EFFECTS OF HOST RESISTANCE, FUNGICIDES, AND COVER CROPS ON *PHYTOPHTHORA CAPSICI*

By

Charles S. Krasnow

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ABSTRACT

EFFECTS OF HOST RESISTANCE, FUNGICIDES, AND COVER CROPS ON *PHYTOPHTHORA CAPSICI*

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The soilborne oomycete *Phytophthora capsici* causes root, crown, and fruit rot of economically important vegetable crops in the Cucurbitaceae and Solanaceae families. *P. capsici* is a persistent problem due to long-lived oospores in soil and plant debris. The effects of host resistance and fungicides on *P. capsici* and susceptibility of *Brassica* spp. to the pathogen were investigated. Eight commercial pepper cultivars and experimental breeding entries (collectively termed entries) were evaluated for field resistance to *P. capsici* with or without fungicides in 2014 and 2015. The susceptible cultivar, Camelot X3R, had > 90% wilt and plant death in the untreated plot each year. All other entries had < 10% of plants with root rot symptoms in 2014, however, 'Aristotle', 'AP4835', '13SE12671', and 'AP4841' displayed 10 to 30% wilt and plant death in 2015. Using fungicides reduced disease incidence and improved yield compared to the untreated plot but there was no entry x fungicide interaction. Marketable yield for untreated 'Paladin' was significantly higher than other entries in both years. Fruit size for '13SE12671' was largest among entries; significantly larger than 'Camelot X3R', 'AP4839', 'AP4841', and 'Aristotle' in 2014 and 'Camelot X3R', 'AP4839', 'AP4841' and 'Paladin' in 2015 ($P = 0.05$). *Brassica* cover crops are recommended as biofumigants to reduce soil infestation and have not been reported as hosts for *P. capsici*. Ten *Brassica* spp. vegetable and biofumigation cover crops were grown in the greenhouse in *P. capsici* infested potting medium. Disease incidence, severity, and foliar fresh weight were recorded, and roots of symptomatic plants were sampled. All *Brassica* spp. tested displayed disease symptoms. 'Bronco' cabbage,

'Pacific Gold' mustard, and 'Groundhog' radish ($P < 0.05$) had significant reductions in fresh weight. *P. capsici* was re-isolated from the roots of all *Brassica* spp. tested.

'Spineless Perfection' zucchini and 'Cougar' straightneck squash considered to be less and more susceptible to root and crown rot, respectively, were investigated for differences in root and crown physical factors; the histology of crown infection by *P. capsici* was also investigated. The pH, titratable acidity, and crude fiber of healthy root and crown tissue were not significantly different between cultivars ($P > 0.05$). However, dry matter (%) was highest for 'Cougar' ($P = 0.05$). Whole mounts and histological sections of healthy and infected crown tissue revealed that vascular bundles and metaxylem vessels were most abundant in crowns of 'Spineless Perfection'. Twelve to 48 hours post inoculation (hpi), mycelia in the crown of each cultivar was limited to the cortex and hypodermal tissue. By 72 hpi, hyphae were observed in the cortex and endodermal tissue of 'Cougar' and were concentrated in the phloem and parenchyma cells surrounding vascular bundles. Mycelia were limited to the outer cortex in 'Spineless Perfection'. Tyloses, mycelia, and occluding material were present in the majority of metaxylem vessels of 'Cougar' but not 'Spineless Perfection' at 92 hpi. Dissolution of parenchyma cells surrounding vascular bundles were apparent in 'Cougar'. Additional squash and pumpkin (*Cucurbita pepo*) cultivars were evaluated in greenhouse studies for resistance to root and crown rot. Straightneck, crookneck, scallop, and acorn squash cultivars (*C. pepo* ssp. *ovifera*) were significantly more susceptible ($P < 0.0001$) to root and crown rot than zucchini, marrow, and pumpkin (*C. pepo* ssp. *pepo*).

To my parents

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INTRODUCTION

Phytophthora capsici Leonian is a soil-borne oomycete that causes significant losses to vegetable crops worldwide (Erwin and Ribeiro 1996, Hausbeck and Lamour 2004, Hwang and Kim 1990, Sholberg et al. 2007, Tamietti and Valentino 2001). The pathogen was first reported in 1922 as the causal agent of chile pepper blight in New Mexico (Leonian 1922). During the following two decades numerous other hosts, including melon, cucumber, tomato, and squash, were reported as susceptible to the pathogen (Kreutzer 1937, Kreutzer et al. 1940, Tompkins and Tucker 1937, Wiant 1939, Wiant and Tucker 1940). *P. capsici* has since been reported to infect over 50 plant species in 15 families (Erwin and Ribeiro 1996, Sholberg et al. 2007, Tian and Babadoost 2004). In the early 2000s, *P. capsici* was observed causing disease on lima bean (Davidson et al. 2002) and snap bean (Gevens and Hausbeck 2005) for the first time. Leguminous crops have traditionally been used in rotational programs in vegetable production and were considered to be non-hosts of the pathogen (Hausbeck and Lamour 2004). *P. capsici* was also found to infect Fraser fir (Quesada-Ocampo et al. 2009) and soybean foliage (Gevens and Hausbeck 2005) under controlled conditions. The wide host range of *P. capsici* and ability to cause significant losses on numerous commercially grown vegetable crops stresses the importance of understanding this pathogens biology to improve disease management.

PATHOGEN BIOLOGY

Phytophthora capsici is an oomycete organism in the Kingdom Stramenopila. Unlike fungi, oomycetes contain primarily cellulose in their cell walls, require exogenous sterols to sporulate, produce oospores and bi-flagellate zoospores, and have a diploid life cycle (Erwin and Ribeiro 1996). Oomycetes are closely related to heterokont algae (Rossman 2006).

P. capsici is heterothallic and requires an A1 and A2 mating type to produce oospores (Lamour and Hausbeck 2000, Leonian 1922, Satour and Butler 1968). Composed of an ooplast housed within a thick oogonial wall, oospores can remain viable in soil for more than ten years and are resistant to many adverse environmental conditions (Erwin and Ribeiro 1996). Oospores require an indeterminate time period after formation prior to germination (Hord and Ristaino 1991) and are stimulated to germinate by root exudates and certain chemical compounds when environmental conditions are favorable (Erwin and Ribeiro 1996). The oospore germinates by the formation of a germtube that develops into a sporangia or hyphae (Hord and Ristaino 1991).

P. capsici hyphae are coenocytic and grow optimally between 24-33°C (Babadoost 2004, Islam et al. 2005). When environmental conditions are favorable, the hyphae differentiate into sporangia that are borne on long caducous pedicels (Erwin and Ribeiro 1996). Sporangia are usually papillate, ellipsoid or pyriform, depending on light, nutrients, and other environmental conditions (Erwin and Ribeiro 1996). Once sporangia are mature, they are disseminated readily in water, on infected plant material, or by soil movement (Schlub 1983). Wind is not a factor in dispersal of *P. capsici* (Cafe and Duniway 1995, Granke et al. 2009). When sporangia come in contact with plant tissue or free-water they can germinate directly with a germtube or indirectly with 20-40 motile reniform zoospores (Erwin and Ribeiro 1996, Satour and Butler 1968). Zoospores differentiate from the sporangial cytoplasm (Hardham 2001), and are released through an aperture formed at the papillum (Blackwell and Waterhouse 1931). Indirect germination occurs in a wide range of temperatures (Neher and Duniway 1992) and zoospores can remain motile for up to several days in free water (Bimpong and Clerk 1970, Hickman 1970).

Zoospores are not only actively motile, but can target roots using electro-taxis (van West et al. 2002) and respond chemotactically to amino acids, sugars, and other simple molecules

(Erwin et al. 1983). Calcium is required for zoospore encystment, adhesion, and germination (Donaldson and Deacon 1992). Immediately after coming in contact with a suitable surface, such as a plant root, zoospores dock, encyst, and adhere to the surface with the aid of glycoproteins secreted from vesicles on the peripheral membrane of the zoospore (Donaldson and Deacon 1992, Hardham and Gubler 1990). The zoospore orients itself with the ventral groove in direct contact with the surface or root and a germtube emerges through the ventral groove during germination (Mitchell and Deacon 1986).

P. capsici may penetrate susceptible hosts directly or through natural openings such as stomata (Hausbeck and Lamour 2004). *P. capsici* produces non-pectolytic enzymes that are active during the infection of plants (Yoshikawa et al. 1977), and cause a general breakdown of host tissue (Lamour et al. 2012). During moist and humid environmental conditions sporangia are produced on infected plant tissue (Hausbeck and Lamour 2004). A single infected cucurbit fruit can support anywhere from half a million (Granke et al. 2009) to an estimated three billion (Lamour et al. 2012) sporangia. Sporangia release zoospores when environmental conditions are favorable that readily infect susceptible plants during saturated field conditions (Ristaino and Johnston 1999). The polycyclic production of sporangia and zoospores is considered responsible for the pathogens epidemic potential (Ristaino 1991). *P. capsici* zoospores are able to cause disease at a wide range of temperatures (Granke and Hausbeck 2010) and incited epiphytotics in pepper fields (Cafe and Duniway 1995).

Phytophthora capsici causes severe symptoms on susceptible host plants and can attack crops at any stage of growth (Hausbeck and Lamour 2004, Islam et al. 2002). The pathogen can cause root and crown rot, foliar blight, seedling damping-off, and fruit rot on susceptible cucurbit and solanaceous crops. Symptoms may vary based on tissue type and host maturity, but have

general similarities among hosts. Plants infected at the crown or lower stem display water soaked, brown lesions and constriction at or near the soil-line (Aguirreolea et al. 1995). Cortical parenchyma tissue and epidermal cells of pepper stems were completely degraded after infection by *P. capsici* (Kim and Hwang 1989). Wilt is a commonly observed symptom on cucurbits and pepper infected by *P. capsici*. Wilt can develop on cucurbits after infection by pathogens that occlude or rupture vascular tissue (Main and Walker 1971, Martyn and McLaughlin 1983). In a susceptible pepper cultivar infected by *P. capsici* xylem vessels were occluded (Kim and Kim 2009). Disruption of xylem tissue is known to increase resistance to water flow and cause wilt of tobacco infected by *P. nicotianae* (Powers 1954) and similar disruption may cause wilt in plants infected with *P. capsici*.

Fruit rot of cucurbitaceous hosts can progress rapidly, resulting in complete breakdown of mature fruit in a few days (Babadoost and Zitter 2009, Meyer and Hausbeck 2013). Initial symptoms of fruit rot on cucurbit and pepper appear as circular watersoaked lesions that are sunken into the fruit surface. Advanced lesions support characteristic pathogen sporulation that has the appearance of powdered sugar (Islam et al. 2002). Eventually, the fruit collapses and desiccates (Hwang and Kim 1995). Infected host tissue can also support the production of oospores when both mating types are present, which are transferred to the soil with plant debris (Lamour and Hausbeck 2000). Knowledge of the symptoms characteristic of Phytophthora blight are important for proper identification and disease management.

The population structure of *P. capsici* has been studied as a means to understand the pathogen's virulence and genetic variation (Quesada-Ocampo et al. 2011). In the United States, *P. capsici* populations are outcrossing and genetic diversity appears to be driven by sexual reproduction and the production of oospores (Granke et al. 2012, Jackson et al. 2010). In

Michigan, field populations are genetically isolated and not disseminated field to field (Lamour and Hausbeck 2001), suggesting limited gene flow. The frequent occurrence of both mating types in vegetable fields in the United States (Hausbeck and Lamour 2004, Ristaino and Johnston 1999), and adaptability of *P. capsici* populations (Granke et al. 2012), heightens the importance of disease management strategies that limit the introduction of new isolates into fields (Lamour and Hausbeck 2000). Increases in virulence of progeny from *P. capsici* oospores has been observed *in vitro* (Satour and Butler 1968) and likely occurs in the field (Ristaino 1990). In other vegetable production regions, clonal reproduction appears to drive the population structure of *P. capsici* (Lamour et al. 2012). The year-round cropping in Argentina and Peru may have eliminated the need for sexual reproduction and oospores (Gobena et al. 2012, Lamour et al. 2012).

Isolates can differ in virulence and phenotype based on the geographic location or hosts from which the isolate was collected (Granke et al. 2011, Kim and Hwang 1992). *P. capsici* isolates collected from vegetable hosts were more virulent on tomato and zucchini than isolates collected from hosts grown exclusively in tropical regions, such as macadamia (*Macadamia integrifolia*) and black pepper (*Piper nigrum*) (Granke et al. 2012). When screening crops for resistance, selecting *P. capsici* isolates from diverse hosts and geographic locations can enhance the selection process, as was recommended with cucurbits (Quesada-Ocampo et al. 2011).

Contaminated irrigation water has been indicated as a significant contributor to the increase in *P. capsici* infested fields over the past 20 years in Michigan (Granke et al. 2012). The bi-flagellate zoospores passively or actively disperse in water and can spread *Phytophthora* throughout fields or growing regions (Gevens et al. 2007, Stanghellini et al. 1996). Growers in Michigan and other vegetable-growing states usually use surface water to irrigate their vegetable

crops (Granke and Hausbeck 2010, Roberts et al. 2005, Shokes and McCarter 1979). Growers in Michigan try to provide 2.5 cm of water a week to their crops during the summer months and *P. capsici* recovery was found to be at the highest in surface water sources during late July and August when growers irrigate their crops frequently (Gevens et al. 2007). *P. capsici* zoospores can remain infective in water for five days (Granke and Hausbeck 2010) making infested water a potential threat during the season and heightening the importance of selecting uninfested irrigation water sources. The use of well water has been recommended as a means to prevent the introduction of *P. capsici* isolates into fields as *P. capsici* was not detected in well water (Gevens et al. 2007). *P. capsici* did not appear to overwinter in Michigan surface-water sources, suggesting that oospores or propagule movement from upstream initiated disease at the start of each season (Gevens et al. 2007). Disinfesting surface water used for irrigation has been recommended (Granke et al. 2012). Even in fields previously infested with *Phytophthora*, well water should be used as introduced isolates may be more virulent than the field population (Gevens et al. 2007, Granke et al. 2012).

FUNGICIDES AND CULTURAL MANAGEMENT

Fungicides are a key component of a *P. capsici* management program (Hausbeck and Lamour 2004), although, there are limited fungicide options for oomycete control (Bird et al. 2014). The dissimilarities between oomycetes and true fungi create challenges for agricultural producers managing *P. capsici*, as many traditional fungicides are not effective towards the pathogen (Ristaino and Johnston 1999). Two active ingredients, metalaxyl and its isomeric enantiomer mefenoxam, have been widely used to control *P. capsici*. Released in 1977, metalaxyl was one of the premier oomycete fungicides (Cohen and Coffey 1986). Metalaxyl belongs to the phenylamide (PAF) class of fungicides and inhibits incorporation of uridine into

RNA in sensitive oomycetes (Davidse et al. 1991). The xylem mobility of the fungicide (Erwin and Ribeiro 1996, Jeffers 2003) allows for multiple application methods such as soil drenches and seed treatments (Babadoost and Islam 2003). However, due to the heavy usage of metalaxyl and mefenoxam, field populations of *P. capsici* resistant to the PAF fungicides have developed (Davidse et al. 1991, Hausbeck and Lamour 2004, Lamour and Hausbeck 2000, Ploetz et al. 2002, Ristaino 1990). Mefenoxam resistance is conferred by a single incompletely dominant gene (Lamour and Hausbeck 2000). Bruin and Edgington (1981) found that cross resistance was exhibited in metalaxyl-resistant *P. capsici* isolates to fungicides with similar modes of action. Resistance to newer fungicides such as cyazofamid have also been reported (Jackson et al. 2012). *Phytophthora capsici* isolates resistant to fluopicolide were generated using genetic mutation techniques (Lu et al. 2011), suggesting that field resistance is possible if resistance management recommendations are not followed (Chabane et al. 1993). Additional fungicides that target oomycetes, such as dimethomorph, mandipropamid, and zoxamide, (Erwin and Ribeiro 1996, Granke et al. 2012, Islam et al. 2005) are important in a disease management programs. Spraying metalaxyl directly at the lower stems of peppers gave effective control of *P. capsici* (Simons et al. 1990) and soil- drenches of oomycete fungicides limited Phytophthora blight of squash (Meyer and Hausbeck 2013) and pepper (Foster and Hausbeck 2010). Fungicides should be used in alternation with fungicides of a different mode of action to delay the onset of resistance in *P. capsici* populations (Staub and Sozzi 1984).

Biological controls and biofumigation have been researched to control *P. capsici* (Ahn and Hwang 1992, Ji et al. 2012). Products formulated with *Bacillus* spp. have shown promise in oomycete control (Jacobsen et al. 2004, Smith et al. 1993). For example, cottony leak of cucumber caused by *Pythium aphanidermatum* was suppressed by a strain of *Bacillus cereus*

(Smith et al. 1993). In the former study, suppression of *P. capsici* in-vitro by *B. cereus* was comparable to *Py. aphanidermatum*. *Muscodor albus*, a fungus with biofumigant properties (Mercier and Manker 2005), was able to reduce disease caused by *P. capsici* to commercially acceptable levels in a tolerant pepper cultivar when added to infested potting soil in a greenhouse study (Camp et al. 2008).

Brassica biofumigation can reduce soil-borne pathogens that are difficult to control in both conventional and organic vegetable production. The ability of *Brassica* spp. to reduce pathogen inoculum density is attributed to glucosinolates, thioglucoside compounds in the vacuoles of many *Brassica* spp. (Marschner, 1995). Mustard and canola biofumigation cover crops reduced Phytophthora blight of squash in greenhouse and field trials when incorporated into infested soil (Ji et al. 2012). However, cucurbit crops had reduced germination when planted immediately following soil-incorporation of flail mowed *Brassicas* (Ackroyd and Ngouajio 2011) and additional testing will be necessary to optimize this management practice. The use of *Brassica* biofumigant soil amendments may prove beneficial in organic production systems where limited fungicide options are available, but may not provide economical control as a stand-alone management tool (Ngouajio et al. 2008).

Changes in vegetable markets such as increased consumer demand and merchant size (Dimitri et al. 2003) and advances in agricultural technology, have made the use of raised-bed black plastic culture possible. Raised bed culture is now widely used in the production of fresh market vegetables such as squash, melon, pepper, cucumber, and tomato (Johnson et al. 1979, Meyer and Hausbeck 2012, Nesmith 1993). These high input management tools can reduce water and fungicide use, enable alternative fungicide application methods, decrease weed pressure, improve yields, and reduce soil saturation in the root zone (Hausbeck and Lamour

2004, Johnson et al. 1979, Springer and Johnston 1982). Raised beds and black plastic have also improved control of *P. capsici* by reducing soil saturation and limiting standing water in the root zone (Ristaino and Johnston 1999). Ristaino (1991) found that location of the drip emitter and frequency of irrigation affected *Phytophthora* blight of pepper. Disease was more severe on the side of the plants closest to the drip emitter. For growers who use drip irrigation, this should be the preferred side to apply fungicide drenches (Ristaino et al. 1992). In California pepper fields, placing the drip tape 15 cm deep into the soil reduced *P. capsici* disease incidence, without affecting yield (CafeFilho and Duniway 1996). Production of susceptible vegetables in dry climates, such as the southwestern U.S., makes control of soil moisture more dependent on irrigation practices as opposed to rain events, and presents opportunities to disrupt the *P. capsici* life cycle (Cafe et al. 1995). In growing regions with higher moisture, planting into well drained fields, avoiding poorly drained areas, disking symptomatic plants with a surrounding margin of healthy plants, and cleaning farm equipment between fields can help to limit the spread of *P. capsici* during the growing season and increase the likelihood of producing a successful crop (Babadoost and Zitter 2009, Ristaino and Johnston 1999). Crop rotation has long been recognized as a means to reduce disease pressure from soil-borne plant pathogens (Greaves 1918). The long-term survival of *P. capsici* oospores make this practice ineffective in eliminating *P. capsici* from vegetable fields and growers practicing rotations with non-susceptible hosts longer than 5 years have still experienced crop loss (Hausbeck and Lamour 2004).

Cultural management strategies that improve air flow and growing varieties with upright canopy architecture have been studied as possible additions to an integrated *P. capsici* management program (Ando and Grumet 2006). A cucumber accession which held its fruit off

the ground had significantly less Phytophthora fruit rot than accessions and cultivars which had fruit form along the vine (Ando and Grumet 2006). Trellising cucumbers resulted in significantly lower disease compared with standard spaced rows, possibly due to a reduction in direct fruit contact with the soil and increased airflow. In commercial pickling cucumber production, humid conditions can develop under a closed canopy, increasing the time required for the fruit and soil to dry. The humidity beneath the canopy can promote Phytophthora fruit rot (Ngouajio et al. 2004, Ngouajio et al. 2006), and research has found that row spacing can be increased to improve airflow, without a corresponding loss in yield (Ngouajio et al. 2006). Additionally, closely spaced rows and a closed canopy makes it difficult to apply foliar fungicides to protect the fruit (Hausbeck et al. 2006).

Growing Phytophthora resistant vegetable crops is an important management strategy that may help to decrease fungicide use and increase yields (Hwang and Kim 1995, Ristaino and Johnston 1999). Pepper cultivars with resistance to Phytophthora root rot are available (Wyatt et al. 2013) and have increased in use in production areas where Phytophthora blight is prevalent (Dunn et al. 2014, Foster and Hausbeck 2010). Foster and Hausbeck (2010) identified pepper cultivars and accessions with resistance to virulent *P. capsici* isolates from Michigan. Resistance of stems, leaves and roots to *P. capsici* are considered to be under the action of separate genetic systems (Foster and Hausbeck 2010, Sy and Bosland 2006) and fruit rot of Phytophthora root rot resistant pepper cultivars can occur if infested soil is splashed onto the plant (Foster and Hausbeck 2010, Naegele et al. 2013). Preventing soil splash will remain important when *P. capsici* resistant peppers are grown (Ristaino and Johnston 1999). Phytophthora resistant cucurbit crops are not commercially available (Cafe et al. 1995). Certain squash cultivar-groups, such as zucchini, have field resistance to Phytophthora root rot, and growing these cultivars can

increase the chances of successful production in infested fields (Meyer and Hausbeck 2012). Pickling cucumbers also display partial resistance of stems and roots to *P. capsici*, heightening the importance of fungicide protection of the susceptible fruit. A hard-rind pumpkin cultivar with partial resistance to Phytophthora fruit rot has been identified (McGrath 2007). Incorporating resistance, cultural control strategies, and fungicides remains essential to effectively manage Phytophthora blight.

Developmental resistance to pathogens is a general phenomenon in the plant kingdom (Develey-Riviere and Galiana 2007) and is frequently observed with pythiaceous organisms (Endo and Colt 1974, Jeun and Hwang 1991, Kennelly et al. 2005, Mellano et al. 1970, Meyer and Hausbeck 2013, Swiecki and MacDonald 1988). *Phytophthora megasperma* var. *sojae* was restricted to a small necrotic lesion at the epidermal cell layer of soybean hypocotyls expressing age-related resistance (ARR) (Stossel et al. 1981). Immature soybean plants developed watersoaked lesions and collapsed 24 hours post-inoculation with the same pathogen (Paxton and Chamberlin 1969). Although the phytoalexin gycelolin was associated with resistance of immature soybean plants to incompatible *P. megasperma* var. *sojae* races (Paxton and Chamberlin 1969), glycelolin production correlated more strongly with necrosis than resistance after ARR was expressed in soybean hypocotyls (Lazarovits et al. 1981) and other mechanisms of resistance may be more important. Many crops become resistance to Pythium root rot as the seedlings mature to adult plants and may be the result of cell wall development that precludes mechanical penetration by *Pythium* spp. (Dow and Lumsden 1975, Endo and Colt 1974). Mature watermelon have a thick rind and stone cells in the exocarp that prevented fruit rot caused by *Pythium* spp. (Drechsler 1939).

ARR to *P. capsici* has been observed to varying extents in commercial cucurbit and pepper cultivars (Ando et al. 2009). Pickling cucumbers become resistant to *P. capsici* fruit rot ~2 weeks after anthesis, with resistance coinciding with the completion of the fruits' elongation phase (Gevens et al. 2006). Processing squash fruit have a longer maturation period than cucumber, and were found to acquire resistance to *P. capsici* 21 days post pollination (Meyer and Hausbeck 2013). Cultivated varieties of *Cucumis sativus, C. melo, Citrullus lanatus, Cucurbita pepo* and *C. moschata* all showed an age-related decrease in susceptibility to *P. capsici* (Ando et al. 2009). Fruit were highly susceptible when green and waxy early in development and displayed varying levels of resistance as the fruits matured (Ando et al. 2009). Visible changes in exocarp properties during development, such as waxiness, coincided with changes in resistance (Ando et al. 2009). Additional morphological and biochemical features such as cuticle thickness (Biles et al. 1993), sugar content (Jeun and Hwang 1991), soluble solids and exocarp firmness (Meyer and Hausbeck 2013) have been implicated as factors affecting ARR of *Cucurbita* spp. and peppers to *P. capsici*. Most squash and pumpkins reach full size by 20 to 24 dpp (Loy 2004), and although resistance in certain fruit coincides with this period, maximum size cannot be relied on as an indicator of the onset of ARR (Meyer and Hausbeck 2013). Information on cucurbit species that develop ARR should be considered during cultivar selection, as this can reduce the number and timing of fungicide applications necessary to protect the fruit during the season (Ando et al. 2009). Integration of ARR and fungicide management is exemplified in commercial pickling cucumber production: Fungicides are recommended to be applied to the fruit when 1", 3", and 5", in length, during the cucumbers' susceptible early growth stages (Hausbeck and Lamour 2004).

CONCLUSION

Phytophthora capsici affects numerous crops from diverse host families and can be a significant limiting factor in the production of vegetables worldwide. The difficulties encountered with cultural and fungicide management (Hausbeck and Lamour 2004), and the intractable nature of the pathogen, (Gevens et al. 2007, Hausbeck and Lamour 2004) create numerous challenges for vegetable producers. The objectives of this research were to determine factors responsible for age-related resistance of winter squash to *P. capsici*, evaluate fungicide and cultivar resistance as management options for Phytophthora blight of bell pepper, and investigate factors involved in partial resistance of zucchini (*Cucurbita pepo*) to Phytophthora root and crown rot.

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CHAPTER 1: EVALUATION OF FRUIT ROT RESISTANCE IN CUCURBITA GERMPLASM RESISTANT TO *PHYTOPHTHORA CAPSICI* CROWN ROT

ABSTRACT

Krasnow, C.S., Naegele, R.P., and Hausbeck, M.K. 2014. Evaluation of fruit rot resistance in Cucurbita germplasm resistant to *Phytophthora capsici* crown rot. HortScience 49:285-288.

Phytophthora blight is a destructive disease of cucurbits affecting the fruit, leaves, crown, and/or roots. Ten cucurbit plant introductions with known partial resistance to *Phytophthora capsici* root and crown rot were evaluated for resistance to Phytophthora fruit rot. Unwounded fruit from field grown plants of *Cucurbita moschata* and *C. pepo* were inoculated in a controlled environment at 7-10 or 21-24 days post pollination (dpp) with virulent *P. capsici* isolates to examine the effect of fruit age on disease development. Inoculated fruit were rated for lesion area and pathogen mycelial growth 7 days post inoculation (dpi); fruit length, diameter, and pericarp thickness were also rated. Two *C. pepo* accessions (PI 169417 and PI 181761) had significant resistance to Phytophthora fruit rot at both 7-10 dpp and 21-24 dpp. All accessions evaluated displayed reduced disease susceptibility as the fruit aged.

INTRODUCTION

The oomycete plant pathogen *Phytophthora capsici* Leonian affects the cucurbit industry annually, in some cases causing 90-100% crop loss (Babadoost 2000, Meyer and Hausbeck 2012). Michigan is a leading producer of processing squash, pumpkins, and cucumbers in the United States with more than 68,500 acres of vegetable crops susceptible to *P. capsici* grown annually (Anonymous 2014). In the Midwest and eastern U.S., *P. capsici* commonly causes a fruit rot on cucurbits, and is a limiting factor in production (Babadoost 2004, Hausbeck and Lamour 2004, McGrath 2000, Meyer and Hausbeck 2012). Entire truck loads of processing squash, pumpkins, and cucumbers can be rejected at the processing facility due to the fruit

becoming infected just prior to or during harvest and rotting during transit (Hausbeck and Lamour 2004). The susceptibility of all commonly cultivated cucurbits (Cafe et al. 1995, McGrath 2000), and the rapidity with which epidemics on squash and pumpkin fruit can develop, makes growers vulnerable. Specifically, in Michigan, fruit rot of processing cucurbits can be a major issue (Hausbeck and Lamour 2004, Meyer and Hausbeck 2012), as well as crown and root rots of squash and other vegetables (Hausbeck and Lamour 2004, Meyer and Hausbeck 2012, Quesada-Ocampo and Hausbeck 2010). Young fruit are especially susceptible during the first week following anthesis. Gevens et al. (2006) observed in a detached fruit assay a high susceptibility to *P. capsici* in cucumber fruit within 7 days post pollination (dpp). Other researchers (Ando et al. 2009, Hausbeck and Lamour 2004, Meyer and Hausbeck 2012) noted that diverse cucurbit fruit including squash, pumpkin, melon, and cucumber were most susceptible to *P. capsici* at 3 dpp. Protecting the fruit throughout development is crucial, but is difficult to achieve due to fungicide cost, a dense foliar canopy, and the long maturation time needed by some cucurbit cultivars. Depending on the nature of plant types (i.e. bush or vining) the fruit may lay directly on bare soil (Ando and Grumet 2006), increasing the chances of *P. capsici* infection. Even when cucurbits are grown on black polyethylene plastic, vining cucurbit types will likely develop fruit along the vines that have trailed off of the plastic mulch. Although root and crown rot can be serious issues in certain cucurbit growing systems (Café-Filho and Duniway 1995, Ristaino 1991), fruit infection poses the most serious management challenge (Babadoost and Zitter 2009).

Phytophthora capsici can survive in soil for five years or more via oospores in the absence of a susceptible host (Hausbeck and Lamour 2004). This feature, along with the ability to produce large numbers of sporangia and zoospores in wet field conditions, is responsible for

the pathogens high infection potential. Control of *P. capsici* is not always sufficient with the use of traditional fungicide and cultural management practices. Growers of processing cucurbits (i.e. hard squash and pie pumpkins) are limited in cultural management options because the fruit are harvested mechanically and there is a relatively low profit margin. Therefore, raised plant beds, plastic mulch, and drip irrigation are not routinely used by growers of cucurbits for processing, even though they are used with limited success by growers of cucurbits for the fresh market (Jackson et al. 2010). Fungicide use can be a limiting factor economically during seasons with high disease pressure due to the long maturity period of hard squash and pumpkins. In addition, the vining nature of the plants quickly cover the fields' surface making any sprays applied with a ground rig after the vines have filled in the rows difficult to achieve without damaging the crop. Due to the difficulties encountered in managing *P. capsici* on processing cucurbits, alternative control methods have been sought.

Ontogenetic resistance (age-related resistance) is the ability of plants or plant organs to more aptly defend themselves against biotic and abiotic factors as they mature (Ficke et al. 2002). Cucurbit fruit develop resistance as they mature, and that resistance coincides with the completion of the fruit elongation phase, approximately 1-3 weeks after anthesis, depending on species (Ando et al. 2009, Gevens et al. 2006). This form of resistance to pathogen infection may provide an opportunity to improve fungicide application timing (Ando et al. 2009, Gadoury et al. 2003, Kim et al. 1989, Roberts 2000). For example, age-related resistance in commercial pickling cucumbers has facilitated optimal timing of fungicide application during the crop's susceptible early growth stages. Fungicides are recommended to be applied when fruit are 1", 3", and 5", in length (Hausbeck and Lamour 2004).

Identifying cucurbit accessions with Phytophthora fruit rot resistance to *P. capsici* would be a useful addition to cucurbit breeding programs attempting to integrate resistance into commercial varieties. Sources of root rot resistance have been identified and studied in wild cucurbit relatives (Chavez et al. 2011, Padley 2008), and observations in the field indicate that commercial cucumber cultivars have tolerance to *P. capsici* root infection (Hausbeck and Lamour 2004). However, fruit rot resistance has yet to be identified. Although fungicide and cultural management options have expanded, host resistance is especially desirable for low input systems. The objectives of this study were i) to identify resistance to fruit rot in wild germplasm previously identified as having crown/root rot resistance, ii) to test ontogenetic fruit resistance to two *P.capsici* isolates 7-10 dpp and 21-24 dpp in 10 *C. moschata* and *C. pepo* accessions, and iii) to determine if resistance correlates with changes in fruit size and pericarp thickness.

MATERIALS AND METHODS

Squash accessions were obtained from the United States Department of Agriculture Germplasm Resources Information Network (USDA-GRIN, www.ars-grin.gov). Four accessions were *Cucurbita moschata* and the remaining seven were *Cucurbita pepo,* including the control (Table 1). The accessions were chosen based on previous studies where crown and root rot resistance was demonstrated (Chavez et al. 2011, Meyer and Hausbeck 2012, Padley 2008). The commercial acorn squash cultivar 'Table Ace' (*C. pepo*, Harris Seed Co., Rochester, NY) was used as the control (Enzenbacher and Hausbeck 2012). Seeds of each accession were planted into 72-cell trays containing soilless peat mix (Suremix Michigan Grower Products Inc, Galesburg, MI) and grown for four weeks in a polyethylene greenhouse with a mean temperature of 22°C (±4°C). Up to ten seedlings of each accession were transplanted into the field at the first true leaf stage into Capac loam (fine-loamy, mixed, active, mesic Aquic Glossudalfs) at the

Michigan State University Plant Pathology Research Farm, East Lansing, MI. The field site had no previous history of *P. capsici* infestation and had been previously cropped to pumpkin. Plants were grown in raised beds covered with black polyethylene plastic and irrigated with trickle irrigation. Plant beds were 12.7 cm in height, spaced 91 cm apart, and plants were spaced 61 cm apart within beds. Plants were irrigated and fertilized according to local commercial standards. Once flowers reached anthesis, the female flowers were tagged and hand pollinated. Squash fruit were harvested 7-10 dpp or 21-24 dpp.

Two virulent *P. capsici* isolates were used in this study. Isolates were characterized by compatibility type (CT), mefenoxam sensitivity, and host (Lamour and Hausbeck 2000). Isolate 12889 is an A1 CT, mefenoxam resistant, and was isolated from pepper fruit. Isolate OP97 is also an A1 CT, mefenoxam sensitive, and was isolated from pickling cucumber fruit. The isolates were obtained from the culture collection of Dr. Mary Hausbeck and were passed through pepper fruit prior to the study to ensure virulence. Throughout the study the isolates were maintained on unclarified V8 juice agar (143ml V8 juice, 3g CaCO3, 16g agar, 850ml distilled water). The study was organized in a randomized design with three fruit forming a biological replicate, per isolate, per fruit age. The experiment was conducted three times. Two fruit were used as controls for each isolate by age replication. Due to poor fruit set, PI 458740, PI 442262, and PI 634693 could not be inoculated with isolate 12889.

Harvested fruit were washed in 10% bleach for five minutes, rinsed with sterile distilled water, and allowed to dry under a laminar flow hood. Fruit were measured lengthwise from the peduncle to the blossom end and fruit circumference was measured at the greatest dimension of the fruit. A 1.2 cm core was aseptically removed near the blossom end of each fruit using a

Table 1.1: Accessions and cultivated variety listed by species and country of origin.

Species and Accession	Country of Origin
Cucurbita moschata	
PI 458740	Paraguay
PI 442266	Mexico
PI 442262	Mexico
PI 634693	India
Cucurbita pepo	
PI 169417	Turkey
PI 615142	Kazakhstan
PI 209783	Germany
PI 512709	Spain
PI 615132	Mexico
PI 181761	Lebanon
'Table Ace'	USA

sterile cork borer, and the pericarp thickness was measured (Naegele et al. 2013). A 7 mm plug of actively growing mycelia was placed 12.7 cm from the peduncle of the fruit, and covered with a sterile screw cap (Axygen Inc., Union City, CA) using petroleum jelly as a fixative (Ando et al. 2009). Control cucurbits were inoculated with uncolonized V8 agar plugs. Fruit were then placed in clear plastic bins (Sterilite, Townsend, MA) lined with moist paper towel and covered to maintain high relative humidity. The fruit were incubated at room temperature (22±2°C) under constant fluorescent light. 'Table Ace' squash were also used in a wounded fruit assay, in which fruit were punctured with a sterile pin before mycelial plug inoculation to determine the effect of wounding on disease incidence.

After seven days post inoculation (dpi) the length and width of pathogen growth and watersoaked lesion on each fruit were measured to obtain the total affected area. Pathogen growth was rated based on mycelial growth and percentage of the fruit infected using a 0-4 scale adapted from Meyer (2013), with 0 = no visible pathogen growth, 1 = watersoaking only, 2 = light visible mycelial growth, 3 = moderate mycelial growth, 4 = dense mycelial growth (Meyer

and Hausbeck 2013). After disease assessment, 1-2 mm sections removed from the margin of symptomatic fruit tissue were plated onto BARP (0.05g benomyl, 2ml ampicillin, 2ml rifampicin, and 0.1g PCNB per liter)-amended V8 agar plates. Recovered isolates were confirmed by pathogen morphology (Waterhouse 1963) and mefenoxam sensitivity (Lamour and Hausbeck 2000).

All analyses were completed using SAS v9.3 (SAS Institute, Cary, NC). Data were analyzed using ANOVA. Fisher's least significant difference ($P = 0.05$) was used to measure differences between means. Significant accession by isolate interactions were measured using Proc Glimmix. Correlations between fruit age and pathogen growth rating were made using Pearson's Correlation Coefficient at $P = 0.05$.

RESULTS

Most fruit that were 7-10 dpp when inoculated showed disease symptoms by seven dpi (Fig. 1). Symptoms including watersoaking and external white mycelial growth characteristic of *P. capsici* were usually evident within two days (Fig 2). Accessions with a mean rating value ≤ 1 were considered resistant (R), and accessions with a mean value $1 > x < 2.5$ were considered intermediately resistant (IR). Occasionally, only watersoaking was present, but this occurred with a minority of the fruit. Some of the fruit developed a watersoaked appearance resulting from the permeation of the petroleum jelly. This was more often visible with the light skinned accessions, even at 21-24 dpp. This discoloration was confirmed negative for *P. capsici* using the isolation method described above. Accessions PI 169417 and PI 181761 were significantly resistant ($P = 0.05$) to Phytophthora fruit rot at both 7-10 and 21-24 dpp. These two accessions showed consistent resistance to *P. capsici* 7-10 dpp, and were the only two which performed better than the control, 'Table Ace'(Table2.1). The accession PI 512709 and 'Table Ace' showed

Table 1.2: Mean growth ratings and proportion of fruit infected with *Phytophthora capsici* 7-10 and 21-24 days post-pollination.

Accession	Growth rating[z]	Fruit infected	Lesion size (cm)	Size increase (%)[y]	Pericarp increase (%)[x]
PI 442262	3.8 a[w]	0.82	12.69	398	125.0
PI 458740	3.7 a	0.73	11.06	194	69.2
PI 442266	3.3 ab	0.73	8.58	297	90.0
PI 615132	3.2 ab	0.69	11.21	66	39.2
PI 634693	2.9 ab	0.78	8.86	304	87.1
PI 615142	2.9 ab	0.54	9.64	114	61.7
PI 209783	2.7 abc	0.62	9.05	26	0.0
PI 512709	2.4 bc	0.41	6.22	178	98.9
'Table Ace'	1.6 cd	0.39	3.60	29	8.5
PI 181761	1.0 d	0.09	3.35	60	29.3
PI 169417	0.9 d	0.09	3.07	43	27.2

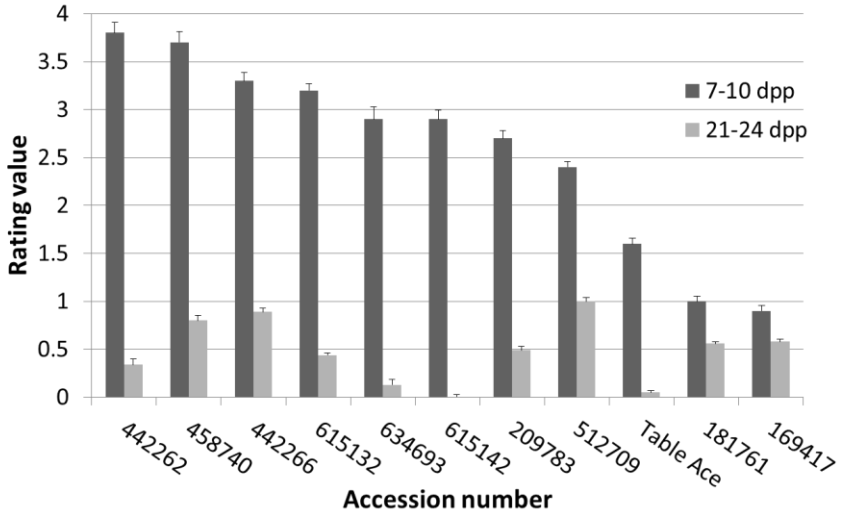
[z] Average growth rating, proportion of accessions infected with visible mycelium, and lesion size (cm), at 7-10 dpp.

[y] Increase (%) in fruit size from 7-10 to 21-24 dpp, calculated from average fruit area (length x diameter) differences between ages.

[x] Increase (%) in pericarp thickness from 7-10 to 21-24 dpp.

[w] Values followed by the same letter are not significantly different based on Fisher's LSD test, $P = 0.05$.

Figure 1.1: Average Phytophthora fruit rot disease ratings for 10 accessions and the control based on assessment of pathogen growth. Average pathogen growth ratings based on 0-4 scale. Error bars represent standard error from the mean. * Indicates significant difference in rating between age-ranges based on Fisher's LSD test ($P = 0.05$).



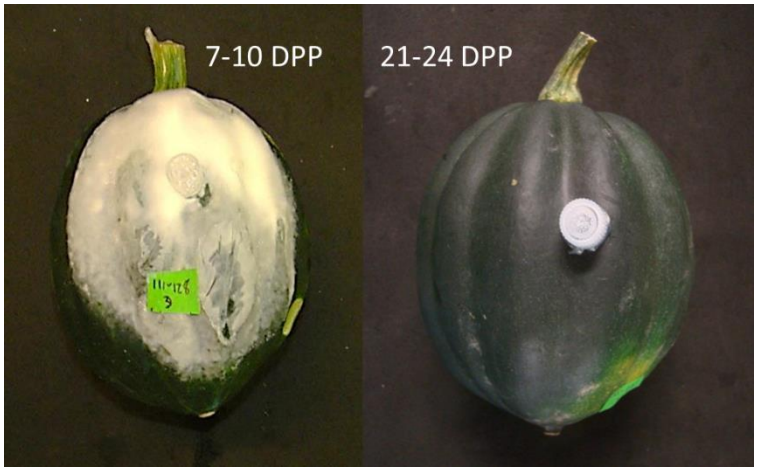
the isolation method described above. Accessions PI 169417 and PI 181761 were significantly resistant ($P = 0.05$) to Phytophthora fruit rot at both 7-10 and 21-24 dpp. These two accessions showed consistent resistance to *P. capsici* 7-10 dpp, and were the only two which performed better than the control, 'Table Ace'. The accession PI 512709 and 'Table Ace' showed intermediate resistance (rating < 2.5) at 7-10 dpp. The three most resistant accessions in this study, PI 169417, PI 181761, PI 512709, and 'Table Ace' are all *C. pepo* species. Average pathogen growth ratings for *C. moschata* species (rating=3.4) and *C. pepo* species (rating=2.0) were significantly different at 7-10 dpp ($P < 0.05$).

Table 1.3: Correlations between fruit age and disease assessments of Phytophthora fruit rot on ten accessions and a commercial variety, Table Ace acorn squash.

	Lesion diameter (cm)		Pathogen growth rating	
Accession	**R^2**	**P-value**	**R^2**	**P-value**
PI 442262	-0.84	0.0007	-0.96	<.0001
PI 458740	-0.82	0.0002	-0.88	<.0001
PI 442266	-0.7	0.0004	-0.83	<.0001
PI 615132	-0.81	<.0001	-0.81	<.0001
PI 634693	-0.61	0.0215	-0.82	0.0003
PI 615142	-0.74	<.0001	-0.79	<.0001
PI 209783	-0.67	0.0017	-0.71	0.0007
PI 512709	-0.41	0.0318	-0.48	0.017
'Table Ace'	-0.44	0.007	-0.55	0.0006
PI 181761	-0.34	0.0453	-0.24	0.1541
PI 169417	-0.28	0.1131	-0.18	0.3125

Most accessions included in this study became significantly more resistant to *P. capsici* as they aged from 7-10 to 21-24 dpp ($P < 0.05$). Disease incidence was significantly greater at 7-10 dpp than at 21-24 dpp for each accession evaluated other than PI 169417 and PI 181761. All fruit were resistant to *P. capsici* (rating ≤ 1) by 21-24 dpp. There was a negative correlation between pathogen growth and lesion size for all fruit tested from the 7-10 to 21-24 dpp age range. (Table 3). The negative correlation between pathogen growth and fruit age was highest in the four *C. moschata* accessions. Most accessions, with the exception of a single infected fruit each for PI 442266, PI 209783, and PI 512709, exhibited no symptoms of *P. capsici* when inoculated 21-24 dpp. Differences between the two isolates were not significant in this study ($P = 0.295$), and there were no significant differences between replications ($P = 0.883$). Accession by isolate interactions were not significant ($P = 0.313$).

Figure 1.2: Differences in susceptibility of acorn squash cv. 'Table Ace' at 7-10 and 21-24 days post pollination (dpp).



Morphologically, the fruit changed in size, thickness, and color as they matured from 7 to 21 dpp. On average there was a threefold increase in squash fruit area from 7-10 to 21-24 dpp. There was no significant difference in pericarp thickness between species at the age ranges tested (P=0.737). In addition, pericarp thickness did not correlate with resistance (P= 0.124). Accessions PI 442262 and PI 512709 showed the largest increase in pericarp thickness as they aged from 7-10 to 21-24 dpp, while accession PI 209783 showed no increase between the two age ranges.

DISCUSSION

An understanding of age-related resistance to *P. capsici* has the potential to reduce fungicide inputs and growers' costs. Previous studies have looked at many aspects of plant and fruit age-related resistance (ARR) (Gadoury et al. 2003, Gevens et al. 2006). Cucurbit powdery mildew ARR has been observed, and it was postulated that genes for resistance are activated or suppressed during plant growth (Kristkova and Lebeda 1999). Age-related resistance has been

noted in association with *P. capsici* infection of pepper and tomato plants (Develey-Riviere and Galiana 2007, Kim et al. 1989, Roberts 2000). Pepper fruit also became increasingly resistant to *P. capsici* as they matured, with resistance being positively correlated with an increase in cuticle thickness (Biles et al. 1993). In grapes, resistance to powdery mildew (*Uncinula necator*) occurs rapidly after fruit-set, even though the fruit are susceptible immediately after anthesis (Gadoury et al. 2003). The grape downy mildew pathogen, *Plasmopara viticola,* was found to be unable to penetrate the cuticle of grape fruit only after resistance became established 1-2 weeks post-bloom (Kennelly et al. 2005). As with grapes and peppers, the resistance to *P. capsici* in mature cucurbits could be due to physical factors in the exocarp, pericarp, or cuticle (Ando et al. 2009). In this study, we observed that 'Table Ace' (acorn squash) was more susceptible to *P. capsici* at 21-24 dpp after being punctured with a 1mm sterile needle, with an average pathogen growth rating of 2.5 compared with a rating of 0.05 for nonwounded fruit (*data not shown*). This supports the idea that physical entry into the underlying tissue of mature fruit is one of the barriers for *P. capsici*. This effect has also been observed in peppers and cucumbers, where wounding fruit negated any observable ARR (Biles et al. 1993, Granke and Hausbeck 2010). During the season, physical damage to the fruit's surface via mechanical damage, insects, rodents, or growth cracks, could increase susceptibility to *P. capsici* of cucurbit cultivars which have acquired resistance (Enzenbacher and Hausbeck 2012).

Age-related resistance to *P. capsici* was observed in most squash fruit tested in this study as they matured from 7-10 to 21-24 dpp. Pumpkins and squash reach maximum fruit size by 20 - 24 dpp (Loy 2004), and in this study, disease incidence at 21-24 dpp was < 12.5% for all accessions tested, with most accessions remaining healthy. This is consistent with previous work on ARR in cucumbers and other cucurbit fruit (Ando et al. 2009, Gevens et al. 2006, Meyer and

Hausbeck 2012), which demonstrated the transition from the end of the fruit elongation phase coinciding with the onset of resistance. In a recent study, the exocarp of two commonly grown processing cucurbits were found to become more dense as the fruit aged, which correlated with reduced Phytophthora fruit rot disease severity (Meyer and Hausbeck 2012). In another study, a decrease in infection due to physical factors may have been observed with a hard rinded pumpkin (*C. pepo*) cultivar, which shows resistance to *P. capsici* (McGrath 2007). The resistance observed may relate to the pathogen's ability to enter the fruit (Biles et al. 1993, Kennelly et al. 2005). In our study, however, pericarp thickness did not correlate with fruit resistance ($P = 0.124$), but thickness does not necessarily indicate firmness. Future studies could investigate how pericarp firmness and sugar accumulation affect pathogen growth, as Meyer et al. observed that exocarp firmness and fruit resistance increase with age in processing pumpkin (Meyer and Hausbeck 2012).

In addition to physical factors, there is the possibility of the presence of a biochemical factor in the outer surfaces of cucurbit fruit, as was discussed for pepper fruit and the phytoalexin capsidiol (Hwang and Kim 1990). This compound was present in greater amounts in the resistant pepper cultivars, as well as the mature tissue, and was associated with ARR in the pepper. Cucurbits are known to produce compounds which affect insect feeding (Tallamy D.W. 1989), and the possibility of compounds which affect *P. capsici* opens another avenue for research. In addition, it would be useful to study the wounding effect observed in this study on other cultivars as well as fruit attached to a growing plant (Lau et al. 1986). Since processing squash are often infected in the field while laying on infested soil (Babadoost 2000), observing these resistance mechanisms in the field using resistant and susceptible accessions would aid in better quantification of physical versus biochemical resistance factors.

All accessions evaluated have shown resistance to Phytophtora root/crown rot (Chavez et al. 2011, Meyer and Hausbeck 2012, Padley 2008). However, based on our study, not all accessions were resistant to Phytophthora fruit rot. Our results agree with findings which show that fruit rot compared with other *P. capsici* blights must be considered separately (Sy and Bosland 2006). The four *C. moschata* cultivars evaluated were susceptible to *P. capsici* fruit rot at 7-10 dpp (>73% infection) even though these accessions have shown crown rot resistance in previous studies (Chavez et al. 2011, Padley 2008). Resistance in the fruit most likely is conferred by different genes than those for root resistance (Sy and Bosland 2006, Walker and Bosland 1999), and the genes for all resistance mechanisms must be incorporated to have fully resistant cucurbit crops. This separation effect has also been seen in pepper, snap beans, and potato to *Phytophtora spp*. (Bonde et al. 1940, Gevens et al. 2006, Kim et al. 1989). The accessions with fruit rot resistance found in our study also exhibit crown and root rot resistance and may be a useful source of material for breeding. In addition to winter squash and pumpkins, breeding resistance into summer squash (*C. pepo*) would be useful in disease management. When temperatures are favorable during the harvest period, summer squash may be harvested daily, and the re-entry interval of many fungicides becomes prohibitive. Having cultivars available which contain genetic resistance to *Phytophthora* could enable growers to maintain a longer interval between fungicide sprays.

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CHAPTER II: EVALUATION OF WINTER SQUASH AND PUMPKIN CULTIVARS FOR AGE-RELATED RESISTANCE TO *PHYTOPHTHORA CAPSICI* FRUIT ROT

ABSTRACT

Krasnow, C.S., and Hausbeck, M.K. 2016. Evaluation of winter squash and pumpkin cultivars for age-related resistance to *Phytophthora capsici* fruit rot. Hortscience (in-press).

Phytophthora capsici annually threatens production of cucurbit and solanaceous crops. Long-lived oospores produced by the pathogen incite primary infection of susceptible plants when conditions are wet. Limiting the rot of winter squash and pumpkin (*Cucurbita* spp.) fruits is difficult due to the long maturation period when fruits are often in direct contact with infested soil. Genetic resistance to fruit rot is not widely available within *Cucurbita* spp., however, age-related resistance (ARR) to *P. capsici* fruit rot develops in specific cultivars during maturation. The objective of this study was to evaluate the fruits of twelve cultivars of *Cucurbita pepo*, *C. moschata*, and *C. maxima* for ARR to *P. capsici* using a mycelial inoculation method. All *C. pepo* and *C. moschata* cultivars displayed ARR; limited lesion development occurred on fruits 22 days post pollination (dpp) and lesions did not develop at 56 dpp. Both *C. maxima* cultivars tested became infected at 7, 14, 22 and 56 dpp. The exocarp firmness of all cultivars included in the study increased during maturation, however, there was no correlation between exocarp firmness and disease incidence among cultivars at 22 dpp (R^2 = -0.01, P = 0.85). When fruits of cultivars expressing ARR at 22 dpp were wounded prior to inoculation, fruit rot developed.

INTRODUCTION

Phytophthora capsici is a destructive pathogen of cucurbit and solanaceous vegetables. Losses in winter squash and pumpkin production may exceed 50% (Babadoost 2000, Isakeit

2007, Meyer and Hausbeck 2013). The pathogen overwinters in the soil as long-lived oospores that serve as primary inoculum and polycyclic production of sporangia and zoospores occurs on infected plant tissue. The movement of *P. capsici* in surface water used for irrigation (Gevens et al. 2007) contributed to the dispersal of the pathogen in Michigan. Managing Phytophthora root and crown rot requires an integrated approach that includes raised plant beds in conjunction with fungicides applied via drip irrigation or soil-directed sprays (Foster and Hausbeck 2010, Jones and McGovern 1994, Meyer and Hausbeck 2013). Tolerance to root rot has been identified in cultivars of summer (*Cucurbita pepo*) and winter squash (*C. moschata*) and cucumber (*Cucumis sativus*)(Hausbeck and Lamour 2004, Meyer and Hausbeck 2012, Ppoyil 2011). Raised-bed culture with plastic mulch-covered plant beds limits soil-splash onto fruit (Kousik et al. 2011), however, vines of winter squash and pumpkins typically grow off of the plastic mulch coming into direct contact with the soil between the plants beds. Foliar fungicides to protect against fruit rot are limited by a dense foliar canopy and an inability to cover the fruit surfaces in contact with the soil (Newhall and Wilkinson 1949). Further, raised plant beds are not economical for growers of winter squash for processing where profit margins are narrow. Over 40,000 acres of winter squash and pumpkin are grown in the Midwest (Anonymous 2014) and highlight the importance of developing effective strategies to limit fruit rot.

The ability of plants to acquire resistance to pathogens as they mature has been studied in many host-pathogen systems (Gadoury et al. 2003, Gerlach et al. 1976, Kennelly et al. 2005, Kim et al. 1989), especially the development of seedling resistance to damping off pathogens (Koh et al. 1987, Lazarovits et al. 1981, McClure and Robbins 1942). Vegetable crops in the Cucurbitaceae and Solanaceae families develop age-related resistance (ARR) to *P. capsici* fruit rot (Ando et al. 2009, Biles et al. 1993, Gevens et al. 2006, Meyer and Hausbeck 2013) that has

been studied to enhance disease management programs (Ando et al. 2009, Hausbeck and Lamour 2004, Krasnow et al. 2014, Meyer and Hausbeck 2013). The fruits of cucurbit crops including acorn squash, pumpkin, and cucumber are highly susceptible to *P. capsici* during early fruit formation, but become increasingly resistant as they mature (Ando et al. 2009, Gevens et al. 2006). Watermelon, muskmelon, and summer squash do not appear to have appreciable levels of resistance (Ando et al. 2009). Meyer and Hausbeck (2013) found differences in the onset and magnitude of ARR to *P. capsici* fruit rot between 'Dickenson Field' (*C. moschata*) and 'Golden Delicious' (*C. maxima*) processing squash. While both cultivars were susceptible to the pathogen up to 14 days post pollination (dpp), 'Dickenson Field' developed ARR at 21 dpp (< 15 % fruit rot) whereas 'Golden Delicious' remained susceptible (approximately 80 % fruit rot). The large acreage and low profit margin of squash grown for the processing market necessitates novel control methods. Mechanical harvesting of processing squash with incipient *P. capsici* infections can result in spread of the pathogen to surrounding fruit post-harvest and during transportation, resulting in potential loss of entire truckloads (Hausbeck and Lamour 2004, Kousik et al. 2014).

The differences in the onset of ARR among cucurbits (Ando et al. 2009, Gevens et al. 2006, Krasnow and Hausbeck 2015) and the lack of ARR in cultivars of C*itrullus lanatus* and *Cucurbita maxima*, (Kousik et al. 2012, Krasnow and Hausbeck 2015, Meyer and Hausbeck 2013) have made it difficult to incorporate this feature into disease management programs. Fungicides are applied to pickling cucumbers during the period of rapid fruit growth when the fruit are highly susceptible to *P. capsici* (Hausbeck and Lamour 2004). Identifying winter squash and pumpkin cultivars that express ARR could help growers make cultivar selections and time fungicide applications to protect developing fruit.

The objectives of this study were i) to evaluate winter squash and pumpkin cultivars (*Cucurbita spp*.) for ARR to *P. capsici,* and ii) to determine the effect of morpho-physiological changes during winter squash and pumpkin fruit development on ARR. A brief report of this work has been published (Krasnow and Hausbeck 2015).

MATERIALS AND METHODS

Plant culture and fruit inoculation: Twelve winter squash and pumpkin cultivars representing the three most economically important *Cucurbita* spp. were selected (Table 1). Seeds were planted into 72-cell flats containing soilless media (Suremix Michigan Grower Products Inc, Galesburg, MI) and grown for three wk in a greenhouse with day/night temperatures of 27°/25°C. Squash seedlings were transplanted into 15 cm raised plant beds covered with black polyethylene plastic at the Michigan State University Plant Pathology Farm in Lansing, Michigan. The soil-type was a Capac loam that was previously cropped to pumpkin and had no history of *P. capsici* infestation. Watering was accomplished with trickle irrigation and plants were grown according to local commercial production standards for fertilizer and pest management (Bird et al. 2014). Once female flowers reached anthesis, male flowers were removed and used to pollinate female flowers of the same cultivar. Female flowers were tagged with the date of pollination and desired harvest age. Fruit were harvested 7, 14, 22, and 56 days post pollination (dpp), ages selected based on developmental changes in fruit color, firmness, and size (Loy 2004, Meyer and Hausbeck 2013). Following harvest, fruit were surface sterilized in 10% bleach for 5 minutes, rinsed with tap water, and air dried on a laboratory bench. Fruit length from the apex to blossom end and the width at the fruit's widest point were measured. Two *P. capsici* isolates obtained from the culture collection of Dr. M. Hausbeck were used for fruit inoculation; OP97 (A1 mating type, sensitive to mefenoxam, isolated from pumpkin) and

Table 2.1. Cultivars, market use, and days to maturity of winter squash and pumpkin evaluated for age related resistance to *P. capsici* fruit rot.

Cucurbita species	Cultivar	Intended use	Days to maturity
C. pepo			
Acorn squash	Autumn Delight[a]	Fresh market	90
Acorn squash	Table Ace[a]	Fresh market	70
Acorn squash	Table Gold[a]	Fresh market	80
Pie pumpkin	Chucky[a]	Fresh market	85
Pumpkin	Diablo[a]	Ornamental	100
Mini-pumpkin	Gold Dust[a]	Ornamental	95
Spaghetti squash	Vegetable Spaghetti[a]	Fresh market	100
C. moschata			
Butternut squash	Avalon[c]	Fresh/processing market	90
Butternut squash	Early Butternut [a]	Fresh market	82
Butternut squash	Waltham Butternut [a]	Fresh market	110
C. maxima			
Hubbard squash	Hubba Hubba[b]	Fresh market	95
Pumpkin	Lumina[c]	Ornamental	100

[a] Siegers Seeds, MI

[b] Johnny's Selected Seeds, ME

[c] Seedway, PA

12889 (A1 mating type, insensitive to mefenoxam, isolated from pepper). The isolates were grown on V8 juice agar (143 ml V8 juice, 3 g CaCO3, 16 g agar L-1). To ensure isolate virulence, the isolates were used to inoculate squash fruit and subsequently recovered from the diseased fruit prior to the initiation of the study (Quesada-Ocampo and Hausbeck 2010). To inoculate fruit, a 7-mm agar plug from the margin of an actively growing colony was placed mycelial side down in the middle of each fruit on unwounded epidermal tissue. The agar plug was covered with a sterile plastic screw cap (Axygen Inc., Union City, CA) using petroleum jelly as a fixative to prevent plug desiccation. Control fruit were inoculated with sterile V8-agar

plugs. The inoculated fruit were incubated in large clear plastic bins (Sterilite, Townsend, MA) lined with moist paper towel to maintain high relative humidity (RH). WatchDog Dataloggers (Spectrum Technologies, Inc., Aurora, IL) were used to monitor temperature and RH within the bins. The average temperature and RH was 24.0°C and 99.7 %, respectively, during the study.

Four days after inoculation, fruit were removed from the bins and lesion diameter measured on two axis. Pathogen growth and sporulation was rated on a 0 to 4 scale adapted from Meyer and Hausbeck (Meyer and Hausbeck 2013) where 0 = no visible pathogen growth; 1 = watersoaking only; 2 = light visible mycelial growth; 3 = moderate mycelial growth; and 4 = dense mycelial growth. Fruit receiving a mean rating value ≤ 1 were considered resistant (R), and fruit with a mean rating value >1 but < 2 were considered intermediately resistant (IR) (Foster et al. 2013). After disease assessment, 1-2 mm tissue sections were removed from the margin of diseased tissue and plated onto BARP (50 ppm benomyl, 100 ppm pentachloronitrobenzene, 100 ppm ampicillin, and 30 ppm rifampicin)-amended V8 agar plates. Recovered isolates were confirmed as *P. capsici* by pathogen morphology on V8-agar (Waterhouse 1963). Mefenoxam sensitivity (Lamour and Hausbeck 2000) was determined to verify similarity to the isolate used for inoculation. Control fruit were observed for symptoms and tissue cultured to confirm the absence of *P. capsici* infection. There were four fruit per replication per isolate with one control. The experiment was conducted twice.

Fruit firmness testing and wound assay. Pericarp and exocarp firmness were measured using a fruit pressure tester (model FT 327, QA Supplies LLC, Norfolk, VA) with a 5-mm-diameter press. The measurement was taken from squash or pumpkin tissue (approximately 25-cm^2) after rating pathogen growth. Exocarp firmness was measured by using the fruit pressure tester to directly penetrate the exocarp. Pericarp tissue firmness was measured by removing the

exocarp (0.5-1.0 mm depth) with a sterile scalpel prior to the measurement. For the wound assay, 22 dpp fruit from five cultivars representing each *Cucurbita* spp. were selected. Each fruit was wounded with a sterile needle to 1 cm depth prior to inoculation, incubated and then and assessed for disease as previously described. The isolate 12889 was used in all experiments in which fruit were wounded prior to inoculation.

Data analysis. Data analysis was accomplished using SAS v9.3 (SAS Institute, Cary, NC). Differences among the variables including pathogen growth rating, lesion size, exo- and pericarp firmness, and fruit age were analyzed using analysis of variance (ANOVA) in SAS Proc Mixed. Mean differences were separated using Fisher's LSD ($P = 0.05$). Correlations among morphological features and disease incidence and severity at the four selected ages were analyzed with Pearson's Correlation Coefficient ($P = 0.05$). Homogeneity of variance between isolates was assessed by residual analysis and data from each isolate was pooled as there were no significant differences in pathogen growth rating, lesion size, and disease incidence. Isolate OP97 was not included in the assay of 56 dpp fruit as there were not an adequate supply of the large fruited *Cucurbita* spp.. Control fruit did not display symptoms after inoculation with sterile agar and were not included in the analysis.

RESULTS

The fruits of all winter squash and pumpkin cultivars tested increased in size and exo- and pericarp firmness as they matured from 7 to 56 dpp (Table 2.1). From 14 to 21 dpp, fruits increased in width for 'Diablo' (47 %), 'Hubba Hubba' (25 %), 'Lumina' (19 %), and 'Vegetable Spaghetti' (14 %); fruits from all other cultivars increased < 5%. The length of the fruits of all cultivars increased < 15 % from 14 to 21 dpp, with the exception of 'Diablo' (29 %) (*data not*

shown). At 22 dpp 'Hubba Hubba' and 'Lumina' (*C. maxima*) had the least firm exocarp while 'Gold Dust' and 'Table Ace' (*C. pepo*) had the firmest exocarp (Table 2.1). There was no correlation between exocarp firmness and disease incidence among cultivars at 22 dpp (r = -0.01; P = 0.85). Exocarp and pericarp firmness was negatively correlated with disease incidence and pathogen growth when analyzed across all ages tested (r = -0.53, P < 0.0001). The exocarp of 'Table Ace' and 'Gold Dust' were the most firm among cultivars at 14, 22, and 56 dpp.

All cultivars were susceptible to *P. capsici* at 7 dpp with fruit rot incidence ranging from 69 to 100 % (Table 3); pathogen growth was similar among cultivars (P = 0.241). 'Autumn Delight', 'Vegetable Spaghetti', 'Avalon', 'Early', and 'Waltham' were IR at 14 dpp with an average growth rating < 2 (Table 3); 'Autumn Delight' and 'Vegetable Spaghetti' had the lowest disease incidence (50 %)(Table 3). At 22 dpp, average disease ratings for all but two cultivars were < 1 (Table 3) and disease incidence was > 20 % for six cultivars. Fruits from the two *C. maxima* cultivars Lumina and Hubba Hubba were the only ones to become infected at 56 dpp, with 63 and 25 % fruit rot, respectively (*data not shown*).

Table 2.2. Exocarp firmness during development of select winter squash and pumpkin cultivars.

Cultivar	Exocarp firmness (kg)[x]			
	7	**14**	**22**	**56**
Autumn Delight	3.6	5.5	10.2	12.8
Chucky	3.3	6.3	10.0	12.3
Diablo	3.3	4.1	8.0	11.1
Gold Dust	3.1	8.2	12.7	13.0
Table Ace	3.2	7.9	11.9	13.0
Table Gold	3.8	7.2	11.1	12.5
Vegetable Spaghetti	2.7	5.2	10.0	12.7
Avalon	3.6	5.8	8.5	12.3
Early Butternut	3.1	5.9	9.0	11.6
Waltham Butternut	3.5	6.4	9.1	11.8
Hubba Hubba	3.4	5.0	7.2	10.1
Lumina	2.8	4.8	6.0	11.7

[x] Measurement made using a model FT 327 fruit penetrometer with 5 mm plunger. Value represents pressure (kg) required to puncture fruit surface.

Table 2.3. Growth rating and disease incidence four days after inoculation with *P. capsici* of winter squash and pumpkin cultivars 7, 14, and 22 days post pollination.

Cultivar	*P. capsici* growth rating[x]			Infected fruit (%)		
	7	14	22	7	14	22
Autumn	3.4 [y]	1.5	0.2 d[z]	94	43	6
Chucky	3.1	2.7	2.3 a	76	75	75
Diablo	3.6	2.1	0.4 cd	94	63	19
Gold Dust	3.8	3.3	1.1 b	94	100	63
Table Ace	3.1	2.6	0.2 d	81	81	6
Table Gold	3.6	2.5	0.4 bcd	94	69	25
Vegetable Spaghetti	2.7	1.3	0.0 d	69	50	0
Avalon	3.6	1.4	0.2 d	94	75	19
Early Butternut	3.8	1.3	0.2 d	94	56	19
Waltham Butternut	3.6	1.7	0.4 bcd	100	88	44
Hubba Hubba	3.5	2.7	0.9 bc	88	75	31
Lumina	4.0	2.9	0.9 bc	100	94	56

[x] Rated 4 dpi on a scale of 0-4, where 0=no growth; 1=watersoaking only; 2=light pathogen growth; 3=moderate pathogen growth; 4=dense pathogen growth. Values represent the mean of two experiments with 8 fruit per age.

[y] Column means for *P. capsici* growth rating without a letter are not significantly different ($P = 0.05$).

[z] Column means with a common letter are not significantly different based on Fisher's LSD test ($P = 0.05$).

Figure 2.1: Effect of inoculation on unwounded (**A** and **C)** and puncture wounded (**B** and **D**) 'Table Ace' (**A** and **B**) and 'Vegetable Spaghetti' (**C** and **D**) winter squash four days post-inoculation with *P. capsici*. Note the lack of sporulation on diseased tissue in (**D).**

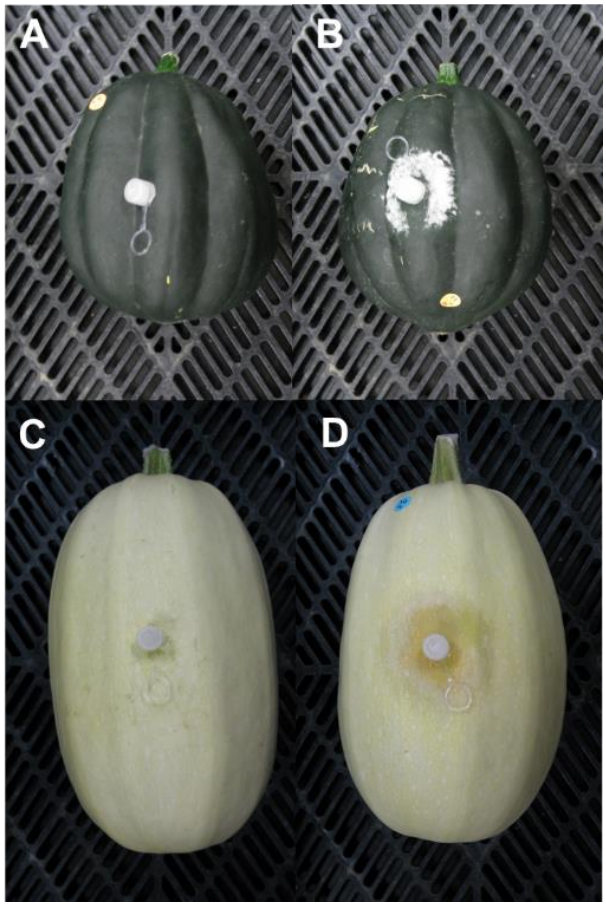
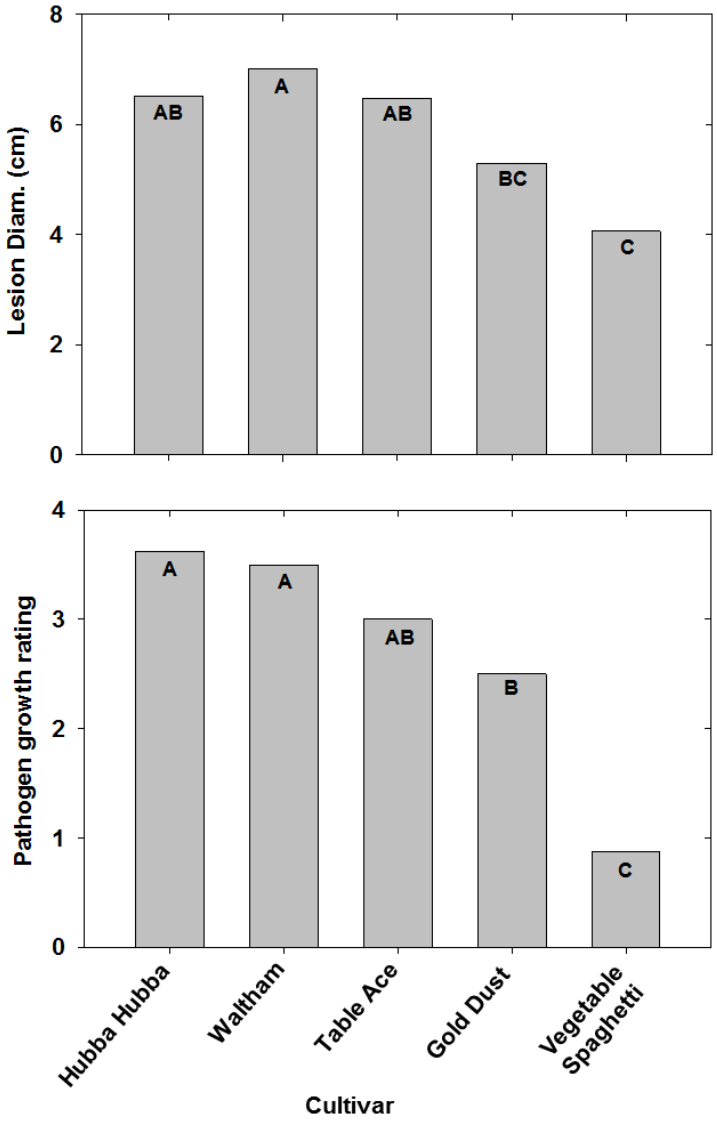


Figure 2.2: Lesion diameter and pathogen growth rating four days post-inoculation for puncture wounded winter squash and pumpkin cultivars at 22 dpp. Fruits were wounded with a sterile needle to 1 cm depth prior to inoculation. Each column represents the mean of two trials with four replicate fruits per isolate per trial. Columns with a letter in common are not significantly different based on Fisher's LSD ($P < 0.05$).



Wounding the fruits of five cultivars prior to inoculation significantly increased disease incidence and pathogen growth ($P = < 0.0001$) compared with the unwounded inoculated fruits. 'Vegetable Spaghetti' had an average rating < 1 after wound inoculation due to a lack of pathogen sporulation and mycelial growth (Fig. 1), however, average lesion size was 4.1 cm, with 88 % fruit rot incidence (Fig. 2). 'Table Ace', 'Gold Dust', 'Waltham', and 'Hubba Hubba' exhibited 100 % fruit infection following inoculation of the wounded fruits, with an average rating of 3.0, 2.5, 3.5, and 3.6, respectively (Fig. 2). Superficial wounding of 'Table Ace' fruit by removing a thin piece of the exocarp < 1.0 mm thick with a scalpel prior to inoculation resulted in 100 % infection (*data not shown*).

DISCUSSION

The relatively long maturation time and growth habit of winter squash and pumpkin increases the risk of Phytophthora fruit rot in growing regions with frequent rainfall and infested soil. Representative cultivar-types of *C. pepo*, *C. moschata*, and *C. maxima* include jack-o-lantern pumpkin, butternut squash, spaghetti squash, and processing squash and pumpkin and all are susceptible to *P. capsici* fruit rot (Babadoost 2000, Isakeit 2007, McGrath 2000, Meyer and Hausbeck 2013). Cultural practices including trellising and choosing varieties with a compact plant size can help prevent *P. capsici* fruit rot by avoiding contact with infested soil (Ando and Grumet 2006). Large-fruited *Cucurbita* spp. offer unique challenges that are difficult to address using cultural practices. Exploiting ARR to *P. capsici* fruit rot offers a valuable opportunity to improve disease management when integrated with other strategies.

Most winter squash and pumpkin cultivars reach full size by 20-24 dpp (Loy 2004) and the development of ARR in many of the *Cucurbita* spp. cultivars tested coincides with this

period of growth (Krasnow and Hausbeck 2015, Meyer and Hausbeck 2013). Similar to the results of experiments by Meyer and Hausbeck (2013), the fruits of *C. maxima* cultivars in this study displayed a greater incidence of infection than the fruits of *C. pepo* and *C. moschata* cultivars. The *C. maxima* cultivars were the only *Cucurbita* spp. to develop *P. capsici* lesions at 56 dpp. Exocarp firmness of Golden Delicious' (*C. maxima*) and 'Dickenson Field' (*C. moschata*) processing squash increased as the fruit matured, but firmness was not correlated with *P. capsici* lesion size on 'Golden Delicious', a cultivar highly susceptible to fruit rot. In this study there was no correlation between exocarp firmness and disease incidence among winter squash and pumpkin cultivars 22 dpp. Changes in surface wax as cucurbit fruit develop have been implicated as influencing resistance to *P. capsici* (Ando et al. 2009). *Cucurbita maxima* begins to accumulate epicuticular wax at 14 dpp (Sutherland and Hallett 1993). Watermelon fruit also develop a thick wax layer at 14 dpp that covers the fruit surface and stomates (Frankle and Hopkins 1993). Fruits of *C. maxima* and *Citrullus lanatus* cultivars are susceptible to *P. capsici* at all maturity stages (Kousik et al. 2012, Krasnow and Hausbeck 2015, Meyer and Hausbeck 2013), and changes in surface wax likely have a limited effect on ARR and fruit rot. Recent studies have identified *C. pepo* and *Citrullus lanatus* germplasm accessions with resistance to *P. capsici* fruit rot as early as 7 dpp during the period of fruit elongation (Kousik et al. 2012, Krasnow et al. 2014) that provide additional evidence for the limited role of surface wax in resistance to fruit rot.

Biles et al. (1993) found that the cuticle of pepper increased in thickness as the fruit matured from green to red and developed resistance to *P. capsici* fruit rot. Similarly, the thicker cuticle and epidermal cells of the stem end of tomato were suggested to prevent infection of the fruit by *P. capsici* (Simonds and Kreutzer 1944). The stylar end did not possess these

characteristics and infection occurred within 70 to 90 min after inoculation. The cuticle of *Cucurbita* spp. contains trichomes and stomata (Barber 1909, Sutherland and Hallett 1993) and differences in the quantity and morphology of these structures may influence ARR to *P. capsici*. Zoospores were observed to accumulate preferentially over stomates of a *C. maxima* cultivar susceptible to *P. capsici*, but not a *C. moschata* cultivar (C. Krasnow and M. Hausbeck, *unpub. data*). Micro-cracks in the fruits' surface occur due to growth and water influx (Schaffer and Boyer 1984) and may also influence the susceptibility of winter squash and pumpkin cultivars to fruit rot. The exocarp of cucurbit fruit is likely the location where ARR is expressed as wounding the fruits prior to inoculation negates ARR resistance (Gevens et al. 2006).

Phytophthora fruit rot has long been a limiting factor in winter squash and pumpkin production. Cultivars that express ARR to *P. capsici* as early as 14 dpp could be selected as part of an integrated management program with fungicide sprays timed for the onset of fruit formation when protection is most needed. Winter squash and pumpkin may be stored post-harvest prior to transporting and marketing. Infection and disease development of the fruit during post-harvest storage is especially costly to growers due to the added expenses associated with disposing of the rotted produce (M. Hausbeck, *pers. obs*.). The use of cucurbit cultivars that express ARR may limit post-harvest losses since ARR decreases the risk of fruit rot developing as the crop reaches maturity.

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CHAPTER III: MECHANISMS OF RESISTANCE TO PHYTOPHTHORA ROOT AND CROWN ROT IN *CUCURBITA PEPO* L.

ABSTRACT

Krasnow, C.S., and Hausbeck, M.K. 2016. Mechanisms of resistance to Phytophthora root and crown rot in *Cucurbita pepo* L. Plant Disease (in-revision).

Root and crown rot incited by *Phytophthora capsici* causes considerable annual losses in squash producing regions in the United States. 'Spineless Perfection' zucchini and 'Cougar' straightneck squash considered to be less and more susceptible to root and crown rot, respectively, were investigated for differences in root and crown physical factors and the histology of crown infection by *P. capsici*. The pH and titratable acidity of healthy root and crown tissue from tissue extracts were not significantly different between cultivars ($P = 0.05$). Crude fiber content (%) of blended and oven-dried root and crown tissue from healthy plants was similar between cultivars. However, dry matter (%) was highest for 'Cougar' ($P = 0.05$). Colonies of *P. capsici* grown from mycelial plugs in root exudates collected from each cultivar were similar in diameter. Whole mounts and histological sections of healthy and infected crown tissue revealed that vascular bundles and metaxylem vessels were more abundant in crowns of 'Spineless Perfection' than 'Cougar'. Twelve to 48 hours post inoculation (hpi), mycelia in the crown of each cultivar was limited to the cortex and hypodermal tissue. By 72 hpi, hyphae were observed in the cortex and endodermal tissue of 'Cougar' and were concentrated in the phloem and parenchyma cells surrounding vascular bundles. Mycelia were limited to the outer cortex in 'Spineless Perfection'. Mycelia and occluding material were present in the majority of metaxylem vessels of 'Cougar' but not 'Spineless Perfection' at 92 hpi; dissolution of parenchyma cells surrounding vascular bundles was apparent in 'Cougar'. The vascular

occlusions observed in ‘Cougar’ may be responsible for plant wilting, a common disease symptom. Additional straightneck, crookneck, scallop, and acorn squash (*C. pepo* ssp. *ovifera*) and zucchini, marrow, and pumpkin (*C. pepo* ssp. *pepo*) cultivars were evaluated in a greenhouse study for resistance to root and crown rot. *C. pepo* ssp. *ovifera* cultivars were significantly more susceptible than ssp. *pepo* to root and crown rot ($P < 0.0001$). Growing ssp. *pepo* cultivars may be beneficial in an integrated Phytophthora management program.

INTRODUCTION

Root and crown rot incited by *Phytophthora capsici* is an annual threat to squash production in Michigan, a crop valued at 19.5 million dollars (Anonymous 2015). All commercially available squash and pumpkin cultivars are considered susceptible (Babadoost and Islam 2003, Cafe et al. 1995). Severe disease outbreaks have occurred in years with frequent heavy rainfall and temperatures favorable for *P. capsici* (Hausbeck and Lamour 2004). Oospores serve as the primary inoculum source and require specific edaphic conditions for germination (Hausbeck and Lamour 2004, Lamour and Hausbeck 2000). Disease foci frequently develop where soils remain saturated for extended periods and often include poorly drained sections of fields. Motile zoospores are released from sporangia that form on infected plant tissue when free water is present and are a secondary infective propagule (Biles 1995, Granke and Hausbeck 2010). Raised-bed plant culture used in fresh market vegetable production has reduced losses by improving drainage and limiting soil splash onto above-ground plant parts (Hausbeck and Lamour 2004, Meyer and Hausbeck 2012). However, adherence to strict irrigation schedules and proper placement of drip lines remains important to prevent excessive water and disease (Cafe-Filho and Duniway 1996). In addition, fungicides can provide protection from root and crown rot

when applied to the soil at transplant and via drip irrigation (Kuhn et al. 2011, Meyer and Hausbeck 2013).

Straightneck, crookneck (yellow squash) and zucchini are among the primary squash cultivar groups grown for fresh and processing market sales in Michigan (Zandstra et al. 1986) and are of high economic importance (Paris et al. 2006, Ploetz and Haynes 2000). These cultivar groups have exhibited qualitative differences in susceptibility to Phytophthora root and crown rot (Camp et al. 2009, Holmes et al. 2002, Meyer and Hausbeck 2012). Meyer and Hausbeck (2012) observed lower levels of root rot on 'Payroll' zucchini than 'Cougar' straightneck squash grown on raised and flat plant-beds, and suggested the use of cultivars with some degree of resistance as a component of an integrated management program. Appreciable levels of resistance to root rot were also observed in trials in New York and North Carolina with zucchini cultivars compared to yellow squash (Camp et al. 2009, Holmes et al. 2002).

Zucchini releases greater levels of organic acids under phosphorous depletion (Gent et al. 2005) and has fewer trichomes (Xiao and Loy 2007) than yellow squash, suggesting that certain biochemical or morphological differences among *C. pepo* cultivar groups may influence susceptibility to Phytophthora root rot. A pepper cultivar resistant to *P. capsici* contained higher levels of carbohydrates, macroelement nutrients, and dry matter in stem tissue than a susceptible pepper cultivar (Jeun and Hwang 1991). Additionally, *P. capsici* growth is limited in exudate materials and mucigel at the root surface of resistant peppers (Kim and Kim 2009), while the roots and stems of susceptible cultivars are rapidly penetrated (Hwang et al. 1989, Kim and Kim 2009). Identifying traits affecting susceptibility of zucchini and yellow squash to *P. capsici* may provide information useful in squash breeding as all *C. pepo* cultivars cross freely (Erwin and Haber 1929, Paris 1986). The objectives of this study included the following: i) Determine

morphological and physiological factors that affect resistance of zucchini to Phytophthora root rot and ii) Evaluate squash and pumpkin cultivars for resistance to *P. capsici*.

MATERIALS AND METHODS

Plant culture, inoculum production, and inoculation. 'Spineless Perfection' zucchini and 'Cougar' straightneck squash previously determined to be less and more susceptible to Phytophthora root rot, respectively, were selected for the study (Meyer and Hausbeck 2012). Plants were grown from seed in 10-cm pots containing coarse vermiculite (Sun Gro, Agawam, MA) or peat potting mixture (Suremix Michigan Grower Products Inc., Galesburg, MI) in a research greenhouse located on the campus of Michigan State University in East Lansing, MI. Vermiculite was used to grow plants for tissue analysis and exudation assays so that roots could be rinsed free from soilless mixture. The greenhouse day/night temperatures were 27/26°C and supplemental lighting was provided from sodium lamps for 16 h per day. Plants were watered to maintain adequate soil moisture with a 20-20-20 complete fertilizer (Peters, Dublin, OH). In all experiments, 21- to 27- day-old plants (3 to 4 true leaves) were used. *P. capsici* isolate SP98 (A2 MT originally isolated from pickling cucumber) was selected for inoculum from the culture collection of M. Hausbeck at Michigan State University and maintained on V8-juice agar (140 ml V8 juice, 3 g $CaCO_3$, and 16 g agar L^{-1}). Zoospores were produced from 5- to 7-day-old cultures grown under constant fluorescent light by flooding the agar plate with sterile distilled water (SDW), chilling at 4°C for 30 min, and returning to ambient temperature (21 ± 1°C) to permit synchronous release of zoospores. To inoculate plants, a 15 ml zoospore suspension (1 x 10^5) was poured onto the soil around the base of each plant.

Root and crown tissue analysis. Tissue from uninoculated (healthy) or inoculated zucchini 'Spineless Perfection' and straightneck squash 'Cougar' was obtained by gently uprooting the plants, rinsing the roots under running tap water, rinsing again with SDW, and blotting the tissue dry with paper toweling. Dry matter (%) was determined from 1 to 2 g of healthy root and crown tissue that was dried on pre-tared aluminum dishes in a gravity oven at 60°C for 24 h. Crude fiber content (%) of roots was determined by blending 10 g fresh-weight of healthy root and crown tissue in 50 ml of SDW in a Sorval Omni-mixer for 30 sec, vacuum-filtering the residue through miracloth (Millipore, Billerica, MA), and rinsing with SDW. The crude fiber residue was dried at 60°C to a constant weight and the dry weight was recorded. Root and crown tissue pH and titratable acidity from healthy and infected plants were determined. Plants were inoculated as described above and symptomatic roots developed 2 to 3 days post inoculation (dpi). Healthy or infected root and crown tissue (5 g) was triturated 1:6 in SDW using a mortar and pestle and the pH of the extract was measured with a glass electrode pH meter (Mettler Toledo, Columbus, OH). The extract was poured through two layers of cheesecloth into a 125 ml flask and the residue extracted with two more volumes of SDW. The extract volume was increased to 100 ml with SDW then rapidly titrated with 0.005 NaOH using phenolthalien as an indicator. Tissue acidity was recorded as μeq of NaOH required to titrate the equivalent of 1 g of root tissue to pH 9. There were 5 to 10 healthy or infected plants of each cultivar per replication and each assay was conducted 3 to 4 times. Plants for each replication were harvested on the same day.

A method adopted from LaMondia (1995) was used to collect root exudates. Roots of healthy plants of 'Cougar' and 'Spineless Perfection' were harvested, rinsed as previously described and then excised from the plant at the apex of the crown, immediately below the soil-

line. The roots (2 g) were soaked for 2 h in 35 ml of SDW in a sterile acid-washed deep petri dish. Exudate (6 ml) was filter sterilized through a 0.45 µm Millipore filter (EMD Millipore, Billerica, MA) into a sterile 60-mm petri dish and a 5-mm mycelial plug of *P. capsici* taken from the margin of a 5-d-old corn meal agar culture (17 g corn meal L^{-1}) was placed into the exudate. Filter-sterile SDW was used as a control. Colony growth was measured on two axes 72 hours post inoculation (hpi) and the plug diameter was subtracted from the mean. The exudate was confirmed free from bacterial contamination by streaking drops of exudate onto V8 agar. There were seven plates per replicate and the experiment was conducted twice.

Cultivars of squash from the extant *C. pepo* cultivar groups (Paris 1986) were selected to evaluate host resistance to *P. capsici* (Table 3.1). The squash plants were grown in peat potting medium in 10-cm pots and plants were inoculated 22 to 23 days post seeding by making a 1-cm deep depression in the potting medium 2 cm from the crown of the plant and pouring 15 ml of zoospore suspension (1×10^5) into the depression. The plants were rated for disease severity 10 dpi using a scale adapted from Meyer and Hausbeck (2012) where 1 = healthy appearing plant; 2 = lower leaves wilted with water-soaked tissue observed at the crown; 3 = all leaves wilted with water-soaked tissue and constriction at the crown; 4 = all leaves wilted with crown tissue rotted and necrosis and pathogen sporulation observed on crown and lower stem; and 5 = dead plant. An average disease severity ≤ 2 was considered to represent partial resistance (Kim et al. 2012). The trial was organized in a completely randomized design with seven plants per cultivar and was conducted twice. Following the termination of each trial, approximately 10% of plants were arbitrarily selected to isolate *P. capsici* from the root system. Plants were uprooted and the root systems were rinsed with tap water to remove adhering potting medium. Small sections (5 mm) of symptomatic roots were excised, dipped into 70% ethanol for 3 sec, blotted dry with paper

towels, and plated onto BARP-amended V8-agar (Krasnow and Hausbeck 2015). There were three segments of root tissue plated from each plant. Colonies that developed on the amended media were transferred to V8-agar and confirmed as *P. capsici* using sporangial morphology and the key of Waterhouse (1963). The mating type and mefenoxam sensitivity of the recovered isolates was determined (Lamour and Hausbeck 2000) to confirm phenotypic similarity to the isolate used for inoculation.

Light microscopy and histology. Healthy and infected plants of each cultivar were studied to observe differences in the infection process and crown tissue morphology. Root and crown tissue from plants of each cultivar grown in peat potting medium were harvested 24, 48, 72, and 92 hpi and rinsed as described above. Transverse and tangential whole mounts from ~ 1 cm of symptomatic crown tissue at the crown-primary root region were excised and stained with 0.005% acid fuchsin in 1:1 SDW:lactic acid and viewed using brightfield microscopy. For histological examination, plants were harvested 12, 24, 48, 72, and 92 hpi and crown tissue pieces were fixed in formalin, acetic acid, alcohol, and water (10:5:50:35) and dehydrated through a tertiary butyl alcohol series. The tissue was embedded in paraffin (m.p. 52°C), and 12-µm sections were made using a rotary microtome. Sections were affixed to glass microscope slides and stained with safranin and fast green (Jensen 1962). Controls included tissue that was harvested from uninoculated plants. For each time point, crown samples from 5 to 10 plants were prepared and multiple sections from each sample were observed. Photomicrographs were taken with a microscope camera (U-CMAD3, Olympus, Tokyo, Japan).

Statistical analysis. Data were analyzed using the Statistical Analysis System v. 9.4 (SAS Institute, Cary, NC). Dry matter (%), crude fiber content, tissue pH, and titratable acidity were compared between ‘Spineless Perfection’ and ‘Cougar’ with ANOVA using the Proc

Mixed procedure. The diameter of *P. capsici* mycelial growth in root exudate was analyzed using ANOVA ($P = 0.05$). Data from SDW controls were not included in the analysis because there was no measurable mycelial growth in this treatment. Differences in disease severity values for *C. pepo* cultivars, cultivar groups, and subspecies in the greenhouse cultivar resistance evaluation were analyzed with Proc Mixed. Normality of residual data was assessed using Proc Univariate and Proc Gplot. Data from each trial was pooled prior to analysis as assumptions for homogeneity of variance were met. A slice statement was used when interactions of simple main effects were found to be significant. The likelihood of a subspecies having an average disease severity value ≥ 2 was determined with Chi-square analysis and odds ratios using Proc Freq and Cochran-Mantel-Haenszel test statistics. *C. pepo* cultivar group comparisons were made using Proc Freq.

RESULTS

The roots of healthy plants harvested for root assays were white and turgid and symptomatic roots were brown or discolored with water-soaking evident on tissues including the taproot, crown, and lateral roots at the point of emergence from the crown. The dry matter (%) of healthy roots of 'Cougar' was significantly higher than 'Spineless Perfection' with values of 7.6 and 7.2 %, respectively ($P = 0.04$; *data not shown*). Crude fiber content was not significantly different between cultivars ($P = 0.39$) averaging 3.1 and 3.5 % for 'Cougar' and 'Spineless Perfection', respectively (*data not shown*). pH values for healthy and

Table 3.1: Disease severity ratings for squash and pumpkin (*Cucurbita pepo*) cultivars evaluated for resistance to *Phytophthora capsici* root rot.

Cultivar name	Cultivar group[x]	*C. pepo* sub-species	Seed source[y]	Disease severity[z]
Early Summer Crookneck	Cn	ovifera	Ris	5.0
Goldstar	Cn	*ovifera*	Rg	5.0
Cougar	Sn	*ovifera*	Ris	5.0
Multipik	Sn	*ovifera*	Ris	5.0
Superpik	Sn	*ovifera*	HM	5.0
Table Queen	Ac	*ovifera*	Rup	5.0
Gold Dust	P	*pepo*	Sie	5.0
Taybelle	Ac	*ovifera*	Rup	4.9
Bennings Green Tint	Sc	*ovifera*	Jon	4.9
Magic Lantern	P	*pepo*	Ris	4.9
Table Ace	Ac	*ovifera*	SW	4.7
White Bush Scallop	Sc	*ovifera*	Ris	4.7
Payroll	Z	*pepo*	Ris	4.1
Orange Rave	P	*pepo*	Sie	4.1
Spineless Perfection	Z	*pepo*	Ris	4.1
Fordhook	Z	*pepo*	Bur	3.3
Tivoli	M	*pepo*	Rup	2.9
Diablo	P	*pepo*	Sie	2.9
Vegetable Spaghetti	M	*pepo*	Rup	2.3
Dark Green	Z	*pepo*	FM	1.4
Magda	M	*pepo*	Jon	1.2
Hurikan	M	*pepo*	HM	1.2

[x] *Cucurbita pepo* cultivar type based on Paris (1986). A = acorn, Cn = crookneck, M = marrow, P= pumpkin, Sc = scallop, Sn = straightneck, Z = zucchini.

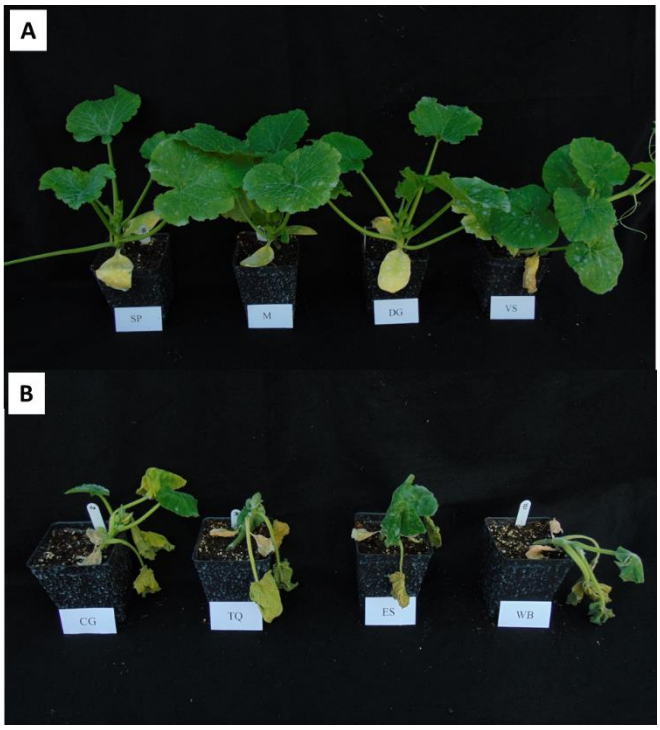
[y] Bur = W.Atlee Burpee & Co, Warminster, PA; FM = Ferry-Morse, Norton, MA; HM = Harris Moran, Modesto, CA; Jon = Johnny's Selected Seeds, Winslow, ME; Rg = Rogers Seeds, Syngenta Co., Boise, ID; Ris = Rispens Seeds, Inc., Beecher, IL; Rup = Rupp Seeds Inc., Wauseon, OH; Sie = Siegers Seed Co., Holland, MI; SW = Seedway, Elizabethtown, PA.

[z] Disease severity rated 10 dpi on a scale where 1 = healthy plant; 2 = lower leaves wilted with watersoaked tissue observed at the crown; 3 = all leaves wilted with watersoaked tissue and

Table 3.1 (cont'd).

constriction at the crown; 4 = all leaves wilted with crown rotted and necrosis and sporulation present on crown and lower stem; and 5 = dead plant. Value represents the mean of two trials.

Figure 3.1: *Cucurbita pepo* ssp. *pepo* (**A**) and ssp. *ovifera* (**B**) plants 8 days post inoculation with *Phytophthora capsici* in greenhouse evaluation for root and crown rot resistance. SP = 'Spineless Perfection', DG = 'Dark Green', M = 'Magda', VS = 'Vegetable Spaghetti', CG = 'Cougar', TQ = 'Table Queen', ES = 'Early Summer Crookneck', WB = 'White Bush Scallop'.



diseased roots of 'Spineless Perfection' and 'Cougar' were not significantly different between cultivars (mean pH 6.7; *data not shown*). Titratable acidity of healthy roots of each cultivar was lower than that for diseased roots (*data not shown*). Diseased roots contained 6.4 and 24.5 % greater acidity than healthy roots for 'Spineless Perfection' and 'Cougar', respectively. However, differences between cultivars for healthy (P = 0.13) and diseased (P = 0.78) roots were not significant.

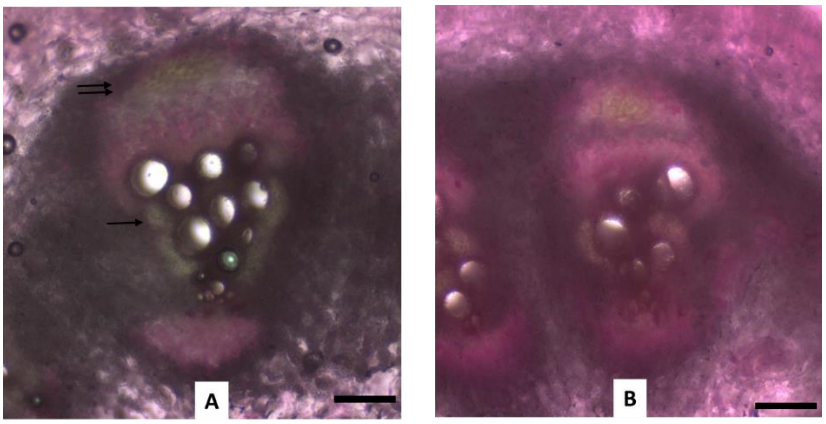
When *P. capsici* was grown in root exudate of 'Cougar' and 'Spineless Perfection' the average diameter of mycelial growth was 7.3 and 6.7 mm, respectively (*data not shown*). These

differences were not significant when analyzed using ANOVA ($P = 0.41$), however, they were greater than the growth observed for the SDW control (mean 0.0 mm diameter).

Wilt developed rapidly on *C. pepo* ssp. *ovifera* cultivars in the greenhouse screen for resistance (Fig. 3.1). By 3 dpi, 50% of the ssp. *ovifera* cultivars had at least one plant with wilt symptoms (*data not shown*). At 4 dpi, all ssp. *ovifera* cultivars had plants displaying symptoms of wilt, while plants of only one ssp. *pepo* cultivar developed symptoms. The average disease severity for ssp. *ovifera* cultivars was 4.9 at 10 dpi (Table 3.1). *C. pepo* ssp. *pepo* cultivars displayed lower disease severity, averaging 3.1 among cultivars. 'Dark Green', 'Magda', and 'Hurikan' had the lowest disease severity values at 1.4, 1.2, and 1.2, respectively (Table 3.1). Zucchini cultivars had an average disease severity of 3.2, significantly lower than the average for crookneck squash cultivars (5.0) and straightneck squash (5.0) when the cultivar groups were compared using ANOVA ($P = 0.05$). The mean disease severity for ssp. *ovifera* cultivars was significantly higher than for ssp. *pepo* cultivars ($P < 0.0001$). Cultivars of ssp. *pepo* were less likely to have disease severity ≥ 2 compared to ssp. *ovifera* ($\chi^2 = 62.0$; $P < 0.0001$).

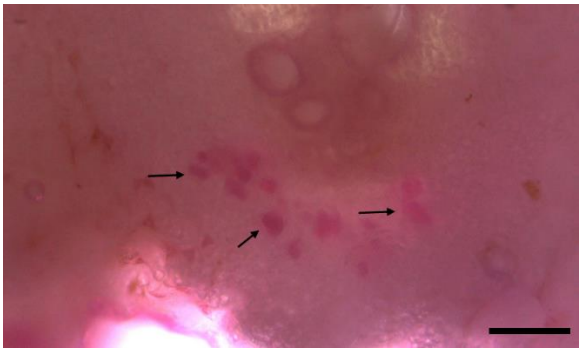
Symptom development and infection process. The lower leaves of 'Cougar' began to wilt 2 to 3 dpi when plants were inoculated with a zoospore suspension of *P. capsici*. Water-soaking of crown tissue was usually observed concurrently with the initial wilt symptoms. By 4 dpi, wilt was severe in 'Cougar', and sunken lesions and constriction were observed at the crown and lower stem. Plants often lodged 5 to 6 dpi and constriction and necrosis was advanced to the lower stem. Plant death occurred by 8 dpi; leaves withered with constriction and stem necrosis

Figure 3.2: Photomicrograph (40x) of healthy vascular bundle from whole-mount section of crown tissue of (**A**) 'Spineless Perfection' zucchini (field resistant) and (**B**) 'Cougar' straightneck squash (susceptible). Note the quantity of metaxylem vessels (arrow) and thick bundle sheath (double arrow) of 'Spineless Perfection'. Bars = 20 µm.



evident from the soil-line to the apical meristem and base of the lowermost petioles. *P. capsici* sporulated profusely at the soil-line on crown and lower stem tissue. Yellowing of lower leaves of 'Spineless Perfection' was observed 6 dpi with no additional symptoms; incipient wilt was occasionally observed 8 dpi. Crown tissue of 'Cougar' contained 6 to 7 vascular bundles and 'Spineless Perfection' contained 8 to 10 (*data not shown*). Individual vascular bundles of 'Spineless Perfection' contained a greater number of metaxylem elements and a thicker bundle sheath than 'Cougar' (Fig. 3.2). In whole mounts of infected plants, mycelium of *P. capsici* was observed in hypodermal tissue of 'Cougar' and 'Spineless Perfection' and at the point of emergence of lateral roots by 24 hpi. By 48 hpi, the epidermal tissue of 'Cougar' exhibited orange pigmentation and the discoloration extended into the cortex at the point of infection. Mycelium and occluding material were present in many of the metaxylem vessels of 'Cougar' at 72 hpi and hyphae had ramified through the tissue and were observed in the medulla. Some

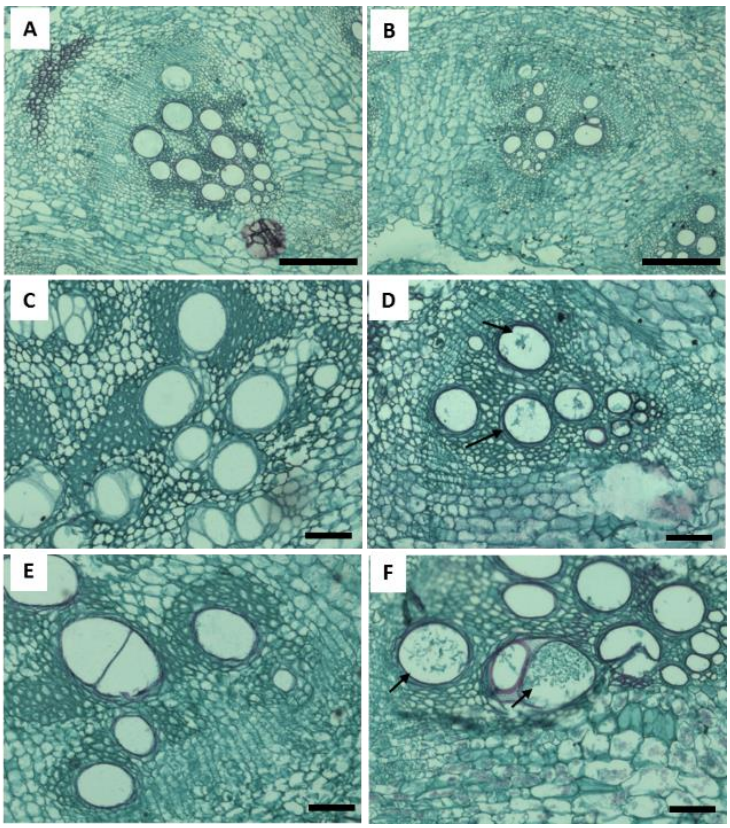
Figure 3.3: Infection and development of hyphae of *Phytophthora capsici* in the inner phloem tissue of 'Cougar'. Dense staining (arrows) due to *P. capsici* mycelium. Bar = 10 µm.



metaxylem vessels of 'Spineless Perfection' contained occluding material and mycelia, but occlusion was less frequent than in 'Cougar'. Mycelia were observed exiting stomata on the crown surface of tangential sections of both cultivars at 72 hpi. Mycelial growth was not dense in the cortex of either cultivar at 72 hpi, but was abundant in the phloem and parenchyma surrounding vascular bundles of 'Cougar' (Fig. 3.3). At 92 hpi, mycelia were present through the crown tissue of 'Cougar'. Host cell walls were thin, some were broken, and the walls had lost birefringence. Dissolution of cortex cell walls was apparent and cortex tissue was compressed. Vessels were almost completely occluded with mycelium and occluding materials. The cortex cells of 'Spineless Perfection' were not compressed or broken and the majority of vessels were not occluded. Vascular bundles and bundle sheaths appeared structurally unaffected at 92 hpi in both cultivars.

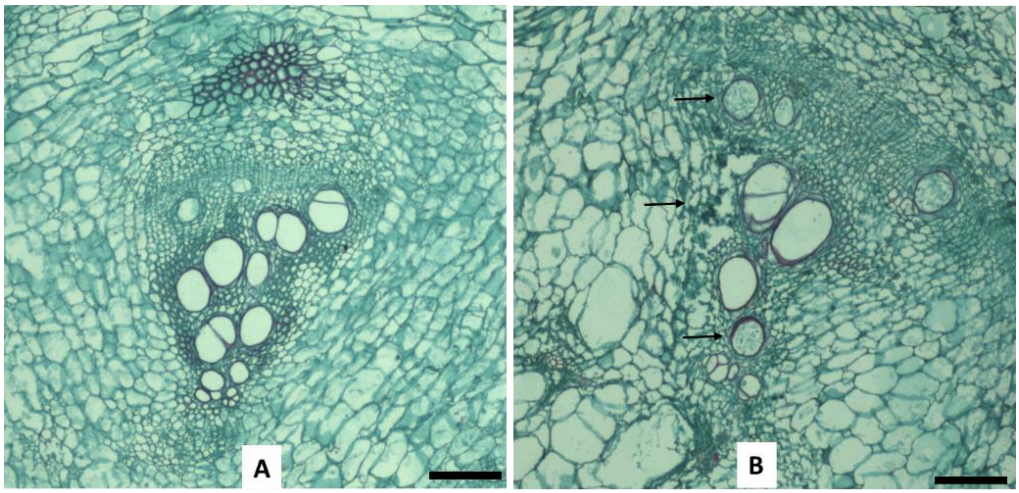
In histological sections, xylary fibers adjacent to xylem vessels appeared more compact in 'Spineless Perfection' than 'Cougar' (Fig. 3.2-A,B). Mycelium and occluding substances were difficult to observe at early stages of infection and sections of each cultivar appeared similar until 48 hpi (Fig. 3.4-A-D). At 72 hpi, dissolution of phloem cells and cells surrounding xylem

Figure 3.4: Crown sections of 'Spineless Perfection' zucchini (**A, C, E**) and 'Cougar' straightneck squash (**B, D, F**). **A, B**) Healthy crown tissue. Bars = 40 µm. **C**, **D**) Crown tissue 48 hours post inoculation with *Phytophthora capsici.* Mycelium and occluding substances (arrow) present in xylem in **D**). Bars = 10 µm. **E, F**) crown tissue 72 hours post inoculation with *P. capsici.* Dense mycelium (arrows) present in xylem vessel in **F**). Bars = 10 µm.



vessels of 'Cougar' was apparent and some vessels were filled with mycelia (Fig. 3.4-E). Vascular tissue and surrounding parenchyma tissue of 'Spineless Perfection' appeared unaffected (Fig. 3.4-F). Tyloses, occluding material, and mycelia were present in the majority of 'Cougar' metaxylem vessels by 92 hpi, but were absent from most vessels of 'Spineless Perfection' (Fig. 3.5). Xylem vessels and bundle sheaths stained with safranin and in both cultivars were similar in appearance at 92 hpi to the healthy controls.

Figure 3.5: Sections of crown tissue of (**A**) 'Spineless Perfection' zucchini and (**B**) 'Cougar' straightneck squash 92 hours post inoculation with *Phytophthora capsici*. Note the occluded vessels and apparent deterioration of parenchyma tissue surrounding metaxylem (arrows) in **B**). Bars = 20 μm.



DISCUSSION

The Phytophthora crown and root rot epiphytotics on yellow squash (Cafe and Duniway 1995, Crossan et al. 1953, Jones and McGovern 1994, Tompkins and Tucker 1941) corroborate the high susceptibility of the crop relative to other summer squash cultivar groups observed in research trials (Camp et al. 2009, Holmes et al. 2002, Meyer and Hausbeck 2012). Differences reported among zucchini and yellow squash cultivar groups in trichome size and abundance (Xiao and Loy 2007), cucurbitacin content (Sharma and Hall 1971), and ability to uptake nutrients (Gent et al. 2005), suggest that there may be biochemical or morphological differences that relate to susceptibility to *P. capsici*. 'Cougar' and 'Spineless Perfection' had similar fiber content (%) in this study although dry matter (%) was highest for 'Cougar'. NeSmith (1993) determined that root and shoot dry weight for 'Senator' zucchini was significantly higher than 'Dixie' crookneck, however, the earlier growth stage at harvest in the current study may have influenced dry matter accumulation. Acidity and pH of diseased and healthy root and crown tissue of 'Cougar' and 'Spineless Perfection' were similar. *C. pepo* is known to produce numerous organic acids in the root system (Kursanov and Kulaeva 1957) and although zucchini was able to exude higher levels of citric acid under phosphorus depletion than yellow squash, acid concentrations in root tissue was not different among cultivar groups grown under normal nutrition (Gent et al. 2005). Some fungal pathogens are known to change tissue pH during pathogenesis (Venning and Crandall 1954) and the limited pH change in *P. capsici* infected squash roots may be due to differences in infection processes or plant parts infected. Infected tissue from the margin of a *P. capsici* lesion on zucchini fruit had a pH approximately 1.5 units higher than non-infected fruit (pH 6.4) (C. Krasnow and M. Hausbeck, *unpub. data*.).

Cucurbita pepo is a species highly polymorphic for fruit shape that has traditionally been grouped based on fruit morphology and color (Castetter 1925, Whitaker and Davis 1962). The cultivar grouping has been refined more recently using fruit shape (Paris 1986). Phenotypes of six isozyme systems present throughout root, hypocotyl, and leaf tissue of *C. pepo* cultivars (Ignart and Weeden 1984) differentiated major cultivar groups into two sub-species, *C. pepo* ssp. *ovifera* and ssp. *pepo* (Decker 1988, Paris et al. 2006). The sub-species designation was additionally confirmed with genetic markers (Bates et al. 1990). *C. pepo* ssp. *ovifera* contains cultivar groups scallop, acorn, crookneck, and straightneck squash, while marrow, zucchini, cocozelle, and pumpkin are within ssp. *pepo* (Paris et al. 2006). Crookneck, straightneck, and acorn squash cultivars in ssp. *ovifera* are highly susceptible to *P. capsici* (Cafe and Duniway 1995, Crossan et al. 1953, Holmes et al. 2002, Jones and McGovern 1994, Krasnow and Hausbeck 2015, Meyer and Hausbeck 2012, Tompkins and Tucker 1941) and *C. pepo* ssp. *pepo* cultivars of zucchini and vegetable spaghetti (marrow) often display field resistance (Camp et al. 2009, Holmes et al. 2002, Krasnow and Hausbeck 2015, Meyer and Hausbeck 2012). The relationship of *C. pepo* sub-species and field resistance to *P. capsici* may be specific to oomycete pathogens as *Didymella bryoniae* (Keinath 2014), *Fusarium* spp. (Martyn and McLaughlin 1983, Sumner 1976), *Cladosporium cucumerinum* (Strider and Konsler 1965), *Erwinia tracheiphila* (Rand and Enlows 1916), and root knot nematodes (Thomason and McKinney 1959) cause disease on *C. pepo* cultivars without a relation between disease severity and the *C. pepo* sub-species. In contrast, another oomycete, *Pseudoperonospora cubensis*, has been observed to cause lower levels of disease on ssp. *pepo* than on ssp. *ovifera* (Holmes et al. 2015). *C. pepo* ssp. *ovifera* and ssp. *pepo* have different centers of origin and likely have separate wild progenitor *Cucurbita* spp. (Bates et al. 1990) which may be a factor in susceptibility to oomycete pathogens.

The isozyme phenotypes of pumpkin cultivars are more variable than other ssp. *pepo* cultivar groups (Ignart and Weeden 1984) and the presence of small fruited gourds in ssp. *ovifera* (Decker 1988, Paris et al. 2006) may relate to the high disease levels observed on 'Magic Lantern' and 'Gold Dust' mini pumpkin. Additionally, further testing of *C. pepo* ssp. *pepo* cultivars under field conditions would be beneficial as prolonged inoculum contact is known to influence disease severity (Barksdale et al. 1984).

Wilt develops on cucurbits after infection by pathogens that occlude or rupture vascular tissue (Main and Walker 1971, Martyn and McLaughlin 1983, Palodhi and Sen 1979). In the current study, mycelia and occluding substances were observed in vessels of 'Cougar' squash plants at the initiation of wilt symptoms induced by *P. capsici* and were present more frequently than in infected 'Spineless Perfection' plants. Vascular occlusions and mycelial growth in xylem vessels of resistant squash cultivars infected by *Fusarium oxysporum* is also limited (Martyn and McLaughlin 1983). The phloem tissue, parenchyma, and meristem cells adjacent to vascular bundles of 'Cougar' and to a lesser extent in 'Spineless Perfection' were apparently preferential for *P. capsici* growth due to the intensity of hyphal staining in these regions. Phloem parenchyma has been suggested to be involved in solute storage in *C. pepo* (Duloy et al. 1962). Nutrients located in the phloem parenchyma and the thin cell walls of vascular meristem cells (Esau 1965) may be preferential for ramification of *P. capsici* hyphae in infected *C. pepo* roots and crown. The dense parenchyma cells in the endodermal region and numerous lignified metaxylem vessels of 'Spineless Perfection' may provide structural support of infected crown tissue preventing collapse and mechanical breakage of xylem tissue that would increase resistance to water flow and cause wilt (Powers 1954). Cell deterioration of parenchyma cells surrounding xylem vessels in 'Cougar' may have a role in symptom development if vessels break

under the weight of the above ground plant due to reduced cellular support. Marks and Mitchell (1971) observed that alfalfa tolerant of *P. megasperma* had a thicker central stele and greater lateral root production than cultivars susceptible to root rot. If occlusions or mechanical blockage are the cause of wilt in *P. capsici* infected squash, they are likely localized at the site of infection; severely wilted squash plants fully recovered in 1 to 2 h after the stems of infected, symptomatic plants were excised above the crown lesion and placed into distilled water in 50 ml flasks (C. Krasnow and M. Hausbeck, *unpub. data*). Localized obstruction of vascular tissue of *P. nicotianae* infected tobacco was also observed. (Powers 1954).

The field resistance of *C. pepo* ssp. *pepo* may be beneficial in an integrated *Phytophthora* management program as resistant squash cultivars are not commercially available. Repeat fungicide applications are frequently made during fresh market squash production to limit Phytophthora root rot, however, the resistance of the cultivar planted is usually not considered when determining application intervals (C. Krasnow, *pers. obs.*). Yeh and Kim (1991) recommended basing spray intervals to control pepper Phytophthora blight on the resistance of the cultivar planted. Applying fungicides at an increased interval may reduce fungicide costs and improve plant health and yield for ssp. *pepo* cultivars with field resistance to Phytophthora root rot.

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CHAPTER IV: PATHOGENICITY OF *PHYTOPHTHORA CAPSICI* TO *BRASSICA* VEGETABLE CROPS AND BIOFUMIGATION COVER CROPS (*BRASSICA* SPP.)

ABSTRACT

Krasnow, C.S., and Hausbeck, M.K. 2015. Pathogenicity of *Phytophthora capsici* to Brassica vegetable crops and biofumigation cover crops (*Brassica* spp.). Plant Disease 99:1721-1726.

The soilborne oomycete *Phytophthora capsici* causes root, crown, and fruit rot of many vegetable crops in the Cucurbitaceae and Solanaceae families. *Phytophthora capsici* is a persistent problem in vegetable fields due to long-lived oospores that survive in soil and resist weathering and degradation. Vegetable crops in the Brassicaceae family have been considered non-hosts of *P. capsici* and are planted as rotational crops in infested fields. *Brassica* spp. are also grown as biofumigation cover crops to reduce inoculum levels of *P. capsici* and other soilborne pathogens, and this use has increased concurrent with restrictions on soil fumigation. Oriental mustard (*B. juncea*), oilseed rape (*B. napus*) and oilseed radish (*Raphanus sativus* var. *oleiferus*) contain high levels of glucosinolates and are widely recommended for biofumigation and as cover crops. The objective of this study was to evaluate vegetables and biofumigation cover crops in the Brassicaceae family for susceptibility to *P. capsici*. *Brassica* spp. used as vegetable crops and for biofumigation were grown in *P. capsici* infested potting soil in the greenhouse and disease incidence and severity were recorded. In greenhouse trials, infection by the pathogen reduced the fresh weight of all *Brassica* spp. tested and resulted in plant death of 44% of plants of *B. juncea* 'Pacific Gold'. *Phytophthora capsici* isolates exhibited differences in virulence ($P < 0.0001$), and were re-isolated from the roots of all *Brassica* spp. included in the study. The biofumigation cover crop 'Pacific Gold' mustard may not reduce populations of *P. capsici* in soil and instead may sustain or increase pathogen levels. Further research is necessary to test this possibility under field conditions.

INTRODUCTION

The Brassicaceae family includes vegetables and cover crops considered important due to their diversity and adaptability to variable soil types and growing conditions (Nieuwhof 1969). In Michigan, over 2,500 hectares of cabbage, turnip, radish, broccoli and other *Brassica* vegetables are grown annually for the fresh and processing markets (Anonymous 2012). In addition to production as vegetable crops, *Brassica* spp. are widely planted as cover crops and for biofumigation in both horticultural and agronomic crop production (Kirkegaard and Sarwar 1998, Ngouajio and Mutch 2004, Snapp et al. 2006). Numerous studies have reported that *Brassica* biofumigation can reduce soil-borne pathogens that are often an intractable problem in both conventional and organic vegetable production (Lewis and Papaviza 1971, Mattner et al. 2008, Mayton et al. 1996, Muehlchen et al. 1990). The use of *Brassica* spp. for this purpose has increased in recent years concurrent with the phase-out of the fumigant methyl bromide and an emphasis on sustainable disease management alternatives (Ackroyd 2010, Gardiner et al. 1999, Kirkegaard and Sarwar 1998, Lazzeri and Manici 2001). The ability of *Brassica* spp. to reduce pathogen inoculum density is attributed to glucosinolates, which are hydrophilic, thioglucoside compounds that are stored in the cell vacuoles of all *Brassica* spp. for use in sulfur assimilation and storage (Clossais-Besnard and Larher 1991, Larsen 1980, Marschner 1995). At vegetative maturity, *Brassica* cover crops are incorporated into the soil via flail mowing and disking, and the disruption of cellular content facilitates the hydrolysis of glucosinolates by the enzyme myrosinase (Fahn 1982, Marschner 1995, Morra and Kirkegaard 2002). Volatile isothiocyanates and other hydrolysis products that possess biocidal properties are produced during this process (Kirkegaard and Sarwar 1998, Larsen 1980, Smolinska et al. 1997), and come into direct contact with pathogen propagules following soil incorporation (Lazzeri and Manici 2001, Lewis and

Papaviza 1971, Snapp et al. 2006). Certain *Brassica* varieties have been developed and marketed specifically for the quantity and composition of glucosinolates produced by the plant (Anonymous 2014, Baysal and Miller 2009, Charron and Sams 1999, Morra and Kirkegaard 2002). Disease control recommendations using *Brassica* biofumigation have not been optimized, however, as glucosinolate production is environmentally and ontogenetically influenced (Greenhalgh and Mitchell 1976, He et al. 2003, McGregor 1988, Rosa et al. 1996, Sarwar and Kirkegaard 1998).

Phytophthora root and crown rot of *Brassica* crops caused by *Phytophthora drechsleri* and *P. megasperma* has been reported on cabbage, broccoli, cauliflower, and turnip, and occasionally causes major economic loss (Downes and Loughnane 1969, Geeson et al. 1990, Hamm and Koepsell 1984, Tompkins et al. 1936). Symptoms typical of Phytophthora root rot of *Brassica* crops include wilt, purple discoloration of the stem and older foliage, and eventual plant death (Downes and Loughnane 1969, Tompkins et al. 1936). *Phytophthora* spp. also cause post-harvest rots during storage of cabbage, Chinese cabbage, and swede (Geeson 1976, Geeson et al. 1990, Hermansen and Hoftun 2005, Semb 1969). Management practices to control Phytophthora rots affecting *Brassica* spp. include planting into well drained soil, fungicide application, and temperature control post-harvest (Hermansen and Hoftun 2005, Kontaxis and Rubatzky 1983). *Phytophthora capsici* has been reported to be pathogenic to seedlings of cauliflower, radish, and turnip in studies conducted under controlled environmental conditions (Hartman and Huang 1993, Ji et al. 2012, Satour and Butler 1967, Tian and Babadoost 2004). In the same studies, *P. capsici* was non-pathogenic on cabbage, broccoli, mustard, rape, kale, and kohlrabi. Reports of *P. capsici* affecting traditional non-host crops including Fraser fir (Quesada-Ocampo et al. 2009), herbaceous ornamental plants (Enzenbacher 2011), and snap

bean (Gevens et al. 2008), and weed species such as *Portulaca oleracea* (French-Monar et al. 2006), highlight this pathogen's virulence and adaptability to diverse hosts. *Phytophthora capsici* is known to increase in virulence as a result of genetic exchange during oospore formation, (Satour and Butler 1968) and the frequent occurrence of both mating types in vegetable fields (Lamour and Hausbeck 2000) heightens the importance of control methods to reduce inoculum pressure and pathogen spread. *Brassica* vegetables such as cabbage, broccoli, and radish, and *Brassica* spp. used for biofumigation, are often planted into fields infested with *P. capsici* with the assumption that these crops are not affected by the pathogen and will reduce inoculum levels (M. K. Hausbeck, *pers. comm*.). The potential for *P. capsici* to cause disease on *Brassica* spp. or to survive and reproduce in debris remaining in the field post-harvest, would negatively affect growers facing limited crop rotation, fumigation, and fungicide control options (Hausbeck and Lamour 2004). The objective of this study was to evaluate select vegetables and biofumigation cover crops in the Brassicaceae family for susceptibility to *P. capsici*.

MATERIALS AND METHODS

Isolate selection and inoculum preparation. *Phytophthora capsici* isolates originally collected from cucurbitaceous, solanaceous, and fabaceous hosts were selected from the culture collection of Dr. M. K. Hausbeck. The isolates were previously characterized for mating type (MT) and mefenoxam sensitivity (Lamour and Hausbeck 2000). Isolate 12889 (A1 MT) is mefenoxam insensitive and 10193 (A1 MT) and 14110 (A2 MT) are mefenoxam sensitive. The cultures were maintained on V8 agar media (143 ml V8 juice, 3 g $CaCO_3$, 16 g agar, 850 ml distilled water). Prior to the study, the isolates were inoculated onto pepper fruit and subsequently recovered from the diseased fruit to ensure virulence of the isolates (Quesada-Ocampo and Hausbeck 2010). Millet inoculum (Quesada-Ocampo and Hausbeck 2010) was

prepared by autoclaving millet seed (100 g), distilled water (72 ml), and L-asparagine (0.08 mg) in mushroom bags (RJG Sales, Port Richey, FL) twice consecutively, and adding seven 7-mm agar plugs colonized by a single *P. capsici* isolate. Infested millet seed was grown under constant fluorescent light for 3 to 4 weeks and mixed weekly prior to use as inoculum.

Pathogenicity testing of *P. capsici* on *Brassica* spp. *Brassica* vegetables and cover crops (Table 1) were sown into 288-cell flats in the Plant Science Research Greenhouses at Michigan State University, East Lansing, MI. Three grams of millet infested with a single isolate and prepared as described previously was deposited into each transplant hole in 10-cm pots containing autoclaved peat potting mixture (Suremix Michigan Grower Products Inc, Galesburg, MI) and gently mixed to incorporate with the soil. Eight-day-old seedlings were used for the study and were transplanted into the infested peat potting mixture. Control pots received 3 g of millet prepared with sterile V8 agar plugs. The quantity of inoculum was selected based on previous studies (Quesada-Ocampo et al. 2009), and preliminary inoculum-density experiments with 'Bronco' cabbage (*data not shown*). A single plant was grown in each pot and was considered an experimental unit, with six pots per isolate for each cultivar. All plants in the experiment were inoculated on the same day. Plants were watered to maintain adequate soil moisture, and plant height from the soil-line to the tallest expanded foliage and width at the widest point were measured weekly during the experiment. Visual disease severity was rated on a 0 to 4 scale adapted from Glosier et al. (2008), where 0 = healthy; 1 = minor wilting, chlorosis, or stunting; 2 = moderate wilting, chlorosis, and stunting; 3 = severe wilting, chlorosis, and stunting; and 4 = plant death. Plants of 'Pacific Gold' and 'Florida Broad Leaf' mustard and 'Rover' and 'Groundhog' radish were harvested 3 weeks after inoculation, and all other crops

Table 4.1. *Brassica* spp. used as vegetables and biofumigation cover crops evaluated in *Phytophthora capsici* pathogenicity experiments.

Brassica species	Cultivar	Intended use	Days to maturity
Brassica juncea L.			
Mustard	Florida Broad Leaf[a]	Fresh market	40
Indian mustard	Pacific Gold[b]	Biofumigation	65-80
B. napus			
Rape	Dwarf Essex[b]	Biofumigation[d]	60-80
B. oleracea var. botrytis			
Cauliflower	Snow Crown[c]	Fresh market	50
B. oleracea var. capitata			
Green cabbage	Bronco[c]	Fresh market	78
Red cabbage	Buscaro[c]	Fresh market/processing	100
B. oleracea var. italica			
Broccoli	Emerald Crown[c]	Fresh market	60
B. rapa			
Turnip	Purple Top White Globe[a]	Fresh market	58
Raphanus sativus			
Radish	Rover[c]	Fresh market	25
R. sativus var. *oleiferus*			
Oilseed radish	Groundhog[c]	Biofumigation[d]	60-90

[a] Rispens Seeds, IL

[b] Johnny's Selected Seeds, ME

[c] Seedway, PA

[d] Oilseed radish and rape are also used as traditional cover crops without mowing and incorporation of green tissue that is frequently practiced with biofumigation.

were harvested 4 weeks after inoculation. Above-ground fresh weights were recorded at harvest for all crops. Root weights of 'Groundhog' and 'Rover' radish and 'Purple Top White Globe' turnip were also recorded. At the completion of the experiment, roots were rinsed in tap water to remove adhering potting mix and approximately 50% of the plants of each cultivar and isolate

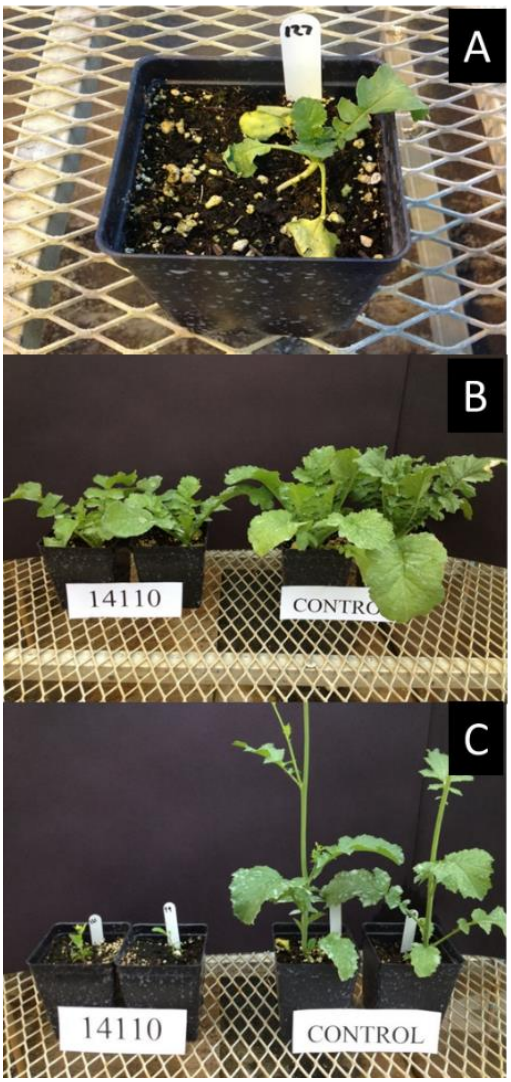
were arbitrarily selected for pathogen re-isolation. Necrotic or water-soaked root tissue was rinsed in SDW and surface sterilized in 70% ethanol for 5 s prior to plating onto BARP-amended V8 agar (50 ppm benomyl, 100 ppm pentachloronitrobenzene, 100 ppm ampicillin, and 30 ppm rifampicin). Isolated colonies were transferred to V8 agar and confirmed as *P. capsici* by pathogen morphology (Waterhouse 1963). Temperature and relative humidity were measured using a WatchDog data logger (Spectrum Technologies Inc., Plainfield, IL). The experiment was organized in a completely randomized design and was conducted twice.

Data were analyzed using SAS version 9.4 (SAS Institute, Cary, NC). Response variables were analyzed by ANOVA using the Proc Mixed procedure. *Phytophthora capsici* isolate and cultivar were considered fixed variables and trials were considered random variables. Interactions were sliced when found to be significant at $P = 0.05$ in an analysis of variance (ANOVA) to analyze simple main effects. The response variables fresh weight, root weight, plant height, and plant width, were analyzed separately for each cultivar. Fisher's protected least significant difference was used to separate treatment differences using the SAS pdmix800 macro (Saxton 1998). Residuals were plotted against predicted values using Proc Gplot and checked for normality and equal variance to ensure statistical assumptions were met. Data from non-inoculated control plants were not included in the analysis because disease symptoms were not observed and root isolations did not yield *P. capsici*. Pearsons correlation coefficients between incidence of disease and the independent variables plant height, width, and above-ground weight were analyzed using Proc Corr to determine the degree of association between disease incidence and plant size. The likelihood of disease incidence due to specific isolate was analyzed using logistic regression.

RESULTS

Disease symptoms observed for inoculated *Brassica* plants included stunting, wilting, chlorosis, and plant death (Fig. 1). Severely affected cauliflower and broccoli plants also exhibited purple discoloration of the foliage. Initial symptoms of wilting and chlorosis were typically evident within three days post inoculation. Discolored roots and constriction of the stem at the soil-line were apparent upon harvest of symptomatic plants. The results were similar between the two trials and data were pooled for analysis because a significant trial x treatment interaction was accounted for by magnitude of plant growth, not rank. Differences in temperature and relative humidity were observed between the two experiments, with a mean temperature/relative humidity of 26.6°C/19% and 29.4°C/41% in trials 1 and 2, respectively. Disease symptoms were noted on all inoculated *Brassica* cultivars included in this study (Table 4.2). There was a negative correlation between fresh weight and disease incidence for all cultivars (R^2 = - 0.391, $P < 0.0001$) and a similar correlation with height and width measurements was observed (*data not shown*). Using logistic regression, the probability of disease incidence was highest in 'Pacific Gold' ($P < 0.0001$) and lowest in 'Essex' ($P = 0.79$). 'Essex' displayed the lowest disease severity when compared to the other *Brassica* spp. (Table 4.2). The *Brassica* spp. used for biofumigation tended to exhibit a more drastic decrease in fresh weight than the vegetable crops (Table 4.3); for example, 'Pacific Gold' and 'Groundhog' displayed a 78% and 24% reduction in fresh weight, respectively, compared to the control ($P < 0.001$). The reduction in fresh weight for the *Brassica* spp. used as vegetable crops was significant for 'Bronco' ($P < 0.01$) and 'Buscaro' ($P < 0.05$) cabbage, and was not significant for the remaining cultivars (Table 4.3). *Phytophthora capsici* also reduced root weight of the three

Figure 4.1: Symptoms of disease caused by *Phytophthora capsici* on *Brassica* spp. including **(A)** wilting of 'Groundhog' radish (**B**) stunting of 'Groundhog' radish, and (**C)** plant death of 'Pacific Gold' mustard.



Brassica spp. grown for their large root size ($P = 0.1$), most notably with 'Purple Top White Globe' (Fig. 4.2). The three *P. capsici* isolates selected for this study caused varying degrees of

Table 4.2: Disease severity of select *Brassica* spp. used as vegetables and biofumigation cover crops when inoculated with *Phytophthora capsici* in greenhouse trials and frequency of pathogen recovery from diseased roots.

Cultivar[a]	Disease severity[bc]			Recovery frequency[e]
	P. capsici isolate[d]			
	10193	12889	14110	
Bronco	0.25	0.33	0.50	+++
Buscaro	0.42	0.25	0.58	+++
Emerald Crown	0.33	0.50	0.33	+
Dwarf Essex	0.25	0.17	0.08	++
Florida Broad Leaf	0.67	0.25	0.50	++
Groundhog	0.80	0.40	0.56	+
Pacific Gold	2.25	3.00	3.25	++
Purple Top White Globe	0.33	0.25	0.75	++
Rover	0.0	0.08	0.33	+
Snow Crown	0.58	0.33	0.42	+
Mean	0.59	0.56	0.73	

[a] Cultivar information and intended crop use presented in Table 1.

[b] Disease severity values represent final visual rating of inoculated plants using a 0-4 scale; 0 = healthy; 1 = minor wilting, chlorosis, or stunting; 2 = moderate wilting, chlorosis, and stunting; 3 = severe wilting, chlorosis, and stunting; 4 = plant death. Values represent the mean of two trials with six replications per trial.

[c] Disease was not observed on nor was *P. capsici* recovered from control plants, which were not included in the analysis.

[d] *Phytophthora capsici* isolates originally recovered from three different host families. Isolate designation from the culture collection of Dr. M. K. Hausbeck.

[e] Frequency of *P. capsici* recovery from roots of diseased *Brassica* spp.. + = < 33%; ++ = 33 – 66%; +++ = > 66%. Percentage recovery represents the mean of two trials with six replications per trial.

Table 4.3. Effect of *Phytophthora capsici* isolate on above-ground fresh weight of select *Brassica* spp. used as vegetables and biofumigation cover crops in greenhouse pathogenicity trials.

Cultivar[a]	Reduction in above-ground fresh weight (%)[b]				
	P. capsici isolate[c]				
	10193	12889	14110	Mean[d]	
Bronco	16	24	22	21	**
Buscaro	13	22	29	21	*
Emerald Crown	11	17	15	14	
Dwarf Essex	6	0	0	2	
Florida Broad Leaf	19	4	10	11	
Groundhog	37	11	23	24	**
Pacific Gold	66	78	90	78	**
Purple Top White Globe	17	3	19	13	
Rover	0	13	2	5	
Snow Crown	9	11	19	13	

[a] Cultivar information and intended crop use presented in Table 1.

[b] Decrease in above-ground fresh weight (%) compared to control plants. Fresh weight (g) measured at the conclusion of each trial. Values represent the mean of two trials with six replications per trial.

[c] *Phytophthora capsici* isolates originally recovered from three different host families. Isolate designation from the culture collection of Dr. M. K. Hausbeck.

[d] Asterisks * and ** indicate significant treatment differences at $P < 0.05$ and $P < 0.01$, respectively, according to Fisher's protected LSD test.

Figure 4.2: Effect of *Phytophthora capsici* isolate on root weight of three *Brassica* cultivars grown for their large root size. Each column represents the mean of 2 trials with 6 replicate plants per isolate per trial. Columns with a letter in common are not significantly different within each cultivar based on Fisher's protected LSD ($P < 0.05$). Error bars represent the standard error of the mean.

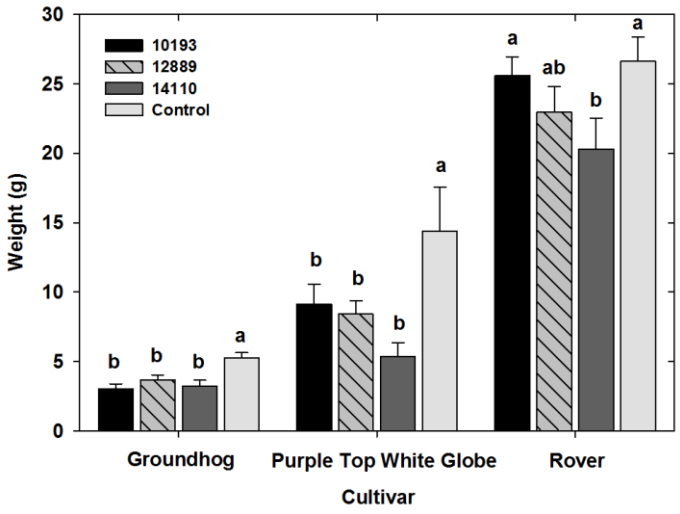
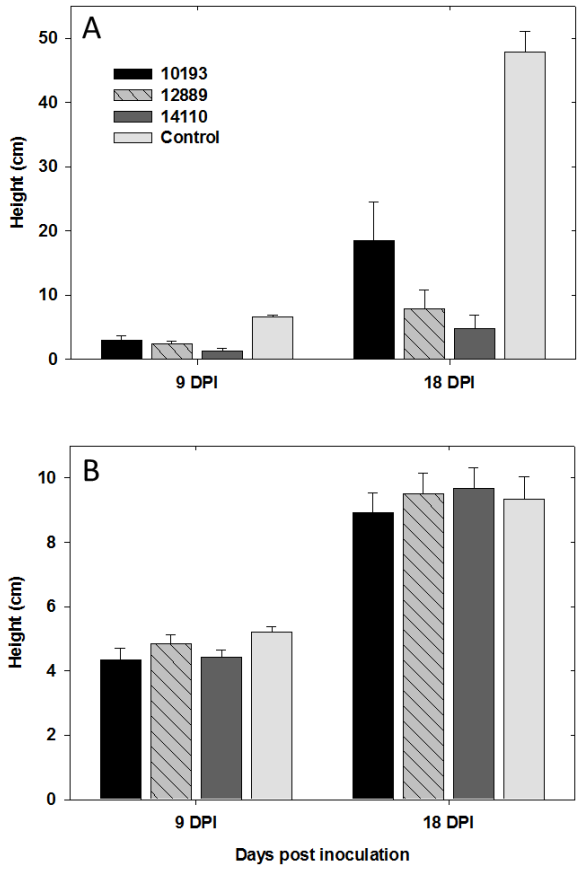


Figure 4.3: Differences in height of (**A**) 'Pacific Gold' and (**B**) 'Florida Broad Leaf' at 9 and 18 days post inoculation (DPI) with three Phytophthora capsici isolates. Each column represents the mean of 2 trials with 6 replicate plants per isolate per trial. Error bars represent the standard error of the mean.



disease severity on the *Brassica* spp. tested. Differences in the cumulative disease severity values between isolates 14110 and 10193 for 'Rover' and 'Pacific Gold' and between isolates 14110 and 12889 for 'Purple Top White Globe' were significant ($P < 0.05$, *data not shown*). However, at the final disease severity rating, differences among isolates were not significant ($P = 0.159$, Table 2). Isolate 14110 was the most virulent isolate tested based on disease incidence (*P*

< 0.001, *data not shown*) as determined by binary logistic regression. All *P. capsici* isolates tested reduced the height of 'Pacific Gold' plants ($P < 0.001$, Fig. 3) while significant differences among *P. capsici* isolates and the control were not observed with 'Florida Broad Leaf' ($P = 0.213$). The differences in mean disease severity among *Brassica juncea* cultivars were larger than differences observed among other crops of the same species (Table 2). *Phytophthora capsici* was successfully re-isolated from the roots of all *Brassica* cultivars tested (Table 2). Each of the three isolates included in the study were successfully recovered from at least one infected plant in both trials (*data not shown*).

DISCUSSION

Cover crops are often grown in annual vegetable production systems primarily to improve soil drainage, suppress weeds, increase soil organic matter, capture nutrients, and reduce erosion (Hartwig and Ammon 2002). *Brassica* spp. developed for biofumigation are planted in vegetable production systems when an additional goal is to decrease populations of soilborne pathogens (Ackroyd 2010, Charron and Sams 1999, Ngouajio and Mutch 2004, Snapp et al. 2006). The cover crops are usually seeded, grown to vegetative maturity, and incorporated prior to planting an economically important vegetable crop or planted post-harvest and killed by freezing temperatures (Snapp et al. 2006, Sundermeier 2008). Our greenhouse study indicates that *P. capsici* can colonize the roots of diverse *Brassica* spp. and can cause plant death of 'Pacific Gold' mustard (*B. juncea*) and 'Groundhog' radish (*Raphanus sativus* var. *oleiferus*) used specifically for biofumigation. There have been conflicting studies about the ability of *Brassica* cover crops to suppress Oomycete plant pathogens (Larkin and Griffin 2007, Mattner et al. 2008, Wiggins and Kinkel 2005). Wiggins (2005) did not find a significant difference in Phytophthora root rot of alfalfa planted into pots of infested field soil that had been cropped to

and amended with chopped canola (*B. napus*) tissue 20 days prior or left fallow (Wiggins and Kinkel 2005). Similarly, a commercial biofumigation cover crop mixture of *B. napus* and *B. rapa* that was mowed and incorporated at anthesis into field soil did not decrease the viability of *P. cactorum* that was buried in the soil for 3 days following *Brassica* incorporation. Growth of the isolates on selective media after recovery from the soil, however, was reduced by 20% compared with *P. cactorum* buried and recovered from fallowed soils (Mattner et al. 2008). Control of pathogenic *Pythium* spp. and *Aphanomyces* spp. with *Brassica* tissue and associated glucosinolate compounds has been more consistent than with *Phytophthora* spp. (Charron and Sams 1999, Lazzeri and Manici 2001, Lewis and Papaviza 1971, Muehlchen et al. 1990, Smolinska et al. 1997), although, lack of suppression of *Pythium* spp. has been noted in studies on apple replant disease (Mazzola et al. 2007, Mazzola et al. 2009). Studies on the biofumigation potential of *Brassica* spp. to *P. capsici* have reported decreases in pathogen populations or disease severity upon incorporation of fresh or dry *Brassica* residues into soil, potting mix, or other media (Cohen et al. 2008, Demirci and Dolar 2006, Fan et al. 2008, Ji et al. 2012, Ludwig et al. 2004, McGrath and Menasha 2013, Ppoyil 2011, Sanogo and Schaub 2012, Wang et al. 2014). In a greenhouse study, the proportion of 'Dickenson' pumpkin plants infected by *P. capsici* crown rot was limited by adding macerated *Brassica* tissue to the soil around the crown of inoculated seedlings (Ppoyil 2011). McGrath (2013) and Ji (2012) found that mustard cultivars used for biofumigation were able to reduce the incidence of Phytophthora blight of pumpkin and squash, respectively, in field trials where the mustard cover crops were incorporated prior to planting (Ji et al. 2012, McGrath and Menasha 2013). Additionally, pepper seeded into *P. capsici* infested soil amended with *Brassica juncea* seed meal had a 46% germination rate as opposed to 6.7% in the infested control (Cohen et al. 2008). The sensitivity

of *P. capsici* to *Brassica* tissue observed in previous studies may be related to the use of isolated volatile compounds, extracts, seed meal produced from Brassicaceous tissues, and macerated plant material (Charron and Sams 1999, Demirci and Dolar 2006, Mazzola et al. 2009, Morales-Rodriguez et al. 2014, Ppoyil 2011, Smolinska et al. 1997) to decrease pathogen levels. Differences exist among *Brassica* spp. between above-ground tissue and roots in quantity and composition of glucosinolates, with root tissue and seedlings usually containing higher quantities than shoots (Gardiner et al. 1999, Kirkegaard and Sarwar 1998, Muehlchen et al. 1990, Rosa 1997). Although, higher concentrations of glucosinolates in shoot tissue have been noted with *Sinapis alba* and *B. juncea* (Anonymous 2014). *Brassica juncea* cultivars, including 'Pacific Gold' used in this study, contain the highest concentration of glucosinolate compounds among *Brassica* spp. used for biofumigation (Anonymous 2014, Charron and Sams 1999, Larkin and Griffin 2007, Smolinska and Horbowicz 1999, Snapp et al. 2006) and concentrations of glucosinolates are highest during the seedling stage (Fahey et al. 2001, He et al. 2003). As *Brassica* spp. used for biofumigation and as cover crops are direct or broadcast seeded into vegetable production fields (Ngouajio and Mutch 2004, Sundermeier 2008), colonization of roots by *P. capsici* may increase pathogen populations in soil, nullifying the ability of glucosinolate compounds to significantly decrease pathogen levels. Additionally, *P. capsici* caused lesions on the foliage of seedlings and mature plants of cabbage, mustard, and other *Brassica* spp. when inoculated in the laboratory and greenhouse with zoospores or mycelia and incubated under high relative humidity (*data not shown*). The ability of the pathogen to infect diverse tissue types raises the possibility that the residues of *Brassica* spp. incorporated into the soil may sustain or increase *P. capsici* population densities in the field instead of reducing inoculum levels. *Phytophthora capsici* does not appear to be affected by glucosinolate compounds in *Brassica*

tissue, and the disease reduction noted in previous studies using incorporated *Brassica* plant tissue to reduce *P. capsici* (Coelho et al. 1999, Cohen et al. 2008, Demirci and Dolar 2006, Ji et al. 2012, Ludwig et al. 2004, McGrath and Menasha 2013, Ppoyil 2011) may have been due to increases in antagonistic soil microorganisms (Mazzola et al. 2007, Wiggins and Kinkel 2005), or variations in *P. capsici* isolate sensitivity to the *Brassica* tissue and related volatile compounds (Morales-Rodriguez et al. 2014, Ppoyil 2011).

The difference in susceptibility of *B. juncea* 'Pacific Gold' and 'Florida Broad Leaf' suggests that tolerance to *P. capsici* may exist among *Brassica* spp. Field tolerance to *P. capsici* has been observed with other hosts such as *Cucurbita pepo* (Meyer and Hausbeck 2013) and *Capsicum annuum* (Foster and Hausbeck 2010), and host resistance is often considered an optimal disease management strategy (Granke et al. 2012, Ristaino and Johnston 1999). Using fungicides to keep biofumigant cover crops healthy is not desirable so screening *Brassica* cover crops for resistance to *P. capsici* isolates from diverse geographic locations is important. A similar screening has been successful in identifying tolerance in cauliflower to Phytophthora blight caused by *P. megasperma* (Hamm and Koepsell 1984, Kontaxis and Rubatzky 1983). *Phytophthora* spp. that affect *Brassica* vegetables have been observed during wet years or when fields with heavy soils that are prone to waterlogging are utilized (Tompkins et al. 1936). *Phytophthora drechsleri* and *P. megasperma* caused significant losses to *Brassica* crops both in the field (Hamm and Koepsell 1984, Thompson and Phillips 1988, Tompkins et al. 1936) and in storage (Geeson 1976, Geeson et al. 1990, Hermansen and Hoftun 2005), often after periods of soil saturation and wet conditions during harvest. The similarity of the reported symptoms of Phytophthora blight of *Brassica* vegetables to those observed in this study stress the need for accurate identification of diseased plants. Fungicide recommendations have been made for

controlling *Phytophthora* spp. on *Brassica* vegetables and include the systemic fungicide metalaxyl (Anonymous 1996, Kontaxis and Rubatzky 1983). However, widespread resistance to this fungicide and its racemic isomer, mefenoxam, have been reported for *P. capsici* (Hausbeck and Lamour 2004). Soil directed fungicide applications of newer systemic fungicides are effective in controlling *P. capsici* root rots (Foster and Hausbeck 2010, Meyer and Hausbeck 2013) and trials are in progress to test the effectiveness of fungicides for management of *P. capsici* root rot of *Brassica* vegetables.

Phytophthora capsici has the potential to infect and cause significant losses on over 30,000 hectares of susceptible vegetables grown annually in Michigan (Anonymous 2012), and uninfested land used for vegetable production that is available for rotation is becoming increasingly scarce (Hausbeck and Lamour 2004). The widespread utilization of *Brassica* spp. as vegetable crops and for biofumigation in vegetable production, (Larsen 1980, Ngouajio and Mutch 2004, Snapp et al. 2006) and the prevalence of *P. capsici* in vegetable fields (Hausbeck and Lamour 2004), highlights the importance of conducting further studies into the etiology of this pathogen on *Brassica* spp. Even though severe disease was not observed on some of the vegetable crops tested in this study, roots infected at the subclinical level may enable survival of *P. capsici* in soil (Shishkoff 2007) and result in significant reductions in yield (Stanghellini and Kronland 1986). The identification of *P. capsici* as a potential pathogen of *Brassica* spp. and the ability to cause severe disease on the biofumigation cover crop 'Pacific Gold' means that these crops should be monitored for Phytophthora root rot when planted into fields with a history of *P. capsici*. In addition, recommendations for Phytophthora blight prevention and management (Hausbeck and Lamour 2004) should be strongly adhered to when considering planting *Brassica* vegetables into infested fields.

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CHAPTER V: EVALUATION OF PEPPER CULTIVAR RESISTANCE IN AN INTEGRATED PHYTOPHTHORA BLIGHT MANAGEMENT PROGRAM

ABSTRACT

Phytophthora blight incited by *Phytophthora capsici* is an important and limiting disease in bell pepper production in many vegetable producing areas of the United States. Soil-borne oospores initiate the disease when conditions are favorable and polycyclic production of sporangia and zoospores occurs on infected plant tissue during the production season. Raised-bed plant culture and oomycete specific fungicides are commonly used to control Phytophthora blight, however, there are limited commercially available resistant cultivars. The objective of this study was to evaluate pepper cultivars and experimental breeding entries (collectively termed entries) for resistance to *P. capsici* in Michigan, and to determine the effect of a fungicide program on plant health and yield. The susceptible 'Camelot X3R' had > 90% wilt and plant death in the untreated plot both years of the study. All entries other than 'Camelot X3R' had < 10% of plants with root rot symptoms in 2014, however, 'Aristotle', 'AP4835', '13SE12671', and 'AP4841' displayed between 10 and 30% wilt and plant death in 2015. The fungicide program reduced plant death and improved yield of most entries, however, there was no entry x fungicide program interaction in both years. Marketable yield for the 'Resistant Standard' was significantly higher than other entries in both years. Fruit size for '13SE12671' was the largest among entries.

INTRODUCTION

Phytophthora blight incited by *P. capsici* is a destructive disease of pepper that causes significant annual losses throughout the United States (Hausbeck and Lamour 2004). Michigan is an important producers of fresh market bell peppers in the Great Lakes region with 1,000 ha

planted annually (Anonymous 2015). Yield loss can be severe when environmental conditions are suitable for disease (Hausbeck and Lamour 2004, Lamour and Hausbeck 2000). Infected plants support abundant sporangial production and motile zoospores liberated in free water contribute to the high disease potential and polycyclic spread of the pathogen (Bowers and Mitchell 1991, Ristaino 1991). Irrigation or surface run-off water infested with *P. capsici* propagules serves as a source of secondary inoculum (Gevens et al. 2007). Infected pepper plants display irreversible wilt and plant death develops rapidly when temperatures are warm and soils saturated (Ristaino and Johnston 1999). A necrotic lesion encircling the base of the crown is often apparent at advanced stages of infection. Disease may develop at any stage of growth, however, young plants are considered most susceptible (Cafe-Filho and Duniway 1996, Ristaino 1991).

Raised bed plant culture and trickle irrigation are used in fresh market pepper production to improve yields and reduce Phytophthora root rot (Bosland and Votava 2012, Hausbeck and Lamour 2004, Ristaino 1991). Judicious application of fungicides via drip lines, or as soil-directed sprays can provide additional control (Foster and Hausbeck 2010, Kuhn et al. 2011, Meyer and Hausbeck 2013). A drip-injected fungicide program that contained Revus (mandipropamid) and Actigard (acibenzolar-S-methyl) or Presidio (fluopicolide) provided 50 and 75 % control of *P. capsici* root and crown rot of pepper and squash, respectively (Kuhn et al. 2011). When Revus, Presidio, or Forum (dimethomorph) were applied weekly as soil drenches to summer squash, the incidence of Phytophthora blight was reduced to $\leq$ 10% compared with 100% for the control (Meyer and Hausbeck 2013). Despite the effectiveness of soil-applied fungicides in Phytophthora management, many fungicides efficacious towards *P. capsici* are not labeled for this method of application (Bird et al. 2016, Sanogo and Ji 2012, Wyenandt 2016).

Additionally, the fungicide metalaxyl and its active enantiomer mefenoxam that were once highly effective in Phytophthora management (Hausbeck and Lamour 2004) are currently limited in use due to widespread resistance in field populations of *P. capsici* (Café-Filho and Ristaino 2008, Hausbeck and Lamour 2004, Ploetz and Haynes 2000). The paucity of fungicides that can be applied as soil-drenches or via drip irrigation can negatively affect anti-resistance management strategies that encourage tank mixing and rotation of fungicide classes (Skylakakis 1981, Staub and Sozzi 1984).

Planting Phytophthora resistant and/or tolerant bell peppers has been considered an optimal method to decrease fungicide use and improve yields (Hwang and Kim 1995, Ristaino and Johnston 1999, Wyatt et al. 2013). However, there are a limited number of pepper cultivars available that have high levels of resistance (Foster and Hausbeck 2010). Incorporating resistance to *P. capsici* is a difficult and complex process. Root, stem, and foliar blight are considered to be under the action of separate genetic systems (Sy and Bosland 2006, Walker and Bosland 1999) and multiple genes must be introgressed to breed resistance to each disease syndrome. Seedling screens have been used to evaluate breeding lines and accessions of pepper and other vegetable crops for root rot resistance and can be performed relatively quickly in a greenhouse (Bolkan 1985, Bosland and Lindsey 1991, Foster and Hausbeck 2010, Kim et al. 2012). However, pepper seedlings are more susceptible to *P. capsici* than mature plants (Café-Filho and Duniway 1995, Hwang and Kim 1990) and differences in the magnitude of age-related resistance to *P. capsici* among pepper cultivars can affect disease severity (Hwang and Kim 1995). Variations in *P. capsici* isolate virulence based on geographical location and the host that the isolate was recovered from (Foster and Hausbeck 2010, Islam et al. 2005) heighten the importance of selecting isolates that represent field populations in resistance screening (Granke

et al. 2012, Kim and Hwang 1992). A study evaluating 12 Korean pepper cultivars for resistance to a worldwide collection of *P. capsici* isolates found significant differences in disease severity due to isolate (Kim and Hwang 1992). Additionally, *P. capsici* isolates recovered from vegetable hosts were shown to sporulate more on zucchini fruit than isolates from tropical hosts (Granke et al. 2012). The development and adoption of resistant cultivars is important as land suitable for vegetable production that is not infested with *P. capsici* is becoming increasingly limited (Hausbeck and Lamour 2004, Quesada-Ocampo and Hausbeck 2010) and effective soil fumigants are strictly regulated or have been phased out of production (Ristaino and Johnston 1999).

The objectives of this study were to evaluate select experimental entries and bell pepper cultivars with varying levels of resistance to Phytophthora root rot and a single fungicide program on root rot control.

MATERIALS AND METHODS

Entry selection and experimental design. Seeds of selected pepper cultivars and experimental breeding entries (collectively referred to as entries) were obtained from Seminis Seeds Inc. (Table 5.1). The commercial cultivars used in this study represented either high or intermediate level of resistance to *P. capsici* root and crown rot (Foster and Hausbeck 2010). 'Camelot X3R' represented a susceptible cultivar. Experimental breeding entries were known to have intermediate to high resistance based on preliminary field testing (A. Wyenandt, M. Hausbeck, and B. Carey, *unpub. data*). In both years, the trials were located at the South West Michigan Research and Extension Center (SWMREC) in Benton Harbor, MI. In each year, transplants were grown in 128 cell flats at the Michigan State University research greenhouses

Table 5.1. Pepper entries evaluated for resistance to Phytophthora root rot at SWMREC.

Entry	*Phytophthora* resistance
Aristotle[y]	Intermediate
Archimedes[y]	High
Camelot X3R[y]	Low
Resistant Standard[z]	High
13SE12671[y]	Intermediate to high
AP4841[y]	Intermediate to high
AP4835[y]	Intermediate to high
AP4839[y]	Intermediate to high

[y] Seminis Seeds Inc, St. Louis, MO.

[z] Syngenta AG, Greensborough, NC; Resistant Standard represents a Phytophthora blight resistant cultivar from Syngenta AG that cannot be listed by name due to contract agreement between Syngenta AG and Seminis Seeds Inc.

in East Lansing, MI. The field site consisted of a loamy fine sand that was previously cropped to squash with a history of Phytophthora. On 6 June 2014 and 28 May 2015, pepper seedlings that were 7-8 wk old were transplanted 30.5 cm apart into 15 cm raised plant-beds covered with black polyethylene plastic and spaced 1.7 m apart. Each treatment contained 18 plants with a 3.1 m buffer between treatments within the row. 'Camelot X3R' was planted in the buffer to increase disease pressure within the plot. Fertilizer was broadcast preplant at the rate of 112 kg 19N-19P-19K and 56 kg 46N-0P-0K/ha, and the herbicides Command 3ME (1.16 L/ha), Sandea 75W (0.03 L/ha), and Dual Magnum 7.62 EC (0.6 L/ha) were applied preplant between rows. Additional fertilizer was applied via drip irrigation every 1 to 2 weeks as urea (46N-0P-0K) and liquid potassium (0N-0P-28K) at 11.1 and 3.5 kg/ha, respectively. Weekly fertigation was done using 2.2 kg 20N-20P-20K during the season. Insects were controlled when necessary with drip applications of Admire Pro (1.0L/ha). The trial was arranged as a completely randomized split

plot design with fungicide program as the main plot and entry as sub plot with 4 replications per treatment. In both years, the fungicide program consisted of Presidio SC (0.3 L/ha) injected into the drip lines at transplant and 30 and 60 days post planting (dpp), and Revus SC (0.6 L/ha) applied as a directed foliar spray 14 dpp. Presidio SC was injected into the irrigation line of a single row with 172 kPa CO_2 pressure from 11.5 L canisters while irrigation was operating. Revus SC was applied at 345 kPa using a backpack sprayer with a 2 nozzle spray boom and XR8002 nozzles (TeeJet, Spraying Systems Co., Wheaton, IL) directed at a 45° angle towards the plant crown. The trial was conducted in 2014 and 2015.

The plants were inoculated two and a half weeks after planting with *P. capsici* infested millet seed. Millet inoculum was prepared as previously described (Quesada-Ocampo and Hausbeck 2010) using *P. capsici* isolates OP97 (A1 mating type, mefenoxam sensitive, isolated from pickling cucumber fruit) and 12889 (A1 mating type, mefenoxam resistant, isolated from pepper fruit). The isolates were obtained from the culture collection of Dr. M. Hausbeck at Michigan State University and were inoculated into cucumber fruit and subsequently recovered from the diseased fruit to ensure isolate virulence prior to the study. Pepper plants were inoculated by placing 2 g of infested millet into a depression made in the soil 2 to 3 cm from the crown of a plant and covering with soil. Infested millet of each isolate was mixed 1:1 prior to inoculation.

Disease rating and harvest.The number of plants killed by *P. capsici* were counted each week and AUDPC values and the percentage of plants killed were calculated for each main plot. Fruit from all plants in a treatment row were harvested by hand and graded based on weight and

Table 5.2. Mean air temperature and precipitation at SWMREC during Phytophthora root rot evaluations in 2014 and 2015.

Location		Air temperature (°C)[z]			Precipitation (cm)		
	Year	Jun.	Jul.	Aug.	Jun.	Jul.	Aug.
SWMREC	2014	69.2	66.6	70.4	14.7	9.7	6.1
	2015	68.0	70.8	70.2	8.6	9.9	7.1

[z] Air temperature and precipitation were measured using a weather station located at SWMREC

appearance using a scale developed to represent commercial standards for fresh market pepper sales in Michigan. Plants were harvested 5 and 4 times in 2014 and 2015, respectively. Peppers were graded as Jumbo = >230 g; extra-large = 190-229 g; large = 160-189 g; medium = 130-159 g; small = <130 g; No. 2 = irregular shape, minor blossom end rot, or superficial blemish; cull = *P. capsici* or fungal fruit rot, bacterial spot, or insect damage. Size-graded fruit were unblemished, primarily green, with three to four lobes. No. 2 fruit were considered marketable to a processing or secondary market, and cull fruit were unmarketable.

The Statistical Analysis System (SAS v9.3) was used to analyze trial data. Area under disease progress curve (AUDPC) values were calculated using disease incidence data and the method of Shaner and Finney (1977). Differences among AUDPC values were analyzed with Proc GLM ($P = 0.05$). Disease incidence data was analyzed with ANOVA and Fisher's LSD test ($P = 0.05$). Differences in yield among treatments based on data taken as total fruit weight, total count, and mean marketable weight for individual fruit were analyzed with Proc Mixed. Fungicide and entry were considered fixed effects and block a random effect. There was no interaction of fruit weight with fungicide treatment within years and data were pooled prior to analysis. Normality of residual data was assessed with Proc Univariate and Proc Gplot.

RESULTS

The inoculation method used in this study provided uniformly high disease pressure throughout the plot; symptoms of Phytophthora blight were observed 2 to 3 wk post-inoculation on ‘Camelot X3R’ in the untreated plot both years of the trial. Symptoms included wilt, sunken black lesions on the lower stem and crown, and girdling of the lower stem at the soil-line. ‘Camelot X3R’ had a significantly higher AUDPC value than the other entries in 2014 (Table 5.3). In 2015, the AUDPC values for ‘Camelot X3R’, ‘Aristotle’, and ‘AP4835’ were higher than the other entries (Table 5.3). Final disease incidence was highest for ‘Camelot X3R’ in treated and untreated plots in 2014 and 2015 (Table 5.3). In 2014, all entries other than ‘Camelot X3R’ had < 10% incidence of wilt and plant death in fungicide treated and untreated plots. ‘AP4835’, ‘Aristotle’, ‘13SE12671’ and ‘AP4841’ had > 10% plant death in the untreated plot in 2015. The fungicide program reduced disease incidence of all entries (Table 5.3), however, there was no fungicide program x entry interaction ($P = 0.05$).

The ‘Resistant Standard’ yielded the largest number of marketable fruit in 2014 and 2015 in untreated plots (Table 5.4). In 2014, all entries in the fungicide treated plots other than ‘Camelot’ and ‘AP4841’ yielded similarly, with an average of 155 to 177 fruit per row. The ‘Resistant Standard’ produced 212 fruit per row in 2015 in the ‘fungicide treatment’ plot, significantly higher than the other entries (Table 5.4). The ‘Resistant Standard’ and ‘13SE12671’ yielded the most marketable fruit by weight in 2014 and 2015 with 21 to 25 Kg fruit/ 5.5 m row (Fig. 5.1). ‘13SE12671’ had the largest mean marketable fruit size both years (Table 5.5). The average fruit size of ‘Camelot X3R’, ‘AP4839’, ‘AP4841’, and ‘Aristotle’ in 2014, and ‘Camelot X3R’,

Table 5.3. Mean area under disease progress curve (AUDPC) values and incidence of plant death for pepper entries evaluated at SWMREC for resistance to Phytophthora root rot.

Entry	AUDPC[x]				Disease incidence[y]							
					Untreated				Treated			
	2014		2015		2014		2015		2014		2015	
Camelot X3R	5353.9	a[z]	2814.0	a	98.6	a	91.6	a	83.3	a	87.3	a
Aristotle	401.4	b	950.9	b	8.3	b	29.4	b	4.2	bc	24.0	b
AP4841	386.0	b	276.5	cd	4.2	b	12.8	dc	4.3	bc	4.2	c
AP4835	367.6	b	541.1	c	8.3	b	21.1	bc	7.0	b	7.5	c
Archimedes	340.7	b	75.0	d	2.8	b	4.3	d	1.4	bc	0.0	c
AP4839	296.0	b	166.8	d	2.8	b	8.5	dc	2.8	bc	00	c
13SE12671	203.1	b	257.6	cd	8.3	b	11.1	dc	1.4	bc	2.8	c
Resistant Standard	0.0	b	23.3	d	0.0	b	1.4	d	0.0	c	0.0	c

[x] AUDPC values were calculated from disease incidence data according to the method of Shaner and Finney (1977). Data from fungicide treated and untreated plots were combined as there was no significant fungicide x entry interaction ($P = 0.05$).

[y] Disease incidence values based on final visual rating of plants for symptoms of Phytophthora blight. Values represent the mean of two trials with four replicate rows of 18 plants.

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

Table 5.4. Mean total count of marketable fruit from pepper entries evaluated for resistance to Phytophthora root rot at SWMREC.

Entry	Total marketable fruit count[x]							
	2014				2015			
	Untreated		Treated		Untreated		Treated	
Resistant Standard	169.8	a	174.3	ab	201.0	a	212.5	a
AP4839	162.8	a	165.0	ab	136.3	c	164.5	b
AP4841	160.0	a	145.5	b	155.3	bc	145.5	b
Archimedes	157.0	a	177.5	a	169.5	ab	166.0	b
AP4835	153.8	a	155.5	ab	123.3	c	153.8	b
13SE12671	150.5	a	159.0	ab	170.8	ab	164.3	b
Aristotle	148.5	a	168.8	ab	144.5	bc	153.3	b
Camelot X3R	26.0	b	54.5	c	41.5	d	49.3	c

[y] Mean count of marketable fruit (culls excluded) per 18 ft row harvested 5 and 4 x in 2014 and 2015, respectively.

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

'AP4839', 'AP4841' and the 'Resistant Standard' in 2015 were significantly smaller than '13SE12671'. 'Archimedes' and 'AP4839', and 'AP4841' and 'AP4839', had the greatest proportion of fruit graded as No. 2 in 2014 and 2015, respectively (*data not show*). Over 50% of fruit harvested from 'Camelot X3R' were graded as cull in both years; there were < 15 and 30 % cull fruit for all other entries in 2014 and 2015, respectively.

Figure 5.1: Marketable fruit (size graded and No. 2 fruit) harvested from pepper entries evaluated for resistance to Phytophthora root rot at SWMREC in 2014 and 2015. Totals represent kg fruit per 5.5 m row.

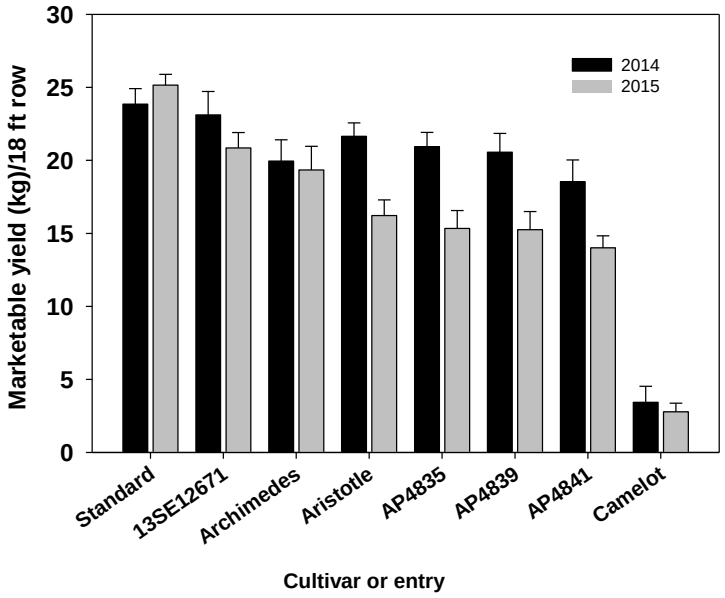

Table 5.5. Mean weight of individual marketable fruit harvested from pepper entries evaluated for resistance to Phytophthora root rot at SWMREC.

Entry	Marketable fruit wt (g)[x]			
	2014		2015	
13SE12671	194.0	a	182.0	a
Archimedes	187.8	ab	172.8	ab
AP4835	185.2	ab	174.0	ab
Resistant Standard	183.9	ab	168.5	b
Camelot X3R	178.5	bc	167.9	b
AP4839	177.4	bc	164.3	b
Aristotle	177.2	bc	171.9	ab
AP4841	166.6	c	165.4	b

[y] Mean weight (g) of individual marketable fruit per 18 ft row harvested over 5 and 4 x in 2014 and 2015, respectively.

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

DISCUSSION

Cultivars and experimental entries with resistance to Phytophthora root rot displayed a range of disease responses under heavy pathogen pressure in this study. Limited disease developed on the 'Resistant Standard' and 'Archimedes' each year, and resistance levels were comparable to the experimental entries. In recent field and greenhouse experiments, high levels of resistance to Phytophthora root rot in bell pepper breeding lines and commercial cultivars were observed (Dunn et al. 2014, Foster and Hausbeck 2010, Wyatt et al. 2013). In New York field trials, 'Paladin', 'Intruder', and 'Archimedes' were resistant to Phytophthora blight (Dunn et al. 2014). Foster and Hausbeck (2010) inoculated pepper breeding lines and cultivars at the 3-4 true leaf stage with *P. capsici* isolates from Michigan, and found four breeding lines resistant to all isolates tested. 'Revolution', 'Paladin', and 'Declaration' were the most resistant cultivars, however, were killed by a single virulent isolate. Root rot of commercial pepper cultivars considered resistant to Phytophthora has been observed in NJ field production (Johnston et al. 2002). The resistance genes of the pepper are known to affect the rate of resistance breakdown (Palloix et al. 1988) and Granke et al. (2012) suggested that shifts in virulence of *P. capsici* populations may occur where resistant cultivars are widely planted. *P. capsici* is known to increase in virulence after sexual reproduction and genetic recombination of parent isolates (Satour and Butler 1968) and A1 and A2 mating types of *P. capsici* are present in major pepper growing regions of the U.S., often in the same field (Hausbeck and Lamour 2004, Ploetz et al. 2002, Ristaino 1990). Additionally, temperatures unfavorable for plant growth (Café-Filho and Duniway 1995), length of contact time with inoculum (Barksdale et al. 1984), and splashing of infested soil onto stems and foliage (Elenkov 1977, Schlub 1983) may result in disease development on resistant cultivars. Further, resistance of stems, leaves and roots to *P. capsici*

are considered to be under the action of separate genetic systems (Foster and Hausbeck 2010, Sy and Bosland 2006, Walker and Bosland 1999). Large lesions developed on fully expanded detached leaves of mature plants of all entries in this study after inoculation with a *P. capsici*-colonized V8-agar plug and incubation for 3 d in a moist chamber (C. Krasnow, *unpub. data*.). Additionally, the fruit of Phytophthora root rot resistant pepper cultivars are susceptible to *P. capsici* (Foster and Hausbeck 2010, Naegele et al. 2013). Limiting soil splash will remain important when resistant peppers are grown.

In addition to root rot resistance, large fruit size and uniform quality are desired for fresh market sales (Ristaino and Johnston 1999). Small fruit size has precluded adoption of some resistant cultivars (Ristaino and Johnston 1999, Wyatt et al. 2013) especially where Phytophthora blight is perceived as a minor problem (Hwang and Kim 1995). Breeding lines tested in a field trial in New York had high levels of root rot resistance, however, total yield and fruit size was lower than for the commercial cultivars tested (Wyatt et al. 2013). In the current study, Paladin was the highest yielding cultivar, however, fruit were smaller than other entries. The total yields of '13SE12671' in the untreated plot was comparable to the commercially resistant cultivars and '13SE12671' had the largest average fruit size among entries. Phytophthora root rot resistance has also been correlated with the physiological disorder silvering, a separation of the cuticle from underlying epidermal cells of the fruit (Wyenandt and Kline 2006). Silvering of fruits from resistant entries was noted infrequently in the current study (C. Krasnow and M. Hausbeck, *unpub. data*.).

In this study, the fungicide program limited plant death and increased yield of the resistant cultivars. Foliar fungicide sprays have traditionally been used to manage *P. capsici* diseases (Hausbeck and Lamour 2004) however are considered less effective than soil-directed

sprays and applications via drip irrigation (Foster and Hausbeck 2010, Meyer and Hausbeck 2013). In a greenhouse trial, soil-drenches reduced pepper root rot more than foliar sprays of the same fungicides (Foster and Hausbeck 2010). Foliar sprays of fluopicolide did not protect tomato plants from *P. capsici* (Jiang et al. 2014). However, fluopicolide effectively reduced Phytophthora blight of summer squash in field trials when applied as foliar sprays or through drip injection (Jackson et al. 2010). The reduction in root rot realized with the relatively long application interval of the fungicide program in the current study was similar to our observations with a drip-applied fungicide program to protect winter squash (*Cucurbita* spp.) from *P. capsici* root rot (Hausbeck and Krasnow 2014). Yeh (1991) suggested basing the number of fungicide applications on the resistance of the pepper cultivars planted. As '13SE12671' has high levels of root rot resistance and yielded similar to commercially available resistant cultivars in this study, performance in the Great Lakes region could be expected. Additional research on the use of *P. capsici* specific fungicide programs that can optimize the yield of resistant cultivars would be beneficial.

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