Susceptibility of Pistachio Male Cultivars to Botrytis Blossom and Shoot Blight Caused by *Botrytis cinerea*

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ABSTRACT

Michailides, T. J. 1991. Susceptibility of pistachio male cultivars to Botrytis blossom and shoot blight caused by *Botrytis cinerea*. Plant Dis. 75:410-415.

The pistachio (*Pistacia vera*) male cultivars 02-16 and 02-18 showed higher levels of Botrytis blossom and shoot blight caused by *Botrytis cinerea,* had more inflorescences 1 mo after blooming, and retained more inflorescences 10 mo after blooming than did the cultivar Peters. When shoots were inoculated, more shoots of 02-16 and 02-18 than of Peters became blighted, and significantly larger cankers developed. Inoculation of inflorescences with either a spore suspension or mycelial plugs of *B. cinerea* caused more shoots on 02-16 than on Peters to become blighted, and cankers that originated from the inoculated inflorescences were significantly larger on 02-16. In both experiments (inoculation of shoots and inflorescences), the cultivar 02-18 was intermediate in susceptibility to infection by *B. cinerea.* Agar media amended with ground, unwashed or washed inflorescences of 02-16 supported better growth and sporulation of *B. cinerea* than media amended with unwashed or washed inflorescences of Peters. The inflorescences of 02-16 and 02-18 are significantly larger, contain more pollen grains, retain significantly more water, and dry more slowly than those of Peters. Saturated inflorescences of 02-16 and 02-18 required 4 days to dry to 1–2% moisture, compared with 2 days for inflorescences of Peters. In addition, inflorescences of 02-16 and 02-18 are bulkier and more firmly attached to the supporting shoots than are those of Peters. The greater susceptibility of 02-16 and 02-18 to infections by *B. cinerea* stems from more extensive colonization of shoots, more available infection sites that are richer in nutrients, and more favorable conditions for infection.

Additional keywords: gray mold, resistance

Pistachio (*Pistacia vera* L.) is a dioecious plant in the Anacardiaceae. To assure full pollination and good yields, male and female trees are usually planted in the ratio of one male to eight females (8). More than 95% of the female trees now grown in California are the cultivar Kerman; the male cultivars most commonly used for interplanting are Peters and two Russian selections, 02-16 and 02-18.

Pistachio is a well-established crop in California, with more than 20,500 ha planted and 18,000 ha in production; the annual yield of nuts exceeds 42 million kilograms (3). More than 90% of the state's pistachio acreage is in the San Joaquin Valley; the rest of the plantings are in the Sacramento Valley, usually in smaller, dispersed orchards. As the area of bearing pistachio in California has increased, fungal diseases have become a major threat to the industry, since they cause significant losses.

In the spring of 1983, after a period of heavy and prolonged rains and cool weather, a new disease of pistachio, characterized by blighted blossoms and shoots, was observed. The disease was described in 1984 as Botrytis blossom and shoot blight caused by *Botrytis cinerea* Pers.:Fr. (2). Shoot blight was significant on both male and female pistachio trees during the wet springs of 1983 (2) and 1986 (11) but was insignificant during 1987–1989 because of very dry conditions (12,13).

A number of pistachio growers commonly use Peters and 02-16 and/or 02-18 trees in the same plantings to extend the period of availability of pollen and thus achieve more efficient pollination of the female flowers of the Kerman trees (5). Botrytis blight occurs more frequently on male than on female trees (2,11). In a pistachio orchard in Butte County (in northern California), the average number of shoots blighted by *B. cinerea* was significantly higher on 02-16 and 02-18 trees than on Peters trees (12). Similarly, in an orchard in Solano County, 02-16 and 02-18 trees had an abundance of blighted blossoms and shoots, whereas nearby Peters trees had only a few (*personal observation*), suggesting that these cultivars may differ in susceptibility to *B. cinerea.* However, no reports have been published on the relative susceptibility of pistachio male cultivars to *B. cinerea.* The objectives of this study were to determine whether 02-16, 02-18, and Peters differ in susceptibility and to investigate the nature of any differences in susceptibility.

Accepted for publication 18 October 1990 (submitted for electronic processing).

MATERIALS AND METHODS

Symptomatology and isolation of the pathogen from shoot cankers. To determine the pathogen, isolations were made from 10–25 infected shoots collected in the spring of 1988 and 1989 from three pistachio orchards with histories of Botrytis shoot blight. Five pieces 3–5 mm^3 in area were cut from the lower margins of the cankers, surface-sterilized in a 0.08% solution of chlorine (household Clorox containing 5.25% sodium hypochlorite), rinsed once in sterile water, dried on sterile filter papers, and plated on acidified Difco potato-dextrose agar (APDA) (2.5 ml of a 25% [v/v] solution of lactic acid per liter of medium). The dishes were incubated at room temperature (23 ± 1 C) for 6 days, and fungi that grew from the plant tissues were identified.

A previously undescribed symptom caused by *B. cinerea* was observed, primarily on trees of the male cultivars 02-16 and 02-18, in orchards in Butte, Solano, Yolo, and Merced counties. Because pistachio inflorescences develop in 1-yr-old shoots, cankers that originate from inflorescences that continue hanging on the trees result in blighting of current-season shoots.

To determine the time required for the blighting of current-season shoots at the distal end of cankers, five shoots with young cankers that had developed during the spring and five shoots without evident cankers were flagged on five replicate 02-16 trees in a pistachio orchard at the University of California Wolfskill Experimental Orchards (UC-WEO) in Solano County. The experiment was repeated in a commercial orchard in Butte County. One year later, blighted shoots at the distal ends of girdling cankers were recorded.

Relationship between persistent inflorescences and incidence of Botrytis blossom and shoot blight. To determine the number of persistent inflorescences on the three cultivars, three 1-min counts (on nine to 10 shoots) were done in early May on three sites on three replicate trees of each cultivar at UC-WEO. The total number of blighted blossoms and shoots was also counted on randomly selected trees in early May in 1988 and 1989. After the counting, samples of blighted blossoms and shoots were collected to isolate the pathogen(s) involved. In addition, on 20 February 1989 and 1990

(about 10 mo after bloom), the total number of inflorescences still hanging on the trees was counted on three replicate trees of each cultivar at UC-WEO.

Shoot inoculation experiments. To determine the relative susceptibility of the three cultivars to infection by *B. cinerea,* 10 current-season shoots on three trees of each cultivar were wounded on 6 March 1988 with a cork borer, and a plug of bark 5 mm in diameter and about 1 mm thick was removed from each shoot. A plug of agar 5 mm in diameter with mycelium and spores from a 7-day-old culture of *B. cinerea* (conidial isolate Bc #37-1 or sclerotial isolate Bc #7-1) was placed in each wound and covered with laboratory film (Parafilm "M", American Can Co. Dixie/Marathon, Greenwich, CT). The shoots were examined after 10 days, canker length was measured after shoots were pruned 3 mo later, and isolations were made from pieces of cankered wood tissue as described previously. The experiment was repeated in April 1988, except that only the conidial isolate (Bc #37-1) of *B. cinerea* was used, since there were no significant differences between the two isolates in the first experiment.

Inflorescence inoculation experiments. To determine the relative susceptibility of inflorescences of the three cultivars to infection by *B. cinerea,* 10 inflorescences with pollen sacs not dehisced on three trees of each cultivar were inoculated on 22 April 1988 with a mycelial plug of a 5-day-old culture of the conidial isolate (Bc #37-1) grown on APDA and immediately wrapped with laboratory film. Similarly, 10 inflorescences (with pollen sacs not dehisced) on three trees of each cultivar were spray-inoculated with a suspension of 1.5×10^5 spores of the Bc #37-1 isolate per milliliter and wrapped with laboratory film. Shoots and inflorescences inoculated with an APDA plug or sprayed with water and wrapped with laboratory film served as controls.

Inoculated shoots and inflorescences and uninoculated controls were examined 1 wk and again 4 mo after inoculation, and cankers that developed on pruned-out shoots were measured. To recover the pathogen, isolations were made on APDA from the margins of 10 cankers from each cultivar.

In another experiment, 10 inflorescences were removed from each of three trees of each cultivar, and inflorescence scars on the shoots were inoculated with a mycelial plug of the Bc #37-1 isolate of *B. cinerea.* Canker length was measured 3 mo after inoculation.

Effect of nutrients from male inflorescences on growth and sporulation of *B. cinerea*. To determine whether male pistachio inflorescences provide a substrate for the development of *B. cinerea,* inflorescences were collected after pollen sac dehiscence from trees of Peters and 02-16 (and/or 02-18) at UC-WEO that had not been treated with pesticides. Air-dried inflorescences (200 g) were ground in a blender for 1–2 min and incorporated in 1 L of distilled water containing 15 g of Bacto agar. Similarly, inflorescences were washed in glass jars with running tap water for 6 hr then ground and incorporated in 1 L of water agar (WA). The density of pollen grains in the water suspensions before and after the inflorescences were washed was determined with a hemacytometer.

In another experiment, 5-g samples of dry inflorescences (about seven to 10 inflorescences) from both Peters and 02-16 trees were placed in plastic bottles containing 250 ml of distilled water and shaken with a reciprocating, fixed two-speed shaker (Eberbach Corp., Ann Arbor, MI) for 2 hr. Washings were filtered through Whatman no. 1 filter paper and then through an 8-μm Millipore filter (Millipore Filter Corp., Bedford, MA) to eliminate pollen grains and other floral parts. The filtrates were used to prepare an agar medium containing 15 g of Bacto agar per liter of filtrates, and the medium was distributed among plastic petri dishes. Four dishes each of ground inflorescences, inflorescence filtrate agar (IFA), WA, and APDA were inoculated centrally with a mycelial plug obtained from the margins of a 5-day-old culture of *B. cinerea* (conidial isolate Bc #37-1 and sclerotial isolate Bc #7-1 growing on WA) and incubated at 20 ± 1 C. Mycelial growth after incubation for 3 and 4 days was recorded as the mean of the two orthogonal diameters per colony.

In addition, numbers of conidia produced on media amended with washed inflorescences of 02-16 and Peters were determined as follows. Five agar plugs (7 cm in diameter) were taken randomly from each 20-day-old culture, placed in test tubes (17 × 2 cm) with 10 ml of distilled water and one drop of Triton X-100, and shaken vigorously for 20–30 sec. Conidia were counted with a hemacytometer on two counts per dish. Four replicate dishes were used for each treatment, and the experiment was repeated twice.

In another experiment, conidial germination and growth of germ tubes of the two isolates of *B. cinerea* were determined on APDA, WA, and IFA prepared with filtrates of inflorescences of 02-16 and Peters. The dishes were seeded with 0.5 ml of a suspension containing 2.5×10^4 spores of each isolate per milliliter and incubated for 10 hr at 21 C. Germination was determined on 50 spores and germ tube length was measured on 10 germinated spores per dish. Four replicate dishes were used for each treatment, and the experiment was repeated twice.

Dry weight, water-retaining capacity, and water loss of inflorescences. To determine the dry weight of inflorescences of the three cultivars, 30 inflorescences were collected at random from each of three 02-16, 02-18, and Peters trees in May of 1988 and 1989, allowed to dry to constant weight on a laboratory bench at 23 ± 1 C for 10 days, and weighed. The amount of water retained by the inflorescences was determined by placing one inflorescence in each of 30 95-cm^3 paper cups (James River Corp. Dixie Products Business, Norwalk, CT), saturating them with 50 ml of distilled water for 24–48 hr, draining off the excess water, and weighing the inflorescences immediately after draining and once daily afterward until weights became stable. Water-retaining capacity (grams of water per gram of dried inflorescence) and rate of water loss were determined for each cultivar.

RESULTS

Isolates of *B. cinerea*. Both conidial and sclerotial isolates of *B. cinerea* were isolated from blighted shoots bearing cankers that had originated from male pistachio inflorescences at an incidence of 57–88% in the three orchards sampled. One conidial isolate (Bc #37-1) and one sclerotial isolate (Bc #7-1) were used in these studies. Although during subculturing the conidial isolate produced some sclerotia and the sclerotial isolate produced some conidia, these two isolates did not lose their initial characteristic morphology; that is, the conidial isolate always produced large numbers of conidia and the sclerotial isolate numerous sclerotia. To minimize the chances of these isolates switching from one type to another, subcultures were always made using only the PDA slants of the initial isolates on APDA.

Symptomatology and isolation of *B. cinerea* from shoot cankers. The blight of current-season infected shoots on both male and female pistachio trees, often covered by buff-colored masses of conidiophores and conidia of *B. cinerea,* has been described (2). In addition, a blight of current- or past-season shoots caused by girdling from enlarged cankers that originated from male inflorescences was observed (Fig. 1). These cankers were longitudinal, sunken, and dark brown and extended equally above and below the inflorescences (Fig. 1A). Often, more than one canker developed on the same shoot (Fig. 1B).

The infected inflorescences were firmly attached to the shoot and, when they were sectioned, abundant sporulation of *B. cinerea* was evident. *B. cinerea* did not sporulate on the cankers but did sporulate among the flower bud scales, in inflorescences on the trees, and in 33–55% of inflorescences on the ground. These symptoms and signs were common on 02-16 and 02-18 trees but very sporadic on Peters trees.

B. cinerea was consistently isolated from cankers that originated from male flowers collected from two orchards in northern California. Other fungi

recovered from the cankers included *Alternaria alternata* (Fr.:Fr.) Keissl., *Cladosporium herbarum* (Pers.:Fr.) Link, and species of *Diplodia, Dothiorella, Fusarium,* and *Phomopsis.*

In one orchard, about 1 yr after shoots bearing cankers originating from inflorescences had been marked, 80% of the shoots were blighted. Only 10% of the shoots without cankers at the time they were marked were blighted; all cankers on the blighted control shoots originated from inflorescences. In a second orchard, only 6.3% of the shoots with cankers originating from inflorescences and none of the controls were blighted.

Fig. 1. Male pistachio inflorescences infected by *Botrytis cinerea.* **A,** Typical canker originating from an infected inflorescence on a shoot of the male cultivar 02-16. **B,** Multiple cankers on a shoot of the cultivar 02-18. (Arrows indicate margins of cankers.)

Table 1. Frequency of inflorescences and of blighted blossoms and shoots on three male pistachio cultivars[w]

Cultivar	Inflorescences[x] (no./min)	Inflorescences retained[y] (no./tree)	Blighted blossoms and shoots[z] (no./tree)
02-16	81 a	54 a	> 156 a
02-18	71 a	20 b	28 b
Peters	18 b	13 c	18 c

[w]Numbers in each column followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[x]Average of three 1-min counts on each of three replicate trees.
[y]Total number of inflorescences retained until February of the following year.
[z]Average count for three replicate trees.

Table 2. Susceptibility of shoots of pistachio male cultivars to inoculation with *Botrytis cinerea*

Isolate of *B. cinerea* and date of inoculation[x]	Cultivar	Blighted shoots[y]	Mean length of cankers after 3 mo[z] (mm)
Bc #7-1, 6 March 1988	02-16	3	44 a
	02-18	1	30 a
	Peters	0	13 b
Bc #37-1, 6 March 1988	02-16	7	72 a
	02-18	0	19 b
	Peters	0	12 b
Bc #37-1, 22 April 1988	02-16	6	51 a
	02-18	4	42 ab
	Peters	0	31 b

[x]Inoculum was a mycelial plug 5 mm in diameter obtained from a 7-day-old culture of each isolate of *B. cinerea.*
[y]Average number of blighted shoots out of 10 current-season shoots on three trees of each cultivar that were wounded with a cork borer (5 mm in diameter) and inoculated with a mycelial plug. The inoculation sites were covered with Parafilm.
[z]For each date, numbers followed by different letters are significantly ($P = 0.05$) different according to Duncan's multiple range test.

Relationship between persistent inflorescences and incidence of Botrytis blossom and shoot blight. Significantly more inflorescences were counted per minute (on nine to 10 shoots) on 02-16 and 02-18 trees than on Peters trees (Table 1). After 10 mo, significantly more inflorescences still hung on trees of 02-16 and 02-18 than on Peters trees. In addition, more blighted blossoms and shoots were recorded on 02-16 and 02-18 trees than on Peters trees.

Shoot inoculation experiments. Seven to 10 days after shoots were inoculated with a mycelial plug of *B. cinerea,* only one out of 10 inoculated shoots of Peters showed wilting leaves, compared with 10 out of 10 shoots of 02-16 and nine out of 10 shoots of 02-18. No shoots from any of the cultivars inoculated with a spore suspension of *B. cinerea* showed evidence of infection 7 days after inoculation. However, after 3 mo, zero, zero to four, and three to seven shoots of Peters, 02-18, and 02-16, respectively, were blighted (Table 2). Moreover, cankers were significantly shorter on Peters shoots than on 02-16 shoots. Cankers on 02-18 shoots were intermediate in length between those of 02-16 and Peters. In one experiment, following inoculation with Bc #37-1, cankers on shoots of 02-18 and Peters were not significantly different in length (Table 2).

Inflorescence inoculation experiments. Inoculation of inflorescences of the three cultivars did not result in any leaf wilting 7–10 days after inoculation except on two shoots of 02-16. Four months after inoculation of inflorescences with mycelial plugs of isolate Bc #37-1 of *B. cinerea,* none of the shoots of Peters and 02-18 and only three shoots of 02-16 were blighted (Table 3). However, cankers at the infection sites were significantly longer on 02-16 and 02-18 than on Peters. Inoculation of inflorescences with a spore suspension of isolate Bc #37-1 resulted in wilting of two shoots of 02-16 7 days after inoculation and of five, three, and three shoots of 02-16, 02-18, and Peters, respectively, 4 mo later (Table 3). Cankers that developed on shoots of 02-16 were significantly longer than those that developed on shoots of Peters and 02-18.

Seventy to 80% of the inflorescence scars inoculated with a mycelial plug of isolate Bc #37-1 were infected 3 mo after inoculation. Cankers that originated from these inflorescence scars were 17–19 mm long, and canker length did not differ significantly among cultivars.

Effect of nutrients from male inflorescences on growth and sporulation of *B. cinerea*. Both isolates of *B. cinerea* grew

best on APDA and poorest on WA (Tables 4 and 5). The fungus grew better on WA amended with unwashed than with washed inflorescences (Table 4). Both isolates usually grew slower on WA amended with washed or unwashed inflorescences of Peters than on WA amended with washed or unwashed inflorescences of 02-16 (Table 4). Sporulation of *B. cinerea* (isolate Bc #37-1) was significantly better ($F = 72.9$, 1 df; $P < 0.01$) in agar dishes amended with washed inflorescences from 02-16 trees (121×10^6 conidia per dish) than in those amended with washed inflorescences from Peters trees (22.4×10^6 conidia per dish).

Both isolates of *B. cinerea* grew better on media amended with filtrates of inflorescences than on unamended WA (Table 5), and both isolates grew better on APDA than on WA amended with these filtrates. After 4 days of incubation, growth of *B. cinerea* isolates was not significantly different in media amended with filtrates from inflorescences of Peters and 02-16 (Table 5).

Inflorescences of 02-16, 02-18, and Peters contained 24.8, 18.2, and 10.6×10^6, respectively, pollen grains per gram of dry inflorescence before washing and 16.0, 15.5, and 3.1×10^6, respectively, pollen grains per gram of dry inflorescence after washing for 6 hr. Washing significantly reduced the number of pollen grains per gram of dry inflorescence ($F = 57.1$, 1 df; $P < 0.01$).

Germ tubes of conidia from both isolates of *B. cinerea* seeded in dishes containing WA amended with filtrates of inflorescences from 02-16 trees were significantly longer than those of conidia on dishes containing the other agar media (Table 5).

Dry weight, water-retaining capacity, and water loss of inflorescences. Inflorescences of 02-16 and 02-18 are larger and more firmly attached to the supporting shoot than those of Peters (Fig. 2). The dry weights of inflorescences of 02-16 and 02-18 (0.72 and 0.82 g, respectively) were significantly ($P < 0.05$) greater than that of inflorescences of Peters trees (0.34 g). Inflorescences of 02-16 and 02-18, when saturated, retained more than twice as much water (10.9–11.8 g of water per inflorescence) as inflorescences of Peters (4.9 g). The water-retaining capacity (grams of water per gram of dry inflorescence) of inflorescences was about the same for all three cultivars, although the inflorescences of Peters, being smaller, dried faster (after 48 hr) than those of 02-16 and 02-18 (after 96 hr) (Fig. 3).

DISCUSSION

The omnivorous plant pathogen *B. cinerea* requires cool temperatures and wet conditions to develop (7). In California, Botrytis blossom and shoot blight of pistachio develops only in cool, rainy springs (13).

Table 3. Susceptibility of inflorescences of pistachio male cultivars to inoculation with *Botrytis cinerea*[w]

Inoculum[x]	Cultivar	Blighted shoots[y] (no.)	Mean length of cankers after 4 mo[z] (mm)
Mycelial plug	02-16	3	46 a
	02-18	0	32 a
	Peters	0	13 b
Spore suspension	02-16	5	54 a
	02-18	3	13 b
	Peters	3	14 b

[w] Ten inflorescences (with pollen sacs not dehisced) were inoculated on each of three trees of each cultivar.
[x] Mycelial plugs 5 mm in diameter were obtained from a 5-day-old culture of *B. cinerea* (isolate Bc #37-1) grown on acidified potato-dextrose agar (APDA). The spore suspension contained 1.5×10^5 spores per milliliter and was prepared from a 5-day-old culture of the fungus on APDA.
[y] Average number of blighted shoots that developed from the terminal vegetative buds of the 10 shoots bearing the inoculated inflorescences, recorded 4 mo after inoculation.
[z] For each kind of inoculum, numbers followed by different letters are significantly ($P = 0.05$) different according to Duncan's multiple range test.

Table 4. Mycelial growth of *Botrytis cinerea* on water agar (WA) amended or not amended with male pistachio inflorescences

Medium[x]	Isolate of *B. cinerea*[y]	Mean diameter of colony[z] (mm) after 3 days	4 days
APDA (control)	Bc #37-1	53.0 a	81.0 a
WA, 02-16		41.3 b	59.8 b
WA, Peters		35.8 c	57.0 b
WA, 02-16, washed		30.8 d	51.3 c
WA, Peters, washed		25.3 e	43.8 d
WA (control)		22.3 e	41.8 d
APDA (control)	Bc #7-1	54.8 a	81.0 a
WA, 02-16		35.8 b	58.8 b
WA, Peters		34.3 bc	54.8 c
WA, 02-16, washed		30.0 cd	48.3 d
WA, Peters, washed		26.5 de	42.8 e
WA (control)		21.5 e	38.5 f

[x] WA was amended with 20 g of dry inflorescences per liter of water. APDA = acidified potato-dextrose agar.
[y] Both isolates of *B. cinerea* were from pistachio. Analysis of variance indicated that growth of the conidial isolate (Bc #37-1) was significantly greater than that of the sclerotial isolate (Bc #7-1) after 4 days' incubation of the dishes ($F = 6.0$, 1 df; $P < 0.05$).
[z] Values are the average of four replicate dishes (two experiments). Numbers in each column for each isolate followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Table 5. Mycelial growth of *Botrytis cinerea* on water agar (WA) media amended or not amended with filtrates from male pistachio inflorescences

Medium[v]	Isolate of *B. cinerea*[w]	Mean diameter of colony[x,y] (mm) after 3 days	4 days	Spore germination[y] (%)	Length of germ tubes (μm)[y,z]
APDA (control)	Bc #37-1	53.0 a	81.0 a	96.5 a	302.7 b
WA, Peters		40.6 b	66.8 b	83.5 b	227.8 c
WA, 02-16		40.3 b	65.3 b	85.5 b	504.3 a
WA (control)		22.3 c	43.8 c	93.0 ab	195.8 c
APDA (control)	Bc #7-1	54.8 a	81.0 a	94.0 a	207.4 b
WA, Peters		37.3 b	58.9 b	78.0 b	202.2 b
WA, 02-16		35.7 b	56.7 b	95.0 a	337.3 a
WA (control)		21.5 c	38.5 c	97.5 a	211.2 b

[v] WA was amended with filtrates from inflorescences (20 g of dry inflorescences per liter of water). APDA = acidified potato-dextrose agar.
[w] Both isolates of *B. cinerea* were from pistachio. Analysis of variance (ANOVA) indicated a significant difference in growth of the conidial (Bc #37-1) and sclerotial (Bc #7-1) isolates after 4 days ($F = 83.1$, 1 df; $P < 0.01$).
[x] Values are the average of four replicate dishes (two experiments).
[y] Numbers in each column for each isolate followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.
[z] Mean of four 10-spore counts per dish after 10 hr at 21 C. ANOVA indicated a significant difference between isolates Bc #37-1 and Bc #7-1 in length of germ tubes ($F = 12.5$, 1 df; $P < 0.01$).

Although Bolkan et al (2) described symptoms of Botrytis blight in 1984, infections of male inflorescences resulting in canker development along the shoot (Fig. 1) and subsequent shoot blight have not previously been reported. In the spring of 1986, cankers originating from male inflorescences were very common not only in shoots from 1985 but also in shoots from 1983 (which was another wet year in California). These cankers, frequently as long as 10 cm, resulted in girdling of the shoot parts above the cankers in the following year. Two to four cankers per shoot enlarged and coalesced, resulting in shoot blight during April and May. The results obtained in this study explain the more frequent occurrence of these cankers on male pistachio cultivars 02-16 and 02-18 than on Peters.

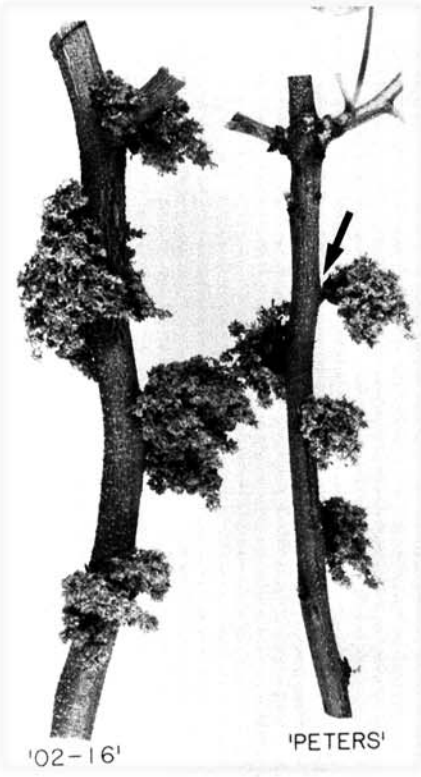

Fig. 2. Staminate inflorescences of pistachio cultivars 02-16 (left) and Peters (right). The arrow indicates the loose attachment of an inflorescence. Inflorescences of cultivar 02-18 resemble those of 02-16.

Results from experiments in the field showed that inoculation of shoots with either conidial or sclerotial isolates of *B. cinerea* resulted in significantly smaller cankers in Peters than in 02-16 and 02-18 (Table 2), indicating less intrinsic susceptibility to infection by *B. cinerea*. All shoots from all cultivars were the same age, although shoots of 02-16 and 02-18 grow more vigorously than those of Peters.

Fewer inflorescences were counted hanging on Peters trees during the spring and about 10 mo later than on trees of 02-16 and 02-18, which could explain the lower incidence of Botrytis blossom and shoot blight on Peters (Table 1), since a large percentage of shoot blight is associated with infected blossoms. In addition, inflorescences of 02-16 and 02-18 have twice as many pollen grains per gram of dry weight as inflorescences of Peters.

Propagules of *B. cinerea* were found in at least 25% of pistachio flower and vegetative buds (12). Spores of *B. cinerea* germinate better in suspensions of pollen from strawberry or raspberry than in water (1,6). Chou and Preece (4) reported that addition of pollen grains induced the development of lesions caused by *B. cinerea* on bean leaves, while removal of all anthers, the source of pollen, markedly affected the speed and severity of infections of strawberry fruits. Similarly, pollen with filaments and anthers found in pistachio inflorescences may favor the germination and growth of conidia of *B. cinerea*.

The role of nutrients in predisposing pistachio inflorescences to infection by *B. cinerea* is further indicated by the fact that the fungus grew better on media amended with inflorescences or filtrates from inflorescences than on WA (Tables 4 and 5). Because *B. cinerea* grew equally well in agar media amended with inflorescence filtrates from 02-16 and Peters trees, the faster growth of the fungus on agar media amended with unwashed inflorescences of 02-16 can be explained by the greater number of pollen grains per gram of inflorescence. Similarly, although washing the inflorescences significantly reduced growth of the fungus, growth on agar media amended with washed inflorescences of 02-16 was still significantly better than that on media amended with inflorescences of Peters (Table 4). Even after being washed with tap water for 6 hr, inflorescences of 02-16 and 02-18 contained significantly more pollen grains than those of Peters.

Cultivars 02-16 and 02-18 bloom about 4–7 days later than does Peters (5). The inflorescences of 02-16 and 02-18 are bulkier, are more firmly attached to the supporting shoot (Fig. 2), and hang on to the shoots longer than those of Peters. When it rains in the spring, these inflorescences are ideal for development of *B. cinerea*. Because inflorescences of 02-16 and 02-18 contain more pollen grains and are more compact than those of Peters, they are more prone to infection by *B. cinerea*. Similarly, cultivars of castor bean (*Ricinus communis* L.) with compact inflorescences are more susceptible to Botrytis capsule mold, caused by *Amphobotrys ricini* (Buchw.) Hennebert (syn. *B. ricini* Buchw.), than cultivars with less compact inflorescences (15), and dense almond blossoms are also more susceptible to Botrytis infections (14). A comparable situation has been reported for grape cultivars, such as Cabernet Sauvignon and Muscat of Alexandria, that have looser clusters and are less susceptible to bunch rot caused by *B. cinerea* under field conditions than are other cultivars (16). Under laboratory conditions, however, inoculated berries from these cultivars are as susceptible or more susceptible than those of cultivars with tighter clusters (16).

Before inflorescences dry after a spring rain, conidia of *B. cinerea* can germinate better and the germ tubes can grow faster and can infect more inflorescences of 02-16 or 02-18 than of Peters. Although

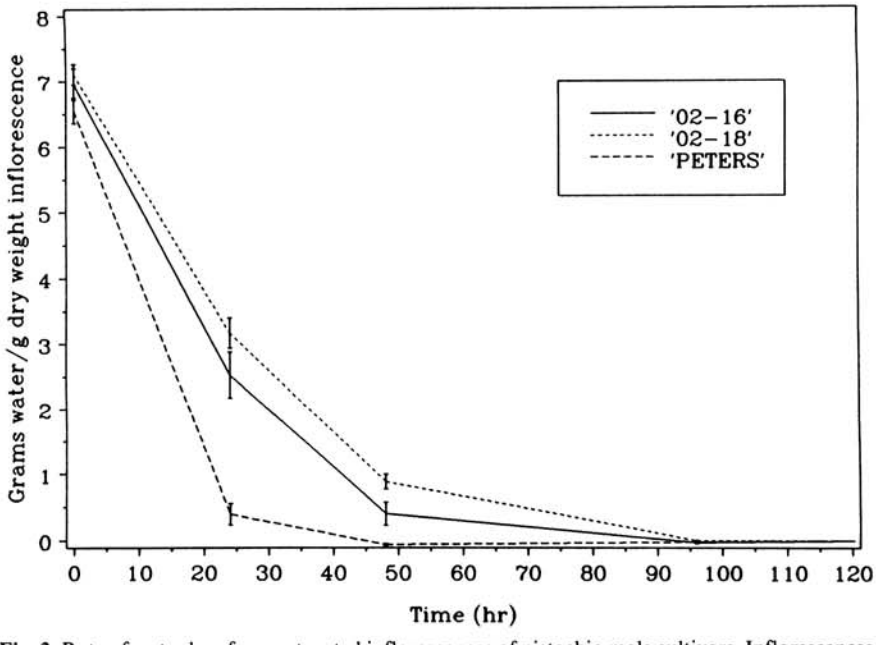

Fig. 3. Rate of water loss from saturated inflorescences of pistachio male cultivars. Inflorescences were soaked in distilled water for 24–48 hr, the excess water was drained, and inflorescences were allowed to dry at 23 ± 1 C. Data are the average of 30 inflorescences per cultivar. Vertical bars represent standard deviations.

the water-retaining capacity of inflorescences of all three cultivars is about the same, inflorescences of Peters, because they are smaller, dry faster than inflorescences of 02-16 and 02-18 (Fig. 3) and thus probably do not allow sufficient time for germination and infection by the conidia of *B. cinerea*.

All these data suggest that the biological basis for susceptibility of 02-16 and 02-18 pistachio trees to Botrytis blossom and shoot blight lies in both intrinsic and morphological characteristics of the inflorescences of these cultivars. Their inflorescences are bulkier, more compact, richer in nutrients, and more firmly attached to their supporting shoots than the inflorescences of Peters trees, and they dry more slowly.

Cultivar 02-18 usually was intermediate in susceptibility to *B. cinerea* between the more susceptible 02-16 and the more resistant Peters. In addition, some other predisposing agronomic features of 02-18 were rated as intermediate between those of 02-16 and Peters (i.e., number of inflorescences per tree and of pollen grains per inflorescence).

In summary, these results suggest that trees of pistachio male cultivars 02-16 and 02-18 are more sensitive to infection by *B. cinerea* than Peters trees, and growers should take this into account when they plan to establish new pistachio orchards. Although 02-16 and 02-18 have been extensively propagated and distributed throughout the pistachio production area (5), some growers, especially in northern California, are considering replacing them, because the numerous cankers that originate from infections of inflorescences by *B. cinerea* can serve as infection courts for *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not. (10), a very destructive pathogen of pistachio (9), which may spread to the female Kerman pistachio trees and damage the nuts.

ACKNOWLEDGMENTS

I thank D. P. Morgan and J. Dunai for their help in this project, Z. K. Punja for reviewing the manuscript, D. Hutfless (Ord Bend Farms, Inc.) for his cooperation, and the California Pistachio Commission for financial support of the project.

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