HOST SUSCEPTIBILITY TO TWO PERONOSPORALES AMONG CUCURBITS AND BEANS

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ABSTRACT

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The Order Peronosporales, a group of fungal-like organisms commonly known as the water molds, incite many of the most devastating plant diseases worldwide. Two particular Peronosporales, *Phytophthora capsici* Leonian and *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov., are a major threat for many Michigan vegetable crops. *Phytophthora capsici,* a limiting factor mainly for cucurbitaceous and solanaceous production, was confirmed to infect snap bean (*Phaseolus vulgaris* L.) in Michigan fields. Since dry edible beans are a variant of the snap bean species, we assessed eight dry edible bean cultivars for susceptibility to *P. capsici*. Different plant parts were inoculated independently with each of two *P. capsici* isolates. Results indicated that dry edible beans are susceptible to *P. capsici* under controlled conditions.

Cucurbit downy mildew, caused by *Pseudoperonospora cubensis* is the most devastating foliar disease for cucurbits. Forty-one cucurbit cultigens (i.e., commercial cultivars and plant introductions) from five different genera were assessed for their susceptibility to natural infection by *P. cubensis* in a Michigan research field during 2010 and 2011. Results confirmed that *Cucumis* is the most susceptible genus to downy mildew. *Citrullus* cultigens presented small necrotic lesions, and no visible infection was found on cultigens of *Cucurbita moschata* and *C. pepo*.

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LITERATURE REVIEW

INTRODUCTION

The Order Peronosporales is comprised of a group of fungal-like organisms commonly known as the water molds, which were previously classified as true fungi (99). More recently, this group has been placed within the Kingdom Straminipila, which also includes diatoms and algae. Thus, the order Peronosporales is more closely related to the heterokont algae than true fungi (99). Organisms belonging to the order Peronosporales incite many of the most devastating plant diseases worldwide (3, 46). The most notorious examples include *Phytophthora infestans* (Mont.) de Bary, the causal agent of potato late blight that was responsible for the mass emigration from Ireland during the great famine, and *Plasmopora viticola* (Berk. & M.A. Curtis) Berl. & De Toni, the causal agent of downy mildew on grapes, which caused massive economic losses during the 19th century in Europe (50, 104). Today, growers worldwide struggle with frequent and significant economic losses caused by Peronosporales (43, 55).

In the current study, the pathogenicity of *Phytophthora capsici* Leonian on dry edible beans and *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostov. on cucurbits was investigated. In Michigan, these pathogens place vegetable crops at significant risk (62).

THE PATHOGEN: *Phytophthora capsici* Leonian

During the past few years, growers around the world have discovered the challenges of controlling Phytophthora symptoms in numerous economically important crops (63, 70). *Phytophthora capsici* is a devastating pathogen on a broad range of plants, including vegetable crops as well as tropical and woody ornamental plants (63, 65, 69, 70). Increased production costs and pathogen resistance to fungicides have resulted from the intensive chemical input required to control this disease (13). Although the frequency of fungicide sprays may be high, the efficacy is often moderate (30, 72). As a result, cultural management practices have been implemented in addition to chemical control to mitigate the losses caused by *P. capsici* (81, 124). Among the cultural practices applied, rotational schedules with nonhost plants are typically ineffective due to the persistence of *P. capsici* oospores in the field and the limited number of nonsusceptible vegetable crops (82). Highly resistant cultivars of susceptible crops are not yet available for all *P. capsici* symptoms (107). Moreover, breeding for resistance in this pathosystem is challenging, primarily due to the multiple disease symptoms caused by *P. capsici* (i.e. foliar and stem blight, and fruit, root and crown rot) and the inheritance mechanism of the genes that govern resistance in each plant tissue (136). In Michigan, *P. capsici* is a threat to a total of 81,400 acres (32,941 ha) of vegetables and woody ornamentals valued at $179.264 million dollars in 2011 (Table 1.1) (10).

TAXONOMY

Phytophthora capsici taxonomy has not significantly changed since the establishment of the oomycetes as a group apart from the Eumycetes. Currently, there are some ambiguities in naming certain taxa within *Phytophthora*. According to the most rigorous rules under the International Code of Botanical Nomenclature (ICBN), the mislabeled Class Oomycetes should be renamed Peronosperomycetes (102), as a generic name like Oomycetes cannot be adopted as a name for taxa above family. In the most recent Peronosperomycetes taxonomy (17), *Phytophthora capsici* belongs to the Kingdom Straminipila (syn. Chromista); Phylum Heterokonta; Class Peronosporomycetes (syn. Oomycetes); Order Peronosporales and Family

Peronosporaceae along with the other most economically significant plant pathogens such as *Phytophthora infestans, Phytophthora ramorum* and *Plasmopora viticola*. Nevertheless, in most recent studies, researchers have refused to adopt the new Peronosperomycetes nomenclature in favor of the traditional Oomycetes name (17). Although there are molecular data that suggested a revision of some taxonomical changes proposed by Dick (17), this document follows the most recent taxonomy to comply with the rules of the ICBN.

MORPHOLOGY AND PHYSIOLOGY

Microscopic structures that identify *P. capsici,* such as caducous sporangia that are papillated and possess large pedicels (greater than 50 μm), are easily detectable using a compound light microscope (51). The size and shape of *P. capsici* sporangia are variable, but are typically classified as ovoid to ellipsoidal and are generally 57 μm long and 32 μm wide. Sporangia are produced on the tips of sympodial sporangiophores that are hyaline and coenocytic (151). In contrast with some *Phytophthora* spp., *P. capsici* chlamydospore (asexual resting spore) production is rare. As a heterothallic species, *P. capsici* requires physical contact between two different thalli, designated A1 and A2, to form oospores (151). Plerotic oospores (filling the oogonium) with an approximate diameter of 29 μm are produced as result of plasmogamy and karyogamy processes following fertilization of spherical oogonia (approx. 35 μm) by amphigynous antheridia (14 μm) (151).

Phytophthora capsici and *P. tropicalis* Aragaki & J.Y. Uchida are morphologically similar (150). Traditionally, the criteria to separate these two species were based on host range and physiological traits (5). For instance, isolates obtained from vegetables were *P. capsici,* whereas isolates from tropical plants such as macadamia, cacao, and black pepper were *P.*

tropicalis (145). However, the distinction based on host range is not conclusive due to hosts being susceptible to both of these two species of *Phytophthora* (11). Although there are some slight differences regarding the sporangia shape, a major physiological criterion to differentiate these two species is the growth temperature (51). The majority of *P. capsici* isolates grow at 35ºC whereas many *P. tropicalis* isolates show sparse or no growth at this temperature (11).

Among Peronosperomycetes, *P. capsici* is one of the few species that can be readily cultivated on artificial media. Hence, *P. capsici* colonies on V-8 media are characterized by a white mycelial growth and lemon-shaped sporangia production that varies in abundance depending on temperature and light conditions as well as the isolate (71). Various colonial patterns can be formed depending on the strain, but petalloid and stellate patterns are more common (71).

MOLECULAR IDENTIFICATION TECHNIQUES

Currently, a rapid and reliable technique for identifying plant pathogens such as *P. capsici* is imperative for successful management of plant diseases (100). Molecular-based tools have recently been routinely used for quick identification (100, 131). Molecular-based techniques are valuable for diagnosis, especially when pathogen isolation is difficult to perform (131). Molecular techniques may be able to overcome difficulties in isolating *P. capsici* from infections that occur on woody and fibrous tissues such as pepper stems or Fraser fir roots or when the amount of pathogen is low such as in samples from infested bodies of water (53, 117, 154).

In addition to detection and identification, molecular techniques are currently used to define species within Peronosperomycetes and to study pathogen populations (118, 134). For these purposes, traditional molecular markers such as internal transcribed spacers (ITS) have been frequently used (155). However, in closely related species, more than one molecular marker or more specific primers are necessary to generate conclusive results. For example, between *P. capsici* and *P. tropicalis* the ITS sequence has the same length but differs in just 10 nucleotides (155). Hence, a set of nested primers has been used to improve the specificity of *P. capsici* detection in order to reduce the possibility of amplification of the host ITS region (154).

Recently the *P. capsici* genome has become available (83). As a result, a better understanding of molecular interactions between host and pathogen as well as population studies that contribute to evolutionary knowledge can be enriched through application of the genome information (40). A total of four *Phytophthora* genome sequences are accessible: *P. capsici, P. infestans, P. sojae* and *P. ramorum*. The *P. capsici* genome is approximately 65 Mbp with an estimated of 12,011 encoded genes (83).

DISEASE CYCLE

Phytophthora capsici produces sexual and asexual propagules that play important roles in dispersal and host infection. Depending on environmental conditions, mycelia, sporangia, zoospores and/or oospores are produced (75). Moreover, the primary inoculum varies among regions (69, 81). In temperate regions, thick-walled oospores are the primary source of inoculum due to their persistence in soil and ability to survive under adverse environmental conditions, whereas in tropical areas asexual propagules such as zoospores and sporangia that can survive on wild hosts or plant debris from previous seasons are the primary inoculum (69, 81).

Sporangia and zoospores are propagules generated asexually in copious numbers to disseminate the pathogen (46). These two propagules have highly active metabolism, making them extremely sensitive to environmental changes (75). Therefore, sporangia and zoospores cannot survive moderate changes in solar radiation or nutrient deprivation (86). A single sporangium can germinate directly producing a germ tube or indirectly producing between 20 to 40 reniform zoospores through cytoplasmic cleavage (150). Depending on temperature, age of sporangium, and substrate matric potential, germination can occur directly via a germ tube or indirectly through zoospore production (20, 22). Direct sporangial germination in *P. capsici* occurs at temperatures higher (>10ºC) than that required for zoospore production (63). Sporangia are less vulnerable to desiccation than nonencysted zoospores. The thin walls of sporangia, composed of cellulose and ß-glucans contribute to their longer lifespan in soil (4 to 8 weeks) compared to zoospores that do not have walls (16, 86). Zoospores are produced in larger quantities than sporangia and account for higher levels of disease infection (137), but can only survive up to 5 days in water within a temperature range of 9 to 32ºC (57). Biflagellate zoospores can actively swim toward the host through chemotaxis or are passively transported via irrigation or rain splashing (2, 124). Upon reaching a susceptible host, zoospores encyst (lose their flagella) and form a germ tube to infect and colonize the plant tissue (123).

Phytophthora capsici can persist for extended periods of time in the soil as thick-walled structures called oospores (63). In temperate regions, oospores are considered the source of primary inoculum (81). Once a field is infested with oospores, eradication is difficult (63). A dormancy period of approximately 30 days is required for oospores to germinate (80). When a susceptible host is planted and the environmental conditions are favorable, oospores can germinate directly by forming a germ tube or indirectly by producing sporangia (123).

HOSTS

Approximately, 28 different plant families are affected by *P. capsici* worldwide (47). Economically important vegetable crops produced in Michigan include members of the Solanaceae, Cucurbitaceae and more recently, Fabaceae family are threatened by this pathogen (Table 1.1) (46, 63). Additionally, a few ornamental plants within the Solanaceae and Fabaceae family and a woody ornamental in the Pinaceae family are potentially susceptible to *P. capsici* in Michigan greenhouses and fields respectively (44, 117).

Table 1.1 Cultivated area and production value of susceptible hosts to *Phytophthora capsici* for Michigan in 2011 according to USDA National Agricultural Statistics Service (10).

Commodity	**Cultivated area acres (ha)**	**Harvested area acres (ha)**	**Value of production (1,000 dollars)**
Solanaceae			
Bell pepper	1,400 (567)	1,300 (526)	$12,636
Tomato			
Fresh	2,100 (850)	2,000 (809)	$17,600
Processing	3,500 (1,416)	3,500 (1,416)	$11,340
Cucurbitaceae			
Cucumber			
Fresh	3,800 (1,538)	3,700 (1,497)	$16,169
Processing	32,400 (13,112)	31,600 (12,788)	$45,125
Squash	6,500 (2,630)	6,400 (2,590)	$25,536
Pumpkin	7,200 (2,914)	6,800 (2,752)	$16,762
Fabaceae			
Snap bean			
Fresh	3,000 (1,214)	2,900 (1,174)	$8,800
Processing	15,900 (6,435)	15,600 (6,313)	$14,736
Woody ornamental			
Fraser fir [z]	NA	7,600 (3,076)	$10,560
Total Vegetables	**75,800 (30,675)**	**73,800 (29,866)**	**$168,704**
Total Vegetables + Fraser fir	**NA**	**81,400 (32,941)**	**$179,264**

[z] Data from 2004-2005 published in USDA Nursery Crops and Christmas tree rotational survey (7). NA: Not available data

Peppers. *Capsicum annuum* L. was the first reported species infected by *P. capsici* (96). Incidence and severity of *P. capsici* has noticeably increased on susceptible vegetables worldwide since 1922, when it was first described causing severe disease on chili pepper in New Mexico during 1918 (63, 70). Symptoms caused by this pathogen depend upon the plant part infected. Water splashing disperses soilborne *P. capsici* inoculum to aerial parts of the plant; thus fruit rots and foliar blights are more common in moderate to high-precipitation regions, whereas root and crown rots prevail in warm and saturated soils (4, 148). Infected roots become girdled resulting in plant decay; young plants may succumb in few days. As infection extends to the soil line, black lesions appear on the stem and a discoloration of the vascular system occurs, resulting in collapse of the entire plant (116).

Due to the soilborne nature of *P. capsici*, plant tissues close to the soil line are more prone to infection (124). Lesions on aerial parts of the plant (leaves and fruits) are most likely to occur by human dispersion during harvest or during periods of heavy rain where water splashing disperses the pathogen (4, 124). Foliar lesions appear water-soaked initially and then turn brown in color. Fruit lesions may begin near the pedicel with dark, water-soaked zones. As lesions enlarge and the fruit rots, it remains attached to the stem. During wet and warm periods, sporangia form and appear as a white powdery substance covering the lesion. Fruit rot symptoms may develop after harvest (116).

Genetic studies have determined that different genes govern *P. capsici* resistance for different disease symptoms in peppers (136, 148). For instance, the genes involved in stem blight resistance are different from those involved in root rot and foliar blight (136, 148). Hence, breeding for resistance for all disease symptoms is challenging. Although there are no pepper cultivars that are completely resistant to *P. capsici*, the commercial cultivar Paladin is tolerant to some selected pathogen isolates (107). However, research by Foster and Hausbeck proved that 'Paladin' plants succumbed to selected Michigan isolates of *P. capsici* (49). The cultivar Criollo de Morelos 334 displays a high level of resistance to *P. capsici* root rot and foliar blight (138).

Tomatoes. In Michigan, yields of *Solanum lycopersicum* L. plants grown for processing have been negatively affected by *P. capsici*. In total, fresh and processing tomatoes generate yearly revenue of $28.9 million USD in Michigan (Table 1.1) (10). *Phytophthora capsici* was first reported in Michigan tomato fields in 2003, when approximately 100 acres (40.5 ha) of processing tomatoes could not be harvested due to the disease (63, 82). High relative humidity, abundant soil moisture and warm weather favor symptom development. Symptoms resulting from *P. capsici* infection vary depending upon the plant age. Infected seedlings succumb rapidly to *P. capsici* and die, whereas infected mature plants develop fruit, crown, and root rot (125). Fruits that are in contact with the soil are at high risk of becoming infected regardless of fruit maturity. Initially, fruit rot lesions appear as dark brown and water-soaked spots that enlarge over time forming concentric rings of light and dark brown bands (74). Mature ripe fruits that are infected by *P. capsici* are also likely to become infected by secondary pathogens; when young green fruits become infected they may become mummified (135).

Stem blight and root rot lesions on tomatoes are somewhat similar to those produced on bell pepper, another solanaceous crop; however, tomato plants are more tolerant to root rot than bell pepper plants (35). Root rot tolerance is governed by quantitative genes that are involved in secondary root regeneration, which compensate for the infected roots resulting in healthy canopies (21). Two commercial tomatoes, 'Jolly Elf' and 'Tallega' were moderately resistant to infection by each of four highly virulent *P. capsici* isolates under greenhouse conditions (121). An accession of *Solanum habrochaites,* a wild relative of cultivated tomato, was found to be resistant to crown and root rot caused by *P. capsici*. Thus, *S. habrochaites* may offer potential in a tomato-breeding program (121).

Cucurbits. The vast majority of economically important cucurbit crops in Michigan are susceptible to *P. capsici* (63). The first report of *P. capsici* devastating a cucurbit planting was published in 1937 when an entire cucumber harvest was lost due to fruit rot (77, 144). Since then, enormous economic losses caused by this pathogen have occurred on several cucurbitaceous crop plants (12, 63). In Michigan an estimated area of 48,500 acres (21,732 ha) of cucurbit crops is threatened by this pathogen every season (Table 1.1) (10). Depending upon the cucurbit species, all plant parts are potentially susceptible to infection. Cucumber plants are more tolerant to root rot, whereas squash plants are highly susceptible (63). As a soilborne pathogen, the tissues that are frequently infected include roots, crown, vines and fruits laying on the soil (124). Although foliar lesions rarely occur in Michigan, leaf blight has been observed during periods of heavy rain and warm weather (48). Fruits of cucumber are especially susceptible to *P. capsici* (52). Fruit lesions begin as sunken, irregular, water-soaked spots that become dark and circular over time (52). Eventually, the whole fruit is covered by white powdery sporangia and mycelia and may decay rapidly under environmental conditions that are optimal for the pathogen (52). Like peppers, latent infections that develop during crop transport may cause rejection of semi-loads of fruits (63).

Contrary to pepper fruits that are potentially infected at any stage of development, cucurbit fruits become more resistant as they mature (6, 52). However, wounding appears to break the age-resistance mechanism in cucumber fruits; susceptibility also increases in wounded pepper tissue (2, 58). Although there are no commercial cultivars resistant to all of the symptoms caused by *P. capsici*, there are a couple of tolerant cultivars that have been identified within each cucurbit type. For example, the pumpkin 'Danmatmaetdol' is highly resistant to root and crown rot (95). Cucumber fruits of cultivars Discover, Excel and Vlaspik developed lesions that were reduced in diameter and sporulation compared with the other cultivars tested (52). Within *Cucurbita moschata* Duchesne ex Poir. (squash), five plant introductions (PI 176531, PI 458740, PI 442266, PI 442262 and PI 634693) have been identified as highly resistant to crown rot (27). Moreover, a *Cucurbita* breeding line resulting from the introgression of two wild *Cucurbita* spp., *C. lundelliana* and *C. okeechobeenesis* subsp. *okeechobeenesis,* exhibited high levels of resistance to crown rot caused by *P. capsici* (111).

Ornamentals. Reports of *P. capsici* infecting ornamentals were reported before 2001 when the distinction between *P. capsici* and *P. tropicalis* was unclear (11, 37). When the first report of *P. capsici* causing disease on *Calibrachoa* spp. Cerv. was researched further by using sequencing data it was determined that the causal agent was actually *P. tropicalis* (37, 119). As a result, there are no reports of natural infections of *P. capsici* on ornamental plants. However, artificial inoculations show that seven million bells cultivars (*Calibrachoa* x *hybrida*), lupine (*Lupinus polyphyllus* Lindl.), flowering tobacco (*Nicotiana* x *sanderae*), and sweet pea (*Lathyrus latifolius* L.) were susceptible to *P. capsici* root rot under greenhouse conditions (44). For this reason, scouting for this disease would be wise since there is a possibility that *P. capsici* could endanger floriculture plants and many Michigan greenhouses that are producers of both floral crops and vegetable transplants (44). In Michigan, possible damage caused by this pathogen on floral crops would negatively affect the state's economy since floriculture represents the fourth largest commodity in cash receipts. Michigan ranks third in the U.S. for total wholesale value of floriculture crops after California and Florida in 2011 (8, 73).

Fraser Fir. Besides the flowering plants mentioned above, Fraser fir (*Abies fraseri* (Pursh) Poir.), a woody ornamental, is susceptible to *P. capsici* infection (117). In nature, this popular Christmas tree is mainly infected by *Phytophthora cinnamomi*; however, other *Phytophthora* spp. including *P. capsici*, can cause root rot and shoot blight (26). As demonstrated by Quesada-Ocampo et al., *P. capsici* was able to cause disease on Fraser fir under controlled and field conditions, through artificial and natural infections, respectively (117). A high percentage of root rot incidence caused by *P. capsici* was obtained when seedlings were planted in a naturally-infested field (117). Infected roots exhibited reddish to black coloration and a distinctive "root sloughing" symptom where the inner root core remained after the outer layer had pulled away (26). Root rot infections caused by *P. capsici* on Fraser fir trees resulted in reddish to brown needles that remained on the branches causing a generalized bronzing of the tree foliage as the disease progressed (26).

Beans: A novel *P. capsici* host

Historically, snap beans and dry edible beans have been included as rotational crops for *P. capsici*-susceptible vegetables. However, a study showed that snap beans can be a host for *P. capsici* and they are no longer recommended as a rotational crop (54, 63). *Phytophthora capsici* is commonly found infecting solanaceous and cucurbitaceous crops, but over the last decade, *P. capsici* has been reported on lima beans (*Phaseolus lunatus* L.) in Delaware in 2000 (38), and on snap beans (*Phaseolus vulgaris* L.) in Michigan in 2003 (54), New York in 2008 (101) and Connecticut in 2010 (78). Bean roots do not appear to be susceptible to the pathogen; however, stems, leaves, and pods become infected under warm and wet conditions (54). *Phytophthora capsici* symptoms on bean plants include reddish to brown stem lesions that rapidly expand resulting in plant wilting, water-soaked foliage, and dark brown and sunken lesions on pods that become covered with the sporulating pathogen in a matter of days (54, 78, 101).

Growers have long speculated that soybean plants (*Glycine max* (L.) Merr.) may be a host for *P. capsici* based on increased disease incidence observed when *P. capsici*-susceptible vegetables followed soybeans in the cropping rotation (M.K. Hausbeck, personal communication, 2011). However, there are no data available that support *P. capsici* infection of soybeans under field conditions (54). Studies performed under controlled conditions showed that soybean leaves, especially in early stages of growth, are susceptible to different *Phytophthora* spp. including *P. capsici* (149). More recently, a study performed by Gevens et al. (54) showed that one-month-old soybean plants develop leaf blight caused by *P. capsici*, but roots did not become infected.

DISEASE MANAGEMENT

Management of *P. capsici* is achieved via combination of cultural and fungicide-based strategies. Fungicides must be applied frequently to suppress *P. capsici*, a practice that is not sustainable because the profit margin is low for many susceptible crops (12, 63, 114). Once the pathogen is established in the field it is difficult to eliminate it due to the persistence of the oospore in the soil (63, 70, 79). There is an increasing lack of uninfested fields in Michigan hampering the production of cucurbitaceous and solanaceous vegetable crops (63). Currently, alternative measurements including adjusting cultural practices and judicious use of fungicides have been recommended.

Cultural practices to manage *P. capsici* include planting in raised beds or staking tomatoes to avoid direct contact of the fruits with the soil (124); however, these practices may not be feasible for processing vegetable growers (63). Additional cultural strategies include good water drainage, use of uninfested water for irrigation, and increased spacing among plants and rows (105). A minimum of a two-year rotation with nonhost crops is recommended, but due to prolonged survival time of the *P. capsici* oospore and limited profitability of rotational crops, this practice is not a stand-alone tool (79).

Planting resistant cultivars is the most attractive and viable control strategy to limit losses resulting from plant diseases. However, since each *P. capsici* disease symptom appears to be governed by different genes, it is difficult to develop a completely resistant cultivar (136), and the genetic mechanisms for some hosts are unknown. However, there are some commercially available pepper lines tolerant to the syndromes caused by *P. capsici*. Gene introgression using wild parents in breeding programs has shown successful results for some cucurbits (27, 95, 111) and few solanaceous crops (107, 121, 138).

Before methyl bromide was phased out in 2005 for contributing to the ozone layer depletion, this chemical was the best alternative to control numerous soilborne pathogens in different crops including *P. capsici* in peppers and cucurbits (24). Application methods for some of the fungicides registered to manage *P. capsici* have recently been reevaluated. Products registered for foliar use to control *P. capsici* in cucurbits may have improved efficacy when applied as drenches (103). Michigan growers rarely observe foliar blight in cucurbits, but fruit, root and crown rot commonly occur (48). Because of the diploid nature of *P. capsici* and the dominant or semidominat genes involved in fungicide resistance, there is a high risk of *P. capsici* to develop resistance to several fungicides (64). Additionally, the ratio for each mating type, A1 and A2, in Michigan is 1:1, which contributes to the fast pace at which populations genetically change (79). Phenylamides, such as mefenoxam, are no longer recommended for disease management because of mefenoxam-insensitive isolates that are widespread in many fields in the U.S. including Michigan (63, 124). Interestingly, resistance to newly registered products, such as fluopicolide, has been detected in *P. capsici* isolates that were never exposed to this active ingredient (72, 97)

CUCURBIT DOWNY MILDEW: *Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev

Cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, is widely distributed from temperate to tropical regions wherever cucurbits are cultivated (112, 113). Warm and humid regions with annual precipitations greater than 300 mm provide optimal conditions for *Ps. cubensis* to thrive (29, 142). Particularly in the eastern U.S., cucurbit downy mildew is an annual disease affecting economically important crops such as cucumber, squash, cantaloupe, melon and watermelon (93). In regions south of 30 degrees latitude, such as Florida, the lack of seasonal frost promotes the presence of *Ps. cubensis* year-round as long as a host is present (29, 106).

TAXONOMY

Pseudoperonospora cubensis (Berk. & M.A. Curtis) Rostovzev belongs to the Class Peronosporomycetes (formerly Oomycetes) (39), which includes some of the most destructive plant pathogens, such as other downy mildew causal agents and members of the *Phytophthora* genus. Historically, *Ps. cubensis* has been associated with a series of misleading synonyms since 1868 when it was first described in Cuban plant material preserved in a herbarium (19). Berkeley and Curtis in 1868 initially named *Ps. cubensis* as *Peronospora cubensis* because of its resemblance to the sporangiophore, haustoria and sporangia color of the *Peronospora* genus (19). Further descriptions performed by Humphrey in 1891, renamed *Ps. cubensis* as *Plasmopara cubensis*, due to its similar reproduction strategy (biflagellate zoospore production via cytoplasmic cleavage of the sporangia) to the *Plasmopara* genus (68). Finally, the genus received its current name in 1903 when Rostovzev decided to classify it as *Pseudoperonospora*.

Taxonomically, *Ps. cubensis* belongs to the Kingdom Straminipila (syn. Chromista); Phylum Heterokonta; Class Peronosporomycetes (syn. Oomycetes); Order Peronosporales and Family Peronosporaceae (39).

DISEASE CYCLE

The principal dispersal structure of *Ps. cubensis* is the sporangium, which can be transported long distances by wind and thus introduced to cucurbit fields (85, 98). Once a sporangium reaches the leaf of a host plant, free moisture is crucial for the indirect germination of the sporangium into 2 to 15 biflagellate zoospores that are discharged through a papillum (84, 85). Morphologically, *Ps. cubensis* sporangia are ovoid to ellipsoid in shape and range from 20 to 40 μm long and 14 to 25 μm wide (157); zoospores measure 10 to 13 μm long (157).

Once the zoospores are discharged, they swim toward open stomata where they lose their flagella, encyst (85), form a germ tube that penetrates the stomata and colonizes the host cells by producing intercellular hyphae (112, 113). The pathogen takes up nutrients from plant cells through specialized hyphae called haustoria (112, 113). Once the infection cycle in the plant ends, new sporangiophores emerge from the leaf epidermis via stomatal openings (113). Once the sporangia are formed at the sporagiophore tip, a short dry period is required for the sporangiophore to twist thereby releasing the sporangia into the atmosphere (84). The reproduction of *Ps. cubensis* is mainly asexual. However, oospores (sexual propagules) have been sporadically reported in some countries outside the U.S. (14, 18, 36, 66). Thus, the role of oospores in the disease cycle of *Ps. cubensis* remains unclear mainly because of the low frequency with which they have been found and the stringent conditions under which they are produced (33).

Overwintering. Due to the biotrophic nature of *Ps. cubensis*, the survival of the pathogen in the absence of the host is impossible. Thus, a series of hypotheses about overwintering have been proposed to explain the occurrence of cucurbit downy mildew in areas above 30 degrees latitude, where frost destroys cucurbit crops (42, 142). Thick-walled oospores, which are resilient within hostile environments, have been sporadically reported in China (28), India (14), Iran (153), Israel (31) and Italy (36). However, overwintering of sexual spores has not yet been proven (32), although Cohen and Rubin (33) demonstrated that under specific conditions *Ps. cubensis* oospores are able to infect some cucurbit plants (33). Besides possible oospore formation, *Ps. cubensis* may potentially overwinter on a naturally occurring perennial plant host (126). Bryony or wild hop (*Bryonia dioica* Jacq.*)* is the only perennial cucurbit native to temperate zones in Europe that has developed downy mildew symptoms when it was artificially inoculated with *Ps. cubensis* (126). Runge and Thines (127) demonstrated that *Ps. cubensis* is able to infect hop leaves (*Humulus lupulus* L.), and proposed that there is a possibility of *Ps. cubensis* overwintering on hops rhizomes.

Epidemics of cucurbit downy mildew in areas with frost depend on the wind dispersion of the sporangia from year-round production sites such as subtropical regions or greenhouses (106, 108, 113). In central Europe, the inoculum spreads from southeast Europe (89), whereas in the eastern U.S. the inoculum comes from sites in Florida or Mexico (41, 106). In Michigan it is possible that the initial inoculum comes from cucumber production greenhouses located on the border with Canada, because *Ps. cubensis* sporangia are often detected in the air in Michigan prior to disease development in states located to its south (108). In addition, genetic analysis of *Ps. cubensis* populations showed similarities between the genetic cluster occurring in Ontario (Canada) and the cluster observed for Michigan isolates (120).

HOSTS

Many species within Cucurbitaceae, including cultivated, semi-cultivated and wild genera, are infected by *Ps. cubensis* under natural conditions (76, 112, 129). Species within approximately 20 genera in the Cucurbitaceae have been reported as hosts of *Ps. cubensis* (112, 113). Specifically, within the *Cucumis* genus, a total of 19 species are susceptible to downy mildew (87, 93). Among cucurbits, cucumber (*Cucumis sativus* L.) is the crop most commonly infected by *Ps. cubensis* followed by cantaloupe (*Cucumis melo* L.), whereas other economically important cucurbits such as pumpkin (*Cucurbita maxima* Duchesne), squash (*Cucurbita pepo* L.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) are less susceptible (91, 109). Additionally, less cultivated species such as loofahs (*Luffa* spp.), wax gourds (*Benincasa hispida* (Thunb.) Cogn.), bottle gourds (*Lagenaria* spp.) and chayotes (*Sechium edule* (Jacq.) Sw.) are also susceptible to *Ps. cubensis* infection (76, 93, 112). Besides cucurbitaceous plants, artificial inoculations performed under laboratory conditions have proved that *Ps. cubensis* may infect hop (*Humulus lupulus* L.), a member of the Cannabaceae family (127).

Symptoms of this foliar disease differ depending upon the crop. In cucumber, squash and loofah, the symptoms occur on the upper (adaxial) side of the leaf, and include irregular, pale-yellow lesions restricted by leaf veins. In cantaloupe and watermelon, lesions are not limited by the leaf veins and are more circular and regular (Figure 1.1). Eventually these lesions coalesce resulting in dried leaves that cup downwards (157). On the lower (abaxial) side of the leaf (Figure 1.1 B and E) lesions develop as gray-brown to deep purple spots with a dirty appearance that corresponds to sporulation of the pathogen (157). Although it is uncommon to observe sporulation on the upper (adaxial) side of the leaf, during heavy infections, sporulation is conspicuous on highly susceptible hosts (Figure 1.1 C and F) on both sides of the leaves (34).

Although *Ps. cubensis* infection is confined to the leaves the defoliation caused by this pathogen results in low productivity or poor quality fruit (113).

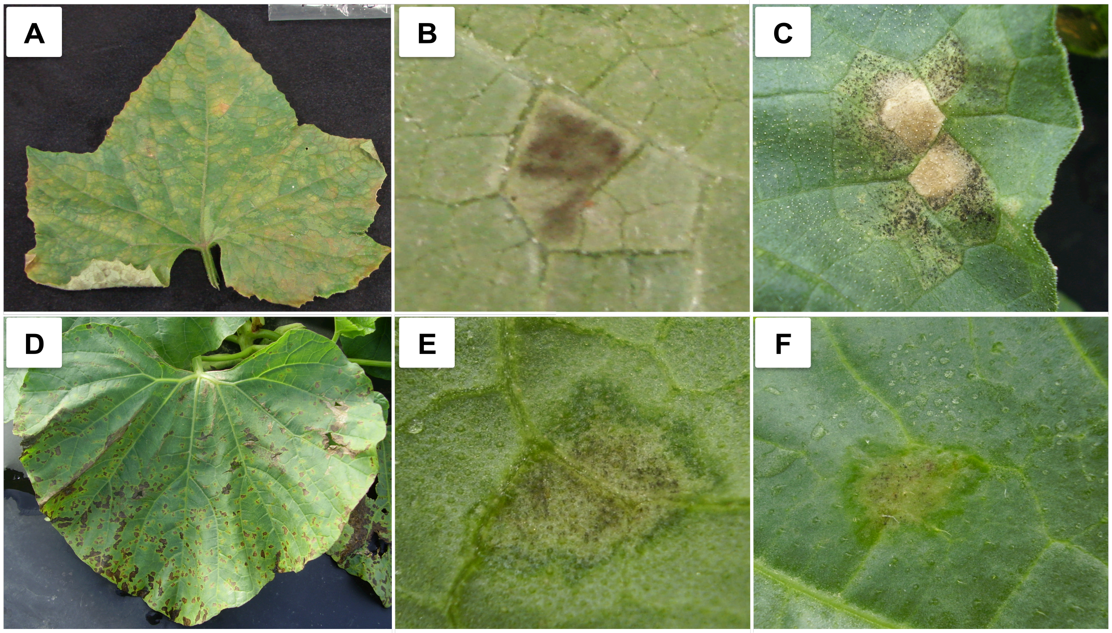

Figure 1.1 Symptoms of downy mildew caused by *Pseudoperonospora cubensis* on leaves of cucumber (**A** to **C**) and leaves of cantaloupe (**D** to **F**). **A,** symptoms on the upper side of a cucumber leaf. **D,** symptoms on the upper side of a cantaloupe leaf. **B** and **E** close-up of the underside of the leaf showing dark brown sporangia. Note the angular lesion restricted by the veins on the cucumber leaf (**B**) compared with the irregular lesion with no vein restriction on the cantaloupe leaf (**E**). **C** and **F** show uncommon sporulation on the upper side of the leaf, characteristic of heavy infections.
For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

DISEASE MANAGEMENT

Epidemics of downy mildew occur when large quantities of *Ps. cubensis* sporangia are available and the susceptible host is unprotected. Preventive precautions such as avoidance of overhead irrigation for field and greenhouse management are recommended since prolonged leaf wetness favors downy mildew (89). In addition, frequent ventilation and blue-pigmented polyethylene sheets significantly inhibit *Ps. cubensis* sporangial production in greenhouse and in plasticulture vegetable systems (122). However, once the disease is established, downy mildew can rapidly defoliate the crop before it is detected (60). Due to the biotrophic nature of *Ps. cubensis* and its aerial dispersion, two major disease control methods are used (89). One strategy is to limit downy mildew by selecting moderately resistant cucurbit cultivars; currently there are few options (88). The second disease management tool, and the most widely utilized, is frequent fungicide applications; however, this approach is expensive and pathogen resistance may develop (146). It is necessary to continuously scout for disease, since *Ps. cubensis* may quickly develop resistance to the most commonly used fungicides (128).

Breeding for Resistance. Since *Ps. cubensis* isolates may develop resistance to commonly-used fungicides, research in breeding for genetic resistance has been encouraged (25, 128). Although resistant cultivars have been developed, these cultivars are susceptible to the recently-emerged pathogen (23, 67). Such cultivars were developed in *Cucumis sativus,* but finding resistant sources in this species is difficult due to its low genetic variability (94). In the U.S., various resistant cultivars of cucumber have been released. For instance, cultivar Palmetto, released in 1948, developed few yellow lesions with sparse pathogen sporulation that classified this cultivar as highly resistant to downy mildew (15). However, the resistance in 'Palmetto' was overcome a couple of years after its release (45). In 1966, the cultivar Poinsett 76 was released and its resistance to downy mildew was attributed to a single recessive gene, *dm (dm-1)*, formerly designated as *p* (88, 147). This cultivar owes its resistance to the introgression of the plant introduction PI 197087, into its pedigree. Currently, most of the commercial cultivars that resulted from crosses with the PI 197087 (i.e. cultivars Gy 4, M21 and Poinsett 76) are intermediately resistant (152). In 1992, Doruchowsky and Lakowska-Ryk found three recessive genes mediating resistance in cucumbers in the cultivar Wisconsin-4783: *dm-1*, *dm-2* and *dm-3*

(42). Although some genes have been identified, the full mechanism of resistance to downy mildew in cucumber is not yet fully understood (1). Today, two downy mildew resistant slicing cucumber hybrids are available for U.S. and Canada growers. The cultivars SV3462CS and SV4719CS offered by Seminis® showed a good fruit shape, abundant foliage and an intermediate resistance to downy mildew when they were tested under high downy mildew pressure (9).

The inheritance mechanism for resistance in *C. melo* is not as well studied as cucumber. Early studies suggested that in melon is controlled by two partially dominant genes designated *Pc1* and *Pc2* (87, 138). More recently, it has been proposed that the inheritance of resistance to downy mildew in melon is quantitative and mediated by quantitative trait loci (QTL) (115, 132). Information about breeding for resistance to *Ps. cubensis* in other genera of Cucurbitaceae is limited, mostly because of the sporadic occurrence of *Ps. cubensis* infection on these plants (94). A few sources of resistance have been reported in some species within the *Cucurbita* and *Citrullus* genera (88, 92, 139).

The existence of pathotypes (defined as the variability of pathogenicity among genera) and physiological races (defined as the variability of pathogenicity within individuals from the same genus) are important aspects that should be taken into account when assessing disease resistance (90, 93). Differences in resistance levels between geographical points may be associated with the presence of local specialized isolates (133, 142). Moreover, the breakdown of resistance in some cultivars is attributed to the appearance of new pathotypes in the population of *Ps. cubensis* (31). Initially, Thomas in 1987 unified the researches on host range available at that time to propose the first set of cultivars for pathotype determination (141). This first set was based on the reaction of *Ps. cubensis* to three genera of the Cucurbitaceae (*Cucumis*, *Cucurbita* and *Citrullus*) by which five pathotypes were described in Japan, Israel and the U.S. (141). Based on this set, the presence of a new pathotype was detected in *Ps. cubensis* populations in Israel, which was able to infect *Cucurbita* spp., in addition to the previously reported species *Cucumis sativus* and *Cucumis melo* (31). Currently, an extension of the previous set proposed by Thomas et al., which had not included important host genera such as *Lagenaria*, *Luffa* and *Benincasa* (141), has been proposed. This new system is based on the compatible (disease)/incompatible (no disease) reaction of a certain *Ps. cubensis* isolate to 12 differentials that belong to six genera of the Cucurbitaceae family (*Cucumis, Cucurbita, Citrullus, Benincasa, Luffa and Lagenaria)* (93). Currently, a total of 67 pathotypes have been determined in the Czech Republic based on the reaction of *Ps. cubensis* to individuals of this new differential set (90). The shift to more virulent strains is observed by the establishment of the disease on species that were not previously reported in the Czech Republic, such as *Cucurbita* spp. and *Citrullus lanatus* (90).

Fungicides. In the absence of resistant cucumber cultivars, downy mildew control relies on frequent fungicide sprays (146). Historically*, Ps. cubensis* was controlled using protectant fungicides such as copper-based products, which were rapidly replaced by dithiocarbamates due to phytotoxicity produced by copper products (113). The inhibition of zoospore germination by protectant fungicides is highly effective, but the frequent applications that are required to protect the crop, substantially increase the production costs (146).

The development and use of systemic fungicides have recently contributed to the limitation of downy mildew (e.g., cymoxanil, aluminum tris, phenylamides, propamocarb, dimethomorph, cyazofamid, zoxamide, mandipropamid and fluopicolide) (109). Some of these fungicides have a single-site mode of action, which puts them at high risk of *Ps. cubensis* developing fungicide resistance. The efficacy of fungicides with a single-site mode of action is rapidly broken once there is a mutation in the specific metabolic pathway where the fungicide is directed within pathogen. In addition to this, *Ps. cubensis* has been classified by the Fungicide Resistance Action Committee (FRAC) as one of the pathogens that represent a high risk of developing resistance to fungicides (128). Resistance to phenylamides, strobilurins, phosphonates, carbamates and carboxylic acid amides (CAA) has been reported in many countries (56, 156).

In order to mitigate the risk of fungicide resistance, a prescribed spray program should be followed. Additionally, due to the variability among fungicide efficacy across U.S. regions due to differences in climate conditions, *Ps. cubensis* local populations and susceptibility of cultivars, a spray program directed to a state level is recommended (109). Common compounds used for downy mildew control include the highly effective fluopicolide, a pyridinyl methyl-benzamide, followed by propamocab (carbamate) and cyazofamid (quinone inside inhibitor) (109). In Michigan, a downy mildew spray program for cucumbers includes alternation of systemic fungicides (i.e. propamocarb, cyazofamid or flucopicolide) mixed with protectant fungicides (i.e. mancozeb or chlorathalonil) applied at a seven-day intervals before the disease occurs and intensified to a five-day interval once the disease is present. On the other hand, the same fungicide program is recommended for other cucurbits but in a seven- to ten-day application interval before disease occurs and intensified to a seven-day interval once the disease is established (61). Alternating among fungicides with different FRAC groups helps to delay the buildup of resistant *Ps. cubensis* isolates and to prolong the efficacy of the fungicide over time (128).

Forecasting. In 1996, the global cost of fungicides to control diverse downy mildews was determined to be $1.24 billion (approx. 1.2 billion SFr) (55, 146). Controlling downy mildew caused by *Ps. cubensis* constitutes 10% of this amount (55, 146). In order to reduce unnecessary fungicide costs and to determine the most effective timing for fungicide sprays (25), a forecasting system is needed for growers.

In the U.S. and Ontario, Canada, a forecasting system developed at North Carolina State University is available to monitor and alert the growers about cucurbit downy mildew outbreaks. This web-based system (http://cdm.ipmpipe.org/) is available at no cost. This system integrates forecasts based on meteorological data and reports of outbreaks (110). Factors such as dew periods, temperature, relative humidity, ultraviolet radiation and precipitation are taken into account at each phase of the epidemic development to track outbreaks and alert growers of any spore influx at the state level and providing guidance regarding the timing for fungicide applications (110).

In addition to this web-based forecasting system, cucurbit growers in Michigan have another warning system, which was established in 2006 (59). This system is based on monitoring atmospheric concentrations of sporangia to alert growers when to initiate a fungicide spray program (59). For this purpose, spore traps are placed in selected cucurbit fields in the major cucumber growing regions in Michigan (59). These spore traps have a small orifice where air and airborne particles such as *Ps. cubensis* sporangia are sampled and the particles impacted onto a tape covered in petroleum jelly (59). Subsequently, the tape is processed in the laboratory where identification and quantification of the pathogen can be accomplished using a compound microscope (59).

Pseudoperonospora cubensis is a major threat for cucurbit producers worldwide. With data obtained from genome sequencing, questions about host-pathogen interactions can be resolved and may help to elucidate better ways to control this pathogen (143). In the meantime, screening cucurbit cultivars at a regional level for resistance to downy mildew is needed.

Overall, losses caused by the water molds, *Phytophthora capsici* and *Pseudoperonospora cubensis,* have negatively impacted Michigan vegetable production. Registered chemical products which are available to growers to manage these two pathogens are expensive and do not offer complete control. Thus, the objectives of this thesis research include (i) screening cucurbit cultigens for resistance to the re-emergent pathogen *Pseudoperonospora cubensis* and (ii) assessing commercial common bean (*Phaseolus vulgaris*) types for resistance to *Phytophthora capsici* blight.

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CHAPTER I: Assessment of commercial bean cultivars for susceptibility to *Phytophthora capsici* blight

ABSTRACT

Snap bean (*Phaseolus vulgaris* L.), a frequent choice as a rotational crop for *P. capsici*-susceptible vegetables, was confirmed as a host for *P. capsici* for the first time in Michigan commercial fields in 2003. Dry edible beans, a different variety of *Phaseolus vulgaris*, and soybeans (*Glycine max* (L.) Merr) are also used as rotational crops for *P. capsici*-susceptible vegetables. We assessed the susceptibility of eight bean cultivars representing the most economically important market types for Michigan, and a Pioneer soybean variety, 92M91. Roots, stems, petioles and pods were inoculated independently with each of two highly virulent isolates of *P. capsici*. Both isolates were pathogenic and sporulated on most bean tissues at 28ºC and relative humidity above 90%. All bean plant tissues except roots and soybean pods were susceptible to *P. capsici*. Differences among dry edible bean cultivars suggested that 'Merlot' (small red bean) was the most susceptible cultivar for all aerial parts of the plant. Snap bean cultivars were significantly more susceptible to *P. capsici* than dry edible bean cultivars and soybean variety 92M91. Development of *P. capsici* symptoms on beans required specific conditions. Temperatures above 28ºC and high relative humidity favored the development of *P. capsici* symptoms and sporulation on beans. Results obtained in this study indicate that dry edible beans might become infected under natural conditions when planted in *P. capsici*-infested soils and grown under environmental conditions conducive for disease development.

INTRODUCTION

Phytophthora capsici is a devastating pathogen on a broad range of plants. It was initially described infecting chili peppers in New Mexico and today, around 46 plant species distributed across 28 evolutionarily distinct families are susceptible to this organism (8, 9, 17). Members of the Solanaceae and Cucurbitaceae are the most severely infected (3, 12). *Phytophthora capsici* can affect multiple host tissues at varying developmental stages. Thus, specific screening methods for evaluating all susceptible tissues to *P. capsici* are necessary to determine resistant cultivars.

Currently, disease management relies heavily on the use of fungicides. However, increased production costs and the elevated risk of *P. capsici* to develop resistance to fungicides are some of the limitations of a successful disease management program (1). Cultural practices have been implemented in addition to chemical control to mitigate the losses caused by *P. capsici* since fungicides alone do not provide the desired efficacy (14, 19). Among cultural practices used for *P. capsici* management, rotational schedules with nonhost plants are particularly ineffective due to the long persistence of *P. capsici* oospores in the field and the limited number of nonsusceptible vegetable crops (15).

A relevant example of a failure in rotational schemes occurred in 2003 when fields, which previously produced cucurbits were planted with snap beans as a rotational crop, resulted in a significant snap bean loss due to *P. capsici* infection (11). The first field report of *P. capsici* on the Fabaceae occurred in an Argentinean lima bean field in 1950 (10). In the U.S., *P. capsici* on lima beans was confirmed in the Mid-Atlantic region in 2000 (5). Thereafter, subsequent occurrences of *P. capsici* on snap bean were reported in the U.S. in Michigan in 2003, New York in 2008 and Connecticut in 2010, and internationally in Brazil in 2011 (6, 11, 13, 16). Symptoms on snap bean include foliar blight appearing as rapidly expanding water-soaked lesions on leaves and petioles resulting in plant wilting, stem blight appearing as red-brownish lesions, and pod rot appearing as water-soaked lesions that become covered with white mycelial growth in a matter of days (11, 13, 16). Unlike susceptible cucurbitaceous and solanaceous crops, root rot lesions caused by *P. capsici* have not been reported on members of the Fabaceae (11).

Visual assessment of bean fields during a *P. capsici* epidemic in Michigan showed that green beans ('Romano') did not present *P. capsici* symptoms, while yellow wax beans planted in the same field the year before were severely diseased (11). Growers have long speculated that soybean plants (*Glycine max* (L.) Merr.) may be a host for *P. capsici* based on increased disease incidence when *P. capsici*-susceptible vegetables followed soybeans in the cropping rotation (M.K. Hausbeck, personal communication, 2011). Studies performed under controlled conditions showed that soybean leaves, especially in early growth stages, were susceptible to different *Phytophthora* spp. including *P. capsici* (27). More recently, a study conducted by Gevens et al. (11) showed that one-month-old soybean plants developed leaf blight after inoculation with *P. capsici*, but the roots did not get infected. Currently, there are no data available that support *P. capsici* infection of soybeans under field conditions (11). The epidemiology of *P. capsici* on beans is not well understood. Since snap bean (*Phaseolus vulgaris* var. *vulgaris*) is another variant of the same dry bean species, our hypothesis is that dry beans are also susceptible to *P. capsici*. Thus, the objectives of this study were (i) to determine and compare the susceptibility of nine commercial bean cultivars widely grown in Michigan to

P. capsici under controlled conditions and (ii) to examine the effect of isolate and inoculated tissue on disease development.

MATERIALS AND METHODS

Isolates and inoculum production. Two highly virulent isolates of *Phytophthora capsici* Leonian from Michigan fields were used in this study. Isolate 12889, recovered from infected peppers (A1 mating type), and 10193, isolated from snap beans (A1 mating type), were obtained from the culture collection at Dr. Hausbeck's laboratory at Michigan State University, East Lansing, MI. Mycelial plugs of each isolate were taken from the margin of 7-day-old cultures grown on unclarified V-8 juice agar (1.6% agar, 0.3% $CaCO_3$ and 16 ml canned V-8 juice per litter) and transferred onto great northern bean agar (GNBA; 50 g of great northern beans per 500 ml of water were autoclaved for 10 min, the liquid was passed through a 4 layers of cheesecloth and an autoclaved solution of 17 g of agar and 2.5 g of glucose per 500 ml was added). All the plates were incubated at room temperature (21± 2 ºC) under continuous fluorescent light for a maximum of 7 d. In order to avoid loss of virulence (degree of damage caused to a host by the pathogen), disinfected pods of snap bean 'HyStyle,' a highly susceptible snap bean cultivar, were inoculated with each isolate. Then, small slices from infected pods were transferred onto GNBA and incubated under the same conditions described above. After five transfers onto GNBA media, a new reinfection process was performed. Two different inoculum production procedures were performed to ensure infection of a particular tissue: mycelial plugs were used for stem, pod, and petiole inoculation and infested millet seeds were used for root inoculation. (i) A 7-mm-diameter mycelial plug obtained from the margin of an actively growing *P. capsici* culture was transferred onto a new GNBA plate, and incubated for 7 d. (ii) For millet seeds inoculum, 8 mycelial plugs of *P. capsici* were added to a flask containing

100 g of millet seeds with 72 ml of water amended with 0.08 g of asparagine and sterilized twice (24 hours apart). Flasks were incubated at room temperature for 4 weeks and periodically shaken to ensure homogenous infestation.

Plant material. A total of nine cultivars, eight common bean cultivars (*Phaseolus vulgaris* L.) from different market types, and a single soybean cultivar (*Glycine max* (L.) Merr.) were used in this study. Seeds of six market types of dry edible beans released by Michigan State University: 'Matterhorn' (great northern type), 'Merlot' (small red type), 'Red Hawk' (kidney bean type), 'Santa Fe' (pinto type), 'Vista' (navy type), and 'Zorro' (black bean type); two processing snap bean 'Hercules' and 'HyStyle;' and a soybean cultivar, 92M91, from Pioneer were either obtained from commercial sources or kindly donated by Dr. James Kelly (Department of Plant and Soil Microbial Sciences, MSU) or a collaborator extension agent, Vic Shank.

Two to three seeds per each cultivar were sown in 10.2-cm (0.46 liter) plastic pots containing a peat and perlite substrate (Suremix; Michigan Grower Products, Inc., Galesburg, MI). The substrate was enriched with 1 g of slow-release fertilizer, Osmocote™ (14:14:14; NPK) once the plants germinated and were thinned to one plant per pot. Plants were kept in the greenhouse until they reached the growth stage required for each tissue inoculation method.

Root inoculation and assessment. Root inoculations were performed on bean plants at two different growth stages. Six bean plants per cultivar per isolate at the vegetative stage 4 (V4; fourth fully developed trifoliate leaf) were inoculated with 1 g of infested millet seeds adjacent to the root system and then covered with a layer of noninfested soil in order to prevent direct contact of cotyledons and lower leaves with the inoculum and to prevent inoculum splashing.

Plants were immediately watered to ensure moisture for the inoculum to develop. A second experiment using six bean plants per cultivar per isolate at the vegetative stage cotyledon (VC; cotyledons are completely unfold and unifoliates are visible) was performed. Bean plants were germinated on 72-well trays and transplanted to 10.2-cm pots filled with a 1:1 mixture of peat and perlite substrate and infested millet seeds. A layer of noninfested soil was added. Noninoculated controls received 1 g of non-infested millet seeds or a 1:1 mixture of substrate with non-infested millet seeds for each experiment respectively. Yellow squash 'Cougar' was included as a susceptible control. Disease incidence was determined by the number of dead plants 2 weeks after the susceptible control succumbed to *P. capsici* root infection. All the plants were arranged following a complete randomized design in a dew chamber. The experiments were conducted twice. Root susceptibility assessments were performed at 28±3ºC and high relative humidity (99.7±0.3%) in a dew chamber with 16 h of photoperiod.

Stem inoculation and assessment. Stem inoculations were performed on bean plants in a V4-V5 stage. A seven-day-old mycelial plug (7 mm in diameter) was placed on the natural wound that is left when cotyledons drop off and covered with Parafilm® to prevent desiccation. A sterile GNBA media plug was placed on the stems of negative control plants. One negative control per cultivar was used. Disease severity for stems was assessed following a 0 to 5 scale (0 = no visible symptoms, 1 = small brownish lesion at the inoculation point, 2 = stem lesion expanded 1 to 3 cm from the inoculation point with no sporulation present, 3 = stem lesion expanded up to 10 cm from the inoculation point with slight to heavy pathogen sporulation present, 4 = up to 90% of the plant collapsed but younger leaves do not present visible *P. capsici* symptoms and 5= plant dead), according to the results of a pilot test. Ratings were performed at 4, 6, 8, 12, and 15 dpi. Seven plants per cultivar per each isolate were completely randomized in a dew chamber. This experiment was conducted three times. Stem susceptibility assessments were performed at 28±3ºC and high relative humidity (99.7 ±0.3%) in a dew chamber with 16 h of photoperiod.

Petiole inoculation and assessment. Petiole inoculations were performed by placing a 7-mm mycelial plug on the leaflet junction of first trifoliate leaf of plants in a V4-5 stage. Negative controls were inoculated with sterile GNBA media plugs. Plugs were covered with Parafilm®. For petiole/leaflets lesion assessment, an adapted disease scale from 0 to 5 proposed by Gevens et al. (11) was used were 0 = no visible symptoms, 1 = <70% petiole necrotic, 2 = 100% petiole necrotic, 3 = 100% petiole necrotic and 25% leaflets necrotic, 4= 100% petiole necrotic and 50% leaflets necrotic and 5= 100% petiole and leaflets necrotic. Ratings were performed at 4, 6, 9, and 12 dpi. Seven plants per cultivar per each isolate were completely randomized in a dew chamber. This experiment was conducted three times. Petiole susceptibility assessments were performed at 28±3ºC and high relative humidity (99.7±0.3%) in a dew chamber with 16 h of photoperiod.

Detached pod inoculation and assessment. In a separate experiment, the nine bean cultivars were grown in a field with no history of *P. capsici* infestation and in the greenhouse. In order to obtain pods at the same maturity stage the seeds were differentially sown according to their days to mature. Dry bean and soybean pods in a reproductive stage 5 (R5; seeds filling half of the space in the pod), and 12-cm snap bean pods were manually harvested and placed separately in plastic bags by variety. Detached pods were inoculated in the middle of the pod with a 7-day-old mycelial plug (7 mm in diameter). A negative control per cultivar was inoculated with a sterile GNBA media plug. The plug was covered with Parafilm®. All pods were arranged in a complete randomized block design in 7 humid chambers. Disease rating for pods was performed measuring the diameter of the mycelial growth and the whole lesion (mycelial growth and water-soaked lesion) at 6 dpi. This experiment was conducted twice. Pod assessments were performed under laboratory conditions at 21±2°C, 93±4% relative humidity and continuous light.

Isolate identity confirmation. Isolations were performed on all the plants to confirm the presence of *P. capsici* in symptomatic plants, and to determine whether symptomless infection occurred in asymptomatic plants. Petioles and leaves were disinfected with 10% sodium hypochlorite for 30 sec., while stem and root tissue was disinfected for 1 min. Tissue was rinsed 3 times with double distilled water and blotted dried with sterile paper towels. Small tissue pieces were placed onto benomyl, ampicillin, rifampicin and PCNB amended V-8 media (25 ppm of benomyl, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene) and incubated at room temperature. Suspected *P. capsici* colonies were transferred onto regular V-8 agar plates and incubated for 7 d at room temperature and 35°C for morphological identification of *P. capsici* following the key presented by Waterhouse (28).

Statistical analyses. The conditional severity data, obtained by dividing the mean severity of all assessed plants by the proportion of those diseased ones, were used to calculate the area under the disease progress curve (AUDPC) according to the formula proposed by Shaner and Finney (22). Analyses of variance (ANOVA) on AUDPC values, mean mycelial growth, and whole lesion diameters were performed using the PROC MIXED function of SAS (v. 9.2; SAS Institute, Cary, NC). Mean separation was conducted using the Fisher's protected least significant test ($P \leq 0.05$). Data from repetitions were pooled together when no statistical differences were found in the interaction between treatment and repetitions ($\alpha = 0.05$).

RESULTS

In a preliminary pilot study, the plants were kept 24 h prior to stem and foliar inoculations and for an additional 72 h period post inoculation in a dew chamber to ensure *P. capsici* infection before they were moved back to the greenhouse. *Phytophthora capsici* lesions were found on the stems and leaves of snap beans 'HyStyle' and 'Hercules' after the incubation period in the dew chamber. Reddish brown lesions, 7 mm long were found on the stems, along with chlorotic lesions of 12 mm in diameter on leaves of snap beans cultivars. After two weeks under greenhouse conditions, *P. capsici* lesions ceased to develop (data not shown). No *P. capsici* lesions were found on dry edible bean and soybean cultivars.

Root inoculation results. After 3 weeks post inoculation the two inoculated stages (i.e., VC and V4 stages) did not become infected. Conversely, the susceptible yellow squash cultivar Cougar died after 7 days post inoculation with *P. capsici* isolates 12889 and 10193. Attempts to reisolate *P. capsici* from bean roots failed, whereas the frequency of reisolation from yellow squash roots was 100%.

Stem inoculation results. All cultivars showed reddish brown symptoms on stems in response to inoculation with the isolates 12889 and 10193. However, no pathogen sporulation was observed on dry beans 'Matterhorn,' 'Zorro,' 'Santa Fe' and soybean variety, 92M91 (Table 2.1). When inoculated with isolate 12889, snap beans 'Hercules' and 'HyStyle' were significantly more susceptible than the other cultivars. Among dry edible beans, small red bean 'Merlot' was significantly more susceptible than the other dry edible bean market types. No significant differences were found among dry beans 'Red Hawk,' 'Matterhorn,' 'Vista,' 'Zorro,' and soybean 92M91. Pinto bean 'Santa Fe' was the least susceptible to *P. capsici* isolate 12889.

When inoculated with isolate 10193, snap beans 'Hercules' and 'HyStyle' were also significantly more susceptible than the other cultivars. Stems of small red bean 'Merlot' were significantly more susceptible than the other dry edible bean market types. Soybean 92M91 and pinto bean 'Santa Fe' were significantly least susceptible to *P. capsici* isolate 10193 (Table 2.1). Significant differences were found between isolates ($P = 0.008$). Isolate 10193 was significantly more virulent on bean stems than 12889. No *P. capsici* colonies were obtained from asymptomatic bean plants, whereas the frequency of recovery of *P. capsici* colonies from lesions in the symptomatic plants varied from 86 to 100%.

Table 2.1 Mean area under disease progress curve (AUDPC) per *Phytophthora capsici* isolate and pathogen sporulation on stems of 9 bean cultivars.

			Isolate 12889			**Isolate 10193**		
Cultivar/ Variety	**Class**	**Source**	**Mean AUDPC**[y]		**Spor.**[z]	**Mean AUDPC**[y]		**Spor.**[z]
Hercules	Snap processing	Seminis	36.88	a	+	47.33	a	+
HyStyle	Snap processing	Harris M	40.95	a	+	43.00	a	+
Merlot	Small red	MSU	21.35	b	+	36.64	b	+
Red Hawk	Kidney bean	MSU	11.67	c	+	29.68	c	+
Matterhorn	Great northern	MSU	10.88	c	-	26.17	c	-
Vista	Navy	MSU	14.58	c	+	16.00	d	+
Zorro	Black bean	MSU	11.07	c	-	12.83	d	-
92M91	Soybean	Pioneer	8.72	c	-	9.50	e	-
Santa Fe	Pinto	MSU	6.05	d	-	7.71	e	-

[y] Mean area under disease progress curve (AUDPC) calculated from a disease severity rating based on a 0 to 5 scale. Mean separation performed by year using LSD ($\alpha = 0.05$): Numbers followed by the same letter are not significantly different from each other. High values represent high levels of susceptibility. No visible symptoms were observed on negative control plants.

[z] Spor. = sporulation. (-) = no pathogen sporulation was visible under experimental conditions; (+) = *Phytophthora capsici* visible sporulation and morphological pathogen confirmation.

Petiole inoculation results. All the tested cultivars showed symptoms after inoculation with isolates 12889 and 10193 (Table 2.2). No sporulation was observed on 'Zorro,' ‘Matterhorn,’ ‘Red Hawk,’ ‘Santa Fe,’ ‘Vista,’ and soybean 92M91. Cultivars Hercules and HyStyle were more susceptible than all other cultivars evaluated. Petioles of ‘Merlot’ were more susceptible to *P. capsici* than petioles of the other dry edible beans, but less susceptible than the petioles of the two snap bean cultivars. No significant differences were found in cultivars Zorro, Matterhorn, Red Hawk, Santa Fe, Vista and the soybean variety, 92M91. Isolates 12889 and 10193 were not significantly different from each other in virulence. No *P. capsici* colonies were obtained from asymptomatic bean plants, whereas the frequency of recovery of *P. capsici* colonies from petiole lesions in the symptomatic plants varied from 79 to 100%. Frequency of *P. capsici* from infected leaflets was lower compared with other infected tissues (data not shown).

Table 2.2 Mean area under disease progress curve (AUDPC) and *Phytophthora capsici* sporulation on petiole/leaves of 9 bean cultivars inoculated with the *P. capsici* isolates 12889 and 10193.

		Isolate 12889			**Isolate 10193**		
Cultivar/ Variety	**Class**	**Mean AUDPC**[y]		**Spor.**[z]	**Mean AUDPC**[y]		**Spor.**[z]
Hercules	Snap processing	25.61	a	+	29.65	a	+
HyStyle	Snap processing	24.86	a	+	26.36	a	+
Merlot	Small red	13.29	b	+	16.21	b	+
Zorro	Black bean	3.30	c	-	6.89	c	-
Matterhorn	Great northern	3.35	c	-	5.42	c	-
Red Hawk	Kidney bean	3.12	c	-	5.25	c	-
Santa Fe	Pinto	2.88	c	-	5.18	c	-
92M91	Soybean	2.32	c	-	5.15	c	-
Vista	Navy	3.12	c	-	4.43	c	-

[y] Mean area under disease progress curve (AUDPC) calculated from a disease severity rating based on a 0 to 5 scale. Mean separation performed by year using LSD ($\alpha = 0.05$): Numbers followed by the same letter are not significantly different from each other. High values represent high levels of susceptibility. No visible symptoms were observed on negative control plants.

[z] Spor. = sporulation. (-) = no pathogen sporulation was visible under experimental conditions; (+) = *Phytophthora capsici* visible sporulation and morphological pathogen confirmation.

Detached pod inoculation results. *Phytophthora capsici* mycelial growth and lesion development were observed on all cultivars except soybean (Table 2.3). Small water-soaked lesions were observed on both snap bean cultivars after 3 dpi and most pods were completely covered by mycelia after 6 days post inoculation with isolates 12889 and 10193. When inoculated with isolate 12889, ‘HyStyle’ had significantly more mycelial growth than the rest of the cultivars. No significant difference in mycelial growth was found among dry bean cultivars Red Hawk, and Matterhorn and the soybean variety, 92M91. The largest lesions were observed on snap beans ‘HyStyle’ and ‘Hercules,’ followed by the dry bean cultivars Merlot and Santa Fe. No significant differences were found between ‘Vista’ and ‘Matterhorn.’ When isolate 10193 was used, mycelial growth was significantly more severe on ‘HyStyle’ and ‘Hercules’ than the other cultivars. No significant differences in mycelial growth diameter were observed on ‘Hercules,’ ‘Merlot,’ ‘Santa Fe,’ and ‘Zorro.’ The largest lesion was observed on ‘HyStyle’ and ‘Hercules,’ followed by ‘Merlot,’ ‘Santa Fe,’ ‘Red Hawk,’ and ‘Zorro.’ Dry edible bean cultivars ‘Matterhorn’ and ‘Vista’ were significantly less susceptible than the other dry edible and snap bean cultivars, except ‘Zorro’.

Table 2.3 Mean *Phytophthora capsici* mycelial growth and whole lesion (water-soaked and mycelial growth) diameter on pods of 9 bean cultivars after 6 days post inoculation.

Cultivar	Class	Isolate 12889		Isolate 10193	
		Mycelial growth^z	Lesion^z	Mycelial growth^z	Lesion^z
HyStyle	Snap processing	11.33 a	12.26 a	9.17 a	10.41 a
Hercules	Snap processing	9.24 b	10.78 a	8.73 ab	9.92 a
Merlot	Small red	6.93 b	7.45 b	6.63 b	6.94 b
Santa Fe	Pinto	7.81 b	8.23 b	5.94 b	6.53 b
Red Hawk	Kidney bean	0.76 e	1.54 e	3.41 cd	5.99 b
Zorro	Black bean	4.64 c	5.27 c	4.81 bc	5.59 bc
Matterhorn	Great northern	0.82 e	2.48 de	2.75 d	4.09 c
Vista	Navy	2.91 d	3.82 cd	3.10 d	3.89 c
92M91	Soybean	0.0 e	0.00 f	0.0 e	0.00 d

^{z} Mean *Phytophthora capsici* mycelial growth and whole lesion (mycelial growth and water-soaked lesion) measured at 6 dpi. Mean separation was performed using Fisher's LSD test ($\alpha = 0.05$). Numbers followed by the same letter are not significantly different from each other. No visible symptoms were observed on negative control plants.

DISCUSSION

In this study, the susceptibility of nine bean cultivars to *P. capsici* infection was investigated using different screening methods for specific tissue responses. These results support our initial hypothesis that dry edible beans were also susceptible to *P. capsici* since dry edible beans and snap beans are the same species (i.e. *Phaseolus vulgaris*) and this latter plant was already reported as susceptible to *P. capsici* (11); however, snap bean cultivars were significantly more susceptible than dry edible beans. Previous studies have shown that dry edible beans and snap beans are susceptible to *Phytophthora nicotianae* and *P. phaseoli*, whereas soybeans are susceptible to *P. sojae* and *P. megasperma* (20, 21). In this study, it was shown that both of these bean plants are also susceptible to *P. capsici* under controlled conditions. Both

P. capsici isolates were pathogenic (defined as the capacity to cause disease) on all the bean cultivars tested. However, isolate 10193, originally obtained from infected snap bean stems, was significantly more virulent on stems than isolate 12889, which was obtained from infected pepper fruits.

Plant tissues and cultivars reacted differently in response to *P. capsici* isolates. Mycelial growth, lesion development and sporulation were observed on most inoculated tissues except on soybean pods and roots of all bean cultivars. In addition, it was found that tissue reactions to *P. capsici* were different. Stems of cultivar Santa Fe were less susceptible to *P. capsici* than pods whereas, aerial parts (i.e. leaves, petioles and stems) of soybean variety, 92M91, were more susceptible than soybean pods. These differences could be explained by different genetic mechanisms that govern the resistance for specific plant tissues to *P. capsici*. This phenomenon has also been observed in resistance to *P. capsici* on pepper and in other pathosystems such as potato and *Phytophthora infestans* (2, 23, 26). Moreover, the resistance of soybean pods to *P. capsici* infection may be attributed, in part to the high density of trichomes present in this tissue. It has been demonstrated that the presence of soybean pod trichomes on soybean conferred resistance to various herbivores through the production of secondary chemicals such as terpenoids (4, 18). In peppers, it was shown that the presence of trichomes on stems and leaves was associated with resistance to *P. capsici* (7).

Phytophthora capsici lesions on beans ceased to expand under greenhouse conditions, in accordance with the results obtained by Gevens et al. (11) while no *P. capsici* infection was detected when the plants were kept in the greenhouse (data not shown). Contrary to Gevens et al. (11), in which no *P. capsici* lesions were developed at temperatures greater than 23ºC, in this study *P. capsici* lesions were developed and heavy sporulation was observed for the most susceptible bean cultivars at 28ºC and >90% of relative humidity. Assessments with variable temperature and relative humidity are needed to determine whether these two variables affect disease initiation and *P. capsici* sporulation on bean plants.

Some researchers have argued that differences in disease response between different regions is due to the presence of different *P. capsici* physiological races (24); however, another hypothesis that accounts for the variability in these results is the difference in the inoculation method. The present study and the one conducted by Gevens et al. (11) showed a successful infection of soybean plants under controlled conditions when plants were inoculated using mycelial plugs on leaves and petioles. Conversely, Tian and Babadoost (25) found that 12 different soybean cultivars were resistant to *P. capsici* when using infested soil as an inoculation method. Moreover, factors such as plant age and environmental factors also influence the infection development. A study performed on soybeans documented the possible development of ontogenic or age-related resistance to *P. capsici* as the plants mature (27). These results emphasize the importance of evaluating all *P. capsici* symptoms (pod rot and stem and foliar blight) when performing screening methods to distinguish *P. capsici* resistance from susceptible individuals.

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LITERATURE CITED

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CHAPTER II: Assessing susceptibility of cucurbit hosts to *Pseudoperonospora cubensis* under field conditions

ABSTRACT

Cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, is a severe foliar disease of many commercial cucurbitaceous crops worldwide. In this study, 41 cucurbit cultigens (i.e., commercial cultivars and plant introductions) from five different genera (*Cucumis*, *Citrullus*, *Cucurbita*, *Lagenaria,* and *Luffa*) were assessed for their susceptibility to natural infection by *Ps. cubensis* in a Michigan research field. Eight of these 41 cultigens that belong to the differential set for pathotype determination were used to identify changes in the predominant *Ps. cubensis* populations in Michigan between 2010 and 2011. No pathotype differences were found. Disease severity data in 2010 and 2011 indicated a single *Cucumis melo* cultigen, MR-1, was less susceptible to Michigan *Ps. cubensis* populations than other *C. melo* cultigens. No visible infection was detected on cultigens of *Cucurbita moschata* and *C. pepo.* Although disease onset in 2011 was later than 2010, high disease pressure was observed in both years (>90% disease severity in pickling cucumber 'Vlaspik'). These results confirmed that the genus *Cucumis* is the most susceptible to downy mildew among the Cucurbitaceae. *Citrullus* and *Cucurbita* cultigens were more suited than *Cucumis* to endure infection by Michigan *Ps. cubensis* populations. All *Citrullus* plant introductions were slightly infected and no sporulation was detected under field conditions; thus, they are potentially good sources for breeding programs for downy mildew resistance in this crop. Since less susceptible cultigens demand less fungicide input than highly susceptible cultigens, *Cucumis melo* cultivars Edisto 47, Primo, Athena, Strike, Ananas, Banana and Tam-Dew, which have low disease progression values, would be recommended for Michigan production.

INTRODUCTION

Cucurbit downy mildew, caused by the peronosperomycete (oomycete) fungal-like *Pseudoperonospora cubensis* (Berk. and M.A. Curtis) Rostovzev), is one of the most devastating foliar diseases of a wide range of cucurbits (15). Although this pathogen is found in many cucurbit production areas, *Ps. cubensis* particularly thrives in areas where elevated humidity and warmer conditions favor its establishment (3, 13, 28). Due to the biotrophic nature of *Ps. cubensis,* the disease emerges annually in temperate regions through wind dispersion from year-round cucurbit growing sites in subtropical regions or greenhouses (18, 19). In central Europe, the inoculum spreads from southeast Europe (13), whereas in the eastern U.S. the inoculum is suspected to come from fields located in Florida or Mexico (16, 18). In Michigan, the number one producer in pickling cucumbers in the U.S., it is believed that the initial inoculum comes from greenhouses located along the border with Canada (23).

Many Cucurbitaceae genera, including cultivated, semi-cultivated and wild genera, are affected by *Ps. cubensis* under natural conditions (12, 20, 24). Species within approximately 20 genera have been reported as hosts of *Ps. cubensis;* however *Cucumis*, *Cucurbita* and *Citrullus* are the most economically important genera affected by cucurbit downy mildew (15). Symptoms of this foliar disease vary depending on the cucurbit host. In cucumbers (*Cucumis sativus* L.), squashes (*Cucurbita pepo* L. or *C. moschata* Duchesne ex Poir.), and loofahs (*Luffa* spp.) the symptoms on the upper side (adaxial) of the leaf are irregular, pale-yellow lesions restricted by leaf veins that become necrotic over time; whereas in cantaloupe (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai), lesions are not limited by the leaf veins and are more circular (30). Although *Ps. cubensis* is confined to the leaves, the defoliation caused by this pathogen results in low productivity and poor fruit quality (21).

During the late 1960s, a completely resistant cucumber cultivar, Poinsett, was released in the U.S. and no fungicides were required to control the disease until 2004, when *Ps. cubensis* reemerged in North Carolina (9). Subsequent losses were reported in other states in the eastern part of the U.S. the following year (9). In Michigan, the first report of *Ps. cubensis* occurred in 2005; since then, cucurbit downy mildew has been reported annually on cucumbers and other economically important cucurbit crops such as squash and melon (8). The resurgence of *Ps. cubensis* in Europe and in the U.S. has been attributed to the appearance of a more aggressive pathotype in *Ps. cubensis* populations (4, 9). Recently, an expansion of the initial set proposed by Thomas et al. in 1987 for pathotype determination is used. This new system includes 12 differentials that belong to six Cucurbitaceae genera (*Cucumis, Cucurbita, Citrullus, Benincasa, Luffa and Lagenaria*) to characterize variability in virulence and host range of *Ps. cubensis* (16, 27). Based on the interaction of *Ps. cubensis* with this current differential set, a total of 67 pathotypes were determined for the Czech Republic pathogen populations (14).

Since the options of resistant cultivars that are commercially available are limited, disease management relies heavily on the use of fungicides. Approximately, $1.2 billion were spent globally for downy mildew control in 1996, 10% of the total cost was allocated solely for cucurbit downy mildew control (7, 29). In Michigan, growers spend about $8 million USD annually on fungicides alone. This value does not take into account related expenses associated with disease management such as labor and equipment costs (8). Hence, genetic resistance is of paramount importance for successful disease management. Breeding a resistant cultivar with desirable traits may take years. Meanwhile, available sources for disease resistance need to be identified. Due to reported differences in the genetic structure of the pathogen within and between countries (23), continuous cultigen screening on a regional level for downy mildew is imperative. Therefore, the objectives of this study were to (i) screen 41 cultigens (cultivars and plant introductions) for susceptibility to *Ps. cubensis*; (ii) validate the susceptibility level of melon and muskmelon cultivars in Michigan fields; and (iii) determine the host range of the most predominant pathotype in Michigan natural populations.

MATERIALS AND METHODS

Cucurbit cultigens. Forty-one cultigens (plant introductions (PI) and cultivars) belonging to five naturally susceptible *Ps. cubensis* host genera (i.e. *Cucumis*, *Citrullus*, *Cucurbita*, *Lagenaria,* and *Luffa*) were evaluated for disease response to natural populations of *Ps. cubensis* in Michigan. This set of cultigens included 24 commercial melon and cantaloupe cultivars provided by Dr. Chandrasekar S. Kousik (Research Plant Pathologist, Vegetable Laboratory USDA-ARS), 16 plant introductions provided by the U.S. plant germplasm collection at the Plant Genetic Resources Conservation Unit at Griffin, GA, and a cultivar of pickling cucumber (Vlaspik) included as susceptible control and as source of inoculum. Cultigens were chosen because they were either previously tested in South Carolina (24 melon and cantaloupe cultivars), belong to the differential set of taxa for *Ps. cubensis* pathotype determination or had not yet been screened for downy mildew (Table 3.1).

Field trials. Field experiments were conducted at the Plant Pathology Research Farm (Michigan State University, East Lansing at 42º 41' 32" N, 84º 29' 13" W). All 41 cultigens were planted in a sandy loam soil (54.2% sand, 35% silt, and 10.8% clay) late in the season (July 13 of 2010 and 2011) to ensure *Ps. cubensis* presence in the field. Additionally, pickling cucumber 'Vlaspik' was planted along the edge of the plot as a source of inoculum. Rows were sprayed with Roundup Ultra [active ingredient (a.i.) glysphosate] at 5.85 liter/ha two months prior to planting as weed management; thereafter, the plots were kept weed-free by weekly hand weeding. A standard chemical management was applied three times during the cropping season for powdery mildew control with Quintec (a.i. quinoxyfen) at 438.5 ml/ha, and Asana XL (a.i. esfenvalerate) at 658 ml/ha was applied once for cucumber beetle (*Acalymma vittatum*) control. All the applications were made using a manual backpack airblast sprayer. A 20:20:20 (N:P:K) fertilizer was injected through the drip irrigation system on a weekly basis at 5.6 kg/ha. Plants were drip irrigated twice a week for a total of 6 h per week. To promote infection, overhead irrigation was used 44 days after planting in addition to the standard irrigation schedule during two consecutive weeks in 2010. In 2010, all cultigens were directly sown and thinned to 5 plants per cultigen per block while in 2011, three-week-old seedlings of each cultigen were transplanted into the soil at the same plant density as in 2010. All the cultigens were planted in raised beds covered with black polyethylene mulch and spaced 2.4 m apart on center. Spacing between plants was 32 cm. The experiment was set up as a randomized complete block design (RCBD) with four replicates. Eight hosts belonging to five of the six genera proposed for pathotype determination (Table 1) were included in the previous experiment to determine differences between years at the pathotype level.

Disease Assessment. Plants were scouted twice a week to determine the date when the first symptoms appeared. Disease severity (% of the total necrotic and chlorotic leaf area of fully expanded leaves) was visually assessed from all the plants of each cultivar in each block (20 plants total per cultivar). Data were recorded once per week for a total of seven measurements, starting at the first date on which symptoms were observed in 2010. One affected leaf per plant per rating day was detached, independently bagged and brought to the laboratory for *Ps. cubensis* identification. Symptomatic leaves without obvious pathogen sporulation were placed in a moisture chamber for 3 days to promote sporulation. *Pseudoperonospora cubensis* was morphologically identified based on spore dimensions and sporangiophore branching. Disease severity data were used to calculate the area under the disease progress curve (AUDPC) according to the following formula:

$$AUDPC = \sum_{i=1}^{n} \left(\frac{(x_i + x_{i-1})}{2} \right) (t_i - t_{i-1}),$$

where n is the number of observations, x_i is the severity at the time of evaluation, and $(t_i - t_{i-1})$ is the interval between two evaluation dates. The level of sporulation was rated in 2010 on a scale from 0 to 2, where 0 = no sporulation, 1 = minimum sporulation and 2 = maximum sporulation. Final percentage disease ratings were transformed into a 0 to 9 ordinal scale for agronomical purposes as highly resistant: ≤3; moderately resistant: 3.1 to 4; intermediate: 4.1 to 6; moderately susceptible: 6.1 to 7; or highly susceptible: ≥7.1 according to Call. et al. (2). Data were analyzed independently for each year. Mean procedures (PROC MEANS) of SAS (v. 9.2; SAS Institute, Cary, NC) were performed to calculate final rating means per cultivar per year. Analysis of variance (ANOVA) on AUDPC values was performed using PROC MIXED of SAS. Cultigens with no visible downy mildew infection were removed from the analysis in order to fulfill the assumptions of normality and homogeneity of variances. Mean separation was conducted using the Fisher's protected least significant test (P ≤0.05). An additional t-test was performed to compare the differences between mean AUDPC values and zero.

RESULTS

Disease response. *Pseudoperonospora cubensis* was first detected and confirmed on pickling cucumber 'Vlaspik.' Cultivar Vlaspik exhibited the characteristic small pale-yellow water-soaking (initial downy mildew symptoms) 28 and 42 days after planting in 2010 and 2011 respectively (Figure 3.1). Significant differences between years due to differences in environmental conditions (data not shown) were found (P =0.019); however disease pressure was high (final ratings >90% on the susceptible control 'Vlaspik') in both 2010 and 2011. Final disease rating values were significantly higher in 2011 than 2010 for all *C. melo* cultigens whereas lower values were observed in 2011 for *C. lanatus* cultigens (Table 3.1). The disease progression graph for 'Vlaspik' in 2010 presented a stepped shape whereas in 2011 data resulted in a sigmoid shape (Figure 3.1). Final disease ratings for the selected cultigens in host range determination were considered as compatible interactions when values were >0. No differences between 2010 and 2011 regarding the predominant pathotype in Michigan *Ps. cubensis* populations were found (Table 3.2).

Susceptible levels and cultigen comparison. Significant differences were found among AUDPC values in all the cultigens evaluated (P <0.0001) for both years. More disease levels (i.e. R, MR, I, S and HS) were determined in 2011 whereas the majority of *C. melo* cultigens (92%) were classified as intermediate in 2010 (Table 3.1). Final disease ratings and AUDPC values in the *Cucumis melo* cultigens Ambrosia Hybrid, Aphrodite, Grand Slam, Planters Jumbo, Topmark, Atlantis, Mainstream, and Tekos were higher in 2011 than in 2010. Among *C. melo* cultigens, 'Ambrosia Hybrid' and 'Hale's Best Jumbo' were the most susceptible in 2010 and 2011, respectively (greater AUDPC values). The cultivar Banana was significantly more resistant than 'Ambrosia Hybrid,' but it was not significantly different from the moderately resistant cultigen MR-1 during 2010. In 2011, cultivar Hale's Best Jumbo was not significantly different from the susceptible control 'Vlaspik,' but it was significantly more susceptible than moderately resistant cultivars Primo, Edisto 47 and the partially resistant control MR-1. Slight pathogen sporulation was observed on all *Cucumis* cultigens (data not shown). Conversely, no sporulation was detected under field conditions on *Citrullus lanatus* var. *lanatus* cultigens; however, under elevated humid conditions in incubation chambers the sporulation was observable after 3 d of incubation and *Ps. cubensis* identity was confirmed. There were no significant differences within *Citrullus lanatus* var. *lanatus* cultigens, and between *Lagenaria siceraria* and *Luffa aegyptiaca* cultigens. Overall, *Cucumis* cultigens were significantly more susceptible to cucurbit downy mildew compared with *Citrullus*, *Cucurbita*, *Lagenaria* and *Luffa* genera. Neither of the individuals from *Cucurbita* genus (i.e. *C. moschata* and *C. pepo*) showed downy mildew lesions; however, a heavy infection with powdery mildew was observed on *Cucurbita pepo* cultigens PI 508468 and PI 171622.

Final disease severity data showed a similar trend to AUDPC values for all the cultigens, except for the *Cucumis melo* cultigens 'Mainstream,' 'Melon Gold Bar,' and 'Primo.' According to the final rating values in 2011, the cultigen Primo was classified as susceptible (71%), but had significantly lower AUDPC values compared with the susceptible control. Four *Citrullus lanatus* var. *lanatus* cultigens, PI 171584, PI 612145, PI 595218 and PI 171392, were significantly less susceptible than the partially resistant *Cucumis melo* cultigen MR-1 according to AUDPC and final rating values.

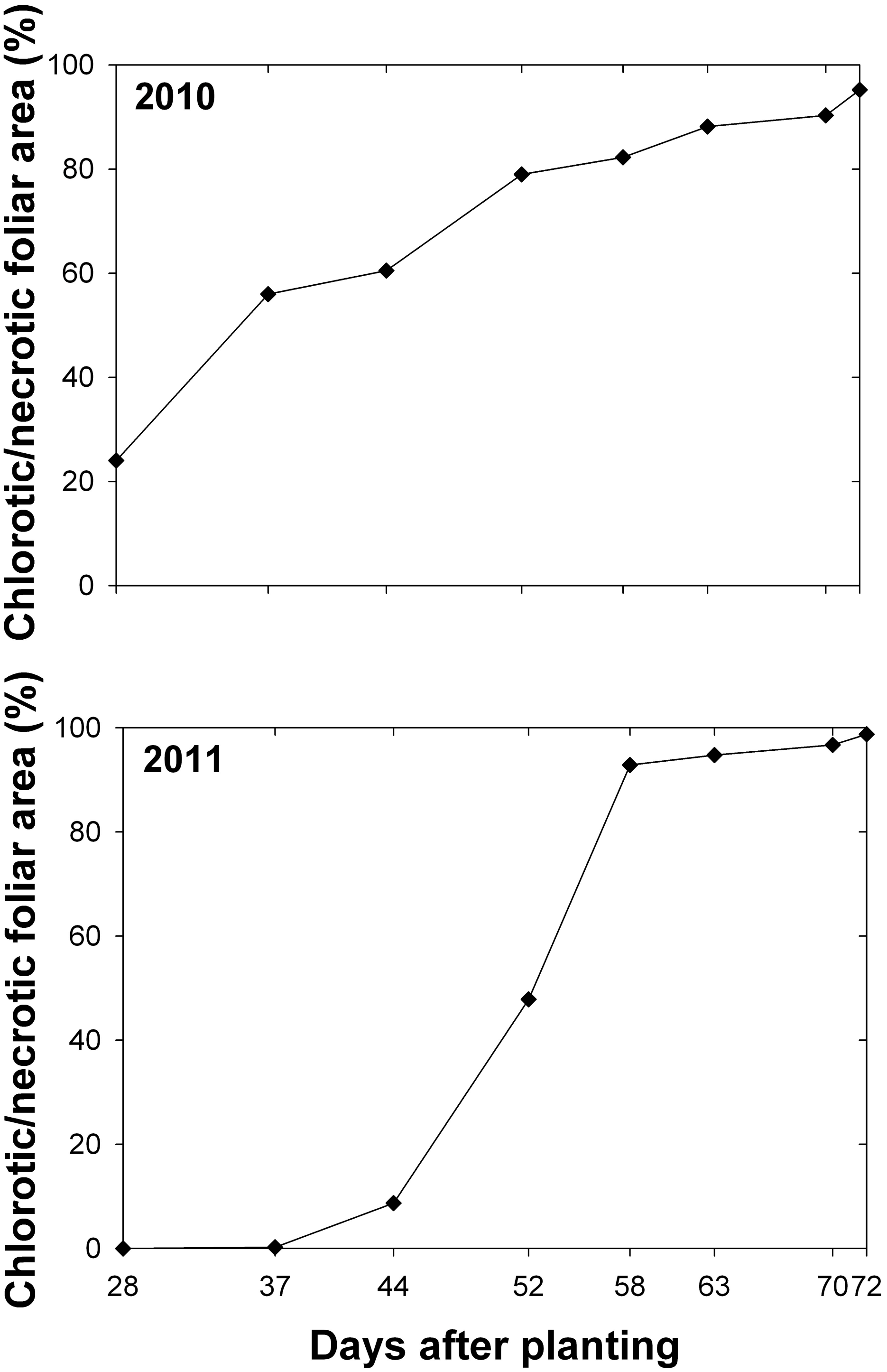

Figure 3.1 Progression of total percentage of chlorotic/necrotic foliar area caused by natural populations of *Pseudoperonospora cubensis* on pickling cucumber cultivar Vlaspik (susceptible control) in 2010 and 2011. Note stepped shape in 2010 and sigmoid shape in 2011. Seeds were planted on July 13 of 2010 and 2011.

Table 3.1 Final disease rating and area under disease progress curve (AUDPC) of the 41 cultigens assessed for field resistance to natural populations of *Pseudoperonospora cubensis* in Michigan.

		Final ratings[x]				**AUDPC**[y]			
Species/cultigen	**Origin**	**2010**		**2011**		**2010**		**2011**	
Cucumis sativus									
Vlaspik[w]	U.S.	95	HS	99	HS	3238	a	2174	a
Cucumis melo									
Hale's Best Jumbo	U.S.	50	I	89	HS	1329	b-d	1931	ab
Crete	U.S.	54	I	92	HS	1556	bc	1906	bc
Atlantis	U.S.	47	I	90	HS	1329	b-d	1872	bc
Ambrosia Hybrid	U.S.	59	I	97	HS	1668	b	1815	bc
Planters Jumbo	U.S.	43	I	96	HS	1347	b-d	1812	bc
Topmark[w]	U.S.	53	I	91	HS	1346	b-d	1806	b-d
Grand Slam	U.S.	52	I	85	S	1408	b-d	1681	b-e
Python	U.S.	47	I	83	S	1278	b-d	1565	c-e
Toscana	U.S.	38	I	83	S	1232	b-d	1553	de
Mainstream	U.S.	46	I	90	HS	1236	b-d	1547	de
Aphrodite	U.S.	45	I	85	S	1413	bc	1496	de
Tekos	U.S.	46	I	84	S	1176	b-d	1492	de
Minerva	U.S.	35	I	77	S	1212	b-d	1464	de
Sun Jewel	U.S.	33	I	82	S	1014	b-e	1397	d-f
Odyssey	U.S.	36	I	70	I	1345	b-d	1369	d-g
Melon Gold Bar	U.S.	49	I	75	S	1120	b-d	1325	e-h
Tam-Dew	U.S.	37	I	68	I	1190	b-d	1159	f-i
Banana	U.S.	31	I	57	I	827	c-f	1131	f-i
Ananas	U.S.	33	I	54	I	1160	b-d	1096	g-i

Table 3.1 (cont'd)

Strike	U.S.	39	I	55	I	1168	b-d	1077	hi
Athena	U.S.	44	I	64	I	1172	b-d	1069	hi
Primo	U.S.	47	I	71	S	1351	b-d	1061	i
Edisto 47	U.S.	32	I	41	I	1031	b-e	784	j
MR-1	India	15	MR	19	MR	309	e-g	326	k
Lagenaria siceraria									
PI 642042[w]	U.S.	11	R	20	MR	514	d-g	410	k
Citrullus lanatus* var. *lanatus									
PI 169290	Turkey	9	R	9	R	250	e-g	140	kl
PI 490378	Mali	5	R	7	R	185	fg	107	kl
PI 629106	U.S.	4	R	7	R	148	fg	105	kl
PI 438676	Mexico	5	R	5	R	165	fg	67	l
PI 171584[w]	Turkey	6	R	3	R	194	fg	55	l
PI 612145	U.S.	5	R	3	R	179	fg	46	l
PI 595218	U.S.	5	R	3	R	209	fg	42	l
PI 171392	Africa	5	R	3	R	123	fg	36	l
Luffa aegyptiaca									
PI 527331[w]	China	9	R	9	R	265	fg	119	kl
***Cucurbita moschata*[z]**									
PI 169410	Turkey	0		0		0	h	0	m
PI 358507	U.S.	0		0		0	h	0	m
***Cucurbita pepo*[z]**									
PI 171622[w]	Turkey	0		0		0	h	0	m
PI 508468	S. Korea	0		0		0	h	0	m

Table 3.1 (cont'd)

***C. pepo* subsp. *fraterna*^{z}**					
PI 532355^{w}	Mexico	0	0	0 h	0 m
***C. pepo* var. *texana*^{z}**					
PI 614687^{w}	U.S.	0	0	0 h	0 m

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[w] Subset of cultigens for the determination of host range and variability between 2010 and 2011.

[x] Mean percentage took at the final rating; agronomical levels of disease HS= highly susceptible (>87%), S= susceptible (51 to 86%) , I= intermediate (26 to 50%), MR=moderately resistant (13 to 25%), R= resistant (<12%).

[y] Mean area under disease progress curve (AUDPC) calculated from % of the total chlorotic and necrotic leaf for each year. Mean separation performed by year using LSD ($\alpha = 0.05$) and an additional t-test performed to compare the differences between AUDPC means and zero (mean AUDPC in *Cucurbita* spp.): Numbers followed by the same letter are not significantly different from each other. High values represent high levels of susceptibility.

[z] No visible infection was observed in these cultigens.

Table 3.2 Interaction of *Pseudoperonospora cubensis* and selected cultigens for the determination of host range variability in 2010 and 2011.

		***Ps. cubensis* reaction** [z]	
Taxa host range	**Common name**	**2010**	**2011**
Cucumis sativus	Cucumber	+	+
C. melo subsp. *melo*	Cantaloupe	+	+
Cucurbita pepo var. *pepo*	Zucchini	-	-
C. pepo var. *texana*	Texas gourd	-	-
C. pepo var. *fraterna*	Gourd	-	-
Citrullus lanatus	Watermelon	+	+
Luffa aegyptiaca	Loofah	+	+
Lagenaria siceraria	Bottle gourd	+	+

[z] (-)= Incompatible host reaction, no visible symptoms; (+)= Compatible reaction based on final ratings >0, evident downy mildew symptoms and *Ps. cubensis* morphological identification.

DISCUSSION

Different levels of resistance to *Ps. cubensis* were detected among cucurbit cultigens. The most susceptible cultigens were observed in the *Cucumis* genus; 'Vlaspik,' a pickling cucumber cultivar, was the most severely infected by *Ps. cubensis* (highest AUDPC value). Overall, downy mildew was less virulent on cultigens in the *Citrullus*, *Lagenaria* and *Luffa* genera and absent in the *Cucurbita* genus. These results have statistically corroborated what empirical studies indicated about the high susceptibility of some *Cucumis sativus* cultivars compared with other Cucurbitaceae species (17, 29). Among *Cucumis melo* cultigens, the breeding line 'MR-1' was significantly less susceptible to *Ps. cubensis,* followed by the cultigen 'Edisto 47.' Low levels of susceptibility to downy mildew in 'MR-1' and 'Edisto 47' are attributed to the inclusion of PI 124111 and PI 124112 into their pedigrees respectively (10, 11, 26). Mechanisms governing the resistance to downy mildew in *C. melo* are associated with quantitative trait loci (QTL) (22). Hence, differences in disease responses may be explained by environmental influence on expression of the QTL. Environmental differences between 2011 and 2010 may have contributed to prolonged leaf-wetness periods favoring *Ps. cubensis* infection; more precipitation events and rainfall amount occurred during the planting season in 2011 (30 events and 280.6 mm) than in 2010 (23 events and 172.3 mm). Thus, the homogeneity (fewer numbers of cultigens significantly different from each other) observed in 2010 for AUDPC values could have resulted from less favorable environmental conditions for pathogen infection in this year compared to the conditions in 2011.

Cucumis melo cultigens 'Tam-dew' and 'Ananas' were cataloged as intermediately resistant in this study; however, when tested in South Carolina they were highly susceptible to *Ps. cubensis* infection (C.S. Kousik, personal communication, 2010). Incidence and severity of *Ps. cubensis* on watermelons were higher in South Carolina compared with Michigan. Differences found in this study in disease response among cultigens between Michigan and South Carolina may indicate differences in the genetic structure of pathogen populations and environmental conditions (23). Low *Citrullus lanatus* var. *lanatus* production in Michigan may also contribute to the low AUDPC values seen in this crop, since Michigan *Ps. cubensis* populations have not been adapted to this host yet. Differential taxon *Benincasa hispida* was not tested due to its unavailability at the time of study. Cucurbit downy mildew symptoms and signs have been reported under natural conditions in Delaware and South Carolina in 2007 with no reports in Michigan (5). No pathogenicity differences were found on the subset of differential taxa for pathotype determination tested in Michigan predominant *Ps. cubensis* populations during the 2010 and 2011 planting seasons. Similar results were obtained at the genetic level when no differences were found among years in Michigan *Ps. cubensis* populations (23). It has been proposed that Michigan downy mildew initial populations originate either the from the Georgia, South Carolina and North Carolina area or from greenhouses located along the border with Canada. However, divergent results between South Carolina and Michigan and genetic analyses performed by Quesada-Ocampo et al. (23) strongly support the "green bridge" hypothesis whereby the primary *Ps. cubensis* inoculum in Michigan comes from greenhouses located along the Canadian border in the Great Lakes area.

Cucurbitaceous plants can become infected by *Ps. cubensis* at any developmental plant stage. Symptoms on young and developing leaves are very rare, whereas cotyledons are even more susceptible than fully developed true leaves (21). Cucurbit downy mildew symptoms differed depending on the host. Chlorotic and necrotic leaf lesions were angular and restricted by the leaf veins in cucumber and loofah, whereas lesions were more circular and not restricted by the leaf veins in cantaloupe, watermelon and bottle gourd. Although many studies evaluate disease as the percentage of chlorotic and necrotic leaf area independently, in this study both traits were evaluated altogether since chlorosis and necrosis are highly correlated to each other (6). Other traits for disease evaluation include plant stunting, lesion size and sporulation (1, 6). In this study, sporulation was not taken into account as a trait for cultigen resistance discrimination as it was subject to environmental conditions. For instance, sporulation on watermelon cultigens was not visible under field conditions; however, when leaf samples were incubated at high relative humidity under laboratory conditions, slight *Ps. cubensis* sporulation was detected. According to Thomas (25) at least 5 hours of dew and low air currents are necessary for maximum sporulation on a susceptible watermelon cultivar. Plant stunting was not measured due to the absence of free-disease plants for comparison and the intrinsic genetic variability in each cultigen that accounts for plant architecture differences. Although Call et al. (1) proposed measuring lesion size for cultigen discrimination and especially to determine hypersensitive responses, this trait was omitted in this study since the polycyclic nature of the pathogen contributes to repeated infections on leaves resulting in coalescent lesions that reduce photosynthetic area. Instead, we measured the area under disease progress curve (AUDPC) to determine the susceptibility of each cultigen to *Ps. cubensis*. For instance, *C. melo* 'Primo' had a final percentage rating of 71% (susceptible), with significantly lower AUDPC values compared with the control. This suggests a delay in cucurbit downy mildew development.

The cultivar Melon Gold Bar, a *Cucumis* hybrid resulting from the interspecific hybridization of *Cucumis sativus* (cucumber) x *Cucumis hystrix* Chakr. (wild melon), was classified as intermediate in 2010 and susceptible in 2011. However, this cultivar yielded high numbers of fruit compared with other *Cucumis melo* cultivars. Although further yield evaluations should be performed to determine differences among production, it is possible that the short maturity time of this cultivar (68 days) compared with the others (78 to 100 days) allows the plant to yield before the disease pressure affects fruit production.

Since cucurbits with higher resistant values demand less fungicide applications than susceptible cultigens, the recommended cantaloupe cultivars for Michigan would be 'Edisto 47,' 'Strike,' 'Banana,' 'Primo,' 'Athena,' 'Tam-dew' and 'Ananas,' classified as intermediate resistant. Moreover, growers will benefit from vigorous and early season plants that may further overcome the damages caused by downy mildew and reduce the necessity of frequent fungicide inputs.

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