SUSCEPTIBILITY OF CUCURBIT FOLIAGE AND FRUIT TO *PHYTOPHTHORA CAPSICI*

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ABSTRACT

SUSCEPTIBILITY OF CUCURBIT FOLIAGE AND FRUIT
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Phytophthora capsici is a destructive pathogen that affects the foliage, fruit, crown, and roots of cucurbit crops. To date, research has focused on cucurbit fruit rot caused by *P. capsici*, whereas little is known about foliar blight. Laboratory experiments were conducted to evaluate foliar blight incidence and severity associated with zoospore concentration (ranging from 1×10^3 to 1×10^6 zoospores/ml), zoospore condition (encysted or motile), inoculation technique (mycelial plugs, zoospore suspension droplets, or zoospore suspension spray), *P. capsici* isolate, and leaf position (cotyledons, first or second true leaves). Incidence and severity of foliar blight in cucumber cotyledons were positively correlated ($P < 0.0001$) with zoospore concentration. Disease severity differed significantly for the interaction between inoculation technique and isolate. Pathogen isolates varied in their ability to cause disease on foliage of cucurbit crops tested. Cotyledons of cucumber, yellow squash, and green zucchini were more susceptible to *P. capsici* isolates than second true leaves and first true leaves showed an intermediate level of susceptibility. When the susceptibility of cotyledons and fruit of cucumber and yellow squash to select *P. capsici* isolates was compared, all *P. capsici* isolates caused disease on all samples, but virulence varied. Significantly ($P \leq 0.0025$) larger lesions and more sporangia were observed on squash than cucumber cotyledons. Lesions and mycelial growth on fruit varied when inoculated with different isolates; significantly ($P = 0.0034$) more sporangia were produced on cucumber fruit than on squash fruit. Overall, cucurbit cotyledons exhibited a different susceptibility to *P. capsici* isolates than that observed in fruit, which has important implications for cucurbit growers.

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INTRODUCTION

The United States is an important producer of several cucurbit crops, including cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai), squash (*Cucurbita pepo* L.), and pumpkin (*Cucurbita maxima* Duchesne). The total annual production and value of cucurbit crops has been increasing (Table 1), but many cultivated cucurbit crops are susceptible to various diseases, caused by many pathogens, that lead to significant reductions in overall production and fruit quality and thus profitability (93). Diseases such as Phytophthora crown, root, and fruit rot (6,41), powdery mildew (67), downy mildew (18), Fusarium wilt (40,61), and various viral diseases (20,26,91), are often limiting factors in domestic cucurbit production. Of many pathogens, *Phytophthora capsici* Leonian is of particular importance because it causes destructive fruit rot (41).

Phytophthora capsici was first identified as the causal agent of chili pepper (*Capsicum annuum* L.) wilt in New Mexico in 1922 (58). The pathogen was later reported to cause crown, root, and fruit rot in many cucurbit crops (28,41). *P. capsici* significantly threatens not only the fresh market and processing industries of cucurbit crops but also the production of Solanaceous and select Fabaceous crops (21,28,34,41). The broad host range and lack of known disease resistance in vegetables complicate effective management of disease caused by *P. capsici* (41).

Foliar blight is observed infrequently in the field compared to Phytophthora crown, root and fruit rot (M. K. Hausbeck, personal observation). It has been reported that foliar blight is favored by warm temperatures (25 to 30 ℃) and prolonged periods of heavy rainfall (5,19). Early studies on cucumber fruit reported that there were no

significant variations in virulence among four *P. capsici* isolates (33) and that fruit becomes less susceptible as it matures (1). Similar information regarding cucurbit foliage is not yet available, but this information may become available by assessing foliar disease incidence and severity.

Table 1. The production of cucurbit crops in the United States during 2007 and 2009 according to USDA National Agricultural Statistics Service (4).

	Year 2007		Year 2009	
Cucurbit crop	**Production (1,000 kg)**	**Value ($1,000)**	**Production (1,000 kg)**	**Value ($1,000)**
Cucumber (F)[z]	439,985	238,925	395,941	220,761
Cucumber (P)[z]	490,996	175,822	492,238	180,845
Cantaloupe	926,500	300,578	902,240	359,082
Pumpkin	519,726	123,519	422,431	102,730
Squash	284,220	222,718	327,450	203,464
Watermelon	1,694,110	313,458	1,819,890	460,778
Total	4,355,537	1,375,020	4,360,190	1,527,660

[z] F = fresh market, P = processing market.

CUCURBIT CROPS

The family Cucurbitaceae is an important group of domesticated plants with tropical or subtropical origins; most of the species are cultivated as vegetables or supplementary food (69). The family is composed of about 118 genera and 825 species (48); seven of these species are of great economic importance, including watermelon, cucumber, cantaloupe, summer squash, winter squash, pumpkin, and bottle gourd (*Lagenaria siceraria* (Molina) Standl) (10,79). Based on world production of cucurbit crops in 2007, as estimated by the Food and Agriculture Organization (3), watermelon is the most widely cultivated, followed by cucumber, melon, pumpkin, squash, and gourd.

The United States is one of the top five cucurbit-producing countries along with China, Iran, Turkey, and Russia.

There are seven major cucurbit crops in the United States, including fresh market and processing cucumber, pumpkin, squash, and melons including cantaloupe, honeydew, and watermelon (15). The total annual value of these crops in 2009 was $1.59 billion, with field production of approximately 4,527,928,000 kg harvested from 182,757 ha (4). Florida, California, Georgia, Texas, Michigan, and North Carolina were the top producers of cucurbit crops in the United States in 2009 (4). Data from the top cucurbit-producing states are listed in Table 2.

Table 2. Comparison of the top cucurbit crops producing states in the United States during 2009 according to USDA National Agricultural Statistics Service (4).

Cucurbit crop	States	Area harvested (ha)	Production (1,000 kg)	Value ($1,000)
Cucumber (F)[y]	Florida	4,573	120,474	78,618
	Georgia	4,047	113,398	59,000
	Michigan	1,740	43,908	18,586
	North Carolina	2,914	34,292	12,852
	California	1,295	21,047	11,693
	Texas	445	5,307	3,159
Cucumber (P)[y]	Michigan	13,152	171,004	49,010
	Florida	2,833	44,452	22,932
	North Carolina	3,764	38,809	12,192
	Texas	2,833	34,927	19,674
	California	——[z]	——	——
	Georgia	——	——	——
Cantaloupe	California	15,540	531,247	166,310
	Georgia	2,023	62,369	39,188
	Texas	971	10,886	6,960
	Florida	——	——	——
	Michigan	——	——	——
	North Carolina	——	——	——
Squash (F and P)	Michigan	2,630	61,915	11,739
	Florida	3,561	51,891	51,480
	California	2,347	55,248	32,160
	Georgia	2,145	48,081	29,892
	North Carolina	1,295	15,966	11,264
	Texas	567	6,350	6,412
Watermelon	Florida	10,441	370,993	135,771
	California	5,018	314,975	86,106
	Georgia	9,308	312,979	67,620
	Texas	8,458	265,442	47,986
	North Carolina	2,711	69,899	15,410
	Michigan	——	——	——

[y] F = fresh market, P = processing market.

[z] —— = no data.

PHYTOPHTHORA SPECIES

The genus *Phytophthora* includes biflagellate and heterokont organisms classified in the Kingdom Stramenopila, the Phylum Oomycota, the Class Oomycetes, the Order Pythiales, and the Family Pythiaceae (17,27,42). There are more than 60 *Phytophthora* spp., many of which cause severe disease in economically important crops (28,37). Although morphologically and physiologically similar to true fungi, *Phytophthora* spp. are more closely related to brown algae and diatoms (8). *Phytophthora* spp. differ from true fungi in three primary ways. First, they have cell walls that consist mainly of cellulose and β-glucan, as compared to chitin and/or chitosan for true fungi. Second, they occur predominantly in vegetative diploidy, whereas true fungi occur primarily in haploid or dikaryon. Third, they use exogenous sources of sterols for sporulation and sexual reproduction, whereas true fungi synthesize sterols for reproduction (28,49).

Within the genus *Phytophthora*, traits such as the size of sporangia, sporangium length to width ratio, sporangium morphology (i.e., nonpapillate, semipapillate or papillate), antheridium formation (i.e., amphigynous or paragynous), and mating type (i.e., homothallic or heterothallic) have historically been used to differentiate species (31,86,89). Other morphological criteria (e.g., sporangium caducity, pedicel length, and ontogeny) have been accepted more recently as means for morphological identification (44,66). Molecular techniques including phylogenetic analysis of protein patterns, isozymes, and restriction fragment length polymorphism of mitochondrial and nuclear DNA are newer methods used to aid identification (29,60,63). Currently, the polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP), a DNA

fingerprint technique, provides the highest resolution to date for species identification as well as population diversity and ecology assay within the genus *Phytophthora* (31).

PHYTOPHTHORA CAPSICI

Phytophthora capsici is a devastating soilborne pathogen that affects many crops under favorable conditions (41). This pathogen was first identified on chili pepper in New Mexico in 1922 (58). *Phytophthora capsici* infection in a cucurbit crop was first reported in Colorado in 1937, when 100% of fruit in a 3.2-ha cucumber field were infected (52). To date, *P. capsici* has been found to infect at least 49 plant species worldwide (28). Notably, *P. capsici* severely threatens fresh market and processing industries of many vegetable crops including Cucurbitaceous, Solanaceous, and Fabaceous crops in the United States (21,28,41); in cucurbits, this includes cucumber (6,41,76), cantaloupe (6), squash (6,19,41), pumpkin (5,6,7,41), and watermelon (6). Recently, various other plants have been identified as hosts for *P. capsici* including snap bean (*Phaseolus vulgaris* L.) (34), lima bean (*Phaseolus lunatus* L.) (21), and Fraser fir (*Abies fraseri* (Pursh) Poir.) (72). Tian and Babadoost (85) observed that cucurbit crops were more susceptible to *P. capsici* than other hosts. Therefore, cucurbit crops were chosen to further the characterization of disease response to *P. capsici*.

Phytophthora capsici is recognized as part of the Waterhouse Group II of morphospecies based on the presence of papillate sporangia and amphigynous oospores that form when A1 and A2 compatibility types (CTs) pair (90). Both CTs have been found in a pepper field in New Jersey (68), pepper and squash fields in North Carolina (76), a pepper field in Ohio (62), and cucurbit fields in Michigan (53), which indicates

that the presence of oospores in vegetable production fields is likely. The A1 and A2 CTs exhibit a ratio of approximately 1:1 in surveyed states (41,53), which could allow for frequent sexual recombination. Therefore, offspring may gain traits such as increased virulence on additional hosts (55) and increased resistance to fungicides that target specific sites of metabolic activity, allowing a population to adapt to the environment with genetic diversity created by sexual recombination (54,71). Thick-walled oospores can survive for a long period in the soil as overwintering structures, and may serve as the primary inoculum for disease (28,41). One study demonstrated that 8 to 9% of tested *P. capsici* oospores were viable in the soil after 27 weeks in a pepper field (12). In another study, *P. capsici* oospores caused significant squash yield loss after lying dormant in the soil for 5 years, which suggests that oospores make possible long-term survival of the pathogen outside of host tissue (41). Oospores mature in two weeks to three months, depending on environmental conditions, and are then able to germinate directly or form sporangia to infect plant hosts (28,46,81).

Following infection, asexual propagules (i.e., sporangia and zoospores) can be produced on host tissues. Abundant formation of sporangia is responsible for repeated cycles of disease during a growing season (41). Water favors disease caused by *P. capsici* by transporting caducous sporangia and causing zoospore release from sporangia by turgor pressure (35), thereby playing a key role in *P. capsici* dispersal (14,36,77,82). Once in contact with water, sporangia can liberate 20 to 40 biflagellate, motile zoospores, which can spread via water to infect hosts (9,12,43).

Zoospores, surrounded only by a plasma membrane, have two flagella in a ventral groove (23). There are many factors that may allow zoospores to serve as the major

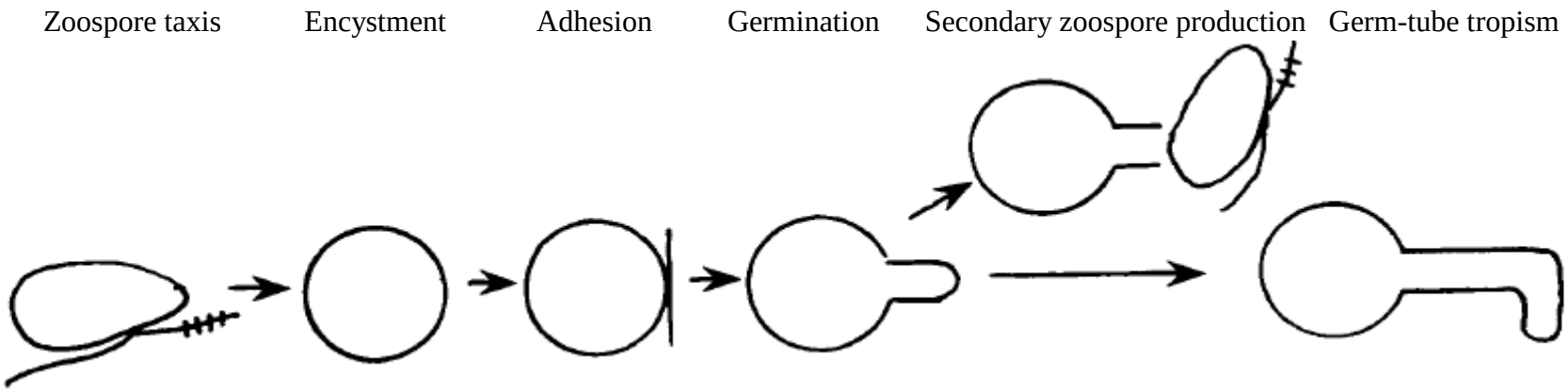
dispersal units and secondary inoculum of *P. capsici*, such as their pattern of negative geotaxic migration in water (16), accumulation on plant surfaces (chemotropism [16], electrotaxis [64,87], and auto-aggregation [75]) and ability to colonize host tissues after encystment (27,87). Taxis of zoospores provides an opportunity for directional dispersal in soil, and helps the pathogen locate an optimal infection site, especially the anticlinal walls of root epidermal cells (23,39).

Zoospores detach flagella and encyst on the surface of plant tissues to germinate directly or invade through natural openings in the host tissues (e.g., stomata) (23). This process is accompanied by a sequence of responses to external amino acids, sugars, calcium, electrical fields, and other factors (Figure 1), which may be necessary for successful infection (23). Thus, disruption of active zoospore movement, taxis, or encystment may prevent *Phytophthora* infection (51); however, whether, and to what extent, the pathogenicity of *Phytophthora* spp. is related to zoospore taxis and encystment has been debated (43,74). Hickman (43) concluded that motile zoospores as inoculum more successfully infect hosts than cysts because taxis of *P. capsici* zoospores provide an opportunity for rapid attraction and accumulation in the elongation zone behind the root tip. However, Raftoyannis and Dick (74) found no significant correlation between the pathogenicity of *Phytophthora* spp. and zoospore taxis or encystment.

Many pathogens produce cell wall-degrading enzymes capable of depolymerizing the polysaccharides in the plant host wall during the colonization of host tissue (56). A nonpectolytic extracellular enzyme from *P. capsici* culture filtrate has been identified that can macerate cucumber mesocarp, resulting in penetration of the host epidermis that contributes to invasion of susceptible host tissue; secretion of this enzyme may also cause

the water-soaked lesions typically seen in *P. capsici*-infected tissue (92). Once host tissue is colonized, hyphae ramify through living plant tissue, developing feeding relationships that involve haustoria (59). Cell wall degrading enzymes have been reported to be effective elicitors of defense responses in pepper-*P. capsici* interactions (65).

Plants have developed a diversity of defense responses triggered by the pathogen (56). For example, an initial quantitative trait loci (QTL) analysis proposed that three QTLs were responsible for resistance of pepper to *P. capsici* (57). However, little is known about the genetic, cellular, and molecular mechanisms underlying the defense of cucurbit crops against *P. capsici* infection.



Factors	Chemical diffusates: amino acids, sugars, aldehydes, alcohols, and isoflavones	Some chemical diffusates: amino acids, and isoflavones	Orientation of zoospore	Amino acids and sugars	Absence of specific nutrients	Amino acids, alcohols, and aldehydes
	Electrical fields	Surface recognition of polysaccharides: fucose, cellulose, chitin, and polyuronates	Release of glycoprotein adhesive	Calcium	Automatic in some Saprolegniales	Electrical fields
	Auto-aggregation	Surface topography	Release of calcium	Autonomous signal		Aeration
	Can be host-specific	Can be host-specific				

Figure 1. The homing responses and external stimuli of oomycete zoospores during infection (22,23).

CROP SUSCEPTIBILITY

Phytophthora capsici can cause symptoms on all plant tissues of cucurbitaceous and solanaceous hosts including foliage, fruit, stems, crowns, and roots (41). Susceptibility to *P. capsici* infection is modulated by host species or cultivar, the type of host tissue, and the age of the plant (27). For example, summer squash was found to be more susceptible to *P. capsici* than 26 other species of common rotational crops and 9 weed species studied (85). To date, fruit have been the most extensively studied tissue of cucurbit hosts for susceptibility to *P. capsici* (1,2,33,45). Screens of cucumber fruit germplasm showed that no commercial cucumber cultivars have significant resistance to *P. capsici* infection (33). Cucurbit fruits become less susceptible to *P. capsici* as they mature (1,2). Cucurbit fruits are particularly susceptible compared to other tissues such as crowns and roots, especially when in contact with infested soil or splash-dispersed zoospores (41).

Isolates of *P. capsici* can differ in how they affect diverse hosts, since separate genes or gene systems have been reported to control virulence for each; fourteen different pathogenic strains were identified among 23 isolates as early as 1972 (70). Ristaino (76) observed significant intraspecific variation and differential virulence among *P. capsici* isolates in cucurbits and pepper. In Italy, no less than 13 pathogenic groups, derived from 26 isolates of *P. capsici* found on pepper and zucchini plants, were identified on nine separate vegetable crops; these were separated based on differences in virulence (84). Foster and Hausbeck (30) assessed the virulence of four Michigan *P. capsici* isolates in pepper lines screened for crown and root rot resistance, and found four different physiological races. Variation in virulence among four Michigan *P. capsici* isolates was

also observed in tomato plants (73). However, whether various *P. capsici* pathogens have differential virulence in cucurbit foliage remains unknown.

Environmental conditions affect *P. capsici* disease incidence and severity (25). Rainfall has a significant effect on sporangial dispersal (36,82) and disease progression (82). For example, rainfall has contributed to *P. capsici* dispersal in pepper fields (82) and commercial cucurbit fields (36). Water also plays a key role in the development and dissemination of sporangia and zoospores produced by *P. capsici* (14,36,77,82); laboratory and field observations indicate that sporangial dispersal occurs in water with capillary force, but not in response to wind or a reduction in relative humidity (36). Therefore, because long-distance dispersal of sporangia through wind is unlikely, water management may be essential to prevent the occurrence of plant diseases caused by *P.capsici* in places far from areas of known pathogen establishment (36).

The mortality of plants infected by *P. capsici* can worsen under conditions of high soil moisture and/or standing water (12). Sporangial production on cucumber fruit was greater at 60 and 80% relative humidity than at > 90% (41). Zoospores were capable of surviving for weeks in surface water at ~25 ℃ (78), making *P. capsici* infection possible in susceptible hosts. Temperature also plays a role; 24 to 32 ℃ and 24 to 27 ℃ were optimal for vegetative growth (47) and sporangial production (24) by *P. capsici* isolates, respectively. Disease incidence and lesion length on pepper fruit were greatest when inoculated fruit were incubated at 27 ℃ as compared to those incubated at 15, 25, 30, and 35 ℃ (11). In addition to temperature and water, other factors can affect disease incidence; for example, over-crowding or over-fertilization with nitrogen favored the infection of pepper fruit (11).

PHYTOPHTHORA FOLIAR BLIGHT

Foliar blight in cucurbits occurs infrequently in the field (M. K. Hausbeck, personal observation), compared to destructive fruit rot. In Michigan, emerging plants in a 24.3-ha cucumber field were killed after a rainstorm transported *P. capsici*-infested soil onto their cotyledons (41). Long periods of heavy rain and warm weather were reported to favor the occurrence of Phytophthora foliar blight in multiple crops (5,41). Severe foliar blight in processing pumpkins was associated with frequent and heavy rainfall, and the incidence of foliar blight was found to be highly correlated with frequency of fruit rot (5). In order to better understand this pathosystem and prevent future crop destruction, it would be beneficial to determine the susceptibility of cucurbit foliage to *P. capsici* and the factors that contribute to foliar disease development.

Zoospores are an important source of inoculum in the *P. capsici* disease cycle (41). Katsura and Miyazaki (51) observed that *P. capsici* zoospores penetrate the epidermal cell wall of the pepper leaf either directly or through the stomata. This penetration occurs approximately 2 hours after zoospore germination and is completed in 4 hours. Typical lesions on pepper leaves develop approximately 14 hours post inoculation at 28-29°C (51). This infection process makes *P. capsici* a destructive, hard-to-manage pathogen.

Foliar infection has been reported in association with many diseases caused by oomycete pathogens (32,80,88). Research on the behavior of *P. infestans* zoospores has shown an orientation of germ tubes toward potato leaf stomata (32). Leaf position significantly contributes to potato resistance, with apical leaves being more resistant to *P. infestans* than basal leaves (88). Zoospores of *Pseudoperonospora humuli* (Miy. and Tak.)

Wilson, located hop stomata and then encysted on the guard cells in response to stomatal topography (80); zoospores of *P. humuli* accumulated at open leaf stomata more frequently than at closed ones (80). These observations may be related to leaf surface pH around the stomata (13,80), CO_2 partial pressure (38), ionic forms of resins (83), or microclimatic conditions (88). *Phytophthora capsici* may use a similar mechanism for foliar infection.

OBJECTIVES OF THE THESIS

To date, research on cucurbits has been focused on the resistance of fruit to *P. capsici* (1,2,33,45). However, it remains unclear whether the foliage of cucurbit crops displays differential susceptibility to *P. capsici* as compared to the fruit. In this research, the study of *P. capsici* in cucurbits was extended to assess foliar disease incidence and severity in relation to factors that may affect disease response and to compare the development of foliar blight and fruit rot in cucumber and yellow squash. The objectives of this research were to (i) determine the effect of zoospore concentration of *P. capsici* on disease incidence and severity using cucumber cotyledons, (ii) assess the response of cucumber cotyledons to disease incited by encysted zoospores compared to that incited by motile zoospores, (iii) investigate the effect of inoculation technique and pathogen isolate on disease severity in cotyledons of different cucurbits, (iv) test the virulence of *P. capsici* isolates on cotyledons of various cucurbits, (v) examine the effect of leaf position and pathogen isolate on foliar disease incidence and severity, and (vi) compare the susceptibility of cucumber and squash cotyledons and fruit using different *P. capsici* isolates.

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CHAPTER I

EFFECTS OF ZOOSPORE CONCENTRATION AND CONDITION, INOCULATION TECHNIQUE, ISOLATE, AND LEAF POSITION ON DEVELOPMENT OF PHYTOPHTHORA FOLIAR BLIGHT IN CUCURBITS

ABSTRACT

Phytophthora capsici is a destructive pathogen that affects the foliage, fruit, crown, and roots of cucurbits. Laboratory experiments were performed to evaluate foliar blight caused by *P. capsici* on cucumber cotyledons in relation to zoospore concentration (ranging from 1×10^3 to 1×10^6 zoospores/ml), zoospore condition (encysted or motile), inoculation technique (mycelial plugs, zoospore suspension droplets, or zoospore suspension spray) and isolate. The effect of leaf position (cotyledons, first and second true leaves) on the susceptibility of cucurbit foliage to four *P. capsici* isolates was also evaluated. Cotyledons of cucumber, yellow squash, and green zucchini were more susceptible to *P. capsici* isolates than second true leaves, and first true leaves showed an intermediate level of susceptibility. Incidence and severity of foliar blight on cucumber cotyledons were positively correlated with higher zoospore concentrations. Both encysted and motile zoospores caused disease on cucumber cotyledons. Disease severity differed significantly for the interaction between inoculation technique and isolate. Lesion diameter was greater with increasing incubation time, and sporangial density reached the greatest level 9 days post inoculation. Virulence differed significantly among isolates; isolates 12889 and SP98 were more virulent than isolates OP97 and SF3 on the three cucurbits tested. Thus, various factors, including zoospore concentration, inoculation method, isolate, and leaf position, affect development of cucurbit foliar blight caused by *P. capsici*.

INTRODUCTION

The United States is one of the leading producers of cucurbit crops in the world (4). Michigan ranks first in the United States for the production of squash (*Cucurbita pepo* L.) and pickling cucumber (*Cucumis sativus* L.) (5). The fresh market and processing industries are dependent on long-term, viable production of cucurbit crops (20), which is threatened by *Phytophthora capsici* Leonian, a soilborne oomycete pathogen (16). *P. capsici* causes rot of the roots, crown, and fruit, and foliar blight, resulting in extensive crop loss (6,16). Management of *P. capsici* is complicated by a lack of known host resistance mechanisms (16). Conventional strategies, including the use of fungicides and crop rotation, do not provide complete protection against this pathogen when environmental conditions favor disease development (16). Isolates of *P. capsici* have developed resistance to mefenoxam, a key fungicide that has previously been effective in disease control (21,28). The efficacy of crop rotation is limited because *P. capsici* has a wide host range (12), and oospores, the primary inoculum, can survive for long periods of time in soil (22).

P. capsici sporangia and zoospores serve as secondary inoculum, resulting in polycyclic disease development during a growing season (16). Biflagellate zoospores released from sporangia may move to uninfested soil via irrigation water, resulting in the spread of *P. capsici* (16,36). Following contact with the host, zoospores encyst, adhere, germinate, and penetrate host tissues (10,12). Disruption of active zoospore taxis or encystment may prevent *Phytophthora* spp. infection (19). However, whether the pathogenicity of *Phytophthora* spp. is correlated with zoospore taxis or encystment remains to be elucidated (17,32).

Symptoms of foliar blight on cucurbit crops include irregular, tan to brown necrotic lesions on foliage or a rapid wilt (25). Environmental conditions can affect the development of these symptoms in cucurbit foliage; rainfall and warm weather favor foliar blight occurrence (6,16,25). Although foliar blight occurs less frequently than fruit rot in the field (M. K. Hausbeck, personal observation), foliar blight has been reported to cause severe crop losses on processing pumpkin in Illinois (6) and cucumber in Michigan (16).

In contrast to foliar blight, fruit rot has been thoroughly investigated. Specifically, the resistance of cucurbit fruit to *P. capsici* in relation to host species or cultivar (14,18) and fruit age (2,3,14) has been studied. Some factors, such as *P. capsici* zoospore concentration, have been shown to have a significant effect on cucumber fruit infection (15). Other factors, such as differences in isolate virulence, have not significantly affected occurrence of cucumber fruit rot (14). The extent to which these results carry over to cucurbit foliage has not yet been investigated.

In the research presented here, the study of *P. capsici* in cucurbits was extended to assess foliar disease incidence and severity in relation to factors that may affect disease response. The objectives of this study were to (i) determine the effect of *P. capsici* zoospore concentration on disease incidence and severity using cucumber cotyledons, (ii) assess the response of cucumber cotyledons to disease incited by encysted zoospores compared to that incited by motile zoospores, (iii) investigate the effect of inoculation technique and pathogen isolate on disease severity in cotyledons of different cucurbits, (iv) test the virulence of *P. capsici* isolates on cotyledons of various cucurbits, and (v)

examine the effect of leaf position and pathogen isolate on foliar disease incidence and severity.

MATERIALS AND METHODS

***Phytophthora capsici* isolates and inoculum preparation.** Actively growing cultures of *P. capsici* isolates 12889, and 13351, OP97, SF3, and SP98 were used (isolate notation refers to the culture collection maintained in the laboratory of M. K. Hausbeck at Michigan State University [MSU]). Isolates were characterized for sexual compatibility type and sensitivity to the fungicide mefenoxam as previously described (21). The geographic origin, source crop from which isolates were originally obtained, and phenotype of *P. capsici* isolates used are listed in Table 3. Isolates were recovered from long-term stock cultures (stored at 20 ℃ in sterile microcentrifuge tubes containing 1 ml of sterile water and one sterile hemp seed), transferred to unclarified V8 juice agar (UCV8: 16 g agar, 3 g $CaCO_3$, 160 ml V8 juice, and 840 ml distilled water) amended with 100 ppm of ampicillin and 30 ppm of rifampicin, and incubated at room temperature (21 ± 2 ℃) under continuous fluorescent lighting (1). Isolates were subsequently inoculated onto cucumber fruit to confirm their pathogenicity using the method of Quesada-Ocampo et al. (30) and isolates obtained from infected cucumber fruits were used.

Isolates were grown on UCV8 under temperature and light conditions described above and were subcultured on new UCV8 weekly by hyphal transfer. Zoospore suspensions were created by flooding cultures with 5 ml of sterile distilled water and incubating at 2 ℃ for 1 h, followed by 30 min at room temperature to induce zoospore release (15). Zoospore concentration was determined using a hemacytometer and adjusted to 1×10^6 zoospores/ml using sterile distilled water. Serial dilution (×10) in sterile distilled water was performed to obtain the desired concentrations described below.

Eight-mm-diameter mycelial plugs were excised using a sterile cork borer from the actively growing edge of cultures for inoculation.

Table 3. Origin, source crop, and phenotype of *Phytophthora capsici* isolates used in inoculations.

			Phenotype	
Isolate	**Origin[x]**	**Source crop**	**CT[y]**	**MS[z]**
OP97	Michigan	Pickling cucumber	A1	S
SF3	Michigan	Pickling cucumber	A1	S
SP98	Michigan	Pumpkin	A2	S
12889	Michigan	Bell pepper	A1	I
13351	New York	Eggplant	A1	S

[x] Origin = the state from which isolate was originally collected.
[y] CT = compatibility type, designated A1 and A2 and determined by crossing the isolate to be screened with OP97 (A1) and SP98 (A2) standard isolates (21).
[z] MS = mefenoxam sensitivity, where I = insensitive and S = sensitive to the mefenoxam, calculated by comparing growth in mefenoxam-amended V8 media to control media as conducted by Lamour and Hausbeck (21).

Plant material. Experiments included five different types of cucurbits susceptible to *P. capsici* (Table 4). Seeds were sown into 72-cell (6 × 12) flats (TLC Polyform, Inc., Plymouth, MN) containing soilless potting media (BACCTO Professional Planting Mix, Michigan Peat Company, Houston, TX), and were grown in a greenhouse with a mean temperature of 24 ℃ and a 14-h photoperiod. Seven-day-old seedlings were transplanted into 1.5-liter plastic pots containing soilless potting media and were grown in the greenhouse as above. Plants were watered daily as needed. For cotyledon experiments, 14-day-old cucumber, yellow squash, and green zucchini plants at the first true leaf stage and 21-day-old watermelon and cantaloupe plants at the second true leaf stage were used for the experiments. One day before inoculation, individual plants of each cultivar were placed into sealed plastic bags (20 cm × 10 cm × 46 cm) or nine plants were placed into a

transparent moisture chamber that was composed of a plastic humi-dome (22 cm × 12 cm × 7 cm) (Hummert International, Earth City, MO) and a germination tray (20 cm × 11 cm × 3 cm) (Hummert International), lined with saturated paper towels to maintain high humidity. For leaf position experiments, plants were incubated and watered daily until cotyledons, first true leaves, and second true leaves were fully expanded. One day before inoculation, 27-day-old plants of each cultivar were individually placed into plastic bags. Twenty ml of distilled water was placed at the bottom of the bag to maintain high relative humidity.

Table 4. Plant materials used in this study.

Host[z]	Cultivar	Source
Cucumber	Vlaspik	Seminis Vegetable Seed Inc., Oxnard, CA
Cantaloupe	Athena	Seedway LLC, Hall, NY
Yellow squash	Cougar	Harris Moran Seed Company, Modesto, CA
Yellow squash	Superpik	Harris Moran Seed Company
Green zucchini	Tigress	Harris Moran Seed Company
Watermelon	Sugar Baby	Seedway LLC

[z] Yellow squash and green zucchini are summer squash.

Effect of zoospore concentration on disease incidence and severity using cucumber cotyledons. Four zoospore concentrations (1×10^3, 1×10^4, 1×10^5, and 1×10^6 zoospores/ml) of *P. capsici* isolates SP98 and 12889 were each used to inoculate cucumber cotyledons. The proportion of motile zoospores from each serial suspension was determined for two concentrations using a hemacytometer; motile zoospores accounted for 42% and 48% of the total number of zoospores at concentrations of 1×10^5 and 1×10^6 zoospores/ml, respectively. The proportion of motile zoospores was not

determined for concentrations of 1×10^3 and 1×10^4 zoospores/ml. A 20-µl droplet of each spore concentration was placed on the center of one cotyledon using a sterile micropipette. Five plants were inoculated per concentration, and five plants were inoculated with sterile distilled water as a control. Plants were arranged in a split-plot design in individual sealed bags, with pathogen isolates as the whole-plot factor, zoospore concentrations as the sub-plot factor, and incubation time as the repeated measure.

Severity of Phytophthora foliar blight was assessed at 1, 3, 5, and 7 days post inoculation (dpi) on a 0 to 5 scale, where 0 = no symptoms; 1 = cotyledons with chlorotic, water-soaked lesions appearing on ≤ 25% leaf area; 2 = cotyledons with 26 to 50% leaf area symptomatic; 3 = cotyledons with 51 to 75% leaf area symptomatic; 4 = cotyledons with 76 to 100% leaf area symptomatic; 5 = symptoms expanded from cotyledons to first true leaves (Figure 2). The area under the disease progress curve (AUDPC) was computed according to the methods of Shaner and Finney (39) to obtain the cumulative disease severity (%) throughout the experiment. Lesion diameter (mm) was measured at 3 and 6 dpi using a ruler, and was estimated as the average of two perpendicular measurements of each lesion. Disease incidence was defined as the percentage of symptomatic cotyledons at 7 dpi. The experiment was conducted three times.

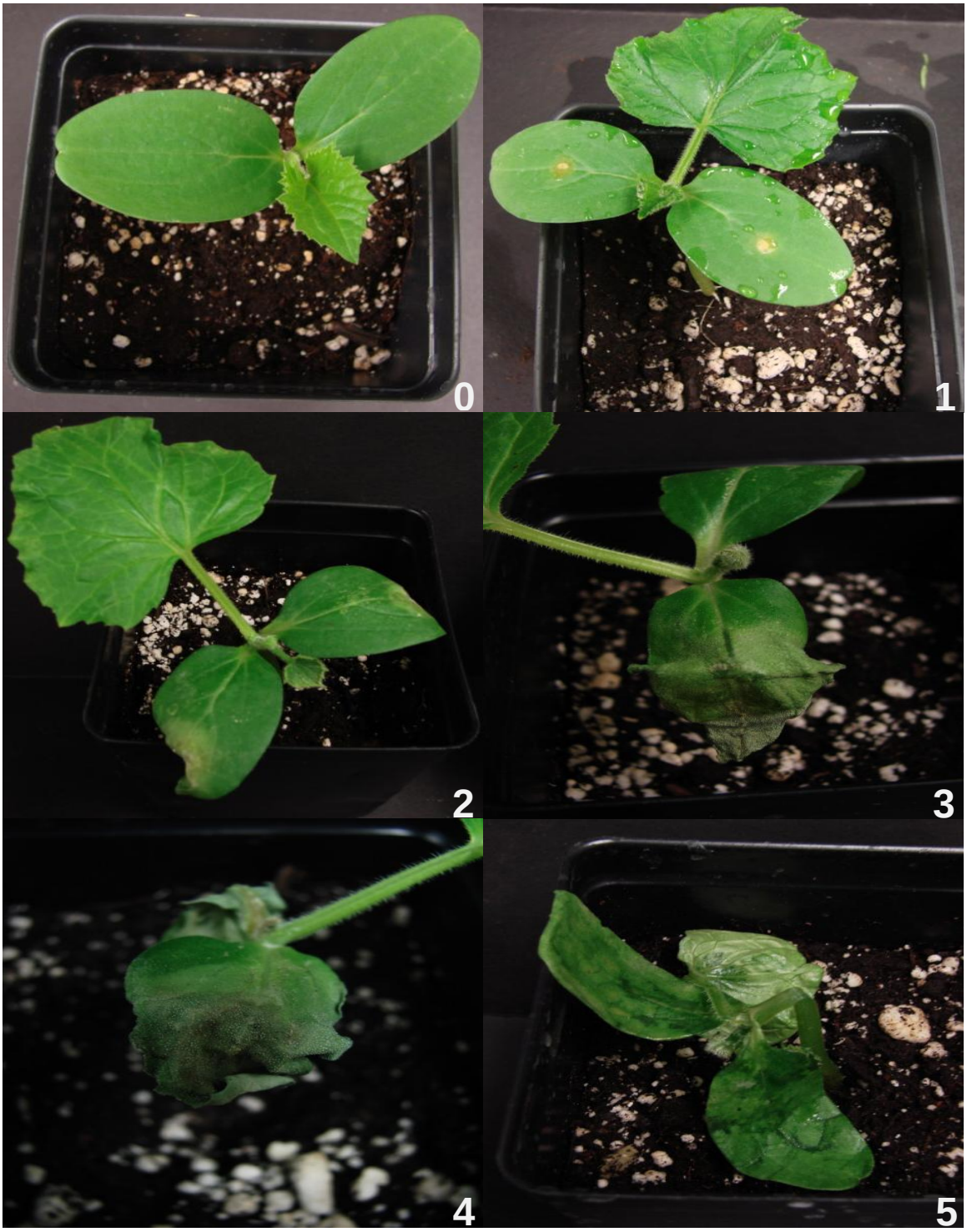

Figure 2. Symptoms used for disease scale of 0 to 5, where 0 = no symptoms, 1 = < 25% cotyledon area symptomatic, 2 = 25 to <50% cotyledon area symptomatic, 3 = 50 to <75% cotyledon area symptomatic, 4 = 75 to 100% cotyledon area symptomatic, and 5 = symptoms expanded from cotyledons to first true leaves. Pictures show cotyledons of cucumber cv. Vlaspik inoculated with a 20-µl zoospore suspension droplet (1×10^6 zoospore/ml) of *Phytophthora capsici* isolate 12889. "For interpretation of the references

to color in this and all other figures, the reader is referred to the electronic version of this thesis."

Sporangial density on cucumber cotyledons. One cotyledon on each of 25 cucumber plants was inoculated with a 20-µl droplet of zoospore suspension (1×10^6 zoospores/ml) of *P. capsici* isolate 12889 and incubated in sealed plastic bags as described above. Five plants were arbitrarily sampled for evaluation of lesion development and sporulation at 1, 3, 5, 7, and 9 dpi. Pathogen growth and development were observed using stereomicroscopy (Leica M165C, Wetzlar, Germany). Lesion width (mm) and length (mm) were measured using a ruler. The number of sporangia in each lesion was counted four times using a hemacytometer after lesions were excised and sporangia were collected as described below.

Single lesions were excised with a razor blade and placed into a sterile 2.2-ml microcentrifuge tube containing 1 ml of sterile water. Sporangia were suspended by vortexing the tubes for 70 s on a vortex mixer before removing the plant tissue with forceps. Resulting sporangial suspensions were concentrated by centrifugation at 18,407 $\times$g for 5 min, removing the supernatant, and resuspending the pellet in 50 µl of sterile distilled water. Lesion area was calculated based on the following formula: $A = \pi \times r_1 \times r_2$, where r_1 = length/2 and r_2 = width/2. Sporangial density was estimated by dividing the number of sporangia by the total lesion area (mm^2). The experiment was conducted three times.

Evaluation of disease response to encysted zoospores on cucumber cotyledons. Encystment was confirmed using microscopic observation after mechanical agitation of

fresh zoospore suspensions on a vortex mixer 70 s as described by Dijksterhuis and Deacon (11). An unvortexed zoospore suspension of 1×10^6 zoospores/ml and sterile distilled water were used as positive and negative controls, respectively. Five plants for each cucumber by pathogen isolate combination were inoculated with zoospore suspensions (1×10^6 zoospores/ml) of *P. capsici* isolates 12889 and SP98 as described above. Plants were arranged in a split-plot design in individual sealed bags with pathogen isolates as the whole-plot factor, zoospore condition (encysted or motile) as the sub-plot factor, and incubation time as the repeated measure.

Foliar blight severity was evaluated at 1, 3, 5, and 7 dpi on the 0 to 5 scale described above and the AUDPC was calculated. Lesion diameter (mm) was measured at 3 and 6 dpi. Disease incidence was determined at 7 dpi and was expressed as the percentage of the inoculated cotyledons showing symptoms. Forty percent of symptomatic plants were arbitrarily sampled for pathogen isolation to confirm the presence and phenotype of each *P. capsici* isolates. The experiment was conducted three times.

Effect of inoculation technique and pathogen isolate on disease severity on cucumber cotyledons. Inoculation techniques (mycelial plug, zoospore suspension droplet, or zoospore suspension spray) were compared using *P. capsici* isolates OP97, SP98, 12889, and 13351 to determine the efficiency and consistency of different techniques in inciting disease in cucumber cotyledons. Each plant was inoculated in the center of both expanded cotyledons. Ten plants were used for each inoculation technique by pathogen isolate combination. Cotyledons were inoculated one of three ways, by placing an 8-mm-diameter mycelial plug with the mycelium side in direct contact with

the surface of both cotyledons, by placing a 20-µl droplet of a zoospore suspension (1×10^6 zoospores/ml) on the center of both expanded cotyledons, or by spraying cotyledons with a zoospore suspension (1×10^6 zoospores/ml) using a handheld sprayer until runoff (~15 ml). Five plants were inoculated with sterile 8-mm-diameter plugs of V8 agar or sterile distilled water droplets or sprayed with sterile distilled water as controls. Plants were arranged in a split-plot design in moisture chambers as described above with inoculation techniques as the whole-plot factor, and pathogen isolates as the sub-plot factor. Each chamber contained nine plants including two inoculated plants per isolate and one control plant. Saturated paper towels were placed in the chambers to maintain high humidity. Plants were incubated under the conditions above for 8 days. Air temperature and relative humidity inside moisture chambers were monitored using Watchdog data loggers (Model 450, Spectrum Technologies, Inc., Plainfield, IL). The average temperature and relative humidity were 20.8 ℃ and 86.2%, respectively.

Severity of foliar blight was assessed daily for 8 days using the 0 to 5 scale described above. An AUDPC was calculated. Forty percent of symptomatic plants were arbitrarily sampled for pathogen isolation to confirm the presence and phenotype of each *P. capsici* isolates. The experiment was conducted twice.

Effect of inoculation technique and pathogen isolate on disease severity on cotyledons of yellow squash and watermelon. Yellow squash cv. Cougar and watermelon cv. Sugar Baby were selected to evaluate the effect of two of the inoculation techniques described in the previous experiment, mycelial plug and zoospore suspension droplet, on disease development on different cucurbit types caused by *P. capsici* isolates OP97, SP98, 12889, and 13351. Ten plants were inoculated using each inoculation

technique by pathogen isolate combination; plants were inoculated by the mycelial plug or zoospore suspension droplet methods described in the previous experiment. Five plants were inoculated with sterile 8-mm-diameter plugs of V8 agar or sterile distilled water as controls. Plants were arranged in a split-plot design for each crop in moisture chambers as described above, with inoculation techniques as the whole-plot factor and pathogen isolates as the sub-plot factor. Disease severity was assessed for 8 days on the 0 to 5 scale described above. AUDPC was calculated. Forty percent of symptomatic plants were arbitrarily sampled for pathogen isolation to confirm the presence and phenotype of each *P. capsici* isolates. The experiment was conducted twice.

Evaluation of isolate virulence on cotyledons of various cucurbit crops. Inoculation with zoospore droplets was selected as an efficient means of inoculation based on the results of previous experiments. Seedlings of cantaloupe cv. Athena, yellow squash cvs. Cougar and Superpik, green zucchini cv. Tigress, and watermelon cv. Sugar Baby were selected for evaluation of isolate virulence. Both cotyledons of ten seedlings were inoculated with a 20-µl droplet of zoospore suspension (1×10^6 zoospores/ml) of individual *P. capsici* isolate, OP97, SP98, 12889, or 13351. Five plants were inoculated with sterile distilled water as a control. Plants were arranged in a randomized complete block design for each crop in moisture chambers as described above. Disease severity was assessed and the AUDPC was calculated. Forty percent of symptomatic plants were arbitrarily sampled for pathogen isolation to confirm the presence and phenotype of each *P. capsici* isolates. Each experiment was conducted twice.

Effect of leaf position and pathogen isolate on foliar disease incidence and severity. Cucumber cv. Vlaspik, cantaloupe cv. Athena, yellow squash cv. Cougar, and

green zucchini cv. Tigress were inoculated by placing a 20-µl droplet of zoospore suspensions (1×10^6 zoospores/ml) containing single isolate in the center of cotyledons, first true leaves, or second true leaves. Five plants per crop were used for each treatment (pathogen isolate by leaf position) and five plants were inoculated with sterile distilled water as a control. Plants were enclosed in plastic bags and incubated under the conditions described above. Plants were arranged in a split-plot design for each cucurbit crop with pathogen isolates as the whole-plot factor, leaf positions as the sub-plot factor, and incubation time as the repeated measure. Lesion diameter (mm) was measured at 3 and 6 dpi. Disease incidence was determined as described above at 7 dpi. Air temperature (℃) and relative humidity (%) inside the plastic bags were monitored (Table 3) using Watchdog data loggers. The experiment was conducted three times.

Table 5. Air temperature and relative humidity inside plastic bags in which the effect of leaf position and pathogen isolate on foliar disease incidence and severity were evaluated.

	Air temperature (℃)			Relative humidity (%)		
Experiment	**Ave[z]**	**Min**	**Max**	**Ave**	**Min**	**Max**
1	21.5	20.9	22.1	76.6	45.6	83.5
2	21.4	20.2	22.1	66.0	53.1	78.5
3	21.6	20.2	22.5	91.8	75.1	98.7

[z] Ave = average, Min = minimum, and Max = maximum.

Pathogen isolation. At the end of the observation period, whole plants for cotyledon experiments or cotyledons, first true leaves, and second true leaves for leaf position experiments were rinsed in distilled water and surface disinfected with a 70% ethanol solution for 2 min and air dried. Three small sections from the margin of lesions were plated onto UCV8 plates amended with 25 ppm of benomyl, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene (BARP). When plants

were asymptomatic, four sections of tissue were excised around the inoculation points and placed onto BARP-amended UCV8. All isolates were identified using morphological characteristics of *P. capsici* (43) after incubation at room temperature under constant fluorescent lighting for 4 days. Hyphal tips of suspected *P. capsici* isolates were subcultured to new BARP-amended UCV8 plates. Resulting *P. capsici* isolates were screened following a 5-day incubation period for compatibility type and mefenoxam sensitivity using the methods of Lamour and Hausbeck (23) to confirm isolate phenotype.

Statistical analyses. For cotyledon experiments, data on disease incidence, lesion diameter, AUDPC values, and sporulation density from repetitions of the same experiment were pooled for statistical analysis after no significant differences were found between repeated experiments. Data were tested by analysis of variance (ANOVA) using the PROC MIXED procedure of SAS version 9.2 (SAS Institute Inc., Cary, NC). Data from the control plants were removed prior to analyses since no symptoms occurred. Residuals were tested for normality and homogeneity of variance using PROC UNIVARIATE. Outlier data were deleted and data were transformed to meet the assumption of normality distribution when necessary. Disease incidence, AUDPC, and lesion diameter data from the zoospore concentration study were square root-transformed to meet the assumption of normality. Sporangial density data also were square root-transformed. AUDPC data from virulence screens were averaged first from two inoculated cotyledons in the same plant and next from two plants in the same chamber and then normalized by ln (yellow squash) and square-root (watermelon) transformation. The relationships between log zoospore concentration and disease incidence, log zoospore concentration and mean AUDPCs, and log zoospore concentration and lesion

diameter were analyzed using linear regression analysis. The relationship between incubation time and lesion diameter was also determined using linear regression analysis.

For the leaf position experiments, data for disease incidence and lesion diameter were subjected to analyses of variance using the PROC MIXED and GLIMMIX procedures of SAS version 9.2. Data from control plants and plants inoculated with isolate SF3 were removed from the data set prior to statistical analyses since no or occasional ($\leq 2\%$) symptom production occurred, respectively. Residuals were checked for normality and homogeneity of variances. Data transformation and outlier data deletion were applied to satisfy normality assumptions when necessary. Data of disease incidence were square-root transformed for cantaloupe and green zucchini. Data of lesion diameter for cucumber and yellow squash were cubic root- and ln- transformed, respectively. Multiple comparisons among the means were conducted using ANOVA and Fisher's protected Least Significant Difference (LSD) was used for separation of means when effects were statistically significant at $\alpha = 0.05$.

RESULTS

Effect of zoospore concentration on disease incidence and severity using cucumber cotyledons. Lesions developed when cotyledons were inoculated with zoospore concentrations ranging from 1×10^3 to 1×10^6 zoospores/ml. *Phytophthora capsici* isolates SP98 and 12889 both caused expanding chlorotic, water-soaked lesions. Symptoms did not appear on cotyledons inoculated with sterile distilled water. All *P. capsici* isolates recovered from the symptomatic cotyledons matched the phenotype of the isolate used for inoculation. Pathogens were not isolated from asymptomatic cotyledons (*data not shown*).

Disease incidence differed significantly ($P < 0.0001$) for zoospore concentration; at 7 dpi disease incidence ranged from 16 to 100% (Figure 3A). Incidence was 100% when cotyledons were inoculated with concentrations of 1×10^5 or 1×10^6 zoospores/ml of either isolate. The pathogen isolate and the interaction between pathogen isolate and zoospore concentration did not significantly affect disease incidence ($P = 0.6214$ and $P = 0.4940$, respectively).

Mean AUDPCs significantly ($P < 0.0001$) increased as *P. capsici* zoospore concentration increased from 1×10^3 to 1×10^6 zoospores/ml (Figure 3B). Suspensions containing 1×10^6 zoospores/ml of either isolate SP98 or 12889 resulted in the most severe disease. In the linear regression analysis, a strong positive relationship was observed between log zoospore concentration and mean AUDPCs for both isolates ($Y = 7.19x - 22.95$, $R^2 = 0.95$). Pathogen isolate and the interaction between pathogen isolate and zoospore concentration were not significant ($P = 0.8767$ and $P = 0.3394$, respectively).

Lesion diameter differed significantly ($P < 0.0001$) for zoospore concentration and incubation time. The interaction between zoospore concentration and incubation time ($P = 0.0069$) was also significant. Lesions increased as zoospore concentration increased at both 3 and 6 dpi (Figure 3C). Lesions were the largest on cotyledons inoculated with 1×10^6 zoospores/ml of both isolates. Lesion diameter did not differ significantly ($P = 0.7947$, $P = 0.1068$, $P = 0.2714$, and $P = 0.4618$, respectively) for pathogen isolate, two-way interactions between pathogen isolate and zoospore concentration, pathogen isolate and incubation time, or the three-way interaction among pathogen isolate, zoospore concentration, and incubation time.

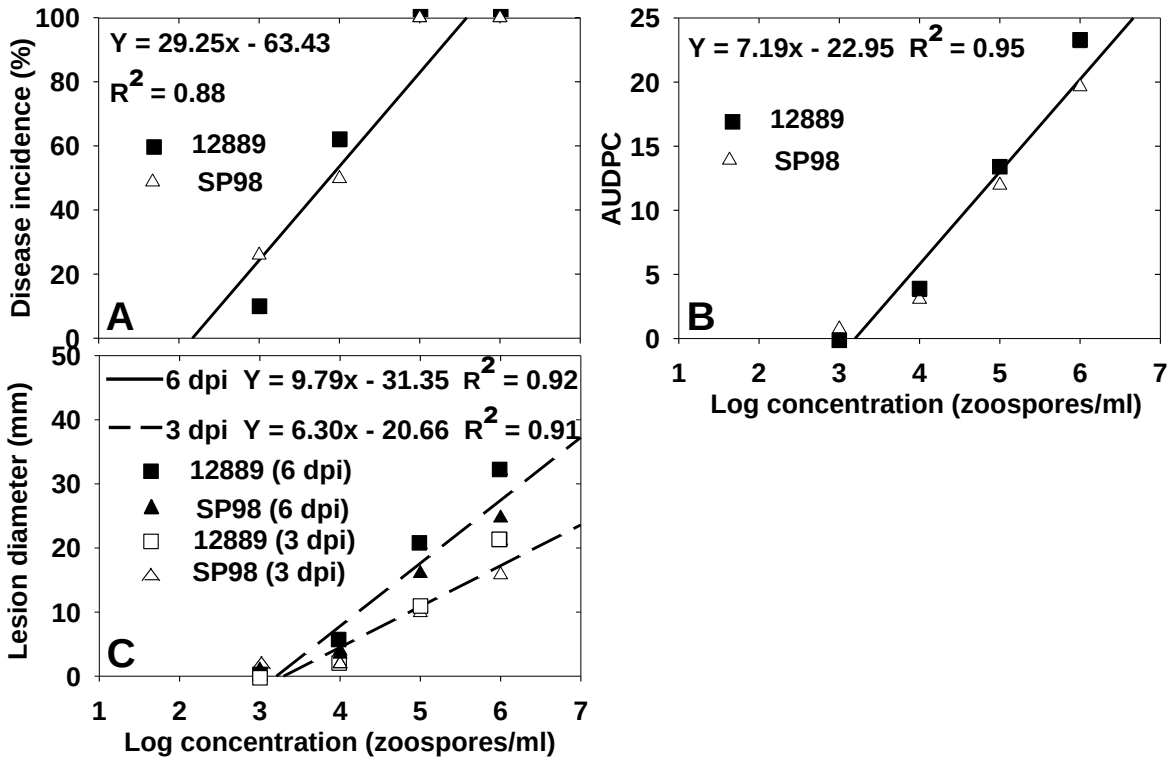

Figure 3. Effect of zoospore concentration of *Phytophthora capsici* isolates SP98 (triangles) and 12889 (squares) on disease incidence (**A**), mean area under the disease progress curve (AUDPC) values (**B**), and lesion diameter (**C**) 3 and 6 days post inoculation (dpi) of foliar blight on cotyledons of cucumber cv. Vlaspik. For lesion diameter, open forms represent 3 dpi and solid forms represent 6 dpi. Each point represents the average of three repeated tests with five replicate cucumber seedlings per treatment per test.

Sporangial density on cucumber cotyledons. A zoospore concentration of 1×10^6 zoospores/ml was selected to inoculate cucumber cotyledons because it produced consistently high disease severity in previous experiments (see above); with this concentration, mycelial growth and sporangial formation in the lesions was detected 3 dpi and continued through the last observation (9 dpi). Disease symptoms were not observed on cotyledons inoculated with sterile distilled water.

Lesion diameter and sporangial density differed significantly ($P < 0.0001$ and $P = 0.0340$, respectively) for incubation time. Lesions increased significantly ($P \leq 0.0354$) with incubation increasing from 1 to 7 dpi (Figure 4A). Sporangial density (sporangia/mm^2) did not differ significantly ($P \geq 0.0644$) from 1 to 7 dpi (Figure 4B). However, the sporangia density was significantly ($P = 0.0199$) greater at 9 dpi than at$\leq$ 7 dpi.

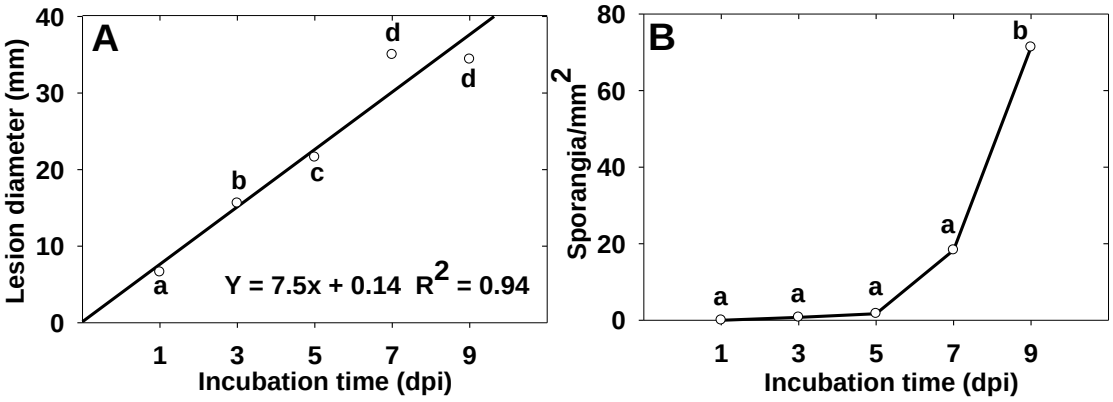

Figure 4. Effect of incubation time (days post inoculation, dpi) on lesion diameter (**A**) and sporangial density (**B**) of lesions on cotyledons of cucumber cv. Vlaspik inoculated with a 20-µl zoospore suspension droplet (1×10^6 zoospore/ml). Each point (**A** and **B**) represents the average of three repeated tests with five replicate cucumber seedlings per time point per test at which lesions were evaluated (1 to 9 dpi). Points with the same letters are not significantly different according to Fisher's LSD ($\alpha = 0.05$).

Evaluation of disease response to encysted zoospores on cucumber cotyledons. Cotyledons were infected by both encysted and motile zoospore suspensions. Sterile distilled water-inoculated cotyledons remained asymptomatic. All *P. capsici* isolates recovered from the symptomatic cotyledons matched the phenotype of the pathogen isolate used for inoculation. Pathogens were not isolated from asymptomatic cotyledons (*data not shown*).

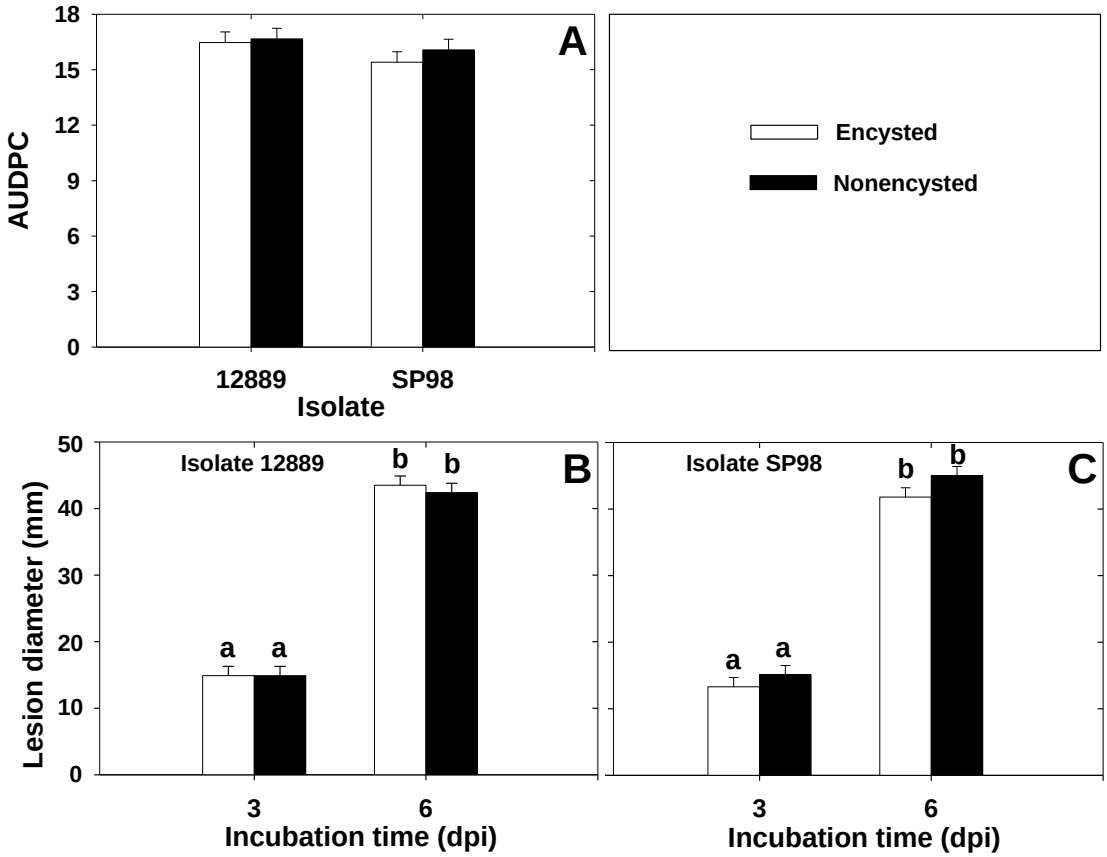

Figure 5. Effect of zoospore condition (encysted or motile) on mean area under disease progress curve (AUDPC) values (**A**) and incubation time (days post inoculation, dpi) on lesion diameter (**B** and **C**) of foliar blight on cotyledons of cucumber cv. Vlaspik inoculated with a 20-µl zoospore suspension droplet (1×10^6 zoospore/ml) of *Phytophthora capsici* isolates 12889 (**A** and **B**) and SP98 (**A** and **C**). Each bar represents the average of three repeated tests with five replicate cucumber seedlings per treatment per test. Bars with different letters are significantly different between incubation time at each zoospore condition (**B** and **C**) (lowercase letters) according to Fisher's LSD (α = 0.05). Error bars represent standard error.

Mean AUDPCs did not differ significantly ($P = 0.4541$, $P = 0.1527$, and $P = 0.6864$, respectively) for pathogen isolate, zoospore condition, or the interaction between pathogen isolate and zoospore condition (Figure 5A). Lesion diameter did not differ significantly ($P = 0.9188$ and $P = 0.4279$, respectively) for pathogen isolate or zoospore condition. A significant ($P < 0.0001$) difference was found for incubation time; lesions

significantly increased with incubation time from 3 to 6 dpi (Figure 5B and C). Lesion diameter was not significantly affected by any between-factor interactions.

Incubation time ($P < 0.0001$) significantly affected lesion diameter, but pathogen isolate ($P = 0.9188$) and zoospore condition ($P = 0.4279$) did not. Lesions significantly increased with incubation time from 3 to 6 dpi (Figure 5C and D). Lesion diameter did not differ significantly for two-way interactions between pathogen isolate and zoospore condition ($P = 0.2445$), or pathogen isolate and incubation time ($P = 0.4144$), or zoospore condition and incubation time ($P = 0.8962$), or the three-way interaction among pathogen isolate, zoospore condition, and incubation time ($P = 0.3752$).

Effect of inoculation technique and pathogen isolate on disease severity on cucumber cotyledons. Disease symptoms appeared 1 dpi and increased with a different rate for each pathogen isolate over the 8-day observation period (Figure 6A to C). Seedlings exhibited chlorotic, irregularly-shaped lesions on cotyledons when inoculated with zoospore suspensions of select *P. capsici* isolates (Figure 2B to F). Lesions developed on the newly-expanding leaves and the stem (Figure 2F), and symptomatic plants eventually wilted (Figure 2E and F). Sterile agar plug- or water-inoculated cotyledons remained asymptomatic (Figure 2A). All *P. capsici* isolates recovered from symptomatic cotyledons had the same phenotype as the pathogen isolate used for inoculation. Pathogens were not isolated from asymptomatic cotyledons (*data not shown*).

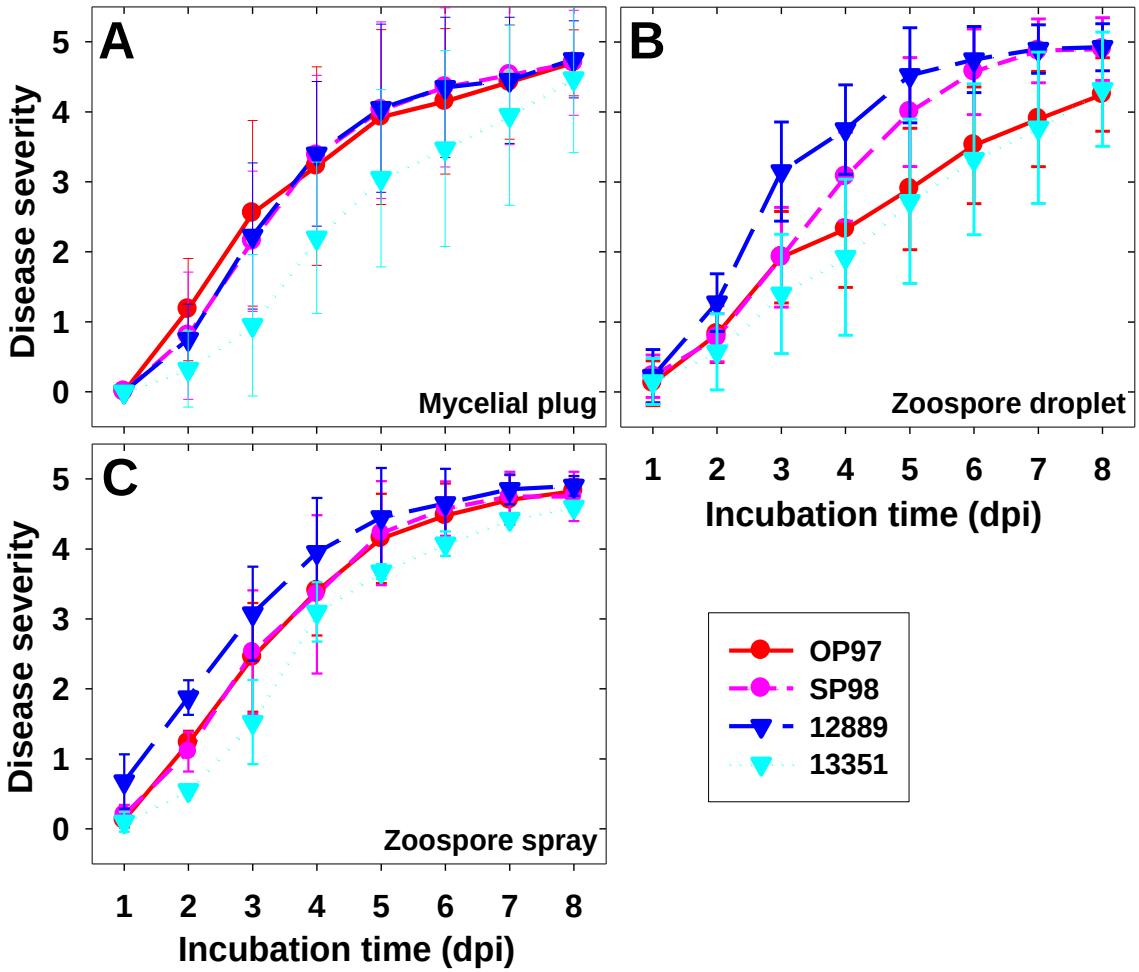

Figure 6. Progression of foliar blight caused by *Phytophthora capsici* isolates on cotyledons of cucumber cv. Vlaspik over incubation time (days post inoculation, dpi). Each point represents a mean ± standard deviation of two repeated tests with ten replicate cucurbit seedlings per treatment per test. Comparison of inoculation of four isolates of *P. capsici* by mycelial plug (**A**), zoospore suspension droplet (**B**), and zoospore suspension spray (**C**).

There was a significant (P = 0.0302) interaction between inoculation technique and pathogen isolate for mean AUDPCs. When isolate OP97 was used as inoculum, both mycelial plug and zoospore suspension spray techniques resulted in more severe disease than the zoospore suspension droplet technique (Figure 7). When isolates SP98 and 13351 were used as inoculum, inoculation techniques were not significantly different according to mean AUDPCs. When isolate 12889 was used as inoculum, the zoospore

suspension droplet technique was not significantly different from the other two techniques in mean AUDPCs. However, the zoospore suspension spray technique caused significantly greater disease severity than using a mycelial plug. When the mycelial plug and zoospore suspension spray techniques were used, isolates OP97, 12889, and SP98 caused significantly more disease than isolate 13351. When the zoospore suspension droplet technique was applied, isolate 12889 caused the most disease followed by isolate SP98 ($P = 0.0301$). Isolates OP97 and 13351 ($P < 0.0001$) caused the least, with no significant difference between each other.

P. capsici OP97 caused significant variation of disease severity on cucumber cotyledons when using the mycelial plug and zoospore suspension droplet techniques. Thus, both techniques were used on cotyledons of yellow squash and watermelon for further comparison.

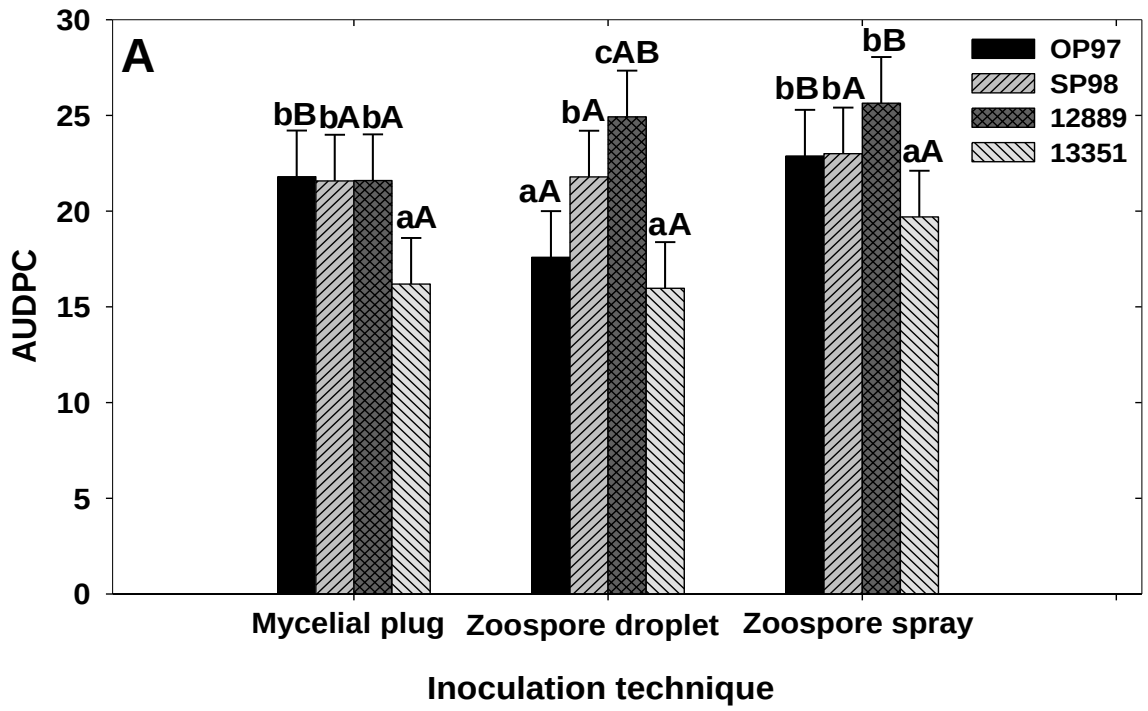

Figure 7. Mean area under disease progress curve (AUDPC) values of foliar blight on cotyledons of cucumber cv. Vlaspik for the interaction between inoculation technique and pathogen isolate. Each bar represents the average of two repeated tests with ten replicate cucumber seedlings per treatment per test. Bars with the same letters are not significantly different among pathogen isolates using each inoculation technique (lowercase letters) or among inoculation techniques for each isolate (uppercase letters) according to Fisher's LSD ($\alpha = 0.05$). Error bars represent standard error.

Effect of inoculation technique and pathogen isolate on disease severity on cotyledons of yellow squash and watermelon. Various levels of disease severity were observed with four isolates of *P. capsici* on yellow squash and watermelon using either mycelial plug or zoospore suspension droplet for inoculation. Sterile agar plug- or water-inoculated cotyledons remained asymptomatic. All *P. capsici* isolates recovered from symptomatic cotyledons matched the phenotype of the pathogen isolate used for inoculation. Pathogens were not isolated from asymptomatic cotyledons (*data not shown*).

Pathogen isolates significantly ($P < 0.0001$ and $P = 0.0044$, respectively) affected mean AUDPCs on yellow squash and watermelon, but inoculation techniques ($P = 0.9840$ and $P = 0.5031$, respectively) did not. Isolate 12889 caused the most severe disease on yellow squash followed by isolates 13351 and SP98; isolate OP97 caused the least. On watermelon, isolate SP98 caused significantly more severe disease than isolates 12889, OP97, and 13351 that were not significant different among them.

The interaction between pathogen isolate and inoculation technique significantly ($P < 0.0001$ and $P = 0.0187$, respectively) affected mean AUDPCs on yellow squash and watermelon. When the mycelial plug technique was applied on yellow squash, isolates OP97 and 12889 produced significantly higher mean AUDPCs than isolates SP98 and 13351. When the zoospore suspension droplet technique was applied, isolates 12889 and 13351 caused the most severe disease followed by isolate SP98; isolate OP97 caused the least. When individual isolates tested were compared between two techniques, isolate OP97 caused significantly more severe disease using the mycelial plug technique than the zoospore suspension droplet technique. In contrast, isolates 12889, 13351, and SP98 caused significantly more severe disease using the zoospore suspension droplet technique (Figure 8A) than the mycelial plug technique. When the mycelial plug technique was applied on watermelon, isolates OP97 and SP98 resulted in significantly more severe disease than isolate 13351; neither was significantly different from isolate 12889. Isolates SP98 and 12889 applied by the zoospore suspension droplet technique caused significantly more severe disease than isolate OP97 (Figure 8B). When individual isolates were compared between two techniques, mean AUDPCs did not differ significantly for pathogen isolates.

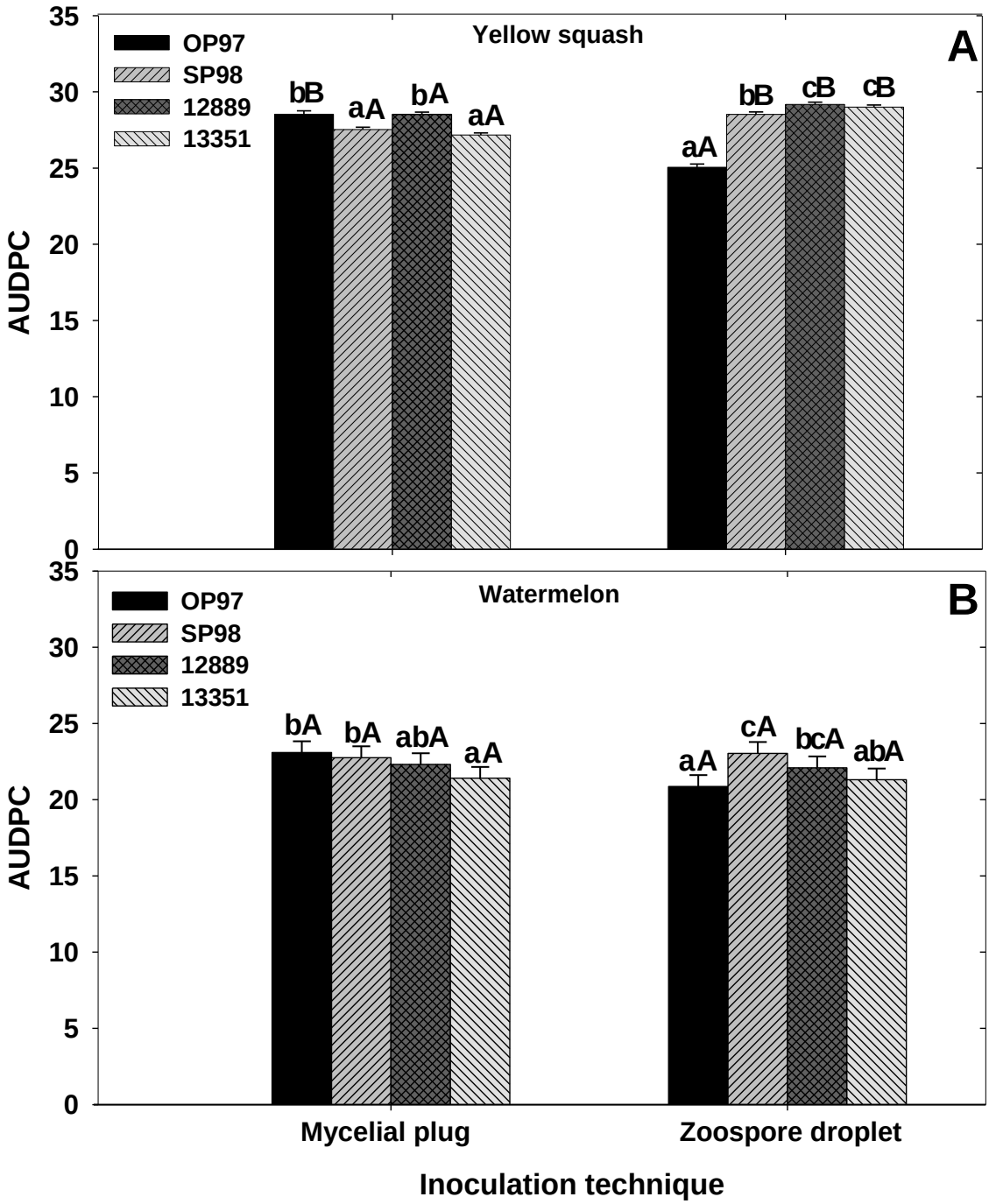

Figure 8. Mean area under disease progress curve (AUDPC) values of foliar blight on cotyledons of yellow squash cv. Cougar (**A**) and watermelon cv. Sugar Baby (**B**) for the interaction between inoculation technique and pathogen isolate. Each bar represents the average of two repeated tests with ten replicate cucurbit seedlings per treatment per test. Bars with the same letters are not significantly different among pathogen isolates using each inoculation technique (lowercase letters) or between inoculation techniques for each isolate (uppercase letters) according to Fisher's LSD (α = 0.05). Error bars represent standard error.

Evaluation of isolate virulence on cotyledons of various cucurbit crops. All pathogen isolates (OP97, SP98, 12889, and 13351) caused typical disease symptoms. Disease severity varied among cucurbit crops. Four isolates completely colonized cotyledons of both yellow squash cultivars and green zucchini in 5 to 6 dpi (Figure 9B to D). However, only isolate 12889 was able to colonize the entire cotyledon of cantaloupe by 6 dpi (Figure 9A). Isolates 12889 and SP98 caused symptoms on the whole cotyledon of watermelon in 7 days (Figure 9E). Sterile water-inoculated cotyledons remained asymptomatic. *P. capsici* isolates recovered from inoculated symptomatic cotyledons were confirmed to have the same phenotype as the pathogen isolate used for inoculation. Pathogens were not isolated from asymptomatic cotyledons (*data not shown*).

In general, select *P. capsici* isolates were highly virulent on cotyledons of five tested cucurbit cultivars (Figure 9A to E); virulence differed significantly among *P. capsici* isolates when using zoospore suspension droplets (Figure 10A to E).

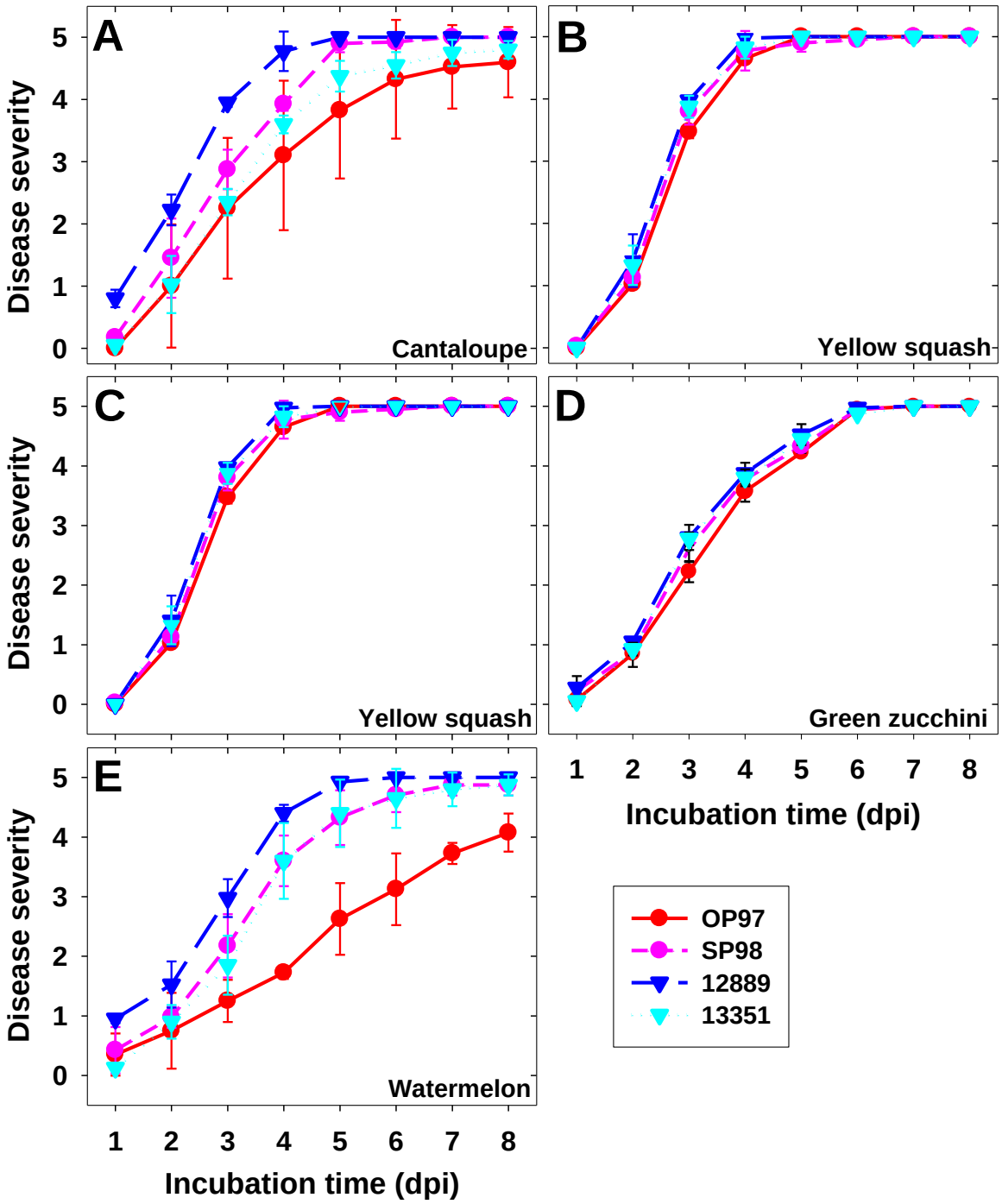

Figure 9. Progression of foliar blight caused by *Phytophthora capsici* isolates on various cucurbit crops over incubation time (days post inoculation, dpi). Each point represents a mean ± standard deviation of two repeated tests with ten replicate cucurbit seedlings per treatment per test. Comparison of inoculation of four isolates of *P. capsici* on cantaloupe cv. Athena (**A**), yellow squash cvs. Cougar (**B**) and Superpik (**C**), green zucchini cv. Tigress (**D**), and watermelon cv. Sugar Baby (**E**) by zoospore suspension droplet.

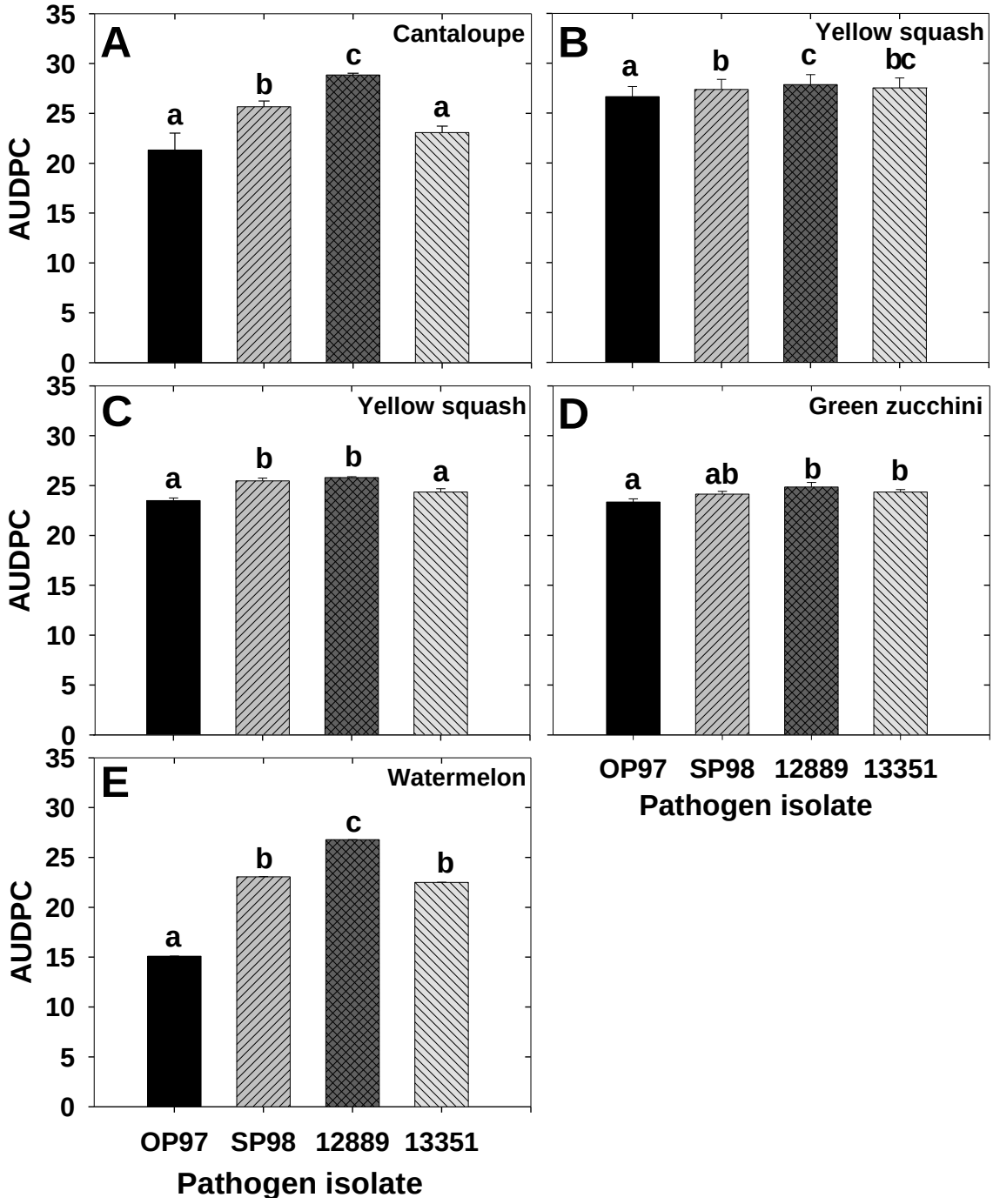

Figure 10. Mean area under disease progress curve (AUDPC) values of foliar blight of various cucurbit crops including cantaloupe cv. Athena (**A**), yellow squash cvs. Cougar (**B**) and Superpik (**C**), green zucchini cv. Tigress (**D**), and watermelon cv. Sugar Baby (**E**) for different *Phytophthora capsici* isolates. Each bar represents the average of two repeated tests with ten replicate cucurbit seedlings per treatment per test. Bars with the same letters are not significantly different among pathogen isolates according to Fisher's LSD (α = 0.05). Error bars represent standard error.

Effect of leaf position and pathogen isolate on foliar disease incidence and severity. Cotyledons, first true leaves, and second true leaves of cucurbits tested showed symptoms of disease including wilting and necrotic lesions (Figure 11A and B) following inoculation with *P. capsici*; uninoculated control plants remained asymptomatic. Reisolation of *P. capsici* isolates from symptomatic leaf tissue was difficult (Table 6). *P. capsici* was not isolated from asymptomatic leaf tissue.

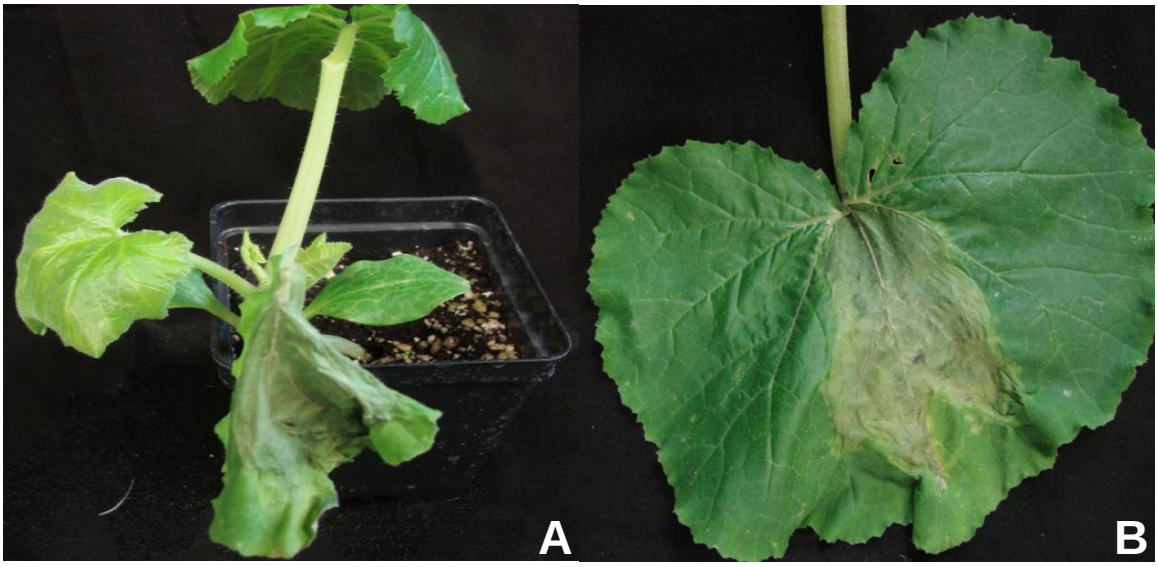

Figure 11. Wilting (**A**) and a lesion (**B**) observed on a first true leaf of yellow squash cv. Cougar 5 days post inoculation with a 20-µl zoospore suspension droplet (1×10^6 zoospore/ml) of *Phytophthora capsici* isolate 12889.

Table 6. *Phytophthora capsici* isolates obtained from leaves of each cucurbit crop.

Cucurbit crop	Leaf position	Isolates obtained[z]	Sampled leaves		
			Symptomatic	Asymptomatic	Total
Cucumber	Cotyledon	2	20	3	23
	First true leaf	7	13	6	19
	Second true leaf	6	8	10	18
Cantaloupe	Cotyledon	0	15	4	19
	First true leaf	1	12	5	17
	Second true leaf	0	13	3	16
Yellow squash	Cotyledon	0	11	6	17
	First true leaf	0	10	7	17
	Second true leaf	0	6	10	16
Green zucchini	Cotyledon	0	8	9	17
	First true leaf	0	12	5	17
	Second true leaf	0	5	12	17

[z] Determined by the recovered *P. capsici* isolates at the end of the experiment.

Table 7. Analysis of variance for effects of pathogen isolate, leaf position, and incubation time on disease incidence and lesion diameter in cucurbit crops inoculated with 20-µl zoospore suspension droplet (1×10^6 zoospore/ml) of *Phytophthora capsici* isolates.

		***P* value**[z]	
Cucurbit Crop	**Source**	**Disease incidence**	**Lesion diameter**
Cucumber	Pathogen isolate	0.0038*	0.0020*
	Leaf position	0.0255*	0.0125*
	Incubation time	——[y]	< 0.0001*
	Pathogen isolate × leaf position	0.1066	0.0855
	Pathogen isolate × incubation time	——	0.0141*
	Leaf position × incubation time	——	0.0084*
	Pathogen isolate × leaf position × incubation time	——	0.0908
Cantaloupe	Pathogen isolate	0.0016*	0.0056*
	Leaf position	0.2464	0.2240
	Incubation time	——	< 0.0001*
	Pathogen isolate × leaf position	0.9825	0.6997
	Pathogen isolate × incubation time	——	< 0.0001*
	Leaf position × incubation time	——	< 0.0001*
	Pathogen isolate × leaf position × incubation time	——	< 0.0044*
Yellow squash	Pathogen isolate	0.0011*	0.0011*
	Leaf position	0.0122*	0.0142*
	Incubation time	——	< 0.0001*
	Pathogen isolate × leaf position	0.0617	0.0660
	Pathogen isolate × incubation time	——	0.0003*
	Leaf position × incubation time	——	0.0244*
	Pathogen isolate × leaf position × incubation time	——	0.1771
Green zucchini	Pathogen isolate	0.0481*	0.0400*
	Leaf position	0.2362	0.0022*
	Incubation time	——	< 0.0001*
	Pathogen isolate × leaf position	0.5814	0.0394*
	Pathogen isolate × incubation time	——	< 0.0001*
	Leaf position × incubation time	——	0.0870
	Pathogen isolate × leaf position × incubation time	——	0.3842

[y] —— = no data.

[z] * Effect of the factor is statistically significant at $\alpha = 0.05$ according to Fisher's LSD.

Disease incidence differed significantly for pathogen isolates on all cucurbit crops tested (Table 7) when zoospore suspension droplets were applied; isolates 12889 and SP98 caused more disease than isolate OP97 (Table 8). Disease incidence differed significantly for leaf positions on cucumber cv. Vlaspik and yellow squash cv. Cougar (Table 7). Disease incidence on cotyledons was greater than that observed on second true leaves; first true leaves showed intermediate disease susceptibility (Table 8). However, leaf positions did not significantly affect disease incidence on cantaloupe and green zucchini (Table 8). The interaction between pathogen isolate and leaf position did not significantly affect disease incidence on all cucurbit crops tested (Table 7).

Table 8. Disease incidence and lesion diameter of foliar blight in cucurbit crops caused by *Phytophthora capsici* isolates for pathogen isolate, leaf position, and incubation time.

Cucurbit crop	Factor	Disease incidence (%)	Lesion diameter (mm)
Cucumber	Pathogen isolate		
	12889	80 b[z]	17 b
	OP97	13 a	2 a
	SP98	73 b	12 b
	Leaf position		
	Cotyledon	71 b	15 b
	First true leaf	53 ab	8 a
	Second true leaf	42 a	8 a
	Incubation time		
	3 dpi[x]	——[y]	7 a
	6 dpi	——	13 b
Cantaloupe	Pathogen isolate		
	12889	95 b	28 b
	OP97	6 a	1 a
	SP98	84 b	26 b
	Leaf position		
	Cotyledon	57 a	16 a
	First true leaf	55 a	22 a
	Second true leaf	42 a	17 a
	Incubation time		
	3 dpi	——	11 a
	6 dpi	——	25 b
Yellow squash	Pathogen isolate		
	12889	67 b	23 b
	OP97	7 a	1 a
	SP98	82 b	32 b
	Leaf position		
	Cotyledon	64 b	20 b
	First true leaf	53 ab	21 b
	Second true leaf	38 a	14 a
	Incubation time		
	3 dpi	——	12 a
	6 dpi	——	25 b
Green zucchini	Pathogen isolate		
	12889	69 b	19 b
	OP97	0 a	0 a
	SP98	48 b	19 b
	Leaf position		
	Cotyledon	32 a	18 b
	First true leaf	31 a	13 a
	Second true leaf	20 a	7 a
	Incubation time		
	3 dpi	——	9 a
	6 dpi	——	17 b

[x] dpi = days post inoculation.
[y] —— = no data.
[z] Values with the same letters in a column within each factor are not statistically different at $\alpha = 0.05$ according to Fisher's LSD.

The three-way interaction among pathogen isolate, leaf position, and incubation time significantly affected lesion diameter on cantaloupe cv. Athena (Table 7). In general, isolates 12889 and SP98 resulted in significantly larger lesion diameter than isolate OP97 on foliage at all leaf positions tested at 3 and 6 dpi when the zoospore suspension droplet technique was used (Table 9). Over incubation time, lesion diameter caused by isolates 12889 and SP98 significantly increased on foliage at all leaf positions tested. However, lesion diameter caused by isolate OP97 was not significantly different between 3 and 6 dpi.

Table 9. Lesion diameter on cantaloupe foliage caused by *Phytophthora capsici* isolates as affected by the three-way interaction among pathogen isolate, leaf position, and incubation time.

	Lesion diameter[z] (mm)					
Pathogen	**Cotyledon**		**First true leaf**		**Second true leaf**	
Isolate	**3 dpi[y]**	**6 dpi**	**3 dpi**	**6 dpi**	**3 dpi**	**6 dpi**
12889	22 bA	31 b B	16 bA	47 b B	15 bA	36 b B
OP97	1 a A	1 a A	1 a A	1 a A	1 a A	1 a A
SP98	17 bA	28 b B	16 bA	49 b B	13 bA	35 b B

[y] dpi = days post inoculation.

[z] Each value represents the average of three repeated tests with five replicate leaves at different leaf positions per treatment per test. Values with the same letters in a column and a row are not significantly different among pathogen isolates at each time period at each leaf position (lowercase letters) or between incubation time at each leaf position for each isolate (uppercase letters) at $\alpha = 0.05$ according to Fisher's LSD.

The interaction between pathogen isolate and incubation time significantly affected lesion diameter on cucumber cv. Vlaspik and cantaloupe cv. Athena (Table 7). The interaction between leaf position and incubation time also significantly affected lesion diameter on both crops. Isolates 12889 and SP98 resulted in significantly larger lesions than isolate OP97 on cucumber and cantaloupe at both 3 and 6 dpi (Figure 12A

and C). When lesion diameter was compared for individual isolates between 3 and 6 dpi, isolates 12889 and SP98 resulted in larger lesions on both crops as incubation time increased from 3 to 6 dpi, but lesions were not significantly larger at 6 dpi than at 3 dpi when foliage was inoculated with isolate OP97. On cucumber, lesions on cotyledons at 3 and 6 dpi were the largest, followed by those on first true leaves, and then second true leaves; Lesions significantly increased on foliage of cucumber and cantaloupe at all leaf positions tested with incubation time from 3 to 6 dpi (Figure 12B and D). Lesion diameter did not differ significantly for cantaloupe leaf position at 3 dpi, but lesions on first true leaves were significantly larger than those on cotyledons and second true leaves at 6 dpi.

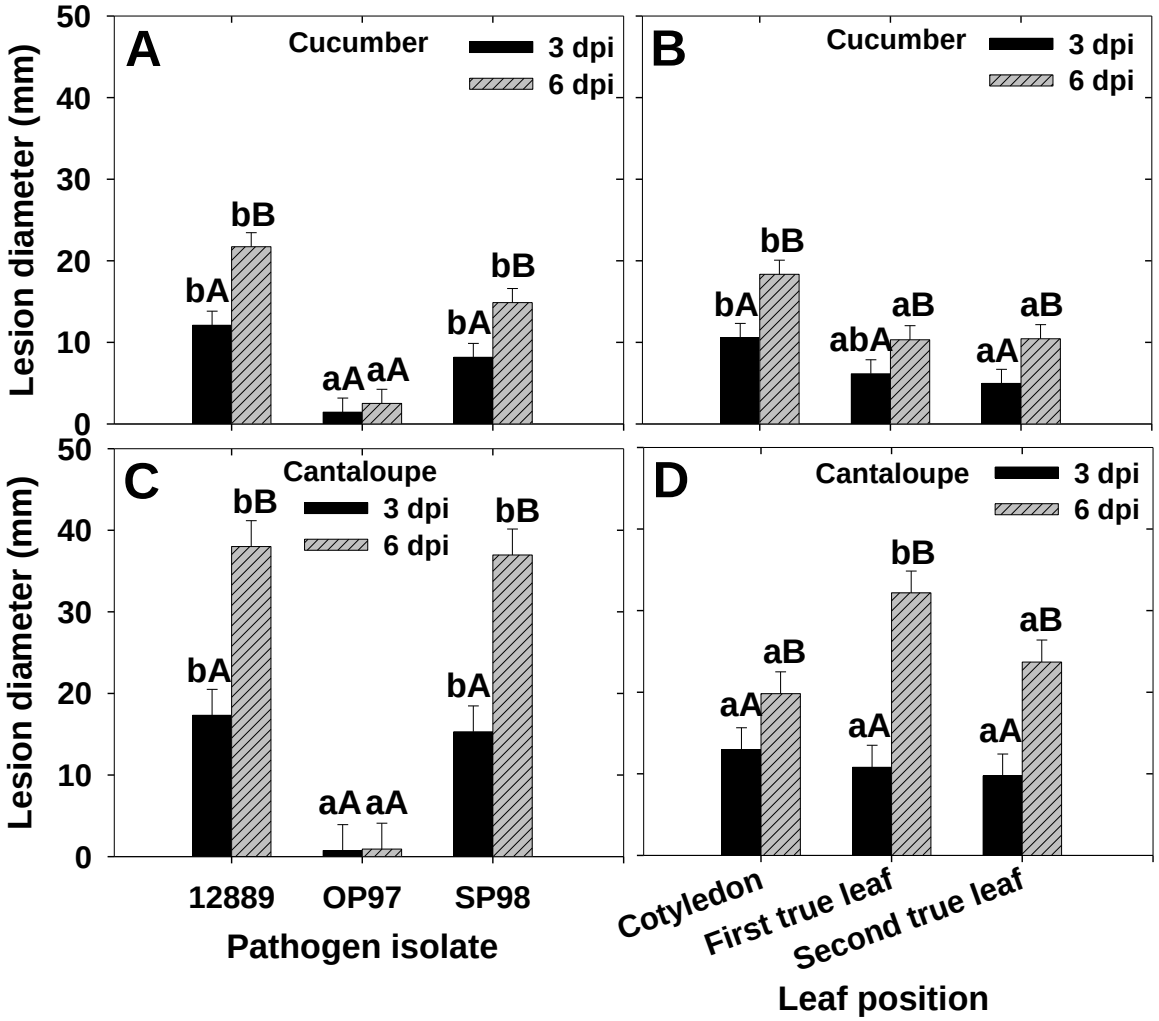

Figure 12. Lesion diameter on cucumber cv. Vlaspik (**A** and **B**) and cantaloupe cv. Athena (**C** and **D**) foliage inoculated with a 20-µl zoospore suspension droplet (1×10^6 zoospore/ml) of *Phytophthora capsici* isolates 12889, OP97, and SP98 for the interaction between pathogen isolate and incubation time (days post inoculation, dpi) (**A** and **C**), and the interaction between leaf position and incubation time (**B** and **D**). Each bar represents the average of three repeated tests with five replicate leaves at different leaf positions per treatment per test. Bars with the same letters are not significantly different among pathogen isolates (**A** and **C**) or leaf positions (**B** and **D**) at each time period (lowercase letters) or between incubation time for each isolate (**A** and **C**) or at each leaf position (**B** and **D**) (uppercase letters) according to Fisher's LSD ($\alpha = 0.05$), respectively. Error bars represent standard error.

Two-way interactions between pathogen isolate and incubation time and leaf position and incubation time significantly affected lesion diameter on yellow squash cv. Cougar (Table 7). Two-way interactions between pathogen isolate and incubation time, and pathogen isolate and leaf position were significant for lesion diameter on green zucchini cv. Tigress (Table 6). On yellow squash and green zucchini, isolates 12889 and SP98 caused significantly larger lesions than isolate OP97 regardless of the leaf position at 3 and 6 dpi. Lesions caused by isolates 12889 and SP98 significantly increased with incubation time (Figure 13A and C). On yellow squash, lesions on cotyledons were significantly larger than those on second true leaves; neither was significantly different from those on first true leaves at 3 dpi (Figure 13B). Lesions on first true leaves and cotyledons were significantly larger than those on second true leaves at 6 dpi, and lesions on first true leaves and second true leaves significantly increased with incubation time. On green zucchini, isolates 12889 and SP98 caused significantly larger lesions than isolate OP97 on cotyledons and first true leaves (Figure 13D). There were no significant differences in lesion diameter among pathogen isolates on second true leaves. Lesions caused by isolates 12889 and SP98 on cotyledons were significantly larger than those on second true leaves with lesions on first true leaves being intermediate. Lesion diameter caused by isolate OP97 was not significantly different among leaf positions.

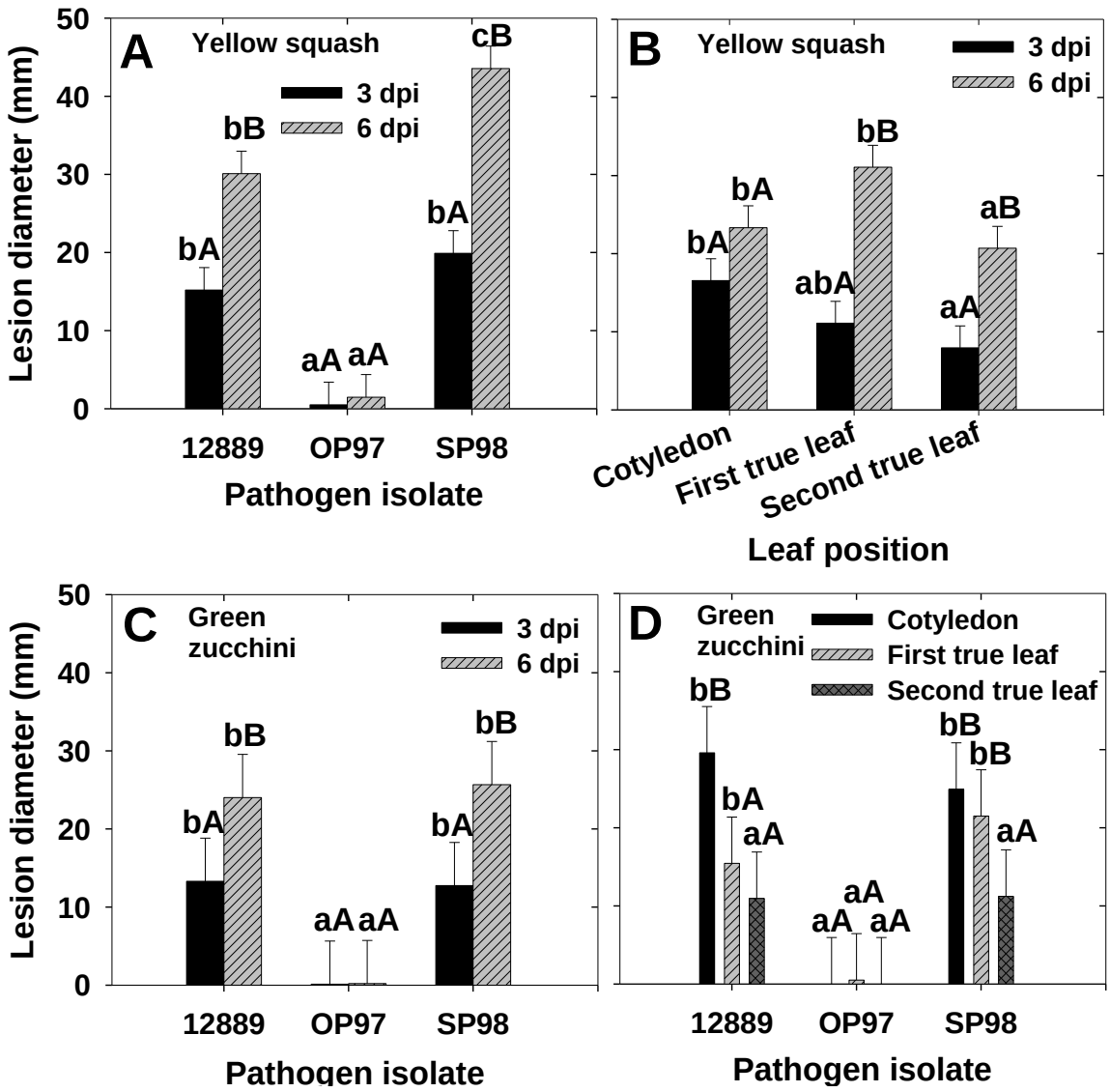

Figure 13. Lesion diameter on yellow squash cv. Cougar (**A** and **B**) and green zucchini cv. Tigress (**C** and **D**) foliage inoculated with a 20-µl zoospore suspension droplet (1×10^6 zoospore/ml) of *Phytophthora capsici* isolates 12889, OP97, and SP98 for the interaction between pathogen isolate and incubation time (days post inoculation, dpi) (**A** and **C**), the interaction between leaf position and incubation time (**B**), and the interaction between pathogen isolate and leaf position (**D**). Each bar represents the average of three repeated tests with five replicate leaves at different leaf positions per treatment per test. Bars with the same letters are not significantly different among pathogen isolates (**A** and **C**) or leaf positions (**B**) at each time period or among pathogen isolates at each leaf position (**D**) (lowercase letters), or between incubation time for each isolate (**A** and **C**) or at each leaf position (**B**) or among leaf positions for each isolate (**D**) (uppercase letters) according to Fisher's LSD ($\alpha = 0.05$), respectively. Error bars represent standard error.

DISCUSSION

Zoospore inoculum concentration significantly affected disease incidence and severity on cucumber cotyledons. Specifically, increasing zoospore concentration from 1 $\times 10^3$ to 1 $\times 10^6$ zoospores/ml increased disease severity. Sporangial production was observed on symptomatic cotyledons 3 dpi and continued throughout the observation period of 9 days. A previous study using pickling cucumber fruit showed a similar strong positive correlation between zoospore concentration and fruit infection and a similar trend in sporangial production (15), although significantly higher levels of fruit rot were observed as zoospore concentration was increased from 1 x 10^2 to 1 $\times 10^4$ zoospores/ml. In the current study, the concentrations of 1 $\times 10^5$ and 1 $\times 10^6$ zoospores/ml resulted in significantly higher disease incidence on cucumber cotyledons than other concentrations studied. Inoculation with 1 $\times 10^6$ zoospores/ml consistently resulted in the most severe cotyledon disease over the incubation period studied. All fruit inoculated with a zoospore concentration of 5 x 10^3 zoospore/ml became infected, compared with cotyledon disease occurring only at comparatively high concentrations of 1 x 10^5 zoospore/ml. Low amount of the pathogen inoculum may help explain why fruit rot occurs more frequently than foliar blight in commercial fields.

Zoospore infection involves a pre-penetration sequence of zoospore taxis, attachment, encystment, cyst germination, and orientation of the germ tube (10). Studies investigating whether the pathogenicity of *Phytophthora* spp. is related to zoospore taxis or encystment have produced inconsistent results (17,32). Hickman (17) concluded that motile zoospores as the inoculum are more successful in infection than cysts because

taxis of *P. capsici* zoospores provide an opportunity for rapid attraction, accumulation, and encystment in the elongation zone behind the root tip. However, Raftoyannis and Dick (32) found no significant correlation between the pathogenicity of *Phytophthora* spp. and zoospore taxis or encystment on roots. Thus, the existence of a direct correlation between zoospore taxis or encystment and pathogenicity of *P. capsici* was questioned. The results of the current study indicated that disruption of zoospore taxis did not prevent infection of cotyledons when *P. capsici* was placed directly on cotyledons. *P. capsici* isolates caused significant disease, regardless of whether inoculum was encysted or motile zoospores, suggesting that pathogenicity of *P. capsici* is not affected by zoospore taxis or encystment on cotyledons.

It has been proposed that inoculation techniques may contribute to the ability of various isolates to infect hosts (37). In the current study, zoospore inoculation by droplet or spray yielded foliar symptoms of chlorotic lesions similar to those caused by a mycelial plug. The interaction between inoculation technique and pathogen isolate resulted in significantly different levels of disease severity. When inoculated by mycelial plug, isolate OP97 was more virulent in tested cucurbit crops compared to inoculation by zoospore suspension droplet. The similar result was reported for Fraser fir seedlings inoculated with isolate OP97, when comparing soil infestation with zoospore suspensions and infested millet seeds with mycelial plugs (30). However, in the current study, isolate SP98, 12889, and 13351 resulted in similar or more severe disease on cucurbit crops tested using the zoospore suspension droplet technique compared to the mycelial plug technique. This may be because the zoospore suspension droplet technique simulates natural processes and allows quantification of *P. capsici* inoculum (33).

Different *P. capsici* isolates varied in virulence across cucurbit leaf positions tested. Isolates 12889 and SP98 were consistently the most virulent when leaves were inoculated with a droplet of zoospore suspension. Variations in virulence among *P. capsici* isolates on pepper lines and other vegetable crops have been reported since 1972 (13,24,27,29,31,34,40). Recently, studies on pepper fruit (13) and tomato plants (31) that were inoculated with a droplet of zoospore suspension and *P. capsici*-infested millet seeds containing mycelia and sporangia, indicated that significant variations in virulence exist among *P. capsici* isolates 12889, OP97, SP98, and SFF3, with isolate 12889 being the most virulent. Studies on cucumber fruit (14) and Fraser fir (30), however, showed no significant variations in virulence among isolates when V8 agar plugs containing mycelia and sporangia were used as inocula. A droplet containing only zoospores was used to inoculate cucurbit cotyledons in the current study, compared with the use of a V8 agar plug for cucumber fruit (14) and infested millet seeds for Fraser fir seedling (30) inoculation. Zoospore inoculum does not have an associated food base that the agar plug contains. It is possible that the agar plug inoculum produced new growth to infect cucumber fruit and Fraser fir seedlings when conditions were favorable (30).

Leaf position-related resistance has been reported in different plant and oomycete pathogen interactions such as potato and *Phytophthora infestans* (7,8,26,41,42) and broccoli and *Hyaloperonospora parasitica* (9). Fruit position-related differences in susceptibility to *P. capsici* were also found between the stem and the blossom end of cucumbers (2). In the current study, the foliage of cucumber, yellow squash, and green zucchini varied in their levels of susceptibility to *P. capsici* isolates; cotyledons were significantly more susceptible than second true leaves. Results were similar to studies on

potato (7,8,26,41,42) and broccoli (9), which showed that basal leaves were more susceptible to *P. infestans* and *H. parasitica*, respectively, than apical leaves. Basal leaves are closer to pathogen-infested soil, which may result in a more humid microclimate that is conducive for disease development (9).

One interesting observation was the difficulty in recovering *P. capsici* from symptomatic plants in the experiment investigating the effect of leaf position, compared to pathogen isolation performed successfully in other experiments. This could be the result of opportunistic organisms (e.g., *Alternaria* spp.) growing on the infected cucurbit leaf tissue, either during the incubation period or while the samples were being stored, and affecting the ability to isolate *P. capsici*. Other studies have also reported difficulty isolating from different tissue types, including mature pepper stems (M. K. Hausbeck, unpublished data), asparagus crowns (38), and Fraser fir seedling tissue (30). More sensitive detection techniques such as PCR may assist culture-dependent methods to confirm *P. capsici* infection (31,35), especially when conventional culturing isolation techniques are not successful.

In summary, increased incidence and severity of foliar blight was observed on cotyledons of cucurbit crops compared to true leaves, and on leaves exposed to a higher zoospore concentration of *P. capsici* isolates. Zoospore encystment was did not significantly affect the pathogenicity of *P. capsici* on cucurbit cotyledons. Rather, the incidence and severity of infection were influenced by zoospore concentration, *P. capsici* isolate virulence, and leaf position.

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LITERATURE CITED

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CHAPTER II

SUSCEPTIBILITY OF CUCURBIT COTYLEDONS AND FRUIT TO *PHYTOPHTHORA CAPSICI*

ABSTRACT

The susceptibility of pickling cucumber and yellow squash cotyledons and fruit to *Phytophthora capsici* isolates was compared in a growth chamber study. Five *P. capsici* isolates from bell pepper (12889), eggplant (13351), pickling cucumber (OP97 and SF3), and pumpkin (SP98) were used. Cotyledons of 16-day-old plants and medium-sized fruit harvested from the field were inoculated with each *P. capsici* isolate using 20 µl zoospore suspension droplets (1×10^6 zoospores/ml). Lesion area and sporangial density were measured on cotyledons 5 days post inoculation (dpi). Similarly, fruit were assessed for lesion area, visible mycelial growth, and sporangial density 5 dpi. All *P. capsici* isolates caused disease on both cotyledons and fruit of cucumber and squash but virulence varied. Significantly larger lesions and more sporangia were observed on squash than cucumber cotyledons, but lesions and mycelial growth on fruit varied when inoculated with different isolates and significantly more sporangia were produced on cucumber fruit than on squash fruit. Overall, cucurbit cotyledons exhibited a different susceptibility to *P. capsici* isolates than that observed in fruit, which has great implications for cucurbit growers.

INTRODUCTION

Cucurbits are an important group of vegetable crops cultivated worldwide (26). In the United States, major cucurbit crops include fresh market and processing cucumber (*Cucumis sativus* L.), cantaloupe (*Cucumis melo* L. var. *cantalupensis* Naudin), honeydew (*Cucumis melo* L. var. *inodorus* Naudin), pumpkin (*Cucurbita maxima* Duchesne), squash (*Cucurbita pepo* L.), and watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) (9). The total annual value of these crops in the United States in 2009 was $1.59 billion, with field production of approximately 4,527,928,000 kg harvested from 182,757 ha (4), but these crops are susceptible to various diseases that cause significant reductions in overall production and fruit quality and thus profitability (36).

Phytophthora foliar blight and crown, root, and fruit rot, caused by *Phytophthora capsici* Leonian, is of particular importance because it can destroy entire crops (10,17). *Phytophthora capsici* has been found on cucurbit crops in many of the states that lead the country in cucurbit production including Florida (12), Georgia (21), Illinois (20), Michigan (17), North Carolina (29), and Texas (19). Foliage, crown, roots, and fruit of susceptible crops can become infected; cucurbit fruit are especially susceptible (17). Thus, fruit rot is a common symptom in *P. capsici*-infested fields when the weather is favorable for development (17). Foliar blight is observed less frequently than fruit rot (M. K. Hausbeck, personal observation). Nonetheless, foliar infection has caused severe disease on processing pumpkin plants in Illinois (5) and killed emerging cucumber seedlings in Michigan (17).

Susceptibility to *P. capsici* infection is affected by host species or cultivar (8,18,33), the age of the plant tissue (2,3,13), and type of host tissue (6,11,17,24,32,34).

Cucurbitaceous crops, especially summer squash, are more susceptible to *P. capsici* than other families including Solanaceous crops (e.g., tomato, eggplant, and tobacco) and Fabaceous crops (e.g., lima bean, green bean, and snow pea) (33). Yellow summer squash cultivars are more susceptible to *P. capsici* than green zucchini (8). Additionally, research results have indicated that there are no commercial cucumber cultivars that are significantly resistant to *P. capsici* infection based on germplasm screening of fruit (13). Younger, smaller cucurbit fruit are more susceptible to *P.capsici* than older, larger fruit (2,3,13), and cucumber fruit are more susceptible to infection than crowns and roots (17). Different levels of disease resistance in pepper are expressed in various plant tissues (6,11,24,32,34); fruit are more susceptible to *P. capsici* than roots and crowns (11). Research on peppers suggests that resistance to root rot, stem blight, and foliar blight is controlled by different genetic mechanisms (6,24,32,34). The objective of this study was to compare the susceptibility of cotyledon and fruit of cucumber and squash using different *P. capsici* isolates.

MATERIALS AND METHODS

Inoculum production. Cultures of *P. capsici* isolates collected from infected Cucurbitaceous and Solanaceous crops were obtained from long-term culture collection stored in Dr. M. K. Hausbeck's laboratory at Michigan State University (MSU). Isolates selected were characterized for sexual compatibility type (CT) and mefenoxam sensitivity (MS) as previously described (22). The geographic origin, source crop from which isolates were originally obtained, and phenotype are listed in Table 10. Isolates were transferred from long-term stock cultures (stored at 20 ℃ in sterile microcentrifuge tubes containing 1 ml of sterile water and one sterile hemp seed) to unclarified V8 juice agar (UCV8: 16 g agar, 3 g $CaCO_3$, 160 ml V8 juice, and 840 ml distilled water) amended with 100 ppm of ampicillin and 30 ppm of rifampicin and incubated at room temperature (21 ± 2 ℃) under continuous fluorescent lighting (1). Isolates were subsequently inoculated onto cucumber fruit to confirm their pathogenicity and isolates obtained from infected cucumber fruits were used (27). Isolates were maintained under temperature and light conditions described above by weekly hyphal transfer to new UCV8. Zoospore suspensions were made by flooding *P. capsici* cultures with sterile distilled water and incubating the cultures at 2 ℃ for 1 h, followed by 30 min at room temperature (16). Zoospore concentration was determined using a hemacytometer and diluted to 1×10^6 zoospores/ml using sterile distilled water.

Table 10. Origin, source crop, and phenotype of *Phytophthora capsici* isolates used in inoculations.

			Phenotype	
Isolate	**Origin[x]**	**Source crop**	**CT[y]**	**MS[z]**
12889	Michigan	Bell pepper	A1	I
13351	New York	Eggplant	A1	S
OP97	Michigan	Pickling cucumber	A1	S
SF3	Michigan	Pickling cucumber	A1	S
SP98	Michigan	Pumpkin	A2	S

[x] Origin = the state from which isolate was originally collected.
[y] CT = compatibility type, designated A1 and A2 and determined by crossing the isolate to be screened with OP97 (A1) and SP98 (A2) standard isolates (22).
[z] MS = mefenoxam sensitivity, where I = insensitive and S = sensitive to mefenoxam, calculated by comparing growth in mefenoxam-amended V8 juice media to non-amended media (22).

Plant material. One cultivar from each of two commercial cucurbit crops susceptible to *P. capsici*, pickling cucumber cv. Vlaspik (Seminis Vegetable Seeds Inc., Oxnard, CA) and yellow squash cv. Cougar (Harris Moran Seed Company, Modesto, CA), were used in this experiment. Cucurbit seeds were individually sown into 72-cell flats (TLC Polyform, Inc., Plymouth, MN) containing soilless potting media (BACCTO Professional Planting Mix, Michigan Peat Company, Houston, TX), and were grown in a greenhouse with an average temperature of 24 ℃ and a 14-h photoperiod. Seven-day-old seedlings were transplanted into 1.5-liter plastic pots and were grown in a greenhouse as above. Plants were watered daily as needed. Sixteen-day-old plants of each cultivar were individually placed into plastic bags (20 cm × 10 cm × 46 cm) containing wet paper towels to maintain high relative humidity prior to inoculation.

Cucumber fruit were obtained from a field site of the MSU Plant Pathology Farm, East Lansing, MI, without any history of *P. capsici* infestation. Squash fruit were obtained from a field site of the Southwest Michigan Research and Extension Center,

Benton Harbor, MI that was free of *P. capsici* infestation. Cucumber and squash fruit were hand-harvested when fruit were in the size range of 3.0 to 4.5 cm diameter × 12 to 14 cm long and 4 to 6 cm diameter × 16 to 20 cm long, respectively. Fruit of certain size were selected according to the work of Ando et al. (3) and Gevens et al. (13) to represent the similar level of age-related resistance to *P. capsici.* Cucurbit fruit were rinsed to remove surface debris, surface disinfested with a 1.24% NaClO solution for 5 min, rinsed with distilled water 3 times, air-dried, and individually placed into plastic bags as described above.

Susceptibility of cucurbit cotyledons and fruit. Cotyledons and fruit were inoculated by placing a 20-µl droplet of a zoospore suspension (1×10^6 zoospores/ml) from one of five *P. capsici* isolates (12889, 13351, OP97, SF3, or SP98) onto the center of a cotyledon or fruit. Five cotyledons or fruit were used for each isolate and five cotyledons or fruit were inoculated with sterile distilled water as a control. Cotyledons or fruit were individually incubated in sealed plastic bags for 5 days in growth chambers (Conviron CMP3244, Pembina, ND) with a 24-hour photoperiod (~95 mE of light intensity) at 21 ℃. Air temperature and relative humidity inside the plastic bag were monitored using Watchdog data loggers (Model 450, Spectrum Technologies Inc., Plainfield, IL) during the incubation period. The average temperature and relative humidity were 22 ℃ and 98.4%, respectively.

Cotyledons and fruit were evaluated for disease 5 days post inoculation (dpi) by measuring the length (cm) and width (cm) of chlorotic (cotyledons only) or water-soaked lesions, visible white mycelium in the lesion (fruit only) and sporangial density. Lesion area on cotyledons and fruit of cucumber and squash and mycelial growth area on fruit of

both crops were calculated based on the following formula: $A = \pi \times r_1 \times r_2$, where r_1 = length/2 and r_2 = width/2. Sporangial density in each lesion on cotyledons and fruit was determined as follows: single cotyledon lesions and fruit lesions that displayed visible mycelial growth were excised with a razor blade and placed into a sterile 2.2-ml microcentrifuge tube containing 1 ml of sterile water. The number of sporangia were estimated using a hemacytometer, after vortexing the tubes for 70 sec and removing the plant tissue with forceps. If sporangia observed by stereomicroscopy (Leica M165C, Wetzlar, Germany) could not be detected via the method above, the sporangial suspension was concentrated by centrifugation for 5 min at 18,407 × g and the pellet was resuspended in 50 µl of sterile distilled water after removing the supernatant. Sporangial density was estimated by dividing the number of sporangia by the lesion area for cucurbit cotyledons and dividing the number of sporangia by the mycelial growth area for cucurbit fruit. The experiment was conducted three times.

Pathogen isolation. At the end of the observation period, 40% of cotyledons and fruit were arbitrarily sampled, washed in distilled water for 1 min, surface disinfested with a 70% ethanol solution for 2 min, and used to isolate the pathogen from the tissues. Three small sections from the margin of lesions were plated onto UCV8 plates amended with 25 ppm of benomyl, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene (BARP). When tissues were asymptomatic, four small sections were excised around the inoculation points and placed onto BARP-amended UCV8. Isolates were identified using morphological characteristics of *P. capsici* (35) after incubation at room temperature under constant fluorescent lighting for 4 days. Hyphal tips of suspected *P. capsici* isolates were transferred to new BARP-amended UCV8

plates. Resulting *P. capsici* isolates were screened following a 5-day incubation period for compatibility type and mefenoxam sensitivity using the methods of Lamour and Hausbeck (23) to confirm isolate phenotype.

Statistical analyses. A randomized complete block design was used for each tissue type with crops and pathogen isolates as two factors. Each test was treated as a block factor. Data from control plants and cucumber cotyledons inoculated with isolate SF3 were removed from the data set prior to statistical analyses since no visible symptoms occurred. Disease incidence was not included for statistical analysis because the residuals did not meet the assumptions required for statistical tests. Data of lesion area and mycelial growth area and sporangial density from multiple runs of the same experiment were pooled for statistical analysis after no significant differences were found between repeated experiments. Data were subjected to analysis of variance (ANOVA) using the PROC MIXED procedures of SAS version 9.2 (SAS Institute Inc., Cary, NC). Sporangial density data were square root- transformed to fulfill the assumption of normally distributed residuals. Variances were grouped by the pathogen isolate factor to evaluate sporangia density on cucurbit fruit. Multiple comparisons among the means were conducted using ANOVA and Fisher's protected Least Significant Difference (LSD) was used for separation of means when effects were statistically significant at $\alpha = 0.05$.

RESULTS

Symptoms and signs of *P. capsici* were observed on inoculated cotyledons and fruit of both cucumber and squash (Figure 14) and became more pronounced as incubation time increased. Water-soaked lesions were the first symptom observed on squash cotyledons and fruit of both crops. On cucumber cotyledons, chlorosis was observed around the inoculation sites before the appearance of water-soaking. On cotyledons, lesions quickly expanded onto the stems of both crops. Subsequently, a white, cottony mycelium was observed, and a powdery growth resulting from sporangial production occurred across the mycelia on the surface of infected fruit. However, mycelial growth and sporangial production on cotyledons of either cucumber or squash were not as visible as on the fruit of these hosts.

Cucumber and squash fruit and squash cotyledons exhibited disease symptoms when inoculated with all *P. capsici* isolates tested. Isolates 12889 and OP97 caused 100% disease incidence on cotyledons and fruit of both crops. Additionally, isolates 13351, SF3, and SP98 caused 100% disease incidence on cucumber fruit. Isolates 13351 and SP98 caused 100% disease incidence on both cotyledons and fruit of squash. However, no symptoms occurred when cucumber cotyledons were inoculated with isolate SF3 (Figure 15). None of the sterile-water inoculated cotyledons and fruit of either crop showed disease symptoms. All *P. capsici* isolates recovered from symptomatic cotyledons and fruit matched the phenotype of the pathogen isolate used for inoculation. Pathogens were not isolated from asymptomatic tissues (*data not shown*).

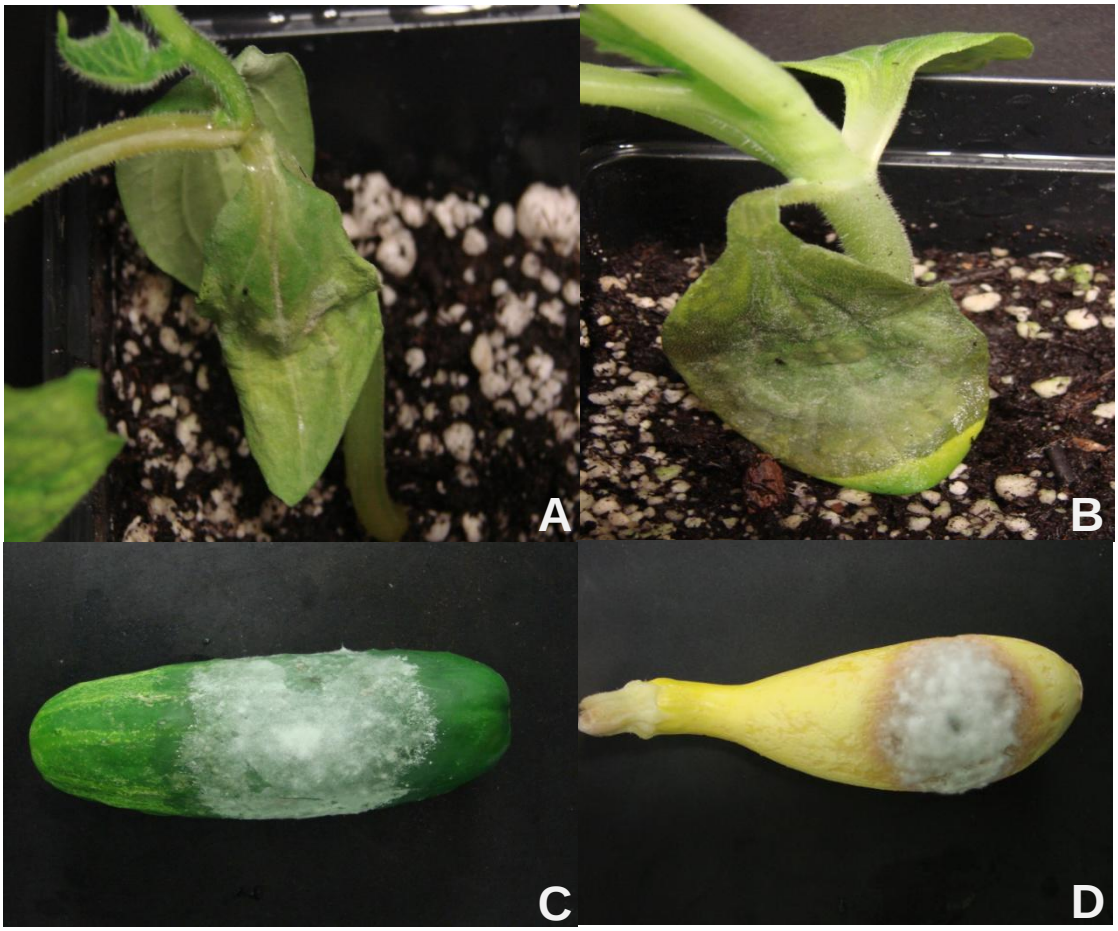

Figure 14. Symptoms and signs on inoculated cotyledons and fruit of cucumber cv. Vlaspik (**A** and **C**) and yellow squash cv. Cougar (**B** and **D**) 5 days post inoculation with a 20-µl droplet of a *Phytophthora capsici* zoospore suspension (1×10^6 zoospores/ml).

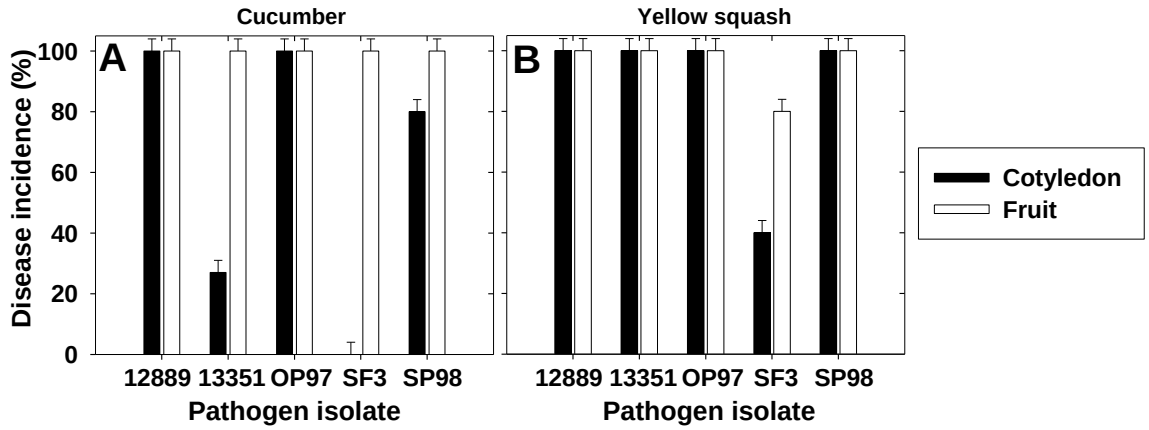

Figure 15. Disease incidence on cucumber cv. Vlaspik (**A**) and yellow squash cv. Cougar (**B**) cotyledons and fruit when inoculated with a 20-µl zoospore suspension droplet (1×10^6 zoospores/ml) of *Phytophthora capsici* isolates obtained from pepper (12889), eggplant (13351), pickling cucumber (OP97 and SF3), or pumpkin (SP98). Each bar represents the average of three repeated tests with five replicate cotyledons or fruit of cucumber and yellow squash per treatment per test. Error bars represent standard error.

Lesion area on cotyledons differed significantly ($P = 0.0012$ and $P = 0.0204$, respectively) for crop and pathogen isolate, but no significant difference ($P = 0.2895$) was found for the interaction between crop and pathogen isolate. Squash cotyledons had significantly larger lesions than cucumber cotyledons, regardless of isolate (Figure 16A). When inoculated with a droplet of zoospore suspension, isolates 12889, OP97, and SP98 caused significantly larger lesions on cotyledons of both crops than isolate 13351, regardless of crop (Figure 16B). In addition, sporangial density on cotyledons differed significantly between cucumber and squash; the interaction between crop and isolate was also significant (Figure 16C). Isolates SP98, 12889, and 13351 produced significantly more sporangia on squash cotyledons than isolate OP97. There were no significant differences in sporangial density on cucumber cotyledons across the isolates tested. All of the isolates produced significantly more sporangia on squash cotyledons than on cucumber cotyledons.

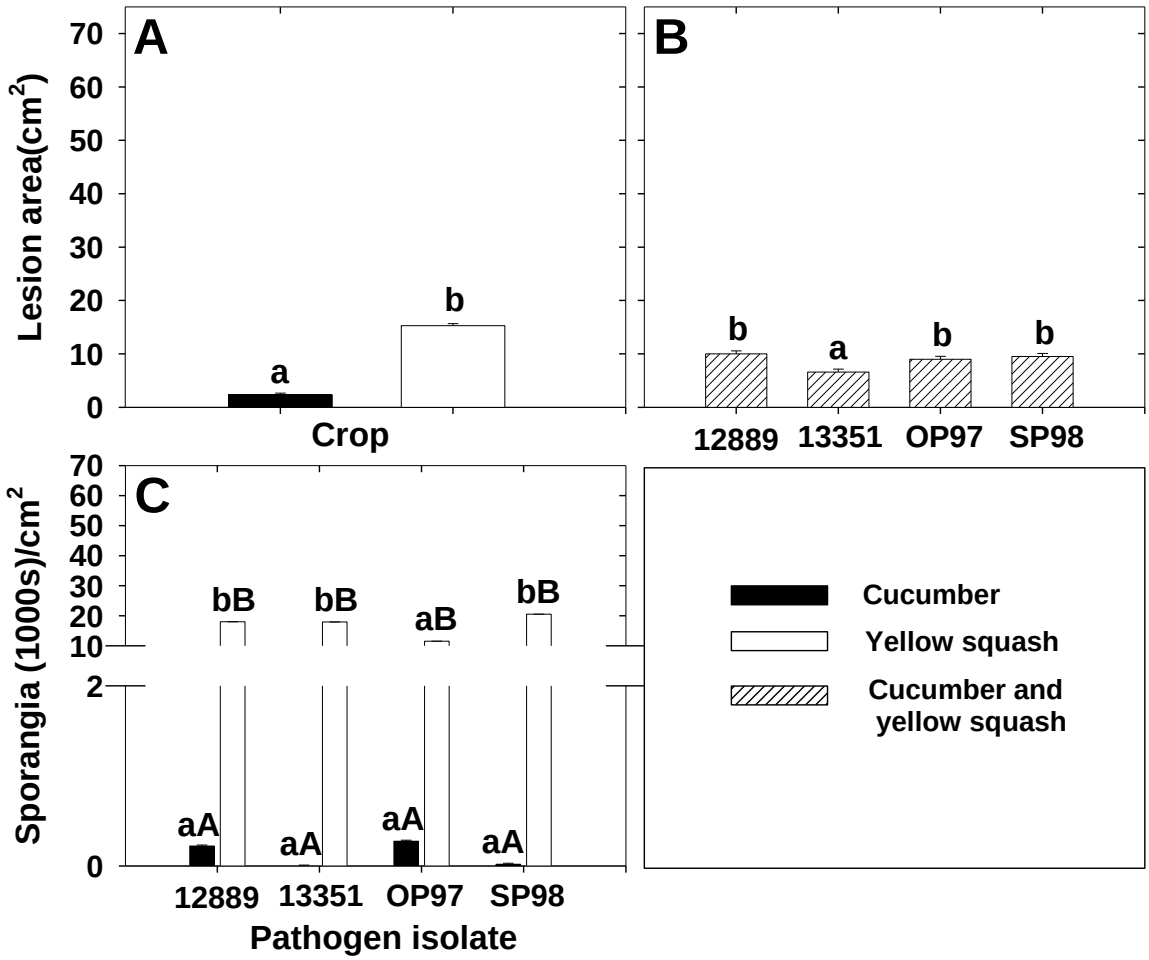

Figure 16. Lesion area (**A** and **B**) and sporangial density (**C**) produced on cotyledons of cucumber cv. Vlaspik and yellow squash cv. Cougar when inoculated with a 20-µl zoospore suspension droplet (1×10^6 zoospores/ml) of *Phytophthora capsici* isolates obtained from pepper (12889), eggplant (13351), pickling cucumber (OP97), and pumpkin (SP98). Each bar represents the average of three repeated tests with five replicate cotyledons of cucumber and yellow squash per treatment per test. Bars with the same letters are not significantly different between crops (**A**) or among pathogen isolates (**B**) or among pathogen isolates for each crop (**C**) (lowercase letters) or between crops for each isolate (**C**) (uppercase letters) according to Fisher's LSD ($\alpha = 0.05$), respectively. Error bars represent standard error.

Lesion area, mycelial growth area, and sporangial density on fruit differed significantly ($P < 0.0001$, $P < 0.0001$, and $P = 0.0015$, respectively) for pathogen isolate. Sporangial density also differed significantly ($P = 0.0034$) for crop, but lesion area and mycelial growth did not ($P = 0.3857$ and $P = 0.6591$, respectively). In addition, lesion area, mycelial growth, and sporangial density on fruit differed significantly ($P = 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively) for the interaction between crop and isolate. Isolates 12889, OP97, and SP98 resulted in significantly larger lesions (Figure 17A) and greater mycelial growth (Figure 17B) on cucumber fruit than isolates 13351 and SF3. Isolates 12889, OP97, and SP98 caused the largest lesions (Figure 17A) and greatest mycelial growth (Figure 17B) on squash fruit, followed by isolate 13351; isolate SF3 caused the least. When lesion and mycelial growth were examined across crops, isolates 13351 and OP97 caused significantly larger lesions on squash fruit compared to cucumber fruit (Figure 17A and B). Only isolate OP97 caused significantly greater mycelial growth on squash fruit than on cucumber fruit (Figure 17B). Isolate SF3 caused significantly larger lesions (Figure 17A) and greater mycelial growth (Figure 17B) on cucumber fruit than on squash fruit.

When sporangial density was examined across pathogen isolates or crops (Figure 17C), isolates 13351 and SP98 produced significantly more sporangia on cucumber fruit than isolates OP97 and 12889. Isolates 13351 and OP97 produced significantly more sporangia on squash fruit than isolates SP98 and SF3, with no difference between isolates OP97 and 12889. Cucumber fruit had significantly more sporangia than squash fruit in response to all isolates tested.

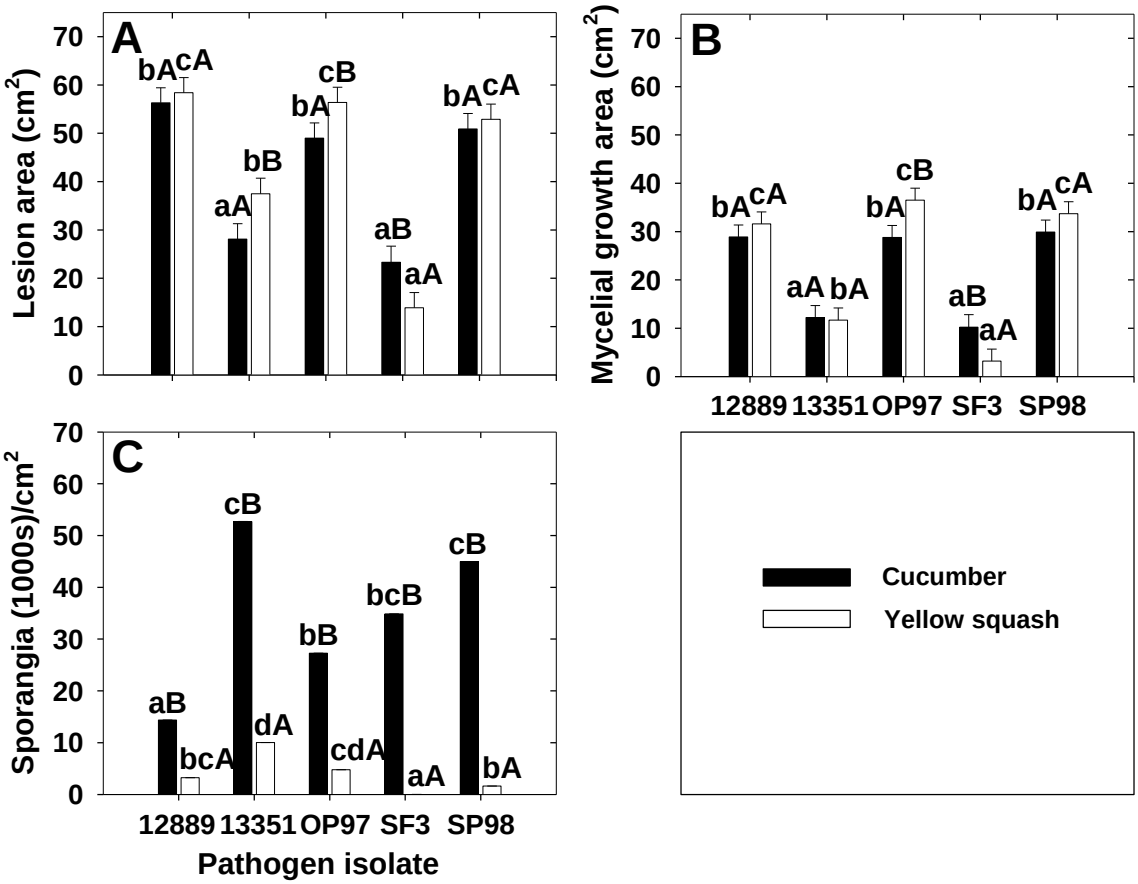

Figure 17. Lesion area (**A**), mycelial growth area (**B**), and sporangial density (**C**) produced on fruit of cucumber cv. Vlaspik and yellow squash cv. Cougar when inoculated with a 20-µl zoospore suspension droplet (1×10^6 zoospores/ml) of *Phytophthora capsici* isolates obtained from pepper (12889), eggplant (13351), pickling cucumber (OP97 and SF3), and pumpkin (SP98). Each bar represents the average of three repeated tests with five replicate fruit of cucumber and yellow squash per treatment per test. Bars with the same letters are not significantly different among isolates for each crop fruit (**A**, **B**, and **C**) (lowercase letters) or between crop fruit for each isolate (**A**, **B**, and **C**) (uppercase letters) according to Fisher's LSD ($\alpha = 0.05$), respectively. Error bars represent standard error.

DISCUSSION

In this study, the susceptibility of cotyledons and fruit of picking cucumber and yellow squash to *P. capsici* infection was compared using five different isolates. Isolates varied in their virulence, but all caused disease on both cotyledons and fruit of cucumber and squash. Significantly larger lesions and more sporangia were observed on squash than cucumber cotyledons when inoculated with *P. capsici*. In contrast, significantly more sporangia were produced in the lesions of inoculated cucumber fruit compared with that of squash. These data suggest that while cucumber cotyledons do not appear to be a favorable substrate for growth and reproduction of *P. capsici*, cucumber fruit provide an especially favorable substrate.

Infection was reliably produced when cotyledons were inoculated with zoospore suspensions and incubated at high humidity for 5 days. This infection technique may simulate field conditions where *P. capsici*-infested water may splash onto the lower portion of cucurbit plants. Progression of foliar blighting from the inoculated cotyledons to the plant stems of both cucurbit crops was observed by 5 dpi, which ensures seedling damping-off and plant death.

Fruit of both cucumber and squash exhibited susceptibility to *P. capsici* isolates, consistent with previous reports (2,13). Specifically, susceptibility indicated by the watersoaked lesion area and mycelial growth on both fruit types varied in response to different *P. capsici* isolates. This differed from the results of Ando et al. (3), who reported that squash and zucchini fruit were the most susceptible of 8 cucurbit crops including cucumber, as evidenced by watersoaked lesions appearing 24 hours post inoculation (hpi). Ando et al. (3) used a 6-mm-diameter mycelial agar plug of a single *P.*

capsici isolate and rated the disease 24 hpi on fruit of various cucurbits, while the current study used a zoospore suspension of 5 isolates for inoculum and rated the disease 5 dpi on fruit using the same cucumber cultivar but a different squash cultivar. A large number of zoospores (~20,000 zoospores/fruit) in our inoculum may also have increased the potential for disease progression (7,13). In addition, significantly more sporangia were produced on cucumber than squash fruit 5 dpi. The production of large quantities of sporangia observed on fruit of cucumber (~33,376 sporangia/cm^2) and squash (~2,911 sporangia/cm^2) 5 dpi suggests that removing diseased cull fruit from the field could help reduce inoculum.

Differences in virulence among the *P. capsici* isolates tested for lesion size, mycelial growth, and sporangial production on cotyledons and fruit were observed. Isolates 12889, OP97, and SP98 caused significantly larger lesions on cotyledons of cucumber and squash than isolate 13351. Isolate 13351 (from eggplant) caused small lesions and sparse mycelial growth on cotyledons of squash and fruit of both crops, despite displaying relatively high sporangial production. On fruit of both crops, isolates 12889, OP97, and SP98 caused significantly larger lesions and greater mycelial growth than isolates 13351 and SF3. Isolates 13351 and SP98 produced more sporangia on cucumber fruit than isolates OP97 and 12889, in agreement with the results from Granke and Hausbeck (16). Isolates 13351 and OP97 produced significantly more sporangia on squash fruit than isolates SF3 and SP98. Recent studies on pepper (11) and tomato (28) also found significant differences in virulence among *P. capsici* isolates 12889, OP97, and SP98; however, a previous study performed by Gevens and colleagues (13) on cucumber fruit showed no differences among isolates OP97, SP98, and SF3.

This inconsistency may have resulted from the use of different inocula (13) or different sized cucumber fruit (3,13). Gevens et al (13) used a V8 agar plug containing mycelia and sporangia as inocula rather than zoospore suspension droplets, thus providing an associated food base and perhaps promoting pathogen growth (27). In addition, fruit size has been reported to affect cucurbit susceptibility (3,13); Gevens et al. (13) reported that at approximately 3 cm diameter, cucumber fruit transitioned from susceptible to more resistant. Thus, the narrower size range (3.0 to 4.5 cm diameter) used in the current study may have ensured a susceptible disease response across fruit.

Phytophthora capsici has been detected in rivers, ponds, and other surface water sources used for irrigation (14,31), and represents a significant means by which the pathogen may be introduced into uninfested fields. Managing *P. capsici* solely through frequent applications of fungicides has practical limitations and raises environmental concerns (16). Integrating the host resistance to *P. capsici* into present management practices would increase control and decrease costs (12,16). Disease severity on cucurbit plant tissues such as crowns (8,25) and fruit (13,18) has been used as a measure of host resistance to screen cultivars and develop an effective disease management strategy (17,30). In the current study, cucurbit cotyledons exhibited a different susceptibility to *P. capsici* isolates than the fruit. For example, enhanced mycelial growth and inoculum production occurred on squash cotyledons compared with those of cucumber; however, significantly more inoculum was produced on cucumber fruit than squash.

In summary, various *P. capsici* isolates exhibit virulence differences in cotyledons and fruit of cucurbit crops tested. Cucurbit fruit seems to be a more favorable substrate for *P. capsici* infection and sporulation than cotyledon. Cucurbit fruit that exhibit the

symptoms of *P. capsici* infection should be removed immediately from the field to reduce pathogen sporulation and subsequent field infestation, which may be beneficial in managing field disease. Also, it has implications for the disease control to further elucidate what factors resulted in the significant difference in sporangial production between tissues of cucumber and squash.

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LITERATURE CITED

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