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MANAGEMENT TOOLS TO CONTROL *PHYTOPHTHORA CAPSICI* IN PEPPER AND EGGPLANT

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EVALUATING MANAGEMENT TOOLS TO CONTROL *PHYTOPHTHORA CAPSICI* IN PEPPER AND EGGPLANT

By

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ABSTRACT

EVALUATING MANAGEMENT TOOLS TO CONTROL *PHYTOPHTHORA CAPSICI* IN PEPPER AND EGGPLANT

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Phytophthora capsici is an important pathogen of pepper and eggplant. Effective management requires a multifaceted approach including both chemical and cultural control methods. Greenhouse, laboratory, and field experiments were conducted to evaluate resistance in pepper and eggplant to *P. capsici*. Select fungicides were tested to determine their efficacy in controlling Phytophthora crown and root rot in pepper. Results from the greenhouse study, indicated that four Michigan *P. capsici* isolates differed in virulence on the roots and crowns of 31 pepper lines. The roots and crowns of pepper lines CM334, NY07-8001, NY07-8006, and NY07-8007 were resistant to the four *P. capsici* isolates. Isolate 12889 was more virulent on pepper fruit than the other two isolates, OP97 and SP98. When fungicides were applied in the field to the resistant cultivar Paladin and the susceptible cultivar Red Knight they effectively controlled crown and root rot on Paladin but adequate levels of control were not achieved on Red Knight. In the greenhouse, drench applications had significantly lower area under the disease progress curve values than foliar applications; treatments applied every 7 days had reduced plant death compared to treatments applied every 14 days. When eggplant lines were screened for resistance to *P. capsici*, line EG195 was resistant to fourteen isolates. The interaction between zoospore concentration and *P. capsici* isolate significantly influenced lesion size on eggplant fruit.

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LITERATURE REVIEW

INTRODUCTION

The oomycete pathogen *Phytophthora capsici* Leonian infects many cultivated and wild plants worldwide. Oomycetes belong to the phylum Oomycota within the recently erected kingdom *Stramenopila* (1,100). Oomycetes have been found to be more closely related evolutionarily to the brown algae and diatoms, rather than fungi (42). *Phytophthora* contains more than 60 species, many of which are plant pathogens (17,37). *Phytophthora capsici* was first identified in 1922 on chile pepper in New Mexico (80). Since then, *P. capsici* has become distributed globally (3,4,5,11,24,25,30,33,35,41,51,57, 61,80,82,83,90,130,131,137). In Michigan, the most important hosts of *P. capsici* belong to the *Cucurbitaceae* and *Solanaceae* families (Table 1) (49). Recently, plants in the *Fabaceae* (44) and *Pinaceae* (104) families have also been identified as susceptible hosts. Successful management of diseases caused by *P. capsici* has been challenging and requires a multifaceted approach that includes using raised beds, black plastic mulch, proper irrigation, host resistance, fungicides, and fumigants (49). When weather favors disease, even integrated management tactics can fail, resulting in field scale epidemics (49,108).

Table 1. Common hosts and diseases of *Phytophthora capsici* in found in Michigan.

Host		Disease	Reference
Cucurbitaceae			
Citrullus sp.	Melon	Collar and fruit rot	25
Citrullus lanatus	Watermelon	Brown fruit rot, vine and leaf wilt	40,65,138
Cucumis melo	Cantaloupe, honeydew	Fruit rot, postharvest decay	26
Cucumis sativus	Cucumber	Fruit rot	65
Cucurbita maxima	Blue Hubbard squash	Wilt and basal stem rot	65
Cucurbita pepo	Yellow squash, zucchini	Leaf and stem blight, crown and fruit rot	132
Fabaceae			
Phaseolus vulgaris var. *humilis*	Snap bean	Leaf and pod blight	44
Solanaceae			
Capsicum annuum	Red or sweet pepper	Root, crown and fruit rot, stem and leaf blight	80,127
Lycopersicon esculentum	Tomato	Roots, crown and fruit rot, seedling damping off	65,110,111
Solanum melongena	Eggplant	Brown rot, fruit rot	30,58

PHYTOPHTHORA CAPSICI ON SOLANACEOUS HOSTS

Infection of crops in fields infested with *P. capsici* is generally initiated by sexually produced oospores (Figure 1) (37). The thick walls of oospores (2 to 6 μm) can withstand salinity and temperature extremes (64). Consequently, oospores are regarded as the primary survival and overwintering structure of *P. capsici* (49) as well as the primary source of inoculum (37). Oospores mature within two weeks to three months, depending on environmental conditions. Mature oospores may germinate indirectly to produce sporangia or directly, generating a germ tube and hyphae (37,53,111). Oospore germination is induced by host seed and root exudates that also encourage chemotactic growth of hyphae towards the host (37,134). Germinated oospores can penetrate and colonize host tissue, producing the primary thallus of *P. capsici* which is composed of coenocytic mycelia that differentiates under favorable conditions to form fully-papillate, limoniform sporangia (12,36,37).

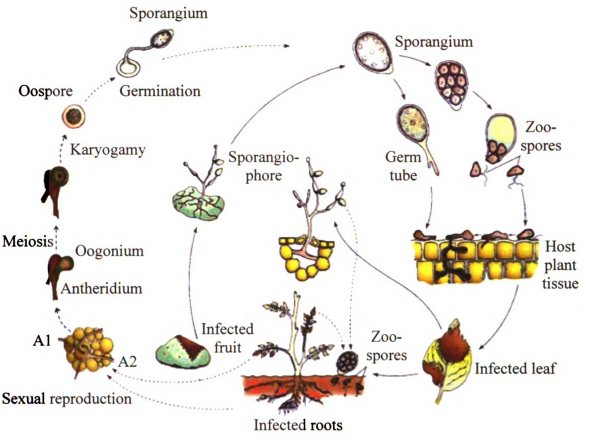

Figure 1. Lifecycle of *Phytophthora capsici* infection on a *Solanaceae* host (2).

Abundant asexual production of sporangia is a key physiological trait that allows *P. capsici* to exhibit rapid polycyclic disease development under appropriate environmental conditions. Sporangia are multi-nucleate and can germinate directly, producing multiple germ tubes that may penetrate host tissue or generate ectotrophic mycelial colonization of host tissue (36,134). When flooded, sporangia differentiate into 20 to 40 biflagellate motile zoospores (16,36,67,73,118). Zoospores are chemo- and electro-trophic towards plant roots (36,134). Once in contact with plant tissue, zoospores encyst, germinate, and may enter the plant through natural openings or may penetrate tissue directly with the aid of a macerating enzymes or mechanical pressure

(12,16,36,50,59,67,72,88,118,140). Ansani and Matsuoka found that sporangia or zoospores of *P. capsici* are unlikely to overwinter since these two propagules can only survive a maximum of 75 days in the soil (8,9).

After penetration, haustoria may form inside host cells as a means to obtain nutrients (37). The first symptom of *P. capsici* infection—water-soaked lesions—is visible after penetration of host tissue when the pathogen is secreting large quantities of macerating enzymes (16,59,80). Although the roots, stems, foliage and fruit of peppers and eggplants are susceptible to *P. capsici*, these dark green water-soaked lesions usually appear as stem lesions at the soil line or as fruit and foliar lesions (80,112,127,136).

As mycelia ramify internally, lesions on the stem turn brown and necrotic and may eventually girdle the stem of pepper plants (80). Foliar lesions turn a light brown color and develop white mycelial growth during times of high moisture (136). Mycelia also develop in and on infected fruit that become dried and shrunken, while remaining attached to the stem (44,112). In addition to stem girdling, the roots may also be infected resulting in plant wilt (27).

Once mycelial colonization becomes extensive in and on the host, profuse sporulation may follow and has been observed on stem, leaf and fruit tissue (80,112,136). These sporangia can go on to further propagate disease within and among host plants. Pepper fruit, for example, may become infected either through the stem or via rain splash containing spores (112). Despite the susceptibility of roots, stems, leaves, and fruits to *P. capsici* infection, in Michigan root and crown rot of pepper is observed more frequently than foliar blight or fruit rot (49). Eggplant fruit rot is observed more frequently in the

field than root or crown rot and can be seen as tan lesions that may become covered in white mycelia and sporangia as disease progresses (30,44,58).

Mycelial growth of *P. capsici* may culminate in the production of oospores which is dependent on the presence of different compatibility types (37). *Phytophthora capsici* is heterothallic with two compatibility types: A1 and A2 (92,133). Hyphal tips of A1 and A2 strains interact in response to hormone signals, producing amphigynous antheridia and spherical oogonia, which unite to form oospores (6,53). In many Michigan fields, the A1 and A2 compatibility types of *P. capsici* occur in a 1:1 ratio (67,68), which allows the frequent production of oospores and sexual recombination (49,54). Considering that it is not uncommon to have multiple genotypes infecting individual plants (113), oospore formation can take place in soil or on plant tissue concomitantly infected with A1 and A2 isolates (68). The frequency at which this occurs is currently unknown.

Sexual recombination creates a population with new genotypes that can adapt to their environment (103), such as resistance to site-specific fungicides (67,69), more virulence, a greater capacity to disperse, or differences in disease development (87). This occurs because the pathogen continues to adapt to its environment (103) and, through asexual reproduction, further perpetuates these genotypes in a population within a given season. For example, if a population of *P. capsici* is controlled with a site-specific fungicide such as mefenoxam, propagules resistant to mefenoxam will become more prominent in the population over time. Thus, the propagules that survive may produce oospores, which germinate the following spring, and create a new population that is more resistant to mefenoxam than the previous season, as was reported in Michigan (67).

PATHOGENICITY, PLANT BREEDING, AND HOST RESISTANCE

The susceptibility of hosts, cultivars and tissue types to *P. capsici* vary and depend on the isolate (10,45,49,52,55,77,93,96,105,119,120,135). *Phytophthora capsici* may infect plants in 27 families, and includes Fraser fir seedlings in Michigan (104). Holmes et al found cucurbits were more susceptible to Phytophthora crown and root rot than solanaceous hosts, with summer squash being the most susceptible host evaluated (52). Tian and Babadoost found cucurbits and peppers were more susceptible to *P. capsici* than other hosts studied, including 26 species of common rotational crops and nine weed species (128). Other vegetables, such as carrots and beets, are susceptible to *P. capsici*, but symptoms are observed less frequently than on solanaceous or cucurbit hosts (127,128). In fields, these vegetable hosts and weed species may act as reservoirs for *P. capsici*, even though they may be asymptomatic or of less economic concern. Ristaino determined that, on average, cucurbit isolates were less virulent on pepper than pepper isolates, but some of the cucurbit isolates were equally as virulent as the pepper isolates, indicating the initial host may not directly affect isolate virulence (105). Also, the host organ type may affect the susceptibility of the host itself. The fruits of pickling cucumber, for example, have been reported to be more susceptible than the roots, even though *P. capsici* has been isolated from the latter (49).

Some pepper lines are resistant to *P. capsici* such as Criollo de Morales 334 (CM334), a landrace with small fruit that is considered resistant to all known isolates of *P. capsici* (121,123,135). Hence, CM334 is the primary source of resistance to *P. capsici* used in plant breeding (121). Despite this, the inheritance of resistance in CM334 is not completely understood. Guerro-Moreno and Laborde found that two recessive genes

provided resistance (47). Later, Ortega et al. suggested that a three-gene, multi-alleic system provided resistance (95). An initial quantitative trait loci (QTL) analysis proposed that three QTLs were responsible for resistance (78). Other studies confirmed a single major QTL that spanned the entire length (107 cM) of chromosome 5, while the other two QTLs were located on chromosome 11. In 2003, it was discovered that six regions on chromosomes 4, 5, 6, 11, and 12 were involved in some part of resistance (79,102,123). Unfortunately, no breeding program has created a pepper with levels of resistance comparable to that of CM334 without losing other desirable horticultural characteristics (122,124,125).

Pathotypes and physiological races of *P. capsici* affecting pepper have been proposed on the basis of isolate virulence which is sometimes determined on different host tissue types (55,77,93,96,105,119,120,121). Sy et al. created 26 recombinant inbred lines (RILs) by crossing the resistant CM334 with the susceptible 'Early Jalapeno' and screened these with isolates from New Mexico, California, and the Netherlands (120). Thirteen physiological root rot races differentiated out of 17 isolates screened (120). In a separate study, nine root rot races were distinguished from ten isolates when zoospores were inoculated into the root zone of 18 pepper varieties (93). Oelke et al. also found four out of four isolates to be separate foliar blight races (93). In another study, thirty-four isolates from California, New Mexico, North Carolina, and Turkey differentiated into 14 physiological races of *P. capsici* on 11 pepper genotypes. The isolate races were not specific to geographical origin (45). Isolates were clearly distinguished on the basis of virulence in all of the studies, however different statistical methods were used to

determine physiological races and it would be difficult to compare results among studies and regions.

The existence of physiological races of *P. capsici* could have significant implications for plant breeding. To define a breeding line as resistant, it would need to be screened against a wide range of physiological races. Also, researchers have suggested that different genetic mechanisms may be responsible for resistance to Phytophthora root rot and Phytophthora foliar blight of pepper (10) which explains the conflicting results in understanding the genetic basis for resistance (47,78,79,95,96,102,123,124,125). Breeders must propagate plants that resist four symptoms in pepper: root rot, fruit rot, stem blight, and foliar blight (10,93). If the primary problem in a field is root rot, a producer would want to grow a cultivar that demonstrates the greatest level of resistance to root rot. However this information is currently not advertised by seed companies and the cultivar is simply labeled as resistant or tolerant to *P. capsici*. Traditional breeding is time consuming because both high levels of resistance and desired agronomic characteristics need to be expressed (98). Molecular markers and genetic maps enable breeders to locate regions of the genome that are involved in polygenic resistance (122). By using molecular techniques and by screening with a variety of isolates from different races, new cultivars could be developed that are resistant to multiple diseases caused by a wide range of *P. capsici* isolates.

When available, host resistance is often at the core of an integrated management strategy for disease control. Breeding efforts to obtain tolerant cultivars of bell peppers have been ongoing (Table 2) (10,62,96). Initially, breeding lines showed low levels of resistance, with little cumulative resistance to *P. capsici* (122). 'Adra' (Abbott and Cobb

Seed Co.) and 'Emerald Isle' (Harris Moran Seed Co.) were two of the first bell pepper cultivars marketed with resistance, yet neither cultivar had desirable fruit characteristics, which resulted in very few seed sales (21,108). The newer variety 'Paladin' is intermediately resistant to crown rot, however, under times of high disease pressure, the cultivar fails to perform adequately in Michigan (49). Also, it is thought that cultivars resistant to *P. capsici* exhibit skin silvering, a fruit blemish which lowers the pepper's grade (39,139). Wyenandt and Kline found that susceptible pepper varieties had a lower incidence of silvering than peppers with resistance to Phytophthora crown and root rot (139). The cause of silvering and its association with *P. capsici* is currently unknown.

Table 2. Commercially available bell pepper cultivars tolerant or resistant to *Phytophthora capsici.*

Cultivar	Company
Aristotle	Seminis Inc., St. Louis, MO
Declaration	Harris Moran Seed Company, Modesto, CA
Intruder	Syngenta Seeds, Boise, ID
Paladin	Syngenta Seeds
Revolution	Harris Moran Seed Company

WATER MANAGEMENT

Phytophthora capsici zoospores may swim or get translocated through surface water (43) and irrigation lines in production fields (13,16,21,22,106). It has been shown that the level of disease incidence and the distribution of *P. capsici* can be directly correlated to high soil moisture (73,106,107). Larkin et al. reported that plants in field sections with higher water potential had a greater incidence of plant death even when the inoculum was evenly distributed across a field (72). Because processing peppers are

produced without mulch, they may be planted into ridges so water will drain away from the crown and roots, decreasing crown rot incidence (108). However, Ristaino and Johnston determined that poorly formed ridges can allow water to pool and increase crown rot incidence (108); even fields with proper bed construction can become infected when heavy rainfall (>2 cm in 30 min) occurs (49).

Phytophthora capsici may also be splash dispersed. In Michigan processing vegetable fields, many producers use a traveler irrigation system, which produces large water droplets that may splash contaminated soil onto susceptible plants (49,81). If overhead irrigation is used, strategic timing (especially near harvest) has been reported to reduce the spread of *P. capsici* in a field without significantly limiting yield (20,21,22). To further reduce the incidence of splash dispersal, black plastic mulch limited the spread of sporangia and zoospores from the soil onto the plants (116).

Irrigation water from surface water sources may contain *P. capsici* propagules (37). In Michigan, Gevens et al. baited *P. capsici* from rivers, creeks, and naturally fed irrigation ponds (43). Several different isolates and both compatibility types were found, which could enable *P. capsici* to establish itself in a field for a long term basis. These surface water sources may have become contaminated by runoff from infested field soils or by waste water from vegetable processing facilities, which is sometimes disposed of in local river systems (43).

CROP ROTATION

Although crop rotation is a standard for disease management, it is difficult to employ in fields infested with *P. capsici* due to the longevity of the organism's survival

in soil. Bowers et al. reported that the density of soilborne propagules is greatly reduced in the absence of a susceptible host because sporangia and zoospores have a limited survival capacity (16). Even though sporangia and zoospores are the primary means of dissemination, this study did not take into account the affects of the long term survival capabilities of oospores (49,68). In a different study, the *P. capsici* population persisted during a cucumber-corn-corn-tomato rotation and caused disease in tomato following two seasons of corn production (68). Because the availability of suitable, uninfested land for vegetable production is declining, the average rotation between host crops is now one to two years, further perpetuating the infestation (49,108).

Some crops have demonstrated the ability to reduce soilborne diseases in other cropping systems (18,63,74,91,94,115). The glucosinolates from *Brassica* spp., for example, break down into isothiocyanates that have a reported toxicity to many soil organisms (74). The use of Brassica cover crops to generate isothiocyanates is referred to as biofumigation (109). Biofumigation has been used in several pathosystems to reduce the population of pathogens in the soil (18,63,91,94,115), but has yet to demonstrate effectiveness for controlling *P. capsici.*

CHEMICAL MANAGEMENT

In Michigan, growers have used the fumigant methyl bromide in conjunction with raised beds and black plastic mulch to manage *P. capsici* (49,116). Compliance with the Montreal protocol of 2005 required the phase-out of methyl bromide in the United States (7). Growers have been able to obtain early market opportunities with methyl bromide because vegetables could be transplanted relatively quickly into the fumigated soil when

compared to new fumigants. In a field scale trial, fumigants designed to replace methyl bromide provided adequate control when used in combination with raised beds covered in black plastic mulch. Despite this, the resulting fruit could not be harvested as early as fruit from the methyl bromide treatment and resulted in economic losses (49).

The fungicides registered to control *P. capsici* in peppers (Table 2) have not provided an economic level of control when used alone (49). In 1977, metalaxyl (Ridomil Gold SL, Syngenta Crop Protection, Greensboro, NC) was registered to control oomycete pathogens (19). Metalaxyl is a site-specific systemic fungicide that can be applied as a directed spray to peppers prior to or following transplanting. Metalaxyl killed the sporangia and zoospores of *P. infestans* in vitro, but had little to no effect on propagules germination (19,29,32). Resistance to metalaxyl in oomycetes was first noted in the 1970s in Ireland and in 1981 in the Netherlands. It has since been reported in several other areas of the world (31,34,38,46,71,76,129). With *P. capsici,* resistance is transferred by a single, incomplete dominant gene (69). Once a field population of *P. capsici* becomes insensitive to mefenoxam, there is little to no economic benefit to using the fungicide (69,99). Ridomil Gold SL now contains the active ingredient mefenoxam, an isomer of metalaxyl, and has the same site-specific mode of action (19,29).

Table 3. Chemicals registered for use on field pepper for controlling *Phytophthora capsici* (14).

FRAC code	Classification	Mode of action	Fungicide common name	Disease controlled
4	Phenyl amides	RNA polymerase I	mefenoxam	Phytophthora crown rot
11	Quinone outside Inhibitors (QoI)	Complex II of fungal respiration, ubiquinol oxidase, Qo site	fenamidone	Phytophthora blight of fruit and foliage
27	Cyanoacetamide-oximes	Unknown	cymoxanil	Phytophthora blight of fruit and foliage
40	Carboxylic acid amides	Phospholipid biosynthesis and cell wall deposition	dimethomorph	Phytophthora blight
			mandipropamid	Phytophthora blight
43	Unknown	Novel	fluopicolide	Phytophthora blight
M1	Inorganic	Multisite contact activity	copper hydroxide	*P. capsici*
M3	Dithiocarbamates and relatives	Multisite activity	maneb	Phytophthora blight
NC	Not classified		potassium phosphite	Phytophthora root rot

Two fungicides classified as carboxylic acid amides (CAA) are effective at controlling *Phytophthora* spp. Dimethomorph (Acrobat 50WP, Forum 4.08SC, E.I. duPont, BASF, Greensboro, NC), a systemic protectant with observed curative properties against *Phytophthora* spp., was registered in 2002 (23). Shishkoff et al. found dimethomorph's activity in vitro reduced mycelial growth, the formation of sporangia, and germination of zoospore cysts (114). According to Stein and Kirk there is little risk of *P. infestans* developing resistance to dimethomorph under normal field conditions and there has been no documented resistance in *P. capsici* (117). Mandipropamid (Revus 2.08SC, Syngenta Crop Protection, Greensboro, NC) is another CAA that was registered for use in 2008 and has shown greater inhibition of *P. infestans* spore germination and greater trans-laminar activity than dimethomorph (28). Similar in vitro studies have not been published for *P. capsici.*

Fungicides in other classes, such as cymoxanil (Tanos 50WP, E.I. duPont), potassium phosphite (ProPhyt 4.2EC, Helena Chemical Company, Collierville, TN), and fluopicolide (Presidio 4FL, Valent U.S.A. Corporation, Walnut Creek, CA) also control oomycete pathogens. Cymoxanil, which inhibits zoospore production and germination, was registered to control only the foliar blight phase of *P. capsici* on pepper in 2003 (60). Potassium phosphite is classified as a biopesticide by the Environmental Protection Agency (EPA) (Cohen and Coffey, 1986). This product worked systemically to inhibit mycelial growth, reduce membrane metabolism, sporulation, germination; affect the phosphorylation reactions in the pathogen; and activate plant defense responses (48,97). The most recent fungicide registered for use on oomycete pathogens is fluopicolide—its mode of action is currently unknown.

Many of these products have been tested in the greenhouse and field for efficacy in controlling diseases of *P. capsici* on pepper. In a greenhouse study, soil drench applications of mefenoxam, fluopicolide, dimethomorph, and mandipropamid significantly increased the survival of pepper plants grown in soil naturally infested with *P. capsici* (85). In another related greenhouse study, plants drenched with dimethomorph survived significantly longer than the untreated control plants (84). The effectiveness of fungicides as drenches in the field at controlling crown and root rot is relatively unknown. The efficacy of several fungicides was tested for control of the foliar phase of *P. capsici* on field-grown peppers. The fungicides fluopicolide, mandipropamid, and mefenoxam significantly reduced the development of foliar blight symptoms; potassium phosphite was statistically similar to the untreated control (86). Few studies have evaluated the effectiveness of foliar application for controlling Phytophthora crown and root rot even if its the only means of application permitted for several fungicides.

GRAFTING

Grafting has been used in multiple plant industries to increase disease tolerance, obtain certain horticultural traits, increase plant vigor, and provide producers with an effective way to manage root and crown rot diseases (26,66,75,101). Cucurbits have been grafted to resistant rootstock in Europe and Asia, where the phase-out of methyl bromide has limited the ability of melon producers to manage *Fusarium* spp. Watermelon scions, for example, were grafted onto resistant squash rootstocks to lessen susceptibility to Fusarium root rot and reduce disease incidence by 85 to 100% at certain test sites (75,101). However, Cohen et al. found that low levels of infection still occurred

in grafted watermelon due to adventitious roots from the scion coming into contact with infested soil (26). Grafting is also used to increase other qualities such as fruit traits, plant vigor, harvest time, and partial disease resistance (26). In the case of watermelon, fruit quality was positively affected when the crown rot-resistant rootstocks produced a crispy, more desirable texture of the fruit flesh (75). Additionally, graft transmission of resistance was reported by Jenns and Kuc in cucumber plants that were grafted and screened for resistance to anthracnose and tobacco mosaic virus (56). The cucumber scion remained susceptible to anthracnose when grafted onto pumpkin or squash, but was resistant when grafted onto watermelon rootstock (56).

Solanaceous crops have been successfully grafted across species and may provide potential rootstocks for bell pepper field production (e.g., eggplant rootstocks). Greenhouse tomatoes, for example, are often grafted for disease resistance and for tolerance to environmental stresses (15,66). In Italy, pepper cultivars have been screened for rootstock resistance to *P. capsici* and the cultivar 'Grafito,' was a promising and potential candidate (89). It should be noted that some pepper cultivars bred for crown rot resistance to *P. capsici* may lack desirable fruit qualities, but could be valuable rootstock candidates.

Since 1922, growers and researchers have been trying to control *P. capsici* in pepper fields. Significantly more information is currently available in pepper production regarding *P. capsici* than exists for eggplant production. Some bell pepper cultivars are tolerant to Phytophthora crown and root rot and may be best used if combined with fungicide applications. Grafting has been used successfully in cucurbit field production

in Asia, and in greenhouse tomatoes in North America, but has yet to be explored in field peppers. The research objectives of this thesis include the following:

i) screen pepper lines for resistance to Michigan isolates of *P. capsici*,

ii) evaluate fungicides and application methods to control Phytophthora root and crown rot in pepper, and

iii) screen eggplant lines for resistance to isolates of *P. capsici* and investigate the virulence of select *P. capsici* isolates on eggplant roots, crowns and fruits.

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CHAPTER I

EVALUATION OF PEPPER LINES FOR RESISTANCE TO PHYTOPHTHORA CROWN, ROOT, AND FRUIT ROT

ABSTRACT

Different physiological races have been proposed in the pepper-*Phytophthora capsici* system, however, the reaction of peppers to Michigan *P. capsici* isolates has not been elucidated. Greenhouse and laboratory experiments were conducted to determine the virulence of four *P. capsici* isolates on 31 pepper lines. Millet seed inoculum was used for the crown and root rot assessment and a zoospore solution (1.75×10^6 zoospores/ml) was used for the fruit rot assessment. The four Michigan *P. capsici* isolates differed in virulence among the pepper lines screened for crown and root rot resistance and were considered to be four different physiological races. The pepper lines CM334, NY07-8001, NY07-8006, NY07-8006 were resistant to all four isolates. None of the commercial cultivars had adequate resistance to *P. capsici* isolate 12889, but several cultivars were resistant to the three other isolates. The *P. capsici* isolates varied in their ability to cause infection on the fruits of the different cultivars. Overall, it appeared that pepper fruit were more susceptible to *P. capsici* than the roots and crowns. Greater knowledge of cultivar resistance to local isolates of *P. capsici* will assist growers in making management decisions and cultivar choices for their fields.

INTRODUCTION

Phytophthora capsici is a soil-borne pathogen that was first identified on pepper (*Capsicum annuum* L.) in New Mexico in 1922 (17). Since then, the host range of *P. capsici* has been expanded to include over 50 plant species worldwide (7,8,21), including many Solanaceous and Cucurbitaceous vegetable crops (12,23). *Phytophthora capsici* is a destructive pathogen of peppers in the United States, where the roots, crown, stem, leaves and fruits can all become infected (17). The pathogen may enter the roots or base of the stem with symptoms first appearing as water-soaked lesions, then rapidly progressing to cause stem girdling, plant wilting, and death (23). In 2008, Michigan producers grew over 25,000 ha of vegetables susceptible to *P. capsici*, including 730 ha of peppers (2). Control of Phytophthora crown and root rot requires a multifaceted approach that combines the integration of cultural practices, fungicides, fumigants, and genetically resistant varieties (12,23).

Several commercial bell pepper cultivars are available that have some level of tolerance to *P. capsici*, but no cultivar provides resistance to a wide range of isolates (18). The cultivars 'Paladin' (Syngenta Seeds Inc., Boise, ID), 'Aristotle' (Monsanto Company, St. Louis, MO), 'Declaration' (Harris Moran Seed Company, Modesto, CA), 'Karisma' (Harris Moran Seed Company), 'Intruder' (Syngenta Seeds Inc.) and 'Revolution' (Harris Moran Seed Company) are regarded as resistant or tolerant to *P. capsici* by seed distributors. Growers are reluctant to use resistant or tolerant cultivars because of an increased incidence of silvering—the separation of the cuticle from the epidermis (33)—and poor fruit shape (6,23), which results in a high quantity of unmarketable fruit.

The pepper line Criollo de Morales 334 (CM334), a landrace with small fruit, is considered resistant to all known isolates of *P. capsici* (28,29,31). It has been reported that CM334 is the primary source of root rot resistance currently used in pepper breeding programs (28). The inheritance of resistance in CM334 is not completely understood. Guerro-Moreno et al found two recessive genes provided resistance (11) and later, Gil Ortega et al. thought a three-gene, multi-allelic system was the source of resistance (9). Initial quantitative trait loci (QTL) analysis proposed three QTLs were responsible for resistance; other studies confirmed a major QTL, reported to span the entire length (107 cM) of chromosome 5 (15,16,19). In 2003, six regions on chromosomes 4, 5, 6, 11, and 12 were determined to be involved in some part of resistance (29). The QTLs may provide an easy way to determine if new breeding lines are resistant to *P. capsici* (30). Unfortunately, a pepper with levels of resistance comparable to that of CM334 with desired horticultural characteristics has not been developed (29,30).

Recent studies have indicated that physiological races exist in the pepper-*P. capsici* system, which implies that pepper breeders need to test breeding lines against a wide range of *P. capsici* isolates (10,18,27). Furthermore, researchers have suggested that different genetic mechanisms may be responsible for resistance to root rot and foliar blight of pepper (3). Instead of breeding for one symptom in pepper, breeders must now breed for four: root rot, fruit rot, stem blight, and foliar blight (3,18,26).

In Michigan, foliar symptoms, including leaf blight and fruit rot, are observed in the field less frequently than root and crown rot symptoms (12). Hence, growers in Michigan would benefit most from a cultivar that is primarily resistant to root and crown rot and stem blight. When foliar blight does occur on pepper in Michigan, it may be

easier to protect against foliar and fruit disease symptoms with fungicide sprays than it is to protect the lower plant stem and crown. To our knowledge, Michigan isolates have not been employed when screening pepper lines for root and crown resistance to *P. capsici*. In addition, the interaction of Michigan *P. capsici* isolates with pepper cultivars and breeding lines is relatively unknown. The objective of this study was to evaluate cultivars and breeding lines of pepper for resistance to Phytophthora crown and root rot using isolates of *P. capsici* from Michigan and to investigate the virulence of three Michigan isolates of *P. capsici* on pepper fruit.

MATERIALS AND METHODS

***P. capsici* isolate selection and inoculum preparation.** Isolates of *P. capsici* obtained from infected plants in Michigan were selected from the long term culture collection of Dr. Mary Hausbeck (Michigan State University, MSU). The isolates were classified according to mating type (MT) and sensitivity to mefenoxam. The isolates OP97 (A1 MT) and SP98 (A2 MT) are sensitive to mefenoxam and were originally isolated from pickling cucumber and pumpkin, respectively. The isolates 12889 (A1 MT) and SFF3 (A2 MT) are insensitive to mefenoxam and were isolated from pepper and pickling cucumber, respectively.

The *P. capsici* isolates were grown on unclarified V8 agar (16 g agar, 3 g $CaCO_3$, 160 ml V8 juice and 840 ml distilled water) under constant fluorescent light at room temperature (21 ± 2°C) for seven days. Millet seed medium (100 g millet seed, 72 ml deionized water and 0.08 g asparagine) was prepared in a 500-ml Erlenmeyer flask that was autoclaved twice in a 24-h time period. The millet seed medium was inoculated with

four 7-mm-diameter agar plugs of actively growing *P. capsici*. Millet seed inoculum was incubated at room temperature under constant fluorescent lighting and shaken regularly. Isolates OP97, SP98 and 12889 were used to screen the harvested fruit for resistance to zoospore infection. Zoospore inoculum was prepared by flooding actively sporulating cultures with sterile distilled water and incubating at 4°C for 1 h followed by 30 min at room temperature to initiate zoospore release. Zoospore concentration was estimated using a hemacytometer and adjusted to 1.75×10^6 zoospores/ml.

Phytophthora root and crown rot screen. Two experiments were designed for the pepper root and crown rot screen and included 28 (experiment 1) and 14 (experiment 2) pepper lines (Table 4). Nine commercially available cultivars ('Alliance,' 'Aristotle,' 'Brigadier,' 'Camelot,' 'Declaration,' 'Paladin,' 'Red Knight,' 'Revolution,' and 'Snapper') were included in both experiments. Seeds were sown into 72-cell flats filled with potting media (BACCTO Professional Planting Mix, Michigan Peat Company, Houston TX) and placed into a greenhouse with a 14-h photoperiod. When seedlings developed three and four true leaves, they were transplanted into 1.5-liter pots filled with potting media (described above) and arranged in a complete randomized design in a greenhouse with a 14-h photoperiod at MSU's Horticulture Teaching and Research Center, East Lansing, MI.

Table 4. *Capsicum annuum* lines screened for root, crown and fruit rot resistance to *Phytophthora capsici* during 2008 and 2009.

Pepper line[x]	Seed Company/Provider
9925776[y]	Monsanto Company, St. Louis, MO
9931126[y]	Monsanto Company
9941819[y]	Monsanto Company
9943084[y]	Monsanto Company
9943095[y]	Monsanto Company
Alliance[yz]	Harris Moran Seed Company, Modesto, CA
Aristotle (non-pelleted seed)[yz]	Monsanto Company
Aristotle (pelleted seed)[y]	Monsanto Company
Brigadier[yz]	Syngenta Seeds Inc., Golden Valley, MN
Camelot[yz]	Monsanto Company
CM334 (serrano type)[z]	Cornell University, Ithaca, NY
Declaration[yz]	Harris Moran Seed Company
Karisma[z]	Harris Moran Seed Company
NY07-8001[z]	Cornell University
NY07-8006[z]	Cornell University
NY07-8007[z]	Cornell University
Paladin[yz]	Syngenta Seeds Inc.
Plato[y]	Monsanto Company
PRO3-13x14R-4[y]	Pepper Research Inc., Belle Glade, FL
PRO3-15x16R-5[y]	Pepper Research Inc.
PRO4T-11x12[y]	Pepper Research Inc.
PRO5-C71x72[y]	Pepper Research Inc.
PRO5-C81x82[y]	Pepper Research Inc.
PRO5-C85x86[y]	Pepper Research Inc.
PRO5-C87x88[y]	Pepper Research Inc.
Prophet[y]	Monsanto Company
PX9942595[y]	Monsanto Company
Red Knight[yz]	Monsanto Company
Revelation[y]	Monsanto Company
Revolution[yz]	Harris Moran Seed Company
Snapper[yz]	
XPP2548 (poblano type)[y]	Sakata Seeds, Morgan Hill, CA

[x]Bell pepper line unless otherwise indicated.

[y]Pepper line included in experiment one, replicated twice in 2008

[z]Pepper line included in experiment two, replicated in 2008 and 2009.

All isolates and a non-inoculated treatment were replicated eight times for each pepper line that was screened. The potting medium was infested using 1 g of infected millet seed per pot, which was inserted into the media directly adjacent to the pepper seedling's root mass one day after transplanting. The plants were watered daily to maintain adequate moisture for plant growth and disease development. In the first six weeks of growth, the pots were fertilized weekly with Peter's (Scott's Company, Marysville, OH) 20-20-20 soluble fertilizer at 200 ppm. For the remainder of the experiment, the pots were fertilized three times a week with the same solution. Irrigation water was amended with phosphoric acid at 132 ppm once a week to maintain the media pH at approximately 6.0 to 6.5. The pH was checked monthly by collecting random media samples and using a pH meter (Hanna Instruments, Woonsocket, RI).

Phytophthora crown and root rot was evaluated every two days following the first symptom of plant wilting and continued until the fruit were harvested (average 83 days post inoculation). Plants were graded on a 1 to 5 scale (1 = healthy, 2 = minor wilting, 3 = moderate wilting, 4 = severe wilting, 5 = plant death), adapted from Gloser et al. (10). The area under the disease progress curve (AUDPC) was calculated according to the methods of Shaner and Finney (25) to demonstrate the cumulative plant infection (%) throughout the growing period. Approximately 10% of the symptomatic plants were returned to the laboratory to isolate the pathogen. The root and crown area were rinsed with deionized water and surface sterilized using 70% ethanol solution. Three sections of root and crown tissue were excised and plated onto UCV8 plates amended with 25 ppm of benomyl, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene (BARP). Plates were incubated at room temperature under

constant fluorescent lighting for three days and checked microscopically (200X) to confirm *P. capsici* using morphological characteristics according to the *Phytophthora* spp. key by Waterhouse (32). Hyphal-tips of *P. capsici* cultures were transferred onto new BARP-amended UCV8 agar plates. After seven days, each resulting isolate was screened for mefenoxam sensitivity and mating type to confirm the isolate phenotype (14).

Phytophthora fruit rot screen. In the first crown and root rot experiment, the pepper fruits were harvested when the average fruit diameter was between 7 and 10 cm, and stored for a maximum of five days at 2°C. Prior to inoculation, fruits were returned to room temperature, surface sterilized with approximately 10% bleach solution for 10 min and rinsed with distilled water. Fruits were placed into disinfested humidity chambers and moistened paper towel was placed into each chamber to maintain humidity at ~100%. A 10 µl drop of the zoospore suspension was placed onto the surface of each fruit. Chambers were sealed and maintained at room temperature (21 ± 2°C). The fruits were incubated in the dark for 60 h, followed by 24 h under constant fluorescent lighting to induce sporulation.

The lesion area that developed on the fruits was estimated by measuring the diameter of water-soaked tissue, pathogen sporulation, and/or mycelia growth. To estimate the density of sporulation, a tape mount was prepared from an area of the lesion with active pathogen sporulation. The average number of sporangia in five fields of view at 400X was extrapolated to the number of sporangia in the entire lesion area.

Statistical analysis. The cumulative AUDPC values and fruit screen measurements were subjected to analysis of variance (ANOVA) using the PROC MIXED

procedure of SAS v 9.1 (SAS Institute Inc., Cary, NC). Fisher's protected LSD was used for separation of means when effects were found to be statistically significant in ANOVA analysis (P=0.05). The pepper line was considered resistant to the isolate if it received an average disease score (DS) < 2, adapted from methods previously described (1,5,10). AUDPC data from both crown and root rot experiments were log transformed in order to avoid violating normality assumptions of the test. To evaluate the lesion area data, all asymptomatic fruits (no lesions) were removed from the analysis. The fruit area with water-soaked tissue, pathogen mycelial growth, and sporulation was square-root transformed in order to avoid violating normality assumptions of the test. Satterthwaite's approximation was used to account for the unbalanced design in all fruit data analyses. Non-inoculated control plants and fruits were removed from analysis to avoid violating variance assumptions of the test.

RESULTS

Phytophthora root and crown rot screen. Susceptible pepper plants exhibited crown rot and stem lesions and eventually wilted and died when inoculated with infested millet seed from one of the four Michigan *P. capsici* isolates (Figure 2). All isolates of *P. capsici* obtained from inoculated plants were confirmed to have the same phenotype as the isolate used as inoculum (*data not shown*). None of the non-inoculated control plants showed disease symptoms. Significant differences ($P \leq 0.05$) were found among AUDPC values calculated for the isolates and pepper lines, but not their interaction.

In both experiments, the mean AUDPC values were statistically different among *P. capsici* isolates; isolate 12889 was more virulent than OP97 and both were more

virulent than SP98 and SFF3 (Figure 2, Table 5). Isolate 12889, originally obtained from pepper, was virulent to most of the pepper lines tested; except for CM334, NY07-8001, NY07-8006, and NY07-8007 (Table 6). In the first experiment, 19 of the 26 pepper lines tested were susceptible to the pickling cucumber isolate OP97 (DS $\geq$ 2). In the second experiment, six of the fourteen pepper lines were susceptible to OP97 (DS $\geq$ 2). No pepper lines were susceptible to SP98 in experiment one (DS < 2) and only one was susceptible in experiment two (DS = 2.0). One pepper line was susceptible to SFF3 in experiment one (DS = 2.1) and all were asymptomatic in experiment two (DS < 2).

Figure 2. Symptoms produced on cultivars **A-E**, Red Knight and **F-J**, Paladin six weeks post inoculation with 1 g millet seed inoculum prepared with one of four isolates of *Phytophthora capsici*, **B**,**G**, SP98, **C**,**H**, SFF3, **D**,**I**, OP97, and **E**,**J**, 12889. Controls, **A**,**F**, were inoculated with sterilized millet seed.

Table 5. Cumulative area under the disease progress curve (AUDPC) values for *Phytophthora capsici* isolates causing crown and root rot symptoms in 27 pepper lines (Experiment 1) and 14 pepper lines (Experiment 2).

Isolate	Host	CT[x]	MS	AUDPC[y]	
				Experiment one[z]	Experiment two
SFF3	pickling cucumber	A2	I	94 a	97 a
SP98	pumpkin	A2	S	95 a	100 a
OP97	pickling cucumber	A1	S	186 b	135 b
12889	bell pepper	A1	I	308 c	276 c

[x]The isolate phenotypes are indicated by compatibility type (CT) and mefenoxam sensitivity (MS, I = insensitive, S = sensitive).

[y]The AUDPC was calculated from scores determined every two days using a 1 to 5 rating scale (1 = healthy, 2 = minor wilting, 3 = moderate wilting, 4 = severe wilting, 5 = plant death).

[z]Column means with a letter in common are not statistically different according to Fisher's LSD (P=0.05).

Table 6. Pepper lines screened for resistance to four Michigan *Phytophthora capsici* isolates obtained from pepper (12889), pickling cucumber (OP97, SFF3), and pumpkin (SP98).

Pepper line[vw]	Disease response[x]				AUDPC[y]				Death (%)			
	12889	OP97	SP98	SFF3	12889	OP97	SP98	SFF3	12889	OP97	SP98	SFF3
Experiment one												
9925776	S	S	R	R	339	202	100	91	100	63	13	6
9931126	S	R	R	R	239	111	87	93	81	25	0	13
9941819	S	S	R	R	335	201	86	86	100	69	0	0
9943084	S	S	R	R	330	171	107	86	100	44	13	0
9943095	S	S	R	R	358	306	125	87	100	94	19	0
Alliance	S	S	R	R	363	283	101	94	100	94	19	6
Aristotle (non-pelleted)	S	S	R	R	332	174	98	100	100	44	6	6
Aristotle (pelleted)	S	S	R	R	354	148	89	96	100	31	0	13
Brigadier	S	S	R	R	362	301	118	99	100	94	25	13
Camelot	S	S	R	R	361	259	94	108	100	94	13	19
Declaration	S	S	R	R	305	119	101	86	100	25	19	0
Paladin	S	R	R	R	200	105	87	87	63	6	0	0
Plato	S	S	R	R	347	252	95	104	100	81	13	6
PRO3-13x14R-4	S	R	R	R	227	90	88	90	63	0	6	6
PRO3-15x16R-5	S	R	R	R	136	93	87	95	38	6	0	13
PRO4T-11x12	S	S	R	R	315	130	91	90	100	25	6	6
PRO5-C71x72	S	S	R	R	321	159	86	86	100	31	0	0
PRO5-C81x82	S	R	R	R	225	92	86	88	81	13	0	6
PRO5-C85x86	S	S	R	R	329	143	96	88	100	56	6	6
PRO5-C87x88	S	S	R	R	342	184	86	100	100	63	0	13
Prophet	S	S	R	R	335	204	86	94	94	50	0	6
PX9942595	S	S	R	R	329	213	86	86	100	63	0	0
Red Knight	S	S	R	R	353	283	113	112	100	94	13	25
Revelation	S	S	R	R	351	283	95	86	100	88	13	0
Revolution	S	R	R	R	326	98	89	101	100	25	0	13

Table 6 (cont'd).

Pepper line[vw]	Disease response[x]				AUDPC[y]				Death (%)			
	12889	OP97	SP98	SFF3	12889	OP97	SP98	SFF3	12889	OP97	SP98	SFF3
Snapper	S	S	R	S	368	299	112	106	100	100	19	25
XPP2548 (poblano type)	S	R	R	R	124	113	89	88	25	13	13	0
Experiment two												
Alliance	S	S	R	R	371	165	105	104	94	44	6	6
Aristotle (pelleted)	S	R	R	R	389	117	109	90	100	13	6	0
Brigadier	S	S	R	R	405	195	125	106	100	44	25	13
Camelot	S	S	R	R	391	173	103	90	100	38	6	0
CM334 (serrano type)	R	R	R	R	90	90	90	90	0	0	0	0
Declaration	S	R	R	R	255	90	91	90	88	0	0	0
Karisma	S	S	R	R	369	144	91	107	94	25	0	6
NY07-8001	R	R	R	R	126	90	90	90	13	0	0	0
NY07-8006	R	R	R	R	90	90	90	90	0	0	0	0
NY07-8007	R	R	R	R	90	90	90	90	0	0	0	0
Paladin	S	R	R	R	208	90	90	90	44	0	0	0
Red Knight	S	S	S	R	368	252	128	128	94	69	25	19
Revolution	S	R	R	R	313	90	90	90	88	0	0	0
Snapper	S	S	R	R	392	216	110	104	100	63	6	6
Cultivars[z]												
Alliance	S	S	R	R	359	217	96	92	97	69	13	6
Aristotle	S	R	R	R	363	125	92	86	100	22	3	6
Brigadier	S	S	R	R	374	241	116	96	100	69	25	13
Camelot	S	S	R	R	367	209	92	92	100	66	9	6
Declaration	S	R	R	R	282	98	89	81	94	13	9	0
Paladin	S	R	R	R	197	91	82	82	53	3	0	0
Red Knight	S	S	R	R	353	261	114	113	97	81	19	22
Revolution	S	R	R	R	312	87	83	89	94	13	0	6
Snapper	S	S	R	R	372	251	104	98	100	81	13	13

Table 6 (cont'd).

[v]The potting media was inoculated with 1 g of prepared millet seed inoculum per plant.

[w]Bell pepper line unless otherwise indicated.

[x]The disease response was determined based on the average plant score at the end of the experiment. If the score was <2, the pepper line was considered resistant (R); if the score was ≥2, the pepper line was considered susceptible (S).

[y]The area under the disease progress curve (AUDPC) was calculated from scores evaluated every two days using a 1 to 5 ratings scale 1 = healthy, 2 = minor wilting, 3 = moderate wilting, 4 = severe wilting, 5 = plant death).

[z]Data was combined and averaged for analysis of the nine cultivars included in experiment one and two.

Four of the nine cultivars showed different disease responses to the isolates when comparing the two experiments (Table 6). In the first experiment, 'Aristotle,' 'Declaration,' and 'Red Knight' were susceptible to isolates 12889 (DS = 5.0) and OP97 (DS = 2.1 to 4.8), but resistant to SP98 (DS = 1.1 to 1.9) and SFF3 (DS = 1.1 to 1.8). In the same experiment, 'Snapper' was susceptible to isolates 12889 (DS = 5.0), OP97 (DS = 5.0) and SFF3 (DS = 2.1), but resistant to SP98 (DS = 1.9). In the second experiment, 'Aristotle,' and 'Declaration' were susceptible to only 12889 (DS $\leq$ 4.3) and resistant to OP97 (DS $\leq$ 1.5), SP98 (DS $\leq$ 1.3), and SFF3 (DS = 1.0). 'Red Knight' was resistant only to SFF3 (DS = 1.8) and 'Snapper' was resistant to SP98 (DS = 1.3) and SFF3 (DS = 1.3).

When data from the nine cultivars were combined from both experiments, 'Aristotle' and 'Declaration' were only susceptible to 12889 (DS $\leq$ 4.6), and 'Red Knight' and 'Snapper were susceptible to 12889 (DS $\leq$ 4.9) and OP97 (DS = 4.3). The plant death (%) caused by isolates 12889 and OP97 progressed rapidly over time compared to SP98 and SFF3 on 'Red Knight' (Figure 3). Isolate 12889 caused more plant death than OP97, SP98, and SFF3 on 'Paladin'.

The average air temperature recorded in the greenhouse experiments was 19.4°C (minimum 1.0°C, maximum 39.7°C) and the relative humidity was 62.6% (minimum 20.7%, maximum 96.2%).

Phytophthora fruit rot screen. The pepper fruits did not have any disease symptoms (rot, water soaking, mycelial growth, or pathogen sporulation) at harvest. All lesions expanded from the point of zoospore inoculation and were elliptical and elongated from stem end to blossom end of the fruit (Figure 4). Some lesions appeared water

soaked only, whereas other lesions exhibited pathogen mycelia growth and in some cases pathogen sporulation (Figure 5). The greatest percentage of healthy fruit was observed with isolate SP98 whereas inoculations with 12889 resulted in the least. The greatest percentage of fruit with water soaked lesions, pathogen mycelial growth, and pathogen sporulation occurred when the *P. capsici* isolate 12889 was used as inoculum. Inoculations with isolate SP98 had the lowest incidence of fruit with water soaked lesions, and pathogen mycelial growth, and OP97 had the least percentage of fruit with pathogen sporulation. The frequency distribution of fruit within a pepper line that supported *P. capsici* sporulation was different among isolates (Figure 6). When isolates 12889 and OP97 were used, more pepper lines supported pathogen sporulation on fruits than when isolate SP98 was used as inoculum.

Isolate 12889 produced water-soaked lesions that were larger than those caused by OP97, but similar to SP98 (P=0.05) (Figure 7). No statistical differences in area with pathogen mycelial growth or sporulation were noted among the three isolates. Of those fruit with pathogen sporulation, isolate OP97 produced significantly more sporangia/cm^2 than isolate 12889. Statistical differences were not observed in lesion size and sporangial density among the cultivars (Table 7).

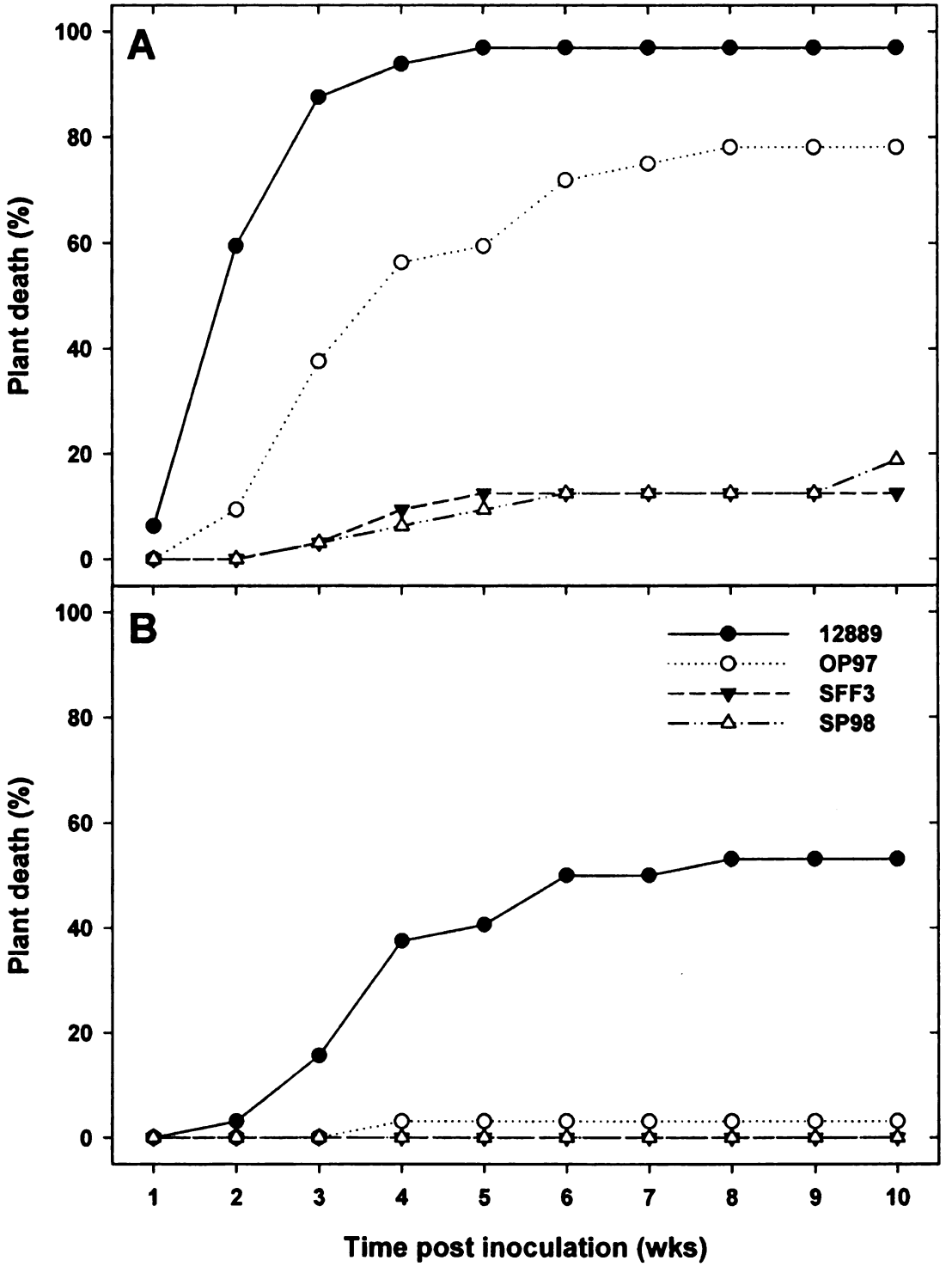

Figure 3. The plant death (%) recorded weekly of **A**, pepper cultivar Red Knight, and **B** pepper cultivar Paladin, caused by *Phytophthora capsici* isolated from pickling cucumber (OP97, SFF3), pumpkin (SP98), and pepper (12889).

Figure 4. Pepper fruit 84 h post inoculation with a 10-µl drop of *Phytophthora capsici* zoospore solution (1.75×10^6 zoospores/ml).

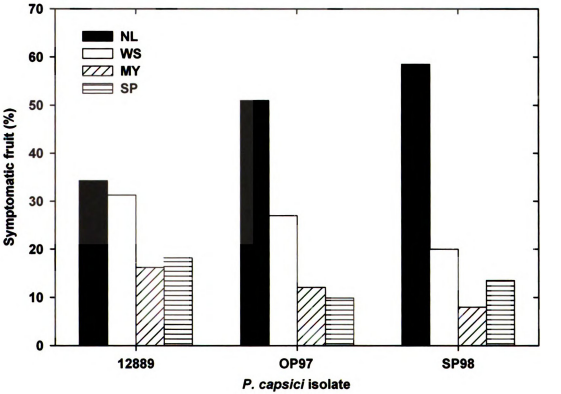

Figure 5. Pepper fruit (%) with or without disease symptoms averaged across all pepper lines when inoculated with a 10-µl drops (1.75×10^6 zoospores/ml) of *Phytophthora capsici* isolates obtained from pepper (12889), pickling cucumber (OP97), and pumpkin (SP98). NL = no lesion, WS = water-soaking only, MY = mycelial growth and water-soaking, SP = sporulation, mycelial growth, and water-soaking.

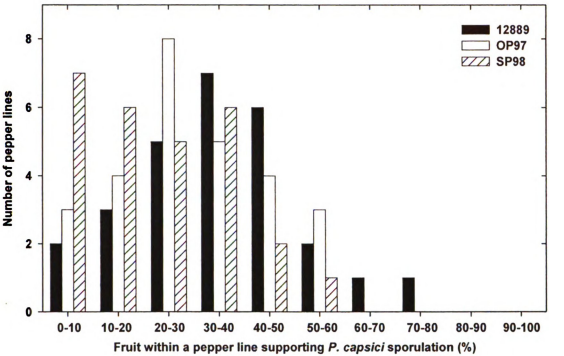

Figure 6. Frequency distribution of the number of pepper lines for which the fruit supported sporulation when inoculated with 10-µl drops (1.75×10^6 zoospores/ml) of *Phytophthora capsici* isolates 12889, OP97 or SP98, obtained from pepper, pickling cucumber, and pumpkin, respectively.

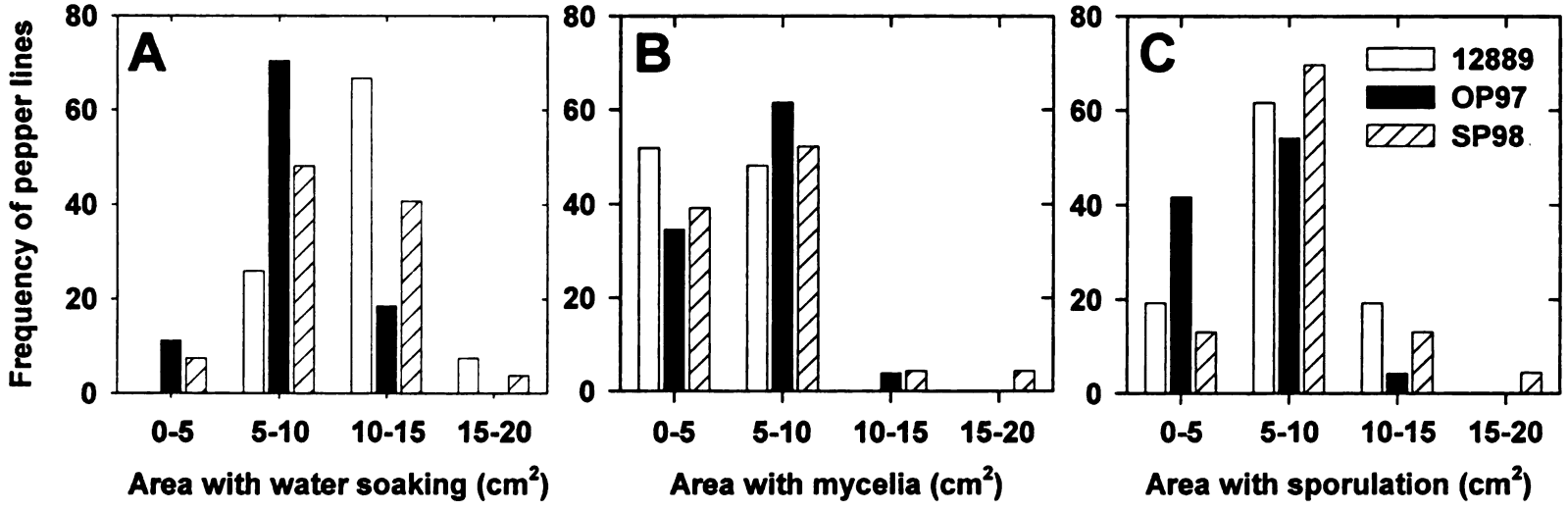

Figure 7. Area of **A** water soaking **B** mycelial growth and **C** pathogen sporulation on pepper fruit inoculated with 10-µl drops (1.75×10^6 zoospores/ml) of *Phytophthora capsici* isolates 12889, OP97, and SP98. The areas were calculated by measuring two diameters perpendicular to each other across the fruit lesion.

Table 7. The mean lesion area (cm^2) and sporulation density (sporangia/cm^2) calculated with water soaking, mycelial growth, and pathogen sporulation on pepper lines.

Pepper line[xy]	Mean lesion area (cm^2)[z]			Sporangia/ cm^2
	Water soaking	Mycelia	Sporulation	
9925776	9.3	9.7	3.1	21706
9931126	8.0	5.2	3.6	8486
9941819	7.8	10.6	4.9	9635
9943084	8.5	6.3	2.5	12624
9943095	8.3	3.8	1.7	5540
Alliance	11.4	6.4	3.1	8993
Aristotle (non-pelleted)	9.0	3.2	2.3	8741
Aristotle (pelleted)	8.0	3.8	2.9	7932
Brigadier	9.9	4.9	2.3	11984
Camelot	6.6	1.4	1.4	14269
Declaration	12.3	4.3	3.5	18151
Paladin	10.5	7.1	2.8	11063
Plato	10.5	5.8	1.6	9197
PRO3-13x14R-4	10.1	4.3	3.5	7377
PRO3-15x16R-5	8.9	5.0	3.4	15239
PRO4T-11x12	13.1	4.2	3.5	9582
PRO5-C71x72	10.1	5.2	2.1	7656
PRO5-C81x82	8.0	6.2	3.1	15842
PRO5-C85x86	11.1	4.0	2.0	3395
PRO5-C87x88	8.2	4.2	2.3	7994
Prophet	9.9	5.2	2.6	9467
PX9942595	7.7	4.7	2.1	13314
Red Knight	9.3	7.2	4.9	7098
Revelation	7.7	6.4	2.4	11835
Revolution	10.2	7.1	2.8	14566
Snapper	10.0	5.1	3.1	5188
XPP2548 (poblano type)	9.6	6.4	1.6	11755

[x]Pepper lines were inoculated with a 10 µl drop of *Phytophthora capsici* zoospore solution (1.75×10^6 zoospores/ml).

[y]Bell pepper line unless otherwise indicated.

[z]Fruit which developed no lesion were removed from the analysis. Data were combined for the three *P. capsici* isolates (12889, OP97, and SP98). None of the parameters were statistically different among cultivars according to Fisher's LSD (P=0.05).

DISCUSSION

Pepper producers in Michigan would benefit from a cultivar that is resistant to Phytophthora crown and root rot. In our study, the susceptibility of the pepper lines to

root and crown rot differed significantly when four Michigan isolates of *P. capsici* were used. None of the cultivars included in our screen had resistance to the *P. capsici* isolate 12889, but several cultivars were resistant to the three other isolates.

Several methods have been developed to screen pepper seedlings for resistance (5,10,18,27). The Chi-squared method has been used to make comparisons with a standard resistant pepper, such as CM334, to determine disease responses of pepper lines (27). However, this method could not be used in our study because the resistant standard CM334 was not included in our first experiment. We used a method adopted from Glosier et al. who used a 0 to 5 disease rating scale (0 = no symptoms, 1 = leaf yellowing, 2 = minor stem necrosis, 3 = moderate stem necrosis and some leaf wilt, 4 = severe stem necrosis and severe wilt, 5 = plant death) and considered plants with an average disease score of < 1 as resistant (27). Both Sy et al. (27) and Glosier et al. (10) determined physiological races by the patterns in resistant and susceptible pepper lines to specific *P. capsici* isolates. Methods of Sy et al. (27) and Glosier et al. (10) are helpful, but determining if the physiological races are the same among regions is difficult because the statistical methods and cultivars used were different between the two experiments. Considering only those pepper lines that exhibited no symptoms of infection as resistant may be prudent. Also, using the currently acceptable methods, it appears as though a high number of physiological races can be determined from a relatively low number of isolates (10,18,27).

There appeared to be a range in virulence among the *P. capsici* isolates and susceptibility among the pepper lines. Polach and Webster (20) observed different levels of virulence among *P. capsici* isolates and others have made similar observations in

studies with pepper (10,18). However, *P. capsici* isolates 12889, OP97, SP98, and SFF3, did not differ in virulence when inoculated onto Fraser fir seedlings (21) and cucumber fruit (8), respectively. Recent studies with tomato, however, have demonstrated significant differences in virulence among the *P. capsici* isolates 12889, OP97, SP98, SFF3 (Quesada, 2009 *unpublished data*). The cumulative AUDPC also differed significantly among the tomato lines. Tomato is Solanaceous and therefore more likely to have resistance mechanisms similar to pepper, than cucurbits or Fraser fir. Differences in incidence of *P. capsici* infection were observed in the fruit screen and isolate virulence occurred in the order: 12889 > OP97 > SP98. Although significant differences in virulence were observed among the isolates, they cannot be considered different Phytophthora fruit rot races because they caused infection on fruits of all pepper lines.

Our results indicate that pepper fruits are more susceptible to *P. capsici* than the roots and crowns. Other reports have indicated different levels of resistance exist in foliar blight and stem blight of pepper (13,18,26). Different mechanisms are responsible for resistance to Phytophthora root rot and foliar blight in pepper (3). Similarly, potato breeders found plants exhibiting tuber resistance to *P. infestans* did not necessarily have resistance to foliar or vine infection by the same *P. infestans* races (4,24). These results, along with differing physiological races of *P. capsici*, have great implications for pepper breeders. Instead of breeding for one symptom, breeders must now potentially breed for four in pepper: root rot, fruit rot, stem blight, and foliar blight (3,18).

The pepper isolate used in our study was highly virulent on the crown, roots, and fruits of pepper. Ristaino found cucurbit isolates were, in some cases, just as virulent on pepper as pepper isolates (22). By using several isolates they were able to establish a

representative sample of the *P. capsici* population. Once a small standard set of cultivars and pepper lines are established, a greater number of isolates can be screened to determine those groups of cultivars that may provide the highest level of resistance in a particular region. This information could be pooled across regions, which would help pepper producers manage Phytophthora crown and root rot more effectively and further facilitate the development of resistant pepper cultivars. Also, producers may consider growing several cultivars in their field to determine which has the highest level of resistance to the *P. capsici* isolates. Information from other regions may not accurately reflect the pathogen population in their targeted fields.

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CHAPTER II

USING FUNGICIDES AND HOST RESISTANCE TO MANAGE *PHYTOPHTHORA CAPSICI* IN BELL PEPPER

ABSTRACT

Phytophthora capsici is an important pathogen of pepper and management requires an integration of cultural and chemical control methods. A resistant cultivar and nine fungicides were tested in both the field and greenhouse for their ability to limit *P. capsici* in pepper. In the field, the susceptible pepper 'Red Knight' and the resistant pepper 'Paladin' were sprayed with fungicides. In the greenhouse, 'Red Knight' and 'Paladin' were treated with fungicides applied as drenches or foliar sprays and reapplied at 7- or 14-day intervals. In both experiments, each plant was inoculated with 1 g of prepared millet seed infected with *P. capsici* inoculum. The cultivar 'Paladin' had significantly less plant death than 'Red Knight.' In the field, plants treated with fluopicolide or mandipropamid had significantly less plant death than the untreated control plants. In the greenhouse, all fixed effects including cultivar, fungicide treatment, application method, and application interval were significant. The interactions among fungicide × cultivar, application method × cultivar, application method × fungicide, and fungicide × cultivar × application method were significant. The potential of resistant cultivars and registered fungicides to be used in managing Phytophthora crown and root rot were demonstrated. In the greenhouse, treatments applied as drenches provided greater control of *P. capsici* than those applied to the foliage.

INTRODUCTION

Michigan producers grow both hot and bell pepper types for fresh and processing markets with a farm gate value of $12 million (1). *Phytophthora capsici* Leonian is a major pathogen of pepper that causes root, crown and fruit rot, as well as stem and leaf blight (12,16,19). The zoospores of *P. capsici* can spread rapidly throughout a field when periods of heavy rainfall occur and has been identified in surface irrigation water (6,9).

The cultural method used to control *P. capsici* in fresh market production includes raised planting beds covered in black plastic mulch with drip irrigation (19). The raised planting beds reduce soil saturation and the black plastic mulch and drip irrigation prevents rain from splashing infested soil onto susceptible plants (3,4,18,21,26). Processing peppers are grown on flat planting beds to reduce costs. In this system, field-scale epidemics can occur frequently because above-ground tissues are not protected from infested soil (9). *Phytophthora capsici* can overwinter for lengthy periods in the soil (> 10 years), which consequently reduces the efficacy of crop rotation (9,11).

Several commercial bell pepper cultivars have a level of resistance to certain isolates of *P. capsici.* Unfortunately, this resistance is not universal as physiological races of *P. capsici* have been reported in the pepper system (7,16,23). This was recently observed by Sy et al., who created 26 recombinant inbred lines (RILs) by crossing the resistant CM334 with the susceptible 'Early Jalapeno' and screened them against isolates from New Mexico, California, and the Netherlands (23). They found that thirteen root rot races differentiated out of the 17 isolates screened, and concluded that resistance in the RILs was dependent on the *P. capsici* isolate or race (23).

Pepper symptoms may be placed into the categories of root rot, stem blight, leaf blight, and fruit rot (16,22,24). Root rot and foliar blight resistance are controlled by two different dominant genes (24). Similarly, Sy et al. demonstrated that resistance in pepper to Phytophthora stem blight, root rot, and foliar blight are controlled by separate genetic systems (22). Therefore cultivars resistant to root rot may not be resistant to foliar blight. As a result, fungicides may be needed in conjunction with cultivars resistant to root rot to control foliar blight symptoms. McGrath and Davey applied foliar fungicides to the root rot resistant 'Aristotle' and the susceptible 'Red Knight;' all foliar fungicides reduced incidence of Phytophthora fruit and crown rot in both cultivars compared to the untreated control (15). The efficacy of fungicides applied as a drench for controlling Phytophthora root rot of pepper have been tested (2,8,14,15). Matheron and Porchas found that drench applications of fungicides provided excellent control of *P. capsici* in 'Aristotle,' and extended the lifespan of the plant compared to untreated control plants under greenhouse conditions (14).

The main objective of our research was to compare the effects of select fungicides when applied as a drench or foliar treatment on susceptible and resistant bell peppers to control Phytophthora root and crown rot caused by *P. capsici.*

MATERIALS AND METHODS

Inoculum preparation. Cultures of *P. capsici* isolate 12889 (mating type A1 and insensitive to the fungicide mefenoxam isolated from a pepper fruit in Michigan) were obtained from long-term stock cultures (stored at 20°C in sterile microcentrifuge tubes with 1 ml of sterile water and a sterile hemp seed) in the laboratory of Dr. Mary

Hausbeck (Michigan State University, MSU). Agar plugs were transferred from the stock cultures onto unclarified V8 (UCV8) agar (16 g agar, 3 g $CaCO_3$, 160 ml unfiltered V8 juice, and 840 ml distilled water) and maintained at room temperature (21±2°C) under constant fluorescent light for seven days. Millet seed medium (100 g millet seed, 72 ml deionized water, and 0.08 g asparagine) was prepared in a 500-ml Erlenmeyer flask that was autoclaved twice in 24-h increments. The medium was inoculated with four agar plugs (7-mm diameter) of actively growing *P. capsici* (12889) and incubated at room temperature under fluorescent light for four weeks and shaken regularly.

Field experiments. The trial was established at two sites: the Muck Soil Research Farm (MSRF) in Clinton County, MI on clay loam soil and the Southwest Michigan Research Center (SWMREC) in Berrien County, MI on loamy fine sand. Flats (128-cell) of *P. capsici* susceptible 'Red Knight' and *P. capsici* resistant 'Paladin' bell pepper transplants were obtained from Keitzer Farms (Hartford, MI). Pepper transplants were nine weeks old with three true leaves and were placed outside for three days prior to transplanting to acclimate the seedlings to the outdoors. The seedlings were planted into raised plant beds (0.6 m wide, 15.24 cm high) covered with black plastic mulch (1.25 mm thickness). Plant beds were spaced 1.5 m apart on center with drip irrigation. Each treatment plot was 12.2 m long and contained a row of each cultivar spaced 0.3 m apart, using staggered planting. Each plant was placed 0.3 m apart within their row, resulting in 80 pepper plants per treatment plot. The plots were arranged in a randomized complete block design, with blocks replicated four times.

Fourteen days after transplanting, 1 g of millet seed inoculum was inserted 2.5 cm into the soil directly beside the transplant plug. Weeds within the planting beds were

removed by hand; weeds between planting beds at the MSRF were controlled with clomazone (Command 3ME, FMC Corporation, Philadelphia, PA) at 0.12 kg a.i./ha and S-metolachlor (Dual Magnum, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.66 kg a.i./ha prior to transplanting. At SWMREC, weeds were controlled between the planting beds with halosulfuron-methyl (Sandea, Gowan Company, Yuma, AZ) at 0.01 kg a.i./ha and S-metolachlor at 0.66 kg a.i./ha. Insects were controlled at both locations with two applications of imidacloprid (Admire Pro, Bayer CropScience, Greensboro, NC) at 0.11 kg a.i./ha applied through drip irrigation emitters calibrated to deliver 473 liter/ha. Four days after planting (9 June), maneb (Maneb 75DF, Cerexagri-Nisso, King of Prussia, PA) at 0.51 kg a.i./ha and copper hydroxide (Champ Formula 2, Nufarm Americas Inc., Burr Ridge, IL) at 0.33 kg a.i./ha were applied to the pepper foliage at SWMREC. No fungicides were applied thereafter aside from experimental treatments. Both sites were fertilized once a week with Mora-Leaf 20-20-20 soluble fertilizer at 5.7 kg/ha (Wilbur Ellis, Fresno, CA) for the first four weeks after planting and twice weekly for the remainder of the experiment. At four and eight weeks after transplanting, plots were fertilized with 1.2 liter/ha of calcium.

Drench treatments (Table 8) were applied to the transplant tray prior to planting using a 7.6-liter watering can at 945 liter/ha. Foliar treatments (Table 8) commenced at planting and were reapplied every seven days using a backpack sprayer and a 3-nozzle boom with 50 mesh screens and 8003XR nozzles, calibrated to deliver 473 liter/ha. The outer two nozzles were aligned at a 45° angle towards the pepper crown, with the middle nozzle positioned directly over the canopy.

Crown and root rot were evaluated weekly following inoculation and continued for 12 weeks until the final harvest. The number of plants per row from each cultivar exhibiting symptoms of infection including wilting, stems lesions, and plant death were counted weekly. Phytotoxic effects of fungicides on pepper plants were evaluated on a 1 to 5 scale per plot (1 = no phytotoxicity, 2 = slight stunting/chlorosis, 3 = moderate stunting/chlorosis, 4 = major stunting/chlorosis, 5 = plant death due to phytotoxicity). The area under the disease progress curve (AUDPC) was calculated according to the methods described by Shaner and Finney (20) to demonstrate the cumulative plant infection (%) throughout the growing period. Marketable-sized fruits ($\geq$ 7.5 cm in width, $\geq$ 8.9 cm in length) were harvested from the entire length of row per plot and weighed.

Table 8. Fungicide treatment, rate, and application schedules applied weekly to pepper plants inoculated with *Phytophthora capsici*.

Treatment	kg a.i./ha	Product information
Untreated uninoculated		
Untreated inoculated		
Mandipropamid[w]	0.15	Revus 2.08SC (Syngenta Crop Protection, Greensboro, NC)
+ nonionic surfactant[w]	0.15	Activator 90 8.33EC (Loveland Products Inc., Greeley, CO)
Fluopicolide[w]	0.10	Presidio 4FL (Valent U.S.A. Corporation, Walnut Creek, CA)
Dimethomorph[w]	0.22	Acrobat 50WP (BASF Corporation, Greensboro, NC)
Potassium phosphite[x]	2.47	Prophyt 4.2EC (Helena Chemical Company, Collierville, TN)
potassium phosphite[y]	3.71	
Fenamidone[w]	0.19	Reason 500SC (Bayer CropScience, Greensboro, NC)
Dimethomorph[w]	0.22	Forum 4.16SC (BASF Corporation)
Famoxadone/cymoxanil[w]	0.28	Tanos 50WG (E.I. du Pont Corporation, Wilmington, DE)
Copper hydroxide[w]	1.21	Kocide 2000 54DF (E.I. du Pont Corporation)
Potassium phosphite[x]	2.47	Prophyt 4.2EC
fluopicolide[z]	0.10	Presidio 4FL
dimethomorph[z]	0.22	Acrobat 50WP
potassium phosphite[z]	3.71	Prophyt 4.2EC

[w]Foliar treatments were applied with a backpack sprayer and 3-nozzle hand-boom (473 liter/ha) and continued every 7 days for 12 weeks.

[x]Drench treatments were applied directly to the transplant tray at planting (945 liter/ha) at planting.

[y]Foliar treatments commenced 14 days after planting and continued every 7 days for 12 weeks.

[z]Foliar treatments commenced 7 days after planting. Products were applied alone and alternated every 7 days for 12 weeks.

Greenhouse experiments. Pepper seeds, 'Red Knight' and 'Paladin,' obtained from Seedway (Hall NY) were sown in 128-cell flats containing potting media (BACCTO Professional Planting Mix, Michigan Peat Company, Houston TX) and placed in a greenhouse with a 14-h photoperiod. When seedlings developed three to four true leaves, they were transplanted into 1.5-liter pots filled with potting media (BACCTO Professional Planting Mix) and arranged in a completely randomized design in a greenhouse with a 14-h photoperiod at the MSU Horticulture Teaching and Research Center, East Lansing, MI. Pots were irrigated individually using a hose and a breaker every one to two days and fertilized three times a week with 200 ppm of Peters 20-20-20 (Scott's Company, Marysville, OH). Irrigation water was amended with phosphoric acid at 132 ppm twice weekly to maintain the media pH at approximately 6.0 to 6.5. The pH was checked monthly by collecting random media samples and using a pH meter (Hanna Instruments, Woonsocket, RI).

Each pot was inoculated with 1 g of millet seed inoculum (as previously described) 24 h after transplanting. Twenty-four pots of each cultivar were untreated and non-inoculated and 24 pots of each cultivar were untreated and inoculated with *P. capsici*. Fungicides and application rates were identical to the field experiment, but included mefenoxam (Ridomil Gold 4SL, Syngenta Crop Protection) at 0.56 kg a.i./ha as an additional treatment. Fungicide treatments were applied immediately following inoculation. Foliar applications were made using a backpack sprayer as previously described. Drench applications were made by hand, applying enough fungicide solution to each pot at a rate equivalent to 945 liter/ha (~80 ml). Each fungicide treatment, application method, and treatment interval combination was replicated six times; the trial

was conducted twice. The experiment was arranged with treatments in a completely randomized design.

Disease was assessed using a 1 to 5 scale (1 = healthy, 2 = minor wilting, 3 = moderate wilting, 4 = severe wilting, 5 = plant death) modified from Glosier et al. (7) every two days following the first symptom of plant wilting and continued for 14 days after the final fungicide application. The AUDPC was calculated according to the methods of Shaner and Finney (20) to demonstrate the cumulative plant infection (%) throughout the growing period.

Pathogen confirmation. Approximately 10% of the symptomatic plants from both the field and greenhouse experiments were returned to the laboratory to isolate the pathogen. The plants were rinsed with deionized water and the roots and crowns were surface sterilized with a 70% ethanol solution. Three sections of root and crown tissue were excised and plated onto UCV8 plates amended with 25 ppm of benomyl, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene (BARP). Plates were incubated at room temperature (21±2°C) under continual fluorescent light for 7 days and checked microscopically (200X) to confirm *P. capsici* using morphological characteristics according to the *Phytophthora* spp. key by Waterhouse (25).

Weather monitoring. Hourly measurements of air temperature, relative humidity, and rainfall were recorded using a Watchdog data recorder (Model 450, Spectrum Technologies, Inc Plainfield, IL) and tipping-bucket rain collector (Spectrum Technologies, Inc.) in the field. In the greenhouse, air temperature and relative humidity were recorded using a Watchdog data recorder.

Statistical analysis. Along with the cumulative AUDPC values from both the field and greenhouse experiments, plant death (%) and yield data were subjected to analysis of variance (ANOVA) using PROC MIXED of SAS (SAS Institute Inc., Cary, NC). Fisher's protected LSD was used for separation of means when effects were found to be statistically significant in ANOVA analysis ($P \leq 0.05$). Greenhouse AUDPC data were log transformed in order to avoid violating normality assumptions of the test. To elucidate significant interactions ($P \leq 0.05$), effects were sliced into single factors or factor combinations. Significant interactions were separated with Fisher's protected LSD. The non-inoculated control plots and plants were removed from analysis due to a violation in variance assumptions.

RESULTS

Field experiments. Disease pressure from *P. capsici* was high and disease symptoms, including wilting and stem lesions, were observed seven days post-inoculation (DPI) in the inoculated control plots at both sites. Significant differences ($P < 0.05$) occurred in plant death incidence and AUDPC values calculated for the cultivars and fungicides, but not the interaction (cultivar × fungicide) (Table 9). Marketable yield differed significantly for cultivars, but not for the fungicide treatments and there was no interaction (cultivar × fungicide) (*data not shown*). In the untreated plots, plant death at the final evaluation was 8.6% and 84.1% for 'Paladin' and 'Red Knight,' respectively. The fungicides fluopicolide, mandipropamid, and the program treatment (potassium phosphite, fluopicolide, and dimethomorph 50WP) had significantly less plant death (%) than the inoculated untreated control (Table 10). Applications of the fungicides

fluopicolide, mandipropamid, dimethomorph 50WP, potassium phosphite, and the program treatment had statistically lower cumulative AUDPC values than the inoculated untreated control. No significant differences were observed in marketable yield. Applications of potassium phosphite, alone and in the program treatment, appeared to cause phytotoxicity on 'Paladin' but not 'Red Knight' (*data not shown*). The visual appearance of phytotoxicity, including plant stunting, browning and curling of leaf margins, and brown speckling on the leaves, only occurred at MSRF.

Table 9. Analysis of variance for effects of cultivar and fungicide treatment on the cumulative area under the disease progress curve (AUDPC) value and plant death (%) caused by *Phytophthora capsici*, from two field sites.

	AUDPC		Plant death (%)	
Effect	**F value**	**P > F**	**F value**	**P > F**
Cultivar	1993.88	< 0.0001	2272.45	< 0.0001
Fungicide	3.35	0.0010	2.30	0.0192
Cultivar x fungicide	0.79	0.6256	0.96	0.4754

Table 10. The plant death (%), area under the disease progress curve (AUDPC), and marketable yield of bell peppers 'Red Knight' and 'Paladin' treated with fungicides.

Treatment and rate (kg a.i./ha)[u]	Plant death (%)			AUDPC			Marketable yield (kg/12.2 m of row)		
	Both cv.	'Red Knight'	'Paladin'	Both cv.	'Red Knight'	'Paladin'	Both cv.	'Red Knight'	'Paladin'
Untreated inoculated	57.8c[v]	92.8	20.0	3228c	5494b-d	963	14.7	2.5	26.8
Fluopicolide 0.15[w]	43.9a	84.1	3.8	2518a	4838ab	198	14.9	1.8	28.0
Mandipropamid 0.10[w]	47.3ab	90.9	3.8	2632ab	5062a-c	202	14.3	2.1	26.5
Potassium phosphite 2.47[x] fluopicolide 0.10[y] dimethomorph 50WP 0.22[y] potassium phosphite 3.71[y]	47.8ab	86.9	8.8	2883a	4632a	291	9.7	5.0	14.4
Dimethomorph 50WP 0.22[w]	51.4a-c	91.9	10.9	2848ab	5217a-c	478	12.7	1.7	23.6
Potassium phosphite 2.47[x] potassium phosphite 3.71[z]	51.6bc	94.7	8.4	2883ab	5474b-d	291	10.0	5.3	17.7
Copper hydroxide 1.21[w]	52.5bc	94.1	10.9	3103bc	5587cd	619	16.1	1.1	31.0
Dimethomorph 4.18SC 0.22[w]	52.7bc	96.9	8.4	3053bc	5699cd	408	13.2	1.8	24.6
Fenamidone 0.19[w]	52.8bc	95.9	11.6	3197c	5719cd	674	13.9	1.4	26.5
Famoxadone/cymoxanil 0.28[w]	55.8c	95.6	15.9	3383c	5947d	819	14.8	1.2	28.5

[u]Each plant was inoculated with 1 g of millet seed inoculum prepared with *Phytophthora capsici* isolate 12889 from Michigan.

[v]Column means with a letter in common or with no letter are not significantly different (Fisher's Method; P=0.05).

[w]Treatments were applied as foliar applications calibrated to deliver 473 liter/ha, weekly commencing at transplanting.

Table 10. (cont'd)

[x]Treatments were applied as a drench application directly to the transplant tray.

[y]Treatments were alternated every seven days, commencing seven days after transplanting.

[z]Treatment was applied as foliar applications calibrated to deliver 473 liter/ha, weekly commencing 14 days after transplanting.

Total rainfall was 442.2 and 480.6 mm at the MSRF and SWMREC, respectively (Table 11). Mean air temperatures at MSRF and SWMREC were 18.7 and 20.2°C, respectively.

Table 11. Precipitation and soil and air temperature (average, minimum, and maximum) at the Muck Soil Research Farm Farm (MSRF) and Southwest Michigan Research and Extension Center (SWMREC) in Michigan.

Field	Month	Air temperature (°C)			Soil temperature (°C)			Precipitation
		Ave.	Min.	Max.	Ave.	Min.	Max.	(total mm)
MSRF	June	19.6	4.5	32.6	19.9	11.1	30.4	91.1
	July	20.7	3.6	31.4	21.7	13.0	29.8	93.7
	August	18.8	2.3	30.6	21.5	12.2	32.9	39.6
	September	15.6	1.0	32.6	18.2	9.7	30.4	217.8
SWMREC	June	20.2	8.2	31.0	21.9	14.4	30.3	59.1
	July	21.9	9.3	31.9	24.9	17.8	32.2	92.2
	August	20.6	10.3	31.4	24.5	17.9	32.6	36.3
	September	17.9	7.6	32.9	20.6	14.7	31.9	293.0

Greenhouse experiment. The average temperature recorded during the greenhouse experiments was 16.0°C (minimum 1°C, maximum 38.4). The average relative humidity was 65.7% (minimum 20.7%, maximum 93.1%).

Pepper plants in the first and second replications began showing symptoms of infection, including wilt and lesions at the base of the stem at 7 and 20 DPI, respectively. The untreated non-inoculated control plants remained asymptomatic. The ANOVA for fixed effects was significant for the AUDPC values of cultivar, fungicide treatment, treatment interval, and application method (Table 12). 'Paladin' (14.4% plant death) had a significantly lower mean AUDPC value than 'Red Knight' (67.9% plant death) (Table 13). Fungicides applied at 7-day intervals had statistically lower AUDPC values than those applied at 14-day intervals. Drench applications of fungicides had a significantly lower mean AUDPC value than foliar applications. Drench treatments resulted in 21.8%

plant death, compared to 56.5% of plants that received foliar applications. All fungicide treatments, except copper hydroxide, had lower AUDPC values than the untreated inoculated control plants (Table 15).

The *P* value for interactions among the effects fungicide × cultivar, application method × cultivar, and application method × fungicide were also significant ($P = 0.05$) (Table 12). Regardless of fungicide treatment, 'Paladin' had statistically lower AUDPC values than 'Red Knight' (Figure 8). However, the differences between the cultivars' AUDPC were not of the same magnitude. Plants treated with potassium phosphite, for example, had a greater difference in mean AUDPC values between 'Paladin' and 'Red Knight' than plants treated with famoxadone/cymoxanil. Differences in magnitude were also observed between application method and cultivar (Figure 9). All combinations of 'Red Knight' and 'Paladin' with drench and foliar applications were statistically different, but it appears as though drench applications had a greater effect on 'Red Knight' than on 'Paladin.' The interaction between application method and fungicide differed in magnitude and drench applications always had statistically lower AUDPC values than foliar applications for all fungicides except copper hydroxide (Figure 10).

Table 12. Analysis of variance for the effects of cultivar, treatment, application timing, and application method on the cumulative area under the disease progress curve value, in a greenhouse experiment with bell peppers inoculated with *Phytophthora capsici.*

Effect	F value	*P* > F
Cultivar	552.12	<0.0001
Fungicide	11.06	<0.0001
Fungicide x cultivar	3.99	0.0001
Application timing	6.86	0.0090
Application timing x cultivar	1.12	0.2912
Application timing x fungicide	0.65	0.7363
Application timing x cultivar x fungicide	1.85	0.0642
Application method	422.90	<0.0001
Application method x cultivar	241.08	<0.0001
Application method x fungicide	4.84	<0.0001
Application method x cultivar x fungicide	1.97	0.0470
Application method x application timing	0.29	0.5929
Application method x cultivar x application timing	0.15	0.6943
Application method x fungicide x application timing	1.15	0.3253
Cultivar x fungicide x application timing x application method	1.10	0.3637

Table 13. The cumulative area under the disease progress curve (AUDPC) and plant death (%) for application timing, application method, and cultivar of bell pepper inoculated with *Phytophthora capsici.*

Effect	AUDPC[z]		Plant death (%)
Application timing			
7-day interval	79	a	36.3
14-day interval	84	b	41.9
Application method			
Drench	60	a	21.8
Foliar	103	b	56.5
Cultivar			
Paladin	58	a	14.4
Red Knight	105	b	67.9

[z]AUDPC means within the same effect that are followed by the same letter are not statistically different according to Fisher's LSD (*P*=0.05).

Table 14. Mean area under the disease progress curve (AUDPC) and plant death (%), caused by *Phytophthora capsici*, calculated over eight weeks in a greenhouse.

Treatment and rate (kg a.i./ha)[x]	Mean AUDPC			Plant death (%)		
	Both cv.	'Red Knight'	'Paladin'	Both cv.	'Red Knight'	'Paladin'
Untreated inoculated	103 f[y]	141 j[z]	64 b-d[z]	59.4	100.0	18.7
Famoxadone/cymoxanil 0.28	62 a	75 de	49 a	19.8	35.4	2.1
Fluopicolide 0.15	74 b	92 ef	55 a-c	26.0	43.8	8.3
Mandipropamid 0.10	79 bc	102 fg	56 a-c	32.3	54.2	10.4
Dimethomorph 4.18SC 0.22	80 bc	108 gh	52 ab	34.4	66.7	2.1
Dimethomorph 50WP 0.22	81 bc	104 fg	57 a-c	35.4	56.3	12.5
Mefenoxam 0.56	84 cd	111 g-i	58 a-c	47.9	85.4	8.3
Fenamidone 0.19	87 c-e	104 fg	69 cd	56.3	68.8	18.8
Potassium phosphite 2.47, 3.71	93 de	125 hi	61 a-d	44.8	77.1	12.5
Copper hydroxide 1.21	96 ef	126 i	65 b-d	57.3	91.7	25.0

[x]Treatments were applied for six weeks on a 7- or 14-day schedule as drenches or foliar applications.

[y]Mean AUDPCs within a column followed by the same letter or with no letter, are not statistically different according to Fisher's LSD (P=0.05)

[z]Mean AUDPCs followed by the same letter or with no letter, are not statistically different according to Fisher's LSD (P=0.05)

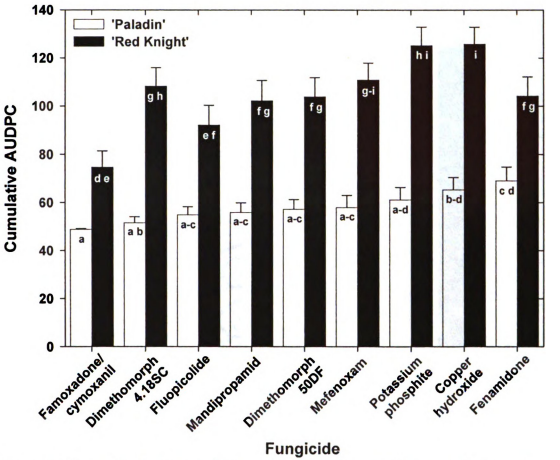

Figure 8. Interaction between cultivar and fungicide in a greenhouse. Plants were inoculated with an isolate of *Phytophthora capsici.* Fungicides were applied six times. Plants were evaluated every two days on a 1 to 5 scale (1 = healthy, 2 = minor wilting, 3 = moderate wilting, 4 = severe wilting, 5 = plant death) and area under the disease progress curve (AUDPC) values were calculated over eight weeks. Bars noted with letters in common are not statistically different according to Fisher's LSD (P=0.05).

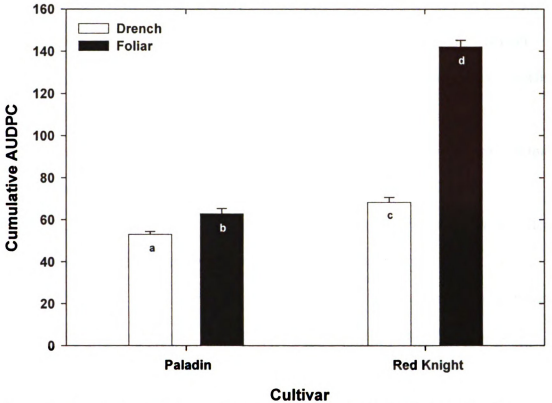

Figure 9. Interaction between cultivar ('Red Knight' and 'Paladin') and fungicide application method (drench and foliar) in a greenhouse. Cumulative area under the disease progress curve (AUDPC) were calculated over eight weeks. Each plant was inoculated with 1 g of millet seed inoculum infested with *Phytophthora capsici*. Bars noted with letters in common are not statistically different according to Fisher's LSD (P=0.05).

A three-way interaction among application method × cultivar ×fungicide was significant and differed in magnitude (P=0.05) (Table 13). The effects of drench treatments of famoxadone/cymoxanil, fluopicolide, mandipropamid, fenamidone, dimethomorph 50WP, and dimethomorph 4.16SC were statistically similar for 'Paladin' and 'Red Knight' (Table 15). Foliar applications of all fungicides on 'Paladin' resulted in statistically lower AUDPC values than the same fungicide applied to 'Red Knight.' Treatments applied to 'Paladin' had statistically similar AUDPC values when applied as

either a drench or foliar application. The application method had a greater effect on 'Red Knight,' as all drench applications had lower AUDPC values than when the same product was applied to foliage. In general, fungicides applied to the foliage of 'Red Knight' resulted in statistically higher AUDPC values than foliar applications made to 'Paladin' and drench applications made to both cultivars. Also, drench applications made to 'Paladin' had lower AUDPC values than foliar applications or drench applications made to 'Red Knight,' although this trend was not always statistically significant.

Repeated, drench and foliar applications of mefenoxam resulted in phytotoxicity. The phytoxicity first appeared as chlorotic and bleached areas on the leaves and subsequently turned leaf margins white (Figure 11). The white areas eventually became holes and the plants defoliated, leaving an apparently healthy green stem (Figure 11). Drench applications of fenamidone also resulted in visual phytotoxicity. Small yellow to brown spots appeared on the leaves, causing eventual plant wilt without death (Figure 12).

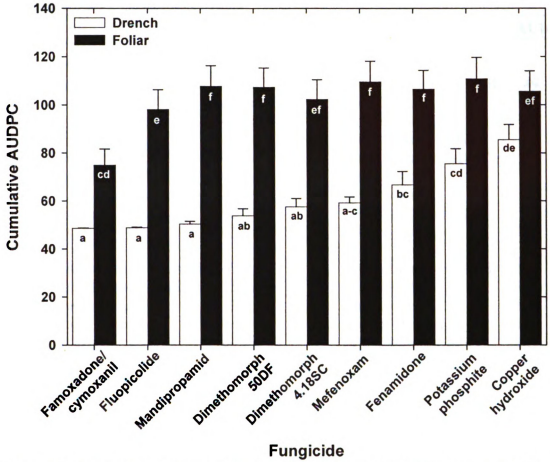

Figure 10. Interaction between application method (drench and foliar) and fungicide treatment in a greenhouse. Cumulative area under the disease progress curve (AUDPC) was calculated over eight weeks. Each plant was inoculated with 1 g of millet seed inoculum infested with *Phytophthora capsici*. Bars noted with letters in common are not statistically different according to Fisher's LSD (P=0.05).

Table 15. Interaction in area under the disease progress curve (AUDPC) between cultivar, 'Red Knight' and 'Paladin,' application method × drench vs. foliar × fungicide treatment.

Treatment[y]	AUDPC[z]		Treatment	AUDPC[z]	
'Paladin'			'Red Knight'		
Drench			Drench		
Famoxadone/cymoxanil	49	a	Famoxadone/cymoxanil	49	a
Dimethomorph 4.18SC	49	a	Fluopicolide	49	a
Fluopicolide	49	a	Mandipropamid	52	ab
Mandipropamid	49	a	Dimethomorph 50WP	59	a-d
Dimethomorph 50WP	49	a	Dimethomorph 4.18SC	66	a-d
Mefenoxam	49	a	Mefenoxam	69	b-d
Potassium phosphite	59	a-d	Fenamidone	72	cd
Fenamidone	62	a-d	Potassium phosphite	92	ef
Copper hydroxide	64	a-d	Copper hydroxide	106	f
Foliar			Foliar		
Famoxadone/cymoxanil	49	a	Famoxadone/cymoxanil	101	f
Dimethomorph 4.18SC	55	a-c	Fluopicolide	136	g
Fluopicolide	61	a-d	Fenamidone	137	g
Mandipropamid	63	a-d	Copper hydroxide	145	gh
Potassium phosphite	64	a-d	Dimethomorph 50WP	149	gh
Dimethomorph 50WP	66	a-d	Dimethomorph 4.18SC	150	gh
Copper hydroxide	66	a-d	Mandipropamid	152	gh
Mefenoxam	67	a-d	Mefenoxam	152	gh
Fenamidone	76	de	Potassium phosphite	158	h

[y]Each plant was inoculated with 1 g of *Phytophthora capsici* infested millet seed.

[z]Mean AUDPC values with letters in common are not statistically different according to Fisher's LSD (P=0.05).

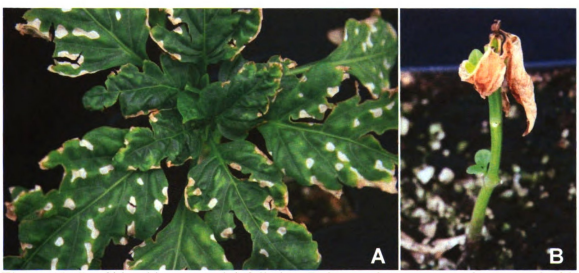

Figure 11. Leaf bleaching, **A**, and defoliation, **B**, incurred on a pepper plant treated with 0.56 kg a.i./ha mefenoxam.

Figure 12. Necrotic lesions on leaf from a pepper plant drenched with 0.19 kg a.i./ha fenamidone.

DISCUSSION

In infested fields, failure to use a disease management program to limit root and crown rot on pepper can have severe economic consequences. *Phytophthora capsici* can infect the root, crown, stems, leaves, and fruit of pepper. Management is dependent on the symptom observed most frequently in the field. Since fungicides are expensive, they must effectively control *P. capsici*, and be applied in a manner that will provide adequate coverage of the targeted plant parts.

Cultivars with demonstrated tolerance to local *P. capsici* isolates are a useful tool in managing crown and root rot. In this study, 'Paladin' was significantly more tolerant to the Michigan pepper isolate 12889 than 'Red Knight.' Others have noted the importance of screening cultivars with local isolates (7,23). Glosier et al. found 14 distinct virulence groups out of 34 isolates from California, New Mexico, and the Netherlands (7). A significant interaction was observed between isolate and cultivar;

some cultivars were resistant to certain isolates, but susceptible to others. Several pepper lines resistant to *P. capsici* are available, but few have demonstrated resistance to a wide range of isolates (5,7,23). This has great implications for growers because cultivars with proven resistance to *P. capsici* isolates in one region may have little or no resistance to the isolates in their fields.

Historically, the fungicide mefenoxam has been used to control *P. capsici* on pepper. However, resistance of *P. capsici* to mefenoxam has now been documented throughout the United States (17), including several regions of Michigan (10), which has created an urgent need for new fungicides. Although foliar applications in the field of fungicides directed at the base of the pepper plant limited crown and root rot compared to the untreated control, the level of control was not satisfactory. Plants treated with fluopicolide and mandipropamid had statistically lower plant death than the untreated control, but still had > 40% plant death. Producers often experience large-scale epidemics, especially in low-lying areas of the field (4,9,18,19), and have not seen a fungicide demonstrate effectiveness when disease pressure has been high (9,15). McGrath and Davey evaluated the efficacy of six different fungicides, including mefenoxam, for controlling Phytophthora blight (15). All fungicide applications resulted in statistically lower plant death (%) than the untreated control, while the best treatment on 'Red Knight' still had 25% plant death. When the fungicides were used in combination with the resistant bell pepper, 'Aristotle,' plant death (%) in the best treatment was reduced to 7%. In our product evaluation, when resistant bell peppers were used in combination with fungicides, plant death decreased significantly in both the field and greenhouse.

In the greenhouse study, products had statistically lower AUDPC values when applied at 7-day versus 14-day intervals. Also, products applied as drenches were more effective than when applied as a foliar treatment. There was no interaction between treatment interval or application method, implying both foliar and drench applications were most effective at 7-day intervals. Matheron and Porchas found that drench applications provided greater control of *P. capsici* than the untreated control, yet no direct comparison between drench and foliar applications were made (14). In our experiment, additional *P. capsici* inoculum was not introduced into the pots after the initial inoculation, which may not adequately reflect field conditions where the soil may be re-infested following each rainfall. Despite this, results from both studies clearly indicate that fungicides applied as drenches to the crowns and roots of peppers will significantly reduce Phytophthora root and crown rot compared to foliar applications. Babadoost made soil drench applications at transplanting with fluopicolide and mandipropamid, and found that both fungicides had significantly less *P. capsici* symptoms than the untreated control (2). Currently, mefenoxam and potassium phosphite (and other salt derivatives) are the only products registered for soil applications.

The efficacy of currently registered fungicides applied as pre-plant soil applications, in drip irrigation lines, or as transplant-tray drenches is relatively unknown and should be further investigated. Also, the compatibility of registered fungicides with irrigation equipment is unknown. If drenches applied through drip irrigation were to require less fungicide than a standard 7-day application program, they may be more cost effective. However, the most effective rate of registered fungicides when applied as a drench via irrigation equipment has not been studied. The application rates of drenches

used in combination with foliar applications were evaluated by Hausbeck and Cortright (8). They applied drench applications of fluopicolide/propamocarb directly to the planting bed at two different application rates and continued treatment 16 days later with a 7-day foliar application program. The plant death (%) was statistically similar for both application rates (8).

In summary, when fungicides are used in combination with host resistance, Phytophthora crown and root rot caused by *P. capsici* can be reduced. Fungicides applied as a drench may provide greater control of *P. capsici*, but further research is warranted to investigate different application methods and treatment rates in the field.

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CHAPTER III

EVALUATION OF EGGPLANT LINES FOR RESISTANCE TO PHYTOPHTHORA CROWN AND ROOT ROT AND VIRULENCE OF *PHYTOPHTHORA CAPSICI* ON EGGPLANT FRUIT

ABSTRACT

An important component of an effective control regimen of *Phytophthora capsici* is plant resistance. For peppers there is only one known line with demonstrated resistance to all *P. capsici* isolates and many of the tolerant cultivars exhibit undesirable horticultural traits. Grafting susceptible pepper cultivars onto resistant root stocks could be a useful approach for generating plants that are resistant to *P. capsici* root and stem rot. The goal of this study was to identify potential eggplant rootstock candidates that are resistant to *P. capsici*. Greenhouse experiments were performed to evaluate two eggplants, EG195 and EG203, with pepper lines of known resistance levels for resistance to fourteen *P. capsici* isolates. The isolates varied in virulence among pepper and eggplant lines that exhibited varied levels of susceptibility. The eggplant line EG195 was resistant to all *P. capsici* isolates screened. A laboratory experiment was conducted to evaluate the virulence of five isolates on eggplant fruit inoculated with a zoospore solution (1.0×10^4 and 1.0×10^6 zoospores/ml). In the fruit screen, the higher zoospore concentration had a significantly higher lesion incidence and size than the lower inoculum concentration. The interaction between the zoospore concentration and isolate was significant ($P < 0.05$) and isolate 13351 had statistically larger lesions than isolate 13566 when inoculated with the higher zoospore concentration. The lesion size was not significantly different among isolates when using the low zoospore concentration. Although the research is preliminary, resistance within eggplant rootstocks was observed, with line EG195 exhibiting resistance to all *P. capsici* isolates tested. Further research is warranted to test line EG195 for resistance to a wider range of *P. capsici* isolates and to evaluate it for potential rootstock compatibility to pepper or eggplant.

INTRODUCTION

In 2008, Michigan farms produced over 25,000 ha of vegetables susceptible to the pathogen *Phytophthora capsici* Leonian, including species in the *Cucurbitaceae, Fabaceae,* and *Solanaceae* families. Pepper production accounts for over $12 million in farm revenue in Michigan (2). There are four different disease symptoms in the pepper-*P. capsici* pathosystem including root and crown rot, stem, and foliar blight, and fruit rot (19,22,26,30,34). Root and crown rot is the predominant disease symptom observed in Michigan bell pepper fields, with fruit rot occurring rarely (12).

Pathotypes and physiological races, distinguished by host tissue type and virulence, exist in the pepper-*P. capsici* system (11,22,31). Races do not seem to be restricted to particular geographic regions (11) and an isolate from Michigan seems to be highly virulent to the roots, crowns and fruits of pepper (9) and the roots of tomato (Quesada-Ocampo and Hausbeck, *unpublished data*). This, in conjunction with the prevalence of the root and crown rot symptoms among peppers in Michigan, necessitates research that focuses on the root and crown rot syndrome. Use of resistant plants is a critical aspect of any *P. capsici* management plan, however no cultivar has demonstrated resistance to all isolates (9) and resistant cultivars have been associated with fruit defects (36).

Grafting susceptible pepper scions to resistant rootstocks of either resistant pepper or other Solanaceous species is a potential method to reduce *P. capsici* infection. Grafted plants are used extensively in Europe (8) and Asia (4,18). In Italy, pepper cultivars have been screened for rootstock resistance to *P. capsici*; the cultivar 'Grafito,' is a promising and potential candidate for grafting pepper plants (21). In North America it is estimated

that 40 million grafted plants are being used in greenhouse tomato production and usually eggplants are used as rootstocks (16). The eggplant lines EG195 and EG203 are resistant to bacterial wilt (28), root-knot nematode, and tomato Fusarium wilt, and have been used in the greenhouse as rootstocks for tomato scions (4). Due to the advancement in grafting technology, a Solanaceous rootstock resistant to *P. capsici* could be implemented in fields infested with *P. capsici.*

Although eggplants can be susceptible to root and crown rot, the primary symptom observed in the field is fruit rot (10,14,15). Eggplant root and crown rot is infrequent in Michigan and rarely limit production even though *P. capsici* can be a major limiting factor in Michigan pepper production and to eggplant production in other parts of the United States (10). Managing *P. capsici* in pepper has been the focus of several research studies (7,20,26,31), whereas similar research on the susceptibility of eggplant is limited.

The objectives of this research were to: (i) evaluate breeding lines of eggplant for resistance to Phytophthora root and crown rot and (ii) investigate the virulence of *P. capsici* isolates on eggplant fruits.

MATERIALS AND METHODS

Isolate selection and inoculum preparation. Fourteen isolates were selected from the long-term culture collection of Dr. Mary Hausbeck (Michigan State University) and Dr. Christine Smart (Cornell University). The isolates were classified according to mating type (A1 or A2), sensitivity to mefenoxam (S = sensitive and I = insensitive), and

host plant. Isolates OP97 (A1, S, pickling cucumber) SP98 (A2, S, pumpkin) 12889 (A1, I, pepper), SFF3 (A2, I, pickling cucumber), and 13566 (A1, S, eggplant) are from Michigan and isolates, 13351 through 13360 (A1, S, eggplant) are from New York.

P. capsici isolates were maintained on V8 agar (16 g agar, 30 mM $CaCO_3$, 160 ml unfiltered V8 juice and 840 ml distilled water) and grown under constant fluorescent light at room temperature (21 ± 2°C) for seven days. For the root and crown rot screen, millet seed medium (100 g millet seed, 72 ml deionized water and 0.08 g asparagine) was prepared in 500-ml Erlenmeyer flasks. The flasks were autoclaved twice on two consecutive days. The millet seed was inoculated with four 7-mm-diameter plugs of actively growing *P. capsici* and incubated at room temperature under constant fluorescent light and agitated daily. For the fruit screen, V8 plates of actively sporulating *P. capsici* cultures (OP97, SP98, 12889, 13351, and 13566) were flooded with 1 ml sterile distilled water. The cultures were incubated at 4°C for 1 h followed by 30 min at room temperature to initiate zoospore release. The concentration of zoospores was estimated using a hemacytometer and adjusted to 1×10^6 and 1×10^4 zoospores/ml.

Phytophthora root and crown rot screen. Two experiments were conducted twice in the greenhouse to evaluate pepper and eggplant seedlings for susceptibility to Phytophthora root and crown rot. In the first screen four *P. capsici* isolates (OP97, SP98, SFF3, and 12889) were tested on four pepper and two eggplant lines (Table 16). A second trial included eleven *P. capsici* isolates (12889, 13351 to 13360) against one pepper and three eggplant lines (Table 16). Commercial eggplant and pepper seeds were purchased from Seedway, LLC (Hall, NY). Experimental eggplant and pepper lines were provided by Dr. Richard Hassel (Clemson University) and George Moriarty (Cornell

University). Seeds were sown into 72-cell flats containing potting media (BACCTO Professional Planting Mix, Michigan Peat Company, Houston TX) and grown in a greenhouse with a 14-h photoperiod. Transplants were grown until all pepper plants and eggplants developed three to four and two to three true leaves, respectively.

Table 16. *Capsicum annuum* and *Solanum melongena* L. var. *esculentum* lines screened for root and crown rot resistance to *Phytophthora capsici* during 2008 and 2009.

Line	Seed Company/Provider	Experiment
Camelot[x]	Monsanto Company, St. Louis, MO	1
Classic[y]	Harris Moran Seed Company, Modesto, CA	2
CM334[z]	Cornell University, Ithaca, NY	1
EG195[y]	Clemson University, Clemson, SC	1, 2
EG203[y]	Clemson University, Clemson, SC	1, 2
Paladin[x]	Syngenta Seeds Inc., Golden Valley, MN	1
Red Knight[x]	Monsanto Company	1, 2

[x]Indicates that the line produces bell pepper type fruit
[y]Indicates that the line produces eggplant type fruit
[z]Indicates that the line produces serrano pepper type fruit

Pepper and eggplant seedlings were transplanted into individual 1.5-liter-pots filled with potting media. Pots were arranged in a complete randomized design on raised benches in a greenhouse and far enough apart to exclude pathogen splash dispersal at MSU's Horticulture Teaching and Research Center, East Lansing, MI. All isolates were replicated eight times among all plant lines, including a negative control consisting of sterile non-inoculated millet seed. The pot media was inoculated with 1 g of millet seed inoculum, which was inserted into the media 2.5 cm below the surface, directly beside the pepper or eggplant's root mass, 24 h after transplanting. Each plant was individually irrigated every one to two days and fertilized three times a week with 200 ppm of Peters 20-20-20 (Scott's Company, Marysville, OH). Irrigation water was amended with

phosphoric acid at 132 ppm twice weekly to maintain the media pH at approximately 6.0 to 6.5. The pH was checked monthly by collecting random soil samples and using a pH meter (Hanna Instruments, Woonsocket, RI).

Approximately 10% of the plants exhibiting *P. capsici* symptoms were returned to the laboratory to isolate the pathogen. The root and crown area of each plant were washed with deionized water and sprayed with a 70% ethanol solution. Three portions of root and crown tissue were excised and plated onto BARP-amended V8 agar plates. Cultures were incubated at room temperature under constant fluorescent lighting for three days and checked microscopically (200X) to confirm *P. capsici* using morphological characteristics according to the *Phytophthora* spp. key by Waterhouse (35). Hyphal-tips of *P. capsici* cultures were transferred onto new BARP-amended V8 agar plates. After seven days, each resulting isolate was screened for mefenoxam sensitivity and mating type to confirm the isolate phenotype matched that of the isolate originally used to inoculate the plant (17).

Phytophthora crown and root rot was evaluated every other day following inoculation until the pepper fruit was 7 to 10 cm in diameter (average 73 days post inoculation). Plants were graded on a 1 to 5 scale (1 = healthy, 2 = minor wilting, 3 = moderate wilting, 4 = severe wilting, 5 = plant death). The area under the disease progress curve (AUDPC) was calculated according to the methods of Shaner and Finney (29) to demonstrate cumulative infection (%) and rate of plant death throughout the growing period.

Phytophthora fruit rot screen. Eggplant fruit free of external blemishes were purchased from a commercial supplier (Meijer, Grand Rapids, MI). All eggplants were

surface sterilized with a 10% bleach solution for 10 minutes and rinsed with distilled water. Fruits were placed into disinfected humidity chambers and moistened paper towel was placed into each chamber to maintain humidity at ~100%. Three 25-µl drops of zoospore suspension (1.0×10^6 and 1.0×10^4 zoospores/ml) of one of five isolates (12889, OP97, SP98, 13351, and 13556) were placed on the surface of each fruit. After inoculation, the chambers were maintained at room temperature (21 ± 2°C) under constant fluorescent lighting for 72 hours. Each zoospore concentration × isolate treatment (including a non inoculated negative control, inoculated with sterile water) was replicated seven times. The experiment was replicated three times and treatments were arranged in split-plot design with whole plots in a complete block design.

Lesion incidence and diameter, incidence of mycelial growth and pathogen sporulation was recorded upon concluding each assay. The quantity of sporangia per lesion was determined by placing the mycelia from the lesion into a 1 ml of sterile water. The solution was vortexed to dislodge sporangia and enumerated using a hemacytometer.

Evaluation and data analysis. The cumulative AUDPC values and fruit screen measurements were subjected to analysis of variance (ANOVA) using the PROC MIXED procedure of SAS v 9.1 (SAS Institute Inc., Cary, NC). Fisher's protected LSD was used for separation of means when effects were found to be statistically significant in ANOVA analysis ($P \leq 0.05$). The eggplant and pepper lines were considered resistant to crown and root rot if it received an average score < 2, based on methods previously described (1,6,11). AUDPC data from the second crown and root rot experiment that included eleven *P. capsici* isolates and the data from fruit lesion incidence were square-root transformed in order to avoid violating normality assumptions of the test. Non inoculated

control plants and fruits were removed from analysis to avoid violating variance assumptions of the test.

RESULTS

Phytophthora root and crown rot screen. Susceptible pepper plants exhibited crown rot and stem lesions and eventually wilted and died when they were inoculated with millet seed infected with *P. capsici* isolates from Michigan and New York (Figure 13). Stem lesions and crown rot was not apparent on susceptible eggplants inoculated with *P. capsici*; wilt was observed that progressed to plant death (Figure 13). All isolates of *P. capsici* obtained from inoculated plant material were confirmed to have the same phenotype as the isolate used for the inoculum (*data not shown*). None of the non inoculated control plants showed symptoms of *P. capsici* infection (*data not shown*). Significant differences ($P \leq 0.05$) were found among AUDPC values for the isolates, pepper/eggplant lines, and the isolate × line interaction.

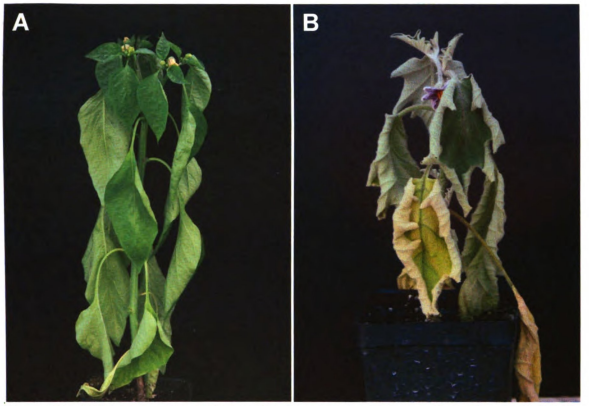

Figure 13. Wilting symptoms observed on **A**, pepper, and **B**, eggplant when inoculated with an isolate of *Phytophthora capsici*.

Mean AUDPC values were statistically different among *P. capsici* isolates. Isolate 12889 was the most virulent isolate in the first trial, followed by OP97 that was more virulent than SP98 and SFF3 (Table 17). The pepper CM334 and eggplants EG195 and EG203 were resistant to all four isolates (Table 18). All other lines tested were susceptible to 12889 and OP97 with the exception of 'Paladin' that was resistant to OP97. None of the lines were susceptible to SP98 and SFF3. In the second experiment, isolates 13353, 13351, 13356, and 13355 were more virulent than 13352, 13357, 12889, and 13358 (Table 17). 'Red Knight' was susceptible to all isolates, while eggplant EG195 was resistant to all eleven isolates (Table 19). 'Classic' was resistant to isolates 13352 and 12889; eggplant EG203 was resistant to all isolates except 13356.

Table 17. Cumulative area under the disease progress curve (AUDPC) values for *Phytophthora capsici* isolates from Michigan and New York causing crown and root rot symptoms on eggplant and pepper in two experiments.

Isolate[u]	CT[v]	MS	AUDPC[w]	
Experiment one[x]				
SP98	A2	S	72	a[y]
SFF3	A2	I	77	a
OP97	A1	S	113	b
12889	A1	1	164	c
Experiment two[z]				
13352	A1	S	102	a
13357	A1	S	132	ab
12889	A1	S	136	bc
13358	A1	S	138	bc
13359	A1	S	148	b-d
13360	A1	S	163	c-e
13354	A1	S	164	c-e
13353	A1	S	172	de
13351	A1	S	176	e
13356	A1	S	178	e
13355	A1	S	180	e

[u]SP98 was isolated from pumpkin, SFF3 and OP97 from pickling cucumber, 12889 from bell pepper, and 13351-13360 from eggplant.

[v]The isolate phenotypes are indicated by compatibility type (CT) and sensitivity to mefenoxam (MS, I = insensitive, S = sensitive).

[w]The AUDPC was calculated from scores evaluated every two days using a 1 to 5 ratings scale (1 = healthy, 2 = minor wilting, 3 = moderate wilting, 4 = severe wilting, 5 = plant death).

[x]Experiment included two eggplant lines (EG195 and EG203) and four pepper lines ('Camelot,' CM334, 'Paladin,' and 'Red Knight').

[y]AUDPC values within the same experiment with letters in common are not statistically different according to Fisher's LSD (P=0.05).

[z]Experiment included three eggplant lines (EG195, EG203, and 'Classic') and one pepper line ('Red Knight').

The average temperature in the greenhouse was 18.6°C (minimum 1.0°C, maximum 41.6°C). The average relative humidity was 59.7% (minimum 20.7%, maximum 96.2%).

Table 18. Pepper and eggplant lines and cultivars screened for resistance to four Michigan *Phytophthora capsici* isolates obtained from pepper (12889), pickling cucumber (OP97, SFF3), and pumpkin (SP98).

Line/cultivar	Disease response[y]				AUDPC[z]				Plant death (%)			
	12889	OP97	SFF3	SP98	12889	OP97	SFF3	SP98	12889	OP97	SFF3	SP98
Eggplant												
EG-195	R	R	R	R	71	71	71	71	0	0	0	0
EG-203	R	R	R	R	71	71	71	71	0	0	0	0
Pepper												
Camelot	S	S	R	R	311	180	72	73	94	56	0	6
CM334	R	R	R	R	71	71	71	71	0	0	0	0
Paladin	S	R	R	R	139	72	71	71	63	6	0	0
Red Knight	S	S	R	R	321	217	104	73	100	69	19	0

[y]The disease response was based on the average plant score at the end of the experiment. If the score was < 2, the line was considered resistant (R); if the score was ≥ 2, the line was considered susceptible (S).

[z]The AUDPC was calculated from scores evaluated every two days using a 1 to 5 rating scale (1 = healthy, 2 = minor wilting, 3 = moderate wilting, 4 = severe wilting, 5 = plant death).

Table 19. The disease response, AUDPC, and plant death of pepper ('Red Knight') and eggplant ('Classic,' EG195, and EG203) lines and cultivars screened for resistance to eleven *Phytophthora capsici* isolates obtained from pepper (12889) and eggplant (13351 to 13360).

Line/isolate	Disease response[x]	AUDPC[y]	Plant death (%)	Line/isolate	Disease response	AUDPC	Plant death (%)
Classic				EG203			
13352	R	76 a[z]	0	12889	R	76 a	0
12889	R	89 ab	19	13352	R	76 a	0
13358	S	126 bc	50	13357	R	76 a	0
13357	S	129 b-d	25	13358	R	76 a	0
13359	S	146 c-e	44	13360	R	83 ab	6
13354	S	164 c-e	56	13353	R	84 ab	6
13360	S	191 ef	100	13354	R	85 ab	6
13355	S	209 fg	100	13351	R	88 ab	19
13351	S	217 fg	94	13356	S	89 ab	25
13353	S	217 fg	94	13359	R	91 ab	6
13356	S	222 fg	100	13355	R	98 ab	13
EG195				Red Knight			
12889	R	76 a	0	13352	S	179 d-f	38
13351	R	76 a	0	13357	S	249 gh	75
13352	R	76 a	0	13358	S	273 hi	88
13353	R	76 a	0	13359	S	279 h-j	94
13354	R	76 a	0	13360	S	301 i-k	100
13355	R	76 a	0	12889	S	303 i-l	94
13356	R	76 a	0	13353	S	311 i-l	94
13357	R	76 a	0	13351	S	325 j-l	94
13358	R	76 a	0	13356	S	326 j-l	100
13359	R	76 a	0	13354	S	329 kl	100
13360	R	76 a	0	13355	S	336 l	100

Table 19 (cont'd).

[x]The disease response was based on the average plant score at the end of the experiment. If the score was < 2, the line was considered resistant (R); if the score was ≥ 2, the line was considered susceptible (S).

[y]The AUDPC was calculated from scores evaluated every two days using a 1 to 5 rating scale (1 = healthy, 2 = minor wilting, 3 = moderate wilting, 4 = severe wilting, 5 = plant death).

[z]Means with letters in common are not statistically different according to Fisher's LSD (P=0.05).

Phytophthora fruit rot screen. Fruit lesions were circular and appeared within three days of inoculation (Figure 14). Symptomatic fruit exhibited lesions with browning (40.8%), mycelial growth (7.6%), and pathogen sporulation (1.7%). Fruits inoculated with *P. capsici* suspensions containing 1.0×10^4 zoospores/ml had a reduced lesion incidence, size, and mycelial growth incidence that was significantly different than fruit inoculated with 1.0×10^6 zoospores/ml (*data not shown*). The lesion located closest to the stem end was statistically smaller in area than the other two lesions on the fruit (*data not shown*). The interaction between isolate and zoospore concentration on lesion size was significant (Table 20). The lesion size was statistically similar among all isolates inoculated with the reduced zoospore concentration. Isolate 13351 had statistically larger lesions than 13566 and OP97 inoculated with an increased zoospore concentration. The lesion incidence and size were not statistically different among isolates (*data not shown*).

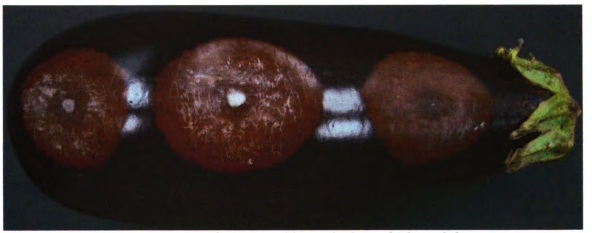

Figure 14. Lesions on eggplant fruit caused by an isolate of *Phytophthora capsici* inoculated with a 25-µl drop of zoospore solution (1.0×10^6 zoospores/ml).

Table 20. The mean number of lesions and mean lesion area (cm^2) with skin browning on eggplant fruit caused by select *Phytophthora capsici* isolates.

Treatment[y]	Lesion incidence	Area browning (cm^2)[z]
1.0×10^4 zoospores/ml		
SP98	0.5	1.7 a
13351	0.5	1.9 a
OP97	0.6	2.7 a
13566	0.7	2.8 a
12889	0.6	3.2 a
1.0×10^6 zoospores/ml		
13566	1.7	6.4 b
OP97	1.6	7.4 bc
SP98	1.8	8.0 b-d
12889	2.2	11.1 cd
13351	2.0	11.3 d

[y]The eggplant fruit were inoculated in three locations with a 25 µl drop of zoospore solution (1.0×10^4 to 1.0×10^6 zoospores/ml) and incubated for 72 hrs.
[z]Column means with letters in common or no letter at all are not statistically different according to Fisher's LSD (P=0.05).

DISCUSSION

In this study, the eggplant line EG195 was resistant to all *P. capsici* isolates tested. The pepper CM334 is resistant to all known isolates of *P. capsici* (31,32,33,34) and EG195 and EG203 appeared to have equivalent resistance to CM334 in the first experiment. However, in the second experiment, EG203 was susceptible to eggplant isolate 13356. Because host resistance is an important component in controlling *P. capsici*, identifying EG195 as being resistant to 14 isolates can be useful in breeding programs or as a grafting rootstock. Its compatibility with pepper lines as a rootstock is unknown, but it has been used as a rootstock in tomato production (4). Nevertheless, EG195 should be screened with more isolates, from a greater geographic region, due to

the known differential in *P. capsici* isolates among pepper (11,31) and tomato (Quesada-Ocampo and Hausbeck, *unpublished data*) lines with resistance.

When cultivars and breeding lines of pepper and eggplant were screened against 14 Michigan isolates of *P. capsici*, the isolates differed in virulence. This observation has been made previously in pepper (9,11,22,23) and in other Solanaceous hosts, such as tomato (Quesada-Ocampo and Hausbeck, *unpublished data*). Both Foster and Hausbeck (9) and Quesada-Ocampo and Hausbeck (*unpublished data*) found Michigan isolate 12889 to be more virulent on pepper and tomato, respectively, than OP97, SP98, and SFF3. The cumulative AUDPC was statistically different among pepper (9) and tomato (Quesada-Ocampo and Hausbeck, *unpublished data*) lines, where the pepper line CM334 and wild tomato line LA407 were found to be resistant to all isolates.

As shown in pepper studies by Oelke et al. (22), Glosier et al. (11), and Sy et al. (31), a significant isolate, host genotype, and isolate × host genotype effects indicate differential disease interactions. Polach and Webster found a significant interaction in virulence among isolates and pepper lines with different levels of resistance (23). As a result, Sy et al. has suggested assigning physiological races to the different levels in *P. capsici* virulence among pepper lines (31). If physiological races were assigned in our experiment, three and four isolates would be assigned in the first and second experiment, respectively. Four physiological races were distinguished when pepper lines were inoculated with the same isolates from the first experiment (9). The same four *P. capsici* isolates may have separated into more physiological races because Foster and Hausbeck (9) used more plant lines than in our experiment.

Differences in lesion incidence on eggplant fruit were not observed but an interaction in lesion size was found between zoospore concentration and isolate. This difference did not appear to have any association with the isolate's original host, as 13566 and 13351 were both from eggplant and had the smallest and largest lesion size, respectively. Ristaino did not find a significant association among isolates from cucurbit or pepper hosts with virulence to pepper (25).

Zoospore concentrations of 1.0×10^4 and 1.0×10^6 zoospores/ml were used to inoculate eggplant fruit. Reifschneider et al. found *P. capsici* zoospore concentrations $\geq 1.0 \times 10^4$ zoospores/ml incited infection on a greater number of pepper lines than concentrations of 1.0×10^3 and 1.0×10^2 zoospores/ml (24). Granke and Hausbeck (*unpublished data*) found concentrations $\geq 1.0 \times 10^4$ zoospores/ml did not significantly increase disease incidence on cucumber fruit. Zoospore concentrations, including 1.0×10^2, 1.0×10^3, 1.0×10^5 zoospores/ml, may have elucidated greater differences among the isolates in lesion size and incidence on eggplant. Also, the eggplant fruit's cultivar was unknown and may have even been a mixture of cultivars. Foster and Hausbeck did find differences in the incidence of lesions among *P. capsici* isolates when several pepper cultivars were inoculated with a zoospore concentration containing 1.75×10^6 zoospores/ml (9). Further studies on zoospore concentration, droplet size, eggplant cultivar, and *P. capsici* isolate involving direct observation assays may produce better understanding virulence of *P. capsici* isolates on eggplant fruits.

The fruits of eggplant appear to be more susceptible to *P. capsici* than the roots and crowns because all isolates caused infection on the eggplant fruit but not all caused

crown and root rot symptoms. The fruits of pepper (9) and the fruits of cucumber (12) appear to be more susceptible than the roots and crowns. In pepper, root rot, stem, and leaf blight are considered distinct diseases (3,22). The roots and crowns of a pepper cultivar, for example, may be resistant to a certain isolate, but the leaves are susceptible to blight (22). In potato, tubers resistant to *P. infestans* did not necessarily have resistance to foliar infection by the same *P. infestans* race (5,27).

Eggplant appears to be more tolerant to root and crown rot than pepper. In the second experiment, the eggplant 'Classic' had lower AUDPC values than the pepper 'Red Knight.' In a field study, eggplant had lower AUDPC values than all other vegetables studied, including pepper, zucchini, pumpkin, and summer squash (13). The fruits of eggplant were less susceptible to infection than the cucurbits, but equally susceptible as tomato; pepper fruit were not evaluated (13). The roots and crowns of eggplant are relatively woody which may increase tolerance compared to other *P. capsici* hosts such as cucurbits. The fruits, however, are composed of soft tissues similar to tomato and pepper, which appear to be more susceptible to *P. capsici* infection than the roots and crowns. Fruit rot in eggplant is rarely observed in Michigan likely because many growers stake the plants to provide support and prevent the fruits from touching the infested soil surface. Eggplants are also relatively tall, compared to cucurbits, so splash dispersal from infested soil to the fruit is less likely.

In summary, the eggplant EG195 was resistant to all *P. capsici* isolates screened. The *P. capsici* isolates that caused crown and root rot had a wide range of virulence, although they were collected from the same field; all of the isolates were virulent on the

fruit of eggplant. Further research is warranted to test EG195 for resistance to additional *P. capsici* isolates and to evaluate it for potential rootstock compatibility to pepper.

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APPENDIX A

SUMMARY OF FUNGICIDE APPPLICATION INFORMATION FOR FIELD STUDIES IN 2008

Table 21. Application dates and weather data recorded at time of application from the Michigan State University Muck Soils Experimental Farm in 2008.

Date	Growth stage of pepper	Wind speed (kph)	Direction (in plot)	Air temperature (°C)	Hours after rain	Hours before rain	Cloud cover (%)	Soil temperature (°C)
10 June	4 to 5 TL	11.2	401 to 101	23.1	2	0	50	21.1
17 June	1 to 2 OF	3.6	401 to 101	15.6	0	0	90	15.6
23 June	1 to 2 OF	1.6	101 to 111	25.2	0.5	0	100	23.6
30 June	1 in. fruit	2.4	411 to 101	20.3	0	0	40	18.3
7 July	2 in. fruit	2.4	401 to 101	26.7	0	0	0	26.1
14 July	3 in. fruit	0.8	401 to 101	23.3	0	0	0	23.1
21 July	Harvestable	0	NA	21.7	0	0	30	20.7
28 July	Harvestable	0	NA	21.0	0	0	10	22.2
4 August	Harvestable	0	NA	29.4	3	0	0	28.9
11 August	Harvestable	1.6	401 to 101	27.4	0	0	10	27.0
18 August	Harvestable	0	NA	21.7	0	0	0	21.0
25 August	Harvestable	3.1	401 to 101	19.9	0	0	0	19.5

Table 22. Application dates and weather data recorded at time of application from the Michigan State University Southwest Michigan Research and Extension Center in 2008.

Date	Growth stage of pepper	Wind speed (kph)	Direction (in plot)	Air temperature (°C)	Hours after rain	Hours before rain	Cloud cover (%)	Soil temperature (°C)
5 June	3 to 4 TL	1.6		29.2	0	0	100	23.9
12 June	1 OF	4.0	101 to 401	24.4	0	0	100	23.4
19 June	1 OF	5.6	101 to 111	23.3	0	0	0	24.3
26 June	1 to 2 OF	8.8	101 to 111	27.5	0	0	0	27.2
3 July	1 in. fruit	4.0	401 to 111	21.1	0	0	70	22.2
10 July	1 in. fruit	2.9	101 to 111	28.4	0	0	100	28.5
17 July	2 in. fruit	4.8	101 to 411	27.0	0	0	100	25.5
24 July	3 in. fruit	4.8	111 to 105	28.3	0	0	80	27.8
31 July	3 in. fruit	5.6	101 to 111	28.9	0	0	0	29.4
7 August	Harvestable	3.2	101 to 111	24.7	0	0	0	25.5
14 August	Harvestable	5.2	111 to 401	23.8	0	0	50	21.2
28 August	Harvestable	1.2	101 to 111	20.6	0	0	0	20.3

APPENDIX B

FUNGICIDE EFFICACY ON SQUASH AND PEPPER HOST RESISTANCE STUDIES IN 2007 AND 2008 IN THE FIELD

BELL PEPPER (*Capsicum annuum*)
Phytophthora crown rot; *Phytophthora capsici*

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Evaluation of bell pepper cultivars and experimental lines for tolerance to Phytophthora crown and root rot, 2007.

This trial was established on a commercial farm in Oceana County, MI in a field with a history of *Phytophthora capsici*. Four replicates of 12 cultivars and breeding lines were transplanted in a randomized complete block design. Four *P. capsici* susceptible cultivars were planted as disease checks and included; 'Camelot,' 'Red Knight,' and 'Brigadier.' The commercially available tolerant varieties 'Revolution,' 'Paladin,' 'Alliance,' 'Aristotle,' and 'Conquest,' were included for comparison. The experimental lines, PRO3-13x14 R-4, PRO5-C71x72, PRO5-C81x82, and PRO5-C85x86, were provided by Pepper Research Inc. Seven-week-old greenhouse grown seedlings were planted 12 Jun with 0.3 m spacing onto 0.6-m-wide raised beds equipped with drip irrigation and covered with black plastic mulch. Plots were 6.1 m long and spaced 1.7 m apart. Weeds were controlled using conventional chemical methods and hand weeding. Symptomatic plants were sampled and tested for *P. capsici* infection. Plants were evaluated weekly and the number of surviving plants was noted. Fruits were harvested from the entire row and evaluated for fruit rot caused by *P. capsici* infection and a coloration defect on the fruit skin referred to as silvering (separation of the cuticle from the flesh of the fruit).

Plant death occurred as a result of crown rot and ranged from 1.3% (PRO3-13x14 R-4) to 28.5% (PRO5-C81x82). Overall, the susceptible lines did not exhibit more plant death than the tolerant or experimental lines. The experimental line PRO3-13x14 R-4 had significantly less plant death than the tolerant cultivar Alliance and PRO5-C81x82; but was similar to all other cultivars. Silvering was most pronounced in the experimental lines of PRO3-13x14 R-4 (19%) and PRO5-C71x72 (7.2%). There was no fruit rot observed among any of the cultivars and experimental lines.

Table 23. Effect of cultivar selection on plant death caused by *Phytophthora capsici* and fruit silvering.

Cultivar	Plant death (%) 23 Aug[z]		Fruit with silvering (%) 27 Aug	
Susceptible				
Camelot	3.8	ab	0.0	a
Red Knight	6.6	a-c	0.0	a
Brigadier	13.3	a-d	0.0	a
Tolerant				
Aristotle	3.9	ab	0.0	a
Paladin	7.6	a-c	2.9	a
Revolution	8.9	a-c	0.4	a
Conquest	15.5	a-d	0.0	a
Alliance	21.7	cd	0.0	a
Experimental				
PRO3-13x14 R-4	1.3	a	19.0	c
PRO5-C85x86	8.9	a-c	0.0	a
PRO5-C71x72	17.7	b-d	7.2	b
PRO5-C81x82	28.5	d	0.0	a

[z]Column means with a letter in common are not significantly different according to Fisher's LSD (P=0.05).

YELLOW SQUASH (*Cucurbita pepo* 'Sunray')
Crown, root and fruit rot; *Phytophthora capsici*

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Evaluation of fungicides for control of Phytophthora blight of yellow squash grown on flat plant beds, 2007.

The trial was conducted on a commercial farm in Oceana County, MI with sandy soil and a history of *Phytophthora capsici* infestation. The field was previously planted to various cucurbit and solanaceous crops. Yellow squash 'Sunray' seeds, commercially treated with Thiram, were sown 0.6 m apart within the row. The plots were 9.1 m long and arranged in a randomized complete block design and replicated four times. Water misters were installed throughout the plot to provide irrigation as needed. Weeds were controlled using commercial herbicides and by hand. Powdery mildew was controlled with Nova 40W (0.21 kg/ha), applied 9 and 16 Aug. Insects were managed by conventional methods using foliar applications of insecticides. Treatments were applied using a CO_2 backpack sprayer and a 3-nozzle boom with 50 mesh screens and 8003XR nozzles, calibrated to deliver 473 l/ha. The outer two nozzles were aligned at a 45° angle towards the squash crown, with the middle nozzle positioned over the plant canopy. Treatments commenced at the one true leaf stage and continued until harvest concluded. Eleven chemical treatments were applied every 5-7 days on 5, 12, 19, 26 Jul, 2, 9, 16, 21, 23, 27 Aug, and 6 Sep. If rainfall exceeded 1.3 cm in a 1-hour period, treatments were applied in addition to the regular spray schedule to prevent increased infection from *P. capsici* inocula moving through rain splash. Symptomatic plants were observed and counted weekly. Approximately 25% of the infected plants were isolated onto selective media using sterile technique to confirm infection by *P. capsici*. Fruits were harvested from the entire row, weighed, and evaluated for *P. capsici* infection on 7, 9, 13, 16, 20, 24, 27, 30 Aug, and 4, 7 Sep. Healthy fruits were stored 4-5 days under ambient conditions for and subsequently evaluated for symptoms of postharvest disease. Data were analyzed using SAS PROC MIXED and statistical differences were compared using the Fisher's Least Significant Differences test (P=0.05).

At the end of the trial, >50% of the untreated plants were dead. Although the treatments included in this trial did not significantly limit disease for any parameter tested, trends were noted. Total yield was greatest in plots treated with Ridomil Gold MZ 76.5WP, Presidio 4FL, or Gavel 75DF. Ridomil Gold MZ and Presidio 4FL had the lowest AUDPC values. Plants treated with Revus 2.08 had the least amount of death. In the untreated control, 9.1% of the fruit showed postharvest disease symptoms. Three treatments completely prevented postharvest infection and included Reason 4.13SC, Gavel 75DF, and Revus 2.08SC. Phytotoxicity was not observed for any of the treatments.

Table 24. Effect of fungicide treatments on crown, root and fruit rot caused by *Phytophthora capsici* on 'Sunray' squash grown on flat plant beds in 2007.

Treatment and rate/ha, applied at 5-7 day interval	Dead (%)[z]	AUDPC	Harvested yield (kg/9.1 m row)	Postharvest infection (%)
Untreated	54.0	418	27.5	9.1
Revus 2.08SC 0.58 liter	26.5	428	30.2	0.0
Presidio 4FL 0.29 liter	28.8	209	41.6	1.5
Ridomil Gold MZ 76.5WP 2.24 kg	32.5	96	45.4	0.6
ProPhyt 4.2EC 4.7 liter	34.8	360	29.8	0.2
Forum 4.16SC 0.47 liter	36.0	326	37.8	2.9
Captan 80WDG 6.73 kg	44.5	275	37.5	0.2
Gavel 75DF 2.24 kg	44.8	376	41.3	0.0
Ranman 3.6SC 0.22 liter.	45.5	252	35.6	0.3
Tanos 50WG 0.56 kg	47.5	410	34.2	2.9
Previcur Flex 6EC 1.4 liter	53.3	639	34.5	1.9
Reason 4.13SC 0.40 liter	56.0	405	30.3	0.0

[z]There were no significant differences among treatments according to Fisher's LSD (P=0.05).

YELLOW SQUASH (*Cucurbita pepo* 'Sunray')
Crown, root and fruit rot; *Phytophthora capsici*

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Evaluation of fungicides for control of Phytophthora blight of yellow squash grown on raised plant beds, 2007.

The experiment was conducted in a commercial vegetable grower's field in Cass County, MI on sandy clay loam soil with a history of *Phytophthora capsici*, previously planted to cucurbits. Summer squash 'Sunray' seeds, commercially treated with Thiram, were sown 0.6 m apart on 0.6-m-wide, 15-cm-high raised beds covered with black plastic mulch. Beds were laid with drip irrigation that was controlled by the grower cooperator. The plots were 6.1 m long and replicated four times in a randomized complete block design. Weeds were controlled using conventional herbicide measures, and by hand around the plants. Insects and powdery mildew were controlled using commercially available foliar pesticides. Treatments were applied using a CO_2 backpack sprayer and a 3-nozzle boom with 50 mesh screens and 8003XR nozzles, calibrated to deliver 473 l/ha. The outer two nozzles were aligned at a 45° angle towards the squash crown, with the middle nozzle positioned over the plant crown. Treatments were initiated when the crop had developed one true leaf and continued until the final harvest. Eleven chemical treatments were applied every 5-7 days on 6, 13, 20, 27 Jul, and 3, 8, 15, 21, 24 Aug. If rainfall exceeded ½ in. in a 1 hour period, treatments were applied in addition to the regular spray schedule to prevent increased infection from *P. capsici* inocula moving through rain splash. The Southwest Michigan Research and Extension Center at Benton Harbor, located 24 km away from the commercial field, received a total of 29.5 cm of rainfall in Aug. Heavy rainfall also occurred during the first two weeks of Sep with a total of 15 cm documented. Plants with signs of *P. capsici* infection, including severe wilt, crown rot, and plant death, were counted weekly. Approximately 25% of the infected plants were isolated onto selective media using sterile technique to confirm infection by *P. capsici*. Fruits were harvested from each row, weighed and evaluated for *P. capsici* infection on 8, 14, 17, 21 and 24 Aug. Healthy appearing fruits were stored for 4-5 days under ambient conditions and subsequently evaluated for symptoms of postharvest disease. Data were analyzed using SAS PROC MIXED and statistical differences were compared using the Fisher's Least Significant Differences test (P=0.05).

Treatments of Captan 80WDG, Revus 2.08SC, Gavel 75DF, Ranman 3.6SC, Presidio 4FL, and Ridomil Gold MZ 76.5WP limited plant death compared to the untreated control. Among these treatments, Captan had the lowest plant death (20%), and provided significantly better control than Ranman (53%), Presidio (54.1%), and Ridomil Gold MZ (66.7%). AUDPC values produced no statistical differences among treatments. Presidio had statistically higher yields than the untreated control. All treatments significantly prevented fruit infection at harvest compared to the untreated control. There were no statistical differences in fruit infection postharvest, but all treatments had ≥ 8.3% infection. Prophyt 4.2EC resulted in plant stunting and yellowing of foliage.

Table 25. Effect of fungicide treatments on crown, root and fruit rot caused by *Phytophthora capsici* on 'Sunray' squash grown on raised beds covered in black plastic in 2007.

Treatment and rate/ha	Plants/6.1 m of row 30 Aug[y]		Yield (kg/6.1 m of row)		
	Dead or wilted (%)	AUDPC	At harvest		Postharvest
			Total	Infected (%)	Infected (%)
Untreated	100.0 e[z]	458	5.9 b-d	6.9 d	27.0
Captan 80WDG 6.73 kg	20.0 a	412	9.5 a-c	0.2 a	9.9
Revus 2.08SC 0.58 liter	34.1 ab	299	7.1 bc	1.3 a-c	16.3
Gavel 75DF 2.24 kg	38.9 ab	255	9.2 a-c	1.4 a-c	20.2
Ranman 3.6SC 0.22 liter	53.0 bc	403	7.7 a-c	0.6 ab	10.7
Presidio 4FL 0.29 liter	54.1 bc	446	11.5 a	2.7 a-c	8.3
Ridomil Gold MZ 76.5WP 2.24 kg	66.7 cd	698	8.4 a-c	1.3 a-c	12.0
ProPhyt 4.2EC 4.7 liter	85.0 de	1079	2.7 d	0.9 a-c	20.0
Forum 4.16SC 0.47 liter	88.8 de	845	5.4 cd	1.9 a-c	14.1
Previcur Flex 6EC 1.4 liter	92.5 e	814	5.2 cd	3.4 c	35.7
Reason 4.13SC 0.40 liter	92.5 e	971	9.9 ab	2.8 bc	17.5
Tanos 50WG 0.56 kg	100.0 e	851	7.9 a-c	2.4 a-c	19.1

[y]Total number of emerged plants was statistically similar among all plots.

[z]Column means with a letter in common or with no letter are not significantly different (Fisher's Method; P=0.05).

YELLOW SQUASH (*Cucurbita pepo* 'Sunray')
Crown, root and fruit rot; *Phytophthora capsici*

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Evaluation of fungicides for control of Phytophthora blight of yellow squash in fumigated beds, 2007.

The trial was conducted in a commercial field in Cass County, MI on a sandy clay loam soil with a history of *Phytophthora capsici*, previously planted to cucurbits. Yellow squash 'Sunray' seeds, commercially treated with Thiram, were sown 0.60 m apart in 0.60-m-wide, 15-cm-high raised beds covered in black plastic mulch following fumigation with Telone C-35 at 35 GPA on 31 May. Beds were laid with drip irrigation that was operated by the grower cooperator. The plots were 6.1m long and arranged in a randomized complete block design. Weeds were controlled using conventional herbicide measures and by hand weeding around the plants. Insects and powdery mildew were controlled using commercially available pesticides. All treatments were applied with a CO_2 backpack sprayer and a 3-nozzle swivel boom with 50 mesh screens and 8003XR nozzles, calibrated to deliver 473 l/ha. The outer two swivel nozzles were aligned at a 45° angle towards the squash crown, with the middle nozzle positioned over the plant crown. Applications were initiated when the crop had developed one true leaf and continued until harvest concluded. Eleven chemical treatments were applied every 5-7 days on 6, 13, 20, 27 Jul, 3, 8, 15, 21, 24, 31 Aug and 7 Sep. If rainfall exceeded 1.3 cm in a 1-hour period, supplemental treatments were applied in addition to the regular spray schedule to prevent increased infection from *P. capsici* inocula moving through rain splash. The Southwest Michigan Research and Extension Center at Benton Harbor, located 15 miles away from the commercial field, received a total of 29.5 cm of rainfall in Aug. Heavy rainfall also occurred during the first two weeks of Sep with a total of 15.2 cm documented. Symptomatic plants were observed and noted weekly until 14 Sep. Periodically, tissue from infected plants was sampled and placed onto selective media using sterile technique to confirm infection by *P. capsici*. Fruits were harvested from the entire treatment row and evaluated for *P. capsici* infection on 8, 14, 17, 21, 24, 31 Aug, and 7, 14 Sep. Healthy fruits were stored for 4-5 days under ambient conditions and subsequently evaluated for post-harvest disease. Data were analyzed using SAS PROC MIXED and statistical differences were compared using the Fisher's Least Significant Differences test (P=0.05).

In the untreated control plots, 87.5% of the plants were wilted or dead by 14 Sep. Gavel 75DF, Captan 80WDG, Ridomil Gold MZ 76.5WP, Presidio 4FL, Revus 2.08SC, Tanos 50WG, and Previcur Flex 6EC limited plant death compared to the untreated control. Among these treatments, Gavel significantly limited plant death (10.6%), compared with Tanos (53%) and Previcur Flex (55.4%). According to AUDPC values, there were no statistical differences among treatments. Only Ridomil Gold MZ 76.5WP produced significantly higher yields than the untreated control. There were no statistical differences in fruit infection at harvest or postharvest. Across all treatments, infected fruit was ≤2.7%, but was ≥10.4% at postharvest evaluation. Prophyt 4.2EC caused plant

stunting and yellowing of the foliage, resulting in significantly lower yields than the untreated control.

Table 26. Effect of fungicide treatments on crown, root and fruit rot caused by *Phytophthora capsici* on 'Sunray' squash grown on fumigated raised beds covered in black plastic in 2007.

Treatment and rate/ha	Plants/6.1 m of row[y]		Harvest/6.1 m of row		
			At harvest		Postharvest
	Dead or wilted (%)	AUDPC	Yield (kg)	Infected fruit (%)	Infected fruit (%)
Untreated	87.5d[z]	998	17.1bc	2.5	18.0
Gavel 75DF 2.24 kg	10.6a	128	23.8ab	1.5	18.3
Captan 80WDG 6.73 kg	12.6ab	116	21.8a-c	0.9	18.0
Ridomil Gold MZ 76.5WP 2.24 kg	30.0ab	396	25.6a	0.9	18.7
Presidio 4FL 0.29 liter	32.1ab	554	21.7a-c	0.7	12.2
Revus 2.08SC 0.58 liter	33.0ab	424	21.3a-c	1.7	14.1
Tanos 50WG 0.56 kg	53.0bc	538	24.2ab	1.7	11.8
Previcur Flex 6EC 1.4 liter	55.4bc	351	20.8a-c	2.1	23.3
Reason 4.13SC 0.40 liter	60.0b-d	608	18.3bc	2.0	10.4
Ranman 3.6SC 0.22 liter	63.4cd	966	16.2cd	2.7	21.1
Forum 4.16SC 0.47 liter	68.6cd	898	20.7a-c	2.7	16.4
ProPhyt 4.2EC 4.7 liter	75.0cd	947	10.7d	0.6	12.9

[y]Total number of emerged plants was statistically similar among all plots.

[z]Column means with a letter in common or with no letter are not significantly different (Fisher's Method; P=0.05).

ACORN SQUASH (*Cucurbita pepo* 'Table Ace')
Crown, root and fruit rot; *Phytophthora capsici*

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Evaluation of fungicides for control of Phytophthora blight of acorn squash, 2008.

The trial was conducted in a commercial field in Kent County, MI on muck soil with a history of *Phytophthora capsici* infestation, previously planted to sweet corn. On 24 Jun, acorn squash 'Table Ace' seeds were sown on 0.6-m-wide raised beds 0.3 m apart within each row. Plots were one row wide and 6.1-m-long in a randomized complete block design with four replications. Weed management and fertilization were maintained by the grower according to standard commercial practices. Powdery mildew was controlled with Cabrio EG (0.84 kg/ha) applied 26 Aug. Insects were managed with Asana XL (0.67 kg/ha) applied 12 and 19 Aug. Treatments were applied using a CO_2 backpack sprayer and a 3-nozzle boom with 50 mesh screens and 8003XR nozzles spaced 0.46 m apart, calibrated to deliver 473 l/ha. The outer two nozzles were aligned at a 45° angle towards the squash crown, with the middle nozzle positioned over the plant canopy. Treatments commenced when plants had 3 to 4 true leaves and were applied on 22, 29 Jul; 5, 12, 19, 26 Aug; 1, 5, and 12 Sep. Treatments of mancozeb (Ridomil Gold MZ 76.5WP and Gavel 75DF) were terminated one week prior to the first harvest, due to concerns with pre-harvest intervals. Disease evaluations consisted of counting wilted and dead plants at onset of first disease symptoms. Market-size fruits were harvested from the entire row weekly and stored under ambient conditions for 4 days, before being weighed and evaluated for symptoms of disease. During the first two weeks of Sep, 22.4 cm of rain was documented at the Michigan Celery Cooperative, located approximately 19 km away from the grower cooperator's field. Data were analyzed using SAS PROC MIXED and statistical differences were compared using the Fisher's Least Significant Differences test (P=0.05).

All treatment programs that included Gavel significantly limited plant death compared to the untreated control. Although statistical differences did not occur among treatments for the remaining three parameters evaluated, trends were noted. Programs that included Presidio 4FL had the lowest AUDPC values and highest yields. Revus 2.08SC alternated with Presidio limited postharvest disease incidence to 0.8%. Phytotoxicity as a result of treatment application was not observed.

Table 27. Effect of fungicide treatment on crown, root and fruit rot caused by *Phytophthora capsici* on 'Table Ace' squash in 2008.

Treatment and rate/ha applied at 5 to 7-day intervals	Plants/6.1 m of row		Yield/6.1 m of row	
	Dead or wilted (%)	AUDPC	kg	Infected fruit (%)
1 Untreated	58.5c[y]	852.0	21.8	3.5
2 Gavel 75DF 2.24 kg (1,3,5[z]) Acrobat 50WP 0.18 kg + Kocide 2000 54DF 2.24 kg (2,6,8) Presidio 4FL 0.29 liter + Kocide 2000 54DF 2.24 kg (4,7,9)	6.5a	23.5	27.2	2.2
3 Gavel 75DF 2.24 kg (1,3,5) Ranman 3.6SC 0.22 liter + Kocide 2000 54DF 2.24 kg (2,6,8) Revus 2.08SC 0.58 liter + Kocide 2000 54DF 2.24 kg (4,7,9)	21.8ab	532.5	24.4	3.0
4 Gavel 75DF 2.24 kg (1,3,5) Revus 2.08SC 0.58 liter + Kocide 2000 54DF 2.24 kg (2,6,8) Presidio 4FL 0.29 liter + Kocide 2000 54DF 2.24 kg (4,7,9)	26.8ab	201.5	28.0	0.8
5 Ridomil Gold MZ 76.5WP 2.80 kg (1,3,5) Acrobat 50WP 0.18 kg + Kocide 2000 54DF 2.24 kg (2,6,8) Revus 2.08SC 0.58 liter + Kocide 2000 54DF 2.24 kg (4,7,9)	33.3bc	547.5	22.2	4.3
6 Ridomil Gold MZ 76.5WP 2.80 kg (1,3,5) Ranman 3.6SC 0.22 liter + Kocide 2000 54DF 2.24 kg (2,6,8) Presidio 4FL 0.29 liter + Kocide 2000 54DF 2.24 kg (4,7,9)	35.0bc	315.5	26.1	2.5

[y]Column means with a letter in common or with no letter are not significantly different according to Fisher's LSD (P=0.05).

[z]Numbers within the parentheses represent the application schedule. Fungicides were applied every 7 days.