-beetle prevalence rather than to weather or to time of planting.

At East Marion (L. I.), N. Y., in 1916 (figs. 1 and 3) the maximum of the striped-beetle curve for the first brood occurred about June 26, while for the corresponding Fields, II and IIa, the wilt-curve maximum occurred, respectively, around July 27 and 29, about one month later. As shown in the graph, downy mildew (*Pseudoperonospora cubensis*) appeared early in August and during the rest of the month, while the summer brood of striped beetles was making its appearance, gradually destroyed the vines in our experimental fields. No evidence of another brood was

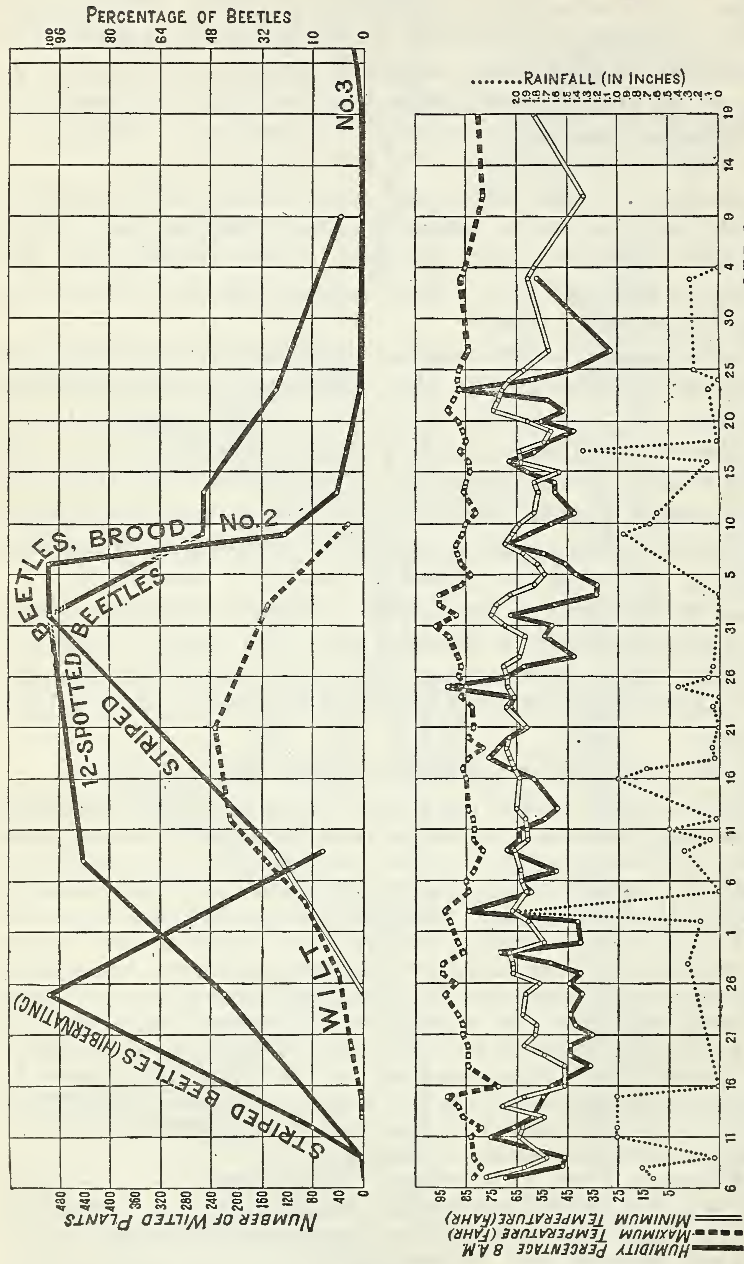

FIG. 4.—Comparison of the prevalence of wilt with that of striped cucumber beetles and with meteorological phenomena in an experimental field, Tuxedo, Md., 1917. (The curve of 12-spotted cucumber beetles is also shown. In all the beetle curves, maximum prevalence is indicated at 100 per cent, regardless of the actual numbers present.)

found in this locality. Referring to the beetle curve for the hibernating brood (fig. 3), it will be seen that on June 14 and again about June 21 there was a sudden drop in the number of striped beetles. On looking over the daily notes after plotting this curve, it was found that on June 13 the beetles were so numerous as to threaten total destruction to the young plants, and an application of a dust insecticide and lime mixture was made to all the plants in the field. Just before the second drop in the curve the first Bordeaux and lead-arsenate spray treatment of the season had been carried out, and in addition the same insecticide had been applied to all control plots. At the same time that the number of beetles decreased in Fields II and IIa a decided increase was noted in Field IV, about a quarter of a mile distant. In Fields II and IIa the maximum wilt came just after a period of heavy rain and high humidity, while in Field II and one other field during 1915 the maximum wilt came just before the period of heaviest rainfall and after a period of comparatively low humidity and rainfall. Again the relation between beetle and wilt curves holds, the one following the other at an interval of about one month.

During the same season (curve not plotted) records of the number of beetles and the amount of wilt were also kept for two other experimental fields in East Marion. In Field I (fig. 1) the maximum of the beetle curve occurred about June 28 and that of the wilt curve about August 1. In Field IV the maximum of the beetle curve occurred near June 26 and the wilt maximum about July 27.

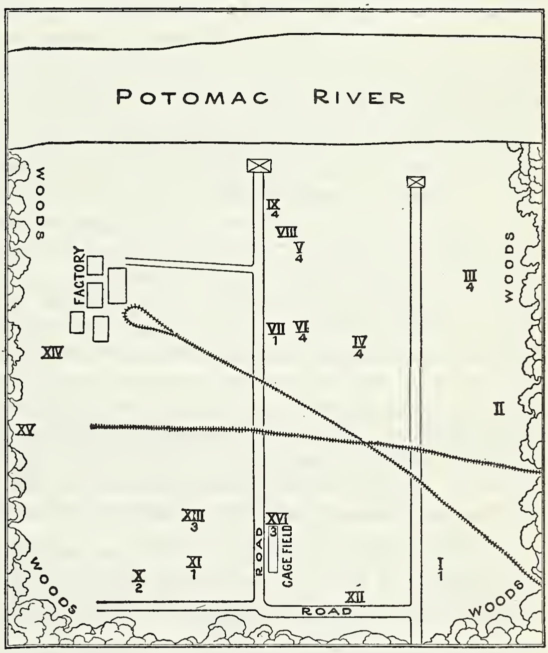

FIG. 5.—Map of a portion of Giesboro Point, D. C., where wilt-sequence records were kept during the spring of 1916. The roman numerals show the location of the fields; the arabic numerals show the sequence of the wilt.

During the season of 1916 the records at Giesboro Point, D. C. (fig. 5), showed this same relation. In Field XI, planted about April 25, the maximum of the first brood of beetles occurred June 12, while the maximum of wilt prevalence came exactly one month later. In another of our experimental fields (XVI) the maximum of the beetle curve came between June 15 and 20, while the corresponding wilt-curve maximum occurred about July 21. Here the first planting was made April 25, but only a few seeds came up and most of the vines were from a second planting made May 21. The month preceding the period of maximum wilt prevalence had been very dry, with only light showers, but this period was immediately followed by several days of heavy rain, during which time the downy mildew obtained a foothold, so that after July 28 no additional wilt records could be kept.

Partial records in this locality were also kept for about a dozen other fields of cucumbers, cantaloupes, and varieties of summer and winter squashes. While the data for complete graphs of both beetles and wilt are not available in these last cases, the portions of the curves made show the same relations as in the instances where complete records were kept. In general, the earlier planted fields developed their maximum wilt prevalence before the later planted fields, but with a few notable exceptions. Thus four out of six cucurbit fields planted between April 25 and May 1 developed their maximum wilt between June 23 and 30, while four out of five fields planted after the first of May (mostly between May 15 and 20) developed their maximum wilt about the middle of July. Of the exceptions, one field planted April 25 and one planted May 15, each gave their maximum wilt on July 5. Reference to the notes in these cases showed the striped-beetle maximum in both fields to have come early in June. Furthermore, one cucumber field planted April 20 to 25 had its maximum beetle prevalence June 12, and its maximum wilt came July 12; another cucumber field planted May 21, with beetle maximum between June 15 and 20, had its wilt maximum July 21. While wilt prevalence usually reached its maximum first in the earliest planted fields, there were these instances where it did not, and in these exceptional cases it was found the beetles had been late in making their appearance. Thus again the wilt prevalence has followed the striped-beetle prevalence rather than the time of planting or the weather conditions.

Finally, during the season of 1917, careful counts were kept of beetle and wilt prevalence (fig. 4) in our experimental field near Tuxedo, Md. Here the hibernating brood of striped cucumber beetles reached its maximum numbers June 25, while the wilt in this field of 1½ acres reached its greatest prevalence July 21. A period of heavy rains preceded and followed the time of maximum wilt prevalence, and rain was fairly well distributed throughout the season. About August 10, the cucumber plants reached maturity and died. The field was replanted, but only about one-fifth of the seeds germinated. The young of the summer brood of striped beetles fed freely on these scattered seedlings, but no wilt was found. The young of a fall brood of striped cucumber beetles began to appear about the middle of September, but at this time all our cucurbits were either ripe or dead, so that their relation to the wilt was not observed. No attempt was made to determine the number of broods of the 12-spotted cucumber beetles, but the curve shows their general prevalence throughout the season. Except for short periods between the broods of the striped beetles, *they were at no time present in as large numbers as the striped species.*

As will readily be seen from the preceding statements and from a comparison of the three sets of graphs (figs. 2 to 4), the wilt curves always bear a definite relation to the beetle curves, approximately one month occurring between the maxima of beetle and wilt curves. Wilt prevalence does not in these cases show any relation to time of planting, humidity, or rainfall, and little or none to temperature.

About one month appears in all these sets of curves between the maxima of beetle and of wilt prevalence. Yet cucumber plants *usually* wilt within less than two weeks after they are inoculated, and the results of a spray test (cf. p. 39) in one of the fields where these beetle and wilt records (fig. 3) were kept show that the greatest number of infections actually did take place within the preceding 2-weeks period. Why, then, are the maxima of the beetle and wilt curves so far apart? Collating all our experimental data and observations concerning beetle and wilt relations, the explanation appears to be that early in the spring only a very small percentage of the beetles are wilt carriers. As the season advances and larger and larger numbers of cucurbit plants become wilted a correspondingly larger and larger number of beetles have the opportunity to feed on diseased plants and so to become possible wilt carriers. Thus the maximum number of actual wilt carriers comes not simultaneously with the maximum beetle prevalence, but after that maximum; that is, at some time during the downward progress of the beetle-prevalence curve.

STOMATAL INFECTION.

Since the last publication on the subject by the writers[1] further attempts at stomatal infection have given uniformly negative results. Unless the epidermis is wounded and the wilt organism introduced, infection apparently never takes place.

SOIL INOCULATIONS.

As reported in a previous article,[2] the only cases in which we have been able to transmit wilt through the soil have been those where heavy tap-water suspensions of virulent bacteria had been poured directly around the roots of potted plants. In these cases sufficient water was used so that it would immediately percolate through the soil to the roots, and some of the plants were root pruned on one side. Only a small percentage, even of the root-pruned plants, became infected, and examination of the roots of nonroot-pruned plants which had wilted usually showed injuries from transplanting, from nematodes, or from other causes.

During 1916 and 1917 several further series of soil inoculations on potted cucumber plants were carried out in a similar manner. The virulence of the heavy tap-water suspensions of the bacteria was established in each case by needle-puncture inoculations made just before pouring into the soil. Briefly, the results are as follows:

March 31, 1916. All had been recently transplanted. (1) Not root pruned, 36 pots, 2 plants to a pot; 22 per cent wilted. (2) Root pruned, 24 pots, 2 plants to a pot; 25 per cent wilted.

April 19, 1916. All had been transplanted. (1) Not root pruned, 30 pots, 2 plants to a pot, 23 per cent wilted. (2) Root pruned, 18 pots, 2 plants to a pot; 39 per cent wilted.

April 19, 1916. All these plants were grown in the pots without transplanting. (1) Not root pruned, 36 pots, 2 plants to a pot, 2.8 per cent wilted. (2) Root pruned, 24 pots, 2 plants to a pot, 29 per cent wilted.

December 7, 1916. All these were old plants and not recently transplanted. (1) Not root pruned, 4 pots, 1 plant to a pot, no wilt. (2) Root pruned, 4 pots, 1 plant to a pot, 50 per cent wilted.

December 7, 1916. All these were young plants recently transplanted. (1) Not root pruned, 27 plants, 3.7 per cent wilted. (This plant showed the roots badly gnawed. Examination of the roots of 8 of the nonwilted plants failed to show any root injury.) (2) Root pruned, 9 plants, 55 per cent wilted. (Examination of the roots of the 4 nonwilted plants failed to show root injury. In running a knife into the soil the main roots at least must have been missed.)

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[1] Rand, F. V., and Enlows, Ella M. A., 1916. Op. cit., p. 425.

[2] Rand, F. V., and Enlows, Ella M. A., 1916. Op. cit., p. 423.

April 11, 1917. Not root pruned, but recently transplanted, and soil suspension poured on base of stem as well as into soil around plant; 72 plants, 61 per cent wilted.

April 13, 1917. Not root pruned, but recently transplanted; 114 plants, 25 per cent wilted.

May 27, 1917. Not root pruned, not transplanted; 20 plants, 2 plants to a pot, none wilted.

Averaging the percentages of infection for all these series of soil inoculations to potted plants, it is found that 17 per cent of all the nonroot-pruned plants became infected, as against 39 per cent of infection among the root-pruned plants. But most of the cucumbers had been recently transplanted, and where in these cases examinations were made of nonroot-pruned wilting plants, root injuries of some sort were usually found. In those cases where the plants had neither been root pruned nor recently transplanted, infections even in the presence of such enormous numbers of bacteria were rare. This evidence, together with that given in other portions of this bulletin, points to the conclusion that the wilt organism does not gain access to the uninjured roots of cucurbits and that under field conditions no infections at all come from the soil.

VIABILITY OF THE WILT ORGANISM IN THE SOIL.

In order to determine how long the wilt organism would remain viable in ordinary garden soil, heavy tap-water suspensions of the bacteria were poured on the soil in one corner of a greenhouse bed. Then at intervals tap-water suspensions of the upper 2 to 4 inches of this soil were made and sprinkled over the needle-punctured leaves of young potted cucumber plants. In no case did any of these plants contract wilt, although the control plants inoculated by needle punctures with the original suspensions promptly succumbed to the disease. Details of these experiments follow:

December 4, 1916. Eight plants were inoculated within one-half hour after pouring the bacterial suspension on the soil. Up to December 30 no wilt had occurred on any of them, although all plants inoculated with the original suspension used in inoculating the soil became infected.

January 8, 1917. Within one-half hour after pouring the bacterial suspension on the soil the thin mud thus made was directly transferred to all the leaves of eight young cucumber plants and numerous needle pricks made into each leaf. At the same time the tips of two other young plants with several pricked leaves were inverted in some of the water suspension of soil and bacteria, left in it for 24 hours, and then held for observation. Three days after the soil inoculation another thin-mud suspension from it in tap water was made and sprinkled over the pricked leaves of 13 young cucumber plants. All these plants were under observation for several weeks, but not a single case of wilt occurred. The control plants inoculated with the original bacterial suspension all wilted.

January 18, 1917. Soil-suspension inoculations into the pricked leaves of eight young cucumber plants, made one-half hour after inoculating the soil, all gave negative results. The control inoculations from the original suspension all gave positive results.

March 26, 1917. Some soil in a greenhouse bed was inoculated to the consistency of thin mud with a thick, milky suspension of wilt bacteria. After 12 hours 10 young cucumber plants were thoroughly wet down with a tap-water suspension of this soil and the leaves all needle punctured. No wilt occurred in any case, although the six control plants inoculated with the original bacterial suspension promptly wilted.

For some reason the bacteria placed in our greenhouse soil have not retained their power to infect. This might be due to a too-great dilution of the bacteria, to their adsorption by the soil particles, or to injury or death from other antagonistic organisms, or from toxic substances in the soil. This phase of the problem is being further investigated.

DO INSECTS BRING WILT FROM THE SOIL TO THE PLANT?

Although cucurbit plants do not contract wilt directly from the soil under field conditions, the question was raised whether the wilt organism might not be brought up from the soil on the bodies of insects and introduced into wounds made by insects or other agencies. Such a method of infection is against conclusions drawn from careful field observations during several seasons in many localities. Furthermore, insects other than the Diabroticas have in all our tests failed to transfer the disease; and mechanical injuries to the vines resulting from storms, cultivation, and other causes in fields badly infested with wilt during the current and preceding seasons have shown no relation to wilt infection. However, for the sake of clearing up this point definitely, the following greenhouse and field experiments were made.

October 15, 1916. The soil in a large greenhouse bed was divided into eight compartments, each approximately 4 feet square, with solid board partitions at the base extending 2 feet into the soil and each compartment covered with 18 mesh wire netting over a skeleton frame (Pl. III). The soil was sterilized for one hour at 15 pounds steam pressure by the steam-pan method, to kill all insects present. This sterilization was repeated one week later to make the result doubly sure, and care was taken that every part of the soil was reached. The soil was allowed to stand three weeks and was then raked over with sterilized tools and thoroughly wet down in seven compartments with a heavy tap-water suspension of *Bacillus tracheiphilus* from 1-week-old beef-agar cultures grown in large flat culture flasks. The soil in compartment 4 was sprinkled with tap water only and held as a control. In the inoculation suspension 10 different isolations of the wilt organism were used. Before inoculation of the soil the virulence of this suspension was proved by needle-prick inoculations in the leaves of 13 potted cucumber seedlings, all of which promptly wilted. Arlington White Spine cucumber seeds were then sown in the soil still wet with the bacterial suspension. Insects were then introduced as follows:

Compartment 1. A total of 116 striped cucumber beetles previously shown to be free from wilt by feeding on cucumber plants which did not subsequently contract the disease.

Compartment 2. Miscellaneous insects (species of flies, lady bird, etc.) and angle worms and wireworms.

Compartment 3. Squash bugs.

Compartment 4. Control, without soil inoculation or insects.

Compartment 5. Nematodes (*Heterodera radicicola*). Two buckets of soil from around nematode-infested tomato plants were thoroughly mixed with the soil, and in addition the infested roots and stems of several tomato plants were cut up and raked into the soil.

Compartment 6. Seven wilt-free 12-spotted cucumber beetles.

Compartments 7 and 8. Control on insects. Soil inoculated with *Bacillus tracheiphilus*, but no insects introduced.

Until the seeds had germinated, the beetles were fed on cut stems and leaves of healthy cucumber plants introduced into the compartments on pieces of heavy manila paper to keep the pieces from possible contamination by the soil.

Eight days after sowing the seed the soil was reinoculated in the same manner as before with a suspension of *Bacillus tracheiphilus* proved virulent by needle-prick inoculations into 31 potted cucumber plants, all of which wilted.

On December 4, 1916, the young cucumber plants in all the compartments were thinned out and the soil loosened. In compartment 1, 85 plants showed beetle gnawings on the true leaves, as did also several plants in No. 6.

Again on December 7 the soil was inoculated with *Bacillus tracheiphilus* as before, using great care, however, to prevent the liquid from coming in direct contact with the plants. All control plants inoculated from this fluid by needle puncture wilted.

About the middle of December, 25 striped beetles were counted feeding on the plants in No. 1, and 4 more wilt-free 12-spotted beetles were added to No. 6, where they were later seen feeding.

This experiment was under almost daily observation from the time the seeds were planted (November 15, 1916) until January 11, 1917, and not a single case of bacterial wilt or anything suggesting it occurred during the whole time in any of the compartments. At this date the first crop of plants was pulled, and cucumber plants grown in sterile soil in pots were transplanted to the bed cage—six hills of three to four plants each to every compartment. The soil was then reinoculated with *Bacillus tracheiphilus* as before. Up to January 29 the plants were fed upon by both species of cucumber beetles, but no wilt had occurred in any section of the cage. Six more small plants were then set out in compartment 1, cucumber seeds planted in all the compartments, and the soil reinoculated with *Bacillus tracheiphilus* as before. Up to February 9 no wilt had appeared, although plants in Nos. 1 and 6 had been fed upon, and Diabroticas were still alive in both compartments. Powdery mildew was now getting bad, so all plants in the bed were pulled and fresh plants grown in sterilized soil were set in each compartment. There were now six beetles in compartment 1 and one beetle in compartment 6. One week later the soil was reinoculated as before and, as in each preceding case, the virulence of the bacterial fluid was proved by needle-puncture inoculations. At this date 6 pots of cucumber plants were placed in compartment 1 and 13 in compartment 6 and left for five days, at which time the leaves on every plant had been gnawed by the beetles. The plants were then removed for observation. Three more potted plants were then placed in compartment 1, and after the leaves had been gnawed these also were removed and held for observation. No wilt occurred on any of these plants.

On February 17 and again on March 8 the soil was all respaded and each compartment set to plants grown in sterile soil and the soil in the bed reinoculated with *Bacillus tracheiphilus* as before. In no case did a single plant in any of the compartments contract wilt, although the suspensions used in inoculating the soil were virulent, as shown by needle-punctured control inoculations.

This set of experiments was discontinued about March 25, 1917, and the whole cage was taken up and removed to one of our experimental fields near Tuxedo, Md., where with the slight variations noted below the experiment was repeated.

Compartment 1. Control. Soil not inoculated, but cucumber seeds sown.

Compartments 2 and 3. Soil inoculated with a well-water bacterial suspension from 4-day-old beef-agar cultures and sown to cucumber seeds while the soil was still moist.

Compartment 4. A large number of cucumber vines freshly wilted from pure-culture inoculation were buried in the soil just before sowing cucumber seeds.

Compartments 5 and 6. Controls. Not inoculated, but cucumber seeds sown and wilt-free striped and spotted cucumber beetles introduced.

Compartments 7 and 8. Soil inoculated and planted to cucumber seed as in Nos. 2 and 3, but in these cases the soil had been drenched with formalin (1–320) two weeks previously to kill any insects and insect eggs and pupæ present in the soil.

In due season the seeds germinated, and the plants in all the compartments continued healthy and vigorous. On June 15 the soil was again inoculated with *Bacillus tracheiphilus* as at first, and wilt-free striped and spotted cucumber beetles were introduced into all the compartments except the control, No. 1.

Up to June 27 no wilt had occurred anywhere in the compartments and all the plants were pulled; the soil was reinoculated as before, immediately replanted with cucumber seed, and two days later more wilt-free cucumber beetles were added.

Again on July 23 the soil was very heavily reinoculated. The cucumber plants were now in full bloom. No wilt had yet appeared.

On August 22 the soil was again inoculated. Beetles were present in all the compartments and the vines had been fed upon freely. The cage was under constant observation up to September 15, but at no time did any wilt develop.

In this same field similar tests were carried out at varying intervals throughout the season, using three of the large cloth-covered cages (Pl. II, fig. 1). In two of these cages the soil was inoculated by bacterial suspension and in the third by burying 24 wilted vines in the soil about 1 inch below the surface. In the latter case cucumber seeds were planted directly among these wilted vines. Wilt-free beetles were added to each cage and the soil was inoculated several times during the season. No wilt developed in these cages at any time.

In all these experiments the virulence of the bacterial suspensions used was proved by needle-puncture inoculations into cucumber seedlings.

In these numerous greenhouse and field tests where the soil was heavily inoculated with virulent bacteria or when freshly wilted vines were buried in the soil and where wilt-free cucumber beetles and other insects, nematodes, etc., were introduced with the cucumber seeds sown in these soils no single case of bacterial wilt developed. These results fully corroborate the findings from careful field observations, namely, that bacterial wilt of cucurbits develops under field conditions only when introduced into the plant by human agencies or by cucumber beetles which have previously fed upon plants infected with the wilt organism.

WINTER TRANSMISSION OF WILT.

BEETLE HIBERNATION EXPERIMENTS.

As previously reported,[1] in some cases, at least, striped cucumber beetles are capable of carrying the wilt organism during six weeks' artificial hibernation in cold storage. Several similar cold-storage tests have been carried out since that report. Striped beetles both of the hibernating and summer broods were used, and the conditions of temperature and humidity were varied in different sets. However, in no case have we been able to keep the beetles alive for a longer period than six weeks.

Experiments of 1916.—It was thought possible that better success might be obtained if the beetles were allowed to hibernate under more natural conditions. Accordingly, in eastern Long Island during September and early October, 1916, about 4,000 cucumber beetles (mostly *Diabrotica vittata*) were collected, and 150 to 300 were introduced into each of 20 large wire-covered cages (Pl. II, fig. 2). These cages were 4 feet cubes, were provided with solid board bases without bottoms, and were set 15 inches into the soil. A pile of dry sticks and chips was placed in one corner of each cage, and the imprisoned beetles were fed upon wilted cucumber and squash vines until the 1st of November, at which time settled cold weather had come and all the beetles had disappeared from view.

This experiment was duplicated the same fall at Giesboro Point, D. C., using about 2,500 striped and 12-spotted cucumber beetles, the two species being caged separately. From 50 to 150 beetles were introduced into each of 15 cages provided in this case with a double covering of wire netting and fine cheesecloth. Boxes filled with hay or excelsior were placed in part of the cages and in a few cages a pile of dry chips, sticks, and bark was also added.

In the following spring cucumbers were planted in the cages in both localities, and the plants were kept under careful observation from the first appearance of warm weather until cucumber beetles had long been present in the surrounding fields. In all this time not a single cucumber beetle nor a beetle-injured leaf was found inside any of the 20 cages on Long Island or of the 15 at Giesboro Point, D. C.

During September, 1916, a supplementary test was started, using 11 pint glass jars with 2 inches of fine leaf mold in the bottom and covered at the top with fine cheesecloth. From 18 to 50 cucumber beetles were placed in each of these jars, fed upon wilted cucumber plants, and kept over winter in an open shed. Many of the beetles were still alive 38 days after imprisonment, but in the spring following all were dead.

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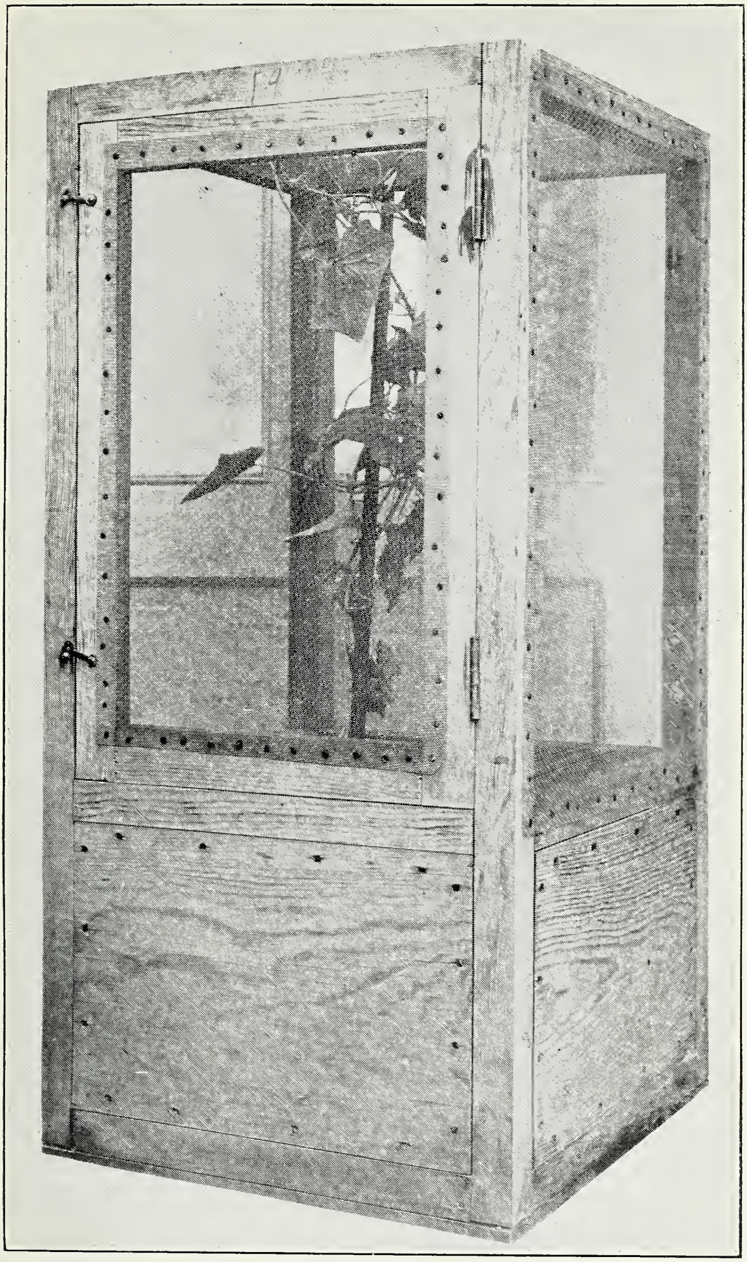
[1] Rand, F. V., and Enlows, Ella M. A., 1916. Op. cit., p. 417.



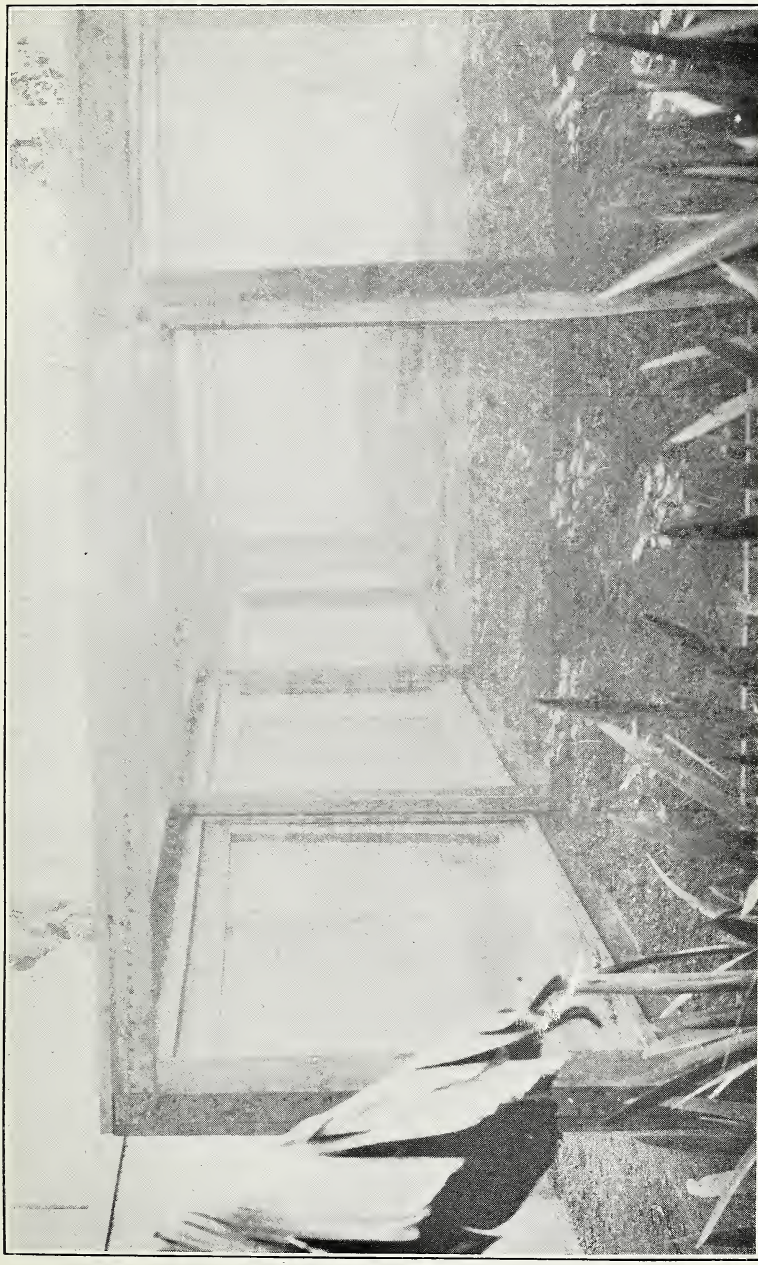
ONE OF THE SMALL CAGES USED IN THE INSECT-FEEDING EXPERIMENTS.

Photographed by J. F. Brewer.

FIG. 1.—LARGE INSECT CAGES IN THE EXPERIMENTAL FIELD AT TUXEDO, MD. THE METEOROLOGICAL INSTRUMENT SHELTER IS SHOWN AT THE LEFT.

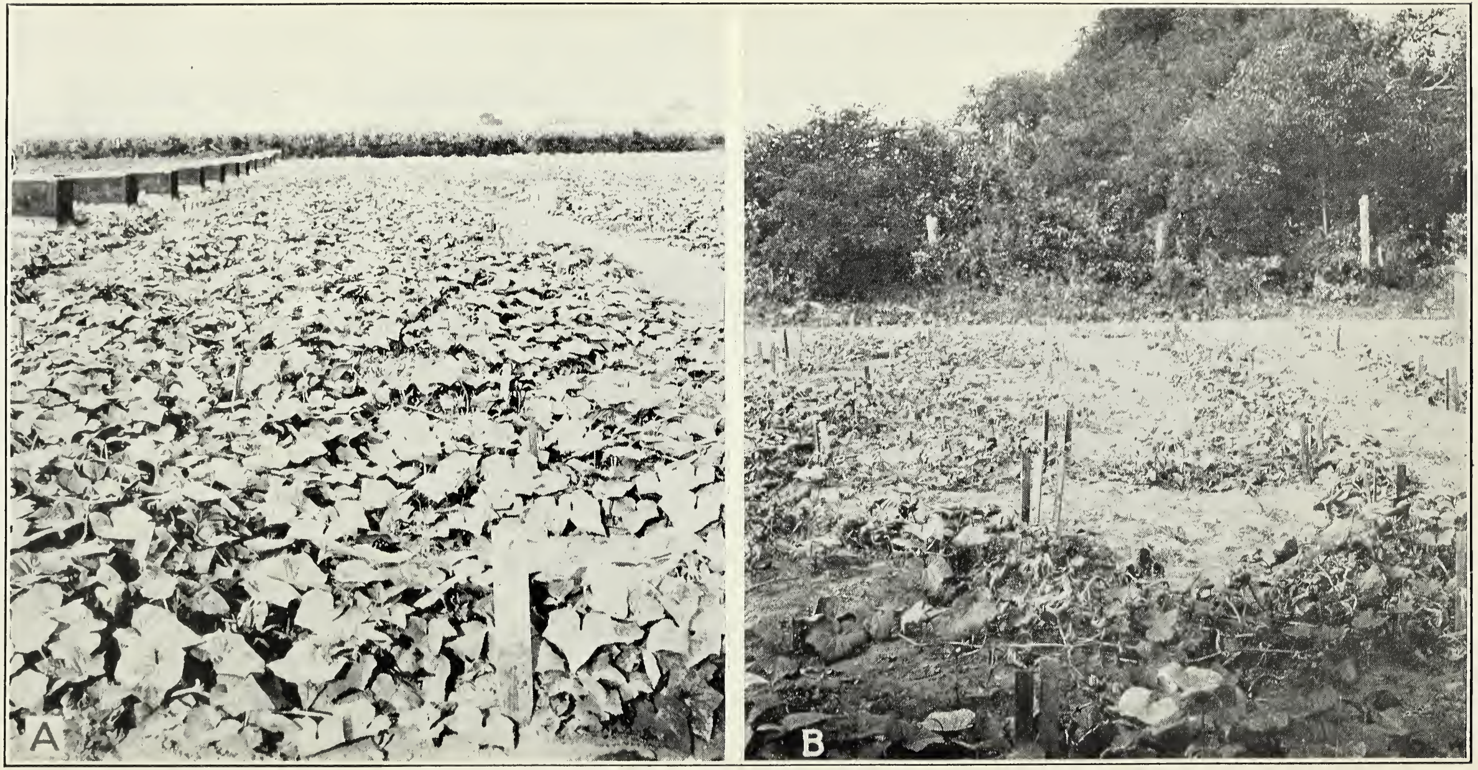
FIG. 2.—CUCUMBER SPRAY TEST PLAT NO. 11, SPRAYED WITH LEAD ARSENATE ALONE, EAST MARION, LONG ISLAND, N. Y. EACH STAKE REPRESENTS A CASE OF WILT.

Photographed by F. V. Rand.



LARGE 8-COMPARTMENT CAGE USED IN THE EXPERIMENTS RELATIVE TO INSECT TRANSFER OF WILT FROM THE SOIL.

Photographed by J. F. Brewer.



CUCUMBER SPRAY TEST AT EAST MARION, LONG ISLAND, N. Y.

Each stake represents a case of wilt. *A*, Plat No. 6, season treatment with Bordeaux and lead-arsenate mixture (2-3-50-2); *B*, plat No. 1, one of the controls, unsprayed. Photographed by F. V. Rand, August 25, 1916.

Experiments of 1917.—In August, September, and October, 1917, at Tuxedo, Md., about 5,000 of these two species of cucumber beetles were collected. Special care was taken not to injure the beetles in any way, and they were placed at once in the cages (Pl. II, fig. 1) standing in the fields where they were caught. Three species of cucurbits were growing in these cages and also corn in a few of them. Some of the cucurbits were inoculated with the wilt organism and others were left uninoculated. After the growing plants in the cages had died, squashes and other cucurbit fruits were fed to the beetles until November, 1917. A few of these cages were destroyed by winds during the succeeding winter, but in the 13 remaining intact not a single beetle appeared in the spring of 1918. It might be added, however, that in one of these cages into which several squash lady bird (*Epilachna borealis*) had been introduced the preceding fall, three live individuals were found in May, 1918.

These attempts at hibernation of wilt-fed cucumber beetles under experimental conditions have been rather discouraging, since, so far as ascertained, none have lived longer than six weeks under the hibernation conditions used.

On the other hand, striped cucumber beetles collected at the same time and place for the direct wilt-transmission experiments in the warm air of the greenhouse have frequently been kept alive and active in small cages (Pl. I) for three to five months, and in two cases for more than six months (Oct. 4, 1916, to Apr. 28, 1917). These beetles were fed upon potted young cucumber plants which were frequently changed. and they were under almost daily observation.

DO THE WILT BACTERIA WINTER OVER IN THE SOIL?

Experimental carrying of the wilt organism through the entire winter by the beetles has failed, on account of our inability to keep the beetles alive for that length of time under the hibernation conditions given. However, the problem has been attacked from other angles and some further evidence has been accumulated to show that the striped cucumber beetle may be a winter carrier of *Bacillus tracheiphilus*.

In eastern Long Island the previously reported [1] cage experiments were repeated during the summer of 1916. In the original experiments (1915) 50 large, wire-covered cages (Pl. II, fig. 2) were set in two fields where during the preceding season about 75 per cent of the cucumber plants had been infected with wilt. In September, 1915, and again in May, 1916, the soil in one-half of these cages was heavily inoculated with well-water suspensions of tested virulent wilt bacteria. No beetles were introduced, and not a single

[1] Rand, F. V., 1915. Op. cit.

case of wilt occurred throughout the summer of 1916 in the cucumbers growing in this artificially infected soil in any of the 50 cages, although, as in the preceding season, the disease was abundant on the plants outside the cages.

At Giesboro Point, D. C., a second experiment, using 20 similar cages, was carried out during 1916 on land planted to Hubbard squashes the preceding season. Wilt was very prevalent in these vines during the fall of 1915, as verified by personal observations and by pure-culture isolations. Many hills bearing wilted vines were marked at this time, and 12 of the cages were set directly over such hills. A wire netting slightly coarser (16 meshes to the inch) than that used in the Long Island experiments was employed for these cages, with the result that some of the smaller beetles, especially of the second brood, gained entrance. Until the first of July all of the cages were gone over every day except Sunday and any beetles or gnawed leaves found within were removed. Wilt in this vicinity first appeared on June 3 in four near-by fields not in cucurbits the preceding season and on June 13 in the cucumbers around the cages.

On July 5, over a month after the first appearance of wilt in this vicinity, a gnawed leaf which had been overlooked in one of the cages began to wilt around the points of injury. With the appearance of many small beetles of the second brood in July it became impossible to keep them entirely out of all the cages. On that account, after July 25, wilt occurred in single plants in 4 other cages out of the 20. In all these cases striped cucumber beetles had gained entrance, and the first leaves to wilt were those showing beetle injuries. There was no infection from the soil in any case, but direct and positive evidence of infection from cucumber-beetle injuries and absence of infection in the absence of such injuries.

SPRING SEQUENCE OF WILT.

In addition to the cage experiments during the early part of the season of 1916, a complete record was kept of the first appearance and spread of bacterial wilt of early cucurbits in all the field and garden plats in these two localities.

At East Marion, Long Island, the area selected comprised the land between Long Island Sound and Greenport Harbor and extending about 2 miles east and west. (Fig. 1.) No wild cucurbits occurred within at least 10 or 15 miles of this locality.[1]

A careful inspection on June 8, 9, and 22 of all cultivated cucurbits within this area gave no evidence of wilt.

The first wilt of the season was found on June 29 on a cucumber plant (fig. 1) in our experimental Field IV, planted May 24, 1916, and not in cucurbits the preceding season. The plants were just breaking through the ground on June 5, but on account

[1] Rand, F. V., and Enlows, Ella M. A., 1916. Op. cit., footnote 2, p. 419.

of rather cool weather during early June they had at this time (June 29) attained a height of only 2 to 4 inches with two to four true leaves developed.

The following day, June 30, seven cases were found in two half-acre commercial fields (V) of cucumbers planted May 15 (not in cucurbits in 1915) and 19 cases in a large garden plat of cucumbers and squashes (XII) likewise planted May 15 (possibly in cucurbits the preceding year).

The next cases of wilt were found July 11 in our experimental Blocks II and IIa (IIa was not in cucurbits in 1915). These blocks were first planted on May 8, but on account of cold, damp, weather and a consequent poor stand they were replanted June 13.

Cucurbits were planted in experimental Blocks I and III on June 21, and wilt first developed here July 20.

In all these instances wilt started from beetle-injured leaves. Subsequent cases developed in similarly injured plants and more or less in widening circles around the original centers.

The record at Giesboro Point, D. C., covered an area approximately one-half by three-quarters of a mile in extent, bounded on one side by the Potomac River and on three sides by woods. (Fig. 5.)

Up to June 1, 1916, no wilt had appeared within this area. At the next date of observation, June 3, one case of wilt was found in a cantaloupe field planted May 10, one case in a crookneck-squash field planted about May 1, and three and six cases, respectively, in two cucumber fields planted April 20 to 25. At least three of these fields were not in cucurbits the preceding season. Pure cultures of *Bacillus tracheiphilus* were obtained from several of these plants.

On June 6 one plant began to wilt in a cymling field planted April 20 to 25 and not in cucurbits during 1915.

On June 13 seven plants began to wilt in a cymling field planted about May 15 and not in cucurbits during 1915, and four cases developed in cucumber plants outside the wire-covered insect cages in the experimental field where the main part of the stand was planted May 31. Wilt was very prevalent in this field during 1915.

At the time of the next observation, June 23, wilt was becoming rather prevalent in all the fields where wilt had occurred before the middle of the month, and many cases were also found in four other fields.

Observations throughout the season on wild cucurbits (*Sicyos angulatus*) growing abundantly along the Potomac shore failed to show any signs of bacterial wilt, although a few striped cucumber beetles were now and then found feeding upon the leaves. On two occasions a slight but general wilting was noted during a hot sunny day on plants growing in the sand high up on the shore. The wilting was plainly due to excessive transpiration in a dry soil. However, plates were poured from several of these plants and a microscopical examination also was made. There was in no case any evidence of bacterial invasion.

During the spring of 1917 a similar set of observations was made in a locality near Tuxedo, Md. On account of the press of other work the records were not quite as complete, but the results were entirely in line with those obtained in the two preceding localities.

It should be noted with regard to the bacterial wilt in these three localities that in all cases the leaves first showing wilt in the spring had typical cucumber-beetle injuries, that the wilt had plainly started from these injured points, and that in many of the fields such beetle injuries had not at this time become general.

There is a tendency for wilt to spread in groups around the plants first developing the disease, and in all observed cases it always starts from beetle-injured plants. The facts that a careful record was kept of all cucurbits in these localities and that the first wilt to appear was from beetle injuries on the earliest plantings of the season preclude the possibility of a transfer of the disease from previously wilted cucurbits of the current season. These results, added to the data presented in our previous papers, negative the possibility of transmission of bacterial wilt of cucurbits through the soil from season to season and give strong circumstantial evidence in favor of the cucumber beetles as winter carriers.

Until the striped or 12-spotted cucumber beetles can be hibernated successfully in considerable numbers and under experimental conditions, absolute experimental proof that these insects are winter carriers can not be obtained. However, it would appear from the evidence given that either the beetles must have carried the wilt organism over winter or must have brought it directly from previous cases of the current season. The second assumption is eliminated so far as cucurbits are concerned by the facts that in neither locality were cucurbits grown under glass, that our observations covered a considerable territory, and that the wilt appeared on the very earliest plantings of the season only after the plants were bitten by the beetles. In one locality no wild cucurbits were present within at least 10 miles.

Apparently the only loophole remaining is the possibility that the organism may live over winter in some perennial noncucurbitaceous host. In his early work upon this organism, Dr. Erwin F. Smith obtained infections by pure-culture inoculations on various species of Cucumis and Cucurbita and on *Benincasa cerifera*, *Sicyos angulatus*, and *Micrampelis lobata*, and failed to infect or obtained only local injury on the following cucurbits: *Melothria scabra*, *Cucumis erinaceus*, *Luffa acutangula*, *Momordica balsamina*, *Lagenaria vulgaris*, *Trichosanthes cucumeroides*, and *Apodanthera undulata*.[1] He states further:

"Inoculations into noncucurbitaceous plants such as *Solanum tuberosum*, *Lycopersicum esculentum*, *Datura stramonium*, *Passiflora incarnata*, *Vigna catjang*, *Nicotiana tabacum*, *Pyrus orientalis*, and *Hyacinthus orientalis* yielded only negative results. The disease is not known to occur outside the Cucurbitaceæ, and probably many species of plants within the limits of this family are not subject to it."

The present writers have no data concerning the possible occurrence of this disease outside the Cucurbitaceæ.

[1] Smith, Erwin F. Bacteria in relation to plant diseases, v. 2, p. 209. Washington, D. C., 1911. (Carnegie Inst., Washington. Pub. 27, v. 2.)

SEED TRANSMISSION.

Our earlier experiments relative to seed transmission [1] all gave negative results. One further planting of seeds from a wilting cucumber vine gave healthy plants which at no time showed signs of wilt. Numerous plantings have also been made with cucumber seeds soaked in heavy tap-water suspensions of virulent wilt bacteria immediately before sowing. These tests likewise all gave negative results.

RELATION OF THE WILT BACTERIA TO CUCUMBER BEETLES.

SUCCESSIVE INFECTIONS.

The objection has been raised that it would be impossible for the cucumber beetles to feed for several weeks in the early spring upon noncucurbitaceous plants and yet infect cucurbits when they appeared above ground. It is a well-known fact that these insects are rather omnivorous and in the absence of cucurbits will feed on a wide range of host plants. In a locality where cucurbits are planted late in the season the beetles feed for some time on other vegetation. If the wilt organism is carried only on the mouth parts or other external portions of the body, one might think that after feeding two or three weeks on plants not infected with cucurbit wilt none of the bacteria would be left. However, early spring infection *does* occur, and the following experiments were designed to throw further light on this question.

The small cages used in these experiments (Pl. I) were covered with 18-mesh wire netting, and the bases and joints were made insect proof. After allowing the beetles to feed on the plants, the latter, unless otherwise stated, were removed and held for observation in similar cages free from insects. The details of these experiments are as follows:

In July, 1915, at East Marion, Long Island, several striped cucumber beetles were inclosed over a hill containing wilting young cucumber plants and were allowed to feed on them for five days. The beetles were then removed and allowed to feed for 24 hours each on three healthy and uninjured young cucumber plants successively caged with them. These three plants were then kept under observation in a cage free from beetles, and between the seventh and tenth days all had shown evidence of wilt. The cut stems showed the typical, stringy ooze, and microscopical examination showed the xylem elements to be full of bacteria. As no culture media were at that time available, isolations were not attempted.

No further attempts of this kind were made until the following year (Sept. 26, 1916), when several striped cucumber beetles were fed for three days on plants wilted from pure-culture inoculations. The diseased plants were then removed and the beetles allowed to feed 5 and 15 days, respectively, on two large potted cucumber plants successively introduced. The plants were afterwards kept under observation for 27 days, but no wilt developed in either case.

[1] Rand, F. V., and Enlows, Ella M. A., 1916. Op. cit., p. 424–425.

Nine striped beetles were fed constantly on wilted cucumber plants from January 7 to February 12, 1917. On the latter date all wilted plants were removed from the cage (No. XXII) and from then until March 10 they were fed on healthy young cucumber seedlings which were so small that the leaves were entirely consumed. On March 10 the three beetles left alive were caged with a large potted cucumber plant grown in a greenhouse free from wilt. Two weeks later one beetle-gnawed leaf was found wilting and after three weeks nearly the whole vine was involved. *Bacillus tracheiphilus* was isolated from this plant and tested by successful inoculations into cucumbers. The cage used for this test was covered both by wire netting and cheese cloth, and had not been used for beetles since the preceding fall.

(Cage XXIV.) From January 11 to February 5, 1917, 25 striped beetles were fed on wilted cucumber plants, then all diseased plants were removed and until February 26 the beetles were fed on very young cucumber seedlings, which were completely riddled, as in the preceding test. At the latter date a large potted cucumber plant was placed in the cage and left for three days, after which it was removed and held for observation. On March 10 this plant was wilted and the vascular system was plugged with the typical stringy masses of bacteria. No isolations were made from this plant.

(Cage XVIII.) Wilted cucumber plants from pure-culture inoculations were fed to three striped cucumber beetles on January 25, 29, and February 8. All wilted plants were removed on February 16 and a healthy young cucumber plant was introduced. Two days later this plant was removed and a second introduced, and so on until the beetles had been allowed successively to feed on four healthy plants. On February 19, plant No. 1 was starting to wilt around the beetle injuries on one leaf. Six days later this plant was showing wilt on several leaves and plant No. 3 was starting to wilt around the gnawed places on several leaves. On March 1, Nos. 1 and 3 were entirely wilted and No. 4 was starting; plant No. 2 showed no signs of wilt. However, the leaves on this plant were scarcely gnawed at all by the beetles. Isolations were made from plant No. 4 on March 1 and inoculations from this source to six cucumber plants gave typical wilt in every case.

(Cage XVIII.) On March 20, 1917, a wilted cucumber plant was fed to the same three beetles, and on March 28 it was removed and a healthy plant introduced. Two days later this was removed and a second one introduced. On April 13, plant No. 1 showed typical wilt, but No. 2 remained free from the disease.

This last experiment was duplicated on the same date in two other cages (XIII and XXI), but in these cases neither of the two plants contracted wilt.

(Cage XXII.) On the same date (March 20, 1917) wilted cucumber plants from pure-culture inoculations were fed to several striped cucumber beetles. On March 28 these plants were removed and a healthy plant introduced. Two days later, plant No. 1 was removed and a second plant introduced. Both plants contracted wilt from the beetle injuries and on April 13 were wilted throughout and dying.

Plant No. 2 was allowed to remain in the cage with the beetles. On April 28 the plant had long since died, but one beetle was found still alive in the cage and a healthy cucumber plant was introduced. On May 7 two leaves had started to wilt next to the margins, where they had been slightly gnawed. The beetle had died since the last preceding observation. The wilt was a typical case, and the vascular bundles of the leaf petioles were filled with bacteria.

From the results of these "successive infection" tests it is demonstrated that striped cucumber beetles are sometimes capable of successively infecting three or more healthy cucumber plants after one initial wilt feeding and that after one such feeding they may in some cases in the active state retain their infecting power for over three weeks.

DISSECTION EXPERIMENTS.

It is clear from the foregoing that striped beetles in the active state are sometimes capable of harboring the wilt organism for considerable periods of time. With the idea of determining in what part of the body the bacteria are carried, the following beetle dissection experiments were carried out.

Six 12-spotted cucumber beetles were fed for three weeks on wilted cucumber plants in pots and finally for five days on cut stems and leaves of wilted plants. After this last feeding (Aug. 8, 1917) the heads and legs of the beetles were removed separately with sterile instruments. Then the anal region was cut off with a hot scalpel, and the intestinal contents were carefully squeezed out with sterile instruments on a sterile Petri dish, using care that none of the contents should come in contact with the exterior of the insect body. Young potted cucumber plants were then inoculated separately with heads and mouth parts, legs, intestinal contents, and reproductive systems. In each case the body parts named were pricked into the young leaves without moistening the surfaces. Five days later one leaf inoculated with intestinal contents had started to wilt. After two days more, wilt had started in one leaf inoculated with beetle legs and in two additional leaves inoculated with intestinal contents. This infection from beetle legs is not to be wondered at, as the beetles were allowed to feed on cut stems and leaves of wilted plants for several days prior to dissection. These plants had been kept throughout the test in a large 6-foot square beetle-free cage (Pl. II, fig. 1) and were entirely free from insect gnawings. A hundred or more uninoculated cucumber seedlings of the same age and variety held as controls in the same cage remained free from the disease.

On August 16, 1917, the preceding experiment was duplicated, using six striped and twelve 12-spotted cucumber beetles. Five days later one of the plants inoculated with intestines and one inoculated with legs of the 12-spotted species were found with wilt. In this case none of the plants inoculated with head parts became infected.

During the summer of 1918 four more sets of dissection tests were carried out. On June 15, after a 7-day's feeding on wilted plants, forty striped and three 12-spotted cucumber beetles were dissected as in preceding experiments, and separate cucumber seedlings were inoculated with heads and with intestinal contents. Ten of the forty striped beetles were feeding on wilted leaves when taken. The other thirty were not feeding when taken for dissection. In these cases the body parts were crushed in a drop of tap water and pricked into the cucumber leaves. In 11 days two plants and next day three more plants showed wilt starting from leaves inoculated with intestinal contents. Of these five wilted plants only one was infected from one of the ten beetles feeding on wilt when taken; the other four wilted plants were from four of the thirty beetles not feeding when taken. Cultures made from one of these plants gave *Bacillus tracheiphilus*, and all five showed typical wilt symptoms. No infections resulted from the three 12-spotted beetles.

This experiment was repeated on June 28, using 45 striped beetles. In eight days two plants were wilting from beetle-intestine inoculations and the following day two more from intestines and one from head and mouth parts. Cultures were made from the first two plants showing wilt, and *Bacillus tracheiphilus* was isolated and proved to be virulent by successful inoculations.

Sterile instruments were used and great care was taken in all these tests not to allow the intestines to come in contact with external body parts. However, in order completely to eliminate all possible source of external contamination, the beetles in the following two tests were immersed for five minutes previous to dissection in a 1 to 1,000 solution of mercuric chlorid in 25 per cent alcohol. The alcohol was added to facilitate reaching all parts of the insect body, and the solution was also constantly shaken during the exposure.

Approximately 35 striped beetles were dissected (June 29) after a 7-day feeding on wilted cucumber plants in pots, and inoculations were made to the leaves of as many cucumber seedlings by mixing the intestinal contents of each beetle in a drop of tap water and pricking the mixture into the leaves. These plants were kept in beetle-free cage with 20 or more uninoculated control plants. Two of the inoculated plants developed typical cases of wilt starting from the points of inoculation with intestinal contents.

Finally, on July 5, 1918, the last experiment was repeated, using sixty striped and thirty 12-spotted cucumber beetles and fifteen squash lady birds. All had been previously fed for 10 days on wilted cucumber plants in pots and had been treated for five minutes before dissection with mercuric chlorid and alcohol, as in the preceding test. Five plants out of the 60 inoculated with intestines of striped beetles promptly developed wilt. All these cases showed the typical stringy slime from the cut ends of the vascular bundles, and cultures made from one of these plants gave *Bacillus tracheiphilus*. Inoculations in this experiment with intestines of the other two insect species gave negative results.

These dissection experiments prove definitely that both striped and 12-spotted cucumber beetles may harbor the wilt organism in their intestinal tracts. In fact, a much larger percentage of infections took place from intestinal contents than from mouth parts, although this may have been due to the fact that the mouth parts were harder to break up and mix thoroughly with the water on the leaves. They also show that only a small proportion of the beetles gave infection from the intestines even after feeding seven days or more on wilted plants. Thus, in the two tests with 12-spotted beetles, the intestines gave infection in about 50 and 8 per cent of the individuals, respectively. In the four tests with striped beetles the proportion varied in round numbers between 6 and 12 per cent. There was very little difference in the proportion of wilt carriers among individuals taken while feeding directly on wilted plants and those not feeding at all when taken for the test.

BACTERIAL ISOLATIONS FROM BEETLES.

One attempt was made (Dec. 10, 1917) at direct isolation of *Bacillus tracheiphilus* from the intestinal tracts of wilt-fed striped cucumber beetles.

Ten individuals were fed for two weeks upon cucumber plants wilted from pure-culture inoculation. They were then dipped for several seconds into 95 per cent alcohol, for 60 seconds in 1 to 1,000 mercuric chlorid, quickly washed in sterile water, and then given five more washings of several minutes each in sterile water. The intestinal tracts were then carefully dissected out as described under "Dissection experiments," without allowing the body contents to come in contact with the surface of the beetle. The intestines in each case were crushed in sterile distilled water with a sterile glass rod and allowed to stand for 20 minutes, when four series of dilutions in beef bouillon were made. From these tubes one series of poured plates was made at once and another the next morning after the bouillon had become slightly cloudy. From most of the plates developing colonies, pure cultures of one type of bacteria (*A*) arose, which at first somewhat resembled *Bacillus tracheiphilus*. Later, on beef-agar slants, this type gave a dense, opaque, somewhat yellowish and rapidly growing colony. A second type (*B*) was constantly found in the plates poured from one set of dilutions. This isolation (*B*) more closely resembled the wilt organism, and young colonies on beef agar were at first scarcely to be distinguished. Even the rate of growth was about the same as for *Bacillus tracheiphilus*. However, old colonies are somewhat more opaque and also have a very slight yellowish cast not present in the wilt organism. The organism also produces more alkali than *B. tracheiphilus*. Inoculation tests on cucumber plants have given negative results with both these types of bacteria. Occasional colonies of other bacterial and fungus types came up on the plates, but on account of their rarity were considered as contaminations. The wild organism was not obtained.

Portions of intestines left from each beetle dissected were retained and in each case were used in inoculating a single young cucumber plant by needle puncture. One plant only out of the ten wilted, but isolation was not attempted. There were at the time a large number of other cucumber plants in the greenhouse, all of which were free from wilt, and there is little doubt that the plant in question had become infected with the true bacterial wilt.

It was thought worth while to test the types of intestinal bacteria in mixet culture with authentic isolations of *Bacillus tracheiphilus* for possible antagonism. After three weeks in mixed cultures (beef agar plus 1 per cent cane sugar) young cucumber plants were inoculated by needle punctures in the leaves; and other plants were inoculated as checks with intestinal types *A* and *B* alone and with the wilt organism alone. Of ten plants inoculated with cultures of *A* plus wilt, and of the two plants with *A* and *B* alone, none became infected; but of four plants inoculated with *B* plus wilt all contracted wilt, showing the typical stringy ooze from the cut stems and the vessels filled with bacteria. All plants inoculated with *B. tracheiphilus* alone showed wilt. The plants were under observation one month.

In another experiment using four plants with type *B* alone, four plants with the wilt organism alone, eight plants with *A* plus wilt, and four plants with *B* plus wilt, all from 2-weeks-old cultures, the results were identical—growth of *B. tracheiphilus* and infection in the presence of *B;* none in the presence of *A*.

The wilt organism itself was not isolated on agar-poured plates made from beetle intestines, but evidence was adduced that the organism was present in the intestines of one of the ten beetles used and that in the presence of cane sugar the intestinal flora of at least one beetle was not antagonistic to the wilt organism, but that in the majority of the cases the intestinal flora (type *A*) was under these conditions antagonistic to the wilt organism.

These results, while not sufficient to warrant far-reaching conclusions, suggest the possibility that a careful study of the bacterial flora of cucumber-beetle intestines will afford an explanation of the fact that only a small proportion of wilt-fed beetles are wilt carriers.

VIRULENCE TESTS.

During the interval since August, 1914, several hundred isolations of *Bacillus tracheiphilus* have been made from cucumber, cantaloupe, winter squash, crookneck summer squash, and cymling, and from an area extending from Canada to southern Georgia and Long Island to Iowa. Of these isolations from various hosts and localities, over one hundred have been carried continuously in culture for considerable periods—in some cases for nearly four years.

It was early noted by Dr. Erwin F. Smith[1] that his isolations of *Bacillus tracheiphilus* from squash would always infect cucumber, but that his isolations from cucumber usually gave no infection at all on squash and at most only a slight local wilting near the point of inoculation. It seemed possible from these results that there might be at least two true biological strains of the organism.

With a large number of isolations from different hosts and localities at hand it seemed well worth while to investigate this question further. For this purpose relative virulence tests were made of large numbers of isolations grown on the same medium and in cultures of the same age inoculated in as nearly an identical manner as possible into one of the host varieties as an indicator. Chicago Pickling cucumbers were selected as being a representative variety of one of the most susceptible host species; and in each series of inoculation tests plants 12 to 18 inches high and of like age and similar vigor were inoculated simultaneously with cultures of the different isolations. From two to eight series of these tests were made, and in each series two or more (usually two) plants were inoculated with each isolation under study. Experience showed that within a variety a fairly constant reaction was given to any one isolation of the wilt organism, provided the plants were of comparable age and vigor. Occasionally an individual plant would show a wide variation from its fellows in reaction to a particular isolation, but since considerable numbers of plants were used these comparatively rare aberrant cases did not alter the end results of the study.

In each series of tests careful observations were made of the absolute and relative progress of the disease, special attention being given to three cardinal points—(1) the number of days after inoculation when incipient wilt was noted, (2) the total number of days after inoculation required for actual wilting of the larger part of the inoculated leaf, and (3) the total time after inoculation for the wilting of the entire plant. By "incipient wilt" is meant either a slight loss of turgor without actual wilting or a slight indication of wilt immediately around the point of inoculation. For purposes of ready comparison a "virulence index" was worked out for expressing the degree of relative virulence of each isolation to the indicator used. This virulence index was secured in each instance by adding together the three cardinal points above mentioned. These figures as they stand express the relative virulence in inverse ratio, the smaller figure expressing the greater virulence. In order to show the relation more clearly, expressing the greater virulence by the larger number, the numerator "1" was placed over each of the figures so obtained

[1] Op. cit., p. 284.

and the resulting fraction reduced to a decimal. In making up the relative-virulence graphs these fractions were used in the abscissæ to express varying degrees of relative virulence between possible instant death (unity) and no infection at all (zero). For example, one isolation gave (1) incipient wilt in 4 days, (2) wilt of the inoculated leaf in 5 days, and (3) wilting of the whole plant in 11 days after inoculation. Added together, these three figures equal 20, the number used as the basis of comparison. The expression 1 over 20 reduced to a decimal equals 0.05, which is the figure taken as the final index expressing the degree of relative virulence for this particular isolation of *Bacillus tracheiphilus*. This happens to be one of the most virulent isolations found thus far in our study.

The relation in number of days between 1 and 2 and between 2 and 3 tended to be similar in the different isolations tested. That is, if the first signs of wilt appeared late a longer time usually elapsed before complete wilting than when the first signs appeared early. However, this rule was not strictly true in all cases, so it was felt that a truer comparison could be made by adding together the three cardinal points above mentioned rather than by taking any one of them alone as the basis for comparison.

In the graphs the ordinates show the number of isolations found by numerous inoculation tests to fall at any particular degree of relative virulence. In plotting the curves the average virulence index for all tests with any one isolation was in each case taken.

One hundred and three different isolations were tested as to relative virulence in Chicago Pickling cucumber, and this list included isolations from cucumber, squash, and cantaloupe. Referring to the graph (fig. 6-B), it will be seen that most of the isolations are extremely virulent. However, a few isolations worked very slowly, and one very weak isolation never caused anything more than incipient though undoubtedly true bacterial wilt. It will be noted that most of the isolations (84 in number) fall above the virulence index 0.033, and it may be added that all the isolations from squash are within this group.

As a supplementary test, 62 of these isolations from cucumber, squash, and cantaloupe were further tested as to relative virulence when inoculated into White Bush Scallop squash, the method used being the same as in the cucumber tests above described. Reference to the graph (fig. 6-A) shows the opposite kind of a curve from that obtained with cucumber as indicator. Only 12 out of the 62 isolations tested caused wilting of entire squash plants, and a lower degree of relative virulence was shown here than when these same isolations were tested by inoculation into cucumber. Of these 12 highly virulent isolations it happens that 6 were originally obtained from cucumber and 6 from squash. Eighteen other isolations tested on squash exhibited various shades of virulence from incipient wilt only up to the wilting of one to several leaves, but never caused wilting of an entire plant. Of this group, 14 isolations were from cucumber, 2 from squash, and 2 from cantaloupe. The third and last group, made up of 23 isolations from cucumber, 2 from squash, and 7 from cantaloupe, falls upon virulence index zero—that is, none of these isolations gave any apparent infection at all on White Bush Scallop squash, although all had caused infection in cucumber. In cases of recovery of a squash plant after partial wilting, *Bacillus tracheiphilus* may continue to live in the vascular tissue of the apparently healthy plant. In one such instance plates were poured from the fruit of an inoculated squash plant about one month after apparently complete recovery from wilt,

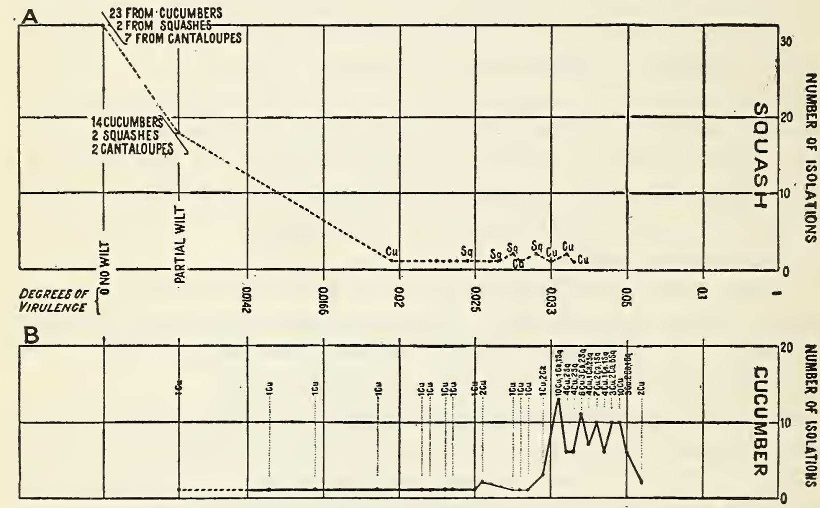


FIG. 6.—Curves showing the varying degrees of virulence among different isolations of *Bacillus tracheiphilus* from the three common hosts and from many sections of the country as demonstrated by large numbers of inoculations into squashes (*A*) and into cucumbers (*B*), 62 different isolations being tested in this way on squashes and 103 on cucumbers. The letters *Cu, Ca,* and *Sq* refer to the source of the isolation as being cucumber, cantaloupe, or squash, and the figures preceding refer to the total number of isolations from each host possessing a similar degree of virulence.

and *B. tracheiphilus* was recovered from these plates and proved infectious by inoculation into cucumber.

A large proportion of all the isolations were retested on both cucumber and squash in April, 1918, and essentially the same relations have continued to hold after long periods. For example, two isolations (*R 230*, New York; *R 235*, Michigan) from cucumber carried continuously in culture since August, 1914, and tested from time to time by inoculations, have preserved approximately the same virulence to Chicago Pickling cucumber and the same relation to each other during all this time, *R 230* being considerably more virulent than *R 235*.

Furthermore, in 1915 these two isolations were inoculated into cucumber plants and then reisolated. The reisolations (*En 14* from *R 230* and *En 15* from *R 235*) were found still to retain approximately the same virulence relations as the originals. The relative virulence indexes of these four isolations as deduced from many series of cucumber inoculations are, respectively, 0.0384 and 0.0366 for *R 230* and *En 14* and 0.0218 and 0.0229 for *R 235* and *En 15*.

The least virulent isolation (*En 160* B) of the series which at any time caused complete wilting in cucumbers had a relative virulence index of 0.0149. This was one of four isolations made from slightly wilted cucumber vines collected at Goldsboro, N. C., in June, 1916. One of these cucumber isolations (*R 317*) made in the field caused wilting of only a few cucumber leaves, never of entire plants. The other three isolations (*En 160* A, B, C) were from cucumber material collected in this same locality and sent to the laboratory at Washington, D. C., for isolation. These three isolations sometimes caused wilting of entire plants after a comparatively long time and in other cases caused only partial wilting. The bacterial wilt was rare in the vicinity of Goldsboro, and only after careful search were these few cases found. With the exception of this Goldsboro locality all the southern isolations possessed a comparatively high degree of virulence.

Leaving out the one very exceptional case at Goldsboro, N. C., the average indexes of relative virulence for all cucumber isolations grouped according to geographical source were as follows: Ten isolations from Norfolk, Va., southward, 0.042; 15 from the District of Columbia, 0.041; 17 from Long Island, 0.040; 7 from middle New York to Canada, 0.036; and 9 from Michigan, Wisconsin, and northern Iowa, 0.032. As will be readily noticed, the average virulence is highest in the South and gradually decreases northward. A study of the figures making up these averages shows that the southern isolations as a whole tended to higher virulence, while the northern isolations contained examples of both high and low virulence instead of decreasing as a unit. The higher virulence of the southern isolations is shown also by the fact that out of seven isolations from cucumbers obtained in North Carolina, South Carolina, Georgia, and Alabama, six of them (*R 318, 320, 321, 323, 324,* and *325*) were found capable of causing wilt in White Bush Scallop squash. Of the northern isolations from cucumber, the larger number obtained have caused no apparent infection when inoculated into squash.

The isolations from squash and cantaloupe when grouped according to geographical source have shown this same general tendency, but not in quite so marked a degree, since all isolations from these hosts have fallen within the more highly virulent group.

Viewing the general results of these relative virulence tests, it seems possible that instead of having only two distinct and well-differentiated biological strains of *Bacillus tracheiphilus* we may be dealing with a long series of "races" or "pure lines" of the organism exhibiting among themselves closely intergrading degrees of virulence for their various hosts. Wilt goes from cucumber to squash as well as from squash to cucumber. Furthermore, although among cucumber isolations the larger percentage are extremely virulent when inoculated into cucumber, while the larger percentage do not cause any signs of the disease at all in squash, the considerable number of cucumber isolations remaining which do cause appreciable infection in squash exhibit all grades of host reaction—the minutest signs alone, wilt of only a few square millimeters around the inoculation points, wilt of a considerable portion of the inoculated leaf, wilt of one to several leaves, and finally, in the case of the more virulent isolations, wilt of the entire squash plant.

However, the factors causing resistance or susceptibility to the wilt disease in cucumbers may be more or less distinct from the factors causing resistance or susceptibility in squash. Thus, there may still be a distinct cucumber strain represented by those of our isolations which never caused infection in squash and a squash strain possessing not only the factors necessary for infection in cucumber but additional factors enabling it to attack squash also. If this is the case, in all probability some means will be found of distinguishing the two strains by morphological or cultural characters of the parasite. Thus far, however, no constant morphological or cultural differences have been found between cucumber and squash isolations or between isolations of greater or less relative virulence. The only constant differences as yet noted among the various isolations have been differences in relative virulence to a given host. The fullest tests have been made upon Chicago Pickling cucumber, but smaller tests have also been carried out upon Arlington White Spine cucumber and upon White Bush Scallop and Yellow Crookneck squashes. While in these tests the individual isolations have not preserved exactly the same order of relative virulence when inoculated into the different host species and varieties, the tendency has been in this direction. That is, isolations most or least virulent to Chicago Pickling cucumber tended to be, respectively, most or least virulent to Arlington White Spine cucumber; and many though by no means all isolations highly virulent to cucumbers have been found capable of infecting squashes to a greater or less degree, while isolations of low virulence to cucumbers have invariably given no infection at all on squashes. This last group Dr. Smith called *Bacillus tracheiphilus* forma *cucumis*. Apparently the factors in the parasite causing high virulence to cucumbers are necessary to infection in squashes, for none of the isolations weak in virulence to cucumbers has caused any infection at all in squashes. But not all of the isolations highly virulent to cucumbers will cause infection in squashes. Thus additional qualities in the parasite may be necessary for squash infection, and it is entirely possible that besides the many races or pure lines of the organism showing various degrees of relative virulence to any particular host there may be also distinct cucumber and squash strains.

SOME HOST RELATIONS.

On the other side of the host-parasite relation the species and varieties of cucurbits themselves differ in susceptibility to the wilt organism as a whole and to its various races. Thus cucumbers constitute the most susceptible host species so far tested, and most of the isolations have proved highly virulent to them. Some differences in varietal susceptibility have been observed, but none have shown marked resistance, and apparently little hope of control through resistant varieties is given here.

At the other extreme are watermelons, the most resistant of our economic cucurbits. No authentic report of natural infection to this host has come to our notice, and our inoculations have given negative results or at most a very slight local wilt immediately around the points of inoculation.

The squash group stands next to the watermelon in resistance. Unlike the cucumber, most isolations tested on squash have given no infection or only slight local wilting. The few remaining were highly virulent, but slower in developing the disease than when inoculated into cucumber. Much greater differences in varietal resistance were shown within the squash group than among cucumbers.

Aside from the differences in resistance inherent in the species and varieties of cucurbits, a further cause of variability in the host reaction to the parasite appears to be directly connected in some way with the growth condition of the host at the time of inoculation.

It has long been thought by many growers that the spread of wilt and the extent of damage done are in some way connected with weather conditions. In two different papers, W. G. Sackett [1] states that growers seem to think that wilt is worse during wet weather and just after a heavy rain, especially if the sun comes out hot. He says further that if this is true it is probably due to the fact that these conditions favor growth of the germs and bring about a more rapid distribution of the bacteria through the plant.

G. H. Coons [2] thought it probable that hot, dry summers, such as the summer of 1911, might do much to check the spread of the disease by drying out the wounds made by insects before the bacteria could obtain a foothold. He stated further that perhaps the temperature conditions alone are sufficient to check the growth of the disease.

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[1] Sackett, W. G. Some bacterial diseases of plants prevalent in Michigan. Mich. Agr. Exp. Sta. Bull. 230, p. 217, 1905.

——— Some bacterial diseases of plants. Colo. Agr. Exp. Sta. Bul. 138, p. 22–23, 1909.

[2] Coons, G. H. Cucumber and muskmelon wilt. *In* Mich. Farmer, v. 140, no. 1, p. 1–2, illus. 1913.

Reviewing our own work and observations, these assumptions apparently have considerable truth in them, but they do not appear to express the whole truth. Among the enormous number of inoculations made during the past four seasons, several common factors have been observed running through them all. In our greenhouse experiments during the winter months very little or no difference in the percentage of infection or the rate of progress of the disease has been observed between plants sprayed with tap water immediately preceding inoculation and other plants of the same age and vigor inoculated when dry. The same infection relations also have held between greenhouse inoculations made in sunlight and those made on cloudy days or under shade.

On the other hand, striking differences are often shown among plants in varying states of vigor or age. After inoculation, badly stunted or old plants have in general shown a very much lower percentage of infection and slower progress of the disease than young vigorously growing plants of the same variety. These facts have repeatedly been observed during the progress of our studies, and for this reason great care has been exercised in selecting plants for virulence tests.

From these observations, together with the beetle and wilt curves and the meteorological data obtained in field experiments (figs. 2 to 4), it appears that within rather wide limits weather conditions have very little direct effect on the percentage of infection. However, a rapid, sappy growth of the vines favors infection and spread of the disease through the plant; whereas senility in the host, whether due to normal maturity or to unfavorable conditions such as drought or lack of plant food, furnishes conditions unfavorable to infection and spread of the bacteria within the plant. Thus, by inducing rapid succulent growth of the vines rainy weather may indirectly raise the percentage of infection and increase the reproduction of the bacteria inside of the plant. If, then, following such a rainy period the sun suddenly comes out hot, any tendency to wilt will obviously become evident at once. Indeed, under these circumstances, the excessive transpiration from too rapidly growing tissues often causes transitory wilting where no parasitic organisms are present. On the other hand, after a long period of drought the prevalence of wilt often decreases, not directly because of the weather conditions but because the vines have hardened up and no longer favor infection. Furthermore, periods of drought often come at midseason when senility is normally approaching, and the weather conditions serve only to hasten and intensify the natural process of ripening. A decrease in number of cucumber beetles between broods often comes simultaneously with these midseason drought conditions and this again reduces the number of infections at their source. Undoubtedly an extremely dry atmosphere coupled with prolonged temperatures at or above the maximum for growth of the wilt bacteria will reduce the percentage of infection and rate of progress of the disease, and in extreme cases the wilt may be even practically stamped out. However, under ordinary field conditions in the Northern and Middle States, the limiting factors appear to be (*a*) the presence of wilt-carrying beetles and (*b*) a vigorous, succulent growth condition in the host.

Certain data relative to the incubation period of the wilt organism have been compiled from our inoculation experiments and field records. Among 295 sets of greenhouse inoculations on cucumber seedlings, in each case using two or more plants 6 to 18 inches high, the great majority showed signs of wilt in 4 to 6 days after needle-prick inoculation, wilting of the first leaf in 5 to 8 days, and complete wilting of

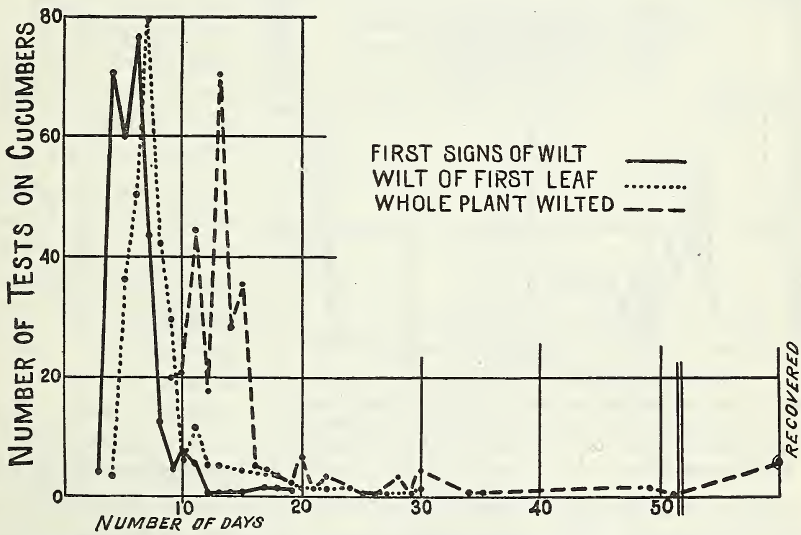

FIG. 7.—Diagram showing the progress of wilt in 295 inoculation tests on cucumbers (mostly the Arlington White Spine variety), with two or more plants in each test and in all using 103 different isolations of *Bacillus tracheiphilus*.

the plant in 11 to 15 days (fig. 7). Comparatively few of these cucumber plants took more than three weeks for complete wilting, and out of the whole 295 experiments only 6 exhibited any cases of recovery after once showing signs of the disease. Upon reference to the notes it was found that our 5 least virulent isolations (*En 15*, *R 235*, *R 311–a*, *R 317*, and *En 160*) had been used for inoculation in these 6 experiments. These 5 isolations were all low in virulence, but, with the exception of *R 317*, they usually caused complete wilting of a plant in the end.

In 51 inoculation tests on squash seedlings 6 to 12 inches high most of the plants showed incipient wilt in 5 to 8 days, wilting of the first leaf in 6 to 13 days, and complete wilting, where it occurred at all, in 15 to 20 days after inoculation (fig. 8). However, in 26 out of the 51 sets of inoculations the plants all recovered after partial wilting. It will be noted that the first signs of the disease and wilting of the first leaf were only a little slower in appearing than in cucumber, but in general a much longer time elapsed before the whole plant became involved. A far greater percentage of recovery was exhibited than in cucumber.

Field records during 1916 in the spray plat at East Marion, Long Island (fig. 1, Field IIa), showed that among the cucumbers where wilting of the first leaves appeared on July 12 the larger number of plants had completely succumbed six days later (July 18). The vines at this time were 1 to 2 feet in length, which was approximately the size of those used in the greenhouse inoculation tests above noted, and it will be seen at once that the period between the wilting of the first leaves and the total wilting of the plant corresponds very closely under the field conditions and the greenhouse conditions.

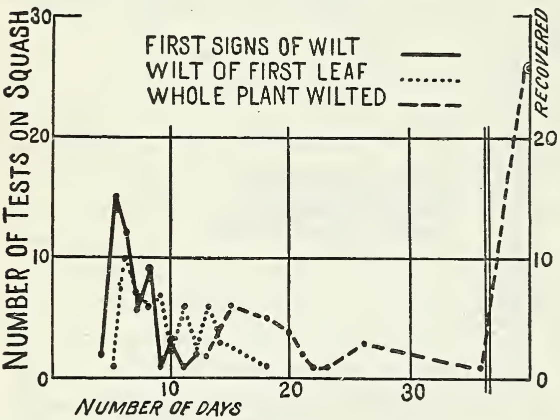

Fig. 8.—Diagram showing the progress of wilt in 51 inoculation tests on squash (mostly the White Bush Scallop variety), with two or more plants to a test and in all using 21 different isolations of *Bacillus tracheiphilus*.

Of the plants which showed wilting of the first leaves on July 18 the larger number had completely wilted 11 days later, while of those with first wilt on July 29 the larger number lived for 17 days longer. Complete records of later infections could not be obtained, on account of the appearance of downy mildew.

As would be expected, the larger the plant the longer the period of time elapsing between initial and total wilting. However, the two are not directly proportional, since in the older plants the absolute progress of the disease is slowed down through some relation to the condition of senility in the host. (Cf. pp. 31 and 32.)

DISTRIBUTION OF BACTERIAL WILT.

Bacterial wilt of cucurbits was reported by Dr. Erwin F. Smith[1] as occurring (fig. 9) in Canada, Massachusetts, Vermont (?), Rhode Island, Connecticut, New York, New Jersey, Delaware, Maryland, District of Columbia, Virginia, West Virginia, Pennsylvania, Ken-

[1] Op. cit., p. 209–211.

tucky, Ohio, Indiana, Illinois, Michigan, Wisconsin, Missouri, Iowa, Nebraska, and Colorado, and as having been reported also from Germany, Russia, and the Transvaal. He states that "nothing is known of its occurrence south of Virginia." On account of the low thermal death point of the organism and the lack of reports or specimens of the disease from the States south of Virginia he was rather of the opinion that it would not be found to occur in the far South, except in the more mountainous parts.

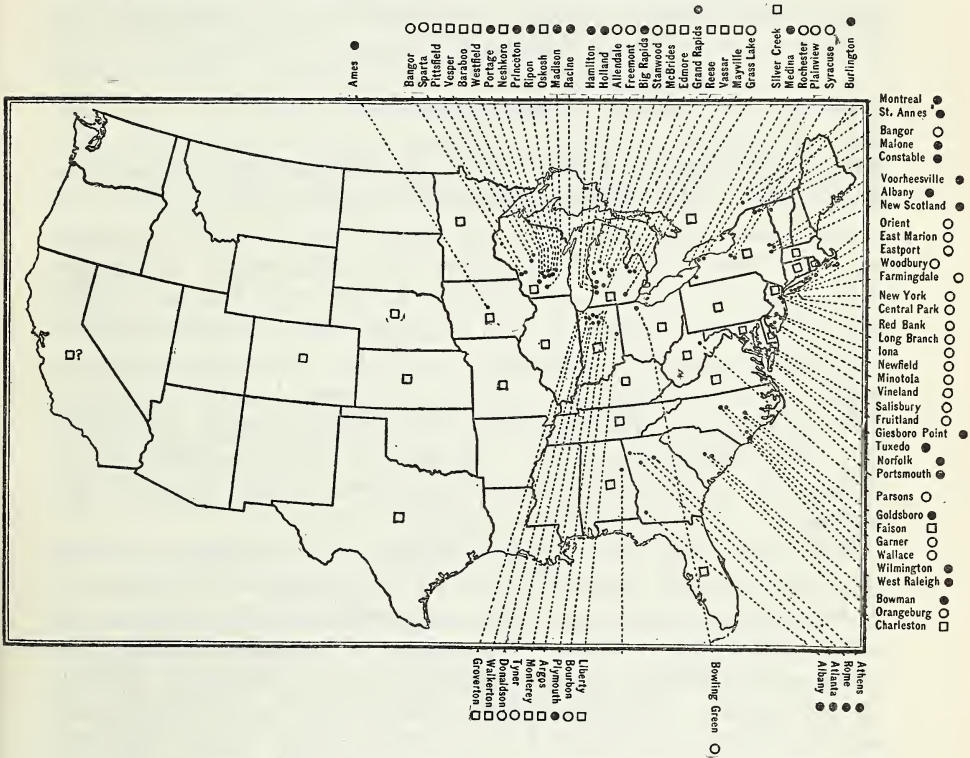

FIG. 9.—Map of the United States, showing the distribution of the bacterial wilt of cucurbits. ○=Bacterial wilt seen by one or both of the writers. ●=Isolations of *Bacillus tracheiphilus* made by one or both of the writers. □=Bacterial wilt reported by other pathologists, either with or without specific locality.

In 1914, F. M. Rolfs [1] reported this disease as causing serious losses to cucumbers in the vicinity of Charleston and Beaufort, S. C. With this exception no published reports of its occurrence south of Virginia and east of the Mississippi River had been found, and letters to experiment station pathologists from North Carolina to Louisiana had given no other definite information.

Accordingly, during June and July, 1916, a scouting trip was made by the senior writer through North Carolina and South Carolina,

[1] Barre, H. W. Report of the botanist and plant pathologist. S. C. Agr. Exp. Sta., 27th Ann. Rpt., [1913]/14, p. 23. [1915.]

Georgia, and parts of Alabama for the purpose of obtaining further information as to the southern distribution of bacterial wilt of cucurbits. The disease was found and pure cultures of *Bacillus tracheiphilus* were obtained from all four States above named. Later inoculations with these cultures into cucumbers in the greenhouse at Washington, D. C., gave typical wilt infection in every case, thus fully establishing the presence of the disease in these four Southern States. Specifically, natural infection was found on cucurbits in the field at West Raleigh (cucumber isolation *R 316–a*), Wilmington (cucumber isolation *R 318*), Goldsboro (cucumber isolations *R 317* and *En 160* and cantaloupe isolation *En 161*), and Garner, N. C.; at Orangeburg and Bowman (cantaloupe isolation *R 319*), S. C.; at Albany (cucumber isolation *R 320*), at Athens (cucumber isolation *R 325*), Atlanta (cucumber isolation *R 323*), and Rome (cucumber isolation *R 322*), Ga.; and at Anniston (cucumber isolation *R 321*), Ala. The southernmost location visited was Albany, Ga., and here bacterial wilt was found on both cucumbers and cantaloupes. The worst damage in any of these cases occurred at Anniston, Ala., and Rome, Ga., where in some cases as high as 25 per cent of the cucumber or cantaloupe vines had succumbed. In the far southern or coastal localities wilt was doing very much less damage, but this may have been partly due to the lateness of the cucumber season at the time the observations were made. In some of these localities (e. g., Charleston, S. C.), the cucumber season was entirely past, so that nothing but ripe fruit and dead vines were left.

Reference to the map (fig. 6) will show all the localities in which we have found this disease and from which we have obtained cultures, as well as the States from which the disease has been reported by other pathologists. Most of the reports of "specific localities" by other pathologists are from unpublished notes of Mr. W. W. Gilbert, of the Bureau of Plant Industry. During 1917 and 1918 bacterial wilt of cucurbits was reported by the Plant-Disease Survey [1] not only from portions of the United States where it had hitherto been known to occur, but also from Alabama, Florida, Tennessee (Essary), Texas (J. J. T.), Kansas (Melchers), and probably also from California. The disease was also reported as never having been seen in the State of Maine.

CONTROL.

Relative to the problem of control, at least four courses lie open—(1) the finding or developing of resistant varieties, (2) spraying the plants with a bactericide, (3) eliminating the beetles through poisons or repellents, and (4) removing wilted plants as sources of spread through the beetles.

[1] U. S. Department of Agriculture, Bureau of Plant Industry, Plant-Disease Survey, Plant-Disease Bulletin, v. 1, no. 5, p. 92; no. 6, p. 101, 1917; v. 2, no. 4, p. 58; no. 10, p. 181, 1918.

VARIETY TESTS.

The results of the tests already published [1] give little hope of control in cucumbers or cantaloupes through resistant varieties. A second variety test on Long Island (1916) and a third at Tuxedo, Md., (1917) have not changed this conclusion. Wilt infection is rarely as bad in squashes as in cucumbers or cantaloupes and it is not often serious enough to require special control measures. The variety tests showed greater differences in resistance among squashes, and should control become necessary it is possible that fairly immune varieties might be developed.

SPRAY TESTS.

The earlier spray tests [2] were repeated (1916) at East Marion, Long Island, on a larger scale and with some modifications. On May 12,

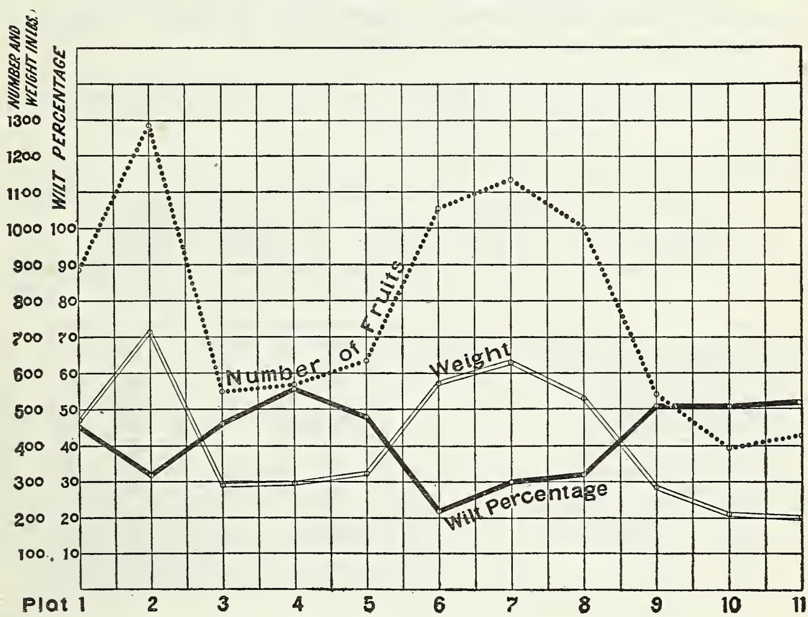

FIG. 10.—Curves showing the percentage of bacterial wilt and the weight and number of fruits in 11 plats of the cucumber spray test, East Marion, Long Island, N. Y., season of 1916.

1916, Woodruff's Hybrid cucumbers were planted in hills 3 by 4 feet apart on a level and uniform strip of land about one-half acre in extent. This area was divided into 11 plats, each containing 81 hills. Two of these plats were left untreated as controls, and one plat was treated to equal parts of lime and tobacco dust, while the remaining 8 plats were given spray applications varying as to number and ingredients. The dates of application of the various treat-

[1] Rand, F. V., and Enlows, Ella M. A., 1916. Op. cit., p. 426–428.
[2] Rand, F. V., and Enlows, Ella M. A., 1916. Op. cit., p. 429–433.

ments were June 19, 28, July 6, 13, 18, 28, August 3, 13, and 16. Table I gives the details as to the number of plants, kind and number of treatments, and wilt records for each plat in the field, and figure 10 shows graphically the relative effect of the various treatments as compared with the control plats. Photographs of plats Nos. 1, 6, and 11, taken August 25, 1916 (Pls. II and IV), show the appearance of the vines with all the applications of Bordeaux mixture and lead arsenate, with lead arsenate alone, and also without any treatment. Each stake shown in these photographs represents one case of wilt. In all cases the cucumbers were picked when about 6 inches in length. No new or special combinations of insecticides were used in this test, as the insect control, per se, is a matter left to the Bureau of Entomology. In the earlier tests, Bordeaux mixture with lead arsenate gave a better control than either mixture alone, so that 2 pounds of lead arsenate paste were added to each 50 gallons of Bordeaux mixture used in this experiment.

Table I.—*Bacterial wilt record of the spray experiment in Field IIa at East Marion, Long Island, 1916.*

Treatment.	Plat No.	New cases of wilt at different dates.						Number of plants at the start.	Wilt for the season.	
		July—			August—				Total.	Percentage.
		11	18	29	9	15	23			
Control, unsprayed	1	6	12	56	25	11	1	248	111	45
Bordeaux mixture plus lead arsenate:										
1–2–50–2, all applications	2	7	7	50	29	6	4	325	103	32
2–3–50–2, first two only	3	8	21	48	12	14	3	230	106	46
2–3–50–2, first only	4	3	26	52	43	5	2	234	131	56
2–3–50–2, all but first four	5	3	8	58	33	6	2	227	110	48
2–3–50–2, all applications	6	1	6	21	17	7	8	267	60	22
2–3–50–2, all but first	7	3	5	30	24	15	5	268	82	30
2–3–50–2, all but first and second	8	9	12	36	20	4	9	282	90	32
Control, unsprayed	9	2	32	41	26	14	3	231	118	51
Lime and tobacco dust, all applications	10	7	48	51	18	8	2	260	134	51
Lead arsenate, 2–50, all applications	11	11	19	66	16	18	7	264	137	52
Total	......	60	196	509	263	108	46	2,836	1,182	......

It will be noted that the reduction of wilt was greater with the 2–3–50 Bordeaux mixture than with the 1–2–50 mixture, although the number and weight of fruit picked was slightly greater for the weaker mixture. The wilt records show the effect of the treatments on the wilt alone, while a comparison of the number and weight of fruit for the various plats shows the combined control against both wilt and downy mildew (caused by *Pseudoperonospora cubensis*). The latter disease became prevalent during the month of August (fig. 3). For control of wilt the last five treatments had no appreciable effect. Of the remaining four applications the two most effective were made July 6 and 13, respectively, and it thus seems apparent that the greater portion of wilt infections took place during the first half of July. Reference to the beetle and wilt curves for this field (fig. 3) shows that this was during the period when the striped cucumber beetles were becoming less prevalent; but when it is known that wilt usually appears within less than two weeks after inoculation it is seen that this period of greatest probable infection corresponds with the curves showing the actual appearance of wilt (fig. 3, II and IIa) in these two contiguous spray and cage-test blocks.

During the following season (1917) another spray test was carried out near Tuxedo, Md., using Arlington White Spine cucumbers. The purpose of this experiment was to compare different strengths of Bordeaux mixture, as well as to gain further general evidence along the line of control. Applications of each mixture were made on June 13, 18, 25, July 2, 9, 17, 23, 30, and August 6, making nine treatments in all. Reference to Table II will show the different mixtures used and the numbers and percentages of wilted plants in the different plats. Two pounds of lead arsenate paste were added to each 50 gallons of Bordeaux mixture used. The total number of plants in each plat is based on the assumption of three to a hill, since they were thinned to approximately this number early in the season. Wilt in this field was not quite as serious as in the Long Island locality.

TABLE II.—*Spray experiment at Tuxedo, Md.*

Plat No.	Treatment.	Number of plants at the start.	Number of cases of wilt.	Percentage of wilted plants.
I	Bordeaux mixture plus lead arsenate, 2–3–50–2	225	37	16.4
II	Control, unsprayed	225	94	41.7
III	Bordeaux mixture plus lead arsenate, 3–4–50–2	225	35	15.5
IV	Bordeaux mixture plus lead arsenate, 4–5–50–2	225	26	11.5
V	Control, unsprayed	225	80	35.5
VI	Bordeaux mixture plus lead arsenate, 4–2–50–2	177	5	2.8
VII	Control, unsprayed	99	30	30.3

The percentage of cases in the three unsprayed plats varied from 30 to 42 per cent, while in the four sprayed plats only 3 to 16 per cent of the plants wilted. The fungicide was progressively more effective as the amount of copper sulphate was increased, the 4–2–50 formula reducing the wilt to about 3 per cent. However, the excess of copper sulphate in this last mixture seriously burned the foliage, so that its general use can not be recommended. The 4–5–50 Bordeaux mixture with 2 pounds of lead arsenate paste gives the best control of any treatment we have thus far used in our experiments.

PULLING TESTS.

Since the cucumber beetles originally become carriers through feeding on wilt-infected plants, it was thought possible that by pulling the wilted plants during the early part of the season an appreciable measure of control might be effected. Accordingly at East Marion, Long Island (May 24, 1916), 200 hills of Woodruff's Hybrid cucumber were planted in an isolated block, the location of which is shown in figure 1, Field IV. There were 800 plants in the field after thinning and at the time the first wilt appeared (June 29). Wilted plants were pulled and removed from the field 10 times through the season, on the following dates: June 29, July 11, 13, 18, 21, 27, 31, and August 11, 18, and 25. During the course of the season 249 plants wilted, or 31 per cent of the total number left after thinning. In the control, untreated plats of spray-test Field IIa in this same locality (fig. 1) the amount of the disease varied from 45 to 56 per cent, while of about 1,000 unsprayed plants in Field II (fig. 1) 60 per cent wilted. Thus the control by pulling of wilted plants was entirely as effective as that by the weaker Bordeaux mixture used.

This experiment was repeated in 1917, near Tuxedo, Md., using Arlington White Spine cucumbers. The number of plants left after thinning was 1,046, while 332, or a little less than 32 per cent, contracted wilt and were pulled during the season. The average wilt in the three untreated plats of the neighboring spray test was about 36 per cent. Thus a slight difference is to be noted in favor of pulling the wilted plants, but the result is by no means so well marked as in the Long Island test. However, the Tuxedo plat was located in a field of cucurbits and was separated from other cucumbers and cantaloupes only by a strip of watermelons 3 rods wide, while the test plat on Long Island was about one-eighth of a mile from any other cucurbits, and corn and other noncucurbitaceous crops occupied the intervening area.

Clearly some measure of control is effected by the pulling of wilted plants, but the presence of a neighboring field where this control method is not practiced may largely nullify the good results.

CONTROL IN GREENHOUSES.

The senior writer has in some cases found serious damage from wilt on cucumbers in commercial greenhouses. In these instances the striped cucumber beetle has clearly been the first source of infection, though the greenhouse workers have sometimes continued the spread of the disease with much greater efficiency than the original carrier. Cases have been noted where wilt infection has followed down the row on one side of a greenhouse bed, taking nearly every plant, while on the other side of the same bed scarcely a single case could be found. Here the evidence is clear that pruning instruments were the carriers of infection.

SUMMARY.

As a result of our studies on bacterial wilt of cucurbits during the period 1914–1918, considerable new information regarding the disease and its relations to insects has been obtained.

The disease occurs in 31 States, including the territory from Vermont and Canada to Florida and west to Minnesota, Nebraska, Colorado, and Texas. It also probably occurs in parts of California. Of our common domestic cucurbits the disease affects cucumbers, cantaloupes, summer and winter squashes, and pumpkins, but not watermelons.

The severity of the disease varies widely in different seasons and localities from an occasional wilted plant up to a destruction of 75 to 95 per cent of the crop. There is very little direct relation between percentage of infections and severity of the disease and ordinary weather conditions in the North, but there is a direct relation to prevalence of cucumber beetles and condition of vigor in the host plant.

Careful and extensive experiments have shown that infection does not come through soil or seed; that the squash bug (*Anasa tristis*), squash lady bird (*Epilachna borealis*), melon aphis (*Aphis gossypii*), potato flea-beetle (*Epitrix cucumeris*), and honeybee (*Apis mellifera*) are not wilt carriers; but that the striped cucumber beetle (*Diabrotica vittata*) and the 12-spotted cucumber beetle (*D. duodecimpunctata*) are both summer carriers and probably the only means of summer transmission of the disease in the localities studied. Infection through the breathing pores of the plant does not occur, introduction of virulent bacteria into the interior plant tissues being necessary for infection.

It has been definitely proved that bacterial wilt of cucurbits does not winter over in the soil, and all seed tests have given negative results. The disease has been carried experimentally for six weeks by striped cucumber beetles hibernated artificially in cold storage. We have thus far been unable to hibernate the beetles for a longer period.

Considerable circumstantial evidence, however, points to cucumber beetles as winter carriers. For example, among the first collections of beetles in the spring a small percentage were found to be wilt carriers. The earliest cucurbits were still very small and no wilt was anywhere in evidence. Futhermore, a careful record of spring sequence of wilt was kept in all field and garden plats of cucurbits in two localities and in all cases the leaves first showing wilt in the spring had typical cucumber-beetle injuries; the wilt had plainly started from these injured points, and there was a tendency for wilt to spread in groups around the original cases, in each new plant starting from beetle-injured leaves. In many of the cases such beetle injuries had not become general at this time throughout the fields.

After one initial feeding, striped cucumber beetles are sometimes capable of infecting at least four healthy cucumber plants successively fed upon, and after one wilt feeding some individuals in the active condition have still been capable of giving infection as long as 23 days afterwards.

Dissection and inoculation experiments have shown virulent wilt bacteria present in the intestines of a portion of the beetles fed on wilted plants, and isolations of intestinal bacteria so far as tried showed in most cases an acid-forming and gas-forming flora antagonistic to the wilt organism; but in 10 per cent (one experiment only) of the beetles used the intestinal bacteria were not inimical to the wilt organism, the latter remaining alive and causing infection in cucumber plants after three weeks' growth in mixed culture with the intestinal bacteria.

In the extensive virulence tests the various isolations of *Bacillus tracheiphilus* from different hosts and localities have shown wide and fairly constant differences in relative virulence to a given host variety. While in these tests the individual isolations have not preserved exactly the same order of relative virulence when inoculated into the different host species and varieties, the tendency has been in that direction. That is, isolations highly or weakly virulent to one variety of cucumbers have tended, respectively, to be highly or weakly virulent to another variety, and many though not all isolations highly virulent to cucumbers have been found capable of infecting squashes to a greater or less degree, while isolations of low virulence to cucumbers have invariably given no infection at all in squashes. It is entirely possible that in addition to the many races of the parasite exhibiting varying degrees of relative virulence to any particular host species there may be also two distinct strains for cucumber and squash.

Cucumbers are the most susceptible host species thus far tested and watermelons the most resistant of any species which has shown any host reaction at all. No authentic report of natural infection on watermelon has been found, and inoculations have given either negative results or at most a very slight local wilting. Cantaloupes are slightly more resistant than cucumbers, while the squash group stands next to watermelons in order of resistance.

In greenhouse tests, given similar plants to start with, very little difference in the percentage of infection or progress of the disease occurs between wet versus dry inoculations or between those made in sunlight versus shade. On the other hand, badly stunted or old plants have in general shown a much lower percentage of infection and slower progress of wilt than young, vigorously growing plants of the same variety. Ordinary variations in weather conditions have very little direct effect on percentage of infection, but may possibly have some effect on the rate of wilting. The principal essentials to maximum damage in cucumbers from wilt disease in the Northern and Middle States consist in maximum numbers of wilt-carrying cucumber beetles together with succulent, rapidly growing vines. Given these conditions, the injury will be severe with either wet or dry weather and within a considerable range of temperature.

Where the disease is likely to be severe, spraying with strong Bordeaux mixture and lead arsenate powder (4–5–50–2) is recommended. Treatments should begin as soon as the cucumber plants develop their first true leaves and should continue at intervals of about a week until the cucumber beetles practically disappear from the field. In localities where downy mildew is also prevalent the treatments should be continued later as a partial insurance against this disease. The beetles prefer unsprayed plants as food, and undoubtedly the efficiency of wilt control would be enhanced if a slightly earlier trap crop, such as squash, were planted along the edges of the cucumber field. The beetles could be poisoned there with a strong insecticide.[1]

The pulling of wilted vines during the first part of the season, or as long as it can be done without mechanically injuring the healthy plants, will greatly assist in controlling bacterial wilt if consistently done in all neighboring fields. The diseased vines should be buried or otherwise removed from access by the beetles.

Where a few plants only are grown in garden plats, screening the hills with fine mosquito netting will prevent the appearance of the disease. On a large scale this method of control obviously would be impractical.

For control in greenhouses the beetles should be kept out in the first place if possible. Do not grow cucurbits nor pile cucurbit refuse in the immediate vicinity of greenhouses, as this attracts the beetles and many will later find their way into the houses. If the beetles once gain entrance to a house filled with growing plants hand picking is the only remedy to be recommended until some fumigant is found that will kill the beetles without injuring the cucumber plants. Besides destroying the cucumber beetles, great care must be exercised in disinfecting all instruments used in pruning wilted vines before using them again on healthy plants. This may easily be done with a bottle of 1 to 1,000 mercuric chlorid and a sponge. In some cases it may be advantageous to keep the beetles out by screening doors, ventilators, and other openings into the greenhouse. For this purpose a netting with 18 meshes to the inch will be necessary.

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[1] Directions for the control of the striped cucumber beetle are given in Farmers' Bulletin 1038. (Chittenden, F. H. The striped cucumber beetle and its control. U. S. Dept. Agr. Farmers' Bul. 1038, 20 p., 15 figs. 1919.) This publication may be obtained free on application to the Secretary of Agriculture, Washington, D. C. The most approved methods as given include covering young plants, planting an excess of seed, clean culture, the use of trap plants, spraying with arsenate of lead and other arsenicals with or without Bordeaux mixture or the dry dusts, stimulation of growth, keeping the plants in good condition, and cooperation with neighboring growers of cucurbits in the use of control methods.

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