

Role of *Botryosphaeria* Species in Peach Tree Gummosis on the Basis of Differential Isolation from Outer and Inner Bark

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ABSTRACT

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Nonwounded stems of 1-yr-old peach trees were inoculated with *Botryosphaeria* spp. in May, and fungal colonization of outer bark (rhytidome), inner bark (living secondary phloem), and xylem were evaluated after 18 mo. Trees inoculated with an isolate of *B. obtusa* or *B. rhodina* from peach or an isolate of *B. dothidea* from apple were symptomless, whereas trees inoculated with an isolate of *B. dothidea* from peach were dead (69%) or severely diseased. The same fungal species used in inoculations were isolated from the outer bark of symptomless stems (following surface-sterilization with 70% ethanol) at frequencies of 35–100% but from inner bark or xylem at frequencies of 0–13%. Sequential isolations were made from outer to inner bark of commercially grown peach trees that varied in age (4–18 yr) and symptom expression in central Georgia. The progression from outer to inner bark usually resulted in an increase in the proportion of *B. dothidea* isolated on potato-dextrose agar relative to *B. obtusa*. *B. dothidea* was consistently predominant in diseased inner bark associated with lenticels. *Botryosphaeria* spp., which inhabit only dead outer bark as saprophytes, may have been disproportionately represented in previous studies on the cause of peach tree gummosis.

A fungal gummosis disease of peach trees (*Prunus persica* L.) was first noticed in central Georgia in the 1960s (13,17,18) and has since appeared in peach-production areas throughout much of the southeastern United States (12,13). Symptoms include sunken necrotic lesions (1–2 cm in diameter) in bark around lenticels, resin exudation from diseased lenticels, and blisters associated with lenticels on young branches (17,19). These same symptoms on peach have been reported in Japan (1,2), China (5), and more recently, Australia (J. Slack, *personal communication*). Because of the characteristic swelling of the bark, the disease is known in Japan as “peach blister canker.” The swelling is due to hyperplasia of peridermal and cortical cells surrounding diseased tissue near the lenticel opening (1; P. L. Pusey, *unpublished*).

Botryosphaeria dothidea (Moug.:Fr.) Ces. & De Not. consistently has been associated with necrotic tissue around lenticels, and pathogenicity was demonstrated by inoculation of nonwounded trees (2,5,11,12,19). *B. berengeriana* De Not., the pathogen name used in Japan (7), is considered a synonym for *B. dothidea* (6).

Peach tree gummosis in Georgia is reported (3,4) to be caused by three

species of *Botryosphaeria*: *B. dothidea*, *B. obtusa* (Schwein.) Shoemaker, and *B. rhodina* (Cooke) Arx. These fungi were isolated from cankers on twigs, scaffold limbs, and tree trunks of peach. A description of the cankers and their association with either wounds or natural openings in the bark was not provided. When one isolate of each of the three *Botryosphaeria* species was used to inoculate wounds in peach bark (3), gumming cankers that developed were indistinguishable after 6 or 12 mo. However, other wound-inoculation studies with peach that extended to 17 mo (12) revealed differences in symptoms caused by *Botryosphaeria* species. In this report, intraspecific variation was tested for *B. dothidea*, but other species were represented only as single isolates from peach. After 17 mo, cankers caused by all 13 isolates of *B. dothidea* from peach were still active as indicated by gum production, whereas gumming induced by an isolate of either *B. obtusa* or *B. rhodina* had nearly stopped, and lesions were delimited by newly formed bark.

Trees wound-inoculated with *B. dothidea* also were distinguished after 17 mo (12) from those inoculated with *B. obtusa* and *B. rhodina* by the presence of numerous localized lesions at lenticels located below the inoculated site. Weaver (17) earlier reported that gummy cankers developed on peach within 3 mo at sites wound-inoculated with *B. dothidea*; however, 14–18 mo were required for the development of localized infections at lenticels below inoculations. Infections associated with lenticels in these tests (12,17) were presumed to be secondary infections resulting from spores pro-

duced at wound-inoculation sites and carried downward by water.

Weaver (17) identified *B. dothidea* but not other *Botryosphaeria* species among fungi isolated from blisters and gumming lesions on peach trees in central Georgia. Reilly and Okie (13) isolated *B. dothidea* from gumming lesions sampled in 95% of peach orchards with a medium-to-heavy disease rating in Georgia and nearby states. Of all isolations of *Botryosphaeria* made, 89% were *B. dothidea* and 11% were *B. obtusa* (C. C. Reilly, *personal communication*). In contrast, Britton and Hendrix (3) reported higher isolation frequencies of *B. obtusa* than of *B. dothidea*, and *B. rhodina* also was detected but was rare. These workers later reported (4) that cankers are dominated by *B. dothidea* in summer and *B. obtusa* in winter; *B. dothidea* was not detected during January in either 1983 or 1984. This finding also differs from that of Reilly and Okie (13); of 37 positive isolations of *Botryosphaeria* in January 1981, 36 were *B. dothidea* and one was *B. obtusa* (C. C. Reilly, *personal communication*). Such inconsistencies may result from differences in the tissue sampled or in the isolation method used. One major difference in the isolation technique used is that Weaver (17) and Reilly and Okie (13) removed outer bark following surface-sterilization of samples, whereas Britton and Hendrix (3,4) did not.

The purpose of this study was to assess through isolation methods whether *Botryosphaeria* species detected in association with symptoms of peach gummosis, particularly those at bark lenticels, are invading living tissues or merely residing in the outer bark (rhytidome).

MATERIALS AND METHODS

Isolation from inoculated trees. Trees (1-yr old) of peach cultivars Candor and Summergold were obtained from a nursery and placed in 23-cm-diameter pots with peat, vermiculite, and soil (1:1:1) in February 1986. Each cultivar was inoculated on 28 May 1986 with different isolates of *Botryosphaeria* species, using eight trees per isolate in a randomized complete block design. Fungi used were *B. obtusa* (isolate Bo-52), *B. rhodina* (isolate Br-48), and *B. dothidea* (isolates Bd-67 and Bd-68), which were collected near Byron, Georgia, from diseased bark of peach trees, except Bd-68 came from

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diseased bark of an apple tree. The fungi were cultured on Difco (Difco Laboratories, Detroit, Michigan) oatmeal agar for 13–23 days under 15-hr diurnal light at 25 C. Intact bark was inoculated as described previously (11,12) by applying an aqueous suspension of 10^5 conidia per milliliter with a brush to the stem between the bud union and first branch (15–30 cm). Uninoculated control trees were brushed with water only. All stems were wrapped with moist cheesecloth and Parafilm, which were removed after 7 days. Trees were maintained in a greenhouse for 5 mo, then transferred to an outside lathhouse.

On 17 November 1987, trees were examined for disease symptoms, and recovery of the fungi was attempted with five methods. In method A, stem segments 2.5 cm long were split into halves and surface-sterilized as described by Britton and Hendrix (4). Each half segment was immersed in a solution of 0.5% NaOCl and 10% ethanol for 1 min and then placed with the cut surface down on Difco potato-dextrose agar (PDA) in one 9-cm-diameter petri dish. For methods B through E, a 4-mm-diameter cork borer was used to cut through the bark, and scalpel and forceps were used to separate tissues and transfer five disks per method from each tree to a single PDA dish. Each disk was placed with the abaxial surface on the medium. In method B, disks of outer bark were cut from the nonsterilized stem surface and placed on the medium. Methods C, D, and E involved sequential isolation from progressively deeper tissues taken from the same stems used in A and B. In method C, the stem was wiped with 70% ethanol, and the surface was allowed to dry before disks of outer bark were collected. In method D, 70% ethanol was applied to the surface of inner bark exposed in method C. After the alcohol evaporated, a disk of living secondary phloem was collected and transferred to PDA. Method E involved cutting below the cambium in the same area used for D, removing tissue composed mainly of xylem, and transferring it to PDA. Culture dishes were incubated under 15-hr diurnal light at 25 C.

Dishes were examined periodically, and *Botryosphaeria* species were identified according to conidial morphology (8–10). If the fungi did not produce spores on the PDA, they were transferred to oatmeal agar, which generally promoted sporulation under the same incubation conditions.

Isolation of naturally occurring *Botryosphaeria* species. Bark samples were collected in October 1989 when the expected population of *B. dothidea* would be high and in January 1990 when it would be comparatively low or undetectable based on Britton and Hendrix (4). Samples were from diseased bark on scaffold branches of declining peach trees 13–

18-yr old in four orchards in central Georgia. Cultivars, tree ages, and orchard locations were as follows: Harvester, 17-yr old, Peach Co.; Harvester, 16-yr old, Houston Co.; Coronet, 17-yr old, Peach Co.; and Coronet, 13-yr old, Peach Co. The diseased areas of bark were 20–40 cm in length, irregular in shape, accompanied by profuse gumming, and had a dark scaly surface. These areas represented the most severe bark necrosis on the tree. In general, an association with wounds or lenticels was not apparent. When outer bark was removed, live and necrotic tissue was interspersed, giving the exposed area a mosaic or mottled appearance. The necrosis was mainly in the secondary phloem but often extended to the xylem. One section of diseased bark 8–10 cm² was removed with a knife (by cutting below the cambium) from each of 20 trees in each orchard. The samples were placed in 0.5% NaOCl and 10% ethanol for 1 min (4). From each sample, three 3-mm² sections of outer bark were removed with a scalpel and placed with the abaxial surface on acidified PDA (APDA) in a single dish. APDA was prepared from fresh potatoes (16) to more closely follow Britton and Hendrix (3) and to improve fungal sporulation. After surface sterilization as described above, a second isolation was performed by removing the outer bark, cutting three 3-mm² sections of inner bark consisting of both live and necrotic tissue from each sample, and placing the sections with the abaxial surface on APDA in one dish. Culture dishes were incubated as described above and examined periodically for growth of *Botryosphaeria* spp. Isolation frequencies for *B. dothidea* and *B. obtusa* were compared by means of *t* tests ($P \leq 0.05$).

Two relatively young commercial peach orchards in central Georgia were also studied—a 5-yr-old planting of Summergold in Houston Co. and a 4-yr-old planting of Springbrite in Peach Co. Bark samples were collected October 1989 and January 1990. Eight different types of samples were cut with a knife from each of 12 trees in each orchard. The first sample type was healthy bark from the trunk or lower part of the scaffold, and the second was from diseased bark similar to that described for older trees. These diseased areas, about 10–20 cm in length, represented the most extensive bark necrosis and gum exudation found on the trees. For the other six sample types, symptom characteristics and bark age were as follows: 1) blisters 1–3 mm diameter (with necrotic lesions <1 mm diameter) on 1-yr-old twigs (developed during most recent season), 2) blisters 4–5 mm diameter (with necrotic lesions 2–3 mm diameter) on 2- or 3-yr old branches, 3) sunken necrotic lesions 7–12 mm diameter with gum exudate on branches 2- or 3-yr old, 4) same as “3” but with no gum visible,

5) sunken necrotic lesions 14–20 mm diameter with gum exudate on trunk 4- or 5-yr old, and 6) same as “5” but with no gum visible. The same two isolation methods described for trees 13- to 18-yr old were used for all sample types collected from trees 4- to 5-yr old. In addition, a third method was used with the latter samples. After surface-sterilization and removal of outer bark, the sample was again placed in 0.5% NaOCl and 10% ethanol for 1 min and then rinsed in sterile distilled water. Samples of inner bark were then collected and placed with the abaxial surface on APDA. Three 3-mm² sections from the margin of necrotic tissue were taken by each isolation method and from each sample type from each tree. The three tissue sections were generally from the same bark sample and were incubated in the same APDA plate. An exception to the above was the 1- to 3-mm diameter blisters; three 2-mm² sections were cut from different blisters. For these infections, necrotic inner bark was only slightly visible around the lenticel at the apex of the blister. Isolation frequencies were compared based on *t* tests and least significant difference ($P \leq 0.05$).

RESULTS

Isolations from inoculated trees. After 18 mo, trees inoculated with the peach isolate of *B. obtusa* or *B. rhodina* or the apple isolate of *B. dothidea* had no visible symptoms. Trees inoculated with the peach isolate of *B. dothidea* were either dead (69%) or severely diseased. Infections were manifested initially as blisters and later as sunken lesions surrounding lenticels. Lesions on the surviving trees often were accompanied by profuse gumming and had coalesced.

B. obtusa was isolated from the outer bark of all trees including uninoculated trees of both cultivars (Table 1). *B. dothidea* and *B. rhodina* were isolated at frequencies of 80–100% from outer bark of trees on which peach isolates of these fungi were introduced but seldom were isolated from other trees. *B. dothidea* was isolated more frequently from outer bark of trees inoculated with the peach isolate (80 and 100% for the two cultivars) than from outer bark of trees inoculated with the apple isolate (3 and 40%) of this species. Isolation was most frequent for stem segments that had been immersed in 0.5% NaOCl and 10% ethanol for 1 min and then split longitudinally. Surface-sterilization of the stem with 70% ethanol tended to reduce, but did not eliminate, fungi from the outer bark. Isolation from inner bark or xylem of the surviving trees inoculated with the peach isolate of *B. dothidea* was not feasible using the above technique; disks of tissue could not be separated because of the uneven swelling and extensive necrosis. *B. obtusa* and *B. rhodina* were recovered from surface-sterilized outer

bark (of trees on which they were inoculated) at frequencies of 35–60% and 78–100%, respectively. The isolation frequencies of these fungi for inner bark were only 5–13%, and for xylem, 0–3%.

Isolation of naturally occurring *Botryosphaeria* species. *B. dothidea* was more prevalent in October 1989 than in January 1990 in isolations from cankers on trees 13- to 18-yr old (Table 2). The opposite was observed with *B. obtusa*. Results with two cultivars, each at two sites, were similar. In October, both species were common in outer bark, but *B. dothidea* was always predominant in inner bark. In January, *B. obtusa* was generally isolated more often than was *B. dothidea* from outer bark, but not from inner bark.”

Botryosphaeria species isolated from infected young trees were similar for the cultivars Springbrite and Summergold. Only data for Springbrite are presented (Table 3). As with cankers on the older trees mentioned above, *B. obtusa* generally was detected more often in January than in October. There was no significant difference among the frequencies of *B. obtusa* in bark collected in October with various symptoms or from inner bark sampled in January; there were few

differences among outer bark samples taken in January.

When outer bark of young trees was plated on PDA, the frequency of *B. dothidea* seldom was different from that of *B. obtusa* (Table 3). The few cases in which differences occurred involved blisters or small nongumming lesions where *B. dothidea* was predominant. *B. dothidea* was consistently predominant in inner bark of lenticels associated with various symptoms whether or not tissue was surface-sterilized after the removal of outer bark.

DISCUSSION

The separation of outer bark from inner bark with a scalpel and forceps was neither complete nor precise. A certain amount of contamination during the isolations was expected from organisms present on the bark surface or in adjacent tissues. Consequently, the outgrowth of a *Botryosphaeria* sp. on PDA in this study does not indicate positively that the fungus came from the tissue being sampled. However, differences in isolation frequencies of *Botryosphaeria* species from different tissues do indicate adaptation of these species to different habitats.

Botryosphaeria spp. probably survive in or on the outer bark of peach trees as saprophytes. This was apparent in nonwound inoculations with *B. rhodina*, which was recovered at high rates from the outer bark of symptomless trees after 18 mo but which was rarely isolated from inner bark (Table 1). It is likely that the strain of *B. rhodina* isolated was the same one used in the inoculations, since *B. rhodina* was seldom detected on other trees in the experiment. *B. obtusa*, on the other hand, was common on all trees regardless of inoculation treatment. Its general occurrence may have been due to its spread from inoculated trees and/or natural inoculum in the lathhouse.

All trees inoculated without wounding were symptomless except those inoculated with the isolate of *B. dothidea* from peach, which caused severe necrosis or tree death. The isolate of *B. dothidea* from apple persisted in the outer bark but did not cause disease. Although intraspecific variability was not tested, results are consistent with previous pathogenicity tests (2,12) indicating that a physiologic race of *B. dothidea* causes disease symptoms associated with lenticels. On trees 13- to 18-yr old, *B. obtusa* was generally more frequent in January

Table 1. Isolation of *Botryosphaeria* spp. from non-wound-inoculated peach trees of two cultivars^a

Inoculum Tissue sampled ^b	Isolation frequency ^c (%)					
	Candor			Summergold		
	<i>B. dothidea</i>	<i>B. obtusa</i>	<i>B. rhodina</i>	<i>B. dothidea</i>	<i>B. obtusa</i>	<i>B. rhodina</i>
Control						
Half stem	0	81 ± 9	0	19 ± 13	56 ± 11	6 ± 6
Outer bark-N	0	73 ± 9	0	0	48 ± 11	3 ± 3
Outer bark-S	0	28 ± 9	3 ± 3	0	28 ± 5	3 ± 3
Inner bark	0	10 ± 8	0	0	8 ± 5	0
Xylem	0	0	0	0	0	0
Bd-67 ^d						
Half stem	100 ± 0	50 ± 50	0	83 ± 17	67 ± 17	0
Outer bark-S	100 ± 0	0	0	80 ± 0	7 ± 7	0
Bd-68						
Half stem	9 ± 16	69 ± 16	0	19 ± 13	19 ± 9	6 ± 6
Outer bark-N	18 ± 18	38 ± 15	0	10 ± 4	10 ± 8	0
Outer bark-S	40 ± 10	3 ± 3	0	3 ± 3	0	0
Inner bark	15 ± 8	0	0	5 ± 3	0	0
Xylem	0	0	0	5 ± 5	0	0
Bo-52						
Half stem	0	88 ± 13	0	0	88 ± 8	0
Outer bark-N	0	75 ± 12	0	0	40 ± 9	0
Outer bark-S	0	60 ± 11	0	0	35 ± 11	3 ± 3
Inner bark	0	13 ± 4	0	0	8 ± 5	0
Xylem	0	3 ± 3	0	0	0	0
Br-48						
Half stem	0	38 ± 16	100 ± 0	0	13 ± 8	100 ± 0
Outer bark-N	0	13 ± 8	93 ± 5	0	23 ± 10	80 ± 10
Outer bark-S	0	13 ± 6	100 ± 0	0	8 ± 5	78 ± 12
Inner bark	0	3 ± 3	5 ± 5	0	5 ± 3	0
Xylem	0	0	0	0	0	0

^a Eight trees of each cultivar were sampled 18 mo after inoculation with isolates of *B. dothidea*, *B. obtusa*, and *B. rhodina*. Fungi used were collected from diseased bark of peach trees: *B. obtusa* (isolate Bo-52), *B. rhodina* (isolate Br-48), and *B. dothidea* (isolate Bd-67), except *B. dothidea* (isolate Bd-68), which came from apple.

^b A 2.5-cm section of each stem was immersed in 0.5% NaOCl and 10% ethanol for 1 min, and each of two longitudinal halves was placed in separate potato-dextrose agar dishes. Also, 4-mm-diameter disks of outer bark were removed before (N) and after (S) surface-sterilization with 70% ethanol. Stems were again wiped with ethanol before removing disks of inner bark and xylem. Five disks of each tissue type were plated per tree.

^c Values are means ± standard errors.

^d Isolation data for Bd-67 are based on two Candor and three Summergold trees, which were the numbers remaining alive. Because of severe swelling and necrosis, isolations from tissue disks were attempted only with outer bark. All trees inoculated with other isolates were asymptomatic.

than it had been the previous October. In outer bark sampled in January, *B. obtusa* was clearly predominant over *B. dothidea*, which was detected at only 1% (Table 2). This is consistent with results of Britton and Hendrix (4), who detected no *B. dothidea* in January. However, in the present study, when the outer bark was removed and isolations were made from the diseased inner bark, *B. dothidea* was detected at frequencies not different ($P \leq 0.05$) from those of *B. obtusa* (Table 2).

The progression of isolations from

outer to inner bark tissues usually resulted in an increase in the proportion of *B. dothidea* compared with *B. obtusa*. *B. dothidea* was predominant in the diseased inner bark on old trees sampled in October (Table 2). It was also predominant in the diseased inner bark of young trees (Table 3), regardless of the time of isolation or the symptom observed. *B. dothidea* was isolated most frequently from the youngest infections, which were generally on the youngest bark, and least frequently in the oldest infections. It is expected that time and

extent of necrosis would afford greater opportunity for secondary organisms to invade older infections. Also, the thicker rhytidome of older bark may support greater numbers of saprophytic and opportunistic fungi.

In Britton and Hendrix's studies (3,4), *Botryosphaeria* species were isolated from cankers on twigs, scaffold limbs, and trunks of peach trees; however, a description of the cankers was not given. They did not state whether the gummosis symptoms were the same as or different from those reported earlier by Weaver (17-19), who basically described localized symptoms associated with lenticels. Cankers referred to by Britton and Hendrix (3) possibly were associated with wounds, since they inoculated trees with *Botryosphaeria* by wounding. Weaver (17) found that *B. dothidea* can invade through wounds, but in describing disease symptoms that typically occur in peach orchards, he did not mention an association with wounds.

In the present study, efforts were made to sample bark from all parts of the tree where profuse gumming occurred. Gumming lesions were generally localized and not connected with wounds. Where necrosis and gumming on the oldest bark was extensive, there was little clue as to how or where the causal agent may have entered. Because tissue was typically interspersed with patches of live bark, diseased areas probably resulted largely from a coalescence of localized lesions initiated at lenticels.

The most common wounds on trees studied were those resulting from pruning. The extent of necrosis occurring immediately below pruning cuts was not unusual for peach trees in general. Prun-

Table 2. Isolation of *Botryosphaeria* spp. from diseased scaffold limbs of 13- to 18-yr-old peach trees in commercial orchards in central Georgia

Cultivar (age) Bark ^b	Isolation frequency ^a (%)			
	October 1989		January 1990	
	<i>B. dothidea</i>	<i>B. obtusa</i>	<i>B. dothidea</i>	<i>B. obtusa</i>
Harvester (17 yr)				
Outer	0*	19 ± 7	2 ± 2*	42 ± 9
Inner	42 ± 10*	4 ± 4	12 ± 6	20 ± 7
Harvester (18 yr)				
Outer	2 ± 2	0	0	16 ± 8
Inner	25 ± 8*	0	12 ± 6	9 ± 3
Coronet (18 yr)				
Outer	24 ± 9*	16 ± 8	0*	27 ± 9
Inner	21 ± 6*	2 ± 2	7 ± 3	17 ± 6
Coronet (13 yr)				
Outer	0	5 ± 3	4 ± 3*	27 ± 5
Inner	35 ± 9*	0	19 ± 8	11 ± 5
Combined . . .				
Outer	7 ± 3	10 ± 3	1 ± 1*	29 ± 4
Inner	31 ± 4*	1 ± 1	13 ± 3	14 ± 3

^a Bark samples from each of 20 trees per orchard were immersed in 0.5% NaOCl and 10% ethanol for 1 min, and then three 3-mm² sections of each tissue type were transferred to potato-dextrose agar. Sections were cut at margins of necrosis.

^b Values are means ± standard errors. Asterisk indicates difference ($P \leq 0.05$) between means for *B. dothidea* and *B. obtusa* for a given tissue and isolation date based on Student's *t* test.

Table 3. Isolation of *Botryosphaeria* spp. from peach bark of various ages and symptomatology on 4-yr-old commercial trees of the cultivar Springbrite^a

Symptom or lesion type (size) ^b	Bark age (yr)	Isolation frequency ^c (%)							
		October 1989				January 1990			
		Outer bark		Inner bark		Outer bark		Inner bark	
		<i>B. dothidea</i>	<i>B. obtusa</i>	<i>B. dothidea</i>	<i>B. obtusa</i>	<i>B. dothidea</i>	<i>B. obtusa</i>	<i>B. dothidea</i>	<i>B. obtusa</i>
Healthy	4	0	6	11	0	0	11	0	0
Blisters ^d (1-3 mm)	1	46*	0	97*	3	55*	3	64*	0
Blisters ^e (4-5 mm)	2-3	33*	0	89*	0	36	11	86*	11
Sunken (7-12 mm)									
No gum	2-3	30	3	85*	0	28*	17	75*	0
Gumming	2-3	25	6	69*	0	17	0	53*	3
Sunken (14-20 mm)									
No gum	4	8	11	48*	0	6	19	53*	3
Gumming	4	10	0	33*	3	12	15	58*	8
Extensive necrosis ^f	4	0	0	52*	3	8	6	56*	6
LSD ($P \leq 0.05$)		25	NS	29	NS	26	17	25	NS

^a Bark samples were immersed in 0.5% NaOCl and 10% ethanol for 1 min, and then sections of outer and inner bark were transferred to potato-dextrose agar dishes. From each of 12 trees, three 3-mm² sections from the margin of necrosis were plated per sample type and isolation method.

^b Numbers in parentheses are lesion diameters in millimeters.

^c Asterisk indicates difference ($P \leq 0.05$) between means for *B. dothidea* and *B. obtusa* for a given sample type and isolation method based on Student's *t* test. Mean separations in each column are based on least significant difference ($P \leq 0.05$); NS indicates no significant difference.

^d Blisters consisted of hyperplastic tissue surrounding necrotic tissue (lesion) at margin of lenticel. Lesion diameter is ≤ 1 mm.

^e Lesion diameter is 2-3 mm.

^f Areas of disease described as follows: profuse gumming, irregular shape, roughly 10-20 cm in length, and extensive necrosis but with living bark interspersed.

ing stubs (often only a few millimeters in length) on major branches had died back to the collar, as is typical after woody plants are pruned (14,15), and older cuts had been partially or completely walled off by new bark. *Botryosphaeria* species had colonized the pruning stubs, as evidenced by the presence of fruiting bodies, but usually did not invade the collar of the supporting branch. Localized lesions frequently clustered in areas below, but not connected with, old pruning wounds and may have resulted when fungal spores produced on the dead stubs were carried downward by rainwater.

On the basis of the isolation study reported here and on previous pathogenicity tests (2,5,11,12,19), *B. dothidea* is considered to be the primary agent causing lenticel-associated symptoms on peach bark. Peach gummosis, as characterized by these symptoms, is unique and should be distinguished from peach diseases caused by other *Botryosphaeria* species. If *B. obtusa* sometimes succeeds *B. dothidea* as a pathogen, as Britton and Hendrix (4) have suggested, an interaction involving these fungi could be

tested by sequential or combined inoculations of trees.

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