Inoculum Sources and Characterization of Isolates of *Gilbertella persicaria* from Peach Fruit in South Carolina

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ABSTRACT

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In 1991 and 1992, *Gilbertella persicaria* was more common than *Rhizopus* spp. on rotted peaches found on the orchard floor in the major peach production areas of South Carolina. *G. persicaria* was isolated frequently from soil and surface organic debris in commercial peach orchards in five counties. *Rhizopus* spp. were isolated much less frequently from these sources. Also, *G. persicaria* was commonly found along with *Rhizopus* spp. in soil and debris collected from harvest bins before and during harvest, in hydrocooling water and dump tank water, and on packing line belts. Dicloran and iprodione were both active in vitro to inhibit growth of mycelium, but chlorinated water dips were required for control of fruit decay caused by *G. persicaria*. Selected isolates of *G. persicaria* grew and sporulated at 10 to 40°C and induced symptoms within 4 days at 22 to 34°C. External nutrients were required for spore germination, and wounding was required for infection of peach fruit.

Postharvest decay of peaches (*Prunus persica* (L.) Batsch) associated with *Monilinia* and *Rhizopus* spp. is important in all production areas of the United States, including South Carolina. Many other fungi also cause decay of peach fruit, and sometimes economic loss is significant. Recently, *Gilbertella persicaria* (E.D. Eddy) Hesseltine was isolated frequently in South Carolina peach orchards (4). *G. persicaria* causes fruit decay of apricot (*Prunus armeniaca* L.) (14), pear (*Pyrus communis* L.) (8), apple (*Malus* × *domestica* Borkh.) (9), and tomato (*Lycopersicon esculentum* Mill.) (7) in addition to peach (12), but it has not been of major concern to the fruit industry except in California (3,14). Many reports of fruit decay caused by *Rhizopus* spp. do not identify the fungus to species. It is not clear whether these reports have carefully distinguished Rhizopus decay and Gilbertella rot, because symptoms and signs of these two diseases are easily confused. However, accurate diagnosis is important because Ogawa et al. (16) reported that variants of *G. persicaria* could tolerate high concentrations of dicloran, a fungicide recommended until recently for postharvest decay control. These reports indicated a need for careful evaluation of the importance of *G. persicaria* in fruit decay in South Carolina.

Objectives of this study were to evaluate the occurrence of *G. persicaria* in peach production areas and to determine the possible significance of this pathogen in postharvest decay in South Carolina. Sensitivity of isolates to recommended fungicides and chlorinated water was measured to evaluate the efficacy of current decay-control practices. Requirements of exogenous nutrients and wounding for infection were examined to characterize the probable mode of infection for local isolates. The effects of temperature on growth, sporulation, and infection were measured to further characterize South Carolina isolates.

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MATERIALS AND METHODS

Sources of inoculum. *Orchard floor.* The prevalence of pathogenic isolates of *G. persicaria* on rotting peaches collected from the ground in commercial orchards was determined during two growing seasons. During 1991, four to 10 decaying fruit were collected from each of 14 orchards distributed among five counties representing two major peach production areas in the state. Selection of decayed fruit was based on the occurrence of sporangiophores on the fruit that were typical of the Mucorales. Fruit were collected during June and July and placed in plastic bags that were packed in a cool container for transport to the laboratory. In 1992, a random sample of 100 fruit from the orchard floor was collected from each of 10 orchards distributed among four counties. The fruit were handled and processed similarly to the previous year. Fungi were isolated from collected fruit in the laboratory by transferring sporangia and sporangiophores to potato-dextrose agar (Difco, Detroit, MI) acidified with 1.5 ml (85% vol/vol) of lactic acid per liter of medium (APDA).

Soil and surface debris under two or three randomly selected peach trees for each of 11 orchards were sampled to determine the occurrence of *G. persicaria* and *Rhizopus* spp. Three orchards were sampled during July and August of the 1991 season, and seven orchards were sampled from April to July in 1992. Three 5-cm-deep soil cores (2 cm diameter) were removed from each of four quadrants under each tree sampled. Cores were combined in a plastic bag to provide a composite sample for each tree. Surface debris was collected in plastic bags from the same sites as soil samples. All samples were placed in a cool container for transport to the laboratory. Fresh and dry weights (105 to 110°C for 15 h) were determined to calculate moisture content. Fungal populations were estimated by dilution-plating techniques. Subsamples were blended in sterile water for 2 min, and 0.1 ml each of 10^{-2} and 10^{-3} dilutions for soil or 10^{-2}, 10^{-3}, and 10^{-4} dilutions for debris were plated on four replicate plates of potato-dextrose agar supplemented with Rose Bengal (40 µg/ml), streptomycin (60 µg/ml), and chloramphenicol (60 µg/ml) (PDA-RSC). Colonies were identified and counted after 2 and 3 days at 25°C. The count determined from the daily observations for each plate was used to calculate the number of colony-forming units per gram of dry matter averaged over the replicate platings.

Orchard air. Air was sampled with an Anderson spore sampler (Anderson Samplers Inc., Atlanta, GA) on 6 days for spores in an orchard at the Clemson University Experiment Station, Pendleton, SC, from 22 June through 4 August 1992. One to 3 days prior to spore collection, fruit infected with *G. persicaria* and covered with sporangia were distributed over a 34 × 53 m area around the spore sampler. The number of fruit distributed in the orchard varied from 100 to 344. For three sampling days in August, an additional 60 fruit infected with *Rhizopus* sp. were distributed over a 27 × 27 m area around the spore sampler 1 day before spore collection for the next 3 days.

The spore sampler was operated at 28.3 liters/min of air for 5 or 10 min beginning at 8:00, 13:30, or 20:00 h each sample day. In general, two sample positions or times of day were selected for each day of sampling. Most commonly, two samples per day (at 13:30 and 20:00 h for 5 min) at 1 m above the soil surface were collected. On two occasions, sampling was done at 8:00 h, and once a height of 2 m was compared with the standard 1-m vertical level of spore collection. All exposed plates (PDA-RSC) were incubated at 25°C for up to 5 days. Fungal colonies were identified and counted daily after the first 2 days of incubation. Duplicate samples were collected at each sampling so that the total number of samples collected was 22.

Packing lines. Five packing houses were visited before harvest in the 1992 season. Six packing houses were visited in 1991 and four in 1992 during the harvest. For most houses visited, harvest bins and three sites along packing lines were swabbed with sterile cotton tips to detect viable propagules of *G. persicaria* and *Rhizopus* spp. Each site was swabbed with five cotton tips, which then were placed in 50 ml of sterile distilled water. In summary, 13 bins and 15 sites along the packing line were swabbed prior to harvest in 1992, and 24 bins and 89 sites along the packing line were swabbed during the harvest in 1991 and 1992.

Soil and plant debris also were collected from bins and packing lines during the preharvest visits in 1992. Samples (100 ml) of hydrocooling water were collected from packing lines that were in operation at the time of the visits. For two packing houses, water samples (100 ml) were collected from the dump tanks. Overall, 24 water samples were collected over the two seasons. All samples were transported in a cool container to the laboratory for testing. For hydrocooling and dump tank water samples, the pH was measured and total available chlorine was determined with a ferrous ammonium sulfate titration kit (LaMotte Chemical Products Co., Chestertown, MD). When chlorine was detected, it was neutralized with sodium thiosulfate prior to attempts to isolate fungi. Water samples and swab samples were diluted and plated on PDA-RSC to determine the propagule densities of the fungi. Soil and debris samples were processed as described above. Four replicate plates were incubated at 25°C for 3 days before the fungal colonies were identified and counted.

Fruit was collected from packing lines from six packing houses during the 1991 harvest season (80 fruit per location) and from four packing houses in 1992 (60 fruit per location). The fungicides used on the packing lines were recorded for each packing house. Peaches collected were incubated at 23 to 26°C for 5 days. Causes of fruit decay were identified from symptoms, and sporulation was detected on the surface of developing lesions.

Characterization of isolates. Isolates were grown on synthetic Mucor agar (SMA) (5) for identification to genus (6). Identification of *G. persicaria* was based on description of the species (5) and comparison with known isolates (NRRL 1546, a – mating type, and NRRL 2357, a + mating type) of *G. persicaria* obtained from K. L. O'Donnell, Northern Regional Research Center, USDA-ARS, Peoria, IL. No attempt was made to determine the species of isolates that were not identified as *Gilbertella* sp. Eight isolates of *G. persicaria* were selected as representative cultures from sampled regions of the state, and these isolates were evaluated and characterized.

Spore suspensions. For experiments that required spore suspensions, sporangiospores were collected in the following manner. Spores from sporulating cultures (4 to 6 days at 28°C) were suspended in a sterile aqueous solution of Tween 80 (1 µl/ml) by agitation of the solution on cultures. Suspensions were filtered through several layers of sterile cheesecloth to remove mycelium and sporangiophores. Inoculum was calibrated by counting spores with a hemacytometer under a microscope.

Pathogenicity tests. Firm, ripe peach fruit (cv. Cresthaven, Springcrest, or Springbrite) were washed, submerged in sodium hypochlorite (5.3 µl/ml), and then rinsed in sterile distilled water. A 20-µl drop of inoculum (1×10^4 spores per ml) was placed on a 2-mm-deep wound caused by pressing a 4-mm-diameter glass rod into the fruit. Five inoculated fruit per isolate were incubated at 21 to 26°C on wire mesh in covered plastic boxes for 5 days. Symptoms and signs were recorded daily during incubation.

Mating types. Eight isolates of *G. persicaria* were mated according to the methods of O'Donnell et al (11) with NRRL 1546 and NRRL 2357 to determine mating type and provide zygospores to confirm identifications.

Temperature effects on spore germination. Spore suspensions were prepared as described above from four isolates collected from different counties in South Carolina. Spores were pelleted by centrifugation and rinsed in sterile distilled water twice before being resuspended in potato-dextrose broth to a concentration of 1×10^4 spores per ml. Aliquots (200 µl) were incubated at seven different temperatures in the range of 4 to 40°C on sterile glass microscope slides that were placed in covered plates on glycerol agar (1). The number of spores germinated of the first 100 spores observed was recorded at 4, 8, 12, 24, and 48 h after initiation of incubation. Three observations were made at each temperature, and these were combined for each isolate. Isolates then were treated as replicates for statistical analysis. The experiment was repeated once. The rate of spore germination per hour during each observation period was averaged for observations made prior to 100% germination.

Temperature effects on mycelial growth. Plates with PDA were incubated at seven temperatures in the range of 4 to 40°C for 24 h prior to the introduction of four isolates of *G. persicaria.* Mycelial plugs of 4 mm diameter from 1- or 2-day-old cultures (21 to 24°C) were placed in the center of three culture plates for each temperature. Plates were enclosed in perforated plastic bags and returned to the same temperature as that used for equilibrating the medium. Colony diameter was measured as the average of two perpendicular measurements at 12 and 24 h and then daily for 4 days (six measurements). The test was repeated once. Fungal growth accelerates as a colony forms on petri dishes, so growth rates were standardized by computing the estimated growth rate when the colony reached 30 mm in diameter. Each measurement minus 4 mm, the starting diameter, was divided by twice the number of days since initiation of growth to compute a radial rate of growth. Growth rates for measurements made before and after the colony reached 30 mm in diameter were used to estimate the time required for the colony to reach that size, and the mean of these two estimates was used to compute the standardized rate of radial growth. The standardized growth rate (mm/day) was assumed to be linear with respect to temperature in the range above a minimum temperature for growth and below the injury threshold. The injury threshold was assumed to be above 28°C. Estimation of the rate of growth of mycelium with respect to temperature was accomplished by adopting a degree-day model for expected values, $R(T)$, of the observed average rate of growth (mm diameter per day) of mycelium at incubation temperature T: $R(T) = r(T - \tau)$, where r is the rate constant in the form of mm growth per degree-day, T is the incubation temperature, and τ is the base temperature below which no growth is expected. This model was fitted to standardized rates of mycelial growth by nonlinear least squares analysis, with each fungal isolate treated as a replicate. Four isolates were included in one trial, and three isolates were in a second trial. Asymptotic standard errors were determined for parameters estimated from fitting the model.

Sensitivity to chlorine. To determine the efficacy of chlorine against *G. persicaria,* sporangiospores (1×10^3 spores per ml) were exposed to dilutions in phosphate buffer (pH 6.7 to 6.8) of a commercial sodium hypochlorite solution (52.5 mg/ml) or to buffer alone. After 5- to 20-min exposure, chlorine was neutralized with equal volumes of 1.4 mM sodium thiosulfate (17,18). Aliquots of the resultant spore

suspension (100 µl each) were spread on five replicated petri dishes containing PDA-RSC, and colonies that formed were counted. Pathogenicity of spores in treated suspensions was determined as described previously, except that 40 µl of the final spore suspension (ca. 20 spores) was applied to wounds on peaches.

Sensitivity to fungicides. In vitro sensitivity of mycelium and sporangiospores of *G. persicaria* to fungicides was determined (15) for isolates from Orangeburg (O10) and Edgefield (E1) counties. Dicloran (Botran 75W), iprodione (Rovral 50WP), triforine (Funginex 50WP), vinclozolin (Ronilan 50WG), and captan (Captan 75WDG) were incorporated into cool (50°C) molten SMA, and 27 ml was dispensed in petri dishes (100 mm diameter). Each fungicide was prepared at concentrations of 1, 4, 10, 40, 100, or 400 µg a.i./ml. Unamended SMA provided a control treatment. Mycelial plugs (5 mm diameter) from 1-day-old cultures grown on PDA were placed in the center of three dishes and incubated at 25°C. Colony diameters were recorded at 12-h intervals. The rate of mycelial extension per hour for each replicate was averaged from the time of first detectable growth until the time before coverage of the petri dish. These rates of growth then were treated as replicates to determine treatment means and standard deviations. About 1,000 sporangiospores from isolate E1 were spread over a second set of the fungicide-amended dishes (two replicates), and the morphological appearance of germinating spores was characterized for the first 100 spores observed under a microscope at 4, 8, 24, 48, and 96 h.

Disease suppression was determined on inoculated peach fruit for iprodione, dicloran, and fluazinam. Fruit were prepared and inoculated with one isolate as described for pathogenicity tests, except that the fruit were brushed lightly to remove most trichomes prior to surface disinfestation. Inoculated fruit was allowed to dry prior to fungicide treatment. Three replicated treatments consisting of five fruit each were dipped in suspensions of iprodione (1.2 mg a.i./ml), dicloran (0.9 mg a.i./ml), or fluazinam (312.5 µg a.i./ml) and allowed to air-dry. Treated fruit were incubated at 23 to 26°C in plastic boxes, and lesion diameters were recorded after 3 days. Incidence of fruit rot among treated peaches was recorded after 2, 3, and 4 days.

Requirements for infection. *Nutrients and injury.* External nutrient requirements for spore germination were determined for one isolate. The effects of external nutrients and plant injury on successful infection were determined at the same time. Peach juice was prepared by crushing fresh fruit and straining it through sterile cheesecloth (10). Sporangiospores were collected as described above. Aliquots were washed four times by centrifugation in sterile distilled water or left unwashed. Suspensions of washed spores (1×10^4 spores per ml) were prepared in sterile distilled water or peach juice diluted 1:3 with sterile distilled water (10) and then incubated at 23 to 26°C for 18 h on a shaker or applied to SMA or water agar. Unwashed spores also were incubated on water agar or in sterile distilled water. Spore germination was assessed after 6 and 18 h by scoring the first 100 spores observed with a microscope.

For tests of spore preparation on infection efficiency, washed spores in sterile distilled water or diluted peach juice were prepared as above. Fruit were brushed lightly to remove trichomes and then punctured 1 mm deep with a 4-mm glass rod. Control fruit were brushed but not punctured. Three fruit per treatment were used in the first experiment, and 10 were used in a repetition of the experiment. Additionally, five and 16 uninjured fruit were inoculated in two other tests, respectively, by adding 150 µl of the spore suspensions to plastic rings (10 mm diameter × 3 mm high) placed on the surface of the fruit. After 4 days, spores from water treatments were removed from fruit and placed on APDA to determine incidence of germination.

Additional fruits were selected based on the appearance of harvest injuries on the stem end. Five categories were established: (i) uninjured fruit without the pedicel attached, (ii) uninjured fruit with the pedicel attached, (iii) split endocarp on stem end, (iv) slight injury to exocarp in stem end, and (v) as in the first category, but with a puncture to stem end by insertion of a sterile scalpel blade. Three and 10 fruit in each of these categories were inoculated by placing 40 µl of a spore suspension on the stem end in two separate tests, respectively. Suitability of inoculum was assayed at the time of each experiment by inoculation (1×10^4 spores per ml) in the manner described for tests of pathogenicity. Fruit incubation and assessment of infection were as described for pathogenicity tests.

Single-spore infections. Ungerminated spores from a collection of spores spread on water agar were transferred individually into wounds on 18 peach fruit. Wounds were created in firm, ripe peach fruit by puncturing them with a glass rod as described for pathogenicity tests. Inoculated fruit were incubated at 23 to 26°C in closed plastic boxes. The experiment was repeated once.

Temperature. Tests of pathogenicity were conducted for two isolates at 22, 28, and 34°C in standard incubators without light. Isolates were from Edgefield and Spartanburg counties. Infection was scored as with tests of pathogenicity, and the experiment was replicated 10 times and repeated once.

RESULTS

Sources of inoculum. *Orchard floor. G. persicaria* was found frequently in 1991 and 1992 on selected rotting peach fruit in commercial peach orchards in South Carolina (Tables 1 and 2). No rotted peach fruit was discovered in two orchards in Orangeburg county or an orchard in Oconee county during early-season visits in May 1992. *Rhizopus* spp. also were detected, but less frequently, in some of the same orchards. *Mucor* spp. on fruit were not detected. Viable propagules of *G. persicaria* were found in soil, debris, or both in all orchards sampled. Propagules of

Table 1. Number of rotted peaches from which isolates of *Gilbertella persicaria* or *Rhizopus* spp. were obtained. Fruit were collected from the orchard floor of commercial orchards in five counties of South Carolina in 1991[a]

County	Orchards	*G. persicaria*	*Rhizopus* spp.
Anderson	3	21	1
Edgefield	3	17	0
Orangeburg	4	18	0
Spartanburg	3	18	3
York	1	4	0

[a] Up to 10 rotted peaches were collected from each orchard and fungi were isolated and identified from each.

Table 2. Percentage of peach fruit decayed among approximately 100 fruit examined on orchard floors in four counties in South Carolina during the 1992 ripening season

			Percent fruit decayed			
Date	County	Site	*Gilbertella*	*Rhizopus*	*Monilinia*	Other[a]
10 June	Orangeburg	a	64.9	6.4	18.1	1.1
	Orangeburg	b	7.6	0.0	72.7	1.5
16 June	Edgefield	a	3.0	0.0	6.0	4.0
	Edgefield	b	10.9	10.9	47.8	6.6
	Edgefield	c	11.3	0.0	61.3	1.6
6 July	Spartanburg	a	19.3	1.2	6.0	7.2
	Spartanburg	b	20.0	10.0	29.0	16.0
10 July	Oconee	a	41.1	0.0	35.7	2.7

[a] Other included *Botrytis, Penicillium, Botryosphaeria,* and *Colletotrichum.*

Rhizopus spp. were detected in three of 11 sites sampled during these two seasons.

Orchard air. Only a single propagule of *G. persicaria* was detected (22 June) during 11 periods of air sampling in an orchard where *G. persicaria*–infected fruit were distributed on the orchard floor, and a single propagule of a *Rhizopus* sp. also was detected at this time. But later placement of 60 fruit infected with *Rhizopus* spp. was followed by aerial detection during the succeeding 2 days. One day after placement of infected fruit, 2.5 propagules per 142 liters of air were detected; and on the second day, 4.5 and 8.5 propagules per 142 liters air were detected at 1:30 and 8:00 p.m., respectively.

Packing lines. Prior to harvest in 1992, viable propagules of *G. persicaria* were found by swabbing nine of 13 harvest bins with cotton tips, but propagules were not found on equipment in five packing lines. *Rhizopus* spp. were also found in five of the 13 bins and in the sorting areas of two sites. Both *G. persicaria* and *Rhizopus* spp. were detected frequently in plant debris associated with harvest bins (eight of 11, and six of 11 bins, respectively) and packing lines (four of seven sites each) before the harvest in 1992. Propagule densities of *G. persicaria* in debris ranged from 250 to 16,700 CFU, and those of *Rhizopus* spp. ranged from 250 to 2,500 CFU/g of dry matter. Propagule densities found in soil ranged up to 100 and 500 CFU/g of dry matter for *G. persicaria* and *Rhizopus* spp., respectively. At one site, *Rhizopus* spp. were not detected but *G. persicaria* was.

During both harvest seasons, *G. persicaria* and *Rhizopus* spp. were detected in hydrocooling water, and also on rollers, defuzzers, and sorting belts at some sites. No viable propagules of either fungus were detected in hydrocooling water at three sites where chlorine in the water was measured at or above 3.0 µg/ml. Viable propagules of both fungi occasionally were detected at other sites along the packing lines. When the concentration of chlorine was less than or equal to 0.2 µg/ml, viable propagules were detected frequently in the hydrocooling water and on the packing line components. At two sites visited in 1991, chlorine levels were not measured, but *G. persicaria* was found either in hydrocooling water or on the packing line.

Collections of peach fruit from packing lines rotted from a variety of fungal pathogens, including *G. persicaria* in some cases. Rots caused by *Rhizopus*, *Monilinia*, *Penicillium*, and *Botrytis* spp. were detected. Postharvest treatments used routinely with wax in the packing lines sampled included iprodione and dicloran, iprodione and benomyl, or benomyl and dicloran, but none of the three combinations appeared to prevent occurrence of Gilbertella rot. However, details of rates and methods of fungicide application were not recorded.

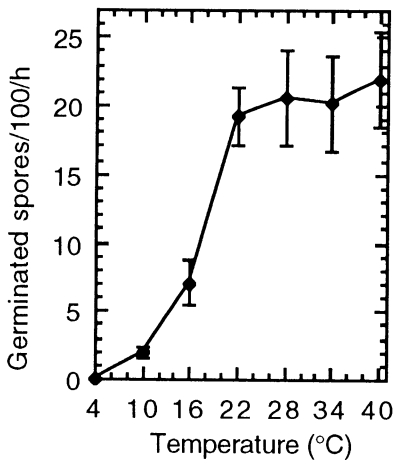

Fig. 1. Germination of sporangiospores of four isolates of *Gilbertella persicaria* at different temperatures (°C). Spores were washed in sterile distilled water and resuspended in potato-dextrose broth for germination. Error bars represent ±1 standard deviation.

Characterization of isolates. All isolates of *G. persicaria* were morphologically similar on SMA and matched published descriptions (2,5,6,19,20). Zygospores were warty, darkly pigmented, globose, or compressed between suspensors, and 54.0 to 100.8 µm in diameter. Of eight selected isolates, two were – mating type and six were + mating type. All were pathogenic on peach, with symptoms appearing in 2 to 3 days. Symptoms were similar to published reports (3,5), except that the exocarp on affected tissues was easily removed from the mesocarp.

Temperature effects. Sporangiospores germinated from 10 to 40°C, with the highest frequency of normal germination at 22 and 34°C. Results of both trials were similar, and only one is illustrated (Fig. 1). At 40°C, germ tubes were swollen, vacuolated, and stunted.

The standardized rate of mycelial extension increased as temperatures increased from 10 to 28°C for both trials, but the relationship differed in the two trials (Fig. 2). No growth occurred at 4°C, and mycelial extension ceased after 1 day at 40°C. The average growth rates, r, were 1.2 ± 0.1 and 1.8 ± 0.1 mm/degree-day with estimated base temperatures, τ, of 6.9°C ± 1.0°C and 7.8°C ± 0.4°C, respectively, for the two trials. The maximum rate of growth occurred above 28°C. Thus, the models for the two trials were: $R(T) = 1.2(T - 6.9)$ and $R(T) = 1.8(T - 7.8)$. The R-square values were 95.2 and 98.5%, respectively. The difference between these models is likely to have resulted from experimental error.

Sensitivity to fungicides. No spores germinated when incubated for 5 or more min in chlorine at 4 µg/ml (Fig. 3). A portion of spores survived treatment periods up to 20 min at lower concentrations. Surviving spores still were able to infect wounded peach fruit, and lesion development was similar to that observed for spores not exposed to chlorine (data not shown).

The growth rate for isolate E1 on unamended media was 29.1 ± 2.3 mm/day when averaged for all treatments and replicates. Therefore, all concentrations of the fungicides tested reduced growth rates (Fig. 4). Captan required much higher doses than did other fungicides for substantial reduction in mycelial growth. Captan appeared to be least toxic and dicloran most toxic to the fungus at the lowest concentration. However, iprodione was the only fungicide that completely inhibited growth at the higher concentrations tested. Similar results were observed for a second isolate (data not shown).

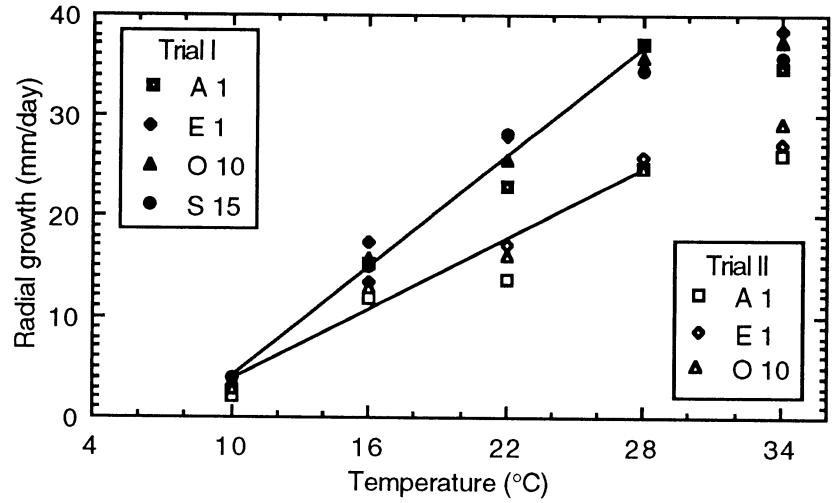

Fig. 2. Growth rate of isolates of *Gilbertella persicaria* mycelium at different temperatures. Symbols indicate standardized values for each isolate. Open and solid symbols indicate the standardized values for two different trials. Solid lines are the predicted responses from a degree-day model based on the response between 10 and 28°C where the model applies. There was no germination at 4°C. The model for trial I was $R(T) = 1.8(T - 7.8)$, and the model for trial II was $R(T) = 1.2(T - 6.9)$. Statistical measures of the fit for these models are provided in the results.

A portion of sporangiospores did not germinate in captan at concentrations of 4 and 10 µg/ml, and no sporangiospores germinated at or above 40 µg/ml. Although almost all spores germinated in the other three fungicides, germ tubes swelled and lysed in dicloran and iprodione at ≥4 µg/ml, and in vinclozolin at ≥40 µg/ml. In dicloran or iprodione at 1 mg/ml and vinclozolin at 4 and 10 µg/ml, germ tubes appeared normal but the density of aerial mycelium was less than in unamended cultures.

None of the fungicides tested (iprodione, dicloran, and fluazinam) limited fruit rot by *G. persicaria* in laboratory tests. Almost all fruit (93 to 100%) were infected after 3 days, and lesion diameters were not significantly different from controls (67 mm; $P = 0.05$).

Requirements for infection. *Nutrients and injury.* When washed in distilled water, sporangiospores of *G. persicaria* germinated well on SMA, water agar, and in peach juice (99 to 100%), but they did not germinate in distilled water. Unwashed spores germinated well on water agar (98%) but not as well in distilled water (42%). In all cases, spores prepared in distilled water and in peach juice infected peach fruit that had been injured by punctures. Peaches injured by brushing lightly to remove most trichomes were infected infrequently (10 to 20%), and the preparation of spores had no effect on results. For fruit that were not injured, the spores prepared in peach juice caused no infections, whereas those in distilled water caused a few infections (20%).

Results of the two infection studies were similar, and only results of the second test are reported here. Spore suspensions confined on the surface of uninjured fruit by plastic rings resulted in 6 and 56% incidence of rot for spores in distilled water or peach juice, respectively. The fungus grew and sporulated in the peach juice, but only short germ tubes formed in the water. When the spores from water suspensions were moved from fruits to APDA after 4 days, the fungus grew well.

All fruit injured by puncture with a scalpel of the stem end became infected, but only two other fruit with natural wounds became infected. Most fruit inoculated with single spores became infected (83 to 90%).

Fruit were infected and rotted rapidly at temperatures of 22, 28, and 34°C. Symptom development appeared to be more rapid at 28°C than at the other temperatures (Table 3). No symptoms were apparent in 4 days for fruit held at or below 16°C or at 40°C. When inoculated fruit were transferred from these temperatures to 22°C, symptoms developed rapidly.

DISCUSSION

During the 1991 and 1992 seasons in South Carolina, *G. persicaria* was found commonly rotting peaches on the orchard floor, in soil and debris associated with harvest bins before and during harvest, and in packing houses. A portion of fruit collected from packing houses developed rot associated with *G. persicaria* after incubation for a short time. Although *Rhizopus* spp. are a significant problem in South Carolina, *G. persicaria* were not reported until recently (4). Gilbertella rot has been reported in California (12) and was common there prior to 1961 (16). The relative economic importance of these two fungi over time is uncertain and should be determined if unexplained epidemics occur.

The requirement of external nutrients for spore germination and a wound for infec-

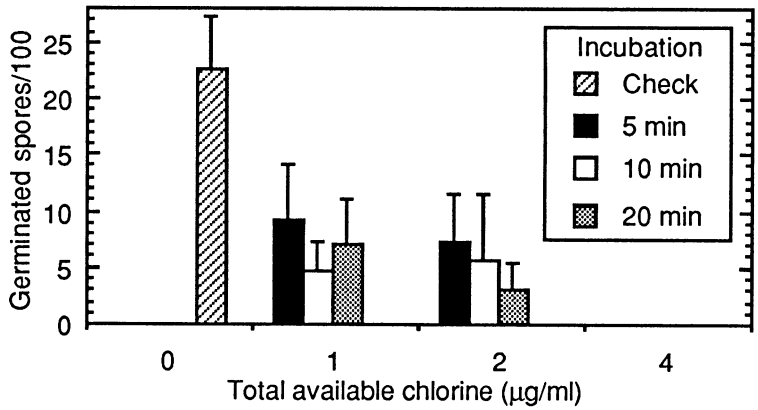

Fig. 3. In vitro sensitivity of *Gilbertella persicaria* sporangiospores to chlorine. The mean numbers of colonies formed on media from spore suspensions untreated or treated for 5 to 20 min with each chlorine solution are indicated. Error bars represent 1 standard deviation. No colonies formed from spore suspensions in chlorine at 4 µg/ml.

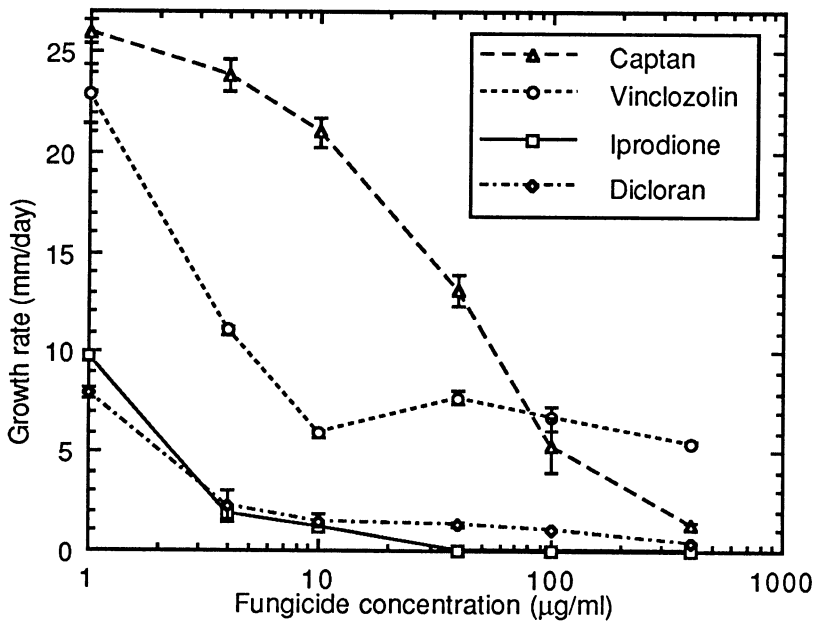

Fig. 4. In vitro sensitivity of *Gilbertella persicaria* (isolate E1) to iprodione, dicloran, vinclozolin, and captan as determined by mycelial growth. Mycelial plugs were transferred to potato-dextrose agar with fungicides added at the concentrations indicated. Mean growth rate on unamended media was 29.1 ± 2.3 mm/day when averaged for all treatments. Error bars represent ±1 standard deviation.

Table 3. Number of peach fruit inoculated with *Gilbertella persicaria* that exhibited decay after 2 and 5 days' incubation at 22, 28, or 34°C

		No. infected/10 fruit					
		22°C		28°C		34°C	
Isolate	Trial	2 days	5 days	2 days	5 days	2 days	5 days
E1	1	10	10	10	10	0	9
E1	2	0	4	3	10	0	5
S15	1	0	10	9	10	10	10
S15	2	0	9	3	10	8	10

tion indicate that the most important time for disease control may be during harvest. Older wounds may not be important for infection, but fresh puncture wounds appear to be ideal infection sites. Careful control of the chlorine levels in hydrocooling water in packing houses may be critical to limiting problems with fruit rot caused by *G. persicaria* and similar pathogens.

Although fungicides have activity against *G. persicaria* in vitro, treatment of fruit after inoculation was ineffective. Gilbertella rot was observed among fruit collected from packing lines that used some of the most active fungicides tested, including dicloran, which in the past has been used extensively for Rhizopus control. Fortunately, *G. persicaria* is sensitive to chlorine, and use of chlorine in water during sorting and grading activities appears to control Gilbertella rot. The identification of *G. persicaria* in debris collected from many locations in the postharvest handling equipment underscores the need for sanitation in transport, storage, and marketing.

Although sporangiospores of *G. persicaria* have been described as airborne (19,20), aerial dissemination appeared to be uncommon under the conditions prevailing in this test. Only one propagule of *G. persicaria* was detected during 11 periods of air sampling with inoculum available on rotting peaches on the ground, whereas *Rhizopus* spp. were readily detected under similar sampling conditions.

The isolates of *G. persicaria* from South Carolina do not appear to differ significantly from previously described isolates in terms of morphology (2,5,6,19,20), sensitivity to temperature (13), or requirements of wounds for infection (14). The disease appears not to be new to South Carolina, but probably is a common disease that has now been recognized. Its significance to peach production may vary from one season to the next, and it may be an important problem in some seasons. More careful monitoring of the fungi associated with fruit rots will eventually provide a clearer picture of the relative importance of the pathogens associated with postharvest decay of peaches in South Carolina.

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