

Occurrence, Dissemination, and Survival of Plant Pathogens in Surface Irrigation Ponds in Southern Georgia

F. M. Shokes and S. M. McCarter

Former graduate assistant and professor, respectively, Department of Plant Pathology and Plant Genetics, University of Georgia, Athens 30602. Present address of first author: Institute of Food and Agricultural Sciences, Agricultural Research and Education Center, P.O. Box 470, Quincy, FL 32351.

Financial support was provided through Department of Agriculture Cooperative Agreement 12-14-7001-518.

We thank Johnny Enfinger for assistance with the sampling of ponds and Jan Fowler for technical assistance with the laboratory assays. We thank J. T. Ratcliffe for help in selecting certain ponds that were sampled. We also thank E. E. Butler for identification of the *Sclerotium* isolate.

Accepted for publication 28 November 1978.

ABSTRACT

SHOKES, F. M., and S. M. McCARTER. 1979. Occurrence, dissemination, and survival of plant pathogens in surface irrigation ponds in southern Georgia. *Phytopathology* 69: 510-516.

Eight surface irrigation ponds were surveyed for plant pathogens in 1976 and 1977 to determine whether water used to irrigate vegetable transplants in southern Georgia is a potential source of plant pathogens reinfesting recently fumigated fields. The sampling techniques were: (i) unfiltered samples from three depths at five or six locations taken with a water sampling bottle, (ii) filtered debris collected after pumping 48–378 L of water through microorganism filters, and (iii) bottom sediment samples taken with an Ekman dredge. Plant pathogenic fungi from samples included *Pythium aphanidermatum*, *P. irregulare*, 20 other *Pythium* spp., *Phytophthora* spp., *Fusarium* spp., and *Rhizoctonia* spp. These fungi were isolated through laboratory assays with general and selective media, baiting techniques, and growth chamber bioassays. One sample taken by filtering water from an irrigation line in the field yielded five *Pythium* spp., a *Phytophthora* sp., and 14 lance nematodes. In pathogenicity tests 27 isolates

representing 13 *Pythium* spp. and 14 isolates representing 10 *Pythium* spp. and two *Phytophthora* spp. differed widely in virulence on cotton, cabbage, tomato, and pea. *P. aphanidermatum* was the most virulent pathogen, but *P. irregulare* was the most frequently isolated pathogen. In a survival study in a pond, *P. aphanidermatum* in infested ryegrass seed and as oospores was recoverable for 185 days after submersion. Isolates from surviving oospores, after overwintering in the pond, were pathogenic on the four hosts. Zoospores were not recovered after 12 days. *Rhizoctonia solani* in infested cotton stem sections was recoverable in declining numbers up to 96 days and as sclerotia up to 85 days but failed to overwinter in the pond. Sclerotia of a *Sclerotium* sp. (probably *S. hydrophilum*) were 92% viable after 133 days in the pond. Results indicate that the use of contaminated irrigation water may be a major factor limiting the success of general purpose fumigants in transplant production.

Additional key words: fungal pathogens, reinfestation, *Rhizoctonia zeae*.

Southern Georgia is a major production area for certified vegetable and ornamental transplants for shipment to other areas of the United States and to Canada. General purpose fumigants such as methyl bromide and methyl bromide-chloropicrin are currently used to control soilborne plant pathogens of field-grown ornamental transplants, and there is increased interest in their use for controlling several soilborne pathogens that cause diseases of vegetable transplants (24,25). However, reinfestation of recently fumigated fields by plant pathogens is a major factor limiting the success of general purpose fumigants for transplant production (24).

Transplants produced in southern Georgia are irrigated with sprinkler systems that use pond water. These ponds often receive runoff from surrounding cultivated fields. Although plant pathogens are present in irrigation waters of the southeastern United States (7,10,18,34,37) and other areas (9,15,20,31,33,35), relatively little information exists on their occurrence, dissemination, and survival in southern Georgia pond waters. Most reports associating disease outbreaks with contaminated water have been largely circumstantial. No systematic studies have been conducted to prove that pathogens in irrigation ponds in Georgia are pumped with irrigation water and subsequently cause diseases in growers' fields.

We conducted a 2-yr study to: (i) survey representative irrigation ponds in the Georgia coastal plain for plant pathogens, (ii) determine if plant pathogens could be pumped from a contaminated water source onto a grower's field, (iii) determine the pathogenicity of suspected plant pathogens from irrigation ponds, and (iv) study survivability of selected fungal plant pathogens in a typical irrigation pond.

MATERIALS AND METHODS

Survey of ponds. Six surface irrigation ponds in southern Georgia were surveyed twice for plant pathogens in the fall of 1976. Two additional ponds were sampled once in the summer of 1977. Two of the initial six ponds were sampled monthly from April to September 1977 to study changes in pathogen populations. Two of the eight ponds sampled were on the Coastal Plain Experiment Station at Tifton, one on the Southeast Georgia Branch Experiment Station at Midville, and five on vegetable transplant farms near Tifton. The two ponds sampled monthly during 1977 were on farms and received surface runoff directly from adjacent fields planted to tomato (*Lycopersicon esculentum* Mill.), cabbage (*Brassica oleracea* var. *capitata* L.), and pepper (*Capsicum frutescens* L.).

Sampling methods. Each pond was sampled by three methods: (i) A 20-L composite water sample (designated unfiltered water) was collected with a van Dorn water bottle sampler (21) at five or six random locations at three depths (surface, intermediate, and bottom). (ii) A filtered sample was collected by forcing 48–378 L of pond water through a microorganism filtering apparatus (MFA) with a small (2.5-HP) gasoline-driven centrifugal pump equipped with 3.8 cm diameter inlet pipe placed in the pond to simulate the irrigation system used by growers. Five filters (53, 35, and 20 μ m nylon mesh, 10 μ m polypropylene, and 5 μ m polyvinyl chloride [PVC] nylon-supported membrane) were stacked in order of decreasing pore diameter from top to bottom on the MFA filter section supports (Fig. 1). Filters with debris and microorganisms were transported in a polypropylene bottle with 2 L of sterile deionized water (SDW) and washed manually in the same water after transport. The resulting suspension (designated filtered water) was assayed for microorganisms. (iii) A bottom sediment

sample (approximately 5,000 g) was collected at six to eight locations with an Ekman dredge (21) and homogenized in a plastic container. All samples were placed on ice for transport to the laboratory and assayed the same day. Irrigation water was sampled once in a grower's field by diverting approximately 150 L through a garden hose connected to an irrigation riser and filtering this water through the MFA. A deep well near one pond was sampled by passing approximately 378 L of water through the filtering apparatus.

Laboratory assay methods. Appropriate dilutions of the water and bottom sediment samples were assayed for total fungi, total bacteria, *Pythium* spp., *Fusarium* spp., and *Rhizoctonia solani* Kuhn. Bottom sediment was prepared for assay by diluting 1:1 v/v with SDW (designated sediment slurry), for 30 sec in a Waring Blender, shaking for 30 min on a wrist-action shaker, and making appropriate dilutions. Population estimates of total fungi were made by plating dilutions of 10^0 and 10^{-1} unfiltered water, 10^{-2} and 10^{-3} of filtered water, and 2×10^{-3} and 2×10^{-4} of bottom sediment on five replicate dilution plates per sample of dextrose-peptone-yeast extract agar (17). Total bacteria were estimated with dilutions of 10^0 and 10^{-1} of unfiltered water, 10^{-3} and 10^{-4} of filtered water, and 2×10^{-4} and 2×10^{-5} of bottom sediment on dilution plates of soil extract agar (17). Plates were incubated in the dark at 25 C and checked at 7 and 14 days; colonies were counted and representative colonies of fungi transferred to appropriate media for identi-

fication.

Assays for *Pythium* spp. were made by spreading 1 ml of unfiltered water, filtered water, and sediment slurry on 10 plates each of selective media. Modified Kerr medium (19) was used in 1976 and in 1977 the P₁₀VP medium of Tsao and Ocana (36) modified by addition of rose bengal at 0.01 g/L to improve colony visibility (P₁₀VPR). Plates were incubated 48 hr at 25 C and washed free of sediment and debris before colonies were counted. Representative colonies were transferred to hempseed agar (30 ml of hot water extract from 500 g of hempseed, 15 g of agar, 750 ml of distilled water) for identification and pathogenicity tests. All samples also were baited for pythiaceous fungi with citrus leaf sections (11) and with potato cubes soaked in pimarinic and streptomycin sulfate (14). Baits were incubated in the samples for 3 days at 25 C, washed, blotted dry, and plated on P₁₀VPR medium. Representative colonies were transferred to hempseed agar for identification (13,28) and pathogenicity tests. Infested citrus leaf pieces or boiled grass blades floating in SDW were used to induce zoosporangial formation when necessary.

Assays for *Fusarium* spp. were made by spreading 1-ml aliquots of 10^0 and 10^{-1} , 10^{-1} and 10^{-2} , and 2×10^{-3} and 2×10^{-4} dilutions of unfiltered water, filtered water, and sediment slurry, respectively, on 10 plates each of the medium of Nash and Snyder (29) modified by adding a 300 mg/L of streptomycin sulfate and 1,000 mg/L of penicillin G to inhibit bacteria in water and sediment. Plates were

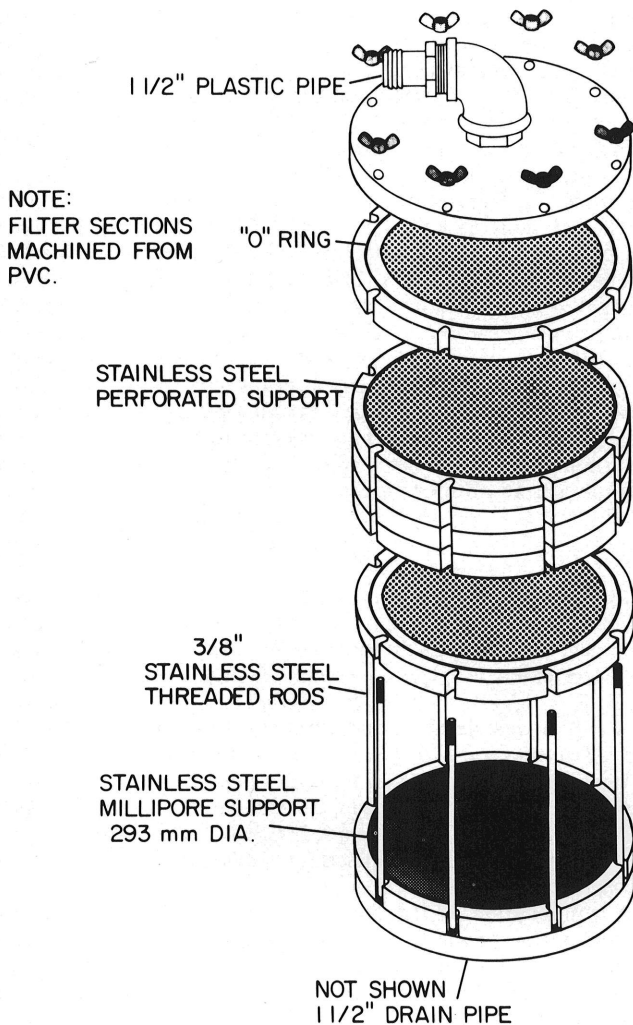
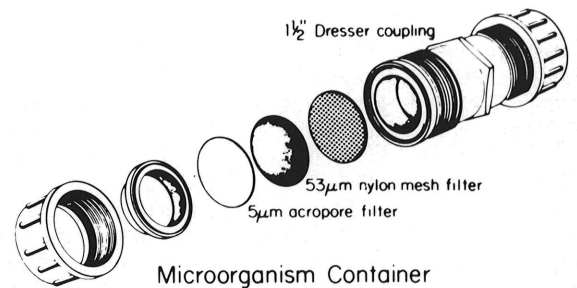
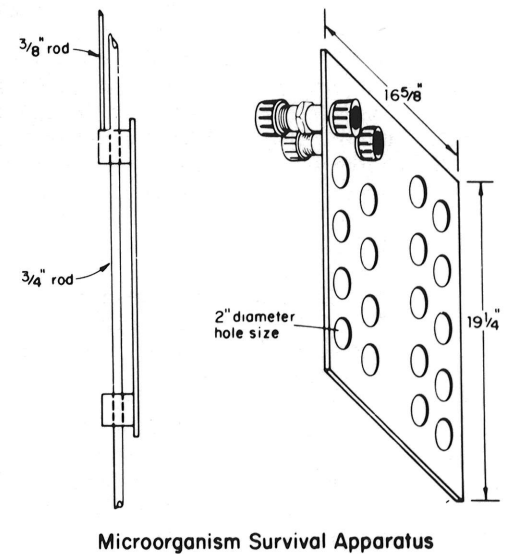


Fig. 1. Exploded view of filtering apparatus used to collect microorganisms from irrigation water. Five filters (53, 35, and 20 μm nylon mesh, a 10- μm polypropylene, and a 5- μm polyvinyl chloride nylon-supported membrane) were stacked in order of decreasing pore diameter from top to bottom on the filter section supports. Sections were sealed with rubber "O" rings.



A



B

Fig. 2. Apparatus used to study the survival of selected propagules of plant pathogens in surface irrigation ponds. **A**, Exploded view of a microorganism container showing filter support, filters, seal, and packing nuts. **B**, An aluminum panel designed to hold 20 microorganism containers. Apparatus slides into pond on 5.1 cm diameter aluminum support rod driven into the pond floor. A 0.95 cm diameter rod raises and lowers panel.

incubated under fluorescent lights. Counts of *Fusarium* spp. were made after 2 wk. Representative colonies were transferred to Difco potato dextrose agar (PDA) and water agar plates for identification (5).

Samples were assayed for *R. solani* with a baiting technique (30) modified for use with water and slurry instead of soil. Unfiltered water, filtered water, and bottom sediment slurry (300 ml each) were added separately to a mixture of sterile washed sand and beet seed (1.5 L of sand with 200 beet seeds) in a 2-L beaker. After incubation at 25 C for 48 hr, the seeds were screened from the sand with running tap water, blotted dry, and placed 10 per plate on the medium of Papavizas et al (30). Plates were examined for growth of *R. solani* after 24 hr in the dark at 25 C, and representative isolates were transferred to PDA for anastomosis grouping and pathogenicity tests. Assays for nematodes were conducted by the Nematology Laboratory of the Cooperative Extension Service at the University of Georgia. Samples of unfiltered (1,500 ml) and filtered water (100 ml) were screened for nematodes and sediment samples were checked by the centrifugal-flotation method (16).

Bioassays. Pots (10 cm diameter) were filled with soil/sand/vermiculite (3:1:1, v/v) that had been fumigated with methyl bromide (454 g/m³). These were seeded with cotton (*Gossypium hirsutum* L. 'McNair 612'), pea (*Pisum sativum* L. 'Early Alaska'), Charleston Wakefield cabbage and Marion tomato, placed in growth chambers at 18 and 30 C, and watered with unfiltered water, filtered water, 1:1 sediment slurry, or tap water (check). Seedlings with postemergence damping-off, root rot, or stem rot were plated on water agar, and the fungi were transferred to appropriate media for identification.

Pathogenicity of fungal isolates from ponds. Representative fungal isolates from water and sediment samples were tested for pathogenicity in the greenhouse. In one test 27 isolates representing 13 *Pythium* spp. from pond water and bottom sediment were used to inoculate cotton, cabbage, pea, and tomato grown in temperature tanks at 18 and 30 ± 2 C. Inocula prepared by blending four 7-day-old cornmeal agar (Difco) cultures with enough water to give a final volume of 1 L were mixed by hand with 8 kg of methyl bromide-fumigated soil. Sterile 3.8-L metal cans were two-thirds filled with fumigated soil and the top was layered with 8 cm of infested soil. Each can was divided into four quadrants, each seeded with 20, 50, 20, and 50 seeds of cotton, cabbage, pea, and tomato, respectively, and the seeds were covered with 2 cm of infested soil. Four replications of each isolate and noninfested controls were arranged in a completely randomized design in each tank. Healthy plants were counted and disease severity was recorded after 3 wk. In another test, 8-day-old tomato and cabbage and 5-day-old cotton seedlings grown in 10 pots (10 cm diameter) each in fumigated soil were inoculated with each of three isolates of *R. solani* and one isolate of *R. zeae* Voorhees in the greenhouse where daytime temperatures ranged from 21–32 C. Fifty milliliters of inoculum (from one 7-day-old PDA culture blended in 200 ml of SDW) was injected with a syringe into each pot. Seedling survival and disease severity ratings were made after 10 days.

Survival of selected plant pathogens in a pond. *Pythium aphanidermatum* Edson (Fitz.), *R. solani*, and a *Sclerotium* sp. (probably *S. hydrophilum* Sacc.), which were selected as representative organisms from ponds, were reintroduced into a typical irrigation pond on a grower's farm to study survival. The organisms were placed in the pond in 200-ml containers (Fig. 2A) constructed from 3.8 cm diameter PVC Dresser couplings modified to hold a 53- μ m nylon mesh and a 5- μ m Gelman acropore filter at each end to retain the organisms but allow some water exchange. The containers were supported on an aluminum panel (Fig. 2B) suspended 1.0–1.6 m deep on an aluminum rod driven into the pond floor. A sleeve of PVC pipe placed over the rod stopped downward movement of the panel. *P. aphanidermatum* propagules as zoospores produced by floating mycelial plugs (27), as oospores produced in Schmitthenner's medium (1), and in infested ryegrass seed (grown on autoclaved seed for 14 days at 30 C) were placed in the containers separately at the rate of 50 zoospores or oospores per milliliter of container water and 100 ryegrass seeds per container. *R. solani* was placed in the containers as sclerotia (75 per container,

picked from 4-wk-old PDA cultures grown at 25 C) or in infested autoclaved cotton stem sections (75 per container, 1–2 cm long, grown for 14 days at 25 C). An undetermined number of sclerotia of the *Sclerotium* sp., harvested from 21-day-old PDA plates by flooding with SDW and scraping with a rubber policeman, were placed in the containers. The study was conducted from September 1977 to March 1978, and each organism-propagule treatment was replicated four times.

All containers were sampled initially when placed in the pond (0 days) and at various intervals until pathogens were no longer detected or until the study was terminated. Containers with zoospores were sampled at 0, 6, 12, and 28 days. Containers with oospores, *Pythium*-infested ryegrass seed, and *R. solani*-infested cotton stem sections were sampled at 0, 6, 12, 28, 53, 71, 96, and 185 days. Containers with sclerotia of *R. solani* and the *Sclerotium* sp. were sampled after 0, 17, 42, 60, 85, 175, and 0, 18, 43, and 133 days in the pond, respectively. Containers with *P. aphanidermatum* were sampled by pipetting 0.5-ml aliquots of zoospores or oospores on 10 plates per replicate or by plating 10 ryegrass seeds, five per plate, onto plates of P₁₀VPR. The ryegrass seeds were removed with forceps, washed in SDW, and blotted dry before plating. Sclerotia of *R. solani* (8–10) and infested cotton stems (8–10) were treated similarly to the ryegrass seed except that they were plated four or five per plate on a selective medium (30). All platings were made at the pond site; the plates were placed in petri dish holders and transported to the laboratory for incubation at the optimum temperature for the organism. Colonies on plates were counted after 1–3 days of incubation and representative colonies were transferred to appropriate media to confirm initial identification. At each sampling all containers were refilled with water from the same depth and location as the container panel to reintroduce resident microflora, and the filters were replaced on the end removed for sampling.

Water from the same depth and location as the container panel was tested each time for pH, alkalinity, dissolved O₂, CO₂, total hardness, Ca hardness, and free acidity with a Hach DR-EL Portable Engineer's Laboratory (Hach Chemical Company, Ames, IA 50010). Water temperature was checked at the surface, intermediate, and bottom depths with a Markson Model 5650 portable digital thermometer equipped with a remote sensing probe (Markson Science Inc., Del Mar, CA 92014). At the end of the survival study, four isolates of *P. aphanidermatum* originating from agar cultures of surviving oospores were tested on 8-day-old Charleston Wakefield cabbage, Marion tomato, Early Alaska pea, and McNair 612 cotton seedlings in a growth chamber at 30–32 C to determine if the organism maintained pathogenicity during the 185-day period in the pond.

RESULTS

Pathogens recovered from ponds. Water and bottom sediment samples collected from the eight ponds and assayed in the laboratory or in growth chamber bioassay tests yielded many common plant pathogens including *Pythium* spp. (*P. irregulare* Buis, *P. aphanidermatum*, *P. dissotium* Drechs., *P. spinosum* Saw., *P. vexans* de Bary, and 19 other *Pythium* spp.), *Fusarium* spp. (*F. oxysporum*, *F. solani*, and 11 other *Fusarium* spp.), *R. solani* and *R. zeae*, and *Macrophomina phaseolina*. The assays also yielded species of *Alternaria*, *Ascochyta*, *Aspergillus*, *Cladosporium*, *Diplodia*, *Phoma*, and *Rhizopus*. Twenty-one genera of purely saprophytic fungi, including *Trichoderma harzianum* and *T. viride* also were isolated.

A combination of the three sampling methods gave a better representation of the total microflora than either method alone, although bottom sediment usually yielded higher populations of most organisms than unfiltered or filtered water samples. For example, populations of *Pythium* spp. ranged from 0 to 0.7 propagules per milliliter in filtered and unfiltered water and from 0 to 78 propagules per milliliter in bottom sediment (Table 1). *Pythium* spp. always were detected by baiting methods even when undetectable with a selective medium. Populations of *Fusarium* spp. ranged from 0.2 to 3.4 and 22 to 6,200 propagules per milliliter

in water and bottom sediment, respectively (Table 1). Counts of total fungi ranged from 3 to 17 and from 560 to 1.1×10^5 propagules per milliliter in water and bottom sediment, respectively, and total bacteria ranged from 8×10^2 to 2.7×10^4 and from 2.4×10^3 to 1.2×10^7 colonies per milliliter in water and bottom sediment.

Random isolates (235 of *Pythium* spp. and 110 of *Fusarium* spp.) were selected during the study, transferred to appropriate media, and identified. *P. irregulare* was the most frequently occurring *Pythium* and accounted for 13.9% of isolates from all eight ponds and 38.2% of isolates from the two ponds sampled monthly. *F. solani*, *F. moniliforme*, *F. oxysporum*, and *F. lateritium* were the most frequently occurring species of *Fusarium*.

The two ponds sampled monthly each had a characteristic microflora, a portion of which remained constant during the sampling period. For example, one pond yielded *P. aphanidermatum*, mainly in bottom sediment, six of the seven times the pond was sampled. The other pond yielded *P. irregulare* consistently from water and sediment. *R. solani*, *R. zeae*, and *M. phaseolina* were isolated infrequently (five, four, and two times, respectively, from the two ponds sampled intensively) and usually occurred in bottom sediment. *R. solani* also was isolated several times from seedlings in the bottom sediment bioassays. Low populations of plant parasitic nematodes (*Hoplolaimus* sp., *Tylenchorhynchus* sp., and *Macroposthonia* sp.) were found infrequently and only in bottom sediment. Water collected from a field irrigation line and filtered through the MFA yielded *P. catenulatum* Matthews, *P. rostratum* Butler, *P. torulosum* Coker and Patterson, two unidentified *Pythium* spp., a *Phytophthora* sp.,

and a low number (14/150 L) of lance nematodes (*Hoplolaimus* sp.). Water from a deep well pumped through the MFA and assayed was free of plant pathogens.

Pathogenicity of isolates. All 27 isolates of 13 *Pythium* spp. from water or bottom sediment samples were pathogenic to one or more of the four hosts (cabbage, cotton, pea, and tomato) in tests in temperature tanks at 18 and 30 C (Table 2). Nine isolates representing *P. aphanidermatum*, *P. dissotomicum*, *P. irregulare*, and *P. spinosum* were pathogenic to all four crops at 18 C, and *P. aphanidermatum* was the most virulent species tested. Damage to the four hosts included various degrees of preemergence and postemergence damping-off, root rot, and stem rot. In another test 12 isolates of 10 species of *Pythium* and two unidentified species of *Phytophthora* (all from filtered water) were tested for pathogenicity at 18 and 30 C on the same four hosts with similar results.

Three AG-4 isolates of *R. solani* from filtered water or bottom sediment from two grower ponds varied in pathogenicity to cabbage, cotton, and tomato in greenhouse tests (Table 3). One isolate caused severe damping-off of cabbage and two were pathogenic to cotton, causing damping-off or stem rot. Two isolates caused damping-off or stem rot of tomato. The single isolate of *R. zeae* tested caused stem lesions on cotton but little or no damage to cabbage or tomato. No extensive testing was done to determine the pathogenicity of the *Fusarium* spp., miscellaneous fungi, and various bacteria isolated from irrigation ponds. However, in a limited test with 17 isolates of *Fusarium* spp. on cotton, corn, cabbage, and tomato, some isolates caused

TABLE 1. Populations of *Pythium* and *Fusarium* spp. in water and bottom sediment samples collected from eight surface irrigation ponds in the coastal plain area of Georgia in 1976 and 1977

Pond designation ^a	Date sampled	Mean number of propagules per ml ^b			
		<i>Pythium</i> spp.		<i>Fusarium</i> spp.	
		Pond water ^c	Bottom sediment	Pond water ^c	Bottom sediment
BSP	5 October 1976	0.0 ^d	0.0 ^d	1.6	110
	1 December 1976	0.0 ^d	5.8	0.9	86
N	30 September 1976	0.0 ^d	0.0 ^d	... ^e	... ^e
	1 December 1976	<0.01	13.0	2.8	260
S	22 October 1976	0.0 ^d	1.0	... ^e	... ^e
	8 December 1976	<0.01	2.0	0.6	300
T2	30 September 1976	0.0 ^d	0.0 ^d	... ^e	... ^e
	10 November 1976	<0.01	0.4	1.8	1800
NP	22 October 1976	0.0 ^d	9.2	... ^e	... ^e
	8 December 1976	<0.01	3.6	0.6	1100
	12 April 1977	0.07	19.0	1.6	6000
	12 May 1977	<0.01	22.0	... ^e	220
	14 June 1977	0.11	23.0	0.5	140
	13 July 1977	<0.01	8.0	0.8	660
	11 August 1977	0.67	78.0	3.4	1000
	14 September 1977	<0.01	28.0	0.8	6200
T1	3 September 1976	0.20	2.4	... ^e	... ^e
	10 November 1976	<0.01	11.0	0.8	4200
	12 April 1977	0.02	78.0	1.2	1400
	24 May 1977	0.10	20.0	... ^e	... ^e
	14 June 1977	<0.01	7.6	0.4	5700
	13 July 1977	0.01	8.0	0.6	5300
	11 August 1977	0.01	9.4	1.6	2200
M	5 August 1977	0.10	34.0	0.9	540
MV	1 June 1977	0.06	32.0	0.2	22

^aBSP = black shank pond, and N = nematode pond, both at the Coastal Plain Experiment Station, Tifton; NP = farm pond, Norman Park; T1 and T2 = farm ponds, Tifton; S = farm pond, Scottdale; M = farm pond, Moultrie; MV = station pond, Southeast Georgia Branch Experiment Station, Midville.

^bPlate counts of *Pythium* spp. were made on modified Kerr medium (19) until December 1976; then P₁₀VPR was used. Counts of *Fusarium* spp. were made on the medium of Nash and Snyder (29).

^cPlate counts for pond water represent the mean number of propagules obtained from filtered and unfiltered water samples.

^dZero indicates a population below the level of detection by plating 1-ml aliquots of water and diluted (1:1) bottom sediment slurry.

^eCounts were not possible due to massive bacterial growth.

discoloration or a mild rot of roots on one or more of the test plants. Several isolates of *Erwinia* spp. from bottom sediment caused no stalk rot of corn but caused rapid decay of raw potato sections and characteristic cuplike depressions in a pectate medium (6).

Survival in ponds. Different kinds of propagules of *P. aphanidermatum* differed in survival rates when placed in a grower's irrigation pond near Tifton in September 1977 and recovered at scheduled intervals until March 1978 (Fig. 3). Zoospores apparently died rapidly, because only 4.2% (based on initial counts at 0 days) produced colonies on P₁₀VPR medium when plated at 6 days and none produced colonies by 12 days. The organism survived throughout the test period on infested ryegrass seed; colony counts from plated seed declined to 42% after 185 days. Numbers of oospores forming colonies (percent of initial propagule counts) declined initially, increased sharply at 28 days, and again declined gradually until 17% produced colonies after 185 days. Four isolates randomly selected from germinating oospores after 185 days in the pond were still pathogenic to cabbage, cotton, pea, and tomato, as determined in a growth chamber test. *R. solani*, in the sclerotial form and in infested cotton stem sections, was progressively less viable throughout the test period and failed to overwinter (Fig. 4). Sclerotia of the *Sclerotium* sp. survived throughout the test period; 92% produced colonies after 133 days in the water.

At midlevel (1.2–1.4 m deep) water temperature dropped from 25.8 C on 14 September to 7.3 C on 13 December, and increased to 11.8 C by the time the final sample was taken on 13 March. The CO₂ content ranged from 2.0 to 24.0 mg/L, dissolved O₂ from 3.2 to 12.8 mg/L, and pH from 5.8 to 7.1 during the test period. Alkalinity showed a general downward trend from September through March. Calcium hardness fluctuated around a midpoint of 14 mg/L and total hardness varied around a midpoint of 25 mg/L. Free acidity was zero at each measurement.

DISCUSSION

Irrigation water from contaminated surface ponds is a probable source of inoculum for reinfestation of fumigated fields in southern Georgia. The pythiaceous fungi appear to be the greatest threat because they are well adapted to aquatic survival, occur commonly in irrigation ponds, and are important causal agents of seedling diseases, root rots, and stem rots of many plant species. *P. irregulare*, *P. aphanidermatum*, *P. spinosum*, and *P. dissoticum* isolated from irrigation ponds were highly virulent in our pathogenicity tests.

The high frequency of *P. irregulare* is especially important since this is also the most commonly occurring *Pythium* sp. in soils in Georgia (12). Although *P. aphanidermatum* occurred less frequently than *P. irregulare*, its presence in farm ponds is also significant because it is devastating to a wide range of hosts (26) and is particularly destructive to tomato transplants grown at high temperatures in southern Georgia (22). *P. aphanidermatum* and two other *Pythium* spp. were isolated from water used to irrigate fields of chrysanthemum in North Carolina (23), and *P. myriotylum* and an unidentified *Pythium* sp. were isolated with a baiting technique from an irrigation pond near Tifton, GA (10). *P. myriotylum* was not isolated, possibly because our assays were not as sensitive as the living plant baiting technique used by Gill (10). Oospores of *P. myriotylum* probably were not detected in our assays because they germinate poorly even after stimulation or shock treatments (2).

Several *Phytophthora* spp. were isolated in our studies. *Phytophthora* spp. also were isolated from irrigation waters in Arizona (35) and California (20) and were linked to an outbreak of brown rot of citrus after sprinkler irrigation in Florida (37). Dukes et al (7) isolated *Phytophthora parasitica* var. *nicotianae*, the tobacco black shank fungus, from surface irrigation ponds in southern Georgia.

TABLE 2. Pathogenicity of 27 *Pythium* isolates from water and bottom sediment collected from irrigation ponds and tested on four hosts at two soil temperatures in water bath tanks

Isolate ^a	Mean number of healthy seedlings (% of control) ^b							
	18 C				30 C			
	Cabbage	Cotton	Pea	Tomato	Cabbage	Cotton	Pea	Tomato
<i>P. acanthicum</i>	93 ^c	72	100	85*	69*	81	103	80
<i>P. aphanidermatum</i>	34**	0**	0**	1**	1**	0**	0**	0**
<i>P. aphanidermatum</i>	47**	0**	98	0**	0**	0**	0**	1**
<i>P. catenulatum</i>	88	35**	100	96	45**	78	103	85
<i>P. catenulatum</i>	82	35**	95	98	72*	92	100	88
<i>P. catenulatum</i>	90	0**	102	86*	44**	95	103	77
<i>P. dissoticum</i>	98	27**	110	103	52**	81	99	93
<i>P. dissoticum</i>	67**	0**	0**	0**	1**	7**	0**	0**
<i>P. dissoticum</i>	75*	35**	103	90	62*	76	96	66**
<i>P. inflatum</i>	85	58**	102	104	41**	78	51**	86
<i>P. inflatum</i>	96	90	104	96	38**	95	76	83
<i>P. irregulare</i>	8**	0**	0**	0**	11**	74	82	26**
<i>P. irregulare</i>	1**	0**	0**	0**	1**	48*	84	31**
<i>P. irregulare</i>	1**	0**	0**	0**	4**	86	84	59**
<i>P. paroecandrum</i>	100	35**	101	97	54**	92	78	86
<i>P. rostratum</i>	91	32**	101	99	82*	65	98	80
<i>P. rostratum</i>	94	42**	101	101	40**	65	73	95
<i>P. spinosum</i>	29**	0**	0**	0**	37**	48*	102	67**
<i>P. spinosum</i>	51**	0**	0**	0**	41**	70	100	69**
<i>P. splendens</i>	53**	0**	0**	1**	48**	83	83	48
<i>P. splendens</i>	80	45**	76*	94	99	95	77	96
<i>P. sylvaticum</i>	86	35**	102	93	86*	102	96	53**
<i>P. sylvaticum</i>	8**	0**	0**	0**	15**	76	76	93
<i>P. sylvaticum</i>	49**	10**	103	48**	56**	81	102	93
<i>P. torulosum</i>	91	28**	100	88	96	81	78	78
<i>P. torulosum</i>	87	38**	100	95	98	74	91	98
<i>P. vexans</i>	47**	38**	104	92	83*	92	72*	87

^a All isolates were obtained from pond water (filtered or unfiltered) or bottom sediment from six surface irrigation ponds in the coastal plain area of Georgia during 1976.

^b Each value is a mean of four replications each consisting of 50 Charleston Wakefield cabbage, 20 McNair 612 cotton, 20 Early Alaska pea, and 50 Marion tomato seeded in 3.8-L metal cans filled with infested soil. Healthy plants were those surviving without lesions or postemergence damping-off.

^c Single and double asterisks denote that values are significantly different from checks at $P=0.05$ and 0.01 , respectively, as determined by the LSD test.

TABLE 3. Pathogenicity of four isolates of *Rhizoctonia* spp. from irrigation ponds on three crops in the greenhouse^a

Isolate ^c	Mean number of healthy seedlings (% of control) ^b		
	Cabbage	Cotton	Tomato
<i>R. solani</i>	61	0** ^d	47**
<i>R. solani</i>	90	75	50**
<i>R. solani</i>	3**	0**	100
<i>R. zeae</i>	95	50*	100

^aThe test was conducted in the greenhouse with 21–32 C temperatures. Each value is a mean of 10 replications consisting of pots with 75 Early Jersey Wakefield cabbage, 20 McNair 612 cotton, and 75 Marion tomato. Plants were inoculated with mycelial fragment suspensions approximately 1 wk after emergence.

^bHealthy plants were those surviving without lesions or postemergence damping-off.

^cIsolates were obtained from filtered water or bottom sediment of irrigation ponds.

^dSingle and double asterisks denote values that are significantly different from checks at $P = 0.05$ and 0.01 , respectively, as determined by the LSD test.

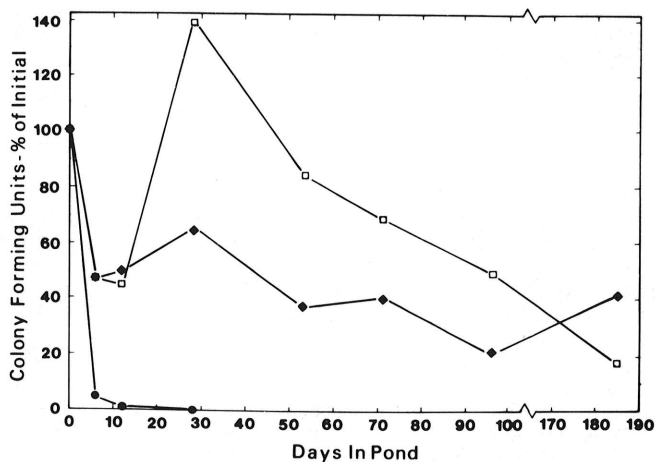


Fig. 3. Survival of *Pythium aphanidermatum* in microorganism containers suspended in a surface irrigation pond near Tifton, GA, from September 1977 to March 1978. Plotted values are percent of zoospores (●—●), oospores (□—□), and infested ryegrass seed (◆—◆) producing colonies on P₁₀VPR medium after various periods in the pond. All survival structures were plated initially (0 days) and all other values are based on initial plate counts.

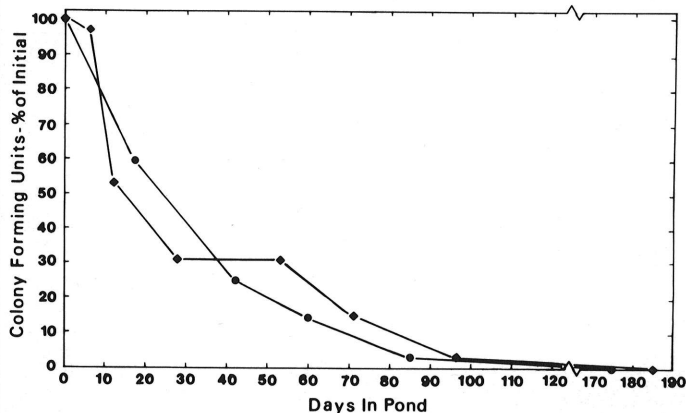


Fig. 4. Survival of *Rhizoctonia solani* in microorganism survival containers suspended in a surface irrigation pond near Tifton, GA, from September 1977 to March 1978. Plotted values are percent sclerotia of *R. solani* (●—●) and *R. solani*-infested cotton stem sections (◆—◆) producing colonies on Papavizas' medium after various periods in the pond. All survival structures were plated initially (0 days) and all other values are based on initial plate counts.

The infrequent recovery of *R. solani* in our survey and its poor adaptation to an aquatic environment probably means that this pathogen is of limited importance in irrigation water except in association with organic debris in bottom sediment for relatively short periods. However, some *R. solani* isolates from ponds were highly virulent on test crops in the greenhouse. *R. solani* was previously found in greenhouse water supplies from streams and ponds in England (3).

Our limited pathogenicity tests do not allow a conclusion concerning the threat of the various *Fusarium* spp. and species of certain other genera isolated from farm ponds. The presence of pathogenic *Fusarium* spp. in ponds suggests that these organisms are disseminated by irrigation systems. Both *Verticillium albo-atrum* (8) and *Whetzelina sclerotiorum*, the bean white mold organism (33), are disseminated in irrigation waters.

Additional research with selective isolation methods is needed to define the role of irrigation water in disseminating plant pathogenic bacteria to transplant fields. Circumstantial evidence suggests that use of contaminated irrigation water in southern Georgia causes bacterial soft rot diseases of corn and of turnip grown for greens (J. D. Gay, unpublished). Irrigation systems have been implicated in disseminating plant pathogenic bacteria attacking bean (31,33), onion (15), and corn (18,34).

We conclude that plant parasitic nematodes do not occur frequently enough or in sufficient numbers to cause a serious reinfestation problem in fumigated fields in Georgia, although they are disseminated from certain irrigation canals (9). Recovery of several plant pathogenic fungi and a few lance nematodes from an irrigation line in a grower's field proves that they are disseminated along with irrigation water.

Plant pathogens are of most concern if they are capable of surviving long enough to be recycled after being washed into surface ponds. *P. aphanidermatum* and *R. solani* both survive long enough to be redistributed as resistant structures. Surface ponds are frequently replenished by runoff, and transplants are irrigated once or twice daily during the early stages of growth. *Pythium* spp. probably survive for long periods in irrigation ponds as oospores or in infested organic matter since zoospores were recovered for only a few days after being released in containers placed in a farm pond. The increase in propagule counts for oospores (Fig. 3) on day 28 may have been due to the after-ripening effect noted by Ayers and Lumsden (2). Propagules of *P. aphanidermatum* apparently survive for shorter periods in ponds than in certain soils (32). *R. solani* survives in soil as sclerotia or in particles of debris as thick-walled hyphae (4) and may survive the same way for shorter periods in water. Adverse temperature, absence of suitable substrates, and microbial activity probably influenced the survival of the various propagules in pond water, because none of the chemical parameters were extreme enough to influence survival capacity greatly.

Use of water contaminated with plant pathogens apparently is a major factor limiting the success of general purpose fumigants in transplant production. Our studies suggest that constructing ponds to prevent undesirable runoff and the use of floating inlet pipes to minimize intake of bottom sediment during pumping will reduce contamination. The feasibility of chemical treatment of water in irrigation systems or holding ponds is being investigated. The use of deep wells as a water source is the most desirable solution but is more expensive than the use of surface ponds.

LITERATURE CITED

- ADAMS, P. B. 1971. *Pythium aphanidermatum* oospore germination as affected by time, temperature, and pH. *Phytopathology* 61:1149-1150.
- AYERS, W. A., and R. D. LUMSDEN. 1975. Factors affecting production and germination of oospores of three *Pythium* species. *Phytopathology* 65:1094-1100.
- BEWLEY, W. F., and W. BUDDIN. 1921. On the fungus flora of glass-house water supplies in relation to plant disease. *Ann. Appl. Biol.* 8:10-19.
- BOOSALIS, M. G., and A. L. SCHAREN. 1959. Methods for microscopic detection of *Aphanomyces euteiches* and *Rhizoctonia solani* and for isolation of *Rhizoctonia solani* associated with plant debris. *Phyto-*

- pathology 49:192-198.
5. BOOTH, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England. 237 pp.
 6. CUPPELS, D., and A. KELMAN. 1974. Evaluation of selective media for isolation of soft rot bacteria from soil and plant tissue. *Phytopathology* 64:468-475.
 7. DUKES, P. D., S. F. JENKINS, Jr., and S. S. THOMPSON, Jr. 1977. Detection and some observations of *Phytophthora parasitica* var. *nicotianae* in ponds used for irrigation of tobacco. *Tob. Sci.* 21:97-100.
 8. EASTON, G. D., M. E. NAGLE, and D. L. BAILEY. 1969. A method of estimating *Verticillium albo-atrum* propagules in field soil and irrigation waste water. *Phytopathology* 59:1171-1172.
 9. FAULKNER, L. R., and W. J. BOLANDER. 1970. Agriculturally-polluted irrigation water as a source of plant-parasitic nematode infestation. *J. Nematol.* 2:368-374.
 10. GILL, D. L. 1970. Pathogenic *Pythium* from irrigation ponds. *Plant Dis. Rep.* 54:1077-1079.
 11. GRIMM, G. R., and A. F. ALEXANDER. 1973. Citrus leaf pieces as traps for *Phytophthora parasitica* from soil slurries. *Phytopathology* 63:540-541.
 12. HENDRIX, F. F., and W. A. CAMPBELL. 1969. Distribution of *Phytophthora* and *Pythium* species in soils in the continental United States. *Can. J. Bot.* 48:377-384.
 13. HENDRIX, F. F., and K. E. PAPA. 1974. Taxonomy and genetics of *Pythium*. *Proc. Am. Phytopathol. Soc.* 1:200-207.
 14. HINE, R. B., and L. V. LUNA. 1963. A technique for isolating *Pythium aphanidermatum* from soil. *Phytopathology* 53:727-728.
 15. IRWIN, R. D., and E. K. VAUGHAN. 1972. Bacterial rot of onion and the relation of irrigation water to disease incidence. (Abstr.) *Phytopathology* 62:1103.
 16. JENKINS, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Dis. Rep.* 48:692.
 17. JOHNSON, L. F., E. A. CURL, J. H. BOND, and H. A. FRIBOURG. 1959. Methods for studying soil microflora-plant disease relationships. Burgess Publishing Co., Minneapolis, MN. 178 pp.
 18. KELMAN, A., L. H. PERSON, and T. T. HEBERT. 1957. A bacterial stalk rot of irrigated corn in North Carolina. *Plant Dis. Rep.* 41:798-802.
 19. KERR, A. 1963. The root rot-Fusarium wilt complex of peas. *Austr. J. Biol. Sci.* 16:55-69.
 20. KLOTZ, L. F., PO-PING WONG, and T. A. DeWOLFE. 1959. Survey of irrigation water for the presence of *Phytophthora* spp. pathogenic to citrus. *Plant Dis. Rep.* 43:830-832.
 21. LIND, O. T. 1974. Handbook of common methods in limnology. C. V. Mosby Co., St. Louis, MO. 154 pp.
 22. LITTRELL, R. H., and S. M. McCARTER. 1970. Effect of soil temperature on virulence of *Pythium aphanidermatum* and *Pythium myriotylum* to rye and tomato. *Phytopathology* 60:704-707.
 23. LUMSDEN, R. D., and F. A. HAASIS. 1964. *Pythium* root and stem diseases of chrysanthemum in North Carolina. N.C. Agric. Exp. Stn. Tech. Bull. 158. 27 pp.
 24. McCARTER, S. M., C. A. JAWORSKI, and A. W. JOHNSON. 1978. Effect of continuous plant culture and soil fumigation on soilborne pathogens and on growth and yield of tomato transplants. *Phytopathology* 68:1475-1481.
 25. McCARTER, S. M., C. A. JAWORSKI, A. W. JOHNSON, and R. E. WILLIAMSON. 1976. Efficacy of soil fumigants and methods of application for controlling southern blight of tomatoes grown for transplants. *Phytopathology* 66:910-913.
 26. McCARTER, S. M., and R. H. LITTRELL. 1970. Comparative pathogenicity of *Pythium aphanidermatum* and *Pythium myriotylum* to twelve plant species and intraspecific variation in virulence. *Phytopathology* 60:264-268.
 27. McCARTER, S. M. and R. H. LITTRELL. 1973. Factors influencing zoospore production by *Pythium aphanidermatum* and *Pythium myriotylum*. *Bull. Ga. Acad. Sci.* 31:183-190.
 28. MIDDLETON, J. T. 1943. The taxonomy, host range and geographic distribution of the genus *Pythium*. *Mem. Torrey Bot. Club* 20:1-171.
 29. NASH, S. M., and W. C. SNYDER. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathology* 52:567-572.
 30. PAPAIVIZAS, G. C., P. B. ADAMS, R. D. LUMSDEN, J. A. LEWIS, R. L. DOW, W. A. AYERS, and J. G. KANTZES. 1975. Ecology and epidemiology of *Rhizoctonia solani* in field soil. *Phytopathology* 65:871-877.
 31. SCHUSTER, M. L. 1959. Relation of root-knot nematodes and irrigation water to the incidence and dissemination of bacterial wilt of bean. *Plant Dis. Rep.* 43:27-32.
 32. STANGHELLINI, M. E., and E. L. NIGH, Jr. 1972. Occurrence and survival of *Pythium aphanidermatum* under arid soil conditions in Arizona. *Plant Dis. Rep.* 56:507-510.
 33. STEADMAN, J. R., C. R. MAIER, H. F. SCHWARTZ, and E. D. KERR. 1975. Pollution of surface irrigation waters by plant pathogenic organisms. *Water Resour. Bull.* 11:796-804.
 34. THOMPSON, D. L. 1965. Control of bacterial stalk rot of corn by chlorination of water in sprinkler irrigation. *Crop Sci.* 5:369-370.
 35. THOMPSON, S. V., and R. M. ALLEN. 1974. Occurrence of *Phytophthora* species and other potential plant pathogens in recycled irrigation water. *Plant Dis. Rep.* 58:945-949.
 36. TSAO, P. H., and G. OCANA. 1969. Selective isolation of species of *Phytophthora* from natural soils on an improved antibiotic medium. *Nature* 223:636-638.
 37. WHITESIDE, J. O., and T. W. OSWALT. 1973. An unusual brown rot outbreak in a Florida citrus grove following sprinkler irrigation with *Phytophthora*-infested water. *Plant Dis. Rep.* 57:391-393.