Susceptibility of *Cyphomandra betacea* to *Pseudomonas solanacearum*

CARLOS MARTIN and URSULA NYDEGGER, Pathology Department, International Potato Center, Apartado 5969, Lima, Peru

ABSTRACT

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Seventy-day-old seedlings of tree tomato (*Cyphomandra betacea*) were inoculated through soil infestation and into the stem with 13 strains of *Pseudomonas solanacearum* from six countries and isolated from six different host species. All three races and the four biotypes of the bacterium were included. In infested soil, only biotypes I and II, which were isolated from potato and tomato, caused tree tomato plants to wilt. When plants were stem inoculated, all four biotypes caused wilt. A strain isolated from *Heliconia* sp. (banana) did not induce wilting by either inoculation method. An increase in temperature from 22 to 32 C increased the percentage of wilted plants. When larvae of the root-knot nematode (*Meloidogyne incognita acrita*) were introduced together with *P. solanacearum* into the soil, the percentage of wilted tree tomato plants increased substantially.

Tree tomato (*Cyphomandra betacea* (Cav.) Sendt.) is a non-tuber-bearing member of the Solanaceae grown in warm regions of South America for its edible fruit, from which fresh juices and marmalades can be prepared. Although it is not an important commercial crop, it provides supplemental income for small farmers in the highland tropics (1,800–3,200 m) (2).

In some places (eg, Antioquia in Colombia and Huanuco in Peru), tree tomato plants are grown where potatoes are produced on a large scale. Tree tomato and potato plants with symptoms of bacterial wilt were observed in Rio Negro, Antioquia, Colombia, in 1975 (2,10). Isolates were studied at the International Potato Center (CIP) and found to be *Pseudomonas solanacearum*, race 3. Bacterial wilt of tree tomato is now considered to be the most important bacterial disease affecting that plant in Colombia. Severity of the disease increased when the root-knot nematode, *Meloidogyne incognita*, was present in the soil (Navarro, *personal communication*).

The susceptibility of tree tomato to *P. solanacearum* was reported in Indonesia in 1941 (6). In a search by Karthaus and Thung for a resistant rootstock on which to graft tomato plants (*Lycopersicon esculentum* Mill.), nine solanaceous plant species including *C. betacea* were planted in a highly infested field. Tree tomato was moderately resistant to bacterial wilt (3.4% wilted plants). *C. betacea* has been ranked as a host with very low susceptibility to *P. solanacearum* (7).

The objectives of this study were to evaluate the susceptibility of *C. betacea* to different strains of *P. solanacearum* and the influence of root-knot nematode on bacterial wilt infection. A preliminary report of this research has been published (9).

MATERIALS AND METHODS

Plants. Seeds of tree tomato obtained at Medellín, Colombia, from a local cultivar (*not named*) with yellow-orange fruits were started in flats in a screenhouse at 14–22 C. Thirty-five days after emergence, seedlings were transplanted to 12-cm-diameter clay pots (one per pot) containing a mixture of soil, peat moss, and Jiffy Mix (Jiffy Products of America, P.O. Box 336, West Chicago, IL) (2:1:1), pH 6.5, and maintained in the screenhouse until 3 days before inoculation. Plants were inoculated 35 days after transplanting.

Isolates. Thirteen strains of *P. solanacearum* representing the four biotypes (3) and from different hosts and countries (Table 1) were used for inoculation. The strains form part of the world collection of *P. solanacearum* maintained at the Pathology Department of the International Potato Center at Lima, Peru. The cultures were stored in sterile tap water in screw-cap tubes at 18–23 C. Prior to preparation of inoculum, cultures were streaked on tetrazolium chloride (TZC) medium and examined for the wild type to ensure that they were free of avirulent colony type (8).

Inoculation. Inoculum was obtained from 48-hr-old cultures of each strain grown at 30 C on TZC medium without tetrazolium salts to avoid pigment formation. Suspensions were made in sterile tap water and adjusted to about 2 $\times 10^7$ colony-forming units (CFU) per milliliter. Three different types of inoculation were used: a) soil infestation, in which 50 ml of the inoculum suspension was poured onto the soil in each pot; b) stem inoculation, in which a drop of the bacterial suspension was placed in the axil of the second leaf from the top and the stem was then punctured through the drop with a sterile needle; and c) addition of suspension through three glass tubes (12 × 1 cm) placed about 9 cm deep around the roots at transplanting and protruding 3 cm above the soil line. Four hours before inoculation with methods a and c, soil in pots was watered to field capacity to distribute inoculum uniformly throughout the potted soil. After inoculation, plants were placed in a heated greenhouse with night/day temperatures of 26/30 ± 1 C until the end of the experiment.

To study susceptibility of *C. betacea* to the four biotypes of *P. solanacearum*, 70-day-old plants were inoculated with each of the 13 strains listed in Table 1. Soil infestation and stem inoculation were used in this experiment. Ten plants per isolate and per inoculation method were inoculated. The experiment was repeated twice. Tomato plants (*L. esculentum* 'Huando') were included as susceptible controls. Three tomato plants per isolate and per inoculation method were inoculated 15 days after transplanting. Inoculated plants were maintained for 45 days in the heated greenhouse at 26/31 ± 1 C. In a different experiment, plants treated as above were inoculated and maintained in a screenhouse at 14–22 C.

Interaction with *M. incognita*. Five of the 13 strains of *P. solanacearum* were chosen for an interaction study with *M. incognita acrita*. A 20-ml volume of bacterial suspension (2×10^7 CFU/ml) and 2,000 freshly hatched larvae of the nematode in 20 ml of water were introduced through the glass tubes as previously explained. Treatments included nematodes alone, bacteria alone, nematode plus bacteria, and a sterilized water control. Inoculated plants were maintained in a greenhouse at 26/30 ± 1 C for 45 days. Ten plants per treatment were used in each of two repetitions.

The number of wilted plants was determined three times a week up to the 45th day. In all three experiments, wilted plants were assayed for the presence of *P. solanacearum* in the vascular system. Stem sections 3 cm long were collected and placed in a test tube containing sterile

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Table 1. Strains of *Pseudomonas solanacearum*, their biotypes, origin, and pathogenicity on tree tomato (*Cyphomandra betacea*) and tomato plants (*Lycopersicon esculentum*) following soil or stem inoculation

CIP[a] no.	Biotype	Source of isolate		Percentage of wilted plants			
				Tree tomato[b]		Tomato[c]	
		Host	Origin	Soil infest.	Stem inoc.	Soil infest.	Stem inoc.
009	I	Plantain	Costa Rica	0	0	0	0
018	I	Potato	Yurimaguas, Peru	0	40	100	100
092	I	Tomato	Paraiso, Costa Rica	80	100	100	100
013	II	Potato	Cajamarca, Peru	50	100	66	100
048	II	Potato	Paraiso, Costa Rica	20	20	100	100
052	II	Tomato	Taiwan	0	100	100	100
071	II	Potato	Sinaloa, Mexico	30	100	66	100
065	III	Chili	La Garita, Costa Rica	0	100	100	100
072	III	Tomato	Lupuna, Peru	0	100	0	100
132	III	Potato	Sri Lanka	0	60	0	0
035	IV	*Datura* sp.	Nairobi, Kenya	0	60	66	100
080	IV	Ginger	Costa Rica	0	20	0	0
121	IV	Potato	Sri Lanka	0	80	66	100

[a] International Potato Center.
[b] Average of two repetitions, 10 plants per repetition, in the greenhouse at 26–30 ± 1 C; evaluated 45 days after inoculation.
[c] Average of two repetitions, 3 plants per repetition, in the greenhouse at 26–30 ± 1 C; evaluated 45 days after inoculation.

Table 2. Pathogenicity of various strains of *Pseudomonas solanacearum* on tree tomato (*Cyphomandra betacea*) in presence of *Meloidogyne incognita acrita*

Treatment	Percentage of wilted plants				
	092 (I)[a]	018 (I)	052 (II)	065 (III)	121 (IV)
Nematodes (larvae) (2,000/ml)	0[b]	0	0	0	0
Bacteria (cells) (2×10^7/ml)	50	10	0	0	0
Bacteria + nematode	70	40	70	20	0
Control (water)	0	0	0	0	0

[a] Roman numbers designate biotypes.
[b] Average of two repetitions after 45 days with 10 plants per repetition. Greenhouse temperature was 26–31 C.

tap water for 15 min. The suspension was streaked on TZC medium. After 48 hr at 30 C, colonies were examined for the presence of *P. solanacearum* (8). Symptom expression was also observed under natural conditions.

RESULTS

The first symptom on fully grown tree tomatoes in the field is wilting (flaccidity) of young leaves, usually on one branch or part of the tree. After 2–4 wk, the leaves drop off and the twigs die. In the next season, few twigs produce leaves that also become infected. By peeling off the bark, the discolored vascular system can be seen as brown, parallel streaks running up the trunk. Generally it takes 2 yr for the entire tree to collapse (Navarro, *personal communication*). Only four strains (one of biotype I and three of biotype II) induced bacterial wilt symptoms on tree tomato plants (Table 1). Bacteria were not isolated from plants showing no visible symptoms of bacterial wilt infection. The highest percentage of wilted plants (80%) was obtained with strain 092 (biotype I) from Costa Rica, followed by strains 013 (50%), 071 (30%), and 048 (20%), all of biotype II. Plants showing initial bacterial wilt symptoms generally died within 15 days. No plants wilted in infested soil with strains of biotypes III and IV and no bacteria were detected in isolations from stem tissue.

By stem inoculation, only one strain—isolate 009—did not wilt a single tree tomato plant (Table 1). It was expected that isolate 009 would not induce wilting because it was isolated from plantain.

Stem inoculations with strains of biotypes III and IV resulted in wilted tree tomato plants. Both types of inoculation induced a large number of wilted tomato plants, which confirmed the pathogenicity of most isolates in the present study. Strain 132 (biotype III, isolated from potato in Sri Lanka) did not wilt tomato plants even by the stem-inoculation procedure, but it did wilt tree tomato plants inoculated by stem puncture.

No visible wilting symptoms appeared in any of the plants inoculated with the 13 isolates by soil infestation and incubated at 14–22 C. After 45 days, plants were moved into a heated greenhouse (26–31 ± 1 C) and maintained for an additional 15 days, during which time only plants inoculated with strain 092 (biotype I) showed bacterial wilt symptoms (20%). No bacteria were isolated from stems of the symptomless plants. With the exception of 009 and 080, all strains had 60–100% wilted plants when they were stem inoculated and maintained at 14–22 C. These were the two strains that wilted none or a very low percentage of plants when they were stem inoculated and kept at the higher temperature.

Wilt symptoms were generally similar following both types of inoculation and were identical to those described for potato, tomato, and tobacco (7). The first symptoms for stem-inoculated plants at 14–22 C developed 15 days after inoculation as compared with 7 days for those at the higher temperatures. On the other hand, plants inoculated by soil infestation and kept in the heated greenhouse showed the first symptoms 3 days later than those inoculated by stem puncture and maintained under the same conditions. The stem-inoculated plants that did not wilt, especially at the lower temperature, usually developed a dark, necrotic spot around the point of needle penetration. This necrosis resembled more an incompatible reaction than mechanical damage caused by needle penetration. Control plants inoculated with sterile water did not show this peculiar symptom.

Interaction with *M. incognita*. Strain 092 (biotype I) was chosen as a positive control for bacterial wilt incidence in tree tomato because it produced the highest percentage of wilted plants (80%) when plants were inoculated by soil infestation (Table 1). Strains 018 (biotype I), 052 (II), 065 (III), and 121 (IV) were selected because under the same conditions they did not wilt any tree tomato plant even though they were highly pathogenic in tomato (Table 1). The number of wilted plants was much higher when nematodes were present than when plants were inoculated only with *P. solanacearum*. Plants inoculated with isolate 121 did not wilt after either treatment (Table 2). Plants inoculated with isolate 052 and 065 did not wilt when the bacteria was used alone, but the percentage of wilted plants was 70 and 20%, respectively, when both organisms were added to the soil together. With isolate 092, there were

also marked differences among the three treatments.

DISCUSSION

Because tree tomato is a perennial and takes almost 1 yr to produce fruits, a significant investment is lost when a plant is killed. Furthermore, tree tomato is one of very few plants known to be a host of biotype II (race 3, the potato strain) under natural field conditions, and it may thus serve as a potential source of inoculum for potato crops. The capacity of other strains to infect tree tomato may not be of significance because only biotype II occurs commonly in the ecologic zones of both the tree tomato and the potato in South America. The differences in disease incidence following inoculation by soil infestation and following stem inoculation are consistent with what is known about *P. solanacearum*. When the inoculum is inserted in the vascular system at the leaf axil, it has a greater probability for initiating an infection process (1). Similarly, damage to the root system by nematodes or other means enhances the probabilities of infection (4,5,7).

This short study has indicated that tree tomato can be readily infected by *P. solanacearum* under natural field conditions. It has also indicated that the infection process can be enhanced by the presence of nematodes in the soil and that the strain infecting tree tomato is of the same race as that infecting potato.

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LITERATURE CITED

1. French, E. R. 1972. Progress and problems in selecting resistance to bacterial wilt. Pages 212-214 in: Prospects for the Potato in the Developing World. E. R. French, ed. International Potato Center (CIP), Apartado 5969, Lima, Peru.
2. Girard, E. O., and Lobo, A. 1977. El cultivo del tomate de árbol (*Cyphomandra betacea* Cav. Sendt.). Pages 195-212 in: Curso sobre Frutales. Inst. Colomb. Agropec. Reg. 14. Compend. 20. Medellín, Colombia.
3. Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. J. Appl. Bacteriol. 27:265-277.
4. Jatala, P., French, E. R., and Gutarra, L. 1975. Interrelationships of *Meloidogyne incognita acrita* and *Pseudomonas solanacearum* on potatoes. (Abstr.) J. Nematol. 7:325.
5. Jatala, P., and Martin, C. 1977. Interactions of *Meloidogyne incognita acrita* and *Pseudomonas solanacearum* on field grown potatoes. (Abstr.) Proc. Am. Phytopathol. Soc. 4:177-178.
6. Karthaus, J. P., and Thung, T. H. 1941. Het verenten van tomaten op voor slijmziekte resistente onderstammen. Natuurk. Tijdschr. Ned. Indie 101:266-270.
7. Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. N.C. Agric. Exp. Stn. Tech. Bull. 99. 194 pp.
8. Kelman, A. 1954. The relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance in a tetrazolium medium. Phytopathology 44:693-695.
9. Martin, C. 1978. Susceptibility of tree tomato (*Cyphomandra betacea*) to different biovars of *Pseudomonas solanacearum*. (Abstr.) Phytopathol. News 12:265-266.
10. Navarro, R. 1975. El tomate de árbol (*Cyphomandra betacea*) hospedante de *Pseudomonas solanacearum*. ASCOLFI (Asociación Colombiana de Fitopatología). Informa 1:5.