Chemical and Cultural Control of Band Canker of Almond Caused by *Botryosphaeria dothidea*

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Objectives:

Etiology, ecology, and epidemiology:

1) Compare isolates of *B. dothidea* collected from tree trunks and upper canopy.
2) Determine sources of inoculum of *B. dothidea* from almond and survey more orchards in search of the sexual airborne stage of the pathogen.

Disease Management:

3) Compare various fungicide treatments by injecting them in trees in (a) a lath house, and (b) in the field.
4) Compare methods of irrigation in controlling band canker of almond.

Introduction. Band canker was reported years ago as a problem in California almonds. Although this is a rare disease affecting the trunks and scaffolds of young almond trees, in the last few years the incidence of the disease has increased in several commercial young orchards in Butte, Glenn, Stanislaus, and Kern counties. The occurrence of the disease is unpredictable; however, its frequency can increase in years with high rainfall, such as those in 2005. When the cankers are large and cover large area of the trunk circumference, tree death occurs. An example of the damage this disease can cause is depicted in a Kern Co. orchard where 1700 trees were removed in 2002 and 2003. Additionally, growers who have this problem in their orchards, year after year until the trees become 7 years and older, will have to replant killed trees due to band canker.

The disease occurs in vigorous Nonpareil, Carmel, and Padre trees and less frequently on other almond cultivars, 4 to 6 years old. Infections probably occur in the spring, and the source of spore inoculum has been unknown up until now. Infections seem to be active only during the growing season in which they first appear. Fall infections may be possible too, since inoculum is present in infected trees in almond orchards throughout the year. The epidemiology of band canker has not been described. This study was initiated to help understand the ecology and epidemiology (sources of inoculum, time and mode of infection, conditions affecting development of the disease) and develop management approaches of band canker in almonds.

Cankers with excessive gumming become obvious in early summer and fall. Unlike other cankers, their long dimension is horizontal, or perpendicular to the long axis of the branch or trunk. Usually the cankers arise from small growth cracks of vigorously growing young trees and elicit abundant gum formation. In very narrow cankers, the cambium survives, and new phloem replaces the outer necrotic tissue. If the infection extends to the wood, the branch above the dead area also dies. Tree death usually occurs when the cankers enlarge or when trees have double or sometimes triple bands of infection. Discolored sapwood often extends longitudinally several centimeters beyond the canker margin. Older literature reports that infections are usually active only during the growing season in which they first appear, but our research suggests that cankers can be perennial since we noticed new active gumming and expansion of some of the cankers for at least two seasons later.

Etiology, ecology, and epidemiology of band canker

Causal organism. Band canker is caused by *Botryosphaeria dothidea* which is a cosmopolitan fungal pathogen. Initially only the asexual stage, a *Fusicoccum* species of the fungus was reported on almond. However, our study showed that both the sexual, *Botryosphaeria dothidea*, and the asexual, *Fusicoccum* sp., are found in the almond bark and riparian plants growing next to almonds orchards.

1. Comparison of isolates of *B. dothidea* collected from tree trunks and upper canopy. In 2004 in an initial test, using a small sample of isolates of *B. dothidea* from almond and pistachio and using the polymerase chain reaction (PCR) primers M13 and T_3B, we found that the almond isolates are different from those of pistachio. Pistachio isolates of *B. dothidea* are very uniform genetically and very similar to isolates collected from hosts such as pecan, walnut, willow, eucalyptus, and blackberry. However, the isolates of *B. dothidea* from almond showed more genetic variability than those of pistachio. This implies that the *B. dothidea* causing band canker is not the same as the one causing panicle and shoot blight of pistachio and it is a more genetically heterogeneous species. In general, pathogens with greater genetic variability may need more aggressive control methods than genetically uniform pathogens.

In 2005, to compare isolates of *B. dothidea* from almond and other hosts, from cankers on trunks and the upper canopy of almond trees, and pycnidial and pseudothecial (see objective #2) isolates, we collected more isolates from various orchards in Butte, Colusa, Glenn, and Kern Counties, and from almond, walnut, blackberry (growing next to almonds), and pistachio. In addition, eight isolates of *B. dothidea* causing problems in almonds in Australia were used as an out-group of isolates for comparative reasons (Table 1). Isolates included single spore pycnidiospores while some were from single ascospores, since pseudothecia with mature ascospores were discovered in some of the sampled almond orchards, in walnuts, and in blackberries grown next to almond orchards in Butte County.

Using four microsatellite primers, 98 isolates of *B. dothidea* from almond trunks and upper canopy and from plants growing next to almonds were fingerprinted. An example of the DNA fingerprints for some isolates from almond, walnut, and pistachio is given in Figure 1. Results of the DNA fingerprints of these isolates indicated that (1) The *B. dothidea* population from almond showed much higher diversity than that of walnut and pistachio in California, and these results

agreed with the initial results of 2004. (2) Some isolates from twigs of the upper canopy and the trunk of almonds, and isolates from pycnidiospores and ascospores from walnut growing next to almonds in Colusa County had identical DNA fingerprints. (3) Some isolates from almonds from Colusa and Butte Counties had identical DNA fingerprints, while all the isolates of *B. dothidea* from Kern County formed a separate group, which suggests that perhaps the strain in Kern Co. is different than the strain occurring in northern Sacramento Valley. And (4) some isolates from pycnidiospores (asexual) and ascospores (sexual) from almond or walnut had identical DNA fingerprints, suggesting that these hosts when planted next to each other can contribute spore inoculum that can infect each other (see also sources of inoculum and spread below).

2. Sources of spore inoculum of *B. dothidea* in almond and other hosts.

A) Sources of inoculum of *B. dothidea*. Isolates of *B. dothidea* were collected from four almond orchards in Butte County, one each in Glenn and Colusa Counties, four orchards in Stanislaus County, and three orchards each in Kern and Madera Counties. Isolations and microscopic observations were made and revealed that some of the orchards bore both the *Fusicoccum* and *B. dothidea* stages of the fungus (Table 2):

Significance of the findings: The occurrence of pseudothecia in almond is very important in the epidemiology of this disease because they produce airborne ascospores that do not need water to move around and can travel through the air perhaps over long distances. The presence of pseudothecia explains why the pathogen was isolated from trees that did not show any disease symptoms as reported in 2004 (Michailides et al. 2004 Annual Report) and why cankers on the upper part of the tree canopy were present and caused infections in some of the surveyed orchards. Also, because pseudothecia represent the sexual stage of the pathogen, it explains the greater genetic variability we observed among the isolates of *B. dothidea* from almond than the isolates from pistachio (where pseudothecia have not been observed).

To determine other possible sources of inoculum, we also collected blighted shoots of other kinds of trees, growing close to almonds. Again in 2005, isolates of *Fusicoccum* sp. were collected from many of the plants growing next to almonds and pseudothecia of *B. dothidea* were found in blackberries bushes next to an almond orchard in Butte County and in walnuts grown next to almonds in an orchard each in Colusa and Stanislaus Counties. Therefore, other hosts can also contribute inoculum to infect almonds. For instance, we observed an increase in symptomatic Nonpareil almond trees in the rows closer to a walnut orchard, which is located on the east side of an almond orchard with severe band canker disease (Figure 2). Disease symptoms, including all but lightly infected trees, decreased from 74% in the row adjacent to the walnuts to 33%, 11 rows away from the walnuts, suggesting that more spore inoculum was available for infection of almond trees next to walnuts than those trees away from the walnut trees. Because the walnut orchard was much older than the almond, it is most likely that inoculum of *B. dothidea* came from the walnuts and not vice versa.

B) Infection courts. As in 2004 and 2005, we also observed cankers associated with pruning wounds and peduncles in the upper canopy of the trees in an orchard in Colusa County. In addition, we collected samples of many blighted twigs as well as samples of discolored bark where the infections initiated from the pruning wounds. Isolations were made to determine the

incidence of *B. dothidea.* All these samples were collected from the same orchard in Colusa County where there is a walnut orchard next to it. Both the *Fusicoccum* and the *B. dothidea* stages of the pathogen were found on fallen walnut shoots, but only the *Fusicoccum* stage in the almonds infected by the disease in this orchard. In contrast to pistachio shoots, almond shoots seem not to be a good substrate for the reproduction of the band canker pathogen. However, the rough bark of the trunk of certain almond cultivars can support large quantities of pycnidia and pseudothecia of the pathogen. In addition, stumps of cut trees in orchards were found to carry these two types of spores, suggesting that they can serve as sources of both water-splashed (pycnidiospores) and air –dispersed (ascospores) inoculum for the disease. Therefore, stumps of cut trees should be removed out of the orchard and destroyed when band-cankered trees are cut down.

Results of isolations showed that peduncles were colonized by *B. dothidea* and the pathogen was isolated from 51 to 83% of the cankers associated with peduncles. The high frequency of isolation (38-50%) of the pathogen from blighted twigs, suggests that the pathogen reaches the upper canopy and causes some shoot blight, killing fruiting wood. *B. dothidea* was also isolated from 40% of the cankers associated with pruning wounds, suggesting that the pathogen can enter through pruning wounds. Pruning wounds with typical cankers were also observed in an orchard in Butte County.

The high frequency of *Fusicoccum* recovery from plated plant tissues suggests that there must be several ways of infection of almond by the band canker pathogen. Our 2004-2005 results show that infections can occur through (1) growth cracks in the trunk, (2) pruning wounds, (3) lenticels, (4) rough bark and cracks at the base of shoots and or suckers, and (5) fruit peduncle. All these modes of infection of the band canker pathogen explain the occurrence of aerial cankers caused by *B. dothidea* that we have been finding more and more in almond orchards in the last few years. Presence of aerial spore inoculum and the excessive rains in 2005 may also explain why we have found these infections, suggesting that under certain conditions (such as excessive rain in spring) the band canker pathogen has the ability to infect almond trees in several ways.

C) Time of infection. The time of infection study is under progress, using greenhouse and lath house experiments; five potted trees have been inoculated periodically and the incidence of infection will be reported after the completion of the experiment.

Disease management

3. Compare various fungicide and biological treatments by injecting trees.
Experiment 1. This experiment involved various chemical and two biological treatments as shown in Table 3 and was performed in a row of Nonpareil trees in an orchard with band canker in Colusa Co. Ten trees were used for each treatment. Approximately 10 ml of each compound was injected in each of four holes per tree using a Sidewinder® Tree Injector. Canker size (% of trunk circumference) was measured just before injection on May 12, 2004, and evaluations of the treatment effects were recorded on July 1, August 23, and October 21, 2004, and again August 25, 2005 (Table 3). On all the recording dates we found no effect of any of the treatments in suppressing canker expansion. However, there was a significant effect of all the treatments in

reducing the defoliation in comparison with the control and among the treatments propiconazole (Break®) was the most effective. As an average, it seems that treatments stopped the growth of the cankers, which resulted in three times less defoliation than the untreated control. No defoliation was observed in any of the untreated trees without symptoms (on May 12, 2004), although some of these trees developed new but small band cankers on their trunks by Aug. 2005 (Table 3).

Experiment 2. In the same orchard in Arbuckle, an additional experiment was set June 17, 2004. Only cankers with active gumming were used in this experiment. After identifying the canker and its perimeter, the site was sprayed with each compound (as shown in Table 3) to run off. Then four layers of cheesecloth pieces about 6"× 9" were folded in thirds, soaked with about 60 ml of each compound, and placed on top of the canker. A bead of silicone seal was then placed around the canker and a 4-mil piece of plastic was placed over the silicon to seal the treated site. The corners of the plastic were stapled to the tree to secure the plastic and create a humid environment. The treatment using soil, involved mixing about 200 cc of soil under the tree canopy with water to field capacity and plastering it over the canker. The effects of the treatments were evaluated on 25 August 2005. Although nothing dramatic was observed during these evaluations, we decided to wait 2 more months to do a final evaluation before terminating this experiment. The preliminary evaluations of all the trees in this experiment also show once again that although these treatments may not cure the band cankers; they might stop the expansion of the cankers resulting in beneficial end results (in general, reduce defoliation and improve tree health).

Experiment 3. The biological control treatments were also performed in another orchard with band canker on Carmel trees located in Kern County. We only used cankers with active gumming in this experiment also. After identifying the canker and its perimeter, the site was sprayed with each compound (Table 4) to run off. The treated cankers were covered and sealed as in experiment #2. Evaluations of the treatments were done on September 21, 2004, and again on August 29, 2005. There were no significant effects of either of the biological treatments on the size of the cankers, the number of gum secretions, and the incidence of tree defoliation. The treatments will be monitored for a few more months.

Experiment 4. Compare methods of irrigation in controlling band canker of almond.
Irrigation manipulation experiment. An orchard with severe band canker on Padre trees was selected for this experiment in Butte County. The orchard was irrigated with high angle sprinklers which wetted the trunks of almost all of the trees. To determine the effect of reducing wetting the tree trunks, on 10 June 2004 the grower installed special metallic splitters, which he designed and had manufactured, that were attached in each sprinkler so that the water was diverted from the trunk of the majority of Padre trees. Splitters were not attached to sprinklers in two areas of 5 and 10 rows located in the middle and the south side of the orchard. All trees in this experiment were evaluated on July 1, 2004 (beginning of the experiment), on August 3 and October 21, 2004 and again on August 25, 2005. Results from the initial, and the October 21, 2004, and August 25, 2005 recordings are reported in Figure 3A and 3B.

As expected, in the initial recording on July 1, 2004, there were no significant differences between the two treatments since the installation of the splitters was done on June 10 and the

first irrigation after the installation on June 17-20, 2004. No effect was expected by this date. However, by the end of the 2004 growing season, on October 21, 2004 there was a significant reduction on the incidence of trees with cankers in comparison with the trees irrigated with sprinklers without splitters (Figure 3A). This significant effect was also measured in the number of gum secretions on the trunk and the incidence of trees with gum secretions, which are indications that the activity of some of the cankers had stopped. The effects of irrigating with sprinklers bearing splitters were more pronounced in 2005 evaluation. Both the incidence of trees with cankers and the size of active cankers have been reduced significantly in comparison with those on trees irrigated using sprinklers without splitters (Figure 3B). Thus reducing wetting of the tree trunks not only prevented the development of new cankers, it also slowed down the growth of existing band cankers with a result of better recovery of the infected trees. This is the first report that manipulation of irrigation was effective in reducing band canker in this orchard and in general trees looked healthier in the orchard in 2005 than in 2004.

Experiment 5. Effects of fungicides mixed with latex paint on band canker symptoms or infections by *B. dothidea*. On November 1, 2005, we painted the trunks of Nonpareil almond trees in an orchard in Butte County. Before applying the paint, the Nonpareil trees were mapped and rated for symptom expression of band canker. Categories included normal trees (healthy), lightly infected (one to six small spots with gum), moderately infected (5 to 10 larger spots with gum), severely infected (large cankers with raised and roughened bark at borders), dead, and replanted. Trees selected for treatment were either free of band canker symptoms, or were lightly infected and showing one to several areas of gumming per tree. Glidden Interior flat latex paint (part number HM 1211 Base 1) was diluted 1:1 with water, and fungicides were incorporated into the paint as described in Table 5. The fungicide/paint mixture was applied at the rate of 10 gallons per acre using hand held compressed air sprayers. Paint was applied to the trunks from the ground up to the scaffolds. The effects of the treatments will be recorded in September 2006.

Experiment 6. Determine the efficacy of bloom time (petal fall) canopy sprays on the control of Botryosphaeria infections. We sprayed the canopies of Nonpareil almonds in Colusa County at petal fall on March 1, 2006. There were 7 replications consisting single-trees per fungicide treatment. Fungicides were applied at the rates shown in Table 6. The effects of treatments will be evaluated in mid summer to early fall 2006.

Conclusions:
Significant progress has been made in understanding the biology, epidemiology, spread, and management of the pathogen causing band canker of almond. A summary of the new findings in the first two years of this project are described below:

1. The pathogen *Botryosphaeria dothidea* was confirmed from several commercial orchards in Butte, Glenn, Colusa, San Joaquin, Stanislaus, and Kern Counties.
2. Both the water-splashed asexual (pycnidia of a *Fusicoccum* sp.) and the airborne sexual (pseudothecia of *B. dothidea*) stages of the pathogen have been discovered in almond.
3. Also both the *Fusicoccum* and the *B. dothidea* stages have been discovered in walnuts and blackberries grown next to almonds with band canker.
4. The pathogen causing band canker seems to be different from and show greater genetic

diversity than the pathogen causing Botryosphaeria panicle and shoot blight of pistachio, although pathogenicity studies showed that almond isolates can infect pistachio and vice versa.

5. The presence of the sexual stage of *B. dothidea* in almonds and plants grown in proximity to almonds can explain why this pathogen shows greater genetic diversity and why it has been isolated even from trees without symptoms and from the tree canopy.
6. In lath-house/greenhouse inoculation experiments, *B. dothidea* from willow and blackberry collected next to almonds infected almond trees, caused large cankers and killed some of these trees.
7. Prunings shredded and left on the orchard floor may provide spore inoculum sources of the pathogen, but almond shoots do not seem to be a good substrate for producing spores of the pathogen.
8. Earwigs did not vector the band canker pathogen.
9. In addition to growth cracks in the trunk of younger trees, *B. dothidea* can also infect cracks on branches, pruning wounds, lenticels, peduncles, and to some extend the rough base of smaller shoots and suckers.
10. Greenhouse experiments showed that propiconazole, azoxystrobin, and iprodione prevented canker formation and stalled band canker development in a field experiment.
11. Manipulation of irrigation to reduce wetting of tree trunk resulted in less canker activity (smaller cankers) and significantly lower incidence of active canker development (protected trees from new canker development).

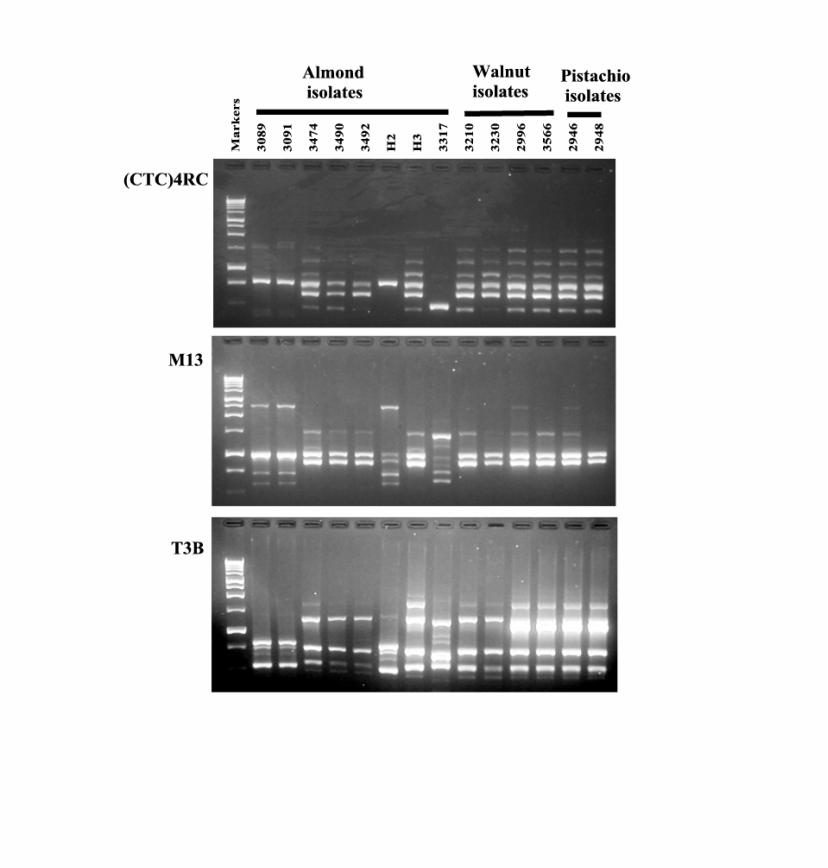

Figure 1. An example of DNA fingerprints of various isolates of *Botryosphaeria dothidea* collected from almond, walnut, and pistachio.

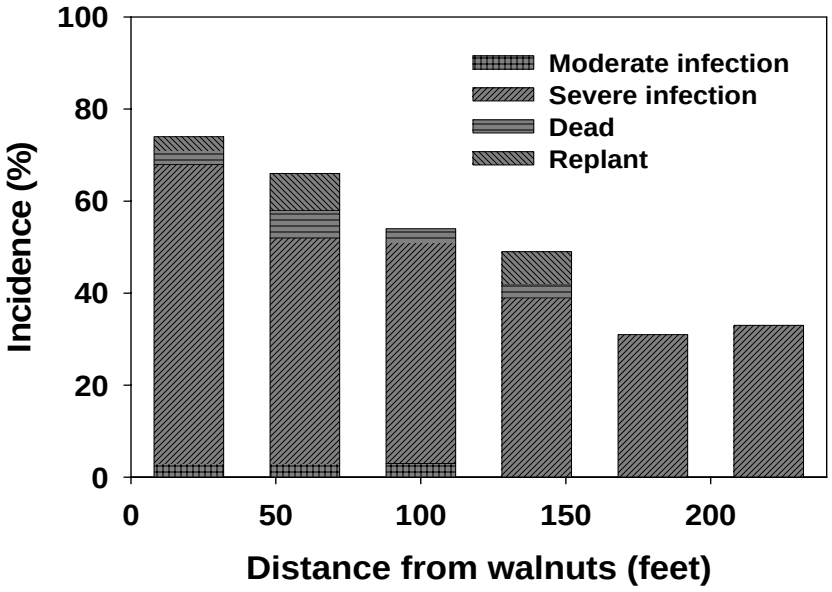

Figure 2. Incidence of band canker symptoms in Nonpareil almond trees in a Butte County almond orchard (cv. Nonpareil) located adjacent to a walnut orchard with branches infected with *Botryosphaeria dothidea.*

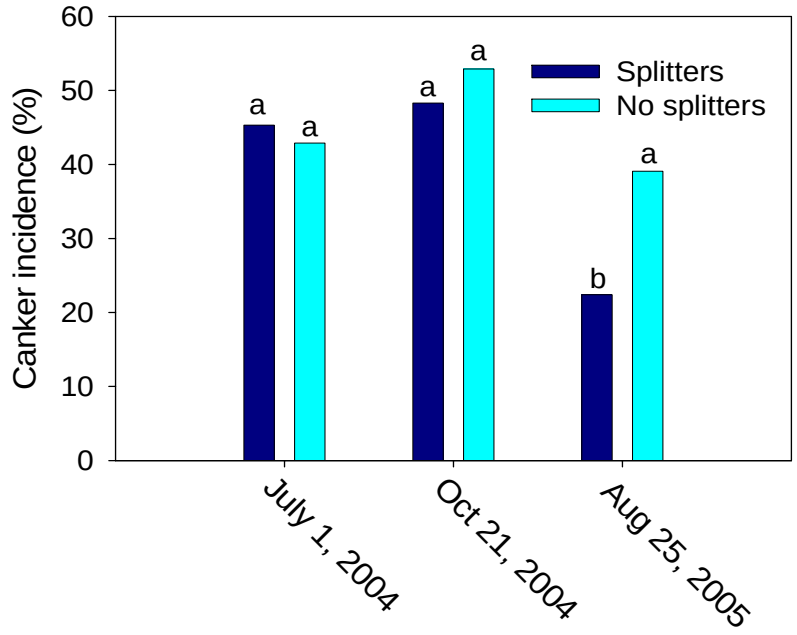

Figure 3A. Effect of installing splitters in the sprinklers on band canker incidence in an almond orchard in Butte County.

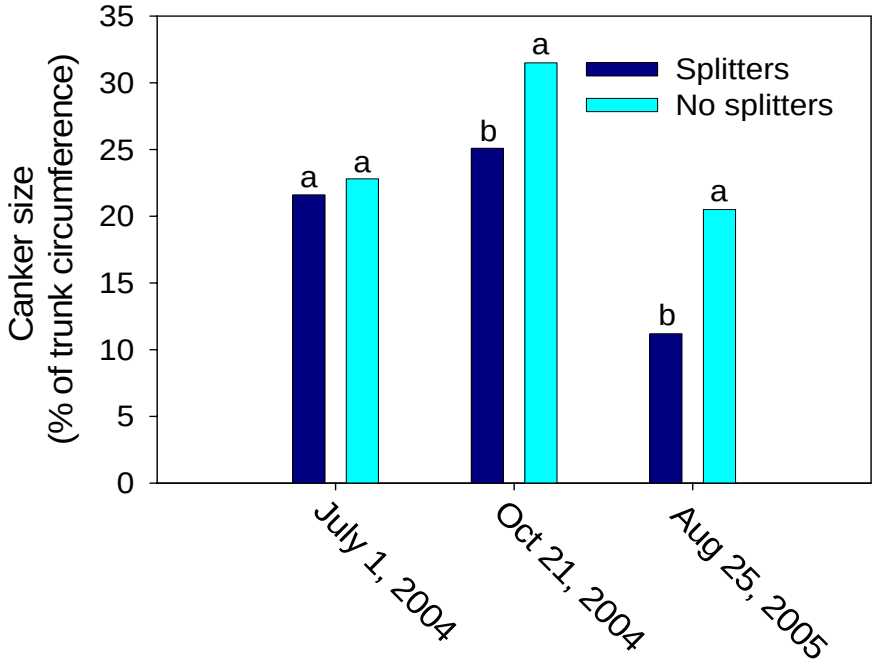

Figure 3B. Effect of installing splitters in the sprinklers on band canker size in an almond orchard in Butte County.

Table 1. Summary of isolates of *Botryosphaeria dothidea* used in the molecular study to compare isolates from almonds obtained from band cankers on the trunk and cankers on the upper canopy of trees and isolates from other hosts.

Location	Host	Isolate type	No. of isolates
Butte, orchard 1	Almond	Ascosporic and Pycnidiosporic	>30
	Walnut	Pycnidiosporic	3
	Blackberry	Pycnidiosporic	2
Colusa, orchard 2	Almond	Pycnidiosporic from band cankers and upper canopy canker	12
	Walnut	Ascosporic and Pycnidiosporic	>30
Stanislaus, orchard 3	Almond	Pycnidiosporic	>5
	Walnut	Pycnidiosporic and ascosporic	8
Kern, orchard 4	Almond	Pycnidiosporic	>30 (2002-04)
KAC	Walnut	Pycnidiosporic	14
	Pistachio	Pycnidiosporic	6
Australia	Almond	Pycnidiosporic	8

Table 2. Occurrence of pycnidia and pseudothecia of *Botryosphaeria dothidea* in the **bark** collected from symptomatic almond trees in various counties at different dates.

Date of sample collection	County	Number of orchards	Pycnidia (pycnidio-spores)	Pseudothecia (Ascospores)	Viability of spores[1]
27 April 2004	Butte	3	+, +, +[2]	+, +, +[2]	+, +, +[2]
26 July 2004	Kern	2	+, +[2]	-, -[2]	+, +[2]
23 August 2004	Butte	1	+	+	+
21 September 2004	Stanislaus	1	+	-	+
8 February 2005	Butte	1	+	+	+
8 February 2005	Colusa	1	+	-	+
3 March 2005	Colusa	1	+	-	+
25 August 2005	Colusa	1	+	-	+
25 August 2005	Butte	1	+	+	+
11 October 2005	Butte	1	+	+	+

[1] Viability was checked by culturing each isolate on potato-dextrose agar using either type of spores.
[2] For each orchard, respectively.

Table 3. Size of band cankers in a Nonpareil almond orchard in Colusa County before (5 May 2004) and 15 months after injection (25 Aug 2005) and effects on tree defoliation.

		Canker size (% of circumference)[1]		
Treatment	**Rate**	**Before**	**15 months later**	**Defoliation (% of canopy)**
Break®	10,000 ppm ai	65 a	48 a	2 b
Abound®	10,000 ppm ai	71 a	28 a	10 ab
Rovral®	10,000 ppm ai	69 a	48 a	12 ab
Trichoderma viride – 36E1	$5x10^7$/ml	68 a	35 a	10 ab
Trichoderma harzianum Plant Shield®	100 mg product /10 ml	66 a	55 a	12 ab
Mean for all the treatments		**68**	**43**	**9**
Untreated	---	58 a	40 a	30 a
Untreated without symptoms	---	0 b	22 b	0 b

[1] Cankers were evaluated based on signs indicating activity of canker such as, new gumming, fresh discoloration of the bark, etc.

Table 4. Biological control treatments for management of band canker on Carmel trees in an

orchard in Kern County (initiated on June 4, 2004 and recorded on August 29, 2005).

Treatment	Rate	Field rate	Canker size (% of circumference)	Number of gum secretions	Trees with defoliation (%)
Trichoderma viride – 36E1	$5x10^7$ cfu/ml	14 plates/600 ml water	34 a	25 a	12 a
Trichoderma harzianum (Plant Shield®)	100 mg product /10 ml	6 g/600 ml water	25 a	25 a	25 a
Untreated band canker-(control)	---	---	28 a	16 a	25 a

Table 5. Effect of fungicides, applied in white latex paint to the trunks of almond trees, on the control of Almond band canker in Nonpareil almonds located in Butte County (November 1, 2005)

Treatment	Rate per acre[1]
Abound	15.4 fl oz
Pristine	14.5 oz
Captan 4L	1.125 Gallon
Plant Shield	2.5 lb
Control with paint[2,3] only	---
Unpainted control	---

[1] We applied about 10 gallons per acre.
[2] The paint was diluted 1 part paint interior latex paint and 1 part water.
[3] We used Glidden Interior flat latex paint (part number HM 1211 Base 1)

Table 6. Fungicides and rates used in canopy sprays to control infections of *Botryosphaeria dothidea* (sprays applied on 1 March 2006).

Treatment (fungicide(s))	Rate per acre (100 Gallons per acre)
Echo 720	4 pints
Echo Ultimate	3.6 lbs
Topsin M 4.5	20 fl oz
Ziram	8 lbs
Topsin M 4.5	30 fl oz
Ziram	8 lbs
Pristine	14.5 oz
Control	Untreated