STUDIES ON THE BACTERIAL WILT OF THE SOLANACEAE IN PORTO RICO

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A bacterial disease of solanaceous crops has been prevalent in Porto Rico for many years, the first apparent report on the occurrence of this disease, having been made by Henricksen in 1906 (3). Outbreaks of this malady in solanaceous vegetable crops have been very severe. Since the demand for these crops had been limited to local consumption the importance of the disease was not fully appreciated until 1926 when shipments of tomatoes, peppers, eggplants and other vegetables began to be made to the United States. No sooner was planting of these solanaceous plants extended than the losses from this disease increased very materially. It was then recognized that, if the growing of tomatoes, eggplant and peppers was to be safeguarded against serious diseases, a study of the bacterial wilt was necessary. The writer was asked in the autumn of 1926 to undertake an investigation of the malady with special reference to its control. It is obvious that certain other points had to be studied to some extent in order to gain a better knowledge of the relations between the pathogen and its suscepts such as would clear the way for the all-important subject of control.

SUSCEPTS

VARIETAL SUSCEPTIBILITY

As a knowledge of the susceptibility of solanaceous crops is indispensable, preliminary trials were made in the growing season of 1926–27 with commercial varieties of tobacco, peppers, tomatoes and eggplant. The potato was added later, as information on the susceptibility of this suscept seemed important, since certain trials had shown it to be a promising new crop for the Island. Seed of all these crops was obtained from various sources in the United States.

The susceptibility of varieties of pepper, eggplant and tomato was determined by seed-bed tests and by studies in the field after transplanting. It was observed during the first year's work that the disease developed to some extent in the seedlings, especially on tomato and eggplant and to a lesser extent on pepper. The seed was

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sown in rows, four inches apart, in flats or beds. The soil used in these contained a large quantity of manure from a source known to be heavily infested with the wilt organism. As soon as any seedlings showed symptoms of the disease they were removed, a record being taken of the number of seedlings destroyed.

Seedlings were pulled from the seedbed when small, and planted in flats at a distance of four inches each way. Here they remained until large enough for transplanting in the field. The plants were removed with a ball of earth, reducing any temporary check in development as is the case when seedlings are transplanted without soil about the roots. A second advantage is that the seedlings suffer less and do not have the tendency to spindling growth, if for any reason transplanting is delayed. The less crowded condition helps in producing a hardier, stronger plant. A third advantage is the fewer chances of loss of seedlings during transplanting. It has been our experience that the mole-cricket attacks but few of the seedlings when they are transplanted according to this method.

The number of plants of each variety grown varied with the crop. The varieties were planted successively using four replications to reduce error.

Final counts were taken at the time when the second picking of fruit was made. Counts of diseased individuals in the seedbed and in the field are recorded together.

The results of incidence of the disease are given in the following tables.

TOMATO

TABLE 1.

INCIDENCE OF DISEASE IN COMMERCIAL VARIETIES OF TOMATOES, FIELD PLANTING

Variety	Population	Healthy	Diseased	% Diseased
Ponderosa	200	51	149	74.5
Pear Shaped Yellow	200	74	126	63.0
Gulf States	200	76	124	62
Louisiana Pink	200	79	121	60.5
Payne's Victory	200	83	117	58.5
Alameda Trophy	200	85	115	57.5
Early Detroit	200	89	111	55.5
Avon Early	200	91	109	54.5
Red Rock	200	94	106	53
San José Canner	200	94	106	53
Dwarf Champion	200	95	105	52.5
Maule's Matchless	200	102	98	49
Morse's Globe	200	102	98	49
Delaware Beauty	200	103	97	48.5
Bonny Best Early (Maule's)	200	104	96	48
Morse's No. 493, Special Early	200	109	91	45.5
Maule's Success	200	112	88	44
Marvana (U. S. D. A.)	300	188	112	37.33
Norton Wilt Resistant	300	189	111	37.0
Morse's Norton	300	192	108	36.0
Marvelosa (U. S. D. A.)	300	203	97	32.33
Morse's Marglobe	300	210	90	30.0
Stoke's Marglobe	300	218	82	27.33

From the above table it is clear that most commercial varieties of tomatoes are very susceptible to bacterial wilt. Ponderosa proved to be the most susceptible, almost three fourths of the population succumbing to the disease. Next in susceptibility is the Pear-Shaped Yellow (63 per cent) and the Gulf States (62 per cent). In a group ranging in susceptibility from 50 to 60 per cent, there are eight varieties. In a similar group from 40 to 50 per cent susceptibility, appear to fall six other varieties and between 30 and 40 per cent diseased plants there are four varieties. The least susceptible of the varieties were, in order of resistance: Marglobe (U. S. D. A.) (Plate XXXVI) with 23 per cent diseased plants; Marglobe (Stoke's) with 27.33 per cent, and Marglobe (Morse's) with 30 per cent diseased individuals. From the grower's standpoint a variety with a susceptibility higher than 30 per cent should not be recommended. It appears, that perhaps the Marglobe is the only tomato which may be grown with a reasonable degree of safety. It happens to be a good type, and the preliminary trials with it on the Island have shown it to be a good marketable tomato. Another tomato which proved to be somewhat resistant (32.33 per cent diseased) is the Marvelosa, distributed by the United States Department of Agriculture. It should be tested further.

A resistance test was also made of eight non-commercial Porto Rican varieties in order to locate resistant plants for breeding with varieties commercially desirable. Each one of these varieties was given a number in our records. They may be described briefly as follows: No. 1, small, about ½ inch–¾ inch in diameter, round; No. 2, slightly larger than No. 1; No. 3, pear-shaped, with longitudinal undulations, about 2 inches–3 inches long; No. 4, more or less oblong, about 2 inches × 1½ inches; No. 5, typically pear-shaped, small; No. 6, flat, large, so-called "platillo" (plate-like), much wrinkled; No. 7, flat, small, smooth; No. 8, flat and smooth, intermediate in size between No. 6 and No. 7.

Two hundred plants of each kind were grown. The symptoms of the disease appeared shortly after transplanting. The final data were collected in July, 1927. The results show that all plants died in all the strains except No. 6 in which 78.5 per cent were diseased.

It is evident that none of the native tomatoes are resistant and, therefore, are not to be used as material for breeding for disease resistance.

PEPPER

The results of tests with commercial varieties of pepper are given in table 2.

TABLE 2.

INCIDENCE OF DISEASE IN ELEVEN COMMERCIAL VARIETIES OF PEPPER.

Variety	Population	Healthy	Diseased	% Diseased
Early Giant	184	135	49	26.63
Ruby Giant	172	129	43	25.00
Tomato Salad	192	146	46	23.96
Large Bell	188	143	45	23.92
Neapolitan	185	143	42	22.70
Improved Ruby King	188	149	39	20.74
Crimson Giant	183	146	37	20.22
Ruby King	178	142	36	20.20
Chinese Giant	191	155	36	18.85
Worldbeater	189	154	35	18.52
Bull Nose Pepper	162	134	28	17.28

It will be seen that the populations are not uniform for the varieties, although 200 plants of each variety were set in the field. The reduction is due to injury by the mole-cricket to the seedlings which were not very large at the time of transplanting.

From the results given in the above table, it may be inferred that only Early Giant and Ruby Giant were heavily affected. However, the differences in susceptibility among the remaining varieties are not very significant. Three varieties Bull Nose, Worldbeater and Chinese Giant appear to have greater resistance than the others. Ruby King, Crimson Giant and Improved Ruby King are almost equally resistant.

Sixteen different forms of Porto Rican pepper were collected from various places in the Island and tested during the summer of 1927 in the same way as the imported commercial varieties.

Briefly described these forms are as follows:[1] No. 1, flat, wrinkled, hot pepper, about 1 inch in diameter; No. 2, hot, ovoid, 2 inches-2½ inches long, pointed at the blossom end; No. 3, long, large, hot, with a few longitudinal undulations; No. 4, "ají caballero", very small, hot, more or less conical and usually known as *Capsicum Baccatum;* No. 5, long, narrow, sweet, smooth, ends pointed, light green color; No. 6, Bull Nose type; No. 7, long, rather broad, angled, with blunt tip, about 6 inches long, light green color, sweet, so-called "Mallorquín"; No. 8, large, short, broad, almost as long as it is broad, dark green color, resembling the imported Chinese

[1] Descriptions for Nos. 10, 11, 12, and 14 are omitted. These forms were all nearly alike and similar to No. 9.

Giant, sweet; No. 9, a peculiar hot pepper with narrowed ends, broadened at about the lower third and pointed at the blossom end, purple toward the peduncle when not ripe, long peduncled, red when mature, so-called "ají platero" in Coamo; No. 14, a medium-sized sweet pepper with blunt tip, light green color; No. 15, rather long and broad, smooth, pointed abruptly at the blossom end, ratio of length to width 2:1, sweet; No. 16, extra long, sylindrical, hot pepper, so-called "ají picante" in Cayey.

One-hundred plants of each form were tested for resistance and the results are given below in the order of susceptibility: No. 6, 29 per cent diseased; No. 8, 27 per cent; No. 10, 23 per cent; No. 5, 21 per cent; No. 7, 21 per cent; No. 2, 19 per cent; No. 14, 18 per cent; No. 1, 17 per cent; No. 13, 17 per cent; No. 3, 16 per cent; No. 9, 16 per cent; No. 12, 16 per cent; No. 15, 16 per cent; No. 11, 15 per cent; No. 4, 12 per cent; No. 16, 12 per cent.

It is evident that the forms Nos. 6 and 8 are even more susceptible than the imported varieties given in table 2. These are, unfortunately, the two most desirable types in shape and size and the ones which would, therefore, offer the most suitable material for breeding work. The most resistant of the native forms are the hot peppers Nos. 4 and 16, both of which showed 12 per cent infection. A group with percentages of infection ranging from 15 to 19 includes forms Nos. 11, 3, 9, 12, 15, 1, 13, 14 and 2. These are given in order of relative resistance. Of the sweet peppers in the group the best in size and shape are Nos. 14 and 15. The remaining three forms, Nos. 5, 7, and 10 are undesirable; the first and third because of their shape and size and the second,—"mallorquín", because of its very light color and tender skin which does not stand much handling.

In general, it may be said that the hot peppers showed much higher resistance than imported sweet peppers, and that native forms of sweet pepper were not significantly higher in resistance than the imported varieties.

EGGPLANT

The following varieties of eggplant have been tested during the four-season period: six imported and five from the Island. Table 3, shows the relative susceptibility of each variety. The forms from Porto Rico are given in bold-face type.

TABLE 3.

INCIDENCE OF DISEASE IN IMPORTED AND PORTO RICAN VARIETIES OF EGGPLANT.

Variety	Population	Healthy	Diseased	% Infection
Black Beauty	500	72	428	85.60
Excelsior	500	85	415	83.00
New York Spineless	250	44	206	82.40
Large Round Purple	250	45	205	82.00
New Orleans Market	250	57	193	77.20
University Pink	250	69	181	72.40
Florida High Bush	500	152	348	69.06
Pompadour Purple Striped White	250	81	169	67.60
Long Purple	250	118	132	52.80
Camuy	500	444	56	11.20
Long Green	500	484	16	3.20

It is very evident that imported varieties of eggplant are extremely susceptible to the bacterial wilt and that, therefore, they can not be planted in infested soils without considerable risk. The varieties from Porto Rico are very resistant with the exception of "Pompadour" and University Pink. It is very unfortunate, however, that these resistant varieties do not possess the qualities of a commercial variety. The Long Green (Plate XXXVII) for instance, the most resistant of all (only 3.20 per cent infection) is a long, gourd-like green fruit and the "Camuy" (11.20 per cent infection), is a small, cylindrical purple fruit. The Long Purple, another form from the Island is of similar shape as the long green, but as will be seen from the table, it is quite susceptible to the disease.

The most susceptible variety was found to be the Black Beauty (Plate XXXVIII) with 85 per cent infection, followed closely by the Excelsior (83 per cent), New York Spineless (83.40 per cent), the Large Round Purple (82 per cent) and the New Orleans Market (77.20 per cent). Of the imported varieties, the most resistant appeared to be the Florida High Bush with 69.06 per cent infected plants, but it is doubtful if this variety can be successfully grown in infested lands on a commercial scale.

POTATO

Four varieties of potatoes of those which are being tested in the Island were planted in order to find out whether they were susceptible to the wilt. These were as follows: Bliss Triumph, Irish Cobbler, Green Mountain, and Spaulding Rose.

Twenty-five hills of each were planted in the field where the tests with eggplant, pepper, and tomato were conducted. The disease appeared on all varieties three weeks after planting and all plants were

infected by the sixth week. This experiment, although not dealing with a large population, indicates the high susceptibility of these varieties to the wilt, so that these potatoes should be planted here with this fact in mind. Infested fields should not be planted to potatoes.

TOBACCO

The following varieties of tobacco have been planted in infested land: "Virginia Blanco", the standard Porto Rican filler; "Ceniza"; "Vuelta Abajo"; a mammonth type labeled Station; Consolation (Yellow); Porto Rico Wrapper No. 1; "País"; Turkish; and two forms imported from Colombia, South America. Not a single case of the disease has appeared in any of the varieties. Wounding of the roots was practiced in order to increase the chances for the entrance of the causal agent. This was done by digging a number of plants of each variety when they were about a foot in height. The roots were then severely pruned and injured and the plants set again in their places. No cases appeared even with this treatment. It should be noted that in these experiments the susceptible Black Beauty eggplant was used as a check.

Another scheme was followed. Black Beauty eggplants which showed unquestionable symptoms of the wilt were dug out and tobacco plants set in their place. Even in this case the tobacco plants failed to become affected with the disease.

One hundred tobacco seedlings in pots and an equal number of plants about one foot in height, also in pots, were inoculated on roots, stems, and leaves. The cultures employed were isolated from eggplants and tomato plants. Six plants from the first lot became badly wilted, but a study of the lesions and a microscopic examination of them showed this wilting to have been caused by *Phytophthora Nicotianae*. All the remaining plants developed normally to maturity, bore seed, and when finally examined revealed no traces of injury by the inoculum.

From the above it seems that this bacterial wilt does not affect tobacco in Porto Rico.

INFECTION OF NORMALLY RESISTANT EGGPLANT VARIETIES

To ascertain whether normally resistant varieties of eggplant will contract the disease if artificially inoculated, a set of fifty plants of the Long Green variety was inoculated in 1928 with masses of bacteria oozing out of the vascular bundles of diseased Black Beauty

eggplants. Inoculation was made on tender twigs and on the woody stems by lifting the bark, injuring the woody tissues and transferring the inoculum into these wounds. Twenty-two of the fifty plants took the disease but it did not become generalized. From this experiment it appears that the disease may be induced on a normally resistant variety of eggplant through inoculation. The Long Green variety was shown in table 4 with a percentage of infection of only 3.2 while here 44 per cent have been infected.

RECOVERY OF DISEASED EGGPLANT

Recovery of diseased plants of resistant eggplant varieties has been found to occur. In the season 1929–30 it was found, for the first time, that a few plants of the Long Green variety which had been labeled as wilted or wilting had completely recovered. A closer inspection of the field was made and all plants that showed symptoms of the wilt were tagged on January 3, 1930. Bi-weekly observations were made recording the new cases of wilt or cases of recovery. The data collected up to March 9th. is as follows: out of 32 wilted plants, seven recovered and 25 died, a recovery of 21.89 per cent. Whether this may be expected in a larger population cannot be said. Similar counts with diseased Black Beauty eggplants were made and it was found that none of the wilted plants recovered. This would indicate that recovery takes place only in the resistant varieties.

GENERIC AND SPECIFIC SUSCEPT REACTIONS

It has been shown in the foregoing that the bacterial wilt of some solanaceous crops in Porto Rico severely affects eggplant (*Solanum Mclongena* L.), pepper (*Capsicum baccatum* L., potato (*S. tuberosum* L.) and tomato (*Lycopersicon Lycopersicon* (L.) Karst.). The higher susceptibility of potatoes, tomatoes, and eggplant as compared to peppers is very clear from the data furnished in the preceding paragraphs.

Wilted zinnias (*Crassina Elegans* (Jacq.) Kuntze), were examined and an organism similar to or identical with that obtained from the eggplant, potato, and tomato, was isolated from them. A few cases of the disease on *Solanum torvum* L., a common weed, have been observed in the Station plots. The organism isolated from it is indistinguishable from that obtained from the aforementioned suscepts. The disease, however, is not very common on this suscept. The disease also occurs on the deadly night-shade (*Solanum Nigrum* L.).

Seaver and Chardón (6) list a wilt disease of solanaceous crops caused by *Bacterium solanacearum* E. F. Sm. and they evidently had in mind the same disease with which we have been dealing. *Solanum Wrightii* Benth., a recent importation from Panamá, has been repeatedly inoculated without success. Tobacco (*Nicotiana Tabacum* L.) has always failed to take the disease either through inoculation or under natural conditions. This is of particular interest in these studies because what is held to be the same disease occurs on tobacco in the United States where it is known as the Granville wilt. The same or a similar wilt is also found in many other tobacco countries.

Smith (l. c.) states that he received a report from Mr. Iorns, of Porto Rico, in which he held that *Solanum Mammosum* is resistant to what was, evidently, the disease under consideration.

The disease, then, appears to affect seven species of the higher plants, in four different genera from two families, the Solanaceae and the Carduaceae.

SYMPTONS

MORPHOLOGIC SYMTOMS

On the eggplant.—The first symptoms of the disease on eggplants are evident as a slight yellowing of the tender leaves of one or several shoots, followed by a severe wilting of these parts. The symptoms may appear on one branch alone or on several or may involve the whole plant. The period elapsing between the appearance of the first symptoms and the complete death of the suscept is usually from 7–10 days in the case of the very susceptible varieties like the Black Beauty, but it may be two weeks or longer in resistant varieties. In rapidly growing plants with abundant tender vegetative parts, the symptoms may have the appearance of lesions produced by scalding with boiling water or scorched by intense sun light.

On resistant varieties the symptoms may appear on one branch only which may be gradually killed while the rest of the plant remains healthy for a long time. Very frequently this is also affected. This is a very peculiar behavior of the eggplant, similar cases not being observed on any of the other suscepts. As discussed elsewhere in this paper a large proportion of the affected individuals of resistant varieties may recover from the disease.

In advanced cases the symptoms are those of the necrotic type, especially during rainy weather, when the tissues are overrun with

saprophytes. The leaves may show a rotting which invades the petiole and later the stem.

When plants are pulled and examined it is found that a large number of the roots have rotted, and upon lifting the cortex, a dirty white to creamy exudation, swarming with bacteria, is discovered.

On tomatoes.—The symptoms on tomatoes appear as a sudden wilting of the twigs and branches as in the eggplant. They resemble wilting produced by a cutting of the vascular bundles at some place below the wilted portion. They also resemble the condition resulting from attack by some sucking insects. The disease seems to start more frequently in the roots, which are much affected, and from there the organism works up the conducting system into the vegetative parts. Incipient roots are extensively developed on the stems of affected plants. It should be noted that this symptom has been reported for the same, or a similar disease, which occurs in other countries. Another symptom often observed, is a swollen condition of the stems. This has been reported by Smith (8) and others.

In the year 1927, during a very rainy season, some tomato plants showed a browning and rotting of the petioles of wilted leaves. Cankers were evident at the axils of the leaves. Isolations of a bacterium were made from the cankers and upon injection into healthy tomatoes, the characteristic wilting was produced, thus linking this symptom with the wilt. It is thought that the cankers were a result of the continuous rainy weather which helped to create a condition in the plant whereby they responded differently toward the organism. These symptoms were not repeated by inoculation, nor were they encountered in the succeeding seasons.

On peppers.—On this suscept the disease is characterized by a yellowing of the leaves at the margins. The discoloration may only include a few leaves, before the complete wilting of the plant occurs. Rolling or curling up of the yellowed leaves may occur, a symptom which has not been found on any other suscept. Peppers appear to be able to live longer than eggplant, tomato, or potato after the appearance of the first symptoms. Affected plants may gradually become defoliated before they finally succumb to infection.

On potatoes.—On the potato the disease is characterized by a yellowing of the leaves of young or immature shoots, or a sudden withering and death of stems and leaves. The disease spreads very rapidly in potato plants, one after the other succumbing to its ef-

fects. The symptoms in advanced stages are similar to those of plants which are undergoing a natural death.

In general, it may be said that the symptoms appear on the underground and all above-ground parts except the flowers and fruits.

HISTOLOGIC SYMPTOMS

Stems of all the suscepts show a browning of the vascular bundles which may extend from the roots to the petiole and main veins of the leaves. This browning of these tissues is very characteristic and is used in diagnosing the disease in those suscepts where morphologic symptoms are not very clearly defined. When infection begins on the leaves, the browning works down the veins into the midrib, thence down the petiole and into the stem. The bacteria which cause the disease are found massed in the vascular bundles and in great abundance in the stem in the region near the surface of the soil and in the roots. When either the affected roots or stems are cut transversely, the bacteria will ooze out in a short time in the form of a dirty white to creamy exudation.

In advanced stages the pith is also involved. In tomatoes and potatoes it soon breaks down into a soft mass; while in the egg-plant and pepper it is slowly decomposed, the stems retaining their rigidity and finally becoming hollow.

In potatoes the pathogen extends into the tuber, resulting in a browning of the bundles there as in the stems. Thus, when an affected tuber is cut transversely, a browned ring will be evident underneath the superficial cork layer. In large tubers which have recently contracted the infection the vascular ring is browned only a short distance beyond the stem end. If these are kept for a few days, however, the browning extends to the opposite ends and in still later stages a rotting of the tubers from the ring inwards with complete destruction is reached in ten to fourteen days.

ETIOLOGY

IDENTITY OF THE PATHOGEN

The identity of the pathogen causing the bacterial wilt of some solanaceous crops in Porto Rico has not been definitely established, although it has been referred to as *Phytomonas solanaceara* (E. F. S.) Com. S. A. B. (*Bacterium solanacearum* E. F. Sm), by Henricksen (**3**), Stevenson and Rose (**9**), Stevenson (**10**), Thomas (**11**), Ló-

pez (5), and Seaver and Chardón (6). It is interesting to observe that Smith (8) basing his opinion on tomatoes and eggplants received from this Island, regarded the disease identical with the brown rot of the Solanaceae which occurs in the United States and other countries. This is probably the first report of the disease in which the organism from Porto Rico was studied.

Suspicion that the pathogen might be an altogether different one was raised when Cook (1) reported that the organism causing a bacterial disease of eggplants at the Insular Experiment Station would infect tomatoes and peppers, but not tobacco, and also by the fact that the author also failed to get infection with it on tobacco.

The organism was isolated from various suscepts, cultural studies made, and cross-inoculations performed in an effort to further elucidate the problem.

CULTURE CHARACTERS

On nutrient glucose agar: A dirty white colony which produces a brown discoloration at the end of four to five days.

On steamed potatoes: A gray white colony producing pronounced browning.

On potato cylinders: A dirty white growth, staining the potato brown and causing a slow rot. The rotted portion becomes browned first and finally blackened.

On glycerin agar: Colonies dirty creamy to dirty white with only very slight browning.

On Litmus milk: Litmus is slightly reduced and curdling does not set in.

On milk: No curdling produced.

On nutrient bouillon (plus peptone): browning occurs and there is also some precipitation.

On peptone water: No browning.

On Dunham's solution: Pnorounced clouding.

On lactose agar: Growth dirty white producing much browning after eight to ten days.

No gas production was observed in fermentation tubes in peptone water or bouillon with the following sugars: dextrose, maltose, lactose, dextrine, laevulose, or saccharose. There was no clouding in the column of medium at the closed end of the tubes which indicates an aerobic organism.

The behavior on the above media was alike for the following cultures:

No. 1 and 4	From eggplant	Experiment Station 1926
No. 6	From eggplant	Manatí 1926
No. 7 and 9	From tomato	Experiment Station 1926
No. 12	From tomato	Manatí 1926
No. 14	From zinnia	Experiment Station 1927
No. 18 and 19	From potato	Cayey 1927
No. 20 and 21	From eggplant	Cayey 1927
No. 24 and 27	From tomato	Experiment Station 1927
No. 28 and 30	From pepper	Experiment Station 1927
No. 33	From eggplant	Experiment Station 1928
No. 36	From *Solanum Torvum*	Experiment Station 1928
No. 37	From tomato	Experiment Station 1928
No. 38	From *Solanum Nigrum*	Experiment Station 1928

These cultures have been given in the order in which they were isolated. The irregularity in numbering is due to the fact that transfers were made and sub-cultures numbered before browning of the medium occurred and before the results of inoculations had been secured when a number of cultures were destroyed, thus accounting for the missing numbers in the series.

The organism proved to be gram negative.

From cultural characteristics alone it would seem that the organism that is responsible for the disease in eggplant, tomato, zinnia, potato, and pepper is probably the same.

PATHOGENICITY STUDIES

A large number of cross-inoculations have been made with the organism from the various sources. Considerable difficulty was experienced in this work since the organism appears to lose its virulence quickly in ordinary culture media and especially because the colonies from poured plates are similar during the first few days to those of non-pathogenic bacteria which also appear in the same plates.

The following scheme was at first adopted: plants were inoculated with the organism from the colony from which the subculture was made, usually five or six days after plating. The organism appeared to be virulent at this age. Later it was found that the organism retains the ability to infect its suscepts if it is carried in potato tubers or milk. The organism was most virulent when transferred directly from the affected parts to the plant to be inoculated. In this case the parts used for isolation were the bases of the stems, cut usually near the soil level. The portions of the stems were sterilized in mercuric chloride, after which the lower end was still further cut back with a sterile knife. These portions were then placed in sterile petri dishes, the bacterial ooze showing on the lower cut surface in a few minutes. Inoculations made with this ooze scarcely ever failed.

Almost invariably the inoculations were made on tender twigs or shoots at the axils of the leaves. It was only in the case of tobacco

that inoculations were made directly on the main stem, a few inches above the surface of the soil.

Infection through the roots was obtained merely by removing large plants from pots on which they had been growing, shaking out the soil around a few of the larger roots, wounding these and then planting in infested soil.

In every case the plants were cut after the wilt symptoms were evident and examined for browning of the vascular bundles, when the inoculations were pronounced as positive or negative.

The following varieties were used for inoculation Black Beauty: eggplant, "Mallorquín" and Ruby Giant peppers, Ponderosa tomato, and Porto Rico Filler tobacco. The potatoes were secured in the market and the variety is unknown.

The results are given in the following tables:

TABLE 4.
RESULTS OF INOCULATIONS AND CROSS-INOCULATIONS ON VARIOUS HOSTS—INOCULUM FROM FRESH COLONIES

Culture		Tomato		Eggplant		Pepper		Potato		Tobacco	
No.	Source	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
1	Eggplant	6	0	6	0	4	2	5	1	0	6
2	Eggplant	0	4	0	4	0	4	0	4	0	4
3	Eggplant	0	4	0	4	0	4	0	4	0	4
4	Eggplant	4	0	4	0	4	0	4	0	0	4
5	Eggplant	0	4	0	4	0	4	0	4	0	4
6	Eggplant	4	0	4	0	3	1	4	0	0	4
7	Tomato	4	0	4	0	3	1	4	0	0	4
8	Tomato	0	4	0	4	0	4	0	4	0	4
9	Tomato	4	0	4	0	2	2	3	1	0	4
10	Tomato	0	4	0	4	0	4	0	4	0	4
11	Tomato	0	4	0	4	0	4	0	4	0	4
12	Tomato	4	0	4	0	3	1	4	0	0	4
13	Tomato	0	4	0	4	0	4	0	4	0	4
14	Zinnia	4	0	4	0	3	1	4	0	0	4
15	Zinnia	0	4	0	6	0	6	0	6	0	6
16	Zinnia	0	4	0	6	0	6	0	6	0	6
17	Zinnia	0	6	0	6	0	6	0	6	0	6
18	Potato	6	0	6	0	4	0	4	0	0	6
19	Potato	6	0	6	0	3	1	4	0	0	6
20	Eggplant	6	0	6	0	.....	.....	.....	.....	0	6
21	Eggplant	6	0	6	0	.....	.....	.....	.....	0	6
22	Eggplant	0	6	0	6	.....	.....	.....	.....	.....	.....
23	Eggplant	0	6	0	6	.....	.....	.....	.....	.....	.....
24	Eggplant	6	0	5	1	.....	.....	.....	.....	0	6
25	Eggplant	0	6	0	6	.....	.....	.....	.....	0	6
26	Eggplant	0	6	0	6	.....	.....	.....	.....	0	6
27	Eggplant	6	0	6	0	.....	.....	.....	.....	0	6
28	Pepper	6	0	6	0	6	0	6	0	0	6
29	Pepper	0	6	0	6	0	6	0	6	0	6
30	Pepper	6	0	6	0	5	1	6	0	0	6
31	Pepper	0	6	0	6	0	6	0	6	0	6
32	Eggplant	0	6	0	6	.....	.....	.....	.....	0	6
33	Eggplant	6	0	6	0	.....	.....	.....	.....	0	6
34	Eggplant	0	6	0	6	.....	.....	.....	.....	0	6
35	Eggplant	0	6	0	6	.....	.....	.....	.....	0	6
36	*Solanum Torvum*	6	0	6	0	6	0	.....	.....	0	6
37	Tomato	6	0	6	0	.....	.....	.....	.....	0	6
38	*Solanum Nigrum*	6	0	6	0	6	0	.....	.....	0	6
39	*Solanum Nigrum*	0	6	0	6	.....	.....	.....	.....	0	6

The results in table 4 show clearly that the pathogen in eggplant, pepper, potato, and tomato is identical. It is striking that in no

case did tobacco become infected, a total of 196 inoculations having failed.

TABLE 5.

RESULTS OF INOCULATIONS WITH OOZE FROM THE STEMS

Source	Eggplant		*Solanum Torvum*		*Solanum Wrightii*		*S. Nigrum*		Zinnia		Tobacco		Tomato	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Eggplant..	24	1	0	36	0	24	12	0	6	4	0	36	12	0
Tomato...	24	0	0	24	0	24	12	0	8	2	0	36	12	0

When the bacterial ooze, both from eggplant and tomato, was used as inoculum, infection was obtained on tomato, eggplant, *Solanum nigrum,* and on zinnia. Tobacco, *S. Torvum* and *S. Wrightii* did not become infected.

TABLE 6.

RESULTS OF INOCULATIONS ON ROOTS

	Eggplant		Tomato		Pepper		*Solanum Torvum*		Tobacco	
	+	−	+	−	+	−	+	−	+	−
Wounded	22	0	22	0	11	1	1	23	0	24
Not wounded	39	11	41	9	28	22	0	24	0	50

From the results in table 6 it may be concluded that invasion through wounded roots takes place readily in infested soils, hence the practical conclusion may be drawn, that these solanaceous crops should be carefully cultivated so that the danger from disturbing the roots may be minimized as much as possible. The amount of infection in the different suscepts was as much as could be expected from susceptible varieties in a heavily infested soil. It is significant that tobacco did not succumb to the disease even when roots were heavily wounded. *Solanum Torvum* did not take the disease, except in one case, a result which is in harmony with that in table 5. This behavior of this wild suscept from which an organism similar in every respect to that recovered from eggplant, tomato, and the other suscepts was obtained, is rather peculiar. That a few plants were once infected by the organism, as must have been the case in the few individuals in which the disease was recorded and from which the organism was isolated, may have been due to a certain set of predisposing conditions within or without the plant or to great virulence of the invading pathogen. This is as far as we are able to explain. It is obvious that further studies on this suscept should be made.

LIFE HISTORY

The pathogen seems to live over in soils for a long time. In the Experiment Station grounds it has existed in the soil for at least ten years. This estimate is based on reports that the author has had from former horticulturists.

Plant remains from previous crops are an important source of inoculum. Manure has been found to be a carrier of the pathogen which appears to be able to live in it. The manure at the Insular Station has been shown to be infested with the organism, hence the infestation of all soils fertilized with it. The infestation of manure was proven by the following experiment. Six tin cans (5–gallon capacity) were filled with well-rotted manure, another six with soil known to have produced healthy eggplants and still a third series with a 1–1 mixture of such soil and manure. Healthy Black Beauty plants about six inches high were transferred with a ball of earth around the roots (these were slightly injured) to these cans which were kept in the greenhouse. All the plants grew to normal development until the first flower bud appeared, when two of those in the cans with soil and manure and three in those with manure alone showed unmistakable wilt symptoms. Three weeks later all the plants in the cans with either manure alone or with the mixture of soil and manure had contracted the disease. The plants in the cans with soil alone remained healthy to maturity.

Invasion occurs under natural conditions through leaves and roots. In plants which have been beaten strongly by the wind the lower leaves are much injured by the constant friction against the soil and it is these leaves which are often the first to be infected. Injured roots also provide entrance for the pathogen. As far as we have been able to ascertain the pathogen is only capable of invading plants through wounds. In the inoculation experiments given in the preceding paragraphs it would seem that this is not the case, because when plants were not injured a large number of them succumbed to the attacks of the malady. But it should not be forgotten that the roots of all the suscepts employed in these experiments were always subject to a certain amount of injury by animal pests.

In the experiment with manure in cans, where these soil inhabitants were obviated, the pathogen, no doubt, gained entrance through the wounded roots. By reason of unsuitable equipment we failed to conduct an experiment where these soil pests could be included.

During the growing season of 1929–30 the green beetle (*Diabro-*

tica graminea Baly) swarmed on our eggplants following which the disease appeared to extend considerably over a large field. In order to determine whether this insect was at all responsible for the dissemination of the pathogen a series of experiments was begun, using wire cages. Six healthy plants (Black Beauty eggplant) were covered with these cages. At the end of ten days they were examined and found to be healthy. The insects (*Diabrotica*), which had been fed for six days with twigs of diseased plants on which the leaves had wilted and the brown stain was evident, were then transferred to these cages, ten to fifteen to each cage. The insects were kept in the cages for two weeks when the first case of wilting was found in one of the cages. A second case appeared four days after the first and two more plants showed the disease twenty days after the insects had been transferred to the cages. Two plants remained healthy throughout the season. Six plants, also covered with cages, but from which the beetle was excluded, did not show any wilt symptoms. It may be concluded that *D. graminea* is a disseminating agent of the disease. (Plate XXXIX).

EPIPHYTOLOGY

The pathogen seems to attack most frequently plants growing in low, wet places, but the disease also appears on higher, well-drained locations. From our observations it may be inferred that the malady is more prevalent in heavy soils; nearly all the cases reported to us have come from sections having heavy soils. The disease is very severe in Río Piedras and in the Cidra-Cayey district where the soils are predominantly of the heavy class. But, in the sandy loams of the intervening section of Caguas no cases have ever been found by the writer or reported by others.

The appearance of the disease is in no way related to the age of the suscepts. We are inclined to believe that it coincides with certain seasonal changes, as for example, periods of continued rains followed by short periods of dryness. In our plantings the first cases were observed during one such rainy period following a dry season.

CONTROL

General eradicatory measures are always helpful in reducing the foci for inoculum and consequently furnish important means of control. However, they do not go very far in preventing heavy losses to our solanaceous vegetable crops. The removal of diseased plants is to be recommended but it must not be overlooked that the causal

agent lives in the soil in which it is capable of existing for a long time. A practical method of soil treatment which will kill the organism is desirable. But such a measure does not seem likely to be developed with our present knowledge of the malady. Preventive measures appear to us the most logical solution for the problem.

BREEDING FOR DISEASE RESISTANCE

The preliminary trials with varieties of eggplant, pepper and tomato, have made it clear that breeding for resistance is not promising for the latter two crops, since native and imported commercial varieties were more or less equally susceptible to this disease. Consequently, development of resistant strains in these must be sought through selection within the commercial varieties. On the other hand, some native varieties of eggplant showed a very remarkable superiority in resistance over any of the imported varieties. Unfortunately their shape, size and color are not suitable for the market thus making it impractical to extend their cultivation for commercial ends but they are suitable for crossing with desirable commercial sorts to secure desirable resistant plants.

Breeding work on tomato and pepper was not started by the author because he was engaged on other problems. It is only on the eggplant that he has endeavored to develop resistant strains.

Several crosses were made in 1926 between the imported susceptible varieties of desirable shape and color and those of Porto Rico showing decided resistance to the malady. Much progress has been made in this line and some of the results will be reported on later. Several strains which combine the desirable characteristics of both parents in each case, have been isolated and are under trial.

It was even thought that strains might be developed from the susceptible types. With this purpose in mind two selections in the Black Beauty variety were made in 1927. One of these was discontinued in 1928. That year two other selections were made from the one retained. Although in 1929 these did not show any higher resistance than the plants raised from imported seed, yet two further selections were made. These were given a final test in 1929–30 when they were abandoned because of their high susceptibility.

GRAFTING ON RESISTANT STOCKS

Grafting the eggplant on *Solanum Torvum* has been suggested by Iorns (4) for the control of diseases. Eighteen grafts of the Black beauty eggplant on *Solanum Torvum* were made in 1929–30 of which

only eight lived. The stock had been planted in our most heavily infested plots. All the grafts developed excellently without showing the symptoms of the disease. Of these plants five were destroyed by heavy winds, breaking the stems at the junction of stock and scion. The three remaining plants grew to maturity, bearing many fruits. They were then inoculated with ooze from Black Beauty diseased plants and all contracted the disease. This shows that as long as infection depended on invasion through the root system the plants were safe; but when the inoculum was introduced into the stems of the scion the disease developed. This seems to indicate that the stock has no influence on scion in respect to resistance.

GENERAL CONTROL MEASURES

Certain control measures which are always helpful should be enumerated here: (*a*) proper drainage of the land, (*b*) careful cultivation to prevent root injury, (*c*) care in transplanting to injure the least number of roots, (*d*) avoiding the use of manure which has given indications of being infested with the pathogen, and (*e*) avoiding, when possible, the heavier soils.

DISCUSSION

A bacterial disease present in Porto Rico on some solanaceous crops was considered as identical to that of the Southern United States for the first time by Henricksen (**3**) who based his conclusion on the similarity of the morphologic symptoms of the Porto Rican disease and that of the continent. Later the pathogen was determined as *Bacterium solanacearum* E. F. S. by Smith (**8**) who had received some diseased plants from Mr. Iorns of the Porto Rico Agricultural Experiment Station at Mayagüez. The disease has been subsequently attributed to Smith's pathogen, but no experimental work has been done previously in Porto Rico to prove that assumption. Cook (**1**) in 1924 raised the question of the identity of the eggplant wilt organism which he believed would not infest tobacco. The author has found that the eggplant organism will not infect tobacco but the disease produced by it on the other solanaceous crops is evidently very similar. Still the doubt remains as to whether it is *B. solanacearum* which is responsible for the disease, since this organism causes a wilt of tobacco in other countries. The fact that the organism isolated in Porto Rico from the other suscepts has never infected tobacco leads to the conclusion that either tobacco is resistant, or that the organism is distinct from *B. Solanacearum* or is a strain of it unable to infect tobacco.

The symptoms of the disease described elsewhere agree in every respect with those of the Porto Rican malady. The description given for *Bacterium solanacearum* E. F. S. is essentially that for the pathogenic organism isolated from the various suscepts. The organism is gram negative; it develops as white to dirty white colonies which produce a brown stain on many media, especially on sugar media; it loses its virulence very readily in most culture media but retains it in potato tubers and in milk; it does not curdle milk or reduce litmus; does not cause the fermentation of sugars and is aerobic. Although the writer admits that much more extensive work should be done with the pathogen before its identity can be definitely established, he is inclined to regard it as a strain of *B. solanacearum*. The only reason why it should not be considered as identical is the fact that tobacco is not infected by it. We think that, in all probability, we are concerned with a strain different from that occurring on the continent. A second possibility is that all tobacco in Porto Rico is resistant to *B. solanacearum*. In the final elucidation of the problem the tobacco from Porto Rico should be tested in the Southern United States, for instance, or the varieties from the continent should be introduced and exposed here to the pathogen. To conclude, we tentatively assign to the Porto Rican organism the rank of a strain which is incapable of infecting Porto Rican tobacco. Since the virulence of the organism is maintained only with difficulty it did not seem possible to obtain cultures from abroad for use in inoculating our tobacco.

SUMMARY

1. A bacterial disease of solanaceous vegetable crops has been prevalent in Porto Rico for many years.

2. The disease affects tomato, potato, eggplant, and pepper; *Solanum torvum, S. nigrum* and zinnias become infected occasionally, while tobacco appears to be immune.

3. All imported varieties of tomato, pepper, and eggplant are more or less susceptible. The Marglobe and the Marvelosa tomatoes and the Bull Nose, World beater, Chinese Giant, Ruby King, Crimson Giant and Improved Ruby King peppers are very resistant. The most resistant of the imported eggplants is the New Orleans Market. The most susceptible of all suscepts used are the Ponderosa tomato, the Early Giant and Ruby Giant peppers, and the Black Beauty eggplant. Four varieties of potato were found susceptible.

4. Peppers are more resistant than either eggplants or tomatoes.

5. Native varieties of eggplant are more resistant than the im-

ported sorts; but all native peppers and tomatoes are very susceptible. Hot peppers proved to be more resistant than the sweet peppers.

6. The disease is very severe on tomatoes and to a less extent on eggplants, at the seedling stage.

7. Normally resistant varieties of eggplant may contract the disease if artificially inoculated.

8. Some diseased plants of the resistant eggplant recovered from the disease.

9. The symptoms of the disease are characterized as a yellowing and withering of the tender shoots and leaves and finally a complete wilting of the affected plant. Sometimes lesions having the appearance produced by scalding water or scorched by strong sunlight are evident. Cankers are formed occasionally in tomatoes. Histologic symptoms consist of a browning of the vascular bundles.

10. The pathogen is culturally indentical with *Phytomonas solanaceara* (E. F. S.) Com S. A. B. *bacterium solanacearum* E. F. S. or very similar to it. In cross-inoculation studies it is also similar to it, but is unable to infect tobacco. We are of the opinion that it is only a strain of that pathogen.

11. The organism loses it virulence very readily on most artificial culture media.

12. Successful inoculations with the pathogen in culture were obtained when the inoculum was used directly from the original isolation on the poured plate. Inoculations with the bacterial ooze obtained in more or less uncontaminated condition from the vascular bundles of diseased plants scarcely ever failed.

13. Natural infection occurs through wounds in the leaves and roots.

14. The pathogen may be disseminated by the green beetle, *Diabrotica graminea* Baly.

15. The pathogen seems to be harbored in manure, plant remains, etc., and appears to exist in soils for a number of years.

16. It is believed that the first appearance of the disease coincides with periods of continued rainfall followed by periods of drought.

17. The production of resistant strains of the various crops seems to offer a logical means of control. Breeding work in the eggplant is in progress.

18. Grafting of susceptible varieties on *Solanum Torvum* only eliminates the chances of root infection.

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Explanation of Plates

Plate XXXVI. A tolerant tomato plant at left and a susceptible (wilted) one at right.

Plate XXXVII. Upper. A susceptible variety of egg-plant.
Lower. A resistant variety of egg-plant.

Plate XXXVIII. An advanced case of wilt in the Black Beauty egg plant.

Plate XXXIX. At left—a wilted tomato plant.
At right—a cage used in the study of insect transmission.

PLATE XXXVI.

PLATE XXXVII.

PLATE XXXVIII.

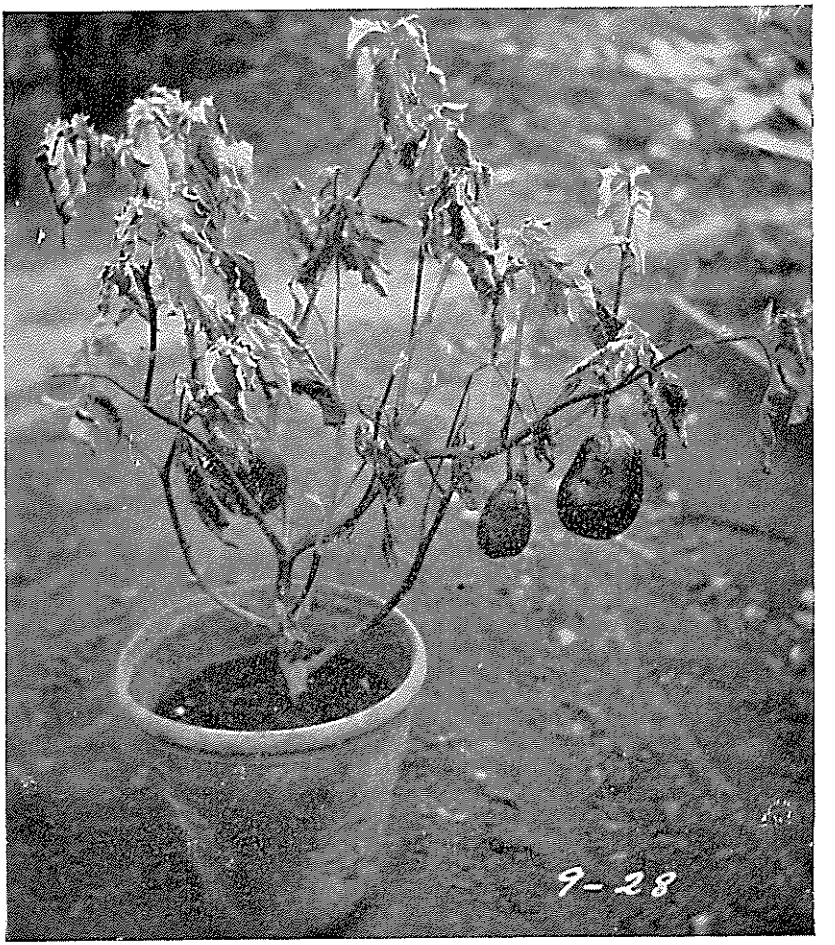

PLATE XXXIX.