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EFFECTS OF ENVIRONMENTAL CONDITIONS ON *PHYTOPHTHORA CAPSICI* DISPERSAL AND DISEASE DEVELOPMENT

By

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ABSTRACT

EFFECTS OF ENVIRONMENTAL CONDITIONS ON *PHYTOPHTHORA CAPSICI* DISPERSAL AND DISEASE DEVELOPMENT

By

Leah L. Granke

The oomycete plant pathogen *Phytophthora capsici* Leonian can infect a variety of hosts including vegetable crops in the Cucurbitaceae and Solanaceae. *P. capsici* has a worldwide distribution and may be a significant limiting factor to vegetable production due to the root, crown, stem, and fruit rot and foliar blighting it causes. Laboratory and field studies were conducted to study the mechanisms of sporangia dissemination. Direct laboratory observations showed *P. capsici* sporangial dispersal occurred in water with capillary force, but did not occur in response to wind or a reduction in relative humidity. When airborne sporangial concentrations and environmental conditions were monitored under field conditions, sporangial concentrations were positively associated with rainfall. Both laboratory and field observations indicated that dispersal of sporangia via wind currents is infrequent, and sporangia are unlikely to be naturally dispersed among fields by wind alone.

Controlled laboratory studies were undertaken to determine the effects of water temperature (2, 9, 12, 19, 22, and 32°C), spore concentration (1×10^2, 1×10^3, 5×10^3, 1×10^4, 2×10^4, and 4×10^4 zoospores/ml), and zoospore suspension age (0, 1, 3, and 5 days old) on infection of pickling cucumbers by *P. capsici*. Zoospore motility and mortality in response to commercial algaecides were also investigated. Cucumbers became infected at all temperatures tested, except 2°C, and the highest infection

incidence was observed for cucumbers incubated in suspensions held at ≥19°C. More fruits became infected when suspensions contained a greater number of zoospores. While the incidence of fruit infection declined with the zoospore suspension age, infection still occurred when 5-day-old suspensions were used. Commercial algaecides inhibited zoospore motility and caused significant zoospore mortality in laboratory assays and show promise for treatment of infested irrigation water.

The effects of temperature (10, 15, 20, 25, 30, and 35°C) and relative humidity (~35, 60, 70, 80, and 100%) on development of Phytophthora fruit rot of pickling cucumber were investigated in controlled growth chamber studies. The effect of wounding on disease development was characterized for small (2.0 to 2.5 x 8 to 9 cm), medium (3.0 to 4.0 x 12.0 to 13.0 cm), and large (>4.5 cm x >14 cm) pickling cucumbers. No lesions developed on cucumbers incubated at 10°C, but lesions were observed on cucumbers incubated at all other temperatures tested. Disease severity was greatest on cucumber fruits incubated at 25°C at 4 days post inoculation (dpi). Lesions formed on cucumbers incubated at all relative humidities tested. The diameter of water-soaking and pathogen growth increased as the relative humidity increased. Wounding was found to lessen size-related resistance in pickling cucumber. The diameter of water-soaking was similar for all wounded cucumbers at 4 dpi regardless of fruit size. While sporangia were formed on wounded large fruits, greater sporangial production was observed in lesions on small and medium fruits. These results indicate *P. capsici* is capable of infecting cucumbers under a wide range of temperature and relative humidity conditions.

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LITERATURE REVIEW

Phytophthora capsici Leonian is an oomycete plant pathogen in the Kingdom Stramenopila (11,23). Although *Phytophthora* spp. resemble fungi morphologically, they are phylogenetically distinct from true fungi. According to their evolutionary history, *Phytophthora* spp. are more closely related to the heterokont algae (2). Oomycetes have traits that are absent in the true fungi including cell walls composed primarily of cellulose (β-glucan), a predominantly diploid life cycle, and the need for an exogenous source of sterols for sporulation (23).

P. capsici was first identified as the causal agent of chile wilt in New Mexico in 1922 (48). Foliar blight and root, crown, and fruit rot of cucumber (*Cucumis sativus* L.), zucchini (*Cucurbita pepo* L.), squash (*Cucurbita pepo* L.), eggplant (*Solanum melongena* L.), tomato (*Solanum lycopersicon* L.), pepper (*Capiscum annuum* L.), pumpkin (*Cucurbita moschata* Duchesne ex Poir.), melon (*Cucumis melo* L.), and watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai), are elicited by *P. capsici.* Erwin and Ribeiro (23) reported that at least 49 plant species have been infected by *P. capsici* worldwide. Recently, snap bean (*Phaseolus vulgaris* L.) (29), lima bean (*Phaseolus lunatus* L.) (20) and fraser fir (*Abies fraseri* (Pursh) Poir.) (57) have been added as new hosts, and weeds may be susceptible (25). Additional hosts that were infected under laboratory conditions included beet (*Beta vulgaris* L.), Swiss chard (*Beta vulgaris* L.), turnip (*Brassica rapa* L.), spinach (*Spinacia oleracea* L.), and velvet-leaf (*Abutilon theophrasti* Medik) (70).

P. capsici overwinters and survives long-term in the soil as thick-walled oospores, which are produced by sexual reproduction (35,41,43). Sexual reproduction is common

in Michigan (42) and other regions of the United States (55) and is the primary source of genetic variation in nature and allows for generation of fungicide resistance (41,43). Since *P. capsici* is heterothallic, both mating types are required to be present for oospore production in the soil or on or within host tissue (39,72). The pathogen can survive as oospores in the soil for years, making crop rotations relatively ineffective for managing the disease (45). Oospores are capable of germinating, even in the absence of nutrients, to give rise to mycelia and/or sporangia (3,23,34). *Phytophthora* oospore germination is affected by length of dormancy period, nutrition, light, temperature, soil water matric potential, chemical treatments, and treatment with enzymes (10,34,59).

P. capsici has coenocytic hyphae (23). When environmental conditions are favorable, the hyphae differentiate into long caducous pedicles, which support mostly papillate, but occasionally semipapillate, limoniform clustered sporangia. Proper water potential, aeration, temperature, light, inorganic salts, exogenous sterols, and protein and RNA synthesis are required for sporangia production (4,23,59). Sporangia are usually limoniform, but may also be subspherical, ovoid, obovoid, ellipsoid, spherical, fusiform, pyriform or distorted depending on light, nutrients, and other environmental conditions. Once mature, the caducous sporangia may be dispersed by wind-driven rain or in irrigation water, but are not likely to be dispersed between fields via the wind (30,42,61,66).

The asexual propagules (sporangia and zoospores) are responsible for rapid polycyclic disease development when the environment is favorable for disease (32). When a sporangium comes into contact with free water, it differentiates to produce 20 to 40 heterokont reniform, uninucleate swimming zoospores, which maintain motility in

water for 5 to 10 h (23). The motility period is affected by temperature and is shortened by temperatures greater than those optimal for growth and lengthened by temperatures below those optimal for growth. Motility is also affected by the concentration of zoospores present, with lengthened motility periods for suspensions with higher concentrations (23).

Zoospores are negatively geotaxic in water and they utilize chemo- and electrotaxis toward the host to favor zoospore contact with the host (15,74). It has been suggested that receptor molecules on the surface of *P. capsici* zoospores receive environmental signals that direct preinfection behavior (7). After zoospore-plant contact is achieved, zoospores encyst (lose flagella and develop a cell wall) and form germ tubes (3). Zoospores store adhesive material in small vesicles, which is released upon encystment to facilitate a firm attachment to the plant surface and to anchor the spores in a favorable position for host penetration (65).

P. capsici may penetrate host surfaces directly or enter through natural plant openings such as the stomata or lenticels. A non-pectolytic protein produced by the hyphae is capable of macerating host tissue and may play an important role in penetration of the host epidermis and invasion of susceptible tissue (77). Hyphae grow intercellularly through plant tissues and develop haustoria within host cells to absorb nutrients (47). Under specific light and temperature conditions, some isolates have been known to produce chlamydospores in some hosts (73), but chlamydospores are not typically produced by *P. capsici* isolates from pepper or cucurbit hosts (60).

Development of disease depends on the three components of the disease triangle being present: a virulent pathogen, a susceptible host and a favorable environment (52).

Differences in virulence and pathogenicity exist between different isolates of *P. capsici* on various hosts (24,40,57,69). The amount of inoculum present also may affect disease severity and whether or not infection occurs (46,58).

Susceptibility of the host is influenced by the cultivar, age, organ affected, and by whether or not the host is wounded. Many attempts have been made to find cultivars that exhibit resistance to *P. capsici*. Screenings of cucumber germplasm have yielded some varieties that exhibit limited lesion development and sporulation, but not complete resistance (27). Two pumpkin cultivars, Danmatmaetdol and Lil Ironsides (Harris Moran Seed Co., Modesto, CA) show quantitative resistance (46). Tolerance has also been identified in a few tomato cultivars with commercially acceptable horticultural traits (8,36), but more work is needed to identify sources of full resistance. Several commercial bell pepper cultivars possess tolerance to *P. capsici*, but growers are reluctant to use these because of marketability problems due to poor fruit shape (24) and a potential correlation with silvering (76), the separation of the fruit cuticle from the epidermis. It is known that host resistance in pepper is controlled by two distinct genes, but little information exists regarding inheritance of resistance to *P. capsici* in most host plants (46,56).

Some plant organs are more susceptible than others. For example, while cucumber plants show a relative tolerance to *P. capsici,* cucumber fruits are very susceptible (32). Previous experiments have shown that age and/or size are important factors for infection of and sporangial production on cucumber (27) and pepper fruits (5), and on pepper plants (38). Generally, disease was more severe on younger/smaller fruits

and plants. Wounding has been shown to negate age-related resistance in pepper fruits (5).

Climatic conditions influence development of diseases caused by *Phytophthora* spp. (22). The minimum, optimal, and maximum growth temperatures for *P. capsici* isolates in culture media range from 6 to12°C, 24 to 33°C, and 32 to >35°C, respectively (23,51,68,71). Schlub found that a greater percentage of detached pepper leaves were infected following incubation at 23°C as compared to 15 or 31°C when sporangial suspensions were removed from the leaves 4 hours post inoculation (66). Disease incidence and lesion length were greatest when inoculated pepper fruits were incubated at 27°C (6). While temperature does affect disease, rainfall is the most important environmental factor affecting sporangial dispersal (30) and disease progress and intensity (9,66).

A number of factors including temperature (21), nutrition (78), light (31), aeration (53), availability of sterols (33), and relative humidity (32,66) are known to affect *P. capsici* sporangial production. Sporangial production is optimal at 24 to 27°C (21). While a limited number of sporangia may be produced in the dark, sporangial production is much greater under conditions of light. An exogenous sterol source is necessary for sporangial production (33) and more sporangia are produced in an aerobic environment (53). Previous work by Lamour and Hausbeck (32) suggested that sporangial production on slicing cucumber fruits was greater at 60 and 80% relative humidity than at >90%. Sporangial production following incubation at 25°C was greater for pepper stem pieces that were incubated at 100 or 97.5% relative humidity than at 92.5 or 87% relative humidity (66).

Recommended management strategies to control the diseases elicited by *P. capsici* involve integrated approaches that focus on using cultural practices to manage water, using cultivars with resistance to the disease, avoiding introduction of the pathogen to new areas and fields, and chemical control via fungicides (63).

Cultural control practices such as water management, crop rotation, bedding and plasticulture, and avoidance of low-lying or infested fields have limited *Phytophthora* infection and dissemination in some situations (32). Since Phytophthora diseases are worsened under conditions of high soil moisture and/or standing water (10), water management strategies are key. Many studies have demonstrated the importance of water in the development (10,66) and spread of disease caused by *P. capsici* (13). Water may be managed within a field by planting into well-drained fields, using raised beds, using black plastic mulch, trellising cucurbit plants, irrigating sparingly and using trickle irrigation (13,32,63). Mowed cover crops on bare soil or straw mulch between rows may be utilized to reduce splash dispersal of soilborne inoculum (50,62). The trellising of smaller cucurbits keeps fruits off the ground and out of standing water, reducing the probability of infection via splash dispersal. The variety of cultural modifications accessible to large-acreage cucumber and winter squash producers in Michigan are limited by reliance on mechanical harvesters (32).

Since *P. capsici* may be found in surface water used for irrigation (28,64,75), susceptible crops should not be irrigated with surface water to avoid pathogen dissemination (28). Zoospores are able to survive for weeks in surface water at ~25°C (64), and detection of *P. capsici* in surface water appears to be related to water temperature (28). While most growers prefer to use surface water to irrigate vegetable

crops because well water is more expensive and less plentiful, water from deep wells is preferable since surface water may be contaminated with *P. capsici* (28).

Crop rotation may be integrated with other methods to control *Phytophthora* diseases, but is not successful because of the long-term survival of oospores even in the absence of a host (45). In practice, even rotations for longer than five years to nonsusceptible crops have not proven successful. A minimum of a three to four year rotation to nonsusceptible crops is recommended, but growers may be reluctant to do this because of the relatively low value of field crops (32,45) and because soybeans may be vulnerable (29). Recent reports of hosts that were previously thought nonsusceptible indicate that rotation strategies may become further limited (20,57).

Very few fungicides provide economically acceptable control of the diseases caused by *P. capsici*. When field conditions are ideal for disease development, none of the available fungicides eliminate *P. capsici*-caused diseases completely (32). The systemic phenylamide fungicide metalaxyl was introduced in 1977 and is widely used to control oomycete diseases (12,16,81). The primary mode of action of metalaxyl (Ridomil) is to inhibit RNA synthesis in sensitive isolates by reducing the incorporation of uridine into RNA (18,19). Metalaxyl is a racemic mixture; mefenoxam is the more potent enantiomer (81). Metalaxyl limits sporulation and mycelial growth inside the host tissue, but has little effect on sporangium and zoospore germination (12,18,19). Resistance to mefenoxam has been observed in some isolates of *P. capsici* in Michigan and other states (44,54). A single incompletely dominant gene controls mefenoxam sensitivity in *P. capsici* on cucurbit hosts, according to in vitro crosses. Isolates may be grouped as fully sensitive, intermediately sensitive, and fully resistant based on their

sensitivity to mefenoxam (44). Once resistance is established, the future usefulness of mefenoxam in a field may be severely limited. A study in Michigan showed that mefenoxam resistance did not decrease in an agriculturally significant time period (2 years) after mefenoxam selection pressure was removed (43). Since no management benefit is expected from applying mefenoxam to a field with a significantly insensitive *P. capsici* population, ineffective applications can increase economic losses to a grower (44,54). Part of designing an effective fungicide strategy to control *P. capsici* should include alternation between fungicides to delay the development of fungicide resistance (32). Other fungicides such as dimethomorph (17,67), zoxamide in combination with mancozeb (79,80), and cymoxanil in combination with famoxadone (37) have been registered for and show efficacy against *P. capsici.*

In addition to fungicide applications, fumigation has been employed to manage reduced *P. capsici* inoculum in the soil. The use of methyl bromide was phased out in 2005 except for critical use exemptions, which have been granted to Michigan vegetable growers for a number of years (1). Treatments containing methyl bromide have been shown to reduce *P. capsici* propagules in the soil (26), resulting in some degree of disease control in the field (32). Various alternatives to methyl bromide have been proposed including other fumigants and biofumigants (14,49).

In summary, while significant advances have been made to understand and manage the diseases elicited by *P. capsici*, this pathogen continues to threaten production in major vegetable growing regions of the United States and elsewhere. To effectively manage this disease, future research needs to focus on determining new sources of host resistance, new fungicide products and application methods, and the relationships

between *P. capsici* and environmental conditions. The research objective of this dissertation was to determine the effects of environmental conditions on *P. capsici* dispersal and disease development.

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CHAPTER I: DISPERSAL AND MOVEMENT MECHANISMS OF *PHYTOPHTHORA CAPSICI* SPORANGIA

ABSTRACT

L. L. Granke, S. T. Windstam, H. C. Hoch, C. D. Smart, and M. K. Hausbeck. 2009. Dispersal and movement mechanisms of *Phytophthora capsici* sporangia. Phytopathology 99:1258-1264.

Understanding the mechanisms of *Phytophthora capsici* sporangial dissemination is paramount to understanding epidemic initiation and development. Direct laboratory observations showed *P. capsici* sporangial dispersal occurred in water with capillary force, but did not occur in response to wind or a reduction in relative humidity. Atmospheric sporangial concentrations were monitored under field conditions using a volumetric spore sampler in a commercial cucurbit field and in an experimental setting where copious sporangia were continuously available in close proximity to the spore trap. Dispersal was infrequent (0.7% of total hours monitored) during sampling in a commercial field; 14 sporangia were detected during a 7.5-week sampling period. In the experimental field situation, dispersal occurred in 4.6% of the hours sampled, and 438 sporangia were impacted onto tapes during a 7-week sampling period. Airborne sporangial concentrations were positively associated with rainfall at both sites, but not vapor pressure deficit. Furthermore, in the experimental field situation, wind speed was not significant in regression analysis. Wind speed was not measured in the commercial field. Hence, both direct laboratory observations and volumetric spore sampling indicate that dispersal of sporangia via wind currents is infrequent, and sporangia are unlikely to be naturally dispersed among fields by wind alone.

INTRODUCTION

The oomycete plant pathogen *Phytophthora capsici* Leonian has a large host range including vegetable crops in the Cucurbitaceae and Solanaceae (19) as well as snap bean (*Phaseolus vulgaris* L.) (23), lima bean (*Phaseolus lunatus* L.) (12), Fraser fir (*Abies fraseri* (Pursh) Poir.) (39) and weed hosts (22). *P. capsici* has a worldwide distribution (19) and may be a significant limiting factor to vegetable production (29). Although *P. capsici* is a soilborne pathogen, it also causes significant disease on aerial plant tissues including rot of the fruit, stem, and crown and blighting of the foliage, in addition to root rot (29,43). *P. capsici* may generate copious amounts of asexually produced caducous sporangia on a host surface after infection has been established. Mature sporangia may germinate directly via formation of one to several germ tubes or, when in contact with free water, will differentiate into 20 to 40 biflagellate motile zoospores (5). Abundant sporangial production is a key ecological trait that allows *P. capsici* to exhibit rapid polycyclic disease development under favorable conditions (29).

Since spores are microscopic and hence difficult to detect, especially at low concentrations (4), most evidence for *P. capsici* dispersal has been indirect, either through inferences based on spatial analysis of disease patterns (40,42) or genetic diversity data (35,36). Studies in which the genetic diversity of *P. capsici* populations in Michigan were examined suggested that long-distance dispersal of sporangia is uncommon (35,36). Elucidation of the mechanisms of sporangial dissemination is critical to fully understanding the progress of an epidemic within a field. Several dispersal mechanisms have been proposed for *P. capsici*, including transfer via (i) water movement down rows, (ii) rain splash or wind-blown rain, (iii) air, or (iv) movement by

humans or invertebrate activity (29,42). Analysis of spatial patterns of disease support water movement as a major dispersal mechanism for *P. capsici* propagules (40,42). Splash dispersal has been observed for several *Phytophthora* spp. including *P. nicotianae*, *P. palmivora*, and *P. infestans* (44,48), but has not been directly examined for *P. capsici* even though it is assumed to be a means of pathogen spread. Although it has been speculated that local aerial dispersal of *P. capsici* propagules may occur (43), previous studies have described aerial dispersal as unimportant (41,46). To our knowledge, the only study that has attempted to directly sample aerially dispersed *P. capsici* propagules used media-containing Petri plates affixed to stakes in a pepper field during an active *P. capsici* epidemic (46). This study found that aerial dispersal occurred rarely, and dispersal in dry air was only detected when plates were within 5 cm of a heavily sporulating source.

P. infestans, the causal agent of potato late blight, is the most well studied *Phytophthora* spp. with regards to aerial dispersal (11,32,34). It has long been known that *P. infestans* sporangiophores display hygroscopic movement as relative humidity decreases during the morning hours and actively release sporangia (14,32,33). Sporangia are subsequently carried away from the inoculum source by wind (3). This mode of dispersal is also seen in downy mildew pathogens such as *Bremia lactucae*, the causal agent of lettuce downy mildew (45). Because *P. infestans* and *P. capsici* both infect and form sporangia on above-ground plant tissue, growers sometimes postulate that *P. capsici* sporangia may be dispersed in a manner similar to that of *P. infestans*. To our knowledge, no studies have directly examined *P. capsici* sporangial detachment and dispersal in response wind and water. The objectives of our study were to: directly

observe whether a change in relative humidity, direct wind, or water combined with capillary force enables detachment and transport of *P. capsici* sporangia, and assess the probability of aerial dispersal in commercial and experimental field situations.

MATERIALS AND METHODS

Growth and maintenance of *Phytophthora* isolates. *P. capsici* isolates SP98 and 0664-1 were maintained at room temperature (25 ± 2°C) under continuous fluorescent lighting on UCV8 (160 ml unclarified V8 juice, 840 ml distilled water, 30 mM $CaCO_3$, and 1.5% agar) and were transferred to fresh UCV8 weekly. SP98 (A2 mating type, sensitive to mefenoxam) was isolated from pumpkin (*Cucurbita pepo* L.) in Michigan, and 0664-1 (A1 mating type, sensitive to mefenoxam) was isolated from pepper (*Capsicum annuum* L.) in New York. Isolate notation refers to the culture collections maintained in the laboratories of M. K. Hausbeck at Michigan State University and C. D. Smart at Cornell University. *P. infestans* clonal lineage US-11 was obtained from the laboratory of W. E. Fry at Cornell University and was maintained on detached tomato leaflets (*Solanum lycopersicum* L. cv. Sunchief).

Inoculation. Detached pickling cucumber fruits (*Cucumis sativus* L., 10 ± 1 cm long) were surface disinfested with a 10% bleach (0.62% NaClO) solution for 10 min, rinsed with distilled water, blotted dry and placed in a sterile humid chamber (23 x 10 x 31 cm lidded clear plastic box with moist paper towels in the bottom to maintain humidity) before inoculation with *P. capsici* zoospores. Zoospore suspensions were prepared by flooding actively sporulating (4-7 day old) *P. capsici* cultures with sterile distilled water and incubating the plates at 4°C for 45 min followed by 30 min at room

temperature. The concentration of zoospores was determined using a hemacytometer and adjusted to 2.5×10^6 zoospores/ml for each isolate. Three 10-µl droplets of zoospore suspension were used to inoculate each cucumber fruit. Cucumbers were incubated at room temperature under continuous fluorescent lighting until copious sporangia were detectable by light microscopy, approximately 72 to 96 h post inoculation (Figure 1.1A-C).

Tomato leaflets were inoculated by firmly pressing healthy leaflets against ones with actively sporulating lesions caused by *P. infestans*. Inoculated leaflets were incubated at 19°C in 100 × 15-mm round plastic Petri dishes with either water agar or moist filter paper in the lids to maintain humid conditions (incubation units) and were used once abundant sporulation was apparent, which was a minimum of 4 days post inoculation.

Fabrication of mini-chambers for direct observation of *P. capsici* sporangia. For preparation of mini-chambers, polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, Midland, MI) was poured over a 100 × 100 × 15-mm plastic Petri dish and cured at 60°C for 1 h. Spacers were used to maintain a PDMS thickness of 5 mm. Following curing, a square of PDMS was cut into an H-shape and released from the Petri dish. To create the bottom half of the chamber, the PDMS and a 51 × 76-mm microscope slide were covalently bonded to each other following exposure to an air plasma (15). Openings were bored through the PDMS, and stainless steel tubing (Small Parts Inc., Miramar, FL) was inserted and sealed with unpolymerized PDMS, which was heat-cured and reinforced with Torr Seal epoxy (Varian Inc., Lexington, MA) to create an inlet and outlet for wind at each end of the chamber. A cover glass was placed on the mini-

chamber and held in place with microscope stage clips to seal the chamber during observation. Wind was generated using a TopFin XP-125 aquarium pump (PetSmart, Phoenix, AZ) and humidified by passing air through moist cheesecloth loosely packed into a humidity chamber to minimize sporangia dehydration. The air flow was adjusted to 1.50 ± 0.06 liters/min using a flow meter (Dwyer Instruments, Michigan City, IN). Vinyl and rubber tubing was used to route the wind from the pump through the humidity chamber and into the mini-chamber.

Direct observation of sporangia exposed to wind and water. Sporangia were directly observed using an Olympus SZX12 dissecting microscope (Olympus America Inc., Center Valley, PA) with an Intralux 4000 fiber optic light source (Volpi, Switzerland) for overhead illumination or an Olympus IMT-2 inverted compound microscope. Images and real-time movies were captured using a CoolSnap Cf camera (Photometrics, Tucson, AZ). MetaMorph software (Universal Imaging, Dowingtown, PA) was used for shutter control, image capture, and subsequent image processing. Individual video frames were obtained from a streaming video sequence.

Tomato leaflets with actively sporulating *P. infestans* were used as a positive control to demonstrate that sporangial dispersal in the laboratory setting could be directly observed. The top of the incubation unit was removed, which allowed low humidity ambient air to enter the unit resulting in a humidity decrease in the unit. Sporangia were directly observed and images were captured as described above. Intact pickling cucumber fruits with *P. capsici* sporangia were removed individually from a moist incubation chamber and placed onto a 103 × 83-mm glass cover slide to observe *P.*

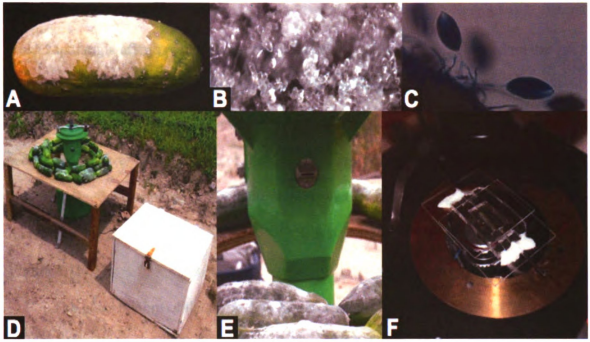

Figure 1.1. A, Pickling cucumbers with actively sporulating *Phytophthora capsici* were used for both laboratory and field experiments. **B,** Close-up of a sporangial lawn and **C,** sporangia stained with trypan blue on a cucumber fruit. **D-E,** Volumetric spore sampler and platform supporting cucumbers with sporulating *P. capsici* in a field. **F,** Mini-chamber (on inverted compound microscope stage) used for direct observations of the effect of wind on *P. capsici* sporangia.

capsici sporangia after a reduction in relative humidity. The fruit were placed on the glass cover slide that was scanned before and after sporangial observation to ensure all dispersal events were detected. Sporangia were observed for 2 min with images captured as described above. A small piece of tissue containing sporangia was excised from the fruit and placed into a mini-chamber. Sporangia were observed using a compound microscope for 2 min while 1.5 liters/min of air was pumped through the mini-chamber (Figure 1.1F).

The glass bottom of the mini-chamber was scanned before and after observation of sporangia to determine if any undetected dispersal occurred. Finally, two small pieces of tissue containing sporangia were excised from the same fruit and placed onto a glass microscope slide 1 to 2-mm apart with the infected surfaces facing each other. A cover slip was placed on top of the tissue pieces and 10 to 50-µl of water was pipetted between the pieces allowing capillary action to draw the water down the length of the pieces. The sporangia were directly observed for dispersal in water as described above. At least 30 observations were made for each potential dispersal event.

Monitoring of atmospheric sporangial concentrations of *P. capsici* under field conditions. Atmospheric concentrations of *P. capsici* sporangia were determined using a Burkard 7-day volumetric spore sampler (Burkard Manufacturing Co Ltd, Rickmansworth, Hertfordshire, UK). The sampling airflow rate was 10 liters/min, and the sampler was set to allow free movement according to wind direction so that the orifice faced the prevailing wind. Sporangia were impacted onto tapes coated with an adhesive mixture of petroleum jelly and paraffin (9:1, wt/wt) dissolved in sufficient toluene to

result in a thick, liquid consistency. Tapes were removed weekly, cut into 48-mm lengths, scored at hourly intervals, stained with aniline blue in glycerol (0.14 mg aniline blue, 20 ml distilled water, 15 ml glycerol, 10 ml of 85% lactic acid), mounted on glass slides under 22 × 50-mm cover slips, and sealed using Cytoseal (Richard-Allan Scientific, Kalamazoo, MI). Slides were scanned, and *P. capsici* sporangia were examined at 400× magnification and identified based on morphological characteristics (50).

Atmospheric sporangial concentrations were monitored in a commercial cucurbit field and in an experimental field situation. Spore sampling was conducted for a period of 7.5 weeks (16 June to 8 August 2006) at a commercial farm in Cass County, MI planted to cucurbits (acorn squash (*Cucurbita pepo* L. cv. Table Ace) followed by cucumber (cv. Cobra)). Fungicides were applied to acorn squash in support of fungicide research that was conducted in this field and to the cucumber crop according to commercial recommendations. Severe disease was observed in the acorn squash, and more than 75% of plants were dead by 26 July when the spore trap was moved to cucumber.

Atmospheric concentrations of sporangia were monitored for 7 weeks (19 July to 16 September 2008) in an experimental field setting at the MSU Muck Soils Research Farm, Laingsburg, MI. At this site, infected cucumbers with sporulating *P. capsici* (isolate SP98) were prepared as described above. Cucumbers were placed on a raised platform approximately 15 to 40 cm from the sampler so that infected tissue with the sporulating pathogen faced the Burkard spore sampler and was level with the sampler orifice (Figure 1.1D and E). This configuration allowed for quantification of dispersal

without the limitation of a canopy and with minimal spatial dilution effects. Fruit were replaced with new infected cucumbers with sporulating *P. capsici* triweekly to ensure that sporangia were constantly available. Cucumbers were checked for sporulating *P. capsici* using a dissecting microscope before and after placement in the field to ensure that copious amounts of sporangia were available for the entire monitoring period.

Collection of weather data and statistical analysis. Hourly meteorological data of temperature (°C), relative humidity (%), and rainfall (mm) were measured by a Watchdog model 450 data logger, and tipping bucket rain gauge (Spectrum Technologies, Plainfield, IL) at the commercial field. Hourly meteorological data of air temperature (°C), relative humidity (%), average and maximum wind speed (m/s) and rainfall (mm) were measured by an automatic weather station belonging to the Michigan Automated Weather Network at the experimental field. Vapor pressure deficit (VPD, kPa) was calculated for both sites using temperature and relative humidity values (38).

Statistical analyses were performed using the SAS statistical package version 9.1 (SAS Institute, Inc., Cary, NC). The PROC CORR procedure was used to determine any positive or negative correlations among daily cumulative atmospheric sporangial concentration, daily cumulative rainfall, and daily average VPD for the commercial field and to determine correlations between daily cumulative atmospheric sporangial concentration, daily cumulative rainfall, daily average VPD, and daily average wind speed prior to regression analysis for the experimental field situation. Autocorrelation in the data set was characterized using ITSM 2000 software and was detected for the experimental field situation (attached to (8)). Regression analysis of data from the commercial field was not completed as the assumptions of regression could not be met.

The PROC AUTOREG procedure of SAS was used for regression analysis of data from the experimental field to appropriately address the autocorrelation detected in this data set. The AUTOREG procedure calculates the equivalent of the least square parameter estimates of a regression model when the data are time series and the error term is an autoregressive process. The AUTOREG procedure uses the two-step full transform estimation method (28) to correct for the lack of independence through time of the error term of time series data. Variables with $P \leq 0.05$ were considered statistically significant. The final day of sampling at each site was not used in statistical analyses because less than 24 h of data were available for these days.

RESULTS

Direct observations on wind and water effects on dispersal. *P. infestans* sporangiophores displayed hygroscopic movement almost immediately after a reduction in relative humidity. In all cases where mature sporangia were present, dehiscence followed the twisting of sporangiophores under nearly still conditions (Table 1.1). All dispersal events took place within 60 s of a reduction in humidity, and could be clearly viewed by microscopy (Figure 1.2). This validated that the methods used in this study were sufficient to directly observe sporangial dispersal. Dispersal of individual sporangia was rapid, often no longer than a single video frame (Figure 1.2 A-B). In contrast, *P. capsici* sporangiophores did not display hygroscopic movement upon a reduction in relative humidity, and dispersal was not observed (Table 1.1). Sporangia on the cucumber fruit surface were not dispersed in wind (Table 1.1, Figure 1.3). However, water in combination with capillary force caused sporangia to readily detach from the fruit surface almost immediately upon wetting the cucumber fruit surface, and sporangia

Table 1.1. Number of direct observations of *Phytophthora infestans* or *Phytophthora capsici* sporangia made with a dissecting or inverted compound microscope. Each cluster of sporangia on an individual leaflet or cucumber was observed until dispersal occurred or up to 2 min.

			Observations (% with dispersal)		
Pathogen	Lineage/isolate	Host tissue	RH[x]	Wind	Water
P. infestans	US-11	Tomato leaflets	40 (100)	n/a[y]	n/a
P. capsici	SP98	Cucumber fruit	30 (0)	30 (0)	30 (100)
P. capsici	0664-1	Cucumber fruit	30 (0)	30 (0)	30 (100)

[x] RH=a reduction in relative humidity

[y] n/a=not applicable

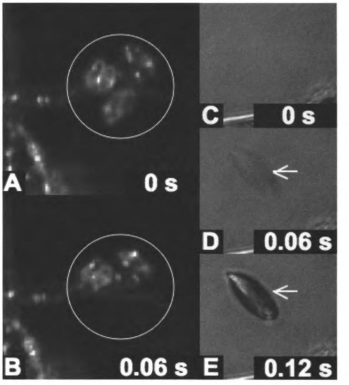

Figure 1.2. A -B, Direct observation of individual *Phytophthora infestans* sporangia being dispersed and **C-E,** settling on the bottom of the incubation unit. A sporangium in the circled cluster of sporangia in **A** is dispersed and absent in the subsequent video frame captured 0.06 s later in **B**. A sporangium (arrow) in **E** attaches to the bottom of the incubation unit after its shadow is seen in the previous frame **(D)**.

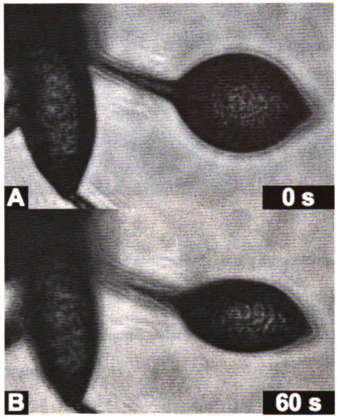

Figure 1.3. A *Phytophthora capsici* sporangium on a piece of cucumber fruit **A,** before and **B,** after being exposed to 1.5 liters/min of wind for 60 s. No dispersal was observed. Note dehydration of sporangium.

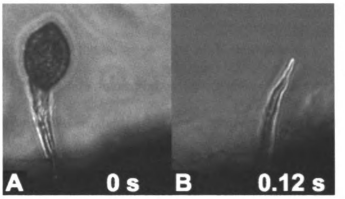

Figure 1.4. A, A *Phytophthora capsici* sporangium before introducing water that is distributed via capillary force along the surface of a cucumber fruit piece with the sporulating pathogen. **B,** The sporangium detaches and is dispersed in the water resulting in an empty sporangiophore.

were transported in the water (Table 1.1, Figure 1.4). Videos capturing these dispersal experiments may be viewed at the home page of M. K. Hausbeck.

Atmospheric *P. capsici* sporangial concentrations under field conditions. When atmospheric sporangial concentrations were monitored in a commercial cucurbit field, dispersal occurred during 0.8% of hours sampled (10 of 1278 h). The concentration of sporangia never exceeded 5 sporangia/m^3/h when dispersal was detected. In total, 14 sporangia were impacted onto tapes during the course of the 7-week sampling period. Only one sporangium was sampled during most (80%) of the hours when dispersal was detected. In the experimental field situation (Figure 1.1D), aerial dispersal occurred in 4.6% of the hours sampled (54 of 1174 h). The concentration of sporangia was low (≤15 sporangia/m^3/h) for 80% of the occasions when dispersal was detected. Only 438 sporangia were impacted onto tapes over the course of the 7-week sampling period. No clear diurnal pattern was observed for sporangial dispersal at either site (*data not shown*).

Rainfall was significantly positively correlated (r=0.57, P<0.0001) with detection of sporangia in the commercial field. Rainfall was the only weather parameter that was significant in regression analysis (ß=3.57, R^2=0.68) for the experimental field situation. Dispersal occurred during 56% (5 of 9) of days with rainfall at the commercial farm and 56% (9 of 16) of days with rainfall at the experimental field (Figure 1.5). Dispersal occurred during 42% (5 of 12) of days without rainfall at the commercial farm and 30% (9 of 30) of days without rainfall at the experimental field (Figure 1.5). Vapor pressure deficit was not significant in correlation or regression analysis, respectively for the commercial or experimental field. Wind speed was not measured in the commercial

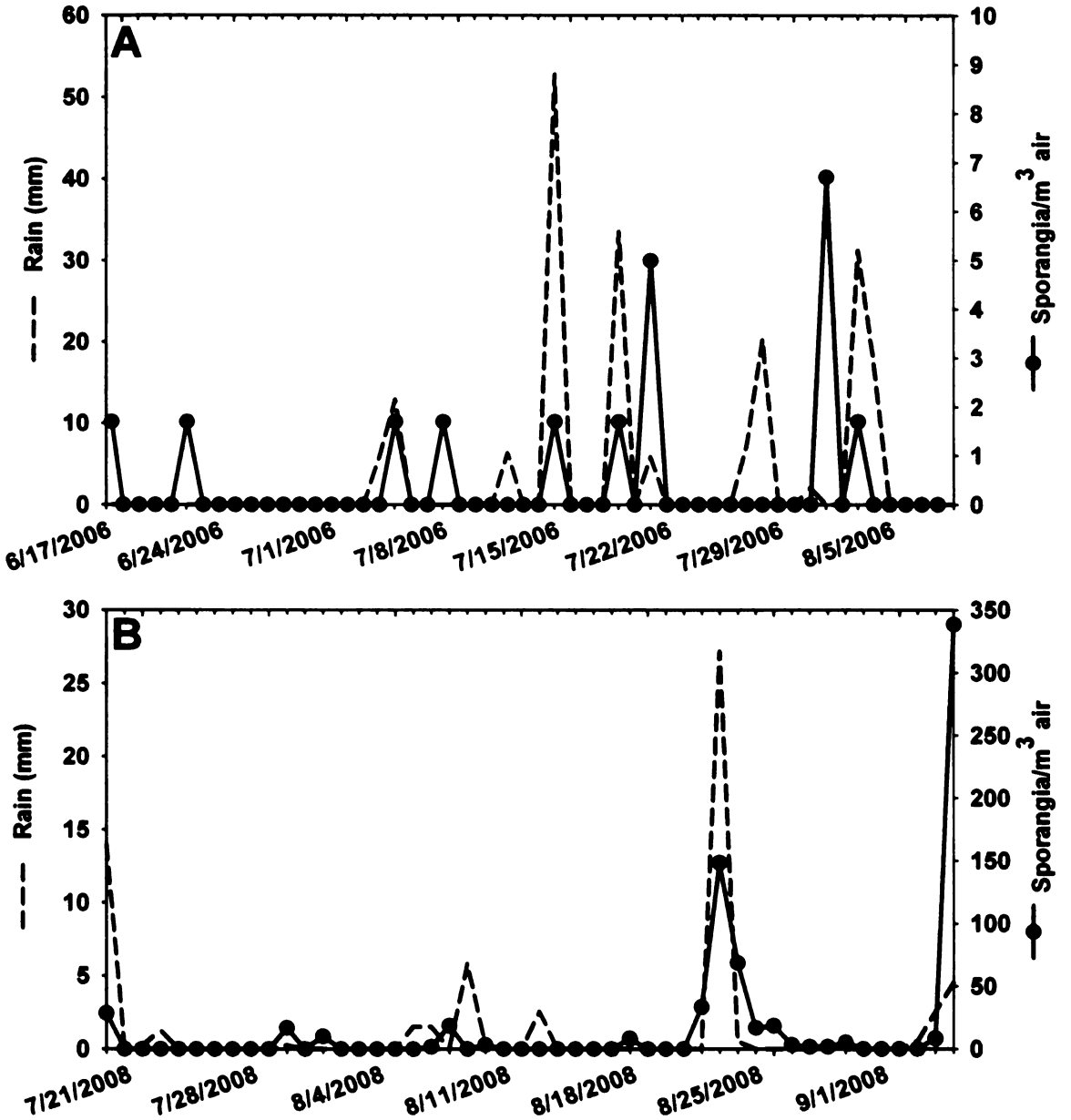

Figure 1.5. Relationship between daily cumulative concentration of airborne *P. capsici* sporangia (sporangia/m^3/day) and the correspondent daily rainfall (mm) for **A,** monitoring in the commercial cucurbit field in 2006 and **B,** monitoring in the experimental field setting in 2008. Note the differences in scale on the y-axes.

field, and was not found to be significant in regression analysis for the experimental field.

DISCUSSION

This study suggests that dispersal of *P. capsici* sporangia via wind currents is infrequent and that sporangia are unlikely to be dispersed among fields by wind. This is in agreement with the conclusions of Lamour and Hausbeck (35,36) from spatiotemporal genetic diversity data of populations of *P. capsici* in Michigan and Schlub (46), who investigated aerial dispersal in a pepper field with an active epidemic of Phytophthora blight.

Direct observations in the laboratory showed that sporangial dispersal occurred in water with capillary force, but did not occur due to wind or a reduction in relative humidity. Laboratory results were confirmed in the field; volumetric spore sampling demonstrated aerial dispersal was a low frequency event, especially under commercial field production conditions. Furthermore, a very small amount of the total available sporangial load was dispersed. In the experimental field situation, a total of 438 sporangia were detected from an estimated minimum of 189 to 567 lesions throughout the 7-week sampling period. In comparison, up to 600,000 sporangia can be dislodged by water and mechanical force from one lesion with *P. capsici* sporulating profusely when a cucumber is inoculated and incubated as described above (*data not shown*). The escape of sporangia is limited by the presence of a canopy, and detached sporangia may be diluted during transport in the wind. These two limitations on detection were intentionally removed in the experimental field setting, but not at the commercial farm. In cucumber, the plant's foliage is typically asymptomatic and sporangia form primarily on fruit or occasionally at the plant's base. Thus, a reduced canopy is not common in

cucumber fields with high disease pressure, and sporangia tend to be low in the canopy where the wind velocity is reduced as compared to the upper canopy (3). While some hosts such as pepper may display sporulation on tissue higher in the canopy (43), sporangia tend to be low in the canopy on the crown and/or fruit tissues of most infected host plants including acorn squash (29). When we sampled aerial sporangial dispersal from infected cucumbers placed on a platform without the limitation of a canopy (experimental field situation) few sporangia were trapped, and often these sporangia were detected in clusters. Clusters of sporangia should deposit close to the original source due to the increased settling speed of these clusters as compared to a single sporangium (20). Based on laboratory and field observations from this study, we suggest that aerial dispersal of sporangia is relatively unimportant for *P. capsici*.

Unlike what is observed for *P. infestans* (3) and some downy mildew pathogens (9,31), atmospheric *P. capsici* sporangial concentrations did not display a clear diurnal pattern in either field. Only rainfall was statistically associated with dispersal. Dispersal was sporadic, and sporangial dispersal was not always detected during rain events at either sampling site. This could be explained by sporangia wash-out during rainy or wet conditions, or sporangial detachment rates may have been low even when moisture was present. Our data is supported by a previous study by Schlub (46) that examined aerial dispersal of *P. capsici* in a pepper field during an active epidemic by using media-filled plates on stakes placed at different distances from sporulating host tissue. Schlub detected dispersal on 5 of 70 sampling occasions. Three of these dispersal events were associated with rain or overhead irrigation, and the remaining two occurred when plates were placed within 5 cm of host tissue with actively sporulating *P. capsici* (46).

Irrigation water infested with *P. capsici* can initiate an epidemic in a field lacking a previous history of infestation (25). Many studies have demonstrated the importance of water in the development and spread of disease caused by *P. capsici* (10,40,46). In this study, we directly observed that sporangia will readily detach and be transported in water combined with capillary force. It is possible that the observed detachment and subsequent dispersal could be an artifact of tissue manipulation in setting up the samples. We maintain that this is unlikely since dispersal was never observed from tissue with *P. capsici* sporangia that was subjected to direct wind, although this tissue was manipulated in a similar fashion. The ability of water to detach and transport sporangia underscores the importance of water as the primary mechanism of pathogen dispersal.

It is known that wind has the potential to move microbes long distances, either as spores or in conjunction with soil particles. While it may be tempting to assume that long-distance atmospheric transport of inoculum is responsible for the sudden appearance of plant diseases in places far from areas of known pathogen establishment, it also important to remember that alternative pathways for pathogen transport exist, such as human-mediated transport (4). Since long-distance dispersal of sporangia via wind is unlikely, preventing long-distance human-mediated transport of *P. capsici* via infested soil and water and infected plant material is key. The current management strategies of exclusion, cultural control, host resistance, and fungicides and fumigants are still warranted in light of this study. Growers can circumvent long-distance spread of the pathogen by cleaning equipment between fields, not spreading cull fruit onto fields, and avoiding the use of surface water that may be contaminated with *P. capsici* to irrigate healthy crops (25,29). Water is known to be a key factor in local spread of *P. capsici*

spores and in the development of epidemics (10,40). Water may be managed within a field by planting into well-drained fields, using raised beds, using black plastic mulch, trellising cucurbit plants, irrigating sparingly and using trickle irrigation. While it is important to use a multifaceted approach to manage the diseases caused by *P. capsici*, managing water appears paramount in light of the results of this as well as other studies (29).

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CHAPTER II: EFFECTS OF TEMPERATURE, CONCENTRATION, AGE, AND ALGAECIDES ON *PHYTOPHTHORA CAPSICI* ZOOSPORE INFECTIVITY

ABSTRACT

Granke, L. L. and Hausbeck, M. K. 2010. Effects of temperature, concentration, age, and algaecides on *Phytophthora capsici* zoospore infectivity. Plant Dis. 94:54-60.

Controlled laboratory studies were undertaken to determine the effects of water temperature (2, 9, 12, 19, 22, and 32°C), spore concentration (1×10^2, 1×10^3, 5×10^3, 1×10^4, 2×10^4, and 4×10^4 zoospores/ml), and zoospore suspension age (0, 1, 3, and 5 days old) on infection of pickling cucumbers (*Cucumis sativus*) by *Phytophthora capsici*. Zoospore motility and mortality in response to commercial algaecides were also investigated. Cucumbers became infected at all temperatures tested, except 2°C, and the highest infection incidence was observed for cucumbers incubated in suspensions held at ≥19°C. Fewer fruit (<40% at ≥19°C, 0% at ≤12°C) became infected when water contained 1×10^2 zoospores/ml. Almost 100% of fruit were infected when water contained ≥5×10^3 zoospores/ml at temperatures ≥12°C. While the incidence of fruit infection declined with the zoospore suspension age, infection still occurred when 5-day-old suspensions were used. Commercial algaecides inhibited zoospore motility and caused significant zoospore mortality in laboratory assays, and show promise for treatment of infested irrigation water. Avoidance of infested irrigation water throughout the growing season is warranted until effective and economically acceptable water treatments are developed for field use.

INTRODUCTION

Michigan is the largest producer of pickling cucumbers (*Cucumis sativus*) in the United States (3). The oomycete plant pathogen *Phytophthora capsici* Leonian has a large host range including vegetable crops in the Cucurbitaceae and Solanaceae (16). Cucumber fruit are especially susceptible to infection even though infected cucumber plants may be relatively asymptomatic. In Michigan, entire fields of cucumbers bearing mature fruit, and semi truck loads of harvested fruit may be rejected due to fruit rot (22). *P. capsici* may generate copious amounts of asexually produced sporangia on the host surface after infection has been successfully established. Mature sporangia may germinate directly, forming one to several germ tubes or, when in contact with free water, will differentiate into 20 to 40 biflagellate motile zoospores (22). *Phytophthora* zoospores may swim electro-(50) and chemotactically or may be carried in flowing water (41), including irrigation water. Once a zoospore comes in contact with host tissue, it encysts, attaches, germinates by forming a germ tube, and penetration and colonization of the host tissue follows (16).

Phytophthora spp., including *P. capsici*, have been detected in surface water used for irrigation in diverse geographic regions of the United States (19,37,42). It has been hypothesized that irrigation water infested with *P. capsici* may initiate a disease epidemic of susceptible host plants within a field lacking a previous history of infestation (19). Zoospores are known to be the primary propagules found in irrigation water (10,46), but the ability of zoospores to cause infection over time at different inoculum densities has not been determined. In a study in Florida, *P. capsici* propagules could be detected for at least 45 days following incubation in tailwater ponds in fine pore membrane covered-

bottles that contained ~1.5 x 10^5 zoospores suspended in tailwater, surface runoff water that is collected in retaining ponds and canals (42).

While the optimal temperature for growth of *P. capsici* has been described (16), the effect of water temperature on infection of cucumbers by zoospores is unclear. Studies in Florida did not find a correlation between temperature and detection of *P. capsici* propagules in tailwater (42), but studies in Michigan did find a significant correlation between temperature and detection of *P. capsici* in surface water irrigation sources (19). Understanding the relationship between water temperature and the ability of zoospores in water to infect cucumber fruits is required for successful management of disease when irrigation water may be infested.

Most growers in Michigan utilize surface water to irrigate vegetable crops since well water is more expensive and less available. Michigan cucurbit growers provide an average of 2.5 cm of water per week to their crops during the summer growing months. Gevens et al. (19) found that the detection of *P. capsici* in surface water irrigation sources was greatest during July and August when the need to irrigate crops was also the greatest.

The increasingly common greenhouse/nursery practices of recycling irrigation water and recirculating nutrient solutions are economically and environmentally beneficial, but also increase the spread of waterborne pathogens including *P. capsici* (46,47). There are several methods currently available for disinfesting contaminated irrigation water in greenhouse and nursery settings including the use of antimicrobial compounds such as copper (48,49) and hydrogen peroxide (23,43), but little research has focused on disinfestants for use in a field setting.

Commercial algaecides contain copper sulfate, chelated copper, or sodium carbonate peroxyhydrate as the active ingredient. It has long been known that copper is suppressive to *Phytophthora* spp. (21,45), and spray tank-mixing copper with other fungicides has been shown to improve management of *P. capsici* in the field (22). Previous studies have shown that increasing copper ion concentrations in nutrient solutions reduces disease caused by *P. cinnamomi* (49) or *P. cryptogea* (48), and recent laboratory studies have shown copper-based algaecides have potential for treating *P. ramorum* in water (11). Sodium carbonate peroxyhydrate (SCP)-based algaecides were developed as a nonpersistent alternative to copper algaecides (27,39), which may build up in aquatic systems and can be toxic to nontarget organisms (30,34). SCP releases hydrogen peroxide upon dissolution in water (53), a compound that is active against a wide variety of organisms (28) and is an effective treatment to control oomycete parasitic infection of fish eggs (5,27). Chlorination and algaecide treatments are already used by some growers to control undesirable algal growth in irrigation systems (36) since algae may serve as a substrate for undesirable bacterial growth and may clog intake screens and filters and other system components, resulting in reduced water flow rates and an inadequate supply of water for irrigated crops (13,35).

The objectives of our study were to elucidate the effects of: (i) water temperature, inoculum concentration, and zoospore suspension age on infection of pickling cucumbers by *P. capsici* and (ii) copper and SCP-based algaecides on zoospore motility and mortality.

MATERIALS AND METHODS

***P. capsici* isolates and production of zoospore inoculum**. Actively growing cultures of *P. capsici* isolates SP98 and OP97 were used (isolate notation refers to the culture collection maintained in the laboratory of M. K. Hausbeck at Michigan State University). The cultures were derived from long-term stock cultures (stored at 20°C in sterile 1.5-ml microcentrifuge tubes with 1 ml of sterile water and one sterile hemp seed) that were transferred to unclarified V8 agar (UCV8, 160 ml unclarified V8 juice, 840 ml distilled water, 30 mM $CaCO_3$, and 1.5% agar). Isolates were maintained on UCV8 at room temperature (21 ± 2°C) under continuous fluorescent lighting and were transferred to new UCV8 weekly. OP97 (A1 mating type, sensitive to mefenoxam) was isolated from cucumber in Michigan and was used for the studies looking at the effects of water temperature, inoculum concentration, and zoospore suspension age on infection of pickling cucumber fruits by *P. capsici*. SP98 (A2 mating type, sensitive to mefenoxam) was isolated from pumpkin (*Cucurbita pepo*) in Michigan and was used for the studies examining the effects of copper and SCP-based algaecides on zoospore motility and mortality. Studies by Gevens et al. (18) demonstrated that SP98 and OP97 are equally virulent on cucumber fruits. Zoospore suspensions were prepared by flooding actively sporulating *P. capsici* cultures with distilled water and incubating the plates at 2°C for 1 h followed by 30 min at room temperature. The concentration of zoospores was determined using a hemacytometer and adjusted to the desired concentration using distilled water at the appropriate experimental temperature.

The effect of water temperature and inoculum concentration on infection. Six temperatures (2, 9, 12, 19, 22, and 32 ± 2°C) and six inoculum densities (1×10^2, $1 \times$

10^3, 5×10^3, 1×10^4, 2×10^4, and 4×10^4 zoospores/ml) were tested to investigate the effects of water temperature and inoculum concentration on infection of pickling cucumbers. Pickling cucumber fruits of a similar size (10 ± 1 cm long) were purchased from a local supplier. For each temperature/concentration pair, six detached unwounded pickling cucumbers were rinsed to remove surface debris, surface disinfested with a 10% bleach (0.62% NaClO) solution for 5 min, air dried, and placed into 11.4-liter plastic tubs containing 3 liters of *P. capsici* isolate OP97 zoospore suspension. The fruits were kept in the zoosopore suspension for 24 h at the desired temperature, rinsed with distilled water, and incubated in a moist chamber at ~100% relative humidity overnight at room temperature. When lesions with water soaking and/or pathogen growth were apparent on fruits, tissue 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 fruits appeared healthy, at least three tissue sections were randomly excised from the fruit and plated onto BARP-amended UCV8. Plates were incubated at room temperature under continuous fluorescent lighting for 3 to 5 days and were observed using a compound microscope. *P. capsici* was confirmed using morphological characteristics according to the *Phytophthora* spp. key by Waterhouse (52). The resulting *P. capsici* colonies were transferred to new UCV8, and about 96% of isolates were characterized for mating type and mefenoxam sensitivity (26) to confirm they were the same as OP97. Six control cucumbers were added to 3 liters of distilled water at room temperature and treated as above to ensure that the cucumber fruits used in the experiment were not infected with *P. capsici* at the onset of the experiment. The temperatures of the zoospore suspensions were monitored throughout

the course of the experiment using Watchdog 100 series temperature loggers (Spectrum Technologies, Plainfield, IL). Three independent experiments were conducted for each temperature/concentration combination.

The effect of zoospore suspension age on infection of pickling cucumber fruits. The effect of zoospore suspension age on infection of pickling cucumber fruits was tested by adding six unwounded pickling cucumbers to 3 liters of OP97 zoospore suspension for each inoculum concentration tested (1×10^2, 1×10^3, 5×10^3, 1×10^4, 2×10^4, and 4×10^4 zoospores/ml) at 0 (immediately), 1, 3, or 5 days following zoospore suspension creation. Cucumbers were removed 24 h later and treated as described above. Six cucumbers were added on day 0 to 3 liters of distilled water and treated as the other fruits for a control. All experiments were conducted at room temperature. Four independent experiments were conducted for each age/inoculum concentration combination.

The effects of algaecides on zoospore motility and mortality. Commercially available algaecides were assessed for effects on zoospore motility and mortality in water. The algaecides tested contained either chelated copper or SCP as the active ingredient and were used at rates allowable in surface water bodies in Michigan (2) (Table 2.1). Fresh stock solutions containing twice the desired rate of algaecides were created immediately prior to experiments. A bleach stock solution containing ~4 ppm chlorine was used to treat zoospores in water at ~2 ppm chlorine as a positive control (24). Distilled water was used as a negative control. The pH of algaecide-treated water was measured three times using a S20 pH meter with InLab Expert Pro electrode (Mettler-Toledo, Columbus, OH).

Table 2.1. Algaecide treatments applied to water containing *Phytophthora capsici* isolate SP98 zoospores and the resulting pH of the water.

Treatment (product/liter)	Active ingredient (%[z])	pH ± SE[y]
Bleach (39 ml)	sodium hypochlorite (6.2)	7.61±0.01
Cutrine-Plus (1.8 ml)	copper ethanolamine (9)	8.55±0.02
Cutrine-Ultra (1.8 ml)	copper ethanolamine (9)	7.87±0.03
Symmetry (2.1 ml)	copper triethanolamine and copper hydroxide (8)	7.16±0.01
Captain (1.8 ml)	copper carbonate (9.1)	8.71±0.01
GreenClean (0.006 mg)	sodium carbonate peroxyhydrate (50)	9.87±0.00
K-Tea (2.1 ml)	copper triethanolamine and copper hydroxide (8)	7.64±0.01
Formula F-30 (6.1 ml)	copper sulfate pentahydrate (2.7)	6.34±0.16
Pak 27 (0.006 mg)	sodium carbonate peroxyhydrate (85)	9.57±0.00
EarthTec (1 ml)	copper sulfate pentahydrate (5)	6.21±0.03
GreenClean Pro (3 µg)	sodium carbonate peroxyhydrate (85)	9.57±0.00
Algimycin-PWF (3.3 ml)	copper citrate and copper gluconate (5)	5.70±0.02
Distilled Water	None	6.67±0.01

[z] Copper (%) is expressed as elemental copper equivalent

[y] SE=standard error

For the zoospore motility experiments, 5 ml of stock solution or distilled water were mixed with 5 ml of distilled water containing 1×10^6 zoospores/ml (isolate SP98) in a 60 × 15 mm plastic Petri dish. The treated suspension was checked using a compound microscope every 3 min for the first 15 min, then every 15 min until 1 h after treatment, and then every 30 min thereafter until no zoospore movement was observed. The amount of time required for all zoospores to cease movement was recorded for three replicate plates of each treatment. The experiment was conducted three times.

Zoospore mortality was assessed after treatment for 5, 10, 30, 60, or 120 min. Algaecide and control treatments were initiated by mixing 1 ml of stock solution or water with 1 ml of a suspension containing 1×10^4 zoospores/ml (isolate SP98) in a 2.2-ml microcentrifuge tube. After the desired contact time was reached, the tube was vortexed briefly, and three 100-µl aliquots of treated zoospore suspension were diluted with 100 ml of distilled water each in 125-ml glass Erlenmeyer flasks to stop the reaction. The resulting treated zoospore suspension was vacuum filtered through quantitative filter paper with 1-µm particle retention (VWR, Batavia, IL), and the filter paper was plated onto BARP-amended CV8 (160 ml clarified V8 juice, 840 ml distilled water, 30 mM $CaCO_3$, and 1.5% agar). The plates were incubated at room temperature under continuous fluorescent lighting for 48 h. The filter papers were removed, colonies were enumerated, and ~50% of colonies were observed via compound microscopy to confirm *P. capsici* using morphological characteristics according to the *Phytophthora* spp. key by Waterhouse (52). Zoospore mortality was calculated by comparing the number of

colonies that formed from treated suspensions to the number of colonies from the untreated control. The experiment was conducted twice.

Data Analyses. All statistical analyses were performed using the SAS statistical package version 9.1 (SAS Institute, Inc., Cary, NC). Prior to analysis, percentage data were normalized by angular (arc sin) transformation. Data were subjected to analysis of variance ($P \leq 0.05$), and Fisher's protected LSD was used for separation of means ($P \leq 0.05$). Unequal variance models were used to evaluate the effects of temperature and zoospore concentration on infection incidence, zoospore suspension age and zoospore concentration on infection incidence, and algaecide treatment and contact time on zoospore mortality. Variances were grouped by the concentration factor, age factor, and treatment factor, respectively. Data from 2°C were removed prior to analysis since no infection occurred at this temperature. Regression analysis was used to determine the relationship between each variable and mean infection incidence. The model that best fit the data was used. The relationship between temperature and infection incidence was analyzed using nonlinear polynomial regression analysis. The relationship between zoospore inoculum concentration and infection incidence was analyzed using logarithmic regression analysis. The relationship between zoospore suspension age and infection incidence was determined using linear regression analysis after data were subjected to analysis of covariance to determine if the same slope could be used for all of the data despite differences in zoospore suspension concentrations.

RESULTS

No infection occurred when cucumbers were incubated at 2°C regardless of zoospore inoculum concentration. Cucumber fruits became infected with *P. capsici* at all other temperatures tested (Figure 2.1). A statistically similar infection incidence ($P>0.41$) was observed for fruit incubated in suspensions at ≥19°C. The results showed a strong positive relationship between water temperature and cucumber fruit infection incidence when analyzed using a second order polynomial regression ($y=-0.2x^2+7.6x-9.8$, $R^2=0.96$). When a suspension containing 1×10^3, 2×10^4, or 4×10^4 zoospores/ml was used, temperature did not significantly affect infection incidence. However, temperature did significantly affect infection incidence for all other zoospore concentrations tested. When a suspension containing 1×10^2 zoospores/ml was used, fewer fruit became infected than at any other concentration tested ($P<0.0008$). When a suspension concentration of 1×10^3 zoospores/ml was tested, fewer fruit became infected than at higher densities ($P<0.0002$). Zoospore suspensions containing 5×10^3 or 1×10^4 zoospores/ml resulted in similar levels of infection ($P=0.1479$); suspensions containing $\geq 1 \times 10^4$ zoospores/ml also resulted in similar levels of infection ($P>0.28$). The results indicate a strong positive relationship between inoculum concentration and cucumber fruit infection when analyzed using logarithmic regression ($y=15.9\text{Ln}(x)-61.7$, $R^2=0.97$). Overall, suspensions with concentrations from 1×10^4 to 4×10^4 zoospores/ml caused the greatest amount of fruit infection at all temperatures tested (Figure 2.1). A significant

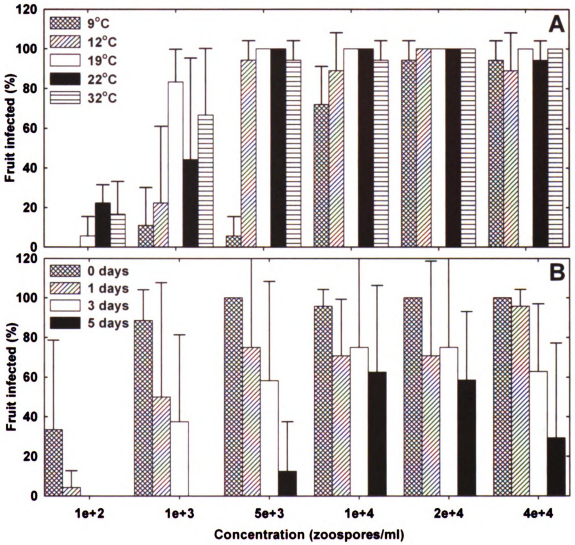

Figure 2.1. Effects of *Phytophthora capsici* zoospore inoculum concentration and **A,** temperature (data from 2°C not shown as no infection was observed), and **B,** zoospore age on infection of cucumbers. Each column represents the average of three (A) or four (B) repeated tests with six replicate pickling cucumbers per treatment per test. Error bars represent standard error.

interaction between temperature and inoculum concentration was noted.

A strong negative relationship between zoospore suspension age and fruit infection incidence was noted when linear regression analysis was applied ($y=-11.0x+82.0$, $R^2=0.96$). More infection occurred when zoospore suspensions were used immediately after creation than when 1, 3, or 5-day-old suspensions were used ($P<0.0016$). The amount of infection was similar for 1 and 3-day-old suspensions ($P=0.3277$), but not for 1 and 5-day-old suspensions ($P=0.0005$) or for 3 and 5-day-old suspensions ($P=0.0244$). Suspensions containing 1×10^2 zoospores/ml caused significantly less infection than any of the other densities tested ($P<0.0094$). Suspensions containing 1×10^3 zoospores/ml caused significantly less infection than suspensions containing 1×10^4 to 4×10^4 zoospores/ml ($P<0.0279$) and similar amounts of infection to suspensions containing 5×10^3 zoospores/ml ($P=0.0575$). Suspensions containing $\geq 5 \times 10^3$ zoospores/ml caused statistically similar amounts of infection ($P>0.47$, Figure 2.1B). There was no significant interaction between zoospore suspension age and concentration. All of the isolates recovered from the inoculated cucumbers at the end of experiments were found to have an A1 mating type and were sensitive to mefenoxam (*data not shown*).

All of the algaecides tested caused zoospore swimming to cease within three min of treatment (*data not shown*). The corresponding control zoospores were motile for 4.5 to 6 h. Algaecide treatments caused significant mortality of zoospores in water (Figure 2.2). A contact time of ≥30 min resulted in greater zoospore mortality than a contact time

of 5 or 10 min ($P<0.0039$). Overall, statistically similar zoospore mortality was observed after treatment for 30, 60, or 120 min ($P>0.69$). Several of the copper-based and one of the SPC-based algaecides showed efficacy similar to bleach, the positive control, in killing zoospores when the contact time was ≥30 min ($P>0.10$, Figure 2.2), and all of treatments were better than the negative control ($P<0.0001$). There was no significant interaction between the algaecide treatment and contact time. The water was more alkaline following treatment with most (8 of 11) of the algaecides tested and bleach (Table 2.1).

DISCUSSION

It is important that use of infested irrigation water is avoided to prevent the introduction of *P. capsici* to uninfested agricultural land, or that effective treatments are found so that water sources may be disinfested and safely used to irrigate susceptible crops. This study found that the incidence of cucumber fruit infection was greater with increasing temperature and inoculum concentration and lower with increasing age of the zoospore suspension. Upon treatment with copper or SCP-based algaecides at rates allowable for Michigan surface waters, zoospore motility was inhibited and significant zoospore mortality was noted.

Zoospores in water at 9 to 32°C were able to infect pickling cucumber fruits, yet no infection occurred at 2°C. Since zoospores in water at 9°C were able to infect fruits, hydrocooling water, which is usually around 10°C (14), may be a source of postharvest *P. capsici* inoculum, but this has not been investigated to date. Hydrocooling water is often recycled, which may lead to a buildup of pathogens in the water (51), but chlorine

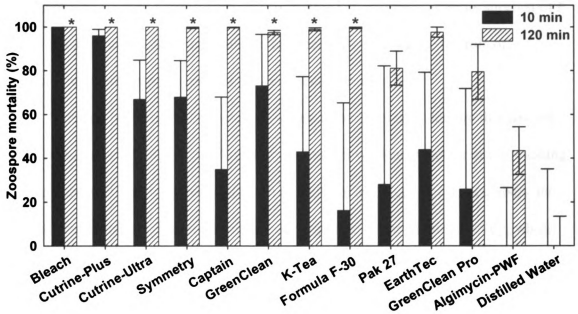

Figure 2.2. Effects of algaecide treatment and contact time on *Phytophthora capsici* zoospore mortality (%). Each column represents the average of two repeated tests with three replicate culture plates per test. Error bars represent standard error. Columns with an asterisk are not significantly different from the positive control, bleach, at ≥30 min contact time (LSD, $P>0.10$).

treatment may be used to limit pathogen spread (17). Chlorine also limits survival of *P. capsici* zoospores (24), and future studies are necessary to elucidate the role of hydrocooling water in postharvest infection of cucumbers.

Washing pits are another potential source of postharvest inoculum that has not been characterized to date, and future studies should investigate whether or not washing pits are important for postharvest infection of fruit. In Michigan, most cucumbers are harvested mechanically into semi trucks for transport to the processing facility, where they are dropped into a washing pit. Washing pits are usually filled with cold well water in the morning and the water warms throughout the day to ambient temperatures (Hausbeck, *personal communication*). Since both water temperature and possibly the concentration of inoculum in the water increases throughout the day as multiple loads of cucumbers are washed in the same pit, these washing pits could potentially be responsible for postharvest infection of washed fruits. The results of our study show that increased temperature and inoculum densities favor zoospore infection of fruits. Furthermore, during harvest and handling, cucumber fruits may be dropped distances of >3 m, which constitutes a major source of mechanical injury for the fruit (32). While mature cucumber fruits are less susceptible to infection by *P. capsici* (18), wounding has been shown to negate age-related resistance in pepper (7), and wounded cucumbers may be more susceptible to infection.

A greater percentage of fruits were infected following incubation in zoospore suspensions held at temperatures ≥19°C in our study; this was especially apparent when water contained a lower concentration of zoospores. A significant interaction was found between temperature and inoculum concentration in our research. A positive correlation

between water temperatures and infection incidence was observed in our study, which agrees with previous studies on *P. capsici* (19) and *P. cinnamomi* (37). Biles et al. (6) found that 27°C was optimal for zoospore infection of pepper fruits, and fruits were not infected at 15 or 35°C in laboratory studies, which is a more limited range than was found in our study. Biles et al. used a different isolate and host from that used in our study, which may explain some of the variation observed. A large volume of inoculum was used for our experiment, which may have increased the potential for infection (6). Previous research with *P. infestans* showed that zoospores are more susceptible to unfavorable environmental conditions than sporangia, and zoospore survival is negatively affected by cool temperatures (38). Gevens et al. (19) did not detect *P. capsici* in surface water irrigation sources via baiting (infection of pear and cucumber fruits) when the water temperature was <14°C or >25°C. It is likely that the concentration of zoospores in surface water sources such as ponds or rivers is lower during much of the growing season than the densities used in laboratory studies, and zoospores may be absent from irrigation surface water sources for part of the growing season. In the field, additional factors may influence zoospore motility and infectivity such as solar radiation, pH, ion, amino acid, sugar, and salt concentrations (9,16,31) and parasitism. Survival of *P. infestans* zoospores was strongly affected by ultraviolet radiation in previous studies (31,38). While solar ultraviolet radiation declines exponentially with water depth (8), motile *Phytophthora* zoospores display negative geotropism placing them toward the surface of a water body (9).

Our study found zoospore inoculum concentration significantly affected infection incidence of pickling cucumbers. There appeared to be a threshold at 1×10^4

zoospores/ml; increasing the concentration beyond this did not result in a greater amount of infection at most of the temperatures tested. This is similar to previous research in pepper that showed that a suspension containing $\geq 1 \times 10^4$ *P. capsci* zoospores/ml resulted in 100% infection of susceptible plants at normal greenhouse temperatures (40). In agreement with a previous study using *P. nicotianae* (4), significantly greater levels of disease were observed with increasing inoculum concentration up to 1×10^4 zoospores/ml. However, it is unlikely that 1×10^4 zoospores/ml would be present in irrigation water, so it significant to note that at temperatures ≥19°C, zoospore suspensions containing 1×10^2 zoospores/ml, the lowest concentration tested, resulted in infection of cucumbers.

Mean survival of zoospores of several *Phytophthora* spp. (not including *P. capsici*) in sterile distilled water or filtered lake water declined to <20% in 48 h in a previous study (10). Shokes and McCarter found that zoospores of *P. aphanidermatum* died rapidly, with only 4.2% surviving at day 6 and 0% at day 12 (44). In this study, it was found that the infection incidence of cucumber fruits was significantly reduced when older zoospore suspensions were used. When 5-day-old suspensions with $\leq 1 \times 10^3$ zoospores/ml were used, no fruit became infected, and few fruit were infected for 5-day-old suspensions containing 5×10^3 zoospores/ml. Since it is thought that *P. capsici*-infested water may initiate an epidemic in a field without a previous history of infestation when planted to a susceptible host (19), it is significant that zoospores can survive and initiate infection for 5 days. Furthermore, complete resistance to *P. capsici* has not yet

been observed for any cucumber cultivar, although reduced pathogen sporulation has been observed on some varieties (18). Roberts et al found zoospores were able to survive for 45 days in tailwater in Florida (42). Neither our study nor the Florida study investigated whether encysted zoospores were the surviving propagules or if the zoospores had germinated to produce mycelial fragments that survived.

Our research suggests that algaecides may be a viable disinfestant for *P. capsici*-contaminated irrigation water. All algaecides tested caused a significant reduction in zoospore motility and mortality. Previous work has shown zoospores are able to remain motile longer at higher cell densities (20). All zoospores ceased to swim after ≤3 minutes of exposure to algaecide in our experiments, despite the high zoospore concentration used. In addition to greatly reduced motility, significant mortality of zoospores was observed after algaecide treatment in our study. Copper-based algaecides also caused significant mortality of *P. ramorum* zoospores in another recent study (11).

Treatment of the water with algaecides modified the pH of the water, usually resulting in an increase in alkalinity. Recently, Kong et al. (25) found that survival of zoospores of various *Phytophthora* spp. was affected by the pH of the solution in which they had been incubated. The optimal pH for zoospore survival varied by species from pH 5 to 9, and survival was generally poor at pH (3 or 11) extremes. Furthermore, *P. palmivora* zoospores in solutions with pH 6 or 8 encysted twice as quickly when compared to zoospores kept in solutions with pH 7 (33). While *P. capsici* was not included in either of these studies, it is likely that zoospores of *P. capsici* in water are affected by pH in a manner similar to other *Phytophthora* spp. Commercial algaecides used to treat algae in irrigation water sources may also be useful to reduce *P. capsici*

propagules, but more research is needed before recommendations can be made. Since algaecides are already used to prevent algae growth and clogging of irrigation equipment (36), this treatment option would not result in additional management costs for many growers.

Further work is necessary to investigate the efficacy of algaecides in field and greenhouse settings and to explore potential problems with using algaecide-treated water for irrigation, such as phytotoxicity in plants and effects on nontarget species such as fish. The effectiveness of copper-based algaecides is affected by the concentration and form of copper applied, the duration of exposure, and characteristics of the water body (34). A copper half-life of 2.6 to 5.7 days was observed in a previous study when algaecides were applied to barrels containing water and sediment with different physical characteristics (34), and elevated copper concentrations were observed for several weeks following algaecide treatment of a reservoir (15). The longest half-life was observed when low hardness, low alkalinity water was used in the barrel experiments (34). The persistence and concentration of copper in water and sediments significantly affects the impact on nontarget species including phytoplankton and fish (30), and this should be considered if algaecides are applied to a water source to reduce the number of *P. capsici* zoospores. Excess copper in irrigation water may lead to phytotoxicity including reduced plant growth and photosynthetic ability (1). Sodium carbonate peroxyhydrate has been less well studied than copper, but it is known that efficacy is affected by the soluble organic matter content of the water body (29). Hydrogen peroxide may be phytotoxic, especially to young plants, when used at rates required to control algae (12). Future studies should

focus on investigating the efficacy and potential phytotoxic effects of algaecides in field, greenhouse, and processing (postharvest treatment) settings.

In summary, zoospores are able to infect cucumber fruits at a wide range of ecologically relevant water temperatures, which suggests that avoidance of *P. capsici*-infested water is necessary throughout the entire growing season. The emergence of new treatment options such as algaecides may provide easy, economically acceptable options for disinfestation of surface water irrigation sources in the future, but further research is warranted to investigate the efficacy of these products in the field setting.

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CHAPTER III: EFFECTS OF TEMPERATURE, HUMIDITY, AND WOUNDING ON DEVELOPMENT OF PHYTOPHTHORA ROT OF CUCUMBER FRUIT.

ABSTRACT

The effects of temperature (10, 15, 20, 25, 30, and 35°C) and relative humidity (~35, 60, 70, 80, and 100%) on development of Phytophthora fruit rot of pickling cucumber were investigated in controlled growth chamber studies. The effect of wounding on disease development was characterized for small (2.0 to 2.5 x 8 to 9 cm), medium (3.0 to 4.0 x 12.0 to 13.0 cm), and large (>4.5 cm x >14 cm) pickling cucumbers. Lesions developed on cucumbers incubated at all temperatures tested, except 10 and 35°C. Disease severity was greatest on cucumbers incubated at 25°C at 4 days post inoculation (dpi). Lesions formed on cucumbers incubated at all relative humidities tested. The diameter of water-soaking and pathogen growth was greater under conditions of higher relative humidity. Wounding lessened size-related resistance in pickling cucumber. The diameter of water-soaking was similar for all wounded cucumbers at 4 dpi regardless of fruit size. While sporangia were formed on wounded large fruits, more sporangial production was observed in lesions on small and medium fruits. These results indicate *Phytophthora capsici* is capable of infecting cucumbers under a wide range of temperature and relative humidity conditions.

INTRODUCTION

The oomycete plant pathogen *Phytophthora capsici* Leonian causes fruit rot, foliar blight, and crown and root rot of many vegetable crops in the Cucurbitaceae and Solanaceae (9), as well as snap and lima bean (*Phaseolus vulgaris* L., *Phaseolus lunatus* L.) (5,11) and Fraser fir (*Abies fraseri* (Pursh) Poir.) (18). Fruit rot of cucumber (*Cucumis sativus* L.) is common when weather is favorable for disease development and *P. capsici* is present (15). Michigan is the largest producer of pickling cucumbers in the United States (2), and many of the major growing areas of the state are affected by *P. capsici*. Entire fields of mature cucumber fruits and semi truck loads of harvested cucumbers may be lost due to fruit rot (15).

Climatic conditions influence development of diseases caused by *Phytophthora* spp. (8). The optimal growth temperature for *P. capsici* in culture media ranges from 24 to 33°C (17). Reports of minimum and maximum growth temperatures vary, but range from 6 to12°C and 32 to >35°C, respectively (9,20,21). Temperature effects on infection of detached pepper leaves by *P. capsici* were previously described; a greater percentage of leaves were infected following incubation at 23°C as compared to 15 or 31°C when the sporangial suspension was removed from the leaves 4 hours post inoculation (19). If the suspension was left on the leaves for 8 or 24 hours post inoculation, disease incidence was similar for leaves incubated at 23 or 31°C (19). Disease incidence and lesion length were greatest when inoculated pepper fruits were incubated at 27°C in a previous study (4).

A number of factors including temperature (7), nutrition (23), light (14), availability of sterols (16), and relative humidity (15,19) are known to affect *P. capsici*

sporangial production. Sporangial production is optimal at 24 to 27°C (7). Lamour and Hausbeck (15) suggested that sporangial production on slicing cucumber fruits was greater at 60 and 80% relative humidity than at >90%. Sporangial production following incubation at 25°C was greater for pepper stem pieces that were incubated at 100 or 97.5% relative humidity than at 92.5 or 87% relative humidity (19).

Previous work has shown that fruit age and size are important factors for symptom severity and sporangial production on cucumbers (12) and peppers (3). Gevens et al. (12) found that oversized pickling cucumbers with a diameter >4.5 cm and length >14 cm were more often symptom-free following inoculation than smaller fruits. Pickling cucumbers with a diameter ≥3.5 cm had much lower disease ratings than smaller fruits in the same study. Size-related resistance was also observed for a slicing cucumber variety, with more water-soaking and sporulation being observed on the smallest fruits tested (12). A similar phenomenon has been observed for pepper fruits, where unwounded young pepper fruits became more severely diseased than mature pepper fruits (3). However, when the peppers were wounded, lesion expansion was similar for all fruits, regardless of age (3). It is currently unknown whether or not wounding is able to negate age-related resistance in cucumber.

The objective of our study was to elucidate the effects of temperature, relative humidity, and wounding on development of Phytophthora rot of cucumber and on pathogen growth and sporulation on infected cucumber fruits.

MATERIALS AND METHODS

Generation of host and pathogen material. Cucumber fruits ('Vlaspik') were grown at the Michigan State University (MSU) Plant Pathology Farm, East Lansing, MI,

a site without a history of *P. capsici*. Cucumber plants were treated weekly with either propamocarb hydrochloride (Previcur Flex, Bayer CropScience, NC) or chlorothalonil (Bravo Weather Stik, Syngenta Crop Protection, CA) to control downy and powdery mildews and were fertilized and watered according to standard production practices. For the experiments investigating the effects of temperature and relative humidity on disease development, fruits were hand-harvested when 2 to 3 cm in diameter, washed in a 2% bleach (0.12% NaClO) solution, rinsed, surface disinfested with a 20% bleach (1.24% NaClO) solution for 5 min, rinsed, air dried, and placed into the incubation chambers prior to inoculation. Incubation chambers consisted of clear polystyrene boxes with lids (23 x 10 x 32 cm, Potomac Display, Hampstead, MD).

For the wounding experiments, cucumbers were hand-harvested at three sizes: small (2.0 to 2.5 cm diameter x 8 to 9 cm long), medium (3 to 4 cm diameter x 12 to 13 cm long), and large (>4.5 cm diameter x >14 cm long). Fruit size was chosen based on the work of Gevens et al. (12) to represent different levels of age/size-related resistance to *P. capsici*. Cucumbers were washed and surface disinfested as described above, and placed into moist chambers prior to treatment and inoculation. Moist chambers consisted of aluminum trays containing wet paper towels, which were sealed using plastic film and tape following inoculation.

Actively growing cultures of *P. capsici* isolates SP98 and OP97 were used (isolate notation refers to the culture collection maintained in the laboratory of M. K. Hausbeck at MSU). The cultures were derived from long-term stock cultures and passed through cucumber fruits according to the methods of Quesada-Ocampo et al. (18) before

use. Isolates were maintained and zoospore suspensions created as described previously (13).

Effects of temperature on disease development. Two incubation chambers were placed into a growth chamber set at 10, 15, 20, 25, 30, or 35°C (16/8 day/night photoperiod and ~95 mE of light intensity). Air from a TopFin XP-125 aquarium pump (PetSmart, Phoenix, AZ) was humidified by pumping it through an aquarium airstone submerged in 1.5 liters of distilled water in a 2-liter Erlenmeyer filter flask. Air exiting the flask was routed to the incubation chambers using clear tygon tubing. A small piece of wet paper towel was added to each box to ensure high relative humidity (~100%) was maintained. Air exited through a 6.4-mm hole that was drilled into the opposite side of the chamber lid.

For each temperature treatment, 24 fruits were used (12 per incubation chamber). Each fruit was inoculated with a single 10-µl droplet of zoospore suspension (isolate SP98). A single 10-µl droplet of water was placed on each of six control cucumbers that were incubated at room temperature (~21 ± 2°C) and high relative humidity to ensure that the cucumber fruits used in the experiment were not infected with *P. capsici* at the onset of the experiment. Six fruits were chosen arbitrarily and removed from each temperature treatment at 1, 2, 3, and 4 days post inoculation (dpi) for evaluation.

Each fruit was evaluated for water-soaked lesion diameter (visible dark discoloration of the fruit surface) and the diameter of pathogen growth in the lesion by taking 2 perpendicular measurements of each lesion for each parameter. The number of sporangia in each lesion was determined as follows. If sporangia could be observed in a lesion via stereomicroscopy (Leica M165C, Wetzlar, Germany), the area of pathogen

growth was carefully excised with a razor blade and placed into 1 ml of water in a 2.2-ml microcentrifuge tube. The tube was vortexed for 70 s, plant material removed using forceps, and number of sporangia in the tube estimated using a hemacytometer. If sporangia could be observed via stereomicroscopy, but could not be detected via the method above, the sporangial suspension was concentrated by centrifuging the tube for 5 min at 14,000 rpm. The supernatant was removed and the sporangia were re-suspended in 100-µl of distilled water and numbers of sporangia estimated using a hemacytometer.

When lesions were apparent on fruits but no sporangia observed, tissue sections from the margin of each lesion were plated onto UCV8 (160 ml unclarified V8 juice, 840 ml distilled water, 30 mM $CaCO_3$, and 1.5% agar) plates amended with 25 ppm of benomyl, 100 ppm of ampicillin, 30 ppm of rifampicin, and 100 ppm of pentachloronitrobenzene (BARP) to confirm infection with *P. capsici*. When fruits appeared healthy, three tissue sections were excised from under the point of inoculation and plated onto BARP-amended UCV8. Plates were incubated at room temperature under constant fluorescent lighting for 3 to 5 days and observed using a compound microscope. *P. capsici* was confirmed using morphological characteristics according to the *Phytophthora* spp. key by Waterhouse (52). The resulting *P. capsici* colonies were transferred to fresh UCV8, and isolates were characterized for mating type and mefenoxam sensitivity (26) to confirm they had the same phenotype as the inoculum. Two independent experiments were conducted.

The temperature and relative humidity in each incubation chamber was monitored for the duration of each experiment using WatchDog model 450 data loggers (Spectrum Technologies, Plainfield, IL). Data loggers were set to record temperature (°C) and

relative humidity (%) at 10 min intervals. The accuracy ratings of the data loggers were ±0.6°C and ±3% relative humidity. Data were downloaded into a computer at the end of each experiment. Since lighting was not continuous, slight photoperiodic fluctuations in temperature and relative humidity were observed.

Effects of relative humidity on disease development. Six incubation chambers were placed into a growth chamber maintained at 25°C with fluorescent lighting (16/8 day/night photoperiod and 95 mE of light intensity). Glycerol/water solutions (60, 70, 80, and 100% relative humidities) and a saturated salt solution (potassium acetate, 35% relative humidity) were prepared as described in Forney and Brandl (10) and Winston and Bates (22). One liter of solution was added to each incubation chamber to control the relative humidity of that chamber (~35, 60, 70, 80, or 100%). Eight 2.5 cm diameter x 3 cm tall pieces of PVC pipe (stilts) were placed into the solution in the bottom of each incubation chamber. A 29 cm x 20 cm screen made of two layers of hardware mesh (0.7 cm squares) was suspended above the glycerol or salt solution by the stilts. A piece of clear tygon tubing was inserted into a 6.4-mm hole that had been drilled into the lid of the incubation chamber and connected to a TopFin XP-125 aquarium pump to circulate the air within the chamber. The temperature and relative humidity (Table 3.1) in each chamber were monitored as described above. For each relative humidity treatment, twelve fruits were inoculated with a single 10-µl droplet of zoospore suspension containing ~2.5 x 10^4 zoospores. Six fruits were chosen arbitrarily and removed from each incubation chamber at 3 and 4 dpi, and evaluated for disease as described above. Six control cucumbers were treated as described above. Three independent experiments were conducted for each isolate (SP98 and OP97).

Table 3.1. Average, maximum, and minimum relative humidities observed in incubation chambers over the course of this study.

Expected RH[a]	Actual RH					
	Isolate SP98			Isolate OP97		
	Mean ± SE[b]	Max.[c]	Min.[d]	Mean ± SE	Max.	Min.
35	32.0±0.2	33.0	20.7	35.7±0.2	47.5	20.7
60	59.3±0.1	69.5	48.9	61.4±0.2	74.3	48.6
70	67.3±0.1	76.2	60.5	67.6±0.1	72.5	56.6
80	81.1±0.1	86.6	72.2	79.1±0.1	85.7	71.6
100	99.1±0.0	100.0	86.6	97.5±0.1	100.0	89.8

[a]RH=relative humidity (%)

[b]Mean ± SE=mean relative humidity ± standard error

[c]Max.=maximum relative humidity

[d]Min.=minimum relative humidity

Effect of wounding on size-related resistance. For each cucumber size chosen, 20 surface disinfested fruits remained unwounded, and a shallow wound (~1-2 mm deep) was created in the center of 20 fruits using a sterilized 1 mm dissecting needle. All of the fruits were inoculated with a single 10-µl droplet of water containing ~2.5 x 10^4 zoospores in the center of the fruit, over the wound, if present. Ten unwounded and 10 wounded cucumbers were inoculated with isolate SP98 and the remaining cucumbers were inoculated with OP97. Inoculated fruits were incubated for 4 days at room temperature in moist chambers under constant fluorescent lighting and evaluated as described above. Six control cucumbers were treated as described above. The experiment was conducted three times.

Statistical analyses. All statistical analyses were performed using the SAS statistical package version 9.1 (SAS Institute, Inc., Cary, NC). Data from 10 and 35°C were removed prior to analysis, as no symptoms or signs of the disease were observed on fruits incubated at these temperatures. Averages were calculated for the two perpendicular measurements of lesion and pathogen growth diameters prior to statistical analysis. A split plot design was used for the temperature studies with temperature as the mainplot effect and time as the subplot effect. Isolate was the mainplot effect for the relative humidity studies and relative humidity was the subplot effect. For the wounding studies, treatment (fruit size x isolate) was the mainplot effect and wounding was the subplot effect. Using the Proc Mixed procedure of SAS, data were subjected to split-plot analysis of variance (ANOVA, $P \leq 0.05$), and Fisher's protected LSD was used for separation of means ($P \leq 0.05$) when effects were significant. Prior to analyses, the number of sporangia produced in each lesion was log transformed for all experiments,

and the water-soaked lesion diameter was log transformed for the wounding experiments to fulfill the assumption of normally distributed residuals.

The Proc LOESS procedure of SAS was used to perform *loess* nonparametric regression analyses to model the relationship between temperature, incubation time, and each of the response variables (water-soaked lesion diameter, pathogen growth diameter, and sporangial production). The correlation between predicted and actual measures of disease severity was used to measure goodness-of-fit of the *loess* nonparameteric regression model.

RESULTS

No symptoms or signs of disease were observed on cucumber fruits incubated at 10 or 35°C, and the pathogen was never recovered from fruits incubated at 35°C. *P. capsici* was recovered from a small (<10%) number of cucumber fruits incubated at 10°C (data not shown). Germination of at least some of the zoospores placed on these fruits could be observed via stereomicroscopy. At 1 dpi, symptoms were not apparent on any of the fruits, regardless of incubation temperature (Figure 3.1A), but *P. capsici* was isolated from fruits, suggesting that infection had already occurred (data not shown). Water-soaked lesions were apparent on fruits incubated at 20 to 30°C at 2 dpi (Figure 3.1B), but pathogen growth and sporulation were not visible. At 3 dpi, pathogen growth and sporulation in lesions could be observed and measured (Figure 3.1C and D). Up to 2.7×10^6 sporangia were quantified from a single lesion at 4 dpi.

The interaction between temperature and time was significant for water-soaked lesion diameter, pathogen growth diameter, and sporangial production in the lesion

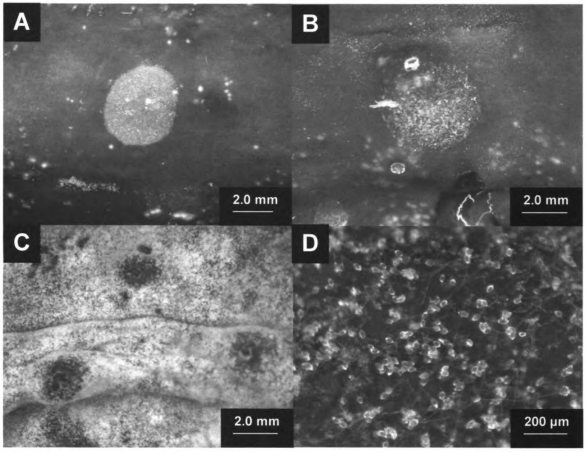
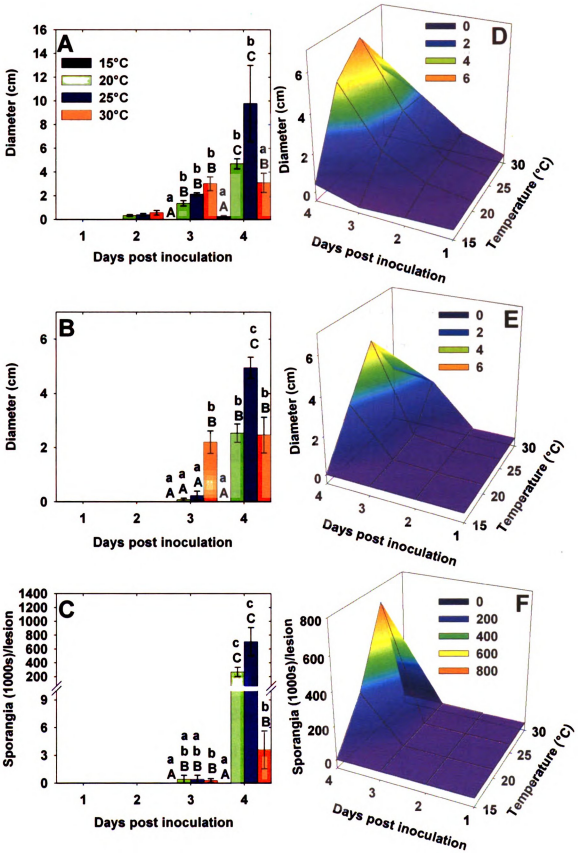


Figure 3.1. A, No symptoms of disease were observed on fruits incubated at any temperature (25°C shown) at 1 dpi (PI=point of inoculation). **B**, Water-soaking (WS) was apparent on fruits incubated at 20 to 30°C at 2 dpi (30°C shown), but pathogen growth outside of the point of inoculation (PI) was not observed. Significant pathogen growth and sporulation was apparent in water-soaked lesions on fruits incubated at 20 to 30°C at 3 **(C & D)** and 4 dpi.

(P<0.0001). Slicing of the interaction showed differences in water-soaked lesion diameter and pathogen growth diameter for the different incubation temperatures for fruits incubated 3 or 4 dpi (P<0.0054), but not on fruit incubated for 1 or 2 dpi (P>0.22). Differences in sporangial production in lesions were only observed at 4 dpi (P<0.0001). The water-soaked lesion diameter was greatest for fruits incubated 4 dpi at 20 or 25°C. At 3 dpi, the water-soaked lesion diameter was similar for fruits incubated at 20, 25, or 30°C (Figure 3.2A). The pathogen growth diameter and sporangial production were greatest for fruits incubated at 25°C at 4 dpi (Figure 3.2B, C). At 3 dpi, the diameter of pathogen growth was greatest for fruits incubated at 30°C, and sporangial production was similar for fruits incubated at 20, 25, or 30°C. The results of *loess* regression suggested that 25°C was the most favorable temperature for disease development at 4 dpi (Figure 3.2E, F, G), in agreement with the results from ANOVA analyses. The main effect of time was significant (P<0.0001) for all of the response variables, but temperature was not (P>0.07). Water-soaked lesion diameter, pathogen growth diameter, and sporangial production were greatest at 4 dpi (Figure 3.2D).

No significant interaction was noted between relative humidity, isolate, and time (P>0.5731), but there was a significant interaction between relative humidity and time for all three measures of disease severity (P<0.0437). The diameter of water-soaking and pathogen growth increased as the relative humidity increased at both 3 and 4 dpi (Figure 3.3A, B). Sporangial production in the lesions was greater for cucumbers incubated at ≥60% (Figure 3.3) than for fruits incubated at 35% relative humidity at 3 or 4 dpi (Figure 3.3C). Sporangia were not produced (Figure 3.3C, E, F), but lesions did form (Figure

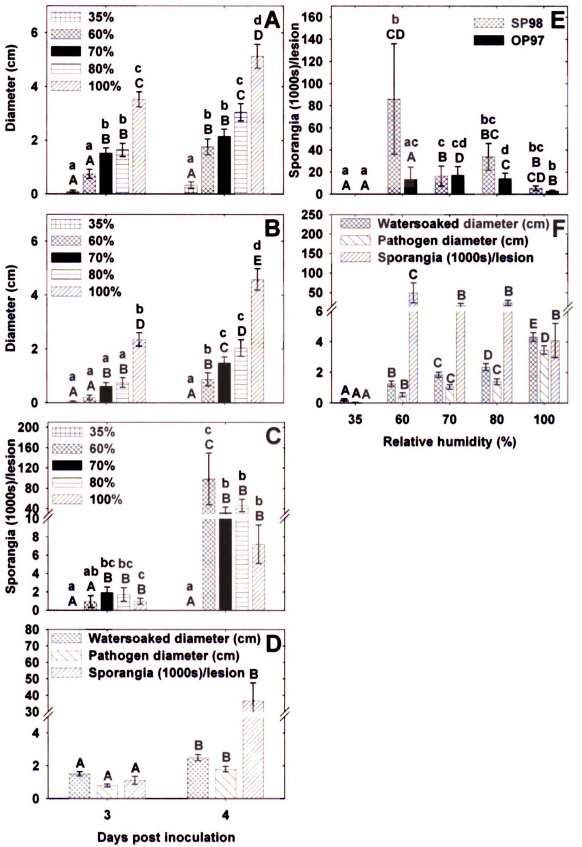
Figure 3.2. **A,** Mean water-soaked lesion diameter **B**, pathogen growth diameter and **C,** number of sporangia produced on pickling cucumbers after 1, 2, 3, or 4 days incubation at 15, 20, 25, or 30°C following inoculation with *Phytophthora capsici* zoospores. Note differences in y axes and break in the y axis from 10-100 in **C**. Error bars represent standard error. Bars that share letters are not significantly different within a time point (days post inoculation, dpi, lower case letters) or between time points (capital letters). Response surface of the **D**, water-soaked lesion diameter, **E**, pathogen growth diameter and **F**, number of sporangia per lesion as a function of temperature and time (dpi). The plots were generated using predicted values from fitted *loess* nonparametric regression models. Models were fitted using 32 observations (two experiments x 16 treatment combinations).


A
15°C
20°C
25°C
30°C
Diameter (cm)
Days post inoculation
B
C
Sporangia (1000s)/lesion
D
0
2
4
6
Temperature (°C)
E
F
0
200
400
600
800

3.3A, F) when inoculated fruits were incubated at 35% relative humidity. The interaction between isolate and time was not significant ($P>0.2424$), nor was the main effect of isolate ($P>0.0813$). The main effect of time was significant ($P<0.0001$) and greater diameters of watersoaking and pathogen growth and sporangia production was observed on fruits incubated for 4 dpi (Figure 3D). The interaction between isolate and relative humidity was not significant for the water-soaked or pathogen growth diameter ($P>0.0750$), but it was significant for sporangial production in the lesion ($P=0.0059$). For isolate SP98, sporulation was significantly lower in lesions on fruits incubated at 35% relative humidity when compared to the other relative humidites tested. For OP97, sporulation was also lowest at 35%, but a statistically similar number of sporangia were produced in lesions on cucumbers incubated at 60% relative humidity (Figure 3.3E). When the main effect of relative humidity ($P<0.0001$) was examined, the water-soaked lesion diameter was greater as the relative humidity level increased (Figure 3.3F). Pathogen growth diameter followed a similar trend, except that the diameter for 70 and 80% relative humidites were statistically similar. Sporangial production was greatest at 60% and lowest at 35% relative humidity, with a statistically similar number of sporangia observed in lesions on cucumbers incubated at the other relative humidity values tested (Figure 3.3F).

The interaction between treatment (fruit size x isolate) and wounding (wounded or not) was significant for water-soaked lesion diameter ($P<0.0001$), pathogen growth diameter ($P<0.0001$), and sporangial production in the lesion ($P=0.0017$). Small and medium fruits inoculated with SP98 displayed the largest water-soaked lesion diameters among the unwounded fruits. The smallest water-soaked lesions were observed on

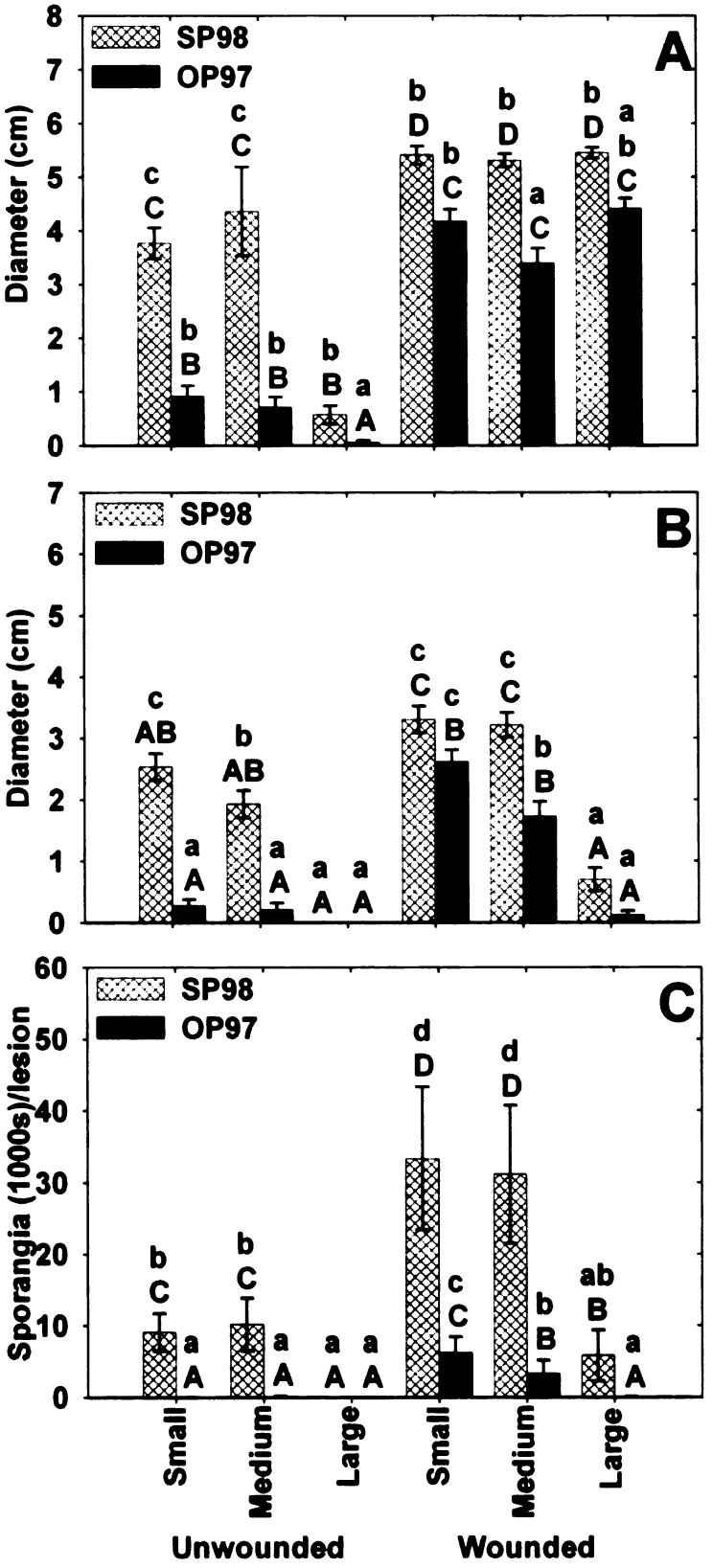
Figure 3.3. Mean **A,** water-soaked lesion diameter **B,** pathogen growth diameter and **C,** number of sporangia produced on pickling cucumber fruits after 3 or 4 days incubation at 35, 60, 70, 80, or 100% relative humidity following inoculation with *Phytophthora capsici* zoospores. **D**, Mean water-soaked lesion diameter, pathogen growth diameter, and number of sporangia produced on pickling cucumbers following 3 or 4 days incubation. Bars that share letters are not significantly different within a time point (days post inoculation, dpi, lower case letters) or between time points (capital letters). **E,** The number of sporangia produced on cucumbers incubated at 35, 60, 70, 80, or 100% relative humidity by isolate. **F**, Mean water-soaked lesion diameter, pathogen growth diameter, and number of sporangia produced on pickling cucumbers following incubation under different conditions of relative humidity. Bars that share letters are not significantly different for a specific isolate (lower case letters) or between conditions of relative humidity (capital letters). Error bars represent standard error.


A
35%
60%
70%
80%
100%
Diameter (cm)
B
C
Sporangia (1000s)/lesion
D
Watersoaked diameter (cm)
Pathogen diameter (cm)
Sporangia (1000s)/lesion
Days post inoculation
E
SP98
OP97
F
Relative humidity (%)

unwounded large fruits inoculated with isolate OP97 (Figure 3.4A). When fruits were wounded, similar water-soaked lesion diameters were observed for fruits of all sizes, and the diameter of water-soaking was greater than for unwounded fruits of the same size and isolate combination (Figure 3.4A). A similar relationship was also observed for pathogen growth in the lesion (Figure 3.4B) and sporulation (Figure 3.4C), with lesions on wounded fruits showing more pathogen growth and sporulation than on corresponding unwounded fruits. While pathogen growth was greater on wounded large fruits than on unwounded large fruits, the pathogen growth diameter was still greater on unwounded small and medium fruits (Figure 3.4B). Pathogen growth diameter and sporulation were greatest on wounded small or medium cucumbers inoculated with SP98 (Figure 3.4B &C). Sporangia were not produced in lesions on large fruits, except for large wounded fruits inoculated with SP98 (Figure 3.4C).

Overall, symptom expression was greater on fruits inoculated with SP98 than with OP97 (Figure 3.4A) and the main effect of treatment (size x isolate) was significant ($P<0.0012$). All of the wounded fruits inoculated with SP98 had a greater water-soaked lesion diameter than any of the unwounded fruits, regardless of isolate (Figure 3.4A). In addition, wounded fruits inoculated with OP97 had lesions of similar size to the largest lesions observed on the unwounded fruits (Figure 3.4A). Interestingly, the difference in virulence observed between isolates on unwounded fruits was less obvious when the fruits were wounded (Figure 3.4A). However, sporangial production was much greater with SP98 than with OP97, regardless of whether or not the fruit were wounded (Figure 3.4C). Wounded fruits showed more severe symptoms and signs of disease than unwounded fruits (Figure 3.4) and the effect of wounding was significant ($P<0.0001$).

Figure 3.4. Mean **A,** water-soaked lesion diameter, **B,** pathogen growth diameter and **C,** number of sporangia produced on pickling cucumber fruits of various sizes (small, medium, or large) that were wounded or not 4 days post inoculation (dpi) with a droplet of water containing *Phytophthora capsici* zoospores. Note differences in the y-axes. Means that share letters are not significantly different for an isolate (lower case letters) or between treatments (size*wounding, capital letters). Error bars represent standard error.


A
SP98
OP97
Diameter (cm)
B
SP98
OP97
Diameter (cm)
C
SP98
OP97
Sporangia (1000s)/lesion
Small
Medium
Large
Small
Medium
Large
Unwounded
Wounded

Hence, wounding lessens size-related resistance.

DISCUSSION

This study showed that *P. capsici* is capable of infecting cucumber fruits under a wide range of temperature and relative humidity conditions. In addition, wounding was found to lessen size-related resistance to *P. capsici* in pickling cucumber. Careful handling, storage, and transport of pickling cucumbers and temperatures <15°C are recommended based on the results of this study. The current recommended storage temperature of ~12°C (6) seems appropriate in light of these results.

Growth chamber studies showed that *P. capsici* is capable of infecting and causing disease on cucumber fruits incubated at 15 to 30°C. By 2 dpi, water-soaked lesions, the first symptom of Phytophthora fruit rot, were present in this study, as has been observed in previous studies in cucumber (15). A slightly longer lag has been observed in pepper with symptom expression at 3 to 6 dpi (4). A limited number of cucumbers were infected, but did not display symptoms when incubated at 10°C in this study. The minimum growth temperature for *P. capsici* in culture medium has been reported as 10°C (9), and it is not surprising that symptoms were not observed at this temperature. Pickling cucumbers were infected in a previous study when incubated in a large volume of zoospore suspension held at 9°C, removed from the suspension, and then incubated at room temperature (13). A previous study in pepper (4) showed that disease incidence was affected by the volume of *P. capsici* zoospore suspension used, even if the same number of zoospores were present in the water. Since the volume of inoculum in our studies was very small (10 µl), this may explain this discrepancy. In addition,

symptoms may have developed during incubation at room temperature. Cucumbers incubated at 35°C did not show symptoms of disease in this study, and the pathogen was not recovered from these fruits, even though *P. capsici* has a growth maximum of >35°C in culture medium (9,20,21). Both of the isolates used in this study grow readily on UCV8 when incubated at 35°C (data not shown).

Disease development was significant for cucumbers incubated at 20 to 30°C in our study and limited lesions formed at 15°C. Symptoms and signs of disease were most apparent on cucumbers incubated at 25°C for 4 dpi in our study, which was similar to what was previously reported as ideal for infection and lesion development on pepper fruits (27°C) (4) and leaves (23°C) (19) . The optimal growth temperature for *P. capsici* isolates in culture media ranges from 24 to 33°C depending on the isolate tested (17). In the pepper study fruits were not infected at 15 or 35°C when 5×10^3 zoospores were placed onto the fruits in a 100-ul droplet of water (4), which was a lower concentration than was used in our study (2.5×10^4 zoospores per 10-ul droplet of water). Sporangia did not form in lesions on cucumber fruits in this study following exposure to temperatures $\leq$ 15°C or $\geq$ 35°C, suggesting limited or no sporangial production at these temperatures. As would be expected, symptoms and signs of disease became more pronounced the longer the cucumbers were incubated.

Lesion expansion and pathogen growth were greater for cucumbers incubated at high relative humidity. When fruits were incubated at 35% relative humidity, no sporangia were detected in the lesions. However, *P. capsici* was able to produce thousands of sporangia in some of the lesions on fruits incubated at ≥60% relative humidity. While similar numbers of sporangia were produced at ≥60% at 3 dpi,

significantly more sporangia were produced at 60% relative humidity than at ≥70% at 4 dpi in our study. Lamour and Hausbeck (15) found that sporangial production was greater at 60% and 80% relative humidity than at >90% at 5 dpi on slicing cucumber fruits, in partial agreement with the current study. It is interesting to note that while 60% was optimal for sporangial production by isolate SP98, this relationship was not as clear for OP97. For OP97, sporulation was greater at 60 to 80% relative humidity and was reduced at 100% relative humidity, in agreement with the results from Lamour and Hausbeck (15) who also used isolate OP97 in a previous study.

In this study, all of the symptoms and signs of disease were greater on wounded fruits than on unwounded fruits. Wounding has been shown to increase *P. capsici* disease severity in Fraser fir (18), pepper plants (1), and pepper fruits (3) as well. In our study, small and medium unwounded cucumbers showed more severe symptoms and signs of disease than large fruits, as has been previously noted for pickling and slicing cucumber (12) and pepper (3) fruits. Gevens et al. (12) found that oversized pickling cucumbers had lower disease incidence, and no sporangia were detected in lesions on pickling cucumbers ≥14 cm long. We did not detect sporangia in lesions on unwounded large fruits at 4 dpi, but sporangia were formed in the large fruits ≥14 cm long that were wounded prior to inoculation. For isolate SP98, sporangial production on wounded large fruits was reduced compared to unwounded or wounded small or medium fruits. Sporangial production was generally poor on fruits inoculated with OP97 as compared to SP98, but wounding increased sporangial production.

When cucumbers were not wounded, small and medium fruits had larger lesion diameters than large fruits. However, wounding appeared to negate the difference in

lesion diameter; water-soaked lesion diameters were similar for wounded fruits inoculated with the same isolate regardless of fruit size. In pepper, wounding has been found to negate age-related resistance and lesion expansion was similar on wounded fruits, regardless of age (3). Interestingly, while differences in virulence by isolate were obvious for unwounded fruits, differences in virulence on wounded cucumbers were more subtle for water-soaked lesion diameter and pathogen growth diameter, suggesting that virulence may be at least partially affected by the penetration ability of the different pathogen isolates used. There were obvious differences between isolates for sporangial production on both unwounded and wounded fruits.

In summary, *P. capsici* is a dynamic pathogen that is capable of infecting and causing disease on pickling cucumbers under a wide range of temperature and relative humidity conditions. Because there is a lag time of days following inoculation before symptom expression and sporulation, storing and transporting potentially infected fruits under cool temperature (<15°C) is necessary to slow disease progression so that fruits will not rot in transit. Wounding has been shown to lessen the effects of age-related resistance; thus, careful handling of the fruits is key for cucumbers of all ages and sizes. Cucumber fruits may be dropped distances of >3 m during harvest and handling, which constitutes a major source of mechanical injury for the fruits (32). If injured fruits are exposed to inoculum after wounding, disease is more likely to occur.

ACKNOWLEDGMENTS

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