Control of Root Rot of Peppers Caused by *Phytophthora capsici* with a Nonionic Surfactant

M. E. Stanghellini, D. H. Kim, S. L. Rasmussen, and **P. A. Rorabaugh,** Department of Plant Pathology, University of Arizona, Tucson 85721

ABSTRACT

Stanghellini, M. E., Kim, D. H., Rasmussen, S. L., and Rorabaugh, P. A. 1996. Control of root rot of peppers caused by *Phytophthora capsici* with a nonionic surfactant. Plant Dis. 80:1113-1116.

Motile zoospores were identified as the sole infectious propagule of *Phytophthora capsici* responsible for spread of the pathogen in a recirculating rock wool cultural system. Amending the nutrient solution with a nonionic surfactant resulted in the elimination of zoospores and 100% control of the spread of the root pathogen from a point source. In the absence of the surfactant, all of the pepper plants within the cultural system, irrespective of plant age, died within 2 weeks following hypocotyl-inoculation of a single plant, which served as the source of secondary inoculum. The potential significance of surfactants for the control of polycyclic soilborne diseases attributed to *Phytophthora* spp. and other zoosporic pathogens is discussed.

Additional keywords: pathogen dispersal, polycyclic diseases, *Pythium aphanidermatum*

In general, soilborne pathogens are associated with monocyclic diseases. Notable exceptions, however, include root diseases caused by certain soilborne species of *Phytophthora,* which have been documented as polycyclic in nature. The latter include, among others, *Phytophthora parasitica* (Breda de Haan) Dastur, *P. cryptogea* Pethybr. & Lafferty, *P. cinnamomi* Rands, and *P. capsici* Leonian. The polycyclic nature of diseases caused by these species has been reported in open-field agriculture, as well as in greenhouse production of vegetable, ornamental, and transplant crops (3–9,11). Although the precise nature of the infectious propagule(s) responsible for spread of these pathogens is not known with certainty, zoospores have been implicated as the most likely candidate (2,3,7,11). In addition to their inherent self-dispersal mechanism (motility), passive and long-distance dispersal is facilitated by their transport in flowing surface water from rain or irrigation. The latter is particularly important in greenhouse industries that employ recirculation of the irrigation water, including hydroponic (8) and ebb-and-flow cultural systems (11). In the latter industry, preventive control measures include incorporation of highly effective chemical fungicides into the irrigation water. However, the continued use and reliance on fungicides could favor the buildup of these chemicals in the nutrient solution and increase the probability of the development of pesticide-insensitive strains of the target pathogen. These potential problems, in addition to the lack of registration of effective pesticides in the hydroponic vegetable industry, have stimulated research directed toward the discovery of alternative strategies for disease control.

Recently, the efficacy of nonionic surfactants in the control of root rot of cucumber caused by *Pythium aphanidermatum* (Edson) Fitzp. in a hydroponic cultural system was reported (9). Surfactants were demonstrated to rapidly lyse zoospores, which were identified as the sole infectious propagule responsible for spread of the fungus in the recirculating nutrient solution. The success of the latter study prompted an evaluation of the efficacy of surfactants for the control of other root diseases caused by zoosporic plant pathogens. These studies evaluated the efficacy of a nonionic surfactant in the control of root rot of peppers (*Capsicum annuum* L.) caused by *P. capsici*.

Corresponding author: Michael E. Stanghellini
E-mail: mstang@ag.arizona.edu

This research was supported by Research Grant Award No. IS-2115-92 from BARD-The United States-Israel Binational Agricultural Research and Development Fund.

Accepted for publication 20 June 1996.

Publication no. D-1996-0725-04R

MATERIALS AND METHODS

Pathogen and host. A virulent pepper isolate of *P. capsici* was employed throughout this study. A stock culture of the fungus was stored in sterile distilled water, and working cultures were reared on 10% V8 agar medium. Peppers plants, *C. annuum* cv. Joe Parker, were employed as the susceptible host.

Hydroponic cultural system. All experiments were conducted in a temperature-controlled greenhouse (24 to 32°C) containing 12 recirculating hydroponic units. Each hydroponic unit consisted of two rock wool slabs connected to a common reservoir containing 50 liters of a nutrient solution (Fig. 1). Unless otherwise specified, all cultural practices were the same as those employed in a previous investigation (9).

Pepper seeds were sown in Grodan Rockwool cubes (2.5 × 5 cm), and developing plants were fertilized daily for 40 days. Four 40-day-old pepper plants were then transplanted onto each rock wool slab (eight plants/hydroponic unit). Distance between plants on individual slabs was 20 cm.

There were two separate experiments, and each experiment ranged in duration

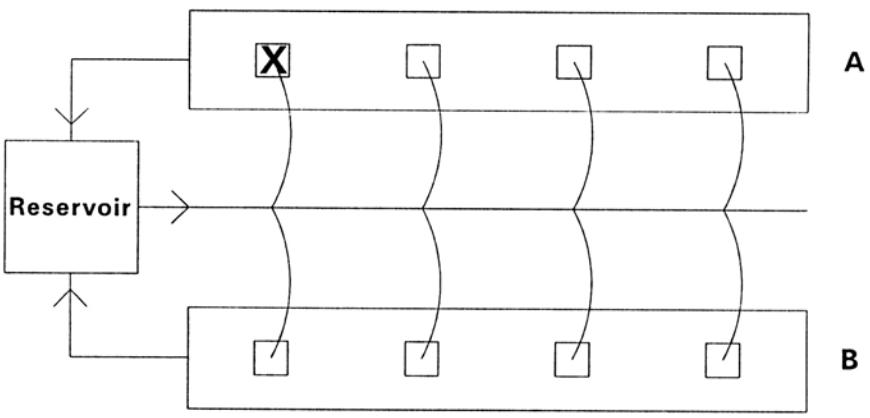

Fig. 1. Schematic of a recirculating hydroponic unit employed to evaluate the efficacy of a nonionic surfactant in the control of root rot of pepper caused by *Phytophthora capsici*. There were four plants on each side of the unit. One plant (X) was inoculated with the pathogen.

from 7 to 8 weeks after transplanting. After completion of each experiment, the entire hydroponic system in the greenhouse was dismantled, surface-sterilized in sodium hypochlorite (10%), and reassembled as previously described (9).

In each experiment, there were three treatments with one to two replications per treatment. Treatments included noninoculated units, inoculated units, and inoculated units in which the nutrient solution was amended with a nonionic surfactant. In addition, plants were inoculated either 1 week after transplanting or 4 to 5 weeks after transplanting. Variation in time of inoculation was conducted to evaluate the influence of plant age on the relative susceptibility of the host crop. The number of treatments and treatment replications (herein called trials) per experiment is summarized in Table 1.

Table 1. Summary of the number of treatments and replications in each of two separate experiments. Each experiment ranged from 49 to 56 days in duration after transplant

Treatments	Experiment 1	Experiment 2
Control	4[a]	4
Seedlings		
Inoc. with *Phytophthora*	2	2
Inoc. with *Phytophthora* + surfactant	1	2
Adult plants		
Inoc. with *Phytophthora*	1	2
Inoc. with *Phytophthora* + surfactant	2	2

[a] The number of treatment replications (trials) per experiment.

Inoculation was conducted as follows: a 5-mm-diameter disk, cut from the advancing margin of a 5-day-old culture of the pathogen, was placed in contact with the lower hypocotyl (immediately below the substrate surface) of one plant on one side (side A) of a hydroponic unit (Fig. 1). The agar disk was removed after 24 h. This method of inoculation permitted us to evaluate pathogen spread within a recirculating hydroponic system subsequent to pathogen colonization and reproduction on a single plant.

A nonionic surfactant (AquaGro 2000L, Aquatrols, Cherry Hills, NJ) was added to the nutrient solution in the reservoir of appropriate treatments 24 h prior to transplant. The final reservoir concentration was 20 µg a.i./ml. Visual foaming in the reservoir was regarded as evidence of the presence of the surfactant. The frequency of surfactant reapplication in individual hydroponic units varied from 14 to 30 days. The variation in the frequency of reapplication is not known with certainty but was probably related to the rapidity of surfactant biodegradation by the resident microflora in the recirculating nutrient solution of individual hydroponic units.

Preliminary studies showed that the surfactant at 20 µg a.i./ml was not phytotoxic (data not shown). Additionally, preliminary in vitro studies showed that motile zoospores of *P. capsici* ceased motility and lysed within 1 min upon exposure to the surfactant and that the surfactant had little or no effect on other life stages of the pathogen such as hyphae, encysted zoospores, or sporangia germinating directly by germ tubes (data not shown).

Monitoring pathogen presence in the recirculating nutrient solution and root infection. A baiting technique employing green tomato fruit (1) was used to monitor pathogen presence (which was most likely motile zoospores) in the nutrient solution as well as root infection.

Table 2. Percent mortality of seedling pepper plants after hypocotyl-inoculation of a single plant on one side (side A) of a two-sided recirculating hydroponic unit with *Phytophthora capsici*

Treatment	Side	Weeks after inoculation[a] 1	2	3	4	5	6	7
Control[b]	A	0	0	0	0	0	0	0
	B	0	0	0	0	0	0	0
Phytophthora[b]	A	62	100					
	B	43	100					
Phytophthora +[c] surfactant	A	25	25	25	25	25	25	25
	B	0	0	0	0	0	0	0

[a] Pepper seedlings were 47 and 48 days old at time of inoculation in experiments 1 and 2, respectively. There were four plants on side A and four plants on side B of each hydroponic unit. Each hydroponic unit of a treatment was considered a trial.

[b] Mean percent mortality from four trials: two trials in experiment 1 and two trials in experiment 2.

[c] Mean percent mortality from three trials: one trial in experiment 1 and two trials in experiment 2.

Fig. 2. Mortality of seedling pepper plants after hypocotyl-inoculation of a single plant (X) on one side of a two-sided recirculating hydroponic unit in the **(A)** absence or **(B)** presence of a nonionic surfactant in the nutrient solution.

The presence of the pathogen in the recirculating nutrient solution was detected as follows: 300 ml of nutrient solution was collected in a glass beaker from the common return line at 24-h intervals subsequent to inoculation of a single plant in appropriate hydroponic units. The samples were transported to the laboratory, and a green tomato fruit was placed in each beaker. The fruit floated approximately 90% submersed in the nutrient solution. After 24-h incubation, the fruit was removed from the beaker, placed in a humid chamber, and incubated at 24°C. Necrotic brown lesions on fruit were observed within 24 to 36 h. Lesions were excised and plated on water agar. Emerging hyphae were transferred to V8 agar. Cultures were subsequently identified by the morphological characteristics of the reproductive structures. Once the pathogen was detected in the nutrient solution, no further sampling was conducted.

Root infection was assessed as follows: sections of rock wool, measuring approximately 2 cm^2, were excised from rock wool slabs at weekly intervals over the cropping season. Ten root segments, 1 to 2 cm long, were extracted from each rock wool section. Five roots were rinsed in sterile distilled water and placed in a beaker containing 300 ml of sterile distilled water. A tomato fruit was added to each beaker and incubated as described above. The remaining five roots were observed microscopically for the presence of hyphae and sporangia.

Data analysis. Mortality data are presented per side A or B of a hydroponic unit. Lack of significant variation, as shown by Bartlett's test of homogeneity, permitted the combining of data from all replications of the same treatments from experiments 1 and 2.

RESULTS

Mortality of seedling pepper plants in the absence or presence of a surfactant. All hypocotyl-inoculated seedlings in both experiments and all treatments wilted and died within 48 h after inoculation (Table 2, Fig. 2). In the absence of a surfactant, all seven of the remaining seedling pepper plants within a recirculating hydroponic unit died within the next 12 days. Sporangia were observed microscopically on roots of the hypocotyl-inoculated plants, and the fungus was first detected in the nutrient solution (as assessed by the baiting technique) 3 days after inoculation. Numerous necrotic brown lesions occurred on all submerged portions of the tomato fruit, and isolations from lesions consistently yielded pure cultures of *P. capsici*. In contrast, no plant mortality or root infection occurred, with the exception of the hypocotyl-inoculated plants, in hydroponic units that were amended with the surfactant over the duration of the experiments (7 weeks postinoculation). Additionally, the fungus was never detected, as assessed by the tomato baiting technique, in the nutrient solution of surfactant-amended units over the duration of the experiments. Sporangia were observed microscopically on roots of the hypocotyl-inoculated plants, but not on roots of any of the other plants, within a surfactant-amended unit.

No plant mortality occurred in noninoculated control units, and the fungus was not isolated from or observed on roots sampled periodically throughout both experiments.

Mortality of adult pepper plants in the absence or presence of a surfactant. All hypocotyl-inoculated adult pepper plants in both experiments and all treatments died within 5 to 7 days after inoculation (Table 3, Fig. 3). In the absence of a surfactant, all seven of the remaining adult plants within a recirculating hydroponic unit wilted and died within the next 12 days. Sporangia were observed microscopically on roots of the hypocotyl-inoculated plants, and the fungus was detected in the nutrient solution by day 3 after inoculation. In contrast, no plant mortality or root infection occurred, with the exception of the hypocotyl-inoculated plant, in hydroponic units that were amended with the surfactant over the duration of the study (3 weeks postinoculation). Additionally, the fungus was never detected, as assessed by the tomato baiting technique, in the nutrient solution of surfactant-amended units over the duration of the cropping periods. Sporangia were observed microscopically on roots of the hypocotyl-inoculated plants, but not on roots of any of the other plants, within a surfactant-amended unit.

Viability of the pathogen in roots of the hypocotyl-inoculated plants in the presence of a surfactant. At the termination of each cropping period, the rock wool block supporting the hypocotyl-inoculated

Table 3. Percent mortality of adult pepper plants after hypocotyl-inoculation of a single plant on one side (side A) of a two-sided recirculating hydroponic unit with *Phytophthora capsici*

Treatment	Side	Weeks after inoculation[a] 1	2	3
Control[b]	A	0	0	0
	B	0	0	0
Phytophthora[c]	A	25	100	
	B	0	100	
Phytophthora +[b] surfactant	A	25	25	25
	B	0	0	0

[a] Adult pepper plants were 75 and 69 days old at time of inoculation in experiment 1 and 2, respectively. There were four plants on side A and four plants on side B of each hydroponic unit. Each hydroponic unit of a treatment was considered a trial.
[b] Mean percent mortality from four trials: two trials in experiment 1 and two trials in experiment 2.
[c] Mean percent mortality from three trials: one trial in experiment 1 and two trials in experiment 2.

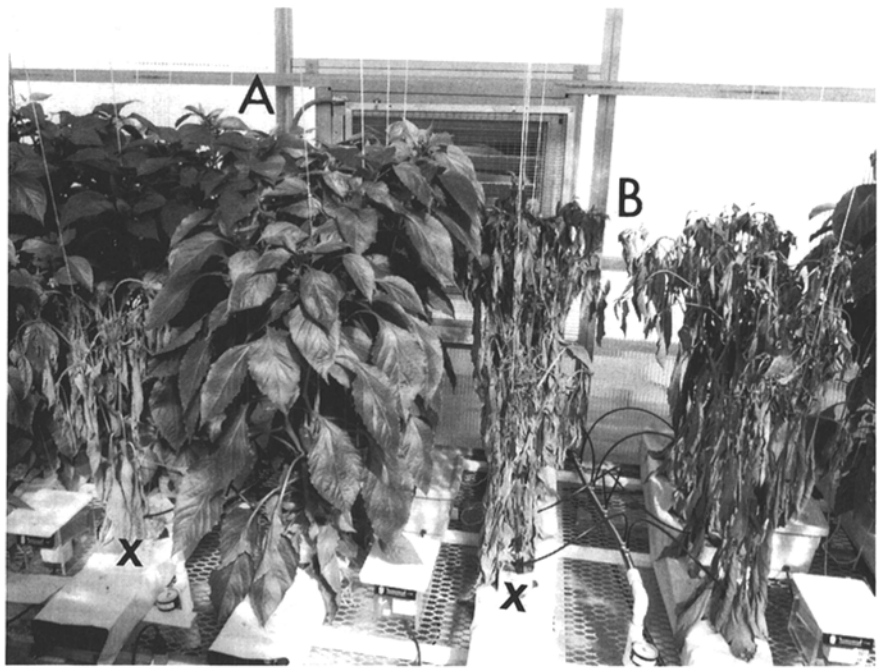

Fig. 3. Mortality of adult pepper plants after hypocotyl-inoculation of a single plant (X) on one side of a two-sided recirculating hydroponic unit in the **(A)** presence or **(B)** absence of a nonionic surfactant in the nutrient solution.

plant was physically removed and split into two equal halves. One half was rinsed in running tap water for 10 min to remove the surfactant and placed in a beaker containing sterile distilled water. The remaining half of each block was placed, without rinsing, in a beaker that contained the surfactant-amended nutrient solution. A tomato fruit was then added to each beaker and incubated as described above. The pathogen infected and was isolated from all tomato fruit that were placed in beakers containing roots in rinsed rock wool blocks but not from any fruit placed in beakers containing roots in nonrinsed rock wool blocks.

DISCUSSION

The results of the above studies support and extend previous findings (9) on the efficacy of surfactants in the control of root diseases caused by zoosporic plant pathogens in cultural systems that employ recirculation of the nutrient solution. Amending the nutrient solution with a nonionic surfactant resulted in the complete suppression of the dispersal of zoospores of *P. capsici* from a point source, the hypocotyl-inoculated plant; whereas in the absence of the surfactant, *P. capsici* was dispersed from the point source and killed all the pepper plants in the hydroponic unit within 12 days. Infectious propagules of the pathogen, which were most likely motile zoospores, were first detected in the recirculating nutrient solution of non-surfactant-amended units on day 3 following hypocotyl-inoculation of a single plant in a rock wool unit. Their presence also coincided with the first observation of sporangia on roots of the hypocotyl-inoculated plant. These observations, coupled with the known selective lytic activity of the surfactant to motile zoospores (10), indicate that motile zoospores were the sole infectious propagule responsible for both pathogen dispersal in the recirculating nutrient solution and plant infection. This conclusion is in contrast to some of our findings in an identical cultural system employing *Pythium aphanidermatum* as the root pathogen and cucumbers as the susceptible host. In the latter interaction, zoospore dispersal in the recirculating nutrient solution was completely suppressed; however, *Pythium aphanidermatum* was also capable of spreading via hyphae, which were not affected by the surfactant, from the hypocotyl-inoculated plant to all plants growing in the same rock wool slab. The lack of spread of *P. capsici* from the hypocotyl-inoculated plant to adjacent healthy plants growing in the same rock wool slab in the presence of a surfactant indicates that *P. capsici*, in contrast to *Pythium aphanidermatum*, is not capable of spreading from plant to plant or root to root via hyphae. This conclusion is supported by the observation that roots of the healthy plant adjacent to the hypocotyl-inoculated plant had grown into the root zone of the latter. Yet no disease or root infection occurred in the healthy plant throughout the entire cropping period, which extended, depending upon the particular experiment, 3 to 7 weeks postinoculation. It could be argued that such prolonged exposure to the surfactant resulted in the mortality of the pathogen in the hypocotyl-inoculated plant. However, the baiting technique showed that *P. capsici* was viable in the roots of the hypocotyl-inoculated plant throughout the duration of the experiments. Further, if any caducous sporangia were present in the recirculating nutrient solution, they either do not function as inoculum or they germinate only indirectly via zoospores. If any directly germinating sporangia or hyphal fragments were present in the recirculating nutrient solution, control of the spread of the pathogen with the surfactant would not have been achieved, since the latter life stages of the pathogen are not subject to the lytic effects of the surfactant.

These results, coupled with those from the previous investigation (9), indicate that surfactants constitute an efficacious method for the control of the spread of root diseases caused by zoosporic plant pathogens in recirculating hydroponic cultural systems. Additionally, our results have significant implications regarding the control of polycyclic diseases of numerous crops caused by zoosporic pathogens in open-field agriculture. Previous investigations indicate that spread of *Phytophthora* spp. and other zoosporic pathogens in surface irrigation water or rainwater is associated with the rapid and long-distance dispersal of inoculum (3,5,7). If zoospores are in fact the specific inoculum being transported in the surface waters in open-field agriculture, incorporation of a surfactant into the irrigation water should eliminate or at least reduce zoospore density and adversely affect the rapidity and long-distance dispersal of the pathogen from a point source. The latter studies are currently underway in the field and include, among others, *P. capsici* on pepper and *P. parasitica* on citrus.

ACKNOWLEDGMENTS

We thank C. Liddell and N. P. Goldberg, New Mexico State University, for providing a culture of *P. capsici* (isolate 6143, A1) and pepper seeds.

LITERATURE CITED

1. Ioannou, N., and Grogan, R. G. 1984. Water requirements for sporangium formation by *Phytophthora parasitica* in relation to bioassay in soil. Plant Dis. 68:1043-1048.
2. Larkin, R. P., Ristaino, J. B., and Campbell, C. L. 1995. Detection and quantification of *Phytophthora capsici* in soil. Phytopathology 85:1057-1063.
3. Neher, D., and Duniway, J. M. 1992. Dispersal of *Phytophthora parasitica* in tomato fields by furrow irrigation. Plant Dis. 76:582-586.
4. Pegg, G. F., and Jordan, M. M. 1991. *Phytophthora cryptogea* root rot of tomato in rockwool nutrient culture. I. Analysis of root infection. Ann. Appl. Biol. 117:525-535.
5. Ristaino, J. B., Larkin, R. P., and Campbell, C. L. 1993. Spatial and temporal dynamics of *Phytophthora* epidemics in commercial bell pepper fields. Phytopathology 83:1312-1320.
6. Ristaino, J. B., Larkin, R. P., and Campbell, C. L. 1994. Spatial dynamics of disease symptom expression during *Phytophthora* epidemics in bell pepper. Phytopathology 84:1015-1024.
7. Shew, H. D. 1987. Effect of host resistance on spread of *Phytophthora parasitica* var. *nicotianae* and subsequent development of tobacco black shank under field conditions. Phytopathology 77:1090-1093.
8. Stanghellini, M. E., and Rasmussen, S. L. 1994. Hydroponics: A solution for zoosporic pathogens. Plant Dis. 78:1129-1138.
9. Stanghellini, M. E., Rasmussen, S. L., Kim, D. H., and Rorabaugh, P. A. 1996. Efficacy of nonionic surfactants in the control of zoospore spread of *Pythium aphanidermatum* in a recirculating hydroponic system. Plant Dis. 80:422-428.
10. Stanghellini, M. E., and Tomlinson, J. A. 1987. Inhibitory and lytic effects of a nonionic surfactant on various asexual stages in the life cycle of *Pythium* and *Phytophthora* species. Phytopathology 77:112-114.
11. Thinggaard, K., and Anderson, H. 1995. Influence of watering frequency and electrical conductivity of the nutrient solution on *Phytophthora* root rot in pot plants of *Gerbera*. Plant Dis. 79:259-263.