Symptomatic Responses of Peach Trees to Various Isolates of *Botryosphaeria dothidea*

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ABSTRACT

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Peach trees (*Prunus persica*) were inoculated with isolates of *Botryosphaeria dothidea* from peach trees inside (PI) and outside (PO) the geographic area in which peach fungal gummosis has been reported and with isolates from nonpeach hosts inside (NI) and outside (NO) the gummosis area. Thirty isolates applied as conidial suspensions to wounds induced gum exudation within 3 mo; however, observations extending beyond 1 yr revealed that all 13 PI isolates except one continued to induce gumming at a high level, whereas all 11 NI isolates except three from *Prunus* spp., 5 NO isolates, and 1 PO isolate ceased to induce gumming during the longer period. When the percentage of trees dead or with lenticel-associated symptoms (blisters or lesions) was analyzed, only the PI group differed from the uninoculated check after 1 yr. Similarly, in nonwound inoculations with one PI isolate, one NI isolate, *B. obtusa*, and *B. rhodina*, only the PI isolate caused gumming and other symptoms associated with lenticels. *B. dothidea* isolates from peach trees with typical gummosis symptoms may represent a new biotype of the fungus.

Fungal gummosis of peach trees was first noticed in Georgia sometime during the 1960s (7,12) and has since appeared in peach-producing areas in Alabama, Florida, and Louisiana (7) and, more recently, in Mississippi (R. A. Haygood, *personal communication*) and South Carolina (R. W. Miller, *personal communication*). Symptoms are associated with lenticels and include sunken necrotic lesions that frequently exude gum on trunks and scaffold limbs and swollen areas on young branches (11,13). The fungus, *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & de Not. (= *B. ribis* Gross & Dugg.), has been isolated consistently from the diseased bark. Initial attempts to reproduce the gummosis symptoms by inoculating bark wounds with *B. dothidea* (11) resulted in the development of a "gummy" canker after 3 mo and infected lenticels and gum deposits typical of gummosis after 18 mo. When nonwounded, 1- to 3-yr-old tree branches were exposed to spores of *B. dothidea*, swollen lenticels and sunken necrotic lesions beneath lenticels were observed after 3 mo, but gumming was not observed during the 5-mo study. The fungi *B. obtusa* (Schw.) Shoem. and *B. rhodina* (Berk. & Curt.) Arx have also been associated with gummosis (1); however, they have not been shown to infect trees through lenticels.

B. dothidea, B. obtusa, and *B. rhodina* (in its conidial state, *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl. (=*Botryodiplodia theobromae* Pat.)) have wide host ranges and wide distributions in the United States (4–6,8). As early as the 1920s, *B. dothidea* was isolated from peach in Florida and from apple in Georgia (10), yet severe gummosis was unnoticed until the 1960s. Possible reasons include a change in environmental factors, a change in cultural practices, the development of new strains of the microorganism(s) involved, or a combination of these. This investigation focuses on the possible adaptation of strains of *B. dothidea* to cause peach fungal gummosis.

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MATERIALS AND METHODS

Thirty isolates of *B. dothidea* were used in inoculations (Table 1). These were obtained from peach inside (PI) and outside (PO) the reported geographic range of peach gummosis and from nonpeach hosts inside (NI) and outside (NO) the same region. Isolates were maintained on Difco potato-dextrose agar, and conidial inoculum was produced on Difco oatmeal agar under continuous light at 26 C for 10–20 days.

In a field test begun in February 1981, 3-yr-old Redglobe peach trees were inoculated with eight *B. dothidea* isolates (two PI, three NI, and three NO isolates). A *B. obtusa* isolate from a peach tree with gummosis was also included. For each treatment, there were five-tree plots replicated four times in a randomized complete block. A mason's hammer was used to make one wound (9 cm^2) on the trunk and one wound on each of two scaffold branches (3–5 cm in diameter). Conidial suspensions (10^5 spores per milliliter) were brushed on the wounds. Uninoculated check wounds were treated with sterile, distilled water. Sites of inoculation were not surface-disinfected before treatment or covered afterward.

Wounds were rated periodically for amount of gum exuded using a scale of 0–4, where 0 = no gum, 1 = slight gum inside wound, 2 = exuded gum mass 0.5–1 cm in diameter, 3 = gum mass 1–1.5 cm in diameter, and 4 = gum mass larger than 1.5 cm in diameter. After the end of the rating period, attempts were made to isolate fungi from each wound as described previously (7). Data for gum exudation and frequency of isolation were subjected to analysis of variance and the Waller-Duncan *K*-ratio *t* test.

In March 1983, 1-yr-old budded Winblo peach trees were inoculated in the greenhouse with 28 isolates of *B. dothidea* (13 PI, 9 NI, 1 PO, and 5 NO isolates). One *B. rhodina* isolate from a gummosis tree was also used. Six replicate trees were placed in separate randomized blocks. Conidial suspensions (10^5 spores per milliliter) were brushed onto five pairs of wounds on each tree (two on the stem and three on a branch) made with pliers as described earlier (3). Trees were transferred in autumn 1983 from the greenhouse to a lathhouse outside where they remained until the end of the experiment. Gum was rated, fungi were isolated, and data were analyzed as in the field test. In addition, incidence of mortality and percentage of trees with lenticel-associated symptoms were noted. The latter data were analyzed using chi-square or Fisher's exact test following the guidelines of Snedecor and Cochran (9).

Another greenhouse experiment was set up in June 1983 to determine if representative isolates of *B. dothidea* or other species of *Botryosphaeria* can initiate infections at lenticels. Two isolates of *B. dothidea* (one from peach and one from plum), one peach isolate of *B. obtusa*, and one peach isolate of *B. rhodina* were used to inoculate 1-yr-old budded Sunland peach trees with a modification of Weaver's procedure (13). Each fungal isolate, as a suspension of 10^5 spores per milliliter, was sprayed onto 25 cm of the nonwounded stem (1.0–1.5 cm in diameter) of four trees. The stem was wrapped with moist cheesecloth and Parafilm. Bark above and below the treated area was marked with latex paint before wrappings were removed 6 days later.

RESULTS

Three months after inoculation of orchard trees, mean gum ratings were lower for two peach isolates than for six nonpeach isolates (except NO-3) (Table 2). Sixteen months after inoculation, the opposite was observed; the two peach isolates had continued to induce gumming but mean ratings for the six nonpeach isolates did not differ from the uninoculated check. Despite this contrast, values of percent isolation for *B. dothidea* isolate groups did not differ. Similarly, gumming induced by *B. obtusa* had stopped in the second year; however, this fungus was recovered at a higher rate ($P = 0.05$) than were any of the *B. dothidea* isolate groups.

A similar trend was observed when wounded trees were inoculated in the greenhouse (Fig. 1). Fifteen months after inoculation, all 13 PI isolates (except PI-7) induced gumming at a high level but only three of the 15 non-PI isolates differed from the uninoculated check. The three non-PI isolates that continued to induce gumming after 1 yr were all from *Prunus* spp. in the gummosis area (NI-5 from almond, NI-8 from apricot, and NI-9 from plum). During the first three ratings in 1983, means for the PO and NO isolate groups were higher than means for the PI and NI isolate groups (Table 3). This was reversed in 1984, 15 and 17 mo after inoculation. Means for PO and NO isolates and the *B. rhodina* isolate did not differ from the check.

Table 1. *Botryosphaeria dothidea* isolates used in peach tree inoculations

Host	Isolate code[a]	Location source[b]
Prunus persica L.	PI-1	Calhoun, LA
	PI-2	Chilton County, AL
	PI-3, PI-4, PI-5	Dooley County, GA
	PI-6–PI-12	Peach County, GA
	PI-13	Pontotoc County, MS
	PO-1	Kearneysville, WV
Ilex sp.	NO-1	North Carolina
Malus pumila Mill.	NI-1, NI-2	Byron, GA
	NO-2	North Carolina
	NO-3	Urbana, IL
P. dulcis (Mill.) D. A. Webb	NI-3	Byron, GA
P. augustifolia Marsh.	NI-4	Byron, GA
P. armeniaca L.	NI-5	Byron, GA
P. davidiana (Carr.) Franch.	NI-6	Byron, GA
P. serotina Ehrh.	NI-7	McDuffee Co., GA
P. (complex plum hybrids)	NI-8, NI-9, NI-10	Byron, GA
Sassafras albidum (Nutt.) Nees	NO-4	McMinnville, TN
Vaccinium ashei Reade	NI-11	Byron, GA
Vaccinium sp.	NO-5	North Carolina

[a] Letters designate peach isolates from inside (PI) and outside (PO) reported range of peach gummosis and nonpeach isolates from inside (NI) and outside (NO) gummosis area.
[b] Isolates NO-1, NO-2, and NO-5 were obtained from D. Ritchie, N.C. State University, Raleigh; isolate PI-12 was obtained from K. Britton, University of Georgia, Athens; isolate PO-1 was obtained from R. Scorza, USDA, Kearneysville, WV; all others were isolated from bark samples by C. C. Reilly, USDA, Byron, GA.

Table 2. Peach tree response to, and recovery of *Botryosphaeria dothidea* isolates after, wound inoculations made in the field in February 1981[a]

	Gum rating[c]		Isolation of *Botryosphaeria* (%)[c]	
Treatments[b]	3 mo (May 1981)	16 mo (Sept. 1982)	*B. dothidea*	*B. obtusa*
Individual				
PI-10	1.33	0.64	51.6	2.1
PI-11	1.28	0.96	37.2	7.8
NI-1	2.70	0.07	27.2	5.8
NI-7	2.63	0.20	53.8	5.5
NI-9	2.88	0.15	35.0	1.7
NO-1	3.18	0.23	37.2	0
NO-3	1.19	0.30	27.6	7.2
NO-5	3.08	0.20	50.4	6.0
BO	2.05	0.02	1.7	67.7
CK	0.56	0.02	1.7	8.1
LSD ($P = 0.05$)	0.42	0.31	20.3	6.8
Group				
PI	1.31	0.80	44.4	5.1
NI	2.74	0.14	38.7	4.3
NO	2.48	0.24	38.4	4.6
BO	2.05	0.02	1.7	67.7
CK	0.56	0.02	1.7	7.2
LSD ($P = 0.05$)	0.27	0.19	19.9	5.6

[a] For each treatment, there were four replicate plots, five trees per plot, and three wounds per tree.
[b] Means are presented for individual isolates and for groups of *B. dothidea* isolates from peach inside (PI) geographic range of peach gummosis and from nonpeach hosts inside (NI) and outside (NO) gummosis area. A *B. obtusa* isolate (BO) and an uninoculated check (CK) were included.
[c] Gum rating on a scale of 0–4, where 0 = no gum, 1 = slight gum inside wound, 2 = exuded gum mass 0.5–1 cm in diameter, 3 = gum mass 1–1.5 cm in diameter, and 4 = gum mass larger than 1.5 cm in diameter. Mean separations for gum ratings and percentage of wounds from which *Botryosphaeria* was isolated after 18 mo are based on Waller-Duncan's *K*-ratio *t* test.

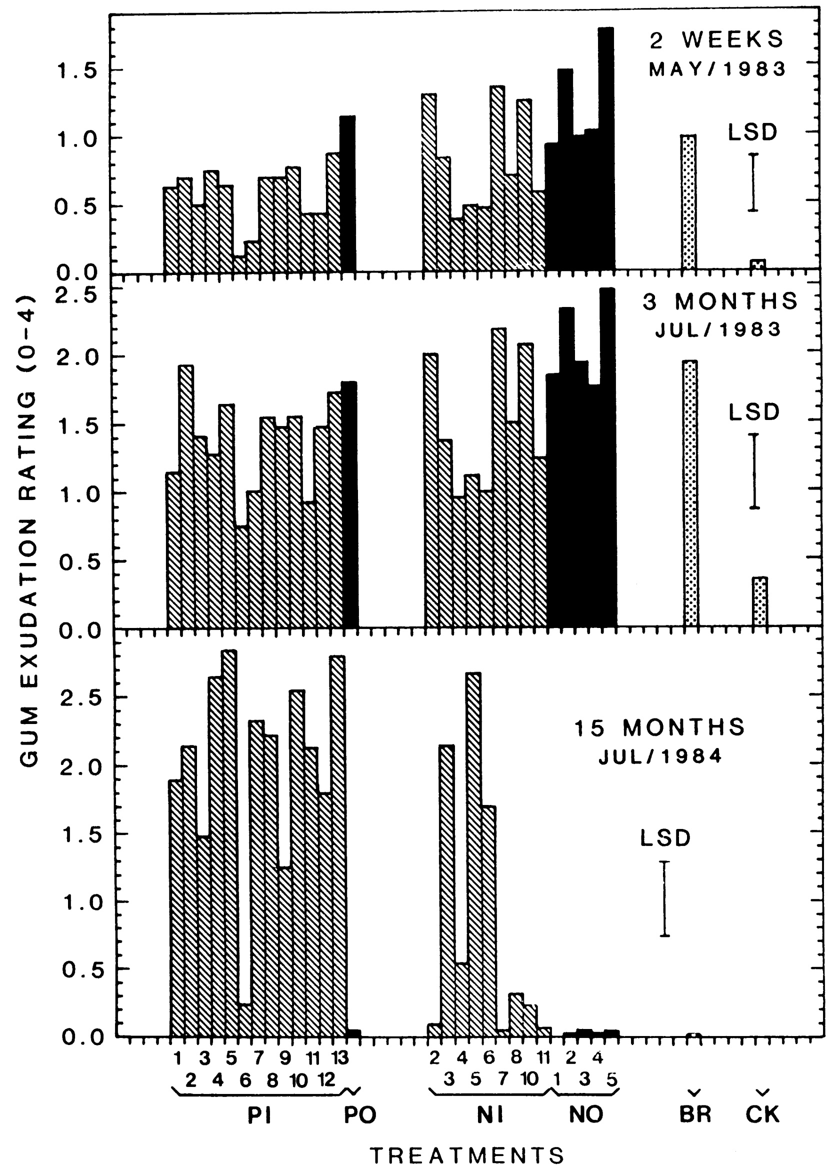

Fig. 1. Gumming responses of peach trees to *Botryosphaeria dothidea* isolates obtained from peach inside (PI) and outside (PO) the reported geographic range of peach gummosis and from nonpeach hosts inside (NI) and outside (NO) the gummosis area. Hatched and solid bars separate isolates from inside and outside gummosis area, respectively. Dotted bars indicate *B. rhodina* isolate (BR) and uninoculated check (CK). Each isolate (code number below bar) was tested on six trees, 10 wounds per tree. Mean separation is based on Waller-Duncan *K*-ratio *t* test ($P = 0.05$).

Seventeen months after inoculation, trees in the above experiment were examined for sunken lesions or blisters associated with lenticels as described previously (11,13). The number of these diseased areas on individual trees ranged from zero to more than 100. Ninety-three percent of the trees inoculated with peach isolates of *B. dothidea* from the gummosis area had lenticel-associated symptoms (Table 3) compared with 0% for the one PO isolate (PO-1) and 12 % for the NO isolates. A difference was shown between NI and PO groups and between NI and the check at $P<0.1$. The 20% shown for *B. rhodina* represented only one or two swollen lenticels on one of five live trees.

The severe wounding combined with the fungal inoculations caused some trees to die during winter or during the 1984 season. Dead trees or branches were not rated for gumming or evaluated for lenticel symptoms. Percent tree death was statistically higher for the PI group than for any other isolate group. None of the uninoculated check trees died.

Nonwound inoculations of peach trees in the greenhouse resulted in no apparent symptoms during a 4-mo period before trees were transferred to the lathhouse. Thirteen months after inoculation, tree stems inoculated with the peach isolate of *B. dothidea* (PI-10) showed lenticel-associated symptoms (Figs. 2 and 3), including profuse gumming at 30–50 sites, 70–100 blisters (2–6 mm in diameter), and 4–8 sunken lesions (5–8 mm in diameter). In contrast, trees inoculated with the plum isolate of *B. dothidea* (NI-10) and with peach isolates of *B. obtusa* and *B. rhodina* had no visible symptoms and appeared no different from the uninoculated check trees.

Removal of outer bark from blisters on stems inoculated with PI-10 revealed necrotic spots (1–3 mm in diameter) surrounded by healthy tissue that protruded above the normal bark.

Table 3. Peach tree responses to, and recovery of *Botryosphaeria dothidea* isolates after, wound inoculations made in the greenhouse in April 1983[v]

		Gum rating[x]							
Treatment groups[w]	No. of treatments	2 wk (May 1983)	2 mo (Jun. 1983)	3 mo (Jul. 1983)	15 mo (Jul. 1984)	17 mo (Sept. 1984)	Isolation[x] (%)	Lenticel-associated symptoms[y] (%)	Tree death[y] (%)
PI	13	0.57	1.02	1.38	2.00	2.10	34	93 a	46 a
PO	1	1.13	1.77	1.80	0.06	0.00	53	0 b	17 b
NI	9	0.81	1.29	1.49	0.65	0.48	36	39 ab	33 b
NO	5	1.23	1.86	2.07	0.03	0.14	52	12 b	13 b
BR	1	0.98	1.80	1.93	0.02	0.00	61	20 ab[z]	17 b
CK	1	0.07	0.33	0.35	0.00	0.00	3	0 b	0 b
LSD ($P = 0.05$)	···	0.30	0.27	0.23	0.40	0.45	20	···	···

[v] For each *B. dothidea* isolate, there were six trees, 10 wounds per tree.

[w] Means are presented for groups of *B. dothidea* isolates from peach inside (PI) and outside (PO) geographic range of peach gummosis and from nonpeach hosts inside (NI) and outside (NO) gummosis area. One *B. rhodina* isolate (BR) and an uninoculated check (CK) were included.

[x] Gum rating on a scale of 0–4, where 0 = no gum, 1 = slight gum inside wound, 2 = exuded gum mass 0.5–1 cm in diameter, 3 = gum mass 1–1.5 cm in diameter, and 4 = gum mass larger than 1.5 cm in diameter. Mean separations for gum ratings and percentage of wounds from which *Botryosphaeria* was isolated after 17 mo are based on Waller-Duncan's *K*-ratio *t* test. Isolation was attempted for six wounds per tree.

[y] Percentage of trees with lesions or blisters associated with lenticels and percentage of trees dead was recorded 17 mo after inoculation. Treatment groups were compared using chi-square or Fisher's exact test following the guidelines of Snedecor and Cochran (9); means in a column followed by the same letter do not differ significantly at $P<0.05$.

[z] Represents only one or two blisters on one of five live trees.

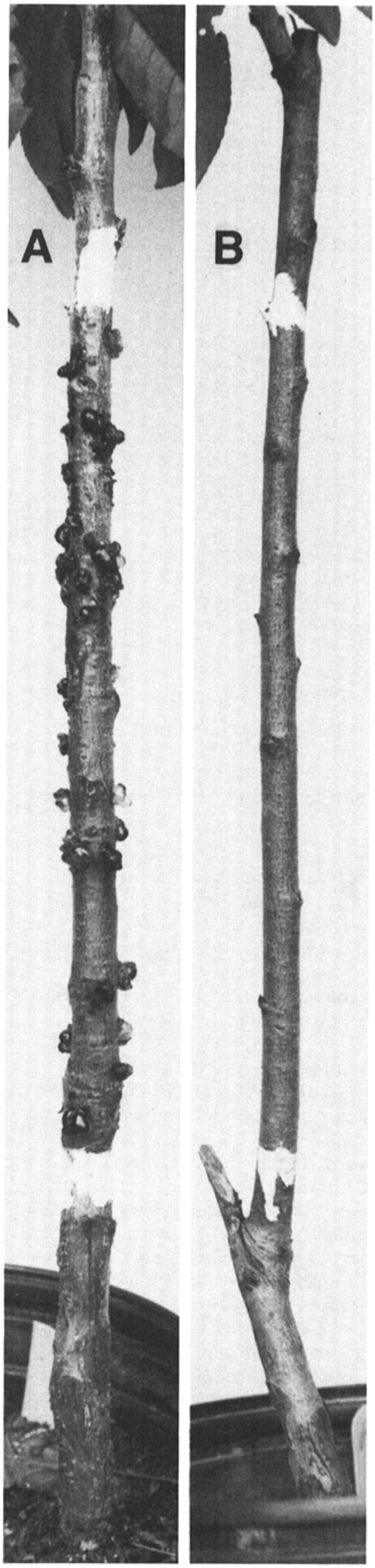

Fig. 2. Responses of potted peach trees 16 mo after inoculation of nonwounded stems. **(A)** Tree inoculated with *Botryosphaeria dothidea* isolate (PI-10) obtained from peach trees showing typical gummosis symptoms. **(B)** Tree inoculated with *B. dothidea* isolate (NI-10) from plum.

Necrotic tissue generally did not extend deeper than 1 mm.

Sixteen months after the nonwound inoculations, isolations from 10 tissue samples from each tree were attempted. Samples of diseased trees were from the necrotic/healthy interface and samples of healthy-appearing trees were from just beneath the periderm at the edges of lenticels. Recovery was 55% for the peach isolate of *B. dothidea*, 90% for the plum isolate of *B. dothidea*, 58% for *B. obtusa*, and 60% for *B. rhodina*. No *Botryosphaeria* spp. were isolated from the uninoculated check trees.

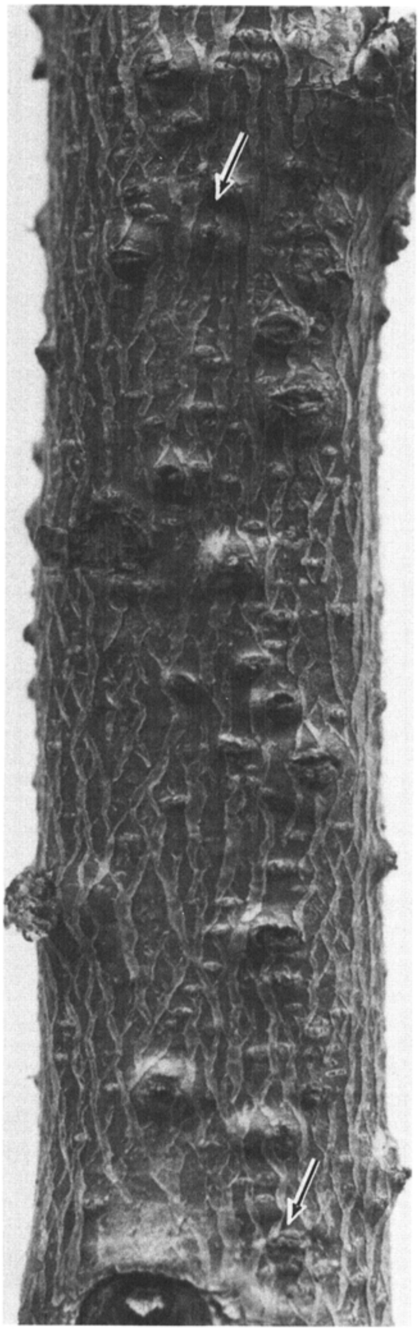

Fig. 3. Peach stem 16 mo after nonwound inoculation with *Botryosphaeria dothidea* isolate (PI-10) obtained from peach trees with typical gummosis symptoms. Shown are lesions (indistinct depressions at arrows) and blisters associated with lenticels.

DISCUSSION

The most significant observation in the two wound-inoculation experiments occurred after 1 yr. All but one of the 13 PI isolates continued to induce gum exudation at a high level, whereas the 16 nonpeach isolates (except three from related *Prunus* spp. in gummosis area) and the one PO isolate ceased to induce gumming at a level different from the uninoculated check. The role of gumming as a response of peach to outside agents is not generally understood, but continued gumming usually indicates an active infection.

Despite the differences in gum exudation after 1 yr, recovery of PI isolates was never higher than that of the other *B. dothidea* isolate groups or the other *Botryosphaeria* spp. Isolates not in the PI group, including *B. obtusa* and *B. rhodina*, were recovered at a high rate after 1 yr even though in many instances gumming had stopped and wounds were closed over with new tissue. The *B. obtusa* isolate was actually reisolated at a higher frequency than were any of the *B. dothidea* isolate groups and *B. rhodina* was reisolated at a higher frequency than PI and NI groups. Similar results occurred with nonwound inoculations. Whether symptoms appeared or not, each fungal isolate tested was recovered at a rate equal to or greater than 55%. Thus, the fungi can persist on the tree without causing any apparent infection; possibly, they reside between the outer dead cork layers or in the intercellular spaces of lenticels. According to Conner (2), *B. dothidea* can reside in lenticels of apple trees and may enter the cortex when moisture stress develops.

Results were affected by the natural occurrence of *B. dothidea* or cross-contamination. This appeared to be at a low level because isolation values for uninoculated checks in the wound-inoculation tests (Tables 2 and 3) were only 1.7 and 2.8%. Also, *B. dothidea* was isolated from wounds inoculated with *B. obtusa* at the low rate of 1.7% and was not isolated from wounds inoculated with *B. rhodina*. Because of this, we assume that the presence of the fungi after 1 yr is due mainly to their persistence over the winter rather than natural reinfestation in the second year.

Cross-contamination could have affected results pertaining to lenticel-associated symptoms more than results for gumming and reisolation at the wound site because trees had many more lenticels than wounds. We are therefore uncertain whether or not abnormal lenticels on trees inoculated with *B. rhodina* or NO isolates of *B. dothidea* were actually caused by these fungi. *B. dothidea* from plum (NI-10), *B. rhodina*, and *B. obtusa* appeared not to infect trees through lenticels in the nonwound-inoculation test, although these fungi might still do so under other conditions or when trees are stressed. The wound and nonwound inoculations did seem to indicate that PI isolates have a greater ability to initiate infections at lenticels.

In an early study by Smith (8), cross-inoculations between different hosts suggested that different pathogenic strains of *B. dothidea* did not occur. Data from our investigation suggest that *B. dothidea* isolates from peach trees with typical gummosis symptoms represent a new biotype of the fungus. Isolates collected from three other *Prunus* spp. at Byron (NI-5, NI-8, and NI-9) caused the same general response in peach as did PI isolates. The new biotype could have developed in a *Prunus* sp. other than peach; however, it seems more likely that it occurred in peach because monoculture tends to favor disease and peach is the only *Prunus* sp. grown commercially on a large scale in Georgia. Also, the fungus is not considered an important pathogen of other *Prunus* spp.

We are unable to predict whether the peach fungal gummosis found in the Southeast will spread elsewhere in the United States. Further spread may be possible because *B. dothidea* currently has a much wider distribution than does the disease.

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