

MANAGEMENT OF *PHYTOPHTHORA CAPSICI* ON SUMMER SQUASH AND AGE-RELATED RESISTANCE ON PROCESSING PUMPKIN AND WINTER SQUASH FRUITS

BY

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ABSTRACT

MANAGEMENT OF *PHYTOPHTHORA CAPSICI* ON SUMMER SQUASH AND AGE-RELATED RESISTANCE ON PROCESSING PUMPKIN AND WINTER SQUASH FRUITS

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Phytophthora blight, caused by *Phytophthora capsici* Leonian, is an economically important disease of cucurbit crops (*Cucurbita* spp.). Michigan accounts for 20% of the squash produced in the U.S., with total cash receipts of \$12 million in 2009. Field and greenhouse trials were conducted to compare soil drenches and foliar sprays of eleven fungicides for control of Phytophthora crown and root rot on summer squash. Soil drenches were more effective than foliar sprays at limiting plant death caused by *P. capsici*. Mean plant death 42 days post inoculation (dpi) was 41% for soil drenches and 92% for foliar sprays. Drenches of fluopicolide, mandipropamid or dimethomorph limited plant death to $\leq 10\%$, and prevented yield loss associated with crown and root rot. Similarly, disease progress was slower and crown rot was less severe following soil drenches compared to foliar sprays in greenhouse trials. Most fungicide treatments were more effective on the cultivar ‘Leopard’ than ‘Cougar’, which is more susceptible to *P. capsici*.

A laboratory study evaluated age-related resistance to Phytophthora fruit rot in ‘Dickenson Field’ processing pumpkin and ‘Golden Delicious’ winter squash. Hand-pollinated fruits were harvested 3, 7, 10, 14, 21, 28, 42, or 56 days post pollination (dpp) and inoculated with *P. capsici*. Susceptibility to Phytophthora fruit rot decreased with fruit age in ‘Dickenson Field’ processing pumpkin, whereas ‘Golden Delicious’ winter squash remained susceptible to fruit rot even as fruit reached full maturity. Lesion diameter and pathogen growth were generally greater on younger fruit than older fruit. Less than 15% of ‘Dickenson Field’ fruit 21 dpp or

older became diseased. Conversely, about 80% of 'Golden Delicious' fruit 21 dpp or older became diseased. Several morphological and physiological changes were observed as fruit matured. Soluble solids content and exocarp firmness of both cultivars increased with fruit age, and were negatively correlated ($p = -0.29$ to -0.73) with lesion diameter and pathogen growth density.

Field trials were conducted to evaluate the effects of bed height, mulches, dried poultry litter, and cultivars on *Phytophthora* crown and root rot of summer squash. Differences in cultivar susceptibility to *P. capsici* accounted for most of the variation in the observed disease levels. Mean incidence of plant death 35 dpi was 87% for 'Payroll' and 99% for 'Cougar'. Plant death of 'Payroll' was greater in flat beds than raised beds. Disease was not affected by bed height, mulches, or rates of dried poultry litter application. Crown rot severity differed significantly among thirty-two summer squash cultivars and ten cucurbit germplasm accessions in a separate greenhouse trial. Mean crown rot ratings were 4.3 on commercial cultivars and 2.2 on germplasm accessions. Crown rot was least severe on the cultivar 'Spineless Beauty'. No disease developed on four accessions of *Cucurbita moschata* (PI 442262, PI 442266, PI 458740, and PI 634693) previously reported to be crown rot resistant.

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LITERATURE REVIEW

CUCURBIT PRODUCTION

Cucurbitaceae is a diverse family of more than 800 plant species (59). Plants in the Cucurbitaceae family, including cucumbers, gourds, melons, pumpkins, and squash, are commonly referred to as cucurbits. There are ten wild and five cultivated species within the genus *Cucurbita* (19). *Cucurbita pepo*, *C. maxima*, and *C. moschata* are the three most widely cultivated species (48). Cucurbits are grown throughout the world for food, ornamental, and pharmaceutical uses. Edible cucurbits are broadly classified by whether their fruit is consumed immature (summer squash [*C. pepo*]) or mature (winter squash [*C. maxima*] and pumpkin [*C. moschata*]).

Michigan is the largest producer of squash in the United States, accounting for 20% of total production (4). Squash is grown on approximately 3,000 ha in Michigan (4). The principal squash producing counties include Berrien, Ottawa, Oceana, Mason, and Newaygo (3). More than 60% of squash grown in Michigan is winter squash. Certain cultivar-groups are exclusively grown for processing or fresh market consumption. The principle types of squash grown in Michigan include acorn, butternut, yellow crookneck, yellow straightneck, and zucchini (3). Two-thirds of squash in Michigan is grown for fresh market. One-third is grown for processing, including freezing and canning. The Gerber Products Company of Fremont, MI is a major processor of winter squash for baby food (52).

Illinois is the leading producer of jack-o'-lantern (*C. pepo*) and processing (*C. moschata*) pumpkins in the U.S. In 2010, pumpkins were grown on more than 6,000 ha in Illinois (4). About 90% of the U.S. processing pumpkin crop is grown in Illinois (6). Most pumpkins are processed by Libby's Pumpkin of Morton, IL to make canned pie filling (52). Michigan is also a

significant producer of pumpkin in the U.S. More than 2,700 ha of pumpkins were grown in Michigan in 2010 (4). Production of processing pumpkin has recently increased in Michigan due to production shortages in other states (N. Myers, *personal communication*).

Squash is planted from May to July in Michigan. Sandy loam soils are best suited for squash production. Squash can be direct-seeded or grown from transplants. Squash grown for processing is direct-seeded and grown on flat ground. Early fresh market plantings may require the use of transplants. Fresh market squash is grown on raised beds covered with plastic mulch. Low tunnels can be used to cover early-planted crops for frost protection. Summer squash is harvested when fruits are immature and tender. Oversized fruits with thick flesh and developed seeds are unmarketable. Winter squash is harvested when fruits are mature and the rind has hardened (74).

PHYTOPHTHORA BLIGHT

Pathogen and host range. Phytophthora blight, caused by the Oomycete *Phytophthora capsici* Leonian, is one of the most economically important diseases of cucurbit crops (6,7,10,31). More than 50 plant species in 15 families are hosts of *P. capsici* (66). Phytophthora blight was first reported on chili pepper (*Capsicum annuum* L.) in 1922 at the New Mexico Agricultural Experiment Station (44). In the 1930s and 1940s, Phytophthora blight was reported on numerous cucurbit crops including cucumber (*Cucumis sativus*), honeydew melon (*C. melo*), cantaloupe (*C. melo* var. *cantalupensis*), and watermelon (*Citrullus lanatus*) (39,67,68,70,71). Among cucurbit crops, summer squash appears to be the most susceptible to *P. capsici* (30).

Disease cycle. *Phytophthora capsici* overwinters in infected plant debris and soil as mycelia and thick-walled oospores. Oospores can remain viable for several years in the absence of a susceptible host (42). *Phytophthora capsici* is heterothallic and requires two compatible

mating types (A1 and A2) for sexual reproduction and oospore formation (7,31). Oospores germinate to form sporangia as soil temperatures increase and soil moisture levels approach field capacity (7). Sporangia are caducous and readily dislodge from the sporangiophore (7). Sporangia may germinate and directly infect a host, or differentiate to form 20 to 40 motile zoospores (31). Biflagellate zoospores are released in the presence of free water, and must encyst before germinating and infecting a host. Both zoospores and sporangia are dispersed by splashing rain, surface water and irrigation water (28). Temperature, zoospore age, and zoospore concentration affect disease development (26). Abundant sporulation on infected tissues results in secondary cycles of infection and disease (7). Phytophthora blight is favored by warm temperatures and excessive rainfall or irrigation (7).

Symptoms and signs. *Phytophthora capsici* primarily causes a root, crown, and fruit rot, as well as a foliar blight on cucurbit crops (5,6,7,30,31,47). Phytophthora crown and root rot is particularly severe because infections result in plant death and significant crop loss. Crown and root rot occurs when root infections progress up the stem resulting in a permanent wilt of the plant (56). Phytophthora fruit rot is initiated when fruit contact *P. capsici*-infested soil, or when propagules are splash dispersed to fruit during rain and irrigation events (7,47). Fruit rot can also occur postharvest (47). Initial symptoms include watersoaking and a sunken lesion (47). Infected fruit eventually collapse as the lesion expands (7,47). Sporangia produced on the fruit surface have a powdered sugar-like appearance (31).

PHYTOPHTHORA BLIGHT MANAGEMENT

Cultural management. Crop rotation and destruction of infected plant debris are not effective as stand-alone management practices because *P. capsici* is capable of surviving in the absence of a host for extended periods of time (42). A Phytophthora blight epidemic on squash

following a five-year rotation with non-host crops indicated that rotations of five years or less are insufficient (41). Furthermore, long-term crop rotations are often unfeasible because of the limited availability of noninfested fields suitable for vegetable production (31). Some rotational and alternative crops including snap bean (*Phaseolus vulgaris*) and Fraser fir (*Abies fraseri*) have been identified as hosts of *P. capsici* (25,53).

Growing plants on raised beds improves water drainage, thereby limiting the conditions favorable for Phytophthora root and crown rot. Phytophthora blight incidence on pepper was 18% in flat beds and 5% in raised beds (32). Similarly, plant death of zucchini in a field naturally infested with *P. capsici* was greater in flat beds than raised beds (31). Covering planting beds with plastic mulch can reduce splash dispersal of *P. capsici* from the soil to susceptible plant tissues (57). However, in some cases, plastic mulches increased the spread of Phytophthora blight within a row because *P. capsici* propagules are readily dispersed in water on the surface of plastic mulches (57,64). Organic mulches (e.g., wheat straw) have also been effective at reducing splash dispersal of *P. capsici* (57).

Some *Phytophthora* species can be readily isolated from bodies of natural water. *Phytophthora capsici* was frequently recovered during a survey of irrigation water sources in Michigan (24). Thus, irrigation with surface water or effluent water from processing facilities should be avoided to prevent the introduction of *P. capsici* (31). Similarly, *P. capsici* can be introduced when culled fruits are discarded in or near noninfested fields (31,47). *Phytophthora capsici* can also be transported on equipment used in infested fields (31).

Frequency and type of irrigation affects the development of Phytophthora blight. Incidence of Phytophthora root rot on chile pepper was lower in plots with alternate-furrow irrigation than in plots with every-furrow irrigation (12). Phytophthora root and fruit rot on

summer squash were greater in plots irrigated every 7 days than in plots irrigated every 14 or 21 days (15). Where possible, drip irrigation is the best method of supplying water while limiting the development of Phytophthora crown and root rot. Incidence of Phytophthora root rot on chile pepper was 1.5% for drip irrigation and 36.8% for alternate row furrow irrigation (72). Drip emitter location and frequency of drip irrigation can affect Phytophthora blight development. Phytophthora blight incidence on pepper was 42% when drip emitters were located on the soil surface near the plant stem and 0% when emitters were positioned below the soil surface (14). Phytophthora blight incidence was greater and pepper yield was lower in plots drip irrigated three times weekly than plots irrigated only to avoid water stress (55).

Host resistance to *P. capsici*. Resistance to *P. capsici* is an important component of Phytophthora blight management for some crops. Resistance to *P. capsici* was identified in the pepper cultivar ‘Criollo de Morelos 334’ (11,38). Different genetic systems confer resistance to the different phases of Phytophthora blight (11,65). Isolate virulence affects the expression of resistance, and breeders must screen germplasm against multiple *P. capsici* isolates (20). Pepper cultivars with resistance to Phytophthora crown and root rot are commercially available. The cultivar ‘Paladin’ is resistant to Phytophthora crown rot and has acceptable fruit quality (8,17,35). Unfortunately, resistance to *P. capsici* is overcome when conditions are conducive for severe disease (31). In tomato, resistance to Phytophthora crown and root rot was recently identified in an accession of *Solanum habrochaites* (54). Moderate levels of resistance were found in the tomato cultivars ‘Ha7998’, ‘Fla7600’, ‘Jolly Elf’, and ‘Talladega’.

Limited research has been done to identify resistance to *P. capsici* in cucurbit crops. A detached fruit assay was used to screen 333 cucumber cultigens for resistance to Phytophthora fruit rot (23). Limited sporulation was observed on some cultigens, but none were completely

resistant to *P. capsici* (23). Fruit age affects susceptibility to Phytophthora fruit rot on several cucurbit crops (2,23,27). Plant architecture can also affect the development of fruit rot. Phenotypes that limit fruit contact with infested soil during their development or have an open canopy that promotes drying could reduce the incidence of rot (1).

At least one *C. pepo* and five *C. moschata* germplasm accessions with resistance to Phytophthora crown rot were recently identified using *P. capsici* isolates from Florida (16,50). Resistance to multiple *P. capsici* isolates was also identified in the Korean pumpkin cultivar ‘Danmatmaetdol’ (*C. maxima*) (43). Resistance to *P. capsici* was identified in the wild species *Cucurbita lundelliana* and introgressed into 19 winter squash breeding lines (37). An inheritance study indicated that resistance to crown rot derived from *C. lundelliana* and *C. okeechobeensis* subsp. *okeechobeensis* is conferred by three dominant genes (49). Major genes for resistance to *P. capsici* have not been incorporated into commercial breeding lines, and all cucurbit cultivars are considered susceptible to Phytophthora crown and root rot (6,7,31).

Chemical management. Growers have traditionally relied on a relatively few number of fungicides to control Phytophthora blight. *Phytophthora capsici* lacks the target sites of most conventional fungicides because unlike true fungi Oomycetes have a diploid nuclear condition, coenocytic mycelia, cell walls composed of cellulose and β -glucans, and lack methods to synthesize sterols (29). The five primary classes of systemic fungicides with activity against Oomycetes include: the phenylamides, the carbamates, the cyanoacetamide oximes, the ethyl phosphonates, and the isoxazoles (61).

The phenylamide fungicides metalaxyl and mefenoxam (metalaxyl-M) have been widely used to manage Phytophthora blight on vegetable crops since the early 1980s (9,34,36,60,63). Crown-directed sprays of metalaxyl significantly reduced crown rot caused by *P. capsici* in bell

pepper (63). Incidence of Phytophthora blight on yellow squash was 0% for plants drenched with metalaxyl and 100% for the nontreated control (36). Seed treatment with metalaxyl or mefenoxam reduced damping-off caused by *P. capsici* in pumpkin seedlings (9). Repeated use of metalaxyl and mefenoxam has resulted in the selection of resistant isolates of *P. capsici*. In Michigan, 45% of sampled *P. capsici* isolates were intermediately or completely insensitive to mefenoxam (40). Nearly 70% of *P. capsici* isolates sampled from pepper and cucurbit fields in North Carolina were insensitive to mefenoxam (51). More than 25% of *P. capsici* isolates sampled from four vegetable growing regions in New York were intermediately or fully resistant to mefenoxam (18).

Newer fungicides registered for control of Phytophthora blight on cucurbits include fluopicolide (Presidio, Valent USA Corp., Walnut Creek, CA) and mandipropamid (Revus, Syngenta Crop Protection, Greensboro, NC). Fluopicolide causes delocalization of spectrin-like proteins, which results in zoospore lysis and inhibition of mycelial growth (69). Mandipropamid appears to inhibit cell wall biosynthesis (13). Fluopicolide applied through drip irrigation at transplanting and as a foliar spray at weekly intervals reduced plant death in yellow summer squash (33). Incidence of Phytophthora blight on pepper was 1.3% and 9.0% when fluopicolide was applied as a soil drench or a foliar spray, respectively (62). Fluopicolide and mandipropamid were more effective as a soil drench than as a foliar spray at limiting plant death of pepper in the greenhouse (21).

Other fungicides used to control *P. capsici* include acibenzolar-*S*-methyl, dimethomorph, fosetyl-Al, and the phosphonates (45,46,73). Pepper plant survival in *P. capsici*-infested soil was greatest when dimethomorph was applied as a soil drench in an evaluation of five fungicides (45). Soil applications of acibenzolar-*S*-methyl increased plant survival and restricted the

development of stem lesions caused by *P. capsici* (46). Phosphonates applied as a soil drench reduced the severity of Phytophthora crown rot on pumpkin and zucchini seedlings (73).

In addition to fungicides, soil fumigation has also been used to manage Phytophthora blight (31). Metam sodium, 66% methyl bromide + 33% chloropicrin, and 61% 1,3-dichloropropene + 35% chloropicrin limited development of Phytophthora blight in field trials (31). No viable *P. capsici* propagules were detected using three assays following soil fumigation with methyl bromide and chloropicrin (22). Although they are effective at controlling *P. capsici*, fumigants such as methyl bromide are being phased out due their detrimental effects on human health and the environment (58).

Phytophthora root, crown, and fruit rot are a major constraint to cucurbit production in Michigan, and growers have few options for managing these diseases. The objectives of this research were (i) to compare soil drench and foliar spray applications of eleven fungicides for managing Phytophthora crown and root rot of summer squash, (ii) to determine the effects of fruit age on susceptibility to Phytophthora fruit rot in processing pumpkin and winter squash fruits, and (iii) to evaluate the integrated use of multiple cultural management practices on Phytophthora crown and root rot of summer squash.

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LITERATURE CITED

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CHAPTER I: USING SOIL-APPLIED FUNGICIDES TO MANAGE PHYTOPHTHORA CROWN AND ROOT ROT ON SUMMER SQUASH

ABSTRACT

Phytophthora crown and root rot (*Phytophthora capsici* Leonian) of summer squash is especially difficult to manage because all commercial cultivars are highly susceptible to *P. capsici*. Producers have traditionally relied on foliar fungicide applications to control Phytophthora crown and root rot despite their limited efficacy. Soil fungicide applications, including via subsurface drip chemigation, have recently gained interest as a method of improving control of *P. capsici* infections. In this study, soil drenches and foliar applications of eleven fungicides were compared for control of Phytophthora crown and root rot of summer squash in replicated field and greenhouse trials. Fungicides were applied at 7-day intervals. Incidence (%) of plant death was assessed from 7 to 42 days post inoculation (dpi) in field trials. Crown rot severity was rated on a 1 (no symptoms) to 5 (plant death) scale from 5 to 21 dpi in greenhouse trials. Results of field and greenhouse trials were similar. Plant death of ‘Cougar’ following inoculation with *P. capsici* isolate 12889 occurred at all growth stages from expansion of the first true-leaf to full maturity in field trials. Plant death 42 dpi differed significantly ($P<0.0001$) among fungicides and application methods. The fungicide x application method interaction also was significant. Some fungicides were ineffective regardless of application method. In general, soil drenches were more effective than foliar applications at limiting plant death, but no treatment completely controlled disease symptoms. Mean plant death 42 dpi was 41% for soil drenches and 92% for foliar sprays. Drenches of fluopicolide, mandipropamid or dimethomorph limited plant death to $\leq 10\%$, and prevented yield loss associated with root and crown rot. Foliar applications generally did not reduce plant death compared to the untreated,

inoculated control, and were unable to prevent yield loss in field trials. In greenhouse trials, crown rot severity differed significantly ($P < 0.0001$) among fungicides, application methods, and cultivars when plants were inoculated with *P. capsici* isolate 12889 or SP98. Disease progress was slower and crown rot was less severe following soil drenches than foliar applications. Some fungicide treatments were more effective on ‘Leopard’, which was less susceptible to *P. capsici* than ‘Cougar’. Soil application methods, including soil drench and drip chemigation, should be evaluated when fungicides are registered for soilborne disease control, as these methods provide better control of Phytophthora crown and root rot than foliar application.

INTRODUCTION

Michigan is the largest producer of squash (*Cucurbita* spp.) for processing and fresh market consumption in the United States, accounting for 20% of total production (3). *Phytophthora capsici* Leonian (23) is an economically important soilborne pathogen of summer squash (*C. pepo* L.) and other vegetable crops (14). Yield losses due to Phytophthora blight can be substantial when soils are heavily infested with *P. capsici*. In Michigan, complete crop failure has been reported in numerous vegetable crops, including summer squash, resulting in considerable financial losses for producers (14). All tissues of the summer squash plant including roots, crowns, fruit, and leaves are susceptible to *P. capsici* (4). Phytophthora crown and root rot is particularly severe, resulting in permanent wilting and rapid plant death (13,14,37).

Successful management of Phytophthora crown and root rot requires an integrated approach (14,15,36). Crop rotation and destruction of infected plant debris are not effective as stand-alone management practices because *P. capsici* is capable of surviving in the absence of a host for extended periods of time (22). Cultural practices including planting on raised beds,

using plastic mulches (42), and irrigation management (6,7,44) limit disease, but do not provide complete control. Resistance to *Phytophthora* crown rot has been identified in germplasm accessions of *Cucurbita pepo* L. (30) and *C. moschata* Duchesne (8), but has not been incorporated into commercial breeding lines. Presently, all commercial summer squash cultivars are highly susceptible to *P. capsici*. Hence, growers have relied on foliar fungicide applications in conjunction with cultural practices to manage *Phytophthora* crown and root rot despite their limited efficacy.

Metalaxyl and mefenoxam (metalaxyl-M) have been widely used to manage *Phytophthora* root, crown, and fruit rot on vegetable crops since the early 1980s (5,19,39,41). Repeated use of metalaxyl and mefenoxam has resulted in the selection of resistant isolates of *P. capsici* (9,21,28,31,32,33). Resistance to metalaxyl-based products necessitates additional fungicides and novel methods of fungicide application to improve control of *P. capsici*.

Soil fungicide application, including via subsurface drip chemigation, has recently gained attention as a method of improving control of *Phytophthora* crown and root rot. Pepper (*Capsicum annuum*) plant survival in *P. capsici*-infested soil was greatest when dimethomorph was applied as a soil drench in an evaluation of five fungicides (26). Similarly, soil applications of acibenzolar-*S*-methyl increased plant survival and restricted the development of stem lesions caused by *P. capsici* (27). Plant death due to *Phytophthora* crown and root rot was 2.5 times greater when fungicides were applied as foliar sprays compared to when the same products were applied as soil drenches in a greenhouse evaluation of nine fungicides on two pepper cultivars (11). Phosphonates applied as a soil drench reduced the severity of *Phytophthora* crown rot on pumpkin and zucchini seedlings, whereas foliar applications did not (45). Fluopicolide applied

through drip irrigation at transplanting and as a foliar spray at weekly intervals reduced plant death in yellow summer squash (16).

Although some studies, primarily on pepper, have evaluated soil applications of fungicides for managing *Phytophthora* crown and root rot, additional research is needed to develop effective methods for fungicide use on summer squash in Michigan's production fields. The objective of this study was to evaluate the effects of eleven fungicides and two application methods (foliar spray and soil drench) for managing *Phytophthora* crown and root rot, and to compare the effects of fungicide treatments on two summer squash cultivars differing in susceptibility to *P. capsici*.

MATERIALS AND METHODS

Inoculum preparation. Two Michigan isolates of *P. capsici* originally collected from pumpkin (SP98) and pepper (12889) and maintained in the culture collection of M. K. Hausbeck at Michigan State University were used as inocula in this study. Isolates SP98 and 12889 are highly virulent and have been used in previous studies screening for *P. capsici* resistance (10,12,35). Mating type and sensitivity to mefenoxam differed among the isolates. Isolate SP98 (mating type A2) is sensitive to 100 ppm of mefenoxam. Isolate 12889 (mating type A1) is insensitive to mefenoxam. Cultures were grown on unclarified V8 juice agar (UCV8) at room temperature ($21 \pm 2^\circ\text{C}$) under constant fluorescent light. *P. capsici*-infested millet seed was produced for each isolate as described by Quesada-Ocampo et al. (34).

Field evaluation. Two separate trials were conducted at the Michigan State University Southwest Michigan Research and Extension Center in Benton Harbor. Plots were previously planted to a cover crop of cereal rye (*Secale cereale* L.) and hairy vetch (*Vicia villosa* Roth). The soil type was Spinks loamy fine sand (sandy, mixed, mesic Lamellic Hapludalfs).

Susceptible yellow straightneck squash ‘Cougar’ (Harris Moran Seed Co., Modesto, CA) was grown in both trials. Planting dates were 25 May 2010 (trial 1) and 29 July 2010 (trial 2). Seeds were planted in raised beds (15-cm-high x 60-cm-wide) covered with black, plastic mulch. Beds were spaced 1.7 m apart. Plants were spaced 46 cm apart within beds. Plants were irrigated twice weekly for 3 to 4 h using drip irrigation. The experimental design was a randomized complete block with four replicates. An experimental unit was one 6.9-m-long bed with 15 plants.

Plants were inoculated with *P. capsici* isolate 12889 at the first true-leaf stage. A 3-cm-deep hole was made approximately 2 cm from the crown of each plant. One gram of *P. capsici*-infested millet seeds was placed in each hole and covered with soil. Fungicides were applied immediately after inoculation and at 7-day intervals thereafter until 42 days post inoculation (dpi). Fungicides and application rates are listed in Table 1.1. Control plots were not treated with fungicides. Foliar applications were applied using a CO₂-pressurized backpack sprayer and hand-held boom equipped with three TeeJet XR8003 flat-fan nozzles spaced 46 cm apart. One nozzle was positioned directly above the canopy and two lateral nozzles were positioned downward at a 45° angle towards the plant crown. All foliar sprays were applied in a volume of 468 liter per ha at 345 kPa. Soil drenches were applied using a CO₂-pressurized backpack sprayer and spray wand equipped with one TeeJet XR8010 nozzle calibrated to deliver 80 ml per plant at 89 kPa. The soil drench treatment was applied at the soil line near the crown of each plant.

Table 1.1. Fungicides and rates of application evaluated for managing *Phytophthora* crown and root rot caused by *Phytophthora capsici* on summer squash in field and greenhouse trials

Trade name	Active ingredient (a.i.)	Manufacturer ^a	FRAC ^b code	a.i./ha (kg)
Forum 4.17SC	dimethomorph	BASF	40	0.22
Gavel 75DF	mancozeb + zoxamide	Gowan	M3 / 22	1.50 / 0.19
Kocide 3000 46.1DF	copper hydroxide	DuPont	M1	0.65
Presidio 4SC	fluopicolide	Valent	43	0.14
Previcur Flex 6EC	propamocarb hydrochloride	Bayer	28	1.01
ProPhyt 4.2L	potassium phosphite	Helena	33	3.53
Ranman 3.6SC	cyazofamid	FMC	21	0.08
Reason 500 4.13SC	fenamidone	Bayer	11	0.20
Revus 2.08SC	mandipropamid	Syngenta	40	0.15
Ridomil Gold SL 4EC	mefenoxam	Syngenta	4	1.12
Tanos 50WG	famoxadone + cymoxanil	DuPont	11 / 27	0.18 / 0.18

^a BASF = BASF Corp., Research Triangle Park, NC; Gowan = Gowan Co., Yuma, AZ; DuPont

= E. I. du Pont de Nemours and Co., Wilmington, DE; Valent = Valent USA Corp., Walnut

Creek, CA; Bayer = Bayer CropScience LP, Research Triangle Park, NC; Helena = Helena

Chemical Co., Collierville, TN; FMC = FMC Corp., Agricultural Products Group, Philadelphia,

PA; Syngenta = Syngenta Crop Protection, Inc., Greensboro, NC.

^b FRAC = Fungicide Resistance Action Committee.

Disease incidence, defined as the percentage of wilted or dead plants in each plot, was measured at 7-day intervals from 7 to 42 dpi in field trials. Area under the disease progress curve (AUDPC) values for disease incidence were calculated according to the formula presented by Shaner and Finney (40). Squash fruit were hand harvested from healthy plants in each plot and weighed. Plants were harvested every 3 or 4 days over a 3-week period. Relative yield of fungicide treated plots was calculated as a proportion of the yield obtained in uninoculated

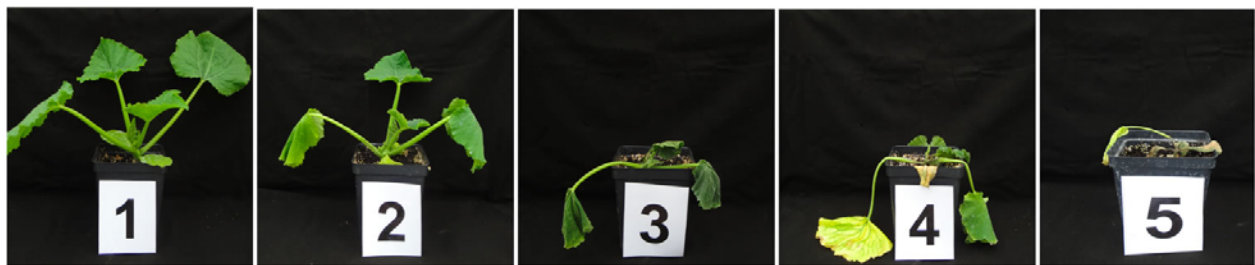
control plots within replicates, where relative yield = (yield of fungicide treated plot ÷ yield of uninoculated control plot).

Greenhouse evaluation. Greenhouse trials were done in May (trial 1) and September (trial 2) 2010 at the Michigan State University Horticulture Research and Teaching Center in Holt. Susceptible yellow straightneck squash ‘Cougar’ and green zucchini ‘Leopard’ (Harris Moran Seed Co., Modesto, CA) were grown in both experiments under natural light. The experimental design was completely randomized with six replicates. An experimental unit was one plant grown in a 10-cm-diameter pot containing soilless potting mix (BACCTO High Porosity Professional Potting Mix; Michigan Peat Co., Houston, TX). A single fungicide x application method combination was assigned randomly to each plant.

Plants were inoculated with *P. capsici* as described above for field trials. Isolates SP98 and 12889 were evaluated in separate, concurrent trials. Fungicides were applied immediately after inoculation and at 7-day intervals thereafter until 21 dpi. Fungicides and application rates were the same for greenhouse and field trials (Table 1.1). Application methods were as described above for field trials with the exception that soil drenches were applied by hand using a plastic bottle.

Disease severity was assessed at 2- or 3-day intervals from 5 to 21 dpi. Each plant was visually assessed using a 1 to 5 scale similar to that described by Yandoc-Ables et al. (45), where 1 = no symptoms; 2 = lower leaves wilted with slight constriction of the stem; 3 = all leaves wilted with constriction and slight discoloration of the stem; 4 = all leaves wilted and crown rotted with visible sporulation on the stem surface; and 5 = plant dead (Figure 1.1). AUDPC values were calculated using disease severity ratings.

Figure 1.1. Scale used to rate crown rot severity on summer squash plants following inoculation with *Phytophthora capsici* isolate 12889 or SP98 in greenhouse evaluations of eleven fungicides and two application methods for *Phytophthora* crown and root rot control, where 1 = no symptoms; 2 = lower leaves wilted with slight constriction of the stem; 3 = all leaves wilted with constriction and slight discoloration of the stem; 4 = all leaves wilted and crown rotted with visible sporulation on the stem surface; and 5 = plant dead. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.



Pathogen confirmation. Approximately 5% of symptomatic plants from field and greenhouse trials were sampled for pathogen confirmation. Symptomatic plants were arbitrarily selected from treatment plots and sheared at the soil line with hand pruners. Crown sections were surface disinfested with a 70% ethanol solution and blotted dry with paper toweling. Four pieces of tissue were excised from each crown and plated on UCV8 amended with 25 ppm of benomyl, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene (BARP). Colonies were identified as *P. capsici* using morphological characteristics and a key developed by Waterhouse (43). Mating type and sensitivity to mefenoxam was determined for each isolate and compared to the phenotype of isolates used as inoculum.

Statistical analysis. For field trials, final incidence of plant death, AUDPC, and relative yield were analyzed separately by analysis of variance (ANOVA) using the Proc Mixed procedure of SAS, version 9.2 (SAS Institute, Cary, NC). Trials and blocks were considered random variables. Fungicides and application methods were considered fixed variables. Pearson correlation coefficients between relative yield and disease incidence were calculated using the Proc Corr procedure of SAS. For greenhouse trials, final crown rot severity and AUDPC were analyzed separately for each isolate by ANOVA using the Proc Mixed procedure of SAS. Trials were considered random variables. Fungicide treatments, application methods, and cultivars were considered fixed variables. Slice statements were used to test simple main effects, when two-way interactions were statistically significant. Fungicide treatment means were separated by Fisher's protected least significant difference (FLSD) using the pdmix800 SAS macro (38). Residuals were tested for normality using the Shapiro-Wilk statistic in the Proc Univariate procedure of SAS. Residuals were plotted against predicted values using the Proc Gplot procedure of SAS to assess homoscedasticity.

RESULTS

Field evaluation. Plant death associated with *Phytophthora* crown and root rot occurred at all growth stages from expansion of the first true-leaf to full maturity. *P. capsici* with the same phenotype as 12889 was consistently isolated from symptomatic plants. Incidence of plant death increased rapidly from 7 to 21 dpi (Figure 1.2). Plant death 42 dpi in inoculated control plots was 100% (Figure 1.3). Little or no plant death occurred in uninoculated control plots. Results of analyses of plant death incidence and AUDPC were similar. A significant trial x treatment interaction was primarily accounted for by changes in magnitude among soil drenches rather than in rank; therefore, data were pooled for analysis. Plant death 42 dpi and AUDPC

differed significantly ($P<0.0001$) among fungicides and application methods. The fungicide x application method interaction also was significant ($P<0.0001$). No treatment combination (fungicide x application method) completely controlled disease symptoms. In general, treatment combinations that reduced plant death also limited AUDPC (Table 1.2; Figure 1.3). Plant death and AUDPC were high regardless of application method for some fungicides with less activity against *P. capsici*. For example, mefenoxam was ineffective regardless of application method because *P. capsici* isolate 12889 is mefenoxam-resistant (Table 1.2; Figure 1.3).

Soil drenches were more effective in limiting disease than foliar sprays. Among foliar sprays, plant death 42 dpi ranged from 53 to 100% and averaged 92% for the eleven fungicides. Foliar sprays of fluopicolide, mandipropamid, and dimethomorph reduced plant death compared to the inoculated control (Figure 1.3). Mean plant death 42 dpi was 84% following foliar sprays of these fungicides. Fluopicolide sprays also reduced AUDPC (Table 1.2). Plant death 42 dpi varied among soil drenches, ranging from 0 to 100% and averaging 41% for the eleven fungicides. Soil drenches of nine fungicides reduced plant death compared to the inoculated control (Figure 1.3). These fungicides, with the exception of copper hydroxide, also reduced AUDPC (Table 1.2). Plant death 42 dpi was <10% when dimethomorph, mandipropamid, or fluopicolide was applied as a soil drench (Figure 1.3).

Relative yield of inoculated control plots was zero because all plants died following inoculation with *P. capsici*. Relative yield differed significantly ($P<0.0001$) among fungicides and application methods. The fungicide x application method interaction also was significant ($P<0.0001$). Some fungicides were unable to prevent yield losses regardless of application method. Mean relative yield was 17% and 72% for foliar sprays and soil drenches, respectively (Table 1.3). No fungicides prevented yield losses when applied as a foliar spray (Table 1.3).

Soil drenches of cyazofamid, fluopicolide, mancozeb + zoxamide, mandipropamid, dimethomorph, or fenamidone prevented significant yield losses (Table 1.3). Plant death was $\leq 36\%$ in plots treated with these fungicide drenches and yield loss was zero.

Figure 1.2. Incidence (%) of plant death in summer squash ‘Cougar’ grown in field plots treated with fungicides applied as a foliar spray or soil drench to control *Phytophthora* crown and root rot caused by *Phytophthora capsici*. Control refers to *P. capsici*-inoculated plots without fungicide application. Data points are the means of eleven fungicides and eight replicates from two trials. Error bars represent the standard error of the mean.

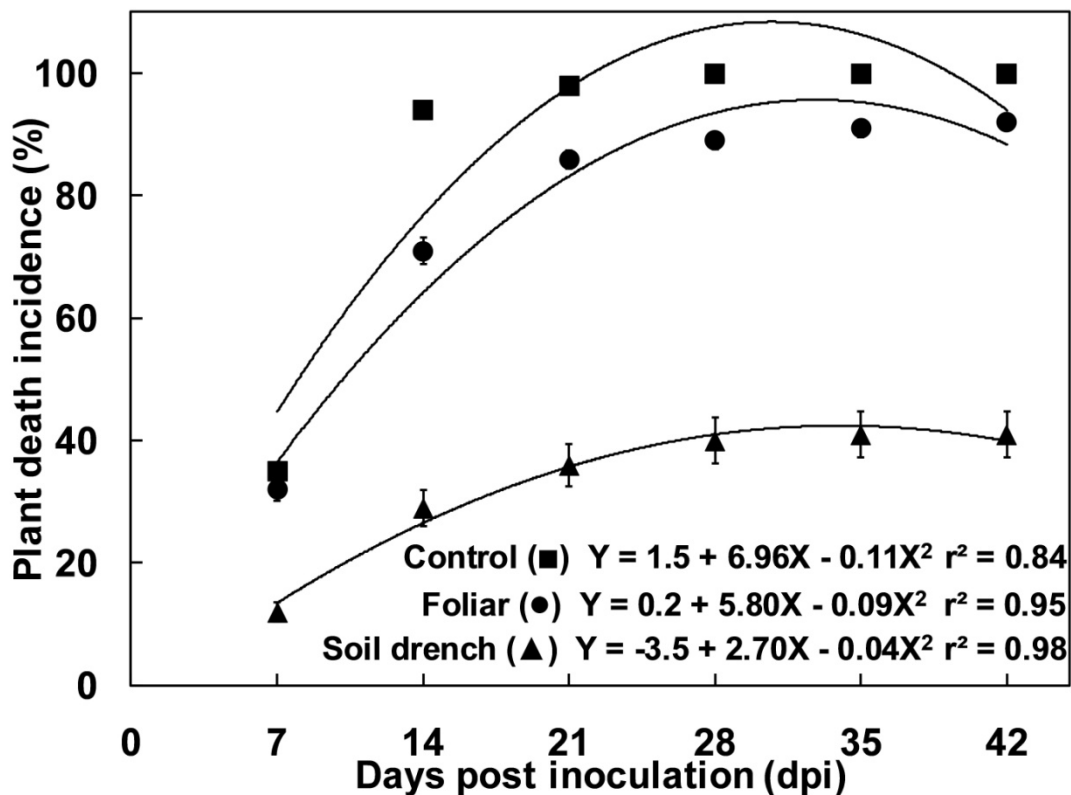


Figure 1.3. Incidence (%) of plant death 42 days post inoculation (dpi) in summer squash ‘Cougar’ grown in field plots treated with fungicides applied as a foliar spray or soil drench to control *Phytophthora* crown and root rot caused by *Phytophthora capsici*. Values represent the mean of eight replicates from two trials. Error bars represent the standard error of the mean. Within an application method, bars with a common letter do not differ significantly based on Fisher’s protected least significant difference test at $\alpha = 0.05$. For each fungicide, an asterisk indicates that application methods differed significantly based on single degree of freedom slice statements used to partition the fungicide x method interaction at $\alpha = 0.05$

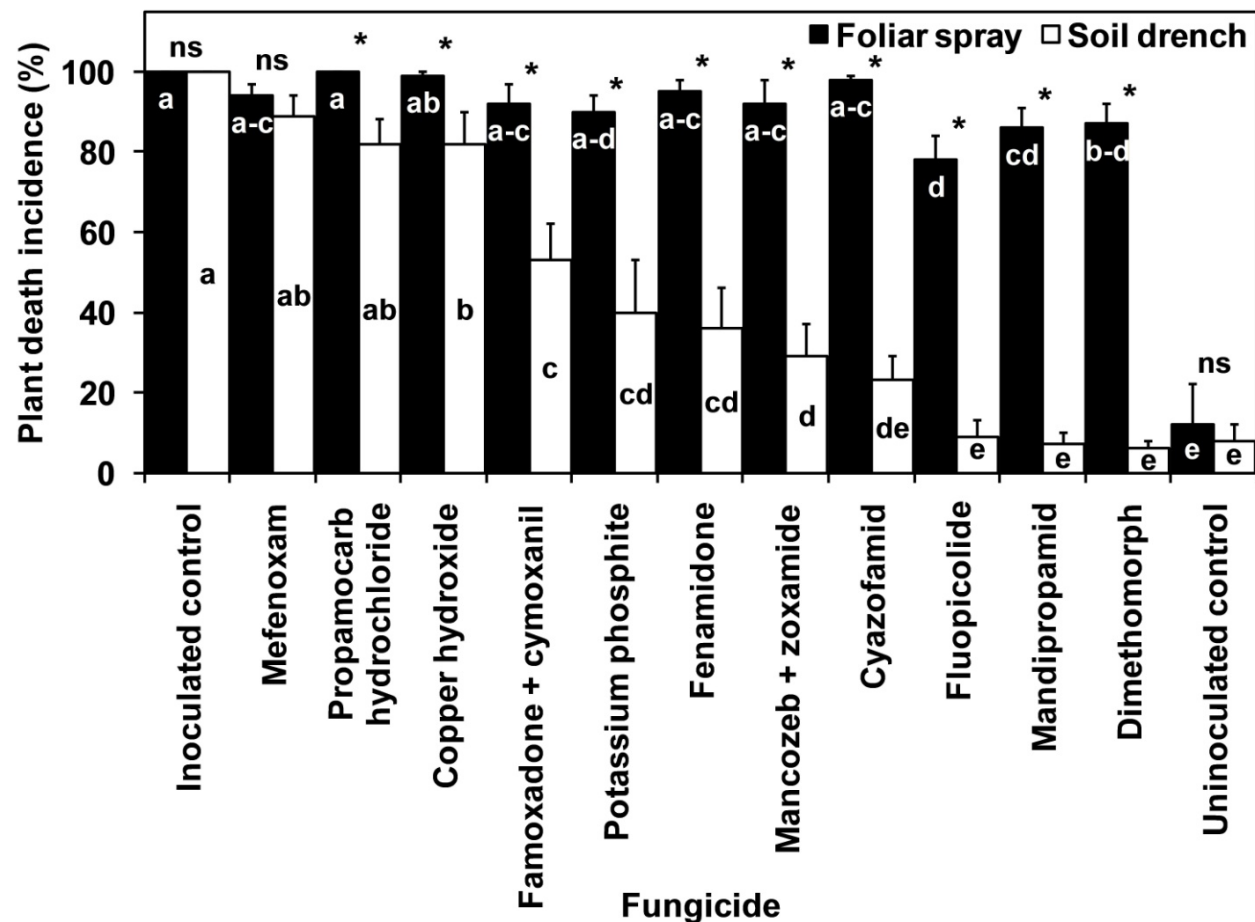


Table 1.2. Area under the disease progress curve (AUDPC) values for plant death on summer squash ‘Cougar’ grown in field trials evaluating fungicides applied as a foliar spray or soil drench to control *Phytophthora* crown and root rot caused by *Phytophthora capsici*

Fungicide treatment ^v	AUDPC ^u		P-value ^w
	Foliar spray	Soil drench	
Inoculated control	2812 a-c ^x	2841 a	0.8877
Mefenoxam	2892 a-c	2576 ab	0.1239
Copper hydroxide	3094 ab	2559 ab	0.0095
Propamocarb hydrochloride	2874 a-c	2041 bc	<0.0001
Famoxadone + cymoxanil	2674 cd	1672 cd	<0.0001
Fenamidone	2947 a-c	1105 de	<0.0001
Potassium phosphite	2675 cd	1094 e	<0.0001
Mancozeb + zoxamide	2772 a-c	897 e	<0.0001
Cyazofamid	3130 a	730 ef	<0.0001
Fluopicolide	2375 d	210 fg	<0.0001
Mandipropamid	2580 cd	173 fg	<0.0001
Dimethomorph	2727 b-d	146 g	<0.0001
Uninoculated control	187 e	130 g	0.7810
Mean ^y	2795	1200	...
FLSD ^z at $\alpha = 0.05$	393	575	...

^u AUDPC was calculated for plant death incidence assessed at 7-day intervals from 7 to 42 days post inoculation (dpi).

^v Fungicides were applied as a foliar spray or soil drench following inoculation with *P. capsici* isolate 12889 and at 7-day intervals thereafter until 42 dpi.

^w Indicates statistical significance of the comparison of application methods for each fungicide.

^x Within columns AUDPCs with a common letter do not differ significantly based on Fisher’s protected least significant difference (FLSD) at $\alpha = 0.05$.

Table 1.2 (cont'd).

^y Untreated control plots were excluded from the calculation of the mean.

^z FLSD = Fisher's protected least significant difference.

Table 1.3. Yield of summer squash ‘Cougar’ expressed as a percentage of the uninoculated control yield in field trials evaluating foliar spray and soil drench applications of eleven fungicides for managing *Phytophthora* crown and root rot caused by *Phytophthora capsici*

Fungicide treatment ^a	Relative yield (%) of uninoculated control	
	Foliar spray	Soil drench
Fluopicolide	46* ^b	108
Mandipropamid	30*	101
Dimethomorph	29*	94
Potassium phosphite	23*	67*
Mancozeb + zoxamide	13*	104
Fenamidone	12*	88
Mefenoxam	9*	9*
Famoxadone + cymoxanil	9*	59*
Cyazofamid	7*	109
Copper hydroxide	3*	32*
Propamocarb hydrochloride	2*	21*
Inoculated control	0*	0*
Mean ^c	17	72
FLSD ^d at $\alpha = 0.05$	18	24

^a Fungicides were applied as a foliar spray or soil drench following inoculation with

Phytophthora capsici and at 7-day intervals thereafter until 42 days post inoculation.

^b Within an application method, values followed by an asterisk differed significantly from the uninoculated control based on Fisher’s protected least significance test at $\alpha = 0.05$.

^c Untreated control plots were excluded from the calculation of the mean.

^d FLSD = Fisher’s protected least significant difference.

Greenhouse evaluation. Disease symptoms began to develop 5 dpi. Most inoculated control plants died by 21 dpi, whereas uninoculated control plants remained asymptomatic. The phenotype of *P. capsici* isolated from symptomatic plants matched that of the isolate used for inoculum. In general, crown rot was more severe following inoculation with *P. capsici* isolate 12889 than SP98. Results from analyses of crown rot severity and AUDPC were similar. Crown rot severity differed significantly ($P<0.0001$) among application methods, fungicides, and cultivars in the ANOVAs for both isolates (Table 1.4; Figure 1.4A and B). The fungicide x application method and the fungicide x cultivar interactions also were significant ($P<0.0001$) for both isolates. Crown rot on both cultivars was less severe for soil drenches compared to foliar sprays for nine fungicides in both trials (Table 1.4). Crown rot was not affected by application method for copper hydroxide or mefenoxam, regardless of cultivar (Table 1.4). In general, treatment combinations (fungicide x application method) were more effective on ‘Leopard’, which is less susceptible than ‘Cougar’ (Table 1.4; Figure 1.4A and B). For isolate SP98 only, the application method x cultivar and the fungicide x application method x cultivar interactions were significant ($P<0.05$). When the untreated controls, copper hydroxide, and mefenoxam were excluded from the analysis these interactions were not significant.

Table 1.4. Mean crown rot severity on summer squash ‘Cougar’ and ‘Leopard’ following inoculation with *Phytophthora capsici* isolate 12889 or SP98 in greenhouse trials evaluating foliar and soil drench applications of eleven fungicides for controlling *Phytophthora* crown and root rot

Fungicide treatment ^c	Crown rot severity 21 days post inoculation (dpi) ^a							
	<i>P. capsici</i> isolate 12889 ^b				<i>P. capsici</i> isolate SP98			
	‘Cougar’		‘Leopard’		‘Cougar’		‘Leopard’	
	Foliar spray	Soil drench	Foliar spray	Soil drench	Foliar spray	Soil drench	Foliar spray	Soil drench
Inoculated control	5.0	4.7 ns ^d	4.8	4.9 ns	4.9	5.0 ns	4.2	4.3 ns
Copper hydroxide	5.0	4.7 ns	4.8	4.5 ns	4.7	5.0 ns	3.8	3.5 ns
Mefenoxam	5.0	4.3 ns	3.5	2.4 ns	1.7	1.2 ns	1.0	1.0 ns
Dimethomorph	5.0	3.8 *	4.6	2.5 *	4.8	1.7 *	3.9	1.7 *
Fenamidone	4.8	3.3 *	4.4	2.2 *	4.7	1.8 *	4.1	1.4 *
Cyazofamid	4.8	3.2 *	4.5	3.3 *	4.8	3.9 *	4.0	1.3 *
Mancozeb + zoxamide	4.8	2.8 *	4.3	2.3 *	4.6	2.2 *	4.0	1.8 *
Mandipropamid	4.9	2.3 *	5.0	1.6 *	4.8	1.3 *	3.9	1.3 *
Potassium phosphite	4.9	1.4 *	3.7	1.3 *	4.8	1.3 *	3.8	1.2 *
Fluopicolide	4.9	1.1 *	4.5	1.0 *	4.4	1.0 *	3.8	1.0 *
Propamocarb hydrochloride	3.6	1.0 *	3.1	1.0 *	3.9	1.3 *	2.1	1.0 *
Famoxadone + cymoxanil	4.7	1.0 *	3.2	1.0 *	4.5	1.0 *	3.2	1.0 *
Uninoculated control	1.0	1.0 ns	1.0	1.0 ns	1.0	1.0 ns	1.0	1.0 ns
Mean ^e	4.8	2.6	4.2	2.1	4.3	2.0	3.4	1.5
FLSD ^f at $\alpha = 0.05$	0.4	0.9	0.9	1.0	0.7	0.6	1.0	0.7

Table 1.4 (cont'd).

^a Crown rot severity was rated from 5 to 21 dpi on a 1 to 5 scale, where 1 = no symptoms; 2 = lower leaves wilted with slight constriction of the stem; 3 = all leaves wilted with constriction and slight discoloration of the stem; 4 = all leaves wilted and crown rotted with visible sporulation on the stem surface; and 5 = plant dead.

^b Plants grown in separate, concurrent trials were inoculated with *P. capsici* isolate 12889 or isolate SP98.

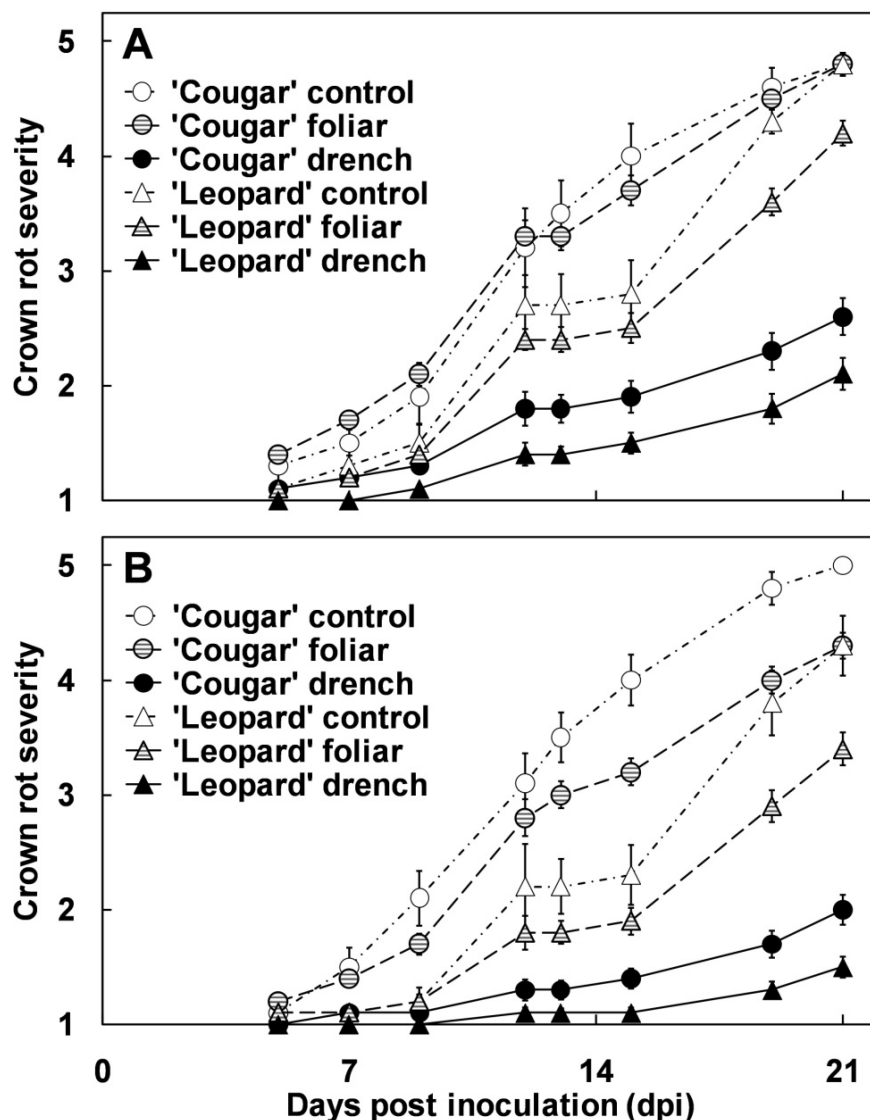
^c Fungicides were applied as a foliar spray or soil drench following inoculation with *P. capsici* and at 7-day intervals thereafter until 21 dpi.

^d For a given isolate x cultivar x fungicide combination, an asterisk indicates that application methods differed significantly based on single degree of freedom slice statements used to partition the fungicide x method interaction at $\alpha = 0.05$. Values represent the mean of twelve replicates.

^e Untreated control plots were excluded from calculation of the mean.

^f FLSD = Fisher's protected least significant difference.

Figure 1.4. Development of crown rot on summer squash ‘Cougar’ and ‘Leopard’ inoculated with *Phytophthora capsici* **A**, isolate 12889 or **B**, isolate SP98 in greenhouse trials evaluating the efficacy of foliar spray (foliar) and soil drench (drench) applications of eleven fungicides. Crown rot severity was rated from 5 to 21 dpi on a 1 to 5 scale, where 1 = no symptoms; 2 = lower leaves wilted with slight constriction of the stem; 3 = all leaves wilted with constriction and slight discoloration of the stem; 4 = all leaves wilted and crown rotted with visible sporulation on the stem surface; and 5 = plant dead. Ratings are the means of eleven fungicides and twelve replicates. Error bars represent the standard error of the mean.



DISCUSSION

Applying fungicides to the soil represents a significant change in management strategy since growers have traditionally managed Phytophthora crown and root rot using foliar fungicide applications. In this study, fungicides with activity against *P. capsici* were most effective at controlling Phytophthora crown and root rot on summer squash when applied as a soil drench. It should be noted that drench applications were evaluated in a plasticulture system and in a greenhouse where the possibility of rain leaching the fungicides from the root zone was relatively low. Foliar applications did not adequately reduce AUDPC or the final incidence of plant death and were unable to prevent yield losses. These results are in agreement with previous research. In a greenhouse study, Foster and Hausbeck (11) demonstrated that soil drenches were more effective than foliar applications at reducing AUDPC and pepper plant death caused by *P. capsici*. In a separate study, phosphonate drenches reduced the severity of crown rot on zucchini and pumpkin seedlings, whereas foliar applications did not (45). Fungicide drenches may be more effective than foliar applications due to increased contact with the plant root system and *P. capsici* propagules in the soil. Furthermore, the dense canopy of summer squash leaves may prevent adequate coverage of lower portions of the plant when fungicides are applied as a foliar spray.

Among the fungicides evaluated in this study, fluopicolide, mandipropamid, and dimethomorph consistently reduced disease progress and symptoms associated with Phytophthora crown and root rot in both field and greenhouse trials. In the greenhouse, levels of control were similar for both *P. capsici* isolates used as inoculum. Recently, fluopicolide was registered for application through drip irrigation systems (i.e., subsurface drip chemigation) to control “soilborne infections” on cucurbits and other vegetable crops (2). Field trials in Georgia

have demonstrated the efficacy of fluopicolide application through drip irrigation systems for controlling *P. capsici* on yellow summer squash (16). Results from this study confirm that soil applications of fluopicolide are highly effective at preventing plant death associated with Phytophthora crown and root rot. Resistance to fluopicolide, mandipropamid or dimethomorph has not been reported in *P. capsici* populations from Michigan. However, *P. capsici* isolates from Michigan were less sensitive to fluopicolide compared to isolates from four southeastern states (20). Lu et al. (24) were able to generate fluopicolide-resistant mutants of *P. capsici* in the laboratory, and determined the risk of *P. capsici* developing resistance to fluopicolide in the field to be moderately high. To delay the development of resistance, fluopicolide should be used in rotation with fungicides with different modes of action such as mandipropamid or dimethomorph. Cross-resistance to multiple CAA fungicides including dimethomorph and mandipropamid has been reported in some *P. capsici* populations (17,25). Therefore, these fungicides should not be used exclusively or in tank mixtures.

Subsurface drip chemigation of fungicides to manage soilborne diseases including Phytophthora crown and root rot is expected to increase as vegetable producers discontinue the practice of soil fumigation with methyl bromide. In addition to accurately delivering fungicide to the plant root system, drip chemigation may reduce applicator exposure, application costs and fossil fuel use compared to application with ground rig sprayers (1,18). Also, drip chemigation can be performed during poor field conditions that prohibit application by ground rig (1,18). Among the fungicides evaluated in this study, only fluopicolide (Presidio 4SC) and mefenoxam (Ridomil Gold 4SL) are registered for drip chemigation. Numerous factors affect the efficacy of soil applications including fungicide concentration, formulation and placement, as well as soil characteristics including texture, moisture and organic matter content (29). Additional studies

are necessary to determine whether fungicides that suppressed *Phytophthora* crown and root rot as a soil drench in this study are also effective when applied by drip chemigation. Furthermore, the optimal interval between soil fungicide applications needs to be determined. Fungicides were applied at 7-day intervals in this study; however, soil applications may need to be applied less frequently than foliar applications.

Currently, few fungicides are registered for application by soil drench or drip chemigation. Therefore, foliar fungicide sprays will continue to be necessary, especially for managing *Phytophthora* fruit rot and foliar blight, which did not occur in this study. Crown-directed applications may penetrate the canopy better than broadcast applications, resulting in improved coverage of the lower portions of the plant. Some of the fungicides evaluated may be more or less effective at controlling foliar blight and fruit rot than root and crown rot. For example, copper hydroxide has been recommended in fungicide tank-mixes for *Phytophthora* fruit rot control (14). However, results from this study indicate it does not control root and crown rot.

Fungicides are most effective when used in combination with host resistance and cultural practices (11,14,15). ‘Cougar’ and ‘Leopard’ differed in susceptibility to *P. capsici* in this study; however, plant death and severe crown rot occurred on both cultivars by 21 dpi. These cultivars likely do not possess levels of field resistance that are of commercial significance for *Phytophthora* crown and root rot management. Research to identify sources of *P. capsici* resistance in cucurbit germplasm has been limited (8,12,30). Additional studies are necessary to screen for *P. capsici* resistance genes and incorporate them into commercially available cultivars. In the meantime, growers should integrate fungicide use with cultural practices including the use

of dome-shaped raised beds, plastic mulches, and drip irrigation to manage Phytophthora crown and root rot in production fields.

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CHAPTER II: AGE-RELATED RESISTANCE TO PHYTOPHTHORA FRUIT ROT IN 'DICKENSON FIELD' PROCESSING PUMPKIN AND 'GOLDEN DELICIOUS' WINTER SQUASH FRUITS

ABSTRACT

Phytophthora fruit rot, caused by *Phytophthora capsici* Leonian, is a major constraint to cucurbit production for the processing industry in Michigan. Age-related resistance to Phytophthora fruit rot has been identified in pepper (*Capsicum annuum*) and some cucurbit fruits. In this study, 'Dickenson Field' processing pumpkin (*Cucurbita moschata* Duchesne ex Poir.) and 'Golden Delicious' winter squash (*C. maxima* Duchesne) were evaluated for age-related resistance to Phytophthora fruit rot. Hand-pollinated fruit were harvested 3, 7, 10, 14, 21, 28, 42, or 56 days post pollination (dpp), and inoculated with *P. capsici* isolate 12889. Susceptibility to Phytophthora fruit rot decreased with fruit age in 'Dickenson Field' processing pumpkin, whereas 'Golden Delicious' winter squash remained susceptible to fruit rot even as fruit reached full maturity. Less than 15% of 'Dickenson Field' fruit 21 dpp or older became diseased. Conversely, about 80% of 'Golden Delicious' fruit 21 dpp or older became diseased. Lesion diameter and pathogen growth density ratings differed significantly ($P < 0.0001$) among fruit ages for both cultivars, and were negatively correlated ($\rho = -0.37$ to -0.88) with fruit age. Lesion diameter and pathogen growth were generally greater on younger fruit than older fruit. Lesion diameter was greatest on 7 and 10 dpp old fruit of 'Dickenson Field' and 'Golden Delicious', respectively. Pathogen growth density ratings were greatest on 3 dpp old fruit of both cultivars. Several morphological and physiological changes were observed as fruit matured. Soluble solids content and exocarp firmness of both cultivars increased with fruit age. Lesion

diameter and pathogen growth density were negatively correlated ($\rho = -0.29$ to -0.73) with soluble solids content and exocarp firmness.

INTRODUCTION

Phytophthora fruit rot, caused by *Phytophthora capsici* Leonian, is a major constraint to cucurbit production for processing and fresh market consumption (3,4,5,6,13). Production of processing pumpkin (*Cucurbita moschata* Duchesne ex Poir.) for canned pie filling has recently increased in Michigan due to production shortages elsewhere (N. Myers, *personal communication*). Winter squash (*C. maxima* Duchesne) is also grown commercially in this region for use in baby food purees. In 2010, Phytophthora fruit rot incidence was greater than 90% in a 32 ha field of ‘NK 580’ winter squash in Mason County, MI (M. Meyer and M. Hausbeck, *personal observation*). Similarly, severe epidemics of Phytophthora fruit rot have occurred on processing pumpkin in Illinois, where a majority of the crop is produced (3).

Phytophthora fruit rot is initiated when fruit contact *P. capsici*-infested soil, or when propagules are splash dispersed to fruit during rain and irrigation events (5,19). Infected fruit eventually collapse and cannot be harvested (5,19). Processing pumpkin and winter squash plants have a vining growth habit and fruit are grown in direct contact with the soil, which favors fruit rot development. Management of Phytophthora fruit rot is particularly difficult because all commercial cucurbit cultivars are highly susceptible to *P. capsici* (3,4,6,13). Therefore, Phytophthora fruit rot is primarily managed with fungicides. Resistance to the fungicides metalaxyl and mefenoxam (metalaxyl-M) is widespread in *P. capsici* populations from the primary vegetable growing regions in Michigan (17). Furthermore, foliar fungicides can be ineffective when the crop canopy prevents adequate coverage of the fruit surface or when applications do not reach the undersides of fruit that are in direct contact with the soil.

Fruit age affects susceptibility to *P. capsici* in pepper and some cucurbit fruits (2,7,10,12,15). The degree of ontogenic or age-related resistance to *P. capsici* varies among cucurbit cultivar-groups, but taxonomic classes are not correlated with susceptibility to *P. capsici* (2). Age-related resistance to *P. capsici* is most apparent in cucumber (*Cucumis sativus*) (2,10,12). Immature, elongating cucumber fruits are more susceptible to *P. capsici* than mature fruits (2,10,12). Cucumber fruits harvested 14 days post pollination (dpp) and inoculated with *P. capsici* rarely developed symptoms (10). Seven cucurbit crops from four species including *Cucumis melo*, *Citrullus lanatus*, *Cucurbita moschata*, and *C. pepo* exhibited similar age-related responses to *P. capsici* inoculation using detached fruits under laboratory conditions (2). Wounding fruits prior to inoculation reduces age-related resistance to *P. capsici* (10,12).

The morpho-physiological changes that convey age-related resistance to *P. capsici* are not well understood. Changes in exocarp color and waxiness during fruit development were associated with age-related resistance in acorn squash (*C. pepo*), butternut squash (*C. moschata*), and pumpkin (*C. pepo*) (2). In pepper, cuticle thickness was greater and lesion length was smaller on mature fruits than on immature fruits (7). However, *P. capsici* mycelial growth was greater in extracts from mature pepper fruits, which contained higher levels of sugar than immature fruits (7). Accumulation of dry matter increased and macroelement content decreased as pepper stems aged and became more resistant to *P. capsici* (15).

Phytophthora fruit rot is a major constraint to cucurbit production for the processing industry in Michigan, and growers have few options for managing this disease. Relatively little is known about the reactions of processing pumpkin and winter squash fruits to inoculation with *P. capsici*, and these fruits were not included in previous evaluations of age-related resistance to Phytophthora fruit rot. The objectives of this study were to determine the effect of fruit age on

susceptibility to *P. capsici* in processing pumpkin and winter squash, and to determine if susceptibility to *P. capsici* is associated with soluble solids content and exocarp firmness.

MATERIALS AND METHODS

Inoculum. *Phytophthora capsici* isolate 12889 (mating type A1) obtained from the culture collection of M. K. Hausbeck at Michigan State University was used to inoculate fruit in this study. Isolate 12889 was originally isolated from bell pepper, and is highly virulent on various host fruits (8,9). All cultures were grown on unclarified V8 juice agar (UCV8) at room temperature ($21 \pm 2^{\circ}\text{C}$) and under constant fluorescent light.

Fruit production and measurement. Processing pumpkin and winter squash fruits were grown at the Michigan State University Plant Pathology Research Farm in East Lansing. *Phytophthora* blight had not previously occurred at this location. The soil type was a Capac loam (fine-loamy, mixed, active, mesic Aquic Glossudalfs). Seedlings of ‘Dickenson Field’ processing pumpkin (Rispen Seeds Inc., Beecher, IL) and ‘Golden Delicious’ winter squash (Stokes Seeds Inc., Thorold, ON, Canada) were transplanted at the second true-leaf stage into four raised beds covered with black plastic mulch. Beds were 30.5 m-long and spaced 3.7 m apart. Plants were spaced 61 cm apart within beds. Female flowers were tagged at anthesis and hand-pollinated. Fruit were harvested 3, 7, 10, 14, 21, 28, 42, or 56 dpp. Ages were selected based on previous research of age-related resistance in other cucurbit fruits (2,10). All replicates of a single fruit age were harvested on the same calendar date.

Fruit length was measured from the stem-end to the blossom-end of each fruit. Width was measured perpendicular to the stem-blossom axis at the greatest dimension of the fruit. Fruit weight and exocarp color also were determined. A 1.2 cm-diameter core was aseptically removed near the stem of each fruit using a cork borer. The resulting hole was covered with a

piece of clear tape. Fruit cores were placed into individual plastic bags and frozen at -20°C for 24 to 48 h. Fruit cores were thawed at room temperature ($21 \pm 2^{\circ}\text{C}$) for about 1.5 h and hand-pressed to extract juices. Soluble solids content was determined using a temperature-compensated refractometer (model r^2 Mini; Reichert, Inc., Buffalo, NY). At the end of the experiment, firmness of a ca. 25 cm^2 exocarp (rind) section was determined using a penetrometer (model FT 327; QA Supplies LLC, Norfolk, VA) mounted to a manual press and equipped with a 5 mm-diameter plunger. Firmness was expressed as the force in newtons (N) required to puncture the exocarp.

Experimental design and inoculation procedure. The experimental design was completely randomized, with eight replicates of each fruit age. The experiment was conducted twice. Prior to inoculation, fruit were surface disinfested in a 10% bleach solution (0.62% NaClO) for 5 min and rinsed with tap water for 1 min (8). Seven fruit were inoculated with a 7 mm-diameter agar plug removed from the edge of an actively growing culture of *P. capsici* isolate 12889. One fruit was inoculated with a sterile agar plug as a control. Fruit were placed into 53 cm x 33 cm x 28 cm transparent, plastic moisture chambers and incubated at room temperature ($21 \pm 2^{\circ}\text{C}$). Moisture chambers were lined with moist paper toweling to maintain high humidity and exposed to constant fluorescent light. A WatchDog[®] data logger (model A150; Spectrum Technologies Inc., Plainfield, IL) recorded temperature and relative humidity in the moisture chamber during incubation. Conditions in the moisture chambers during incubation were similar among experiments and favorable for disease development. Mean temperature was 23.9°C in experiment 1 and 23.5°C in experiment 2 (Table 2.1). Mean relative humidity was 97.7 and 99.0% in experiments 1 and 2, respectively (Table 2.1).

Table 2.1. Minimum (Min.), maximum (Max.), and mean air temperature and relative humidity in moisture chambers containing ‘Dickenson Field’ processing pumpkin or ‘Golden Delicious’ winter squash fruits inoculated with *Phytophthora capsici* in experiment (Expt.) 1 and 2

Expt.	Air temperature (°C) ^a			Relative humidity (%)		
	Min.	Max.	Mean	Min.	Max.	Mean
1	21.9	26.7	23.9	93.4	99.9	97.7
2	21.4	27.6	23.5	70.5	99.9	99.0

^a Air temperature and relative humidity were measured in the moisture chambers during a four day incubation period using a WatchDog[®] data logger (model A150; Spectrum Technologies Inc., Plainfield, IL).

Disease assessment and statistical analysis. Disease incidence was recorded for each fruit as a binary response, where 0 = not diseased and 1 = diseased. Lesion diameter and pathogen growth diameter were measured 4 days post inoculation (dpi). Pathogen growth density was visually assessed 4 dpi on a 1 to 4 scale, where 1 = no external signs of pathogen growth; 2 = light growth; 3 = moderate growth; and 4 = dense growth. Uninoculated control fruit were excluded from the analyses. Data from both experiments were combined before analysis. All analyses were done using SAS, version 9.2 (SAS Institute, Cary, NC). Disease incidence was analyzed by logistic regression using the Proc Logistic procedure. Logistic regression was also used to calculate the predicted probability of disease for each cultivar. Lesion diameter, pathogen growth diameter, and pathogen growth density ratings were analyzed separately for each cultivar by analysis of variance (ANOVA) using the Proc Mixed procedure. Fruit age was considered a fixed effect. Soluble solids content and exocarp firmness were regressed against fruit age using the Proc Reg procedure. Spearman-rank correlation coefficients

were calculated between fruit measurements and disease assessments for each cultivar using the Proc Corr procedure.

RESULTS

A range of reactions to *P. capsici* developed on ‘Dickenson Field’ and ‘Golden Delicious’ fruits differing in age (Figure 2.1 and 2.2). Soft, water-soaked lesions formed on 53% and 88% of ‘Dickenson Field’ and ‘Golden Delicious’ fruits, respectively. Most lesions contained visible signs of *P. capsici*, including mycelia and sporangiophores with sporangia. About 10% of lesions were water-soaked, but lacked external signs of pathogen growth. These lesions formed only on fruit that were 21 dpp or older.

Disease incidence varied among cultivars and fruit ages, but generally decreased as age increased (Figure 2.3A and B). The logistic regression of disease incidence on fruit age was significant ($P < .0001$) for both cultivars. On ‘Dickenson Field’, disease incidence was 100% when fruit were 3 dpp old and 0% when fruit were 56 dpp old (Figure 2.3A). On ‘Golden Delicious’, disease incidence was 100% when fruit were 3 dpp old and 79% when fruit were 56 dpp old (Figure 2.3B). Less than 15% of ‘Dickenson Field’ fruit 21 dpp or older became diseased. Conversely, about 80% of ‘Golden Delicious’ fruit 21 dpp or older became diseased. The predicted probability of disease on ‘Dickenson Field’ decreased substantially as fruit age increased, and was ≤ 0.45 when fruit were 21 dpp or older (Figure 2.4). The predicted probability of disease on ‘Golden Delicious’ was ≥ 0.60 regardless of fruit age (Figure 2.4).

Figure 2.1. Signs and symptoms four days post inoculation with *Phytophthora capsici* on ‘Dickenson Field’ processing pumpkin fruit harvested 3 to 56 days post pollination (dpp).

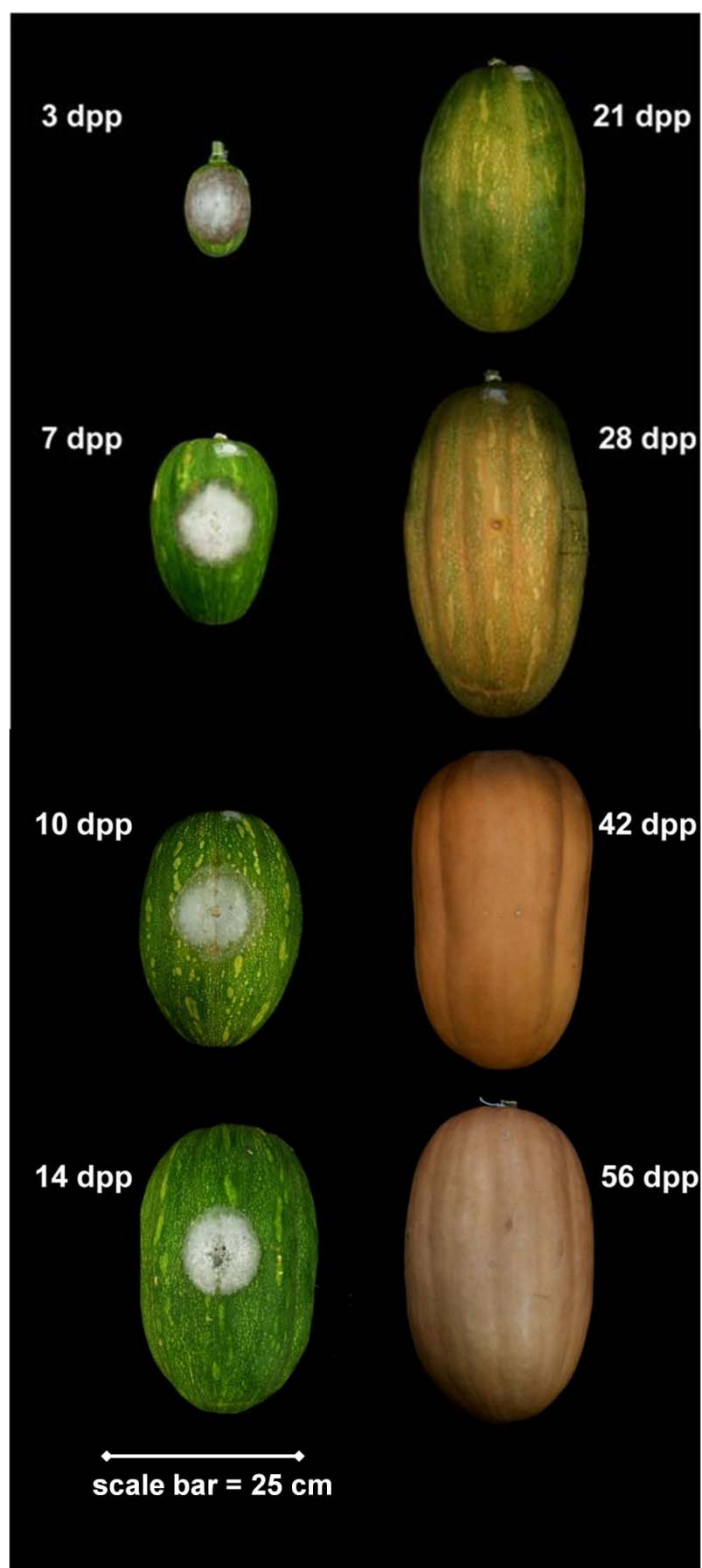
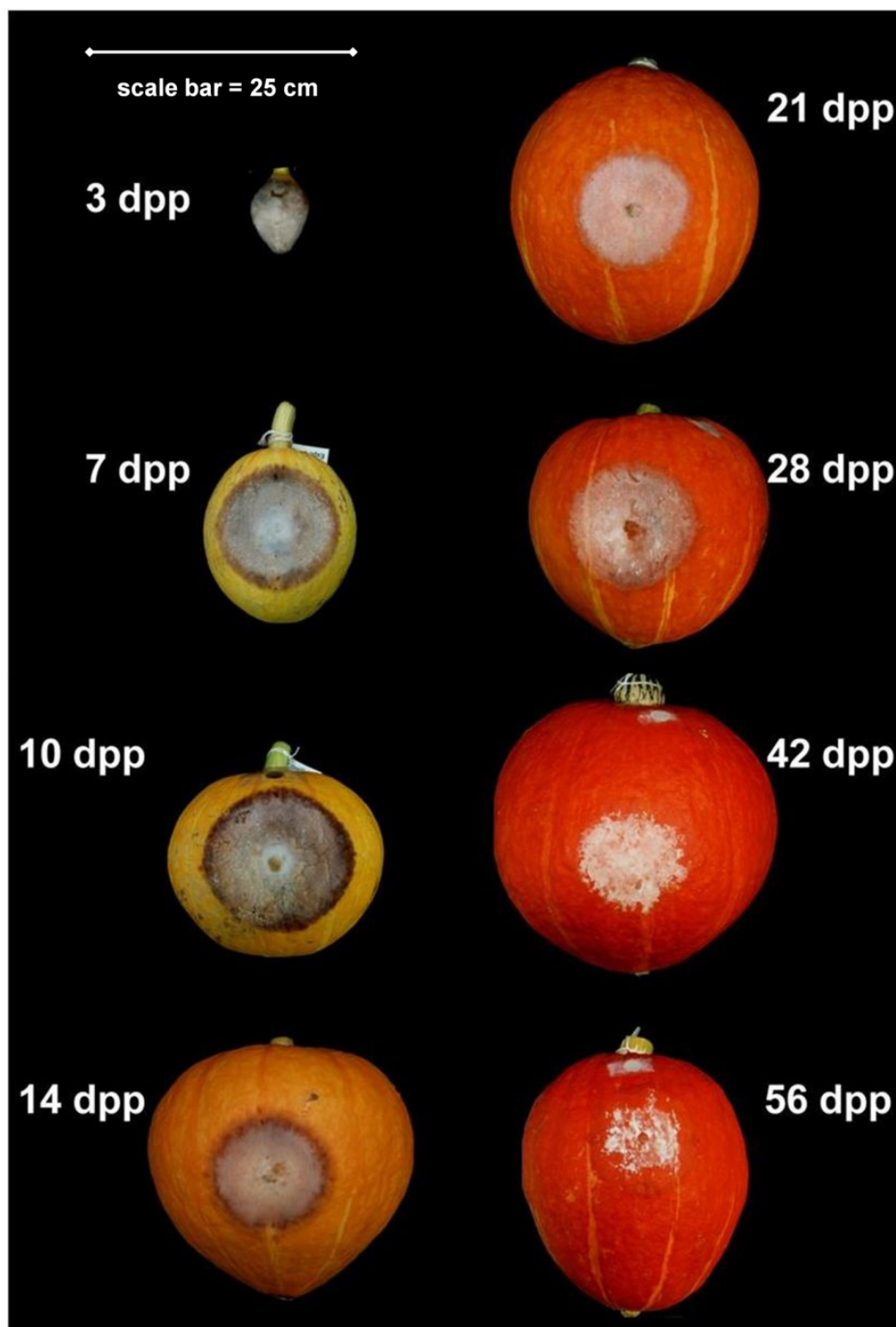


Figure 2.2. Signs and symptoms four days post inoculation with *Phytophthora capsici* on ‘Golden Delicious’ winter squash fruit harvested 3 to 56 days post pollination (dpp).



Analyses of lesion diameter and pathogen growth diameter were similar. Therefore, only lesion diameter results are presented. Lesion diameter and pathogen growth density ratings differed significantly ($P < 0.0001$) among fruit ages for both cultivars. Fruit age was negatively correlated ($\rho = -0.37$ to -0.88) with lesion diameter and pathogen growth density ratings (Table 2.2). Lesion diameter and pathogen growth were generally greater on younger fruit than on older fruit (Figure 2.3C and D; 2.5A and B). Lesion diameter was greatest on 7 dpp fruit of ‘Dickenson Field’ and on 10 dpp fruit of ‘Golden Delicious’ (Figure 2.3C and D). Pathogen growth density was greatest on 3 dpp old fruit of both cultivars (Figure 2.5A and B). Lesions that developed on ‘Dickenson Field’ fruit 21 dpp or older were ≤ 1.0 cm in diameter and contained little or no pathogen growth (Figure 2.3C and 2.5A). Lesions on ‘Golden Delicious’ fruit 21 dpp or older were < 5.0 cm and contained light pathogen growth (Figure 2.3D and 2.5B).

Several morphological and physiological changes were observed during maturation in fruits of both cultivars. Fruit size and fresh weight generally increased as age increased (Table 2.3). Exocarp color changed from pale green to beige in ‘Dickenson Field’ fruit, and from pale yellow to dark orange in ‘Golden Delicious’ fruit (Figure 2.1 and 2.2). Soluble solids varied among fruit ages, ranging from 2.6 to 6.7°Brix in ‘Dickenson Field’ and from 3.1 to 7.3°Brix in ‘Golden Delicious’. Soluble solids content slightly decreased after pollination, and then increased when fruit were 14 dpp or older (Figure 2.6A and B). Soluble solids content was negatively correlated with lesion diameter ($\rho = -0.33$ to -0.57) and pathogen growth density ($\rho = -0.29$ to -0.38) (Table 2.2). Exocarp firmness ranged from 2.1 to 13.0 N in ‘Dickenson Field’ and from 2.9 to 13.0 N in ‘Golden Delicious’. Exocarp firmness increased linearly with fruit age (Figure 2.6C and D). Exocarp firmness was also negatively correlated with lesion diameter ($\rho = -0.21$ to -0.73) and pathogen growth density ($\rho = -0.52$ to -0.75) (Table 2.2).

Figure 2.3. Incidence of diseased fruit (**A** and **B**) and mean lesion diameter (**C** and **D**) four days post inoculation with *Phytophthora capsici* on ‘Dickenson Field’ processing pumpkin and ‘Golden Delicious’ winter squash fruits harvested 3 to 56 days post pollination (dpp). Values are the mean of fourteen fruits. Error bars represent the standard error of the mean.

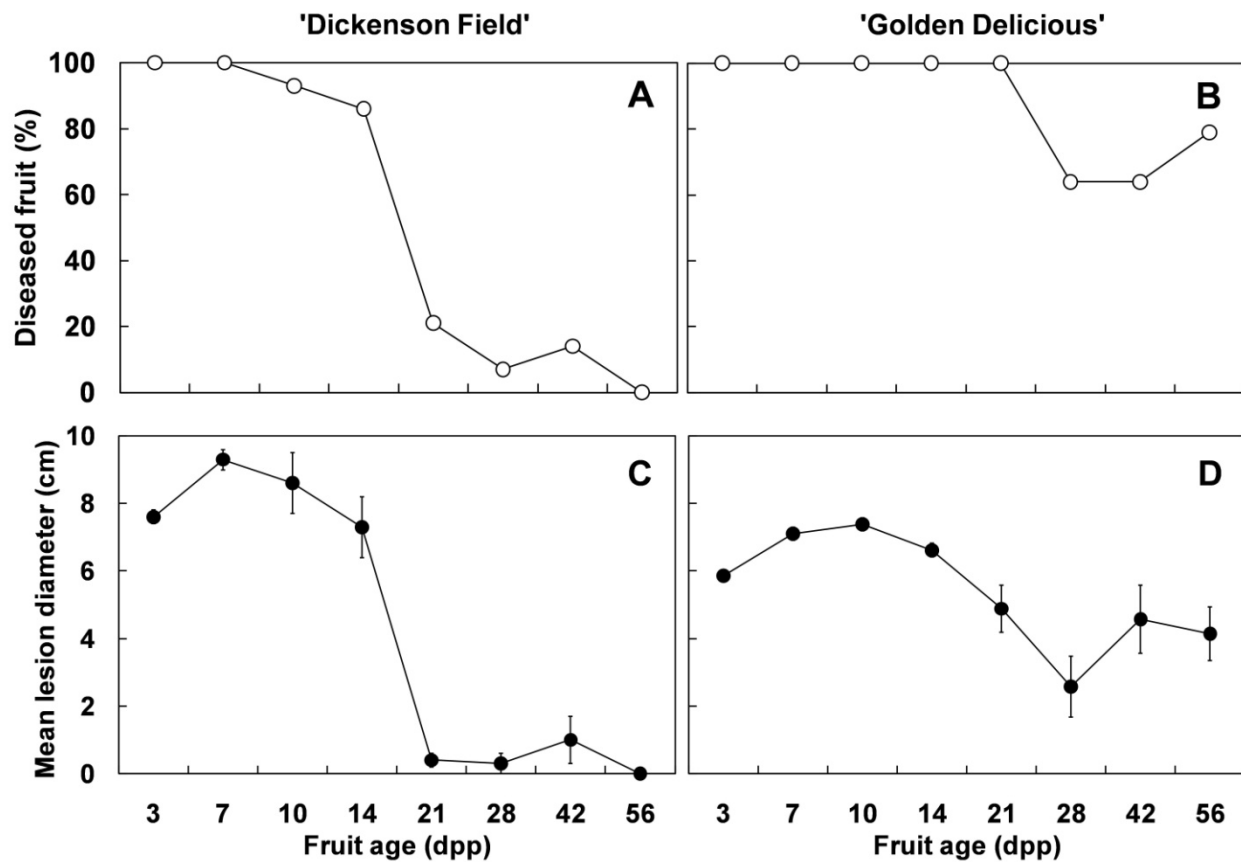


Figure 2.4. Predicted probability of disease four days post inoculation with *Phytophthora capsici* on ‘Dickenson Field’ processing pumpkin and ‘Golden Delicious’ winter squash fruits harvested 3 to 56 days post pollination (dpp).

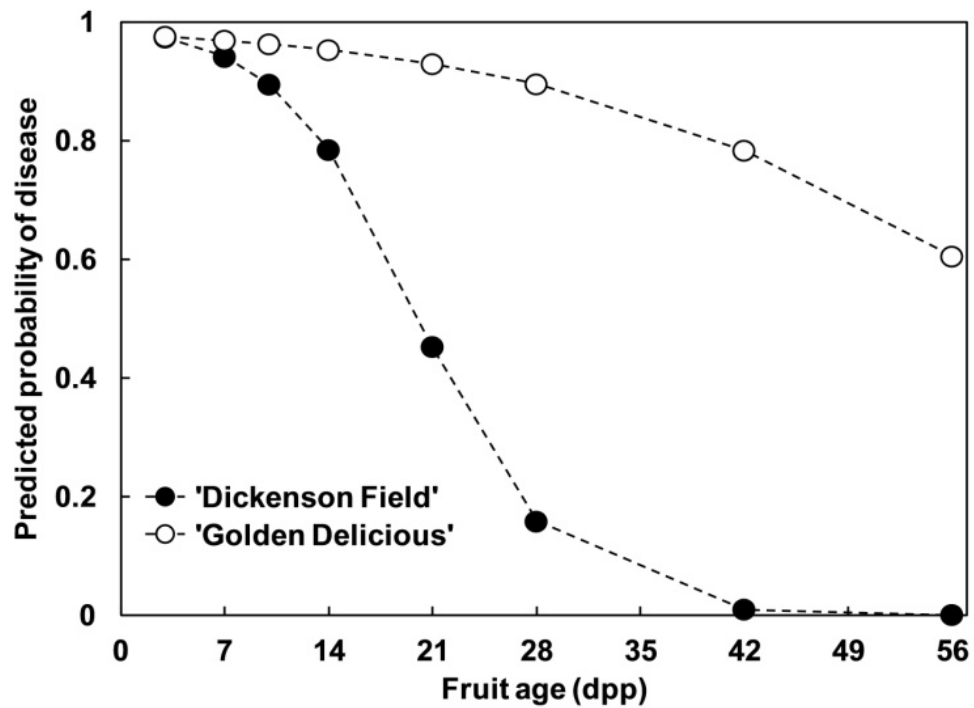


Figure 2.5. Pathogen growth density ratings four days post inoculation with *Phytophthora capsici* on **A**, ‘Dickenson Field’ processing pumpkin and **B**, ‘Golden Delicious’ winter squash fruits harvested 3 to 56 days post pollination (dpp). Pathogen growth density was visually assessed on a 1 to 4 scale, where 1 = no external signs of pathogen growth; 2 = light growth; 3 = moderate growth; and 4 = dense growth. Values are the mean of fourteen fruits. Error bars represent the standard error of the mean.

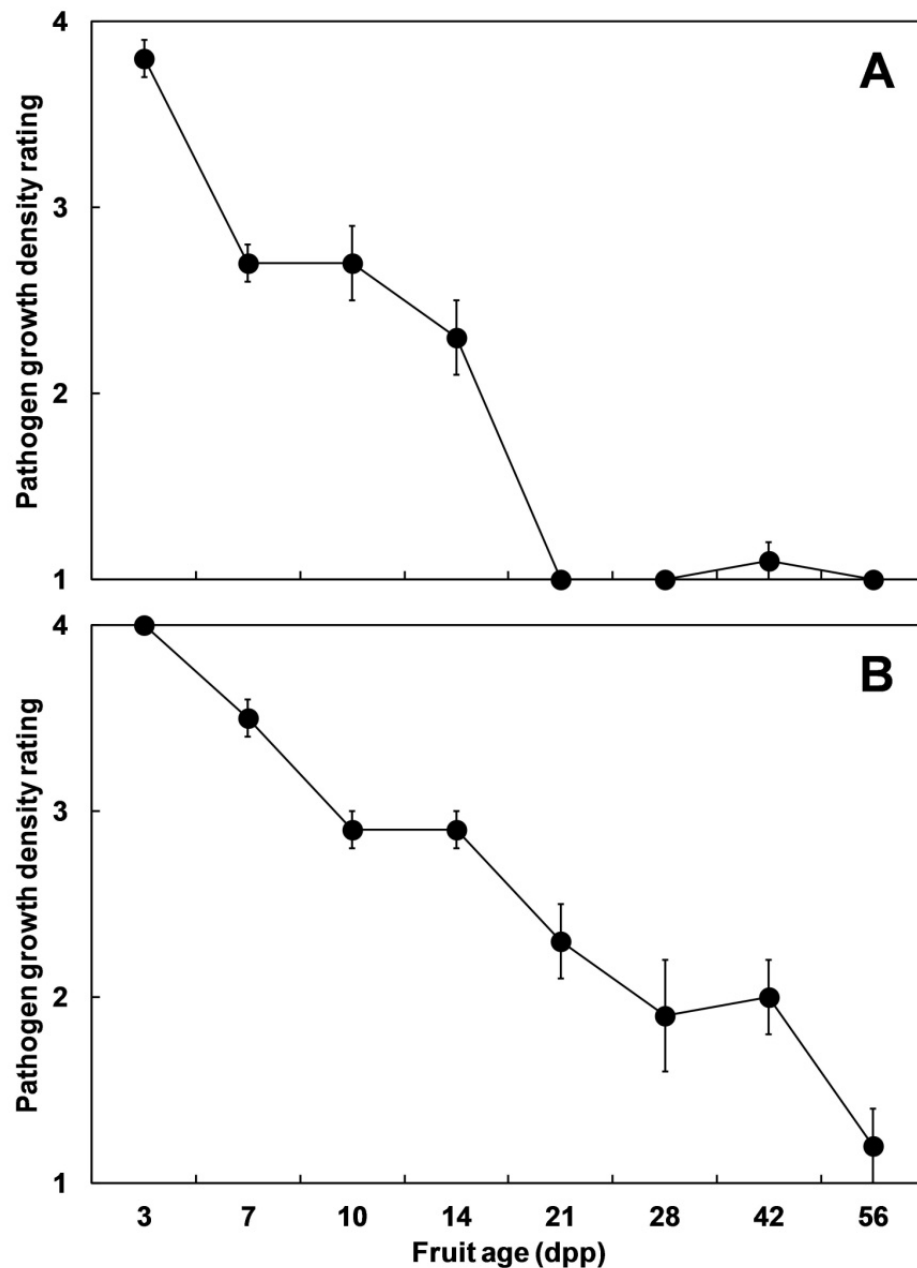


Table 2.2. Spearman-rank correlations between fruit characteristics and disease assessments in experiments evaluating age-related resistance to *Phytophthora* fruit rot caused by *Phytophthora capsici* in ‘Dickenson Field’ processing pumpkin and ‘Golden Delicious’ winter squash

Cultivar, measurement	Lesion diameter (cm)		Pathogen growth density rating	
	ρ^a	P-value	ρ	P-value
‘Dickenson Field’				
Age (dpp) ^b	-0.74	<0.0001	-0.87	<0.0001
Soluble solids (°Brix)	-0.57	<0.0001	-0.38	<0.0001
Exocarp firmness (N)	-0.73	<0.0001	-0.75	<0.0001
‘Golden Delicious’				
Age (dpp)	-0.37	<0.0001	-0.80	<0.0001
Soluble solids (°Brix)	-0.33	0.0004	-0.29	0.0017
Exocarp firmness (N)	-0.21	0.1451	-0.52	<0.0001

^a Rho (ρ) = Spearman-rank correlation coefficient.

^b Days post pollination = dpp.

Table 2.3. Characteristics of ‘Dickenson Field’ processing pumpkin and ‘Golden Delicious’ winter squash fruits harvested 3 to 56 days post pollination (dpp)

Cultivar, fruit age (dpp)^a	Length (cm)^b	Width (cm)^c	Fresh weight (kg)
‘Dickenson Field’			
3	10.6 ^d	7.0	0.4
7	19.9	13.1	2.0
10	25.2	16.5	2.8
14	28.9	20.9	5.1
21	27.9	20.7	4.8
28	30.5	22.4	6.2
42	31.1	23.5	7.0
56	31.8	23.1	7.0
‘Golden Delicious’			
3	6.0	4.9	0.1
7	10.6	9.8	0.8
10	13.6	13.1	2.1
14	15.8	14.5	2.3
21	19.1	18.4	2.8
28	19.2	18.8	2.6
42	22.1	19.7	3.1
56	22.0	19.6	3.1

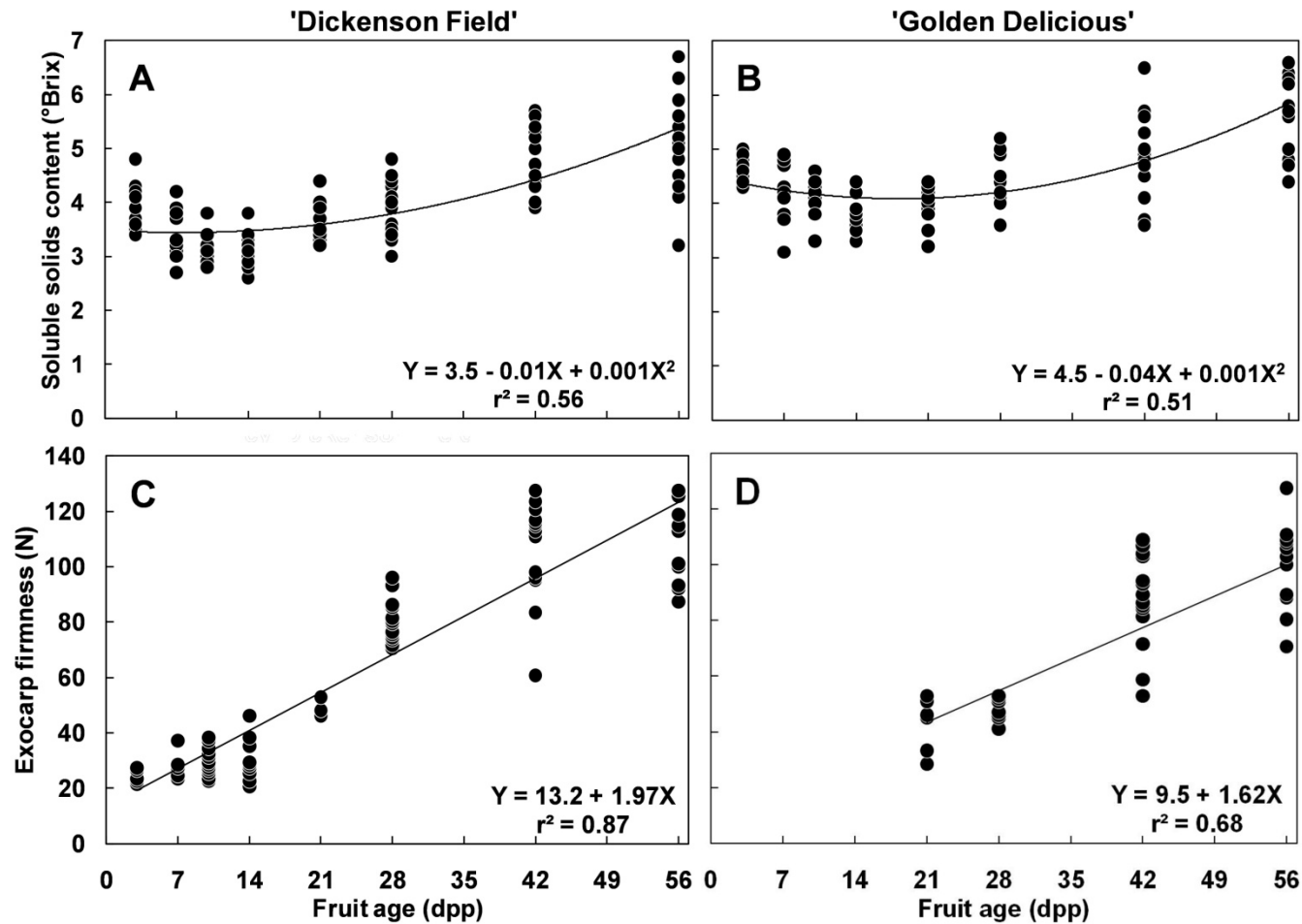
^a Fruit age expressed as days post pollination (dpp).

^b Length was measured from the stem end to the blossom end of each fruit

^c Width was measured at the greatest dimension of the fruit at a 90° angle to the stem-blossom axis.

^d Values represent the mean of fourteen fruits.

Figure 2.6. Soluble solids content (A and B) and exocarp firmness (C and D) of ‘Dickenson Field’ processing pumpkin and ‘Golden Delicious’ winter squash fruits regressed against fruit age. Soluble solids content was determined using a temperature-compensated refractometer. Exocarp firmness was determined using a handheld penetrometer, and is expressed as newtons (N) force required to puncture the exocarp. Fruit age is expressed as days post pollination (dpp).



DISCUSSION

This is the first evaluation of age-related resistance to *Phytophthora* fruit rot in pumpkin and winter squash cultivars grown primarily for processing. Previously, age-related resistance to *Phytophthora* fruit rot was reported in eight cultivar-groups from five cucurbit species (2,10,12). Results from this experiment demonstrate that ‘Dickenson Field’ processing pumpkin fruit become resistant to *Phytophthora* fruit rot as they mature. Conversely, ‘Golden Delicious’ winter squash fruit remain susceptible to fruit rot even as they reach full maturity. Avoiding cultivars that are highly susceptible to *Phytophthora* fruit rot, and scheduling fungicide applications according to host growth stage could be used to improve management of this disease. Additional studies are necessary to determine if age-related resistance occurs under field conditions, and whether fungicide applications can be scheduled according to host growth stage on cultivars with age-related resistance.

Cucurbit fruits undergo numerous morphological and physiological changes as they mature. The onset of age-related resistance to *Phytophthora* fruit rot in multiple cucurbit species appears to occur when fruits reach maximum size (2,10). Large-fruited cucurbits reach maximum fruit size about 20 to 24 dpp, whereas small-fruited cucurbits reach full size about 15 to 20 dpp (18). In this study, less than 15% of ‘Dickenson Field’ fruit 21 dpp or older became diseased. Cucumber fruit 14 dpp or older rarely became diseased in a similar study (10).

Changes in nutrient content (15), cuticle thickness (7), exocarp color and waxiness (2) have been associated with age-related resistance to *P. capsici* in various host fruits. In this study, soluble solids content was negatively correlated with lesion diameter and pathogen growth density. However, a significant correlation does not necessarily indicate a cause and effect relationship. Pathogen growth can be inhibited or enhanced by the sugar content of host tissues

(14). The extracts of mature pepper fruits contained higher levels of sucrose and enhanced the growth of *P. capsici* (7). Thus, Phytophthora fruit rot may not be inhibited by increases in soluble solids content. Exocarp firmness also was negatively correlated with Phytophthora fruit rot development in this study. Thick cell walls can serve as a physical barrier to pathogen growth because they are more difficult for a fungus to penetrate and colonize (1). Hard-rinded Jack-o-lantern pumpkin (*C. pepo*) cultivars have recently been developed that are less susceptible to Phytophthora fruit rot (20). Incidence of Phytophthora fruit rot in field trials was 51% on ‘HMX 5681’ and 100% on ‘Magic Lantern’, which are hard- and soft-rinded pumpkin cultivars, respectively (20). Unfortunately, exocarp firmness of these pumpkin cultivars was not determined. Breeding processing pumpkin and winter squash cultivars with the hard rind (*Hr*) gene could reduce the incidence of Phytophthora fruit rot in the field, but it is unclear how this would affect the fruit’s ability to be processed.

The effects of inoculum concentration, incubation period, and wounding on age-related resistance to Phytophthora fruit rot in processing pumpkin and winter squash are unknown. A similar inoculation method and a four day incubation period were used in this and previous experiments evaluating age-related resistance to Phytophthora fruit rot (2,10). Expression of age-related resistance to Phytophthora stem and foliar blight in pepper was affected by *P. capsici* zoospore concentration and inoculation method (16). Increasing *P. capsici* zoospore concentration resulted in greater levels of Phytophthora fruit rot on pickling cucumber fruits of similar age (11). Thus, increasing *P. capsici* inoculum concentration or using a different method of inoculation could affect age-related resistance to Phytophthora fruit rot in processing pumpkin and winter squash fruits. Similarly, wounding fruits could reduce age-related resistance, as has been demonstrated for cucumber (10,12). Additional studies are necessary to determine if age-

related resistance is overcome under high disease pressure associated with field conditions. For example, when fruits rest on *P. capsici*-infested soil for extended periods of time and when fields are flooded during heavy rainfall or irrigation events. In the meantime, cucurbit growers should apply fungicides with activity against *P. capsici* when conditions favor disease development, ensure fields drain properly, and irrigate sparingly.

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CHAPTER III: USING CULTURAL PRACTICES AND CULTIVAR RESISTANCE TO MANAGE PHYTOPHTHORA CROWN AND ROOT ROT ON SUMMER SQUASH

ABSTRACT

The effects of bed height, mulches, dried poultry litter, and cultivars on *Phytophthora* crown and root rot (*Phytophthora capsici* Leonian) of summer squash (*Cucurbita pepo* L.) was evaluated in the absence of fungicide applications. The experimental design was a split-split-split plot arrangement of a randomized complete block. Bed height (flat or raised) was the main plot treatment. Mulches (bare soil, wheat straw, or plastic) were subplot treatments. Poultry litter applications (0 or 4.5t ha⁻¹) were sub-subplot treatments. Squash cultivars ('Cougar' or 'Payroll') were sub-sub-subplot treatments. Incidence of plant death (%) was assessed from 0 to 35 days post inoculation (dpi) with *P. capsici*. Plant death 35 dpi and AUDPC differed significantly ($P<0.0001$) between the cultivars 'Cougar' and 'Payroll'. Mean plant death 35 dpi was 87% for 'Payroll' and 99% for 'Cougar'. The bed height x cultivar interaction was also significant ($P=0.0018$) in the ANOVAs for plant death and AUDPC. Plant death incidence and AUDPC for 'Payroll' was greater in flat beds than raised beds. Disease was not affected by bed height, mulch type, or poultry litter treatments. Thirty-two summer squash cultivars and ten germplasm accessions were also evaluated for resistance to *Phytophthora* crown and root rot in a separate greenhouse trial. Crown rot severity was rated on a 1 (no symptoms) to 5 (plant death) scale at 18 dpi. Crown rot severity differed significantly ($P<0.0001$) among cultivars and germplasm accessions. Crown rot severity averaged 4.3 on commercial cultivars and 2.2 on germplasm accessions. Crown rot was least severe on the commercial cultivar 'Spineless Beauty' (mean rating = 2.8). No disease developed on four accessions of *Cucurbita moschata* previously reported to be crown rot resistant. Results from this experiment demonstrate the

importance of genetic resistance for managing *Phytophthora* crown and root rot. Additional studies are necessary to identify and incorporate sources of crown and root rot resistance from unadapted cucurbit germplasm into commercial cultivars.

INTRODUCTION

Phytophthora capsici Leonian is an economically important soilborne pathogen of summer squash (*Cucurbita pepo* L.) and other vegetable crops in many areas of the world (1,2,3,11,12). *P. capsici* causes a fruit, crown, and root rot, as well as a foliar blight (1,2,3,11). *Phytophthora* crown and root rot is particularly severe because infections result in plant death and significant crop loss. Management of *Phytophthora* crown and root rot requires an integrated approach (2,11,12,25). Modifying cultural management practices, including growing resistant cultivars, has been used to successfully manage other diseases caused by *Phytophthora* spp. including, root rot of red raspberry (*Rubus idaeus* L.) caused by *P. fragariae* var. *rubi* (32), root rot of papaya (*Carica papaya* L.) caused by *P. palmivora* (30), and leather rot of strawberry (*Fragaria* × *ananassa* Duchesne) caused by *P. cactorum* (18).

Host resistance is generally considered the most efficient means of controlling plant diseases. Resistance to *P. capsici* has been identified in pepper (*Capsicum annuum* L.) (4,14), and cultivars with resistance to *Phytophthora* crown and root rot are available. Unfortunately, few studies have screened unadapted cucurbit (*Cucurbita* spp.) germplasm for resistance to *Phytophthora* crown and root rot (7,21). At least one *Cucurbita pepo* and five *C. moschata* germplasm accessions with resistance to *Phytophthora* crown rot were recently identified using *P. capsici* isolates from Florida (7,21). Resistance to multiple *P. capsici* isolates was also identified in the Korean pumpkin cultivar ‘Danmatmaetdol’ (*C. maxima*) (16). Resistance to *P. capsici* was identified in the wild species *Cucurbita lundelliana*, and introgressed into 19 winter

squash breeding lines (13). An inheritance study indicated that resistance to crown rot derived from *C. lundelliana* and *C. okeechobeensis* subsp. *okeechobeensis* is conferred by three dominant genes (20). Resistance to *P. capsici* has not been incorporated into commercial breeding lines, and all cucurbit cultivars are considered susceptible to Phytophthora crown and root rot (1,2,11). However, cultivars may differ slightly in their reactions to *P. capsici*. Growing resistant squash cultivars in combination with other cultural management practices could be used to improve control of Phytophthora crown and root rot.

Cultural management practices that reduce soil moisture or prevent splash dispersal of *P. capsici* propagules can affect Phytophthora root and crown rot development. Growing plants on raised beds improves water drainage, thereby limiting the conditions favorable for Phytophthora root and crown rot. Phytophthora blight incidence on pepper was 18% in flat beds and 5% in beds raised 45 cm (12). Similarly, plant death of zucchini in a field naturally infested with *P. capsici* was greater in flat beds than raised beds (11). Covering planting beds with plastic mulch can reduce splash dispersal of *P. capsici* from the soil to susceptible plant tissues (26). However, in some cases, plastic mulches increased the spread of Phytophthora blight within a row because *P. capsici* propagules are readily dispersed in water on the surface of plastic mulches (26,29). Organic mulches have also been effective at reducing splash dispersal of *P. capsici*. Chopped wheat straw dispersed between planting rows reduced the spread of Phytophthora blight on pepper (26). Modifying cultural practices may not affect Phytophthora blight development on vining crops like watermelon, which grow off of raised beds and come into contact with the soil between rows (15). Similarly, the raised bed plasticulture system may not be feasible in crops grown for processing because crop values are lower.

Organic soil amendments, such as composted animal manures, have been used to suppress diseases caused by various soilborne pathogens including *P. capsici* (33). Compost water extracts from livestock manures inhibited zoospore germination, germ tube formation, and mycelial growth of *P. capsici* (27). In a separate study, manure application reduced the viability of *P. capsici* oospores (19). Applications of compost water extracts increased the expression of numerous pathogenesis-related genes in pepper plants, and reduced disease caused by *P. capsici* (27). Amending potting mix with composted sewage sludge reduced the incidence of Phytophthora crown rot on pepper by 42% in a greenhouse trial (17). Application of semicomposted horse and poultry manure followed by plastic mulching increased soil microbial activity and reduced the incidence of Phytophthora crown and root rot on pepper in Spain (19).

Modifying cultural management practices, including planting cultivars with resistance to *P. capsici*, could be used to improve the management of Phytophthora crown and root rot on summer squash. Few large-scale field trials have evaluated the integrated use of multiple cultural practices to manage Phytophthora crown and root rot of summer squash. The objective of this field study was to evaluate the effects of bed height, mulches, dried poultry litter, and cultivars on Phytophthora crown and root rot of summer squash in the absence of fungicides. Thirty-two summer squash cultivars and ten germplasm accessions were also evaluated for their reaction to Phytophthora crown and root rot in a separate greenhouse study.

MATERIALS AND METHODS

Inoculum preparation. *P. capsici* isolate 12889 (mating type A1) obtained from the culture collection of M. K. Hausbeck at Michigan State University was used to inoculate plants in this study. *P. capsici* isolate 12889 was originally collected from bell pepper, and is insensitive to mefenoxam. Cultures were grown on unclarified V8 juice agar (UCV8) at room

temperature ($21 \pm 2^\circ\text{C}$) under constant fluorescent light. *P. capsici*-infested millet seed was produced as described by Quesada-Ocampo et al. (22).

Field evaluation. Two separate field experiments were conducted at the Michigan State University Southwest Michigan Research and Extension Center in Benton Harbor. The previous crop was yellow straightneck summer squash (*C. pepo* L.). The soil type was Spinks loamy fine sand (sandy, mixed, mesic Lamellic Hapludalfs). The experimental design was a split-split-split plot arrangement of a randomized complete block with four replicates. Bed types (flat or raised) were main plots. Beds were spaced 1.7 m apart. Mulches (none, ca. 8 cm of wheat straw, or black plastic) were applied to subplots. Dried poultry litter (Nature's Supreme Poultry Fertilizer Crumbles; Herbruck Poultry Ranch Inc., Saranac, MI) was applied at a rate of 0 or 4.5 t ha^{-1} to sub-subplots. Poultry litter was incorporated to a soil depth of 5 to 8 cm prior to mulch application. Summer squash cultivars 'Cougar' (Harris Moran Seed Co., Modesto, CA) and 'Payroll' (Rogers Brand Vegetable Seeds, Syngenta Seeds, Inc., Boise, ID) were grown in sub-sub-subplots. Seeds were planted 0.46 m apart within beds. An experimental unit was one 4.6 m-long bed with ten plants. Planting dates were 3 June 2011 (trial 1) and 13 June 2011 (trial 2). Plants were inoculated at the first true-leaf stage with one gram of *P. capsici*-infested millet seeds. Infested millet seeds were placed in a 3-cm-deep hole near the crown of each plant and covered with soil. Plants were irrigated twice weekly for 6 to 7 hours to promote disease development. Disease incidence, defined as the percentage of wilting and dead plants in each plot, was measured at 7-day intervals from 0 to 35 days post inoculation (dpi). Area under the disease progress curve (AUDPC) values for disease incidence were calculated according to the formula presented by Shaner and Finney (28). Squash fruits were harvested from healthy-appearing plants in each plot every 3 or 4 days over a 2-week period and weighed.

Greenhouse evaluation of cultivars and germplasm accessions. Thirty-two summer squash cultivars and ten germplasm accessions were evaluated for their susceptibility to *Phytophthora* crown and root rot in replicated greenhouse trials. Accessions were obtained from the USDA-ARS Plant Genetic Resources Conservation Unit in Griffin, GA and the USDA-ARS North Central Regional Plant Introduction Station in Ames, IA. Accessions were selected based on previous studies of *Phytophthora* crown rot resistance (7,21). Trials were conducted in May and June 2011 at the Michigan State University Horticulture Research and Teaching Center in Holt. The experimental design was completely randomized with eight replicates. An experimental unit was one plant grown in a 10-cm-diameter pot containing soilless potting mix (BACCTO High Porosity Professional Potting Mix; Michigan Peat Co., Houston, TX). Plants were inoculated with one gram of *P. capsici*-infested millet seeds as previously described for field trials. Disease severity was assessed at 18 dpi. Each plant was visually assessed using a 1 to 5 scale, where 1 = no symptoms; 2 = lower leaves wilted with slight constriction of the stem; 3 = all leaves wilted with constriction and slight discoloration of the stem; 4 = all leaves wilted and crown rotted with visible sporulation on the stem surface; and 5 = plant dead.

Pathogen confirmation. Approximately 5% of symptomatic plants in field and greenhouse trials were sampled for pathogen confirmation. Symptomatic plants were arbitrarily selected, and sheared at the soil line with hand pruners. Crown sections were surface disinfested with a 70% ethanol solution, and blotted dry with paper toweling. Four pieces of tissue were excised from each crown and plated on UCV8 amended with 25 ppm of benomyl, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene (BARP). Colonies were identified as *P. capsici* using morphological characteristics and a key developed by

Waterhouse (31). Mating type and sensitivity to mefenoxam was also determined for each isolate and compared to the phenotype of isolate 12889.

Statistical analysis. All analyses were done using SAS, version 9.2 (SAS Institute, Cary, NC). For field trials, final incidence of plant death 35 dpi and AUDPC were analyzed separately by analysis of variance (ANOVA) in the Proc Mixed procedure. Trials and blocks were considered random variables. Bed height, mulch type, poultry litter rates, and cultivars were considered fixed variables. For greenhouse trials, final crown rot severity ratings 18 dpi were analyzed by ANOVA using the Proc Mixed procedure. Cultivars were considered fixed variables. All effects were declared significant at $P \leq 0.01$ unless otherwise stated. Slice statements were used to test simple main effects when two-way interactions were significant. Residuals were tested for normality using the Shapiro-Wilk statistic in the Proc Univariate procedure. Residuals were plotted against predicted values using the Proc Gplot procedure to assess homoscedasticity.

RESULTS

Field evaluation. Plants were highly susceptible to *P. capsici* at all growth stages from expansion of the first true-leaf stage to full maturity. Plants with Phytophthora crown and root rot rapidly wilted and died (Figure 3.1A). Crown rot symptoms were also observed in the absence of root rot. The primary growing point of plants with crown rot only was killed, but lower leaves remained green and turgid (Figure 3.1B). All plants with Phytophthora crown and root rot symptoms eventually died. *Phytophthora capsici* with the same phenotype as isolate 12889 was consistently isolated from symptomatic plants.

Figure 3.1. Disease symptoms on summer squash plants inoculated with *Phytophthora capsici*:

A, typical wilting preceding plant death and **B**, death of the primary growing point (enlarged in the inset) while lower leaves remain unaffected.



No combination of cultural practices adequately controlled *Phytophthora* crown and root rot. Incidence of plant death approached 100% in all plots (Figure 3.2). Plant death 35 dpi and AUDPC differed significantly ($P<0.0001$) among cultivars (Table 3.1). Disease was not affected by bed height, mulches, or poultry litter applications (Table 3.1). Plant death 35 dpi was 87% for ‘Payroll’ and 99% for ‘Cougar’ averaged across all other treatments (Table 3.2). The bed x cultivar interaction term also was significant ($P=0.0018$) in the ANOVAs for plant death and AUDPC (Table 3.1). Complete death of ‘Cougar’ occurred regardless of bed height (Table 3.2). Plant death of ‘Payroll’ was greater in flat beds than raised beds (Table 3.2). Yield data were not analyzed because almost all plants in both trials died before bearing fruit.

Greenhouse evaluation of cultivars and germplasm accessions. Disease symptoms began to develop by 7 dpi with *P. capsici* isolate 12889. Disease severity ratings were correlated ($r = 0.73$, $P<0.0001$) among trials, and data were pooled prior to analysis. Crown rot severity differed significantly ($P<0.0001$) among squash cultivars and germplasm accessions. None of the cultivars were completely resistant to *Phytophthora* crown and root rot. Crown rot was most severe on the cultivar ‘Cougar’ (mean rating = 4.9), which is known to be highly susceptible to *P. capsici* (Table 3.3). Crown rot was least severe on the cultivar ‘Spineless Beauty’ (mean rating = 2.9) (Table 3.3). Mean crown rot severity was 2.2 on germplasm accessions of *C. pepo* and *C. moschata* (Table 3.4). Crown rot severity ratings ranged from 2.9 to 3.8 among *C. pepo* accessions (Table 3.4). Most plants of *C. pepo* accessions had necrotic stem lesions, but leaves generally remained unwilted. No disease symptoms (rating = 1.0) developed on four *C. moschata* accessions previously reported to be resistant to *Phytophthora* crown rot (Table 3.4). *Phytophthora capsici* was not isolated from the crown sections of *C. moschata* plants.

Figure 3.2. Plant death of summer squash cultivars ‘Cougar’ and ‘Payroll’ following inoculation with *Phytophthora capsici*. Plants were grown in flat or raised beds covered with no mulch (Bare), ca. 8 cm of wheat straw (Straw), or black plastic (Plastic). Dried poultry litter (Nature’s Supreme Poultry Fertilizer Crumbles; Herbruck Poultry Ranch Inc., Saranac, MI) was applied a rate of 0 or 4.5 t ha⁻¹ prior to mulch application.

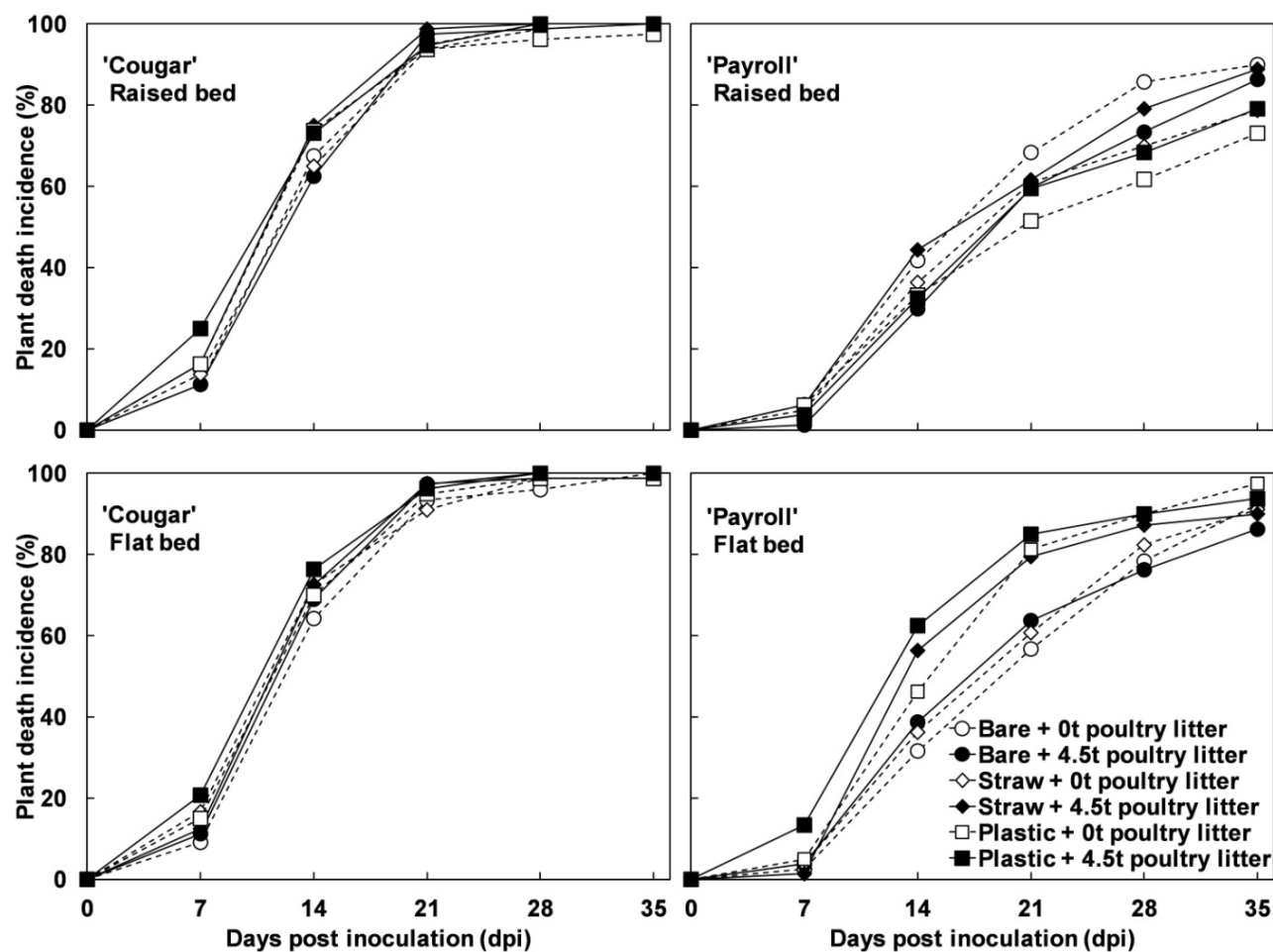


Table 3.1. Mean squares for treatment sources of variation from analyses of variance of plant death incidence 35 days post inoculation (dpi) and area under the disease progress curve (AUDPC) in field trails evaluating the effects of bed height, mulches, dried poultry litter, and cultivars on *Phytophthora* crown and root rot of summer squash

Source of variation	df	Mean square ^a	
		Plant death (%) 35 dpi	AUDPC ^b
Bed ^c	1	954	706,645
Mulch ^d	2	59	201,433
Mulch x bed	2	396	470,689
Litter ^e	1	9	310,498
Litter x bed	1	204	109,491
Litter x mulch	2	101	155,682
Litter x mulch x bed	2	25	64,856
Cultivar ^f	1	7,154**	21,404,059**
Cultivar x bed	1	1,045*	786,944*
Cultivar x mulch	2	15	2,320
Cultivar x mulch x bed	2	320	439,519
Cultivar x litter	1	2	3,614
Cultivar x litter x bed	1	165	92,796
Cultivar x litter x mulch	2	80	89,620
Cultivar x litter x mulch x bed	2	20	7,500
Coefficient of variation (%)	...	13	27

^a Corresponding mean square is statistically significant at $P \leq 0.01$ (*) or $P \leq 0.0001$ (**).

^b AUDPC was calculated for plant death incidence assessed at 7-day intervals from 0 to 35 dpi.

^c Bed height (flat or raised) was the main plot treatment.

^d Mulches (none, wheat straw, or black plastic) were the subplot treatment.

^e Dried poultry litter (Nature's Supreme Poultry Fertilizer Crumbles; Herbruck Poultry Ranch Inc., Saranac, MI) application was the sub-subplot treatment.

^f Squash cultivar ('Cougar' or 'Payroll') was the sub-sub-subplot treatment.

Table 3.2. Effects of bed height and cultivar on incidence of plant death 35 days post inoculation (dpi) with *Phytophthora capsici* and area under the disease progress curve (AUDPC) averaged over mulches and poultry litter applications

Bed height^y	Plant death (%) 35 dpi		AUDPC^x	
	‘Cougar’	‘Payroll’	‘Cougar’	‘Payroll’
Flat	99 a ^z	92 a	2299 a	1760 a
Raised	100 a	83 b	2306 a	1510 b
Mean	87	99	2303	1635

^x Beds were flat or raised 15 cm above the existing soil line.

^y AUDPC was calculated for plant death incidence assessed at 7-day intervals from 0 to 35 dpi.

^z For a given bed height by cultivar combination, values with a common letter do not differ significantly at $P \leq 0.01$ based on differences of least squares means.

Table 3.3. Crown rot severity ratings for summer squash cultivars inoculated with *Phytophthora capsici* in greenhouse trials

Cultivar	Fruit type^a	Fruit color^b	Seed source^c	Crown rot severity 18 dpi^d
Cougar	stra	Y	HM	4.9
Spineless Perfection	zuc	G	Rog	4.9
Golden Rod	zuc	Y	HM	4.9
Payroll	zuc	G	Rog	4.8
Golden Dawn III	zuc	Y	Rog	4.8
Zucchini Elite	zuc	G	HM	4.7
Fortune	stra	Y	Rog	4.6
Multipik	stra	Y	HM	4.6
Sunray	stra	Y	Sem	4.6
Superpik	stra	Y	HM	4.6
Felix	zuc	G	HM	4.6
Noche	zuc	G	Rog	4.6
Golden Glory	zuc	Y	Rog	4.6
Golden Delight	zuc	Y	Rog	4.6
Cheetah	stra	Y	Har	4.5
Paycheck	zuc	G	Rog	4.5
Supersett	croo	Y	HM	4.4
Radiant	zuc	G	Sem	4.4
Tigress	zuc	G	HM	4.4
Zucchini Select	zuc	G	HM	4.4
Senator	zuc	G	Sem	4.4
Jaguar	zuc	G	Har	4.3
Ishtar	mar	G	Sto	4.3
Lioness	stra	Y	HM	4.2
Bobcat	zuc	G	HM	4.2
Leopard	zuc	G	HM	4.2
Gold Rush	zuc	Y	Sem	4.0
Reward	zuc	G	HM	3.6
Cashflow	zuc	G	Rog	3.2
Black Beauty	zuc	G	Sto	3.1
Clarita	mar	G	Sto	3.1
Spineless Beauty	zuc	G	Rog	2.9
Mean	4.3
Standard deviation	0.53
FLSD ^e $\alpha = 0.05$	0.99

Table 3.3 (cont'd).

^a Squash type, where stra = straightneck; zuc = zucchini; croo = crookneck; mar = vegetable marrow.

^b Fruit color, where Y = yellow; G = green.

^c Rog = Rogers Brand Vegetable Seeds, Syngenta Seeds Inc., Boise, ID; HM = Harris Moran Seed Co., Modesto, CA; Sem = Seminis Vegetable Seeds, Inc., St. Louis, MO; Har = Harris Seeds, Rochester, NY; Sto = Stokes Seed Co., Thorold, ON, Canada.

^d Crown rot severity was rated 18 days post inoculation (dpi) on a 1 to 5 scale, where 1 = no symptoms; 2 = lower leaves wilted with slight constriction of the stem; 3 = all leaves wilted with constriction and slight discoloration of the stem; 4 = all leaves wilted and crown rotted with visible sporulation on the stem surface; and 5 = plant dead. Values are the mean of two trials.

^e FLSD = Fisher's protected least significant difference.

Table 3.4. Crown rot severity ratings for *Cucurbita pepo* and *Cucurbita moschata* accessions inoculated with *Phytophthora capsici* in greenhouse trials

Species and accession	Origin	Crown rot severity 18 dpi ^a
<i>Cucurbita pepo</i>		
PI 209783	Germany	3.8
PI 512709	Spain	3.7
PI 615132	Mexico	3.2
PI 169417	Turkey	3.0
PI 181761	Lebanon	2.1
PI 615142	Kazakhstan	1.9
<i>Cucurbita moschata</i>		
PI 442262	Mexico	1.0
PI 458740	Paraguay	1.0
PI 442266	Mexico	1.0
PI 634693	India	1.0
Mean	...	2.2
Standard deviation	...	1.2
FLSD ^b $\alpha = 0.05$...	1.1

^a Crown rot severity was rated 18 days post inoculation on a 1 to 5 scale, where 1 = no

symptoms; 2 = lower leaves wilted with slight constriction of the stem; 3 = all leaves wilted with constriction and slight discoloration of the stem; 4 = all leaves wilted and crown rotted with visible sporulation on the stem surface; and 5 = plant dead. Rating is the mean of two trials.

^b FLSD = Fisher's protected least significant difference.

DISCUSSION

No combination of cultural practices adequately controlled *Phytophthora* crown and root rot in this study. Differences in cultivar susceptibility to *P. capsici* accounted for the most variation in disease levels in the field. In a similar study, red raspberry cultivars had the greatest effect on *Phytophthora* root rot in field trials evaluating multiple disease management practices (32). Results from this experiment demonstrate the importance of breeding summer squash cultivars with resistance to *Phytophthora* crown and root rot, and the difficulty of managing this disease without fungicides. Cucurbit growers could integrate the use of resistant cultivars and fungicide applications to improve control of *Phytophthora* crown and root rot, which has been effective for managing this disease on pepper (10,12). Although raised bed and mulch treatments did not affect disease development in this study, growers should continue to use these practices in fields naturally infested with *P. capsici* because they improve soil drainage (11,12,25), reduce splash dispersal of soilborne inoculum (26), and improve yield (5).

Resistance to *P. capsici* is necessary for the successful long-term management of *Phytophthora* crown and root rot of summer squash. Potential sources of resistance to *P. capsici* have recently been identified in certain *Cucurbita* germplasm accessions (7,21). Stem lesions developed following inoculation with *P. capsici* on the six *C. pepo* accessions evaluated in this study. However, plants did not exhibit characteristic wilting symptoms. No symptoms developed following inoculation with *P. capsici* on four *C. moschata* (PI 442262, PI 458740, PI 442266, and PI 634693) accessions, which appear to possess high levels of *Phytophthora* crown rot resistance. Chavez et al. (7) previously reported these accessions as potential sources of resistance to *Phytophthora* crown rot following inoculation with *P. capsici* isolates from Florida. Disease reactions can differ following inoculation with *P. capsici* isolated from different hosts

due to physiological specialization within the species (16,24). Plants in this study were inoculated with a single Michigan isolate of *P. capsici* originally recovered from pepper. The accessions evaluated in this study could have different reactions to other isolates of *P. capsici*. However, *P. capsici* isolate 12889 is highly virulent on multiple plant species, and has been used in previous studies evaluating resistance to *P. capsici* in squash (8), pepper (9), tomato (*Solanum lycopersicon* L.) (23), and Fraser fir (*Abies fraseri* (Pursh) Poir.) (22). Additional studies are necessary to identify additional sources of *P. capsici* resistance in multiple *Cucurbita* species under greenhouse and field conditions.

Commercial summer squash cultivars differed in their reactions to Phytophthora crown and root rot. The cultivars ‘Cougar’ (yellow straightneck) and ‘Spineless Beauty’ (green zucchini) were the most and least susceptible to *P. capsici*, respectively. Similar results were observed in a field evaluation of summer and winter squash cultivars in New York (6). Although no squash cultivars had complete or monogenic resistance to *P. capsici*, cucurbit growers could use this information to select cultivars that are less susceptible to crown and root rot. Evaluating cultivar reactions to Phytophthora crown and root rot in the greenhouse required less labor and space than field evaluations, which could be a limiting factor when evaluating large, vining cucurbits. Until Phytophthora crown and root rot resistant summer squash cultivars are available, growers should continue to combine fungicide applications and cultural practices to manage this disease.

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CHAPTER IV: CONCLUSION

Phytophthora root, crown, and fruit rot, caused by *Phytophthora capsici* Leonian, are a major constraint to cucurbit production in Michigan, and growers have few options for managing these diseases. The objectives of this research were: (i) to compare soil drench and foliar spray applications of eleven fungicides for managing Phytophthora crown and root rot of summer squash, (ii) to determine the effects of fruit age on susceptibility to Phytophthora fruit rot in processing pumpkin and winter squash fruits, and (iii) to evaluate the integrated use of multiple cultural management practices on Phytophthora crown and root rot of summer squash. Fungicides were more effective at reducing Phytophthora crown and root rot of summer squash when applied as a soil drench than as a foliar spray in field and greenhouse trials. Fluopicolide, mandipropamid, and dimethomorph were the most effective fungicides evaluated. Additional studies are necessary to determine whether these fungicides also effectively control Phytophthora crown and root rot when applied by drip chemigation. Furthermore, the optimal interval between fungicide applications remains to be determined. Application interval could be lengthened beyond 7 days without loss of control if soil drench applications are more effective than foliar sprays. Continued evaluation of soil application methods will be necessary as newer, experimental fungicides are developed by the crop protection industry for managing Phytophthora blight. Susceptibility to Phytophthora fruit rot was affected by fruit age in ‘Dickenson Field’ processing pumpkin but not in ‘Golden Delicious’ winter squash under laboratory conditions. Additional studies are necessary to determine if age-related resistance to Phytophthora fruit rot occurs under field conditions, and whether it is affected by inoculum concentration, incubation period, or wounding. If age-related resistance to Phytophthora fruit rot occurs under field conditions then scheduling fungicide applications according to host growth

stage could be used to improve control of this disease. Differences in susceptibility to *P. capsici* among summer squash cultivars accounted for most of the variation in Phytophthora crown and root rot levels in field trials evaluating multiple cultural management practices. Very few studies have evaluated cucurbit germplasm collections for resistance to *P. capsici*, which is necessary for the long-term management of Phytophthora blight. The location, sequence, and inheritance of *P. capsici* resistance genes in *Cucurbita moschata* accessions reported as resistant to Phytophthora crown rot remains to be determined.