Chemical Control of Botryosphaeria and Botrytis Blights of Pistachio in California in 2005

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Summary

The rainy spring and summer of 2005 resulted in heavy disease pressure. With several exceptions, the best performing fungicides were all strobilurins or treatments either including strobilurins in the spray schedule or formulations of fungicides containing strobilurins along with another compound. When the disease was evaluated on the tree by recording blighted fruit clusters, the four top treatments were three applications of Gem™ 500SC (trifloxystrobin) two sprays of Pristine® (pyraclostrobin and boscalid) and one of Captan®, and two or three applications of Pristine®. Next in effectiveness were three applications of USF2010 and Abound® (azoxystrobin). Non-strobilurin fungicides that also performed well included Polyoxin®, Valent V-10116, and Topsin® (thiophanate-methyl) followed by three of Ziram. We compared sprays based on early-season rain events, and sprays based on hours over a 90° F temperature threshold with those of the grower. The rainy weather provided ample opportunity to test this experiment under heavy disease pressure. A number of these rain events exceeded the minimum temperature and hours of wetness considered necessary for infection to occur (potential infection events). In addition, the last two rain events on June 14 to 16 and July 16 were accompanied by very warm temperatures (13-30° C and 26-35° C, respectively) which were near the optimal temperature for maximum growth rate of this fungus. The most effective spray schedule was the one used by the grower which consisted of six fungicide applications. His sprays were applied shortly before five of the six potential infection events while his sixth spray occurred about a week after the final July 16 rain event and during the period of the highest mean daily temperatures of the season. He reduced disease incidence on fruit by 94-98% compared to the unsprayed trees, and his strategy was much more effective than the temperature threshold treatments. Two of the rain event treatments, consisting of two sprays closely timed to protect the trees from *Botryosphaeria* infections after April or early May rain event, were not quite as effective as the grower's spray schedule, but similar statistically to that of the grower. Disease incidence on fruit was reduced by 76-93% compared to the unsprayed control. Sprays after 20, 40, and 60 hour accumulations of hours over the 90°F threshold called for 3, 3, and 2 sprays, respectively. Because of the cool spring weather, first spray was called for on July 14. The disease incidence increased as the number of threshold hours increased. Even with the 20-hour threshold, however, disease incidence on the fruit was only reduced by 45-67% compared to the unsprayed control. This control was not statistically similar to that of the grower or sprays near early season rain events.

BUDMON (bud monitoring) accurately determined the disease risk potential for the orchard before the growing season started. Analyzing rachises was moderately effective

in determining the risk potential for the orchard before the growing season started. On the other hand, ONFIT (overnight freezing incubation technique), which is performed during the growing season, was very good at estimating final disease development at harvest.

Three fungicides (Vangard®, USF 2010, and Pristine®) consistently and successfully controlled Botrytis blossom and shoot blight in both trials. To follow up on concerns raised by growers and consultants over of the high number of Botrytis blossom and shoot blight strikes during the 2005 season, we collected 89 isolates of *Botrytis* to determine possible resistance to thiophanate-methyl. Surprisingly, 72% of these isolates were resistant to thiophanate-methyl at 10 ppm.

Introduction

Botryosphaeria panicle and shoot blight, caused by a *Fusicoccum* sp. (*Botryosphaeria dothidea*), is regarded as the most serious above ground disease on pistachios grown in California. The disease is still a threat to the California pistachio industry, with the exception of orchards located on the west side of the San Joaquin Valley. The rainy spring weather in 2005 resulted in an epidemic of panicle and shoot blight in unsprayed trees where inoculum was present.

Generally, canker diseases caused by *Botryosphaeria* spp. are difficult to control because the fungus produces pycnidiospores in flask-like structures (pycnidia) embedded in the outer layers of the infected plant tissue. In pistachio, most sources of spore inoculum are retained on trees for years. Fungicides have difficulty in reaching these sources of inoculum because the spores are protected inside the cavities of the pycnidia, and the pycnidia themselves develop under the epidermal layers of infected tissues and can supply viable *B. dothidea* spores for at least 6 years. Therefore, fungicides that can protect young plant tissues from infection by the spores of *B. dothidea* are needed.

The registered strobilurin fungicides have been quite effective in controlling this disease. However, because of the resistance problem with strobilurin fungicides, as demonstrated with Alternaria late blight of pistachio (Ma *et al.*, 2004a) and other crops (Stevenson *et al.*, 2002), it is essential that the efficacy of other classes of fungicides be investigated. Recent research (Ma et al., 2004b, Michailides and Morgan, 2004) has noted that the population structure of *Botryosphaeria dothidea* in California seems quite homogenous. This and the fact that resistant isolates have been rarely detected through the 2003 season (Ma et al., 2004a) suggest that *B. dothidea* may not readily develop resistance to strobilurin fungicides, particularly because we still have never found the sexual (ascosporic) stage on pistachios. On the other hand, we remain concerned about the potential for the development of disease resistance, because *B. dothidea* also causes band canker disease in almonds, and in 2004 and 2005 we have readily found pseudothecia bearing mature ascospores of *B. dothidea* in the bark of trunks of almond trees with severe band canker symptoms, and in stumps of almond trees killed by this fungus in orchards located in the Sacramento Valley (Michailides, 2004 and 2005). Although this almond disease is sporadic in nature, the fact remains that ascospores are genetically diverse. Because almond trees are commonly sprayed with strobilurins, a situation exists for the selection of *B. dothidea* isolates resistant to strobilurins, although on a different crop. This discovery highlights the need for maintaining an active program to identify other effective fungicides from a variety of classes, in order to safeguard the pistachio industry from the possibility of the development of resistance to currently registered fungicides.

Botrytis blossom and shoot blight, caused by *Botrytis cinerea*, is more prevalent during cool, wet springs and causes damage by killing current season shoots, thus reducing fruiting wood for the following season. In addition, these blighted shoots can be colonized by *B. dothidea*. Although it has been known for years that Botrytis is favored by cool wet conditions, some rain during bloom in recent years resulted in very low disease incidence, while in other rainy springs, such as in 2005, the cool wet spring weather resulted in a high incidence of Botrytis blights, even in orchards sprayed with fungicides. This season, we received a number of calls and questions from growers and agricultural consultants who thought that the number of blossom and shoot blights were excessive considering the number and timing of the fungicide applications. The objectives of our 2005 study were to 1) test new fungicides (and fungicide programs) for control of panicle and shoot blight; 2) determine the effect of fungicides applied just before predicted rain events; 3) determine preseason and in season inoculum levels and see which best predict panicle and shoot blight; 4) monitor resistance development in *B. cinerea* isolates from pistachio orchards; and 5) test new fungicides against Botrytis blossom and shoot blight.

Procedures

Efficacy of fungicide against Botryosphaeria panicle and shoot blight. An experimental plot was established for the control of panicle and shoot blight in Glenn County. The orchard was irrigated using drip emitters. Each treatment consisted of five single tree replications. The trade names, active ingredients, and class of the fungicides used in these trials are listed in Table 1. The fungicide treatments, rate of each fungicide and dates of application are listed in Table 2. All bloom sprays were applied with a handgun sprayer at rates recommended by the manufacturer using 100 gal. of water per acre. After bloom time, sprays were applied with a handgun sprayer using 400 gal. of water per acre. Bloom sprays were applied on March 31. The sprays in both locations were applied at different timings throughout the season from bloom to August 1. The disease incidence was very high on the unsprayed trees. On September 13, we recorded the incidence of blighted clusters from 100 fruit clusters on the trees and collected random samples of leaves and fruit clusters for evaluation in the laboratory. In the laboratory, disease symptoms were evaluated on 200 fruit, 25 rachises, and 50 leaves from each replicated tree.

Efficacy of fungicides applied just before or after rain events, or of applications based on temperature thresholds. In this experiment, we compared the effectiveness of early-season rain based fungicide applications which target either preventing the initial infection or eradicating new infections of pistachio tissue by the pathogen (applied either before or after rain events) with the effectiveness of sprays based on ranges of hours above a temperature threshold of 90° F, which are based on the maximum metabolic activity of the pathogen, and with the grower's own spray regime which was a hybrid of these two approaches. This experiment was also established in the orchard in Glenn County. The NOAA weather forecast was monitored closely and fungicides were applied when there was greater than an 80% probability of rain. After a fungicide application, we waited 14 days before considering spraying again, if additional rain events were forecast. Sprays before predicted rain events were applied on March 31 and April 22. Sprays after rain events were applied on April 5 and May 3. The goal was to use Pristine® fungicide for all rain event based sprays. However, the rain event of April 3 and 4, which was much stronger than originally forecast, occurred before we had an opportunity to apply Pristine®, so we had to substitute an application of Topsin 4.5 F, which had originally been applied for another purpose. Fungicides used and dates applied are listed in Table 3. CIMIS #61weather data recorded near the location of this experiment was used to determine temperature thresholds and is shown in Appendix 1. Three treatments were used based on 20, 40, and 60 accumulated hours above 32.2° C (90° F). The 20- and 40-hour threshold treatments called for three fungicide application of Abound®. The 60-hour threshold treatment called for two sprays of Abound®. Dates of these sprays are given in Table 3. Disease incidence on the tree was determined and samples for further disease evaluation were collected on September 13.

Inoculum levels and prediction of panicle and shoot blight. Several methods to assess inoculum potential were compared. Ten unsprayed trees from each of 16 orchards (Table 4) were used in this study. From each orchard early to mid March, 100 buds were collected in early to mid-March and processed according to the BUDMON technique (Michailides *et al.*, 1999) and 250 hanging black rachises were collected at the same time. These were examined with a 10× dissecting for the presence of pycnidia of *B. dothidea.* On June 24, 50 fruit and leaves were collected and processed according to ONFIT (Michailides *et al.*, 1997). Disease incidence on these trees was recorded shortly before harvest by recording shoot blight or blighted panicles from 100 shoots per tree.

Efficacy of fungicides against Botrytis blight. These trials were conducted in the greenhouse using supplemental misting. We did this because in prior years the light disease incidence in our KAC pistachio orchard prevented us from evaluating the effectiveness of fungicides; even though we have tried applying *Botrytis* inoculum immediately before bloom-time rain events and installed patio foggers on trees to provide supplemental misting in this orchard. Two fungicide experiments were performed on potted trees placed in the greenhouse under an overhead misting system. Patio foggers were used to enhance the cool humid greenhouse environment by wetting the leaves. There were five replicate trees per treatment. The trade names and active ingredients of the fungicides used in these trials are listed in Table 1. The fungicide treatments and rates are listed in Tables 5 and 6.

In the first experiment, fungicides were applied at 10 am on March 24. We used pistachio *Botrytis cinerea* isolates 7A6 and 7A7 which were sensitive to iprodione (Rovral) and thiophanate-methyl (Topsin). The spore suspension was sprayed at 3 pm on that day at 5×10^5 spores per milliliter (ml) and sprayed to drip (each tree received about 55 ml of this inoculum). The misting was started at 3:15 pm and ran for thirteen days duration, except the system was turned off during day 6. The misting system, using 1.5 gal. per hour patio foggers, was on for two minutes per 15 minutes during the day, one minute per 15 minutes at night, using a light intensity threshold of 100 W per m^2. Botrytis blossom and shoot blight was recorded on May 5, 2005.

In the second experiment, fungicides were applied at 3:00 pm on April 11 when trees had slightly more leaves emerged than in the first experiment. We accomplished this by using trees which were slow to emerge from dormancy. The same isolates and spore concentration as in the first experiment were used. The misting system, using the same parameters as described above, ran for 15 days duration, though the system was turned off on day 6. Botrytis blossom and shoot blight was recorded on June 1, 2005.

Evaluation of *Botrytis cinerea* isolates for resistance to thiophanate-methyl. A total of 91 isolates of *B. cinerea,* from nine orchards in Fresno, Kern, and Madera Counties, were isolated from blighted pistachio shoots and flower parts in the spring of 2005. Two additional isolates from 1992 were tested. Isolates were grown on acidified PDA and then transferred to three plates each of PDA amended with or without 10-ppm thiophanate-methyl. Colony diameter was measured after 3 days growth. Isolates were considered resistant if growth on fungicide-amended media was greater than 85% of the growth on fungicide free media. Isolates were considered sensitive if growth was less than 10% compared to growth on the fungicide free media.

Results and Discussion

Efficacy of fungicides against Botryosphaeria panicle and shoot blight. The numerous spring and early summer rain events combined with high summer temperatures created conditions ideal for infection and growth of *B. dothidea.* This resulted in heavy disease pressure with about 80% disease incidence for all categories evaluated in the unsprayed control (Table 2). The best performing fungicides were all strobilurins or treatments either including strobilurins in the spray schedule or formulations of fungicides containing strobilurins along with another compound. For instance, two applications of Pristine (Trt 3), three of Gem™ 500SC (trifloxystrobin - Trt 10), and two Pristine® alternated with one Captan® (Trt 15) performed the best (Table 2). Other strobilurin based treatments in this mean separation group ($P < 0.05$) included three applications of USF2010 (Trt 8) and Abound® (azoxystrobin - Trt 14). Non-strobilurin fungicides that also overlapped the top performing treatments included Polyoxin® (Trt 12), Valent V-10116 (Trt 13), and Topsin® followed by three of Ziram (Trt 1). As in earlier research using one spray of benomyl, one spray with Topsin provided about 50% reduction of disease over the unsprayed control when evaluated on the tree. However, lab evaluation of fruit and leaves revealed that symptoms on the fruit and leaves were reduced less, only by 4-38% with the various formulations of this Topsin® M. No formulation consistently outperformed the others. The two applications of Pristine® on 1 June and 1 July were more efficacious than one Pristine® on 1 July, and it seems that the Pristine® applied on 1 August did not have any additive effect against the disease over the two sprays (Table 2).

Disease incidence even with the most effective fungicides was higher than the grower's spray schedule due to the fact that we had fewer sprays than the six sprays applied by the grower and also because there was ample opportunity for early infections to occur before most treatments received their first application on June 1. However, the two important potential infection events occurred in June and July, providing some opportunity to evaluate the fungicides based on their ability to prevent some infection and also reduce growth of the pathogen.

Efficacy of fungicides applied just before or after early season rain events, or of applications based on temperature thresholds. The rainy weather provided ample opportunity to test this experiment under heavy disease pressure. At least six of these rain events (see Appendix 1) exceeded the minimum temperature and hours of wetness considered necessary for infection to occur (Driever et al., 2003). In addition, the last two rain events on June 14-16 and July 16 were accompanied by very warm temperatures (13-30° C and 26-35° C, respectively) which were often near the maximum growth rate of this fungus. These were probably the strongest potential infection events of the season. The most effective spray schedule was the one used by the grower which consisted of six fungicide sprays (Table 3). His sprays were applied shortly before five of the six potential infection events while his sixth spray occurred about a week after the final July 16 rain event and during the period of the highest mean daily temperatures of the season. He reduced fruit symptoms by 94-98% compared to the unsprayed control (Table 3), and his strategy was much more effective than the temperature threshold treatments (Trts 5-7). Although not as effective as the six grower sprays, applying two early season rain event sprays (Trts 3 and 4 in Table 3) were not statistically different from the grower ($P < 0.05$).

Although treatments 3 and 4, consisting of two sprays either before or after the first two potential infection events were not as effective as the six applications of the grower, they were still in the same mean separation group as his ($P < 0.05$) (Table 3). Disease incidence was similar whether the fungicides were applied before or after the rain. Symptoms on the fruit were reduced 72-93% compared to the unsprayed control. In retrospect, treatment 1 only "protected" the fruit from 2 of 12 rainstorms and from none of the more important potential infection events. Treatment 2 which was applied before the second rain storm, protected the pistachio tissue against this marginal potential infection event, but most likely was degraded before the six later potential infection events. Treatments 3 and 4 would have protected the pistachios against the first three of these important potential infection events on May 4-5, May 8-9, and May 17-18, but not from event four on June 8-9, and not the two probably strongest potential infection events on June 14-16 or on July 16.

The narrower the interval triggering temperature threshold (cumulative hours above 90° F) sprays, the more effective was the disease control (Table 3). Although this trend was observed for fruit, rachises, and leaves, there were no significant differences between the spray intervals with the exception of blighted fruit clusters on the tree where the 20-hour interval resulted in significantly less blighted clusters than the 60-hour interval. The analysis of the rain event and temperature based spray treatments is complicated by the fact that the first temperature threshold sprays, based on 20- and 40-hour intervals, were applied shortly before the July 16 rainstorm which had 14 hours of light to moderate rain at temperatures very near the optimum for maximum growth rate of *B. dothidea*. Despite the fact that these two sprays were applied shortly before this likely strong potential infection event, it appears that enough damage was already done by the potential infection events of the spring and especially May and June. Although these sprays were applied after most of the latent infections had occurred, they were still somewhat effective in reducing the growth of *B. dothidea*, and hence reducing symptoms of panicle and shoot blight.

Inoculum levels and prediction of panicle and shoot blight. Very high levels (>45%) of infected pistachio buds were detected by BUDMON from Butte, Glenn, and Tulare Counties (orchard numbers 3, 5, and 12 in Table 4). These orchards had the highest levels of panicle and shoot blight at harvest time. Unfortunately, our trial areas in the Tulare County orchards were accidentally sprayed with fungicides several times after bloom, so we decided to stop recording data from these two sites. The percentage of infected rachises was generally similar to the BUDMON results, but from two orchards the results diverged extremely (Table 4). This could be due to the fact that these growers have pruned out most of the infected rachises, thus successfully lowering the initial inoculum resulting in low BUDMON counts even though a number of the few remaining dead black rachises that we found were infected with *B. dothidea*. The correlation between percentage of infected rachises and infected buds (BUDMON) was not significant at $P < 0.05$. ($R^2 = 0.17$): These results differ from those recorded in 2003 (Mina et al., 2004). The correlation between panicle and shoot blight at harvest and infected buds (BUDMON) in 2005 was highly significant ($R^2 = 0.81$: Figure 1) and was much higher than when disease was regressed against infected rachises ($R^2 = 0.24$: Figure 2).

BUDMON successfully predicted panicle and shoot blight at harvest in individual seasons in 2003 (Mina et al., 2004), 2004, and 2005 (Figure 1), with highly significant R^2 values of 0.73, 0.66, and 0.81, respectively. But when the data were combined for 2004 and 2005 there was a poorer correlation between seasons ($R^2 = 0.17$). This means that BUDMON is very good at predicting the potential for disease development, and that these results are highly significant for a given year. Thus BUDMON results will accurately determine the potential risk for disease development, and will accurately determine the relative risk compared to other orchards in a given season. However, BUDMON may not accurately predict actual panicle and shoot blight at harvest, because disease development is also dependent on other factors such as rain events and temperature, not just initial inoculum potential. While the R-values are highly significant, the linear regression equations are very different from year to year (confer in Mina et al., 2004) with 2004 and 2005 seasons data as shown in Figure 1.

The ONFIT (overnight freezing incubation technique) results show that the correlation between panicle and shoot blight with infected leaves or fruit from the 2004 season (six orchards) were not significant at $P < 0.05$ for both infected leaves ($R^2 = 0.26$) and fruit ($R^2 = 0.02$) in 2004 (Figure 3). In 2005 the correlation between these parameters was significant for fruit but not leaves. Combining the data from 2004 and 2005 results in similar linear regression equations and R-values that are not significant for leaves ($R^2 = 0.17$) and highly significant for fruit ($R^2 = 0.40$, $P < 0.01$). Thus ONFIT results from fruit are a better predictor than leaves. These results support previous work that suggested that ONFIT is a better predictor of panicle and shoot blight than BUDMON (Mila et al, 2004, 2005).

In conclusion, BUDMON accurately determined the disease risk potential for the orchard before the growing season started. Analyzing rachises was moderately effective in determining the risk potential for the orchard before the growing season started. ONFIT, which is performed during the growing season, was very good at estimating final disease development at harvest.

Efficacy of fungicides against Botrytis blight. Three fungicides (Vangard®, USF 2010, and Pristine®) consistently and successfully controlled Botrytis blossom and shoot blight in both trials (Tables 5 and 6). Vangard® also most effectively controlled this disease in our 2004 trials (Michailides et al., 2004). And Pristine® has been a consistent good performer in 2003 and 2004 as well (Michailides et al., 2003 and 2004). Although the other fungicides suppressed disease incidence compared to the unsprayed control; Rovral® did not differ from the unsprayed control according to the LSD test ($P < 0.05$). The level of disease suppression by Topsin® M, Scala™, and the low rates of V-10135 and V-10116 did not differ from the control according to the LSD test ($P < 0.05$) in the first trial (Table 5), but did in the second trial (Table 6). Only the high rates of V-10116 and V-10135 significantly reduced disease ($P < 0.05$) in both trials compared to the control.

Evaluation of Botrytis isolates for resistance to thiophanate-methyl. In a total of 91 isolates of *B. cinerea* collected from several orchards in three counties and tested in 2005, 72% were resistant to thiophanate-methyl at 10 ppm (Table 7). Resistant isolates were detected in all three counties. The percentage varied from 45 to 100% in the orchards that we sampled. In all but one orchard more than 50% of the *Botrytis* isolates were resistant to thiophanate-methyl.

Conclusions and Practical Applications

1. Strobilurin fungicides (Gem™, Pristine®, USF2010, and Abound®) most effectively controlled panicle and shoot blight in the efficacy trial. Other effective fungicides included Polyoxin, V-110116, and Topsin followed by Ziram.

2. Even in a year like 2005 with many potential infection events from bloom through June, applying two fungicide sprays near the early potential infection events after bloom was nearly as effective as the six applications of the grower.

3. The temperature threshold based sprays (90° F threshold), which were applied later in the season, were not as effective as the early season rain event sprays.

4. Botrytis blossom and shoot blight was most effectively controlled by Vangard®, USF 2010, and Pristine®.

5. The percentage of resistance to thiophanate from *Botrytis cinerea* isolates tested from various orchards varied from 45 to 100%.

6. BUDMON (bud monitoring) accurately determined the disease risk potential for the orchard before the growing season starts. Analyzing rachises was moderately effective in determining the risk potential for the orchard before the growing season starts. ONFIT (overnight freezing incubation technique), which is performed during the growing season, was very good at estimating final disease development at harvest.

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Table 1. Trade name, active ingredient, and class of fungicides used in 2005 in trials to control panicle and shoot blight of pistachio caused by *Botryosphaeria dothidea* and Botrytis blossom and shoot blight caused by *Botrytis cinerea*.

Trade name	Active ingredient	Class of fungicide
Abound® *	Azoxystrobin (2.08 lbs per gal.)	Strobilurin
Captan® 80WDG	Captan (80%)	Phthalimide
Endura™	Boscalid (70%)	Carboxyanilide
Gem™ 500 SC	Trifloxystrobin (25%)	Strobilurin
Kasugamycin®	Kasugamycin hydrochloride (2.3%)	Antibiotic fungicide
Polyoxin®	Polyoxorim (2.2%)	Antibiotic fungicide
Pristine® *	Pyraclostrobin (12.8%) + Boscalid (25.2%)	Strobilurin + anilide
Rovral® brand 4 Flowable	Iprodione (4 lbs. per gal.)	Dicarboximide
Scala™ 60 SC	Pyrimethanil (54.6%)	Anilinopyramidine
Topsin® M 70WP*	Thiophanate-methyl (70%)	Benzimidazole precursor
Topsin® M 70WSB*	Thiophanate-methyl (70%)	Benzimidazole precursor
Topsin® 4.5 FL*	Thiophanate-methyl (45%)	Benzimidazole precursor
USF 2010	Not disclosed	Unknown
Valent V-10116	Not disclosed	Unknown
Valent V-10135 9452.05-CAF 216	Not disclosed	Unknown
Vangard®	Cyprodinil	Anilinopyrimidine
Ziram 76DF	Ziram (76%)	Carbamate

* Currently registered for use on pistachios

Table 2. Efficacy of fungicides against Botryosphaeria panicle and shoot blight in a pistachio orchard in Glenn County (blighted clusters recorded and samples collected on September 13, 2005).

Trt	Treatment - fungicide(s)	Rate per acre	100 gal. per acre	400 gal. per acre				Disease incidence on tree	Disease incidence on samples collected at harvest		
			31 March (full bloom)	1 May	1 June	1 July	1 Aug	Blighted fruit clusters (%)[1]	Blighted fruit (%)[2]	Blighted rachises (%)[3,4]	Infected leaves (%)[5]
1.	Topsin M70WP Ziram	2 lbs. 8 lbs.	Topsin		Ziram	Ziram	Ziram	34.3 cdef[6]	45.2 def	18.4 fg	26.0 efg
2.	Pristine	10.5 oz.			Pristine	Pristine	Pristine	15.1 f	24.7 fg	19.2 efg	22.8 efg
3.	Pristine	10.5 oz.			Pristine	Pristine		14.2 f	24.8 fg	6.4 g	13.2 gh
4.	Pristine	10.5 oz.				Pristine		34.4 cdef	45.4 def	35.0 cdef	5.6 h
5.	Topsin M70WP	2 lbs.	Topsin					53.0 bc	57.2 bcde	50.4 bcd	55.6 bc
6.	Topsin 4.5 FL	40 fl. oz.	Topsin					43.1 bcde	73.1 abc	56.8 bc	46.8 cd
7.	Topsin M 70WSB	2 lbs.	Topsin					55.1 bc	79.8 ab	55.2 bc	69.2 ab
8.	USF2010 500 SC	6 fl. oz.			USF2010	USF2010	USF2010	21.5 ef	24.4 fg	28.0 defg	13.6 fgh
9.	Scala 60 SC	18 fl. oz.			Scala	Scala	Scala	63.5 ab	75.6 abc	72.0 ab	47.6 cd
10.	Gem 500 SC	3 fl. oz.			Gem	Gem	Gem	12.7 f	21.7 g	23.2 efg	35.5 de
11.	Kasugamycin	27 fl. oz.			Kasug	Kasug	Kasug	50.5 bcd	62.5abcd	53.3 bc	51.6 cd
12.	Polyoxin	2.2 lbs.			Polyoxin	Polyoxin	Polyoxin	33.0 cdef	37.3 efg	35.2 cdef	29.6 ef
13.	V-10116 50WDG	4 oz.			Valent	Valent	Valent	33.1 cdef	52.8 cde	42.9 cde	16.4 fgh
14.	Abound	12.8 fl. oz.			AB	AB	AB	28.0 def	25.3 fg	27.2 defg	20.4 efgh
15.	Pristine Captan 80WDG	10.5 oz. 5.6 lbs.			Pristine	Captan	Pristine	13.5 f	19.4 g	22.9 efg	10.4 gh
16.	Control	Untreated						78.6 a	83.0 a	83.2 a	75.9 a

1 Fruit clusters where the main rachis was blighted, from a sample size consisting of 100 clusters per five replicated trees.

2 Includes fruit with pycnidia and blighted fruit (dry yellow/beige fruit adhering to portion of blighted rachis) recorded from five replicated samples of 200 fruit evaluated in the laboratory.

3 Includes both partially and totally blighted rachises.

4 Averages of five replicated samples of 25 rachises evaluated in the laboratory.

5 Averages of five replicated samples of 50 leaves evaluated in the laboratory.

6 Numbers followed by different letters are significantly different according to the LSD test at $P = 0.05$.

Table 3. Comparison of various rain event triggered sprays with temperature threshold based sprays in a pistachio orchard in Glenn County (blighted clusters recorded and samples collected on September 13, 2005).

					Disease incidence on tree	Disease incidence on samples collected at harvest		
	Spray **before** first rain	Spray **after** first rain	Spray **before** second rain	Spray **after** second rain	**Blighted fruit clusters (%)**[1]	**Blighted fruit (%)**[2]	**Blighted rachises (%)**[3,4]	**Infected leaves (%)**[5]
	31 March	**5 April**	**22 April**	**3 May**				
1.	Topsin 4.5 F[6]				43.1 ab[7]	73.1 a ab	56.8 a	45.8 b
2.			Pristine[8]		14.9 ef	17.0 c	15.0 bc	14.0 c
3.			Pristine	Pristine	4.6 efg	9.1 c	4.8 bc	18.0 c
4.		Topsin 4.5 F		Pristine	3.7 fg	14.2 c	8.8 bc	3.2 c
5.	Sprayed after 20 hours accumulated above 90° F[9]				17.0 de	33.0 b	18.4 b	10.8 c
6.	Sprayed after 40 hours accumulated above 90° F[10]				29.4 cd	38.6 b	19.2 b	13.6 c
7.	Sprayed after 60 hours accumulated above 90° F[11]				36.3 bc	41.9 b	20.0 b	12.4 c
8.	Untreated				52.2 a	60.0 a	50.4 a	65.9 a
9.	Grower's schedule[12]				0.8 g	3.5 c	1.6 b	8.8 c

1 Fruit clusters with the main rachis blighted from a sample size consisting of 100 clusters per five replicated trees.
2 Includes fruit with pycnidia and blighted fruit (dry yellow/beige fruit on blighted rachises) recorded from five replicated samples of 200 fruit evaluated in the laboratory.
3 Includes both partially and totally blighted rachises.
4 Averages of five replicated samples of 25 rachises evaluated in the laboratory.
5 Averages of five replicated samples of 50 leaves evaluated in the laboratory.
6 Topsin 4.5 F was sprayed at the rate of 40 fl. oz. per acre.
7 Numbers followed by different letters are significantly different according to the LSD test at $P = 0.05$.
8 Pristine® was sprayed at a rate of 12.5 oz. per acre.
9 Abound® at 12.8 fl. oz. per acre was sprayed on July 14, July 29, and August 23, 2005, when 20 hours above 90 F accumulated (3 sprays in total).
10 Abound® at 12.8 fl. oz. per acre was sprayed when 40 on July 15, August 1, and August 23, 2005, hours above 90 F accumulated (3 sprays in total).
11 Abound® at 12.8 fl. oz. per acre was sprayed on July 19, and August 11, 2005, when 60 hours above 90 F accumulated (2 sprays in total).
12 The growers fungicide spray schedule consisted of Topsin® M at 2 lbs per acre on March 26; Abound at 12.8 fl. oz. per acre on April 6, April 26, and May 14; then Pristine @ 12 oz. per acre on June 6 and July 26 (6 sprays in total).

Table 4. Levels of infection by *Botryosphaeria dothidea* of pistachio tissues and final disease at harvest for 2005.

				Latent infections[1]		Disease at harvest[2]
		Infected buds[3] (%)	Infected rachises[4] (%)	Fruit (%)	Leaves (%)	Shoot and panicle blight (%)
1.	Glenn	15	2.0	0	4	0.4
2.	Glenn	5	19.0	14	0	3.4
3.	Glenn	54	40.0	92	34	26.4
4.	Glenn	22	32.0	76	22	6.9
5.	Butte	45	60.0	86	0	34
6.	Colusa	2	0.0	92	8	7.2
7.	Yolo	1	17.0	84	14	1.7
8.	San Joaquin	2	65.0	34	12	1
9.	Madera	5	18.0	100	26	7.5
10.	Madera	2	52.0	94	0	5.1
11.	Fresno	4	38.0	0	0	0.2
12.	Tulare	55	---	---	---	---
13.	Tulare	21	---	---	---	---
14.	Kern	1	3.0	0	0	0
15.	Kern	5	3.0	0	0	0.2
16.	Kern	1	4.0	0	0	0.9

1 Latent infections were determined with the Overnight Freezing and Incubation Technique (ONFIT) on leaves and fruit collected on May 14.

2 100 shoots per tree were recorded shortly before commercial harvest.

3 100 buds were collected from March 1-14 and processed with the BUDMON technique.

4 250 rachises were collected from March 1-14 and 100 were examined for pycnidia of *B. dothidea*.

Table 5. Fungicide efficacy against blossom and shoot blight of pistachio (caused by *Botrytis cinerea*) recorded on May 25, 2005 (Experiment 1: fungicides applied on March 25, 2005).

	Fungicide[1]	Rate per acre	Botrytis blights per tree		
			Flowers	Shoots	Total
1	Topsin M 70WP	2 lbs.	0.2 ab[2]	3.8 ab	**4.0 abc**
2	Topsin 4.5 FL	40 fl. oz.	0.4 ab	4.6 ab	**5.0 abc**
3	Scala 60 SC	18 fl. oz.	0.2 ab	3.4 ab	**3.6 abc**
4	Rovral	32 fl. oz.	0.4 ab	4.8 ab	**5.2 abc**
5	USF 2010 500SC	6 fl. oz.	0.0 b	1.4 b	**1.4 bc**
6	Vangard	10 oz.	0.0 b	1.2 b	**1.2 bc**
7	Pristine	10.5 oz.	0.2 ab	2.0 b	**2.2 bc**
8	V-10135 20 WDG 9452	1.25 lbs.	0.6 ab	4.8 ab	**5.4 ab**
9	V-10135 20 WDG	2.5 lbs.	0.0 b	0.8 b	**0.8 c**
10	V-10116 50 WDG	3.5 oz.	0.2 ab	5.0 ab	**5.2 abc**
11	V-10116 50 WDG	4.0 oz.	0.2 ab	2.8 ab	**3.0 bc**
12	Unsprayed		1.2 a	6.6 a	**7.8 a**

1 Potted pistachio trees (cv. Kerman) were placed in a greenhouse at the Kearney Agricultural Center and inoculated with *Botrytis cinerea* after fungicide applications had dried thoroughly. The canopy of the trees was misted with patio foggers to encourage disease development.

2 Numbers followed by different letters are significantly different according to the LSD test at $P = 0.05$.

Table 6. Fungicide efficacy against blossom and shoot blight of pistachio (caused by *B. cinerea*) recorded on June 1, 2005 (Experiment 2: fungicides applied on April 11, 2005).

	Fungicide	Rate per acre	Botrytis blights per tree[1]		
			Flowers	Shoots	Total
1	Topsin M 70WP	2 lbs.	0.0 b[2]	4.2 bcd[2]	**4.2 bcde**[2]
2	Topsin 4.5 FL	40 fl. oz.	0.0 b	3.2 bcd	**3.2 bcde**
3	Scala 60 SC	18 fl. oz.	0.0 b	6.0 abc	**6.0 bcd**
4	Rovral	32 fl. oz.	0.2 ab	6.0 abc	**6.2 abc**
5	USF 2010 500SC	6 fl. oz.	0.0 b	1.6 cd	**1.6 de**
6	Vangard	10 oz.	0.6 ab	0.6 d	**1.2 e**
7	Pristine	10.5 oz.	0.0 b	2.0 cd	**2.0 cde**
8	V-10135 20 WDG	1.25 lbs.	0.0 b	3.0 bcd	**3.0 bcde**
9	V-10135 20 WDG	2.5 lbs.	0.4 ab	4.4 bcd	**4.8 bcde**
10	V-10116 50 WDG	3.5 oz.	0.0 0b	7.2 ab	**7.2 ab**
11	V-10116 50 WDG	4.0 oz.	0.0 b	4.6 bcd	**4.6 bcde**
12	Unsprayed		1.0 a	9.6 a	**10.6 a**

1 Potted pistachio trees (cv. Kerman) were placed in a greenhouse at the Kearney Agricultural Center and inoculated with *Botrytis cinerea* after fungicide applications had dried thoroughly. The canopy of the trees was misted with patio foggers to encourage disease development.

2 Numbers followed by different letters are significantly different according to the LSD test at P = 0.05.

Table 7. Resistance of *Botrytis cinerea* isolates to thiophanate-methyl.

Orchard	County	Year collected[1]	Tested isolates[2]	Resistant[3] (%)
1.	Fresno	1992	2	50
2.	Fresno	2005	6	67
3.	Fresno	2005	5	60
4.	Fresno	2005	5	60
5.	Fresno	2005	2	50
6.	Madera	2005	23	65
7.	Madera	2005	12	58
8.	Madera	2005	11	45
9.	Kern	2005	2	50
10.	Kern	2005	25	100

1 Isolates from 2005 crop year were collected from blighted shoots which developed after the wet spring weather.

2 Isolates were grown on acidified potato dextrose agar (PDA) and then 5-mm plugs were transferred to three replicate plates of PDA amended with 10 ppm thiophanate-methyl, and three replicate plates of PDA (control) and the growth was measured after 3 days.

3 Isolates were considered resistant if growth on fungicide-amended media exceeded 88% of the fungicide free media, and were considered sensitive if growth was less than 6% compared to growth on the fungicide free media.

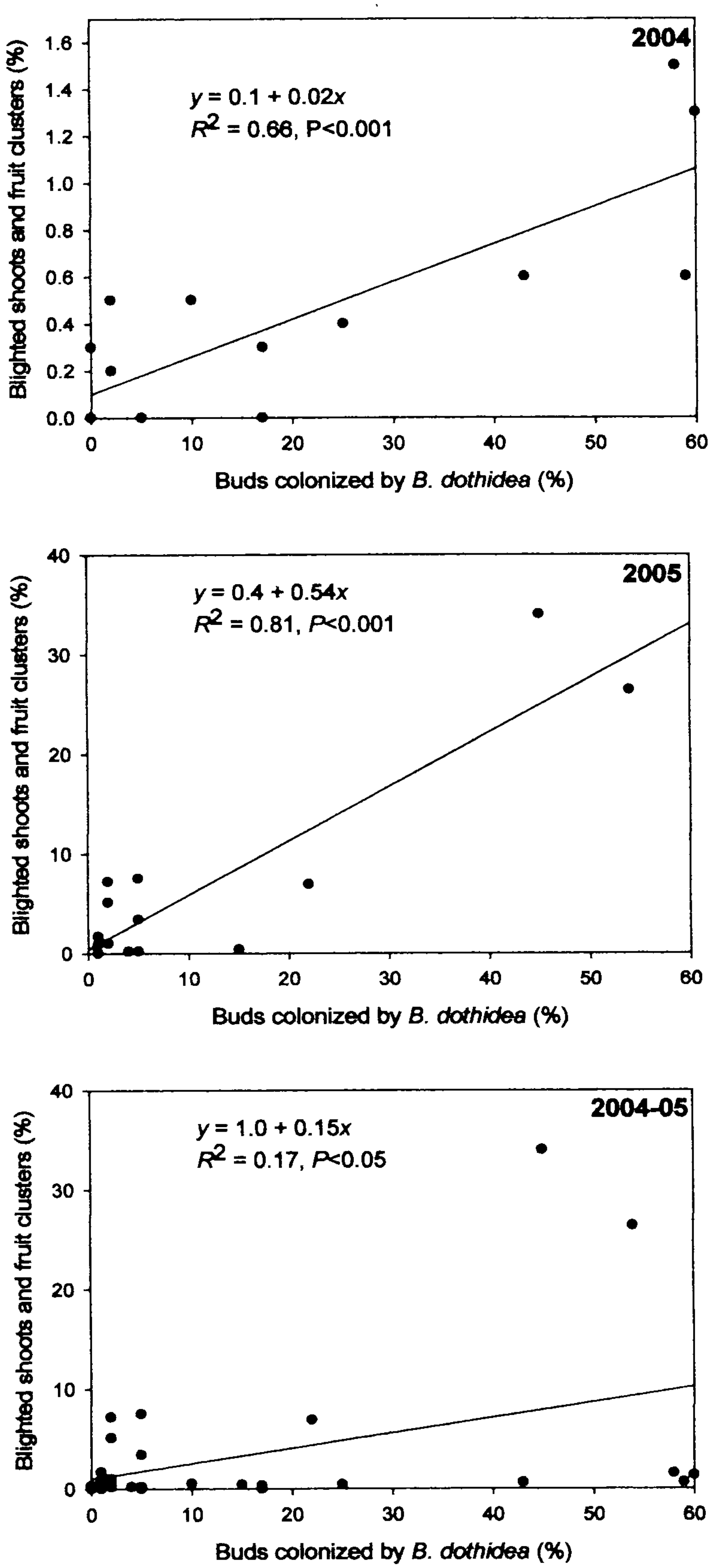

Figure 1. Relationship of incidence of infected pistachio buds (BUDMON) by *Botryosphaeria dothidea* collected in early to mid-March with incidence of panicle and shoot blight disease in orchards before harvest.

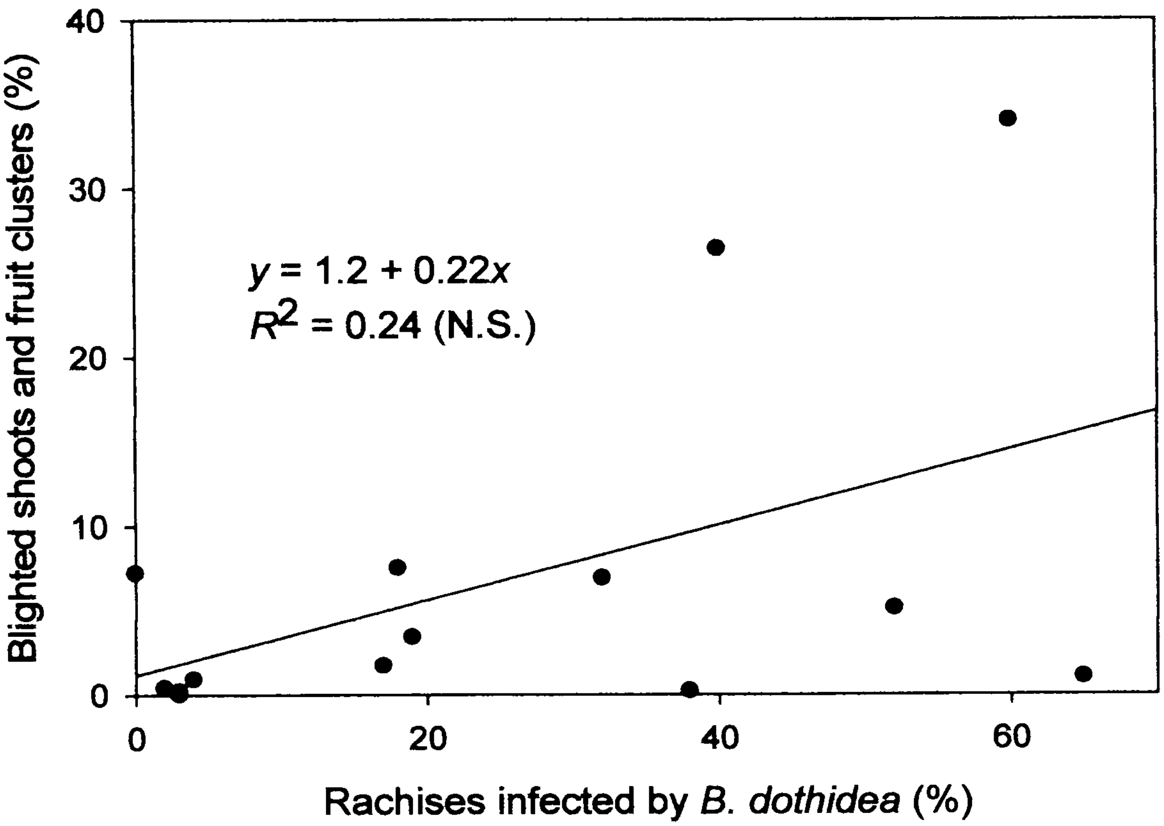

Figure 2. Relationship of rachises infected by *Botryosphaeria dothidea,* which were collected early to mid March 2005, with incidence of panicle and shoot blight disease in orchards before harvest.

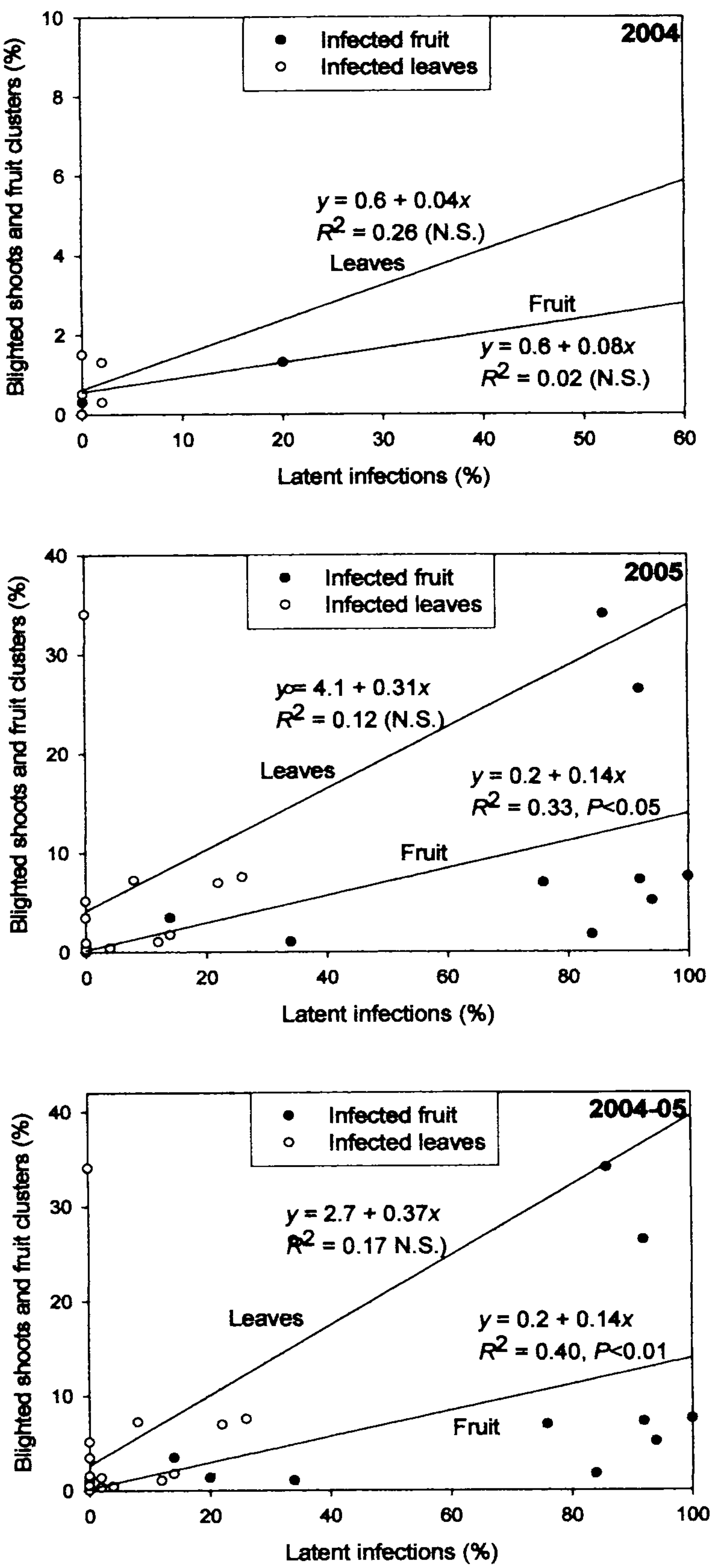

Figure 3. Relationship of latent infections of fruit and leaves (ONFIT) by *B. dothidea* collected on May 24 with incidence of panicle and shoot blight disease in orchards before harvest.

Appendix 1. Weather data in metric units from CIMIS station #61 near Glenn County pistachio fungicide trials (because the rain data for CIMIS #61 was lost due to a malfunctioning sensor, rain data for CIMIS #12 near Durham was substituted).

[redacted]					[redacted]				
4/1/2005	20.4	6	13.1	0	5/13/2005	27.8	12.1	20.1	0
4/2/2005	20.9	4.9	13.8	0	5/14/2005	27.9	13.7	21.8	0
4/3/2005	13.3	6.4	9.4	10.4	5/15/2005	21.3	16.4	18.8	0.6
4/4/2005	16.9	5.7	10.2	0.1	5/16/2005	23.2	9.3	17.1	0.7
4/5/2005	21.9	5.4	13	0	**5/17/2005**	**14.9**	**8.6**	**12.1**	**4.5**
4/6/2005	22.7	5.2	14.6	0	**5/18/2005**	**18.6**	**10.9**	**14.1**	**14.6**
4/7/2005	16.8	4.9	11.7	7.2	5/19/2005	21.6	11	16.5	0
4/8/2005	15	6.6	9	10.1	5/20/2005	23.4	10.8	16.7	0
4/9/2005	19	5.6	12.5	0	5/21/2005	31	11.6	19.8	0
4/10/2005	22.4	7.6	14.8	0	5/22/2005	31.5	15	22.5	0
4/11/2005	20.7	6.4	13.8	0	5/23/2005	29.5	13.1	21.2	0
4/12/2005	18.3	3.3	11.3	0	5/24/2005	31.3	13.9	22.9	0
4/13/2005	15.4	1.9	9	0.3	5/25/2005	34.7	12.5	24.4	0
4/14/2005	18.4	3.1	11.3	0	5/26/2005	32.3	14.7	24.6	0
4/15/2005	25.1	7.3	15.5	0	5/27/2005	31.3	15.2	24.3	0
4/16/2005	25.4	5.3	16.2	0	5/28/2005	24.9	13.4	19.2	0
4/17/2005	21.2	8.8	14.8	0	5/29/2005	23.2	9	16	0
4/18/2005	19.5	10.3	14.2	0	5/30/2005	30.1	10.9	20.8	0
4/19/2005	21	10.6	15.2	0	5/31/2005	30.9	13.9	23.6	0
4/20/2005	21.9	6.3	14.5	0	6/1/2005	28.8	18.5	23.2	0
4/21/2005	24.8	6.3	15.6	0	6/2/2005	28.6	17.7	23	0
4/22/2005	26.4	5.4	16.2	0.8	6/3/2005	31.7	14.9	23.1	0
4/23/2005	16.3	8.7	12.4	1.1	6/4/2005	30	11.3	22.1	0
4/24/2005	18.9	9.6	13.5	3.9	6/5/2005	25.4	11.5	18.8	0
4/25/2005	24.3	9.5	15.4	0	6/6/2005	22.2	7.9	15.3	0
4/26/2005	23.4	8.2	15.7	0	6/7/2005	23	5.5	15.9	0
4/27/2005	22.5	9.9	15.3	3.2	**6/8/2005**	**16**	**11.7**	**14.4**	**17.6**
4/28/2005	21.2	10.7	15.1	0.2	6/9/2005	24.8	14.3	18.6	0.6
4/29/2005	23.2	9.5	16.4	0	6/10/2005	29.3	13.9	22.1	14
4/30/2005	22.7	9.8	16.1	0	6/11/2005	31.4	15.1	22.9	0.1
5/1/2005	24.5	9.4	17.5	0	6/12/2005	31.8	17.6	24.1	0
5/2/2005	26.4	8.5	17.7	0	6/13/2005	34.9	17.4	26.4	0
5/3/2005	25.4	9.5	18.7	0	6/14/2005	32.4	13.8	24.6	7
5/4/2005[1]	**18.3**	**13.3**	**15.7**	**8.4**[1]	**6/15/2005**[2]	**30.7**	**14.6**	**23.4**	**3.3**[2]
5/5/2005	**20.6**	**11.6**	**15.8**	**4.2**	**6/16/2005**	**18**	**11.2**	**13.7**	**13.8**
5/6/2005	20.8	10.9	15.1	0	6/17/2005	20.8	10.2	15.7	0.1
5/7/2005	21	11.9	16.3	0	6/18/2005	21.1	11	16.4	1.1
5/8/2005	**14.9**	**11.8**	**13.2**	**24.3**	6/19/2005	25.2	9	17.8	0
5/9/2005	**18.9**	**7.8**	**13.6**	**8.4**	6/20/2005	28.4	11.3	20.2	0
5/10/2005	20.3	5.1	12.8	0	6/21/2005	28.4	11.6	20.2	0
5/11/2005	25.4	8.1	16	0	6/22/2005	29.6	11.5	20.8	0
5/12/2005	26.5	9.4	17.9	0	6/23/2005	31.3	12.1	22.5	0

Appendix 1 (cont.). Weather data in metric units from CIMIS station #61 near Glenn County pistachio fungicide trials (because the rain data for CIMIS #61 was lost due to a malfunctioning sensor, rain data for CIMIS #12 near Durham was substituted).

[redacted]					[redacted]				
6/24/2005	29.1	13	21.3	0	8/6/2005	36.6	16.5	27.3	0
6/25/2005	29.1	14.1	21.5	0	8/7/2005	35.7	16.3	27	0
6/26/2005	28.3	12.9	21	0	8/8/2005	34.1	17.6	26.6	0
6/27/2005	27.2	13.3	20.1	0.1	8/9/2005	35	16.5	26.4	0
6/28/2005	29.7	13.9	21.7	0	8/10/2005	35.1	15.3	26	0
6/29/2005	37.9	16.5	26.9	0	8/11/2005	36	16.8	26.3	0
6/30/2005	35.8	16.7	27.2	0	8/12/2005	34.5	16.6	26.4	0
7/1/2005	35.3	18.6	27.7	0	8/13/2005	30.2	17.3	23.8	0
7/2/2005	34.7	18.7	27.7	0	8/14/2005	31	13.8	22.7	0
7/3/2005	33.7	17.2	26	0	8/15/2005	28.1	15.3	21.6	0
7/4/2005	35.9	16.6	27.1	0	8/16/2005	32.1	16.4	24.7	0
7/5/2005	34.1	18.9	26.8	0	8/17/2005	32.9	16.4	24.8	0
7/6/2005	34.8	16.9	26.7	0	8/18/2005	28.9	12.8	21.6	0
7/7/2005	31.7	18.1	25.3	0	8/19/2005	29.6	12	21.3	0
7/8/2005	29.6	14.3	22.6	0	8/20/2005	31.9	12.9	22.8	0
7/9/2005	28.4	16.4	21.6	0	8/21/2005	33.3	13.6	23.8	0
7/10/2005	30	15	22.4	0	8/22/2005	35.3	14.2	25.2	0
7/11/2005	35.1	16.8	26.5	0	8/23/2005	34.6	15.8	25.7	0
7/12/2005	35.8	18.4	28	0	8/24/2005	31.3	12	22.1	0
7/13/2005	37.3	21.5	29.4	0	8/25/2005	31.6	--	25.7	0
7/14/2005	38	20.3	29.6	0	8/26/2005	34.1	12.6	23.5	0
7/15/2005	38.7	22.5	30.3	0	8/27/2005	34.8	12.8	24.5	0
7/16/2005	**37.9**	**20.8**	**29.7**	**18.2**	8/28/2005	35	15.8	25.2	0
7/17/2005	40.2	20.8	30.7	0	8/29/2005	32.4	11.6	23.6	0
7/18/2005	36.3	20.8	29.6	1.1	8/30/2005	34	20.6	26.6	0
7/19/2005	34.5	19.4	27.5	0	8/31/2005	40	16.2	27.8	0
7/20/2005	37.3	18.6	28.1	0	9/1/2005	33	12.7	23.5	0
7/21/2005	32.3	18.6	25.1	0	9/2/2005	31.3	13.6	22.4	0
7/22/2005	31.7	13	22.6	0	9/3/2005	30.1	12.1	21.1	0
7/23/2005	36.8	15.6	27.6	0	9/4/2005	27.7	10.8	20.1	0
7/24/2005	36.3	18	28.4	0	9/5/2005	30.4	12.3	21.5	0
7/25/2005	36.2	16.5	27.1	0	9/6/2005	30.2	12.8	21.6	0
7/26/2005	38.3	17.1	28.1	0	9/7/2005	30.3	10.9	20.5	0
7/27/2005	35.5	17.1	27	0	9/8/2005	27.5	11.1	19.3	0
7/28/2005	34.6	15.2	26.4	0	9/9/2005	26.7	12.3	18.5	0
7/29/2005	34.7	17	26.5	0	9/10/2005	25	9.2	17.4	0
7/30/2005	35.1	18	27.1	0	9/11/2005	25	9.4	17.1	0
7/31/2005	35.5	19.3	27.1	0	9/12/2005	26.1	7.3	16.8	0
8/1/2005	35	17	26.2	0	9/13/2005	26.8	8.7	17.5	0
8/2/2005	33.1	16.2	24.8	0	9/14/2005	26.1	9.1	17.2	0
8/3/2005	35.6	14.7	25.6	0	9/15/2005	27.7	9.6	18.3	0
8/4/2005	35.3	16.2	26	0	9/16/2005	24.7	9.7	17.9	0

Appendix 1 (cont.). Weather data in metric units from CIMIS station #61 near Glenn County pistachio fungicide trials (because the rain data for CIMIS #61 was lost due to a malfunctioning sensor, rain data for CIMIS #12 near Durham was substituted).

[redacted]				
9/17/2005	26.3	7.9	17.1	0
9/18/2005	29.8	7.8	18.2	0
9/19/2005	33.7	9.1	19.7	0
9/20/2005	31.7	14.5	23.3	0
9/21/2005	30	14.1	21	0
9/22/2005	29.7	11.9	20.3	0
9/23/2005	26.1	9.1	17.7	0
9/24/2005	26.2	15	20.4	0
9/25/2005	28.7	12.1	20.6	0
9/26/2005	28.8	12.3	20.2	0
9/27/2005	30.9	12.8	20.3	0
9/28/2005	35	13.9	23.9	0
9/29/2005	34.3	10.7	20.5	0
9/30/2005	31.8	9.1	20.8	0
10/1/2005	26.6	10.9	19.2	0
10/2/2005	23.3	8.7	15.8	0
10/3/2005	21.4	5.6	14.2	0
10/4/2005	23.3	7.9	16	0

[1] Entries in bold denote weather conditions especially conducive (**at least 4 hours with >1mm rain and temp above 11° C (52° F)**) to promote potential infection events by *Botryosphaeria dothidea*. Entries in bold and underlined denote unusually strong potential infection events with **rain accompanied by unusually warm temperatures** near the optimal temperature for maximum growth (and infection) rate of *B. dothidea*.