Characterization of *Phytophthora capsici* Isolates From Processing Pumpkin in Illinois

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ABSTRACT

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This study was conducted to investigate pathogenic, morphologic, and genetic variations among *Phytophthora capsici* isolates from processing pumpkin fields in Illinois. Random amplified polymorphic DNA (RAPD) markers were employed to assess genetic variation among twenty-four isolates of *P. capsici* from 10 individual fields at six locations. Unweighted mean pair group analysis clustered isolates into six groups. The genetic distances ranged from 0.03 to 0.45. Inoculation of pumpkin seedlings in the greenhouse revealed that the isolates belonged to six distinct genetic groups differing significantly (P = 0.05) in virulence. Isolates tested exhibited

four growth patterns in cultures: cottony, rosaceous, petaloid, and stellate. *Phytophthora capsici* isolates, including an ATCC isolate (ATCC-15427), with cottony growth pattern did not grow at 36°C. The mean oospore diameter of A1 mating type isolates was greater than that of A2 mating type isolates. Nine of 24 isolates tested produced chlamydospores in V8-$CaCO_3$ liquid medium.

Additional keyword: Phytophthora blight, cucurbit, *Cucurbita muschata*

Illinois ranks first in pumpkin production in the United States (US) producing about 90% of the commercial processing pumpkins in the US (4). Phytophthora blight, caused by *Phytophthora capsici* Leonian, has become one of the most serious threats to processing pumpkin (*Cucurbita moschata* L.) production in Illinois and has caused up to 100% yield losses (3). *Phytophthora capsici* can attack the host plant at any growth stage and causes seedling death, crown rot, foliar blight, and fruit rot. The pathogen also causes severe crop losses in other cucurbits, eggplant, pepper, and tomato (8,11,13,19).

Phytophthora capsici is a soil-borne oomycete. It can survive in soil as oospores for several years, and repeated cultivation of susceptible hosts results in a high density of oospores in soil (8). Free water either in soil or on the foliage favors asexual reproduction of the pathogen with the formation of sporangia and zoospores (5,6). Propagules of *P. capsici* are dispersed by water, soil, and air currents (20). Infection of the foliage of pumpkin occurs when zoospores of *P. capsici* are splashed onto the plant surfaces from the soil during rainfall or irrigation (13).

Variation in virulence among isolates of *P. capsici* has been reported. Lee et al. (13) studied aggressiveness of *P. capsici* isolates from pepper and pumpkin on pumpkin cultivars and found

differences in virulence among the isolates. Ristaino (19) evaluated the relative virulence of isolates of *P. capsici* from cucumber and squash on pepper, and reported variation among the isolates. There is no published report available on pathogenic variation among isolates of *P. capsici* from processing pumpkin.

During the past five years, we observed that the severity of Phytophthora blight and yield losses differed among processing pumpkin fields. In some pumpkin fields, seedling death was so widespread that the growers had to replant fields two or three times. In a survey of affected commercial fields, with the same processing pumpkin cultivars and grown under similar conditions, incidence of vine blight ranged from 4 to 48%, leaf blight ranged from 17 to 68%, and fruit rot ranged from 4 to 71%. Also, we observed variation in growth and sporulation among *P. capsici* isolates from processing pumpkin fields. For example, some isolates grew above 36°C while most of the isolates did not; some isolates produced chlamydospores; and oospores formed in cultures of single mating type. These observations raised the question whether the isolates of *P. capsici* in different processing pumpkin fields vary in virulence.

The objective of this study was to investigate pathogenic, morphologic, and genetic variation among isolates of *P. capsici* from processing pumpkin fields in Illinois. Mating types, chlamydospore formation, and temperature/growth response of the isolates were also investigated.

MATERIALS AND METHODS

Isolates. Fifty-seven single-zoospore isolates of *P. capsici,* collected from 22 processing pumpkin fields in six towns in central Illinois during 2000-2001 (Table 1), were used in this study. *Phytophthora capsici* was isolated from infected plant tissues dipped in 70% ethanol for 3

min, rinsed in sterilized-distilled water (SDW), and plated on PARPH (Corn meal agar, 17 g; pimaricin 10 mg; ampicillin, 250 mg; rifampicin, 10 mg; PCNB, 100 mg; hymexazol, 50 mg; and distilled water, 1000 ml), a selective culture medium for *P. capsici.* Cultures were maintained on lima bean agar (LBA; Difco Lab., Detroit, MI; 23 g/L) slants at room temperature (5). The isolates were identified as *P. capsici* based on colony morphology and sporangial characteristics.

Sporangia were measured by placing a drop of water on a slide and the colony surface of 5- to 6-day-old cultures grown on V8-juice medium under white fluorescent light at 24°C, was touched with the water, covered with a cover slip, and examined using light microscopy (19). Twenty arbitrarily chosen sporangia were examined and measured for each isolate using an optical micrometer.

PCR-based identification of isolates. For DNA extraction, isolates were grown separately in 100 ml of lima bean broth in 250 ml Erlenmeyer flasks for 5 days at 24°C. Mycelia were harvested by filtration and ground to a fine powder in liquid nitrogen using a mortar and pestle. Genomic DNA was extracted from frozen mycelium using the procedure developed by Nelson et al. (17) and dissolved in TE buffer. The identification of collected isolates as *P. capsici* was confirmed by polymerase chain reaction (PCR) using the *P. capsici* specific primer PCAP, and the ITS primer ITS1 according to the method developed by Ristaino et al. (21). The PCAP primer in combination with ITS1 primer amplifies an approximately 172-bp product of ribosomal DNA of *P. capsici* (21). PCR amplification products were separated by electrophoresis in 2% agarose gels stained with ethidium bromide and product size was determined by comparison with a molecular size marker.

Colony characteristics. Plugs (7-mm-diameter) of mycelium in agar, from the edge of 4-day-old culture of *P. capsici*, were placed onto the center of Petri plates containing Difco potato dextrose agar (PDA), and the plates were incubated in darkness at 24°C. After 5 days, the plates were visually examined for colony texture and appearance. For temperature/growth responses, a mycelial disk (7-mm-diameter) was placed in the center of each LBA plate. Inoculated plates were incubated at temperatures of 10, 15, 20, 24, 30, 32, 35, 36, 37, and 38°C (±0.2°C) in a completely randomized design. Each isolate had three replicates. Colony diameter was measured after 3, 5, and 7 days of incubation and data were converted to radial growth in millimeters per day. The experiment was repeated twice.

Mating type. All isolates were tested to determine their mating types by pairing with known A1 (ATCC-15427) or A2 (ATCC-15399) tester isolates of *P. capsici* that were obtained from the American Type Culture Collection (ATCC). Plugs of mycelium in agar (7-mm-diameter) from advancing margins of 4-day-old cultures were placed 2 cm apart on clarified V8-juice agar and incubated at 24°C in darkness for 3 weeks (19). The absence of oospores at the interface between colonies indicated the same mating type, whereas the presence of oospores indicated opposite mating type. The solo culture of each isolate was examined for oospore formation as a control. The positive control was a cross between two tester isolates of opposite mating types. Twenty-four isolates from each A1 or A2 mating type were arbitrarily selected for oospore measurement. For each isolate, twenty-five oospores closest to the A1 isolate (initial inoculum-disk) and those to the A2 isolate, were measured for diameter using an optical micrometer and compared. The experiment was repeated once. The data were analyzed using analysis of variance procedure of SAS (SAS Institute, Cary, NC).

Chlamydospore formation. Twenty-four isolates of *P. capsici* (Table 1), four isolates from each town (location), were selected arbitrarily and cultured on modified cleared V8-$CaCO_3$ liquid medium (V8-juice, 100 ml; filtered 2% $CaCO_3$, 100 ml; and distilled water, 800 ml) under submerged conditions (23). A plug of mycelium on agar (7-mm-diameter), from the actively-growing margin of 4-day-old culture of each isolate, was transferred to a tissue culture flask (275 ml, Corning Inc., New York, NY) containing 25 ml cleared V8-$CaCO_3$ liquid medium. The flasks were incubated vertically at 25°C in darkness for 24 hours. Each flask then was shaken (30 short strokes) to fragment hyphae that had grown out from the inoculum plug. Flasks were then incubated horizontally under the same conditions. After 6 days, 100 ml SDW was added to each flask and the mycelial mat sank to the bottom of the liquid within few seconds. The flasks were further incubated vertically at 18°C in darkness for 10 weeks. Four samples of submerged mycelium were removed from each flask for each isolate and examined for chlamydospore formation by light microscopy. Twenty arbitrarily selected chlamydospores were measured for diameter and wall-thickness for each isolate. In colonies in which chlamydospores were not observed, the entire colony was checked with a dissecting microscope (60X). The experiment was repeated once. Two replications were included in each experiment. Each flask was considered a replication.

RAPD analysis. Twenty-four isolates of *P. capsici* from six different locations (four isolates per location) (Table 1), were assessed for genetic differences by RAPD analysis. A total of 25 decanucleotide primers (Operon Tech., Alameda, CA) were screened using DNA extracted from 4 test isolates of *P. capsici*, selected from among the 24 isolates, which represented different levels of virulence. Seven of the primers (OPA-03, OPA-04, OPA-10, OPA-11, OPB-13, OPC-09, and OPD-04) were selected for this study based on the number of clear, reproducible bands

amplified from the test isolates and the production of polymorphic bands that distinguished the test isolates. The sequences of the primers were OPA-03: AGTCAGCCAC; OPA-04: AATCGGGCTG; OPA-10: GTGATCGCAG; OPA-11: CAATCGCCGT; OPB-13: TTCCCCCGTC; OPC-09: CTCACCGTCC; and OPD-04: TCTGGTGAGG. DNA amplification was performed in a thermal cycler (PTC-200, MJ Research Inc., Waltham, MA) with one cycle at 94°C for 5 min, 36°C for 2 min, and 70°C for 2 min, followed by 44 cycles at 94°C for 1 min, 36°C for 1 min, and 70°C for 2 min, and a final cycle at 70°C for 10 min. Each RAPD reaction was done in a total volume of 25 µl, containing 30 ng of genomic DNA, 1X PCR buffer, 120 µM of each dNTP, 0.4 µM of primer, and 2.5 units of Taq polymerase (KlenTaq, Ab Peptides Inc., St. Louis, MO). Amplified products were separated by electrophoresis in 2% agarose gels (Low EEO, Fisher Scientific, Fair Lawn, NJ) at a constant 70V in 1X TAE buffer for 4 h at room temperature. Fragments were visualized under UV light after staining with ethidium bromide and photographed. RAPD reproducibility was confirmed by repeating the reactions at least twice for each isolate.

A binary data matrix was prepared for each isolate and primer in which a RAPD band was scored as 1 when present or 0 when absent. A matrix of genetic distance coefficients for each pair of isolates was calculated using the Nei and Li (16) equation: $GD_{(ij)} = 1- [(2N_{(ij)}/(N_{(i)} + N_{(j)})]$, where $N_{(ij)}$ is the total number of bands common to isolates i and j, and $N_{(i)}$ and $N_{(j)}$ is the total number of bands present in i and j isolates, respectively. A dendrogram was constructed from the distance matrix by the unweighted pair group method algorithum (UPGMA) cluster analysis using PAUP 3.1 program (developed by D.L. Swofford, Laboratory of Molecular Systematics, Smithsonian Institution, Washington, D.C.). Statistical significance of the dendrogram branches, was assessed through bootstrap with 1,000 replications.

Virulence test. Twenty-four isolates of *P. capsici*, used in the RAPD analysis (Table 1), were tested for their virulence on pumpkin seedlings in a greenhouse. Sporangial suspensions were prepared from 6-day-old cultures of *P. capsici* grown on LBA at 24°C under continuous white fluorescent light. Ten milliliters SDW were added to each plate and the plate was gently shaken to suspend sporangia in water. The suspension was incubated at 20°C for 1 h to allow the sporangia to release their zoospores. Zoospores were separated from the empty sporangia by passing the suspension through a two-layered facial tissue (Kleenex). The concentration of zoospores was adjusted to 2×10^5 zoospores/ml SDW using a hemacytometer.

Seeds of processing pumpkin (cv. Dickinson) were sown in seed flats with 12 holes (6 x 7 x 5 cm each, one seed per hole) containing steamed greenhouse soil mix (soil:peat:perlite; 2:1:1) and grown in a greenhouse at 23 to 25°C. Four-week-old seedlings were inoculated by adding 5 ml of the zoospore suspension (2×10^5 spores/ml SDW) onto soil surface around the seedling in each hole. Seedlings were watered 1 h prior to inoculation to keep the soil wet. After inoculation, the seedlings were watered again. Seedlings were kept on the greenhouse bench in a completely randomized design with three replications. A seed flat containing 12 seedlings was considered as one replicate.

Beginning the day after inoculation, seedlings were watered twice daily. The incidence and severity of disease was evaluated daily for 15 successive days beginning the day after inoculation. Disease severity was evaluated on a scale of 0 to 3, where 0 = no symptoms, 1 = stem lesion without wilting, 2 = wilting and girdled stem without damping-off of seedlings, and 3 = girdled stem with damping-off of seedlings. The experiment was repeated twice. The data were analyzed using analysis of variance procedure of SAS and treatment means were compared using Fischer's protected LSD test ($P = 0.05$).

RESULTS

Sporangial morphology. All isolates produced papillate and deciduous sporangia on long pedicels that were mostly ellipsoid to ovoid. The mean length of sporangia among the isolates ranged from 42.2 to 55.4 µm, and mean breadth of sporangia varied from 24.0 to 39.1 µm. The length/breadth ratio of sporangia ranged from 1.3 to 1.8 among the isolates. Mean pedicel length ranged from 34.3 to 101.4 µm

PCR-based identification of isolates. All of the isolates tested with the *P. capsici* specific primer PCAP, in combination with ITS primer ITS1, yielded the expected 172-bp product. Thus, identification of the isolates as *P. capsici* was confirmed.

Colony characteristics. Isolates of *P. capsici* tested had four different growth patterns: cottony, petaloid, rosaceous, and stellate, with the occurrence of 5, 28, 14, and 53%, respectively (Table 1). Isolates from the same field had similar growth patterns. All of the isolates grew at temperatures of 10, 15, 20, 24, 30, 32, 35, and 36°C, with the exception of isolates Pc1, Pc2-1, Pc2-5, and ATCC-15427, having cottony growth pattern, which did not grow at 36°C. Only isolates Pc24-1, Pc24-2, Pc24-3, Pc24-4, Pc24-5, Pc24-6, Pc31, Pc35-6, Pc35-9, Pc35-10, and Pc38-11 grew at 38°C (approximately 0.4 mm/day). Optimum temperature for growth among the isolates varied from 24 to 32°C.

Mating type. All 57 isolates of *P. capsici* tested were heterothallic. Thirty-one isolates were A1 mating type and 26 isolates were A2 mating type (Table 1). Of seven fields from which multiple isolates were obtained, only A1, only A2, and both A1 and A2 mating types were found in 3, 2, and 2 fields, respectively. All of the oospores examined had amphigynous antheridia. The mean diameter of the oospores ranged from 22.1 to 38.4 µm. The mean oospore diameter in A1

mating type isolates (33.92 µm) was significantly (P = 0.05) greater than A2 mating type (25.55 µm).

Chlamydospore formation. Among the twenty-four isolates tested, nine isolates (Pc1, Pc2-1, Pc10, Pc24-2, Pc24-4, Pc33-1, Pc35-4, Pc36-7, Pc38-12) produced chlamydospores in both of the replicated experiments. Chlamydospores were single, terminal or intercalary, and pale yellow. The diameter of chlamydospores ranged from 20.0 to 27.5 µm. Wall thickness of chlamydospores ranged from 2.0 to 2.5 µm.

RAPD analysis. Seven primers used in the RAPD analysis generated simple banding patterns that revealed DNA polymorphism among the isolates. A total of 65 polymorphic bands were scored in repeated amplifications from the 24 isolates of *P. capsici* (Fig. 1). None of the primers distinguished all the isolates as different genotypes. Cluster analysis separated the isolates into six RAPD groups (Fig. 2). RAPD groups corresponded to pathogenicity traits, not to location of origin. The genetic distance values ranged from 0.03 to 0.45. The first group (A) included four isolates Pc1, Pc2-1, Pc34-7, and Pc34-8 from two towns (Pekin, Machinaw). The second group (B) included eight isolates Pc38-12, Pc38-13, Pc33-1, Pc33-9, Pc33-5, Pc13, Pc35-10, and Pc35-4 from four towns (Manito, Green Valley, Allentown, Pekin). The first and second groups were separated at a genetic distance of 0.33. The third group (C) included three isolates Pc33-3, Pc34-3, and Pc34-11 from two towns (Green Valley, Machinaw) and was separated from groups A and B with a distance of 0.36. Isolate Pc36-5 (Forest City) did not cluster with any isolate and constituted a group alone (D) and was separated from group A, B, and C with a distance of 0.37. The fifth group (E) included three isolates (Pc35-6, Pc35-7, and Pc24-2) from two towns (Allentown, Manito) and was separated from other groups with a distance of 0.40. The last group (F) included five isolates Pc36-7, Pc36-9, Pc36-8, Pc24-4, and Pc10 from three towns (Forest

City, Manito, and Pekin) and was separated with a distance of 0.45 from the other five groups. Five of the groups contained isolates of both mating types.

Virulence of isolates. There was a significant ($P = 0.05$) variation in disease incidence and severity among the isolates irrespective of location. The lowest disease incidence of 8.3% was scored for isolates Pc33-3 and Pc34-3, while the highest of 100% was scored for Pc35-4 and Pc35-10 (Fig. 3). The lowest mean disease severity of 0.01 was scored for isolates Pc34-3, while the highest of 2.52 was scored for isolate Pc35-6 (Fig. 4). Overall, isolates from the Machinaw location were less virulent. The mean disease severity values of 2.52, 2.45, and 2.42 for the isolates Pc35-6, Pc35-7, and Pc24-2, respectively, was significantly higher than those of the rest of the isolates. Based on the disease severity values, the isolates were separated into six groups: I (Pc1, Pc2-1, Pc34-7, and Pc34-8); II (Pc38-12, Pc33-1, Pc33-9, Pc33-5, Pc13, Pc38-13, Pc35-10, and Pc35-4); III (Pc33-3, Pc34-3, and Pc34-11); IV (Pc36-5); V(Pc35-6, Pc35-7 and Pc24-2); and VI (Pc36-7, Pc36-9, Pc36-8, Pc24-4, and Pc10), which correspond to RAPD groups A, B, C, D, E, and F, respectively (Fig. 2).

DISCUSSION

The results of this study showed significant differences in virulence among *P. capsici* isolates from processing pumpkin in Illinois, which agrees with reports by Ristaino (19) and Lee et al. (13) that there are significant differences in aggressiveness among *P. capsici* isolates from cucurbits and peppers.

Cluster analysis separated 24 isolates of *P. capsici* into six RAPD groups, which corresponded to six pathogenicity groups (Fig. 2). Lamour and Hausbeck (12) reported genetic variation among isolates of *P. capsici* from different locations in Michigan. Abu-El Samen et al.

(1) also reported genetic variation among isolates of *P. infestans* from different locations. Relationships between RAPD groups and virulence of isolates of *Aphanomyces euteiches*, another oomycete pathogen, irrespective of geographical origin has been reported (14). In our study, there was no significant relationship between either virulence or genetics of the isolates and locations (towns). The probable reason for the difference between our findings and those reports by other investigators is that all six towns in our studies are located within approximately 30 km radius; thus, the locations are not separated from each other by long distances.

In the present study, both A1 and A2 mating types of *P. capsici* were identified in two of seven processing pumpkin fields. All of the isolates produced oospores when compatible isolates of A1 and A2 mating types were paired in culture. Similar results in squash and pepper fields have been reported by Ristaino (19). The presence of both mating types together in processing pumpkin fields increases the potential of oospore production and genetic recombination of *P. capsici*.

In our study, 37.5% of the isolates of *P. capsici* tested, produced chlamydospores irrespective of mating types. It has been reported that isolates of *P. capsici* from black pepper, macadamia, and cocoa produced chlamydospores (2,24,25). However, there is no report of chlamydospore formation by isolates of *P. capsici* from cucurbits. Thus, this is the first report of chlamydospore formation by *P. capsici* from pumpkin. Chlamydospores may overwinter in the fields and serve as a primary source of inoculum.

It has been reported that diameter of oospores of *P. capsici* varies with pairing different A1 or A2 mating type (19,22). Mchau and Coffey (15) reported that oospore size varied considerably even within a single pair. The usefulness of oospore dimensions for separating different morphological forms or species of *Phytophthora* has been evaluated (7,9,15). In this

study, all of the isolates identified as the A1 mating type produced larger oospores pairing with A2 isolate ATCC-15399 compared with isolates of the A2 mating type which produced oospores pairing with A1 isolate ATCC-15427. According to the existing knowledge, oospore size could not be used to accurately determine mating types or virulence of *P. capsici* isolates from processing pumpkin. Some heterothallic isolates of A1 (Pc1, Pc2-1, Pc9) and A2 (Pc2-5, Pc4, Pc12) mating types produced few homothallic oospores in solo cultures on LBA medium in this study. Similar findings have been reported for isolates of *P. capsici* from pepper, cucumber, squash, cocoa, and other heterothallic species of *Phytophthora* (10,19).

Phytophthora capsici has been characterized by its ability to grow at temperatures higher than 35°C (18). In our study, three isolates (Pc1, Pc2-1, Pc2-5) that have a cottony growth pattern, and an isolate from ATCC (ATCC-15427) with the same growth pattern, did not grow at 36°C. This indicates that *P. capsici* isolates having cottony growth pattern are more sensitive to temperatures higher than 35°C compared with the isolates with stellate, petaloid, or rosaceous growth patterns. Inability of a *P. capsici* isolate from ATCC and a pepper isolate of *P. capsici* to grow at 36°C had been reported previously (19). Therefore, growth at temperature greater than 35°C cannot be a definitive character for identification of *P. capsici.* These findings agree with those of other researches (15,19) who have questioned the reliability of this character for identification of *Phytophthora* species.

The results of this study showed that virulence of *P. capsici* isolates from different processing pumpkin fields varied significantly. The variability in virulence of *P. capsici* may explain some of the difficulties that hinder development of cucurbit cultivars resistant to Phytophthora blight, or developing other effective control measures against *P. capsici.* The results from this study and findings by other investigators (12,13) show that in developing

effective strategies for management of *P. capsici* a large pool of isolates from different locations, must be tested. Development of sensitive DNA-based diagnostic methods for determining virulence of *P. capsici* isolates will be useful.

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Table 1. Mating types, diameter of oospore, and colony characteristics of isolates of *Phytophthora capsici* from processing pumpkin fields in Illinois

Isolate[w]	Field number (town)[x]	Plant part	Year isolated	Mating type	Oospore diameter (µm)[y]	Growth pattern	Colony growth (mm/day)	
							35° C	36° C
Pc1	1 (Pekin)	Seedling	2000	A1	34.16 b[z]	Cottony	8.5	0.0
Pc2-1	2 (Pekin)	Seedling	2000	A1	33.12 b	Cottony	7.2	0.0
Pc2-5	2 (Pekin)	Leaf	2000	A2	27.72 c	Cottony	4.7	0.0
Pc4	3 (Pekin)	Vine	2000	A2	26.12 c	Stellate	5.1	1.8
Pc9	4 (Pekin)	Fruit	2000	A1	37.16 a	Stellate	7.6	2.5
Pc10	5 (Pekin)	Petiole	2000	A2	25.00 cd	Petaloid	10.1	1.8
Pc11	6 (Green Valley)	Petiole	2000	A2	23.54 d	Rosaceous	4.3	1.7
Pc12	7 (Green Valley)	Petiole	2000	A2	-	Stellate	8.8	2.0
Pc13	8 (Pekin)	Petiole	2000	A1	38.40 a	Stellate	7.8	1.9
Pc15	9 (Manito)	Petiole	2000	A2	23.83 d	Stellate	6.2	2.5
Pc16	10 (Manito)	Petiole	2000	A2	27.55 c	Stellate	8.3	2.7
Pc18	11 (Manito)	Petiole	2000	A2	26.00 c	Stellate	8.1	2.8
Pc20	12 (Pekin)	Seedling	2000	A1	32.28 b	Stellate	6.9	2.2
Pc21	13 (Pekin)	Seedling	2000	A2	26.79 c	Stellate	7.5	2.1
Pc22	14 (Pekin)	Seedling	2000	A2	26.87 c	Stellate	8.0	2.3
Pc24-1	15 (Manito)	Fruit	2001	A1	35.00 b	Stellate	9.4	3.8
Pc24-2	15 (Manito)	Fruit	2001	A1	33.13 b	Stellate	6.6	2.3
Pc24-3	15 (Manito)	Fruit	2001	A1	-	Stellate	4.5	2.2
Pc24-4	15 (Manito)	Fruit	2001	A1	-	Stellate	4.8	2.2
Pc24-5	15 (Manito)	Fruit	2001	A1	33.75 b	Stellate	4.3	2.4
Pc24-6	15 (Manito)	Fruit	2001	A1	32.29 b	Stellate	4.3	2.1
Pc31	16 (Green Valley)	Soil	2001	A1	-	Stellate	6.0	2.3
Pc33-1	17 (Green Valley)	Petiole	2001	A1	-	Petaloid	7.0	3.0
Pc33-3	17 (Green Valley)	Petiole	2001	A1	33.12 b	Petaloid	7.2	2.2
Pc33-4	17 (Green Valley)	Petiole	2001	A1	33.54 b	Petaloid	7.4	2.0
Pc33-5	17 (Green Valley)	Petiole	2001	A1	31.46 b	Petaloid	7.6	1.6
Pc33-6	17 (Green Valley)	Petiole	2001	A1	34.00 b	Petaloid	5.8	3.0
Pc33-7	17 (Green Valley)	Petiole	2001	A1	-	Petaloid	6.2	3.1
Pc33-8	17 (Green Valley)	Petiole	2001	A1	33.64 b	Petaloid	7.9	2.4
Pc33-9	17 (Green Valley)	Petiole	2001	A1	33.43 b	Petaloid	5.3	3.2
Pc34-1	18 (Machinaw)	Petiole	2001	A2	27.00 c	Rosaceous	6.8	3.4
Pc34-3	18 (Machinaw)	Petiole	2001	A2	26.34 c	Rosaceous	7.2	3.3
Pc34-4	18 (Machinaw)	Vine	2001	A2	25.41 c	Rosaceous	6.7	2.6
Pc34-5	18 (Machinaw)	Petiole	2001	A2	24.00 cd	Rosaceous	6.2	2.6
Pc34-7	18 (Machinaw)	Petiole	2001	A2	26.79 c	Rosaceous	6.0	2.2
Pc34-8	18 (Machinaw)	Vine	2001	A2	25.78 c	Rosaceous	6.0	2.3
Pc34-11	18 (Machinaw)	Petiole	2001	A2	26.87 c	Rosaceous	5.3	2.3

Table 1. Mating types, diameter of oospore, and colony characteristics of isolates of *Phytophthora capsici* from processing pumpkin fields in Illinois - continues

Isolate[w]	Field number (town)[x]	Plant part	Year isolated	Mating type	Oospore diameter (µm)[y]	Growth pattern	Colony growth (mm/day)	
							35° C	36° C
Pc35-4	19 (Allentown)	Petiole	2001	A2	27.60 c[z]	Stellate	4.8	2.2
Pc35-6	19 (Allentown)	Petiole	2001	A2	27.00 c	Stellate	6.0	2.4
Pc35-7[e]	19 (Allentown)	Leaf	2001	A2	24.79 cd	Stellate	6.3	2.1
Pc35-9[e]	19 (Allentown)	Petiole	2001	A2	23.75 d	Stellate	6.5	2.4
Pc35-10	19 (Allentown)	Petiole	2001	A2	26.77 c	Stellate	6.2	2.8
Pc36-1	20 (Forest city)	Petiole	2001	A2	22.60 d	Stellate	8.9	4.0
Pc36-2	20 (Forest city)	Petiole	2001	A2	22.17 d	Stellate	10.1	6.2
Pc36-3	20 (Forest city)	Fruit	2001	A1	32.92 b	Stellate	8.5	4.0
Pc36-4	20 (Forest city)	Petiole	2001	A2	22.92 d	Stellate	8.7	4.0
Pc36-5	20 (Forest city)	Fruit	2001	A1	38.56 a	Stellate	7.8	3.8
Pc36-7	20 (Forest city)	Petiole	2001	A1	33.02 b	Stellate	8.2	3.9
Pc36-8	20 (Forest city)	Petiole	2001	A1	32.63 b	Stellate	9.6	4.0
Pc36-9	20 (Forest city)	Petiole	2001	A1	35.13 b	Stellate	5.3	3.8
Pc38-8	21 (Manito)	Leaf	2001	A1	-	Petaloid	6.8	2.5
Pc38-11	21 (Manito)	Leaf	2001	A1	32.46 b	Petaloid	6.1	2.5
Pc38-12	21 (Manito)	Petiole	2001	A1	33.00 b	Petaloid	4.1	2.7
Pc38-13	21 (Manito)	Petiole	2001	A1	32.92 b	Petaloid	5.6	2.2
Pc38-14	21 (Manito)	Vine	2001	A1	35.00 b	Petaloid	4.6	2.2
Pc38-15	21 (Manito)	Vine	2001	A1	-	Petaloid	5.1	2.8
Pc39-2	22 (Manito)	Petiole	2001	A2	-	Petaloid	4.0	3.2
ATCC15427	-	-	-	A1	-	Cottony	3.1	0.0
ATCC15399	-	-	-	A2	-	Stellate	4.0	1.7

[w] Isolates marked in bold were used for chlamydospore formation, virulence test, and RAPD analysis. ATCC isolates obtained from American Type Culture Collection Center.

[x] All six towns (locations) are in central Illinois.

[y] Mean of 50 oospores.

[z] Values in the column with a letter in common are not significantly different from each other according to Fischer's protected test ($P = 0.05$).

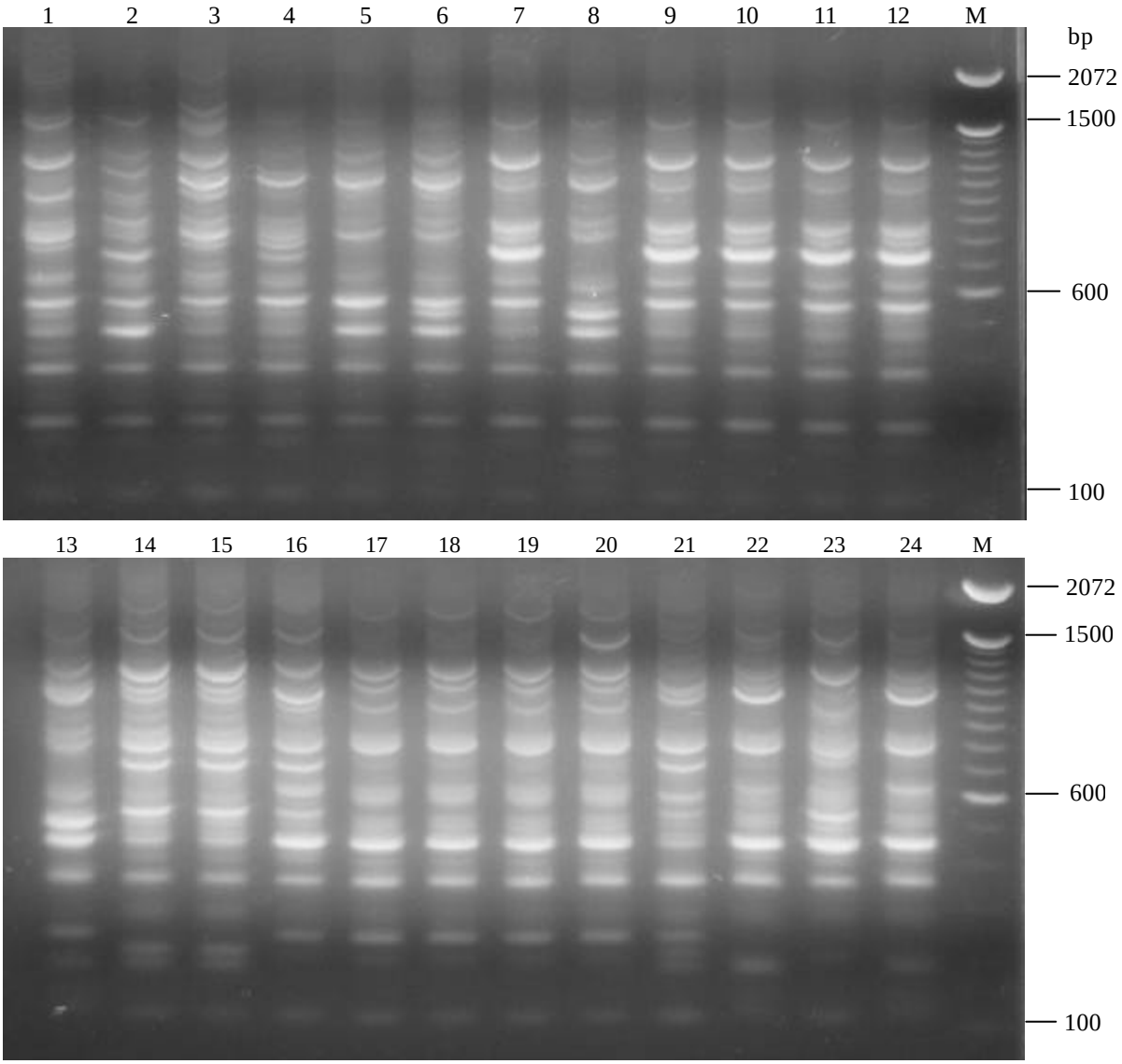
1
2
3
4
5
6
7
8
9
10
11
12
M
bp
2072
1500
600
100
13
14
15
16
17
18
19
20
21
22
23
24
M
2072
1500
600
100

Fig. 1.

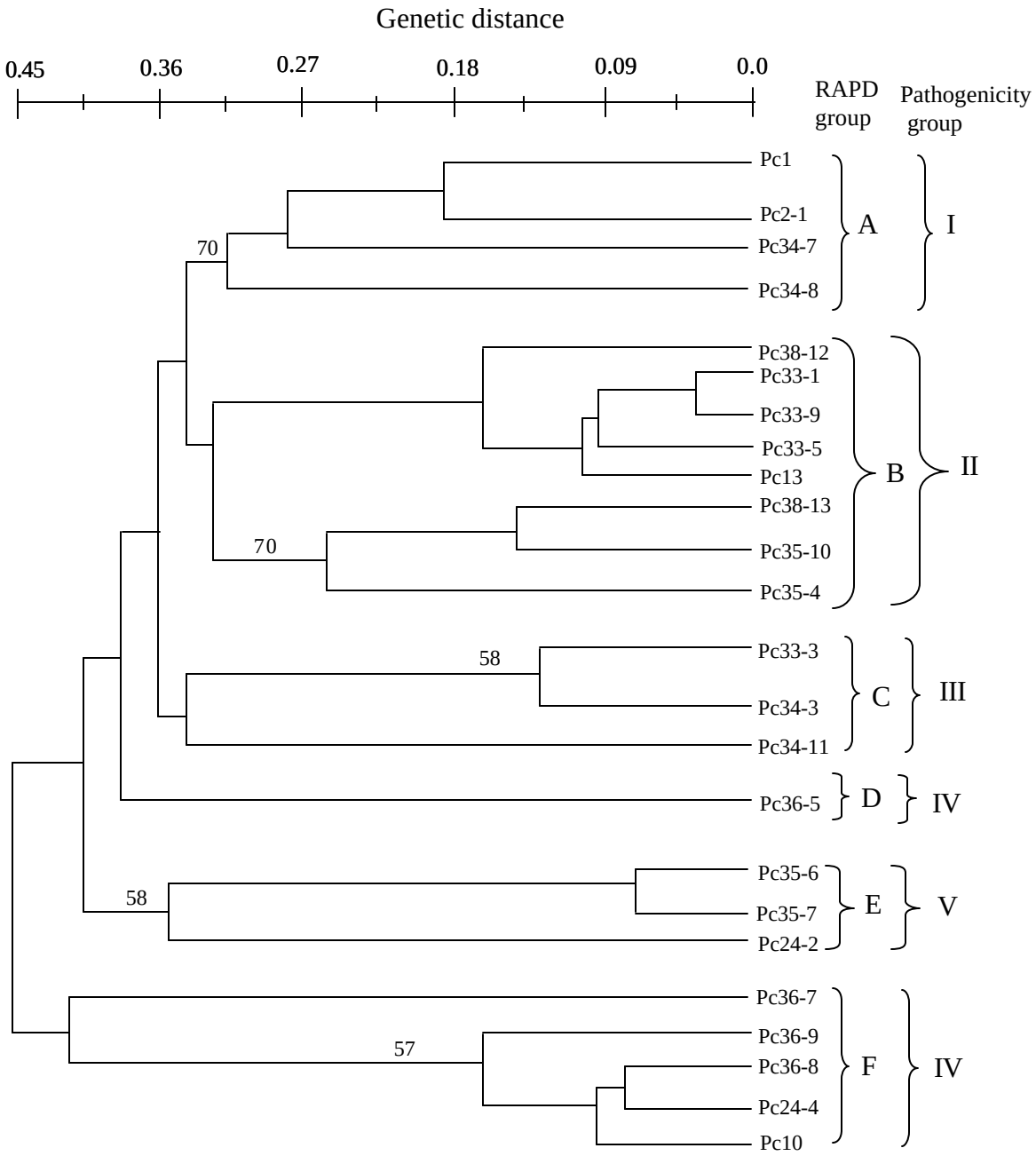
Genetic distance
0.45
0.36
0.27
0.18
0.09
0.0
RAPD group
Pathogenicity group
Pc1
Pc2-1
Pc34-7
Pc34-8
70
A
I
Pc38-12
Pc33-1
Pc33-9
Pc33-5
Pc13
Pc38-13
Pc35-10
Pc35-4
70
B
II
Pc33-3
Pc34-3
Pc34-11
58
C
III
Pc36-5
D
IV
Pc35-6
Pc35-7
Pc24-2
58
E
V
Pc36-7
Pc36-9
Pc36-8
Pc24-4
Pc10
57
F
IV

Fig. 2.

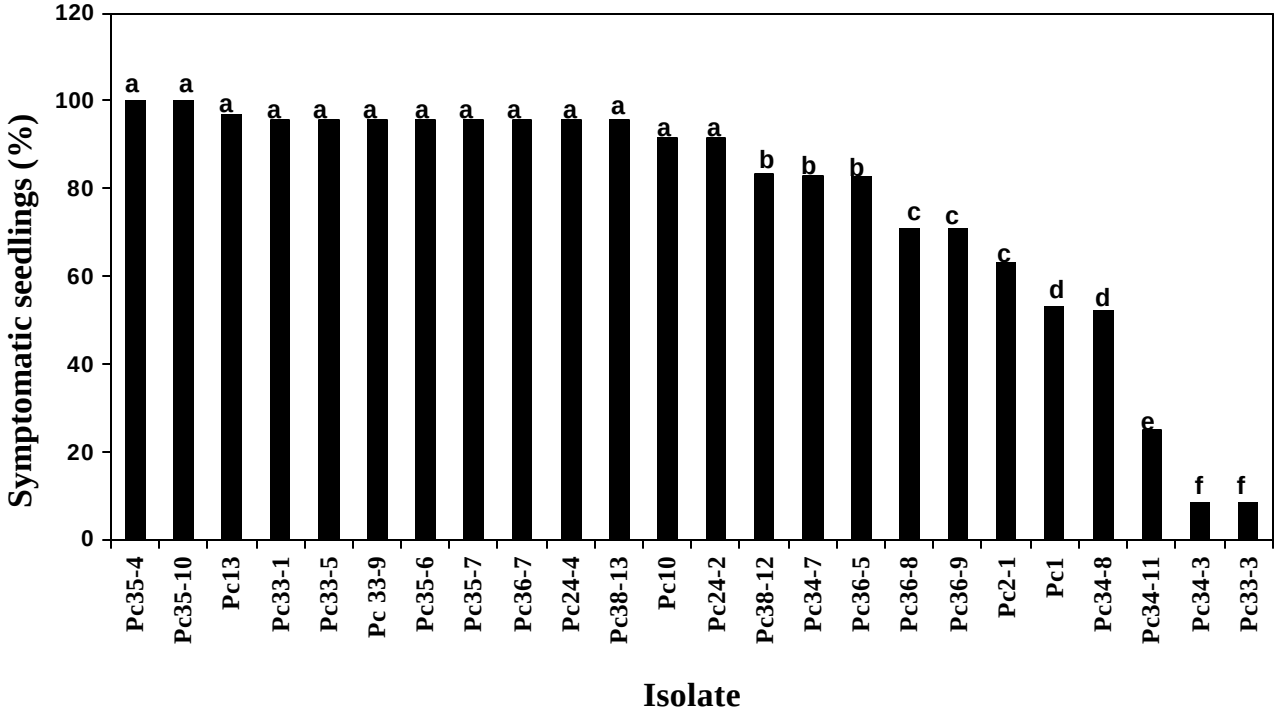
Symptomatic seedlings (%)
120
100
80
60
40
20
0
a a a a a a a a a a a a a a b b b c c c d d e f f
Pc35-4
Pc35-10
Pc13
Pc33-1
Pc33-5
Pc 33-9
Pc35-6
Pc35-7
Pc36-7
Pc24-4
Pc38-13
Pc10
Pc24-2
Pc38-12
Pc34-7
Pc36-5
Pc36-8
Pc36-9
Pc2-1
Pc1
Pc34-8
Pc34-11
Pc34-3
Pc33-3
Isolate

Fig. 3.

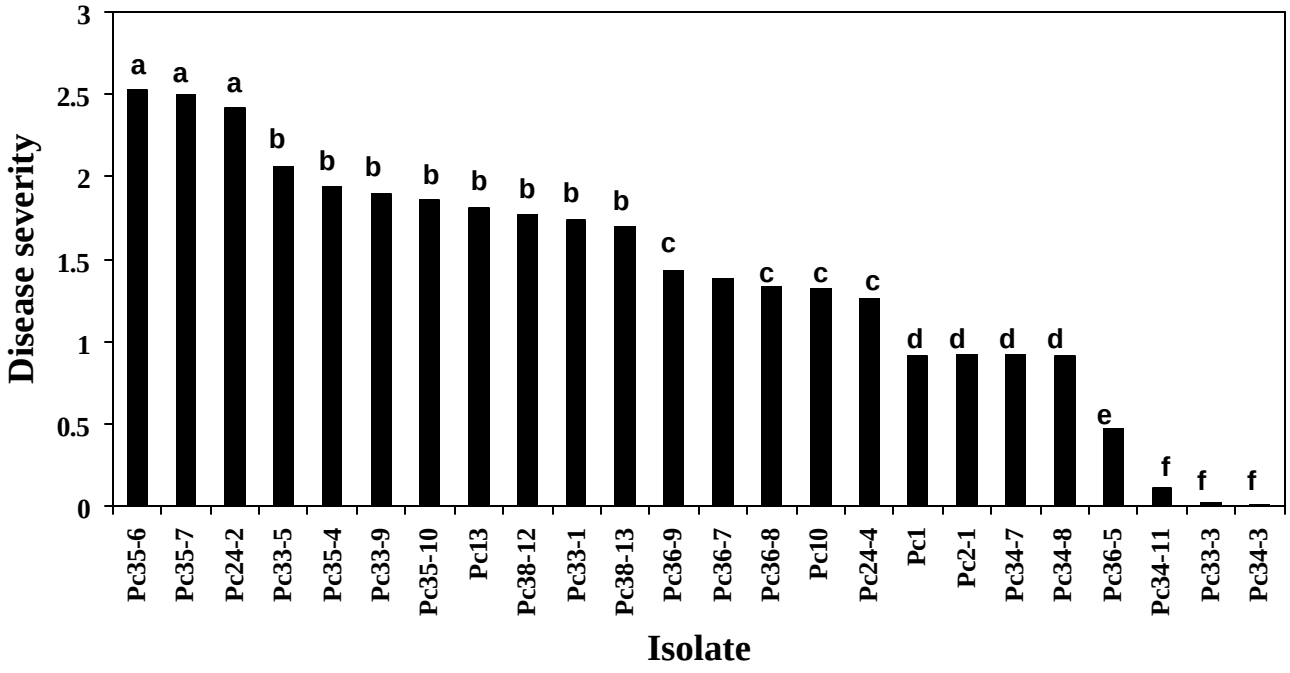
Disease severity
3
2.5
2
1.5
1
0.5
0
a a a b b b b b b b b c c c c d d d d e f f f
Pc35-6 Pc35-7 Pc24-2 Pc33-5 Pc35-4 Pc33-9 Pc35-10 Pc13 Pc38-12 Pc33-1 Pc38-13 Pc36-9 Pc36-7 Pc36-8 Pc10 Pc24-4 Pc1 Pc2-1 Pc34-7 Pc34-8 Pc36-5 Pc34-11 Pc33-3 Pc34-3
Isolate

Fig. 4.

Fig. 1. RAPD profiles generated by primer OPA-11 for 24 isolates of *Phytophthora capsici* from 10 processing pumpkin fields at six locations in central Illinois. Marker (M) is 100-bp DNA ladder (Invitrogen Tech., Carlsbad, CA). Amplified DNA from isolates ("M" suffix is omitted) in the lanes from left to right are Pc1, Pc2-1, Pc10, Pc13, Pc24-2, Pc24-4, Pc38-12, Pc38-13, Pc33-1, Pc33-3, Pc33-5, Pc33-9, Pc34-3, Pc34-7, Pc34-8, Pc-34-11, Pc35-4, Pc35-6, Pc35-7, Pc35-10, Pc36-5, Pc36-7, Pc36-8, and Pc36-9.

Fig. 2. Clustering of 24 isolates of *Phytophthora capsici* from 10 different processing pumpkin fields at six locations in central Illinois using PAUP program. Genetic distance was calculated according to the equation developed by Nei and Li (16). Bootstrap (1000 replicates) values greater than 50% are shown on the branches. Pathogenicity groups are based on disease severity.

Fig. 3. Percent of symptomatic processing pumpkin seedlings inoculated with *Phytophthora capsici* isolates from processing pumpkins. Percent symptomatic seedlings were determined two weeks after inoculation. Bars labeled with the same letter are not significantly different (P = 0.05) according to Fisher's protested LSD test.

Fig. 4. Mean disease severity on processing pumpkin seedlings inoculated with *Phytophthora capsici* isolates from processing pumpkins. Disease severity was determined two weeks after inoculation using a scale of 0-3, where 0 = no symptoms, 1 = stem lesion without wilting, 2 = wilting and girdled stem without damping-off of seedlings, and 3 = girdled stem with damping-off of seedlings. Bars labeled with the same letter are not significantly different (P = 0.05) according to Fisher's protested LSD test.