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NEW FINDINGS ON BAND CANKER OF ALMOND CAUSED BY *BOTRYOSPHAERIA DOTHIDEA*

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Abstract

This study revealed several new findings that may be important in the epidemiology and control of band canker of almond. 1) The disease was found more frequently, and on several almond cultivars in addition to Nonpareil in the last few years. 2) The pathogen *Botryosphaeria dothidea* (Moug.:Fr.) Ces. & De Not. from band canker shows major differences from, and more genetic diversity than, that causing panicle and shoot blight of pistachio. 3) *B. dothidea* from almond can infect and cause panicle and shoot blight on pistachio while *B. dothidea* from pistachio can cause band canker on almond. And 4) inoculum of *B. dothidea* was not found in orchard debris, although there is a possibility that arthropods (i.e., ants) may be involved in the spread of the pathogen.

Introduction

Band canker, otherwise known as Dothiorella canker, was reported years ago as a problem in California almonds (English et al., 1966 & 1975). Its occurrence has been very sporadic in the northern San Joaquin and Sacramento Valleys of California. However, in the last few years several commercial orchards have reported damage by band canker. For instance, in 2001-03 orchards in Stanislaus, San Joaquin, and even as far south as Kern County have been reported with band canker. An example of the damage is depicted in an orchard in Kern County where 1200 trees were removed by the grower in 2002 because of band canker and more than 500 trees were scheduled for removal within 2003. Isolations from the bark of trees

showing symptoms consistently revealed an asexual form of *B. dothidea*, a *Fusicoccum* sp. [initially reported as *Dothiorella* (English et al., 1966 & 1975).

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 horizontal 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. Although tree death is reported to be rare, in the orchard in Kern County where the problem was found in 2001, a large number of infected trees were killed. Discolored sapwood often extends longitudinally several centimeters beyond the canker margin. 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 *Botryosphaeria dothidea* (syn. *B. ribis* Gross. & Duggar) is a cosmopolitan fungal pathogen. It can attack numerous hosts including agricultural, ornamental, and forest crops (Smith, 1934). Thus far, only the *Fusicoccum* sp. (asexual stage) of *B. dothidea* has been found on almond. The pathogen can produce pycnidia in almond tissue and/or media, although a large portion of isolates may not produce any pycnidia in culture media.

When epidemics of the disease occur, *B. dothidea* infection can cause major yield losses in some of the crop. For instance, a disease that kills the fruit clusters and shoots of pistachio caused by *B. dothidea* 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). Considerable research was conducted from 1998 to 2003 to develop control methods for the panicle and shoot blight of pistachio, caused by a *Fusicoccum* sp. of *B. dothidea*. After major efforts of multifaceted research, the growers now have effective chemical and cultural control methods for this disease. In addition, the biology of the pathogen, its sources in pistachio orchards, and the development and epidemiology of the disease are understood much better.

Epidemiology. The epidemiology of band canker has not been described. 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. The disease occurs in vigorous Nonpareil trees, Carmel, 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

1. Isolation of the pathogen and determination of its genetic structure. 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 collection is 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 1). Comparing the pathogen causing band canker on almond with other *B. dothidea* would give us an indication regarding the various sources of the pathogen. 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, since in some areas pistachios and almonds are grown next to each other. In an initial test, using a small sample of isolates of *B. dothidea* from pistachio and almond and using polymerase chain reaction primers M13 and T₃B, we found that the almond isolates are different from those of pistachio (Figure 1). In a previous study, Ma et al. (2001) found that the pistachio isolates are very uniform genetically and very similar with 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 (Figure 1). 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 that may need more

aggressive methods of control than the pistachio pathogen.

2. Cross pathogenicity. Cross pathogenicity studies involve the inoculation of one host with the isolate from another host. When pistachios were challenged with almond isolates of *B. dothidea*, the pistachios became infected and developed panicle and shoot blight. When almond was challenged with pistachio isolates, almonds became infected and developed band canker. Another 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 were inoculation by making a slit of 5 cm long and 2 mm wide on the bark of each tree stem and inserting a longitudinal mycelia strip from a culture of *B. dothidea* (isolate from almond); making five longitudinal slits (5 cm long 2-3 mm wide) and spraying a spore suspension (50,000 spores/ml) 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.

3. 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 on acidified PDA plates. 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, the 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, although not all the samples have yet been examined.

If debris was not the source of the inoculum, we suspected that ants might carry inoculum. We collected about 40 ants crawling on the soil and the trunk of an infected tree, brought them to the laboratory, froze them to kill them, and plated them 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. Although these results are preliminary, they suggest that there is a possibility that arthropods may bring propagules to growth cracks of almond and contribute to the spread of the pathogen. This hypothesis will be investigated further this fall and next spring. We plan to collect more ants or other insects from almond orchards where band canker has been reported, and from surrounding other possible hosts of *B. dothidea* (Table 1) close to almond orchards to compare the almond isolates of *B. dothidea* with those from other hosts. It is possible that the pathogen causing band canker is 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 isolates from almond from different locations and cultivars and from other hosts that are in the proximity of almonds.

Acknowledgments

We thank the following farm advisors, Mario Viveros, UC Coop. Ext., Kern County; Joe Connell, UC Coop. Ext., Butte County; Roger Duncan, UC Coop. Ext., Stanislaus County; and Bill Krueger, Farm UC Coop. Ext. Glenn County; also we thank Dennis McCoy, Bernard Puget, Don Castle, and Dennis Elam, Paramount Farms, for their cooperation in this project, and the California Almond Board for financial support of this project.

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Table 1. 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
Deodar cedar	<i>Cedrus deodara</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>	Fagaceae
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.

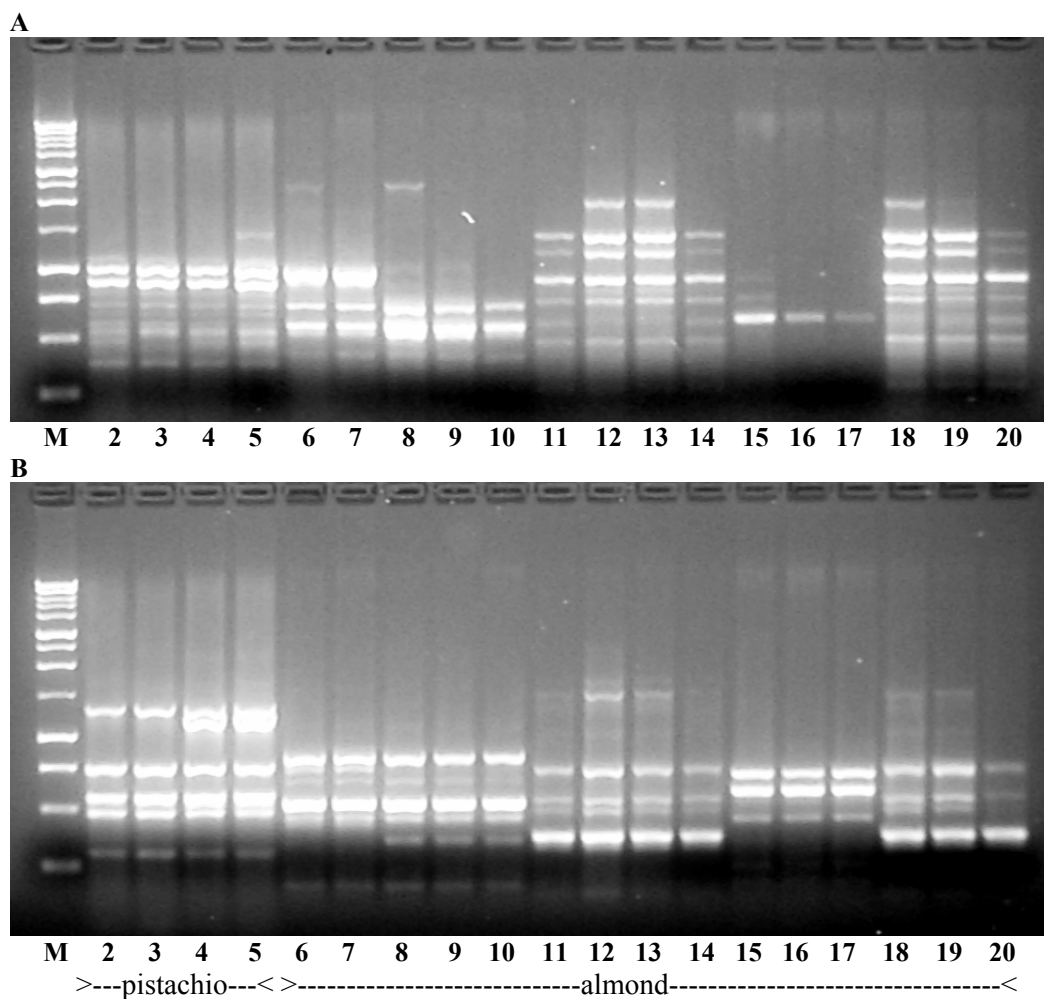


Figure 1. Specificity of the primer pair M13 and T3B, which amplified a fragment from the genomic DNA of *Botryosphaeria dothidea* isolates collected from almonds and pistachios in California. Isolates of *B. dothidea* from pistachio 2-5, and from almonds with band canker from 6 -14, and 18 - 20. Gels 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.)

ABSTRACTS

SOCIETY OF NEMATOLOGISTS, 42nd Annual Meeting, Ithaca, NY, July 12-17 2003.

Nematicidal Activity of Walnut Extracts Against Root-Knot Nematodes.

McKenry, M.V, and S.A. Anwar, Department of Nematology, University of California-Riverside and Kearney Agricultural Center

Reducing branches, limbs, trunks, and roots of *Juglans* spp. to a powder of less than 100-mesh can provide a water extractable tea. The powder exhibits a specific gravity greater than 1.0, enabling easy separation of

particulates from the tea. *Juglans* spp. contain antioxidants, phenolic compounds, tannins, and other soluble ingredients that, in combination, produce a nematicidal effect. A bioassay was performed to investigate the nematicidal activity of walnut extract against second stage juveniles (J2) of *Meloidogyne incognita* at 24, 48, and 144 hours after exposure to various extract concentrations. Freshly hatched J2 were placed into sealed plastic vials at four concentrations of *Juglans* tea, including 50, 25, 10, and 2.5 g/l. The three higher concentrations consistently caused greater mortality, compared to the 2.5 g/l concentration and a water control. Forty-eight hours later, the two higher concentrations resulted in 100% nematode mortality with vacuoles observed in the intestinal region. The 10 g/l concentration took 96 additional hours to produce

100% nematode mortality. By comparison, synthetic juglone provides 100% kill after 48 hr exposure to 1 g/l. In a microplot setting, newly planted grapevines inoculated with *M. incognita* J2 received 50 g/l *Juglans* tea six times over a two-year period. Nematode control approximated 75%, equivalent to the standard phenamiphos comparison. Phytotoxicity was not observed with repeated applications of the tea. This tea product provides the opportunity for delivery of botanically derived nematicidal agents deep into the soil profile around roots of perennial crops.

Mechanisms That Reduce Nematode Development in New Grape Rootstocks.

Anwar, S.A. and M.V. McKenry, Department of Nematology, University of California-Riverside and Kearney Agricultural Center

A decade-long search among *Vitis* spp. for broad and durable nematode resistance culminated in the finding of five new sources from four different parentages. Experiments were initiated to quantify and observe nematode/host interactions during nematode penetration, development, and reproduction. The five sources of resistance were effective against all aggressive *Meloidogyne* populations but also nematodes of other genera. What features were common to the new resistance sources? All the new sources display a plant hypersensitive response (HR) that reduces entry of infective juveniles at the root tip. This HR involves a greater number of plant cells than previously available among commercial *Vitis* rootstocks. Contemporary *Vitis* resistance yields only enough HR to stop the first four or five infective juveniles but aggressive populations enter in greater numbers. The root tip is also a favored feeding site of *Xiphinema index*, another reason to locate adequate HR there. During the period from nematode feeding to reproduction these five new resistance sources also exhibit additional defense mechanisms unavailable in contemporary grape rootstocks. Several resistance sources induced necrosis of feeding tissues within cortical cells, which restricted female development and overall egg production. One source resulted in a dissolving of the adult female within newly formed galls. Several sources limited the size of the syncytium and gall thus limiting long-term feeding by *Meloidogyne* to young roots. Selection for multiple resistant mechanisms in multiple locations along the root and over time provides resistance to a broader collection of nematode genera including aggressive *Meloidogyne* populations, *X. index*, *Pratylenchus vulnus*, and

Tylenchulus semipenetrans. Resistance to this many different nematode species has not previously been available.

STATEWIDE CAPCA CONFERENCE, Sparks NV, October 19-21, 2003.

Development of a Field Key to the Most Common Lygus Species Found in Agronomic Crops of the Central San Joaquin Valley of California

Shannon C. Mueller, Charles G. Summers, and Peter B. Goodell, UCCE Fresno County, Dept. of Entomology, University of California-Davis, UC Kearney Agricultural Center, IPM Advisor, UC Kearney Agricultural Center

Lygus bugs are a major pest of many field, vegetable, and orchard crops grown in the Central San Joaquin Valley. Their feeding causes damage that significantly reduces the yield and quality of commercial crops. There are 43 species of *Lygus* in the world, 34 of which are known to exist in North America. Within the San Joaquin Valley, three species of *Lygus* have been recorded. These are *Lygus hesperus*, *L. elisus*, and *L. lineolaris*.

Proper identification of an insect is the first step in a successful pest management program. Furthermore, as research continues on biological control strategies for *Lygus* and development of resistance, it becomes important to know the species composition in a region.

A survey of agronomic crops was conducted in three different landscapes in Madera, Fresno and Tulare Counties. Changes in species composition related to crop, landscape, and collection period were evaluated. In 2002, surveys showed that 93% of the lygus found in agronomic crops were *L. hesperus*, and 7% were *L. elisus*. There were no *L. lineolaris* found in any of the crops during the production season. Based on 2002 data, there could be some species preference for certain crops, but no change in species composition related to when specimens were collected during the season. The survey was repeated in 2003 and results will be included with those from 2002.

A key has been developed to separate the three *Lygus* species found in the San Joaquin Valley. The key is based on characteristics of the male lygus bug that are easy to observe with minimal magnification. The key is easy to understand and is well illustrated, highlighting the morphological features used to separate the species.

ANNUAL AMERICAN PHYTOPATHOLOGICAL SOCIETY MEETING, Charlotte, NC, August 9-14, 2003.

Best time period for determining latent infection of dried plum caused by *Monilinia fructicola*.

Y. Luo and T.J. Michailides, Dept. of Plant Pathology, University of California-Davis, UC Kearney Agricultural Center

Inoculation experiments were conducted periodically from full bloom to the first harvest stages in 10 California dried plum orchards. In each inoculation, 20 branches were selected, and each fruit was injected with a spore suspension of *M. fructicola*. The incidence of fruit rot per branch developed in inoculated fruit was recorded at different times during the season until before harvest. A linear regression between the rate of development of fruit rot and days after full bloom was obtained. The earlier the fruit inoculation, the slower the development of fruit rot. A diagram was produced to show whether determination of latent infection is not recommended, recommended, or strongly recommended. These recommendations are also based on whether low, moderate, or high levels of spore inoculum potential exist in a dried plum orchard.

Resistance to Azoxystrobin in *Alternaria* Isolates from Pistachio in California

Zhonghua Ma, Dan Felts, David P. Morgan, and Themis J. Michailides, Department of Plant Pathology, University of California-Davis, Kearney Agricultural Center

Azoxystrobin was ineffective in controlling *Alternaria* late blight of pistachio in a fungicide experiment conducted in an orchard in Tulare County and in a commercial pistachio orchard in Kern County, CA, in 2002. All 58 isolates of *Alternaria alternata*, *A. tenuissima*, and *A. arborescens*, causal agents of *Alternaria* late blight of pistachio, collected from these two orchards were highly resistant to azoxystrobin, with the effective dose for 50% inhibition of the conidial germination (EC_{50}) greater than 100 $\mu\text{g/ml}$. However, none of 14 wild-type isolates collected from pistachio orchards without a previous history of strobilurin usage were resistant to azoxystrobin and they had EC_{50} ranging from 0.008 to 0.045 $\mu\text{g/ml}$. Strobilurin resistance in *Alternaria* isolates was conferred by a single change of glycine to an alanine at amino acid position 143 in the

cytochrome b (cyt b) gene. Since there were no differences in the DNA sequence of the partial cyt b gene from *A. alternata*, *A. tenuissima*, and *A. arborescens*, a pair of PCR primers specific to AAF and AAR was developed to amplify a 226-bp DNA fragment containing the mutation site in the cyt b gene. The primers amplified the expected DNA fragment from all tested *Alternaria* isolates, but not from 30 other fungal species isolated from pistachio trees. The restriction enzyme Fnu4H I recognized the sequence GCTGC in the PCR product from resistant isolates only, but not the sequence GGTGC from sensitive isolates. A rapid DNA extraction method and the method of PCR-restriction fragment length polymorphism (PCR-RFLP) were developed to rapidly detect azoxystrobin resistant *Alternaria* isolates from California pistachio orchards.

Identification and Characterization of Benzimidazole Resistance in *Monilinia fructicola* from Stone Fruit Orchards in California

Zhonghua Ma, Michael A. Yoshimura, Yong Luo, and Themis J. Michailides, Dept. of Plant Pathology, University of California-Davis, and UC Kearney Agricultural Center

Low and high levels of resistance to the benzimidazole fungicides, benomyl and thiophanate-methyl, were observed in field isolates of *Monilinia fructicola* in California, causing brown rot of stone fruit. Low resistant (LR) and high resistant (HR) isolates were also cold and heat-sensitive, respectively. Results from microsatellite DNA fingerprints showed that the genetic identities among the populations of sensitive (S), LR, and HR isolates were very high (> 0.96). Analysis of DNA sequences of the beta-tubulin gene showed that the LR isolates had a point mutation at the codon 6, causing substitution of amino acid histidine by tyrosine. The codon 198, which encodes a glutamic acid in S and LR isolates, was converted to a codon for alanine in HR isolates. Based on these point mutations in the beta-tubulin gene, allele-specific PCR assays were developed for rapid detection of benzimidazole-resistant isolates of *M. fructicola* from stone fruit.

Preseason Risk Assessment of Panicle and Shoot Blight of Pistachio in California.

A.L. Mila, D.P. Morgan, D. Felts, and T.J. Michailides. Dept. of Plant Pathology, University of California-Davis and Kearney Agricultural Center.

Panicle and shoot blight, caused by *Botryosphaeria dothidea*, is a destructive disease of pistachios in California. Between 1996 and 1999, the incidence of pistachio buds infected with *B. dothidea* was recorded in several pistachio orchards in February and March. The number of blighted fruit clusters and shoots was also recorded in the same orchards in late summer before harvest. Four classes of *Botryosphaeria* risk (low, moderate, moderate to high, and high) were defined from the number of blighted clusters and shoots. The incidence of infected buds in early spring, the predicted temperature and precipitation from April to September were used as inputs in a Poisson regression to estimate disease risk in the recorded fields. 2^p models were examined, where p is the number of explanatory variables. Estimated risk using the best fitted model differed from the observed disease risk in only 20% of the fields. The system is currently under validation.

ANNUAL INTERNATIONAL RESEARCH CONFERENCE ON METHYL BROMIDE ALTERNATIVES AND EMISSIONS REDUCTIONS, San Diego, CA, November 3-6, 2003

Lethal Temperature-Time Dosages for *Meloidogyne Incognita* in Soil

Tarcisio S. Ruiz, James J. Stapleton, and Michael V. McKenry, University of California, Kearney Agricultural Center

Constant temperature-time dosages were applied to soil infested with cotton root knot (*Meloidogyne incognita* = Mi). Nematodes in soil were extracted after heat treatment (E1). Experimental soil was then bioassayed using grape cuttings, and further extractions from soil (E2), and roots (E3), and a root galling (RG) assessment were done to confirm nematode mortality. Values for lethal dosages were determined for Mi using data from E1. LD₉₅ values were 813, 281, and 32.4 min at 39, 42, and 46 °C, respectively. The E2, E3, and RG ratings showed longer time requirements to achieve LD₁₀₀ than E1. Observed LD₁₀₀ values for Mi in RG were <5, 15, 22, 1440, and 5040 min at 70, 60, 46, 42, and 39°C, respectively.

This study was conducted using 300 g aliquots of natural field soil. The rationale was that, although the presence of a confounding lag time to reach target temperatures was unavoidable, the study was conducted under natural conditions of the experimental soil, with all the physical, chemical, and biological interactions germane to the microenvironment allowed to impact during the entire trial process upon the results obtained. The goal was to provide users of soil solarization, and other heat-based methods of nematode control, with guidelines for treatment applications.

The LD₉₅ information can be a valuable tool for those interested in solarizing open fields. On the other hand, the observed LD₁₀₀ values represents important information for nursery plant production users of “double-tent” solarization, where control of 100% of nematodes is required (Calif. Dept. of Food and Agriculture, 2002).

The temperatures selected for this study are regularly observed in soil when solarization is applied in open fields, or in the “double-tent” technique for soil disinfestation in nursery soils. Temperatures even higher than 70°C have been achieved during summers in the San Joaquin Valley of California, using this technique (Stapleton *et al.*, 2002).

It was not possible to determine the LD₉₅ values for temperatures of 70 and 60°C. At both temperatures, the minimum heat exposure tested was five minutes, and this time-temperature combination was lethal for *M. incognita*.

Portions of this study not reported here confirm that lethal temperature-time data need to be collected for each nematode species, in order to be able to accurately predict efficacy of heat-based methods of nematode control.

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Allium Spp. Amendment, Temperature, And Time Affect Weed Seed Viability In Soil

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A microcosm study was conducted during 2000-01 to evaluate soil-incorporated *Allium* spp. amendments for effects on weed seed populations. Ground and dried residues of onion (*Allium cepa*) and garlic (*A. sativa*), at concentrations of 1% and 3% w/w, were evaluated at soil temperatures of 23 C and 39 C, and exposure times of 0, 2, 4, and 7 days for their herbicidal effects on seeds of barnyardgrass (*Echinochloa crus-galli*), common purslane (*Portulaca oleracea*), London rocket (*Sisymbrium irio*), and black nightshade (*Solanum nigrum*). The 2000 experiment was conducted as a completely randomized split-plot design. Treatments consisted of the amendment concentrations described above and were replicated four times. Plots were split by soil temperature 23 C vs. 39 C. The second experiment was performed in 2001 with modifications: the design was changed to a randomized complete block design and sample number and size were increased.

Results indicated consistently deleterious effects of warm (39 C) soil temperature on seed survival as opposed to ambient temperature (23 C). Significant reductions in seed viability were common when weed seeds were exposed to soil-incorporated onion and garlic residues. No differences in weed seed viability due to soil amendment with onion versus garlic were found in the 2000 experiment, and only in barnyardgrass and black nightshade in 2001. Seed viability differences due to amendment concentration were found in barnyardgrass, black nightshade, and London rocket in the 2000 experiment, but only small viability differences related to concentration were found in the 2001 experiment. In both experiments, barnyardgrass, common purslane, and London rocket seeds were less viable after longer incubation in the microcosms, while black nightshade was not significantly affected by exposure time.

A number of interactions among the tested factors of time, temperature, amendment, and rate produced significant differences in seed viability both years. Interactions of higher temperature x increasing amendment rate and higher temperature x longer exposure time had the most consistently deleterious effects on seed viability.

The results indicated that *Allium* spp. soil amendments, especially at elevated soil temperature, may contribute to decreased populations of viable weed seeds in soil.

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New Chisel Shanks Enable Improved Fumigation Of Finer-Textured Soils

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Production of an adequate supply of fast-growing, budded, nematode-free nursery stock for orchard and vineyard land requires that nematodes in the surface five-feet be controlled prior to seeding. There are difficulties in delivering soil fumigants throughout a five-foot field surface, but successful production of 14 to 26 month nursery crops depends on this level of nematode control. Methyl bromide (MB) properly applied at 300 to 450 lb/ac can achieve this goal. Telone II (1,3-D) properly applied at 330 lb/ac with a tarp or 480 lb/ac as a dual application can also provide success, but only in coarser-textured soils dried to a level of -45 cb moisture tension. This is because the 1,3-D molecule degrades quicker than MB but also has a higher affinity for water. For 1,3-D applications made to sandy loam soils this level of drying translates to less than 12% soil moisture content throughout the surface five-feet. A finer-textured soil deeply dried to -45 cb can commonly hold 12-20% moisture content throughout the surface 5-

feet. Fumigation of these soils can be successful but treatment rates of 500 to 670 lb/ac 1,3-D are essential.

Beginning in 2001, various 1,3-D treatments were applied to clay loam nursery soils of the Sacramento Valley. Compared to MB, the use of 1,3-D in regions north of San Francisco was never popular. Fields near Yuba City and Davis, CA that had not been in permanent crops for two years were selected. Fields were dried with a cropping of winter wheat and then ripped 30-inches deep. To meet California labeling requirements the fields were required to receive ~1.5-ac-in water applied at the field surface. This water delivery is required to impede 1,3-D off-gassing but it actually impedes 1,3-D performance so these field surfaces were not moistened prior to fumigation. Sixty-days after treatment we followed our standard procedure of soil sampling each replicate at one-foot increments to five feet deep. Results depicted for 2001 in Table I revealed that even when soils had been dried to 14-20% soil moisture the addition of higher treatment rates plus a tarp gave inadequate performance. Poorest control occurred deeper in the soil as depicted in Table II. It was also apparent when the tarps were removed from the field that closure of the chisel shank traces was inadequate.

In consultation with the junior authors, new delivery shanks were built. Shanks were designed to deliver fumigant 8-inches deeper than the prevailing depth requirement of 22-inches. Approximately 6 inches above the 30-inch depth a narrow delta wing attachment was welded to each shank. It was positioned so that it scraped soil from the side-walls into the shank trace beneath. A second delta wing was positioned 16 inches

above the delivery depth and a third one above that. In order to pull a series of five shanks through the soil at 24-inch spacings the field had been ripped to 48-inch depth in three directions. Immediately following passage of the shanks a second tractor pulling a disk and ring roller assured complete filling of the shank traces. Compaction and filling of these shank traces was verified by random insertion of a penetrometer across the field surface. Finally, the placement of glued HDPE tarps or application of 20 gpa K-pam in front of a rotavator and press wheel completed the treatments.

Soil samples collected 60 days after treatment are reported in Tables I and II. The 2002 treatment was planted to peach seeds in November 2002. Trees will be grown for two years to evaluate performance of 1,3-D at several rates in addition to various combinations of iodomethane and chloropicrin.

Shortcomings to the realization of this new equipment:

- Larger equipment to pull these designated shanks.
- Deeper soil preparation required.
- CaDPR approval needed for higher 1,3-D treatment rates.
- Verification of these findings in additional field sites.

Attributes and spin-offs of the findings:

- A method of emission reduction that growers might prefer in lieu of current surface moisture requirements (if permitted by CaDPR).
- In 2005 the methodology reported here appears as the only MB alternative for finer-textured vineyard and orchard lands of California.

Table I. Nematode control in clay loam soil expressed as a % of nontreated.

	<u>% Parasitic</u>	<u>% Free-living</u>
UC Davis Trial, 2001-02		
Telone II at 332 lb, tarped	87%	39%
Telone II at 530 lb, tarped	96%	65%
Telone II at 670 lb, tarped	95%	82%
Non-treated, (actual numbers)	(213 nemas)	(114 nemas)
Yuba City Trial, 2001-02		
Telone II at 332 lb, tarped	97.90%	90%
Telone II at 500 lb, tarped	99.00%	99.50%
Telone II at 670 lb, tarped	99.00%	99.60%
Non-treated, (actual numbers)	(107 nemas)	(356 nemas)
	<u>tarped</u>	<u>K-pam*</u>
Yuba City Trial, 2002-04		
Telone II at 332 lb, new shank	98%	98.40%
Telone II at 500 lb, new shank	95.00%	98.80%
Telone II at 670 lb, new shank	98.50%	100.00%
Midas + Pic 200 ea., new shank	98.30%	99.80%
Telone II + Pic 332 lb ea**	99.60%	99.60%
Methyl Bromide 400 lb, 12 inch	96.40%	0
Non-treated (actual numbers)	(37 nemas)	(17 nemas)

* K-pam applied at 20 gpa before rotavator to surface 5 inches

** Pic applied at 30 inches (new shank) and Telone at 20 inch depth

Note: A ring roller and disc followed fumigation shanks except with MB. Each % value above emanates from 20 individual soil samples

Table II. Location of surviving nematodes in the soil profile.

		<u>Mean nematode survival /250 cc soil at five depths*</u>				
		<u>0-1 ft</u>	<u>1-2 ft</u>	<u>2-3 ft</u>	<u>3-4 ft</u>	<u>4-5 ft</u>
Telone II at 332 lb						
old shank	Davis, 2001-Tarp	1.75	0.75	43.5	26.25	7.25
old shank	Yuba City, 2001-Tarp	0	0	0	8	13
new shank	Yuba City, 2002-Trp	2.5	0	0	0	0.25
new shank	Yuba City, 2002-MP	0	0	0.25	0	2
Telone II at 500 lb						
old shank	Davis, 2001-Tarp	0.5	0.25	0.75	15	8
old shank	Yuba City, 2001-Tarp	0	0	0	5	17
new shank	Yuba City, 2002-Trp	0.5	0	0	0.25	6
new shank	Yuba City, 2002-MP	0	0	0	0	1.75
Telone II at 670 lb						
old shank	Davis, 2001-Tarp	0.5	0	1.5	16.75	12.25
old shank	Yuba City, 2001-Tarp	1.5	0.5	0	9	12
new shank	Yuba City, 2002-Trp	0.25	0	0	0	1.25
new shank	Yuba City, 2002-MP	0	0	0	0	0

* each value above is the mean of four soil samples

Note: Soil moisture levels ranged from 14 to 20% across surface five feet of soil

