Pathogenicity and Virulence of *Phytophthora capsici* Isolates from Taiwan on Tomatoes and Other Selected Hosts

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ABSTRACT

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In container experiments, black nightshade, cabbage, cucumber, pepper, potato, tobacco, and tomato plants were inoculated with isolates of *Phytophthora capsici* obtained from either pepper stems or tomato foliage. Inoculum was applied by atomizing foliage or by drenching soil with zoospore suspensions of 1×10^4 zoospores per milliliter. Foliage and stem blight developed on black nightshade, pepper, and tomato plants but not on the other plant species. Tomato and pepper seedlings were inoculated with five isolates of *P. capsici* by spraying foliage or drenching soil. Response to individual isolates varied from no symptoms to 100% blighting and death of foliar-inoculated pepper plants. Regardless of isolate, basal stem blight was not observed on soil-inoculated tomato plants, whereas reaction of pepper plants varied from no symptoms to severe crown lesions causing death. Seedlings of 11 tomato lines foliar-inoculated with one isolate were all susceptible, although the amount of foliage blighted differed among lines. Detached tomato leaves inoculated with a zoospore suspension had more blight at 24 and 28 C than at higher and lower temperatures 3 days after inoculation. In the field, two foliar-inoculated tomato lines developed leaf blight by 3 days after inoculation, and by 14 days, 45–60% of the leaf area was blighted.

Phytophthora capsici Leonian causes blight, root and crown rot, and other symptoms on many hosts. The fungus is soilborne and is difficult to control, partly because of its broad host range and its ability to survive in the soil. On peppers (*Capsicum annuum* L.), the fungus causes cankers on lower portions of stems resulting in plant death (15) and also causes foliar blight and fruit rot (15,23).

In Taiwan, *P. capsici* was first reported on peppers in the Chusan area in 1977 (11). Island-wide surveys in 1981 showed that Phytophthora blight occurred in most hot and sweet pepper fields from May to October during the rainy season (16). In addition to peppers, the fungus has been reported on 13 hosts in Taiwan, including cucumbers (*Cucumis sativus* L.), eggplants (*Solanum melongena* L.), onions (*Allium cepa* L.), and tomatoes (*Lycopersicon esculentum* Mill.) (10).

On tomatoes, *P. capsici* has been reported primarily on roots, lower stems, and fruits (14,24). More recently, *P. capsici* was identified as the causal agent of field-blighted tomato leaves and isolates from peppers and tomatoes were reported to be cross-pathogenic (8). Other than that report, there is a lack of information about the occurrence, pathogenicity, and virulence of *P. capsici* on tomato foliage.

Late blight of tomatoes, caused by *P. infestans* (Mont.) de Bary, was first reported in Taiwan in 1919 (25). Late blight apparently occurs on potatoes and tomatoes in Taiwan, although Ho (10) failed to find *P. infestans* in a survey of *Phytophthora* species and stated that herbarium material collected in 1919 was the last documented occurrence of the fungus in Taiwan. Recently, *P. infestans* was isolated from tomato foliage in Taiwan, and cultures and pathogenicity of *P. capsici* and *P. infestans* were compared (7).

Variability in morphological, cultural, and physiological characteristics as well as in results of tests on pathogenicity and fungicide resistance has been reported for many of the *Phytophthora* species (4). As early as 1972, isolates of *P. capsici* were shown to differ in virulence (20). On pepper seedlings, oospore progeny of pathogenic isolates were shown to vary in virulence (2). Ristaino (22) showed that intraspecific variation of virulence occurred among *P. capsici* isolates from cucurbits and peppers. In studies on host plant resistance, *P. capsici* isolates on pepper were shown to differ in virulence (12,19,21).

P. capsici has been identified by using morphological characteristics, and several taxonomic keys are available to distinguish it from other *Phytophthora* species (5,9,18,27). Recently, a revised species description of *P. capsici* was published (26).

The objective of our study was to compare the pathogenicity and virulence of isolates of *P. capsici* from Taiwan on tomatoes and other selected hosts. A portion of this research was previously reported (6).

MATERIALS AND METHODS

Six isolates of *P. capsici* were used in our experiments. Five isolates were obtained from diseased pepper stems (Pc1, Pc2, Pc3, Pc4, and Pc5) and one isolate (Pc9) from blighted tomato foliage. The fungus was isolated from infected tissue by surface-sterilizing 1- to 2-mm pieces of tissue with 0.525% NaOCl for 3 min, rinsing the tissue in sterile distilled water once for 3 min, then plating the sections on water agar. After 2–3 days, tips of peripheral hyphae from a growing colony were transferred to potato-dextrose agar (PDA). Two additional isolates of *P. capsici* were used for comparison of morphological characteristics. An isolate from pepper was obtained from W. H. Hsieh of National Chung Hsing University and an isolate from eggplant was obtained from H. S. Chang from Academic Sinica in Taiwan. Cultures were maintained on PDA slants at room temperature and were routinely transferred every 3 mo on PDA for maintenance.

To confirm that our isolates were *P. capsici*, we cultured them on V8 agar (17) at 28 C for 7 days in the dark before measuring morphological characteristics. Agar pieces (2 cm^2) with mycelium were cut from colonized areas of the medium, rinsed twice in sterile distilled water, and incubated at room temperature for 2–3 days. Sporangia were dislodged from the agar blocks by agitation, and the length and width of 25 sporangia and the length of 25 pedicels were measured for each isolate. The length: breadth ratio (L:B) of sporangia was calculated. Isolates were identified by comparing our data with known descriptions of *P. capsici* (5,26,27).

Host range. Seedlings (1 mo old) of cabbage (*Brassica oleracea* L. 'KY Cross'), cucumber cv. Chungshih No. 6, pepper cv. Blue Star, tobacco (*Nicotiana tabacum* L. 'Van-Hicks'), and tomato cv. PT4121 planted in 9.5-cm-diameter pots were inoculated by atomizing foliage to the point of runoff or by drenching soil with 50 ml of a suspension of 1×10^4

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zoospores per milliliter of isolates Pc4 and Pc9. Zoospore suspensions were obtained by growing isolates on V8 agar for 1 wk. The agar was cut into four equal pieces, and the pieces were moved to empty culture dishes and cut into approximately 1-cm^2 blocks with a sterilized knife. Agar blocks were soaked twice in sterilized water for 10–15 min, and the water decanted. Sterilized water was poured in the dishes up to the surface of the agar blocks before incubating the blocks at 28 C for 24 hr under continuous light (68 $\mu E \cdot m^{-2} \cdot s^{-1}$) and then moved to a temperature of 4 ± 1 C for 45 min. Drops of the suspension were placed on a hemacytometer and moved to 4 ± 1 C for 5–10 min to stabilize the zoospores for counting.

In a second trial, black nightshade (*Solanum nigrum* L.), eggplant cvs. Farmers Long and Pingtung Long, pepper cv. Blue Star, potato (*Solanum tuberosum* L.), and tomato cv. PT4121 were inoculated with the isolates Pc4 and Pc9 by drenching soil or atomizing foliage as previously described. After inoculation, plants were incubated in a growth room at 28 C, 98 ± 2% RH, and 49.3 $\mu E \cdot m^{-2} \cdot s^{-1}$ of light for 12 hr per day. Plants in both experiments were arranged in a completely random design, with each treatment replicated five times. Disease development was assessed 5 days after inoculation by rating plants as either with or without basal stem and/or foliar blight symptoms.

Virulence of isolates. Seeds of Asian Vegetable Research and Development Center (AVRDC) tomato accession L3975 and pepper cv. Blue Star were planted in 9.5-cm-diameter clay pots. When plants were 30 days old, a suspension of 1×10^3 zoospores per milliliter of each of five isolates—Pc1, Pc2, Pc3, Pc5, and Pc9—was either soil-drenched (50 ml per pot) or atomized on foliage until runoff in separate experiments. Plants were overhead-misted for 40 sec twice an hour in a growth room at 25–28 C. Soil drenched with water and plants atomized with water served as controls. Each experiment comprised five plants (replications) for each host and isolate arranged in a completely random factorial design. Individual plants were rated for disease daily, beginning 2–3 days after inoculation until 7 days after inoculation. Disease severity on foliar-inoculated plants was rated as: 0 = no symptoms, 1 = 1–10% of leaf area infected (LAI), 2 = 11–20% LAI, 3 = 21–40% LAI or 1–10% of stem area infected (SAI), 4 = 41–70% LAI or 11–50% SAI, 5 = 71–90% LAI or 51–100% SAI, and 6 = 91–100% LAI or plant dead. Plants inoculated by soil drench were rated as: 0 = no symptoms, 1 = slight wilting, 2 = 10–30% wilted, 3 = 31–50% wilted, 4 = 51–70% wilted, 5 = 71–90% wilted, and 6 = plants dead. The experiments were repeated with the same factorial arrangement of treatments and number of replications. Data were analyzed by ANOVA as a complete factorial design for experimental effects, using treatment effects (host and isolate) as independent variables and interactions as dependent variables.

Inoculation of tomato lines. Eleven tomato lines (Table 1) were inoculated by atomizing 30-day-old seedlings with a suspension of 10^3 zoospores per milliliter of isolate Pc9. All tomato lines except L3975 were previously described to be resistant to late blight (AVRDC, *unpublished*). Plants were overhead-misted for 40 sec twice an hour in a growth room as previously described. There were 10 plants per line (replications) arranged in a randomized complete block design. Disease was rated 5 days after inoculation on the 0–6 scale previously described. The experiment was repeated.

Detached leaves of 30-day-old seedlings of L3975 were placed on moist filter paper inside 12.5-cm culture dishes. Cut petiole ends were covered with wet sterilized cotton to avoid leaf wilting. Leaves were atomized with a suspension of 1×10^4 zoospores per milliliter of isolate Pc9. Five inoculated leaves representing five replications for each temperature were incubated at 8–32 C (at 4-C intervals) under 12-hr light (68 $\mu E \cdot m^{-2} \cdot s^{-1}$) and 12-hr dark cycles. Atomized water on detached leaves served as a control at each temperature. Disease was rated 3, 7, and 14 days after inoculation on the previously described 0–6 scale for leaf area covered.

In the field, two tomato lines (L1197 and L3975) were transplanted in a randomized complete block design with four replications of 20 plants in a 1 × 2 m bed with 10 plants per bed. Plants were foliar-inoculated with a suspension of 8.2×10^4 zoospores per milliliter of isolate Pc9 1 mo after transplanting. Plants were rated 2 wk after inoculation on a 0–6 scale as previously described. Samples of leaves and fruit were taken to reisolate the pathogen.

RESULTS

The range of the mean measurement of sporangia among isolates was 43.1–65.8 μm in length and 30.0–50.4 μm in width. The mean L:B ratio and the mean pedicel length among isolates ranged from 1.23 to 1.54 and from 33.5 to 98.9 μm, respectively. The isolate obtained from tomato had a mean sporangia length of 53.5 μm and breadth of 37.6 μm, whereas those for the eggplant isolate were 49.1 × 38.7 μm. All eight *P. capsici* isolates had sporangia and pedicel measurements within the range of the revised description of *P. capsici* (26).

Host range. No blight symptoms were recorded on foliar- or drench-inoculated seedlings of cabbage, cucumber, eggplant, potato, or tobacco. Foliar-applied inoculum caused blight on black nightshade, pepper, and tomato leaves, and drench-applied inoculum caused stem blight on black nightshade and pepper plants. All black nightshade plants foliar-inoculated with isolate Pc4 died, whereas those inoculated with isolate Pc9 had some foliar blight but did not die. Each isolate caused stem blight in two of five plants.

Virulence of isolates. There was no significant ($P < 0.05$) experimental effect or interaction of treatments between the two experiments, so data were combined. When pepper plants were soil-drenched with inoculum, isolates Pc9 and Pc5 caused significantly ($P < 0.05$) more disease than the other isolates. The mean ratings at 7 days after inoculation were Pc9 = 5.3, Pc5 = 4.9, Pc2 = 2.3, Pc3 = 0.4, and Pc1 = 0. Tomato plants inoculated in the same way did not develop symptoms regardless of isolate. When plants of pepper and tomato were foliar-inoculated, isolate Pc1 caused no symptoms but isolate Pc3 caused some symptoms on tomato plants. Isolates Pc5 and Pc9 caused significantly more blighting than the other isolates from the initial ratings until 7 days after inoculation (Figs. 1 and 2). There was a significant ($P < 0.05$) interaction between host and isolate.

Inoculation of tomato lines. There were significant ($P < 0.05$) differences in blight ratings among the tomato lines. L418 had significantly ($P < 0.05$) lower blight ratings than all other lines in the first experiment and also in the second experiment, along with three other lines (Table 1).

Blight on detached leaves at temperatures of 24 and 28 C was significantly ($P < 0.05$) greater than at the other temperatures 3 days after inoculation (Table 2). After 14 days, there was no

Table 1. Foliar blight on 10 tomato accessions of the Asian Vegetable Research and Development Center (AVRDC) inoculated with a zoospore suspension of *Phytophthora capsici*

AVRDC accession	PI[a]	Severity of foliar blight[b] Expt. 1	Expt. 2
L418	92865	3.3	3.1
L474	110595	4.5	3.8
L529	118782	4.0	3.8
L587	123538	4.2	5.6
L660	126917	4.1	5.2
L826	128449	4.4	4.3
L1149	146129	4.2	5.1
L1150	147282	4.5	3.0
L1197	162679	4.2	4.9
L1249	169576	4.9	4.8
L3975	CL TK70[c]	4.3	4.4
Average		4.2	4.4
LSD ($P < 0.05$)		0.6	0.9

[a] PI 110595 = *Lycopersicon pimpinellifolium*; all others are *L. esculentum*.
[b] Based on a 0–6 scale, where 0 = no symptoms and 6 = 91–100% of leaf area infected or plant dead.
[c] Commercial line.

significant difference ($P < 0.05$) at 20, 24, 28, and 32 C; the control leaves at all of the temperatures remained green.

In the field, blight severity increased from 0.2 to 1.3, 2.6, and 4.1 for L1197 and from 0.9 to 1.2, 2.4, and 4.2 for L3975. There was no significant difference between lines at the last rating. *P. capsici* was reisolated from blighted leaves and from fruit symptoms. The fungus produced sporangia on incubated fruit but not on leaves.

DISCUSSION

The morphological characteristics of our isolates were within the range of the revised description of *P. capsici* (27) and were similar to the length (51 μm), breadth (34 μm), and L:B ratio (1:5) reported earlier from Taiwan (16). None of our *P. capsici* isolates produced chlamydospores or oospores. According to Ho (10), only the A1 mating type of *P. capsici* occurs in Taiwan.

The isolates we tested infected only black nightshade, pepper, and tomato plants; our inoculation methods did not infect other known hosts such as cucumber and eggplant. Only some black nightshade plants died when roots or foliage were inoculated; this appears to be the first report of *P. capsici* infecting black nightshade. Some crucifers have been reported as hosts (24), but we did not observe symptoms on cabbage. Drench-inoculated tomatoes did not develop crown rot, although it has been reported (24), and we have observed seedling infection on younger inoculated seedlings (*unpublished*).

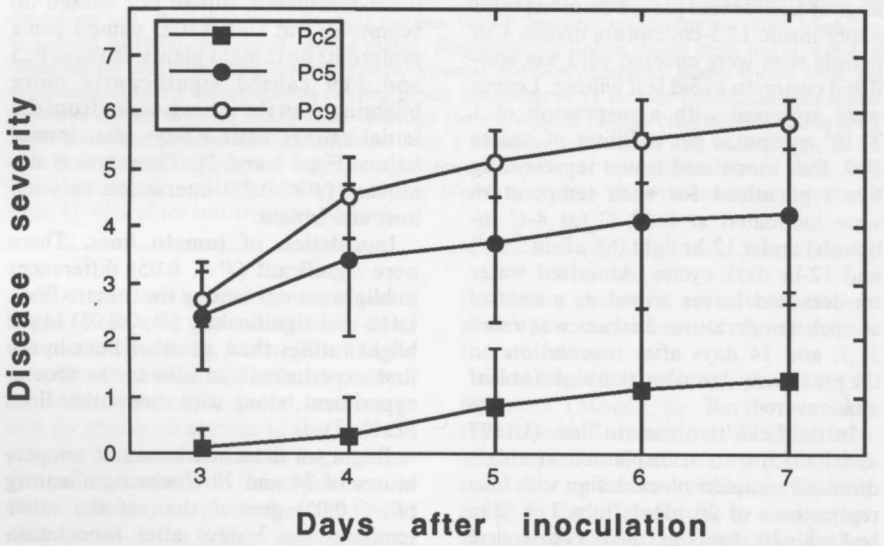

Fig. 1. Development of foliar blight on peppers inoculated with five isolates of *Phytophthora capsici* (Pc1 and Pc3 did not cause blight and are not represented) under growth-room conditions 3–7 days after inoculation. Vertical bars represent the standard error of the mean.

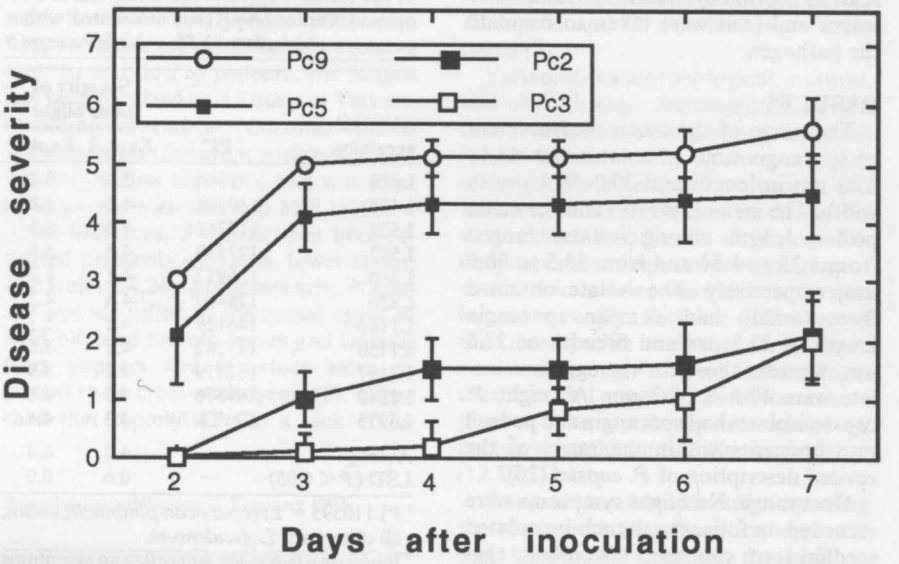

Fig. 2. Foliar blight development on tomatoes inoculated with five isolates of *Phytophthora capsici* (Pc1 did not cause blight and is not represented) under growth-room conditions 2–7 days after inoculation. Vertical bars represent the standard error of the mean.

On the basis of our results, some hosts differ in susceptibility according to method of inoculation, i.e., foliar vs. soil drench. Barksdale et al (1) reported that foliar-inoculated peppers had disease severity ratings similar to peppers planted in infested soil in the greenhouse or field. In another report (13), age-related resistance in pepper was not observed when leaves were foliar-inoculated but was evident when soil was drenched with inoculum. It was also shown that severity of foliar symptoms did not directly compare with severity on basal stems when inoculum was applied to the soil (21).

The isolate Pc9 originated from field-grown tomato foliage that showed typical late blight symptoms. This isolate was generally most virulent in all of our tests, whereas other isolates such as Pc1 did not infect pepper or tomato plants and isolate Pc3 did not infect peppers and was low in virulence on tomatoes. The frequency of occurrence of *P. capsici* on tomato foliage under field conditions is not known, but we have shown under controlled conditions and in field studies that *P. capsici* isolates are capable of causing foliar blight of tomatoes. From our field experiments we noted that *P. capsici* does not sporulate on infected leaves, which differs from the rapid sporulation that occurs when *P. infestans* colonizes leaf tissue. Other reports (14,21) have shown that *P. capsici* causes symptoms on tomato plant parts other than foliage, and we recently reported (7) that an isolate from tomato was pathogenic on foliage of both peppers and tomatoes. No reports have indicated that tomato lines resistant to *P. infestans* are also resistant to *P. capsici*.

Late blight (*P. infestans*) is known to occur in the highland tropical areas throughout Southeast Asia, although details about culturing and properly identifying the fungus are generally lacking. In most of the tropics, *P. infestans* probably does not occur because of temperature restrictions (3). It seems possible that *P. capsici* may frequently

Table 2. Blight of detached tomato leaves inoculated by atomizing a zoospore suspension of *Phytophthora capsici* and incubating leaves at seven temperatures

Temperature (C)	Postinoculation severity of foliar blight[a]		
	3 days	7 days	14 days
8	0	0.6	1.0
12	0.4	1.4	3.2
16	0.8	2.6	4.4
20	3.2	5.6	6.0
24	5.4	6.0	6.0
28	5.2	6.0	6.0
32	1.4	3.8	6.0
LSD ($P < 0.05$)	0.6	0.8	0.6

[a] Based on a 0–6 scale, where 0 = no symptoms and 6 = 91–100% of leaf area infected or dead plant.

cause foliar blight of tomatoes in the highland tropics. In addition, *P. capsici* was isolated from blighted tomato foliage during the spring of 1991 in Louisiana (L. L. Black, *personal communication*). More information is needed to properly identify the causal agent(s) of Phytophthora foliar blight of tomatoes in the tropics.

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