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Amanda Jane Gevens

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Mary K. Hausbeck
Major Professor's Signature

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DOCUMENTING AND CHARACTERIZING PHYTOPHTHORA CAPSICI FROM IRRIGATION WATER AND BEAN IN MICHIGAN AND SCREENING FOR FRUIT RESISTANCE IN CUCUMBER

By

Amanda Jane Gevens

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ABSTRACT

DOCUMENTING AND CHARACTERIZING PHYTOPHTHORA CAPSICI FROM IRRIGATION WATER AND BEAN IN MICHIGAN AND SCREENING FOR FRUIT RESISTANCE IN CUCUMBER

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Amanda Jane Gevens

The fungal-like oomycete *Phytophthora capsici* causes disease on cucurbit, solanaceous, and more recently, leguminous crops worldwide. Surface irrigation sources in three Michigan counties with a history of susceptible crop production were monitored for *P. capsici* during four growing seasons. Pear and cucumber baits were used to detect the pathogen. Recovered *P. capsici* isolates (270) were screened for sensitivity to the fungicide mefenoxam and characterized for compatibility type (CT). Amplified Fragment Length Polymorphism (AFLP) analysis of select isolates was carried out to indicate the persistence of isolate similarity over time and space. *Phytophthora capsici* was not found to overwinter in the eleven Michigan water sources monitored. The consistent detection of *P. capsici* in surface water used for irrigation in Michigan suggests that this is an important means of pathogen dissemination.

Historically, beans (*Phaseolus vulgaris*) have been considered a suitable crop for rotation with *P. capsici*-susceptible crops. Commercial bean fields in three Michigan counties exhibited water-soaked foliage, stem necrosis, and overall plant decline along the surface water drainage pattern. All fields had a history of *P. capsici* infestation. Diseased tissue from stems, petioles, leaves, and pods collected yielded a total of 680 isolates of *P.capsici*. No isolates were recovered from bean roots. Under laboratory conditions, representative bean isolates were pathogenic on cucumber fruit and twelve

different types of bean, including soybean. AFLP analysis was carried out to investigate the genetic diversity among isolates and geographical populations. There was no subdivision in field populations of *P. capsici* based on host or plant tissue. This is the first documentation and etiological report of *P. capsici* on bean in Michigan. At this time, rotating beans with other susceptible hosts is not recommended.

Cucumber fruit rot, caused by *P. capsici*, has become a persistent threat in most commercial production regions. Although mature cucumber plants exhibit some resistance to disease by *P. capsici*, the fruit are particularly susceptible. Identification and utilization of resistance in cucumber fruit would provide a viable disease management strategy for producers. The objectives of this study were to develop a screen for testing detached cucumber fruit for resistance to *P. capsici* and to screen cucumber cultigens for resistance. This is the first report using an unwounded fruit screen to analyze cucumber resistance to *P. capsici*. Although no fruit exhibited complete resistance to the pathogen, some cultigens showed limited pathogen sporulation.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

(Images in this dissertation are presented in color)

LITERATURE REVIEW

INTRODUCTION

Phytophthora is a major genus of plant pathogens within the Phylum Oomycota which contains at least 60 species (14). The oomycetes appear to have closer morphological affinities with algae and higher plants than with true fungi (44, 45). Phytophthora blight, caused by *Phytophthora capsici* Leonian, is an important disease of cucumber (*Cucumis sativus* L.), zucchini (*Cucurbita pepo* L. var. cylindrica Pans), squash (*Cucurbita pepo* L. var. condensa Bailey), eggplant (*Solanum melongena* L.), tomato (*Lycopersicon esculentum* Mill.), pepper (*Capsicum annuum* L.), pumpkin (*Cucurbita moschata*), melon (*Cucumis melon* L.), watermelon (*Citrullus lanatus* L.), and cacao (*Theobroma cacao* L.) (73). Phytophthora blight was first described on bell pepper in New Mexico in 1922 (40). Since then, incidence of *P. capsici* on vegetable crops has been reported in Colorado, Florida, California, Michigan, New York, Georgia, New Jersey, North Carolina, and Europe (26, 66, 75, 76, 77, 120, 134, 135, 141, 143). The incidence and severity of this disease has increased in recent years both within the U.S. and worldwide (5). This pathogen is the limiting factor in the production of susceptible vegetables despite the adherence to recommended control strategies (59). Crown, root, and fruit rot caused by this soilborne pathogen has become a primary concern for Michigan pickling cucumber growers (59, 78). Some producers have been forced to abandon pickle production altogether as a result of increased crop loss from year to year (59). Spread of this disease threatens both fresh market and processing industries, and limits the amount of production land for a large number of Solanaceous, Cucurbitaceous, and more recently, Legumosaceous crops (29, 47, 100, 101).

Prolonged rain or irrigation leading to excessively wet soil conditions favors the spread of the pathogen by splashing (125). Zoospores move in irrigation water, causing spread within and among fields, potentially carrying inoculum from the epidemic site to uninfested ground (59, 120). Narrow row spacing results in early closure of the leaf canopy producing an ideal warm (>25°C), moist environment for disease progression (106). Temperature and the availability of free water drive the initiation of early-season disease and the ensuing production of secondary inoculum from infected tissue. Disease symptoms on cucurbit plants include necrosis and wilting of the roots and crown. Lesions on fruit first appear water-soaked, but quickly mature and take on a powdered sugar appearance from accumulation of sporangia and/or oospores on fruit surfaces (59). Fruit lesions develop beneath the leaf canopy and are the most frequently observed symptom of *P. capsici* on cucumber. Infected fruit quickly break down both in the field and in storage (59).

PATHOGEN LIFE CYCLE

The asexual phase of *P. capsici* includes the mycelial thallus, which produces extracellular enzymes capable of macerating host tissue (37, 38). Mycelia differentiate under ideal environmental conditions into long (35-138 μm) caducous pedicels which give rise to semi- to fully-papillate limoniform sporangia (Fig. 1) (9, 37). At maturity, deciduous sporangia break off and are dispersed by wind-driven rain or irrigation (38, 121). Sporangia are predominantly tapered at the basal end, can have up to three apices, and are shaped variably depending on light, nutrients, and other environmental conditions (3, 38). Sporangial shapes include sub-spherical, ovoid, obovoid, ellipsoid, fusiform,

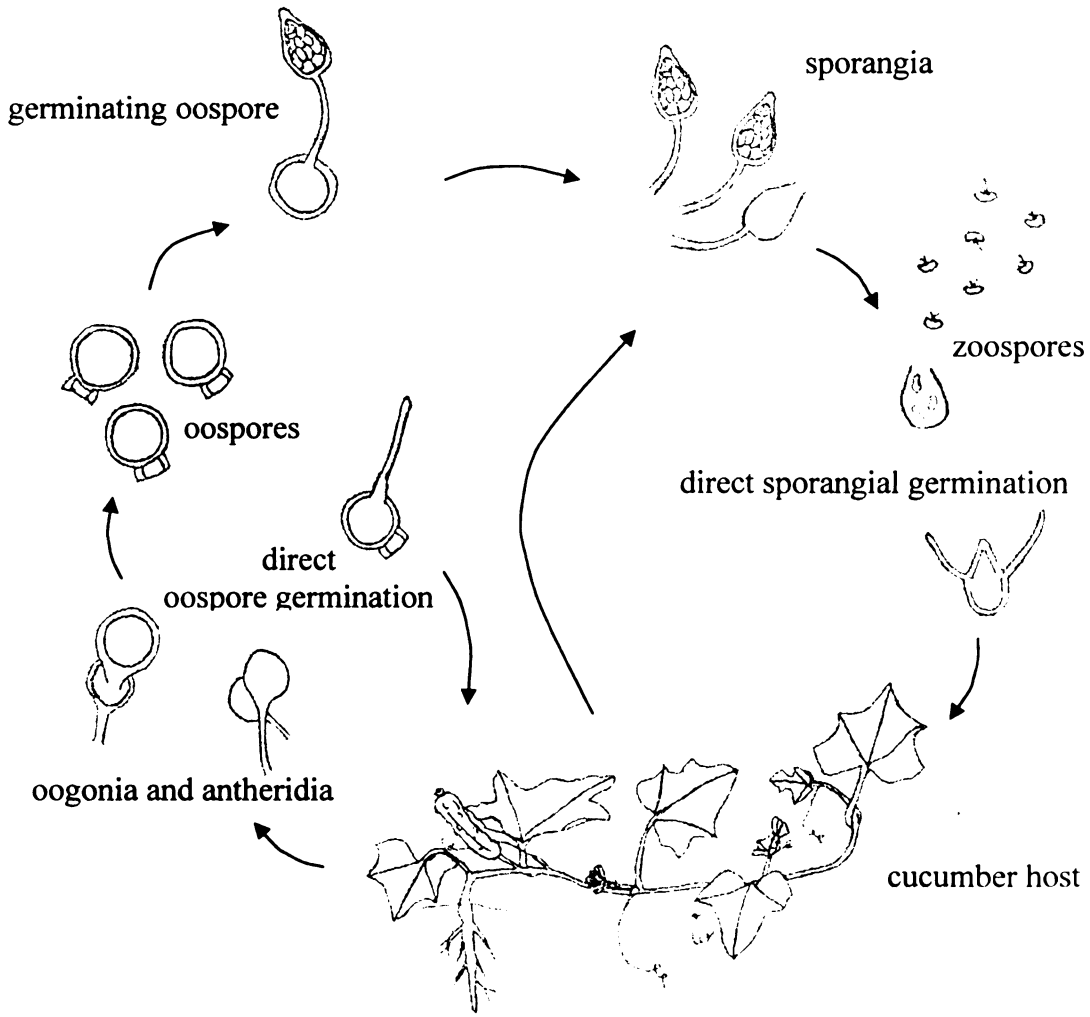

Figure 1. Disease cycle of *P. capsici* on pickling cucumber. Sexual (oospores) and asexual (sporangia and zoospores) spores of *P. capsici* in association with a pickling cucumber host. Oospores are produced when both A1 and A2 compatibility types come into contact.

pyriform, and distorted. Under free water conditions, biflagellate (heterokont), chemotactic, negatively geotropic zoospores are liberated from sporangia (Fig. 1) (13, 37, 78, 84). An individual sporangium can release between 20 to 40 uninucleate zoospores, which maintain motility in water for 5 to 10 hours (37). This increase in inoculum during the early phase of an epidemic occurs rapidly, providing a competitive advantage in host infection. Chlamydospores (hyphal survival structures) are not typically produced by *P. capsici* isolates from pepper and cucurbit hosts. However, isolates from other hosts have been reported to produce chlamydospores under specific light and temperature conditions (38).

Zoospores are predominantly responsible for the spread of the pathogen in water. The negative geotaxis of zoospores in water (tendency to move toward the surface) may be significant in their migration upward in a flooded sill (37). This migration characteristic places them in an ideal position for dispersal by splashing in standing water (37, 118). The attraction of zoospores to plant roots (chemotropism), as well as their ability to stick to host surfaces during encystment, is advantageous to their function as pathogens. The interaction of zoospores with host root exudates within the rhizosphere has been well studied in a variety of host crops such as avocado and eucalyptus (51, 139, 149). More recently, molecules on the surface of *P. capsici* zoospores were shown to be involved in reception of environmental signals that direct pre-infection behavior (12). The root-zoospore interaction is responsible for much of the damping-off and seedling disease associated with this pathogen.

As a heterothallic species, *P. capsici* oospores are produced sexually when hyphae of A1 and A2 compatibility types (CT) interact (Fig. 1) (108, 137). The

production of sexual structures (amphigynous antheridia and spherical oogonia) is induced hormonally in the presence of the opposite CT. Sexual reproduction is the major source of genetic variation in nature (20, 66, 78, 80, 84, 87). Within Michigan and in other U.S. regions, *P. capsici* exhibits an approximate ratio of 1:1, A1:A2 CT (59, 78, 79). An equal balance of the two CTs allows for frequent sexual recombination, and thus, rapid development of resistance to chemical controls that target specific sites of metabolic activity (80). In the U.S., *P. capsici*, *P. cinnamomi,* and *P. infestans* are the only three documented heterothallic *Phytophthora* spp. which have both the A1 and the A2 CT present to complete sexual reproduction (31, 33, 37, 38, 49, 149). However, opportunities for sexual reproduction for *P. infestans* are limited because the CTs are typically separated geographically (49).

Multiple factors affect oospore production. In most *Phytophthora* spp., oospore production is optimal under darkness and temperature of less than 25°C (6, 52, 56, 72, 78, 99). Light becomes necessary for the germination of mature oospores. Yu et al. (148) studied the effects of light and temperature on oospore production and maturation of *P. parasitica*. Light had a significant impact on the induction of oospore production, but was not necessary for maturation. Temperature affected the activity of the hormone needed to induce sexual reproduction. Other factors influencing oospore production in many *Phytophthora* species include nitrogen, the atmospheric gas balance between O_2 and CO_2, phospholipids, plant-derived compounds, vitamins, and sterols (37, 69, 97, 98).

The oospore structure from the central ooplast to the outer surface is comprised of an inner oospore wall, outer wall, periplasm (in which nuclei and mitochondria are

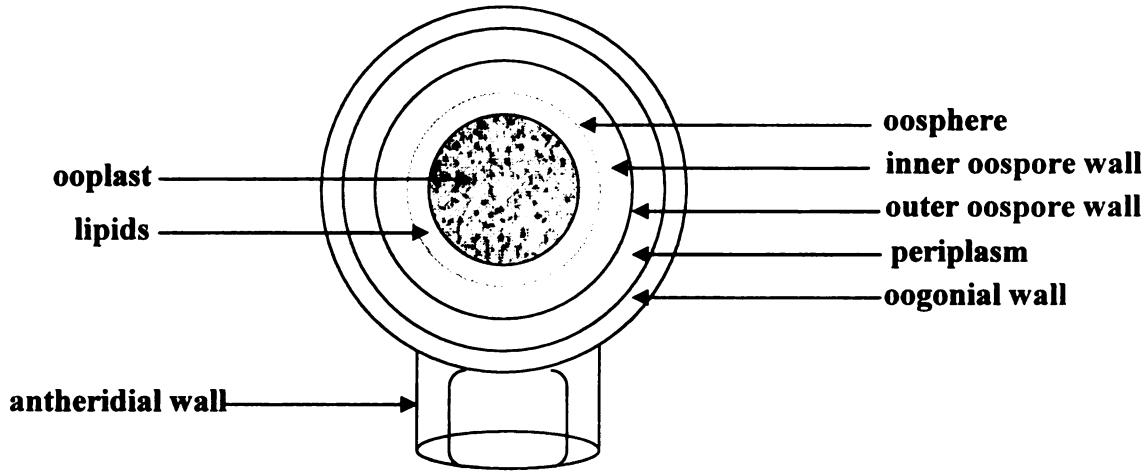

Figure 2. Ultrastructure of a *P. capsici* oospore. The antheridial wall remains attached to the oospore after fusion of the oogonia and antheridia. The average oospore diameter is 20μm. Labeled oospore components include: lipids, oosphere, inner and outer oospore walls, periplasm, oogonial wall, and antheridial wall.

sequestered), and oogonial wall (Fig. 2) (38). Oospores of *P. capsici* are predominantly plerotic (they fill the oogonium), and have thick walls (2 to 6 μm), which confer strong resistance to external factors such as salinity and extreme temperatures. For this reason, the oospores are the most durable and long-lived propagules in the soil (38). Oospores can be formed not only in soil, but also on and within host tissue, wherever A1 and A2 CTs are present (2, 6, 34, 53, 63, 66, 73, 99, 122).

Oospores were hypothesized to be the main *P. capsici* survival propagules in soil in New Jersey (13) and California (123); in Michigan, this hypothesis has been confirmed (80). Oospores mature in 2 weeks to 3 months, depending on environmental conditions, and remain viable for an extended period of time (5+ years). Germination occurs predominantly by forming sporangia in water, root extract, and soil extract; however, oospores can also germinate by forming direct-infecting germ tubes (63, 124). The oospore germination rate in root and soil extracts is asynchronous and increases with time of incubation (63). The ooplast (cortical layer in oospore) is consumed during germination, with some of its lipid bodies moving into the cytoplasm of the emerging germ tube. Most oospores produce a single germ tube that ruptures the thin outer oospore wall and the oogonial wall near the oogonial stalk (37, 124).

PATHOGENICITY AND VIRULENCE

Phytophthora capsici inoculum (zoospores, sporangia, oospores, and mycelial fragments) begins pathogenesis on a susceptible host with an initial chemical recognition step that is often concurrent with the release of adhesive and degradative enzymes (37). Yoshikawa et al. (146) observed that *P. capsici* produced a unique, active, extracellular macerating factor when grown in culture. Propagules can directly penetrate the host plant tissue with germination tubes. Germination tubes can grow into natural plant openings such as stomata or wounds, or can differentiate to form appressoria which create a wound in the plant surface by a penetration peg that uses hydrostatic pressure to puncture host cells (11). Once inside host cells, *P. capsici* produces haustoria which gather plant nutrients (60). After ramification through host tissue, the pathogen can proliferate by producing sporangia and zoospores on host surfaces. Plants infected with *P. capsici* exhibit altered metabolism and are often referred to as 'sinks' because the reduction in photosynthetic potential causes the plant to send resources to sites of infection (37). Infection is chronic and results in plant death. Oospores are often produced on necrotic host tissue and are released into soil after tissue decomposition.

Solanaceous, Cucurbitaceous, and recently Leguminosaceous plant families were all found to be susceptible to *P. capsici* infection (47, 59). Among these families there are differences in susceptibility based on plant variety, age, and organ (37). Developing fruit of a pickling cucumber plant are relatively more susceptible than the vine, crown, and roots (59). However, fully mature fruit exhibit some resistance to infection (1). Compared to other host plants, yellow summer squash exhibit elevated susceptibility and

rapidly succumb to disease (59). Bean roots remain free of disease from *P. capsici,* yet all above-ground parts are susceptible to infection (47). While these differences represent phenotypic variations of resistance in the host, there are also variations in virulence of the *P. capsici* isolates (47, 75, 86, 117, 132).

Polach and Webster (117) suggested the existence of physiological races of *P. capsici* based on the response of different host genotypes (differentials) to pathogen isolates. Strains were identified by a series of inoculations with 23 isolates on 5 different susceptible plants from the Solanaceous and Cucurbitaceous families. Among the isolates tested, fourteen were distinguished by their selective pathogenicity (117). Oelke, et al. (109) also suggested the presence of physiological races of *P. capsici* as the cause of breeding limitations in pepper. The disease response of pepper varieties inoculated with isolates of *P. capsici* indicated that some isolates were responsible for root rot, whereas other isolates only caused foliar blighting. The isolates were described as unique races based on pathogenicity (109).

Although diversity in virulence among *P. capsici* isolates from multiple hosts has been noted, it is questionable whether this diversity reflects true host specificity (68, 75, 86, 117). Studies on resistance of pepper and tomato cultivars to different isolates of *P. capsici* indicate that expression of host resistance is affected primarily by environmental factors such as inoculum dose, temperature, soil moisture, and plant age (66, 75, 111, 119). In the pumpkin- and pepper-*P. capsici* pathosystems, there are putative cases of specific host-parasite interactions or specializations. However, absence of interaction seems to be the rule (86, 111). In general, distinct host specificity among races has not

been confirmed; and host genetics conveying limited quantitative resistance are complex (37, 124).

The host range of *P. capsici* was evaluated extensively by Satour and Butler in 1967 (123). Disease symptoms were observed on 45 species of cultivated plants and weeds, representing 14 families of flowering plants (123). Tian and Babadoost also reported *P. capsici* infection on beet, Swiss chard, turnip, spinach, and lima bean (132). Field reports of *P. capsici* causing disease on bean cultivars have recently been made from Delaware, Maryland, New Jersey, and Michigan, with select Michigan *P. capsici* isolates also causing symptoms on soybean under laboratory conditions (29, 47, 100, 101).

BAITING FOR *PHYTOPHTHORA* SPP.

Methods used to detect, isolate, and quantify *Phytophthora* spp. in soil are numerous, but their success varies depending on the species (37, 38, 84, 96). The most common method is soil dilution plating using a selective medium such as BARP (benomyl-, ampicillin-, rifampicin-, and pentachloronitrobenzene-amended, unclarified V-8 juice agar). Horner and Wilcox (64) optimized a technique called Soil Air Dried And Moistened Chilled And Plated, SADAMCAP, which utilizes basic culturing protocols to increase the number of *P. cactorum* colonies on agar media. This method allowed an accurate and reproducible quantification of *P. cactorum* colony-forming units derived from apple orchard soils of New York. Although this direct soil to agar plating

technique was successful for *P. cactorum*, a similar method had limited success for *P. capsici* (64, 65).

Papavizas, et al. (114) developed a selective medium that inhibited most soil micro-organisms except for oomycetes. This medium was used to develop a dilution plating technique for the direct isolation and quantification of *P. capsici* from soil and infected pepper plants. The isolation technique from peppers was successful. However, in the case of soil, more than 46 propagules per gram of soil needed to be present for *P. capsici* to be detected, while naturally infested soil averaged only 0-24 propagules per gram (114).

Oospores present in soil have been the most difficult propagules to detect and quantify because they do not germinate readily in culture and generally are not accounted for in soil assays (37, 84, 116). In addition, *Phytophthora* oospores are morphologically similar to those of *Pythium*, which are ubiquitous and numerous in agricultural soils. Thus, baiting with susceptible seedlings or fruit remains the most successful tool for the detection of *Phytophthora* spp. in soil (119, 138).

To detect *P. capsici* from soil using a bait method, a susceptible bait from a field without a history of *P. capsici* infestation is needed. With an infected seedling or fruit, the presence or absence of *P. capsici* in a given soil sample can be determined and isolates acquired for further investigation. Some baiting assays detect a lower level of inoculum than may actually be present (37, 38, 84, 96). However, other baiting techniques are more sensitive than selective media methods, and have the capability of detecting just one propagule of *Phytophthora* per gram of soil (125).

Water baiting techniques have been successful for detecting *P. cactorum* and other species in infested irrigation water and for timing application of chemicals for disease control (112, 116, 145). In Washington State (145), green pears were used to bait *Phytophthora* spp. pathogenic to tree fruit. Washington fruit growers regularly begin treating their irrigation water with copper when immature pears, which are suspended in irrigation canals, become infected with *P. cactorum*, the causal agent of sprinkler rot. Infected pear baits were surface sterilized and diseased tissue was excised from lesion margins. Tissue was incubated on selective medium at room temperature. Positively identified *P. cactorum* cultures were then further analyzed (145).

In a study of *Phytophthora* spp. associated with cranberry root rot in surface irrigation water in New Jersey, a lupine baiting technique was utilized in streams, irrigation reservoirs, and drainage canals (112). A 10- to 15-day-old lupine seedling was secured in a Styrofoam boat with its roots extending into the water source. The seedling was then removed 48 hours later and the roots were cut from the seedling. The roots were rinsed and plated onto a selective medium and observed 2 to 4 days later. Positive identification of both *P. cinnamomi* and *P. megasperma* from critical water sources was achieved using this technique (112, 116).

Tomato leaf disks and green tomato fruit were used as baits in a study designed to monitor the spread of *P. parasitica* (causal agent of buckeye rot) in irrigation furrows (107). Baits were floated on the surface of water that was experimentally infested with *P. parasitica*. Both baits were useful for detecting the pathogen at various distances from inoculation source points. In this same study, detection of *P. parasitica* was also carried out by dilution plating but the recovery rate was low (107). Pepper leaf disks have also

been used in assaying flooded soil samples for *P. capsici* presence (83). In these assays, containers of field soil were flooded with water, and the disks were floated on the surface and analyzed 72 hours later. *Phytophthora capsici* successfully colonized leaf disks in the containers with infested soil (83).

Recovery of *Phytophthora* spp. from water has also been achieved without the use of baits. A filter-based method using hydrophilic membranes was carried out with recycled nursery irrigation water (37). Organisms deposited on filters were plated for identification and quantification (61). This technique was used to monitor levels of *Phytophthora* and *Pythium* spp. present in the components of a recycling irrigation system at a perennial container nursery in Virginia over a two-year period (17). The relative occurrence of *Phytophthora* and *Pythium* spp. as well as their taxonomic diversity were documented for three different water sources. To validate the filter-based method, rhododendron leaf disk baits were also implemented (17). Baits were surface washed and placed onto selective media for oomycetes. *Phytophthora* and *Pythium* spp. were identified morphologically and successfully quantified. Results of rhododendron leaf baits confirmed the efficacy of the filter method (17).

MANAGEMENT

Knowledge of field disease, crop, and chemical history is a necessity in controlling *P. capsici* effectively (59). Some crops lend themselves to cultural practices, such as constructing raised beds for hand-picked zucchini. However, control measures need to be heightened for a crop such as pickling cucumbers that is mechanically

harvested. Multi-faceted disease-management practices, combining cultural and chemical control in addition to genetic host resistance are necessary (59).

Cultural Management

Cultural techniques that have aided in limiting *P. capsici* spread and infection have included row spacing, crop rotation, water management, bedding and plasticulture, and avoidance of low-lying or infested fields (18, 59, 66, 127). In Michigan, row spacing for machine-harvested pickling cucumbers varies from 11 to 30 inches with a plant population of 35,000 to over 100,000 plants per acre (103, 105). A closed plant canopy promotes high relative humidity and sustained soil moisture after a rain or irrigation event (105). Under these conditions *P. capsici* rapidly develops sporangia and subsequently releases a large number of zoospores. A 2003 study by Ngouajio (102, 104) examined the effects of widened rows on canopy dynamics and fruit yield. Cucumber canopy remained open during most of the growing season when wide rows (61.0 and 76.2 cm) were used. The optimum row spacing for maximizing the number of fruits per plant was 61.0 cm. With 76.2-cm rows, yield was only slightly reduced. These results suggest that it is possible to significantly reduce cucumber plant density without reducing yield (104, 106).

A two-year rotation with a non-host crop has been suggested to avoid buildup of *P. capsici*; however, planting any host crops into a field with a history of *P. capsici* is risky since non-host rotations do not eliminate the pathogen (59). Lamour and Hausbeck investigated the survival of *P. capsici* in the field following a typical 2-year crop rotation (2 years of production with crops not susceptible to *P. capsici*) in Michigan (81). The potential for dormant inoculum (oospores) to survive a 30-month non-host period was

confirmed, with molecular tools, indicating that management strategies relying on crop rotation may not provide economic control of *P. capsici* (81).

Managing field water includes using fields with soils that allow for efficient drainage, avoiding low-lying areas where water may stand, and limiting irrigation (18, 59). Furthermore, it is recommended that irrigation on pickling cucumbers within a week of harvest be limited for maintaining healthy fruit at a critical, susceptible stage (57, 59). Sources of irrigation water that receive run-off from fields with a history of *P. capsici* should also be avoided (46). Water management remains critical post-harvest in transportation wagons and bulk bin storage since susceptible fruit can still become infected even after removal from the field (57, 59).

When harvest methods allow, planting host crops on raised beds (6 inches in height minimum) greatly reduces the risk and incidence of *P. capsici* infection (59). To complement the beneficial effects of raised beds, black plastic application over beds with the addition of sub-plastic drip-tape irrigation further enhances successful crop production in an infested field (59, 126). These methods facilitate tight control of soil moisture and reduce splashing of water from contaminated soil onto plant parts. In addition, susceptible fruit have increased protection from direct contact with soil, where pathogen propagules reside (59).

Chemical Management

There are few chemical products available that provide economically acceptable control of *P. capsici* (59). Since 1977, metalaxyl has been used to control plant diseases caused by oomycetes (15). Metalaxyl (Ridomil) is a systemic phenylamide fungicide with a site-specific mode of action that inhibits RNA polymerase activity and can be

applied by drench, seed treatment, and by foliar application (15, 24). Mefenoxam (Ridomil Gold) is the active isomeric form of metalaxyl, which comprises 50% of metalaxyl, whereas mefenoxam consists of 100% active isomer (115). Metalaxyl is toxic to sporangia and zoospores *in vitro,* yet has little effect on sporangium and zoospore germination (28). It delays expansion of young lesions and suppresses sporangium production on treated plants (15, 24, 87). Resistance to metalaxyl was first reported in *P. infestans* in Ireland in the late 1970s, in the Netherlands in 1981, and has since been reported frequently in both homo- and heterothallic *Phytophthora* species (27, 32, 41, 49, 82, 87, 133). In *P. infestans*, resistance occurs as a result of a simple change in the target site and there is no correlation between resistance and other phenotypes, such as CT or virulence (30, 48). Lamour and Hausbeck (80) found that *in vitro*, mefenoxam sensitivity in *P. capsici* is conferred by a single, incompletely dominant gene.

When fungicide selection pressure is removed, reversion to mefenoxam sensitivity within a *P. capsici* population does not occur within an agriculturally significant time period (2 years) (80). Application of mefenoxam to a field with a significantly insensitive *P. capsici* population has no management benefit and can increase economic loss to a grower (82, 115). Pathogen insensitivity to mefenoxam has limited the usefulness of this fungicide worldwide (43, 70, 71, 78, 80, 87). Few alternative products are currently available that match the effectiveness that mefenoxam provides against a sensitive pathogen population (59).

Released in 1988, dimethomorph (DMM) (Acrobat), a derivative of cinnamic acid, is a systemic fungicide with supposed protectant, curative, and antisporulant activities against members of the Peronosporaceae and the genus *Phytophthora* (19, 37).

DMM interferes with the assembly of wall polymers in the fungal cell (23). As a result, zoospores fail to produce cell walls, and hyphae stop expanding. There is no cross-resistance between DMM and phenylamide fungicides, such as mefenoxam. *Phytophthora infestans* was recently reported to have little or no risk of developing resistance to DMM in nature (129) and there are no reports of resistance in *P. capsici*. This characteristic may ensure prolonged efficacy of post-infection applications (129). In addition, the fungicide is highly effective at relatively low rates (23). Only DMM was effective in managing disease once mefenoxam insensitivity had been established (5). Further studies by Hausbeck et al. (59) on pickling cucumbers, also support this conclusion.

Zoxamide (Zoxium), a non-systemic benzamide, has been recently introduced as an oomycete fungicide that acts on pathogen microtubules (147). Although field studies suggest that the risk of the development of insensitivity in the pathogen population is much lower in the field for zoxamide than for mefenoxam, it is a site-specific fungicide, and precautions must be taken with its use (147). Zoxamide application, in combination with copper hydroxide (Kocide), provided effective control of *P. capsici* zoospore infection on detached cucumber fruit (Harlan, B.R., and Hausbeck, M.K., unpublished data). However, trials with this product showed a reduced level of protection when compared to DMM (4, 67).

Alternative compounds have also been tested for their *Phytophthora* disease management potential. Simple additions of compounds such as gypsum to field soil was shown to limit zoospore production of *P. cinnamomi* (94). Sodium tetrathiocarbonate (STTC) and water applied to soil was reported to have some fungicidal effects on

Phytophthora spp. in greenhouse trials at high rates of application (90). Soils amended with myxobacteria have been demonstrated to reduce growth of *P. capsici* by enzymatic inhibition (16). The roles of calcium, phosphite, surfactants, and inducers of host plant resistance (chemical and non-pathogen) have also been investigated for their disease management properties (21, 22, 25, 36, 39, 42, 54, 55, 74, 85, 93, 95, 110, 127, 128, 140, 144). Although alternative compounds may provide some management benefit, the methods and rates of application of such products must be consistent, effective, and economical for large scale production.

The multiple propagule types of *P. capsici* are each affected differently by fungicides. In an *in vitro* study, Matheron and Porchas (91), observed the impact of five fungicides on the growth, sporulation, and zoospore cyst germination of *P. capsici.* In addition, effects of these chemicals on the development of root, crown, and fruit rot of chile pepper were studied (91, 92). Inhibition of mycelial growth, sporangial formation, and cyst germination was greatest when the pathogen was treated with DMM. However, DMM had little effect on the inhibition of zoospore motility. Fluazinam had a minimally inhibitory effect on mycelial growth and cyst germination. However, this product inhibited zoospore motility by almost 100% at all concentrations and was equally as effective as DMM in inhibiting sporangial formation. Azoxystrobin had a moderately inhibitory effect on mycelial growth and was most effective at higher concentrations on sporangial formation, zoospore motility and cyst germination. Metalaxyl had a significant limiting effect on the growth of mycelium, sporangial formation, zoospore motility, and cyst formation. The most effective compounds on pepper stems and fruit were mefenoxam and DMM (91).

Genetic Host Resistance

Breeding efforts have resulted in two pumpkin cultivars exhibiting quantitative resistance due to thick rinds: 'Danmatmaetdol' from the National Institute of Agricultural Science and Technology at Suwon, Korea (86) and 'Lil Ironsides' from Harris Moran Seed Company (Modesto, CA). Increased cuticular thickness has also been implicated in elevated disease resistance to *P. capsici* in Mexican-type pepper fruit (10). No pepper cultivars have been shown to have universal resistance to *P. capsici* root and foliar blighting (7, 109), although 'Paladin', a commercially marketed pepper hybrid from Rogers/Novartis is marketed as *P. capsici* tolerant.

Traditional cucurbit breeding for beneficial horticultural traits involves simple cross-pollination of parents with desirable qualities. Compatible cross-pollination, or intercrossibility, can occur only with hard squash, pumpkins, and gourds. Melons, summer squash, and cucumbers are not intercrossible; therefore, resistance present in melons cannot be bred into cucumbers by traditional methods (142).

Pepper plants produce the phytoalexin capsidiol and can upregulate pathogenesis-related (PR) genes known to encode for multiple plant defense proteins following infection (35, 37, 88, 89, 109). Recent research on tomato showed expression of PR-1 proteins when challenged by *P. capsici* (61). These findings provide evidence of plant response systems, which can be exploited for potential use in a breeding program. An example of this application is a marker-assisted selection program, in which four quantitative trait loci (QTL) for *P. capsici* resistance in a small-fruited pepper line were successfully introduced into a susceptible bell pepper line (130, 131). The selection program was initiated from a doubled-haploid line issued from the mapping population

and involved three cycles of marker-assisted backcrossing. Resulting bell pepper plants expressed quantitative resistance to *P. capsici* (131).

Selection of appropriate inoculum and inoculation techniques is critical when screening for disease resistance (113, 136). The rhizosphere of host plants prone to root rots is an important feature to consider. In France, pepper breeders have set up an inoculation method involving incubation of mycelium in the proximity of plant roots in a liquid medium to best mimic the rhizosphere of pepper in a disease situation. Their experimental methods have made it possible to breed for constitutive as well as induced resistance components in controlled conditions at the seedling stage (113). Although inheritance of host resistance to *P. capsici* is understood to be controlled by two distinct genes in pepper, there is little information regarding the inheritance of resistance to *P. capsici* in other host plants (86, 117).

To date, much work has been carried out in effort to curb *P. capsici*. However, no single management practice provides complete control in a reliable and sustainable manner. Crop rotation, sanitation, management of field moisture, and judicious use of fungicides are relied upon to maintain profitability of vine crop production. Although rotation to supposed non-susceptible hosts has been studied for control against this pathogen, it does not provide effective control since the pathogen survives in soil for a long time (>5 years anecdotal evidence, M.K. Hausbeck), and the pathogen has now been isolated from a common rotational crop, snap bean (58). Field moisture, in areas of high relative humidity or rainfall, cannot be effectively managed and is therefore not a stand-alone control option. Because completely resistant cultivars have not been found, research efforts toward control have been focused on the biology of the pathogen. The

research objectives of this dissertation include investigating irrigation water as a means of zoospore spread, documenting and characterizing *P. capsici* on snap bean, and screening cucumber germplasm for fruit resistance to *P. capsici*.

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CHAPTER I

BAITING *PHYTOPHTHORA CAPSICI* FROM MICHIGAN SURFACE IRRIGATION WATER AND CHARACTERIZATION OF ISOLATES

ABSTRACT

The fungal-like oomycete *Phytophthora capsici* causes disease on cucurbit and solanaceous crops worldwide. In free water, *P. capsici* sporangia release zoospores which may be transported by moving surface water. Surface irrigation sources (river system, ponds, and ditches) in three Michigan counties with history of susceptible crop production were monitored for *P. capsici* during four growing seasons (2002 to 2005). Pear and cucumber baits were suspended in water at monitoring sites for 3 to 7 days and water temperature was recorded. Baits were washed and lesions excised and cultured on amended water agar. *Phytophthora capsici* was detected at monitoring sites in multiple years despite nearby crop rotation to non-hosts. Recovered isolates (270) were screened for sensitivity to the fungicide mefenoxam and characterized for compatibility type (CT). Mefenoxam insensitivity of isolates was common in water sources from southwest and southeast Michigan. Most monitoring sites yielded isolates of a 1:1 ratio of A1:A2 CTs. AFLP analysis of select isolates from 2002 to 2004 indicated a lack of similarity groups persisting over time and in specific geographical locations. *Phytophthora capsici* was not found to overwinter in the eleven Michigan surface water sources monitored. The consistent detection of *P. capsici* in surface water used for irrigation in Michigan suggests that this is an important means of pathogen dissemination.

INTRODUCTION

Phytophthora capsici [Leonian] infects Solanaceous and Cucurbitaceous hosts including cucumber, eggplant, tomato, pepper, pumpkin, squash, melon, and zucchini (7). Recently, snap beans have been added to this list of susceptible crops (4). In addition to crown rot, *P. capsici* causes fruit rot symptoms which appear initially as water-soaked lesions that expand rapidly. Sporangia and/or oospores develop in the lesions resulting in fruit surfaces with a powdered sugar appearance. Infected fruit quickly break down both in the field and post-harvest (7).

Oospores overwinter in soil and plant debris and initiate primary plant infections in the spring (11, 12, 14). Oospores are produced when mycelium of A1 and A2 compatibility types (CTs) come into contact (18, 21). Both CTs have been documented in naturally-infested fields in Michigan (11, 12, 13) and other states (7). Mycelia and sporangia develop on infected host tissue under warm (25-28°C), wet conditions. Sporangia and zoospores are secondary asexual inoculum produced during the growing season, which cause rapid escalation of disease (2, 3). In free water, lemon-shaped sporangia release 20-40 motile zoospores, each capable of causing infection (7).

Phytophthora capsici management requires a multi-faceted approach of both cultural and chemical strategies. Planting *P. capsici*-susceptible crops in well-drained fields, on raised beds, and avoiding low-lying regions help to manage disease. Conservative irrigation, especially close to harvest is recommended (7, 20). When applied preventively and frequently, fungicides can also limit disease (7). However, resistance of *P. capsici* to the historically-used fungicide mefenoxam has been widely documented in Michigan and other vegetable production regions (12, 13). Because

current measures, even when used in a comprehensive program are often not adequate, preventing the introduction of *P. capsici* into new sites is critical (7).

Oomycetes have been identified in surface water used in various commercial plant production systems (1, 8, 15, 17, 19, 25). *Phytophthora* and *Pythium* spp. have been detected in recycled water used for irrigation in greenhouse and nursery production in Virginia (1, 8). *Phytophthora cactorum* was identified in surface water used for irrigation of pear in the state of Washington (25). Both *P. cinnamomi* and *P. megasperma* were detected in surface water used in a cranberry production system in New Jersey (19). In North Carolina (15) and California (17), *P. parasitica* was detected in furrow irrigation water which moved the pathogen from loci of infestation to parts of the field that were uninfested. *Phytophthora capsici* has not yet been documented in surface water and many Michigan vegetable growers rely on surface water for irrigation of susceptible crops.

My objective was to determine whether *P. capsici* is present in surface water used for irrigation in Michigan and characterize the CT and sensitivity to mefenoxam of the collected isolates. Representative isolates were subjected to amplified fragment length polymorphism (AFLP) analysis to determine if genetic similarity groups are present over time and among geographical locations. Water and rainfall were also monitored and analyzed for correlation to *P. capsici* incidence.

MATERIALS AND METHODS

Site Selection

Water monitoring sites were either adjacent to a vegetable production field with a history of *P. capsici* infestation or adjacent to a field with a crop infected with *P. capsici* during the 2002 to 2005 growing seasons. Sites were selected in three of the primary vegetable production regions of Michigan: northwest (NW; Oceana Co.), southwest (SW; Allegan Co.), and southeast (SE; Lenawee Co.). Baiting was usually initiated in the spring and terminated at the end of the growing season (Tables 1-3).

Baiting, *P. capsici* Identification, Water Temperature, and Rainfall

Baiting traps were constructed by attaching Styrofoam pool noodles to plastic milk crates with a securable lid (Fig. 1A). Each trap contained two green pears, purchased from a local supplier, and a cucumber produced at the Michigan State University Plant Pathology farm, a site without a history of *P. capsici*. Pears were selected as baits for *P. capsici* because they had previously been shown to be susceptible to *Phytophthora* spp. when used in a water system (25). A WatchDog data logger temperature sensor from Spectrum Technologies, Inc. was attached to the milk crate to measure and record water temperature each hour (Fig. 1A). Each trap was secured to a fixed point and deployed into one of the following water sources: river, creek, pond, or ditch (Fig. 1B). Baits and temperature sensors were replaced once or twice weekly. Hereafter, a baiting period refers to the time that the baits remain in the water (3-7 days). After removal from the traps, cucumbers and pears (Fig. 1C and D) were rinsed in distilled, de-ionized water, dried, and tissue from water-soaked lesions was placed onto water agar amended with rifampicin (2 m/L) and ampicillin (2 ml/L). After 5 days of

Table 1. Time of sampling and *P. capsici* detection for SW Michigan (Allegan County) river sites, 2002 to 2005.

Site:Type	Year	Initiation Date	Date of First Detection	# of periods with *P. capsici*[a]	Crop in nearby field
1: Creek	**2002**	18 Jun[b]	7 Aug	11	cucumber[f]
	2003	30 May[c]	25 Jul	10	potato
	2004	12 May[d]	27 Aug	2	soybean
	2005	3 May[e]	26 Jul	5	corn
2: River	**2002**	18 Jun[b]	19 Aug	3	corn
	2003	22 May[c]	22 Aug	1	cucumber[f]
3: River	**2002**	18 Jun[b]	19 Aug	3	cucumber[f]
	2003	22 May[c]	18 Aug	1	corn
4: River	**2003**	30 May[c]	11 Aug	2	cucumber[f]
	2004	12 May[d]	20 Jul	6	cucumber[f]
	2005	3 May[e]	26 Jul	2	cucumber[f]
5: River	**2003**	30 May[c]	23 Sep	1	cucumber[f]
	2004	12 May[d]	3 Aug	2	cucumber[f]
	2005	3 May[e]	29 Jul	1	soybean
6: River	**2003**	30 May[c]	1 Aug	2	cucumber[f]
	2004	12 May[d]	17 Aug	2	cucumber[f]
	2005	10 May[e]	9 Aug	2	potato

[a]Baiting period was 3-7 days during which baits were in surface irrigation water.
[b]Indicates baiting termination occurred on 8 October 2002.
[c]Indicates baiting termination occurred on 28 October 2003.
[d]Indicates baiting termination occurred on 22 October 2004.
[e]Indicates baiting termination occurred on 21 October 2005.
[f]Indicates that nearby crop exhibited disease caused by *P. capsici*.

Table 2. Time of sampling and *P. capsici* detection for NW Michigan (Oceana Co.) pond sites, 2002 to 2005.

Site:Type	Year	Initiation Date	Date of First Detection	# of periods with *P.capsici*[a]	Crop in nearby field
1: Naturally-fed	**2002**	20 Aug[b]	20 Aug	8	yellow squash[f]
	2003	16 Apr[c]	22 Aug	2	snap bean[f]
	2004	20 Apr[d]	31 Aug	1	carrot
	2005	19 Apr[e]	3 Aug	2	corn
2: Well-fed	**2002**	18 Jun[b]	N/A	0	snap bean
	2003	16 Jul[c]	N/A	0	yellow squash[f]
3: Well-fed	**2002**	25 Jun[b]	20 Aug	1	carrot

[a]Baiting period was 3-7 days during which baits were in surface irrigation water.
[b]Indicates baiting termination occurred on 10 October 2002.
[c]Indicates baiting termination occurred on 28 October 2003.
[d]Indicates baiting termination occurred on 23 October 2004.
[e]Indicates baiting termination occurred on 21 October 2005.
[f]Indicates that nearby crop exhibited disease caused by *P. capsici*.

Table 3. Time of sampling and *P. capsici* detection for Michigan ditch sites (Lenawee and Oceana Cos.), 2004 to 2005.

Site:Type	Year	Initiation Date	Date of First Detection	# of periods with *P. capsici*[a]	Crop in nearby field
1: Run-off SE Michigan (Lenawee Co.)	**2004**	20 Jul[b]	5 Aug	5	cucumber[d]
2: Culvert NW Michigan (Oceana Co.)	**2005**	27 Jul[c]	29 Jul	7	zucchini[d]

[a]Baiting period was 3-7 days during which baits were in surface irrigation water.
[b]Indicates baiting termination occurred on 21 October 2004.
[c]Indicates baiting termination occurred on 7 October 2005.
[d]Indicates that nearby crop exhibited disease caused by *P. capsici*.

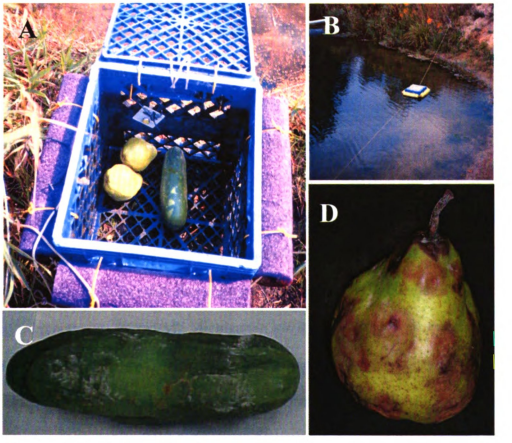


Figure 1. A) Baiting station for *P. capsici* and contents: two green pears, one cucumber from an uninfested source, and a temperature sensor. B) Baiting station deployed in a pond used for crop irrigation. C) Cucumber bait retrieved after one week in water monitoring site. D) Pear bait retrieved after one week in water monitoring site. (Images in this dissertation are presented in color).

incubation, plates were observed with a compound microscope and *P. capsici* was identified based on morphological characteristics according to the *Phytophthora* spp. key by Waterhouse, 1963 (24). Data from temperature sensors were downloaded using Spec Ware 6 data logger software (Spectrum Technologies, Inc., Plainfield, IL) and hourly data were averaged for each baiting period. Rainfall data for each baiting location were collected from nearby weather monitoring stations, totaled for each baiting period, and were analyzed using nonlinear polynomial regression analysis.

Compatibility Type Determination

Compatibility types of *P. capsici* isolates were determined by placing agar plugs (7.0-mm diameter) from the edge of expanding single-zoospore-derived cultures onto a V-8 juice agar plate. At a distance of 3 cm from the baiting isolate plug, a 7.0-mm plug of a known A1 or A2 CT was placed. Known standard isolates of *P. capsici* were OP97 (A1 CT) isolated from pickling cucumber fruit in NW Michigan in 1997; and SP98 (A2 CT) isolated from pumpkin fruit in SW Michigan in 1998. Culture numbers refer to isolates maintained in the *Phytophthora* stock collection in Dr. Mary Hausbeck's laboratory in the Department of Plant Pathology, Michigan State University. After 7 to 10 days incubation at 23-25°C under darkness, plates were observed for the presence of oospores between the two plugs.

Mefenoxam Sensitivity Determination

Mefenoxam sensitivity was determined by placing a 7.0-mm agar plug from a single-zoospore-derived culture on V-8 juice agar and V-8 juice agar amended with 100 ppm mefenoxam (Ridomil Gold EC, 48% a.i. suspended in sterile distilled water and added to cooled agar). Plates were incubated at 23-25°C for 3 days, and colony

diameters were measured. Growth of an isolate on amended media as compared to unamended media was classified as sensitive (S, <30% of the control), intermediately sensitive (IS, 30-90% of the control), and insensitive (I, >90% of the control) (10).

DNA Extraction and Amplified Fragment Length Polymorphism Analysis

Genomic DNA was extracted from approximately 10 mg of freeze-dried *P. capsici* mycelium using DNeasy® mini-kits (Qiagen, Valencia, CA., USA). Mycelium was grown in antibiotic-amended V8 broth. DNA was quantified on an agarose gel with known standards. EcoRI was obtained from Invitrogen (Carlsbad, CA., USA), while MseI and T4 DNA ligase were obtained from Takara (Madison, WI., USA). EcoRI and MseI adapters and primers for ligation and amplification reactions were obtained from Integrated DNA Technologies (Coralville, IA, USA). For sequences of the adapters and primers, see Vos et al. (23). The fluorescently labeled primers were obtained from Proligo (Boulder, CO, USA) and are comprised of the EcoRI core sequence with and without any selective nucleotides and either the WellRED D4-PA label (Proligo) or the WellRED D3-PA label at the 5' end.

Restriction, ligation, preamplification, and selective amplification reactions were carried out as described by Habera et al. (6). Fluorescent products from the selective amplifications were analyzed on a CEQ™ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA) using the manufacturer's protocols.

RESULTS

Pear and cucumber fruit were suitable baits for detecting *P. capsici* in Michigan surface water. Cucumber fruit were especially susceptible and sensitive as baits, with approximately 70% of all *P. capsici* isolates collected from symptomatic cucumber. In addition to *P. capsici*, other oomycete organisms were isolated from baits. Based on morphological characteristics and AFLP analysis, *Pythium* spp. were identified each year. *Pythium* spp. were prevalent during the early (April and May) and late (September and October) portions of the growing season. Additional *Phytophthora* spp. were also observed during the four years of water monitoring.

River System

Prior to 2002, pickling cucumbers were routinely planted in fields nearby the SW Michigan (Allegan Co.) river system (Fig. 2). One or two years of non-host rotations to corn and soybeans were common. *Phytophthora capsici* was detected in the six river sites monitored during the 4-year period. While baiting was initiated in the spring and early summer (prior to 18 June), *P. capsici* was never detected prior to 20 July. The pathogen was detected at each site for multiple years even when a *P. capsici*-host crop was absent in nearby fields. In creek site 1 (Fig. 2), 11 baiting periods (from 7 August to 1 October) during 2002 yielded *P. capsici*. Detection of the pathogen continued for an additional three years despite a nearby crop rotation of potato, soybean, and corn (Table 1). Due to the high number of baiting periods (10) with positive *P. capsici* identification at creek site 1 during 2003, potato plants in the nearby field were sampled, but the pathogen was not detected. At river sites 2-6, when a nearby field was planted to

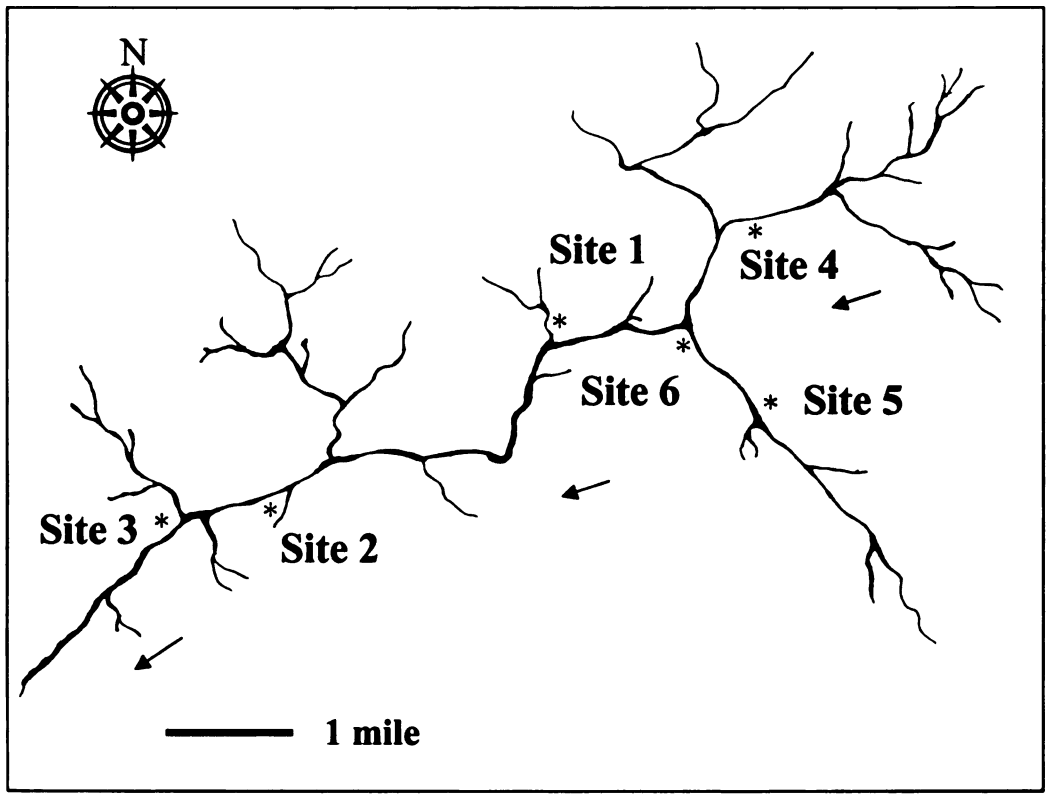


Figure 2. Schematic of six sites along a river and creek system of SW Michigan which were monitored for *P. capsici* infestation during the growing seasons of 2002 through 2005. Site 1 is a creek which flows into the river. Arrows indicate direction of water flow.

cucumber, *P. capsici* disease symptoms were observed and included fruit with water-soaked lesions and pathogen sporulation (Table 1).

Ponds

Phytophthora capsici was detected in a naturally-fed pond (Site 1) and a pond fed by a deep well (Site 2). In a second well-fed pond (Site 3) with nearby field sites historically infested with *P. capsici*, the pathogen was not detected. Baiting in the naturally-fed pond (Site 1) was initiated late in the 2002 growing season in response to crop loss in an adjacent field from *P. capsici*. The pathogen was detected immediately after bait initiation and throughout the remainder of the growing season. Yellow squash was planted in the field nearby the monitoring site in 2002, snap bean in 2003, and non-host crops were planted in the field for the remaining 2 years of the study. *Phytophthora capsici* was isolated from infected yellow squash plants in 2002 and from snap beans in 2003 (Table 2). No *P. capsici* was recovered from sampled carrots in 2004 (Table 2). Well-fed pond site 3 had just a single baiting period with positive *P. capsici* detection. It is likely that this infestation occurred as a result of an influx of run-off water from a neighboring field with diseased zucchini following an irrigation event.

Ditches

Limited data were collected from ditch sites used for irrigation. In 2004, monitoring was initiated in response to widespread and severe disease in a hand-harvested cucumber field in SE Michigan (Lenawee Co.). The detection of *P. capsici* at this site represents the first confirmation of this pathogen in water used for irrigation in SE Michigan. Despite our interest in continuing to monitor this site in 2005, the grower declined. The 2005 ditch site represented a culvert that received run-off from a number

of fields, some with a known history of *P. capsici* infestation. The pathogen was detected immediately following baiting initiation and throughout the remainder of the growing season (Table 3).

Compatibility Types

For all monitoring sites where more than one *P. capsici* isolate was obtained, both A1 and A2 CTs were typically detected (Tables 4, 5, 6). In several sites, the ratio of A1 to A2 was nearly 1:1 when totaled over the monitored years. At sites where the ratio was not close to a 1:1, the A1 CT occurred more frequently when examined across the isolate totals for a particular site (Tables 4, 5, 6).

Mefenoxam Sensitivity

The SW Michigan region (Allegan Co.) surrounding the river system has a history of pickling cucumber production (>40 years) and phenylamide fungicide use for disease management. Each year, river sites yielded *P. capsici* isolates of each of the mefenoxam sensitivity categories (S, IS, and I) (Table 4). Approximately half of the *P. capsici* isolates from the river sites in 2002 were intermediately sensitive with one quarter of the isolates insensitive (Table 4). Most isolates from 2003 and 2004 were insensitive (Table 4). In the last year of monitoring (2005), there was a change in the overall mefenoxam sensitivity of the pathogen population; most (88%) isolates were sensitive (Table 4).

The NW Michigan region (Oceana Co.) surrounding the two monitored ponds has a relatively short history of vegetable production with reports of low reliance on mefenoxam for disease management. Nearly all *P. capsici* isolates collected during 2002 to 2005 were sensitive (Table 5). In 2002, there was a single intermediately sensitive

Table 4. Summary of *P. capsici* baiting isolate characterization for SW Michigan (Allegan Co.) river system, 2002 to 2005.

Site	CT	Mefenoxam (Ridomil Gold) Sensitivity[a]												Total
		2002			2003			2004			2005			
		I	IS	S	I	IS	S	I	IS	S	I	IS	S	
1: Creek	A1	3	8	4	28	5	9	0	2	1	2	0	0	62
	A2	5	6	2	13	11	6	0	0	0	2	1	18	64
2: River	A1	0	0	0	0	0	1	-[b]	-	-	-	-	-	1
	A2	1	2	0	0	0	0	-	-	-	-	-	-	3
3: River	A1	0	0	3	2	0	0	-	-	-	-	-	-	5
	A2	0	0	0	1	0	0	-	-	-	-	-	-	1
4: River	A1	-	-	-	0	3	0	6	1	3	0	1	13	27
	A2	-	-	-	0	0	1	0	0	3	0	0	6	10
5: River	A1	-	-	-	0	0	0	4	2	0	0	0	5	11
	A2	-	-	-	0	0	1	2	2	1	0	0	4	10
6: River	A1	-	-	-	0	0	2	0	0	3	0	0	6	11
	A2	-	-	-	0	0	2	0	0	2	0	1	1	6
Total		9	16	9	44	19	22	12	7	13	4	3	53	211

[a]Indicates mefenoxam sensitivity rated as I (insensitive), IS (intermediately sensitive), and S (sensitive) to 100 ppm mefenoxam.
[b]Indicates that specific site was not monitored in that year of study.

Table 5. Summary of *P. capsici* baiting isolate characterization for NW Michigan (Oceana Co.) ponds, 2002 to 2005.

Site	CT	Mefenoxam (Ridomil Gold) Sensitivity[a]												Total
		2002			2003			2004			2005			
		I	IS	S	I	IS	S	I	IS	S	I	IS	S	
1: Naturally-Fed	A1	0	0	10	0	0	2	0	0	1	0	0	2	15
	A2	0	1	2	0	0	2	0	0	0	0	0	8	13
3: Well-Fed	A1	0	0	1	-[b]	-	-	-	-	-	-	-	-	1
	A2	0	0	0	-	-	-	-	-	-	-	-	-	0
Total		0	1	13	0	0	4	0	0	1	0	0	10	29

[a]Indicates mefenoxam sensitivity rated as I (insensitive), IS (intermediately sensitive), and S (sensitive) to 100 ppm mefenoxam.
[b]Indicates that specific site was not monitored in that year of study.

Table 6. Summary of *P. capsici* baiting isolate characterization for Michigan ditches (Lenawee and Oceana Cos.), 2004 and 2005.

Site	CT	Mefenoxam (Ridomil Gold) Sensitivity[a]						Total
		2004			2005			
		I	IS	S	I	IS	S	
1: Run-off SE Michigan (Lenawee Co.)	A1	3	9	2	-[b]	-	-	14
	A2	3	4	0	-	-	-	7
2: Culvert NW Michigan (Oceana Co.)	A1	-	-	-	0	1	6	7
	A2	-	-	-	0	0	7	7
Total		6	13	2	0	1	13	35

[a]Indicates mefenoxam sensitivity rated as I (insensitive), IS (intermediately sensitive), and S (sensitive) to 100 ppm mefenoxam.
[b]Indicates that specific site was not monitored in that year of study.

isolate collected (Table 5). The culvert ditch monitored in 2005 yielded 14 *P. capsici* isolates, 13 of which were sensitive to mefenoxam (Table 6). The run-off ditch monitored in 2004 in SE Michigan (Lenawee Co.) is surrounded by a region that has history of varied vegetable and agronomic crop production. Mefenoxam has been relied upon in recent years to manage vegetable diseases. The majority of the *P. capsici* isolates collected were intermediately sensitive with approximately 30% also insensitive (Table 6).

Water Temperature

Water temperatures were significantly correlated to positive *P. capsici* detection from the monitoring sites from 2002 to 2005 when analyzed using a non-linear quadratic polynomial regression (R^2=0.9408) (Fig. 3). Most (92%) of the baiting periods yielding positive incidence of *P. capsici* had water temperatures of 15-25°C (Table 7). *Phytophthora capsici* was not identified when water temperatures fell below 14°C or rose above 25°C (Table 7). Average water temperatures at the river monitoring sites during baiting periods with positive *P. capsici* detection were 17 to 19°C. At the pond sites, average water temperatures during baiting periods with *P. capsici* infestation were 20 to 22°C. The run-off and culvert ditch sites had average water temperatures of 21°C during baiting periods with positive *P. capsici* detection.

Rainfall

Rainfall data provided an approximation of the amount of free water present at water monitoring sites and nearby fields. However, these values did not reflect the total precipitation as grower irrigation data was not collected. For the river system, total

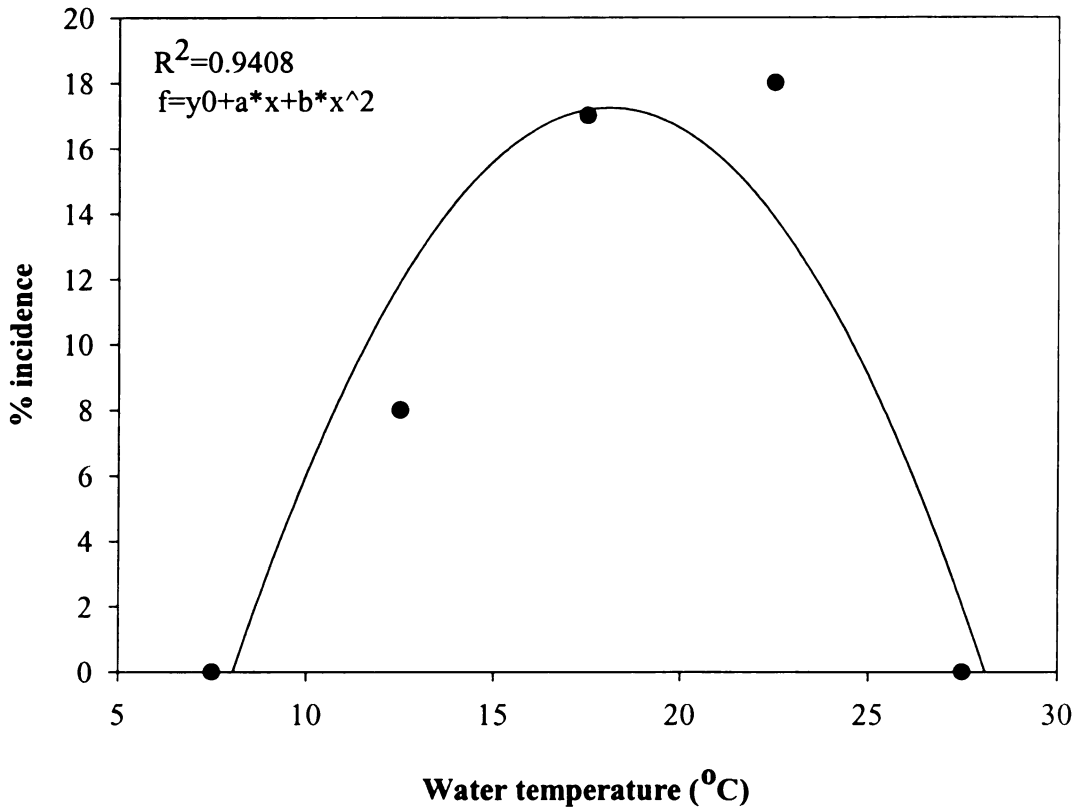
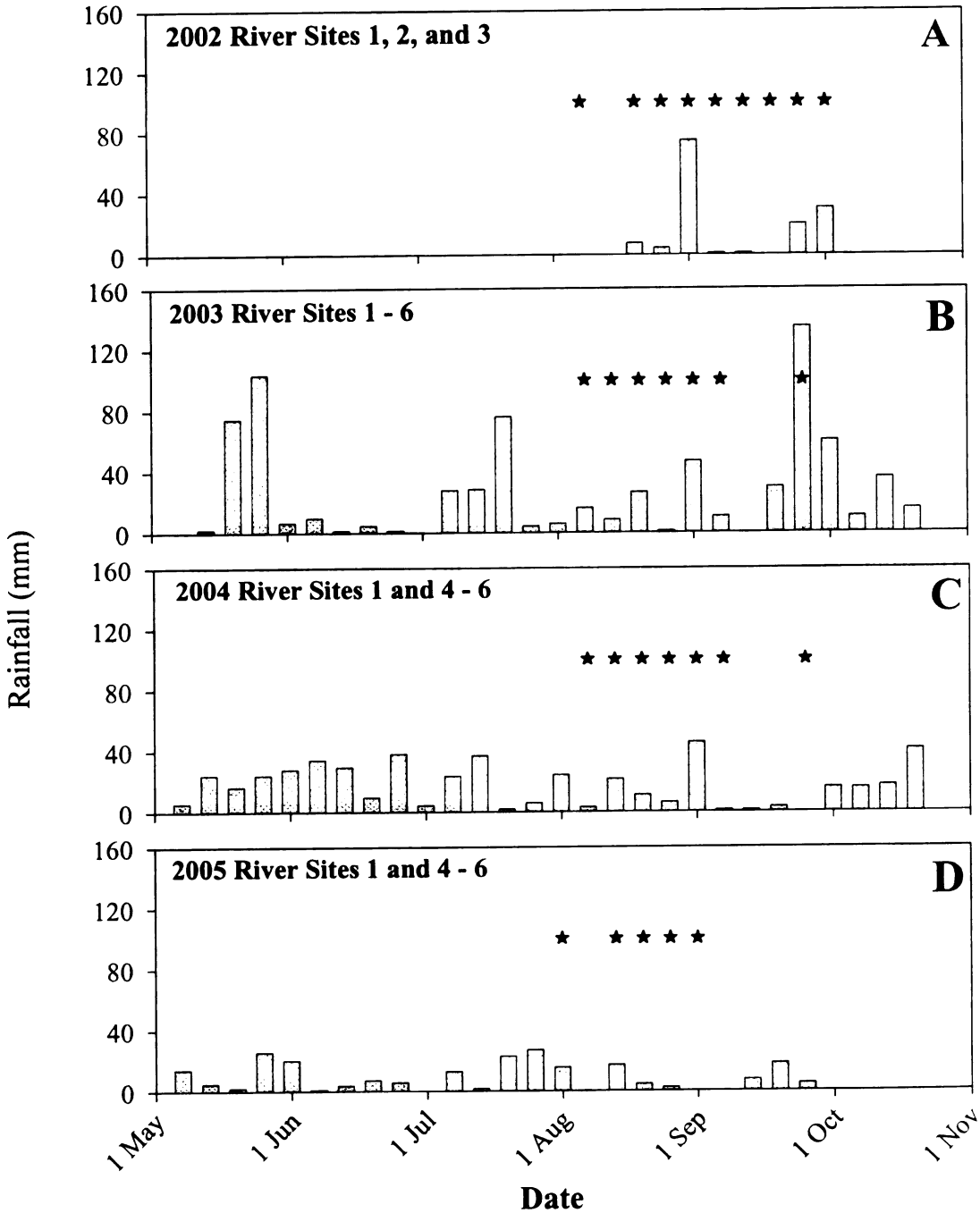


Figure 3. Non-linear, quatratic regression curve of average water temperature (°C) during baiting periods from all sites and years (2002 to 2005) and the percent incidence of *P. capsici* detection.

Table 7. Average water temperature (°C) during baiting periods from all sites and years (2002 to 2005) and the number of baiting periods with positive detection of *P. capsici*.

Temperature during baiting (oC)	Total # of baiting periods	# of baiting periods with *P. capsici*	% incidence
5-10	24	0	0
10-15	114	6	8
15-20	277	47	17
20-25	120	21	18
25-30	8	0	0

Figure 4. Rainfall (weekly totals) and *P. capsici* detection data for river monitoring sites in Allegan Co., Michigan in A) 2002, B) 2003, C) 2004, and D) 2005. Gray vertical bars indicate rainfall (mm). Stars indicate positive *P. capsici* detection.

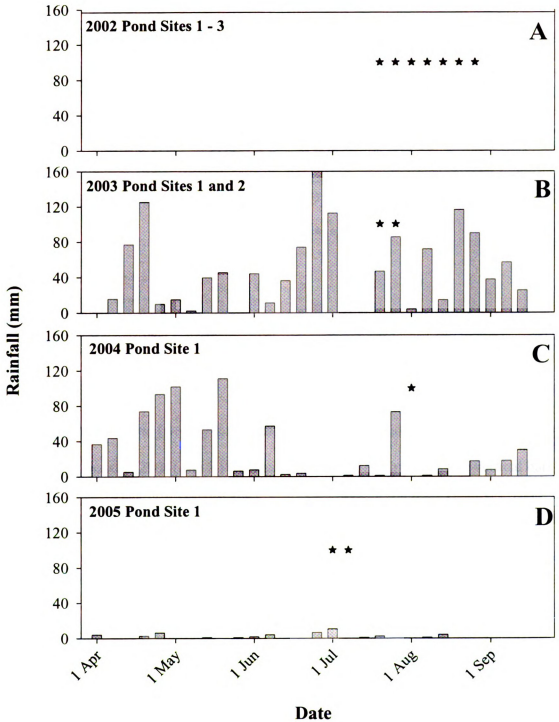

2002 River Sites 1, 2, and 3
A
2003 River Sites 1 - 6
B
2004 River Sites 1 and 4 - 6
C
2005 River Sites 1 and 4 - 6
D
160
120
80
40
0
Rainfall (mm)
1 May
1 Jun
1 Jul
1 Aug
1 Sep
1 Oct
1 Nov
Date

rainfall was greatest in 2003 and 2004 (Fig. 4). Rainfall from all years averaged <40 mm per week during baiting periods with positive *P. capsici* detection at river sites (Fig. 4). No significant rainfall was recorded in 2002 or 2005 at the monitored pond sites in NW Michigan (Figs. 5). Pond site rainfall was greatest in 2003 and 2004 (Figs. 5). Weekly rainfall was <30 mm during baiting periods with positive detection of *P. capsici* at the Lenawee Co. ditch (Fig. 6). No significant rainfall was recorded at the Oceana Co. culvert ditch during baiting periods in 2005 (Fig. 6).

AFLP Fingerprinting

AFLP analysis provided genetic confirmation of *P. capsici* identification to support our morphological determination. Genetic fingerprinting of *P. capsici* isolates collected from water monitoring sites from 2002 to 2004 did not indicate an association between similarity groups and specific locations, or years collected (Fig. 7). Three similarity groups (with >80% homology) were resolved from a cluster analysis of 56 isolates. Group I contained isolates from river, pond, and ditch sites from 2002 to 2004. The second group was comprised entirely of isolates from river sites 1, 3, and 4 from 2002 to 2004. The smallest of the similarity groups, Group III, contained isolates from the Lenawee Co. ditch site from 2004 (Fig. 7).

Figure 5. Rainfall (weekly totals) and *P. capsici* detection data for pond monitoring sites in Oceana county, Michigan, A) 2002, B) 2003, C) 2004, and D) 2005. Gray vertical bars indicate rainfall (mm). Stars indicate positive *P. capsici* detection.


2002 Pond Sites 1 - 3
A
2003 Pond Sites 1 and 2
B
2004 Pond Site 1
C
2005 Pond Site 1
D
Rainfall (mm)
0
40
80
120
160
1 Apr
1 May
1 Jun
1 Jul
1 Aug
1 Sep
Date

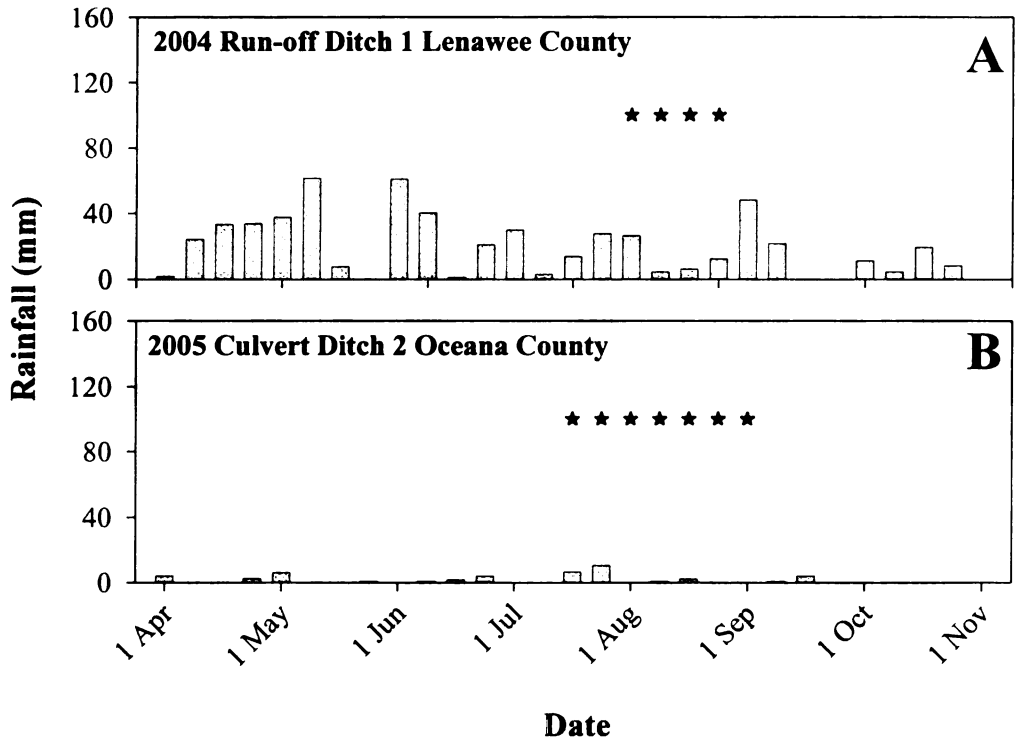


Figure 6. Precipitation (weekly totals) and *P. capsici* detection data for ditch monitoring sites in A) Lenawee (2004) and B) Oceana (2005) Cos., Michigan. Gray vertical bars indicate rainfall (mm). Black stars indicate positive *P. capsici* detection.

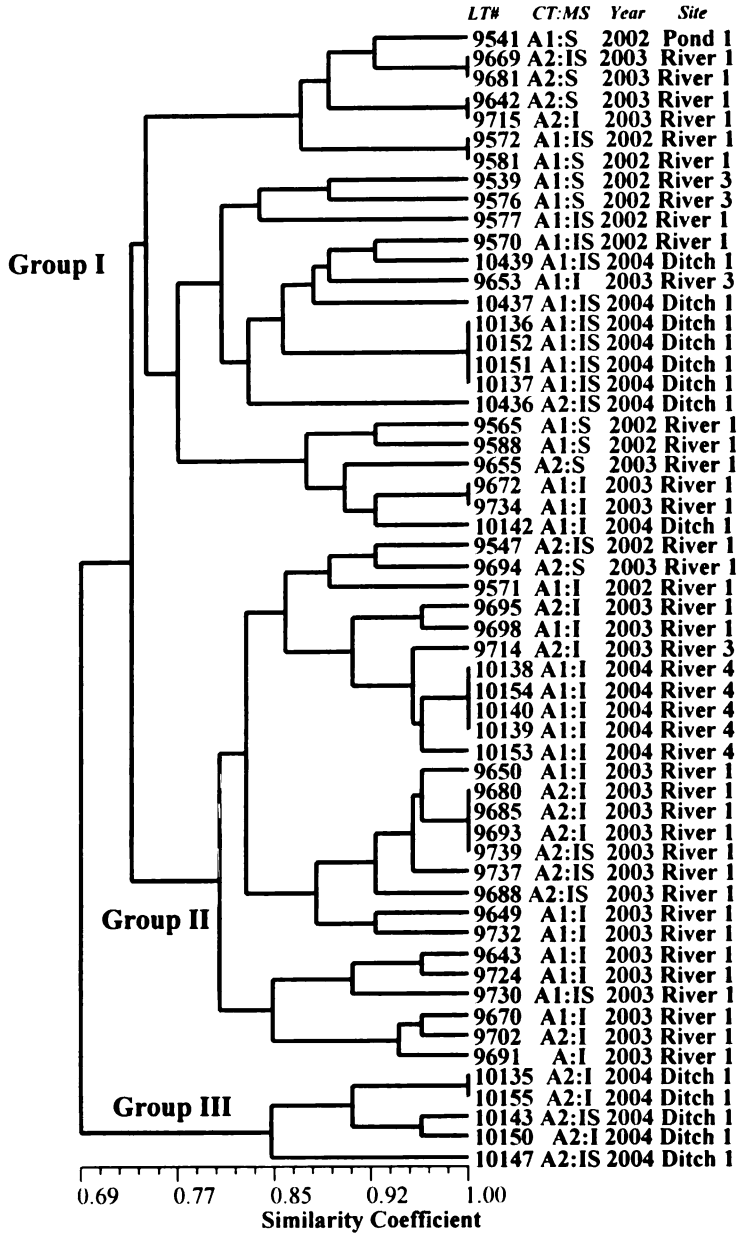


Figure 7. Cluster analysis of *P. capsici* isolated from Michigan surface water. Long term numbers (LT#) refer to culture numbers maintained in the laboratory of Dr. Mary Hausbeck at Michigan State University. Compatibility Types (CT) are either A1 or A2. Mefenoxam sensitivity (MS) characteristics are sensitive (S), intermediately sensitive (IS), or insensitive (I).

DISCUSSION

Phytophthora capsici has a broad host range and many disease outbreaks can be associated with fields with a known history of the pathogen. However, one particular outbreak and crop loss did not fit into the typical model. New pickling cucumber growers with established fields on land never planted to *P. capsici*-susceptible crops experienced widespread, uniform losses in the SW region of Michigan. Explanations including movement of the pathogen via equipment seemed inadequate for the sudden, widespread disease occurring on this new farm. The source of irrigation water (a river) was suspected as the potential means of pathogen movement. Prior to this research, the presence of *P. capsici* in Michigan irrigation water sources had not been reported.

Infested irrigation water is of concern to growers, especially those sharing water sources such as creeks and rivers. In our study, a Michigan river system in a region with a long (>40 year) history of cucumber production yielded 211 *P. capsici* isolates over a 4-year monitoring period of 6 sites. Initial *P. capsici* detection in the river typically followed observation of field disease and a single or a series of rain and/or irrigation events. *Phytophthora capsici* was most frequently detected at monitoring sites with a susceptible host crop in a nearby field, allowing for run-off of infested water to enter the river system. The pathogen was detected less frequently from sites with non-host crops in nearby fields. However, *P. capsici* was still present even after 1-3 years without a host crop nearby. This pathogen activity may indicate a longer range of movement of *P. capsici* within the river system or that there is activity of the pathogen within the environment outside of host plant tissue. In a study of the movement of a *Phytophthora* sp. (interspecific hybrid of *P. cambivora* and an unknown *Phytophthora* related to *P.*

fragariae) on alder in Bavaria, it was determined that once the pathogen was introduced into a river system upstream, it infected alders downstream (9). In addition, area nurseries that had high incidence of infected alder seedlings were found to be using water from infested river courses for irrigation (9). Due to the proximity of the Michigan river system to susceptible crop production, there are numerous potential sources of *P. capsici* infestation. Confirming movement of the pathogen in the river is difficult, as there was no consistent genetic similarity among isolates from specific sites. With a sexually active polymorphic population, we cannot state that isolates from upstream have, in fact, moved downstream using the baiting and AFLP techniques. However, we know that *P. capsici* is present in the surface water and that pathogen propagules likely move with water, as indicated in the Bavarian study (9).

Overhead irrigation with water infested with *P. capsici* is an effective means of field inoculation. A research plot at the Michigan State University Muck Soils Research Farm in Laingsburg, Michigan, was successfully inoculated with *P. capsici* by injecting zoospore and sporangial inoculum into the irrigation system. In just two inoculation/irrigation events, the plot was adequately inoculated, resulting in uniform and severe disease symptoms on established pickling cucumber plants (Lamour and Hausbeck, unpublished results). In the 6 years following initial inoculation of this research plot, infestation of *P. capsici* has been persistent. Susceptible crops planted in this plot have become diseased without additional pathogen inoculation.

The extensive use of phenylamide fungicides for disease management can render *P. capsici* field populations insensitive (10, 11, 12). Southwest Michigan has a long history (>15 years) of *P. capsici* disease incidence and use of phenylamide fungicides,

including mefenoxam. Northwest Michigan has a relatively shorter history of *P. capsici* infestation and use of phenylamides. In particular, mefenoxam application in the NW region has been limited and judicious. Characterization of populations of *P. capsici* collected from SW Michigan from 1997-2001 indicated much mefenoxam insensitivity (12). Isolates from NW Michigan were sensitive to the fungicide (11). This previous work serves as a historic baseline of population resistance to mefenoxam for our regions of investigation.

From the river sites in SW Michigan, there was a change in *P. capsici* isolate sensitivity to mefenoxam during the 2002 to 2005 growing seasons. In the first monitoring year (2002), most isolates were intermediately sensitive; in the following 2 years, approximately half the isolates were completely insensitive. The trend toward insensitivity was not observed in 2005 with all but 10% of isolates sensitive. Previously, a *P. capsici* population from SW Michigan did not revert from mefenoxam insensitive to sensitive during a 2-year crop rotation to non-host crops (12). The isolates collected in 2005 from River (creek) site 1 may have been introduced from a different source. In other studies with *Pythium aphanidermatum*, however, it has been noted that metalaxyl-resistant isolates have declined in number after metalaxyl use was eliminated for at least 3 years. The sensitive isolates of *P. aphanidermatum* then become more prevalent; however, not in numbers large enough to allow the use of metalaxyl (22).

Irrigating from ponds also poses a risk of introducing *P. capsici* infestation. Pond site 1 (naturally-fed) yielded *P. capsici* in each of the four years monitored despite the planting of non-host crops in the nearby field in 2004 and 2005. One of the two well-fed ponds yielded just one pathogen isolate over two monitoring years. Ponds fed by

deep wells may provide a safer option to growers interested in using surface water for irrigation with low risk of pathogen infestation, provided that run-off from nearby fields does not enter the holding pond. A run-off and a culvert ditch yielded *P. capsici* for nearly every period monitored. The ditches received direct run-off of water from numerous infected fields and so had a relatively high potential risk of infestation. Both ditch sites fed into water retention/pumping ponds used for irrigation.

Water temperature, zoospore concentration, and light may determine the length of time zoospores can continue to swim and remain viable (2, 3). When lupines were used to bait for *Phytophthora* spp. in cranberry irrigation reservoirs in New Jersey, a significant positive correlation was observed between water temperature and detection of *P. cinnamomi*; a negative relationship was observed for water temperature and *P. megasperma* (19). In our study, regression analysis determined that water temperatures were correlated with *P. capsici* incidence on pear or cucumber baits (R^2=0.9408). In addition, there appeared to be a lower temperature threshold (14°C) at which *P. capsici* was not detected. *Phytophthora palmivora* and *P. citrophthora* have been shown to remain motile in water at an optimal temperature of 17 and 12.5°C, respectively (2). Further laboratory studies may be needed to elucidate the relationship between water temperature and *P. capsici* zoospore activity. In Michigan, low water temperature typically occurs at a time when crops may not be established (early spring) or have been removed (early fall), thereby impacting the presence of *P. capsici* in surface water.

There was no association between *P. capsici* similarity groups and specific locations or years indicating that populations of the pathogen in the monitored river system were not unique or isolated to specific sites or years. *Phytophthora capsici* does

not appear to be over-wintering in the water sources, as fingerprints are not monomorphic across years at individual sites. In addition, positive detection of the pathogen never occurred prior to mid-July or after late-September, despite water monitoring efforts which began in April and ended in late October at most sites.

This study demonstrated the successful use of pears and cucumbers as *P. capsici* baits in Michigan surface water. The detection of *P. capsici* in water during the growing seasons of 2002 to 2005 appeared to be associated with both incidence of disease in nearby fields, and history of disease in these fields. The pathogen was detected in water when non-host crops were planted near monitoring sites. Monitoring water during the host crop-growing season indicated that irrigation water was often infested when the need to irrigate crops was highest, typically in late-July and August. The concurrence of positive *P. capsici* detection and need for field irrigation poses a great risk for dissemination of the pathogen. Characterization of isolates for sensitivity to mefenoxam and CT both assessed the diversity of the *P. capsici* population present in water and indicated that isolates insensitive to mefenoxam may be disseminated by irrigation. Water temperature exhibited some influence on the frequency of isolation of *P. capsici* from baits. There was, however, a low temperature threshold for pathogen presence which may also coincide with cropping and disease cycles. The reliance of surface water for irrigation of *P. capsici*-susceptible crops should be minimized, and if possible, avoided to prevent further pathogen spread.

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CHAPTER II

IDENTIFICATION AND CHARACTERIZATION OF *PHYTOPHTHORA CAPSICI* ON BEAN (*PHASEOLUS VULGARIS*) IN MICHIGAN

ABSTRACT

Commercial bean fields were observed with water-soaked foliage, stem necrosis, and overall plant decline along the surface water drainage pattern in 3 Michigan counties. All four fields have history of *Phytophthora capsici* infestation. Diseased tissue from stems, petioles, leaves, and pods collected from the fields yielded a total of 680 isolates of *P. capsici*. No isolates were recovered from bean roots. Koch's Postulates were completed with representative pathogen isolates collected in 2003, confirming *P. capsici* as the pathogenic organism. All representative isolates were also pathogenic on cucumber fruit. The majority of *P. capsici* isolates collected were sensitive to the fungicide mefenoxam and were of the A1 compatibility type. Under laboratory conditions, six select *P. capsici* isolates from snap bean (2003) were all pathogenic on twelve bean types, including soybean, causing water-soaked lesions, necrosis, and wilting. Disease on beans was rated (0=healthy, 5=dead) at 6 days post inoculation and ratings averaged ≥4.0. A group of 131 isolates from 2003 and 2004 were subjected to amplified fragment length polymorphism (AFLP) analysis to investigate genetic diversity among isolates and geographical populations. This is the first documentation of *P. capsici* on snap and wax beans in MI. Rotating beans with other susceptible hosts is not currently recommended.

INTRODUCTION

Phytophthora blight caused by the fungal-like Oomycete *Phytophthora capsici* [Leonian] causes foliar blighting, root, crown, and fruit rot on solanaceous and cucurbit crops worldwide (1, 11, 16). Disease symptoms may be first observed in the spring when seedlings exhibited damping-off following infection by overwintering oospores (18, 19). Symptoms can occur throughout the growing season as environmental conditions promote the development of wind and splash-dispersed sporangia and zoospores (5). Crown rot results in irreversible wilt and plant death. Lesions on the foliage and the fruit appear water-soaked and may quickly enlarge. Over time, infected fruit may exhibit a powdered-sugar appearance from accumulation of sporangia and/or oospores (17).

In Michigan, *P. capsici* is a limiting factor in cucumber, squash, zucchini, pepper, and pumpkin production despite adherence to recommended control strategies, including crop rotation, surface water management, and fungicide application (9). The fungicide mefenoxam was typically used to manage *P. capsici*, however, development of insensitivity of pathogen populations has limited its usefulness (17, 20, 21, 25). Reversion to mefenoxam sensitivity in field populations was not observed within a 2-year monitoring period when the fungicide selection pressure was removed (19, 20).

While beans are known to be susceptible to *Phytophthora* spp. such as *P. sojae*, *P. megasperma*, *P. nicotiana*, and *P. phaseoli*, they have not been included in the host range of *P. capsici* (5). Beans have historically been recommended as a suitable rotation crop with *P. capsici*-susceptible vegetables. Laboratory inoculations of *P. capsici* on beans, lima beans, and soybeans resulted in resistant plant responses in a 1967 study (22).

However, in 2000 and 2001, *P. capsici* was detected on lima beans in commercial fields in Delaware, Maryland, and New Jersey (2). In Michigan, snap beans were first diagnosed with *P. capsici* in 2003 from commercial fields with a history of zucchini cropping and *P. capsici* infestation (6, 9).

The pathogenicity and phenotypic characteristics of *P. capsici* isolates within field populations are diverse (12, 23, 24). The concept of host specificity of *P. capsici* has been explored; however, studies have been restricted to fewer than 30 isolates of limited geographical diversity (24, 27). In addition, these studies have relied on a single inoculation technique of delivering zoospores to the soil of seedling differentials (24, 27). Susceptibility of plants to *P. capsici* is greatly impacted by method of inoculation, inoculum density, and environmental conditions (14, 23).

The biology and epidemiology of *P. capsici* on bean (*Phaseolus* spp. and *Glycine max*) is not known. In Michigan and the north-Atlantic vegetable production regions, *P. capsici* is an important disease on many crops (2, 9). Once fields become infested with *P. capsici*, disease may occur in subsequent years when a suitable host is planted and when favorable environmental conditions occur. With the identification of a new host family (Leguminosae), which includes many common rotational crops, it is important to elucidate the etiology of this disease on bean for development of appropriate control strategies. Phenotypic characterization of *P. capsici* isolates from bean can indicate diversity of population and fungicide sensitivity, which may aid in management. Evaluation of the genetic structure of the pathogen population may also detect subdivisions among populations within a particular area and can reveal host or tissue specificity. Our research objectives included 1) confirming pathogenicity of *P. capsici*

isolated from beans, 2) characterizing the isolates through phenotypic and genotypic analyses 3) assessing the range of bean type susceptibility, and 4) comparing virulence of select isolates on cucumber fruit and soybean.

MATERIALS AND METHODS

Sample Collection and Pathogen Identification

Symptomatic and asymptomatic bean plants were collected from commercial bean fields in Michigan, including 2 fields (#1 and #2) in Oceana County (Co.) in 2003, 1 field from Cass Co. in 2004, and 1 field from Van Buren Co. in 2005. Fifty to 200 plants with a range of disease symptoms (from healthy to dead) were collected from each of the 4 fields. Most of the plant samples were taken from areas of the fields with evident disease symptoms of water-soaked foliage and wilting. Asymptomatic plants (approximately 25% of all plants collected) were also obtained for analysis, and were collected by moving through the field in an 'X' configuration and randomly harvesting bean plants.

Leaf, petiole, stem, crown, and root tissues from symptomatic and asymptomatic bean plants were excised, surface sterilized, and plated onto BARP (benomyl, ampicillin, rifampicin, and pentachloronitrobenzene)-amended, unclarified V8 juice agar plates (20). After 3 days of incubation at 24°C under fluorescent lighting at room temperature, microbial growth was assessed by microscopy. Cultures that were morphologically identified as *P. capsici* based on characteristics described in the *Phytophthora* spp. key by Waterhouse, 1963 (31) were transferred to new BARP plates and single-zoospore cultures were generated (17).

Koch's Postulates and Pathogenicity Screen

Single-zoospore (axenic) cultures of *P. capsici* were used to inoculate 10, 3-week-old snap bean plants ('HyStyle') with 7-mm diameter mycelial/sporangial agar plugs on leaf and petiole tissue. After symptoms developed, 5-7 days post inoculation (dpi), tissue at the margin of healthy and diseased was excised, surface sterilized, and plated onto BARP for incubation at 24°C under fluorescent lighting at room temperature for 3 days. Based on morphological characteristics (as stated above), the organism was identified as *P. capsici* and confirmation of pathogenicity on bean was completed. Bean plant inoculations to fulfill Koch's Postulates were conducted two times.

Further pathogenicity testing was conducted on cucumber fruit that were produced at the Plant Pathology Farm at Michigan State University, East Lansing, MI, in a field with no history of *P. capsici* infestation. Fruit were surface disinfested with sodium hypochlorite (5%), rinsed with sterile water, and dried under ambient laboratory conditions (21±2°C). A 7-mm mycelial/sporangial agar plug from each of the 20 *P. capsici* isolates from snap bean (Oceana Co. #1) was placed on the surface of intact fruit, covered with a microcentrifuge tube, and sealed to the fruit with petroleum jelly (7). Fruits were incubated in trays covered with plastic wrap to maintain high humidity. Disease was assessed by measuring 3 lesion parameters: 1) the diameter of the water-soaked region at the leading edge of the lesion, from this point forward referred to as lesion diameter; 2) diameter of the sporulating region (white and powdery) of the lesion, referred to as sporulation diameter; and 3) density of pathogen sporulation (relative number of sporangia/cm^2 fruit surface) rated as 0=none, 1=light, 2=moderate, 3=high, referred to from this point forward as sporulation density. Fruit tests were conducted two

times and results averaged. All fruit disease ratings were taken at 3 days post inoculation. Means were separated and data was analyzed using Tukey's Studentized LSD Test at α=0.05 in SAS (SAS Institute, Cary, NC, USA).

Phenotypic Characterization

Compatibility Types: Seven-mm diameter mycelial/sporangial agar plugs from the edge of expanding single-zoospore-derived cultures were placed at the center of V-8 plates 2 cm away from plugs of isolate OP97 (A1) and SP98 (A2), and incubated at 24°C in the dark for 7 to 10 days. OP97 and SP98 are *P. capsici* isolates collected from Michigan cucurbits which are maintained in the laboratory of Dr. Mary Hausbeck of the Department of Plant Pathology at Michigan State University. OP97 and SP98 have been confirmed to be A1 and A2 using American Type Culture Collection standards. CTs were determined by assessing the presence or absence of oospores between the 2 agar plugs by microscopy (19).

Mefenoxam Sensitivity: Seven-mm diameter mycelial/sporangial agar plugs from the edge of expanding single-zoospore-derived cultures were plated onto V-8 agar and V-8 agar amended with 100 ppm mefenoxam. Plates were incubated at 24°C for 3 days, and colony diameters measured. Percent growth of an isolate on amended agar was calculated by subtracting the inoculation plug diameter (7 mm) from the diameter of each colony and dividing the average diameter of the amended plates by the average diameter of the unamended control plates. Isolates were assigned mefenoxam sensitivities based on percent growth of the control. An isolate sensitive (S) to mefenoxam was <30% of control, an intermediately sensitive (IS) isolate was 30-90% of control, and an insensitive

(I) isolate was >90% of the control (21). All tests were conducted twice and results averaged.

Susceptibility of Bean Types to *P. capsici*

Twelve bean types (Table 1) were either purchased from a commercial seed supplier or were donated by grower cooperators. Three-week-old bean plants were inoculated with six *P. capsici* isolates. Isolates 9948, 9951, and 9974 obtained from Oceana Co. #1 and isolates 9986, 9988, and 9989 from Oceana Co. #2 were chosen. The 6 isolates selected were representative of unique phenotypic groups based on their symptom morphology on cucumber fruit and on V-8 agar. Mycelial agar plugs (7-mm diameter) of 1-week-old, single-zoospore-derived cultures were placed onto bean leaf and petiole tissue, and covered with microcentrifuge tubes sealed with petroleum jelly. One plug was applied to the middle portion of the surface of a leaf and a second to the portion of the petiole which joins the main stem on each plant. Individual plants were enclosed in a sealed plastic bag with a wet paper towel to maintain high humidity. Plants were incubated under ambient laboratory conditions at 21±2°C and rated for disease 6 dpi. The disease rating for leaves was: 0=no disease, 1=<25% necrotic, 2=<50% necrotic, 3=50% necrotic, 4=>50% necrotic, and 5=100% necrotic. The disease rating for petioles was: 0=no disease, 1=<50% petiole necrotic, 2=100% petiole necrotic, 3=100% petiole necrotic and 25% leaf necrotic, 4=100% petiole necrotic and 50% leaf necrotic, and 5=100% petiole and leaf necrotic. Each test was conducted three times. Disease ratings were analyzed by mean separation and significant differences were distinguished using Tukey's Studentized LSD (α=0.05) in SAS (SAS Institute, Cary, NC, USA).

Virulence Comparison of Select Isolates

Six select *P. capsici* isolates (listed above) were compared for virulence on 3-week-old soybean plants. A randomized complete block design with four repetitions of each of the six *P. capsici* isolates plus control was used. Inoculations and disease ratings were made as described above for bean plants. The isolates were also compared for virulence on cucumber fruit. Methods of inoculation and disease rating for cucumber fruit are described above in pathogenicity screening. Soybean plant and cucumber fruit disease ratings were analyzed by mean separation and significant differences were distinguished using Tukey's Studentized LSD (α=0.05) in SAS (SAS Institute, Cary, NC, USA).

DNA Extraction and AFLP Analysis

Genomic DNA was extracted from approximately 10 mg of freeze-dried *P. capsici* mycelium using DNeasy® mini-kits (Qiagen, Valencia, CA., USA). Mycelium was grown in antibiotic-amended V8 broth and DNA was quantified on an agarose gel with known standards. EcoRI was obtained from Invitrogen (Carlsbad, CA., USA), while MseI and T4 DNA ligase were obtained from Takara (Madison, WI., USA). EcoRI and MseI adapters and primers for ligation and amplification reactions were obtained from Integrated DNA Technologies (Coralville, IA, USA). Sequence information for the adapters and primers is published in Vos et al. (30). The fluorescently labeled primers were obtained from Proligo (Boulder, CO, USA) and were comprised of the EcoRI core sequence with and without selective nucleotides and either the WellRED D4-PA label (Proligo) or the WellRED D3-PA label at the 5' end.

Restriction, ligation, preamplification, and selective amplification reactions were carried out as described by Habera et al. (8). Fluorescent products from the selective

Table 1. Bean types included in laboratory *P. capsici* pathogenicity screen.

Genus species	**Cultivar**
Phaseolus lunatus	'Bush Lima'
Phaseolus vulgaris var. *humilis*	'Fordhook Standard' 'Bush Tenderpod' 'Bush Contender'
Phaseolus vulgaris var. *vulgaris*	'Pole Bean' 'Kentucky Wonder Pole'
Phaseolus vulgaris	'Cranberry Soup Bean' 'Gold Mine Wax' 'Black Turtle' 'Blue Lake White Seed'
Glycine max	Soybean

amplifications were analyzed on a CEQ™ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA) using the manufacturer's protocols.

RESULTS

All bean fields sampled had history of *P. capsici*-susceptible crop production and history of disease on those crops due to *P. capsici*. Overall symptoms and field disease pattern on snap and wax beans included localized pockets of plant wilting and death typically along the surface water drainage pattern (Fig. 1A-D). Symptomology included water-soaking, necrosis, and wilting of stem, petiole, and leaf tissue (Fig. 2). In 2004 and 2005, symptomology also included water-soaking, necrosis, shriveling, and pathogen sporulation on pods (Fig.3). Disease symptoms were not observed on roots and *P. capsici* was not isolated from excised root tissue.

The Oceana Co. fields yielded 49 *P. capsici* isolates (Table 2). Most (>85%) of the *P. capsici* isolates collected from snap bean in this county were from stem tissue (Table 3). Leaf symptoms included water-soaking and wilting (Fig. 2A,B). Pod tissue, in 2003, did not exhibit disease symptoms; asymptomatic pods did not yield *P. capsici*. In August 2004, disease symptoms similar to those from 2003 were observed in one commercial snap bean field in Cass Co., Michigan (Fig. 1A,B). Extensive field sampling resulted in the collection of 227 *P. capsici* isolates (Table 2). Stem, leaf, and pod tissues (Fig 3A,B) all yielded *P. capsici*, with most of the isolates from stem tissue (Table 3). This was the initial finding of *P. capsici* on bean pod tissue in this Michigan study. In August 2005, approximately 300 acres of yellow wax beans were identified with disease caused by *P. capsici* in Van Buren Co. in August 2005. Field symptoms of localized

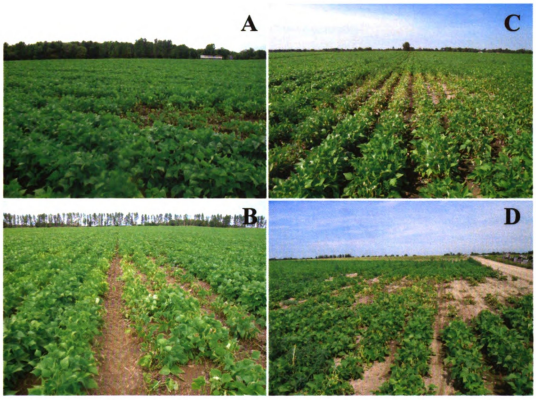


Figure 1. Field symptoms of *P. capsici* on bean. A, B) Snap bean field from Cass Co., Michigan in August 2004. C, D) Yellow wax bean field from Van Buren Co., Michigan in July 2005. A-D) Note localized circular pattern of plant decline and death, and association of plant decline with the surface water drainage pattern. (Images in this dissertation are presented in color).

Figure 2. Symptoms of *P. capsici* on bean leaves, petioles, and stems. A) Snap bean, 'HyStyle,' from Oceana Co., Michigan, 2003. Note water-soaking on leaves. B) Snap bean, 'HyStyle,' leaf from Cass Co., Michigan, 2004. Note water-soaked leaf symptoms observed on leaf underside. C-G) Yellow wax bean, 'Sunrae,' from Van Buren Co., Michigan, 2005. C) Circular necrotic lesion observed from leaf underside. D) Dark brown, necrotic leaf and petiole lesions. E) Necrotic symptoms spreading from junction of leaf and petiole. F) Brown, necrotic stem symptoms. G) Close up of stem lesion with pathogen sporulation on necrotic tissue. (Images in this dissertation are
presented in color).

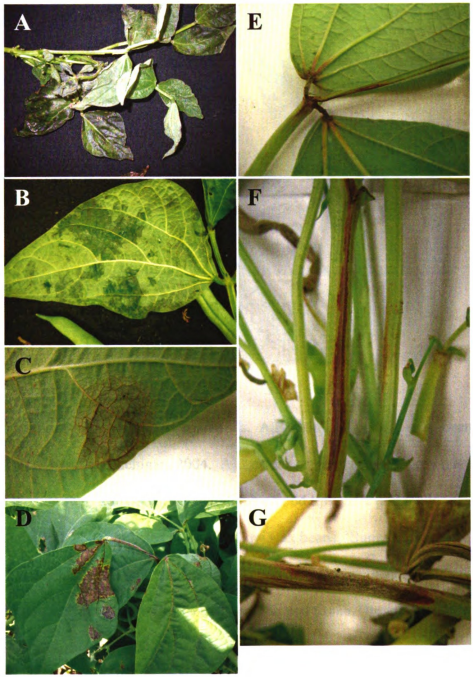

A
B
C
D
E
F
G

Figure 3. Symptoms of *P. capsici* on bean pods. A,B) Infected pods of snap bean, 'HyStyle', from Cass Co., Michigan, 2004. A) Lesions on pods are dark brown and sunken. B) Note brown, water-soaking symptoms on pod tip where pod made contact with the soil. C,D) Infected pods of yellow wax beans, 'Sunrae,' from Van Buren Co., Michigan, 2005. D) Close up of infected pod tip. Note water-soaking and white pathogen sporulation on surface of necrotic pod tissue. (Images in this dissertation are presented in color).

Table 2. *Phytophthora capsici* isolates identified from commercial beans in Michigan from 2003 to 2005. Compatibility Type (CT) and mefenoxam sensitivity are characterized for each isolate.

Year	County	Bean Type	Total # of Isolates	CT[a]	Mefenoxam (Ridomil Gold) Sensitivity[b]			Total
					I	IS	S	
2003	Oceana Field 1	'HyStyle' green snap bean	20	**A1**	0	1	13	14
				A2	0	0	6	6
2003	Oceana Field 2	'HyStyle' green snap bean	29	**A1**	0	3	14	17
				A2	1	1	10	12
2004	Cass	'HyStyle' green snap bean	227	**A1**	5	39	8	52
				A2	40	60	75	175
2005	Van Buren	'Sunrae' yellow wax bean	404	**A1**	92	98	73	263
				A2	31	70	40	141
		Total	653		169	272	239	680

[a]Indicates compatibility type (A1 or A2) of isolate.
[b]Indicates isolate sensitivity to the fungicide mefenoxam: insensitive (I), intermediately sensitive (IS), and sensitive (S).

Table 3. Analysis of *P. capsici* isolates identified from commercial bean fields in 3 Michigan counties during 2003 to 2005 by specific plant tissues. No *P. capsici* was identified on root tissue.

Year/County	**Total # of plants sampled**	**Total # of *P. capsici* isolates**	**# of *P. capsici* isolates from specific plant tissues actual # (% of total)**		
			stem	**leaf**	**pod**
2003/Oceana Field 1	50	20	17 (85%)	3 (15%)	0
2003/Oceana Field 2	50	29	29 (100%)	0	0
2004/Cass	100	227	93 (41%)	59 (26%)	75 (33%)
2005/Van Buren	200	404	137 (34%)	43 (11%)	224 (55%)

plant decline were similar to those observed in 2003 and 2004 (Fig. 1C,D). Sampled wax beans yielded 404 *P. capsici* isolates (Table 2) from stem, leaf, and pod tissues (Figs. 2C-G and 3C,D). Symptoms on wax bean pods were much more severe and frequent than those observed on snap bean in 2004. More than half (55%) of the isolates collected were from infected pods (Table 3).

The average air temperature in Oceana and Cass Cos. during the late-July 2003 and early-August 2004 bean epidemics was 18°C. In late-July 2005, the average air temperature in Van Buren Co. during the period of wax bean infection was 23°C. Under laboratory conditions, *P. capsici* disease on bean could not be initiated at temperatures >23°C. For this reason, all inoculations on bean plants were carried out under ambient laboratory conditions (21±2°C).

Phytophthora capsici was positively identified based on morphological characteristics (26) and AFLP analysis. Other organisms that were isolated from both symptomatic and asymptomatic bean plants included *Pythium* spp., *Alternaria* spp., *Fusarium* spp., and *Phytophthora nicotiana*. *Fusarium* spp. were particularly common from root tissue isolations. On symptomatic plants with positive detection of *P. capsici*, other organisms were rare.

Koch's Postulates and Pathogenicity Screen

Snap bean isolates from Oceana Co. #1 and #2 were pathogenic on 'HyStyle' snap beans and on 'Fanfare' cucumber fruit. Disease symptoms on laboratory inoculated bean plants were identical to those observed in commercial fields: water-soaked foliage, petiole and stem necrosis, and wilting. Cucumber fruit symptoms from bean isolates included water-soaking, and pathogen sporulation (Fig. 4) and were identical to symptoms from fruit

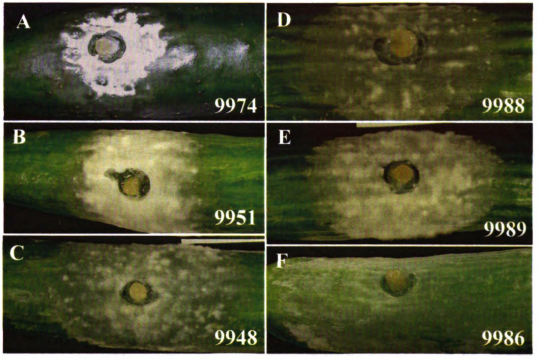


Figure 4. Variation in cucumber fruit lesions (at 4 days post inoculation) from inoculation with 6 select *P. capsici* isolates from Oceana Co. Michigan snap bean, 2003. A-C) Lesions from *P. capsici* from Field 1. Lesions vary from A) sunken and covered in dense pathogen sporulation, which remains appressed to fruit surface, to B) fluffy with dense aerial mycelium. C) Lesion of large diameter with fluffy aerial mycelium. D-E) Lesions from *P. capsici* isolates from Field 2. Lesions with D) moderate and E) dense aerial mycelium. F) Lesion of largest diameter (when compared to A-E) and moderate appressed pathogen sporulation. Numbers in the lower right-hand corners of images indicate the isolate number. (Images in this dissertation are presented in color).

inoculation with cucumber isolates. Phenotypic variation in sporulation density and aerial mycelium morphology was observed on inoculated cucumber fruit (Fig. 4). Isolate 9974 produced fruit lesions that were water-soaked and sunken with dense, appressed pathogen sporulation (Fig. 4A). Isolates (9951 and 9989) (Fig. 4B,E) produced fruit lesions with aerial mycelial and sporangial production that appeared white and fluffy, while lesions from isolates 9948, 9988, and 9986 (Fig. 4C,D,F) were of larger diameter and exhibited ample pathogen sporulation with limited aerial mycelium.

Phenotypic Characterization

Isolates of *P. capsici* from Oceana Co. #1 and #2, and Van Buren Co. were primarily of the A1 CT (Table 2). Cass Co. isolates, however, were more than 75% A2 CT (Table 2). Greater than 80% of *P. capsici* isolates from Oceana Co. #1 and #2 were sensitive to the fungicide mefenoxam (Table 2). Fewer isolates from Cass and Van Buren Cos. were sensitive to mefenoxam; intermediately sensitive isolates comprised >40% of the population sampled, with 20-32% insensitive to mefenoxam (Table 2). Cass and Van Buren Cos. have a history of pickling cucumber production, *P.capsici* incidence, and frequent mefenoxam use.

Susceptibility of Bean Types to *P. capsici*

All 12 bean types (Table 1) inoculated with six select *P. capsici* isolates (listed above) resulted in water-soaking and necrosis of both leaf (Fig. 5) and petiole tissue. Bean types exhibited equal susceptibility to *P. capsici*; no significant differences in disease were determined among bean types (data not shown). On petioles, disease spread rapidly into leaves and stems, resulting in necrosis and plant wilting in just 6 dpi. Under

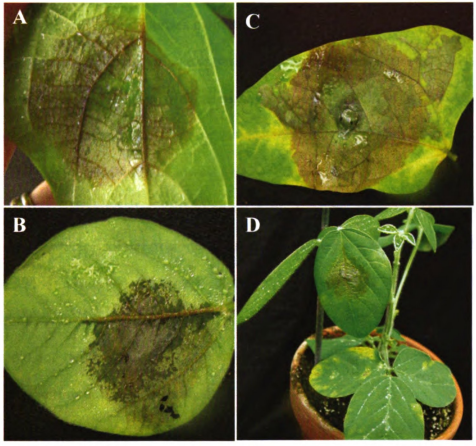


Figure 5. Localized water-soaked foliar lesions on bean types caused by *P. capsici* from Oceana Co., Michigan snap bean in 2003 (Field 1). Lesions are depicted at 5 days post inoculation under laboratory conditions. Symptoms of localized water-soaking and necrosis are evident on A) 'Bush Tenderpod,' B) soybean (leaf underside), C) 'Bush Lima,' and D) soybean (leaf surface). (Images in this dissertation are presented in color).

experimental conditions, the pathogen did not sporulate on bean tissues. It was important to maintain the inoculated plant under conditions of high humidity to initiate disease.

Virulence Comparison of Select Isolates

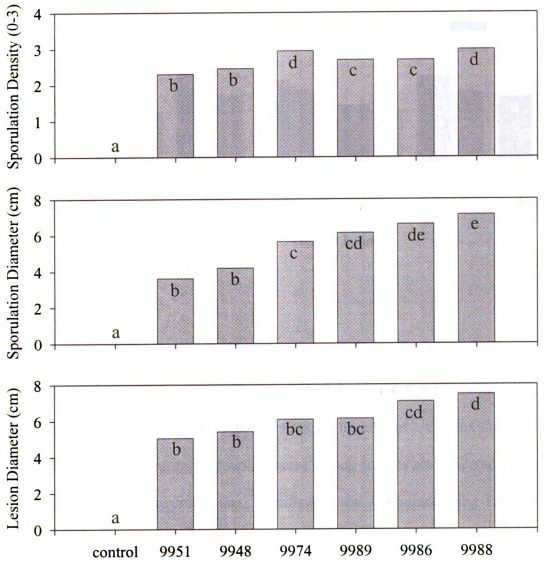
On cucumber fruit, isolates exhibited significant differences for overall lesion diameter, sporulation diameter, and sporulation density (Fig. 6), however, all isolates were highly virulent. For each of these parameters, isolate 9988, from Oceana #2, was consistently the most virulent (Fig. 6). Isolates 9948 and 9951, both from Oceana #1, were consistently rated with the lowest average disease (Fig. 6).

All isolates were also virulent on soybean. Soybean petioles inoculated with the six select *P. capsici* isolates exhibited significantly different disease ratings. However, isolate 9948 (Oceana #1) was most virulent on soybean petioles, with isolates 9988 and 9989 (both from Oceana #2) exhibiting the least virulence (Fig. 7). Inoculated soybean leaves did not yield significant differences among the six *P. capsici* isolates (Fig. 7).

DNA Extraction and AFLP Analysis

Fingerprint analysis of 131 *P. capsici* isolates (from 2003 and 2004) from snap bean indicated that there was genetic similarity of isolates from individual counties and specific years. Groups I and II contain isolates from Cass County (Fig. 8). Within Group I there are 15 subgroups which exhibit 100% similarity based on AFLP analysis; in Group II, just 5 100% similarity subgroups (Fig. 8). Group III contains isolates from Oceana Co. #1 and #2 (Fig. 8) and is comprised of just 3 100% similarity subgroups (Fig. 8). Of the 24 Cass Co. isolates fingerprinted, 14 (58%) were unique (Fig. 8). There were 37 (35%) unique isolates from Van Buren Co. (Fig. 8). There does not appear to be a

Figure 6. Comparison of disease ratings (sporulation density, sporulation diameter, and lesion diameter) for 6 select *P. capsici* isolates from snap bean on cucumber fruit. Isolates 9951, 9948, and 9974 are from Field 1 Oceana Co., 2003. Isolates 9989, 9986, and 9988 are from Field 2 Oceana Co., 2003. Ratings were taken at 3 days post inoculation. Letters indicate significant differences in disease among isolates using Tukey's Studentized Range Test at α=0.05.


Sporulation Density (0-3)
4
3
2
1
0
a
b
b
d
c
c
d
Sporulation Diameter (cm)
8
6
4
2
0
a
b
b
c
cd
de
e
Lesion Diameter (cm)
8
6
4
2
0
a
b
b
bc
bc
cd
d
control
9951
9948
9974
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9986
9988
P. capsici Isolate

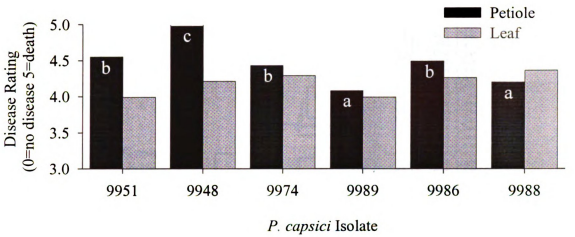

Figure 7. Average disease ratings of 6 *P. capsici* isolates from snap bean on soybean leaves and petioles. Isolates 9951, 9948, and 9974 were from Field 1, Oceana Co., Michigan, 2003. Isolates 9989, 9986, and 9988 are from Field 2, Oceana Co., Michigan, 2003. Significant differences in petiole disease among isolates are indicated by lower case letters within black vertical bars. Significance was determined using Tukey's Studentized Range Test at α=0.05. No significant differences were found in leaf disease among *P. capsici* isolates. Control plants exhibited no disease.

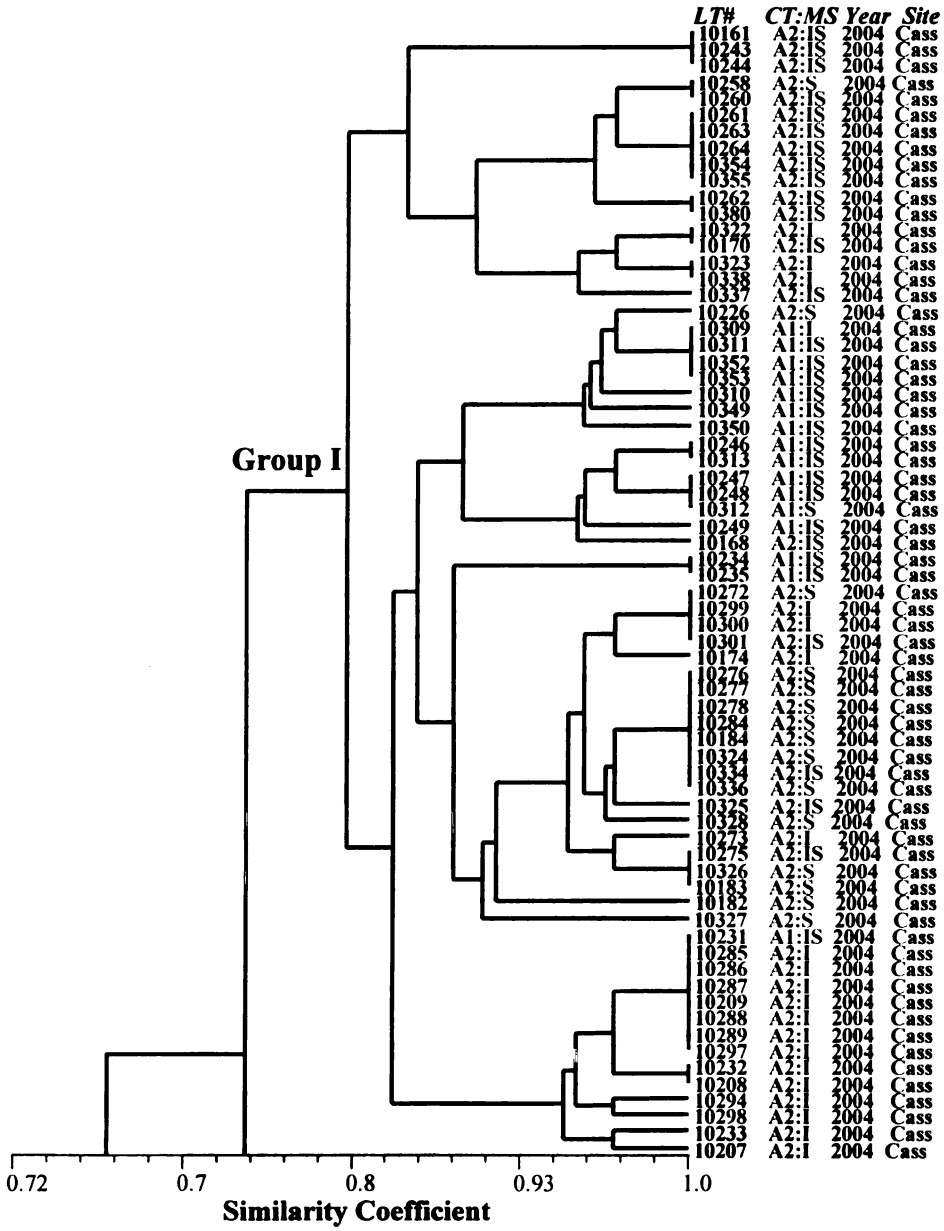

Figure 8. Cluster analysis of *P. capsici* isolated from Michigan snap bean. Long Term Numbers (LT#) refer to culture identification numbers. Compatibility Types (CTs) are either A1 or A2. Mefenoxam Sensitivity (MS) characteristics are sensitive (S), intermediately sensitive (IS), or insensitive (I).

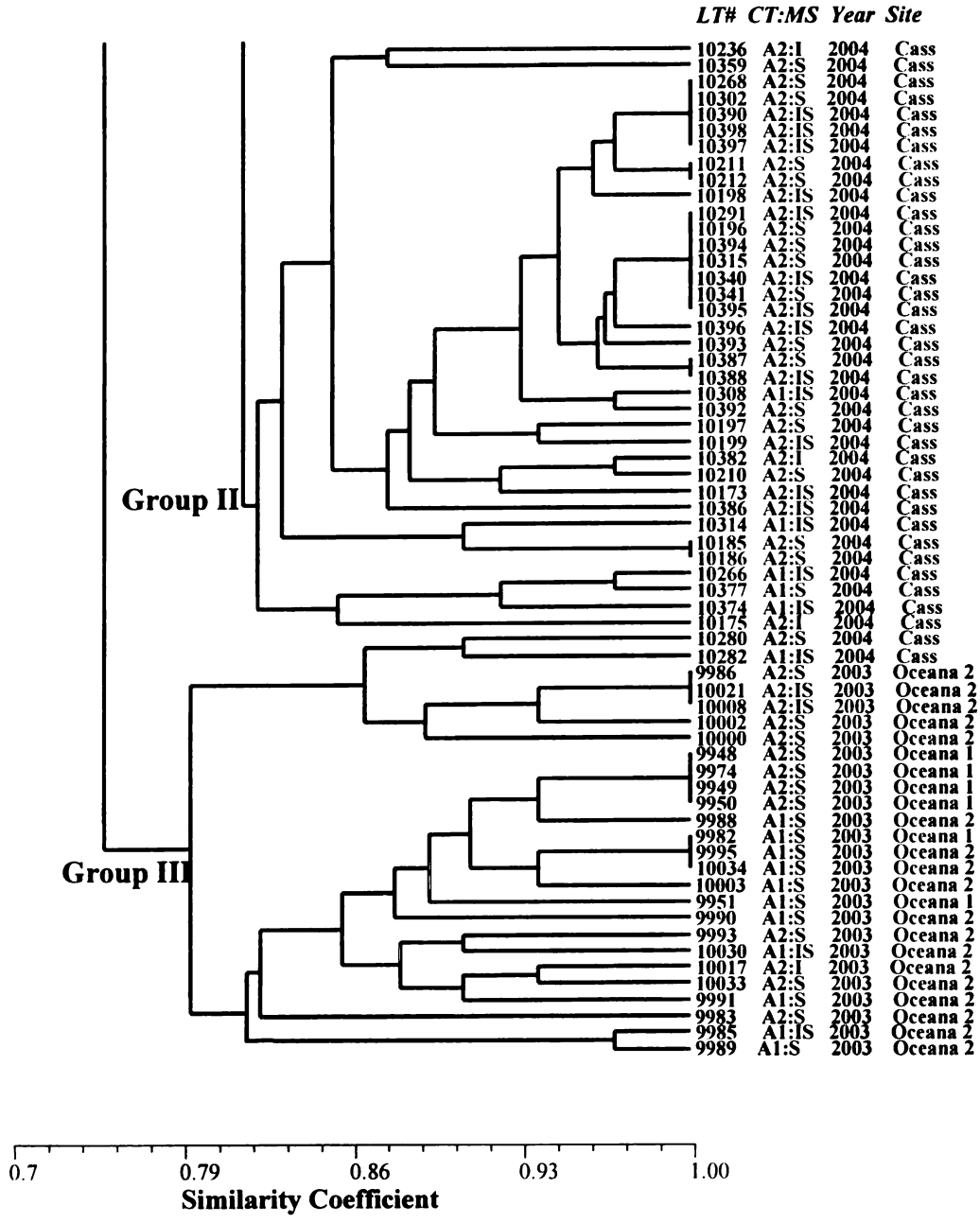

Figure 8. (continued).

Table 4. Bean plant tissue diversity of AFLP similarity groups of *P. capsici* isolates from Oceana and Cass Cos. Michigan in 2003 and 2004. Cluster groups exhibit approximately 80% homology. Similarity subgroups exhibit 100% homology based on AFLP analysis.

Cluster group	Similarity subgroup	Bean plant tissue			Total # of isolates in similarity subgroup
		pod	stem	leaf	
Group I	**1**	1	2	0	3
	2	2	0	0	2
	3	3	0	2	5
	4	2	0	0	2
	5	2	0	0	2
	6	1	1	0	2
	7	0	4	0	4
	8	0	2	0	2
	9	0	3	0	3
	10	0	2	0	2
	11	0	4	0	4
	12	2	6	0	8
	13	1	2	0	3
	14	6	2	0	8
	15	1	1	0	2
Group II	**1**	1	4	0	5
	2	0	2	0	2
	3	6	1	0	7
	4	0	2	0	2
	5	2	0	0	2
Group III	**1**	0	3	0	3
	2	0	4	0	4
	3	0	3	0	3

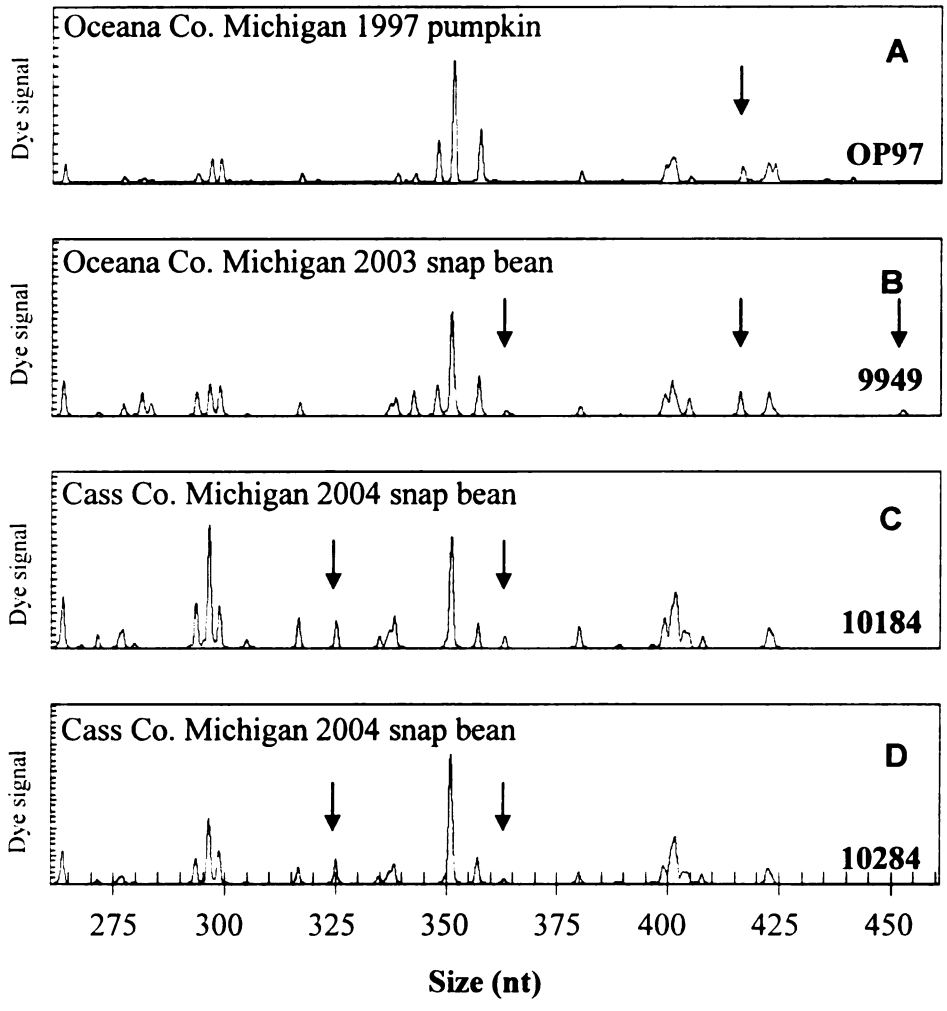

Figure 9. A-D) Segments of electropherograms from amplified fragment length polymorphism (AFLP) profiles of genomic DNA from four *P. capsici* isolates recovered from pumpkin and snap bean in Michigan. The AFLP profiles were produced using a Beckman-Coulter CEQ capillary genetic analysis system with the primer pair E-AC/M-CA and visualized using the CEQ fragment analysis software. Arrows indicate polymorphic markers of 325, 362, 415, and 452 nucelotides.

genetic subdivision in the population of *P. capsici* based on plant tissue or host. Each of the 23 100% similarity subgroups contains *P. capsici* isolates derived from multiple bean plant tissues (Table 4). Segments of electropherograms from AFLP profiles of genomic DNA indicate that a *P. capsici* isolate from pumpkin (Oceana Co., Michigan, 1997) has homology to isolates from snap bean from both Cass and Van Buren Cos., Michigan (Fig. 9). The selection of the 3 bean isolate profiles is representative of the total 131 fingerprinted.

DISCUSSION

The discovery of foliar and pod blighting of bean caused by *P. capsici* in Michigan is alarming. This pathogen poses a potential threat to a range of bean types within the family Leguminosae. Michigan produces approximately 2.2 million acres of beans (snap beans, dry beans, and soybeans) for both processing and fresh market, which may be susceptible (15). Our research goals were to phenotypcially and genotypically characterize *P. capsici* isolates from bean, and to assess *P. capsici* pathogenicity and virulence on additional bean types and cucumber fruit. From these objectives we can make inferences about the etiology of *P. capsici* on bean in Michigan.

The biology and epidemiology of *P. capsici* on cucurbit and solanaceous crops has become well documented in recent years (9, 17-21). In a spatiotemporal study of *P. capsici* in a Michigan commercial field, it was determined that a *P. capsici* epidemic on squash in 1999 was initiated by dormant oospores generated 5 years previously, despite rotation to corn and soybeans (18). The survival of pathogen oospores in soils for long periods of time indicates that seasonal introduction of the pathogen to the field is not

necessary for subsequent disease initiation. Therefore, the introduction of *P. capsici* in Michigan bean fields in 2003 to 2005 was likely due to resident soil populations.

Genetic fingerprinting of *P. capsici* isolates from snap beans in Michigan indicated the absence of host specificity or pathogen population subdivision based on host. Fingerprints of representative isolates from bean indicate homology with an isolate from pumpkin. In addition, all bean isolates were pathogenic on cucumber fruit causing symptoms identical to those isolated from cucurbit hosts. The snap bean isolates are not unique to bean, but are part of resident populations in these fields that have the potential to infect susceptible crops. It has been documented that *P. capsici* field populations include isolates with a range of diversity in phenotypic characteristics; however, genotypic diversity of the representative isolates from the Michigan counties in 2003 and 2004 was minimal. The pathogen populations were also not divided based on plant tissue; similarity subgroups contained isolates from bean pod, stem, and leaf tissue.

Phytophthora capsici spread is limited to short geographical distances, typically within a field (9), however, infested irrigation surface water may also provide a source of longer distance pathogen dissemination (9). History of susceptible cucurbit cropping in the studied bean fields indicates that *P. capsici* had become an annual concern with increasing severity (18, 19). Although *P. capsici* on bean presents new management challenges regarding crop rotation and control options, we are faced with an old pathogen on a new host enabling us to draw from recent studies on established host crops to further understand pathogen epidemiology.

Bean disease symptoms caused by *P. capsici* were only detected on above-ground plant tissues and root sampling yielded no *P. capsici*. In addition, root disease

was not detected when plants were inoculated with a zoospore soil drench under laboratory conditions; disease could only be attained by direct foliar inoculation. In some cases, plant root exudates are essential to chemotactically attract zoospores for encystment to occur (4, 5). Perhaps these exudates are limited or absent in bean roots and are therefore not as readily susceptible. Other environmental or plant resistance factors may also be involved in this phenomenon. During disease epidemics of cucurbit and solanaceous crops, the ideal range of air temperature for *P. capsici* is 25-30°C (9). Lower average air temperature was required for disease initiation and progress on bean hosts. Water is also an important factor in disease initiation and development for cucurbit and solanaceous hosts of *P. capsici*. In our study, bean plants inoculated with *P. capsici* required high humidity, however, little is known about the effect of water on the interaction of *P. capsici* on leguminous hosts under field conditions.

Compared to *P. capsici* disease on green snap beans in 2003 and 2004, disease on yellow wax beans in 2005 was markedly worse, with higher pod incidence and severity. The large-acreage yellow wax bean field was situated adjacent to green 'Romano' beans which exhibited no disease symptoms and did not yield *P. capsici* when sampled. Further examination of cultivar susceptibility is necessary to determine what factors may be responsible for *P. capsici* resistance in bean.

The presence of both CTs in all bean fields studied indicated that sexual recombination is likely occurring and with it, genetic variation within the population. In Oceana Co., isolates were predominantly sensitive to mefenoxam suggesting that although these 2 fields have had application of the fungicide in their management programs, use has been judicious. Lamour and Hausbeck studied a population of *P.*

capsici from Oceana Co. from 1998 to 2000 (19); all but one of the isolates examined were sensitive to mefenoxam. Isolates from Cass and Van Buren Cos. exhibited some sensitivity to mefenoxam, however a large percentage of the populations were intermediately and fully insensitive to the fungicide. The Cass and Van Buren Co. bean fields are known to have had extensive pickling cucumber production in their recent history. The fields were rotated to bean production as a means to avoid and/or limit crop losses to *P. capsici*, as pathogen populations were high and mefenoxam was having reduced efficacy.

Under laboratory conditions, all 6 snap bean *P. capsici* isolates were pathogenic on the 12 bean types inoculated, including soybean, indicating that most bean types grown in rotation with cucurbit or solanaceous crops may be at risk. Although some significant variation in virulence was observed among snap bean isolates on soybean petioles, all isolates were highly aggressive on both leaf and petiole tissues. This petiole response is likely the result of plant structural properties (all petioles are not of equal length and diameter). All isolates were also pathogenic on cucumber fruit and exhibited significant differences in disease severity.

Due to an increase in agricultural land infested with *P. capsici*, it is not economically viable for growers to simply remove fields from host crop production. Instead, they must recognize the biology of the pathogen and focus on factors such as cultural control and water management (9). Commercial bean production is, on average, less profitable than solanaceous or cucurbit crop production, for this reason, fungicide applications may not be an economically viable option for bean disease management. Future evaluation of bean cultivar resistance may provide additional options for

continued bean production in infested fields. Avoiding the introduction of *P. capsici* into uninfested or new fields is the key management strategy. In the past, rotation with a bean cultivar was an option for growers with infested fields, however, this practice is no longer recommended (9).

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CHAPTER III

DEVELOPMENT OF A DETACHED CUCUMBER (*CUCUMIS SATIVUS*) FRUIT ASSAY TO SCREEN FOR RESISTANCE TO INFECTION BY *PHYTOPHTHORA CAPSICI*

ABSTRACT

Cucumber fruit rot caused by *Phytophthora capsici* [Leonian] has become a persistent threat, reducing harvestable yields and rendering fields unsuitable for cucumber production. Identification and utilization of cucumber resistance to *P. capsici* would provide a viable disease management strategy for producers. Therefore, the objectives of this study were to develop a screen for testing detached cucumber fruit for resistance to *P. capsici* and to screen cucumber cultigens for resistance. Four *P. capsici* isolates (differing in their sensitivity to the fungicide mefenoxam and compatibility type) were compared for pathogenicity in 1999 and 2000. No significant differences were found among isolates. From 1999 to 2005, 432 cucumber cultigens (commercial cultivars and plant introductions) were grown according to standard practices at Michigan State University research farms in fields with no history of *P. capsici*. Commercially mature fruit were harvested, inoculated with *P. capsici*, and rated for lesion diameter, sporulation diameter, and density of sporulation. This is the first report using an unwounded fruit screen to analyze cucumber resistance to *P. capsici*. Although no fruit exhibited complete resistance to *P. capsici*, some cultigens showed limited pathogen sporulation.

INTRODUCTION

Fruit rot caused by the fungal-like oomycete pathogen, *Phyophthora capsici* [Leonian] has become a limiting factor for cucumber producers in Michigan and has been reported in several regions of the U.S. (6, 14). *Phytophthora capsici* has been found to infect a wide range of Solanaceous and Cucurbitaceous hosts worldwide (4, 5, 7). Cucumber fruit are especially susceptible. Fields of healthy-appearing vines have been bypassed, and harvested loads rejected at the processor due to widespread fruit rot (6). While the root and crown of the cucumber plant may become infected by *P. capsici*, infection of these tissues is primarily limited to the seedling stage. Fruit may become infected while in the field with the disease progressing during storage and transit; symptoms and/or signs become evident after delivery to the processor or retailer (6).

Pathogen infection is most obvious as white powdery growth resulting from sporangia produced in mass on the surface of infected cucumber fruit. In the presence of free water, sporangia release 20 to 40 motile zoospores capable of causing widespread plant infection in a short period of time (6). Oospores are over-wintering, long-term, primary survival structures that form when the A1 and A2 compatibility types (CTs) of *P. capsici* come together (2, 10). Both the A1 and A2 CTs were present in fields sampled in Michigan, and in other vegetable-producing states (1, 6, 8, 17). Germinating oospores infect the crop in the spring (11).

While crop rotation is the foundation of disease management, the long-term survivability of oospores limits the effectiveness of this strategy for *P. capsici* (6, 11, 17). Historically, growers have relied on the phenylamide fungicide mefenoxam to manage this disease, but resistant *P. capsici* isolates have been identified in Michigan, North

Carolina, and New Jersey (8, 9, 12, 14, 15). While other fungicides are available, they provide varying levels of control, and the required frequent applications increase production costs. In some cases, lengthy pre-harvest intervals limit fungicide use during fruit development (6).

Whenever available, genetic resistance is a preferred disease management tool. Frequently, screening for disease resistance is performed on seedlings because of space and time considerations, as has been done for cucumber with respect to several fungal pathogens (3). However, disease resistance in seedlings is not known to be correlated with fruit resistance, and comparative susceptibility of cucumber vines and fruits in the field suggests that more than one mechanism may be involved. In pepper, resistance to *P. capsici* is under different genetic control in different tissue types (19, 20).

Since the primary impact of *P. capsici* in the field is on fruit, a methodology for screening cucumber fruit for resistance to this pathogen is needed to evaluate cultigens. Our research objectives included 1) developing an effective methodology for screening cucumber fruit for resistance to *P. capsici* and 2) screening the fruit of *Cucumis sativus* PIs and commercial cultivars to identify resistance for breeding.

MATERIALS AND METHODS

Screening Methodology

Isolate maintenance and selection: Fresh cultures of each isolate were obtained by transferring agar plugs from long-term stock cultures (stored at 20°C in sterile micro-centrifuge tubes with 1-ml of sterile water and a sterile hemp seed) onto V-8 juice agar (16 g agar, 3 g $CaC0_3$, 160 ml unfiltered V8 juice, and 840 ml distilled water). Cultures were maintained at room temperature under continuous fluorescent lighting. Seven-mm diameter agar plugs from the margins of actively growing colonies were then transferred to new V-8 juice agar and maintained under the conditions indicated above.

Phytophthora capsici isolates OP97, SP98, SFF3, and SF3 were selected for comparison of cucumber fruit disease response in 1999 and 2000. Isolate notation refers to cultures maintained in the laboratory of Dr. Mary Hausbeck in the Department of Plant Pathology at Michigan State University (MSU). All four isolates were collected in Michigan from infected cucurbit crops. Isolates were characterized according to compatibility type (CT) and sensitivity to mefenoxam as described by Lamour and Hausbeck (8). Both OP97 (A1 CT) and SP98 (A2 CT) are fully sensitive to mefenoxam. Isolate SFF3 is an A2 CT and is insensitive to mefenoxam. Isolate SF3 is an A1 CT with intermediate sensitivity to mefenoxam. For each *P. capsici* isolate, disease was evaluated on 66 cucumber cultigens in 1999, and on 58 cultigens in 2000 at three days post inoculation (dpi). Disease parameters included sporulation density (0=none, 1=light, 2=moderate, 3=heavy sporulation), sporulation diameter (cm, white powdery sporulation, Fig. 1D), and diameter of water-soaking (cm, visible dark discoloration on the fruit surface, Fig. 1D). Data were subjected to analysis of variance using the PROC MIXED

procedure of SAS (SAS Institute Inc., Cary, NC) and means were compared using Fisher's Protected LSD test (P=0.05).

Inoculation evaluation: With the exception of the zoospore method (described below), all inoculation tests were conducted with the following parameters. A 7-mm-diameter plug of actively expanding *P. capsici* mycelia/sporangia was used to inoculate commercially-mature fruit (2.54-5.08 cm diameter) (18) that were placed in aluminum trays and covered with plastic wrap. A wetted paper towel was placed inside each incubation chamber to provide 100% relative humidity (Fig. 1B). Trays were exposed to constant overhead fluorescent lighting at 23-25 °C. Fruit were evaluated for disease 3 dpi by measuring three parameters: water-soaked lesion diameter, sporulation diameter, and sporulation density. In the first test, fruit were not wounded, and a sterile, plastic micro-centrifuge tube (with the cap removed) was placed over the agar plug and fixed to the fruit with petroleum jelly to maintain high humidity during initial infection (Fig. 1C). In a second test, fruit were wounded by penetrating the fruit surface with a sterile syringe needle at the site of inoculation. Plastic micro-centrifuge tubes were placed over the inoculum plug and sealed to the fruit surface as indicated in the non-wounded fruit test.

Placing micro-centrifuge tubes over mycelial/sporangial plugs was compared with inoculation without tubes in a third test. Unwounded fruit were inoculated, as described above, however sterile micro-centrifuge tubes were not placed over the mycelial/sporangial plugs during incubation. All other experimental conditions and disease evaluations were carried out as previously described.

Zoospore inoculum was prepared by placing a 7-mm-diameter plug of a 7-day-old culture in a sterile 1.5-ml plastic micro-centrifuge tube with 1-ml of sterile, de-

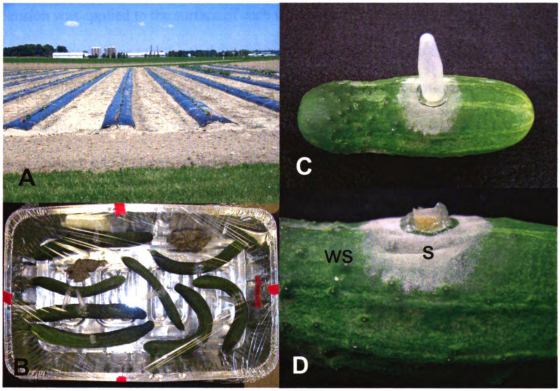

Figure 1. Cucumber fruit production and inoculation technique. A) Field production of cucumber fruit (10 days post planting) at Plant Pathology Farm, Michigan State University. B) Inoculation chamber for screening cucumber fruit for resistance to *P. capsici*. C) Inoculated cucumber fruit 3 days post inoculation (dpi). D) Close up of inoculated cucumber fruit 3 dpi (micro-centrifuge tubes removed for disease evaluation). Note water-soaking (ws) and pathogen sporulation (s). (Images in this dissertation are presented in color).

ionized water. The mixture was incubated at 4 °C for 20-min, followed by a 20-min incubation at 23-25 °C to induce zoospore release. A 50-µl droplet of zoospore suspension was applied to the surface of each unwounded fruit and covered with a sterile 1.5 ml micro-centrifuge tube (with the cap removed), and fixed to the fruit with petroleum jelly. All other experimental conditions and disease evaluations were carried out as described above. Each of the inoculation tests were conducted on 4 fruit per cultigen for each of 5 cultigens and were repeated four times.

Germplasm Screening

Cucurbit germplasm accessions (Plant Introductions or PIs) were provided by the United States Department of Agriculture Agricultural Research Service National Plant Germplasm System (USDA-ARS-NPGS), North Central Regional Plant Introduction Station (NCRPIS), Ames, Iowa. *Cucumis sativus* germplasm was chosen with phenotypic characteristics similar to those of commercial pickling cucumbers (i.e. 'Vlaspik'). In addition, newly-released commercial cucumber hybrids were also screened (Appendix A.1). Germplasm descriptions can be found at ***http://www.ars-grin.gov/npgs***. A total of 432 cultigens were screened during 1999 to 2005 (Table 1, Appendix A.1). The pickling cucumber 'Vlaspik' was grown as a commercial standard in 1999 to 2005.

Cucumbers were grown according to standard management practices in fields with no history of *P. capsici*. In early June, cucumbers were direct-seeded in rows that were 61.0 cm apart with an in-row plant spacing of 30.5 cm. Rows were bedded and covered in black polyethylene mulch with sub-mulch drip irrigation for weed control and overall plant health maintenance (Fig. 1A). Pest control and fertilization were applied according to standard commercial practices. Commercially mature (5.1 to 7.6 cm

Table 1. Cucumber cultigens screened for fruit resistance to *Phytophthora capsici*.

Year	Cultigen			
	Total # of cultigens screened	**# Plant Introductions**	**# Cultivated varieties**	**# Selected for re-evaluation**
1999	78	38	40	18
2000	55	30	25	11
2001	42	13	29	8
2002	60	41	19	10
2003	52	33	19	12
2004	107	77	30	38
2005	38	18	20	0
Total	432	250	182	97

diameter) fruit were harvested, subjected to a 5-minute immersion in a 5% sodium hypochlorite solution, then rinsed in distilled water. Fruit were allowed to dry under ambient conditions and labeled according to cultigen. Fruit were harvested from the field on two or more separate occasions after observation of the first commercially mature fruit. Two to ten fruits were collected for each harvest and were inoculated by placing plugs of mycelial/sporangial inoculum on unwounded fruit. Plugs were covered with sterile micro-centrifuge tubes and fixed to fruit surfaces with petroleum jelly (Fig. 1C). Fruit were incubated in aluminum trays covered with plastic wrap under constant fluorescent lighting at 23-25 °C (Fig. 1B). Disease was evaluated by measuring the water-soaked lesion diameter, sporulation diameter, and sporulation density 3 dpi (Fig. 1D). In all tests, four to eight fruit per cultigen were tested for each of four harvests.

Cultigens whose fruit exhibited limited lesion development and pathogen sporulation were re-screened in subsequent years. Criteria for re-screening included a sporulation density and sporulation diameter of ≤1.0 cm. In some years, lesion diameter was also taken into consideration. Forty-five cultigens were screened in 2 or more years (Table 2). Thirty-one (69%) of the cultigens selected for re-screening were PIs; the remaining 14 were commercial cultivars (Table 2). Of the 25 cultigens selected for re-screening in 1999, 20 (80%) were PIs and only 5 were commercial cultivars (Table 2). In 2000, a total of 29 cultigens were selected; 20 were PIs (69%) and 9 were commercial cultivars. In 2001-2003, 12 to 16 cultigens were re-screened; more than half of these cultigens were PIs. Sixty-seven percent of the 12 cultigens selected for re-screening in 2004 were commercial cultivars (Table 2).

Table 2. Re-screened cucumber cultigens which exhibited reduced pathogen sporulation under fruit inoculation with *P. capsici* from 1999 to 2005.

Cultigen	Source/Year (when available)	Fruit Type	Years screened 99	00	01	02	03	04	05
PI 163213	Koelz, W., USDA Bureau of Plant Industry, 1948	NS[1]	+	+					
PI 197085	Koelz, W., U.S. Embassy, 1951	Pickling			+	+			
PI 197088*	Koelz, W., U.S. Embassy, 1951	NS[1]	+	+	+	+			
PI 209069	H.J. Heinz Co., 1953	Pickling	+	+					
PI 211979	Office of Agricultural Attache, Tehran, 1953	NS[1]	+	+					
PI 227209	Creech, J., USDA ARS, 1955	Pickling	+	+					
PI 249561	Pearson, O., Eastern States Exchange, 1958	NS[1]		+	+				
PI 249562*	Pearson, O., Eastern States Exchange, 1958	NS[1]	+	+	+	+			
PI 257486	Harbin College of Agriculture, 1959	Pickling					+	+	
PI 271326	Harlan, J., USDA ARS, 1961	NS[1]			+	+			
PI 271327*	Harlan, J., USDA ARS, 1961	NS[1]		+	+	+			
PI 271328*	Harlan, J., USDA ARS, 1961	Slicing	+	+			+		
PI 279466	Japanese Seed Growers Cooperatives, 1962	NS[1]	+	+					
PI 279467	Japanese Seed Growers Cooperatives, 1962	NS[1]	+	+					
PI 279468	Japanese Seed Growers Cooperatives, 1962	NS[1]	+	+					
PI 321008*	Taiwan Agricultural Research Institute, 1967	Slicing	+	+	+		+		
PI 330628	Goodbary, W., AID Mission to Pakistan, 1968	NS[1]	+	+					
PI 358813	Bagent, J., Louisiana State University, 1971	NS[1]	+	+					
PI 390240	Komada, H., Veg. and Ornamental Res. Station, 1974	NS[1]	+	+					
PI 390262*	Komada, H., Veg. and Ornamental Res. Station, 1974	NS[1]	+	+	+				
PI 391570	Academy of Agriculture and Forestry Sciences, 1974	Slicing	+				+		
PI 422180	Institute of Genetics and Plant Breeding, 1977	Pickling					+	+	
PI 422182	Institute of Genetics and Plant Breeding, 1977	Pickling	+				+		

PI 426169	Deanon, Jr., J., Vegetable Crops Division, 1978	Pickling	+	+					
PI 426170	Deanon, Jr., J., Vegetable Crops Division, 1978	Pickling	+	+					
PI 432867	Hang, C., Beijing Acad. of Ag. And Forestry Sci., 1979	Pickling	+	+					
PI 618861	Institute of Veg., Agriculture, and Forestry, 1986	Pickling					+	+	+
Ames 13333	Nuez, F., Escuela Tecnica Sup. De Ing. Agronomos, 1985	Slicing				+	+		
Ames 3944*	USDA, ARS, NERPIS, 1963	Pickling				+	+	+	
Ames 3950	USDA, ARS, NERPIS, 1983	Pickling				+	+		
Ames 7744	Groff, D., Asgrow Seed Co., 1987	Pickling				+	+		
Arabian (XP 1914)	Breeder: B. Johnson/Vendor: Seminis Veg. Seeds, Asgrow Seed Co., 1999	Pickling		+	+				
Campbell 4177	Seminis Veg. Seeds,	Pickling					+	+	+
Cross Country (FMX 4318)	Breeder: L. Gautney/Vendor: Ferry-Morse, 1987	Pickling	+					+	+
Discover (XPH 1550)*	Breeder/Vendor: Asgrow Seed Co., 1987	Pickling	+	+	+	+			
Excel (XPH 1682)*	Breeder/Vendor: Asgrow Seed Co., 1993	Pickling	+	+	+	+			
Fancipak (XPH 1369)	Breeder/Vendor: Asgrow Seed Co., 1985	Pickling	+					+	
Fanfare (PSX 113088)	Breeder/Vendor: Petoseed, 1990	Pickling					+	+	+
Lafayette (Sun 3529)	Breeder/Vendor: Sunseeds (Nunhems),	Pickling	+	+					
Stallion (PS 14692)	Breeder: B. Johnson/Vendor: Seminis Veg. Seeds, Asgrow Seed Co., 1998	Pickling		+	+				
Thunder (XPH 1700)	Breeder/Vendor: Asgrow Seed Co., 1993	Slicing		+				+	+
Thunderbird (PS 100395)	Breeder: K. Owens/Vendor: Seminis Veg. Seeds-Petoseed, 1999	Slicing					+	+	
Vlaspik (B-3,SMP,E)*	Breeder: G. Tolla/Vendor: Campbell (Vlasic)-Asgrow Seed Co., 1994	Pickling		+			+	+	+
Vlaspik+M Hybrid	Breeder/Vendor: Asgrow Seed Co.,	Pickling		+	+				
Vlasspear*	Seminis Veg. Seeds,	Pickling		+		+	+	+	+
Total			25	29	13	12	16	12	8

[1]NS: Non-specific fruit type.

Asterisks indicate cultigens that consistently performed with limited pathogen sporulation for more than 2 years.

RESULTS

Screening Methodology

The mycelial/sporangial plug inoculation method using micro-centrifuge caps was an effective screening method for establishing *P. capsici* infection on susceptible cucumber fruits (Fig 1B, C). Wounding was not necessary for successful infection. Covering the mycelial/sporangial plugs with sterile micro-centrifuge tubes was preferred, as uncovered plugs rapidly desiccated and disease did not progress. Zoospore inoculations yielded fruit symptoms similar to those observed with mycelial plugs, however, with less consistency, since zoospores require water. Applying uniformly-sized droplets on the hydrophobic surface of the cucumber fruit was difficult.

The four *P. capsici* isolates representing different CT and mefenoxam sensitivity did not differ significantly for any of the three disease parameters (sporulation diameter, sporulation density, and lesion diameter) (Fig. 2). All four isolates induced sporulating lesions of approximately 2.75 cm in diameter and sporulation density of 1.5 at 3 dpi; similar results were observed for lesion diameter (all approximately 6.0 cm) (Fig. 2). Therefore, a single isolate, OP97, was selected to carry out all subsequent cultigen inoculations.

Germplasm Screening

Of the 432 cultigens evaluated, 250 were PIs and 182 were commercial cultivars. No cultigens exhibited complete fruit resistance to *P. capsici* infection. However, 16% of the PIs and 8% of the commercial cultivars exhibited limited sporulation (e.g., sporulation diameters less than 2.0 cm or sporulation densities less than 1.0) (Fig 3A, B

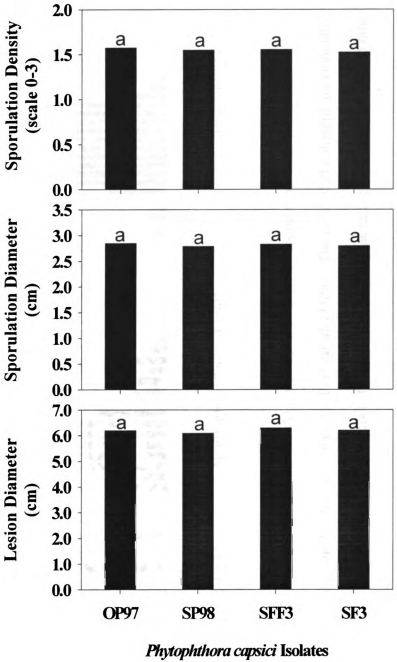

Figure 2. Comparison of disease ratings (sporulation density, sporulation diameter, and lesion diameter) of 4 *P. capsici* isolates (OP97, SP98, SFF3, and SF3) on cucumber fruit during 1999 and 2000. No significant differences were found between isolates using Fisher's Protected LSD test (P=0.05).

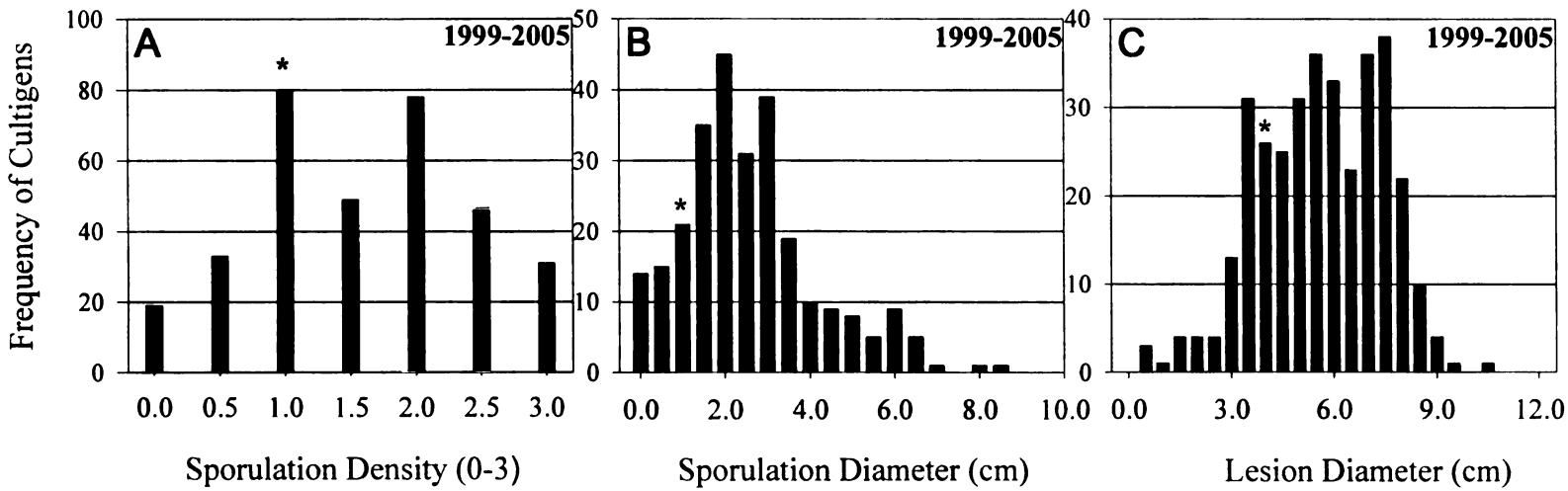

Figure 3. Fruit disease ratings of cucumber cultigens. A) Sporulation density, B) sporulation diameter, and C) lesion diameter measured from cucumber fruit inoculated with *P. capsici* OP97. The number of cultigens are cumulative from 1999 to 2005. Asterisks indicate where the cucumber standard 'Vlaspik' disease ratings fell in comparison to other screened cultigens. Data from 2004 is omitted.

and Table 2). More than half of the cultigens had water-soaked lesions of less than 3.0 cm (Fig. 3C). Promising cultigens that were re-screened either failed to again exhibit limited sporulation and were abandoned, or exhibited limited sporulation and were further evaluated in subsequent years. All cultigens that were screened in more than two years performed consistently with limited sporulation. Some of the least susceptible included the commercially available cultivars Discover, Excel, Vlaspik, and Vlasspear (Table 2). No PIs were reproducibly less susceptible than 'Vlaspik' when retested in multiple years.

DISCUSSION

As *P. capsici* becomes more prevalent on vegetable crops in the U.S. (6), it would be highly valuable to identify host plant resistance to the pathogen for incorporation into a disease management strategy. For cucumber crops, fruit resistance is crucial because fruit, which are the marketable portion of the plant, also appear to be the most susceptible part of the plant. Thus, an essential first step is the establishment of screening procedures specifically for cucumber fruit.

Our screening methodology included unwounded cucumber fruit inoculated with mycelial/sporangial plugs of *P. capsici* covered by micro-centrifuge tubes and incubated at high humidity for three days. This screening method, which was efficient and reproducible, does not require wounding which may interfere with the host-pathogen interaction and result in fruit susceptibility different than that expected under field conditions. In the field, fruit infection occurs after irrigation or driving rain with the splash dispersal of mycelial fragments, sporangia, and zoospores (16). The use of mycelial/sporangial plugs as inocula allowed for more consistent infection than zoospore

inoculum due to the difficulty in quantifying the number of zoospores in each droplet and assuring that each droplet remained intact on the fruit surface for three days.

No significant differences in disease reactions were determined among the four *P. capsici* isolates of differing mefenoxam sensitivity or compatibility type. Therefore, a single *P. capsici* isolate, OP97, was used for fruit inoculations. Isolate OP97 maintained consistent growth and virulence after repeated sub-culturing, and has also been used extensively in previous studies as a robust A1 CT standard (8). Using a single isolate simplified our screening. It is important before initiating a screen, however, to verify the virulence of the selected *P. capsici* isolate on susceptible host tissue.

Over a 6-year period, we evaluated the commercially-mature fruit of 432 cucumber cultigens for resistance to *P. capsici* and found that none exhibited complete resistance to pathogen infection. Some cultigens, including several commercial cultivars, however, showed limited pathogen sporulation on fruit. None of the PIs performed consistently with limited pathogen sporulation, as did the best cultivars. Until a resistant source is developed, using cultivars that limit pathogen sporulation may be beneficial in managing field disease.

Overall disease on cucumber fruit in 2004 was less than that observed during 1999 to 2003 and 2005 making it difficult to differentiate among genotypes; therefore 2004 results were not included in the data summaries. The reduced disease development may be due to weather and/or cultigen selection. The average air temperature during the cucumber-growing season at the Plant Pathology Farm, MSU, was 25.8°C (80°F) for 1999-2003 and 2005, with 19.8 growing degree days (base 50), but only 23.0°C (75°F)

with 16 growing degree days in 2004. Cooler temperatures affect physiological development of cucumber fruit, which may have impacted sensitivity to fruit rot (13).

In summary, we developed a direct cucumber fruit assay to screen for resistance to infection by *P. capsici*. Screening of approximately 400 cultigens did not identify a source of resistance superior to the partial resistance already present within many commercial cultivars.

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APPENDIX

Table A. 1. *Cucumis sativus* cultigens screened for fruit resistance to *P. capsici* during 1999 to 2005.

Cultigen, Provider	Year(s) screened	Cultigen, Provider	Year(s) screened
ACX 18, Abbott & Cobb[S]	99	Bush Baby 141H, Liberty/Stokes[P]	03
ACX 5001, Abbott & Cobb[S]	99	Bush Hyb 141G, Liberty/Stokes[P]	03
ACX 5002, Abbott & Cobb[S]	99	Caipira Hyb Ag-221, Seminis[S]	04,05
Ames 13247, USDA[S]	04	Calypso, Atlas Seeds[P]	99
Ames 13333, USDA	02,03	Campbell 4177, Seminis[P]	03-05
Ames 1760, USDA[S]	02	Carolina, Atlas Seeds[P]	99
Ames 20089, USDA	04	Colt Hyb, Asgrow[P]	00
Ames 21224, USDA[P]	04	Colt, Seminis[P]	02
Ames 23009, USDA[S]	04	Cool Breeze, Harris Moran[P]	04
Ames 3941, USDA[S]	02	Country Fair Hybrid, Park's[P]	05
Ames 3942, USDA[S]	02	Cross Country, Harris Moran[P]	99,04,05
Ames 3943, USDA[S]	02	Cyclone, Asgrow[S]	00
Ames 3944, USDA[P]	02,03,04	Dasher II, Petoseed[S]	99
Ames 3945, USDA[P]	02	Daytona 149 G, Liberty/Stokes[P]	03
Ames 3946, USDA[P]	02	Discover M Hybrid, Asgrow[P]	99,01
Ames 3947, USDA	02	Discover, Seminis[P]	00,02
Ames 3948, USDA	02	Diva, Harris Moran[S]	04,05
Ames 3949, USDA	02	Eclipse 146E, Liberty/Stokes[P]	03
Ames 3950, USDA	02,03	Eureka, Siegers[P]	00
Ames 3951, USDA	02	EX 1911 155633, Seminis[P]	99
Ames 4421, USDA	02	EX 1914 183491, Seminis[P]	99
Ames 4759, USDA	02	Excel M, Asgrow[P]	99,01
Ames 4832, USDA	02	Excel, Seminis[P]	00,02
Ames 4833, USDA	02	Fancipak, Asgrow, Seed Way[P]	99,04
Ames 7118, USDA[P]	99	Fanfare 127G, Liberty/Stokes[P]	03-05
Ames 7730, USDA	02	Feisty, Harris Moran[P]	04-05
Ames 7731, USDA	02	FMX 5020 F1, Harris Moran[P]	99
Ames 7735, USDA	02	General Lee, Harris Moran[S]	99
Ames 7736, USDA	02	Ginga Hyb Ag-77, Seminis[S]	04
Ames 7737, USDA	02	Green Slam, Sieger[S]	04
Ames 7738, USDA	04,05	Greensleeves, Harris Moran[S]	99
Ames 7739, USDA	02	Gy4, NCSU	02
Ames 7740, USDA	02	HMX 3469 F1, Harris Moran[P]	99
Ames 7738, USDA	04,05	HMX 8460 F1, Harris Moran[P]	99
Ames 7739, USDA	02	HMX 8461 F1, Harris Moran[P]	99
Ames 7740, USDA	02	Impact, Sieger[S]	04
Ames 7741, USDA	02	Indio 149F, Liberty/Stokes[P]	03
Ames 7742, USDA	02	Indy, Sieger/Harris/Seedway[S]	04
Ames 7744, USDA	02,03	Intimidator, Seed Way[S]	03
Ames 7742, USDA	02	Jackson, Sun Seeds[P]	99
Ames 7744, USDA	02,03	Jade, Harris Moran[P]	03
Ames 7745, USDA	02	Johnston, NCSU	02
Ames 7749, USDA	02	Lafayette Classic, Sun Seeds[P]	99,00
Ames 7750, USDA	02	Lucia, NCSU	02
Ames 7751, USDA	02	M17, NCSU	02
Ames 7752, USDA	02	Magic Hyb, Seminis[P]	03
Ames 7753, USDA	02	Manteo, NCSU	02
Ames 7785, USDA	02	Marketmore 76, Hollar Seeds[P]	03
Arabian Hybrid, Asgrow[P]	00,01	Meteor, Asgrow[S]	00
Arabian, Seminis[P]	02	NC-43, NCSU	02
Autograph, Seed Way[S]	04,05	Niagra 144W, Liberty/Stokes[P]	03

Table A. 1. Continued.

Cultigen, Provider	Year(s) screened	Cultigen, Provider	Year(s) screened
Orient Express, Harris Moran[S]	04	PI 279467, USDA	99,00
Palomino Hyb, Asgrow[P]	00	PI 279468, USDA	99,00
Palomino, Seminis[P]	02	PI 283899, USDA[P]	04
Panther, Sun Seeds[S]	99	PI 288238, USDA	99
Patio Pickle, Harris Moran[P]	04,05	PI 292012, USDA[S]	04
Patton 9228, SeedWay[P]	03	PI 302443, USDA[S]	04
PI 103049, USDA[S]	04	PI 306179, USDA[S]	04
PI 109482, USDA[S]	04	PI 306180, USDA	04
PI 109484, USDA	04	PI 306785, USDA[S]	04
PI 114339, USDA[S]	04	PI 314425, USDA	04,05
PI 137850, USDA[S]	04,05	PI 321006, USDA	01
PI 163213, USDA	99,00	PI 321007, USDA	01
PI 163214, USDA	00	PI 321008, USDA	99-01,03
PI 167223, USDA	99	PI 321009, USDA	00
PI 169304, USDA[S]	04,05	PI 330628, USDA	99,00
PI 172838, USDA[S]	04	PI 342950, USDA[P]	03
PI 183056, USDA	02	PI 354952, USDA[P]	03
PI 183127, USDA	02	PI 358813, USDA	99,00
PI 183224, USDA[S]	04	PI 358814, USDA	00
PI 197085, USDA	01,02	PI 360939, USDA[P]	03
PI 197086, USDA	99	PI 364472, USDA[S]	04,05
PI 197087, USDA	00	PI 372893, USDA[S]	04,05
PI 197088, USDA	99-02	PI 374694, USDA[S]	04
PI 206043, USDA[S]	04,05	PI 390239, USDA	00
PI 209064, USDA	99	PI 390240, USDA	99,00
PI 209065, USDA[S]	04	PI 390241, USDA	99
PI 209067, USDA	00	PI 390244, USDA	99
PI 209068, USDA	00	PI 390246, USDA	99
PI 209069, USDA	99,00	PI 390251, USDA[S]	04
PI 211979, USDA	99,00	PI 390252, USDA[S]	04
PI 211980, USDA	00	PI 390253, USDA[P]	04
PI 220860, USDA[S]	04	PI 390261, USDA	01
PI 226461, USDA[S]	04	PI 390262, USDA	99,01
PI 227209, USDA	99,00	PI 390263, USDA	00
PI 227210, USDA[S]	00	PI 390266, USDA[S]	04
PI 234517, USDA	99	PI 390529, USDA	99
PI 249561, USDA	00,01	PI 391570, USDA	99,03
PI 249562, USDA	99-02	PI 401732, USDA	01
PI 257486, USDA[P]	03,04	PI 401733, USDA	01
PI 262990, USDA[P]	04	PI 414157, USDA[S]	03
PI 264664, USDA[P]	04	PI 414158, USDA[S]	04,05
PI 264667, USDA[S]	04	PI 414159, USDA[S]	04
PI 267741, USDA[S]	04	PI 414716, USDA[S]	04
PI 267935, USDA[S]	04	PI 418963, USDA[S]	04
PI 267942, USDA	99	PI 418964, USDA	99
PI 271326, USDA	01,02	PI 419009, USDA[S]	04,05
PI 271327, USDA	00-02	PI 420150, USDA[P]	04
PI 271328, USDA	99,00,03	PI 422167, USDA[S]	04,05
PI 271753, USDA[P]	04	PI 422168, USDA[P]	04
PI 271754, USDA[P]	04,05	PI 422169, USDA[P]	04
PI 279466, USDA	99,00	PI 422170, USDA[P]	03

Table A. 1. Continued.

Cultigen, Provider	Year(s) screened	Cultigen, Provider	Year(s) screened
PI 422171, USDA[P]	03	PI 606066, USDA[S]	04,05
PI 422172, USDA[P]	03	PI 618860, USDA[S]	04
PI 422173, USDA[P]	03	PI 618861, USDA[P]	03-05
PI 422174, USDA[P]	03	PI 618862, USDA[S]	04,05
PI 422176, USDA[P]	03	PI 92806, USDA[S]	04
PI 422179, USDA[P]	03	Pioneer, Atlas Seeds[P]	99
PI 422180, USDA[P]	03,04	Prancer, Sieger[S]	04,05
PI 422181, USDA[S]	04,05	Premier Hyb, Seminis[S]	04
PI 422182, USDA	99,03	Raider, Harris Moran[S]	04,05
PI 422183, USDA[P]	03	Raleigh, NCSU	02
PI 422185, USDA[P]	03	Regal F1, Harris Moran[P]	99
PI 422186, USDA[P]	03	Reisenschal B1 SMP, Vlasic[P]	00
PI 422188, USDA[P]	03	Reisenschal B2 SMP+, Vlasic[P]	00
PI 422189, USDA[P]	03	Reisenschal B3 SMPE, Vlasic[P]	00
PI 422190, USDA[P]	03	Reisenschal B4 SMPE+, Vlasic[P]	00
PI 422191, USDA[P]	03	Reisenschal, Vlasic[P]	00
PI 422198, USDA[P]	03	Royal F1, Harris Moran[P]	99
PI 422199, USDA[P]	03	Salad Bush, Harris Moran[S]	04
PI 422200, USDA	04	Salty 142C, Liberty/Stokes[P]	03
PI 426169, USDA	99,00	Sassy, SeedWay	05
PI 426170, USDA	99,00	Shelby, NCSU	02
PI 432851, USDA	99	Spear It 141E, Liberty/Stoke[P]	03
PI 432855, USDA	99	Speedway, Petoseed[S]	99
PI 432865, USDA	99	Spunky, Harris Moran[P]	04,05
PI 432867, USDA	99,00	SRQP 2391, Sun Seeds[P]	99
PI 432868, USDA	00	SRQS 2387, Sun Seeds[S]	99
PI 432890, USDA	99	SRQS 2389, Sun Seeds[S]	99
PI 451975, USDA[S]	04	SS-58137, Sun Seeds	01
PI 451976, USDA[S]	04	SS-58139, Sun Seeds	01
PI 458852, USDA[P]	04	SS-58141, Sun Seeds	01
PI 466922, USDA	99	SS-58142, Sun Seeds	01
PI 483339, USDA	99	SS-58143, Sun Seeds	01
PI 490996, USDA[S]	04	SS-58144, Sun Seeds	01
PI 500359, USDA[S]	04	SS-58145, Sun Seeds	01
PI 504559, USDA[S]	04,05	SS-58146, Sun Seeds	01
PI 504563, USDA[S]	04	SS-58147, Sun Seeds	01
PI 504569, USDA[S]	04	SS-58148, Sun Seeds	01
PI 504813, USDA[S]	04	SS-58149, Sun Seeds	01
PI 508453, USDA[S]	03	SS-58150, Sun Seeds	01
PI 508455, USDA[S]	04	SS-58151, Sun Seeds	01
PI 508456, USDA[P]	03	SS-58152, Sun Seeds	01
PI 508458, USDA[P]	04	SS-58153, Sun Seeds	01
PI 508459, USDA[S]	04	SS-58154, Sun Seeds	01
PI 508460, USDA[S]	04	SS-58155, Sun Seeds	01
PI 511818, USDA[S]	04	SS-58456, Sun Seeds	01
PI 511819, USDA[S]	04,05	Stallion 193782, Seminis[P]	99
PI 512336, USDA[S]	04	Stallion Hyb, Asgrow[P]	00,01
PI 512638, USDA[P]	04	Sumter, Atlas Seeds[P]	99
PI 606000, USDA[S]	04	SVR04504229, Seminis[P]	02
PI 606032, USDA[S]	04,05	SVR04506116, Seminis	02
PI 606058, USDA[S]	04	Sweet Slice, Seed Way[S]	04

Table A. 1. Continued.

Cultigen, Provider	Year(s) screened
Sweet Success, Harris Moran[S]	04,05
SX 2387CW/Lynx, Seed Way[S]	04,05
SXQ 3534, Sieger[P]	04,05
Tamor Hyb, Asgrow[P]	99
Tasty Green, Seed Way[S]	04
Thunder, Asgrow/Sieger[S]	00,04,05
Thunderbird, Seed Way[S]	03,04
Transamerica F1, Harris Moran[P]	99
Transamerica, Sun Seeds[P]	00
Turbo, Harris/Stokes[S]	04,05
Ultra Pak, Stokes[S]	99
Victoria, Sun Seeds[P]	99
Vlaspick, Vlasic[P]	99
Vlaspik B1 SMP, Vlasic[P]	00
Vlaspik B2 SMP+, Vlasic[P]	00
Vlaspik B3 SMPE, Vlasic[P]	00,03-05
Vlaspik B4 SMPE+, Vlasic[P]	00
Vlaspik VGA733, Seminis[P]	99
Vlaspik, Asgrow[P]	00
Vlaspik, Seminis[P]	02
Vlaspik, Vlasic[P]	00
Vlaspik+M Hyb, Asgrow[P]	00,01
Vlasset, Asgrow[P]	99
Vlasset, Seminis[P]	02
Vlasspear Hyb, Asgrow[P]	99
Vlasspear, Seminis[P]	00,02-05
Vlasstar B, Asgrow[P]	99
WI 1983 G, 1997	01
WI 5207, 2000	01
WI 5551, 1994	01
WI 6632 E, 1997	01
WI GY 14, 1998	01
WI SMR 18, 2000	01
Wisconsin, Atlas Seeds[P]	99
XP 1904, Seminis[P]	02
Zapata, Seed Way[P]	03

[P]Indicates that the cultigen exhibits fruit of a pickling cucumber type.
[S]Indicates that the cultigen exhibits fruit of a slicing cucumber type.