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

Project No.: 06-PATH9-Michailides

Project Leader: Themis Michailides
Plant Pathologist
University of California, Kearney Ag Center
9240 South Riverbend Ave.
Parlier, CA 93648
(559) 646-6546
themis@uckace.edu

Project Cooperators: D. Morgan, P. Inderbritzin, D. Felts, H. Reyes, and Z. Ma, Kearney Ag Center; J. Connell, UC Coop. Ext., Butte Co., R. Duncan, UC Coop. Ext., Stanislaus Co.; B. Krueger, UC Coop. Ext. Glenn Co.; J. Edstrom, UC Coop. Ext. Colusa Co.: M. Viveros, UC Coop. Ext., Kern Co.; J. Adaskaveg, UC Riverside, and D. McCoy, B. Puget, D. Castle, and D. Elam, Paramount Farms, and J. Post, PCA

Interpretive Summary:

Significant progress has been made in understanding the biology, epidemiology, spread, and management of the pathogen *Botryosphaeria dothidea*, causing band canker of almond. The pathogen *B. dothidea* was confirmed from several commercial orchards in Butte, Glenn, Colusa, San Joaquin, Stanislaus, and Kern Counties from both band cankers on trunk and canopy of trees. Although some isolates of *B. dothidea* from the canopy resemble those from trunk band cankers, the majority of the isolates from the canopy seem to be different strains and/or species. Limbs killed by the new "disease" Lower Limb Dieback harbor both *B. dothidea* and *Phomopsis* spp. However, because pycnidia of *B. dothidea* were not found on these dead limbs, they cannot serve as active sources of spore inoculum. It was confirmed that peduncles and pruning wounds can be infected by *B. dothidea* and should be protected in orchards where *B. dothidea* inoculum is abundant. Periodic inoculations of potted trees for two years showed that the largest cankers were produced during March to May, which is an indication that this time period may be the "critical" period for infection of almond trees. Among fungicides and biological agents sprayed directly on active band cankers in the field, only a biological treatment (Plant Shield) combined with latex paint was the most effective in arresting the cankers. Among fungicides sprayed at petal fall to control infections by *B. dothidea*, only a mixture of Topsin M4.5 and Ziram was effective in reducing dead shoots or fruit per tree.

Objectives:

1. Collect and compare more isolates of *B. dothidea* from tree trunks and upper canopy from various almond orchards.
2. Continue searching for sources of inoculum of *B. dothidea* in almond orchards and surrounding crops.
3. Determine when infections take place.
4. Compare various fungicide treatments as preventative treatments in (a) the field, and (b) in the lath house/greenhouse.
5. Compare methods of irrigation to control band canker.

Materials and Methods:

Etiology, ecology, and epidemiology of band canker:

1. Comparison of isolates of *B. dothidea* collected from trunk and canopy of trees.

During 2005 and 2006 we continued finding infections by the pathogen in the canopy of trees, causing fruit blight and cankers surrounding the fruit peduncle, in addition to the cankers in the trunks. A large number of isolates were collected and stored for further studies. These isolates currently are being compared for morphological and molecular characteristics as well as for growth differences under various temperature regimes.

2. Continue searching for sources of inoculum of *B. dothidea* in orchards and surrounding crops.

A) Lower limb dieback as source of Botryosphaeria? With the help of several cooperating farm advisors, a large number of samples of killed limbs from lower limb dieback (LLD) were collected in 2005 and 2006 and plated on acidified potato dextrose agar. Plates were incubated at 27°C (81°F) and grown fungi recorded after 5 to 6 days. Representative isolates were kept and will be used in pathogenicity studies using potted almond trees and for inoculations of mature trees in the field.

B) Routes of infection. In 2005, we isolated *B. dothidea* from 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 peduncles, suggesting several routes of infection. In order to determine which of these infection courts are most important in the field, we conducted the following inoculation experiments in an experimental Carmel almond orchard at Kearney Agricultural Center using *B. dothidea* isolates collected from the canopy of almond trees in an orchard from Colusa County.

(a) Inoculations of peduncles: Green peduncles were clipped three millimeters (mm) below the peduncle/nut surface (after removing the nuts) and 50 µl of inoculum were pipetted onto cut end of the peduncle and wrapped with Parafilm. Green peduncles were chosen for this experiment.

(b) Inoculations of leaf scars: Green shoots were stripped of leaves and sprayed with the above inoculum to drip and then wrapped with Parafilm.

(c) Inoculations of pruning wounds: The tips of 2- to 3-year-old, woody shoots (1.5 to 3.0 cm in diameter) were pruned, leaving a cut surface about 1 cm diameter, sprayed with the inoculum, and wrapped with Parafilm.

(d) Inoculations of cracks induced in green and woody shoots. Current season and second year shoots were bent and tied in place overnight to encourage development of cracks on their bark. The shoots were inoculated the next morning (September 27, 2005) by spraying the inoculum of *B. dothidea* and wrapped with Parafilm. Evaluation of cankers was made on October 24, 2006, thirteen months after inoculation.

3. Determine when infections take place.

(i) The study to determine when cankers develop the most and whether almond is susceptible to the disease throughout the year we initiated a serial inoculation experiment. In an earlier study we showed that inoculation of potted trees with *B. dothidea* after making a slit with a knife (simulating growth cracks) was the most successful inoculation procedure (see 2004 Annual Report). Thus, each month we inoculated five Carmel potted trees on Hansen rootstock, after making a slit in the bark and inserting a 2 × 0.5 cm agar strip from a culture of *B. dothidea.* All inoculated areas were wrapped with Parafilm. For these inoculations, we used an ascosporic isolate (Bd #3069) and a pycnidial isolate (Bd #3195) recovered from band cankers of almond. Monthly inoculations were done from December 2004 until December 2006 in order to complete two dormant and two growing seasons. A final evaluation was made on June 25, 2007.

(ii) In a second experiment, we wanted to determine the best time for infection under more natural conditions that involve spore release and infection. We constructed a wooden frame about 6 feet tall with a screen to hold almond trunks infected with B. dothidea (source of natural inoculum). Underneath these frames we placed periodically 5 potted trees that were left for 1 to 2 weeks and then replaced with a new batch of 5 healthy Carmel trees. The trees were removed and placed under drip irrigation and will be recorded for infection levels in the fall 2007.

Disease management:

4. Compare various fungicide and biological treatments.

Experiment 1; biological compounds: In an orchard in Arbuckle, an experiment was set June 17, 2004 to test biological control agents. Only active cankers with fresh 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 runoff. Then four layers of cheesecloth pieces about 6 inches × 9 inches 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 rectangular 4-mil plastic piece 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. This last treatment was based on an empirical method used in Japan to control cankers on trunks of apple trees. Final evaluation of this experiment will be done at the end of 2007.

Experiment 2; fungicide and biological compounds mixed with latex paint: On November 1, 2005 in cooperation with Joe Connell, farm advisor in Butte County, we painted the trunks of Nonpareil almond trees in an orchard with high incidence of band canker. 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 had one to several areas of gumming per tree. Glidden Interior flat latex paint (part number HM 1211 Base 1) was diluted 1:4 with water, and fungicides were incorporated into the paint as described in Table 4. The fungicide/paint mixture was applied at the rate of 10 gallons per acre using hand held compressed air sprayers. The paint with or without the fungicides was applied to the trunks from the ground up to the scaffolds. Canker severity was evaluated on November 1, 2005 and August 23, 2006

Experiment 3; fungicide sprays at petal fall to control infections by *Botryosphaeria* in the canopy: We sprayed the canopies of Nonpareil almonds in Colusa County orchard at petal fall on March 1, 2006. There were 7 single-tree replications per fungicide treatment. Fungicides were applied at the rates shown in Tables 5 and 6. The treatments were evaluated on May 25, 2006 and a number of blighted fruit were collected for plating in agar media and isolating the pathogens involved. In addition, we evaluated "mummies" that were hanging on the trees in winter 2006. The experiment was repeated in spring 2007 and will be recorded in winter 2007.

5. Compare methods of irrigation to control band canker.
The irrigation experiment started 2 years ago in Butte County will be monitored for a final time at the end of 2007 growing season. It was not possible to initiate a new irrigation experiment due to the availability of grower cooperators. However, a new experimental almond orchard was planted at the Kearney Agricultural Center for future disease – irrigation experiments and lower limb dieback research.

Results and Discussion:

Etiology, ecology, and epidemiology of band canker:

1. **Comparison of isolates of *B. dothidea* collected from trunk and canopy of trees.** Morphological comparison of isolates of *Botryosphaeria* recovered from the trunk (causing band canker) and those isolated from the canopy (causing shoot and fruit blight and cankers initiated from pruning wounds) indicated that at least we are dealing with more than two species. Two species were identified and included *B.*

dothidea and *B. rhodina.* Although pseudothecia (sexual stage of *B. dothidea* were found in cankers of the trunk, they were not found in cankers of blighted shoots and fruit in the canopy. Although some of the isolates recovered from the canopy are similar to those recovered from band cankers of tree trunk, the majority of those recovered from the canopy look morphologically different than the trunk isolates. Some of the isolates recovered from the canopy resemble those causing panicle and shoot blight of pistachio. The results suggest that more than one strain and/or species of *Botryosphaeria* may be involved as causal agents of the disease (blights and cankers) on the canopy. Isolates from trunk and canopy as well isolates from neighboring walnut and other kind of trees are being prepared for further molecular studies to determine similarities or differences among them and genetic variability. Earlier results indicated that isolates of *B. dothidea* from almond are more variable than those from pistachio (see 2005 annual report), suggesting that controlling band canker in almond may be more difficult than in pistachio. Although we have developed effective control of Botryosphaeria panicle blight of pistachio, a successful fungicidal control method has not been developed against band canker yet.

2. **Continue searching for sources of inoculum of *B. dothidea* in orchards and surrounding crops.**

   A) Lower limb dieback as source of Botryosphaeria? Pathogens isolated from plated samples of limbs showing dieback (LLD) in relatively high incidence included *Botryosphaeria* and *Phomopsis* spp. **(Table 1).** However, the levels of recovery of these fungi varied with location of the samples, almond cultivar, and season of the year that samples were collected. Although pycnidia of *B. dothidea* were not found in any of the samples, pycnidia of *Phomopsis* sp. were common in most of the samples. Therefore, it is questionable whether these dead shoots can serve as sources of inoculum for *Botryosphaeria* spp. However, other spore sources of *B. dothidea* were investigated in previous years and include the bark of trees with band cankers, stumps of removed trees, and other hosts in the proximity of the almond orchard.

   B) Routes of infection. The results **(Table 2)** showed that all inoculated peduncles and pruning wounds were infected with the cankers being longer from infections of pruning wounds than from those of peduncles. Isolations from the lower margins of cankers developed from inoculated pruning wounds revealed consistently *B. dothidea,* confirming that infections developed quickly. However, no pycnidia developed in any of the pruning wounds, which suggests that these infections, although they can cause significant damage, most likely will not serve as sources of spore inoculum. Only 45% of the cracks of woody shoots were infected and developed very small infections. Therefore, peduncles and pruning wounds can be easily infected while cracks can occasionally infected by *B. dothidea.* Interestingly, cracks in green vigorously growing shoots did not develop, suggesting that green tissues of almonds show resistance to infection by *B. dothidea.*

3. Determine when infections take place.

A) The experiment to determine the time of infection was terminated in December 2006, thus completing two dormant and two growing seasons. The inoculations in March to April (2005) and April to May (2006) resulted in the largest cankers (26 to 28 cm) **(Fig. 1),** suggesting that the period from March to May can be the period when most of the infections take place and develop the most. Since there were no significant differences between the cankers caused by the ascosporic and the pycnidial isolates, the results from both the ascosporic and pycnidial isolates were combined and averaged. Therefore, both types of spores (pycnidiospores which are mainly water splashed and ascospores which become airborne) can cause band canker as shown by the lengthy cankers that developed in the potted trees.

B) The experiment on spore release and infection started in December 2006. We are continuing with this experiment having a continuous spore inoculum source, a spore trap, collecting spore trap samples, and exposing the potted trees biweekly. The first spore collections showed that there was a peak of number of spores (10 spores per day in December 2006 and a second peak of 6 to 7 spores in February 2007 (**Fig. 2**). These peaks corresponded with the amounts of rains occurred during these months.

Disease management:

4. Compare various fungicide and biological treatments.

Experiment 1; biological compounds: There were no significant differences between treated and non treated trees and none of the trees died **(Table 3).** In previous experiments in the lath house, we showed that biological treatments can reduce the length of cankers in inoculated potted trees. However, there was not any obvious activity of these biological agents when applied on actively growing cankers against the pathogen.

Experiment 2; fungicide and biological compounds mixed with latex paint**:** Disease was recorded on August 23, 2006. In this experiment, only Plant Shield®, a commercial formulation of *Trichoderma harzianum*, showed a trend in reducing canker severity **(Table 4).** Trunks treated with paint and Plant Shield® had slightly less gum secretions than the other treatments, but these reductions were not significantly different. Since only trees lightly infected or with no cankers were used in this experiment, it seems that Plant Shield and the latex paint might have protected these trees from infection.

Experiment 3; fungicide sprays at petal fall to control infections by *Botryosphaeria* in the canopy: No *B. dothidea* was isolated from the fruit plated on agar media, but a *Phomopsis* species was very common on these dead fruit on May 25, 2006 **(Table 5).** We did observe a trend towards fewer dead fruit with the fungicide treatments, but only trees sprayed with Echo 720 or Topsin M4.5 and Ziram had significantly (P=0.05) lower numbers of dead fruit. When the fungicide treatments were evaluated again on August 23 for dead shoots and fruit, we observed no significant differences among the

treatments but only the treatment Topsin M4.5 + Ziram showed a trend of lower number of dead shoots or fruit per tree **(Table 6).**

In conclusion, the most significant findings of the 2006-07 season are 1) The pathogen *Botryosphaeria dothidea* can attack both trunk and canopy of almond trees and cause disease with different symptoms. 2) Infections that take place during March to May resulted in the most aggressive band cankers (the largest cankers), suggesting that this could be the period when most of the infections take place. 3) Modifying sprinkler irrigation to reduce wetting the tree trunk significantly reduces incidence and severity of band canker. 4) A biological agent mixed with latex paint showed promise in protecting trees from infection and arresting cankers in the field. 5) A mixture of Topsin M4.5 and Ziram reduced dead shoots and fruits. And 6) the majority of the spores of the pathogen are released during the rainy season.

Recent Publications:

Michailides, T.J. et al. 2006. Chemical and cultural control of band canker of almond caused by *Botryosphaeria dothidea*. Pages 188-197 in: 34th Almond Industry Conference Proceedings, Dec. 6-7, Modesto, CA.

Table 1. Incidence of fungi isolated from shoots with **lower limb dieback (LLD) symptoms** in 10 almond orchards in 2005 and 18 orchards in 2006 from Butte, Colusa, Fresno, Glenn, Kern, and Stanislaus Counties.

Pathogens isolated	Isolation frequency (%)
2005	
Botryosphaeria dothidea	**30**
Botryosphaeria rhodina	**20**
Botryosphaeria sp. (not specified)	**20**
Phomopsis sp.	**60**
Colletotrichum acutatum	10
Ceratocystis fimbriata	10
Aspergillus sp.	10
Alternaria sp.	10
Cladosporium	10
Penicillium sp.	20
2006	
Botryosphaeria dothidea	**33**
Botryosphaeria rhodina	**11**
Phomopsis sp.	**44**
Colletotrichum acutatum	22
Botrytis cinerea	6
Pseudomonas syringe	6
Aspergillus sp.	33
Nattrassia mangiferae	11
Alternaria sp.	17
Fusarium sp	28
Trichoderma sp.	6

Table 2. Inoculations of Carmel almonds on September 26, 2005 to determine the mode of infection by *Botryosphaeria dothidea* isolates.

Inoculated tree part[1]	Treatment description	Infection (%)[1]	Length of canker (mm)[2,3]
Peduncle	Green peduncles were clipped 3 mm below the surface; 50 µl of inoculum were pipetted onto the cut end of the peduncle and wrapped with Parafilm	100	19.5
Leaf scars	Green shoots were stripped of leaves, sprayed to drip with the inoculum, and then wrapped with Parafilm	0	0
Pruning wounds	The tips of woody shoots were pruned, leaving a cut surface about 1.5 to 3.0 cm diameter, sprayed with inoculum, and wrapped with Parafilm	100	112
Cracks on woody shoots	Woody shoots were bent and tied in place overnight to encourage development of cracks on shoots. The shoots were inoculated by spraying on the following morning (Sept. 27, 2005) and wrapped with Parafilm	45	4.1
Cracks on green shoots	Green suckers (green succulent shoots) were bent and tied and inoculated as above	0	0

[1] Averages of 10 inoculated tree parts per treatment.
[2] Tissues were inoculated with a 30,000 spores/ml mixture suspension of *B. dothidea*; isolates collected from the canopy of a Nonpareil almond orchard in Colusa County.
[3] Infection and canker length were recorded on October 24, 2006.

Table 3. Efficacy of *Trichoderma* formulations in a bandage treatment of tree trunks showing band canker in a Nonpareil orchard in Colusa County.

Treatment[1]	Rate	Defoliation (% of canopy	Number of gum secretions	Canker size (% of circumference)[2]
T. viride (Italian isolate – 36E1)	$5x10^7$/ml	5.0 a[3]	0.6 c	0.0 a
Plant Shield	100 mg product /10 ml	0.0 a	4.0 ab	8.2 a
Soil	200 cc wet soil per tree	0.0 a	5.2 a	8.2 a
Untreated		13.8 a	2.0 bc	0.0 a

[1] Treatments were applied on June 17, 2004 and recorded on August 24, 2006.
[2] Cankers were evaluated based on signs indicating activity of canker such as, new gumming, fresh discoloration of the bark, etc.
[3] Numbers followed by different letters are significantly different according to LSD test at $P = 0.05$.

Table 4. Effect of fungicide mixtures with white latex paint[1] in controlling almond band canker in a Nonpareil orchard in Butte County.

Treatment[2]	Rate per acre	Rate per Gallon	Efficacy score[3] (severity of canker)	Number of gum secretions
Abound	15.4 fl oz	91 ml	0.9 a[4]	4.9 a
Pristine	14.5 oz	82 g	0.5 ab	3.0 a
Captan 4L	1.125 Gallon	851 ml	0.6 ab	6.1 a
Plant Shield	2.5 lb	227 g	0.3 b	2.2 a
Control; paint only	---	---	0.7 ab	2.9 a
Control; no paint	---	---	0.5 ab	2.3 a

[1] Flat latex paint was diluted 1 to 1 with water, and sprayed at 10 gallons per acre.
[2] Fungicides were applied on Nov. 1, 2005 and recorded on Aug. 23, 2006.
[3] Efficacy score is the average of ten replicate trees on the basis of 0 = normal healthy tree, 1 = lightly infected tree (one to six small spots with gum), 2 = moderately infected tree (5 to 10 larger spots with gum), 3 = severely infected tree (large cankers with raised and roughened bark at borders, and 4 = dead tree.
[4] Numbers followed by different letters are significantly different according to LSD test at $P = 0.05$.

Table 5. Efficacy of a fungicide application against Botryosphaeria shoot and fruit blight in a Nonpareil almond orchard in Colusa County.

Fungicide treatment [1]	Rate per acre (100 gallons per acre)	Number of dead shoots per tree	Number of dead fruit per tree (*Phomopsis* sp.)[2]
Echo 720	4 pints	0.0 a	8.7 b
Echo Ultimate	3.6 lbs	0.4 a	14.6 ab
Topsin M 4.5 Ziram	20 fl oz 8 lbs	0.0 a	12.0 ab
Topsin M 4.5 Ziram	30 fl oz 8 lbs	0.1 a	6.9 b
Pristine	14.5 oz	0.0 a	11.6 ab
Control	Untreated	0.4 a	20.3 a

[1] Fungicides were applied at petal fall stage on March 1, 2006 and disease was recorded on May 25, 2006.
[2] *B. dothidea* was not found in these fruit at this time; instead a *Phomopsis* sp. was isolated.
[3] Numbers followed by different letters are significantly (P=0.05) different according to LSD test.

Table 6. Efficacy of a March 1, 2006 (at petal fall) fungicide application on dead shoots and fruit of Nonpareil almonds in Colusa County (recorded on August 23, 2006).

Treatment (fungicide(s))	Rate per acre (100 gallons per acre)	Number of dead shoots or fruit per tree[1]
Echo 720	4 pints	8.0 ab
Echo Ultimate	3.6 lbs	15.7 a
Topsin M 4.5 Ziram	20 fl oz 8 lbs	4.4 b
Topsin M 4.5 Ziram	30 fl oz 8 lbs	10.6 ab
Pristine	14.5 oz	8.3 ab
Control	Untreated	13.1 ab

[1] Shoot blights are due mainly to *Botryosphaeria* and *Phomopsis* species and *Colletotrichum acutatum*.

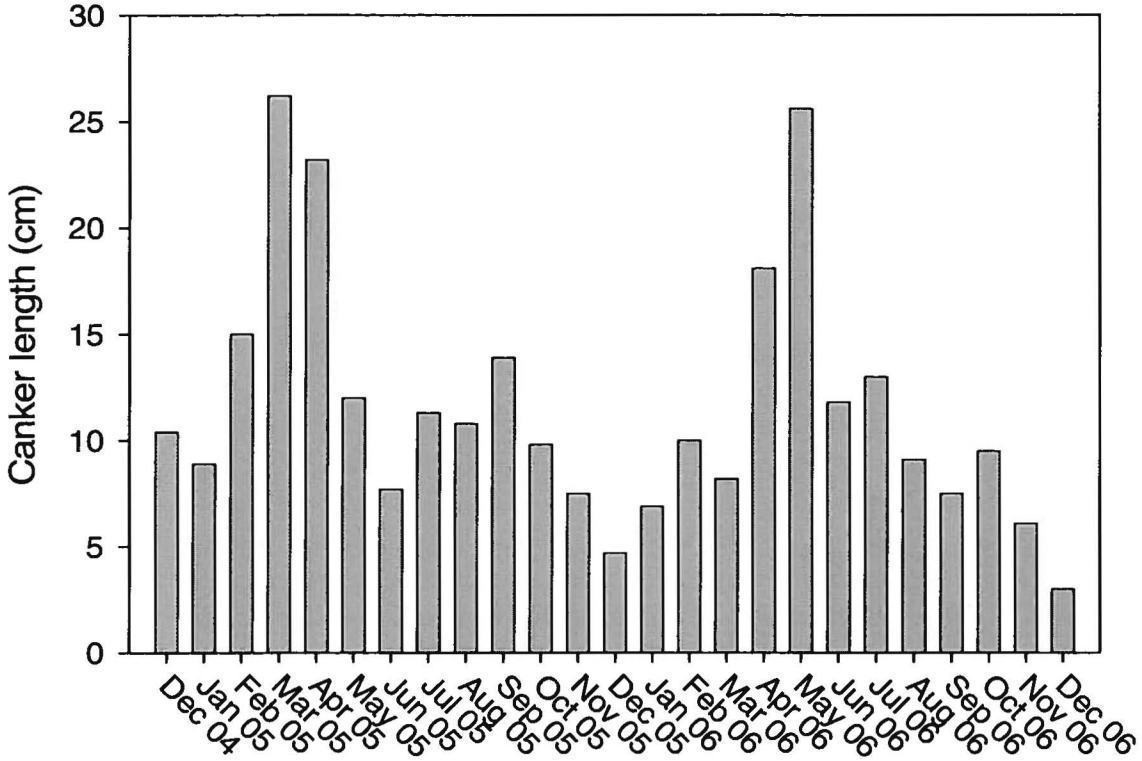

Figure 1. Monthly inoculations of potted Carmel almond trees for two years (2005 and 2006) with an ascosporic and pycnidial isolate of *Botryosphaeria dothidea* isolated from a band-cankered tree.

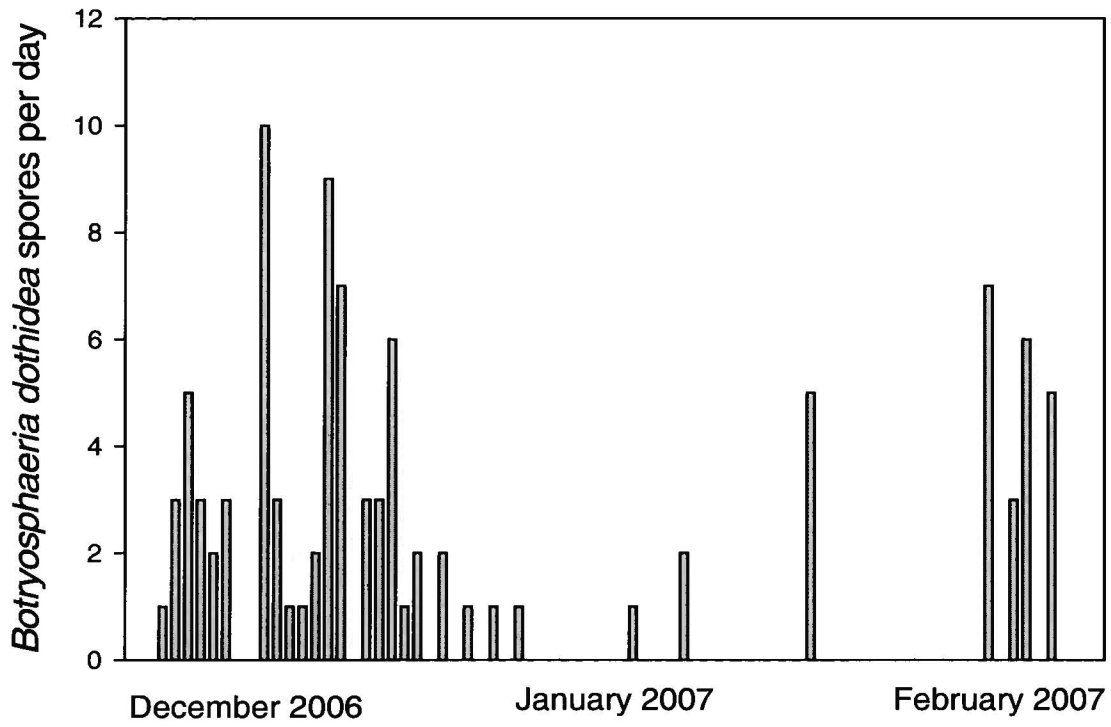

Figure 2. Amounts of spores conidia and ascospores of *Botryosphaeria* collected per day on spore trap tapes recorded daily through December 2006 and every other day thereafter. (This experiment is still in progress but spore trap samples were recorded up to February 2007.)