Spore Release by *Botryosphaeria dothidea* in Pistachio Orchards and Disease Control by Altering the Trajectory Angle of Sprinklers

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ABSTRACT

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To design a disease-control management approach, release of pycnidiospores of *Botryosphaeria dothidea* from infected pistachio was studied in both laboratory and field. In laboratory studies, more cirrhi developed from pycnidia in pistachio rachises infected by *B. dothidea* that were dry in the field than in rachises washed several times during previous sprinkler irrigations. Pycnidia in older rachises produced fewer cirrhi than did younger rachises. Dry rachises released maximum numbers of pycnidiospores (up to 1,000 per milliliter of water) within 2–3 h of irrigation, and only three to four pycnidiospores were recovered after 10 h of sprinkling. In contrast, rachises with exuding pycnidia (cirrhi present on the surface of rachises) released 2.5×10^4, 1.3×10^4, and 63 pycnidiospores per milliliter of water 5, 30, and 450 min, respectively, after activating sprinklers. Released pycnidiospores from blighted shoots and petioles increased within 1–4 h of sprinkling and gradually decreased afterward, within 11–12 h of sprinkling. Released spores from blighted fruit increased gradually until 3 h after sprinkling, maintained high levels 4–9 h after sprinkling, and decreased 10–12 h after the first sprinkling. The numbers of released pycnidiospores decreased significantly with the second, third, fourth, and fifth sprinkling cycles, which were applied at weekly intervals. In field experiments, rachises collected from 1.2 to 1.5 m high in the tree canopy and "washed" in previous irrigations during the season released decreasing numbers of pycnidiospores with increases in sprinkling duration; "unwashed" rachises collected from 2.2 to 2.5 m high in the tree canopy released increasingly high levels of pycnidiospores until 5 h after sprinkler irrigation. In inoculation experiments, higher pycnidiospore concentrations resulted in greater incidence and severity of disease than resulted from lower concentrations, in both male and female pistachio. Altering the irrigation-sprinkler trajectory angle from 23° to 12° significantly reduced the disease in three commercial orchards in California.

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

Pistachio (*Pistacia vera* L.) is a well-established crop in California, with more than 25,500 ha planted and 20,000 ha in production. The average annual yield of pistachio nuts exceeds 50 million kilograms (4). Approximately 90% of the state's pistachio acreage is in the San Joaquin Valley, with smaller, dispersed orchards in the Sacramento Valley. As the growing area of pistachio in California has increased, fungal diseases have become a major threat to the industry, causing significant losses.

Botryosphaeria panicle and shoot blight of pistachio, caused by the pycnidial stage (*Dothiorella* sp.) of the ascomycete *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not., is a destructive disease of pistachio trees in northern California (9). Although the fungus has been reported in San Joaquin and Kern counties (11), disease outbreaks most commonly occur in orchards in the northern part of the state, where pistachios are irrigated mainly by high-angled sprinklers (9).

B. dothidea overwinters in pycnidia developed on pistachio twigs, infected rachises, buds, petioles, and in cankers that remain on trees and provide pycnidiospore inoculum for 1–3 yr (9). Pycnidiospores cause primary infections during early spring and summer, and new pycnidia develop during August–September, causing secondary infections during late summer and fall (9). Although *B. dothidea* produces ascospores in giant sequoia (*Sequoiadendron giganteum*) and coastal redwood (*Sequoia sempervirens*) in California (25), and in blueberry (4), elm (8), peach (16,22), apple (20,21), and willow (24) in the eastern United States, perithecia of the fungus have not been found on pistachio (15). Therefore, disease outbreaks in pistachio orchards depend solely on pycnidiospore availability and dispersal. Under humid conditions, pycnidia of the anamorph *Dothiorella* sp. produce pycnidiospores that are exuded in a gelatinous matrix forming distinct hyaline cirrhi (15).

All pistachio orchards are irrigated; about 5% of the state's acreage is irrigated by sprinklers. In northern California, however, more than 75% of the pistachio acreage is irrigated by sprinklers. Because rain usually does not fall during late spring and summer in California, we hypothesized that water from sprinklers is the means by which pycnidiospores are released and spread in pistachio orchards during spring and summer. This hypothesis is supported by the fact that Botryosphaeria panicle and shoot blight is common only in sprinkler-irrigated orchards, is absent in drip-irrigated orchards, and is sporadic in flood-irrigated orchards.

Winter rains contribute to natural inoculation of apple and peach buds by *Botryosphaeria obtusa* (1,3). In pistachio orchards, spore inoculum of *B. dothidea* can be released and dispersed by winter rains on the plant surface and can be trapped among bud scales (13), leading to infection of vegetative and reproductive buds. This infection can kill buds or lead to blighting of developing shoots and panicles. However, yield is affected most by secondary infections of panicles during summer, caused by sprinkler-spread pycnidiospores. We hypothesized that disease incidence could be reduced by altering sprinkler irrigation to reduce the release and spread of spores.

The objectives of this study were to investigate the effects of 1) sprinkler water on the release and spread of *B. dothidea* pycnidiospores, 2) multiple irrigations on the release of spore inoculum, and 3) inoculum dose on disease incidence and severity on both female (cv. Kerman) and male (cv. Peters) trees. The

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ultimate goal of the study was to investigate ways of managing *B. dothidea* in pistachio by preventing both the release and spread of spore inoculum through proper manipulation of sprinkler irrigation. Parts of this study have been published (11,12,14).

MATERIALS AND METHODS

Experimental plots. All three pistachio orchards (one each in Butte, Tehama, and San Joaquin counties) used in this study had moderate to high levels of Botryosphaeria panicle and shoot blight. Before the initiation of this study (in 1985), all orchards were irrigated by high-angled sprinklers (23°), with trajectories allowing water to reach at least the lower third (~1.8 m high) of the tree canopy. The duration of each irrigation period ranged from 24 h (Butte and Tehama orchards) to 48 h (San Joaquin orchard).

Effects of sprinkler water and age of rachises on the number of cirrhi of *B. dothidea*. To determine the effects of water on the release of pycnidiospores of *B. dothidea*, 10 samples of 1-yr-old blighted rachises were collected in mid-to-late July from areas of the tree canopy (1.2–1.5 m) that water from sprinkler irrigation could reach. These rachises, which had been washed during three previous irrigations (3 May, 27 May, and 3 July) were designated "washed" rachises. Another set of 1-yr-old blighted rachises collected from areas 2.2–2.6 m high in the tree canopy, which water could not reach, were designated "unwashed" 1-yr rachises. In addition, blighted 2-yr-old rachises still hanging on the trees were also collected from a height of 2.2–2.6 m and were designated "unwashed" 2-yr rachises.

Rachises from each sample bearing pycnidia were cut into 2-cm-long pieces, soaked in distilled water for 1 h, laid on wet paper towels over wire screens in covered plastic containers (relative humidity [RH] > 98%), incubated for 72 h at 23 ± 1 C under fluorescent light, and examined with a dissecting microscope 1, 2, 18, 48, and 72 h after completion of incubation. The total number of cirrhi, representing exuding pycnidia per piece of rachis, were counted on the entire 2-cm length. The experiment was repeated once with another set of rachises collected from the same orchard.

Effect of sprinkler water on release of pycnidiospores (laboratory experiments). *From dry and wet rachises.* One blighted rachis was tied firmly with wire to a wooden pot label (12 × 1.5 cm) (Fig. 1). Six labels bearing the plant parts were stapled to stakes (112 × 2 × 2 cm) 9–10 cm apart from each other (Fig. 1). Six plastic funnels (8 cm in diameter), connected to a test tube (18 × 1.5 cm) with a rubber stopper (bearing a 3-mm tygon tube for gas exhaust), were placed beneath each label to collect water. Stakes and funnels were placed 2 m from a part-circle, pulsating water sprinkler (Gilmour Manufacturing Co., Somerset, PA) and were exposed to continuous sprinkling. Cotton blue (0.5 ml of a 1% solution) was placed in each test tube to stain collected spores and to restrict spore germination. Samples consisting of 75–80 ml of run-off water were collected 3, 120, 300, 480, and 600 min after activating the sprinkler. Samples of run-off water collected in each test tube were allowed to settle for 12–24 h and then were reduced to 25 ml by removing a 50- to 55-ml aliquot from the top of each test tube, using a syringe attached to a vacuum line so settled spores would not be disturbed. The remaining 25-ml aliquot was centrifuged at 6,600 *g* for 5 min. The supernatant was removed, sediment and 2.5 ml of water on the bottom of each centrifuge tube were vortexed for 30 s, and spore counts were made with a hemacytometer. Counts were expressed in number of spores per milliliter of aliquot water collected per rachis, shoot, or petiole.

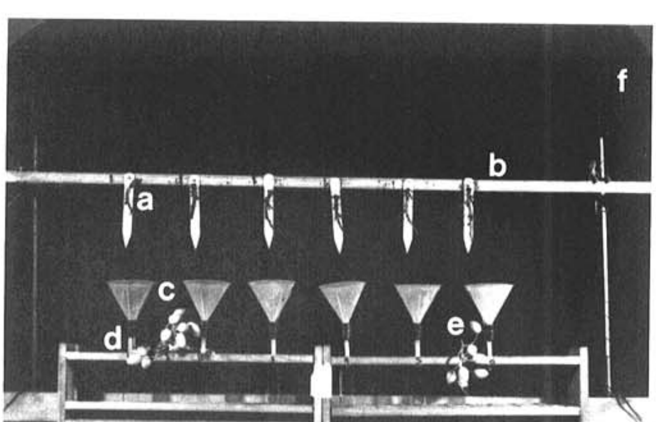

Fig. 1. Construction of a set of pistachio rachises blighted by *Botryosphaeria dothidea* and a funnel/test-tube arrangement used in collecting sprinkler-irrigation water: **A**, blighted pistachio rachis tied on a wooden pot label; **B**, wooden stake on which wooden labels were stapled; **C**, funnel; **D**, test tube; **E**, healthy pistachio clusters on the wooden rack holding funnels/test tubes; and **F**, wire hooks for hanging the set on trees in the field.

The removal of spores from wet rachises bearing exuding pycnidia by sprinkler water was studied on six 2-cm basal portions of blighted rachises bearing exuding pycnidia in the funnel/test-tube arrangement (Fig. 1). However, in this experiment, the collecting tubes were replaced 2 and 5 min and subsequently every 10 min after the onset of sprinkling. Sprinkling lasted 450 min; spore counts were made as described previously. In both experiments, the water pressure was adjusted with a hand valve to 275–285 kPa, the equivalent of that used in the orchard in Butte County. Both of the experiments were repeated once, using a new set of blighted rachises bearing exuding pycnidia. The repetitions yielded essentially the same results.

From infected shoots and petioles. One to three infected shoots or 10 petioles were tied firmly to a wooden pot label in the funnel/test-tube construction (Fig. 1), as described previously for the rachises, and were subjected to continuous sprinkling for 12 h. The water-collecting test tubes were replaced hourly, and spore counts were made as described previously. The experiment was repeated once, using new sets of infected shoots or petioles collected from the orchard in Butte County, and the results were averaged ($P > 0.05$). Ten pistachio fruits with mature pycnidia from the previous growing season were placed in each of six plastic net bags (5 × 20 cm) firmly attached to the wooden pot labels. One set of the infected fruits was placed 0.5 m above the ground and 2 m away from the part-circle, pulsating sprinkler. The fruits were sprinkled five times at weekly intervals for 12 h. To collect the run-off water, test tubes with 0.5–1.0 ml of a 1% cotton blue solution in lactophenol were replaced at hourly intervals. Pycnidiospore counts were made as described previously. The experiment was repeated twice.

Release and spread of pycnidiospores by sprinkler water in the orchard. On 3 August, just before the fourth sprinkler irrigation of the orchard in Butte County, we collected unwashed and washed 1-yr-old rachises with abundant pycnidia. Six unwashed or washed rachises were attached to the funnel/test-tube system, which hung on two pistachio trees at a height of 1.5 m in locations where water from the grower's sprinklers could reach all the rachises. The test tubes were replaced every half hour after sprinkler activation. One milliliter of a 1% cotton blue solution in lactophenol was added, and the test tubes were returned to the laboratory to count pycnidiospores of *B. dothidea* as described previously. At the end of the field experiment, which lasted 5 h, the two sets of six rachises were brought to the laboratory; three rachises from each set were sectioned superficially with a razor blade and were examined with a dissecting microscope to determine the percentage of emptied pycnidia. The experiment in the orchard was repeated once, and values were averaged because trends were the same ($P > 0.05$).

To determine whether pycnidiospores splashed with sprinkler water could cause infections, six healthy 10- to 15-fruit panicles collected from unwashed areas of the trees at a height of 2.5 m were placed on the rack holding test tubes below the attached 1-yr-old rachises (Fig. 1) and were replaced hourly after each test-tube replacement. We used as controls fruit panicles collected from the tree canopy at a height of 2.5 m, placed on a third rack with no blighted rachises hanging, and subjected the controls to sprinkler irrigation for 5 h. All clusters were placed in plastic containers over waxed wire screens, were brought to the laboratory, and were incubated at 25 ± 1 C; disease incidence on the fruits was determined after 12 days of incubation.

Effect of inoculum dose on disease incidence and severity. Ten current-season shoots, each with four to seven leaves, on three Peters (male) and three Kerman (female) trees at the University of California Wolfskill Experimental Orchards (UC-WEO) in Winters, CA, were sprayed with suspensions of 0.5×10^5, 1.0×10^5, and 2×10^5 pycnidiospores of *B. dothidea* per milliliter, prepared from 15-day-old cultures grown on pistachio-decoction agar (10). After spraying, the shoots were immediately covered with a 10-mil polyethylene bag that was sprayed inside with distilled water and was secured to the shoot with masking tape. A brown-paper bag was placed over the polyethylene bag to protect shoots and leaves from sunburn. Control shoots were sprayed with water and were covered similarly with polyethylene and paper bags. Shoots were kept continuously wet by opening the bags and rewetting every 6–10 h as needed. Bags were kept on the shoots for 72 h to insure maximum levels of infection, and symptoms on leaves were evaluated, as previously described (10), 15–20 days after inoculation. Because similar ($P > 0.05$) trends were observed when the experiment was repeated during September of the following year, only results from the first year are presented. In addition, 20–30 random lesions were surface-sterilized in a 0.08% NaOCl solution for 1 min and were plated in dishes containing acidified potato-dextrose agar (2.5 ml of a 25% [v/v] solution of lactic acid per liter of medium) (APDA) for reisolation of the pathogen. Data from the experiments were analyzed by regression using SAS statistics (SAS Institute, Inc., Cary, NC).

Control of disease by altering sprinkler-trajectory angle in a portable system. *Butte County plot.* The orchard in Butte County was irrigated by portable pipes bearing sprinklers with high-trajectory angles (23°) (Rainbird 29BT, 3.2-mm nozzle, Rain Bird Sales, Inc., Agri-Products Division, Glendora, CA) (Fig. 2A), for at least the past 10 yr. A randomized complete-block design was established in late March, before the initiation of irrigation in a 1.4-ha area of the orchard. Six rows of trees with 56 pistachio trees per row were irrigated by the same irrigation pipe, transferred twice every two rows (Fig. 3A). The experimental plot included four replicated blocks of 84 trees, with one half block (42 trees) irrigated by high-angled (23°) sprinklers and the other half (42 trees) irrigated by low-angled (12°) sprinklers (Rainbird M20H, 3.2-mm nozzle) (Fig. 2B). Sprinkler irrigation in the experimental plot was completed in 3 days (three transfers of the irrigation pipe; 24 h in each transfer) (Fig. 3A).

Disease severity was recorded for 10 random shoots (per group) with three- to seven-fruit panicles, each bearing approximately 20–40 fruits, at a height of 1.5–1.8 m on two trees located in the center of the groups of trees irrigated by either the high- or low-angled sprinklers. Because the first symptoms (small black lesions) on fruit appeared 1 wk after the first sprinkler irrigation, severity of disease was determined on 14 May, 4 June, and 10 July. Four (0–3) disease-severity categories were used for fruit, based on density of black lesions on the fruit epicarp: 0 = fruit clusters without black lesions; 1 = fruit clusters with a few black lesions associated with lenticels; 2 = fruit clusters with an intermediate number of lesions; and 3 = fruit clusters with many (dense) black lesions. The disease index (*DI*) was determined as follows:

$$DI = \frac{A \times 0 + B \times 1 + C \times 2 + D \times 3}{A + B + C + D} \tag{1}$$

in which *A, B, C,* and *D* = the number of fruit clusters in the disease categories 0, 1, 2, and 3, respectively.

During the commercial harvest on 10 September, premarked clusters on the experimental trees (four per block) were harvested and brought to the laboratory without removing any rachises or leaves from the shoots. Subsamples of 200 fruits per tree were evaluated for incidence of disease, presence of pycnidia, and weight of the healthy fruit.

The effects of sprinkler-irrigation height on retained rachises and petioles were determined approximately 1 yr later. The number of hanging rachises and petioles on the marked shoots and of killed shoots were recorded, and representative samples were collected and examined with a dissecting microscope for the presence of *B. dothidea* pycnidia.

The effects of altering sprinkler-trajectory angle on levels of pycnidiospores and incidence of the fungus on vegetative and flower buds were determined. On 1 and 15 October, 25 vegetative and flower buds were collected at random from four trees in each block irrigated with either high- or low-angled sprinklers. Buds were placed in 10 ml of sterile water in 125-ml Erlenmeyer flasks and were shaken with a rotary shaker at 200 rpm for 2 h. The water was centrifuged at 6,600 *g* for 10 min, and spores of *B. dothidea* in the sediment were counted with a hemacytometer. No rain or irrigation occurred during the period of 1–15 October.

To determine whether *B. dothidea* pycnidiospores were lodged among the bud scales or had infected the buds, 25 buds from each block irrigated with either high- or low-angled sprinklers in the Butte County orchard were collected from trees, surface-disinfested with a 0.08% NaOCl solution for 1 min, dried over sterile filter papers, and plated on APDA dishes (five buds per dish). The dishes were incubated at 27 C for 5 days, and the number of buds infected by *B. dothidea* was recorded.

The effects of sprinkler-trajectory angle on other disease symptoms were determined on 40 randomly selected current-season shoots, 20 each from male and female trees in each block

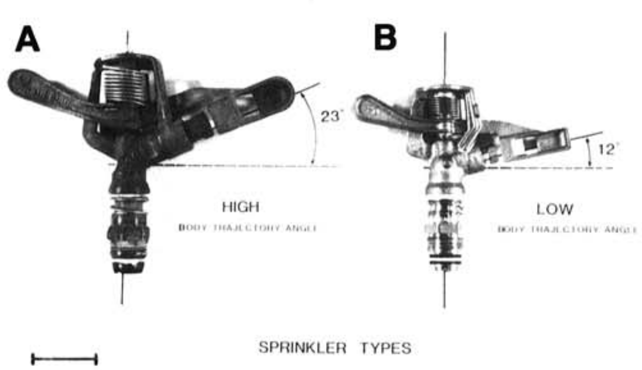

Fig. 2. Types of sprinklers used in the experimental pistachio plot in Butte and Tehama counties. **A,** Sprinkler with high-trajectory angle (23°). **B,** Sprinkler with low-trajectory angle (12°). Bar = 18 mm.

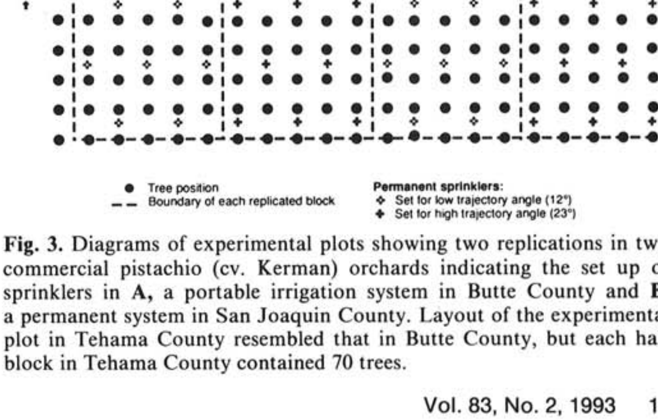

Fig. 3. Diagrams of experimental plots showing two replications in two commercial pistachio (cv. Kerman) orchards indicating the set up of sprinklers in **A,** a portable irrigation system in Butte County and **B,** a permanent system in San Joaquin County. Layout of the experimental plot in Tehama County resembled that in Butte County, but each half block in Tehama County contained 70 trees.

irrigated by either high- or low-angled sprinklers, collected on 1 and 15 October and were evaluated for the incidence of killed buds and the number of infected petioles and leaf and bud scars.

Tehama County plot. An experimental plot similar to the Butte County plot was established in an orchard in Tehama County suffering severe outbreaks of Botryosphaeria panicle and shoot blight. A randomized complete-block design with four replicated blocks was used. Each block included 140 trees (10 rows × 14 trees per row), half (10 rows × 7 trees per row) of which were irrigated by sprinklers with a high-trajectory angle and the remaining half, by sprinklers with a low-trajectory angle. Duration of irrigation per transfer was 24 h, and irrigation was completed in 5 days (five transfers of the irrigation pipe). Fruits were harvested on 4 September from four trees located in the center of each block, and subsamples of 200 fruits were evaluated for disease as previously described. Data were analyzed with ANOVA, and comparison of means was carried out with an LSD test, utilizing SAS statistics.

Altering irrigation in an orchard with a permanent sprinkler system. *San Joaquin County plot.* A third experimental plot was established in a commercial orchard in which Botryosphaeria panicle and shoot blight had been distributed uniformly (determined during the 1988 growing season). In this orchard, the grower used sprinklers (Rainbird L20 VL) with a 2-mm-diameter eccentric-nozzle orifice, which allowed the alteration of the trajectory angle from 23° to 12°, depending on the setting of the orifice. The experimental plot was a randomized complete-block design with three replicated blocks. Each block consisted of 60 trees, (6 rows × 10 trees per row); 30 trees (6 rows × 5 trees per row) were irrigated with sprinklers with the nozzle orifice set at the upper angle (23°), and 30 were irrigated with sprinklers with the nozzle orifice set at the lower angle (12°) (Fig. 3B). Subsamples of 200 mature fruits and 50 leaves were randomly collected from each of four experimental trees located in the center of each replication. Weights of healthy fruits and percentages of infected fruits and rachises, fruits with pycnidia, and leaves with lesions caused by *B. dothidea* were determined. Data were analyzed with ANOVA, and comparisons of means were carried out with an LSD test, utilizing SAS statistics.

RESULTS

Experimental plots. Abundant infected rachises, petioles, and killed shoots were present in all three orchards during spring. Of the rachises, 82–84.4% of those from orchards in Butte and Tehama counties and 70% of those from the orchard in San Joaquin County had pycnidia. Pycnidiospore germination on APDA ranged from 80 to 94% in samples collected from all orchards.

Effects of sprinkler water and age of rachises on the number of cirrhi of *B. dothidea*. No cirrhi from pycnidia in infected rachis pieces were evident after 1 or 2 h of incubation; however, after 18 h, six to eight cirrhi were counted on all sets of rachis pieces (Fig. 4). After 48 and 72 h, significantly higher numbers of cirrhi were counted on unwashed 1-yr-old rachises compared to washed rachises of the same age and to unwashed 2-yr-old rachises (Fig. 4). In contrast, the number of cirrhi counted on washed 1-yr-old rachises and unwashed 2-yr-old rachises did not differ significantly over the sampling period (Fig. 4). Similar results were obtained when the experiment was repeated ($P > 0.05$).

Effect of sprinkler water on release of pycnidiospores (laboratory experiments). *From wet and dry rachises.* Although 10–15 pycnidiospores of *B. dothidea* were collected 3 min after the onset of sprinkling of dry rachises, the maximum number of spores was collected after 2 h (Fig. 5A). Only three to four spores per milliliter of aliquot water per rachis were collected after 10 h of continuous sprinkling (Fig. 5A).

When blighted wet rachises with exuding pycnidia were subjected to continuous sprinkling, approximately 2.5×10^4 spores per milliliter of water per rachis piece initially were collected (Fig. 5B). Five minutes after the onset of sprinkling 1.3×10^4 and 30 min after the onset only 1.0×10^3 spores per milliliter of aliquot water per rachis piece were washed into the test tubes

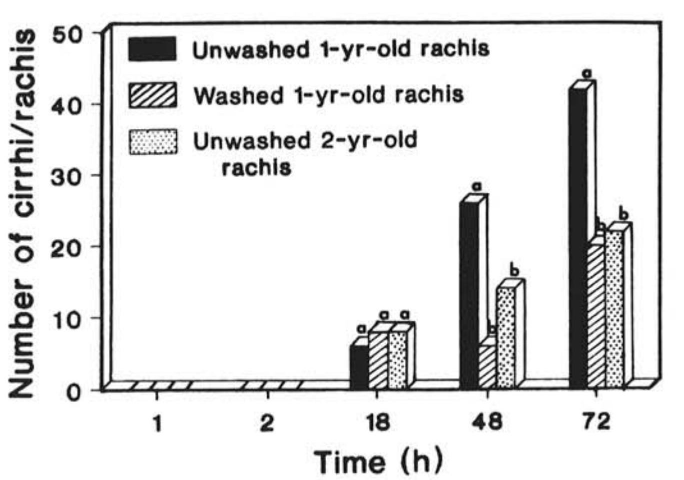

Fig. 4. Number of *Botryosphaeria dothidea* cirrhi developed on 1-yr-old wet ("washed") and dry ("unwashed") rachises and on 2-yr-old unwashed rachises of pistachio (cv. Kerman) after soaking in water for 1 h and then incubating at 98–100% relative humidity for 72 h. Averages from two experiments. Bars for each incubation time topped with different letters are significantly different with LSD ($P < 0.05$).

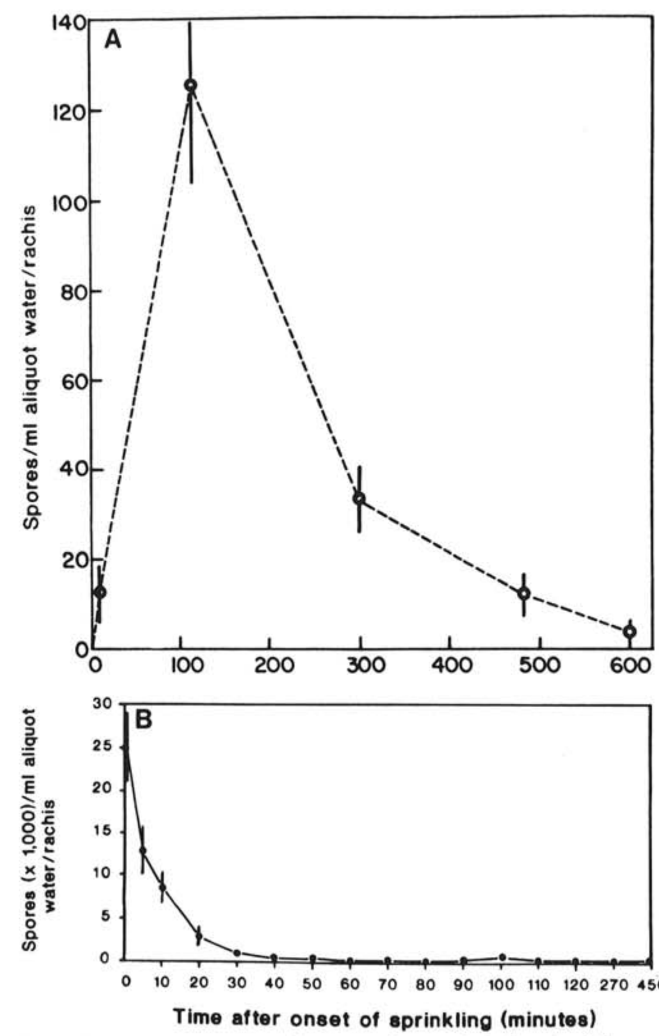

Fig. 5. Release and recovery of *Botryosphaeria dothidea* pycnidiospores from blighted rachises of pistachio (cv. Kerman) using sprinkler water under laboratory conditions. **A,** dry rachises without exuding pycnidia and **B,** wet rachises with exuding pycnidia. Each point is the average of six replications. Standard deviation is illustrated by a vertical line through each point on the graph.

(Fig. 5B). The number of spores washed decreased gradually until 40 min. There were no differences in the number of released spores in subsequent samples (Fig. 5B).

From infected shoots and petioles. The number of released spores from blighted shoots increased gradually until 3 h of sprinkling and decreased afterward until 7 h of sprinkling (Fig. 6). More spores were released when three blighted shoots were used per replication. The maximum number of spores was released after 3 h of sprinkling. Levels of spores from both sets decreased gradually, and after 4 h of sprinkling, the number of released spores was essentially the same until the end of the experiment (Fig. 6). A maximum of 660 spores per milliliter per petiole were released after 1 h of sprinkling; the number of released spores decreased gradually until 5 h of sprinkling and then was maintained between 96 and 200, until 11 h of sprinkling (Fig. 6).

From infected fruits. Release of spores from blighted fruit increased gradually until 3–4 h after onset of sprinkling (Fig. 7). Released spores were maximized after the first sprinkling (750–900 spores per milliliter per fruit), and they decreased to 2–15 spores per milliliter per fruit by the fifth sprinkling (Fig. 7). Levels of spores released during the second and fourth sprinklings (results not shown) were intermediate between those released in the first and third and third and fifth sprinklings, respectively (Fig. 7).

Spread of inoculum in the field. Unwashed rachises had 175 spores per milliliter of aliquot water per rachis at the onset of sprinkler irrigation, and the number of spores remained relatively stable until 3.5 h (Fig. 8). Subsequently, spore release increased significantly, with the maximum number of spores per milliliter of aliquot water occurring 4.5 h after the onset of irrigation (Fig. 8). In contrast, washed rachises released about 180 spores per milliliter of aliquot water per rachis at the onset of sprinkler irrigation, and only a few spores were recovered after 2 h (Fig. 8). Although the level of infection in the healthy clusters placed beneath the rachises during the spore-dispersal experiments varied considerably, all clusters had infected nuts and rachises after 12 days of incubation at 27 C. Approximately 40–45% of the fruit placed beneath the sprinkled, unwashed or washed, rachises were infected, but only 3–5% of the control fruits showed infections by *B. dothidea*. Based on microscopic examination of the rachises at the end of the sprinkler experiment, 93% of the pycnidia in washed rachises and 45% of the pycnidia in unwashed rachises were empty.

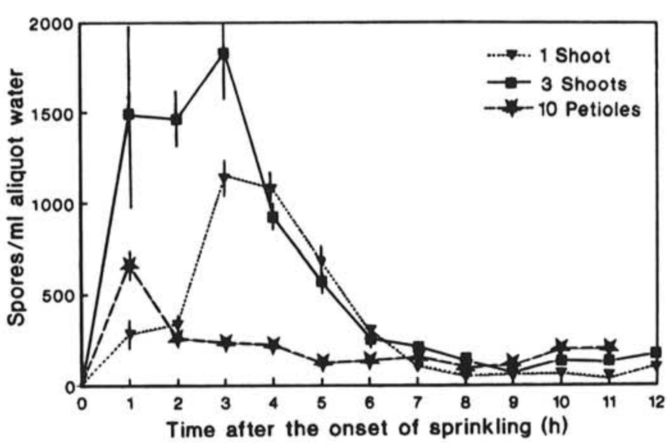

Fig 6. Release and recovery of *Botryosphaeria dothidea* pycnidiospores after sprinkling blighted pistachio (cv. Kerman) shoots and petioles with water for 11–12 h. Each point represents the average of six replications. Experiments were repeated at least once. Standard deviation is illustrated by a vertical line through each point on the graph.

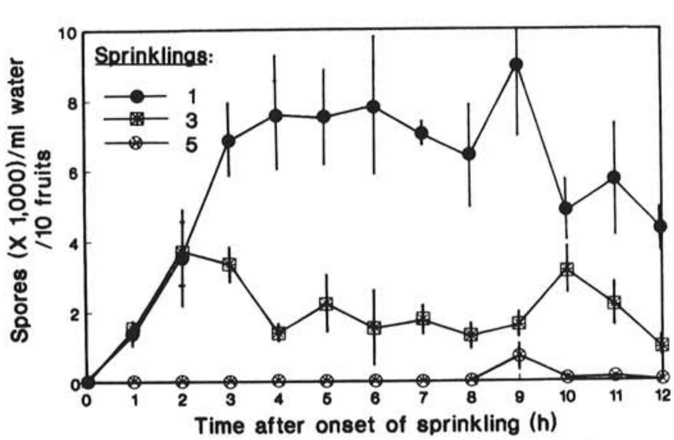

Fig. 7. Release and recovery of *Botryosphaeria dothidea* pycnidiospores after sprinkling blighted, mature pistachio (cv. Kerman) fruits with water five times for 12 h at 7-day intervals. Each point represents the average of six replications (10 fruits per replication). The experiment was repeated once. Standard deviation is illustrated by a vertical line through each point on the graph.

Effects of inoculum dose on disease incidence and severity. Leaves of both male Peters and female Kerman trees inoculated with higher concentrations ($1–2 \times 10^5$ spores per milliliter) of spore suspension developed symptoms more severe than did those trees inoculated with 0.5×10^5 spores per milliliter (Table 1). The incidence of infected leaves on shoots of male trees inoculated with a $1–2 \times 10^5$ spores per milliliter concentration was also higher than the incidence on shoots inoculated with 0.5×10^5 spores per milliliter. In addition, percentages of leaves with more than 16 lesions per leaf were higher with the higher concentrations (Table 1). All lesions were black and 2–3 mm in diameter. Leaves occasionally had infections on petioles and mid ribs. When petioles were infected, they caused leaf blight before the development of any lesions on the leaf blade. The concentration of 2×10^5 spores per milliliter caused a higher percentage (18–22%) of blighted leaves than did the concentrations of $0.5–1.0 \times 10^5$ spores per milliliter (1–14% of leaves were blighted). Results for leaves on female trees were analogous to those on male trees, except that larger percentages of infected leaves of female trees developed more than 16 lesions per leaf after inoculation with a suspension of 0.5×10^5 spores per milliliter (Table 1). In general, leaves of female trees showed a higher disease incidence and severity (Table 1).

Control of disease by altering sprinkler-trajectory angle in a portable system. *Butte County plot.* The effects of trajectory angle of sprinklers on disease were first noticeable 10–15 days after the first irrigation. Disease-severity indices were significantly lower on fruits from trees irrigated with the low-angled sprinklers (12°) than on those irrigated with the high-angled sprinklers (23°) (Table 2). One week after the third sprinkler irrigation 92% of the clusters in the high-angled sprinkler blocks showed signs of severe infections, in contrast to only 20% of the clusters in trees irrigated with low-angled sprinklers (Table 2). Clusters at a height of 2.4 m within unwashed areas were healthy (only 2% were infected), while

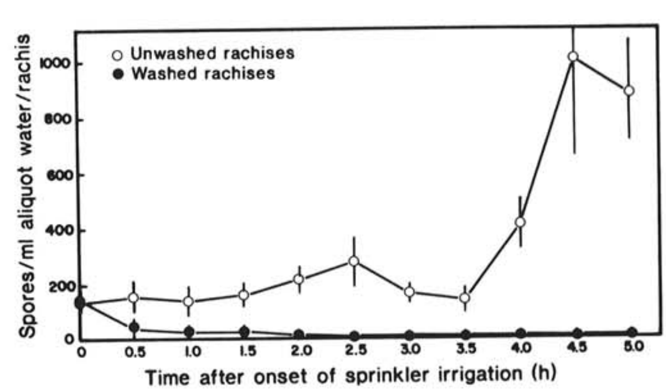

Fig. 8. Number of *Botryosphaeria dothidea* pycnidiospores collected in blighted rachis run-off water for 5 h after the onset of sprinkler irrigation in a pistachio orchard in Butte County. Each point represents the average of six replications. The experiment was repeated once. Standard deviation is illustrated by a vertical line through each point on the graph.

TABLE 1. Effects of inoculum density on incidence and severity of leaf infection of male (cv. Peters) and female (cv. Kerman) pistachio by *Botryosphaeria dothidea*

Cultivar	Spore concentration ($\times 10^5$)[x]	Leaves blighted (%)	Leaves with lesions (%)[v,w] 1–5	6–10	11–15	≥16	Infected leaves (%)	Disease index[w,y]
Peters	0.5	14 ± 2[z]	31 ± 10	22 ± 6	13 ± 6	17 ± 5	83 ± 4	1.8 ± 0.5
	1	14 ± 3	18 ± 7	33 ± 6	17 ± 7	32 ± 5	100 ± 0	2.6 ± 0.2
	2	18 ± 3	3 ± 3	19 ± 5	20 ± 6	56 ± 11	98 ± 2	3.3 ± 0.3
Kerman	0.5	1 ± 1	5 ± 2	9 ± 3	25 ± 5	62 ± 8	100 ± 0	3.4 ± 0.3
	1	7 ± 3	1 ± 1	13 ± 3	20 ± 5	67 ± 8	100 ± 0	3.5 ± 0.2
	2	22 ± 5	0 ± 0	2 ± 2	8 ± 5	90 ± 5	100 ± 0	3.9 ± 0.1

[v] All of the leaves of each shoot were recorded 15–20 days after inoculation.
[w] Results are expressed as an average of three 10-shoot replications.
[x] Inoculations were made on 19 September and 6 October by spraying shoots with the respective spore suspensions until run-off. Only the results of the first year are presented.
[y] Disease index on leaves was determined as previously described (10).
[z] Numbers (±) represent standard errors.

TABLE 2. Effects of trajectory angle of sprinkler irrigation on incidence and severity of disease caused by *Botryosphaeria dothidea* of pistachio (cv. Kerman) panicles in a commercial orchard in Butte County

Sprinkler-trajectory angle (°)[y]	Disease-severity index on[w,x] 14 May	4 June	10 July	Infected panicles after the third irrigation (%)
High (23°)	2.2 ± 0.3[z]	2.4 ± 0.2	2.6 ± 0.2	92.0
Low (12°)	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	20.0
LSD ($P < 0.05$)	...	...	...	8.4

[w] Severity index was based on four disease-severity categories and was determined with equation 1.
[x] Disease was evaluated on 10 random, preflagged shoots with three- to seven-fruit panicles in two trees located in the center of each block. The same panicles were evaluated on all three dates.
[y] Four irrigations were applied during the growing season at nearly monthly intervals (3–6 and 27–30 May, 3–6 July, and 3–6 August). The water pressure in the irrigation pipe was 275–285 kPa.
[z] Numbers (±) represent standard errors.

approximately 85% of the clusters at a height of 1.2 m within washed areas were infected (data not shown).

At commercial-harvest time, only 31.5% of the mature fruits from trees irrigated with low-angled sprinklers were infected, and of those, 3.6% bore mature pycnidia. In contrast, 92.5% of the fruits from trees irrigated with sprinklers with a high-trajectory angle were infected, and 13.5% of those bore pycnidia (Table 3). The weight of healthy fruit harvested from trees irrigated with low-angled sprinklers was approximately 10 times greater than the weight of healthy fruit harvested from trees irrigated with high-angled sprinklers. In addition, irrigating trees with low-angled sprinklers resulted in significantly lower retention of rachises and petioles on the trees and lower frequency of killed shoots than were found on trees irrigated with sprinklers with a high-trajectory angle (Table 3).

Washings of buds collected from both male and female trees from the high-angled sprinkler blocks showed significantly higher conidia per bud compared with those collected from the low-angled sprinkler blocks in the orchard in Butte County (Table 4). The incidence of *B. dothidea* among the scales of surface-disinfected buds was 70.8 and 38.6% for the high- and low-angled sprinkler blocks, respectively (Table 4). In addition, significantly more buds were killed on trees irrigated with high-angled sprinklers than on trees irrigated with low-angled sprinklers (Table 4). Levels of petiole and leaf-scar infections per 20 random twigs were significantly higher for high-angled sprinkler blocks than for low-angled sprinkler blocks in this orchard (Table 4).

Tehama County plot. Altering the trajectory angle of sprinklers in a portable system in the orchard in Tehama County also resulted in significant disease reduction. In fruit clusters, 69% were blighted in trees irrigated with low-angled sprinklers compared to 80% of those in trees irrigated with high-angled sprinklers. In addition, 48% of the leaflets were infected in trees irrigated with high-angled sprinklers compared to 23% of those in trees irrigated with low-angled sprinklers ($P < 0.05$).

Altering irrigation in an orchard with a permanent sprinkler system. *San Joaquin County plot.* At commercial-harvest time (25 September), significantly more infected fruit, rachises, and leaves were recorded in trees irrigated with sprinklers set at the high-trajectory angle compared to those irrigated with sprinklers set at the low-trajectory angle (Table 5). In addition, the majority (87%) of the leaves had three or more lesions per infected leaf in trees irrigated with sprinklers set at a high angle compared with 52% of the leaves in trees irrigated by sprinklers set at a low angle. Weight of healthy fruit harvested from trees irrigated by low-angle sprinklers was 250% higher than the weight from trees irrigated by high-angle sprinklers (Table 5).

DISCUSSION

The results of this study indicate that reduction of Botryosphaeria panicle and shoot blight of pistachio can be accomplished merely by lowering the trajectory angle of sprinklers to prevent the release and spread of pycnidiospore inoculum. Although irrigation management to control root-rot diseases (18) has been given much consideration, this appears to be the first study to report the manipulation of sprinkler-trajectory angles to manage a foliar disease of fruit trees by reducing spore dispersal. Similarly, a disease of avocado (7) and one of banana fruits (17), caused by *Dothiorella gregaria* (*Botryosphaeria ribis chromogena*), were favored by overhead irrigation, while flood irrigation created conditions unfavorable for disease development because of the lack of spore dispersal.

Two-year-old rachises firmly attached to the tree still had pycnidia full of viable pycnidiospores (confirmed by germination on APDA), indicating that blighted rachises can supply fungal inoculum for at least 2 yr (9). Fewer cirrhi developed on sprinkler-washed rachises, probably because pycnidia in these rachises were releasing spores with each irrigation and had been depleted of spores during the previous three irrigations during the summer of 1985. In contrast, significantly larger numbers of cirrhi developed on unwashed than on washed rachises, indicating a higher frequency of fertile pycnidia, even though both categories of rachises had been subjected to equivalent winter rains.

Because *B. dothidea* does not produce perithecia in pistachio orchards, the epidemics must depend on availability and on amounts of pycnidiospore inoculum and dispersal, which is water dependent. Rain did not occur during the growing season (June–August), when these experiments took place, and as a result, dispersal depended entirely on water from sprinkler irrigation. In fact, immediately after the initiation of irrigation, sprinkler water washed pycnidiospores from the rachises in the field. The more the rachises were sprinkled the more pycnidiospores were washed from their pycnidia.

It is believed that rain water (mainly during the fall, winter, and early spring) also can remove and distribute pycnidiospores effectively from any plant part bearing pycnidia. In the orchard in Butte County, a light rain (4 mm) on 9 September wetted the rachises, causing spores to exude from pycnidia in rachises observed in situ on 10 September. Any additional rain would disperse the already exuded spores very effectively. For example, approximately 25,000 pycnidiospores per milliliter per rachis were collected, within 5 min after the initiation of sprinkling, from rachises containing exuding pycnidia in laboratory experiments. However, because most of the crop development occurs in summer, the effect of irrigation is more important than the effect of rain.

In field experiments, the maximal number of spores released from pycnidia in unwashed rachises occurred 4.5 h after the onset of sprinkler irrigation. In laboratory experiments, the highest number of spores was released 2 h after the onset of sprinkling, probably because rachises were sprinkled with approximately the same amounts of water during that period as were those in the field (a half-circle sprinkler was used in the laboratory). Considering this difference, results in the laboratory and field were the same.

Water from sprinklers with a low-trajectory angle (12°) reaches only small areas of the tree canopy, resulting in limited spore release and dispersal and a reduced chance of infection of fruits, leaves, rachises, buds, and shoots. In contrast, water ejected from high-angled (23°) sprinklers reaches much of the lower part of the tree canopy, washes spores from rachises hanging there, and creates ideal conditions for infection and disease development. Spores of *B. dothidea* germinated in water after only 1.5 h, with more than 90% germinating after 5 h (10). Because each irrigation lasted 24–48 h, wetness periods were sufficiently long for spore germination and penetration through lenticels on the fruits and stomata on the leaves. More than 6 h of continuous wetness was necessary for considerable levels of infection of pistachio leaves to develop after inoculation (10).

Generally, bud contamination and natural inoculation of tree buds is common with *Botryosphaeria* fungi (1). In this study, lowering the trajectory angle of sprinklers resulted in the reduction of primary inoculum of *B. dothidea* because fewer buds, leaf and bud scars, rachises, petioles, and shoots were infected; lowering the angle also reduced the inoculum potential for secondary spread of the fungus by reducing the incidence of infected fruit with pycnidia. The improvements in disease control resulting from lowering the trajectory angle of sprinklers almost certainly are the result of fewer released and dispersed spores and less favorable environmental conditions for infection in the tree-canopy environment. Berger (2) considers both effects to be practices that utilize epidemiological principles to achieve plant-disease control.

Disease severity was greater on leaves of female (cv. Kerman) trees than on those of male (cv. Peters) trees (Table 1). Although cultivar differences in disease susceptibility may exist, under the same environmental conditions, female trees may demand greater

TABLE 3. Effects of trajectory angle of sprinkler irrigation on Botryosphaeria panicle and shoot blight of pistachios (cv. Kerman) caused by *Botryosphaeria dothidea*, on the fresh weight of fruit, on retention of rachises and petioles, and on the frequency of killed shoots in a commercial orchard in Butte County

Sprinkler-trajectory angle (°)[y]	Infected fruit (%)[z]	Fruit with pycnidia (%)[z]	Weight of healthy fruit (g)[z]	Retention of[x]		Frequency of killed shoots[x]
				Rachises	Petioles	
High (23°)	92.5	13.5	42.7	32.3	9.7	5.2
Low (12°)	31.5	3.6	405.9	16.3	0.5	2.3
LSD ($P < 0.05$)	8.4	2.3	46.6	8.5	3.7	1.8

[x] Retention of rachises and petioles and frequency of killed shoots were determined 1.5 yr after initiation of the experiment.
[y] Irrigations were done on 3–6 May, 27–30 May, 3–6 July, and 3–6 August. The water pressure of the irrigation pipe was 275–285 kPa.
[z] Values are the average of eight 200-fruit samples harvested on 10 September.

TABLE 4. Effects of trajectory angle of sprinkler irrigation on the number of *Botryosphaeria dothidea* pycnidiospores on vegetative and flower buds of pistachio (cvs. Kerman and Peters), on killed buds, and on the incidence of infected petioles and leaf and bud scars in a commercial orchard in Butte County

Sprinkler-trajectory angle (°)	*B. dothidea*		Dead buds (%)	Total infections per 20 twigs[x]		
	Conidia per bud[y]	Incidence in buds (%)[z]		Petioles	Leaf scars	Bud scars
High (23°)	136	70.8	8.3	3.9	1.3	0.6
Low (12°)	19	38.6	0.4	0.1	0.1	0.0
LSD ($P < 0.05$)	...	14.6	3.3	1.0	0.5	0.6

[x] Total infections of petioles, leaf and bud scars, and percentage of dead buds are the average of four replications. Each replication included 20 randomly selected shoots each, from male and female trees.
[y] Average of four 25-bud samples collected in each low- and high-angled sprinkler block in two experiments on 1 and 15 October.
[z] Average of four trees each in four replications, each including 50 randomly collected buds (25 each from male [cv. Peters] and female [cv. Kerman] trees).

TABLE 5. Effects of sprinkler-trajectory angle on Botryosphaeria panicle and shoot blight of pistachio in an orchard in San Joaquin County during 1989

Sprinkler-trajectory angle (°)[w]	Weight of healthy fruit (g)	Infected fruit (%)[x]	Fruit with pycnidia (%)[x]	Infected rachises (%)[y]	Blighted rachises (%)[y]	Infected leaves (%)[z]
High (≈23°)	212	65.5	0.4	29.0	1.5	93.8
Low (≈12°)	561	6.2	0.0	2.1	0.0	65.2
LSD ($P < 0.05$)	49	4.9	0.5	8.1	2.1	9.1

[w] High = higher-angle setting of nozzle orifice; low = lower-angle setting of nozzle orifice.
[x] Values are the average of 12 200-fruit samples harvested on 25 September.
[y] Average of 12 30-rachis replications.
[z] Average of 12 50-leaf samples each from four trees collected at random from a height of 1.0–1.5 m.

amounts of water and nutrients because they bear fruiting clusters, and as a result, water and nutritional stresses cannot be avoided. The association of water stress with greater susceptibility to *B. dothidea* has been shown in studies of peach (16) and other woody plants (19). Multiple infections of leaves by *B. dothidea* led to defoliation of both male and female trees at the end of August and the beginning of September in the orchards in Butte and Tehama counties.

Because only benomyl (6) is presently registered for control of *B. dothidea* on pistachio, control of the disease can be facilitated greatly by altering sprinkler-trajectory angle, as was shown in this study. Following our recommendation, several growers replaced high-angled with low-angled sprinklers. As a result of this change, the grower in Butte County experienced a 40% increase (16,000 kg) over the yield he harvested the previous year (however, the year when these studies took place was a low-production year because pistachios are alternate-bearing trees) (23). Also, a 46% increase in yield was reported in the San Joaquin County orchard after reducing the trajectory angle of sprinklers. In 1988, a high-production year for pistachio, the grower had the sprinkler nozzles set at a high-trajectory angle to achieve a longer trajectory distance. However, this arrangement created an outbreak of the disease, and he harvested only 5,500 kg net weight of pistachio, while in 1989, a low-production year, he set the sprinklers for a 12° angle, and harvested 10,200 kg from the same orchard (15).

Because of increasing public concern about the excessive use of fungicides in agricultural industries, nonfungicidal methods of disease control, such as the method developed in the present study, should be regarded as environmentally safe and valuable for disease control by the California pistachio industry in the future.

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