HOST RANGE, HOST RESISTANCE, AND POPULATION STRUCTURE OF *PHYTOPHTHORA CAPSICI*

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ABSTRACT

HOST RANGE, HOST RESISTANCE, AND POPULATION STRUCTURE OF *PHYTOPHTHORA CAPSICI*

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Phytophthora capsici is a destructive soilborne pathogen worldwide. *P. capsici* has a broad host range that includes members of more than twenty plant families that contain many economically important vegetable crops. Some vegetable growers in Michigan plant conifers for the Christmas tree market in fields infested with *P. capsici.* To determine the susceptibility of Fraser fir to *P. capsici,* stems or roots of seedlings were inoculated with each of 4 *P. capsici* isolates and incubated in growth chambers. In addition, Fraser fir seedlings were planted in two commercial fields naturally infested with *P. capsici*. All *P. capsici* isolates tested incited disease in the seedlings regardless of incubation temperature or inoculation method. Seedlings (72%) planted in *P. capsici*-infested fields developed disease symptoms and died. Identification was confirmed by species-specific direct colony Polymerase chain reaction. This study suggests that planting Fraser fir in fields infested with *P. capsici* could result in infection and that adjustments in current rotational schemes are needed.

Phytophthora capsici causes root, crown, and fruit rot of tomato, a major vegetable crop grown worldwide. One objective of this study was to screen tomato varieties and wild relatives of tomato for resistance to *P. capsici*. Four *P. capsici* isolates were individually used to inoculate 6-week-old seedlings of 42 tomato varieties and wild relatives in a greenhouse. Plants were evaluated daily for wilting and death. All *P. capsici* isolates tested

caused disease in seedlings but some isolates were more pathogenic than others. A wild relative of cultivated tomato, *Solanum habrochaites* accession LA407, was resistant to all *P. capsici* isolates tested. Moderate resistance to all isolates was identified in the host genotypes Ha7998, Fla7600, Jolly Elf, and Talladega. Amplified fragment length polymorphisms of tomato genotypes showed a lack of correlation between genetic clusters and susceptibility to *P. capsici*, indicating that resistance is distributed in several tomato lineages. The results of this study create a baseline for future development of tomato varieties resistant to *P. capsici*.

Phytophthora capsici Leonian is a destructive soilborne pathogen that can infect economically important solanaceous, cucurbitaceous, fabaceous, and other crops in the United States and worldwide. The objective of this study was to investigate the genetic structure of *P. capsici* isolates assigned to predefined host, geographical, mefenoxam sensitivity and mating type categories. Isolates from 6 continents, 21 countries, 18 United States (U.S) states, and 26 host species were genotyped for four mitochondrial and six nuclear loci. Bayesian clustering analysis revealed population structure by host, geographic origin and mefenoxam sensitivity with clusters occurring more or less frequently in particular categories. Our findings of genetic structuring in *P. capsici* populations highlight the importance of including isolates from all detected clusters that represent the genetic variation in *P. capsici* for development of diagnostic tools, fungicides, and host resistance. This study provides an initial map of global population structure of *P. capsici* but continued genotyping of isolates will be necessary to expand our knowledge of genetic variation in this important plant pathogen.

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CHAPTER I: LITERATURE REVIEW

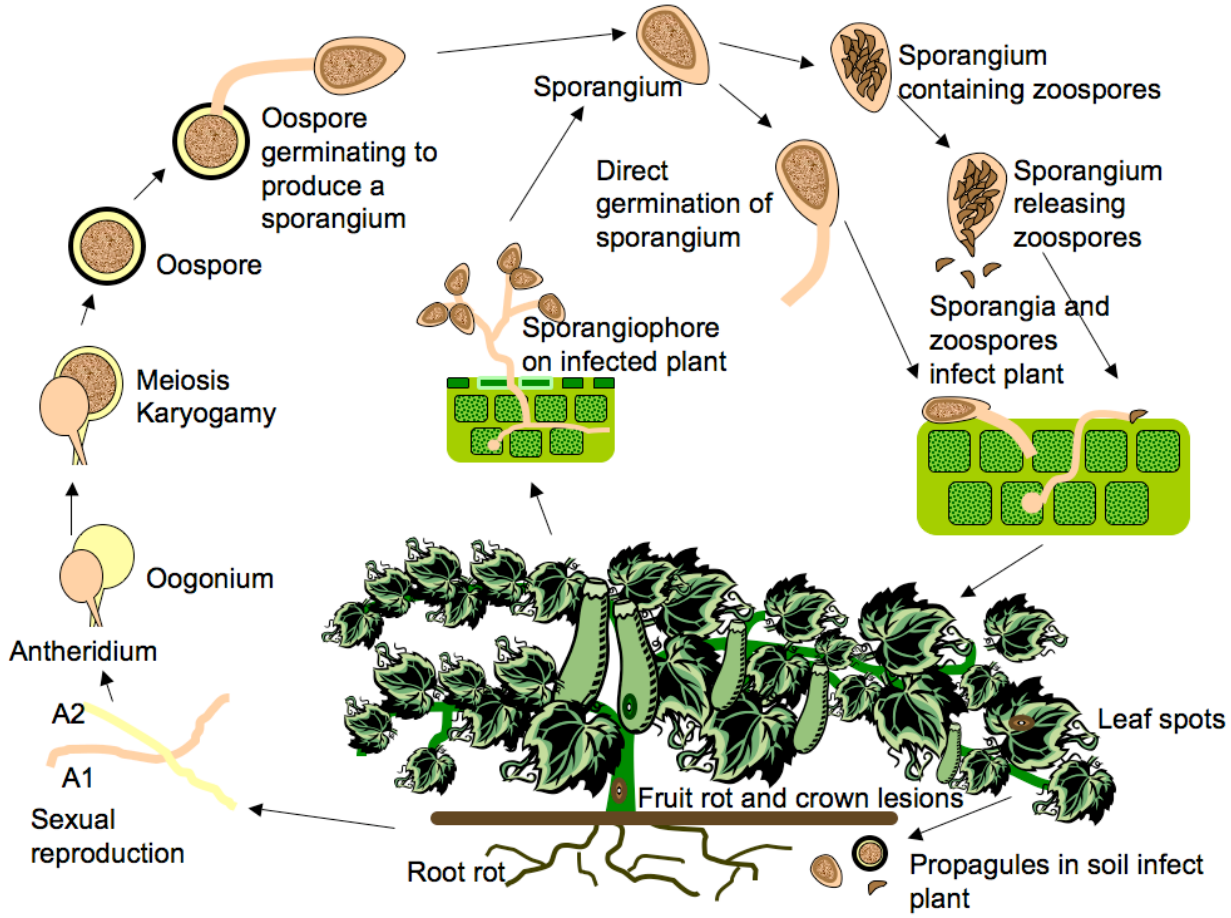
Phytophthora capsici is an important plant pathogen worldwide, affecting several crops in many countries (6, 18, 29, 30, 35) including the United States (U.S.). The genus *Phytophthora* is located within the Kingdom Stramenopila, Phylum Oomycota, Class Oomycetes, Order Peronosporales, Family Phythiaceae (3, 18). There are more than 60 species in the genus *Phytophthora* and most of them are devastating plant pathogens (18, 27). Oomycetes such as *P. capsici* are evolutionarily distant from fungi and close to algae and plants (7). However, the basic biology, the growth habit and plant tissue colonization of oomycetes is similar to that of true fungi and many researchers clasified them as fungi in the past (53, 66). Nonetheless, it is now clear that characteristics such as cell walls composed primarily of β-glucan, a diploid state throughout most of their life cycle, and the inability to produce sterols, make Oomycetes distinct from fungi (18).

Life Cycle

Phytophthora capsici reproduces both asexually and sexually (Figure 1.1). Asexual reproduction occurs by zoospores, which are produced in conspicuously papillate sporangia. Sexual reproduction involves two compatibility types (CT), called A1 and A2, that produce amphigynous oospores when they come into contact (30). The thallus is composed of coenocytic mycelia that give rise to lemon-shaped sporangia (1). When sporangia come in contact with water they release 20-40 zoospores, which have tropism for the plant roots. The inoculum may come into contact with the roots by movement from root to root in the soil, by movement down rows with surface water, and rain or irrigation splash that takes the inoculum to above-ground plant parts (73). After zoospores make

contact with the plant surface, they encyst and germinate, producing germ tubes (9, 31). The penetration of the germ tube into the host can occur directly with the help of macerating enzymes or through natural openings such as stomata (42, 85). The mycelium then grows inside the plant tissue and forms haustoria to obtain nutrients from the host cells (Figure 1.1) (18).

Figure 1.1: *Phytophthora capsici* life cyle. For interpretation of the references to color in this and all other Figures, the reader is referred to the electronic version of this dissertation.



Oospores (Figure 1.1), which are important survival structures (46), overwinter and persist for long periods of time (5 years or more) in soil (30). Sexual reproduction results in genetic variation due to recombination, leading to an increased chance of generating genotypes that may have characteristics such as fungicide resistance or higher levels of virulence. Sexual reproduction likely impacts management and must be considered when developing control strategies (30, 50).

Host Range

Phytophthora spp. infect a wide range of plants including dicot and monocot species; cultivated crops, ornamentals and native plants can be affected (40). Some species of *Phytophthora,* such as *P. infestans,* are associated with specific plant species and have a narrow and defined host range. Others, such as *P. capsici,* have a broad host range including members of very diverse and phylogenetically distant plant families such as solanaceous, cucurbit and leguminous crops (18). The diseases caused by *P. capsici* include damping off, foliar blights, fruit rot, root and stem rot (18). Table 1.1 lists some of the reported hosts and diseases worldwide.

Table 1.1: Known hosts for *P. capsici*, diseases and distribution. Modified from the list of hosts stated by Erwin and Ribeiro (18).

Host	Disease	Distribution and source
Aloaceae		
Aloe sp. (Aloe)	Root rot	Taiwan (32).
Apiaceae		
Daucus carota (Carrot)	Root rot	U.S. (78).
Araceae		
Anthurium andreanum (Flamingo lily)	Spathe blight	Hawai (5).

Table 1.1 (cont'd)

Asteraceae		
Carthamus tinctorius (Safflower)	Root rot	U.S. (69).
Cosmos sp. (Cosmos)		Taiwan (32).
Brassicaceae		
Brassica oleracea var. *botrytis* (Cauliflower)	Root rot	U.S. (69).
Brassica rapa (Turnip)	Damping-off	U.S. (76).
Raphanus sativus (Radish)	Root rot	U.S. (69).
Cactaceae		
Opuntia ficus-indica (Indian Fig)	Cladode rot	Italy (14).
Caryophyllaceae		
Dianthus barbatus (Carnation)		Taiwan (32).
Chenopodiaceae		
Beta vulgaris (Beet)	Damping-off	U.S. (76).
B. vulgaris var. *cicla* (Swiss-chard)	Damping-off	U.S. (76).
Chenopodium amaranticolor (Spinach)	Root rot	U.S. (69).
Spinacia oleracea (Spinach)	Damping-off	U.S. (76).
Cucurbitaceae		
Bryonia dioica (Red bryony, wild hop)	Root rot	Italy (14).
Citrullus. sp. (Melon)	Collar rot	France (12), Spain (82).
C. lanatus (Watermelon)	Brown fruit rot; vine and leaf wilt	Japan (43), U.S. (48).
Cucumis melo (Cantaloupe, Honeydew melon)	Fruit rot; postharvest decay	U.S. (77).
Cucumis sativus (Cucumber)	Fruit rot	U.S. (48), Korea (45), Taiwan (32).
Cucurbita maxima (Blue Hubbard squash)	Wilt and basal stem rot	U.S. (48), Argentina (64), Italy (61), Japan (38).
C. pepo (Yellow squash, zucchini)	Leaf and stem blight	U.S. (81).

Table 1.1 (cont'd)

Ebenaceae		
*Diospyros kak*i (Persimmon)	Fruit rot	Italy (14).
Ericaceae		
Enkianthus quinquefolius		Taiwan (32).
Fabaceae		
Medicago sativa (Alfalfa)	Root rot	U.S. (69).
Phaseolus sp.		Taiwan (32).
P. lunatus (Butter bean, civet bean, lima bean)	Root rot	Argentina (21), U.S. (15).
P. vulgaris var. *humilis* (Snap beans)		Unites States (24).
Pisum sativum (Pea)	Root rot	U.S. (13).
Vicia faba (Broadbean)	Root rot	U.S. (69).
Geraniaceae		
Geranium carolinianum (Carolina geranium)		U.S. (20).
Lauraceae		
Persea americana (Avocado)	Root rot	U.S. (77).
Liliaceae		
Allium cepa (Onion)		Taiwan (32).
Linaceae		
Linum sp. (Flax)	Root rot	U.S. (69).
Malvaceae		
Abelmoschus esculentus (Okra)	Root rot	U.S. (69).
Albutilon theophrasti (Velvet-leaf)	Damping-off	U.S. (76).
Gossypium hirsutum (Cotton)	Root rot; boll rot	U.S. (69).
Moraceae		
Ficus carica (Fig)	Fruit rot	Japan (44).
Orchidaceae		
Vanilla planifolia (Vanilla)	Root rot	French Polynesia (60).

Table 1.1 (cont'd)

Piperaceae		
Piper betle (Betle)	Foot rot	Thailand (79), Taiwan (32).
P. nigrum (Black pepper)	Foot rot	Thailand (79).
Portulacaceae		
Portulaca oleracea (Common purslane)		U.S. (20).
Proteaceae		
Leucospermum (Pincushion flower)	Stem and root rot	U.S. (83).
Macadamia integrifolia (Macadamia nut)	Raceme blight	U.S. (49).
M. ternifolia (Macadamia nut)	Raceme blight	U.S. (49).
Rosaceae		
Crataegus oxyacantha (Hawthorne)	Fruit rot	Italy (14).
Malus pumila (Apple)	Fruit rot	U.S. (78).
Prunus persica (Peach)	Seedling wilt; stem canker	U.S. (77).
Rutaceae		
Citrus spp. (Citrus)	Root rot	U.S. (84).
Solanaceae		
Capsicum annuum (Red or sweet pepper)	Root and fruit rot; stem blight	U.S. (56), Italy (14), Puerto Rico (80), Argentina (58), Venezuela (57), Brazil (16), Japan (44), Bolivia (8), Spain (4), Iran (17), Serbia (2), Taiwan (11).
	Seedling damping off	Argentina (26), Korea (45), China (33).
C. annuum var. *grossum* (Bell or green pepper)	Root rot	U.S. (78).
Datura stramonium (Jimson weed)	Wilt	Italy (14).
Solanum lycopersicon (Tomato)	Fruit rot; root and crown rot; seedling damping off	U.S. (48), Rusia (63), Venezuela (57), Japan (44), Korea (45), Taiwan (32).
Nicotiana glutinosa (Tobacco)	Root rot	U.S. (70).

Table 1.1 (cont'd)

S. americanum (American black nightshade)		U.S. (20).
S. carolinense (Carolina horsenettle)		U.S. (20).
S. marginatum	Fruit rot	Italy (14).
S. melongena (Eggplant)	Brown rot; fruit rot	Italy (14), Argentina (21), Venezuela (65), Japan (41), Korea (45), Taiwan (32).
Solanum nigrum (Black nightshade)		U.S. (20).
Sterculiaceae		
Theobroma cacao (Cocoa)	Black pod	Brazil and Cameroon (86).

Management

Numerous efforts have been made worldwide towards the control of diseases caused by *Phytophthora* spp., but these oomycete pathogens still cause significant losses. *Phytophthora* spp. have caused billions of dollars in damage to many crops in the U.S. (52). One species, *P. infestans*, has been historically recognized for its devastating role in the Irish potato famine and it still constitutes an enormous threat to food security (27).

The recommended control strategies for *P. capsici* include choosing well drained sites, rotating crops and using fungicides, fumigants and resistant varieties (68). Water is important in the pathogen life cycle and planting in well drained fields and using proper irrigation, raised beds and black plastic mulch is recommended (68). Crop rotation is one of the main strategies for managing *P. capsici*; however, this strategy has been limited by the long-term survival of oospores in the soil and plant material (52). Historically, growers have mostly relied on fungicides for the control of *P. capsici*. The most commonly used

product is mefenoxam, but resistance to this fungicide has been documented in *P. capsici* populations (30). Chemical control can be very expensive because of the required frequent applications. Hence, growers whose fields are infected with *Phytophthora* have to manage the losses caused by the pathogen and also the cost of the fungicides (27, 28). Fungicides can have non-target effects on the biology of the pathogen, such as inducing oospore formation and changes in mating type, as observed in *P. infestans* (28). It is desirable to develop new control strategies that reduce growers' reliance on fungicides.

Host Resistance and Pathogen Populations

A desired control method for *Phytophthora* spp., and *P. capsici* in particular, is the use of resistant varieties (23, 39). However, to guarantee the success of strategies including crop rotation, host resistance and chemical control, it is necessary to have detailed knowledge of pathogen populations and evolution (71). Nevertheless, little is known about the genetic diversity and population genetics of *Phytophthora* spp. (27).

Tolerance has been identified in cucumber (23), pumpkin (54), and tomato (10, 37) but no sources of high or complete resistance have been identified. Some pepper varieties show complete resistance to *P. capsici*; nonetheless, they are not widely used by growers due to their lack of commercially appealing characteristics (19). Other pepper varieties such as 'Paladin' have resistance to crown rot caused by *P. capsici* and good horticultural traits, and are grown in *P. capsici*-infested fields. Identifying new sources of resistance to *P. capsici* to use in breeding programs for commercially important hosts is needed.

There is considerable diversity in host-specific pathogenicity. Some isolates are more virulent to certain cultivars or families of plants (18), and distinct physiological races of *P. capsici* have been reported (25, 36, 54, 62, 67, 74). In a differential pathogenicity

study, *P. capsici* isolates were grouped into 13 classes according to their ability to infect different plant species (74). Significant differences in virulence and pathogen-host interactions of *P. capsici* isolates from pumpkin and pepper have been reported (36, 54). In another study of host specificity, Ristaino characterized the morphological variation in field isolates to determine if there were differences between isolates infecting cucurbit and solanaceous hosts (67). The morphological characters observed showed a continuous rather than a discrete distribution within populations according to host type (67). Ristaino concluded that genetic and phenotypic characterization of the isolates would be necessary to delimit groups within the species (67).

Host specificity can be established because of differential fitness of individuals when associated with particular host species (72). Fitness will determine the contribution of an individual to the gene pool of the next generation, leading to the establishment of a genetic group of isolates more successful in a particular host (72). Suassuna et al. reported these events of host specialization in *P. infestans*; two genetic groups are established in Brazil due to differences in fitness of isolates associated with potato and tomato (72). Host differentiation in *P. infestans* has also been reported in Peru in isolates infecting cultivated potato and wild solanaceous hosts (22). It is important to determine what isolates are virulent to which crop or family of plants because a widely used control method is crop rotation. Many growers use crop rotation between solanaceous and cucurbit plants (68). A genetic study to determine if some isolates or populations have host preference infecting cucurbit plants more frequently than solanaceous plants, would help increase the efficiency of crop rotation as a control method.

Genetic variation, sexual reproduction in the field and differences in virulence and fungicide resistance among isolates of *P. capsici* has been reported in several vegetable growing regions (34, 51, 55, 75). Studies of population spatial distribution in *P. capsici* are required to establish control measures regarding the movement of plant material. The dispersal of the pathogen may occur because of the movement of *P. capsici* on infected plants or oospores on soil or dry plant parts. It is important to distinguish what isolates are present in a certain region and which are not, to avoid the introduction of plant tissue infected with isolates that can generate big epidemics by increasing genetic variability and consequently, the probability to generate more virulent strains. Some apparent hybrids of plant pathogens have been identified in nature and it has been seen that they present a wider host range than the species that originated them (47).

A pathogen like *P. capsici* with a mixed type of reproduction has an increased chance of overcoming host resistance and chemical control. New genotypes are created through recombination during sexual reproduction, and then advantageous alleles can be selected and established in populations by asexual reproduction (59). To effectively control *P. capsici*, future research should aim to establish the population structure of this pathogen in particular regions or hosts, identify new sources of host resistance, and identify new products for chemical control. The objective of this dissertation was to determine the susceptibility of Fraser Fir to *P. capsici*, identify sources of resistance in tomato and wild relatives to crown and root rot caused by *P. capsici*, and investigate the global population structure of this important plant pathogen.

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CHAPTER II: SUSCEPTIBILITY OF FRASER FIR TO *PHYTOPHTHORA CAPSICI*

Abstract

Quesada-Ocampo, L. M., Fulbright, D. W., and Hausbeck, M. K. 2009. Susceptibility of Fraser Fir to *Phytophthora capsici.* Plant Disease 93: 135-141.

Phytophthora cinnamomi, *P. drechsleri, P. citricola* and *P. cactorum* limit Fraser fir production, whereas *P. capsici* affects solanaceous, cucurbitaceous, and fabaceous crops. Some vegetable growers in Michigan plant conifers for the Christmas tree market in fields infested with *P. capsici.* To determine the susceptibility of Fraser fir to *P. capsici*, stems (no wound or 1- or 3-mm-diameter wound) or roots (2 or 4 g of infested millet seed or 2 or 5 x 10^3 zoospores/ml of a zoospore suspension) of seedlings were inoculated with each of 4 *P. capsici* isolates and incubated in growth chambers (20°C or 25°C). In addition, Fraser fir seedlings were planted in two commercial fields naturally infested with *P. capsici*. All *P. capsici* isolates tested incited disease in the seedlings regardless of incubation temperature or inoculation method. Seedlings (72%) planted in *P. capsici*-infested fields developed disease symptoms and died. Most of the *P. capsici* isolates obtained from the Fraser fir seedlings infected while in the field were recovered from root tissue. Identification was confirmed by species-specific direct colony Polymerase chain reaction. The pathogen was successfully recovered from stems of all stem-inoculated seedlings, and from roots and stems of all root-inoculated seedlings; the phenotype of the recovered isolate matched the phenotype of the inoculum. This study suggests that planting Fraser fir in fields infested with *P. capsici* could result in infection and that adjustments in current rotational schemes are needed.

Introduction

Each year approximately 33 to 36 million Christmas trees are produced in North America with an estimated farm gate value of $462 million (5). Michigan ranks third in the production of Christmas trees, with 17,000 hectares and an annual value of $4 million (2, 5). Nationally, the most common species used in plantations include Douglas fir (*Pseudotsuga* sp.), Fraser fir (*Abies fraseri*), Noble fir (*A. procera*), and Balsam fir (*A. balsamea*) (5). With its natural Christmas tree shape and excellent postharvest quality, Fraser fir is one of the most valued trees in the Christmas tree industry (5), rapidly becoming the most popular tree among growers in Michigan. In 2005, 3,000 hectares were planted to Fraser fir in Michigan (2).

Phytophthora root rot and shoot blight can limit the production and marketability of Fraser fir (5, 6), as reported in North Carolina (3) and Michigan (1). With the exception of Michigan, *Phytophthora cinnamomi* (11) is typically the causal organism. However, *P. drechsleri* (4), *P. citricola* (35) and *P. cactorum* (1) have also been associated with root rot and shoot blight symptoms in Fraser fir and other *Abies* and conifer species grown for Christmas trees (1, 4, 13, 14, 21, 30). Plant death ranging from 30% to 75% can occur when conditions are favorable (5). Fraser fir is especially susceptible to *P. cinnamomi* and other *Phytophthora* species; symptoms including characteristic reddish-brown needles develop rapidly and most trees die in 4 to 5 weeks (5, 6, 17).

To prevent Phytophthora root rot and shoot blight in Christmas trees, it is important that growers plant pathogen-free seedlings into well-drained fields without a history of root rot organisms (3). In some growing regions, uninfested fields are becoming increasingly scarce, limiting the expansion of production (5). Michigan vegetable growers

are diversifying their crops and including Fraser fir plantings in soils infested with *Phytophthora capsici* Leonian. *Phytophthora capsici* has a broad host range including solanaceous, cucurbitaceous and fabaceous crops (8). Crop rotation is used to manage *P. capsici*, but this strategy is limited by the long-term survival of oospores in soil (27) and an increasing list of *P. capsici*-susceptible hosts (10). The objective of this study was to determine whether *P. capsici* is pathogenic to Fraser fir. Specifically we sought to determine the following: (i) whether or not incubation temperature, inoculation method, or *P. capsici* isolates influence infection and disease development, and (ii) whether or not Fraser fir seedlings could become infected when planted in a field naturally infested with *P. capsici*. A preliminary report of these findings has been published (32).

Methods

Isolate selection, maintenance and inoculum preparation. *P. capsici* isolates collected in Michigan from infected cucurbitaceous and solanaceous crops were selected from the culture collection maintained in the laboratory of Dr. Hausbeck at Michigan State University (MSU). They were phenotypically characterized according to mating type (MT) and sensitivity to the fungicide mefenoxam (24). Isolate OP97 (A1 MT) was isolated from pickling cucumber and SP98 (A2 MT) from pumpkin, and both are sensitive to mefenoxam. Isolate SFF3 (A2 MT) originates from pickling cucumber and isolate 12889 (A1 MT) was obtained from pepper; both are insensitive to mefenoxam.

Actively growing cultures of each isolate were obtained by transferring agar plugs from long-term stock cultures (stored at 20°C in sterile microcentrifuge tubes with 1 ml of sterile water and one sterile hemp seed) onto V8 juice agar (16 g agar, 3 g $CaCO_3$, 160 ml

unfiltered V8 juice and 840 ml distilled water). Cultures were maintained at room temperature (21 ± 2°C) under fluorescent light. To ensure that isolates were virulent, cucumber fruits were inoculated with each isolate. Cucumbers were washed with water, disinfected for 5 min in a 5% sodium hypochlorite solution and dried at room temperature. A small superficial wound was made with a sterile needle in the center of each cucumber. Agar plugs (7 mm diameter) from the margins of actively growing colonies were placed upside down on top of the wound. A sterile microcentrifuge tube (with the cap removed) was placed over each agar plug and fixed to the fruit with petroleum jelly. Control cucumbers were inoculated as described above using a sterile 7-mm-diameter plug of V8 agar. Cucumbers were placed in aluminum trays with wet paper towels and covered with plastic wrap to maintain high relative humidity. Trays were incubated at room temperature (21 ± 2°C). Symptomatic cucumber tissue (5 mm) was excised and transferred to V8 juice agar and maintained under the same conditions described above. Clean cultures of each isolate were obtained from the infected cucumber tissue.
Bare-root Fraser fir seedlings were purchased from a nursery in Holland, MI that obtained the seed from Roan Mountain, NC (lat. 36°09'N, long. 82°05'W). Seedlings were grown in a greenhouse and then transplanted into a field nursery bed for one growing season at the nursery in Holland. Seedlings used in all experiments were 0.15 to 0.3 m in height with 6- to 10-mm diameter stems and grown in 3-liter pots containing soilless medium (Baccto Professional Planting Mix, Michigan Peat Company, Houston, TX).

Three types of inoculum were prepared and included agar plugs, a zoospore suspension, and infested millet seed. Agar plugs (7 mm diameter) of actively growing *P. capsici* containing mycelium and sporangia were used for stem inoculations. Zoospore

inoculum was prepared by pouring 20 ml sterile distilled water into a Petri plate with actively growing and sporulating *P. capsici* on V8 agar. The plates were moved to a 4°C environment for 30 min and then returned to 25°C for 30 min to trigger zoospore release. The number of zoospores in a 1-ml aliquot of the suspension was determined by using a hemacytometer. To facilitate counting, zoospores were first encysted by spinning the suspension at full speed on a vortex mixer for 1 min. Inoculum concentration was adjusted to 2 x 10^3 and 5 x 10^3 zoospores/ml. Viability was confirmed after mixing with a vortex by transferring drops of zoospore suspension onto V8 agar and observing colony growth. *P. capsici*-infested millet seed was also prepared. Millet seed (100 g) was mixed with asparginine (0.08 g) and water (72 ml) in a 500-ml Erlenmeyer flask. The flask was capped with aluminum foil and autoclaved twice consecutively. The flask was shaken to homogenize the mixture. The seeds were inoculated with four 7-mm-diameter agar plugs from an actively growing *P. capsici* V8 culture. The inoculated millet seeds were incubated at room temperature (21 $\pm$ 2°C) under constant fluorescent light for four weeks.

Stem inoculation. Three different methods were used to inoculate each of four Fraser fir seedlings using isolate OP97. Inoculations without wounding involved placing an agar mycelial plug on the stem 2 cm above ground level and covering the plug with parafilm to retain moisture. A second inoculation method included making a small wound (1-mm diameter) in the stem (2 cm above ground level) using a sterile syringe needle, placing the agar mycelial plug over the wound and covering the plug with parafilm. A third inoculation method included making a larger wound (3-mm diameter) in the stem (2 cm above ground level) using a sterile cork borer, placing the agar plug over the wound and covering the plug with parafilm. As a control, four seedlings were each inoculated using the

three inoculation methods as previously described, and sterile 7-mm-diameter V8 agar plugs. The inoculated and control seedlings were placed in a randomized arrangement in each of two growth chambers maintained at 20 or 25°C with fluorescent lighting (14/10 day/night photoperiod and 95 mE of light intensity). Seedlings were watered once a week if the soil was dry. This experiment was conducted twice.

Additional studies were conducted using a small wound inoculation method or no wounding. Four isolates of *P. capsici* (OP97, SFF3, SP98, and 12889) were used as inoculum with four replicate plants used per isolate. Four control plants were inoculated using V8 agar plugs. The seedlings were placed randomized in a growth chamber at 25°C and maintained and watered as previously described. This experiment was conducted twice.

Root inoculation. Roots were inoculated by dispensing 10 ml of a suspension containing 2 x 10^3 or 5 x 10^3 zoospores/ml on the soil surface at the base of each seedling or by mixing the soil with 2 g or 4 g of infested millet seed. Approximately 50 g of soil surrounding the stem was removed, mixed with the millet seed and replaced around the stem. Four Fraser fir seedlings were inoculated with *P. capsici* OP97, and four control seedlings were inoculated with sterile distilled water or millet seed inoculated with sterile V8 agar plugs. The seedlings were placed in a randomized arrangement in a growth chamber at 25°C and maintained and watered as previously described. This experiment was conducted twice.

Millet seed infested with one of four isolates of *P. capsici* (OP97, SFF3, SP98, and 12889) was used to inoculate seedlings after transplanting as previously described. Four replicate seedlings were used per isolate. Four control plants were inoculated with millet

seed with sterile V8 agar plugs. The seedlings were placed randomized in a growth chamber at 25°C and maintained and watered as previously described. This experiment was conducted twice.

Disease assessment and data analysis. Disease assessment was initiated one week following inoculation and continued at weekly intervals for two months or until all seedlings were dead. Disease progression was recorded for each seedling as the percentage of branches with bronze coloration and the area under the disease progress curve (AUDPC) calculated. The AUDPC values were averaged and used for all statistical analyses and their residuals followed the assumptions of all statistical tests performed. The experiment was arranged in a split-plot with whole plots in a randomized complete block design. Data were subjected to analysis of variance using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC) and multiple comparisons among the means were conducted using t-tests (LSD) when effects were found to be statistically significant at 0.05 levels.

Field experiment. In 2007, seedlings were planted in two commercial fields in Michigan known to be infested with *P. capsici*. Previously, the fields had been cropped primarily to cucurbits, including summer and winter squash and cucumber that had become infected by *P. capsici*. Field 1 was located in Cass County and had sandy loam soil, and Field 2 was located in Oceana County and had Benona sand soil. The study was conducted in each field from 29 June to 31 August and repeated from 2 September to 9 November 2007. Soil and air temperatures were obtained from the Michigan Automated Weather Network (MAWN) for the Lawton Station located 14.5 km away from Field 1 and from the Hart Station located 8 km away from Field 2 (Table 2.1).

Table 2.1. Soil and air temperatures of two commercial fields in Michigan naturally infested with *Phytophthora capsici* during the months from June to November, 2007.

		Air temperature (°C)			Soil temperature (°C)		
Field	**Months**	**Average**	**Minimum**	**Maximum**	**Average**	**Minimum**	**Maximum**
1	Jun - Aug	21.6	3.9	35.0	24.3	19.2	29.4
	Sep - Nov	12.3	-8.2	32.6	15.8	12.6	19.0
2	Jun - Aug	19.9	1.6	33.2	26.1	18.5	33.7
	Sep - Nov	10.9	-8.4	32.1	13.3	8.3	18.2

One hundred and fifty Fraser fir seedlings were planted in each field with 0.6 m spacing between seedlings within a row and centered in 10 beds that were 18 m long and 0.6 m wide with spacing of 1.5 m between beds. Yellow squash, a known *P. capsici*-susceptible host, was established in the plant beds between the Fraser fir seedlings. Each row contained approximately 15 Fraser fir and yellow squash seedlings. Eight asymptomatic or 8 symptomatic Fraser fir seedlings were sampled randomly from each field every week for 9 weeks, beginning one week after planting. When symptoms were not observed, only asymptomatic seedlings were collected and sampled. Toward the end of the study when symptoms were observed in most of the planted seedlings, only symptomatic seedlings were collected. Yellow squash showing crown rot was also sampled. The incidence of *P. capsici* recovered from each tissue type (root, stem, branch and needle) was determined for Fraser fir. The incidence of *P. capsici* from each tissue type was used for all statistical analyses and their residuals followed the assumptions of all statistical tests performed. The experiment was arranged in a split-plot with whole plots in a randomized complete block design. Data were subjected to analysis of variance using the PROC MIXED and PROC GLIMMIX procedures of SAS (SAS Institute Inc., Cary, NC) and

multiple comparisons among the means were conducted using t-tests (LSD) when effects were found to be statistically significant at 0.05 levels.

Pathogen isolation. Control and inoculated seedlings from the growth chamber experiments were washed with water to remove the soilless growing medium particles and other debris, dipped into 70% ethanol for 1 min to surface disinfest all tissues and air-dried. The washing and surface sterilization step was accomplished prior to making an incision in the sample to avoid exposing the tissues inside and potentially compromising the success of pathogen isolation. Tissue from root, stem, branch and needle were excised and cut in half with a sterile scalpel and plated onto BARP-(benomyl, ampicillin, rifampicin, and pentachloronitrobenzene) amended, unclarified V8 agar (27). This technique facilitated the growth of the pathogen from the infected tissue into the growth media. The same procedure was used to isolate *P. capsici* from asymptomatic and symptomatic Fraser fir and yellow squash seedlings planted in the field.

Cultures that were suspected to be *P. capsici* based on morphological characteristics were transferred to new BARP plates. Axenic cultures were incubated for 7 days on V8 agar with continual fluorescent lighting under ambient laboratory conditions (21 ± 2°C). Cultures were positively identified as *P. capsici* based on morphological characteristics described by Waterhouse (37). Isolates obtained from seedlings in the growth chambers were characterized for MT and mefenoxam resistance as previously described (24) to compare the phenotype of the isolate obtained with the original inoculum. Isolates obtained from the field were also characterized for MT and mefenoxam resistance.

Molecular confirmation of field isolates. Field isolates suspected to be *P. capsici* and positively identified by morphological characteristics were further confirmed by direct

colony polymerase chain reaction (PCR) (23). Mycelium was prepared by inoculating 1-ml aliquots of cucumber extract amended with antibiotics in sterile 1.5 ml-microcentrifuge tubes. Cucumber extract was prepared by blending 2 kg of fresh cucumbers in 1 liter of water, filtering through cheesecloth, centrifuging the juice at 15,550 x *g* for 20 min and transferring the supernatant to a clean flask. The supernatant was autoclaved and the top layer of the extract was aspirated into a new flask without transferring any precipitate from the bottom. Antibiotics (50 ppm vancomycin, 25 ppm pimaricin and 50 ppm ampicillin) were added to the extract and it was stored in 50-ml falcon tubes for later use. Cultures were grown overnight on a shaker at room temperature (21 ± 2°C) under constant fluorescent light. Cultures were centrifuged at 21,130 *x g* for 2 min and the supernatant was removed. Mycelium was rinsed 3 times with 1 ml sterile water, centrifuged at 21,130 *x g* rpm for 1 min and the supernatant was removed in each washing step. A small amount of mycelium was transferred to PCR tubes and macerated with a sterile micropipette tip. The PCR mix was directly added to the mycelium.

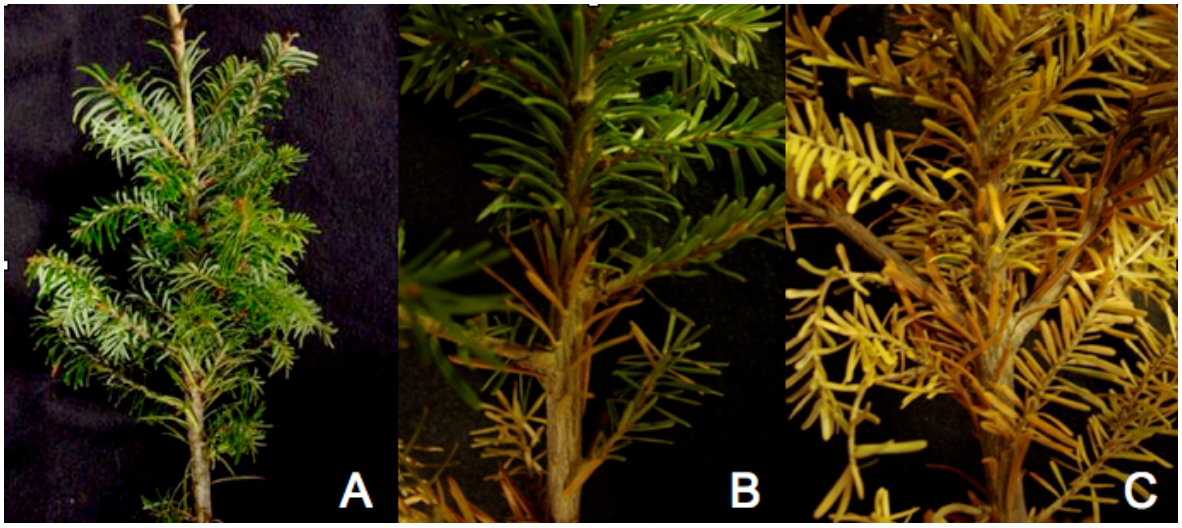
Two specific primers for *P. capsici* were used for PCR: one forward primer (CAPFW; 5´TTTAGTTGGGGGTCTTGTACC3´), and one reverse primer (CAPRV2; 5´TACGGTTCACCAGCCCATCA3´) that were designed by Silvar et al. (36). Direct colony PCR reactions were performed in a total volume of 25 µl containing mycelium, 2.5 µl 10X PCR reaction buffer (Invitrogen, Carlsbad, CA), 14.3 µl sterile water, 1 µl 10 µM $MgCl_2$ (Invitrogen, Carlsbad, CA), 4 µl 1.25 mM dNTP mix (Invitrogen, Carlsbad, CA), 1 µl each primer 10 µM (MSU Macromolecular Structure Facility, East Lansing, MI), and 0.2 µl Taq DNA polymerase (Invitrogen, Carlsbad, CA). The PCR reaction was performed in a programmable Eppendorf mastercycler ep systems thermal cycler (Eppendorf, Westbury,

NY) starting with 5 min denaturation at 95°C to release DNA from mycelium, followed by 30 cycles at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 60 s, with a final extension step of 5 min at 72°C. PCR products were analyzed by electrophoresis in 1% (w/v) agarose gel in 1X Tris-acetate-EDTA buffer (29), stained with ethidium bromide (5 μg/ml) for visualization and compared to a 100 bp ladder (Invitrogen, Carlsbad, CA). A control with extracted DNA was used to ensure that detection of the pathogen was possible using direct colony PCR.

Results

All Fraser fir seedlings inoculated with *P. capsici* developed symptoms and died; wounding was not required. The first disease symptoms occurred approximately 2 weeks after inoculation. Overall disease symptoms included reddish-brown foliage or bronzing and needle death that began in the lower portion of the seedling and progressed upwards until all of the foliage exhibited symptoms; dark, discolored and rotted roots along with seedling death were also observed (Figure 2.1).

Figure 2.1. Symptoms of *Phytophthora capsici* root rot on Fraser fir. A, Normal appearance of the foliage of a control seedling. B, Bronzing of needles starting in the lower part of the seedling that progresses upward to the rest of the foliage. C, Complete bronzing of all the foliage of the seedling.



Significant differences occurred among the stem inoculation methods (ANOVA, *P*=0.0081, Table 2.2). Wounded seedlings developed symptoms sooner than unnonwounded seedlings (Figure 2.2A and B) when inoculated with *P. capsici* OP97 according to AUDPC data. There was not a significant difference in disease resulting from the 1- and 3-mm-diameter wounds. However, they were both different from the inoculation method without wounding.

Although seedlings incubated at 20°C died 3 to 4 weeks later than seedlings incubated at 25°C (Figure 2.2A and B), AUDPC means did not differ significantly between incubation temperatures (ANOVA, *P*=0.5644, Table 2.2). Interaction effects were not found between temperature and the inoculation method (ANOVA, *P*=0.2568, Table 2.2). An incubation temperature of 25°C was used in subsequent growth chamber experiments.

Table 2.2. Effects of inoculum, pathogen isolate, incubation temperature, and inoculation method on the area under the disease progress curve (AUDPC) values for disease incidence on Fraser fir seedlings caused by *Phytophthora capsici.*

Treatment main effect (*P* value)	AUDPC mean[a]
Stem inoculation using OP97	
Temperature (0.5644)	
20°C	219.3 a
25°C	290.7 a
Inoculation method (0.0081)	
No wound	196.8 a
1-mm wound	322.5 b
3-mm wound	245.7 b
Temperature x inoculation method (0.2568)	
Stem inoculation using one of four isolates	
Inoculation method (0.0138)	
No wound	239.6 a
1-mm wound	293.9 b
Isolate (0.4847)	
OP97	249.5 a
SP98	279.5 a
SFF3	266.9 a
12889	271.3 a
Inoculation method x isolate (0.4617)	
Soil infestation using OP97	
Inoculation method (0.0168)	
2 x 10^3 zoospores/ml	61.3 a
5 x 10^3 zoospores/ml	101.9 a
2 g of infested millet seed	375.3 b
4 g of infested millet seed	380.8 b
Soil infestation using one of four isolates	
Inoculation method (0.9603)	
2 g of infested millet seed	285.7 a
4 g of infested millet seed	281.3 a
Isolate (0.1974)	
OP97	205.8 a
SP98	322.8 a
SFF3	314.1 a
12889	291.4 a
Inoculation method x isolate (0.9802)	

[a] Means within a column for each treatment main effect followed by the same letter are not significantly different (LSD).

Significant differences were observed among the methods used to infest the soil (ANOVA, *P*=0.0168, Table 2.2). According to AUDPC data, a zoospore suspension was significantly less effective than using infested millet seed. Most of the seedlings (97%) inoculated with millet seed became infected and died (Figure 2.2E). In contrast, 87% of the seedlings inoculated with a zoospore suspension became infected as determined by pathogen isolation at the conclusion of the experiment, but only 2% died (a maximum of 26.8% showed symptoms). The suspension containing a low (2 x 10^3/ml) or high (5 x 10^3/ml) zoospore concentration was significantly different from soil infestation using 2 or 4 g of millet seed (Table 2.2). Seedlings inoculated using millet seed died 4 to 6 weeks after inoculation, similar to that observed for the stem inoculation experiments. No significant differences were found among zoospore concentrations or millet seed quantities used for soil infestation (Table 2.2).

When four *P. capsici* isolates (OP97, SP98, SFF3 or 12889) were used to inoculate wounded (1-mm diameter) or unwounded seedling stems, significant differences among the isolates were not observed (ANOVA, *P*=0.4847, Table 2.2). Similarly, significant differences were not found among *P. capsici* isolates (ANOVA, *P*=0.1974, Table 2.2) when infested millet was used as inoculum. All isolates were capable of producing disease and death in seedlings regardless of whether they were inoculated via the stem (Figure 2.2C and D) or soil infestation (Figure 2.2F and G) method. There were significant differences between the no wound and 1-mm diameter wound stem inoculation methods according to AUDPC data (ANOVA, *P*=0.0138, Table 2.2), as observed in the previous experiment for isolate OP97. No interaction effects were found between the isolate and the agar plug

inoculation method (ANOVA, *P*=0.4617) or the infested millet inoculation method (ANOVA, *P*=0.9802) (Table 2.2). No significant differences were observed between the two millet seed quantities (ANOVA, *P*=0.9603, Table 2.2) used to infest the soil.

Figure 2.2. Progression of disease caused by *Phytophthora capsici* in inoculated Fraser fir seedlings. Points represent mean values of 8 replicates with the standard deviation. A-B, Comparison of inoculation of *P. capsici* OP97 onto unwounded (none) or wounded (1 or 3 mm wounds) stem and grown at A, 20°C, and B, 25°C. C-D, comparison of inoculations of 4 isolates of *P. capsici* onto C, unwounded or D, wounded (1 mm) stems and grown at 25°C. E, comparison of rates of *P. capsici* OP97-infested millet seeds inserted into the growing media near the stem and concentrations of *P. capsici* OP97 zoospore suspension poured into the growing media near the stem and grown at 25°C. F-G, comparison of inoculum of 4 isolates of *P. capsici* prepared as F, 2 g infested millet seed and G, 4 g infested millet seed inserted into the potting media near the stem and grown at 25°C.

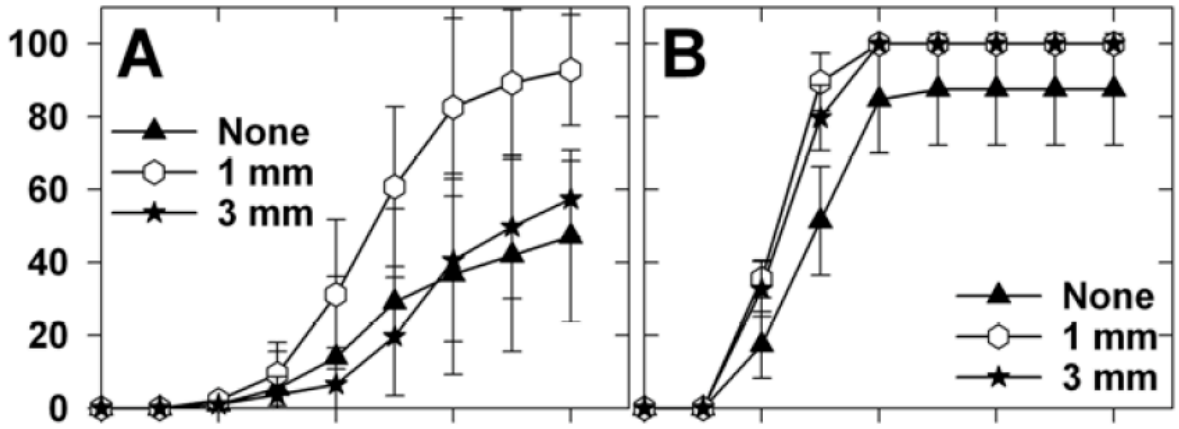
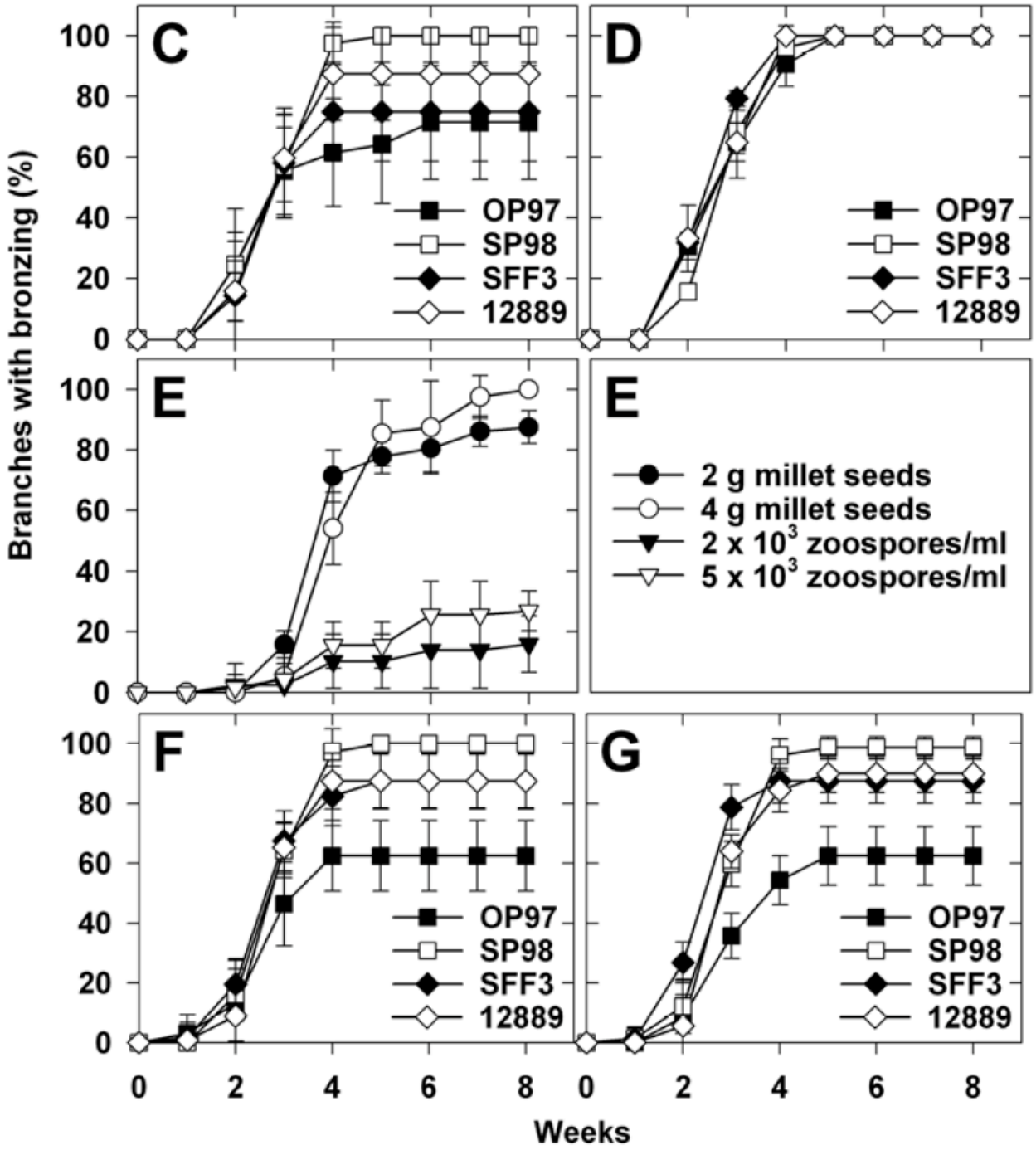


Figure 2.2 (cont'd)



The pathogen was isolated only from inoculated symptomatic seedlings in all growth chamber experiments. The phenotype (MT and mefenoxam resistance) was

confirmed and matched the phenotype of the isolate used as inoculum. The control seedlings remained asymptomatic.

Over 70% of the total number of seedlings planted in both fields naturally infested with *P. capsici* developed disease symptoms and died. Root rot symptoms were observed approximately 2 to 3 weeks after planting in fields 1 and 2 at both planting times. Foliar disease symptoms included bronzing and death of the needles similar to that observed on inoculated plants in the growth chamber experiments. There was not a significant difference in seedling death due to variability between the field sites (ANOVA, P=0.4678) or the planting times (ANOVA, P=0.5277). Seedlings died quickly 3 to 5 weeks after planting in the first field experiment; temperatures were higher in the first experiment relative to the second field experiment (Table 2.1). In the second experiment, Fraser fir seedlings died 4 to 6 weeks after planting. All yellow squash seedlings died 4 to 5 weeks after planting in both experiments and planting times and all isolations made from roots and stems were positive for *P. capsici* growth. Growth of *P. capsici* was observed in all isolations from symptomatic Fraser fir seedlings (Table 2.3), but significant differences in pathogen incidence were found among different plant parts (PROC GLIMMIX, P <0.0001). Field 1 yielded a total of 84 *P. capsici* isolates for both planting times (Table 2.3) obtained from Fraser fir root (87%) and stem (13%) tissue (Table 2.3). Field 2 yielded a total of 87 *P. capsici* isolates for both planting times (Table 2.3) obtained from root (90%) and stem (10%) tissue (Table 2.3).

Table 2.3. Incidence of *Phytophthora capsici* and isolates obtained from different parts of the symptomatic Fraser fir seedlings sampled from naturally infested fields and both planting dates.

Field	Symptomatic seedlings sampled	Total number of isolates	Isolates obtained[a] (%)		Incidence of *P. capsici*[b] (%)			
			Root	Stem	Root	Stem	Branch	Needle
1	144	84	73 (87)	11 (13)	83 (57)	53 (37)	8 (6)	0 (0)
2	144	87	78 (90)	9 (10)	80 (55)	55 (38)	9 (7)	0 (0)

[a]As determined by axenic pathogen isolation at the conclusion of the experiment.

[b]As determined by the total number of tissue pieces that presented *P. capsici* growth.

There was not a significant difference in the number of isolates obtained from roots or stems due to variability between the field sites (ANOVA, P=0.3440 and P=0.2271, respectively) or the planting times (ANOVA, P=0.3390 and P=0.3733 respectively). Although the branches and needles exhibited bronzing in both fields (Figure 2.1), the pathogen was not easily recovered from branches and was never recovered from the needles. The majority of the *P. capsici* isolates collected were sensitive to the fungicide mefenoxam and approximately 50% was of the A2 MT (Table 2.4). Clean cultures were obtained from 60% of the original cultures obtained directly from Fraser fir tissue. The pathogen was not isolated from tissue of asymptomatic Fraser fir seedlings.

Table 2.4. Mating type and mefenoxam sensitivity of *Phytophthora capsici* isolates from symptomatic Fraser fir seedlings grown in two commercial fields in Michigan naturally infested with *Phytophthora capsici.*

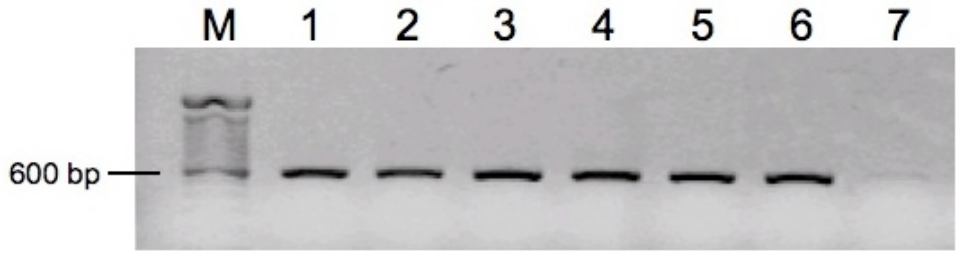
Field	Total number of isolates	MT[a]	Mefenoxam sensitivity[b] (%)			Total (%)
			I	IS	S	
1	84	A1	3 (3.6)	4 (4.7)	32 (38.0)	39 (46.3)
		A2	10 (12.0)	4 (4.7)	31 (37.0)	45 (53.7)
2	87	A1	29 (33.3)	2 (2.3)	11 (12.6)	42 (48.2)
		A2	13 (15.0)	4 (4.6)	28 (32.2)	45 (51.8)
Total	171		55 (32.0)	14 (8.0)	102 (60.0)	171

[a]Indicates mating type (A1 or A2) of isolate.

[b]Indicates isolate sensitivity to the fungicide mefenoxam: insensitive (I), intermediately sensitive (IS), and sensitive (S).

P. capsici was positively identified based on morphological characteristics and was confirmed by direct colony PCR amplification with *P. capsici*-specific primers. The PCR product obtained for all isolates had the expected size of 594 bp (36) when compared to the 100 bp ladder (Figure 2.3). Direct colony and control DNA PCR reactions produced the expected band for positive identification of *P. capsici* (Figure 2.3). Other organisms that were isolated from symptomatic and asymptomatic seedlings included *Pythium* spp., *Alternaria* spp., and *Fusarium* spp. *Pythium* spp. were particularly common from root and stem tissue isolations and *Alternaria* spp. were commonly obtained from branch and needle tissue.

Figure 2.3. Polymerase chain reaction (PCR) confirmation of isolate identification with *P. capsici* specific primers. M, 100 bp ladder, with uppermost band 1,000 bp. 1, 2, 3, 4, PCR product of direct colony PCR of isolates obtained from different symptomatic seedlings sampled in the field experiment. 5, PCR product from DNA extracted from mycelium of *P. capsici* OP97. 6, PCR product from DNA extracted from mycelium of *P. capsici* SP98. 7, negative control where water was added to the PCR reaction instead of mycelium or DNA.



Discussion

Phytophthora root rot and blight incited by *P. cinnamomi* (11), *P. drechsleri* (4), *P. citricola* (35), or *P. cactorum* (1) causes needles and branches of Fraser fir to turn reddish-brown in color (5). Similar symptoms were observed in seedlings infected with *P. capsici*. A wound was not required for infection; however, wounding significantly increased disease incidence. All *P. capsici* isolates tested incited disease in the seedlings regardless of temperature (20 or 25°C) or inoculation method, indicating that *P. capsici* can infect Fraser fir.

Most of the *P. capsici* isolates obtained from the Fraser fir seedlings infected while in the field were recovered from root tissue. In addition, the pathogen was successfully recovered from stems of all stem-inoculated seedlings, and from roots and stems of all root-inoculated seedlings; the phenotype of the recovered isolate always matched the phenotype of the inoculum.

In our study, *P. capsici* was more difficult to recover from Fraser fir seedlings than from the yellow squash. *Phytophthora capsici* can be difficult to isolate from certain tissue

types such as mature pepper stems, but readily isolated from symptomatic pepper fruits (M. K. Hausbeck, unpublished data). Other *Phytophthora* spp. are not readily isolated from the fibrous tissue of asparagus crowns and fern (9, 34) or the woody tissue of oak (7) or various ornamentals (12).

Direct colony PCR was a fast and reliable way to verify the identity of *P. capsici* isolated from plant tissue and allowed the processing of a high number of isolates by eliminating the DNA extraction step (Figure 2.3). Other organisms that were isolated from both symptomatic and asymptomatic Fraser fir seedlings included *Pythium* spp., *Alternaria* spp., and *Fusarium* spp. *Pythium* spp. were particularly common from root and stem tissue and *Alternaria* spp. from branch and needle tissue. *Alternaria* spp. are among the most common needle phylloplane fungi and are commonly isolated from needles of other Pinaceae members (31).

Fraser fir seedlings planted in the *P. capsici*-infested fields died quickly in the first experiment that was conducted during a time of relatively high temperatures compared to the second experiment. However, there was no significant difference in seedling death between fields at different planting times and when inoculated plants were incubated in growth chambers at 25°C or 20°C there was no significant difference in disease. The minimum, optimum, and maximum temperatures for growth of *P. capsici* are 10°C, 28°C, and >35°C, respectively (8). The temperatures used in the growth chamber experiments are close to the optimum temperature for *P. capsici* growth, in the first field experiment the average soil temperature was also close to the optimum growth temperature for *P. capsici*. In the second field experiment, the average soil temperature was close to the minimun growth temperature for *P. capsici*. Since Fraser fir is sensitive to sustained high

temperatures (day temperatures >30°C) (16), the relatively high temperatures in the first field experiment may have stressed the seedlings, increasing their susceptibility to *P. capsici*. Fraser fir naturally occurs on highly acidic soils (pH 3.5) at elevations between 1100 and 2000 m (16, 18) in the Appalachian Mountains (33) where temperatures range from -12 to 26°C depending on the season (16, 28). Day temperatures of 22 to 27°C and night temperatures of 13 to 19°C appear optimum for producing containerized Fraser fir (16).

Once soils become infested with *P. capsici,* oospores allow overwintering and persistence of the pathogen for several years (15, 27). Oospores form when both mating types come into contact; fields in Michigan infested with *P. capsici* have been found to have both mating types present (24, 26). Oospores in soil and plant debris are considered primary inoculum (15), which may directly infect the plant or develop a sporangium that is capable of releasing 20 to 40 motile zoospores when water is present. Mycelium can develop on host tissue under favorable conditions, forming secondary inoculum (sporangia, zoospores) that can be produced repeatedly during the growing season (8). The agar plugs and millet seed used as inoculum in the growth chamber experiments contained mycelium and sporangia, and since only single *P. capsici* isolates were used, sexual reproduction was prevented. The zoospore suspension did not contain other inoculum types. In the field experiment, the Fraser fir seedlings could have been exposed to oospores, mycelium, sporangia, and/or zoospores since both *P. capsici* mating types were found and sexual reproduction was possible. In the growth chamber experiments, the zoospore inoculum did not appear to be as effective as the other inoculum types used in our study. Viability of the zoospore suspension was checked after mixing with a vortex and

it was found to be satisfactory; however, it is possible that the zoospores did not remain viable. This is possibly because the zoospore inoculum did not have an associated food base, as did the agar plugs and millet seed inoculum. The agar plug and millet seed inoculum could produce new growth to infect the seedlings when conditions were favorable.

There were no significant differences in plant death among the *P. capsici* isolates used as inoculum. The *P. capsici* isolates from seedlings planted into *P. capsici*-infested fields exhibited variation in mating type and resistance to mefenoxam. Field populations of *P. capsici* from solanaceous and cucurbitaceous hosts are reported to have variation in virulence and other phenotypic characteristics (19, 20). Historically, Field 2 has had *P. capsici* populations that were primarily sensitive to mefenoxam (M. K. Hausbeck, unpublished data); however, in the present study we recovered 42 isolates that were fully insensitive to the fungicide. Field 1 has a history of a heterogeneous *P. capsici* population with sensitive and insensitive isolates (M. K. Hausbeck, unpublished data), which was also found in this study. The recovery of A1 and A2 mating types in a ratio close to 1:1 from the seedlings is expected from Michigan vegetable fields (26) and suggests that sexual recombination is possible. Genetic diversity is likely being maintained within field populations and there is an increased chance of generating diverse genotypes with new traits such as mefenoxam resistance or increased virulence.

Planting Fraser fir for Christmas tree production is increasing in the northern Great Lakes region due to its desirable traits of foliage color, shape, fragrance and excellent needle retention (33). It is important to use locally or regionally developed guidelines to grow Fraser fir because of the specific environmental conditions they require for good

performance. Christmas tree growers in Michigan perceive Fraser fir to be more sensitive to soil conditions, more likely to experience nutrient disorders, and more responsive to fertilization than other Christmas tree species, restricting production to only optimum sites (33).

The number of fields historically used for vegetable production in Michigan that have become infested with *P. capsici* is raising (25, 26) and growers are planting Fraser fir as a rotational crop. Infested acreage and urban pressure is increasing across vegetable production areas in Michigan, limiting growers' ability to avoid the pathogen (15). *P. capsici* has been documented in many vegetable production regions (15) and Michigan grows over 33,000 hectares of susceptible crops (22). Other species of *Phytophthora* are known to occur on Fraser fir in Michigan, such as *P. cactorum* (1) and *P. citricola* (Fulbright, unpublished data). However, only *P. capsici* was recovered from the infested field sites, probably because they were historically cropped to vegetables, not woody ornamentals.

With the identification of Fraser fir as a newly recognized host for *P. capsici*, it is important to further characterize the disease on Christmas trees for the development of appropriate crop rotation strategies. The present study of *P. capsici* on Fraser fir indicates that adjustments in current rotational schemes are needed as planting Fraser fir in fields infested with *P. capsici* could result in infection and should be avoided.

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CHAPTER III: RESISTANCE IN TOMATO AND WILD RELATIVES TO CROWN AND ROOT ROT CAUSED BY *PHYTOPHTHORA CAPSICI*

Abstract

Quesada-Ocampo, L. M., and Hausbeck, M. K. 2010. Resistance in Tomato and Wild Relatives to Crown and Root Rot Caused by *Phytophthora capsici.* Phytopathology 100: 619-627.

Phytophthora capsici causes root, crown, and fruit rot of tomato, a major vegetable crop grown worldwide. The objective of this study was to screen tomato varieties and wild relatives of tomato for resistance to *P. capsici*. Four *P. capsici* isolates were individually used to inoculate 6-week-old seedlings (1 g *P. capsici*-infested millet seed/10 g soilless medium) of 42 tomato varieties and wild relatives of tomato in a greenhouse. Plants were evaluated daily for wilting and death. All *P. capsici* isolates tested caused disease in seedlings but some isolates were more pathogenic than others. A wild relative of cultivated tomato, *Solanum habrochaites* accession LA407, was resistant to all *P. capsici* isolates tested. Moderate resistance to all isolates was identified in the host genotypes Ha7998, Fla7600, Jolly Elf, and Talladega. *P. capsici* was frequently recovered from root and crown tissue of symptomatic inoculated seedlings but not from leaf tissue, asymptomatic or control plants. The phenotype of the recovered isolate matched the phenotype of the inoculum. Pathogen presence was confirmed in resistant and moderately resistant tomato genotypes by species-specific polymerase chain reaction of DNA from infected crown and root tissue. Amplified fragment length polymorphisms of tomato genotypes showed a lack of correlation between genetic clusters and susceptibility to *P. capsici*, indicating that resistance is distributed in several tomato lineages. The results of this study create a baseline for future development of tomato varieties resistant to *P. capsici*.

Introduction

Tomato (*Solanum lycopersicon* L.) is a major vegetable crop grown worldwide. Each year, between 160,000 to 280,000 hectares of processing and fresh market tomatoes are planted in the United States with an approximate value of $2 billion (2). *Phytophthora capsici* Leonian is a destructive soilborne pathogen with a broad host range that includes solanaceous, cucurbitaceous and fabaceous crops (9). On tomato, the pathogen causes root, crown and fruit rot (28, 54), all of which have been reported in several regions including Michigan (18, 32), California (4, 8, 54), Colorado (28) and Florida (52).

In Michigan, tomatoes are grown for fresh market and processing. Fresh market production utilizes plastic-covered raised beds, trickle irrigation and trellising to hold the plants upright; these management practices also limit disease caused by *P. capsici* (M. K. Hausbeck, unpublished data). Fresh market tomatoes are harvested by hand and have a higher profit margin than processing tomatoes. Since processing tomatoes are mechanically harvested and have a lower profit margin, plants are grown on flat ground where fruit typically come into direct contact with the soil. The growing system used by processing tomato growers is more conducive to *P. capsici* infection than the fresh market system. In Michigan, crop rotation and fungicide applications are commonly used to manage *P. capsici*. The success of crop rotation is limited by the long-term survival of oospores in the soil (32) and the number and diversity of susceptible hosts (15, 45). Applications of the commonly used fungicide mefenoxam may not protect susceptible crops from resistant *P. capsici* populations, which have been documented throughout the U.S. (30, 31, 33, 43). Host resistance should be a key component of a disease management strategy for *P. capsici* on tomato. Regrettably, only a few moderately resistant tomato

cultivars are available with commercially acceptable horticultural traits (4, 20). More sources of resistance from commercial varieties and/or wild species need to be identified. Breeders use wild *Solanum* species as sources of genes controlling traits of economic importance such as fruit characteristics, nutritional content, and general disease resistance (49). Identifying sources of resistant tomato germplasm would aid in the development of cultivars suitable for production in *P. capsici*-infested fields.

The objective of this study was to determine whether different varieties of *Solanum lycopersicon* L., *S. pimpinellifolium, S. pennellii* and/or *S. habrochaites* show resistance to *P. capsici*. Specifically, we sought to determine: (i) whether or not commercial tomatoes or tomato wild relatives could be used as a source of resistance to *P. capsici*, (ii) whether or not infection and disease development were influenced by the *P. capsici* isolates selected, and (iii) the association between lineages of the varieties and resistance to *P. capsici*. A preliminary report of these findings has been published (46).

Methods

Tomato varieties and wild relatives. Forty-two varieties of tomatoes and wild relatives (referred collectively as host genotypes) were selected, which included varieties for fresh market (17 varieties), and processing (17 varieties), breeding lines (1 variety), and 3 wild species (7 accessions) provided by either Dr. D. Francis (The Ohio State University), Redgold, Inc. (Elwood, IN) or Seedway, LLC. (Hall, NY, Table 3.1). Seeds were planted in 72-square cell plastic flats with cell depth of 5.7 cm and cell width of 4 cm (Hummert International, Earth City, MO), containing potting soilless media (Baccto Professional Planting Mix, Michigan Peat Company, Houston, TX) and a top layer of medium grade vermiculite (Hummert International). Plugs were grown in a greenhouse at MSU for

4 weeks, under approximately 14-hr day illumination. They were transferred into 2.5-liter square plastic pots (Hummert International) containing the same potting soilless media once they reached an approximate height of 8 cm and developed three or four true leaves. Plants were allowed to recover from transplant stress for two weeks prior to inoculation. For the duration of the experiment, plants were fertilized weekly with 200 ppm of Peter's 20-20-20 water-soluble fertilizer (The Scotts Company, Marysville, OH) and irrigated as needed to maintain adequate moisture for plant growth and *P. capsici* disease development. Air temperature and relative humidity data (Table 3.2) were collected hourly with a Watchdog data logger 450 series (Spectrum Technologies, Inc., East-Plainfield, IL).

Table 3.1. Disease reaction and mean AUDPC of three experiments in which tomato varieties and wild relatives were screened for resistance to *Phytophthora capsici.*

	Disease reaction[c]				**AUDPC mean**			
Accessions[a, b]	**12889**	**OP97**	**SP98**	**SFF3**	**12889**	**OP97**	**SP98**	**SFF3**
88[Sl, P]	S	M	S	R	114	39.4	114	0
331[Sl, P]	S	S	R	R	112	114	0	0
401[Sl, P]	S	M	S	R	116	13.1	116	0
611[Sl, P]	S	S	S	R	117	118	116	0
818[Sl, P]	S	S	S	S	110	115	114	116
7983[Sl, P]	S	S	S	R	123	107	126	0
9704[Sl, P]	S	R	S	R	113	0	116	0
BHN444[Sl, FM]	S	M	S	R	116	18.8	113	0
BHN591[Sl, FM]	S	R	S	R	118	0	114	0
E6203[Sl, P]	S	S	S	R	119	115	117	0
Fla7600[Sl, FM]	M	R	R	R	45.4	0	0	0
Florida47[Sl, FM]	S	S	M	R	115	119	22.9	0
Florida91[Sl, FM]	S	M	M	R	110	35.6	20	0
Ha7998[Sl, W]	M	R	M	R	37.9	0	20	0
Hunt100[Sl, P]	S	S	R	R	122	120	0	0
Jolly Elf[Sl, FM]	M	M	M	R	30.4	25.9	18	0

Table 3.1 (cont'd)

LA1269[Sp, W]	S	S	S	S	107	111	56.8	105
LA1589[Sp, W]	S	S	S	S	104	110	112	119
LA407[Sh, W]	R[C]	R[C]	R[A]	R	0	0	0	0
LA716[Spe, W]	S	S	S	S	131	130	130	126
M82[Sl, P]	S	S	S	S	114	105	113	117
Mountain fresh plus[Sl, FM]	S	R	R	R	110	0	0	0
Mountain spring[Sl, FM]	S	R	S	R	110	0	118	0
NC23E[Sl, FM]	S	S[B]	M	R	117	115	21.8	0
OH8245[Sl, P]	S	S	S	R	115	122	51	0
OH88119[Sl, P]	S[B]	S[B]	S	R	109	120	106	0
OH9241[Sl, P]	S	S	S	S	116	110	117	120
OH9242[Sl, P]	S	S	S	S	122	117	117	120
Peto95-43[Sl, P]	S	S	S	S	119	122	121	118
PI114490[Sl, W]	S	S	R	R	122	120	0	0
PI128216[Sp, W]	S	S	S	R	121	122	109	0
PI365914[Sp, W]	S[B]	S[B]	R	R	112	114	0	0
Plum crimson[Sl, FM]	S	S	M	R	113	120	45.2	0
Rio Grande[Sl, FM]	S	S	S	S	115	118	120	116
Sebring[Sl, FM]	S	S	S	R	116	115	118	0
Sunbeam[Sl, FM]	S	S	S	R	112	115	57.8	0
Sunleaper[Sl, FM]	S	S	S	M	74.7	118	114	26.7
Sunoma[Sl, FM]	S	S	S	R	107	117	112	0
Super sweet 100[Sl, FM]	S	R	R	R	71.6	0	0	0
Talladega[Sl, FM]	M	M	M	R	44.4	29.4	38.2	0
TR12[Sl, P]	S	S	S	S	117	108	111	119
TSH4[Sl, P]	S[B]	S	R	R	115	115	0	0
Total					103	81.3	71.8	28.6

[a] Species of the accession. Sl, *Solanum lycopersicon*; Sp, *S. pimpinellifolium*; Spe, *S. pennellii*; Sh, *S. habrochaites.*

[b] Type of the accession. P, processing; FM, fresh market; W, wild.

[c] Disease reaction. S, susceptible; M, moderately resistant; R, resistant. Tissue types from which *P. capsici* was recovered in the accession. *P. capsici* was recovered from root and crown tissue unless otherwise noted. A, roots; B, roots, crown and secondary stems; C, *P. capsici* not recovered.

Table 3.2. Air temperature and relative humidity in the greenhouse during each of the three replicated experiments in which tomato plants were screened for resistance to *Phytophthora capsici.*

	Air temperature (°C)			Relative humidity (%)		
Experiment	**Average**	**Minimum**	**Maximum**	**Average**	**Minimum**	**Maximum**
1	18	8	28	75	50	100
2	19	2	36	83	63	103
3	18	1	35	77	64	90

Isolate selection and maintenance. *Phytophthora capsici* isolates originating from diseased cucurbitaceous and solanaceous crops in Michigan were selected from the culture collection maintained in the laboratory of Dr. Hausbeck at MSU. The selected isolates have shown high virulence (defined as the degree of damage caused to a host by a pathogen) and pathogenicity (defined as the capacity of a pathogen to cause disease on a host genotype) in different hosts (12, 14, 45, 53). Isolates were phenotypically characterized according to mating type (MT) and sensitivity to mefenoxam, an oomycete-specific fungicide (30). Isolates OP97 (A1 MT) and SP98 (A2 MT), from pickling cucumber and pumpkin, respectively, were sensitive to mefenoxam, whereas isolates SFF3 (A2 MT) and 12889 (A1 MT) from pickling cucumber and pepper, respectively, were insensitive.

Agar plugs from long-term stock cultures (stored at 20°C in sterilized microcentrifuge tubes containing 1 ml of sterile water and one sterile hemp seed) of each isolate were transferred onto unclarified V8 juice agar (UCV8, 16 g agar, 3 g $CaCO_3$, 160 ml unfiltered V8 juice and 840 ml distilled water) to obtain actively growing cultures, which were maintained at room temperature (21 $\pm$ 2°C) under constant fluorescent lighting. To ensure that isolates could cause disease, cucumber fruits were disinfected for 5 min in a 5% sodium hypochlorite solution, dried at room temperature, and inoculated with each *P.*

capsici isolate. A small, superficial wound was made with a sterile needle in the center of each cucumber. Agar plugs (7 mm diameter) from the margins of actively growing *P. capsici* colonies were placed topside down over each wound. Sterile, capless microcentrifuge tubes were placed over each agar plug and were attached to the fruit with a ring of petroleum jelly. Control cucumbers were inoculated as described above using a sterile 7-mm-diameter plug of V8 agar. Cucumbers were placed in aluminum trays containing wet paper towels and covered with plastic wrap to maintain high relative humidity and incubated at room temperature (21 $\pm$ 2°C). Symptomatic cucumber tissue (0.5 cm) was excised and transferred to UCV8 and maintained under the same culture conditions as described above. Axenic cultures of each isolate were obtained from the infected cucumber tissue and these were transferred to new UCV8 plates weekly until use.

Inoculum preparation and root inoculation. *Phytophthora capsici*-infested millet seed was prepared as inoculum. Millet seed (100 g) was mixed with L-asparagine (0.08 g) and water (72 ml) in a 500-ml Erlenmeyer flask, capped with aluminum foil, autoclaved for two consecutive cycles and shaken to homogenize the mixture. The sterilized millet seed was inoculated with four 7-mm-diameter agar plugs from actively growing *P. capsici* cultures. The inoculated millet seed was incubated at room temperature under constant fluorescent lighting for four weeks.

Inoculation of the plants was achieved by carefully inserting 1 g of millet seed infested with one of four *P. capsici* isolates (OP97, SFF3, SP98, and 12889) directly into the soilless media adjacent to each plant crown, avoiding root or crown injury. For each isolate, five replicate plants of each tomato genotype were inoculated. Five additional

control plants of each tomato genotype were inoculated with uninfested millet seed containing sterile V8 agar plugs. This experiment was conducted three times.

Disease assessment. Disease assessment was conducted daily for five weeks and was initiated one day following inoculation. Disease progression was recorded for each plant using the following scale: 0= no symptoms, 1= 1-30% wilting, 2= 31-50% wilting, 3= 51-70% wilting, 4= 71-90% wilting, and 5= more than 90% wilting or dead plant (Figure 3.1). The area under the disease progress curve (AUDPC) was calculated by inserting the disease score into the equation by Shaner and Finney (56) to describe the cumulative plant susceptibility throughout the experiments. The percentage of death and the incidence of *P. capsici* recovered from roots, crowns, secondary stems (equivalent of branches in a woody plant) and leaves were determined. The plant height was measured at the end of the experiment for host genotypes that appeared resistant based on AUDPC values. Tomato genotypes were considered to be resistant to *P. capsici* if disease, caused by the four isolates used in our study, did not result in serious damage to the plants as indicated by mean AUDPC values of three replicated experiments. Resistant, moderately resistant and susceptible genotypes to each *P. capsici* isolate included those with mean AUDPC values less than 10, between 10 and 50, and higher than 50, respectively.

Pathogen isolation. Control and inoculated plants were gently rinsed to remove the soilless medium and other residues, dipped into 5% sodium hypochlorite for 1 min to surface disinfest, rinsed with sterile water and air-dried. Three sections of root, crown, secondary stem and leaf tissue were excised from each plant and plated onto BARP- (benomyl, ampicillin, rifampicin, and pentachloronitrobenzene) amended UCV8 (32) under sterile conditions.

Colonies suspected to be *P. capsici* were transferred to new BARP-amended UCV8 plates. Axenic cultures were incubated for 7 days on UCV8 with constant fluorescent lighting under ambient laboratory conditions (21 ± 2°C). Cultures were positively identified as *P. capsici* based on morphological characteristics described by Waterhouse (65). Isolates were characterized for compatibility type and mefenoxam resistance as previously described (30) to compare the phenotype of the isolate obtained to the original inoculum.

DNA extraction and molecular confirmation of infection. Samples of root and crown tissue from inoculated resistant and moderately resistant tomato genotypes were collected at the end of the experiment, gently rinsed to remove the soilless medium and other residues, dipped into 5% sodium hypochlorite for 1 min to surface disinfect, rinsed with sterile water, air-dried and ground in liquid nitrogen. Genomic DNA was extracted from approximately 1 g of ground tissue using the DNeasy DNA extraction kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA was quantified using the NanoDrop ND 1000 spectrophotometer and NanoDrop 2.4.7c software (NanoDrop Technologies Inc., Wilmington, DE). Two specific primers for *P. capsici* were used for PCR: one forward primer (CAPFW; 5´TTTAGTTGGGGGTCTTGTACC3´), and one reverse primer (CAPRV2; 5´TACGGTTCACCAGCCCATCA3´) (57). Reactions were performed in a total volume of 25 μl and contained 1ul DNA, 5 μl 5X PCR reaction buffer, 1.25 μl 25 μM $MgCl_2$, 0.5 μl 10 μM dNTP mix, 0.7 μl Taq DNA polymerase (Invitrogen, Carlsbad, CA), 1 μl each 10 μM primer (MSU Macromolecular Structure Facility, East Lansing, MI), and 14.6 μl sterile water. The PCR reaction was performed in a programmable thermal cycler (Eppendorf, Westbury, NY) starting with 3 min denaturation at 94°C, followed by 45 cycles at 94°C for

30 s, annealing at 56°C for 30 s, and extension at 72°C for 60 s, with a final extension step of 10 min at 72°C. PCR products were analyzed by electrophoresis in 2% (w/v) agarose gel in 0.5X Tris-borate-EDTA buffer (35), stained with ethidium bromide (5 μg/ml) for visualization and compared to a 100 bp ladder (Invitrogen) to determine amplicon size. Controls with *P. capsici* DNA and tomato DNA were included.

AFLP analysis. Samples (1 g) of 4-week-old healthy tomato tissue were collected and ground in liquid nitrogen. Genomic DNA was extracted and quantified as described above. All PCR reactions were performed with a programmable thermal cycler (Eppendorf). Approximately 500 ng of DNA was subjected to a restriction/ligation reaction, preselective amplification, and selective amplifications using the AFLP kit for regular plant genomes (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Selective amplifications with the selective primers *Mse*I-CAA and *Eco*RI-ACA, and *Mse*I-CAA and *Eco*RI-AAG were performed. Selective PCR products were purified with ExoSAP-IT (Affymetrix, Inc., Santa Clara, CA) following the manufacturers' instructions. Products labeled with different colored dyes were analyzed at the Michigan State University Research Technology Support Facility (RTSF) using the ABI PRISM 3130 Genetic Analyzer and compared to a 500-carboxy-X-rhodamine (ROX) size standard (Applied Biosystems) following the manufacturer's instructions. Results were prepared for analysis by the RTSF in the form of electropherograms using GeneScan 3.1 analysis software (Applied Biosystems). AFLP fragments were scored manually as present (1) or absent (0) using PeakScanner 1.0 (Applied Biosystems). Only DNA labeled fragments with a size equal to or larger than 70 bp were scored to obtain a binary character matrix. Once fragments of appropriate size were identified, only labeled fragments with a fluorescence signal equal to

or higher than 300 relative fluorescence units (rtf) in at least one variety were scored for presence or absence in the rest of the varieties. The binary matrix, consisting of combined data of both primer sets, was analyzed to generate an unweighted pair-group method with arithmetic mean (UPGMA) distance tree and a pairwise genetic distance matrix using PAUP*4.0b10 (61).

Statistical analyses. All statistical analyses were performed using the SAS statistical package version 9.1 (SAS Institute Inc., Cary, NC). The experiment was arranged in a split-plot with experiment as a blocking factor, isolates as whole plots and host genotypes as sub plots arranged in a randomized complete block design. The AUDPC, incidence, and plant height values of the three replicated experiments were used for statistical analyses. AUDPC data from control plants were removed from the data set prior to statistical analyses since no infection occurred and no symptoms developed. AUDPC and plant height data were normalized by square root transformation, and the residuals followed the assumptions of all statistical tests performed. The percent death was not included for statistical analysis because the residuals did not follow the assumptions required for statistical tests. Data were subjected to analysis of variance (ANOVA) using the PROC MIXED, PROC GLM and PROC GLIMMIX procedures of SAS. Correlation between UPGMA cluster (defined by the genetic distance matrix) and resistance to *P. capsici* was determined using the PROC CORR procedure. Multiple comparisons among the means were conducted using t-tests (LSD) when effects were found to be statistically significant at P=0.05 in ANOVA.

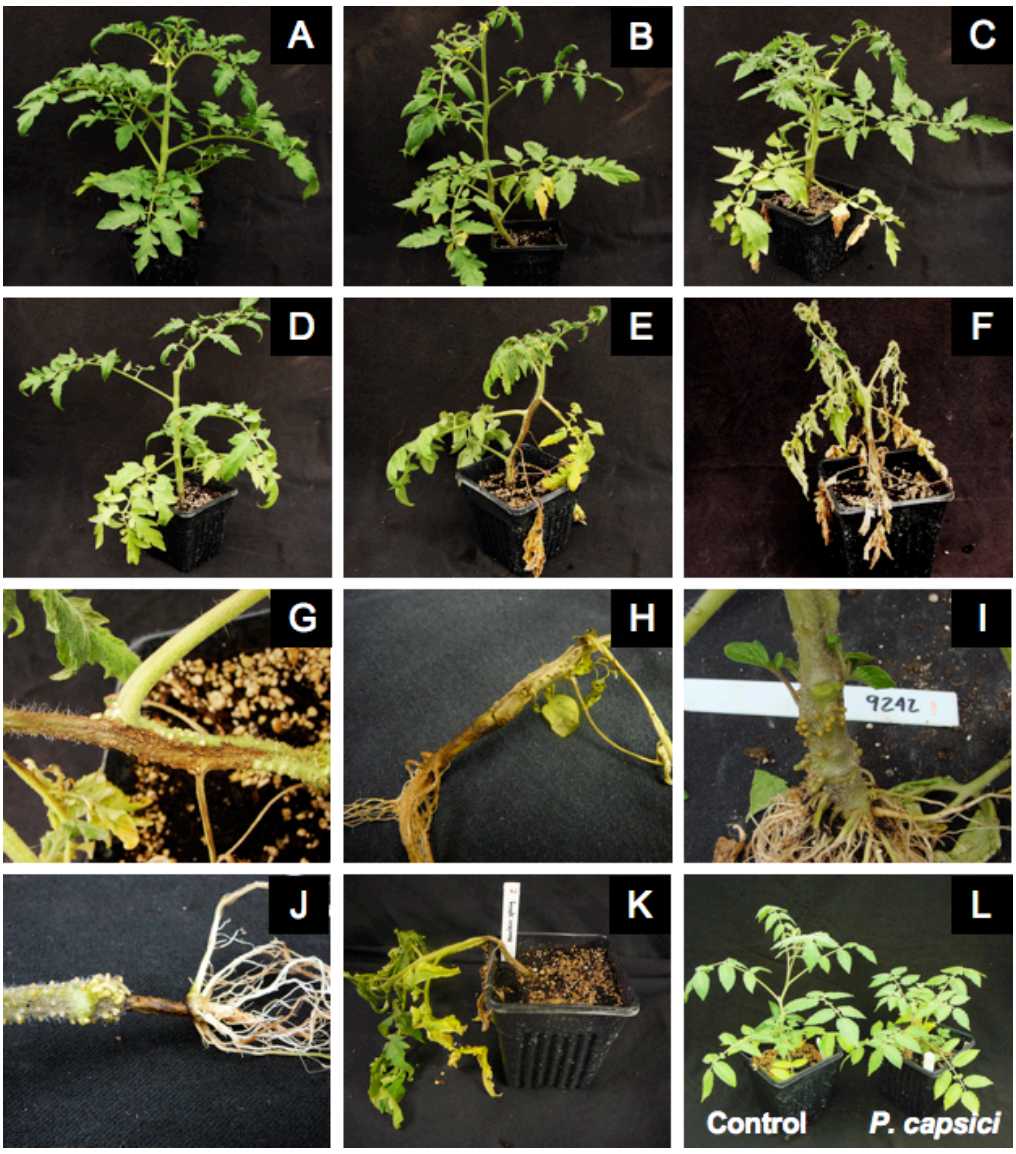
Results

Initial disease symptoms occurred within 4 days following inoculation in susceptible and some moderately resistant tomato genotypes for each *P. capsici* isolate. In susceptible genotypes, wilting was observed in the lower leaves within the first week and progressed upward until the entire plant wilted. External, dark discoloration of the crowns and crown rot were observed on some plants and the root system was often discolored. Subsequently, stem cankers and girdling developed, and some plants shriveled at the soil level (Figure 3.1). Brown, water-soaked lesions were visible on roots and crowns of symptomatic plants within 6 days. Plant death was observed as early as 7 to 8 days following inoculation in some host genotypes, but was not visible until 4 weeks after inoculation for others. Moderately resistant varieties showed mild wilting 7 to 8 days following inoculation but quickly recovered. Some plants continued to grow and maintain healthy tops by regenerating an extensive number of secondary roots (Figure 3.1). Resistant genotypes did not show wilting after inoculation or production of secondary roots. Some resistant and moderately resistant host genotypes also presented significant stunting.

Disease severity differed significantly depending on the interaction effects between isolate and tomato genotype (ANOVA, $P<0.0001$). Responses to individual isolates varied from no symptoms to 100% wilting and death of inoculated plants. Isolate 12889 was more pathogenic on tomato genotypes than other isolates used in plant inoculations according to trends in AUDPC (Table 3.1). Interaction effects were not found between repeated experiments and isolate (ANOVA, $P=0.5178$). Effects due to differences between experiments were not significant (ANOVA, $P=0.2926$). Only one of the genotypes (LA407) did not show wilting or any other symptoms other than stunting following inoculation with

the four different *P. capsici* isolates (Table 3.1). Four of the host genotypes (Fla7600, Ha7998, Jolly Elf and Talladega) developed moderate symptoms with <12% plant death (Table 3.1). LA716 was the most susceptible genotype in our study; exhibiting the highest AUDPC values and 100% plant death (Table 3.1).

Figure 3.1. Symptoms of *Phytophthora capsici* root rot on tomato and wilting scale used in plant evaluations. A, 0= no symptoms, healthy plant. B, 1= 1-30% wilting. C, 2= 31-50% wilting. D, 3= 51-70% wilting. E, 4= 71-90% wilting. F, 5= more than 90% wilting or dead plant. G, Stem canker. H, Water soaked lesions in the stem, rotted crown, and rotted and discolored roots. I, Secondary roots. J, Girlding. K, Damping off. L, Stunting.



The pathogen was isolated from all surviving susceptible, moderately resistant and resistant genotypes at the end of the experiment and from plants that died over the course of the experiment. *P. capsici* was isolated from symptomatic plants of the moderately resistant genotypes Fla7600, Ha7998, Jolly Elf and Talladega, and from the roots of the resistant genotype LA407 (Table 3.1). *P. capsici*-infection in the moderately resistant and resistant genotypes was confirmed by performing *P. capsici*-specific PCR of DNA from surface-sterilized, inoculated root and crown tissue to confirm that the very low recovery of *P. capsici* from LA407 by culturing techniques was not due to an escape. All *P. capsici*-inoculated moderately resistant and resistant genotypes were positive for presence of *P. capsici* in root or crown tissue. Significant differences in pathogen incidence were found among different plant parts (PROC GLIMMIX and LSD, $P<0.0001$). Interaction effects were found between the isolate used and tomato genotype for incidence data (ANOVA, $P<0.0001$). Interaction effects were not found between experiments and isolate (ANOVA, $P=0.9994$). Effects due to differences between repeated experiments were not significant (ANOVA, $P=0.9442$). Although the secondary stems and leaves exhibited wilting in all experiments, the pathogen was rarely recovered from secondary stems and never recovered from leaves (Table 3.3). The phenotype (mating type and mefenoxam resistance) of recovered isolates was confirmed and matched the phenotype of the isolate used as inoculum (data not shown). The control plants remained asymptomatic and the pathogen was not isolated from any control plant tissue. Clean cultures were obtained from 94% of the original cultures isolated from tomato tissue.

Stunting was observed in several tomato genotypes including some of the moderately resistant and resistant candidates (Figure 3.1L). Plant height was measured for

surviving inoculated moderately resistant and resistant genotypes and the corresponding control plants for those host genotypes at the end of the experiment. Generally, plants inoculated with one of the four *P. capsici* isolates were significantly shorter than the corresponding control plants, although the interaction between isolate and tomato genotype was significant (ANOVA, $P<0.0001$). Pairwise comparisons between each tomato genotype inoculated with a particular isolate and the corresponding control plant indicated that genotypes Fla7600 and Jolly Elf did not show significant differences in plant height regardless of isolate (LSD $P>0.05$, Table 3.4). Interaction effects were not found between repeated experiments and isolate (ANOVA, $P=0.5478$). Effects due to differences between experiments were not significant (ANOVA, $P=0.0600$).

The *Mse*I-CAA and *Eco*RI-ACA, and *Mse*I-CAA and *Eco*RI-AAG primer pairs yielded a total of 104 and 120 AFLP markers, respectively. Of the total 224 AFLP markers obtained, 126 corresponded to labeled fragments with a size equal to or larger than 70 bp and a signal equal to or higher than 300 rtf that were scored for presence and absence in the tomato genotypes and included in the analyses. Four of these markers were polymorphic only among *Solanum* species (2% of interspecific polymorphism), 31 markers had variation among *Solanum* species and *S. lycopersicon* varieties (14% of inter and intraspecific polymorphism), 11 markers were polymorphic only for *S. lycopersicon* varieties (5% of intraspecific polymorphism), and the other 80 markers were present in all tomato genotypes (79% monomorphic). Seven similarity groups, including four main clusters (A, B, C, and F) and three clades formed by single genotypes (D, E, and G) with >80% similarity, were obtained after genetic distance analysis of the AFLP data (Figure 3.2). The UPGMA tree topology did not indicate a subdivision of clusters based on susceptibility to *P. capsici*.

Statistical analysis found no significant correlation between cluster and susceptibility to *P. capsici* (*P*>0.05). Table 3.3. Incidence of *Phytophthora capsici* isolates obtained from different parts of the symptomatic tomato plants sampled in each of three replicated experiments.

Trial	Plants sampled	Number of isolates	Isolates[a] (%)		Incidence[b] (%)			
			R	C	R	C	S	L
1	570	910	470 (51.6)	440 (48.4)	488 (50.7)	471 (48.8)	5 (0.5)	0 (0)
2	583	903	458 (50.7)	445 (49.3)	483 (50)	479 (49.6)	4 (0.4)	0 (0)
3	579	890	459 (51.6)	431 (48.4)	488 (51.3)	458 (48.1)	6 (0.6)	0 (0)

[a]Isolates obtained as determined by axenic pathogen isolation at the conclusion of the experiment. R, root. C, crown.

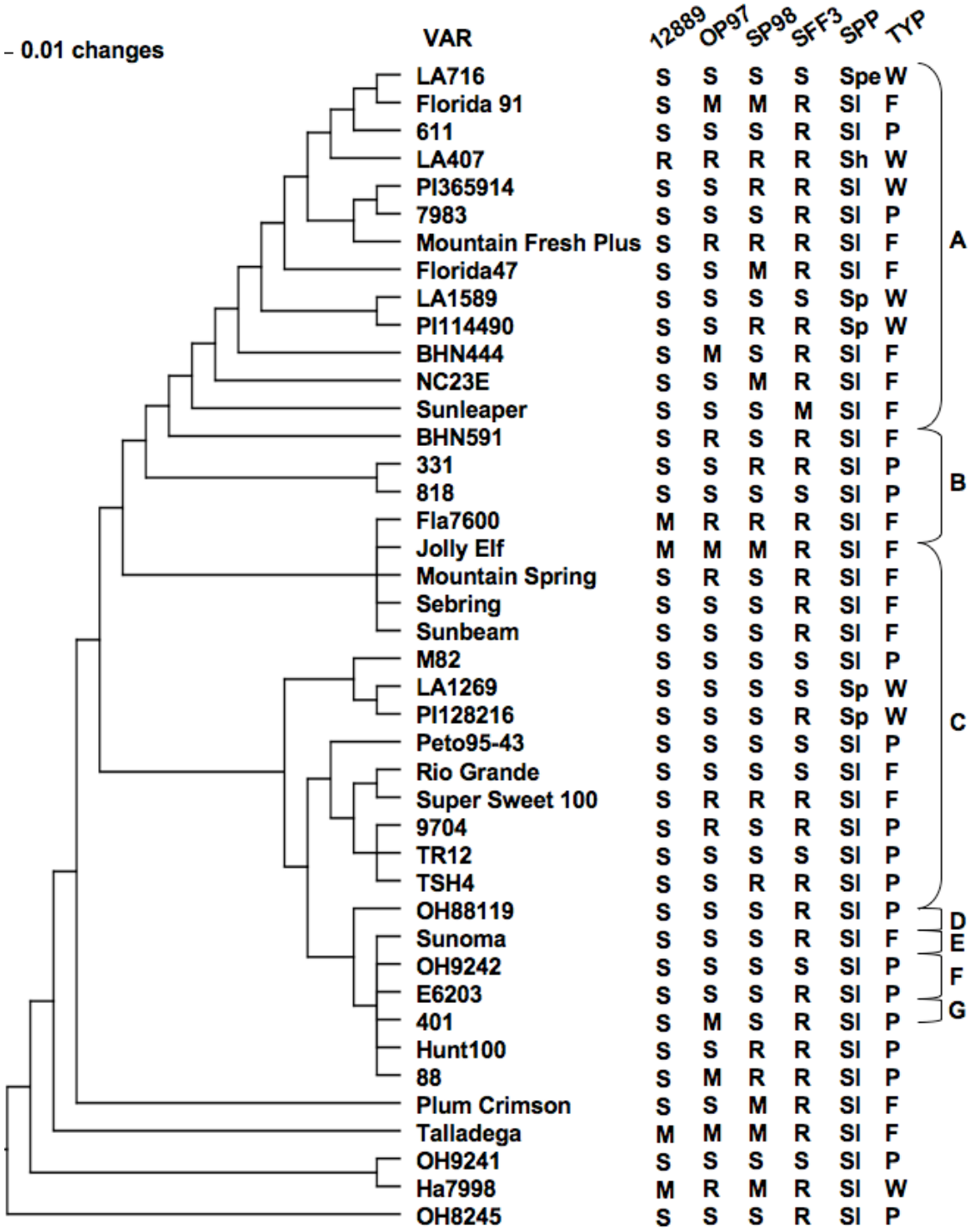
[b]Incidence of *P. capsici* as determined by observation of growth of the pathogen isolated from plant tissue. R, root. C, crown. S, secondary stem. L, leaf.

Table 3.4. Probability (P) values for significance of differences in plant height between inoculated plants and their corresponding controls for tomato genotypes resistant and moderately resistant to *Phytophthora capsici.*

Variety	*P* value*			
	12889	OP97	SP98	SFF3
Fla7600	0.8164	0.5798	0.0744	0.5324
Ha7998	0.0093*	0.0004*	<0.0001*	<0.0001*
LA407	0.0402*	0.0048*	<0.0001*	<0.0001*
Jolly Elf	0.0745	0.1681	0.1567	0.5653
Talladega	<0.0001*	0.0327*	0.0002*	0.8062

* Means significantly different from the control (LSD); analyses included data from three experiments

Figure 3.2. Amplified fragment length polymorphism distance tree of tomato varieties and wild relatives screened for resistance to *P. capsici*. Susceptibility to *P. capsici* 12889, OP97, SP98 and SFF3 (S, susceptible, M, moderately resistant, R, resistant), tomato species (SPP: Sl, *Solanum lycopersicon*, Sp, *S. pinpinelifolium*, Spe, *S. pennellii*, Sh, *S. habrochaites*), and type of variety (TYP: F, fresh market, P, processing, W, wild) are indicated for each variety. A, B, C, D, E, F, G, correspond to similarity groups obtained in UPGMA clustering analysis.



Discussion

Early infections by *P. capsici* on tomato cause plant damping-off, whereas later infections cause root and crown rot on mature plants (21). Phytophthora root rot results in wilting with brown-to-black cankers on the lower stems, blackening of the vascular tissue, and root rot (29), which may initiate the development of secondary roots (4). Infected plants may become stunted and wilted (21), as they did in our study. Plants showing quantitative resistance to Phytophthora root rot have been reported to develop and maintain healthy canopies by regenerating an extensive number of secondary roots, a response that is most likely polygenic (4). In this experiment, several host genotypes produced secondary roots that emerged just above a stem canker or crown rot lesion but many of them still died possibly because the response was not fast enough. The moderately resistant genotypes Talladega and Jolly Elf produced secondary roots in some cases and maintained healthy canopies throughout the duration of the experiment. The resistant variety LA407 did not show this response. Symptoms developed more slowly on the moderately resistant genotypes Fla7600, Ha7998, Jolly Elf, and Talladega than on the other genotypes tested. In general, the response of moderately resistant tomato genotypes to Phytophthora root and crown rot was quantitative rather than qualitative, as observed in the *P. capsici*-pepper interaction (26). In some cases such as the resistant genotype LA407 or the susceptible genotype LA716 the response is more qualitative since there appeared to be no disease in LA407 plants but all LA716 plants died.

The success of greenhouse screening depends on the plant's age and resistance level, inoculum quality/quantity, inoculation technique, and post inoculation environmental conditions. These factors may influence quantitative responses such as

resistance to *P. capsici*. For this study, environmental conditions in the greenhouse were not always ideal for plant and pathogen growth due to low minimum air temperatures (1-2°C) and high maximum air temperatures (35-36°C) in experiments 2 and 3. The minimum, optimum, and maximum temperatures for growth of *P. capsici* are 10°C, 28°C, and >35°C, respectively (9). Tomato plants prefer warmer temperatures (18- 26°C) for normal plant development, growth and fruit set (24), and become sensitive to biotic and abiotic stress at temperatures below 10°C or above 35°C (24). Nonetheless, environmental conditions did not seem to alter disease development and average air temperatures were suitable for plant and pathogen growth. Furthermore, there were no statistically significant interactions with experiment so any effects on disease development appear to have been constant across isolates and host genotypes.

Resistance to *P. capsici* under greenhouse conditions may differ from the responses observed under field conditions due to the quantitative nature of the trait. The soilless medium used in this study may have influenced resistance to *P. capsici,* and the responses observed in the greenhouse may not be consistent in the field depending on soil type, soil microbial community composition, pH and other factors. Other studies on *P. capsici* (27) and other soilborne pathogens (16, 39) have shown variable results, with observations of increased or decreased disease severity depending on the soil type, soilless substrates, or treatments used. To our knowledge, none of these studies have determined the influence of soilless medium on *P. capsici*. However, if soilless medium-effects on *P. capsici* exist, they were consistent throughout the experiments and did not appear to inhibit disease development.

The type and quantity of inoculum used can also affect disease severity in greenhouse and field studies (14, 15, 45). The millet seed inoculation technique was effective for establishing *P. capsici* infection on susceptible tomato genotypes in the greenhouse, as has been reported in previous studies with other host plants (12, 45). The millet seed inoculum for our experiments contained only mycelia and sporangia, while Michigan fields infested with *P. capsici* have been found to contain oospores (30, 31). Oospores allow the pathogen to overwinter, may persist for years (18, 32), and are primary inocula (18). For our experiments, we used *P. capsici* propagules that constitute secondary inocula and cause tomato field epidemics (21). Studies replicating our greenhouse experiment in *P. capsici*-infested fields would help establish the influence of inoculum type on disease severity as well as the transferability of greenhouse results to field conditions.

All *P. capsici* isolates tested caused disease on some plants, although there was a significant isolate by tomato genotype interaction. Isolate 12889 produced symptoms in more host genotypes, followed by OP97, SP98 and SFF3, respectively. When these isolates were tested on pepper in an earlier study (15), 12889 was also found to be the most pathogenic, however no differences were detected when the isolates were tested on cucumber fruit (14) and Fraser fir (45). As early as 1972, field populations of *P. capsici* from solanaceous and cucurbitaceous hosts were shown to differ in pathogenicity (44), other reports of this variation have been published for several hosts (5, 26, 40, 41, 48, 50), including tomato where an interaction between host and isolate was also reported (17, 20). It has been suggested in previous studies that *P. capsici* isolates obtained from the Solanaceae have increased pathogenicity on solanaceous hosts compared to isolates obtained from Cucurbits (19, 34, 50). Further studies that include more isolates are

needed to clarify if *P. capsici* 12889 has increased pathogenicity in tomato and pepper genotypes because it was isolated from another solanaceaous host or if the pathogenicity is due to an isolate-specific effect.

The differences in pathogenicity found between these Michigan isolates have significant implications for local plant breeding programs. Breeding for resistance in pepper against *P. capsici* has been challenging due to the diversity of pathogen populations, the existence of different physiological races within *P. capsici* in the United States (40, 64), and the various complex modes of inheritance of resistance reported for pepper (47). Similarly, for tomato, studies are needed to characterize *P. capsici* populations, define races that infect tomato, and determine their spatial structure in order to intelligently deploy tomato resistance in Michigan. Nonetheless, in this study, tomato genotypes LA407, Fla7600, Ha7998, Jolly Elf and Talladega showed high or moderate resistance to all of the isolates used and could be used as a starting point to find resistance to Michigan *P. capsici* isolates.

Our data indicated that the four isolates tested probably represent different races. Nonetheless, the tomato genotypes should be tested against a larger number of isolates, particularly those coming from tomato to better understand this interaction, and to eventually develop a system with host differentials for identifying races, as has been described for pepper (63). No resistant control was used in our study because, to the best of our knowledge, there is no standard tomato resistant to *P. capsici.* When screening pepper varieties for resistance to *P. capsici*, the Chi-square test has been used to determine relative resistance of pepper lines by comparison to standard resistant peppers such as CM334 (63). Our experiments identified the resistant genotype LA407; thus, a similar

approach to the one used in the *P capsici*-pepper interaction can now be applied to future *P. capsici*-tomato interaction studies.

Significant differences in pathogen incidence were found among different plant parts, possibly indicating that more than one mechanism of host resistance may be involved. In pepper, it has been observed that resistance to *P. capsici* is due to different genetic controls depending on tissue type (62, 64). In our study, the pathogen was successfully recovered from roots and crowns of all symptomatic, root-inoculated seedlings. *Phytophthora capsici* can be difficult to isolate from certain tissue types such as mature pepper stems (M. K. Hausbeck, unpublished data), asparagus (55) or Fraser fir tissue (45), but was easily isolated from symptomatic tomato plants in our experiments. However, PCR was useful because it confirmed that the inability to detect *P. capsici* in LA407 using culture techniques was not due to an escape. In previous studies, PCR was able to detect false negatives after a lack of *P. capsici* detection by culturing techniques in pepper plants when symptoms and signs where not obvious (51). Genotypes Ha7998 and Talladega were both stunted as a result of *P. capsici* infection; yet, when Talladega was inoculated with SFF3, the least pathogenic isolate in our study, the plants did not show stunting. Stunting in resistant and moderately resistant genotypes indicates that the pathogen was directly limiting plant growth. However, it is not known if stunting would occur in nature on a resistant genotype or was the result of the relatively axenic conditions of the experiment.

Wild species of tomato can be a valuable source of economically important traits, including resistance to diverse pathogens for the improvement of cultivated tomato crops (10). In our study, we found that *Solanum habrochaites* LA407 was resistant to the four *P.*

capsici isolates used. Interestingly, LA407 was resistant to bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) (13, 25) and early blight caused by *Alternaria solani* (11). Other genotypes of *Solanum habrochaites* were resistant to late blight caused by *Phytophthora infestans* (6, 7), whitefly infestations (38), whitefly-transmitted geminivirus (36), and *Bemisia argentifolli* (38). This wild species can be crossed with *S. lycopersicon* to generate progeny with relative ease (6, 7, 13), making the species a good candidate for improving tomato disease resistance.

When screening for economically important traits, breeders have performed host samplings under the assumption that taxonomically related plants, or those found in geographic proximity, are likely to share desirable characteristics such as disease resistance (22, 23, 58). The third objective of our study was to determine if resistance to *P. capsici* could be found in diverse tomato lineages or if it was only associated with one or more particular clades. If a breeder can use taxonomic or geographic information to identify the most likely sources of valuable traits, then the efficiency of the search may be improved (22). To determine the relatedness of the tomato genotypes used in this study, we relied on AFLP analysis. AFLP analysis is an appropriate tool to detect polymorphisms in tomato and establish genetic diversity and relatedness among tomato genotypes (42, 60). The obtained UPGMA tree topology agreed with clustering analysis of previous studies (1, 37, 42, 60) and the statistical analysis found no correlation between UPGMA clade and resistance to the pathogen isolates used in this study, indicating that the tree topology obtained cannot be reliably used to predict where additional sources of *P. capsici* resistance may be found. Resistance to isolate SFF3 seems to be more widespread across tomato lineages than for the other *P. capsici* isolates used in our experiment. Spooner et al. (59)

also found that resistance to *Phytophthora infestans* was not associated with lineages of wild potatoes. Our results suggests that tomato germplasm needs to be screened for resistance to diverse *P. capsici* isolates until other methods, such as marker-assisted selection, are developed to identify resistant genotypes. The association between *P. capsici* resistance and geographic origin of tomato genotypes was not analyzed in this study because the origin of most varieties is unknown. Information of geographic origin is only available for LA407 that comes from Ecuador; Ha7998 that comes from Hawaii; and LA1269, LA1589, and LA716 that come from Peru. More accessions of the wild species used in this study need to be included in future experiments to establish if the responses observed occur in other accessions of the same species, and to determine if the lack of association between resistance to *P. capsici* and AFLP-clades is due to the low number of *Solanum* accessions tested or to genetic effects.

P. capsici has been documented in many vegetable production regions in Michigan (18), a state that grows over 33,000 hectares of susceptible crops (3). In Michigan, a grower's ability to grow vegetables in uninfested land is diminished due to a general reduction in farmland caused by urban sprawl and an increase in the number of *P. capsici*-infested fields. In addition, fungicide resistance, an increasing list of susceptible hosts, and the phasing-out of methyl bromide treatments make cost-effective management of *P. capsici* difficult. Disease management strategies that reduce fungicide use without increasing disease related losses, such as host resistance, are needed.

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CHAPTER IV: INVESTIGATING THE GENETIC STRUCTURE OF *PHYTOPHTHORA CAPSICI* POPULATIONS

Abstract

Phytophthora capsici Leonian is a destructive soilborne pathogen that infects economically important solanaceous, cucurbitaceous, fabaceous, and other crops in the United States and worldwide. The objective of this study was to investigate the genetic structure of 255 *P. capsici* isolates assigned to predefined host, geographical, mefenoxam sensitivity and mating type categories. Isolates from six continents, 21 countries, 19 U.S. states, and 26 host species were genotyped for four mitochondrial and six nuclear loci. Bayesian clustering analysis revealed some population structure by host, geographic origin and mefenoxam sensitivity with some clusters occurring more or less frequently in particular categories. Bayesian clustering, split networks, and statistical parsimony genealogies also detected the presence of non-*P. capsici* individuals in our sample corresponding to *P. tropicalis* and isolates of a distinct cluster closely related to *P. capsici* and *P. tropicalis*. Our findings of genetic structuring in *P. capsici* populations highlight the importance of including isolates from all detected clusters that represent the genetic variation in *P. capsici* for development of diagnostic tools, fungicides, and host resistance. The population structure detected will also impact the design and interpretation of association studies in *P. capsici*. This study provides an initial map of global population structure of *P. capsici* but continued genotyping of isolates will be necessary to expand our knowledge of genetic variation in this important plant pathogen.

Introduction

Phytophthora capsici Leonian is a destructive soilborne pathogen that is distributed worldwide. It can infect a broad range of hosts including economically important solanaceous, cucurbitaceous and fabaceous crops in the United States (U. S.) (28, 33, 34, 84). *P. capsici* also causes disease on tropical hosts such as cacao (*Theobroma cacao*), rubber (*Hevea brasiliensis*), macadamia (*Macadamia integrifolia*), papaya (*Carica papaya*), black pepper (*Piper nigrum*) (22) and rocoto pepper (*Capsicum pubescens*) (49), and additional hosts continue to be identified (34, 83, 84).

P. capsici is a diploid oomycete that is heterothallic (22). Hence, sexual reproduction occurs when the A1 and A2 mating types (MTs) come together to produce oospores (59). It is common in vegetable-producing regions in the U.S. for both MTs to be present (44, 57, 88). The success of crop rotation as a management strategy is limited by the long-term survival of oospores in the soil (60), and the number and diversity of susceptible hosts (22). Chemical control of *P. capsici* with mefenoxam, a commonly used fungicide, may not protect susceptible crops from resistant pathogen populations, which have been documented throughout the U.S. (57, 59, 61, 78).

Developing resistant varieties for economically important hosts has been challenging due to the diversity of pathogen populations and the existence of different physiological races within *P. capsici* in the U.S. (73, 108). Host varieties that show resistance in one region may not perform well in another region (28). Also, *P. capsici* isolates that show low or medium virulence on one particular host may show increased virulence on a different host (28). As early as 1972, field populations of *P. capsici* from solanaceous and cucurbitaceous hosts were shown to differ in virulence (80). In previous

studies when *P. capsici* isolates 12889, OP97, SP98, and SFF3 from Michigan were used to inoculate cucumber fruit (33) and Fraser fir (83), differences in virulence were not observed. In contrast, studies on pepper (28) and tomato (84) with these same isolates have indicated significant differences in virulence and pathogenicity, respectively. Other reports of variation in virulence and pathogenicity among *P. capsici* isolates have been published for several hosts (13, 55, 73, 75, 86, 87); however, the genetic basis of these differences is unknown. The differences found between *P. capsici* isolates have significant implications for breeding for resistant hosts since developed varieties should be effective against local pathogen populations. Isolates used in host resistance screenings should incorporate the genetic, phenotypic, and physiological diversity of *P. capsici*. However, the global genetic variation of *P. capsici* populations is still poorly understood.

A detailed knowledge of the population structure and distribution of genetic variation of *P. capsici* is needed to understand the genetic composition of isolates that occur in particular hosts or regions to develop and intelligently deploy host resistance. Characterizing *P. capsici* populations for fungicide resistance is also important to establish if a product will provide effective chemical control against local isolates. Populations of *P. capsici* have been previously studied within states in the U.S. (30, 58, 103, 109), and in countries where this pathogen causes significant losses (65, 95). Such investigations have provided information about local genetic diversity of isolates and the importance of recombination in maintaining genetic variability (58, 59). Some experiments have also used *P. capsici* intra- and inter-specific data to determine whether *P. capsici* and *P. tropicalis*, a sibling species, truly constitute separate species (12, 19, 69, 117) but there is still controversy about the evolutionary relationships between these pathogens (12).

Establishing the population structure of *P. capsici* would help in understanding the genetic variation of this pathogen and better define the species, which will impact the development of species-specific molecular diagnostic tools. Other studies have analyzed *P. capsici* intra-specific data using traditional phylogenetic methods and have established a correlation between phylogenetic cluster and host type, where isolates from woody perennials will group together and separate from isolates obtained from vegetable crops (12, 19). Studies including worldwide samples to examine the global population structure and distribution of genetic variation in *P. capsici* by geography and host are still lacking.

Most studies of plant pathogen diversity are based on sampling from predefined "populations", referred to as categories in this paper. These categories are usually defined by host, geography or physiology and may not reflect underlying genetic relationships. Bayesian clustering can be applied to assign individuals in a sample to subpopulations, referred to as clusters in this paper, based on their distinct allele frequencies (82). This method has been successfully used to visualize overall patterns of genetic structure in several species (9, 24, 26, 29, 54, 90, 102). Nonetheless, Bayesian clustering has not been as extensively used to detect population structure in plant pathogens and explore the distribution of genotypes in different regions or hosts, which may have implications for disease management. In this study we used Bayesian clustering and other methods to investigate the genetic structure of global *P. capsici* populations. Specifically we sought to: (i) establish if we could detect population structure in the sample, (ii) determine whether the predefined grouping of *P. capsici* isolates in host, geography, mefenoxam sensitivity and mating type categories show direct correspondence with inferred genetic clusters, and (iii) examine the distribution of genotypes from each cluster within the predefined categories.

Clustering analysis of *P. capsici* isolates revealed some population structure by host, geographic origin and mefenoxam sensitivity with some clusters occurring more or less frequently in particular categories but showed no direct correspondence between any of the predefined categories and the number of inferred clusters. Bayesian clustering, split networks, and statistical parsimony genealogies also detected the presence of non-*P. capsici* individuals in our sample corresponding to *P. tropicalis* and isolates of a distinct cluster closely related to *P. capsici* and *P. tropicalis*.

Methods

Isolate selection, maintenance and phenotypic characterization. A total of 255 *P. capsici* isolates originating from diverse hosts throughout the world were obtained from colleagues or selected from the culture collection maintained in the laboratory of Dr. Hausbeck at Michigan State University (MSU) (Table 4.1). Type cultures for *P. capsici* 13691 (ATCC.64531, Italy 1927) and 13692 (ATCC.15399, New Mexico 1727), and *P. tropicalis* 13602 (ATCC.76651, Hawaii 2001) were included in the analysis. Actively growing, single-spore cultures were obtained from long-term stock cultures as previously described (83). Agar plugs from actively growing cultures of each isolate were transferred to 50 ml-centrifuge tubes containing 30 ml of unclarified V8 juice broth (UCV8B; 3 g $CaCO_3$, 160 ml unfiltered V8 juice and 840 ml distilled water), which were maintained at room temperature (21 ± 2°C) under constant fluorescent lighting and shaking (0.03 x *g*) to obtain agar-free tissue for DNA extraction. Isolates were characterized according to mating type (MT), and sensitivity to mefenoxam as previously described (57). *P. capsici* was confirmed

using morphological characteristics according to the *Phytophthora* spp. key by Waterhouse (110).

Table 4.1. Isolates of *P. capsici* sensu stricto (SS) and sensu lato (SL) used in this study.

Isolate	Origin[a]	Host	MT[b]	MS[c]	Haplotypes[d]	Source
101	US, MI	*Cucumis sativus*	A1	S	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
102	US, MI	*C. sativus*	A2	I	1-1-1-1-1,5-4,1-7,4-1-1-2	M. K. Hausbeck
135	US, MI	*C. sativus*	A2	IS	1-1-1-1-1-1,11-9,2-1-3,1-5,1	M. K. Hausbeck
455	US, MI	*C. sativus*	A1	S	1-1-1-1-1,5-10,1-2-1-1-5,2	M. K. Hausbeck
1177	US, MI	*C. sativus*	A2	S	1-1-1-1-1-17,1-2,6-1-1,4-1	M. K. Hausbeck
9790	US, MI	*Capsicum annuum*	A1	IS	1-1-1-1-1-11,1-2-1-1-1,3	M. K. Hausbeck
9964	US, MI	*Cucurbita maxima*	A1	S	1-1-1-1-1-1,23-2-1-1-1	M. K. Hausbeck
10044	US, MI	*C. annuum*	A2	S	1-1-1-1-1,4-29,1-4,3-2,1-3-1,2	M. K. Hausbeck
10193	US, MI	*Phaseolus vulgaris*	A1	S	1-1-1-1-1-24,1-7,4-1-1,3-3	M. K. Hausbeck
10213	US, MI	*P. vulgaris*	A1	I	1-1-1-1-1-24,42-2,4-1-1-1	M. K. Hausbeck
10251	US, MI	*P. vulgaris*	A1	S	1-1-1-1-1-30,1-2,4-1-1,4-1,3	M. K. Hausbeck
10412	US, MI	*C. sativus*	A2	I	1-1-1-1-1,8-3,1-2,15-2,1-1,4-1,2	M. K. Hausbeck
10858	US, MI	*P. vulgaris*	A1	S	1-1-1-1-1-3,1-2,4-1-2,24-3	M. K. Hausbeck
11348	US, MI	*C. sativus*	A2	S	1-1-1-1-1-11,1-2,9-2,1-1,2-1,2	M. K. Hausbeck
11457	US, MI	*C. annuum*	A1	IS	1-1-5-1-4,2-3,1-2,7-1,3-1-50,51	M. K. Hausbeck
11469	US, MI	*C. annuum*	A2	IS	1-1-1-1-9-2,1-6,3-1-1-2,3	M. K. Hausbeck
11478	US, MI	*P. vulgaris*	A1	I	1-1-9-1-9-30,1-2,7-1-1-1,17	M. K. Hausbeck
11571	US, MI	*Cucurbita pepo*	A2	S	1-1-1-1-1-3,1-9-2,1-1,3-1	M. K. Hausbeck

Table 4.1 (cont'd)

11780	US, MI	*C. sativus*	A2	IS	1-1-1-1-1,4-11,1-7,4-1-1-1,5	M. K. Hausbeck
11783	US, MI	*C. sativus*	A2	I	1-1-1-1-1,4-11,1-7,4-1-1-55,56	M. K. Hausbeck
11861	US, MI	*C. pepo*	A2	IS	1-1-1-1-1-2,1-2,15-1-1-3	M. K. Hausbeck
11885	US, MI	*C. pepo*	A1	I	1-1-1-1-1-2,1-2,4-2,1-1-3	M. K. Hausbeck
11923	US, MI	*C. pepo*	A1	I	1-1-1-1-1-2,1-6,3-1-1-3	M. K. Hausbeck
12017	US, MI	*C. pepo*	A2	S	1-1-1-1-1-5,1-2,7-2,1-1-1	M. K. Hausbeck
12162	US, MI	*C. maxima*	A2	S	1-1-1-1-1,4-11,1-25,32-2,1-1-5,2	M. K. Hausbeck
12328	US, MI	*C. annuum*	A1	IS	1-1-1-1-4,5-2,1-7,4-1-1-1,5	M. K. Hausbeck
12842	US, MI	*C. annuum*	A2	S	1-1-1-1-4,5-17,1-6,5-1-1-1	M. K. Hausbeck
12889	US, MI	*C. annuum*	A1	I	1-1-1-1-1-2,1-7,4-1,3-1-3	M. K. Hausbeck
13199	US, KY	*C. maxima*	A1	S	1-1-1-1-1,2-18,1-15,3-6-1,2-4,35	K. W. Seebold
13200	US, KY	*Cucurbita* sp.	A2	S	1-1-1-1-1,4-2,12-1,11-2,1-1-3,42	K. W. Seebold
13201	US, KY	*C. pepo*	A2	S	1-1-1-1-4,5-2,12-19,11-2,1-1-3,42	K. W. Seebold
13202	US, NY	*C. maxima*	A2	S	1-1-1-1-9-3,1-4,3-2,1-1-14,6	C. D. Smart
13203	US, NY	*C. annuum*	A1	S	1-1-1-1-1-16,1-4-8-3,7-1	C. D. Smart
13204	US, NY	*C. annuum*	A1	S	1-1-1-1-1-16,1-4-8-3,7-1	C. D. Smart
13205	US, NY	*C. maxima*	A2	S	1-1-1-1-2-31,6-1,3-2,6-10,3-1,11	C. D. Smart
13206	US, NY	*C. maxima*	A1	S	1-1-1-1-1-3,1-4-2,1-1,3-3	C. D. Smart
13207	US, CA	*C. annuum*	A2	S	1-1-1-1-1-10,1-1,8-2-1,9-2,25	C. L. Blomquist
13208	US, CA	*Solanum lycopersicon*	A2	S	1-1-1-1-2,18-2,1-1,8-3-1-1,43	C. L. Blomquist
13209	US, SC	*C. pepo*	A2	S	1-1-1-1-1,5-16,1-1,20-2,1-1,11-1,5	A. P. Keinath
13210	US, SC	*C. pepo*	A2	S	1-1-1-1-1,5-16,1-1,20-2,1-1,11-1,5	A. P. Keinath

Table 4.1 (cont'd)

13211	US, SC	*C. pepo*	A2	S	1-1-1-1-1,5-16,1-1,20-2,1-1,11-1,5	A. P. Keinath
13212	US, SC	*C. pepo*	A2	S	1-1-1-1-1,2-14,12-5,33-2,1-1,11-39,2	A. P. Keinath
13213	US, SC	*C. pepo*	A2	S	1-1-1-1-7,2-14,1-5,10-2,1-3,2-1,68	A. P. Keinath
13214	US, SC	*C. pepo*	A2	S	1-1-1-1-1,5-43,1-5,10-2,1-3,2-4,2	A. P. Keinath
13215	US, SC	*C. pepo*	A2	S	1-1-1-1-1,19-14,1-5,10-2,1-3,2-4,35	A. P. Keinath
13216	US, SC	*C. pepo*	A2	S	1-1-1-1-1,5-14,1-1,20-2,1-1,11-1,5	A. P. Keinath
13217	US, SC	*C. pepo*	A2	S	1-1-1-1-1,5-14,1-5,10-2,1-3,2-35,7	A. P. Keinath
13218	US, SC	*Cucurbita moschata*	A1	S	1-1-7-1-14,15-1,25-2,6-2,1-1,2-44,45	A. P. Keinath
13219	US, SC	*C. moschata*	A1	S	1-1-7-1-14,15-1,25-2,6-1-1,2-44,45	A. P. Keinath
13220	US, SC	*C. moschata*	A1	S	1-1-1-1-1,19-4,1-2,6-2,1-3,2-7,69	A. P. Keinath
13221	US, SC	*C. annuum*	A1	S	1-1-1-1-6-8,1-13,16-2-2-46,21	A. P. Keinath
13222	US, SC	*C. annuum*	A1	S	1-1-1-1-15,12-8,1-13,16-2-2-46,21	A. P. Keinath
13223	US, SC	*C. annuum*	A2	S	2-1-1-1-2-3,1-13,16-2,1-2-19,70	A. P. Keinath
13224	US, SC	*C. annuum*	A2	S	1-1-1-1-6-1,23-1,8-1-2-9,5	A. P. Keinath
13225	US, SC	*C. annuum*	A2	I	1-1-1-1-6-4,1-34,16-2-2-24,21	A. P. Keinath
13226	US, SC	*C. annuum*	A2	I	1-1-1-1-23,14-4,1-1,3-2,4-1,2-1,71	A. P. Keinath
13227	US, SC	*C. annuum*	A2	I	1-1-1-1-6-4,1-13,16-2-2-24,21	A. P. Keinath
13228	US, SC	*C. annuum*	A1	I	1-1-1-1-7,18-4,1-7,4-2,6-10,2-1,72	A. P. Keinath
13229	US, SC	*C. annuum*	A1	IS	1-1-1-1-1-2,1-4,3-2,1-1,13-1,11	A. P. Keinath
13230	US, SC	*C. annuum*	A1	S	1-1-1-1-27,13-2,1-2,9-2,4-1,2-7,73	A. P. Keinath
13231	US, SC	*C. annuum*	A1	S	1-1-1-1-4,5-8,1-5,19-1-2-24,21	A. P. Keinath
13232	US, SC	*C. annuum*	A1	S	1-1-1-1-6-8,1-13,16-2-2-74,21	A. P. Keinath

Table 4.1 (cont'd)

13233	US, LA	*C. annuum*	A1	S	1-1-1-1-2-2,1-4,8-2-35,2-3,75	D. M. Ferrin
13234	US, AR	*C. annuum*	A2	S	1-1-1-1-20-4,1-23,14-3-1,2-2,76	M. E. Matheron
13235	US, AR	*C. annuum*	A2	S	1-1-1-1-28,2-18,1-6,17-18,3-1-1	M. E. Matheron
13236	US, AR	*C. annuum*	A2	S	1-1-2-1-2-4,1-15,1-10,3-1-7	M. E. Matheron
13237	US, AR	*C. annuum*	A2	S	1-1-2-1-2-4,1-15,1-10,3-1-7	M. E. Matheron
13238	US, TX	*C. annuum*	A2	S	4-1-5-1-2-44,1-1-19,20-21,36-7,33	T. Isakeit
13239	US, TX	*C. maxima*	A2	S	1-1-1-1-10,2-32,15-6,4-1-1,2-1,4	T. Isakeit
13240	US, TX	*C. maxima*	A2	S	1-1-1-1-1,2-32,15-6-1-1,2-1,26	T. Isakeit
13243	US, NC	*C. maxima*	A1	S	1-1-1-1-1-2,1-3-2,1-1,13-1,11	K. L. Ivors
13244	US, NC	*C. moschata*	A1	S	1-1-1-1-1-1-11-2,1-1,13-1,11	K. L. Ivors
13245	US, NC	*C. maxima*	A1	S	1-1-1-1-1-1-3-2,1-1,13-1,11	K. L. Ivors
13246	US, NC	*C. maxima*	A1	S	1-1-1-1-1-1-4,21-2,1-1,37-1,27	K. L. Ivors
13247	US, NC	*C. maxima*	A1	S	1-1-1-1-1,2-4,1-9,3-2.1-1,2-3,7	K. L. Ivors
13248	US, NC	*C. maxima*	A1	S	1-1-1-1-1-1-21,3-1-1,13-1,11	K. L. Ivors
13249	US, NC	*C. maxima*	A1	S	1-1-1-1-1-1-3,11-1-1,13-1,11	K. L. Ivors
13334	US, MA	*C. annuum*	A1	S	1-1-1-1-1-18,1-2,35-1,21-1,18-1,43	R. L. Wick
13336	US, MA	*C. moschata*	A2	S	1-1-1-1-1-10,1-12,36-1-1,3-1,5	R. L. Wick
13338	US, MA	*C. moschata*	A2	S	1-1-1-1-1-5,1-27,22-1-1,3-9,17	R. L. Wick
13339	US, MA	*C. moschata*	A1	S	1-1-1-1-1-29,1-27,22-2,1-1,38-5,2	R. L. Wick
13340	France	*Cucumis melo*	A1	S	1-1-4-2-3,11-17,1-5-7,1-8,2-47,4	F. Panabieres
13341	France	*C. annuum*	A1	S	1-1-4-2-3-17,1-5-7,1-8,2-47,4	F. Panabieres
13342	Mexico	*C. annuum*	A1	S	1-1-1-1-1,29-5,6-3-1,3-2-6	F. Panabieres

Table 4.1 (cont'd)

13343	Cameroon	*Teobroma cacao*	A1	S	3-1-2-1-2-1,33-1-5-5-10	F. Panabieres
13344	US, GA	*Citrullus lanatus*	A1	S	1-1-1-1-1-15,26-1,8-1,3-3-2,25	P. Ji
13345	US, GA	*C. lanatus*	A1	S	1-1-1-1-1-15,26-1,8-1,3-3-2,25	P. Ji
13346	US, GA	*C. lanatus*	A1	S	1-1-1-1-1-15,26-1,8-1,3-3-2,25	P. Ji
13347	US, GA	*C. pepo*	A1	S	1-1-1-1-1-45,21-26,3-2,3-2,11-5,14	P. Ji
13348	US, CA	*S. lycopersicon*	A2	S	1-1-1-1-1-3,1-2,9-2,1-1,4-1,2	B. J. Aegerter
13349	US, MI	*C. maxima*	A2	S	1-1-1-1-1-2,1-2-1-1-1	M. K. Hausbeck
13350	Spain	*C. annuum*	A1	S	1-1-1-1-1-9,1-14,10-2,1-1,4-37,14	M. E. Candela
13351	US, NY	*Solanum melongena*	A1	S	1-1-1-1-1,2-27,6-2,6-2,4-3,7-1,4	C. D. Smart
13352	US, NY	*S. melongena*	A1	S	1-1-1-1-1,2-1-1-2,4-1,19-1,12	C. D. Smart
13353	US, NY	*S. melongena*	A1	S	1-1-1-1-1,2-1-1-2,4-3,7-1,12	C. D. Smart
13354	US, NY	*S. melongena*	A1	S	1-1-1-1-1,2-1-1-2,4-3,7-1,12	C. D. Smart
13355	US, NY	*S. melongena*	A1	S	1-1-1-1-1,7-6,13-1-2,4-3,7-1,12	C. D. Smart
13356	US, NY	*S. melongena*	A1	S	1-1-1-1-1,7-6,13-1-2,4-3,7-1,12	C. D. Smart
13357	US, NY	*S. melongena*	A1	S	1-1-1-1-1,7-6,13-1-2,4-3,7-1,12	C. D. Smart
13358	US, NY	*S. melongena*	A1	S	1-1-1-1-1,7-1,13-1-2,4-3,7-1,12	C. D. Smart
13359	US, NY	*S. melongena*	A1	S	1-1-1-1-1,7-6,13-1-2,4-3,7-1,12	C. D. Smart
13360	US, NY	*S. melongena*	A1	S	1-1-1-1-1,7-1,13-1-2,4-3,7-1,12	C. D. Smart
13361	Mexico	*T. cacao*	A1	S	3-1-2-1-2-9,1-7,4-14,5-5-1,4	K. H. Lamour
13362	Mexico	*T. cacao*	A1	I	3-1-2-1-2-9,1-7,4-14,5-5-1,4	K. H. Lamour
13363	Mexico	*T. cacao*	A1	S	1-1-2-1-2-9,1-17,12-2,15-5-1,4	K. H. Lamour
13364	Guatemala	*Piper nigrum*	A1	S	3-1-2-1-2-12,25-1-2-5-36	K. H. Lamour

Table 4.1 (cont'd)

13365	Mexico	*T. cacao*	A2	S	9-4-11-3-30,33-63,64-37-22-39-10[Pt]	K. H. Lamour
13366	Brazil	*T. cacao*	A1	S	13-2-8-7-21-65,66-39,24-25,26-41,42-10[c11]	K. H. Lamour
13367	Brazil	*T. cacao*	A2	S	6-2-8-7-22,21-46-24-16-22,23-81[c11]	K. H. Lamour
13368	Brazil	*T. cacao*	A2	S	6-2-8-4-22,21-47,46-24-16,17-22,23-1,2[c11]	K. H. Lamour
13370	Cameroon	*T. cacao*	A1	S	3-1-2-1-2-1,33-1-5-5-10	K. H. Lamour
13371	Cameroon	*T. cacao*	A1	S	3-1-2-1-2-19,48-1-5-5-10	K. H. Lamour
13377	Mexico	*T. cacao*	A1	S	9-4-11-3-30,31-67,68-37-22-39-80[Pt]	K. H. Lamour
13378	US, CA	*C. annuum*	A1	S	1-1-1-1-1-34,1-1,8-1,3-1,3-4,2	K. H. Lamour
13379	US, CA	*C. annuum*	A1	S	1-1-1-1-1-34,1-1,8-1,3-1,3-4,2	K. H. Lamour
13380	France	*C. annuum*	A1	S	1-1-1-1-3-8,1-5,1-2,6-14,6-15,2	K. H. Lamour
13381	France	*C. annuum*	A1	S	1-1-4-1-3-8,1-5,1-2,6-14,6-15,2	K. H. Lamour
13382	Yugoslavia[e]	*C. annuum*	A1	S	1-1-1-1-3-4,1-5,10-2,10-1,2-77,6	K. H. Lamour
13383	France	*S. melongena*	A1	S	1-1-1-1-3-4,1-5,10-2,10-1,2-16,6	K. H. Lamour
13384	India	*P. nigrum*	A2	S	9-4-11-3-30,31-61-37-22-40-79,85[Pt]	K. H. Lamour
13385	US, HI	*Macadamia integrifolia*	A2	S	10-4-12-6-30,31-59-37-22-39-79,86[Pt]	K. H. Lamour
13386	US, CA	*S. lycopersicon*	A2	S	1-1-1-1-1,2-7,1-1,8-3-1,2-1,2	K. H. Lamour
13387	US, CA	*S. lycopersicon*	A1	S	1-1-1-1-2-7,1-1,8-3-1,2-1,2	K. H. Lamour
13388	US, CA	*S. lycopersicon*	A2	S	1-1-1-1-2-7,1-1,8-3-1,2-1,2	K. H. Lamour
13389	Mexico	*C. annuum*	A1	S	1-1-1-1-1,10-5,6-3-1,3-2-6	K. H. Lamour
13390	Mexico	*C. annuum*	A2	S	1-1-1-1-1,10-5,6-3-1,3-2-6	K. H. Lamour

Table 4.1 (cont'd)

13391	Chile	*C. annuum*	A2	S	1-1-1-1-1,8-2,21-12,1-2-4,2-22,4	K. H. Lamour
13392	US, CA	*S. lycopersicon*	A1	S	1-1-1-1-2-7,1-1,8-3-1,2-1,2	K. H. Lamour
13393	Indonesia	*P. nigrum*	A2	S	1-1-1-1-1-49,50-2-2,1-16,4-23,5	K. H. Lamour
13394	Indonesia	*P. nigrum*	A2	S	4-1-3-1-1-5,1-2-1-3,16-1	K. H. Lamour
13395	Brazil	*C. moschata*	A1	S	1-1-1-1-1-14,51-5,18-2-1-48,49	K. H. Lamour
13396	US, CA	*C. annuum*	A1	S	1-1-1-1-1-7,1-1,8-2-1,3-1,2	K. H. Lamour
13397	US, HI	*M. integrifolia*	A2	S	2-1-2-2-1-35,52-1-2,1-9,2-28	K. H. Lamour
13398	Mexico	*T. cacao*	A2	S	9-4-12-3-34,35-69,70-1-22-39-78,80^{Pt}	K. H. Lamour
13401	US, CA	*C. lanatus*	A2	S	7-1-3-1-1,7-2,1-6,1-2,1-1,2-2,29	K. H. Lamour
13402	US, CA	*S. lycopersicon*	A2	S	1-1-1-1-2-7,1-1,8-3-1,2-1,2	K. H. Lamour
13403	US, CA	*C. annuum*	A1	S	1-1-1-1-1-7,1-1,8-2-1,3-4,2	K. H. Lamour
13405	Mexico	*S. lycopersicon*	A2	S	1-1-1-1-1,10-5,1-3-1,3-2-6	K. H. Lamour
13407	Norway	*C. sativus*	A2	S	1-1-1-1-1-20,1-3,11-2,1-3,4-23,3	M. L. Herrero
13408	Norway	*C. sativus*	A2	S	1-1-1-1-1-36,19-3,11-2,11-3,4-23,5	M. L. Herrero
13409	Norway	*C. sativus*	A2	S	1-1-1-1-1-36,19-3,11-2,11-3,4-1,29	M. L. Herrero
13410	Chile	*S. lycopersicon*	A2	S	1-1-1-1-1,8-2,21-12,1-2-4,2-22,4	E. Chavez
13411	Chile	*S. lycopersicon*	A2	S	1-1-1-1-1,8-2,21-12,1-2-4,2-22,4	E. Chavez
13412	US, TN	*C. pepo*	A1	S	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
13413	US, TN	*C. pepo*	A1	S	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
13414	US, TN	*C. pepo*	A2	S	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
13415	US, TN	*C. pepo*	A2	S	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
13416	US, TN	*C. pepo*	A2	S	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck

Table 4.1 (cont'd)

13423	US, MI	*C. pepo*	A1	S	1-1-1-1-1,8-5,53-7-2,1-1-1	M. K. Hausbeck
13435	US, TN	*C. pepo*	A1	S	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
13436	US, TN	*C. pepo*	A1	IS	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
13437	US, TN	*C. pepo*	A2	S	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
13438	US, TN	*C. pepo*	A2	S	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
13439	US, TN	*C. pepo*	A2	I	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
13440	US, TN	*C. pepo*	A2	IS	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
13441	US, TN	*C. pepo*	A1	S	1-1-1-1-1-2,1-2-2,1-1,4-1	M. K. Hausbeck
13455	France	*C. annuum*	A1	S	2-1-4-2-3-35,37-5,1-12,13-25,2-8	S. Werres
13456	US, MI	*C. melo*	A1	S	4-1-5-1-1,8-38,19-2,4-2,1-1-3	M. K. Hausbeck
13457	US, MI	*C. melo*	A1	S	8-1-6-1-1-5,1-2,28-1-3,16-1	M. K. Hausbeck
13471	US, MI	*C. annuum*	A2	I	1-1-1-1-1,8-20,6-2,7-1,3-1-1,17	M. K. Hausbeck
13479	Spain	*C. annuum*	A1	S	1-1-1-1-1-9,1-14,10-2,1-3,4-37,23	A. Lacasa
13500	US, CA	*C. maxima*	A1	S	1-1-1-1-16,7-22,1-11-2,1-3,11-22,2	B. J. Aegerter
13501	US, MI	*Hedera helix*	A1	S	1-5-13-9-30,36-71,72-38-23-43,44-82^{c11}	M. K. Hausbeck
13588	US, MI	*C. moschata*	A1	S	1-1-1-1-1,5-19,23-2,4-1-1,3-2	M. K. Hausbeck
13602	US, HI	*M. integrifolia*	A1	S	12-4-12-8-32-73,74-37,40-24-40-87,88^{Pt}	ATCC
13603	US, HI	*Carica papaya*	A2	S	10-4-11-6-30,31-59-37-22-39-79,83^{Pt}	J. Y. Uchida
13604	US, HI	*Anthurium andraeanum*	A2	I	10-4-1-6-6,23-2,54-37-22-39-79,83^{Pt}	J. Y. Uchida
13606	Australia	*Annona squamosa*	A1	S	9-4-14-3-30,31-60,62-37-22-39,40-84,3^{Pt}	B. McNeil
13607	Australia	*Mandevilla* sp.	A1	S	11-4-12-3-30-60,62-37-22-39-78^{Pt}	B. McNeil

Table 4.1 (cont'd)

13608	Australia	*Annona reticulata*	A2	S	11-1-12-1-30-59,75-37-22-39-78^{Pt}	B. McNeil
13609	Australia	A. *reticulata*	A2	S	11-4-11-3-30-76,77-37-22-39-78^{Pt}	B. McNeil
13610	Australia	*Fatsia japonica*	A1	S	12-4-15-8-30-60,78-37-22-39-1,27^{Pt}	B. McNeil
13611	Spain	*C. annuum*	A1	IS	1-1-1-1-2-9,1-14,10-1-1,4-14	J. Diaz
13612	Spain	*C. annuum*	A1	IS	1-1-1-1-1-9,1-14,10-2,1-1,4-14	J. Diaz
13613	US, DE	*Phaseolus lunatus*	A1	I	1-1-1-1-1,2-2,1-2,6-2,1-19,7-1,52	G. Majeau
13614	US, DE	*P. lunatus*	A1	S	1-4-1-1-1,2-2,1-37-2,1-39-79,89^{Pt}	G. Majeau
13615	US, DE	*C. annuum*	A1	I	4-1-5-1-1-3,1-37-2,1-45,46-84,3	G. Majeau
13616	US, FL	*S. melongena*	A1	S	2-1-1-1-1-28,1-3,11-2-3,26-18,17	A. J. Gevens
13617	US, FL	*C. annuum*	A1	S	2-1-1-1-1-28,1-2,4-1-1-18,17	A. J. Gevens
13618	US, FL	*C. annuum*	A2	S	2-1-1-1-1-8,39-7,4-1-4,20-18,17	A. J. Gevens
13619	US, FL	*C. lanatus*	A1	S	2-1-1-1-1,5-28,1-7,4-1-4,20-38,3	A. J. Gevens
13620	Italy	*C. annuum*	A1	S	1-1-3-2-3,11-3,1-13,5-2,3-8,12-1,38	R. B. Kung
13621	Italy	*C. annuum*	A2	S	1-1-1-2-2-4,39-6,14-1-8,12-13,2	R. B. Kung
13622	Italy	*C. annuum*	A2	S	1-1-1-2-12-10,1-12,29-2,3-14,2-54,11	G. Tamietti
13623	Italy	*C. annuum*	A2	S	1-1-3-2-3,11-3,1-13,5-2,11-8,12-16	G. Tamietti
13624	Italy	*C. annuum*	A1	S	1-1-1-2-24,12-3,1-14,10-2,1-1,15-15,5	G. Tamietti
13626	Italy	*C. annuum*	A1	S	1-1-1-2-1,2-3,1-13,5-2,1-8,12-8	G. Tamietti
13628	Italy	*C. annuum*	A1	S	2-1-4-2-2-10,1-5,10-2,2-1-8	G. Tamietti
13632	Italy	*C. annuum*	A1	S	2-1-4-2-3,2-10,1-5,10-2-8,12-15,13	G. Tamietti
13633	Italy	*C. annuum*	A2	S	2-1-4-2-3,11-3,1-5,10-12,7-1,14-15,13	G. Tamietti

Table 4.1 (cont'd)

13634	Italy	*C. annuum*	A1	S	1-1-1-2-1-7,1-5,10-1,3-1,2-13,2	G. Tamietti
13635	Italy	*C. pepo*	A1	S	1-1-3-2-3-8,1-5,11-7-15-13,11	G. Tamietti
13636	Italy	*C. pepo*	A1	S	1-1-3-2-3-8,1-1,11-2,1-15-9,18	G. Tamietti
13638	Taiwan	*C. annuum*	A2	S	1-1-1-1-1,17-8,1-2,18-3-1-1,9	T. C. Wang
13639	Taiwan	*C. annuum*	A1	S	1-1-1-1-4,5-10,1-2,9-2,10-1,6-1,27	T. C. Wang
13640	Taiwan	*C. annuum*	A1	S	5-1-2-1-1-2,1-2,1-2,3-1-1	T. C. Wang
13641	Taiwan	*C. annuum*	A1	S	1-1-1-1-1,5-3,1-2,9-2,3-1,6-1,2	T. C. Wang
13642	Taiwan	*C. annuum*	A2	S	1-1-1-1-1,17-1,55-6,5-3-1,6-1,9	T. C. Wang
13643	Taiwan	*C. annuum*	A2	S	1-1-1-1-6,10-4,1-6,5-3-1,10-1,9	T. C. Wang
13644	US, CA	*C. annuum*	A2	S	1-1-1-1-1-7,1-1,8-1-1-30,31	J. P. Prince
13645	US, CA	*C. annuum*	A2	S	1-1-3-1-1-7,1-4,25-1-3,27-30,31	J. P. Prince
13647	US, CA	*C. annuum*	A2	S	1-1-1-1-1-31,15-7,4-3-1-18	J. P. Prince
13649	US, CA	*C. annuum*	A1	S	2-1-4-2-1-18,1-8,3-1-9,2-28,24	J. P. Prince
13650	US, CA	*C. annuum*	A1	S	1-1-1-1-1-40,1-9,19-1-1,3-19,26	J. P. Prince
13651	US, CA	*C. annuum*	A1	S	1-1-2-1-1-56,37-1,19-4,1-9,28-30,31	J. P. Prince
13652	US, CA	*C. annuum*	A2	S	1-1-1-1-1,2-10,1-26,1-2,4-1,9-19,25	N. Kabir
13653	US, NM	*C. annuum*	A1	S	1-1-1-1-1-20,1-6,1-8-10,2-4,2	N. Kabir
13654	Mexico	*C. annuum*	A2	S	1-1-1-1-1-5,1-6,1-2,8-29,2-57,7	N. Kabir
13655	US, FL	*C. annuum*	A2	IS	2-1-3-2-1-27,12-15,3-6,1-8,12-77,7	N. Kabir
13656	US, NM	*C. annuum*	A2	S	4-1-5-1-2-24,6-1-3-4,21-58,7	N. Kabir
13657	Mexico	*C. annuum*	A2	S	1-1-1-1-1,4-40,1-3-2,1-1,4-1,39	N. Kabir
13658	Italy	*C. annuum*	A1	S	2-1-3-2-3-16,1-17,5-2,3-8,14-15,13	N. Kabir

Table 4.1 (cont'd)

13659	Mexico	*C. annuum*	A2	S	1-1-1-1-2-27,1-30,31-2,1-2-22,4	N. Kabir
13660	Korea	*C. annuum*	A1	S	1-1-1-1-2-9,1-21,3-2,3-1,3-1	N. Kabir
13661	China	*C. annuum*	A2	S	1-1-3-1-1,4-17,1-9,5-2,3-1,4-1,26	N. Kabir
13662	Thailand	*C. annuum*	A1	S	1-1-1-1-1,13-4,1-18,22-3-1-9,1	N. Kabir
13663	US, FL	*C. annuum*	A1	S	1-1-1-2-1,2-2,1-23,1-6,11-2,6-1,2	N. Kabir
13664	Taiwan	*S. melongena*	A1	S	1-1-1-1-1-3,1-15,3-2-6,30-2	P. J. Ann
13665	Taiwan	*C. annuum*	A2	S	1-1-1-1-1-29,6-2,9-2,3-1,6-9,32	P. J. Ann
13666	Taiwan	*C. annuum*	A1	S	1-1-1-1-1-20,1-2,9-2,3-1,6-9,32	P. J. Ann
13667	Taiwan	*C. annuum*	A2	S	1-1-1-1-1-4,1-2,9-2,3-1-1,9	P. J. Ann
13668	Taiwan	*S. lycopersicon*	A1	S	1-1-1-1-1,4-3,12-2,9-2,3-1,6-1	P. J. Ann
13669	Taiwan	*S. lycopersicon*	A2	S	1-1-1-1-16,3-4,1-6,5-1-1,10-1	P. J. Ann
13670	Taiwan	*Piper betle*	A1	I	1-1-1-1-4,2-3,1-2,17-5,3-31,5-59,10	P. J. Ann
13671	Taiwan	*P. betle*	A2	IS	1-1-1-1-4,2-3,1-2,17-5,3-5,32-1,27	P. J. Ann
13672	Taiwan	*C. maxima*	A1	S	1-3-1-3-1-3,1-9,3-2-1,1-1,2	P. J. Ann
13673	Taiwan	*C. maxima*	A2	S	1-1-1-1-1-10,1-6,18-2,1-1,6-9,60	P. J. Ann
13674	US, OH	*C. pepo*	A1	A	1-1-1-1-1,4-2,1-1-2,1-1,3-1,26	S. Miller
13675	US, OH	*C. annuum*	A2	S	1-1-1-1-1-3,1-1,3-2,1-1,3-1	S. Miller
13676	US, OH	*C. annuum*	A2	S	1-1-1-1-1-3,1-1,3-2,1-1,3-1,11	S. Miller
13677	US, OH	*C. moschata*	A2	S	1-1-1-1-1-3,1-1,3-2,1-1,16-1,27	S. Miller
13678	US, OH	*C. maxima*	A2	S	1-1-1-1-1-2,1-4,3-9-1,3-16	S. Miller
13689	US, OK	*C. lanatus*	A1	S	1-1-1-1-1,2-2,1-7,4-9-4,6-24,61	J. P. Damicone
13690	China	*C. annuum*	A2	S	1-1-1-1-2-1,57-5,3-1-1,4-19,2	J. Hao

Table 4.1 (cont'd)

13691	Italy	*C. annuum*	A1	S	1-1-3-2-1,25-4,1-23,1-7,13-8,4-16,62	ATCC
13692	US, NM	*C. annuum*	A1	S	1-1-10-1-1,2-38,1-2-2,1-1,33-32,63	ATCC
13693	Uruguay	*C. annuum*	A2	S	1-1-1-1-1,2-5,1-6,1-7,3-1,17-19,13	R. Bernal
13694	Uruguay	*C. annuum*	A2	S	1-1-1-1-1,13-5,1-6,1-7,3-1,17-19,13	R. Bernal
13695	Uruguay	*C. annuum*	A2	S	1-1-1-1-1,2-5,1-6,5-7,3-1,17-64,8	R. Bernal
13696	Japan	*Solanum muricatum*	A2	S	5-1-2-1-1,2-9,1-2,6-2,1-1-8	S. Uemtsu
13697	Japan	*C. lanatus*	A2	S	5-1-2-1-1,2-7,1-2,6-2,1-10-8	S. Uemtsu
13698	Japan	*C. maxima*	A2	S	5-1-2-1-1,2-11,58-2,5-2,3-1-1,29	S. Uemtsu
13699	Japan	*C. annuum*	A2	S	1-1-1-1-1,2-11,12-6,5-10,3-3,9-6,33	S. Uemtsu
13700	Japan	*C. annuum*	A2	S	1-1-1-1-1,2-11,12-6,5-10,3-34,9-6,33	S. Uemtsu
13704	US, NJ	*Phaseolus limensis*	A2	S	1-1-1-1-1,2-41,4-6,1-2,6-2-40,41	N. Gregory
13705	US, NJ	*C. maxima*	A2	S	1-1-1-5-1,2-41,4-6,1-2,6-2-40,41	N. Gregory
13706	US, NJ	*C. maxima*	A2	IS	1-1-1-1-1,2-3,1-7,4-1-1,3-3,34	N. Gregory
13707	US, NJ	*P. limensis*	A2	IS	1-1-1-1-1,2-3,1-7,4-1-1,3-3,34	N. Gregory
13708	US, NJ	*C. maxima*	A2	IS	1-1-1-1-26,2-3,1-7,4-1-1,3-3,34	N. Gregory
13709	US, NJ	*P. lunatus*	A2	S	1-1-6-1-2-5,1-1,3-2,1-1-65,66	N. Gregory
13710	US, NJ	*P. lunatus*	A2	S	1-1-1-1-1,4-2,54-1-2-1,18-1,67	N. Gregory
13711	US, NJ	*P. lunatus*	A1	S	1-1-1-1-4,2-18,1-3,11-2,4-18,3-1	N. Gregory
13712	Peru	*Capsicum pubescens*	A2	S	1-1-1-1-1,4-22,1-12,1-2-4,2-20	K. H. Lamour
13713	Peru	*C. pubescens*	A2	S	1-1-1-1-1,4-22,1-12,1-2-4,2-20	K. H. Lamour
13714	Peru	*C. pubescens*	A2	S	1-1-1-1-1,4-22,1-12,1-2-4,2-20	K. H. Lamour

[a] Geographical origin. US: United States, AR: Arizona, CA: California, DE: Delaware, FL: Florida, GA: Georgia, HI: Hawaii, KY: Kentucky, LA: Louisiana, MA: Massachusetts, MI: Michigan, NJ: New Jersey, NM: New Mexico, NY: New York, NC: North Carolina, OH: Ohio, OK: Oklahoma, SC: South Carolina, TN: Tennessee, TX: Texas.

[b] Mating type (A1 or A2) of an isolate.

[c] I: insensitive, IS: intermediately insensitive, S: sensitive.

[d] Each number represents the haplotype number for both alleles per gene in the following order: Cox1-Cox2-Nad1-Nad5-βTub-EF1A-Enolase-HSP90-TigA-Ura3. Genotype phase is unknown. All isolates are *P. capsici* SS unless otherwise indicated. Pt, *P. tropicalis* and c11, cluster 11.

DNA extraction. Contents of UCV8B-centrifuge tubes with actively growing mycelia were vacuum-filtered through one layer of Whatman grade 1 filter paper. Tissue remaining on the filter paper was transferred to a mortar using a toothpick, and ground with a pestle in liquid nitrogen. All implements were previously sterilized. Genomic DNA was extracted from approximately 1 g of ground tissue using the DNeasy Plant Minikit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA was quantified using the NanoDrop ND 1000 spectrophotometer and NanoDrop 2.4.7c software (NanoDrop Technologies Inc., Wilmington, DE). DNA integrity was analyzed by electrophoresis in 2% (w/v) agarose gel in 0.5X Tris-borate-EDTA buffer (67), stained with ethidium bromide (5 μg/ml) for visualization.

Primer design, DNA amplification and sequencing. Genomic regions to be used as a source of single nucleotide polymorphism (SNP) markers in *P. capsici* were identified in GenBank (Table 4.2). Selected regions were previously used in oomycete coalescence, genetic diversity and phylogenetic studies (10, 19, 38, 76). *P. capsici* sequences from GenBank were input into Primer3 (91) for primer design using default settings. Four regions of the mitochondrial genome (Cox 1, Cox 2, Nad 1, and Nad 5) and six nuclear genes

(β-Tubulin, EF-1α, Enolase, HSP90, Tig A, and Ura 3) were amplified by polymerase chain reaction (PCR). PCR was performed in a total volume of 25 μl and contained 1ul 5 ng/μl DNA, 5 μl 5X PCR reaction buffer (Invitrogen, Carlsbad, CA), 1.25 μl 25 μM $MgCl_2$ (Invitrogen), 0.5 μl 10 μM dNTP mix (Invitrogen), 1 μl each 10 μM primer (MSU Macromolecular Structure Facility, East Lansing, MI), 0.7 μl Platinum Taq DNA polymerase (Invitrogen), and 14.6 μl sterile water. The PCR was performed in a programmable Eppendorf mastercycler ep systems thermal cycler (Eppendorf, Westbury, NY) starting with 3 min denaturation at 94°C, followed by 45 cycles at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 60 s, with a final extension step of 10 min at 72°C. PCR products were analyzed by electrophoresis in 2% (w/v) agarose gel in 0.5X Tris-borate-EDTA buffer (67), stained with ethidium bromide (5 μg/ml) for visualization and compared to a 100 bp ladder (Invitrogen). Controls with no *P. capsici* DNA were included.

PCR products were purified using ExoSAP-IT (Affymetrix, Inc., Santa Clara, CA) following the manufacturer's instructions. Cycle sequencing reactions (1 μl purified PCR product, 3 μl primer, 8 μl sterile water) were done twice directly from the clean PCR products at the Michigan State University Research Technology Support Facility (East Lansing, MI) using the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystem, Foster City, CA). A subset of samples with heterozygote positions (40%) determined from sequence analysis were resolved into haplotypes by cloning the corresponding PCR product using the pGEM-T Easy Vector System (Promega, San Luis Obispo, CA) and Subcloning Efficiency DH5α Competent Cells (Invitrogen) following the manufacturer's instructions. Cloned PCR products were purified using the QIAquick PCR purification kit (Qiagen) following the

manufacturer's instructions, and amplified and sequenced as described above. Obtained haplotypes were confirmed by using PHASE (96) as implemented in DnaSPv5 (64) by running simulations with 5,000 iterations. In all cases the inferred haplotype with cloning and direct sequencing data matched the haplotypes obtained using PHASE. Haplotypes of remaining samples with heterozygote positions were inferred with PHASE.

Table 4.2. Mitochondrial and nuclear genes analyzed in *P. capsici* SS and SL.

Primer	Primer sequence	Product (bp)	Sequence (bp)	Source
Cox1-F	5'GGTGCACCTGATATGGCTTT3'	425	355	AY129166.1
Cox1-R	5'ACAGGATCACCTCCACCTGA3'			
Cox2-F	5'CCAGCAACTCCTGTAATGGAA3'	540	365	DQ365739.1
Cox2-R	5'TTGATTTAAACGGCCAGGAC3'			
Nad1-F	5'CAAAGAAGAAGAGGACCTAATGTTG3'	579	430	DQ361203
Nad2-R	5'TAATGCAAAACCCATTGCAG3'			
Nad5-F	5'GCTATGGAAGGTCCTACACCA3'	341	245	AY423326.1
Nad5-R	5'GCATGGATTACTGCACCTGA3'			
β-Tub-F	5'GGTCAGTGCGGTAACCAGAT3'	597	505	EF495258.1
β-Tub-R	5' GTACAGGGCCTCGTTATCCA3'			
EF-1α-F	5'GACATTGCCCTGTGGAAGTT3'	568	450	EU079545.1
EF-1α-R	5'CAGGCTTGATGACACCAGTC3'			
Enolase-F	5'CGTGAAGAACGTGAACGAGA3'	647	435	EU080622.1
Enolase-R	5'CCGAGATCTTCTCCGACTCC3'			
HSP90-F	5'GCCGATCTCATCAACAACCT3'	542	465	EU079547.1
HSP90-R	5'CTTCTGCGAGTTCAGGTGGT3'			
TigA-F	5'TCAACACTGCCAAAATTCCA3'	516	420	EU080625.1
TigA-R	5'CAGCGTCAGAGGAGACCTTC3'			
Ura3-F	5'GGCTTTCGACCAGCTGAAT3'	570	500	EF617399.1
Ura3-R	5'AGCGTGAAGTCACCGAACTT3'			

Sequence analysis. Manual editing of base calls and sequence alignment were performed using Lasergene SeqMan Pro version 8.0 (DNASTAR Inc., Madison, WI). The alignment was exported as a FASTA file and imported into MacClade (66) to produce a NEXUS file required for subsequent analyses. The two haplotypes within sequences containing heterozygote sites were inferred by haplotype subtraction (38) according to data from direct and cloned-PCR product sequencing or by using PHASE as described above. Sequences from mitochondrial and nuclear loci were analyzed individually and collapsed into unique haplotypes using DnaSPv5. Haplotypes found for each gene were

deposited in GenBank under accession numbers HQ388837 to HQ389193. Haplotype sequences were compared to sequence data publicly available by using nucleotide BLAST (1, 2) to determine that data corresponded to the *P. capsici* target genes. BLAST analyses indicated that some *P. capsici* isolates corresponded to *P. tropicalis* and to another group of isolates with some genes showing high similarity to *P. capsici* and others to *P. tropicalis*. These isolates are referred to in this paper as intermediate. Two separate data sets were created for subsequent analysis to account for the presence of isolates that did not have high genetic similarity to *P. capsici* in all sequenced genes, *P. capsici* sensu stricto (SS), which included isolates with all gene sequences having high similarity to only *P. capsici,* and *P. capsici* sensu lato (SL), which included all isolates. Base substitutions in SS were classified as phylogenetically informative or uninformative, transitions or transversions, and synonymous or non-synonymous substitutions using DnaSPv5.

DNA sequence variability, neutrality tests, recombination and genealogies. Polymorphism, neutrality and recombination analyses were performed for each gene and all SS isolates using the program DnaSPv5. Polymorphism estimates were also calculated for SS isolates grouped in geographic (hemisphere, continent, country, U.S. state, and U.S. or not), host (vegetable crop or not, host family, and host species), mefenoxam resistance and mating type categories. Polymorphism analysis was also performed for SL isolates grouped in a species (*P. capsici* or not) category. Previous work used five individuals as the minimum needed for population genetics studies in *Phytophthora ramorum* (40). We chose to include only categories with at least eight individuals in SS for analyses. Sequence diversity estimates and statistics including the number of polymorphisms (s) and haplotypes (h), haplotype diversity (Hd) (71), Tajima's π (99), the average number of

pairwise nucleotide differences (k) (99), and Watterson's theta (θw) per sequence (111) were calculated. Sequence variation in each gene for all SS isolates was tested for deviations from neutrality by using Fu and Li's D and F, and Tajima's D (31, 100). The recombination parameter (R) per gene (47), and minimum number of recombination events (RM) (48) were also estimated for each gene and all SS isolates. The split network method implemented in SplitsTree v. 4b06 (50) was used to visualize incompatibilities in SL haplotypes by generating a NeighborNet network based on uncorrected P distances. Network support was assessed by running 10,000 bootstrap replicates. Statistical-parsimony genealogies for haplotypes in SL were generated using TCS v. 1.13 (16).

Population subdivision analysis. Population subdivision was assessed with the model-based Bayesian clustering algorithm implemented in Structure 2.3X (82). The values for burnin, chain replication and lambda were set at 300,000, 100,000 and 1, respectively, based on results obtained in preliminary analyses. The optimal number of populations (*K*) was determined by comparing posterior distribution likelihoods among three independent runs of K=1 to K=40 using the established parameters. Data included all loci individually coded as haplotypes and were analyzed under the admixture model with correlated allele frequencies and without previous population information. Population structure figures with prior population information, obtained from the defined geographic, host, mefenoxam sensitivity and mating type categories for SS, and species category for SL and sorted by Q, were generated using the Population Sorting Tool (PST) a graphic editing program created in R (85) to visualize the distribution of clusters in predefined categories (J. J. Morrice, unpublished data). Genetic differentiation indexes (F_{ST}) were calculated for SS data grouped by geographic, host, mefenoxam sensitivity and mating type categories;

and for SL for a species category using DnaSPv5. Statistical significance was determined for each index by running 5,000 permutations (α=0.05).

Results

Haplotype sequences were compared to publicly available *P. capsici* sequences in Genbank through BLAST (data not shown) and several isolates that were not as similar as expected to *P. capsici* were found. Some isolates showed higher similarity to *P. tropicalis* than to *P. capsici* across all genes analyzed. Interestingly, other isolates showed higher similarity to *P. tropicalis* than to *P. capsici* in some genes, but had higher similarity to *P. capsici* than to *P. tropicalis* in others (intermediate genotypes). *P. tropicalis* and intermediate isolates were included in the SL data set and excluded from SS for further analyses.

All genes were polymorphic but nuclear genes were more variable than mitochondrial genes as indicated by polymorphism analysis (Table 4.3) and diversity estimates (Table 4.4). The total number of polymorphisms detected for all SS genes was 120. In general, all nuclear genes presented medium to high values of R and RM. Fu and Li's D and F indicated a significant deviation from neutrality in Nad5, and Fu and Li's D showed a deviation in TigA (Table 4.4). Unique haplotypes occurred in all genes and their frequency range was between 20 (Nad1) and 40% (TigA) (data not shown).

Table 4.3. Polymorphism types for analyzed mitochondrial and nuclear genes in *P. capsici* SS.

Target DNA	PI[a]	S[b]	NS[c]	TS[d]	TV[e]	Sites with three variants[f]
Mitochondrial						
Cox1	2	4	0	2	1	1
Cox2	0	0	1	0	1	0
Nad1	3	7	0	6	1	0
Nad5	0	5	1	6	0	0
Subtotal	5	16	2	14	3	1
Nuclear						
β-Tubulin	8	9	1	9	1	0
EF-1α	17	16	3	16	3	0
Enolase	12	11	2	11	2	0
HSP90	8	7	5	8	4	0
TigA	15	17	1	16	2	0
Ura3	26	22	7	23	6	0
Subtotal	86	82	19	83	18	0
Total	91	98	21	97	21	1

[a] PI, parsimony informative sites

[b] S, synonymous changes

[c] NS, replacement changes

[d] TS, transitions

[e] TV, transversions

[f] Polymorphisms not following the infinite-sites model.

Table 4.4. Diversity estimates, neutrality tests and recombination for mitochondrial and nuclear genes analyzed in *P. capsici* SS.

		Diversity estimates[a]					Recombination estimates[b]		Neutrality[c]		
Target DNA	**s (%)**	**h**	**Hd**	**π**	**θw**	**k**	**R**	**RM**	**T**	**D**	**F**
Mitochondrial											
Cox1	4 (1)	7	0.23	0	0.83	0.38	0	0	-1.02	0.96	0.34
Cox2	2 (0.5)	3	0.07	0	0.33	0.07	0	0	-1.03	-1.26	-1.4
Nad1	7 (1.6)	9	0.33	0	1.16	0.64	0	0	-0.93	-0.81	-1.01
Nad5	6 (2.4)	4	0.18	0	0.99	0.20	0	0	-1.58	-4.22*	-3.94*
Nuclear											
B-Tubulin	10 (2)	27	0.68	0	1.48	2.67	1.3	5	1.69	0.41	1.08
EF1A	19 (4.2)	56	0.82	0	2.82	3.10	14.4	7	0.24	-0.49	0.47
Enolase	13 (3)	35	0.91	0	1.93	2.46	43.1	6	0.6	1.42	1.35
HSP90	12 (2.6)	19	0.73	0	1.78	1.28	16.2	4	-0.06	1.37	0.74
TigA	18 (4.3)	36	0.81	0	2.67	2.89	25.5	6	0.19	1.64*	1.28
Ura3	29 (5.8)	77	0.90	0.01	4.31	5.22	18	13	0.55	1.52	1.34

[a] s: number of polymorphisms, h: number of haplotypes, Hd: haplotype diversity, π: nucleotide diversity, θw: Watterson's theta estimator per gene from sequence, k: average number of nucleotide differences.

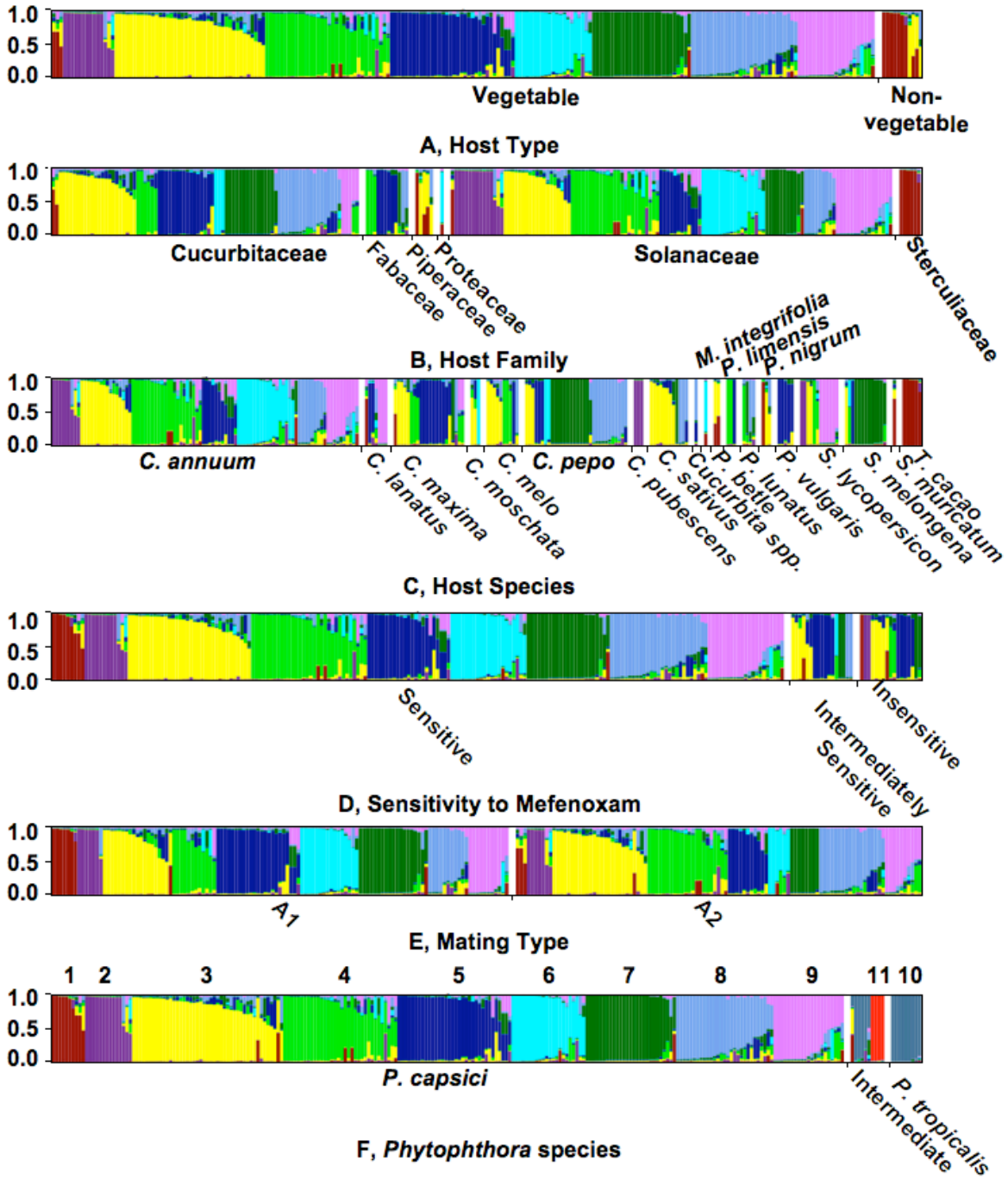
[b] R: recombination parameter, RM: minimum number of recombination events.

[c] T, Tajima's D. D, Fu and Li's D. F, Fu and Li's F. *, significant at 0.05.

The SS structure analysis detected significant population structure by host, geography and mefenoxam sensitivity where some clusters occurred more frequently in some categories than others. However, the analysis showed no direct correspondence between groupings of *P. capsici* isolates in the predefined categories (host, geography, mating type and mefenoxam sensitivity), and inferred genetic clusters. High likelihoods in

SS were observed when the number of clusters was set to nine (K=9, lnP= -5926; K=8, lnP= -6001; K=10, lnP= -6028) (Figure 4.1F). Individual ancestry coefficients were highly consistent across replicate runs. The bar plots indicated that some isolates are highly admixed, while others belong mostly to one particular cluster. Isolates belonging to clusters one and seven presented fewer admixtures than individuals from other clusters. The type culture of *P. capsici* from New Mexico (year 1727) had membership predominantly in cluster three and partial membership in clusters four and eight, which was similar to cluster membership for current New Mexico samples. The type culture from Italy (year 1927) had membership mostly in cluster six and partial membership in clusters two and four, which was similar to cluster membership for current Italy samples.

Figure 4.1. Genetic structure of *P. capsici* SS with isolates grouped by the following predefined categories: host (A host type, B host family, C host species), mefenoxam sensitivity (D), mating type (E) and of *P. capsici* SL by species (F). Each isolate is represented by a thin bar, often partitioned into colored segments each representing the individual's proportionate genetic membership in a given Kth cluster. Cluster colors are indicated in (F) and correspond to: dark red-one, purple-two, yellow-three, light green-four, dark blue-five, aqua-six, dark green-seven, light blue-eight, pink-nine, gray-ten, pink-eleven.



Population structure was detected when the data was analyzed using the host categories as prior population information (Figure 4.1A-C). Isolates from vegetable hosts contained representatives from all clusters, but genotypes belonging to cluster one occurred more frequently in non-vegetable than in vegetable hosts (Figure 4.1A). F_{ST} estimates indicated moderate differentiation between isolates from vegetable and non-vegetable hosts (Table 4.5). Diversity estimates for isolates from non-vegetable hosts were higher than from vegetable hosts (Table 4.6). Cucurbitaceous hosts presented individuals from all clusters, except for cluster two that was only sampled from solanaceous hosts (Figure 4.1B) and was mostly associated to *C. annuum*, *C. pubescens* and *S. lycopersicon* (Figure 4.1C). Solanaceous hosts presented members from all clusters. The Fabaceae had genotypes from clusters four, five, seven and eight. F_{ST} values indicated low differentiation among isolates from hosts in the Fabaceae, Cucurbitaceae and Solanaceae (Table 4.5). The highest and lowest diversity estimates of isolates grouped by host family were observed for isolates from the Solanaceae and Cucurbitaceae, respectively (Table 4.6). The pattern of cluster occurrence among cucurbit species was more similar than among solanaceous species. *C. annuum* contained members from all clusters except for clusters one and seven, while *S. melongena* included several isolates belonging mostly to cluster seven. F_{ST} estimates detected low differentiation among isolates from cucurbitaceous species, but moderate to high differentiation between isolates from cucurbitaceous and solanaceous species and among isolates from solanaceous species (Table 4.5). The highest and lowest diversity estimates of isolates grouped by host species were observed for isolates from *C. annuum* and *C. sativus*, respectively (Table 4.6).

Table 4.5. Minimum, average and maximum genetic differentiation estimates of mitochondrial and nuclear genes for *P. capsici* SL and SS with isolates grouped in predefined host categories.

Category	**Fst**[a]					
Host Type	Vegetable					
Non-vegetable	0,0					
	0.12*,0.18*					
	0.47*,0.36*					
Host Family	Cucurbitaceae	Fabaceae				
Fabaceae	0,0					
	0.01,0.03					
	0.02,0.09*					
Solanaceae	0,0	0,0				
	0.02,0.04	**0.04,0.03**				
	0.04,0.09*	0.08*,0.06*				
Host Species	*C. annuum*	*C. maxima*	*C. moschata*	*C. pepo*	*C. sativus*	*S. lycopersicon*
C. maxima	0,0					
	0.05*,0.03					
	0.08*,0.06*					
C. moschata	0,0	0,0				
	0.1*,0.04	**0.02,0.02**				
	0.17*,0.09*	0.08*,0.07*				
C. pepo	0,0	0,0	0,0			
	0.06*,0.05*	**0,0.03**	**0.03,0.02**			
	0.09*,0.14*	0.01,0.09*	0.09*,0.05*			
C. sativus	0,0	0,0	0,0	0,0		
	0.1*,0.08*	**0,0.05***	**0.03,0.03**	**0.02,0.03**		
	0.17*,0.2*	0,0.15*	0.11*,0.06*	0.03,0.07*		
S. lycopersicon	0,0.02	0,0	0,0	0,0	0,0	
	0.1*,0.05*	**0,0.1***	**0.03,0.14***	**0.02,0.13***	**0,0.17***	
	0.17*,0.1*	0,0.26*	0.11*,0.33*	0.03,0.37*	0,0.44*	
S. melongena	0,0.01	0,0.01	0,0.08*	0,0.06*	0,0.12*	0,0.05*
	0.08*,0.15*	**0,0.18***	**0.03,0.2***	**0.02,0.19***	**0,0.28***	**0,0.22***
	0.17*,0.32*	0,0.37*	0.11*,0.49*	0.03,0.39*	0,0.56*	0,0.59*

[a] The reported values correspond to minimum, average (in bold), and maximum values for mitochondrial (before comma) and nuclear genes (after comma). *, significant at 0.05.

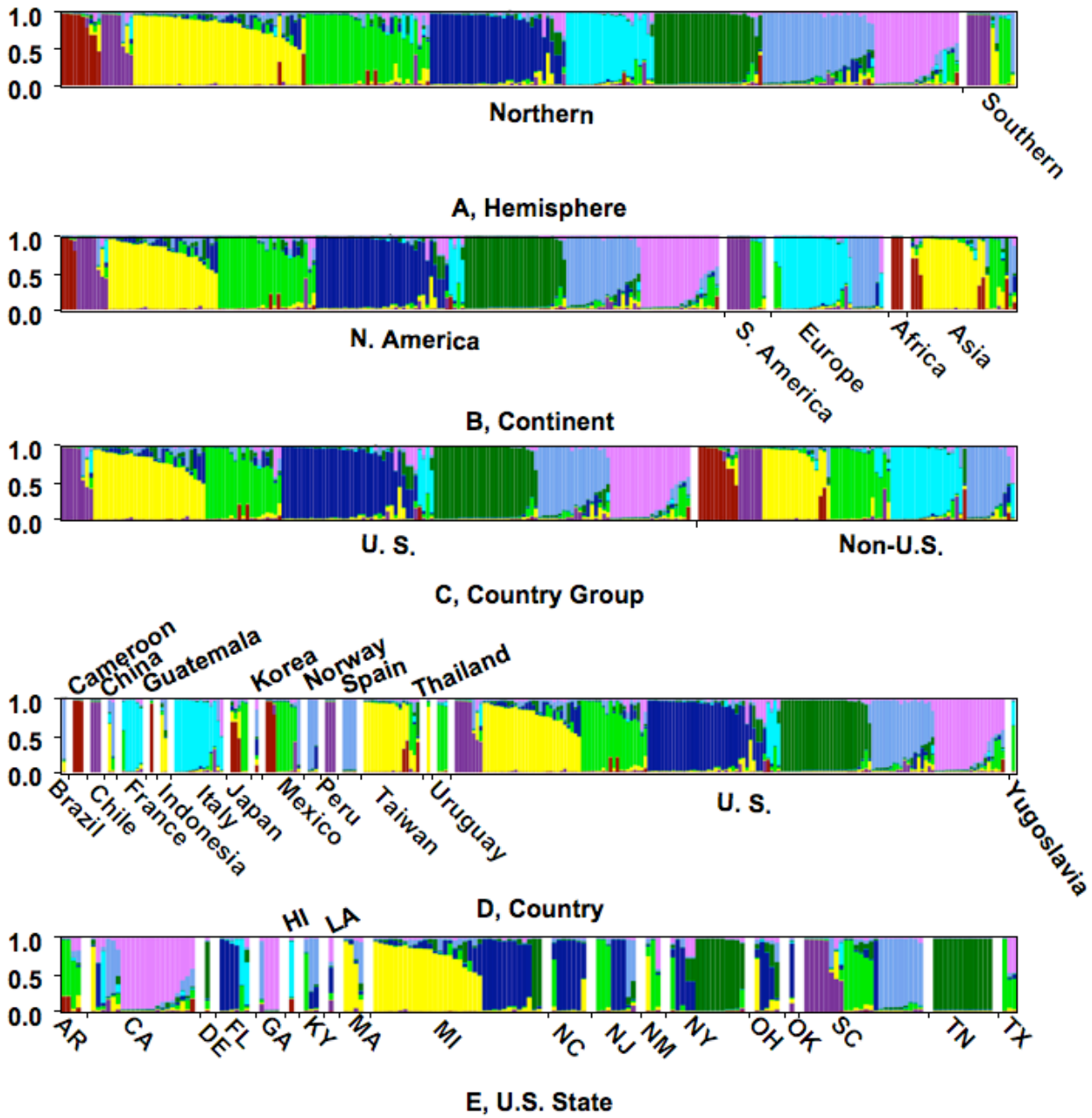
Table 4.6. Minimum, average and maximum diversity estimates of mitochondrial and nuclear genes for *P. capsici* SL and SS with isolates grouped in predefined host categories.

Category	**Isolates**		**Diversity estimates[a]**							
	SL	**SS**	**Hd**		**π**		**θw**		**k**	
			M	**N**	**M**	**N**	**M**	**N**	**M**	**N**
Host Type										
Vegetable	226	224	0	0.67	0	0	0.17	1.5	0	1.16
			0.16	**0.8**	**0**	**0**	**0.79**	**2.4**	**0.27**	**2.88**
			0.28	0.91	0	0.01	1.17	4.19	0.56	5.24
Non-vegetable	29	12	0	0.5	0	0	0.33	1.34	0	2.1
			0.34	**0.72**	**0**	**0**	**0.77**	**2.5**	**0.5**	**2.88**
			0.68	0.88	0	0	1.32	4.02	0.95	3.98
Host Family										
Cucurbitaceae	87	87	0.02	0.5	0	0	0	0	0.02	0.79
			0.12	**0.72**	**0**	**0**	**0**	**0**	**0.19**	**2.56**
			0.22	0.88	0	0.01	0	0	0.37	4.85
Fabaceae	13	12	0	0.59	0	0	0	0	0	0.89
			0.12	**0.76**	**0**	**0**	**0**	**0**	**0.16**	**2.76**
			0.382	0.88	0	0.01	0	0	0.5	5.23
Solanaceae	126	125	0	0.76	0	0	0	0	0	1.39
			0.19	**0.83**	**0**	**0**	**0**	**0**	**0.33**	**3.03**
			0.33	0.93	0	0.01	0	0	0.68	5.42
Host Species										
C. annuum	97	96	0	0.76	0	0	0.19	1.37	0	1.44
			0.23	**0.84**	**0**	**0**	**0.71**	**2.28**	**0.39**	**3.09**
			0.39	0.95	0	0.01	0.97	3.94	0.83	5.75
C. maxima	22	22	0.09	0.56	0	0	0.27	1.38	0.09	0.98
			0.09	**0.73**	**0**	**0**	**0.62**	**2.34**	**0.21**	**2.7**
			0.09	0.88	0	0.01	1.1	3.91	0.36	5.12
C. moschata	10	10	0	0.51	0	0	0.35	0.28	0	0.51
			0.09	**0.74**	**0**	**0**	**0.35**	**2.39**	**0.09**	**2.86**
			0.35	0.95	0	0.01	0.35	5.64	0.36	6.79
C. pepo	32	32	0	0.47	0	0	0.25	0.85	0	0.66
			0.06	**0.66**	**0**	**0**	**0.25**	**2.08**	**0.06**	**2.22**
			0.12	0.8	0	0	0.25	3.38	0.12	3.67
C. sativus	12	12	0	0.37	0	0	0	0.54	0	0.55
			0	**0.66**	**0**	**0**	**0**	**1.74**	**0**	**2.17**
			0	0.85	0	0	0	3.48	0	4.17
S. lycopersicon	12	12	0	0.59	0	0	0	0.54	0	0.99
			0	**0.74**	**0**	**0**	**0**	**2.05**	**0**	**2.57**
			0	0.83	0	0	0	3.75	0	3.75
S. melongena	13	13	0	0.53	0	0	0.64	0.52	0	0.58
			0.04	**0.66**	**0**	**0**	**0.64**	**2.01**	**0.08**	**2.07**
			0.15	0.75	0	0	0.64	3.67	0.31	3.28

[a] The reported values correspond to minimum, average (in bold), and maximum value for diversity estimates in nuclear and mitochondrial genes. N: nuclear, M: mitochondrial.

When geography categories were used as prior population information in the cluster analysis, some population structure was revealed (Figure 4.2A-E). Isolates from the Northern hemisphere were represented in all clusters while isolates from the Southern hemisphere contained less cluster diversity (Figure 4.2A). F_{ST} estimates indicated moderate differentiation between isolates from the Northern and Southern hemispheres (Table 4.7). Diversity estimates for isolates from the Northern hemisphere were higher than those obtained for isolates from the Southern hemisphere (Table 4.8). North America contained genotypes from all clusters and isolates belonging to cluster five were only found in this continent (Figure 4.2B). The U.S. contained isolates from all clusters, except for cluster one, which was associated with Guatemala and Mexico (Figure 4.2D). Mexico presented isolates from clusters four and eight in addition to those from cluster one. Isolates belonging to cluster two were present only in North and South America. South America only presented individuals from clusters two (Chile and Peru), four (Uruguay), and eight (Brazil). Individuals from cluster six were primarily sampled in Europe. Europe presented isolates from clusters four (Yugoslavia), six (France and Italy), eight (Norway and Spain) and nine (Italy). Members from cluster three were only found in North America and Asia, and both continents had the highest diversity of cluster occurrence. Isolates from Asia were present in clusters one (Japan and Taiwan), three (Taiwan, Thailand, and Indonesia), four (Japan and Taiwan), seven (Taiwan) and eight (China).

Figure 4.2. Genetic structure of *P. capsici* SS with isolates grouped by predefined geographic (A hemisphere, B continent, C country group, D country, E U.S. state) categories. Geographic origin abbreviations correspond to U.S.: United States, CA: California, MI: Michigan, NJ: New Jersey, NY: New York, SC: South Carolina, TN: Tennessee. Each isolate is represented by a thin bar, often partitioned into colored segments each representing the individual's proportionate genetic membership in a given Kth cluster. Cluster colors are indicated in Figure1F.



High genetic differentiation was detected between isolates from Europe and from all other continents according to F_{ST} values (Table 4.7). Moderate to high genetic differentiation occurred among isolates from North America, South America and Asia. The highest and lowest diversity estimates of isolates grouped by continent were observed in Europe and South America, respectively (Table 4.8). F_{ST} values showed high genetic differentiation between isolates from Italy and from all other countries and between isolates from Mexico and Taiwan (Table 4.7). Moderate differentiation was observed between isolates from the U.S and Mexico and isolates from the U.S. and Taiwan (Table 4.7). When isolates were grouped by country, isolates from Italy and Taiwan showed the highest and lowest diversity estimates, respectively (Table 4.8).

Genotypes from clusters five, seven and nine were mostly sampled in the U.S., while isolates from clusters one and six were primarily found outside the U.S. (non-U.S.) (Figure 4.2C). F_{ST} estimates indicated moderate differentiation between isolates from the U.S. and non-U.S. regions (Table 4.7). Diversity estimates for non-U.S. isolates were higher than those obtained for U.S. isolates (Table 4.8). Michigan, North Carolina, New York and Ohio; Arizona, New Mexico and Texas; and California and Georgia presented similar patterns of cluster occurrence (Figure 4.2E). California, South Carolina and New Jersey presented more diversity of cluster occurrence than other states. Isolates from California showed high differentiation with isolates from New York and moderate differentiation with isolates from New Jersey and South Carolina according to F_{ST} values (Table 4.7). New Jersey isolates presented low genetic differentiation with isolates from New York and South Carolina (Table 4.7). Michigan and Massachusetts contained a high number of isolates

from cluster three compared to other states. Tennessee only had isolates from cluster seven. F_{ST} estimates indicated moderate to high differentiation between isolates from Michigan and from all other states and isolates from Tennessee and from all other states (Table 4.7). The highest and lowest diversity estimates of isolates grouped by U.S. state were observed in New Jersey and Tennessee, respectively (Table 4.8). South Carolina was the only state with members from cluster two, which was also found in South America. F_{ST} values detected moderate to high differentiation between isolates from South Carolina and New York and low differentiation between isolates from South Carolina and New Jersey (Table 4.7). Genotypes from cluster six that were mostly sampled in Europe were also found within the U.S. in California, Hawaii and Florida. Individuals from clusters three (California, Massachusetts, Michigan, North Carolina, New Mexico, and Ohio), five (Florida, Michigan, North Carolina, New Jersey, New York, Ohio, Oklahoma and South Carolina), eight (California, Georgia, Kentucky, Massachusetts, New Jersey and South Carolina) and four (Arizona, Kentucky, North Carolina, New Jersey, New Mexico, New York, South Carolina and Texas) were sampled across multiple states. Isolates from cluster nine were found in states south of Kentucky (California, Georgia, and Texas). Individuals from cluster five were mostly detected in states east of Texas and isolates from cluster seven were found in states east of Arizona and north of Georgia (Michigan, New York, Ohio, and Tennessee).

Table 4.7. Minimum, average and maximum genetic differentiation estimates of mitochondrial and nuclear genes for *P. capsici* SL and SS with isolates grouped in predefined geographic categories.

Category[a]	Fst[b]				
Hemisphere	Northern				
Southern	0,0.01				
	0.04,0.05*				
	0.07*,0.1*				
Continent	Asia	Europe	North America		
Europe	0,0				
	0.16*,0.11*				
	0.48*,0.24*				
North America	0,0	0,0			
	0.02,0.04	**0.19*,0.09***			
	0.05*,0.13*	0.54*,0.17*			
South America	0,0	0,0.01	0,0.01		
	0.07*,0.13*	**0.27*,0.13***	**0.03,0.08***		
	0.16*,0.25*	0.59*,0.21*	0.07*,0.15*		
Country	Italy	Mexico	Taiwan		
Mexico	0,0.01				
	0.3*,0.11*				
	1*,0.23*				
Taiwan	0,0	0,0			
	0.32*,0.16*	**0.03,0.15***			
	0.8*,0.3*	0.12*,0.26*			
U.S.	0,0	0,0	0,0		
	0.34*,0.12*	**0.04,0.09***	**0,0.08***		
	0.97*,0.3*	0.1*,0.2*	0.01,0.17*		
Country group	U.S.				
Non-U.S.	0,0				
	0.06*,0.04				
	0.12*,0.08*				
U.S. State	CA	MI	NJ	NY	SC
MI	0,0.06*				
	0,0.17*				
	0,0.4*				
NJ	0,0	0,0			
	0.01,0.08*	**0.01,0.16***			
	0.03,0.17*	0.02,0.35*			
NY	0,0	0,0.12*	0,0.02		
	0.03,0.13*	**0.02,0.29***	**0,0.1***		
	0.1*,0.26*	0.05*,0.47*	0,0.22*		
SC	0,0.02	0,0.05*	0,0	0,0.03	
	0.02,0.11*	**0.01,0.19***	**0,0.06***	**0.01,0.15***	

Table 4.7 (cont'd)

	0.09*,0.23*	0.05*,0.32*	0.01,0.11*	0.04,0.39*	
TN	0,0.06*	0,0	0,0	0,0.15*	0,0
	0.03,0.31*	**0.02,0.21***	**0,0.2***	**0,0.39***	**0.01,0.24***
	0.1*,0.73*	0.05*,0.4*	0,0.51*	0,0.86*	0.04,0.5*

[a] Geographic origin, U.S.: United States, CA: California, MI: Michigan, NJ: New Jersey, NY: New York, SC: South Carolina, TN: Tennessee.

[b] The reported values correspond to minimum, average (in bold), and maximum values for mitochondrial (before comma) and nuclear genes (after comma). *, significant at 0.05.

Table 4.8. Minimum, average and maximum diversity estimates of mitochondrial and nuclear genes for *P. capsici* SL and SS with isolates grouped in predefined geographic categories.

Category[a]	**Isolates**		**Diversity estimates[b]**							
	SL	**SS**	**Hd**		**π**		**θw**		**k**	
			M	**N**	**M**	**N**	**M**	**N**	**M**	**N**
Hemisphere										
Northern	235	224	0	0.68	0	0	0.17	1.5	0	1.29
			0.19	**0.81**	**0**	**0**	**0.79**	**2.5**	**0.32**	**2.94**
			0.34	0.91	0	0.01	1.17	4.19	0.66	5.18
Southern	20	12	0	0.59	0	0	0.33	1.07	0	1.07
			0.08	**0.76**	**0**	**0**	**0.33**	**2.1**	**0.08**	**2.65**
			0.17	0.91	0	0.01	0.33	4.28	0.17	4.83
Continent										
Asia	28	27	0.07	0.67	0	0	0.256	1.32	0.07	1.41
			0.21	**0.77**	**0**	**0**	**0.65**	**2.45**	**0.4**	**2.66**
			0.38	0.84	0	0	1.04	4.83	0.62	3.6
Europe	28	28	0	0.74	0	0	0.26	1.52	0	1.52
			0.36	**0.83**	**0**	**0**	**0.51**	**2.32**	**0.6**	**2.88**
			0.63	0.95	0	0.01	0.77	3.92	1.29	4.84
North America	178	168	0	0.63	0	0	0.35	1.41	0	1.1
			0.12	**0.78**	**0**	**0**	**0.76**	**2.42**	**0.22**	**2.84**
			0.26	0.89	0	0.01	1.23	3.91	0.54	5.25
South America	13	10	0	0.49	0	0	0	0.85	0	1.07
			0	**0.74**	**0**	**0**	**0**	**1.97**	**0**	**2.57**
			0	0.88	0	0	0	4.23	0	4.7
Country										
Italy	14	14	0	0.75	0	0	0.63	1.28	0	1.49
			0.28	**0.83**	**0**	**0**	**0.78**	**2.36**	**0.52**	**2.73**
			0.69	0.91	0	0	0.94	3.85	1.22	4.03
Mexico	13	10	0	0.63	0	0	0.35	1.69	0	2.1
			0.21	**0.76**	**0**	**0**	**0.53**	**2.3**	**0.32**	**3.14**

Table 4.8 (cont'd)

			0.47	0.85	0	0.01	0.71	3.66	0.93	5.07
Taiwan	16	16	0.12	0.67	0	0	0.3	1.49	0.12	1.5
			0.12	**0.73**	**0**	**0**	**0.68**	**1.78**	**0.28**	**2.16**
			0.12	0.84	0	0	1.21	1.99	0.5	2.71
U.S.	164	157	0	0.61	0	0	0.35	1.42	0	1.03
			0.11	**0.77**	**0**	**0**	**0.71**	**2.34**	**0.19**	**2.77**
			0.21	0.89	0	0.01	1.24	3.95	0.42	5.2
Country group										
U.S.	164	157	0	0.61	0	0	0.35	1.42	0	1.03
			0.11	**0.77**	**0**	**0**	**0.71**	**2.34**	**0.19**	**2.77**
			0.21	0.89	0	0.01	1.24	3.95	0.42	5.2
Non-U.S.	91	79	0.02	0.76	0	0	0.2	1.6	0.02	1.73
			0.32	**0.85**	**0**	**0**	**0.71**	**2.51**	**0.5**	**3.12**
			0.52	0.95	0	0.01	1.01	4.43	0.97	5.06
U.S. State										
CA	21	21	0	0.54	0	0	0.28	0.7	0	0.99
			0.16	**0.73**	**0**	**0**	**0.56**	**2.01**	**0.24**	**2.65**
			0.35	0.87	0	0	0.83	3.25	0.6	3.96
MI	35	34	0	0.33	0	0	0.49	0.42	0	0.34
			0.08	**0.59**	**0**	**0**	**0.73**	**1.6**	**0.17**	**1.96**
			0.22	0.8	0	0	0.98	2.5	0.5	4.24
NJ	8	8	0	0.69	0	0	0.38	0.9	0	1.08
			0.12	**0.79**	**0**	**0**	**0.58**	**2.36**	**0.19**	**2.93**
			0.25	0.91	0	0.01	0.77	4.82	0.5	5.8
NY	15	15	0	0.55	0	0	0	1.01	0	0.76
			0	**0.67**	**0**	**0**	**0**	**1.85**	**0**	**2.14**
			0	0.77	0	0	0	4.04	0	3.5
SC	24	24	0	0.55	0	0	0.27	0.67	0	0.64
			0.06	**0.78**	**0**	**0**	**0.4**	**2.21**	**0.08**	**3.17**
			0.16	0.94	0	0.01	0.54	4.28	0.17	7.3
TN	12	12	0	0	0	0	0	0.27	0	0
			0	**0.26**	**0**	**0**	**0**	**0.71**	**0**	**0.7**
			0	0.52	0	0	0	1.34	0	2.61

[a] For geographic origin coding refer to table 5.

[b] The reported values correspond to minimum, average (in bold), and maximum value for diversity estimates in nuclear and mitochondrial genes. N: nuclear, M: mitochondrial.

Some structure was identified in isolates intermediately sensitive and insensitive to mefenoxam (Figure 4.1D). The category sensitive to mefenoxam contained individuals from all clusters. The categories insensitive to mefenoxam and intermediately sensitive to

mefenoxam did not contain isolates from cluster nine. F_{ST} values showed low to moderate genetic differentiation between sensitive and intermediately sensitive isolates and sensitive and insensitive isolates (Table 4.9). Genetic differentiation was very low between insensitive and intermediately sensitive isolate (Table 4.9). The highest diversity estimates were found for isolates sensitive to mefenoxam (Table 4.10). Diversity estimates for intermediately sensitive and insensitive isolates were comparable to each other (Table 4.10). Isolates insensitive and intermediately sensitive to mefenoxam were mostly found in North America, except for one isolate from Taiwan that was insensitive to mefenoxam; and one from Taiwan and two from Spain that were intermediately sensitive to the fungicide. Isolates sensitive to mefenoxam were collected from many different regions of the world and hosts (Table 4.1). *P. capsici* solates insensitive and intermediately sensitive to mefenoxam originated from hosts belonging to the Cucurbitaceae, Fabaceae, Piperaceae, Solanaceae, and Sterculiaceae.

No evident population structure was detected when the data were analyzed using mating type (Figure 4.1E) as prior population information. Both mating types contained individuals from all clusters. F_{ST} values indicated very low genetic differentiation between A1 and A2 isolates (Table 4.9). Diversity estimates for A1 isolates were higher than those obtained for A2 isolates (Table 4.10). Mating type distribution in the total sample and in countries including Brazil, Mexico, Taiwan, and the U.S. was approximately 1:1 (A1: 134, A2: 121 for the total sample, Table 4.1). Countries (Cameroon, Chile, China, France, Guatemala, Japan, Korea, Norway, Peru, Spain, Thailand, Uruguay, and the former Yugoslavia) and continents (Africa) with lower sampled isolates (<8) showed a prevalence

of one mating type over the other. Other countries and continents with good sampling (>8) presented an increased number of A2 (South America) or A1 isolates (Europe, Italy). Isolates obtained from cucurbitaceous and solanaceous hosts also presented a 1:1 distribution of mating types. Isolates originating from other host families presented deviations from the 1:1 mating type distribution.

Table 4.9. Minimum, average and maximum genetic differentiation estimates of mitochondrial and nuclear genes for *P. capsici* SL and SS with isolates grouped in predefined mating type, mefenoxam sensitivity and species categories.

Category[a]	**Fst**[b]	
Mating Type	A1	
A2	0,0	
	0.01,0.01	
	0.02,0.02	
Mefenoxam Sensitivity	S	IS
IS	0,0	
	0,0.05*	
	0,0.09*	
I	0,0	0,0
	0.03,0.03	**0,0.01**
	0.08*,0.1*	0,0.04
Species	*P. capsici*	
Not *P. capsici*	0.48*,0.35*	
	0.55*,0.54*	
	0.63*,0.71*	

[a] For mefenoxam sensitivity coding refer to table 6.

[b] The reported values correspond to minimum, average (in bold), and maximum values for mitochondrial (before comma) and nuclear genes (after comma). *, significant at 0.05.

Table 4.10. Minimum, average and maximum diversity estimates of mitochondrial and nuclear genes for *P. capsici* SL and SS with isolates grouped in predefined mating type, mefenoxam sensitivity and species categories.

Category[a]	**Isolates**		**Diversity estimates**[b]							
	SL	**SS**	**Hd**		**π**		**θw**		**k**	
			M	**N**	**M**	**N**	**M**	**N**	**M**	**N**
Mating Type										
A1	73	64	0.01	0.67	0	0	0.18	1.64	0.01	1.4
			0.22	**0.81**	**0**	**0**	**0.83**	**2.54**	**0.37**	**2.99**
			0.39	0.91	0	0.01	1.29	4.43	0.79	5.36
A2	76	66	0	0.68	0	0	0.38	1.5	0	2.46
			0.15	**0.78**	**0**	**0**	**0.69**	**2.31**	**0.23**	**3.18**
			0.26	0.91	0	0.01	0.95	4.18	0.47	5.07
Mefenoxam Sensitivity										
S	218	201	0.01	0.67	0	0	0.17	1.52	0.01	1.32
			0.2	**0.81**	**0**	**0**	**0.76**	**2.51**	**0.33**	**2.93**
			0.35	0.9	0	0.01	1.02	4.1	0.68	5.04
IS	17	17	0	0.41	0	0	0.29	1.47	0	0.7
			0.11	**0.68**	**0**	**0**	**0.59**	**2.4**	**0.2**	**2.75**
			0.23	0.86	0	0.01	0.89	4.16	0.45	5.70
I	19	17	0	0.66	0	0	0.29	1.71	0	1.34
			0.09	**0.77**	**0**	**0**	**0.59**	**2.53**	**0.12**	**2.81**
			0.23	0.86	0	0.01	0.89	5.13	0.35	5.96
Species										
Not *P. capsici*	19	0	0.52	0.4	0	0	1.43	1.66	0.97	1.46
			0.77	**0.72**	**0**	**0.01**	**2.79**	**7.02**	**3.3**	**7.06**
			0.89	0.97	0.01	0.02	4.86	10.95	6.06	10.32

[a] For mefenoxam sensitivity coding refer to table 6.

[b] The reported values correspond to minimum, average (in bold), and maximum value for diversity estimates in nuclear and mitochondrial genes. N: nuclear, M: mitochondrial.

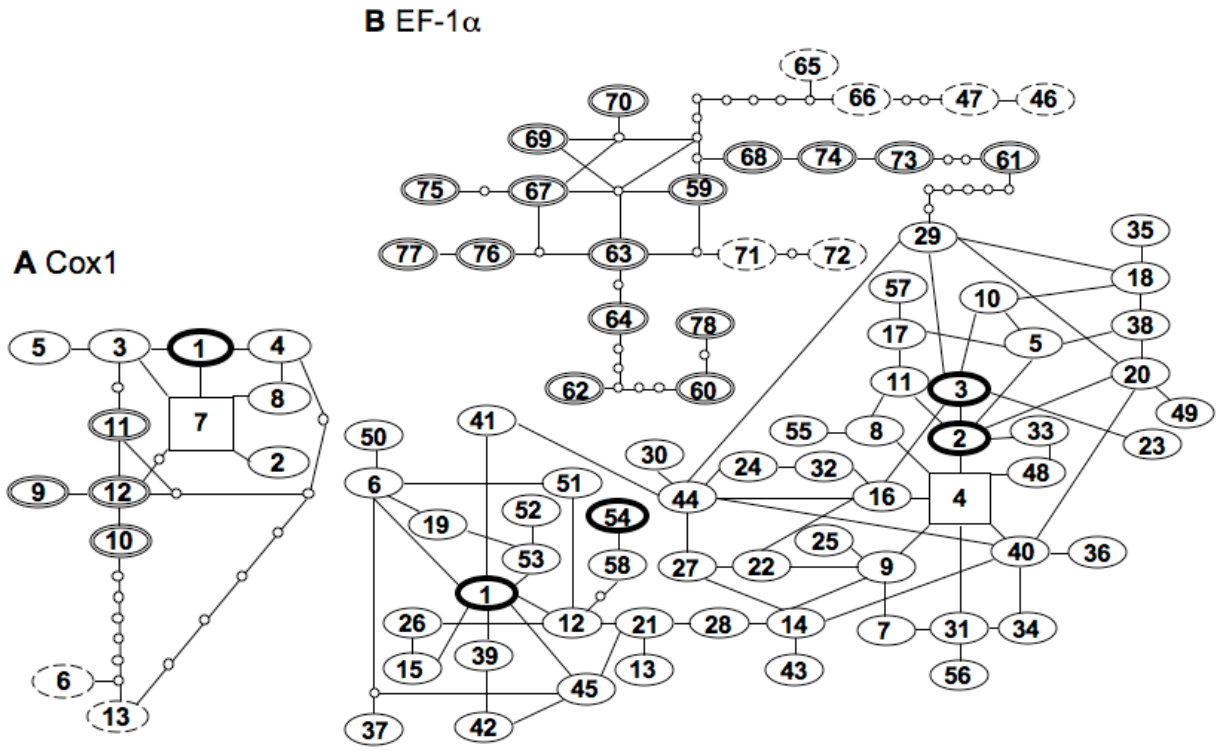
When the structure of the SL data was analyzed, eleven clusters (K=11, lnP= -6580; K=10, lnP= -6787; K=12, lnP= -6659) were detected, two more that were detected in SS (Figure 4.1F). Individual ancestry coefficients were highly consistent across replicate runs, as was observed for SS. Isolates belonging to cluster 10 presented some admixture with SS clusters and genotypes from cluster 11 showed no admixture. More admixture was

observed in clusters generated from the SS group. When the data were analyzed using the categories *P. capsici*, intermediate, and *P. tropicalis* as prior population information, no genetic clustering directly corresponding to the categories was observed. However, some structure was observed in each category. The *P. capsici* category contained the same clusters detected in SS and no individuals from clusters 10 and 11. The intermediate group presented isolates from clusters 10, 11 and some admixed individuals with partial membership in SS clusters and cluster 10. The *P. tropicalis* group only contained genotypes from cluster 10 that showed very little admixture and included the type culture of *P. tropicalis*. Isolates from cluster 10 originated from Mexico, India, Australia, and Hawaii from tropical hosts and from Delaware from lima bean. Isolates from cluster 11 presented no admixture and corresponded to intermediate isolates from Brazil and Michigan obtained from cacao and English Ivy (*Hedera helix*), respectively. Morphological characteristics of all isolates were consistent with the description of *P. capsici* included in the *Phytophthora* spp. key by Waterhouse (110).

The highest values of genetic differentiation observed in this study occurred between *P. capsici* and non-*P. capsisi* isolates (Table 4.9). Diversity estimates for *P. capsici* isolates were lower than those obtained for non-*P. capsici* isolates (Tables 4.4 and 4.10). HSP90 presented deviations from neutrality for non-*P. capsici* isolates (Table 4.7). The NeighborNet networks for genes HSP90, TigA, EF-1α, β-Tubulin, Ura3, and Cox1, showed three groups of haplotypes with moderate (>65) to high bootstrap support (>90). The three groups presented several incompatibilities within each group, but few or no incompatibilities among groups depending on the gene (data not shown). These groups were not contradicted by the other genealogies but grouping of isolates had less resolution.

Isolates belonging to the first group of haplotypes corresponded to *P. capsici* SS, while isolates belonging to the second and third groups of haplotypes included genotypes from clusters 10 and 11, respectively. For *P. capsici* SS isolates, no distinctive groups of haplotypes with high bootstrap support were consistently observed in all or most networks. Networks overall indicated significant incompatibility within nuclear genes, and the highest number of incompatibilities was observed in Ura3. The mitochondrial genes presented a few incompatibilities, and Cox2 showed no networking. Statistical parsimony haplotype genealogies for SL presented a similar topology to the NeighborNet networks (Figure 4.3). *P. capsici* SS haplotypes appeared to be very closely related to each other with only one or two missing haplotypes separating sampled haplotypes. Haplotypes from clusters 10 and 11 were more distantly related to *P. capsici* SS with two or more missing haplotypes separating both clades, which was supported by all genealogies. All genealogies except TigA presented some *P. capsici* SS haplotypes that included one or two alleles of isolates from clusters 10 and 11. In some genealogies, several haplotypes corresponding to clusters 10 or 11 were grouped together and separated from each other and from *P. capsici* by at least one missing haplotype (Cox1, Cox2, Nad1, EF-1α, TigA). Some SS haplotypes were frequently sampled and found throughout the world on diverse hosts, while other haplotypes sampled at lower frequencies originated from specific states, countries, continents and hosts (Table 4.1).

Figure 4.3. Haplotype genealogies of *P. capsici* SL mitochondrial (A Cox1) and nuclear (B EF-1α) genes. One mitochondrial and one nuclear gene are shown as examples. Other genes are not shown but presented similar topologies. The lines in the network represent possible paths of evolution. The haplotype with the highest probability to be ancestral is indicated by a square, other haplotypes are displayed as ovals. The empty dots represent missing or extant haplotypes. Bold lines indicate haplotypes that contain *P. capsici* SS isolates and isolates from clusters 10 or 11. Dashed lines indicate haplotypes that contain isolates from cluster 10. Double-line haplotypes indicate haplotypes that contain isolates from cluster 11. Dotted lines indicate haplotypes that contain both cluster 10 and 11 isolates.



Discussion

Phytophthora capsici is an economically important plant pathogen with a wide geographic and host range, as reflected by the sampling in this study. To detect population structure and determine whether predefined categories based on host, geography, mefenoxam sensitivity or mating type reflect genetic relationships among *P. capsici* isolates, we sequenced ten genes from 255 individuals to perform population structure

analyses. Bayesian clustering of isolates identified nine and eleven major genetically distinct clusters in SS and SL, respectively. Clustering analysis revealed some population structure by host, geographic origin and mefenoxam sensitivity in SS and by species in SL with some clusters occurring more or less frequently in particular categories. However, it showed no direct correspondence between any of the predefined categories and the number of inferred clusters. Pairwise F_{ST} comparisons and genetic diversity estimates among predefined host, geography, mefenoxam sensitivity, mating type and species categories were consistent with the distribution of genetic variation detected by Bayesian clustering and provided additional information on the generic variability within and between categories.

When isolates were grouped by host, differences in frequency of occurrence of certain clusters in particular hosts were observed. Isolates from cluster one occurred more frequently in non-vegetable hosts (cacao and black pepper) than in vegetable hosts. A previous study in *P. capsici* found temperate isolates from vegetable hosts (Solanaceae, Cucurbitaceae and Fabaceae) to be grouped together in the same clade, while tropical isolates appeared to have different genetic lineages (12) in agreement with our findings. Nonetheless, that study did not determine if isolates corresponded only to *P. capsici* or if *P. tropicalis* was present in their sample, so it is not possible to determine if *P. capsici* sensu stricto isolates were truly associated with tropical (non-vegetable) hosts, or if isolates from tropical hosts corresponded only to *P. tropicalis* and isolates assigned to cluster 11. Work by Donahoo and Lamour detected phylogenetic clustering of *P. capsici* sensu stricto isolates from tropical hosts (non-vegetable) in a separated but closely-related clade from isolates from vegetable hosts (19), which further supports our findings of genetic differentiation

between these categories. Tropical hosts of *P. capsici* have more woody tissue if compared to hosts from Cucurbitaceae, Solanaceae and Fabaceae. In previous studies it has been observed that *P. capsici* is difficult to recover from woody tissue of Fraser fir (83), oak (18) and various ornamentals (42). It is possible that isolates from different *P. capsici* clusters are not equally adapted to woody or herbaceous tissue, and this could result in isolates from a specific cluster such as cluster one occurring more frequently in woody hosts. A similar adaptation could be the cause of the almost exclusive association of cluster two with the Solanaceae. Despite the large sampling in the Cucurbitaceae (N=87), no isolates belonging predominately to cluster two were found in hosts from this family. As with the woody hosts it is possible that isolates from cluster two are not able to infect Cucurbitaceae as frequently as Solanaceae.

Evidence of clustering should not be taken as evidence of physiological races or pathotypes since we did not associate virulence data with the genetic clusters in the current study. We only associated occurrence of clusters in a host. Differences in virulence and pathogenicity have been reported between isolates occurring in Solanaceae and Cucurbitaceae (28, 62, 80, 84, 87), but the genetic basis of these differences is unknown. Our current knowledge of differences in virulence of *P. capsici* isolates only includes a few individuals that have been commonly used in host susceptibility experiments and resistance screenings (28, 33, 83, 84). Isolates 101 (known in the literature as OP97), 455 (known as SFF3), 12889, and 13349 (known as SP98) belong mostly to clusters seven, three, five, and seven, respectively. These isolates have been used in experiments with Fraser fir (83), asparagus (L.M. Rodriguez-Salamanca and M.K. Hausbeck, unpublished data), cucumber (33), summer and winter squashes (21), tomato (84) and pepper (28).

However, no virulence or pathogenicity data are currently available for a large number of individuals from different clusters. Experiments that associate virulence and pathogenicity to particular clusters are needed to determine if cluster membership may be used to predict the occurrence of certain isolates in particular hosts and the virulence of the isolates. If such an association is found, cluster information could be applied to determine the most likely sources of virulent strains, which could be used for robust disease screenings in the search for resistant cultivars. Developing economical and high-throughput diagnostic markers for each *P. capsici* cluster would be key for this purpose. Nevertheless, the differences in cluster occurrence by host of origin found in this study have significant implications for the development of resistant varieties. Breeding programs should include isolates that represent the genetic diversity of the pathogen in resistance screenings. Isolates obtained from hosts belonging to the Cucurbitaceae and Solanaceae are commonly used in host resistance screenings (28, 84), but this study suggests that inclusion of isolates from tropical hosts that show high genetic variation is necessary to capture the genetic diversity of *P. capsici*.

Several studies examining samples of *P. capsici* isolates from different geographic locations worldwide have been published; however, clear detection of distinct subgroups in *P. capsici* based on geographic origin through phylogenetic methods remains elusive (12, 19, 27, 117). Some geographic structure was detected in our study. Some clusters occurred in all continents (four and eight), others were only present in select continents (two in North and South America), or were more frequently sampled in certain continents (six in Europe and three in North America and Asia) and countries (five, seven and nine in the U.S.). North America and Asia presented a more diverse occurrence of clusters

compared to other regions. The high diversity of *P. capsici* clusters found in the U.S. may challenge local breeding efforts. Breeding programs usually have a regional focus where resistance for local pathogen populations is incorporated into host varieties. Breeders working in areas with lower genetic diversity of the pathogen may have a higher chance of identifying resistant varieties to local *P. capsici* populations (49). *P. capsici* populations with high levels of genetic variation are likely to adapt more rapidly to resistant hosts than populations with little genetic variation (49, 56). In our study, regions including Cameroon, Chile, Peru, France, Italy, Norway, Spain, Uruguay, and Tennessee presented a predominant cluster. The absence of other clusters is probably due to our sampling, but clonal *P. capsici* populations have been identified in Argentina (36) and Peru (49) in more robust sampling efforts. Local population studies with intensive sampling are needed to characterize and determine the spatial genetic structure of *P. capsici* populations on a smaller scale than examined in the present work. Information on the geographic distribution of genetic variation may be used to decide how best to deploy host resistance to achieve control and how to select isolates to be used as inoculum in resistance screenings. Our results suggest that it is necessary to use isolates from diverse geographic locations to represent the range of genetic diversity observed in this pathogen. Our sampling efforts could be improved upon in future studies and additional isolates from all continents, countries and states will be needed to determine whether additional clusters exist. However, establishing the spatial distribution of the nine *P. capsici* clusters detected in this study provides an initial map of global population structure that will help in isolate selection for resistance screening and will guide future sampling to expand our knowledge of *P. capsici* population structure.

P. capsici is water-dispersed within fields and can be introduced into new fields through infected plant material or infested soil, tools and irrigation water (35, 44). Wind-dispersal is not an important mechanism for large-scale movement of *P. capsici* (41) and human activity likely plays a role in genetic structuring of populations. Migration of few individuals per generation is sufficient to keep F_{ST} values at 0.1 or less (43). The observed F_{ST} values between several regions suggest that movement among locations is rare but historical information indicates it is possible. It has been suggested that *P. capsici* was introduced to the U.S. through contaminated pepper seed that entered New Mexico (1918) (63) and Florida (1932) (112) since some of the earliest reports of this pathogen in the U.S. come from those states (56). Since a type culture of *P. capsici* from New Mexico was included in this study and had membership mostly in cluster three and partial membership in clusters four and eight, we know those clusters were present in New Mexico since 1937. Present samples from New Mexico also had membership in those clusters indicating that cluster variation is maintained within populations but information about the genetic diversity of those isolates is not available since our sample was less than eight isolates. Genetic diversity estimates for New Jersey, South Carolina and California isolates were higher than for other states, which could be due to the longer presence of *P. capsici* in these states compared with Tennessee, New York and Michigan. *P. capsici* was first found in California around 1927-1930 (104, 105) after reports from New Mexico (1918) (63) and Italy (1927) (17, 106) but before it was seen in Colorado (1931) (11, 92), Florida (1932) (112), Arizona (1933) (14), Greece (1933) (93), New York (1935) (114), Chile (1936) (113), Argentina (1940) (37), Michigan (1997) (44), and Illinois (1999) (7). It is estimated

that *P. capsici* became economically important in New Jersey around 1970 (8, 77) and in South Carolina in 1994 (A. P. Keinath, unpublished data) but this pathogen had probably been present both states for a few years. Moderate to low genetic variation was detected in Tennessee, New York and Michigan isolates that could account for the more recent incidence of *P. capsici* in fields of these states. *P. capsici* was first found in New York around 1935 infecting watermelons that came from Colorado in a New York City market (114). It was not seen again in 1937 and 1938 (114) but has only recently (late 1990s) become economically important in New York (20). *P. capsici* was found in Michigan in 1997 in processing tomato fields that were established around 1940 with transplants from Georgia (44). Historically, *P. capsici* has not been a concern in Tennessee; nonetheless, isolates have been recently (2000s) recovered from vegetable hosts (56).

Having accurate historical records of plant pathogen occurrence and maintaining carefully cataloged isolate collections are essential tools to determine the role humans are playing in shaping the current global genetic structure of *P. capsici* populations, which has been suggested in previous work including local Michigan and New York populations (15, 58). Nonetheless, global-scale or inter-U.S. sate migration events in *P. capsici* have not been studied. The type culture from Italy (year 1927) had membership mostly in cluster six and partial membership in clusters two and four, indicating that members of those clusters have been present in Italy since 1927 as reflected by the other Italian samples. Italian samples presented high levels of genetic variation that could be reflecting the long-term presence of *P. capsici* in Italy and subsequent diversification of isolates. Members of cluster six that were mostly sampled in Europe and specifically in Italy, were also found in Hawaii, California and Florida. It is possible that those U.S. isolates belonging to cluster six were

introduced into the U.S. from Europe. Similarly, isolates from cluster two that were only sampled in solanaceous hosts of South Carolina, Chile and Peru could have been introduced into the U.S. from South America. Our analysis does not allow answering these questions but it does provide a global framework to formulate such hypotheses. Evolutionary studies with appropriate markers are needed to determine migration pathways of *P. capsici* as has been done with other pathogens (38, 40). Such studies will provide valuable information for regulatory measures and may also identify the center of origin of *P. capsici,* which could guide samplings of resistant germplasm.

Some structure was detected in isolates grouped by mefenoxam sensitivity, where sensitive isolates contained members from all clusters but intermediately sensitive and insensitive isolates did not contain members from cluster nine. Isolates from Spain, Taiwan, Mexico and the U.S. were found to be intermediately sensitive or insensitive to mefenoxam. Other studies have found isolates insensitive to mefenoxam in Italy (79) and the U.S. (30, 44). Isolates sensitive to mefenoxam were sampled from more regions and hosts than isolates intermediately sensitive or insensitive to mefenoxam. Continued characterization of *P. capsici* populations for mefenoxam insensitivity will help determine whether mefenoxam is still an effective control method in a particular field or if using other products is needed.

No structure was detected when isolates were grouped by mating type and all clusters were sampled in A1 and A2 isolates. A previous study also found no correlation between RFLP lineages and mating type in isolates from New Mexico, Europe and Korea (51). Regions with lower sampling showed the prevalence of only one mating type, while regions with higher sampling contained both A1 and A2 isolates. Only A1 *P. capsici* isolates

have been found in Spain (45), Bulgaria (52), China (98), and Argentina (36) and only A2 isolates in Peru (49) according to previous reports. Spain, China and Peru also had one dominant mating type in our sampling but few individuals were obtained from these countries and additional sampling may detect the other mating type. Reports of *P. capsici* isolates from Brazil (68), Canada (3), Mexico (25), Italy (101), South Africa (70), Taiwan (94) and the U.S. (15, 44, 87, 109) indicate the presence of both mating types. Our study also found both mating types present in Brazil, Mexico, Taiwan, Italy and the U.S. Some regions followed the 1:1 ratio reported in other studies (58) but others were skewed toward one mating type. Since recombination via sexual reproduction generates high genetic variability in *P. capsici* that may result in isolates with increased virulence or fungicide resistance, it is important to know the distribution of mating types in different regions. If only one mating type is present in a field, country or state, efforts should be directed to avoid the introduction of the other mating type so the long-term efficacy of control methods such as host resistance and fungicides is not reduced.

High genetic diversity has been previously reported for *P. capsici* (53, 58, 95). Individuals with partial membership in several clusters and unique haplotypes reported here (20-40%) and in previous studies (70-90%) (58, 59) are consistent with sexual reproduction in *P. capsici* (39). While admixture could be due to factors such as ancestral polymorphism, at least some admixture is likely a result of recombination, as indicated by recombination estimates for nuclear genes (97), incompatibilities observed in the NeighborNet networks, and extensive networking in statistical parsimony genealogies. Although these findings are unsurprising in light of previous studies (57-59), the implications of these results for choosing the best method to analyze populations of *P.*

capsici are significant. Traditional phylogenetic methods have been used to study intraspecific population data of *P. capsici* (12, 19, 117) even though recombination occurs frequently. Yet such methods are only appropriate under the assumption that recombination does not occur (81, 115). If recombination occurs frequently in the plant pathogen of interest, methods such as Bayesian clustering, split networks and statistical parsimony that allow for recombination events should be used (6, 81). Bayesian clustering allows for the reconstruction of ancestral population structure and subsequent admixture in highly recombining species without subjective definitions of population groups or categories (115) and constitutes a useful tool to study population structure in *P. capsici*.

Characterization of genetic structure of *P. capsici* SS populations revealed moderate but significant population structure by host, geography and mefenoxam sensitivity. Beyond the direct application of this knowledge in breeding efforts and development of fungicides, the detection of population structure in *P. capsici* has implications in using association genetics to identify the genetic basis of phenotypes of importance. Association studies have been performed to identify the genetic basis of disease in humans (32) and agronomic traits in plants (46). They have also been successfully used to determine the role of particular genes as virulence and avirulence determinants in plant pathogens such as *Phytophthora infestans* (5) and *Magnaporthe oryzae* (116). Association studies could help uncover the genetic bases of traits such as pathogenicity, virulence or fungicide resistance in *P. capsici*. Nevertheless, even weak population structure obscures the discovery of functionally important variation using association genetics. Since the clusters identified are somewhat structured, stratification of *P. capsici* by geography, host and mefenoxam sensitivity will be important to control for on association analysis of

phenotypic traits of interest. Studies investigating the genetic structure of *P. capsici* populations by host or by mefenoxam sensitivity nested within geography will be key to select appropriate individuals for association studies in *P. capsici*.

Bayesian clustering analysis, split decomposition networks and statistical parsimony genealogies detected the presence of non-*P. capsici* isolates in our sample. Isolates from cluster 10 corresponded to *P. tropicalis* and isolates from cluster 11 corresponded to isolates from Brazil and cacao, and Michigan and English Ivy that were closely related to *P. capsici* and *P. tropicalis* but formed a distinct group in our analyses. Preliminary comparisons of morphology between *P. capsici* and non-*P. capsici* isolates in this study revealed significant differences in some characteristics but most isolates were not readily differentiated by morphology (L. L. Granke et al., unpublished data). Morphological and genetic differences between *P. tropicalis* and *P. capsici* have been documented in previous studies (4, 19, 69, 74, 117) but there is still some debate regarding the appropriateness of designating *P. tropicalis* as a distinct species (12).

Previous studies have used phylogenetic methods to analyze worldwide collections of isolates (69, 74). However, because of the low resolution provided by the markers used, little intra-specific information of lineage-geography or lineage-host associations was gathered. Such methods did prove useful in identifying non-*P. capsici* isolates in the sample as observed in our study. Oudemans and Coffey also found *P. tropicalis* isolates (CAP2) to be separated from *P. capsici* sensu stricto (CAP1) (74). An advantage of the methods used in our study over phylogenetic methods previously used is the ability to characterize admixture between *P. capsici* and *P. tropicalis*. Several studies have detected the separation of *P. capsici* and *P. tropicalis* but no evidence of admixture between them was previously

found (19, 69, 74, 117). Clustering analysis in our study indicates that even though *P. tropicalis* is found as a distinct cluster from *P. capsici*, some admixture has occurred between the species. The observed admixture could be due to ancestral polymorphism or recent recombination events given that is possible to obtain inter-specific progeny in the lab (19) but no hybrids have been characterized from nature. With more samples and using several linked markers it may be possible to detect introgression events or recent gene flow between both species and determine the existence of natural hybrids (23, 72, 107).

Isolates from cluster 11 appear separated from *P. capsici* and *P. tropicalis* in the networks and genealogies and show no admixture in the structure analysis. The lack of admixture in cluster 11 could be due to the few isolates we have in that cluster (three from Brazil and cacao and one from Michigan and English Ivy). With more samples, it is possible that admixture will be detected revealing that cluster 11 corresponds to *P. tropicalis* or *P. capsici*. However, taking into consideration the genealogies and networks that support the separation of haplotypes in three distinct groups, it is also possible that cluster 11 corresponds to a distinct phylogenetic species. Oudemans and Coffey also found in their *P. capsici* study a third distinct clade (CAP3) that contained isolates from Brazil and cacao (74) that likely correspond to cluster 11. Isolates from Brazil and cacao also formed a separate clade in other studies (12, 19). Detection of isolates from cluster 11 seems to be possible by combining information from several markers. In the study by Donahoo and Lamour these isolates appear on the same clade with a few *P. capsici* isolates in an ITS-based phylogeny, but separation of such isolates was observed in other phylogenies based on AFLPs and nuclear genes in the same study (19). This is not surprising since haplotypes

of cluster 11 showed high similarity to *P. capsici* in some genes, while other genomic regions were more similar to *P. tropicalis* according to BLAST. Since the structure analysis uses multilocus genotypes and allelic frequencies for clustering, we believe that it constitutes a valuable tool that could complement phylogenetic studies by providing information of population membership and admixture to aid in the delimitation of phylogenetic species in highly recombining pathogens. However, to truly define if there are genetic barriers to mating among *P. capsici*, *P. tropicalis* and isolates in cluster 11, comparisons of a wide range of inter and intra-cluster crosses between isolates from diverse origins is needed.

Isolates used in this study were obtained from colleagues around the world and from our *P. capsici* collection. Large numbers of isolates could not be obtained from several localities and most isolates come from common vegetable hosts. Isolates from tropical hosts were less represented and no samples from wild hosts or weeds were obtained. Finding non-*P. capsici* isolates in our sample was unexpected so the number of isolates sampled for clusters 10 and 11 was low for a population-based study. Sparse sampling is common in plant pathogen worldwide population studies. One example is work done by Goss et al. in *Phytophthora ramorum* populations (40). Nonetheless, Bayesian clustering analysis can yield valuable information about the population structure of plant pathogen populations. The number and type of markers used can affect the analysis. Loci included in our study were highly informative as indicated by polymorphism, recombination and diversity analysis, and we were able to detect clustering in our samples with the ten genes used. Previous studies have shown that for clustering to be robust less than 40 markers and as low as seven markers (82) can be used if they are highly informative, but using at

least 50 markers is preferred (89). Obviously, structure can only be detected in the samples being analyzed. More clusters likely exist in *P. capsici* since apparent structure may be underestimated when using sparse sampling and few markers (89). Future studies with uniform global sampling and hundreds of markers could determine whether additional genetic clusters are present in *P. capsici*. The approach used in this study could be applied to other plant pathogens where using a large number of markers is not feasible due to high costs or scarce genomic resources available, given that the markers used are highly informative.

P. capsici is a diverse and highly recombining species of economic importance. Our findings of genetic structuring in *P. capsici* populations coupled with observations of differences in virulence of particular isolates when inoculated in diverse hosts (28, 33, 83, 84), highlights the importance of selecting isolates from diverse hosts and geographic origin for breeding programs. When developing diagnostic tools, fungicides, and resistant host varieties, representative isolates from all genetic clusters should be used to account for global genetic diversity. This investigation of genetic structure of *P. capsici* populations may be used to guide isolate selection to improve efficacy of breeding programs. Continued genotyping of *P. capsici* will be necessary to track the diversification of inferred clusters and to identify new ones.

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