

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

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Objectives

- 1) Compare various fungicide treatments by injecting them in trees a) in a lath house, and b) in the field.
- 2) Compare methods of irrigation in controlling band canker of almond.
- 3) Compare tree seals with or without fungicides in controlling band canker.

Abstract

In the first year of this study, we discovered several new findings that seem to be important in the epidemiology and control of band canker of almond. 1) The disease was found frequently on several almond cultivars (Carmel and Padre), in addition to 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 while *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 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 (ascospore 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. 7) *B. dothidea* was isolated from shaker wounds, cankers on the tree crotch, pruning wounds, and woodpecker wounds. 8) Of the insects tested, at least ants can carry and spread *B. dothidea* in almond orchards. And 9) Biological, chemical, and cultural control methods are being investigated.

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 several commercial orchards have seen tremendous damage by band canker. For instance, orchards in Stanislaus, San Joaquin, and Kern County have been reported in 2001 and 2002 with band canker. An example of the damage is depicted in an orchard of the Kern County 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 fungus *B. dothidea* is a cosmopolitan fungal pathogen. It can attack numerous hosts including

agricultural, ornamental, and forest crops. In some of these hosts the damage can be tremendous and very devastating for specific industries. For instance, 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 in the development of control methods of this disease in pistachios, 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 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.

BACKGROUND INFORMATION:

Symptoms. In summer and early fall, narrow bands of asymmetric cankers extend around half or more of the circumference of the trunk or scaffold branches. The unusual characteristic of these cankers is that their greatest dimension is perpendicular to the long axis of the branch or trunk. Usually the cankers seem to arise from small growth cracks and result in abundant gum formation in the infected and/or the area surrounding the canker. If the infection extends to the wood, the branch above the infection dies. The foliage of infected trees becomes chlorotic, defoliates and trees show general decline. Also in one orchard unusual nut drop was observed. Although tree death is reported to be rare, in the orchards in Kern and Butte Counties where the disease was severe, a large number of infected trees were killed and replaced by the grower. Discolored sapwood often extends longitudinally several centimeters beyond the canker margin. The sap initially is light amber, becomes darker with time and during the winter is black, sometimes covered with saprophytes. Under humid conditions, tiny white spore tendrils (cirrhi) can be seen oozing from pycnidia immersed in the outer bark. Pycnidia of the fungus are formed in groups in a stroma and are associated with old lenticel areas of the bark, protruding through old lenticel cracks.

The pathogen. 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 almonds, only the *Fusicoccum* species (asexual stage) of *B. dothidea* has been initially found (English et al, 1975), producing abundant pycnidia in the almond bark. However, in the spring of 2004 (see results of this report), we isolated and recovered both the asexual (*Fusicoccum* sp.) and sexual (*B. dothidea*) stages of the pathogen in three commercial almond orchards located in Butte County.

Epidemiology. The epidemiology of band canker has not been described. Only very limited information is known. Infections probably occur in spring, and the source of spore inoculum is unknown (Teviotdale, 2002). Infections seem to be active only during the growing season in which they first appear. Infections have never been associated with pruning wounds, and the lenticel infections reported in peach (Brown & Britton, 1986) have not been observed in almond. However, in almonds the main avenue of infection seems to be growth cracks as a result of vigorously growing cultivars. The disease occurs in vigorous Nonpareil trees, Carmel, and Padre, and less frequently on other almond cultivars of 4-6 years old.

Because of the recent problems with band canker and the possibility that the disease may expand to epidemic levels in the almond industry, particularly in El Niño years, a study was initiated aiming to help understand and manage band canker in almonds supported financially by the California Almond Board.

Experiments and Results

BIOLOGY AND EPIDEMIOLOGY OF *BOTRYOSPHERA DOTHIDEA* IN ALMONDS:

1. Collection of *B. dothidea* isolates from almonds. Isolates of *B. dothidea* were collected from three orchards in Butte County, one in Colusa County, and three orchards in Kern County. Small pieces of the bark from symptomatic and asymptomatic isolates were collected and isolations from the bark were made using standard isolation procedures. All the isolates were stored 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 (Fig. 1A); although samples from three orchards in Butte County had both pycnidia of *Fusicoccum* intermixed with pseudothecia of *B. dothidea* (Table 1 and Fig. 1B). Finding 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 for longer distances. The presence of pseudothecia explains why we were able to isolate the pathogen from trees that did not show any disease symptoms. Presence of pseudothecia (sexual stage of the *Fusicoccum*) in almond 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 was not found.

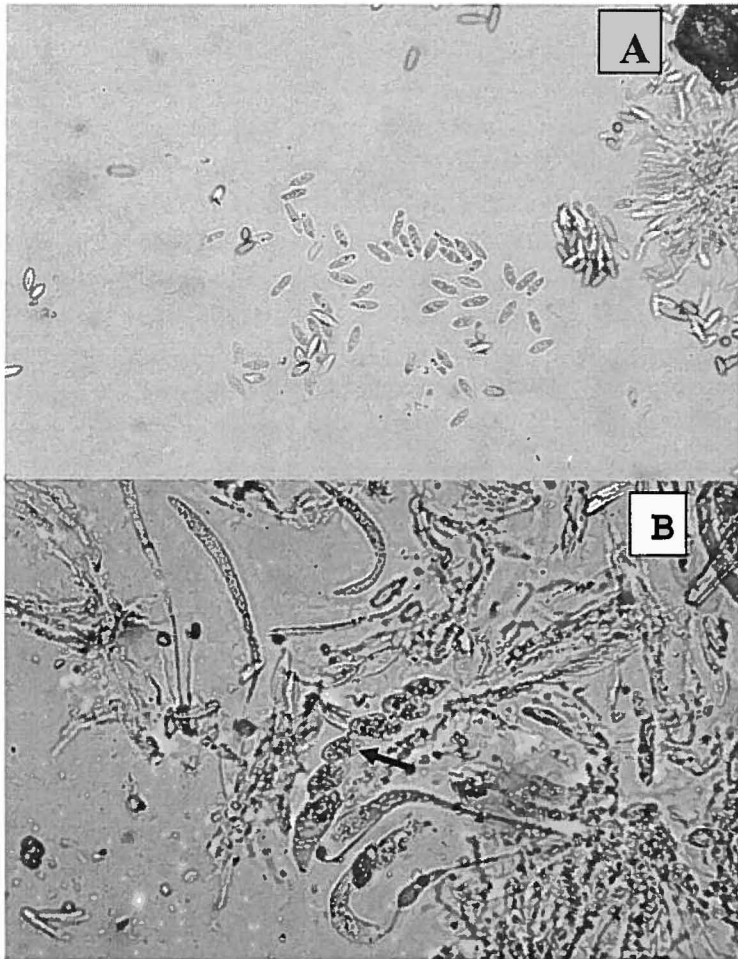


Figure 1. Pycnidiospores of the *Fusicoccum* sp. (A) and ascospores in asci of the *B. dothidea* (B) stages on almond bark collected from orchards in Butte County in 2004.

2. Collection *B. dothidea* isolates from other hosts adjacent to almonds. Isolates of *Fusicoccum* sp. were collected from the following plants growing next to almonds: willow, eucalyptus, blackberry, walnut, sequoia, redwood, and citrus (cumquat trees, close to the almond orchard in Colusa County). Also pseudothecia of *B. dothidea* were found in the blackberry growing next to almond in one orchard in Butte County, and in walnuts grown next to almond in the orchard in Colusa County (Table 1). Isolates of both of the *Fusicoccum* and the *B. dothidea* stages were stored for further studies on comparative pathogenicity and genetic variability among isolates from almond and various hosts.

Table 1. Hosts growing next to almonds from which *Botryosphaeria dothidea* was isolated.

Host	Scientific name	Family	Species found
Almond	<i>Prunus dulcis</i>	Rosaceae	<i>Fusicoccum</i> & <i>B. dothidea</i>
Blackberry*	<i>Rubus ursinus</i>	Rosaceae	<i>Fusicoccum</i> & <i>B. dothidea</i>
Black walnut	<i>Juglans hinsii</i>	Juglandaceae	Not found
English walnut	<i>Juglans regia</i>	Juglandaceae	<i>Fusicoccum</i> & <i>B. dothidea</i>
Eucalyptus	<i>Eucalyptus coccifera</i>	Myrtaceae	Not found
Giant sequoia*	<i>Sequoiadendron giganteum</i>	Taxodiaceae	<i>Fusicoccum</i> sp.
California oak	<i>Quercus</i> sp.	Fagaceae	Not found
California redwood*	<i>Sequoia sempervirens</i>	Taxodiaceae	<i>Fusicoccum</i> sp.
Arroyo willow	<i>Salix lasiolepis</i>	Salicaceae	<i>Fusicoccum</i> sp.
Cumquat	<i>Citrus</i> sp. Kumquat	Citraceae	<i>Fusicoccum</i> sp.
Wild grape	<i>Vitis</i> sp.	Vitaceae	Not found

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

3. Preliminary genetic structure of *B. dothidea* from almond. From 1997 to 2000, we recovered about 50 isolates of *B. dothidea* from almonds collected from San Joaquin and Stanislaus Counties. During 2001 to 2003 we collected more isolates from almonds in Glenn, Kern, and Kings Counties. The purpose of this initial collection was to determine the genetic structure of the population of *B. dothidea* causing band canker and compare it with isolates we collected from more than 40 other native or introduced plants in California (Table 2). Comparing the pathogen causing band canker on almond with other *B. dothidea* would give us an indication regarding the pathogen's sources of origin. In addition, we wanted to see whether the pathogen causing band canker on almond is similar to the one causing panicle and shoot blight of pistachio or blight of walnuts, since in some areas pistachios with severe panicle and shoot blight or walnuts with *Botryosphaeria* blight and almonds are grown next to each other. 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 T₃B, we found that the almond isolates are different from those of pistachio (Fig. 2). 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 (Fig. 2). 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. Greater genetic variability among pathogens may need more aggressive control methods than genetically uniform pathogens.

Table 2. Hosts from which *Botryosphaeria dothidea* was frequently isolated in California.

Host	Scientific name	Family
Almond	<i>Prunus dulcis</i>	Rosaceae
Apple	<i>Malus domestica</i>	Rosaceae
Avocado*	<i>Persea americana</i>	Lauraceae
Blackberry*	<i>Rubus ursinus</i>	Rosaceae
Black walnut	<i>Juglans hinsii</i>	Juglandaceae
Carob seed tree	<i>Ceratonia siliqua</i>	Leguminosae
Incense cedar	<i>Cedrus libani</i>	Pinaceae
Deodor cedar	<i>Cedrus deodora</i>	Pinaceae
Chinese hackberry	<i>Celtis sinensis</i>	Ulmaceae
California redwood*	<i>Sequoia sempervirens</i>	Taxodiaceae
Cotoneaster	<i>Cotoneaster frigidus</i>	Rosaceae
Cottonwood	<i>Populus deltoides</i>	Populaceae
English walnut	<i>Juglans regia</i>	Juglandaceae
Eucalyptus	<i>Eucalyptus coccifera</i>	Myrtaceae
Euonymus	<i>Euonymus fortunei</i>	Celestraceae
Silver dollar eucalyptus	<i>Eucalyptus orbifolia</i>	Myrtaceae
Feijoa	<i>Feijoa sellowiana</i>	Myrtaceae
Fig	<i>Ficus carica</i>	Moraceae
Giant sequoia*	<i>Sequoiadendron giganteum</i>	Taxodiaceae
Juniper	<i>Juniperus occidentalis</i>	Cypressaceae
Jasmine	<i>Jasminum officinale</i>	Jasminaceae
Lemon	<i>Citrus × limon</i>	Citraceae
Sweet gum	<i>Liquidambar styraciflua</i>	Mamamelidaceae
Maple	<i>Acer</i> sp.	Aceraceae
Oak	<i>Quercus</i> sp.	Fagaceae
Olive*	<i>Olea europea</i>	Olivaceae
Orange	<i>Citrus × auranteum</i>	Citraceae
Pistachio	<i>Pistacia vera</i> 'Kerman'	Anacardiaceae
Pear	<i>Pyrus communis</i>	Rosaceae
Pecan	<i>Carya illinoensis</i>	Juglandaceae
Persimmon	<i>Diospyros kaki</i>	Ebenaceae
Pine	<i>Pinus radiata</i>	Pinaceae
Prune	<i>Prunus domestica</i>	Rosaceae
Firethorn*	<i>Pyracantha coccinea</i>	Rosaceae
Raymond ash	<i>Fraxinus augustifolia</i> <i>augustifolia</i> subsp. <i>oxycarpa</i>	Oleaceae
Sycamore maple	<i>Acer pseudoplatanus</i>	Aceraceae
Wax leaf Privet	<i>Ligustrum japonicum</i>	Oleaceae
Western redbud	<i>Cedris canadensis</i>	Leguminosae
Wild rose	<i>Rosa</i> sp.	Rosaceae
White willow	<i>Salix alba</i>	Salicaceae
Arroyo willow	<i>Salix lasiolepis</i>	Salicaceae
Weeping willow	<i>Salix babylonica</i>	Salicaceae

*Hosts where the sexual stage of the pathogen has been found; nut crops in bold.

4. Methods of inoculation of almonds. A study was designed to compare inoculation procedures on almonds. Each treatment utilized four Carmel and four Nonpareil almond trees. There were three treatments and a control. The treatments included, inoculation by making a slit of 5 cm long and 2 mm wide on the bark of each tree stem and inserting a longitudinal mycelium strip from a

culture of *B. dothidea* (isolate from almond) or by making five longitudinal slits (5 cm long 2-3 mm wide) and spraying a 50,000 spores/ml suspension of *B. dothidea*, or by spraying a section of 5 cm length of the trunk with the spore suspension without any wounding. All inoculated sites were wrapped with Parafilm-M to protect the inoculum from drying quickly. Four trees were used as controls. Only the inoculation with mycelial plugs of the pathogen resulted in significant canker development and gumming in either variety, although the cankers on Nonpareil were larger and more active than those on Carmel. This implies that wounding may be required for infection to take place. These results are also supported by the fact that in 2004 we isolated *B. dothidea* from almond trees bearing shaker damage or woodpecker damage of tree trunks.

5. Cross pathogenicity – greenhouse inoculations. Cross pathogenicity studies involved the inoculation of one host with the isolate from another host. One-year old potted Carmel trees kept in the greenhouse of the Kearney Center were inoculated with an isolate of *B. dothidea* from almond and willow and two isolates from blackberry. Inoculations were done by inserting a mycelial plug into a wound on the tree stems as described above. These inoculations were very aggressive and not only resulted in large cankers that ranged from 16 to 21 cm in length, but also killed the majority of the young trees (Table 3).

Table 3. Inoculation of almond trees with various isolates of *Botryosphaeria dothidea* in the greenhouse.

Host from which <i>B. dothidea</i> isolate was obtained	Length of canker (cm)*	Dead trees*
Almond	20.5	3 out of 4
Willow	28.3	3 out of 4
Blackberry - 1	19.3	2 out of 4
Blackberry - 2	16.1	2 out of 4
Control (non-inoculated)	0.0	0 out of 4

* Recorded 25 days after inoculation.

In another experiment potted pistachios were inoculated with mycelial plugs obtained from two isolates of *B. dothidea* from almond, and two isolates from pistachio. Both the almond and the pistachio isolates caused cankers in pistachio that averaged from 13 cm (almond isolate) to 24 cm (pistachio isolate) in length, while the non-inoculated control remained healthy.

In a third experiment, 5-year old branches of mature (older than 15 years) trees were inoculated with mycelial plugs obtained from two isolates of *B. dothidea* from almond, and two isolates from pistachio. The non-inoculated control was wounded and an agar plug was inserted in the wound. Three replicated branches were used in three trees. The development of cankers in these older branches took longer, and 3 months after inoculation, cankers ranged from 8 to 18 cm for the almond isolates of *B. dothidea* and averaged 15 cm in length for the pistachio isolates. The width of these cankers ranged from 2.5 to 6 cm. The non-inoculated control remained healthy. These results suggest that when almond was challenged with isolates of *B. dothidea* from almond, willow, blackberry, or pistachio, it became infected and developed band canker. When pistachios were challenged with almond or pistachio isolates of *B. dothidea*, they became infected and developed panicle and shoot blight. Therefore, although the almond isolates seem to be genetically different than the pistachio isolates, they can cross infect pistachio and vice versa.

6. Source of the pathogen's inoculum. To determine whether the pathogen resides in orchard debris, samples of trunk bark tissues from symptomatic and non symptomatic ("healthy") trees were collected and isolations were made in plates with acidified potato dextrose agar. Interestingly, within 5 days of incubation of Petri plates, *B. dothidea* was isolated not only from the symptomatic (71%) but also from the trees with no symptoms (14%), indicating that the inoculum was present on the bark of "healthy" trees waiting for the right conditions (high humidity and temperatures, growth cracks on the trunk, etc.) to cause infection. The question that remains to be answered is where does the inoculum come from? To answer this question, debris (dead shoots, aborted almond nuts, leaves from the previous season, and solidified gum) under infected and "healthy" trees were collected, brought to the laboratory, and examined for any signs (mycelia, pycnidia, etc.) of *B. dothidea* and direct isolations of suspected structures were made on acidified PDA. In addition, debris was washed with water and the 0.1 ml of the washings was plated in Petri plates with acidified PDA. No isolates of *B. dothidea* were recovered from these samples. However, because we observed pycnidia of the *Fusicoccum* sp. in a piece of almond shoot (2-years old) collected from the ground, it is possible that almond prunings shredded and left on the orchard floor may serve to some degree as sources of inoculum. Additional samples are being examined.

To determine whether invertebrates play a role in carrying and spreading the band canker pathogen, we collected about 40 ants crawling on to the soil and the trunk of an infected tree and 50 earwigs that were gathered under the bark of infected trees. The earwigs were brought to the laboratory, frozen to kill them, and plated on malt extract agar amended with 5 ppm boscalid, which is the active ingredient of BAS 510 fungicide (BASF Corporation). A colony of *B. dothidea* developed from one of 40 ants plated on media while no *B. dothidea* developed from any of the plated earwigs. Although these results are preliminary and very limited, they suggest that there may be a possibility that arthropods can carry propagules to growth cracks of almond and spread the pathogen. However, more invertebrates need to be collected to confirm these results. We plan to collect more ants or other insects from almond orchards where band canker has been reported, and from other possible hosts of *B. dothidea* (Table 2) grown next to almond orchards to determine whether these invertebrates transfer *B. dothidea* from other hosts to almonds or vice versa. It is possible that the pathogen causing band canker may be unique and different from other *B. dothidea* isolates from different host plants. But this will not be known until comparisons include a large number of almond isolates from different locations and cultivars and from other hosts that are in the proximity of almond plantings.

DISEASE MANAGEMENT:

1. 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 4. 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 4. Five replicated trees were used per treatment in each experiment.

Table 4. Effects of fungicide and biological treatments used on almond trees inoculated with *Botryosphaeria dothidea* in the greenhouse.

A. Inoculated and treated 4 days later:			
Treatment	Rate	Field rate	Canker length (mm)
Propiconazole (Break®)	10,000 ppm a.i.	13.8 ml product /600 ml water	91
Azoxystrobin (Abound®)	10,000 ppm a.i.	24 ml product/600 ml water	100
Iprodione (Rovral®)	10,000 ppm a.i.	12.5 ml product /600 ml water	89
<i>Trichoderma viride</i> -36E1	5×10^7 CFU/ml	14 plates/600 ml water	89
<i>Trichoderma harzianum</i> (Plant Shield®)	100 mg product per 10 ml water	6 g product/600 ml water	75
Control (nontreated)	---	---	110
B. Inoculated and treated immediately:			
Propiconazole (Break®)	10,000 ppm a.i.	13.8 ml product /600 ml water	7
Azoxystrobin (Abound®)	10,000 ppm a.i.	24 ml product/600 ml water	0
Iprodione (Rovral®)	10,000 ppm a.i.	12.5 ml product /600 ml water	7
<i>Trichoderma viride</i> -36E1	5×10^7 CFU/ml	14 plates/600 ml water	41
<i>Trichoderma harzianum</i> (Plant Shield®)	100 mg product per 10 ml water	6 g product/600 ml water	43
Control (nontreated)	---	---	60

* Cankers were recorded two and a half weeks after inoculation.

Although final results on the effects of the above treatment will be recorded at the end of September, the initial recordings showed that none of the fungicide or the biological treatments had any effect in curing infections (Table 4A), but the three fungicides were very effective in preventing infections (Table 4B). There was some reduction of canker length by the biological treatments but this reduction was smaller than that of fungicide treatments.

2. Fungicide and biological control treatments in the field.

Experiment 1. This experiment involved various chemical and two biological treatments as shown in Table 5 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 1/4 to 1/2 the way around the trunk then four sites around the perimeter of the canker were injected. Evaluations of the treatments will be done at the end of August or early September and again during the winter and in the spring 2005.

Table 5. Injection trial for the control of band canker on Nonpareil almonds in Arbuckle, Colusa County on 12 May 2004.

#	Treatment/trade name	Rate	Field rate
1.	Propiconazole (Break [®])	10,000 ppm ai	13.8 ml/600 ml water
2.	Azoxystrobin (Abound [®])	10,000 ppm ai	24 ml product/600 ml water
3.	Iprodione (Rovral [®])	10,000 ppm ai	12.5 ml /600 ml water
4.	<i>Trichoderma viride</i> – 36E1	5x10 ⁷ /ml	14 plates/600 ml water
5.	<i>Trichoderma harzianum</i> Plant Shield [®]	100 mg product /10 ml	6 g/600 ml water
6.	Untreated band canker control	---	---
7.	Untreated without band canker (symptomless)	---	---

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. Evaluations of the treatments will be done at the end of August, late September, and again during the winter and in the spring 2005.

Table 6. Biological control treatment for management of band canker on Nonpareil trees in an orchard in Arbuckle, Colusa County (June 17, 2004).

	Treatment	Rate	Field rate
1.	<i>Trichoderma viride</i> – 36E1	5x10 ⁷ /CFU/ml	14 plates/600 ml water
2.	<i>Trichoderma harzianum</i> (Plant Shield [®])	100 mg product/ 10 ml	6 g/600 ml water
3.	Soil mud	---	200 cc wet soil per tree
4.	Untreated band canker control	---	---

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 (as shown in 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 will be done at the end of August, late September, and again during the winter and in the spring 2005.

Table 7. Biological control treatments for management of band canker on Carmel trees in an orchard in Kern County (June 4, 2004).

	Treatment	Rate	Field rate
1.	<i>Trichoderma viride</i> – 36E1	5x10 ⁷ CFU/ml	14 plates/600 ml water
2.	<i>Trichoderma harzianum</i> (Plant Shield®)	100 mg product /10 ml	6 g/600 ml water
3.	Untreated band canker control	---	---

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, the grower designed and had manufactured special metallic splitters which 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 most south side of the orchard. These areas served as controls (without changing the irrigation). On July 17, two rows of 85 to 87 trees each in areas where the irrigation was modified and two rows of the same number of trees in the control areas (where the irrigation was not modified) were recorded for band canker symptoms and the degree of wet surface in the trunk of each tree. Evaluation of the effects of irrigation treatments will be done before harvest, at the end September, and again in the spring of 2005 to determine whether there is any reduction in incidence and severity of the disease.

CONCLUSIONS:

In the first year of this project, significant progress has been made in understanding the biology and aspects of epidemiology of the pathogen causing band canker of almond. A summary of the new findings in the first year 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. Of the insects tested, at least ants may carry and spread *B. dothidea* in almond orchards.
9. In addition to growth cracks, *B. dothidea* can also infect trunks damaged by harvest shakers and woodpeckers as well as pruning wounds.
10. Propiconazole, azoxystrobin, and iprodione prevented canker formation in a greenhouse

experiment.

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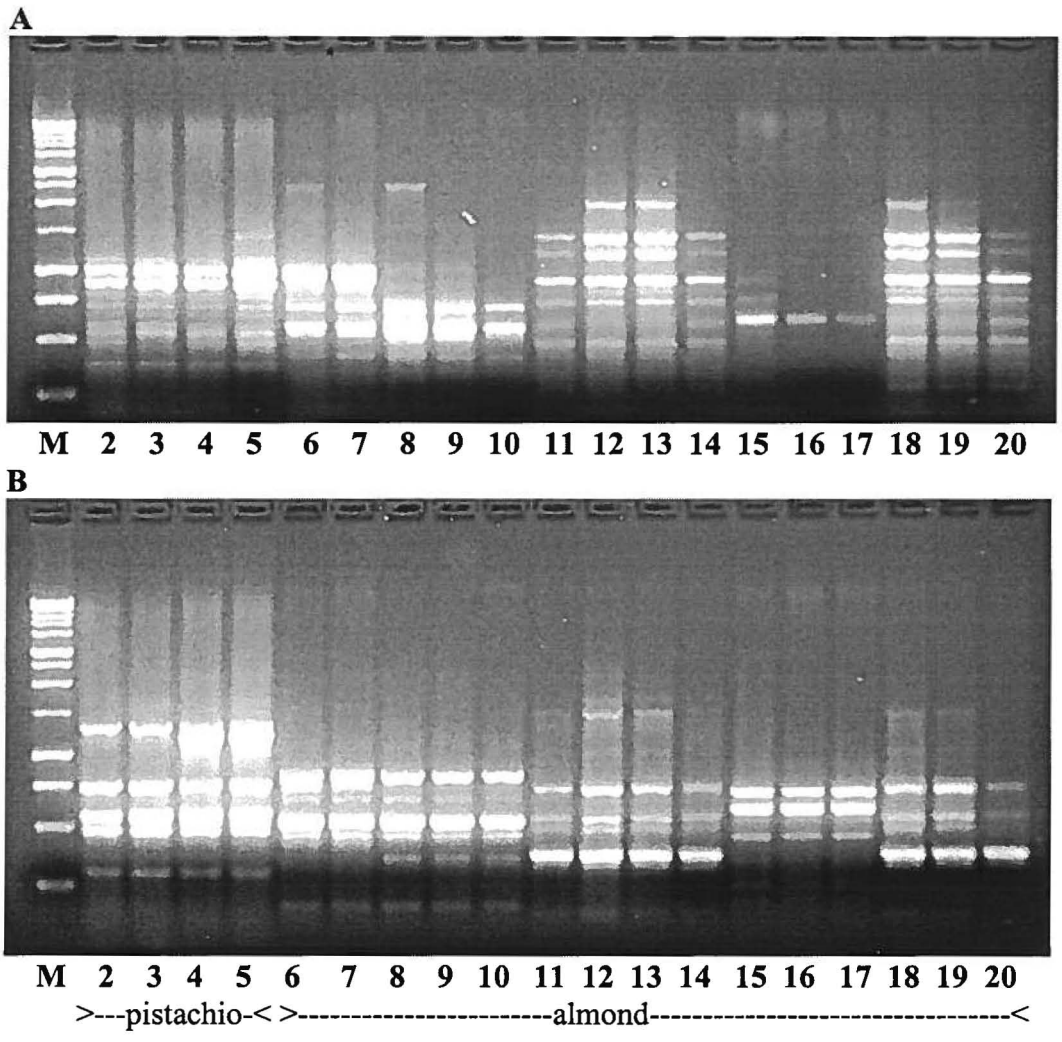


Figure 2. Specificity of the primer pair M13 and T3B, which amplified a fragment from the genomic DNA of *Botryosphaeria dothidea* isolates collected from almonds and pistachios in California. Isolates of *B. dothidea* from pistachio 2-5, and from almonds with band canker from 6 - 14, and 18 - 20. Gels 15, 16, & 17 were from *B. dothidea* isolated from almond trees not showing symptoms of band canker. **A**, using primer M13; and **B**, using primer T₃B. (**M** is a molecular weight marker of 1-kb DNA ladder.)