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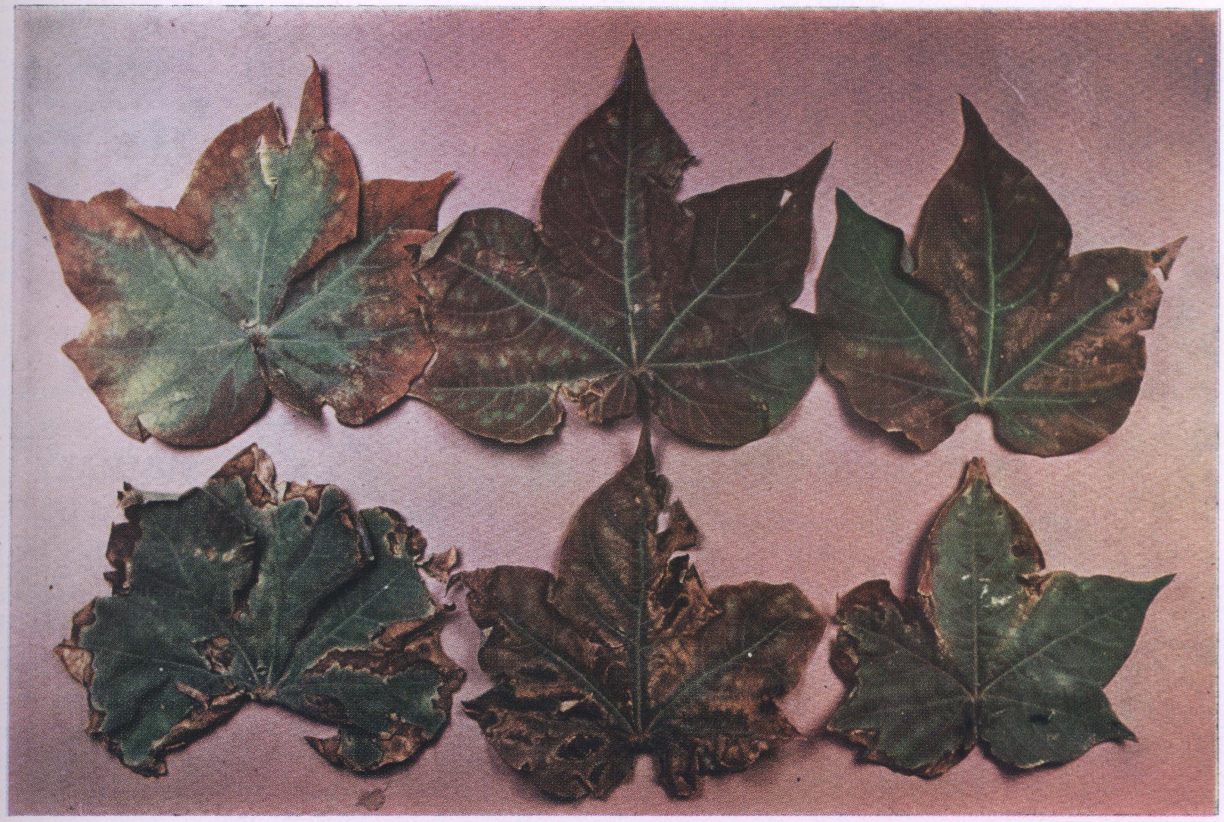
Pseudomonas Wilt of Cotton



THE AGRICULTURAL AND MECHANICAL COLLEGE OF TEXAS
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IN COOPERATION WITH THE
U. S. DEPARTMENT OF AGRICULTURE

CONTENTS

SUMMARY

A hitherto unreported disease of cotton is described. Pseudomonas wilt is the name suggested for the disease.

Host inoculation experiments indicate that the casual organism is *Pseudomonas syringae* van Hall.

Seedling symptoms are stunting and slow emergence combined with root lesions. Plant symptoms are yellowing, reddening and dying of leaves. These leaves usually are shed. The main stem and root have brown to black discolored areas in the pith.

Pseudomonas wilt occurred extensively in Texas during 1958-60. It is considered a major cotton disease of considerable economic importance.

FIGURE 1 – ON THE FRONT COVER

Leaf symptoms: upper left, the leaf margin turned red; upper center, entire leaf is red with brown necrotic spots; upper right, the leaf margins are necrotic. Lower left, the brown necrotic areas become more pronounced and they have thin yellow margins. Lower center and right are additional leaves with the intermediate stages. The leaves are frequently shed before the advanced symptoms develop.

Pseudomonas Wilt of Cotton

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A PREVIOUSLY UNREPORTED BACTERIAL DISEASE of cotton has been under investigation at this laboratory since 1955. The disease caused serious losses in Texas during 1958-60. This publication describes the disease and gives the results of completed experiments. The results of preliminary experiments were reported earlier (2).

BACKGROUND

In a survey (8) began in 1955 of fungi associated with the seedling disease complex of cotton in Texas, a high frequency of association of non-parasitic nematodes with a bacterium was found. The nematodes were identified as being *Aphelenchoides parietinus* (Bastian) Steiner and *Aphelenchus avenae* Bastian. The initial experiment, similar to previously reported work (1, 4), was to determine the role of the nematodes in the seedling disease complex of cotton. The bacterium was used for culturing the nematodes on an agar medium. The results showed that the bacterium, whether the nematodes were present or not, infected cotton seedlings and caused slow emergence, stunting and in some cases destruction of the primary root. The bacterium was reisolated and another pathogenicity test was run. This was repeated and the results of the third test are shown in Figure 2.

A wilt type malady of cotton occurred extensively during August and September 1958 in Southeast, Northeast, North and Northwest Texas. The roots of the plants were sound and there was no vascular discoloration. There were brown to black discolored areas in the pith of the main stems and roots. Leaf and stem symptoms are shown in Figures 1 and 3. A bacterium, similar to the one isolated from seedlings in 1955, was isolated from the stems. This was used for inoculating plants by wounding the stem in old leaf scars. The only symptom resulting from these inoculations was stunting of the new growth of the main stem above the point of inoculation.

Bacterial wilts have been reported on a number of crops but cotton is not among these (3, 5, 12). *Pseudomonas solanacearum* E. F. Sm. isolated from castor bean and tobacco was reported as infecting young cotton seedlings in inoculation experiments (10). Seedlings inoculated by wounding the hypocotyl showed marked stunting and wilting. *Pseudomonas tabaci* (Wolf & Foster) Stevens also infected cotton leaves in inoculation experiments (6). *Pseudomonas solanacearum* was found associated with abyan root-rot of cotton (7). However, results of inoculation experiments have been negative.

As has been pointed out, *Pseudomonas syringae* van Hall has a wide host range, attacking lilac, stone fruits, Hibiscus sp., beans, sorghums, clovers and citrus (3, 5, 12). It is distributed throughout the world and causes leaf spots, die-backs, cankers and wilting. On lilac, *P. syringae* attacks primarily the parenchyma and infection may spread through the vascular system causing wilting of leaves (5).

MATERIALS AND METHODS

Experiments were conducted with acid delinted seed of the Deltapine 15 and Auburn 56 cotton varieties. Pathogenicity tests with seedlings were conducted with clorox sterilized plastic pots and with steam sterilized washed builders sand. The bacteria and agar medium were mixed into the sand used for covering the seed. Medium without the bacteria was used for the control.

Early pathogenicity tests were conducted in a greenhouse where the temperature was maintained at approximately 80°F. Recent tests were conducted indoors under artificial light of about 1,500 foot candles intensity. Pots were alternated between 80 and 60°F. for 24-hour periods for the first 4 days, after which they remained at 80°F.

Individual plants were inoculated by placing a reservoir of inoculum around the main stem. A cone shaped drinking cup was used for the reservoir (Figures 7 and 8). The stem was cut through the bark into the vascular area at a point below the surface of the inoculum. The inoculum was prepared by washing the bacteria from the surface of the agar medium. Potato-carrot-dextrose-peptone agar was the medium used for isolating and culturing the causal organism. Inoculum concentrations were determined with the dilution and plate count method.

Several bacterial isolates and reisolates from cotton were used in the experiments. The culture of *P. syringae* was obtained from James Davis via

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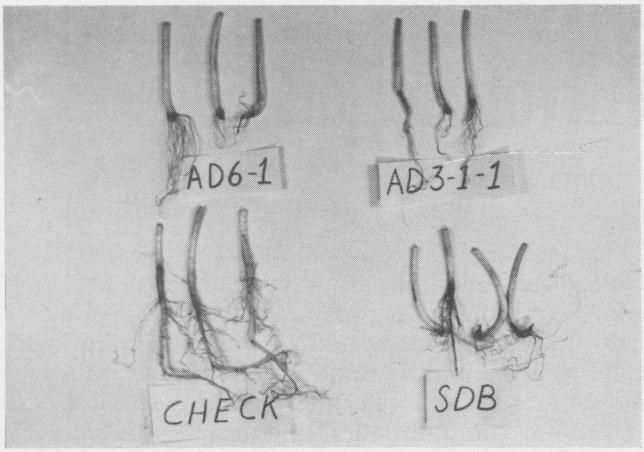

Figure 2. The results of an early pathogenicity test with isolate SDB, a reisolate of it, AD6-1, and a reisolate of AD6-1, AD3-1-1. This also shows the severe seedling phase where the primary root is destroyed. (Check = Control)

Figure 4. A replication of the Auburn 56 seedlings from the test reported in Table 1. The seedlings in the three pots on the left, which were inoculated with P. *syringae* isolates, emerged slower and are stunted.

R. H. Garber, University of California, Davis. The culture was described as follows: D-15, isolated by Davis, 12-17-56, from healthy almond leaves. It had been observed to be pathogenic on red kidney beans, cherry, peach, plum, almond and pears. The culture of *P. solanacearum* was obtained from Arthur Kelman, North Carolina State College, Raleigh.

The laboratory and greenhouse experiments were replicated four and five times. The field experiments with individual plants were replicated 8 to 20 times.

RESULTS AND OBSERVATIONS

A hypothesis was formed that the seedling and plant symptoms were manifestations of the same disease and that infection occurred primarily in the seedling stage. In the first experiment for testing this hypothesis, bacteria were mixed into the soil used for covering the seed. There were no striking seedling symptoms, however, plants growing in the inoculated soil were shorter than the controls and leaf symptoms developed which were similar to those observed in the field. The bacterium was reisolated from the leaves and stems.

Additional seedling tests for determining the pathogenicity of isolates and reisolates were made. The results of two of these are given in Tables 1 and 2. Seedling symptoms from one of these tests are shown in Figures 4 and 5. The stunting of seedlings in these experiments by *P. syringae* is similar to the stunting by *P. solanacearum* reported by Smith and Godfrey (10).

A seedling inoculum potential test was conducted. Measurement data from this test are given in Table 3 and the results are shown in Figure 6.

The cup inoculum reservoir technique for stem inoculation was effective and convenient for rapid pathogenicity tests with plants. This technique was used for conducting an inoculum potential test with field grown plants. The reservoir cups were filled

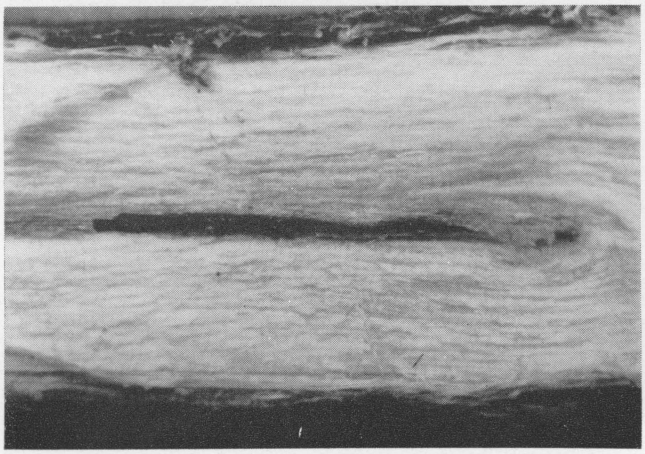

Figure 3. A closeup of a longitudinal split section of a stem showing a black streak in the pith region. This discoloration varies from light brown to black.

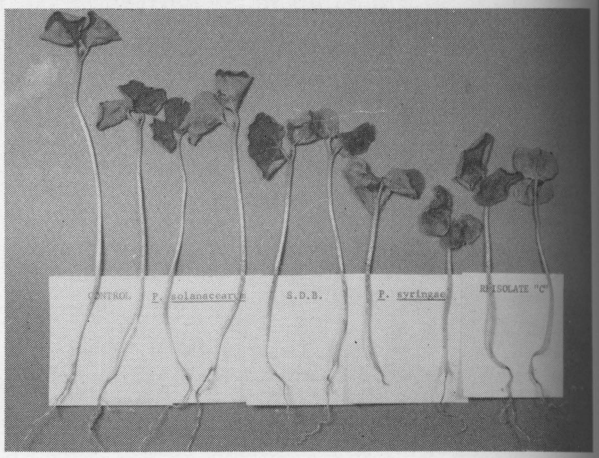

Figure 5. Auburn 56 seedlings, from the test reported in Table 1 and shown in Figure 4, 6 hours after being washed from the potting sand. The brown to black constricted zones are more apparent after the seedlings dry.

TABLE 1. RESULTS OF A SEEDLING PATHOGENICITY TEST WITH SEVERAL ISOLATES AND THE COTTON VARIETIES DELTAPINE 15 AND AUBURN 56[1]

Isolate designation	Seedling height (cm.) 13 days from planting			Seedlings emerging (%)					
				Six days from planting			Nine days from planting		
	D-15	Au. 56	Average	D-15	Au. 56	Average	D-15	Au. 56	Average
Control	7.75	9.13	8.44	67.23	89.08	78.16	73.48	95.33	84.40
P. solanacearum[2]	6.43	8.50	7.21	43.80	81.28	62.54	57.83	89.10	73.46
S.D.B.[3]	5.38	6.98	6.18	56.28	70.33	63.30	70.35	95.35	82.85
Isolate C[4]	4.65	5.53	5.10	25.00	1.58	13.29	68.78	85.95	77.36
P. syringae[5]	3.80	5.20	4.50	21.90	6.25	14.08	65.65	98.45	82.05
Variety averages	5.60	7.07		42.84	49.70		67.21	92.84	
L.S.D. (5% level):									
Isolate averages			0.99			17.49			n.s.d.
Isolates within varieties	1.40			24.70			n.s.d.		

[1]The variety averages for all measurements differed significantly at the 1% level.
[2]Isolate obtained from Arthur Kelman.
[3]Isolate from cotton seedlings.
[4]A reisolate from leaves of a cotton plant inoculated with *P. syringae.*
[5]Isolate obtained from James Davis.

with solutions of "Reisolate *P. syr.*" containing zero, 300 million, 750 million, 1,500 million, 2,250 million and 3 billion bacteria per milliliter. Symptoms appeared within 3 days on leaves, above the point of inoculation, of all plants receiving the bacterial concentrations. No symptoms occurred on the control plants. The symptoms on the plants receiving the lower concentrations disappeared within a few days. Those on the plants receiving the higher concentration, however, were more pronounced and persisted.

A similar test for pathogenicity evaluation of isolates and reisolates was conducted on field grown plants. The results of this test were positive in that all P. *syringae* isolates produced symptoms and *P. solanacearum* did not.

Stem die-back obtained with the cup inoculation technique is shown in Figure 7. A similar die-back of fruiting and vegetative branches was observed on field grown plants naturally infected with *P. syringae.*

Leaf symptoms obtained by using the cup inoculation techniques on leaf petioles are shown in Figure 8. The bacterium was recovered easily from these leaves.

The original isolate obtained from cotton seedlings was identified as *Pseudomonas* sp. This isolate infected leaves of sorghum (5). The isolates from cotton and the *P. syringae* isolate from California gave positive reactions on green lemons (9). The *P. syringae* isolate and reisolates of it from cotton gave positive tests on red kidney beans (11). However, the isolates obtained originally from cotton produced questionable results on the beans. The isolate of *P. solanacearum* did not cause stunting or slower emergence of seedlings. It did cause root lesions, as

TABLE 2. RESULTS OF A SEEDLING PATHOGENICITY TEST WITH SEVERAL ISOLATES AND REISOLATES

Isolate designation		Seedling height (cm.) 14 days from planting	Seedlings emerging (%)	
			Seven days from planting	Nine days from planting
Control		10.50	98.76	98.76
P. solanacearum	(Au56)[1]	10.04	93.76	93.76
P. syringae	(Au56)[1]	5.38	41.26	87.54
Reisolate of *P. syr.*[2]		4.68	27.52	65.04
Reisolate "C"[3]		4.24	13.76	66.28
S.D.B.	(Au56)[1]	3.38	3.76	53.78
Isolate "C"	(Au56)[1]	3.10	2.50	22.52
L.S.D. 1% level		1.68	37.27	39.68
L.S.D. 5% level		1.24	27.42	29.19

[1]Reisolate from seedlings of Auburn 56 grown in the experiment reported in Table 1.
[2]Reisolate from leaves of a cotton plant inoculated with *P. syringae.*
[3]Reisolate from leaves of a cotton plant inoculated with isolate C.

Figure 6. Results of an inoculum potential test 8 days from planting. Back row, left to right, no inoculum, inoculum from 2 plates and 4 plates. Front row, left to right, 6 plates, 8 plates and 10 plates, respectively.

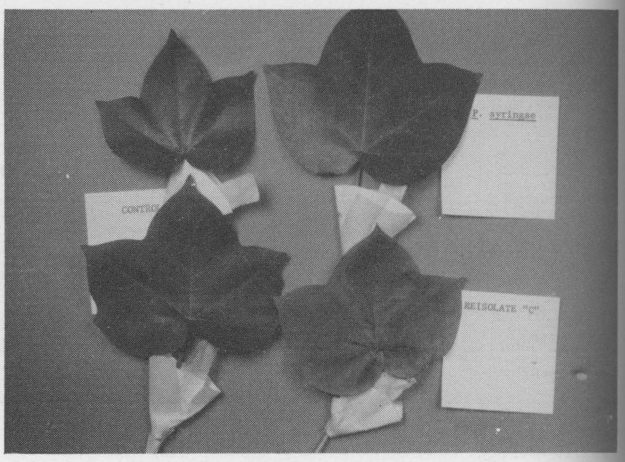

Figure 8. Leaves inoculated on the petioles by using the cup technique. Yellowing of the left lobe of the upper right leaf is a typical early symptom. The yellow mottling of the lower right leaf with the right lobe beginning to die at the lower margin also is a typical early symptom.

shown in Figure 5. It did not induce leaf symptoms on cotton plants. The *P. solanacearum* isolate gave negative results on red kidney beans and green lemons. It, along with the *P. syringae* isolates, gave negative results when inoculated to tobacco. The failure of *P. solanacearum* to infect tobacco in these experiments could have been the result of improper techniques and not the lack of pathogenicity of the isolate.

Preliminary histological studies, as shown in Figure 9, indicate that the bacteria invade the parenchymatous cells of the pith and vascular areas. They also invade the endodermal cells.

Numerous isolations were made during 1959-60 from cotton plants collected from all areas of Texas. *P. syringae* was recovered from about 80 percent of the plants whether they were healthy looking or not. Isolations made from plants having typical Verticillium wilt symptoms recovered *P. syringae* consistently along with *Verticillium albo-atrum* Reinke & Berth. It was not unusual to recover *P. syringae* from these plants without recovering *V. albo-atrum.*

Isolations with cottonseed frequently recovered *P. syringae* from poor quality seed, but not from high quality seed.

Observations made during 1960 in cotton variety tests at several locations suggest that the early maturing varieties are more susceptible to *P. syringae,* while the intermediate and late maturing types are respectively less susceptible.

DESCRIPTIONS

Symptoms of the seedling phase of Pseudomonas wilt are stunting and slow emergence. The plants from these seedlings frequently remain stunted throughout the season, as shown in Figure 10. Under cool conditions the cotyledons tend to remain yellow

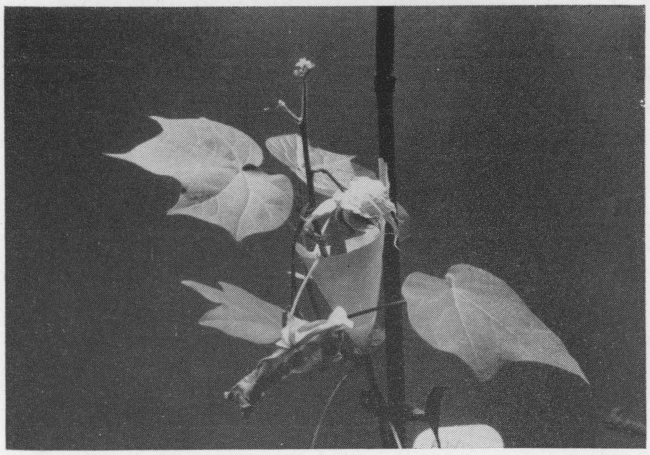

Figure 7. Stem die-back resulting from cup inoculation. The leaves have been shed. Note the boll bracts are dead and the boll is opening prematurely. The healthy leaves are attached below the point of inoculation on the main stem.

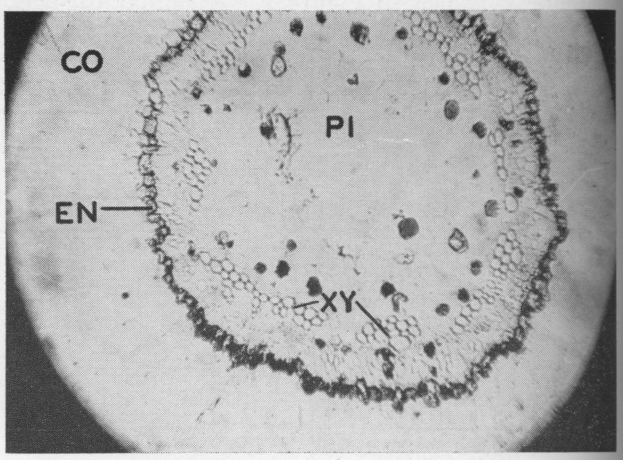

Figure 9. Photomicrograph of a stem cross section in the vascular transition zone. The bacteria are in the endodermal and pith cells which are darker than the surrounding cells. Methylene blue was the stain used. Co, Cortex; en, endodermis; xy, xylem; pi, pith.

for longer periods. The roots may have only slightly constricted lesions at the vascular transition zone or they may become so severe that the primary roots are destroyed. The plants are frequently stunted, however, in some cases the difference is not pronounced. The leaf symptoms vary considerably. Apparently they are influenced by the existing environment, especially light and temperature, and by whether the symptoms develop slowly or rapidly. The leaves may yellow and then become reddish with necrotic areas appearing in the leaf blade and along the margins. Frequently the marginal points of the leaves die first. In other cases the leaves redden along the margins. These areas become necrotic and shatter. Such leaves are shed from the plants. Occasionally leaves may die very fast, turn gray and are not shed. On splitting the main stem of plants lengthwise, brown to black areas will be found in the pith. This usually is more pronounced in the stem-root area.

Another symptom, which has been produced artificially only a few times, is dying of the boll bracts. If this occurs early in boll development the entire young fruit may die and usually is not shed from the fruiting branch. *P. syringae* has been isolated consistently from the bracts and calyx of such bolls that died under natural conditions in the absence of insect injuries.

The causal organism, *Pseudomonas syringae* van Hall, is a rod shaped bacterium 0.75 to 1.5 by 1.5 to 3.0 microns. It is motile with one or two polar flagella. It gives a gram-negative reaction and is an aerobic, facultative parasite. In culture on beef-extract agar, the colonies are circular, grayish-white with a bluish tinge. The colony surface is smooth with entire or irregular edges (3).

DISCUSSION

The results of the host inoculation experiments, and the results obtained with the California isolate and its reisolates from cotton, indicate strongly that Pseudomonas wilt of cotton is caused by *Pseudomonas syringae*. The failure of the isolates from cotton to infect red kidney beans, however, raises some doubts as to whether the identification is correct. The positive results obtained with green lemons lends strength to the identification. Additional identification tests are under way.

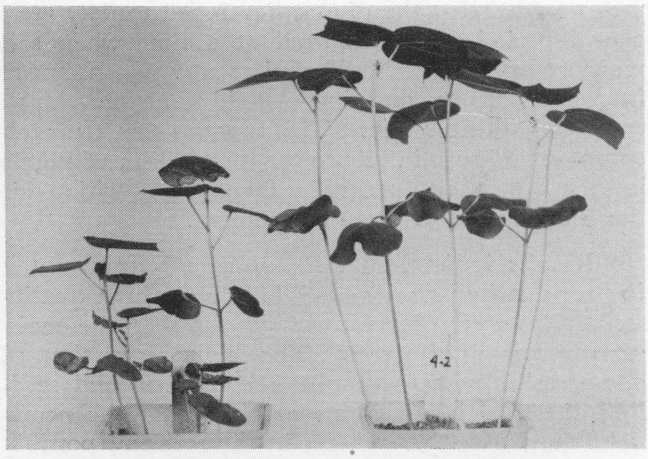

Figure 10. Six-week-old plants grown from inoculated seedlings, left, and uninoculated seedlings, right. The diseased plants on the left frequently do not recover from the initial stunting.

The culture of *P. solanacearum* failed to cause seedling stunting, slow seedling emergence or cause symptoms on cotton plants. It did cause slight lesions on the roots of seedlings. This, along with the fact that *P. solanacearum* type cultures are isolated frequently from cotton, suggests that it may, in some way, be involved in Pseudomonas wilt. This possibility will be investigated further.

The inoculum potential tests emphasized that many bacteria must be present for the disease to develop. In the isolation experiments, *P. syringae* was recovered from a high percentage of the healthy plants. This and the high association of the bacteria with seedlings indicate that under natural conditions the infestation rate of cotton plants is high. Whether the disease develops undoubtedly depends on the host providing a favorable nutrition for a build-up of the bacterial populations to an inoculum level sufficient to cause the disease. Observations in the Lubbock

TABLE 3. RESULTS OF AN INOCULUM POTENTIAL TEST WITH SEEDLINGS

Inoculum level (number of culture plates used)		Seedling height (cm.) 13 days from planting	Seedlings emerging (%)	
			Five days from planting	Eight days from planting
Control		9.30	93.76	95.00
Two plates		8.78	88.82	98.76
Four plates		7.66	58.76	96.28
Six plates		7.72	68.76	98.76
Eight plates		6.36	27.50	95.02
Ten plates		5.28	15.00	73.76
L.S.D.	1% level	2.39	41.12	n.s.d.
	5% level	1.76	30.26	

area revealed that the disease developed rapidly after a cool period which occurred at a time when soil moisture was marginal. A similar situation occurred in the laboratory and inoculated plants developed symptoms within 3 days. This suggests that temperature and moisture influence the metabolism of plants in such a way as to provide a favorable nutrition for the bacteria and disease development.

Results indicate that *P. syringae* is transmitted in poor quality planting seed, but not in high quality seed. However, observations suggest that the main carryover is in the soil. The high association of non-parasitic nematodes with the bacteria and seedling disease points to the possibility that the nematodes may carry the bacteria in the soil and possibly could play a major role in inoculating seedlings.

The results indicate that in many cases there is an association between Verticillium wilt and Pseudomonas wilt. The possibility that these two diseases may form a complex that is more severe than either alone is being investigated.

These results prove that *P. syringae* must be added to the list of organisms causing cotton seedling disease. Initial control experiments, at this location, will involve applying bactericides with fungicides as in-covering soil applications at planting. They also will involve protection of young seedlings from nematodes as a possible indirect control measure. The response of varieties and breeding material to *P. syringae* is under investigation.

ACKNOWLEDMENTS

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