Effects of Temperature, Concentration, Age, and Algaecides on *Phytophthora capsici* Zoospore Infectivity

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ABSTRACT

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Controlled laboratory studies were undertaken to determine the effects of water temperature (2, 9, 12, 19, 22, and 32°C), inoculum 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 $\geq 19^\circ\text{C}$. Fewer fruit ($<40\%$ at $\geq 19^\circ\text{C}$, 0% at $\leq 12^\circ\text{C}$) became infected when water contained 1×10^2 zoospores/ml. Almost 100% of fruit were infected when water contained $\geq 5 \times 10^3$ zoospores/ml at temperatures $\geq 12^\circ\text{C}$. 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.

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, and germinates by forming a germ tube, and penetration and colonization of the host tissue follow (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 $\sim 1.5 \times 10^5$ zoospores (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 disinfecting 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 disinfectants 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 non-target organisms (30,34). Upon dissolution in water, SCP releases hydrogen peroxide (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.

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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 of unclarified V8 juice, 840 ml of distilled water, 30 mM CaCO₃, 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.

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 × 10², 1 × 10³, 5 × 10³, 1 × 10⁴, 2 × 10⁴, and 4 × 10⁴ 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 zoospore 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 margins 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.

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 × 10², 1 × 10³, 5 × 10³, 1 × 10⁴, 2 × 10⁴, and 4 × 10⁴ 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.

Effects of algaecides on zoospore motility and mortality. Commercially avail-

able 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 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).

For the zoospore motility experiments, 5 ml of stock solution or distilled water was mixed with 5 ml of distilled water containing 1 × 10⁶ 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 × 10⁴ 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 of clarified V8

Table 1. Algaecide treatments applied to water containing *Phytophthora capsici* isolate SP98 zoospores and the resulting pH of the water

Treatment (product/liter)	Active ingredient (% ^y)	pH ± SE ^z
Bleach (39 ml)	Sodium hypochlorite (6.2)	7.61 ± 0.01
Citrine-Plus (1.8 ml)	Copper ethanolamine (9)	8.55 ± 0.02
Citrine-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 μl)	Copper sulfate pentahydrate (2.7)	6.34 ± 0.16
Pak 27 (0.006 mg)	Sodium carbonate peroxyhydrate (85)	9.57 ± 0.00
EarthTec (1 μl)	Copper sulfate pentahydrate (5)	6.21 ± 0.03
GreenClean Pro (3 mg)	Sodium carbonate peroxyhydrate (85)	9.57 ± 0.00
Algimycin-PWF (3.3 ml)	Copper citrate and copper gluconate (5)	5.70 ± 0.02
Untreated	None	6.67 ± 0.01

^y Copper (%) is expressed as elemental copper equivalent.

^z SE = standard error.

juice, 840 ml of 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 (arcsine) 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 concentration on infection incidence, and algicide 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 in zoospore suspensions at 2°C regardless of inoculum concentration. Cucumber fruits became infected with *P. capsici* at all other temperatures tested (Fig. 1A). A statistically similar infection incidence ($P > 0.41$) was observed for fruit incubated in suspensions at $\geq 19^\circ\text{C}$. 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

Fig. 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 suspension 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.

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 indicated a strong positive relationship between inoculum concentration and cucumber fruit infection when analyzed using logarithmic regression ($y = 15.9\ln(x) - 61.7$, $R^2 = 0.97$). 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 (Fig. 1A). A significant interaction between temperature and inoculum concentration was noted.

A strong negative relationship between zoospore suspension age and fruit infection incidence was observed 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 ($P > 0.47$, Fig. 1B). There was no significant interaction between zoospore suspension age and concentration. All of the isolates recovered from inoculated cucumbers at the end of the experiments were found to have an A1 mating type and were sensitive to mefenoxam (*data not shown*).

All of the algacides tested caused zoospore swimming to cease within 3 min of treatment (*data not shown*). The corresponding control zoospores were motile for 4.5 to 6 h. Algicide treatments caused significant mortality of zoospores in water (Fig. 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 algacides showed efficacy similar to bleach, the positive control, in killing zoospores when the contact time was ≥ 30 min ($P > 0.10$, Fig. 2), and all treatments were better than the negative control ($P < 0.0001$). There was no significant interaction between the algicide treatment and contact time. The water was more alkaline following treatment with most (8 of 11) of the algacides tested and bleach (Table 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 algacides 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 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

Fig. 2. Effects of algicide 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, $P > 0.10$).

washing pit. Washing pits are usually filled with cold well water in the morning, and the water warms throughout the day to ambient temperatures (M. K. 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 cucumber (L. L. Granke and M. K. Hausbeck, *unpublished data*).

A greater percentage of fruits were infected following incubation in zoospore suspensions held at temperatures $\geq 19^{\circ}\text{C}$ 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 temperature 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 $<14^{\circ}\text{C}$ or $>25^{\circ}\text{C}$. 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. capsici* 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 is significant to note that at temperatures $\geq 19^{\circ}\text{C}$, 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 disinfectant 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 min 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).

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 algacides 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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