Inhibitory and Lytic Effects of a Nonionic Surfactant on Various Asexual Stages in the Life Cycle of *Pythium* and *Phytophthora* Species

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ABSTRACT

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Zoospores of *Pythium aphanidermatum, P. dissotocum, P. intermedium, P. tracheiphilum*, and *Phytophthora nicotianae* ceased motility and lysed within 1 min after placement in a solution containing 20 μg of Agral per milliliter. Zoospores of the same species, with the exception of *P. tracheiphilum*, remained motile for 14 hr or more in the absence of Agral. Solutions containing 20 μg of Agral per milliliter were also totally inhibitory to vesicle formation and zoospore production by the four species of *Pythium* and to zoospore production by *Phytophthora nicotianae*. Vesicles were formed by all *Pythium* spp. except *P. intermedium* in solutions containing 15 μg of Agral per milliliter. All vesicles of *P. aphanidermatum* and *P. dissotocum* and most of those of *P. tracheiphilum*, however, lysed within 10 min after formation. The lytic effect on both vesicles and zoospores indicates that the mode of action of Agral resides in disruption of the integrity and/or permeability of the plasma membrane of fungus structures lacking a cell wall. Agral had little or no effect on mycelial growth or direct germination of encysted zoospores and sporangia of the same fungi. The potential use of Agral for controlling root diseases caused by species of *Pythium* and *Phytophthora* that rely on zoospores for plant-to-plant spread is discussed.

Continuous application of Agral, a nonionic liquid surfactant containing 90% (v/v) alkyl phenolethylene oxide condensate (ICI Plant Protection Division, Fernhurst, Haslemere, Surrey, United Kingdom), to hydroponic nutrient solutions used in commercial glasshouses has controlled melon necrotic spot virus of cucumber (9) and big-vein disease of lettuce (8). Control was attributed to the lethal effects of Agral on zoospores of *Olpidium radicale* Schwartz & Cook and *O. brassicae* (Wor.) Dang., the respective root-infecting fungus vectors of these viruses. During the course of investigations on control of melon necrotic spot of cucumbers in commercial glasshouses, Agral appeared to have an effect on *Pythium aphanidermatum* Edson (Fitzp.). This fungus was isolated from roots of cucumber plants before but not after the addition of Agral at 20 μg/ml to the nutrient solution (7). *P. aphanidermatum* and other zoosporic species of *Pythium* are recognized as destructive root pathogens of various vegetable crops grown hydroponically (1,3–6). No effective fungicides are currently registered for the control of *Pythium* spp. on hydroponically grown vegetables.

This paper reports the results of the effects of Agral on various phases of the life cycle of four species of *Pythium* and an isolate of *Phytophthora nicotianae* Van Breda de Haan (CMI 295909) included in the study for comparative purposes.

MATERIALS AND METHODS

Stock cultures of *P. aphanidermatum, P. dissotocum* Drechsler, *P. intermedium* de Bary, and *P. tracheiphilum* Matta, originally isolated from roots of carrots, lettuce, cucumbers, and carrots, respectively, and *Phytophthora nicotianae* were maintained at 20 C on 10% V-8 juice agar (VJA) medium.

Mycelial growth in the presence of Agral. A 5-mm-diameter disk was cut from the margin of a 1-day-old VJA culture of each fungus and placed at the perimeter of a 9-cm-diameter petri dish containing VJA amended with Agral at 15, 20, and 25 μg/ml. The same medium without Agral was used as a control. Cultures were incubated at 25 C, and colony radii were measured after 24 and 48 hr. There were two replicate plates of each concentration of the chemical, and the experiment was repeated once.

Direct germination of zoospore cysts and sporangia in the presence of Agral. A 0.5-ml suspension of zoospore cysts of each fungus and sporangia of *P. intermedium* and *P. tracheiphilum* were individually dispensed onto the surface of VJA amended with Agral at 15, 20, and 25 μg/ml. The same medium without Agral was used as a control. Cultures were incubated at 20 C, and percentage of germination was determined over a 24-hr period by observing 100 zoospore cysts or sporangia under a compound microscope at 40×. There were two replicate plates per treatment, and the experiment was repeated once.

Zoospore cysts were obtained from a 20-mm-diameter disk cut from the margin of a 7-day-old VJA culture of each fungus and placed in a petri dish containing 15 ml of sterile distilled water. Maximum production of zoospores of *P. tracheiphilum, P. intermedium, P. dissotocum, P. aphanidermatum*, and *Phytophthora nicotianae* occurred after 2, 3, 8, 16, and 20 hr, respectively, of incubation at 20 C. The water containing the zoospores of each fungus was then decanted into centrifuge tubes and shaken vigorously for 60 sec to induce encystment of the zoospores. Cyst population estimates, determined by counts in a hemacytometer, of *P. intermedium, P. tracheiphilum, P. aphanidermatum, P. dissotocum*, and *Phytophthora nicotianae* were 1.2×10^4, 2.2×10^4, 3.4×10^4, 5.1×10^4, and 0.9×10^4 per milliliter, respectively.

Sporangia of *P. tracheiphilum* and *P. intermedium* were obtained from 5 ml of sterile distilled water that was poured onto the surface of a 7-day-old VJA culture of each fungus. The agar surface was then gently agitated with a small brush to dislodge the sporangia. The water containing the sporangia was then decanted into a centrifuge tube. Sporangia population estimates, determined by counts in a hemacytometer, of *P. tracheiphilum* and *P. intermedium* were 1.1×10^4 and 7.2×10^4 per milliliter, respectively.

Vesicle formation and zoospore production in the presence of Agral. A 20-mm-diameter disk was cut from the margin of a 7-day-old VJA culture of each fungus and placed in a 9-cm-diameter petri dish containing 15 ml of sterile distilled water amended with Agral at 10, 15, 20, and 25 μg/ml. Sterile distilled water without Agral served as a control. Cultures were observed microscopically (40×) at frequent intervals over a 24-hr incubation

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period at 20 C. Data recorded included, where applicable, vesicle formation and numbers of zoospores released (counted in a hemacytometer when high numbers were produced or counted in 0.1-ml subsamples of the bathing solution when numbers were low). There were two replicate plates of each treatment, and the experiment was repeated four times.

Zoospore motility and viability in the presence of Agral. The effect of Agral on zoospore motility was determined by comparing the duration of motility in Agral with that of a control preparation. This was done by mixing 1 ml of a zoospore suspension of each fungus (obtained as previously described and containing not less than 1×10^4 zoospores per milliliter) with 1 ml of various concentrations of Agral. The mixtures were immediately poured into a petri dish and observed microscopically at 40×. The maximum time for complete cessation of motility was recorded for each fungus.

Zoospore viability subsequent to complete cessation of motility was assessed 30 min after cessation of motility in the various concentrations of Agral. The mixtures were passed through Millipore filters (0.45-μm pore diam.), and the filters, which retained any zoospores, were placed in centrifuge tubes containing 2 ml of sterile distilled water and vigorously shaken for 1 min. A 1-ml subsample from each tube was dispensed onto the surface of VJA contained in 9-cm-diameter petri dishes. Cultures were incubated at 20 C for 48 hr. All cultures showing fungus growth were recorded and the fungus identified. No attempt was made to quantify the viable zoospore population. The motility and viability experiments were repeated on four separate occasions.

RESULTS

Mycelial growth and direct germination of zoospore cysts and sporangia. Agral, over the range of concentrations tested, had little or no effect on either the rate of mycelial growth (Table 1) or the direct germination of zoospore cysts and sporangia (Table 2) of the fungi assayed.

Vesicle formation and zoospore production. Agral concentrations of 20 and 25 μg/ml were completely inhibitory to vesicle formation by the four species of *Pythium* and completely inhibitory to zoospore production by all fungi assayed (Table 3). Sensitivity to Agral concentrations at less than 20 μg/ml varied with the fungus species involved. For example, vesicles were formed by all species of *Pythium* except *P. intermedium* in the presence of 15 μg of Agral per milliliter. All of the vesicles formed by *P. aphanidermatum* and *P. dissotocum* and about 80% of those formed by *P. tracheiphilum*, however, lysed within 10 min after formation and usually before zoospore cleavage. Zoospores released from the surviving vesicles of *P. tracheiphilum* encysted within 10 min. Relative to control preparations, few zoospores were released from sporangia of *Phytophthora nicotianae* in the presence of 15 μg of Agral per milliliter and those that were released encysted within 10 min. *P. intermedium* showed the greatest sensitivity to Agral. No vesicles or zoospores were produced by this species in any of the concentrations of Agral tested.

Zoospore motility and viability. Zoospores of all fungal species tested ceased motility and lysed within 1 min after being placed in solutions containing 20 and 25 μg of Agral per milliliter. No viable zoospores were recovered (Table 4). When placed in solutions containing 15 μg of Agral per milliliter, zoospores of all species ceased motility within 10 min. Microscopic examination revealed that although numerous zoospores were in various stages of lysis, many had encysted. The encysted zoospores recovered from solutions containing 15 μg of Agral per milliliter were viable. None of the zoospores of any of the species tested lysed when placed in 10 μg of Agral per milliliter, and the only noticeable effect was a decrease in the duration of their motility relative to control preparations.

DISCUSSION

Results of this investigation showed that Agral, a nonionic liquid surfactant with a documented rapid toxicity to zoospores of *O. brassicae* and *O. radicale* (7,9), was also toxic to zoospores of *P. aphanidermatum, P. dissotocum, P. tracheiphilum, P. intermedium*, and *Phytophthora nicotianae*. Zoospores of all fungi tested ceased motility and lysed within 1 min after placement in solutions containing 20 μg of Agral per milliliter. The latter concentration of Agral was also totally inhibitory to vesicle and zoospore formation by the four species of *Pythium* and to zoospore formation by *Phytophthora nicotianae*.

In solutions containing 15 μg of Agral per milliliter, vesicles were formed by all species of *Pythium* except *P. intermedium*. All vesicles formed by *P. aphanidermatum* and *P. dissotocum* and most of those formed by *P. tracheiphilum*, however, lysed within 10 min after formation. The lytic effect on both zoospores and vesicles, i.e., fungal structures surrounded only by a plasma membrane, of diverse fungal genera and species suggests that the mode of action of Agral may reside in alteration of the integrity and/or permeability of the plasma membrane. This hypothesis is supported by the fact that Agral had little or no effect on mycelial growth or direct germination of zoospore cysts and sporangia. The latter fungus structures possess a cell wall that apparently provides protection against Agral. Although Agral was the only surfactant

TABLE 1. Mycelial growth of four *Pythium* spp. and *Phytophthora nicotianae* in the presence of the nonionic surfactant Agral[y]

Agral concentration (μg/ml)	Mycelial growth (mm/24 hr)[z]				
	Pythium dissotocum	*Pythium aphanidermatum*	*Pythium tracheiphilum*	*Pythium intermedium*	*Phytophthora nicotianae*
0	21	40	14	18	4
15	21	37	13	18	4
20	18	34	14	18	4
25	15	35	12	15	3.6

[y] Cultures were grown on V-8 juice agar and transferred onto V-8 juice agar amended with Agral; V-8 juice agar without Agral served as a control. Radial growth was measured after 24 and 48 hr of incubation at 25 C.
[z] Each value is average of data from two experiments.

TABLE 2. Direct germination of zoospore cysts and sporangia of four *Pythium* spp. and *Phytophthora nicotianae* in the presence of the nonionic surfactant Agral[y]

Agral concentration (μg/ml)	Direct germination (%)[z]									
	Zoospore cysts					Sporangia				
	Pythium dissotocum	*Pythium aphanidermatum*	*Pythium tracheiphilum*	*Pythium intermedium*	*Phytophthora nicotianae*	*Pythium dissotocum*	*Pythium aphanidermatum*	*Pythium tracheiphilum*	*Pythium intermedium*	*Phytophthora nicotianae*
0	72	94	90	95	86	nt	nt	100	94	nt
15	74	91	94	96	84	nt	nt	100	90	nt
20	68	87	88	93	80	nt	nt	97	93	nt
25	73	81	83	90	72	nt	nt	98	90	nt

[y] A 0.5-ml suspension of zoospore cysts and sporangia were spread on the surface of V-8 juice agar amended with Agral; V-8 juice agar without Agral served as a control. Cultures were incubated at 20 C for 24 hr.
[z] Each value is average of data from two experiments; nt = not tested.

TABLE 3. Zoospore production by four *Pythium* spp. and *Phytophthora nicotianae* in the presence of the nonionic surfactant Agral[y]

Agral concentration (μg/ml)	Zoospore production (no./ml)[z]				
	Pythium dissotocum	*Pythium aphanidermatum*	*Pythium tracheiphilum*	*Pythium intermedium*	*Phytophthora nicotianae*
0	5.4×10^4	2.5×10^4	3.0×10^4	1.5×10^4	1.2×10^4
10	2.0×10^4	2.0×10^3	2.1×10^4	0	1.0×10^4
15	0	0	1.0×10^4	0	1.5×10^2
20	0	0	0	0	0
25	0	0	0	0	0

[y] A 20-mm-diameter disk was cut from the margin of a 7-day-old V-8 juice agar culture of each fungus and placed in a petri dish containing 15 ml of sterile distilled water amended with Agral; sterile distilled water without Agral served as a control. Maximum zoospore production of *Pythium tracheiphilium*, *P. intermedium*, *P. dissotocum*, *P. aphanidermatum*, and *Phytophthora nicotianae* was recorded after 2, 3, 8, 16, and 20 hr, respectively, of incubation at 20 C.

[z] Each value is average of data from four experiments.

TABLE 4. Zoospore motility and viability of four *Pythium* spp. and *Phytophthora nicotianae* in the presence of the nonionic surfactant Agral[x]

Agral concentration (μg/ml)	Zoospore motility time (min)[y]				
	Pythium dissotocum	*Pythium aphanidermatum*	*Pythium tracheiphilum*	*Pythium intermedium*	*Phytophthora nicotianae*
0	>1,440	>1,440	35	1,000	>1,440
10	840	600	20	400	480
15	8	10	10	5	9
20[z]	1	1	1	0.5	1
25[z]	0.5	0.5	0.5	0.5	0.5

[x] A 1-ml suspension of zoospores of each fungus (see Table 3) containing not less than 1×10^4 zoospores per milliliter was mixed with 1 ml of sterile distilled water amended with Agral. The maximum time for complete cessation of motility, determined microscopically at 40×, was recorded for each fungus.

[y] Each value is average of four experiments.

[z] Lethal to entire zoospore population.

used in our study, its toxicity to zoospores and vesicles is not regarded as a unique or specific property of that chemical. Previous work by Tomlinson and Faithfull (7) showed that among 10 surfactants tested in vitro, seven (which included Agral and other anionic, cationic, and nonionic types) were toxic to zoospores of *O. brassicae*.

The continuous application of Agral, at 20 μg per milliliter, to nutrient solutions used in commercial glasshouse production of lettuce and cucumbers has resulted in the prevention of two fungus-transmitted diseases, lettuce big-vein and melon necrotic spot of cucumber, by controlling the zoospores of the respective root-infecting fungus vectors, *O. brassicae* and *O. radicale* (8,9). The results of our investigation indicate that Agral, because of its lytic effects on vesicles and zoospores, may also be effective in controlling root diseases caused by species of *Pythium* and *Phytophthora* that rely on zoospores for dissemination. Two of the more common *Pythium* spp. responsible for destructive root diseases of commercial vegetable crops produced hydroponically are *P. aphanidermatum* and *P. dissotocum* (1,3–6). Zoospores of these two species have been identified as the fungal propagules mainly responsible for plant-to-plant spread under hydroponic conditions (2). Studies to confirm previous observations (7) of the apparent effectiveness of Agral on the control of *P. aphanidermatum* root rot of cucumbers, in addition to Phytophthora root rot of citrus, are currently in progress.

Agral is a biologically safe, inexpensive surfactant normally used at 60–300 μg per milliliter to improve the wetting or spreading properties of chemical sprays applied to crop foliage. The agent is biodegradable, nonsystemic, and miscible at all temperatures with both hard and soft water and in the United Kingdom is permitted for use in hydroponic nutrient solutions. When Agral has been used at 20 μg per milliliter, no phytotoxic symptoms have been observed in either lettuce or cucumber plants.

LITERATURE CITED

1. Bates, M. L., and Stanghellini, M. E. 1984. Root rot of hydroponically grown spinach caused by *Pythium aphanidermatum* and *P. dissotocum*. Plant Dis. 68:989-991.
2. Gold, S. E., and Stanghellini, M. E. 1985. Effects of temperature on Pythium root rot of spinach grown under hydroponic conditions. Phytopathology 75:333-337.
3. Jenkins, S. F., Jr., and Averre, C. W. 1983. Root diseases of vegetables in hydroponic culture systems in North Carolina greenhouses. Plant Dis. 67:968-970.
4. Stanghellini, M. E. 1984. Yield reduction in lettuce resulting from subclinical infection of feeder-rootlets by *Pythium dissotocum*. (Abstr.) Phytopathology 74:846.
5. Stanghellini, M. E., and Phillips, J. M. 1975. *Pythium aphanidermatum*: Its occurrence and control with pyroxychlor in the Arabian Desert at Abu Dhabi. Plant Dis. Rep. 59:559-563.
6. Stanghellini, M. E., and Russell, J. D. 1971. Damping-off of tomato seedlings in commercial hydroponic culture. Prog. Agric. Ariz. 23:15-16.
7. Tomlinson, J. A., and Faithfull, E. M. 1979. Effects of fungicides and surfactants on the zoospores of *Olpidium brassicae*. Ann. Appl. Biol. 93:13-19.
8. Tomlinson, J. A., and Faithfull, E. M. 1980. Studies on the control of lettuce big-vein disease in recirculated nutrient solutions. Acta Hortic. 98:325-332.
9. Tomlinson, J. A., and Thomas, B. J. 1986. Studies on melon necrotic spot virus disease of cucumber and on the control of the virus vector (*Olpidium radicale*). Ann. Appl. Biol. 108:71-80.