Pathogenicity, Distribution, Sources of Inoculum, and Infection Courts of *Botryosphaeria dothidea* on Pistachio

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ABSTRACT

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A panicle and shoot blight disease caused by *Botryosphaeria dothidea* is reported for the first time on pistachio in California. The disease was prevalent in northern counties (Butte, Tehama, and Glenn) and sporadic in southern counties (Madera, Fresno, Kings, and Kern). In early spring, symptoms of panicle and shoot blight resulted from continued activity of the pathogen in buds infected the previous summer and fall. Shoot blight symptoms included dark brown to black lesions at the base of current-season shoots, followed by the wilting and drying of leaves, which remained attached to their shoots. Panicle and shoot infections caused perennial cankers. The infection of petioles, leaves, fruit, and rachises was evidenced by expanding lesions, which resulted in the death of the structures involved. The infection of panicles usually occurred at the base, causing fruit to shrivel and remain on the tree even after mechanical shaking for harvest. Current-season shoots and panicles were susceptible to artificial inoculations throughout the growing season. Infected rachises, petioles, fruit, and leaf blades were retained longer on the tree than corresponding healthy plant parts and provided inoculum (pycnidiospores) for winter and spring infections. In addition, pycnidia that had formed on current-season infected rachises, fruit, blighted shoots, petioles, and leaf lesions provided inoculum for summer and fall infections. Moreover, the fungus remained active as mycelium in perennial shoot cankers. Light and scanning electron microscopy indicated that germ tubes of conidia of *B. dothidea* entered through the stomata of the upper or lower surfaces of leaves, rachises, and shoots, and also through the lenticels on pistachio fruit.

Additional keywords: *Botryosphaeria ribis*, *Dothiorella* sp., etiology, *Pistacia vera*.

The pistachio (*Pistacia vera* L.) industry in California presently comprises 22,500 ha, of which 18,000 ha is in production (6). The only diseases reported for pistachio trees in California are Verticillium wilt, caused by *Verticillium dahliae* (1); a crown and root rot caused by *Phytophthora parasitica* (14); and a blossom and shoot blight caused by *Botrytis cinerea* (2). In the summer of 1984, an unknown disease characterized by blighted panicles and shoots was initially observed in a commercial pistachio orchard in Butte County and later (during 1985–1986) in other orchards (17). Infected parts consistently showed the pycnidial stage (*Dothiorella* sp.) of the fungus *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not.

B. dothidea, reported previously under the synonym *B. ribis* Gross. & Duggar, causes branch and trunk cankers on a variety of woody plants (19,21). In California, Fawcett (10) reported a *Dothiorella* sp. that causes black cankers in the crotches and limbs and, occasionally, sudden wilting and dying of branches of English walnut (*Juglans regia*) and arroyo willow trees (*Salix lasiolepis*). Smith (20), working mostly with isolates of this fungus from avocado (*Persea americana*), citrus (*Citrus limon*), and walnut, showed that the fungus is pathogenic to 50 plant species representing 34 genera and 20 families.

B. dothidea, first reported on almond (*Prunus dulcis*) cv. Nonpareil in California in 1966, causes a bandlike canker on the trunk or scaffolds of vigorous young trees (8). Both the teleomorph and anamorph phases of *B. dothidea* have been reported on giant sequoia (*Sequoiadendron giganteum*) and on coast redwood (*Sequoia sempervirens*) in California (24).

This is the first study of panicle and shoot blight of pistachio caused by *B. dothidea*. Panicle and shoot blight was chosen as the general (common) name, because the most frequent and destructive phase of the disease is the severe blighting of panicles and shoots. In addition, the fungus infects male pistachio trees and causes blossom and shoot blight.

The objectives of this study were to determine the incidence of the disease in pistachio orchards in California, to establish proof of the pathogenicity of *B. dothidea*, to reproduce the disease symptoms observed in the field, to determine the susceptibility of pistachio throughout the growing season, and to identify the sources of inoculum and infection courts of *B. dothidea* on pistachio.

MATERIALS AND METHODS

Isolation, identification, and distribution of the pathogen. *B. dothidea* was first isolated from pistachio in California after periods of wet weather during the spring of 1984 (17). Diseased plant parts from male and female pistachio trees (i.e., petioles, leaves, panicles, fruit, and buds) were collected periodically from orchards in Butte, Tehama, and Solano counties. The plant parts were surface-sterilized in sodium hypochlorite (0.5%) for 1 min, rinsed in sterile water, and dried on sterile filter paper; tissue pieces were then plated on acidified Difco potato-dextrose agar (APDA) (2.5 ml of a 25% solution of lactic acid per liter of medium). Isolations from buds were made either after cutting them in half or by immersing entire buds into APDA after surface sterilization. All dishes were incubated at 27 C for 5 days.

Isolations were made on APDA from the margins of 1- to 4-yr-old cankers to determine how long *B. dothidea* remains active in shoot cankers and whether the fungus invades pruning wounds.

The incidence of *B. dothidea* was determined by collecting 20 blighted shoots during the spring and summer from both male and female trees in 32 orchards located in 11 counties. Isolations were made from five pieces of tissue from the canker margins.

Pathogenicity tests. To test the pathogenicity of the fungus and determine pistachio susceptibility, shoots, panicles, leaf and bud wounds (scars), and buds were inoculated with fungal inoculum on female pistachio trees (cv. Kerman) at the Wolfskill Experimental Orchards of the University of California in Solano County from May through October. Shoot inoculations were also made at a commercial orchard at Durham in Butte County on 1 and 15 May. Either mycelial-agar plugs (5 mm in diameter, from a 10- to 15-day-old culture of *B. dothidea*) or a spore suspension of *B. dothidea* containing 10^5 conidia per milliliter were used for inoculations. To prepare the spore suspension, dishes containing pistachio nut decoction agar (185 g ground green pistachio fruit and 39 g Difco PDA in a liter of distilled water) were flooded with sterile distilled water, which was then filtered through a four-layer cheesecloth to exclude mycelial fragments, and the spore concentration was adjusted with a hemacytometer.

Shoot inoculations were made by removing a bark plug (5 mm in diameter) from current-season shoots (20–40 cm long) and either placing a mycelial plug on the wound or spraying it with conidia (10^5 spores per milliliter) and then covering it with parafilm. The inoculations were replicated on 10 shoots each of five female (cv. Kerman) trees. Control shoots were inoculated with potato-dextrose agar or sprayed with water.

Leaf and bud wounds were exposed on each of 10 current-season shoots (by removing five to six petioles or buds per shoot), sprayed immediately with spore suspension, and wrapped with parafilm. Controls were sprayed with water.

Ten current-season shoots, each bearing two to four panicles, were sprayed to runoff with approximately 10 ml of a suspension of *B. dothidea* (10^5 spores per milliliter); control shoots were sprayed with water. To prevent moisture loss, shoots and panicles were immediately covered with plastic bags secured with masking tape. Brown paper bags were placed over the plastic bags to protect plant tissues from exposure to direct sunlight. All bags were removed 2–3 days after the inoculations, which were made at 2-wk intervals from 7 May to 20 September. Later in the year, dormant buds on 10 shoots of both male and female trees were inoculated by spraying to runoff with either a spore suspension or water (for the control). Symptoms were recorded at 3, 10, 20, 30, and 60 days postinoculation for all trials and compared with analogous natural infections. The inoculated buds were bisected and compared with naturally infected (blighted) buds.

Basal scale inoculations were made by making three slit wounds (3–4 mm long and 1 mm deep) on shoot bases in areas that had remnants of bud scales. These were sprayed with a suspension (10^5 spores per milliliter) and covered with laboratory film. Similar inoculations were made on shoots where basal scales were either removed or left intact. A water spray was used as the control. These inoculations were performed in May 1985 in the commercial orchard at Durham.

Effects of *B. dothidea* on the retention of naturally infected clusters and petioles. Ten attached shoots, each bearing three to six infected clusters, were marked with flagging tape in September 1985 and 1986 on four replicate trees in the orchard at Durham. Ten shoots with healthy-appearing clusters were also flagged on the same trees and served as controls. In April and August 1986, the rachises and petioles that were still hanging on the marked shoots were recorded. After the final reading the rachises, petioles, and dead shoots were collected, brought to the laboratory, sectioned with a razor blade, and examined with a dissecting microscope for the presence of pycnidia. The experiment was repeated during 1986–1987.

Retention of inoculated fruit clusters and leaves. In an orchard in Solano County, 10 shoots bearing three to six fruit clusters were inoculated by spraying with a suspension of *B. dothidea* (10^5 spores per milliliter). Inoculations were made on 10 different dates from 10 May to 20 September 1985. On each date 10 shoots were also marked but not inoculated and served as controls. Hanging rachises, fruit mummies, and petioles were recorded at the end of January 1986. This experiment was also repeated during 1986–1987.

Sources of inoculum. In April 1985, 1986, and 1987, infected and dead shoots, rachises and petioles, dead buds, and nuts (mummies) hanging on the trees from the 1984 growing season were randomly collected from several trees in three orchards. In August and September, attached leaves with small and large necrotic lesions and infected fruit caused by *B. dothidea* were collected, and in January and February defoliated pistachio leaves were gathered from the orchard floor. All samples were examined with a dissecting microscope after being sectioned with a razor blade. When pycnidia were found, the pycnidiospores were examined to determine the anamorph or teleomorph stages of the fungus.

Mode of infection of pistachio leaves, fruit, and rachises by *B. dothidea*. Leaves, fruit, and rachises from Kerman pistachio trees were collected in July, surface-sterilized for 3 min in a 0.08% sodium hypochlorite solution, dried, and inoculated by spraying with a spore suspension of *B. dothidea* ($\sim 3 \times 10^4$ spores per milliliter). Plant parts were collected 12, 24, and 48 h after inoculation, and 15- to 20-mm^2 sections were prepared for observation under a light microscope or a scanning electron microscope (SEM). For light microscopy, inoculated fruit, leaf, and rachis sections were fixed in 2% glutaraldehyde prepared with Sorenson's phosphate buffer (pH 7.0) for 30 min and then rinsed three times with the buffer. The tissue was stained for 30 min with 1% acid fuchsin or cotton blue in lactophenol, destained in lactophenol for 2 min, and mounted in 50% glycerol.

For the scanning electron microscopy, sections were fixed in 1% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2) for 24 h, rinsed in three half-hour changes of distilled water, dehydrated in ethanol (stepwise at 30, 60, 80, 95, 95, and 95% for 1 h each), and stored in 100% ethanol until used. The tissue sections were then critical-point dried with CO_2, mounted on SEM stubs, coated with 60% palladium and 40% gold, observed with a scanning electron microscope (ISI-DS130 [dual stage], 10 kV, 1.2 A; International Scientific Instruments, Inc., Santa Clara, CA), and photographed.

RESULTS

Isolation and identification of the pathogen and symptomatology of the disease. During the 1985 and 1986 growing seasons, the anamorph (a *Dothiorella* sp.) of *B. dothidea* was consistently isolated from blighted plant parts. In early April 1985, all isolations from lateral and terminal buds killed in 1984 in an orchard in Butte County yielded *B. dothidea* (Table 1). In 1985, the incidence of the pathogen in the various plant parts collected in the spring, summer, and fall ranged from 40% for rachises to 100% for symptomatic twigs, petioles, leaf blades, and fruit (Table 1). In the orchard in Tehama County, fungal incidence ranged from 53% for dead lateral buds to 94% for blighted shoots (Table 1). In the orchard in Solano County, where blighted shoots were found only on the male trees, the incidence of the pathogen ranged from 76 to 86%. Other isolated fungi were *Botrytis cinerea, Alternaria alternata, Cladosporium, Phomopsis, Fusarium* and *Pestalotia* spp., *Diplodia natalensis,* and *Epicoccum purpurascens.* The incidence of *B. dothidea* in isolations from 1- and 2-yr-old cankers was 100% and in 3- and 4-yr-old cankers 98 and 96%, respectively. Pycnidia with viable spores were present in all of the cankers.

Identification of *B. dothidea* was based on comparisons of morphological and cultural characteristics of several isolates from pistachio with those of an isolate of the fungus that causes a bandlike canker of almonds in California (8) and those of an isolate received from Georgia that causes peach tree gummosis (3). Identification was also made through an examination of isolates from pistachio from E. S. Luttrell (Department of Plant Pathology, University of Georgia, Athens).

The first noticeable disease symptoms in early spring were the wilting of young shoots (up to 5 cm long), with leaves that were initially light green turning light brown and remaining on the tree (Fig. 1A and B). Early-season infections of newly emerged shoots originated at their bases from already contaminated buds, and white-gray mycelium was found in the pith of blighted shoots. Developed cankers on current-growth shoots had diffused margins, which became sharpened when the fungus invaded 2-yr-old wood. Symptoms on leaves and petioles consisted of small (2–4 mm in diameter), angular, dark brown lesions, which frequently coalesced and caused death of leaves; pycnidia in leaf lesions were observed in September.

As a result of the infection of flower buds during the previous growing season, emerged panicles became shriveled by the beginning of May. Previous infection of leaf scar also produced panicle blight. After the initiation of sprinkler irrigations, healthy panicles developed black, longitudinal, expanding lesions along the fruit support structure. Such symptoms were not found in nearby flood-irrigated orchards. Affected panicles turned from green to brown, shriveled, and died (Fig. 1B and C). Canker margins often extended beyond the panicle, into the sustaining shoot (Fig. 1C). Fruit borne on such panicles failed to develop.

The fruit was also susceptible to fungal infections, resulting in small black lesions on the green epicarp, which later enlarged and coalesced. By summer or early fall, the infected fruit was gray, with numerous black pycnidia embedded in the epicarp (Fig. 1D). When infections occurred after hull splitting (early to mid-August), the hull cavity was invaded, and white-gray, cottony mycelia of *B. dothidea* developed on the kernels. Infected buds and leaf and bud scars were black, and twig cankers were associated with these infections (Fig. 1E); occasionally gum was produced from the base of the infected buds. By late August to mid-September, pycnidia were evident on these cankers.

Pathogen distribution. *B. dothidea* was isolated from blighted pistachio shoots in a number of counties in California, but the incidence varied considerably (Fig. 2). The fungus was frequently isolated from blighted shoots from orchards in northern counties (Butte, Tehama, Glenn, and Solano) but found only sporadically in southern counties (Madera, Fresno, Kings, and Kern) (Fig. 2).

Pathogenicity tests. In the Wolfskill Experimental Orchards, typical disease symptoms were observed on pistachio shoots within 7 days after inoculation with mycelia and within 10–15 days on shoots inoculated with spores. The incidence of dead shoots ranged from 50 to 100% for mycelium inoculations and from 20 to 100% for spore inoculations (Table 2). Control shoots remained symptomless. Isolation from inoculated shoots yielded *B. dothidea.*

TABLE 1. Isolation of *Botryosphaeria dothidea* from pistachio in three California orchards

Location of orchard[a]	Date of sampling	Plant part[b]	Incidence of *B. dothidea* (%)[c]
Butte County (commercial)	10 Apr.	LB, TB[d]	100, 100[e]
	17 Apr.	T	100
	24 Apr.	T	85
	2 May	BC	56
	10 May	F, R, BC	85, 98, 98
	29 May	P, F, R, BS	98, 100, 40, 93
	5 June	T, L, F	98, 100, 92
	31 July	F, MK, R	100, 70, 96
	20 Aug.	MK	75
	18 Sept.	T, LB, P, T[f]	96, 100, 100, 100
	11 Dec.	T, BLS	96, 100
	8 Jan.	T, P	84, 84
Tehama County (commercial)	2 May	T	74
	10 May	F	85
	5 June	T, F	94, 82
	13 June	T	82
	29 Oct.	LB	53
Solano County (Wolfskill Experimental Orchards)	3 June	T[f]	86
	23 Sept.	T[f]	76
	15 Oct.	T[f]	84

[a]Three orchards with plant parts showing typical symptoms were sampled from spring 1985 to winter 1986. Similar results were obtained from samples collected in 1986 and 1989.

[b]LB = lateral (flower) buds; TB = terminal buds; T = twigs; BC = blighted clusters; F = fruit; R = rachises; P = petioles; BS = bud scales; MK = mycelium in kernels; BLS = bud or leaf scars.

[c]On each date, 50 tissue pieces from 10 random plant samples were plated on acidified potato-dextrose agar medium, as described in the text.

[d]Twenty-five to fifty buds were plated as described in the text.

[e]Percentages respectively correspond to plant parts.

[f]Twigs from male trees.

Leaf wound (scar) inoculations resulted in shoot blight 10–17 days after inoculation (Table 2). Leaves at the tips of inoculated shoots wilted, shriveled, and dried but still remained attached to the shoots. Leaf scars turned black, with lesion margins expanding bilaterally, and gum exuded from some of the infected sites. Abundant pycnidia developed by mid-August from inoculations in May and July. Bud wound (scar) inoculations developed symptoms typical of natural bud-scar infections, and 43% were infected by March of the following year (Table 2).

Within 2–3 days after their inoculation, fruit, rachises, petioles, and leaf blades developed small (1–2 mm in diameter) black lesions. Symptomatic tissues sampled and examined under a dissecting microscope revealed that necrotic tissues were associated with stomates or lenticels. With further incubation (1–2 mo), the individual lesions enlarged, coalesced, and caused fruit cluster, leaf, and shoot blight symptoms (Table 2). The disease symptoms were similar to those observed in natural infections. By mid-September, the fruit surface was covered with mature pycnidia containing spores of *B. dothidea.* Shriveled fruit and blighted leaves remained attached to the tree following fruit harvesting. Uninoculated clusters and leaves on adjacent shoots remained symptomless. After 1 mo, 10% of the buds inoculated in July were killed, and 11–16% of those inoculated in October were infected by March of 1986 (Table 2). The dead buds appeared black, often with gum exuding near the bud base. In partially infected buds, the fungal infection started in the upper part of the bud, and numerous pycnidia developed on the basal portions of the external bud scales. Similar symptoms and signs were also observed in naturally infected buds.

Shoots inoculated on 1 and 15 May with a mycelial plug or spore suspension of *B. dothidea* in the orchard in Butte County were infected in 1–2 mo (Table 3). Basal scale inoculations yielded no symptoms after 2 wk, but 60% yielded dead tissue 1–2 mo after inoculation (Table 3). Uninjured shoots from which basal scales were removed showed 20% shoot blight, whereas shoots with basal scales showed none (Table 3). Canker lengths in 1-yr-old shoots ranged from 8 to 35 mm. Abundant pycnidia developed along all inoculation sites 1–2 mo after inoculation.

Retention of naturally infected fruit clusters and petioles. A significantly greater number of infected rachises and petioles than healthy ones were still hanging on the trees (Fig. 3) 11 mo from the time they were first marked (Table 4). Moreover, the percentages of rachises and petioles with pycnidia were significantly higher than those on shoots that had appeared healthy at the time they were marked. The number of dead shoots was also much higher for infected shoots than for those that appeared healthy (Table 4).

Retention of inoculated fruit clusters and leaves. In the orchard in Solano County, where inoculated clusters were compared with the uninoculated controls, the total number and percentage of rachises hanging on the trees by the end of January was 6–7 times higher than on control trees, and 10 times more fruit mummies were still hanging on inoculated trees (Table 5). Although most of the leaf blades dropped, a high number of petioles were firmly attached to the infected (blighted) shoots. Characteristic pycnidia of *B. dothidea* were found on blighted twigs, rachises, fruit mummies, and petioles sectioned with a razor blade and observed with a dissecting scope.

Sources of inoculum. Pycnidia containing viable pycnidiospores were observed on dead shoots of both male and female trees and on rachises hanging on the trees from previous years. Mature pycnidia (full of pycnidiospores) occurred on rachises still hanging on the trees from the two previous years. The majority of the pycnidia were found close to the base of blighted shoots, rachises, petioles, leaf and bud scars, the centers of cankers, and outer basal scales of blighted buds. Pycnidia were either isolated or in groups of five to eight and were black (but white when sectioned, because of the hyaline, one-celled pycnidiospores). Under the dissecting scope the prominent ostioles of the pycnidia were also evident. No signs of the fungus were found on defoliated leaves collected from the orchard floor in January and February. Inoculum sources of *B. dothidea* during the spring and summer were blighted rachises, dead shoots, petioles hanging on the trees from previous years, dead buds, large leaf lesions with pycnidia, nut

Fig. 1. Symptoms caused by *Botryosphaeria dothidea* on pistachio shoots. **A,** Blighted shoot on male (cv. Peters) tree. **B,** Blighted shoot on female (cv. Kerman) tree. **C,** Panicle (cluster) blight caused by rachis infection and advancing cankers (indicated by arrows). **D,** Infected fruit, showing abundance of pycnidia. **E,** Advancing canker from bud and/or petiole infection.

mummies from previous years (if still present on the trees), and cankers. During the fall and winter the sources of inoculum were all the above (if still present on trees), as well as rachises, shoots, fruit and leaf lesions infected in the previous spring and summer, and cankers that developed during the season. Based on the sources of inoculum (pycnidia), the absence of teleomorph in pistachio orchards, the occurrence of the fungus on the various parts of pistachio trees, the inoculation tests made in this study,

Fig. 2. Frequency of *Botryosphaeria dothidea* on blighted shoots in California pistachio orchards. ● = orchards with *B. dothidea*; ○ = surveyed orchards without the pathogen.

TABLE 2. Inoculations of plant parts of pistachio trees with *Botryosphaeria dothidea*

Plant part[a]	Inoculum[b]	Inoculation date	Parts infected 1–2 mo after inoculation (%)
Shoots	M	5/7, 7/12, 7/26, 8/23, 9/6, and 9/20	100, 100, 50, 100, 100, and 80[c]
Shoots	S	5/16[d], 7/26, 8/23, 9/6, and 9/20	100, 80, 60, 20, and 30
Leaf wounds (scars)	S	5/10[d], 7/5, and 9/20	100, 100, and 90
Bud wounds (scars)	S	10/10	43[e]
Panicles	S	5/7, 5/24, 6/7, 6/21, 7/5, 7/19, 8/2, 8/23, 9/6[f], and 9/20	100, 100, 100, 100, 100, 100, 100, 100, 100, and 100
Buds	S	7/26, 10/10, and 10/14	10, 16[e], and 11[e]

[a]Ten shoots and 10 panicles were inoculated on each date in 1985 at Wolfskill Experimental Orchards of the University of California.
[b]M = inoculations with mycelial plugs (5 mm in diameter) obtained from a 10- to 15-day-old culture of *B. dothidea* on acidified potato dextrose agar; S = spore suspension (10^5 pycnidiospores per milliliter) prepared from cultures of the fungus on nut decoction agar.
[c]Percentages respectively correspond to inoculation dates.
[d]Five shoots only.
[e]Determined in March of the following year.
[f]Seven panicles only.

TABLE 3. Inoculations of pistachio shoots and basal scales with *Botryosphaeria dothidea* in a commercial pistachio orchard in Butte County, California

Inoculation site and method of injury[w,x]	Inoculum[y]	Inoculation date	Samples infected after 1–2 mo (%)
A	M, S	1 May	100 a
A (control)	...	1 May	0 b
A, B	M, S	15 May	100 a[z]
B	S	15 May	60 b
C	S	15 May	20 c
D	S	15 May	0 d
A, B, C, D (controls)	...	15 May	0 d

[w]Ten shoots were inoculated on each date (in 1985) on each of three replicated trees.
[x]A = shoots injured with a cork-borer to remove a plug of bark 5 mm in diameter; B = shoots injured at their base, using a knife and creating three slits (3–4 mm long × 1 mm deep); C = uninjured shoots, with scales at the shoot base removed; D = uninjured shoots, with scales at the shoot base not removed.
[y]M = mycelial inoculum (5-mm-diameter fungal agar plug from a 10- to 15-day-old culture); S = spore suspension (10^5 pycnidiospores per milliliter).
[z]Numbers for each inoculation date followed by the same letters are not significantly different, according to Duncan's multiple range test ($P = 0.05$.).

Fig. 3. Retained pistachio panicle rachises (1R–5R) and petioles (1P–2P) blighted by *Botryosphaeria dothidea.* The basal portions of the rachises and petioles bear the pycnidia of the fungus and serve as sources of inoculum for the following season. Sprouting of lateral young shoots (arrows) results when the terminal shoot is killed.

and the 4-yr observations of the disease in three commercial orchards, a diagrammatic cycle of the disease is proposed (Fig. 4).

Mode of infection of pistachio leaves, fruit, and rachises by *B. dothidea*. The light microscopy showed that germ tubes of pycnidiospores penetrated the leaves through the stomata and the fruit tissues via lenticels. In samples fixed 12 h after inoculation, SEM showed that pycnidiospores of *B. dothidea* germinated with one to four branched germ tubes 150–640 μm in length (Fig. 5A). Germ tubes passing over stomata without penetration were observed (Fig. 5B). In none of these samples did the germ tubes enter the leaf tissue directly through the epidermal layer (Fig. 5A–D). Only occasional penetration through the stomata was observed after 12 h incubation (Fig. 5C [arrow]). However, in leaf samples fixed 24 or 48 h after inoculation, germ tubes frequently entered through the stomata (Fig. 5D). In some cases two or three germ tubes penetrated through stomata of leaves and petioles. The penetration of green rachises was through stomata; the fruit pericarp, through lenticels.

DISCUSSION

The *Botryosphaeria* species isolated from blighted panicles and shoots and from cankers of commercially grown pistachios in California was morphologically similar to an isolate of *B. dothidea* obtained from peaches in Georgia (3). My isolates fit the description of the pycnidial stage of *B. ribis* (16), which is considered a synonym for *B. dothidea* (5). The fungus invaded succulent tissues, producing cankers that eventually girdled entire shoots and fruit clusters. The blighting of shoots and immature fruit clusters in early spring probably resulted from subclinical infections of vegetative and flower buds during the previous fall. This contention is supported by the fact that when apparently healthy vegetative and flowering buds were inoculated, then sectioned 2 mo later and examined with a dissecting microscope, several showed partial infections originating from the outer bud scales, and 11–16% were dead by March of the following year (Table 2). These tissues produced colonies of *B. dothidea* when plated on APDA. In addition, lateral (flower) buds sampled on 18 September from the orchard in Butte County and on 29 October from the orchard in Tehama County revealed a 53–100% incidence of the pathogen (Table 1), indicating that the infection of buds was initiated during fall.

Although *B. dothidea* was isolated from only a few orchards and in a very low incidence in the San Joaquin Valley (except for one orchard in Fresno County), its incidence was very high in samples collected from both male and female pistachio trees in the Sacramento Valley counties of Butte and Tehama (Fig. 2). In an experimental orchard in Solano County, however, blighted shoots occurred only on male trees, whereas nearby female Kerman trees were free of infections, suggesting a potential difference in susceptibility between male and female pistachio trees.

In pistachio, the high incidence of isolation of *B. dothidea* from cankers older than 1 yr suggested that cankers are perennial rather than annual. Although there were instances where 2- to 4-yr-old cankers were isolated (aborted) from healthy tissues, it was still possible to isolate the fungus in areas of the advanced margins, indicating that the mycelium of the fungus remained alive. Moreover, pycnidia of *B. dothidea*, some still containing viable spores, were common in cankers 3–4 yr old. Cankers induced by *B. dothidea* in almond appeared to be primarily annual (9), but in a number of other hosts cankers caused by *B. dothidea* were reported to enlarge year after year (11,19). Britton and Hendrix (4) showed that 7–15% of samples from cankers on scaffold limbs and trunks of peach trees contained *B. dothidea*, and Brown and Britton (5) reported that Bot canker of apple could cause a decline of the entire tree. In California, although Fawcett described a canker disease on scaffolds of English walnut caused by *B. dothidea* (10), the pathogen did not cause cankers on the trunks and scaffolds of pistachio and did not kill the tree.

Shoots inoculated with mycelia were susceptible to the fungus throughout the growing season, but those inoculated with spores were less susceptible in July, August, and September (Table 2). This finding agrees with the fact that blighted shoots were usually not the result of direct spore infections but were caused either by girdling (from cankers developed the previous year from infected rachises or bud and leaf scars) or partial infection of vegetative terminal buds. In general, more newly blighted shoots were noticed in the period from April through June than from July through September (T. J. Michailides, *unpublished*).

TABLE 4. Frequency of dead shoots and retention of naturally infected rachises and petioles of pistachio infected with Botryosphaeria panicle and shoot blight

	Plant parts retained per 10 marked shoots[a]			
	Infected with *B. dothidea*[b,c]		Healthy-appearing[b]	
Plant part	(no.)	(%)	(no.)	(%)
Rachises	45.3*	74.8*	20.0	41.6
Petioles	7.3*	79.0*	0.3	0.0
Dead shoots	8.0*	89.5*	2.3	37.5

[a]Average of four replicate trees of 10 shoots, each bearing three to six clusters.
[b]Ten infected and 10 healthy-appearing shoots per each replicate tree were marked on 18 September 1985 and recorded on 18 August 1986.
[c]Asterisk indicates averages that are significantly different from healthy-appearing, based on a pairwise *t* test at $P \leq 0.05$.

TABLE 5. Frequency of dead shoots and retention of rachises, fruit mummies, petioles, and leaf blades of pistachio inoculated with *Botryosphaeria dothidea*

	Inoculated[a,b,c]		Control[d]	
Plant part	(no.)	(%)	(no.)	(%)
Blighted shoots	70*	70*	0	0
Rachises	168*	42*	26	6.5
Fruit mummies	579*	5.8*	58	0.6
Petioles	46*	9.2*	0	0
Leaf blades	12*	0.8*	0	0

[a]Ten shoots, each supporting three to six clusters, were inoculated with a spore suspension (10^5 pycnidiospores per milliliter) of *B. dothidea* on 10 dates during the period from 10 May to 20 September 1985. Data were recorded on 28 January 1986.
[b]Pycnidia present on all inoculated plant parts.
[c]Asterisk indicates numbers that are significantly different from the control, based on a pairwise *t* test at $P \leq 0.05$.
[d]Shoots, each supporting three to six clusters, were marked but not inoculated and served as controls (total of 100 shoots).

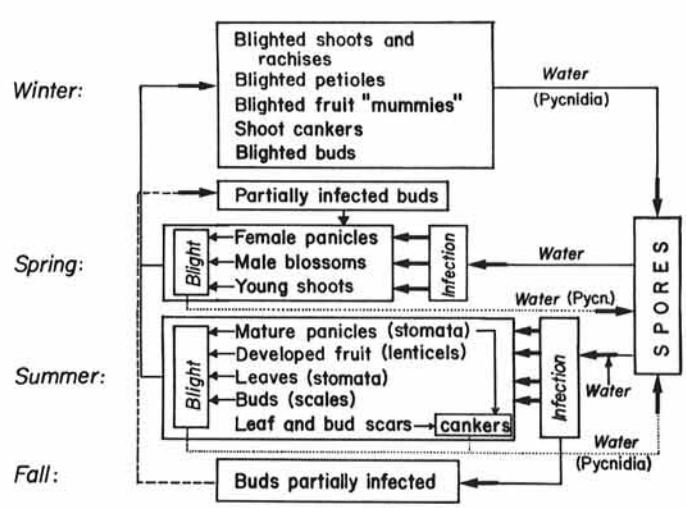

Fig. 4. Disease cycle of Botryosphaeria panicle and shoot blight of pistachio caused by the asexual stage (a *Dothiorella* sp.) of *B. dothidea*.

Fruit clusters were susceptible to the fungus throughout the growing season (Table 2), and newly blighted clusters became evident following irrigation. Localized infections on fruit epicarp, rachises, leaf blades, and petioles were associated with lenticels and open stomata, especially after a 24-h incubation. Pathogenicity tests on other host plants have indicated that *B. dothidea* is a wound pathogen (21–23). However, the pycnidiospores of the fungus have been shown to invade unwounded stems of elm (12) and blueberry (with penetration of germ tubes through stomata on stems) (15). In this study, pycnidiospores of *B. dothidea* were able to invade wounds (created by detachment of petioles, buds, or scales) and aggressively infect uninjured fruit and leaves by entering through stomata openings on leaves (Fig. 5D) and shoots and through lenticels on fruit. However, when basal bud scales of shoots remained intact, infection by pycnidiospores did not occur. Instead, the fungal spores infected and killed intact vegetative and flower buds.

For the abscission of flower buds of pistachio to occur in the same summer that a heavy crop of nuts is produced (an "on" year) is unique, since alternate bearing in other fruit and nut trees is brought about by limited flower bud formation, not abscission, during the heavy crop year (7). The abscission of buds creates several bud scars per shoot in "on" years. This study showed that bud wounds (scars) are susceptible to the invasion and establishment of *B. dothidea*. Because 43% of the artificially created bud wounds were infected after the application of spore inoculum of *B. dothidea*, and because of the high incidence of infections (frequently more than one infected bud scar per shoot was observed), it is suggested that bud scars can be a significant avenue of infection by the fungus, and in a disease management program the protection of these bud scars should be also considered.

The incidence of isolation of *B. dothidea* from naturally blighted shoots and clusters was high throughout the growing season (Table 1), indicating that the fungus was continuously active. Similarly, the isolate of the pathogen that causes bandlike canker of almond was reported to cause infections during the spring, summer, and fall in California (8). Pycnidiospores from pycnidia that develop during the current or prior seasons act as a source for new infections throughout the year.

Gumming is a very characteristic sign of cankers caused by *B. dothidea* on almonds (8), peach (21), and lemon (18). However, because of its sporadic appearance, gum formation was not considered diagnostic for this pathogen on pistachio.

Experiments concerning retention of infected parts were in agreement, regardless of the date of experiment initiation or data collection. Results showed that infected clusters (rachises and fruit) and petioles hang on the trees longer than healthy ones (Tables 4 and 5). Normally, healthy mature fruit of pistachio drops during late fall or after a mechanical shaking, and only the blanks hang longer. Healthy rachises and petioles of pistachio hang longer than the fruit but usually drop in late fall or winter. Infected rachises, fruit, and petioles of pistachio hung on the tree at least until the following summer (Tables 4 and 5). In the orchards in Butte and Tehama Counties, infected rachises were hanging on the trees 2–3 yr later. The retention of infected rachises, fruit, and petioles (sometimes with leaf blades), along with infected and blighted shoots, appeared to be of primary importance in the overwintering and spreading of *B. dothidea*. Since ascocarps of the fungus were not found on blighted shoots and rachises or on leaves dropped on the ground, pycnidia found on infected parts of the trees provided the primary source of inoculum. The retention of petioles was also reported for thornless blackberry infected by *B. dothidea* (13). Most of the pistachio

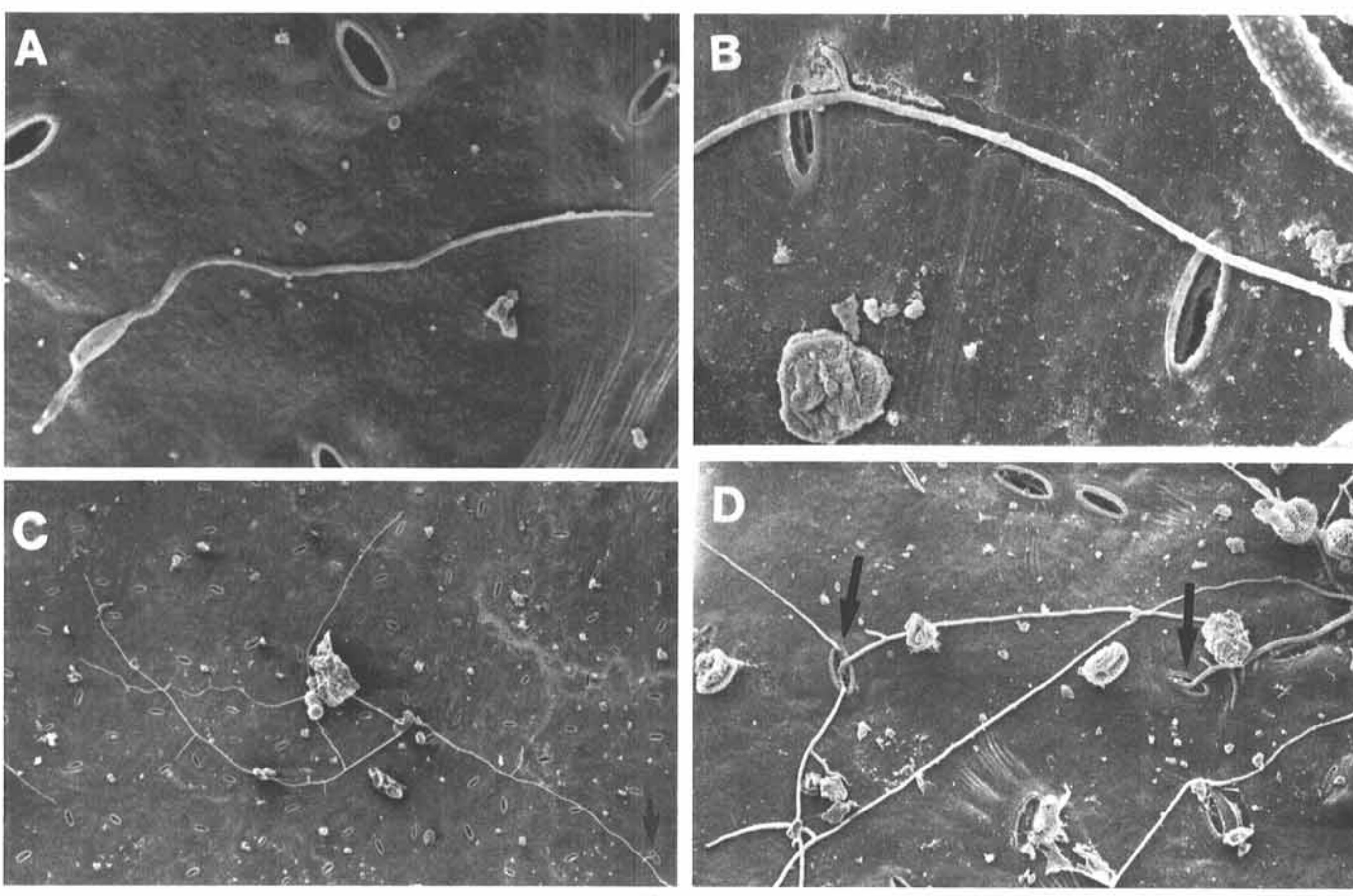

Fig. 5. Scanning electron micrographs showing germination of conidia of *Botryosphaeria dothidea* on pistachio upper and lower leaf surfaces. **A,** Germinated conidium with two germ tubes on the upper leaf surface 12 h after inoculation (×580). **B,** A germ tube passing over stomata on the upper leaf surface, but not penetrating, 12 h after inoculation (×830). **C,** Branched germ tubes on the upper leaf surface not penetrating, except for one case (arrow), 12 h after inoculation (×100). **D,** Germ tubes entering through stomata of the lower leaf surface 24–48 h after inoculation (×400).

nut mummies will drop to the ground by late winter because of winter storms; however, those containing pycnidia will provide inoculum during fall and early winter for the contamination of plant surfaces and buds.

In summary, the disease described in this study became of primary importance and a major concern to pistachio growers, especially in Northern California. Because the fungus attacked and killed fruit clusters, a 40–100% loss in yield was estimated in at least two commercial orchards severely infected by *B. dothidea*. The fact that the fungus also killed male inflorescences before they dehisced, thereby reducing available pollen (which can be critical in some orchards), constituted another serious damage to pistachio production. In addition, the aggressiveness of *B. dothidea*, indicated by its ability to infect any part of the pistachio tree throughout the season (assuming conditions are favorable), resulted in a dynamic disease cycle that involved almost every part of the pistachio tree and provided inoculum (pycnidiospores) for new infections (Fig. 4). Furthermore, the retention of infected rachises, petioles, and leaf blades assured the presence (overwintering) of the pathogen on the tree for new infections in the following spring and summer.

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