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

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Objectives:

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.
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.

Abstract

In the second year (2004/05) of this study, we made new discoveries that helped us understand the band canker disease a little better than before. 1) The disease was found frequently on several almond cultivars (Carmel and Padre), in addition to the cultivar Nonpareil in the last few years. 2) The pathogen *Botryosphaeria dothidea* from band canker showed major differences from and more genetic diversity than *B. dothidea* causing panicle and shoot blight of pistachio. 3) However, *B. dothidea* from almond band canker can infect and cause panicle and shoot blight on pistachio and *B. dothidea* from pistachio can infect almonds. 4) Inoculum of *B. dothidea* was found in some orchard debris (almond shoots shredded and left under the tree canopy), but not in other debris (e.g. remnants of last season's leaves, immature fruit on the ground, etc.). 5) *B. dothidea* was also isolated from walnuts and blackberries grown next to almonds, as well as other hosts grown in proximity with almonds. 6) The airborne (ascosporic phase of the pathogen) was discovered on almond in at least three orchards with severe band canker in Butte County and in walnuts and blackberries grown next to almonds in two counties. 7) *B. dothidea* was isolated from shaker wounds, cankers on the tree crotch, pruning wounds, and woodpecker wounds. 8) Earwigs do not seem to 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 extent the base of smaller shoots/suckers, and small twigs. 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 prevent wetting of tree trunks resulted in less canker activity (smaller cankers) and significantly lower incidence of active canker development.

Introduction

Although band canker was reported years ago as a problem in California almonds, it has been very sporadic until the last few years when a high incidence of this disease developed in 2001 and 2002 in several commercial orchards located in Butte, Glenn, Stanislaus, and Kern counties. An example of the damage is depicted in an Kern County orchard where 1700 trees were removed in 2002 and 2003. Isolations in our laboratory from the bark of trees with symptoms consistently revealed the asexual form of *Botryosphaeria dothidea,* a *Fusicoccum* species [initially reported as a *Dothiorella* species (English et al., 1966 & 1975)].

The pathogen causing band canker. The fungus *B. dothidea* (initially considered as a synonym of *B. ribis* Gross. & Duggar) is a cosmopolitan fungal pathogen. It can attack numerous hosts including agricultural, ornamental, and forest crops (Smith, 1934). In some of these hosts, severe epidemics have developed that have been very devastating for specific industries. For example, a disease that kills the fruit clusters of pistachio was reported initially as a sporadic problem in 1984 (Michailides, 1991), but by 1998 it became an epidemic in California pistachios (Michailides et al., 1999). Major research funds have been spent by the California Pistachio Industry during 1998 to 2003 on the development of methods to control this disease, also caused by a *Fusicoccum* species of *B. dothidea*. After major efforts of multifaceted research, the growers now have effective chemical and cultural control methods for this disease and at the same time, the biology of the pathogen and its inoculum sources and the development and epidemiology of the disease are understood much better. We have now undertaken a major study to investigate the etiology, epidemiology, and management of band canker of almond.

In almonds, only the *Fusicoccum* species (asexual stage) of *B. dothidea* was initially found (English et al, 1975), producing abundant pycnidia in the almond bark. However, in the spring of 2004 and 2005 (see results of last year's and this report), we isolated and recovered both the *Fusicoccum* species (asexual stage) and *Botryosphaeria dothidea* (the sexual stage) of the pathogen in three commercial almond orchards located in Butte County. The discovery of the sexual stage is of major epidemiological significance for the spread of the disease since the Ascosporics produced in pseudothecia become airborne and may travel and spread to significant distances from the source.

The epidemiology of band canker has not been described. Only very limited information is known. The disease occurs in vigorous Nonpareil, Carmel, and Padre trees, and less frequently on other almond cultivars 4-6 years old. Infections probably occur in the spring, and the source of spore inoculum has been unknown (Teviotdale, 2002) up until now (see this report). Infections seem to be active only during the growing season in which they first appear. Infections have never been associated with pruning wounds nor with lenticels (not until now; see this report). Lenticel infections are very common for *B. dothidea* attacking peach trees in Georgia, USA (Brown & Britton, 1986) and have also been reported on pistachios grown in California. However, both in the winter and the summer of 2005, we isolated *B. dothidea* from several pruning wounds in which the fungus seemed to have been actively growing and causing distinct cankers below the pruning wound. This study was initiated to help understand the epidemiology (sources of inoculum, mode of infection, and development of the disease) and management of band canker in almonds.

BIOLOGY AND EPIDEMIOLOGY OF *BOTRYOSPHAERIA DOTHIDEA* IN ALMONDS:

1. Compare 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 (Michailides, 2004). In a previous study, Ma et al. (2001) found that the pistachio isolates are very uniform genetically and very similar to isolates collected from hosts such as pecan, walnut, willow, eucalyptus, and blackberry. Interestingly, 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. 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 isolates, we collected more isolates from various orchards in Butte, Colusa, Glenn, and Kern Counties, and from almond, walnut, blackberry, and pistachio (**Table 1** and also see **Appendix, Table 1**). In addition, 8 isolates of *B. dothidea* causing problems in almonds in Australia were used as an outgroup of isolates for comparative reasons. Isolates included single spore pycnidiospores while some were from single Ascosporics, since pseudothecia with mature Ascosporics were discovered in some of the sampled almond orchards, in walnuts, and in blackberries grown next to an almond orchard in Butte County. Isolation, incubation, sporulation, single sporing, culturing of the isolates, and extracting their DNA were done as described previously following protocols that are used routinely in our plant pathology laboratory at Kearney Agricultural Center.

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 the upper canopy of trees, from almonds at various locations, and from other tree hosts grown in proximity to almonds. In addition, isolates from pycnidiospores were compared with those from ascospores. More details on the isolates are given in Appendix Table 1 at the end of the report).

Location	Host	Type of isolate	No. of isolates
Butte, Sorhney	Almond	Ascosporic and Pycnidiosporic	More than 30
	Walnut	Pycnidiosporic	3
	Blackberry	Pycnidiosporic	2
Colusa, Henderson	Almond	Pycnidiosporic from band cankers and upper canopy canker	12
	Walnut	Ascosporic and Pycnidiosporic	More than 30
Stanislaus	Almond	Pycnidiosporic	More than 5
	Walnut	Pycnidiosporic and ascosporic	8
Kern, Paramount	Almond	Pycnidiosporic	More than 30 (2002-2004)
KAC	Walnut	Pycnidiosporic	14
	Pistachio	Pycnidiosporic	6

Australia	Almond	Pycnidiosporic	8

Using four microsatellite primers, 55 polymorphic bands from a total of 98 isolates of *B. dothidea* were generated. An example of DNA fingerprints, using three of the microsatellites used, and a phenogram are given in Figures 1 and 2. The DNA fingerprint data indicated that 1) The *B. dothidea* population from almond showed much higher diversity than that from 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 pycnidiospores and Ascosporics 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 were grouped separately (Figure 2). And 4) some pycnidiospores and Ascosporic isolates from almond or walnut had identical DNA fingerprints, suggesting that these hosts when planted next to each other can contribute spore inoculum and infect each other.

2. Determine sources of inoculum of *B. dothidea* from almond and survey more orchards in search of the sexual airborne stage of the pathogen.

A) Sources of inoculum of *B. dothidea.* Isolates of *B. dothidea* were collected from three 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. Small pieces of bark from the tree trunks and cankers and blighted shoots when present in the upper canopy of trees were collected and isolations from the bark were made using standard isolation procedures. All the isolates were stored in the Kearney Ag Center fungal pathogen collection and will be used to compare them with isolates of *B. dothidea* collected from other hosts growing adjacent to almonds and non-adjacent hosts. In general most of the samples had pycnidia of the *Fusicoccum* species. Similarly to the 2004 results, samples from the almond orchards in Butte County and samples of walnuts in Stanislaus and Colusa Counties had both pycnidia of *Fusicoccum* and pseudothecia of *B. dothidea* (**Table 2**). The occurrence of pseudothecia in almond is very important in the epidemiology of the disease because pseudothecia produce airborne ascospores that do not need water to spread around and can spread 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 in some of the almond orchards. In addition, the presence of pseudothecia in almond also explains the greater genetic variability we observed among the isolates of *B. dothidea* from almond than the isolates from pistachio in which the sexual stage of the pathogen has not been found (see results in Objective 1 above).

To determine other possible sources of inoculum, we also collected blighted shoots of other kinds of trees (**Table 3**) 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 a blackberry bush growing next to almonds in one orchard in Butte County and in walnuts grown next to almonds in one orchard each in Colusa and Stanislaus Counties (Table 3). Isolates of both of the *Fusicoccum* and the *B. dothidea* stages were stored for further studies.

In another experiment, more than 50 shredded pieces of almond shoots were collected from the orchard floor in one orchard in Colusa County, brought to the laboratory, sectioned superficially, and observed with a dissecting microscope for the presence of *B. dothidea* pycnidia. As in 2004, pycnidia of *Fusicoccum* sp. were found only in one of the shredded shoots and one twig pruned

from the lower portion of a scaffold. Therefore, almond prunings shredded and left on the orchard floor may not be a good source of inoculum of *B. dothidea*. In contrast to pistachio shoots, almond shoots seems not to be a good substrate for the reproduction of *B. dothidea*. However, the rough bark of the tree trunks of certain almond cultivars can support large quantities of pycnidia and sometimes pseudothecia of the pathogen.

In addition to the above samples, we also examined the bark collected from the stumps of five cut trees in one orchard with a high incidence of band canker in Butte County. The bark in all these stumps bore pycnidia of the *Fusicocum* sp. and pseudothecia of *B. dothidea*. Therefore, stumps of cut trees can serve as inoculum sources of both airborne and water-splashed spores of the band canker pathogen.

We continued to examine the possible role of invertebrates in carrying and spreading the band canker pathogen. In 2005, we discovered that earwigs tend to gather under the swollen bark of the trees severely infected by band canker. We collected more than 100 earwigs from three trees and brought them to the laboratory where they were placed in plates containing acidified PDA. None of these produced any colonies of *B. dothidea*, suggesting that earwigs do not seem to transmit the band canker pathogen. Ants and other arthropods were not collected in 2005 because not many were present in the orchards with the band canker since growers have done an excellent job in controlling these pests in these orchards.

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 (pycnidiospores)	Pseudothecia (Ascosporics)	Viability of spores[1]
27 April 2004	Butte	3	+, +, +[2]	+, +, +	+, +, +
26 July 2004	Kern	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	+	+	+

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

Table 3. Hosts growing next to almonds from which *Botryosphaeria dothidea* was isolated.

Host	County	Scientific name	Family	Species found
Almond	Butte	*Prunus dulcis*	Rosaceae	*Fusicoccum & B. dothidea*
Almond	Colusa, Glenn, Stanislaus, Kern	*Prunus dulcis*	Rosaceae	*Fusicoccum*
Pistachio	Glenn	*Pistacia vera*	Anacardiaceae	*Fusicoccum*
Blackberry[1]	Butte	*Rubus ursinus*	Rosaceae	*Fusicoccum & B. dothidea*
Black walnut	Butte	*Junglans hinsii*	Juglandaceae	Not found

English walnut	Colusa, Stanislaus	*Juglans regia*	Juglandaceae	*Fusicoccum* & *B. dothidea*
Eucalyptus	Butte	*Eucalyptus coccifera*	Myrtaceae	Not found
Giant sequoia[1]	Colusa, Madera	*Sequoiadendron giganteum*	Taxodiaceae	*Fusicoccum* sp.
California oak	Colusa	*Quercus* sp.	Fagaceae	Not found
California redwood[1]	Colusa	*Sequoia sempervirens*	Taxodiaceae	*Fusicoccum* sp.
Arroyo willow	Colusa	*Salix lasiolepis*	Salicaceae	*Fusicoccum* sp.
Cumquat	Colusa	*Citrus* sp. Kumquat	Citraceae	*Fusicoccum* sp.
Wild grape	Colusa	*Vitis* sp.	Vitaceae	Not found
Mulberry	Stanislaus	*Morus alba*	Moraceae	Not found
Incense cedar	Stanislaus	*Cedrus libani*	Pinaceae	*Fusicoccum* sp.

[1] Both the *Fusicoccum* sp. and *B. dothidea* stages were found in earlier collections from other locations.

As in 2004, 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 infection 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.

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 (**Table 4**). 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. *B. dothidea* was also isolated from 40% of the cankers associated with pruning wounds (Table 4), suggesting that the pathogen can enter through pruning wounds. Pruning wounds with typical cankers were also observed in an orchard in Butte County but isolations were not made from this orchard.

Table 4. Frequency of *Botryosphaeria dothidea* isolated from fruit **peduncles** and **aerial cankers** associated with peduncles, **blighted twigs, pruning wounds, lenticel lesions,** and **base of shoots/suckers** collected at two different dates from an orchard in Colusa Co. where band canker was present.

Plant organ plated[1]	**Date of sample collection**	***Botryosphaeria* (%)**[2, 3]
Peduncle	2 Feb 2005	9.3
Aerial cankers	2 Feb 2005[2]	83.0
Blighted twigs	2 Feb 2005[2]	50.0
Peduncle	25 Aug 2005[2]	31.4
Aerial cankers	25 Aug 2005[2]	51.3
Blighted twigs	25 Aug 2005[2]	38.1
Pruning wound	25 Aug 2005[4]	40.0
Lenticel lesions	25 Aug 2005	45.3
Infections at the base of shoot to branch	25 Aug 2005	57.5

[1] Ten to 15 plates were prepared for each category per orchard.

[2] Other almond pathogens isolated included *Colletotrichum acutatum* (anthracnose), *Phomopsis amygdali* (Phomopsis blight), and *Monilinia laxa* (brown rot).

[3] Percentage was calculated from the total number of colonies divided by the total number of tissues plated.
[4] Other pathogen isolated was *Phomopsis amygdali.*

Results from Table 4 suggest that there must be several ways of infection of almond by the band canker pathogen: a) ***Pruning wounds.*** In 2005 we found infections of *B. dothidea* starting from pruning wounds in two orchards. ***b) Lenticels.*** In at least one of the orchards used in the band canker project, we noticed a lot of small lesions associated with lenticels, particularly in the lower branches close to the trunk. More such lesions, apparently initiated from lenticels, were observed in the surface facing the ground than on the upper surface of branches. Upon scrapping the bark one could see circular and sometimes irregular brown lesions with gum accumulated in the internal pocket of the lesion. External indication of these lesions was clear or amber gum exuding from the top of the lesion. In some trees, lenticel infections coalesced and formed larger (1 to 2 cm in diameter), discolored lesions of irregular shape. ***c) Cracking of woody tissues.*** Similarly to the infections occurring on the tree trunk starting from growth cracks, we also observed infections on large branches that had started either first from a lenticel and moved along a growth crack or directly at a crack of woody tissues due to wind force. These infections are characterized by the distinct blackening of the cracking and the gumming associated with them. ***d) Rough bark at the base of shoots/sucker.*** Another avenue of infection was found in at least one of the orchards. Infections started from the rough bark that develops around the base of shoots that emerge in an angle from a main branch of trees, and gum was also associated with these infections. And ***e) Fruit peduncle.*** About 200 peduncles with gumming were collected and sectioned longitudinally to observe the cankers around them and after plating, 9 to 31% produced colonies of *B. dothidea* in agar media (Table 4). All these types of mode of infection of the band canker pathogen explain the occurrence of aerial cankers caused by *B. dothidea* we have been finding in almonds 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 (perhaps excessive rain as that in 2005) the band canker pathogen has the ability to infect almond trees in several ways.

DISEASE MANAGEMENT:

3. Compare various fungicide treatments by injecting them in trees in a greenhouse/ lath house and in the field.

A. Fungicide and biological control treatments in the lath house/greenhouse. One-year old potted Carmel trees were inoculated with an aggressive isolate of *B. dothidea* #2180. To determine curative effects of various treatments, 4 days after inoculation, the inoculated sites were treated with the fungicides and biological treatments listed in **Table 5.** After treatment, the inoculated sites were sealed with Parafilm M to prevent desiccation.

To determine if any of the treatments can protect from infection of almond stems by *B.* dothidea, potted Carmel trees were simultaneously inoculated with *B. dothidea* #2180 isolate and treated with the same treatments as shown in Table 5. Five replicated trees were used per treatment in each experiment.

Table 5. Effects of fungicide and biological treatments used on almond trees inoculated with *Botryosphaeria dothidea* in the greenhouse.

A. Inoculated on May 27 and treated on June 1, 2004:		
Treatment	**Band canker size (mm)**	
	July 16	**October 6**
Propiconazole (Break®)	91 a	122 a
Azoxystrobin (Abound®)	100 a	104 a
Iprodione (Rovral®)	89 a	93 a
Trichoderma viride -36E1	89 a	99 a
Trichoderma harzianum (Plant Shield®)	75 a	84 a
Control (nontreated)	110 a	108 a
B. Inoculated and treated simultaneously on June 1, 2004:		
Propiconazole (Break®)	7 c	25 cd
Azoxystrobin (Abound®)	0 c	6 e
Iprodione (Rovral®)	7 c	11 de
Trichoderma viride -36E1	41 b	38 bc
Trichoderma harzianum (Plant Shield®)	43 b	44ab
Control (nontreated)	58 a	58 a

The results in Table 5 suggest that the fungicides Break®, Abound®, and Rovral® protected the trees from infections by *B. dothidea* when applied just before inoculation. However, when the fungicide and the biological treatments were applied after infection took place (post-infection period) by the pathogen, they did not protect the trees and cankers developed to the full extent. The results in the field (see B. below) support this contention.

B. Fungicide and biological control treatments in the field.

Experiment 1. This experiment involved various chemical and two biological treatments as shown in **Table 6** and was performed in a row of Nonpareil trees in an orchard with band canker in Colusa County. 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. If the canker extended 2/3 to completely encircling the trunk, then two sites above the canker and two below were injected. If the canker was ¼ to ½ the way around the trunk then four sites around the perimeter of the canker were injected. 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 6). On all the recording dates we found no effect of any of the treatments, and indeed 15 months after the initiation of the experiment we found that untreated symptomless trees started showing band canker and they had an average of 22% of their circumference infected. 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 symptomless trees (on May 12, 2004), although some of these trees developed new but small band cankers on their trunks by August 2005 (Table 6).

Table 6. Size of band cankers in an Nonpareil almond orchard in Colusa County before (5 May 2004) and 15 months after injection (25 Aug 2005) and effects on defoliation.

Treatment	Rate	Canker size (% of circumference)[1]		Defoliation (% of canopy)
		Before	15 months later	
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 symptomless	---	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.

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 6) 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 treated site. The corners of the plastic, outside of the silicone seal 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 sealing of this treatment was done in the similar way as that for the biological control treatments. 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 evaluations of all the trees in this experiment also show temporarily that again that these treatments may not cure the band cankers; they might though stop the growth of the cankers resulting in beneficial end results (in general, reduce defoliation and improve tree health). Final evaluations of this experiment will be done in late October 2005.

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 7**) to run off. Then four layers of cheesecloth pieces about 6"× 9" were folded in thirds, soaked with about 60 ml of each biological agent, 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, outside of the silicone seal were stapled to the tree to secure the plastic and create a humid environment. Evaluations of the treatments were done on September 21, 2004, and again on August 29, 2005.

Table 7. 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

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 the trees. To determine the effect of avoiding 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 trunks 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 Table 8.

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 (Table 8). 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 (Table 8). Therefore, 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.

Table 8. Effect of altering the irrigation sprinklers by installing metallic splitters and reducing wetting of trunks of Padre almond trees in an orchard in Butte County.

Sprinkler irrigation[1]	Canker incidence (%)[2]	Canker size (% of circumference)[2]	Number of gum secretions on trunk[2]	Incidence of gum secretions on trunk (%)[2]	Incidence of defoliated trees (%)[2]
July 1, 2004					
Splitters	45.3 a[3]	21.6 a	---[4]	---	7.3 a
No splitters	42.9 a	22.8 a	---	---	4.3 a
October 21, 2004					
Splitters	48.3 a	25.1 b	5.8 b	55.8 b	3.6 a
No splitters	52.9 a	31.5 a	9.8 a	71.0 a	4.1 a
August 25, 2005					
Splitters	22.4 b	11.2 b	2.3 a	32.7 a	2.5 a
No splitters	39.1 a	20.5 a	5.2 a	52.3 a	3.6 a

[1] Two splitters were installed on opposite sides of sprinkler heads on June 10, 2004 to reduce the wetting of Padre almond tree trunk.

[2] Averages from four replications of forty-five trees each.

[3] Numbers followed by different letters are significantly different according to the LSD test at $P = 0.05$; comparisons were done separately for each date of evaluation.

[4] Not evaluated on this date.

CONCLUSIONS:

In the second year of this project, significant progress has been made in understanding the biology and epidemiology of the pathogen causing band canker of almond. A summary of the new findings in the first two years of this project is given below:

1. The pathogen *B. dothidea* was confirmed from several commercial orchards in Butte, Glenn, Colusa, San Joaquin, Stanislaus, and Kern Counties.
2. Both the water splash 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 *B. dothidea* causing 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.
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.
8. Earwigs do not seem to 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 base of smaller

shoots/suckers, and small twigs.

10. Greenhouse experiments showed that propiconazole, azoxystrobin, and iprodione prevented canker formation and stall band canker development in a field experiment.
11. Manipulation of irrigation to prevent wetting of tree trunks resulted in less canker activity (smaller cankers) and significantly lower incidence of active canker development.

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APPENDIX:

Table 1. A list of single-spore isolates of *Botryosphaeria dothidea* used in this study.

Isolate	Host	Date of isolation	Location	Notes
3089	Almond	5/5/05	Sohrney	Ascosporic isolate
3091	Almond	5/5/05	Sohrney	Ascosporic isolate
3096	Almond	5/5/05	Sohrney	Ascosporic isolate
3098	Almond	5/5/05	Sohrney	Ascosporic isolate
3099	Almond	5/5/05	Sohrney	Ascosporic isolate
3104	Almond	5/15/05	Sohrney	Ascosporic isolate
3536	Almond	3/3/05	Sohrney	Isolated from canker
3537	Almond	3/3/05	Sohrney	Pycnidiospore isolate
3538	Almond	3/3/05	Sohrney	Isolated from twig
3539	Almond	3/3/05	Sohrney	Isolated from twig
3540	Almond	3/3/05	Sohrney	Ascosporic isolate
3541	Almond	3/3/05	Sohrney	Isolated from twig
3542	Almond	3/3/05	Sohrney	Isolated from twig
3543	Almond	3/3/05	Sohrney	Isolated from twig
3544	Almond	3/3/05	Sohrney	Isolated from twig
3298	Blackberry	6/2/04	Butte	Pycnidiospore isolate
3299	Blackberry	6/2/04	Butte	Pycnidiospore isolate
3473	Almond	2/8/05	Colusa	Isolated from canopy
3474	Almond	2/8/05	Colusa	Isolated from canopy
3475	Almond	2/8/05	Colusa	Isolated from canopy
3476	Almond	2/8/05	Colusa	Isolated from canopy
3477	Almond	2/8/05	Colusa	Isolated from canopy
3489	Almond	2/8/05	Colusa	Isolated from small twig
3490	Almond	2/8/05	Colusa	Isolated from deep canker
3491	Almond	2/8/05	Colusa	Isolated from deep canker
3492	Almond	2/8/05	Colusa	Isolated from deep canker
3493	Almond	2/8/05	Colusa	Isolated from small twig
3157	Walnut	5/11/04	Colusa	Pycnidiospore isolate
3158	Walnut	5/11/04	Colusa	Pycnidiospore isolate
3162	Walnut	5/11/04	Colusa	Pycnidiospore isolate
3163	Walnut	5/11/04	Colusa	Pycnidiospore isolate
3172	Walnut	4/27/04	Colusa	Pycnidiospore isolate
3173	Walnut	4/27/04	Colusa	Pycnidiospore isolate
3209	Walnut	6/25/04	Colusa	Ascosporic isolate
3210	Walnut	6/25/04	Colusa	Ascosporic isolate
3211	Walnut	6/25/04	Colusa	Ascosporic isolate
3212	Walnut	6/25/04	Colusa	Ascosporic isolate
3214	Walnut	6/25/04	Colusa	Ascosporic isolate
3229	Walnut	6/29/05	Colusa	Ascosporic isolate
3230	Walnut	6/29/05	Colusa	Ascosporic isolate
3231	Walnut	6/29/05	Colusa	Ascosporic isolate
3232	Walnut	6/29/05	Colusa	Ascosporic isolate
3233	Walnut	6/29/05	Colusa	Ascosporic isolate
3234	Walnut	6/29/05	Colusa	Ascosporic isolate
3235	Walnut	6/29/05	Colusa	Ascosporic isolate
3236	Walnut	6/29/05	Colusa	Ascosporic isolate
3237	Walnut	6/29/05	Colusa	Ascosporic isolate
3238	Walnut	6/29/05	Colusa	Ascosporic isolate
3239	Walnut	6/29/05	Colusa	Ascosporic isolate
3295	Walnut	6/20/04	Colusa	Ascosporic isolate
3296	Walnut	6/20/04	Colusa	Ascosporic isolate
3305	Walnut	7/23/04	Colusa	Pycnidiospore isolate

3306	Walnut	7/23/04	Colusa	Pycnidiospore isolate
H2	Almond	2005	Henderson	Pycnidiospore isolate
H3	Almond	2005	Henderson	Pycnidiospore isolate
H4	Almond	2005	Henderson	Pycnidiospore isolate
H6	Almond	2005	Henderson	Pycnidiospore isolate
H7	Almond	2005	Henderson	Pycnidiospore isolate
H8	Almond	2005	Henderson	Pycnidiospore isolate
H9	Almond	2005	Henderson	Pycnidiospore isolate
H10	Almond	2005	Henderson	Pycnidiospore isolate
H11	Almond	2005	Henderson	Pycnidiospore isolate
H13	Almond	2005	Henderson	Pycnidiospore isolate
H14	Almond	2005	Henderson	Pycnidiospore isolate
H18	Almond	2005	Henderson	Pycnidiospore isolate
H21	Almond	2005	Henderson	Pycnidiospore isolate
H24	Almond	2005	Henderson	Pycnidiospore isolate
3307	Almond	7/27/04	Paramount	Pycnidiospore isolate
3308	Almond	7/27/04	Paramount	Pycnidiospore isolate
3310	Almond	7/27/04	Paramount	Pycnidiospore isolate
3311	Almond	7/27/04	Paramount	Pycnidiospore isolate
B. obtusa 3317	Almond	7/27/04	Paramount	Isolated from shaker damage tissue
B. obtuse 3319	Almond	7/27/04	Paramount	Pycnidiospore isolate
1542	Almond	2/7/02	Paramount	Isolated from healthy tissue
2175	Almond	5/18/03	Paramount	Isolated from healthy tissue
2181	Almond	5/18/03	Paramount	Isolated from band canker
2996	Walnut	1/15/04	Parlier	Pycnidiospore isolate
2997	Walnut	1/15/04	Parlier	Pycnidiospore isolate
2998	Walnut	1/15/04	Parlier	Pycnidiospore isolate
3001	Walnut	1/15/04	Parlier	Pycnidiospore isolate
3002	Walnut	1/15/04	Parlier	Pycnidiospore isolate
2946	Pistachio	1/29/04	Parlier	Pycnidiospore isolate
2947	Pistachio	1/29/04	Parlier	Pycnidiospore isolate
2948	Pistachio	1/29/04	Parlier	Pycnidiospore isolate
3596	Walnut	5/9/05	Stanislaus	Pycnidiospore isolate
3597	Walnut	5/9/05	Stanislaus	Pycnidiospore isolate
3598	Walnut	5/9/05	Stanislaus	Pycnidiospore isolate
3565	Walnut	5/9/05	Stanislaus	Ascosporic isolate
3566	Walnut	5/9/05	Stanislaus	Ascosporic isolate
3567	Walnut	5/9/05	Stanislaus	Ascosporic isolate
3568	Walnut	5/9/05	Stanislaus	Ascosporic isolate
G.BK1	Almond	2005	Glenn	Pycnidiospore isolate
G.BK2	Almond	2005	Glenn	Pycnidiospore isolate
G.BK4	Almond	2005	Glenn	Pycnidiospore isolate
G.BK6	Almond	2005	Glenn	Pycnidiospore isolate
G.BK7	Almond	2005	Glenn	Pycnidiospore isolate
2804	Almond	12/11/03	Australia	Pycnidiospore isolate
2805	Almond	12/11/03	Australia	Pycnidiospore isolate
2806	Almond	12/11/03	Australia	Pycnidiospore isolate
2807	Almond	12/11/03	Australia	Pycnidiospore isolate
2808	Almond	12/11/03	Australia	Pycnidiospore isolate

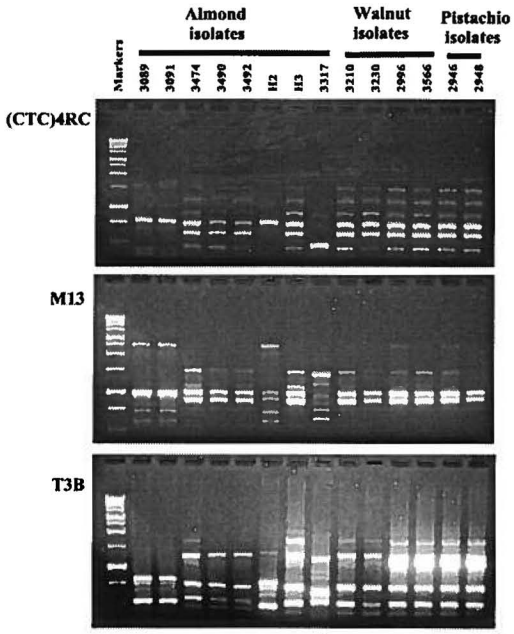

Figure 1. An example of DNA fingerprints of various isolates of *Botryosphaeria dothidea* collected from almond, walnut, and pistachio.

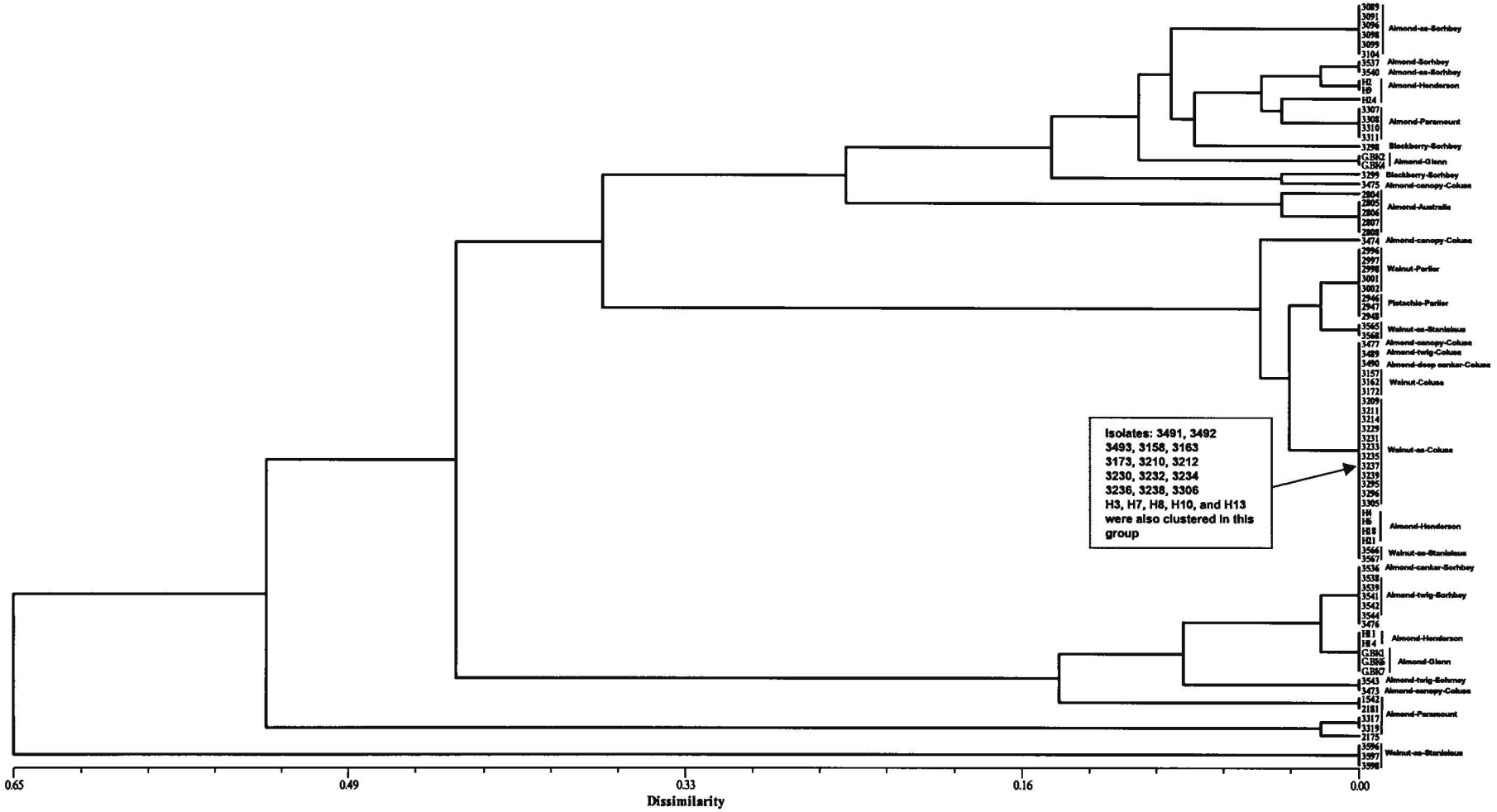

Figure 2. Phenogram generated by the unweighted pair-group method with arithmetic average cluster analysis of random amplified polymorphic DNA and microsatellite-primed polymerase chain reaction data sets from 98 isolates of *Botryosphaeria dothidea*. Dissimilarity shows that the longer the branch the more different the groups held by these branches are.