T H E S I S

A BACTERIAL ROTTING OF CELERY ROOTS.

Submitted by

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THIS THESIS HAS BEEN APPROVED AND RECOMMENDED FOR

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INTRODUCTION

Yellows of celery is a disease now present in most of the celery-growing districts of the United States. It is generally considered to be due to a fungus belonging to the genus Fusarium. A bacterial rotting of the roots of celery has been found, however, associated with the so-called yellows disease common in the Denver celery district. The consistent occurrence of this bacterial rotting, associated with the yellows disease, suggests that perhaps more than one organism is involved, or that the same name is applied to more than one disease.

It is the purpose of the following discussion to present data indicating that part of the symptoms of yellows as found in the Denver district are caused by a bacterium. This organism is not the soft rot bacterium (Erwinia carotovora (Jones) Holland committee S. A. B. 1920) but is a soil form which decomposes the remnants of celery tissue left in the soil and may, under favorable conditions, be weakly parasitic in the roots of young celery plants.

LITERATURE

Celery yellows was first reported in Michigan in 1914 by Coons (4) who discussed a new disease provisionally called the stunting disease of celery. It was first found at Kalamazoo where it had been present for seven years. Mention was made of the fact that the disease had started in a very few limited areas and that it was spreading rapidly . The infested areas were said to consist, for the most part, of spots scattered through the fields, and it was predicted that " in two or three years the majority of the infected fields will be seriously infested."

According to this report work was started on the disease in July, 1914 when specimens were collected and studied. The statements are made that " the result of this study eliminated insects, nematodes, fungi as causal organisms and indicated that some bacterial organism was responsible. The organism has been isolated and is now being tested on seedlings," and "very early we proved in the laboratory the infectious nature of the soil from the fields in question." It was said that soil treatment with chemicals was of very little value but that soil sterilization with steam was being tried.

A brief resume of the subsequent annual reports of the Michigan Agricultural Experiment Station follows. The reports

are discussed in their chronological sequence.

In the 1917 annual report Coons (5), records the rapid spread of the disease which is said to have become too serious to be controlled by steaming the soil. This increase in area and severity is attributed to wet soil and to the transfer of plants and the statement is made that "wet ground in July and August of 1915 especially favored the transfer of the disease by tracking, cultivation, etc." Buying seedlings is said to be poor practice and it is recommended that each grower "grow his own seed on clean ground."

The following paragraph is quoted from the 1918 report (6): "On the stunting disease of celery attention has been concentrated on the growing of plants resistant to the disease. Great difficulty has been met in carrying the selected plants over winter. Healthy plants winter readily but plants grown on diseased soil, although apparently sound, rot in storage, in the trenches and in the cold frame as well."

In 1919 Coons (8) stated that it was impossible to keep any of t e selected plants through the winter, and that the disease had spread to every muck area in the state but that it was localized in all but the Kalamazoo district. It is stated that " The wide spread of the disease has been accomplished by the sale of diseased seedlings."

In 1920 he reports (9) "Excellent progress has been made in the solution of the celery stunt disease, in that Mr. Nelson completed decisive experiments and has shown that this disease is typical Fusarium wilt." "Seed from a resistant plant has been obtained and tests of this seed are at present in progress."

In 1921 the report of the Botanical Section of the experiment station was made by Bessey (2) who says, "In the plant disease work, progress has been made along the study of cause and control of certain celery diseases, viz:- a Fusarium disease of celery known also as Stunt, and Red-heart, for which Dr. Coons and Mr. Nelson have determined the cause and mode of spread and have made progress in developing a disease-resistant strain -------".

Coons (11) in 1922 reports favorable results in "Development of resistant seed" and in 1923 he states (12) that "some 400 plants of known parentage were tested in the field and found resistant to the disease. These plants were brought through the winter in the greenhouse and are now producing seed."

The report for 1924 (13) deals with the increased supply of the seed of the resistant variety.

Following is taken from a discussion by Coons (7) in the 1918 report of the Michigan Academy of Science: "Last year

a field of celery at Byron Center was planted with plants bought at Kalamazoo. The disease was found everywhere in this field. This experience shows clearly how the disaase once prevalent in one locality may become scattered into every celery-growing center. It is natural for growers in times of shortage of plants in one locality to buy plants from a leading celery-growing section not only in their own states but in other state.... It is evident that this is the certain agency for the dissemination of diseases."

In 1921 the following article by Coons and Nelson (10) appeared in Phytopathology: "The stunting disease of celery first discovered at Kalamazoo in 1914, as a serious disease of the Golden Self-blanching variety, has increased in extent so that practically all soil in the immediate vicinity of that city is no longer able to raise this particular variety. The disease is also known from all other extensive districts in the state. It is also a serious disaase in New Jersey, Indiana, Massachusetts and Connecticut. Although first suspected as being of Bacterial origin, and reported by other observers as due to the joint action of bacteria and fusarium, definite proof is now available for assigning to a new species of Fusarium the causal relationship of this disease for which the name Celery Yellows is proposed. The variety Easy Blanching and all of the so-called green varieties

are tolerant to this disease. In the golden varieties excessive stuntingoccurs, accompanied by yellowing and thickening of the foliage, together with reddening of the vascular system.

The above reference to the severity of the disease in New Jersey is very interesting in that a disease of celery called crown rot, is discussed by Krout (17) in the Annual Report of the Department of Plant Pathology of the New Jersey Agricultural Experiment Station for the year ending October 31, 1916. It is noted in this report that the disease had been prevalent for over ten years in that locality, having started in a plot of ground that had a very heavy vegetation of fern growth before clearing. From this beginning it had spread to nearly every farm in that region. The cause was tentatively ascribed to a soil bacterium or to the combined action of a soil bacterium and a fusarium.

The disease is described as follows: "The organism upon its first entrance into the plant through one or several of the main side roots gives the vascular system in the roots and crown a yellowish brown appearance. Once in this system it spreads out laterally rotting the adjoining tissues. As a result of the above method of progress of the organism two or three leaves on one side of the plant are at first observed to be yellowing. This spreads to the entire foliage in from two to four weeks, causing a gradual dying of the plant."

It is further stated that Golden Self Blanching is the most susceptible variety, that "the new Easy Bleacher" is partially resistant and that the green stem varieties are entirely resistant.

The following items are from reports made by the same station in subsequent years. In 1918 Poole (18) reported that " The crown rot disease caused by Bacterium sp., has practically made the growing of Golden Self Blanching celery unprofitable throughout the muck soil districts." Another significant statement is, "In all diseased areas a small percentage of vigorously growing, Golden Self Blanching plants will be found to be immune to the crown rot organism."

In 1920 Cook (3) reports "Crown Rot (Bacerial ?) very severe in many localities", and in 1921 Poole (19) states, "Crown and Heart Rots (Bacterial) very severe in many localities." In the discussion the disease is called root rot and said to be caused by Bacillus carotovorus Jones.

According to Poole (20) "Heart Rot", "Foot Rot", "Leaf Blight" and "Browning of young roots" are the outstanding symptoms. It is also stated that "repeated isolations and inoculation experiments have proven that all these are due to the same organism." "With the exception of a few minor cultural growths, this soft rot Bacillus conforms to the descriptions of Bacillus carotorovus (Jones) and of Bacillus apiovorus (Wormwald)". "Inoculations made with this organism in a partly saturated atmosphere and at temperatures ranging from twenty-five to thirty-five degrees Centigrade have produced soft rots on the

green varieties of celery in 24 hours. Plants inoculated under normal conditions and allowed to dry rapidly gave negative results." "The green varieties previously considered being immune to the bacterium, were found to be slightly susceptible to the organism."

A comparison of the "Stunting Disease", "Celery Stunt" " Stunt", "Red-heart" , or "Celery Yellows" of Michigan with the "Crown Rot," or "Root Rot" of New Jersey indicates, at least, that they are the same disease.

APPEARANCE OF THE DISEASE

In the Field.

In the field the first visible symptoms of the disease, above ground, are stunting and chlorosis. This condition begins to appear about two weeks after the plants are set out. The stunted plants sometimes appear singly but more often in definite areas. These areas or infested spots reappear year after year in the same place and gradually enlarge. This enlarging of the old spots accompanied by the formation of new ones proceeds until in some cases the entire field is involved. The infested areas are readily recognized, the plants being for the most part much smaller and markedly yellowed, with an occasional larger normal plant. It is quite common to see a normal plant surrounded by badly diseased ones or a diseased plant growing among normal ones. This accounts for the irregular though definite borders of the infested areas. The spots of

diseased plants impart to an infested field a quite characteristic appearance.

Individual Plants.

The first evidence of disease manifested by the plants consists of a reddish brown discoloration of the tips, sometimes the sides, of the small lateral roots. This discoloration in more advanced cases, can be traced to the main root and on to the crown and rarely into the petioles. Cross sections of the diseased root show the infection area, macroscopically, as minute red dots in the center of the root. Microscopically, it is seen that the necrosis is confined to the vascular tissues. In older infected areas the entire stele is discolored and the cortex can be removed leaving the brown center. In the crown the decay at first follows the vascular tissue so that the pith appears normal and white as does the cortex while the discoloration follows the conductive tissues on both sides. Later the entire crown is involved.

Some plants show a very interesting variation. In these cases the reddish brown moist necrosis is generally more advanced than the gray brown region.

Both the decay and the yellowing of the leaves may be confined to one side of the plant while the other side appears normal.

EXPERIMENTAL DATA

Isolations from diseased tissues.

In studying the diseased roots of yellowed celery plants isolations were made from various parts of plants showing various stages of the disease. The reddish brown tips of the roots were cultured many times. They were first washed in running tap water, immersed in mercuric chloride (1-1000) for two minutes then washed in sterile water before planting on agar, in Petri dishes. Similarly treated root tips were planted in test tubes and some were dropped in boullion. Beef agar with dextrose or sucrose was used as well as potato dextrose agar. Beef broth with either dextrose or sucrose added was used in all broth cultures. Bacteria only were isolated from these small roots. After 24 hours incubation at room temperature (20-22°C) a good growth of bacteria could be seen on the agar and in the broth.

The larger roots were cultured in several ways. In some cases the piece of diseased root was trimmed and washed in tap water, dipped in mercuric chloride, washed in sterile water and dropped in flasks of sterile water. Later loops of this water was placed on agar where the characteristic bacterial colonies developed. Other roots were dipped in alcohol, then flamed, after which a shallow incision was made along one side of the root and the root torn apart so as to expose the internal lesion without its being touched. With a sterile scalpel small portions of the decayed tissue were transferred

to agar plates. The cultures from larger roots were found to contain a Bacterium and in a few cases a Fusarium.

The crowns were handled in the same manner as the larger roots. Those showing a reddish brown moist necrosis only consistently yielded pure cultures of a Bacterium. The crowns with a reddish brown moist necrosis or the vascular tissues and the gray brown porous decay of the pith yielded both Fusaria and Bacteria. In some plants the vascular infection was from one quarter to one half inch in advance of the center decay. In these plants the advanced edge of the reddish brown area provided pure cultures of a Bacterium while the gray brown area yielded a mixture of a Fusarium and a Bacterium.

Isolations were made from approximately two hundred plants from four fields. Plantings made from the same types of lesions in the same manner as described for the larger roots were made from roots that had remained over winter in the soil and were dug in April. A Bacterium was also obtained from some of these roots.

In addition to the isolation work to determine the constancy of the association of the Bacterium with the diseased plants, prepared slides were made from the necrotic tissue from the crowns. This was done by crushing small portions of the decayed tissue on slides, drying over a flame and staining with carbol fuchsin. For this work 12 plants were taken

from each of 10 fields and a slide made from each plant. When examined Bacteria were found on every slide.

EXPERIMENTAL DATA

The Organism.

In the study of the Bacterium found constantly associated with the rotted roots of cerley plants, the technic of the American Association of Bacteriologists was followed. Bergey's (1) Manual of Determinative Bacteriology was used for the taxonomic work. Three cultures of the organism were used. Throughout all the determinations, one from a root dug from the soil in the field in April, and one from one of three inoculated plants from which the organism was reisolated from the crowns. The characteristics given here were therefore determined in triplicate at the same time and under the same conditions.

(a) Morphology.

The organism is a short rod with rounded ends, measuring .6 -1 x 1.85 -3.7 microns, stained from 24 hr. dextrose agar slants grown at 20°C. It is actively motile both from agar and from broth. The flagella are polar, commonly one, rarely two. Rods occur singly. No spores have been observed. Capsules are present.

(b) Staining reactions.

The organism stains readily with carbol fuchsin, methyleneblue, gentian violet, is gram negative and not acid fast.

(c) Cultural characteristics.

Gentian	Growth scanty in slants and stable, no liquefaction in 10 days at 20°C.
Agar Colonies	Growth slow circular, smooth, convex, undulate, finely granular at 20°C.
Agar Slant	Growth scanty spreading, raised glistening, smooth, opaque, viscid no odor.
Broth	Pellicle, strongly and persistently clouded.
Litmus Milk	Acid in 48 hours, coagulation in three days, no peptonization.
Potato	Growth slow, creamy white
Indol	None in 10 days
Nitrates	Reduced to nitrites in two days
Carbohydrates	Acid and gas from dextrose, sucrose, lactose, maltose and glycerin.
Starch	Not hydrolized.

Facultative anaerobe.

The number becomes 5322-32120-2111

Good growth was obtained in dextrose broth and on dextrose agar over a pH range extending from 5.2 to 8.8. No difference could be seen in the turbidity of the broth but the best growth on the agar was at 8.

The flagella were stained with the method of Ivan V. Schunk (47). The occurrence of more than one flagellum at a pole was extremely rare. The characteristics of this organism do not check with any described in Bergey's manual. The name Phytomonas aerogenes is suggested. The readings of the sugars was 4 plus after 24 hours at 30°C. This aggressive fermentative action suggests the name aerogenes. The organism grew at 10°C and at 37°C.; the best growth was obtained between 25 and 30°C.

Some obvious differences between the organism described here and the soft rot bacterium , Erwinia carotovora (Jones) Holland, Committee S. A. B. 1920, are listed :

Phytomonas aerogenes	Erwinia carotovora.
Flagella polar	Flagella peritrichous
Capsules present	No capsules
Indol none	Indol formed
Starch not hydrolyzed	Starch hydrolyzed

INOCULATION EXPERIMENTS

In an attempt to artificially reproduce the disease the organism, previously isolated was introduced into the crowns of normal unwilted plants. Two flats of plants were used. The plants in one flat were inoculated by running a scalpel thru the crowns then transplanting the bacteria from agar slants to the insides of the crowns with a sterile needle. The control plants were cut with the scalpel but no inoculum was added. Each flat contained 20 seedlings approximately four inches tall. In two weeks all the plants were examined and no infection had resulted. The wounds made by the scalpel had healed and were covered by a thin, dry, brown, corky layer.

The second method of inoculation consisted of mixing prepared inoculum in the soil. The inoculum was prepared by autoclaving celery petioles in a flask then inoculating the sterile tissues with the Bacterium, and incubating for three days at 20 to 22°C. One flask sterilized at the same time but uninoculated, was used for the control flat. Two flats of seedlings were used . The control flat was set out first. Each of the 30 plants in this flat were set in the soil with their roots in contact with sterile celery tissue. The inoculated flat was then prepared in the same manner except for the presence of the organism. The two flats were then set in the greenhouse side by side and held under ordinary greenhouse temperature (22°C) . After two weeks the inoculated plants were noticeably stunted being about two-thirds as large as the

controls. The roots of the inoculated plants were badly decayed and the only healthy roots were the young ones that had grown out after the main root had grown below the inoculum or after the inoculum was completely decomposed. A marked stimulation of the production of fine lateral roots was evident and this resulted in the presence of young white healthy roots mixed through the decayed roots. Some of the decayed roots were cultured and the organism was recovered from lesions that extended as far as one-half inch up the main roots. The controls suffered from a somewhat similar injury in a lesser degree. That is , the fine lateral roots were somewhat decayed but not to the extent found in the inoculated flat, and the decay did not extend as far as the main root in any of them.

As attempts to carry on inoculation work in sterile soil were unsatisfactory because of the poor growth of the plants, unsterilized soil was used in all of the inoculation work. The controls consistently showed some browning of fine roots where they were in contact with the celery tissue in the soil. Examinations of replanted plants from one to two weeks after transplanting showed that merely the transplanting of seedlings results in some browning of the roots, which later disappears.

An investigation of the injury resulting from celery tissue that is in the soil and in contact with the roots of growing plants was made by setting 30 plants in each of two

flats to one of which had been added the same quantity of sterile celery tissue that was used in the inoculation experiments, in such a manner that it was in contact with the roots of the growing plants. The other flat, or control, was filled with soil from the same source and contained plants from the same source as those used in the other flat. After two weeks growth in the greenhouse they were examined and it was found that some of the fine roots of all of the plants growing in the flat to which the celery tissue had been added had suffered about the same injury as described for the controls of the inoculation experiment discussed previously. The roots of all plants in the other flat were white and normal. This information was valuable in the interpretation of the injury found in the control flats of inoculation experiments.

The growers claim that many of the plants that become infected early in the spring recover later in the season. From growers statements and observations it is clear that infection occurs soon after the plants are set out, as badly yellowed plants may be found from two to three weeks after the planting of the field in the spring. Rarely, if ever, are plants infected later in the season. These responses could be due to many factors, as the varying of the plant's susceptibility with age, differences in weather conditions, or the concentration and duration of the deleterious decomposition products of celery tissue in the soil.

The latter possibility was investigated in three ways. First, by using a mixture of autoclaved and uncooked celery petioles for inoculum, secondly, by using uncooked celery roots and crowns for inoculum material and third by using uncooked celery petioles and replenishing the supply in the soil every two weeks. All three of these tests were made at 65°F. and were set up in the same way as described for the preceding inoculation work. The roots of each plant were therefore in contact with celery in the soil. The only difference between the control flats and inoculated ones was the presence of the Bacterium in the inoculated flats. The organism was added to the latter by pouring broth cultures over the plant tissue in the soil just before setting in the seedlings.

The two flats that contained both autoclaved and uncooked celery behaved in much the same way as those previously described in which autoclaved petioles alone were used, that is, at the end of two weeks the inoculatedplants were about two thirds as large as the controls, and showed the same decay but later recovered. The recovery may have been slightly retarded but it was not appreciably so.

Two weeks after being set out the plants in the two flats to which had been added the roots and crowns did not show any difference between the controls and inoculated plants in either size or root decay. The roots of all the plants were discolored where they were in contact with the celery tissue. It may be that the slow decomposition of the more resistant

cortical tissues did not permit a sufficient accumulation of decomposition products to cause much injury to the plants.

The third test, in which inoculum prepared from uncooked petioles was added to the soil, every two weeks, was continued for eight weeks. No difference in size or color of aerial parts existed between the controls and inoculated plants at the end of this time. However, the roots of the plants in the inoculated flat were greatly injured. The fine lateral roots were badly decayed in all plants, and in 26 of the 30 plants the decay had extended into the main root. In three of these the reddish brown necrosis described above had reached the crowns and the organism was recovered from each of these three crowns. The lateral roots of all the plants in the control flat were badly decayed but in no instance did the decay get into the main root.

At ordinary greenhouse temperature raw celery petioles are generally completely decayed in the soil at the end of two weeks. In examining the roots of seedlings growing in inoculated soil it was found that all of the fine lateral roots that were in contact with decaying celery were discolored, and that above these decayed roots wer profuse tufts of young white roots. When inoculum prepared from raw celery was used in the soil an examination after two weeks invariably showed decayed roots of the growing plants in contact with the cortical and epidermal remnants of the inoculum. This condition was constant enough to be termed characteristic. In some

cases there were decayed roots that were not in contact with decomposing tissues but this was unusual, and no instances have been observed in which the roots were in contact with the inoculum and were normal. In the flats to which inoculum prepared from raw celery had been added for a longer time (eight weeks), many roots were found necrotic that were not touching or near any inoculum. This is significant in that it indicates that the continued decomposition had a more lasting effect. In these flats a very small number of the lateral roots were normal while in tests of shorter duration there were more normal roots and fewer discolored ones.

Other attempts to produce the disease in the greenhouse w re made in two ways. First, crowns of diseased plants dug in the field were used as inoculum, and second, normal plants were set in soil from infested spots in the field. In the first test 10 plants were set in flats in the greenhouse with their roots in contact with the diseased crowns. None of these plants became yellowed or stunted. However, when they were dug up after a month, the lateral roots of allplants were decayed and in two of them the decay had reached the crown. Pure cultures of the organism described above isolated from these crowns. In the second attempt two flats were filled with soil from the field, one with soil from an infested spot and one with soil from a part of the field where the presence of the disease was not noticeable. The soil from the infested

part of the field was dug from around the roots of badly diseased plants. The soil for both flats was screened through a coarse screen and seedlings from the same stock were grown in the two flats for six weeks. No yellowing or stunting occurred and the roots were normal and healthy at the expiration of the test.

To determine whether diseased plants would recover when removed from infested fields 10 diseased plants were dug and taken to the greenhouse. When these plants were dug their roots with as much soil as could be retained were wrapped in wet burlap. The plants with the adherent soil were set in pots in the greenhouse and kept under observation for eight weeks. At the end of that time all of the yellow leaves had fallen off and new ones had grown out. The plants appeared normal in every respect.

DISCUSSION.

The consistency with which bacteria are found associated with the rotted roots and crowns of yellowed celery is strong evidence that they are, at least, a contributing if not the causal factor in the yellows disease as manifest in the Denver district. The consistent presence of an organism in the advancing edges of lesions in diseased plants is strongly indicative of pathogenicity. The inoculation experiments show that the bacterium discussed in this paper may enter the roots of plants and progress up into the crowns producing a necrosis of the invaded tissues. It has also been shown that

decomposing celery tissues in contact with the roots of growing plants cause an injury that is similar in appearance to that found in the field.

The dead celery tissues that accumulate in the soil are broken down by soil organisms. The bacterium is very active in this decay and is apparently well adapted to living on dead celery tissue and may become established in soil in which large quantities of crowns and roots of celery plants have accumulated. As the soil is cropped to celery year after year conditions for the growth of this organism become more and more favorable. As the plants that are sufficiently diseased to be valueless are left in the place in which they grew and are incorporated in the soil mass the degree of infestation in an area in the field becomes cumulative. In this way the spots enlarge slowly. Infection occurs in the spring when the rate of decomposition of the celery tissues remaining in the soil from the crop of the preceding year is at its maximum.

Many organisms are known to be weakly parasitic so that they cannot affect living tissues except under particular conditions. Many so called saprophytes are known to bring about the death of living tissues in an indirect manner. These organisms are said to "kill in advance." In their work on Rhizopus rots of sweet potatoes, Harter, Weimer, Lauretzen and Adams (14, 15, 16, 21) have described such a

situation. In this work the presence of the organism and the character stic lesions indicated that Rhizopus was the causal organism but inoculations were always negative. When either the spores or the mycelium of the fungus were placed in the sweet potatoes the freshly cut surfaces became corked over and the fungus could not establish itself. It was found, however, that infection and decay followed if the fungus was grown in a sweet potato decoction for a time and a "well" dug in the potato and filled with this decoction and some of the mycelium. Careful study of the advance of the lesion showed that the tissues were killed in advance of the tips of the hyphae. Additional work showed that the need of a start was a matter of enzyme production. A similar association may exist between the roots of growing celery plants and the decomposing celery tissue in the soil.

SUMMARY

A bacterial rotting of celery roots and crowns has been found in the celery growing districts of Denver, Colorado.

The disease shows differences from the Fusarium yellows of celery.

A new species of bacterium has been isolated from the diseased plants. It is here described and the name Phytomonas aerogenes suggested.

Though the organism produces a rotting of the roots it differs from the soft rot bacterium, Erwinia carotovora (Jones)

Holland, Committee S. A. B. 1920., in morphological as well as physiological characteristics.

Inoculation studies have shown that the organism is weakly pathogenic to celery.

Accumulation of celery tissues in the soil favors the development of the root rot.

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