University of California Division of Agricultural Sciences

Progress report

Project Year 2024 Anticipated Duration of Project _____

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Project Titles: Detection of Fungicide Resistance in Populations of *Venturia pirina* in California Pear Orchards; Identification, Distribution, and Management of Fungal Species associated with Branch Canker and Dieback Disease of Pear in California

Keywords: Branch canker, Dieback, Scab, Fungicide Resistance

Commodity(s) Pear

Relevant AES/CE Project No.

OBJECTIVES

Objective 1. Identification and distribution of fungal species associated with branch canker and dieback disease in California's pear orchard regions.

Objective 2. Evaluate the effectiveness of various registered synthetic and biological pruning wound protectants in pear orchards.

PROCEDURES

Field Sampling and Fungal Identification. In the fall of 2023, field surveys were conducted in eleven Northern Californian ‘Bartlett’ pear orchards throughout Mendocino County (*n* = 4), Lakeport County (*n* = 4), and Sacramento/Delta County (*n* = 3). The average age of the trees in all the orchards was 60 years. In each orchard, approximately 10 trees were samples during each sampling period with branches showing cankered tissue. A total of 302 branches were collected and brought back into our laboratory for further evaluation. Small pieces of tissue from lesion margins were placed onto acidified potato dextrose agar (APDA) media and incubated at 25°C in the dark for 7-10 days. Morphological assessments were used to determine fungal identity using colony morphology and conidial characteristics. Additionally, molecular identification, through a

molecular phylogenetic approach using a multilocal approach of at least three informative gene regions (ITS, *tef1,* and *tub2,*) was used to confirm the species identity of the fungi.

Pruning Wound Protectant Field Trial. Field trials were conducted in two different pear orchards in two locations (Mendocino and Sonoma County, respectively) using a completely randomized block design to protect pruning wounds using the following synthetic and biological products that have shown efficacy on other crops (Table 1). For each treatment, branches were freshly pruned and sprayed with each product at their label rates. Five days later, mycelial fragment suspensions of selected canker fungi were artificially inoculated onto treated pruning wounds. The selected fungi for this trial were *Diplodia malorum*, *D. seriata*, and *D. mutila*. Three months later the branches were collected and brought to the lab four months later for further evaluation. Each branch was evaluated based on vascular discoloration and recovery of the fungal pathogens on APDA media (Blundell and Eskalen 2022). The efficacy of the treatments controlling the fungal pathogens was then calculated as the mean percent infection (MPI) and means comparisons were evaluated using Fisher's least significant difference test ($P < 0.05$).

Table 1. List of pruning wound protectants used for this study, including their application rate, active ingredient, and manufacturer.

No.	*Product name*	*FP/Acre*	*Active ingredient*	*Company*
1	Unsprayed control	-	-	-
2	Botector	6 oz	*Aureobasidium pullulans* strain DSM14940/14941 1	SAN Group Biotech USA, Inc
3	Bio-Tam	2 lbs	*Trichoderma asperellum* (ICC 012) + *Trichoderma gamsii* (ICC 080)	Gowan Company, LLC
4	PureSpray Green	3.0 gal	Mineral oil	PureSpray
5	Lime Sulfur Solution	6.0 gal	Calcium Polysulfide	Ag Formulations Inc.
6	Luna Sensation	7.6 floz	fluopyram (17.5%), tebuconazole (17.5%)	Bayer CropScience
7	Topsin M	1.25 lbs	Triophanate-methyl	United Phosphorus, Inc.

FP = formulated product

RESULTS

Field Survey and Fungal Identification

A total of 165 isolates were recovered from 302 symptomatic branch samples (Table 2). Preliminary results from our field sampling surveys based on conidial morphology and

molecular analysis showed several fungal species, including species of Botryosphaeriaceae (*n* = 119), *Eutypa lata* (*n* = 9)*, Phaeoacremonium minimum* (*n* = 12), and *Kalmusia variispora* (*n* = 24) were associated with pear branch canker and dieback in the pear orchards (Figure 1, Table 2). The most abundant fungal group was Botryosphaeriaceae spp., recovering 119 isolates from 302 branch samples (Table 2). Interestingly, *Neofusicoccum parvum*, a common and aggressive wood canker pathogen, was the least recovered among the Botryosphaeriaceae (*n* = 3). Among these species, *Diplodia malorum* (*n* = 38)*, D. seriata* (*n* = 22), and *D. mutila* (*n* = 27) were the most prevalent with this disease (Figure 2). The multi-loci phylogenetic analysis based on *ITS, tef1*, and *tub2* confirmed the identity of species of Botryosphaeriaceae using 1,000 replicates and 100 random sequence additions to test branch strength (Figure 3).

Table 2. List of isolates recovered from field sampling survey, including number of orchards sampled and isolates recovered per county.

County	***Orchards Sampled***	***D. malorum***	***D.mutila***	***D. seriata***	***N. parvum***	***D. iberica***	***E. lata***	***K. variispora***	***P. minimum***
Lakeport	4	1	9	7	0	4	0	11	2
Sacramento	3	37	5	4	2	5	6	10	6
Mendocino	4	0	13	11	1	20	3	3	4
Total	11	38	27	22	3	29	9	24	12

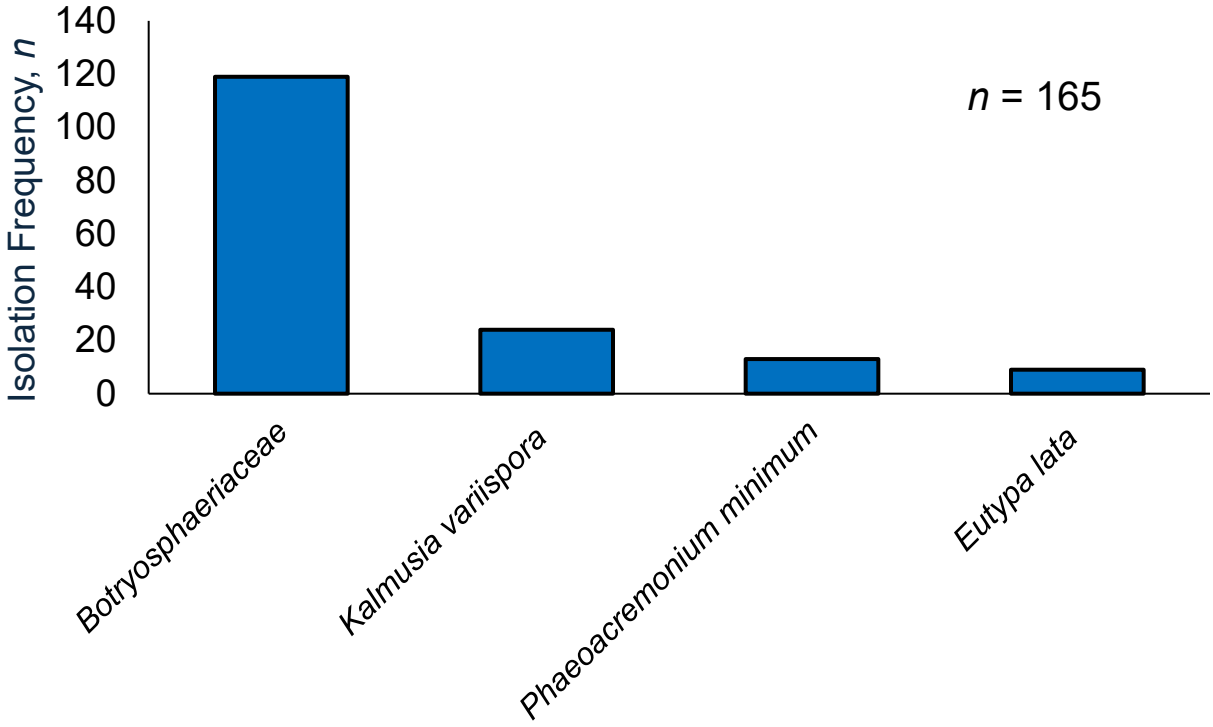

Figure 1. Isolation frequency, *n*, of fungal species associated with pear branch canker and dieback disease based on morphology and molecular analysis

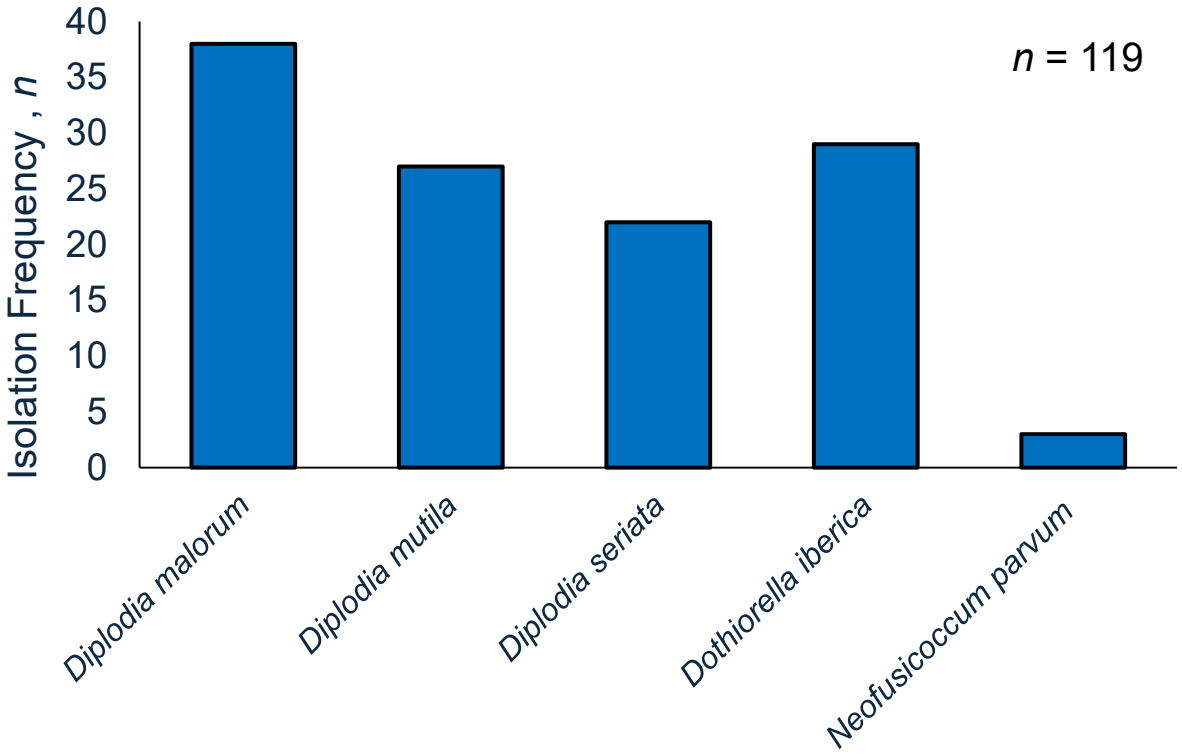

Figure 2. Isolation frequency, n, of Botryosphaeriaceae spp. Recovered from symptomatic samples associated with pear branch canker and dieback disease based on morphology and molecular analysis

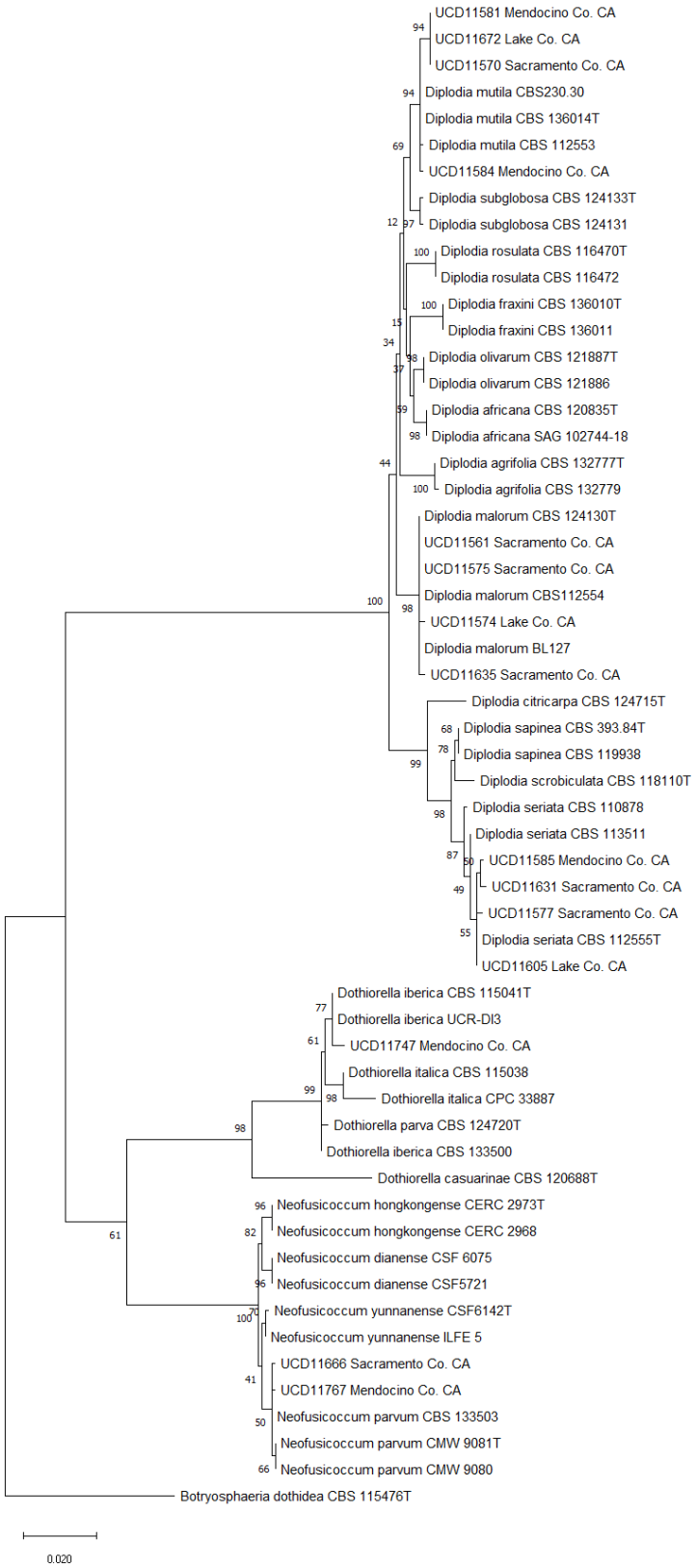

Figure 3. Maximum likelihood (ML) of phylogenetic tree of selected isolates of fungal species of Botryosphaeriace representing pear producing regions associated with pear branch canker and dieback with strains of closely related species. Numbers above branches represent nonparametric bootstrap values from 1,000 replicates.

Pruning Wound Protectant Field Trial

There was no interaction between the three *Diplodia* species and treatments on mean percent infection based on analysis of variance (P>0.05). While the three species did not affect the mean percent infection (MPI) (P>0.05) pruning wound protectant treatments did according to analysis of variance (P<0.05). Thus, Fisher's least significant difference (LSD) was used to evaluate the combination of *Diplodia* spp. and pruning wound protectants. Out of the six treatments, most of the treatments protected the pruning wounds against *Diplodia* spp. Between the biological control products Biotam and Botector, resistance to the combination of *Trichoderma* spp. was low, having recovered a lower MPI, approximately 3.0%, while resistance of *Diplodia* spp. to Botector was present, having a higher MPI, approximately 22%. The synthetic pruning wound protectants (Luna Sensation and Topsin M) effectively controlled the pruning wounds against the fungal pathogens(MPI =3.0%), while resistance to Lime sulfur Solutions was higher (MPI = 16%). PureSpray Green, a mineral oil-based product, showed the least controlled effect out of the six treatments. Resistance of *Diplodia* spp. to this pruning wound protectant was moderately high, obtaining the highest MPI out of the six treatments (MPI = 36%) and compared to that of the inoculated untreated control (MPI= 33%) (Figure 4).

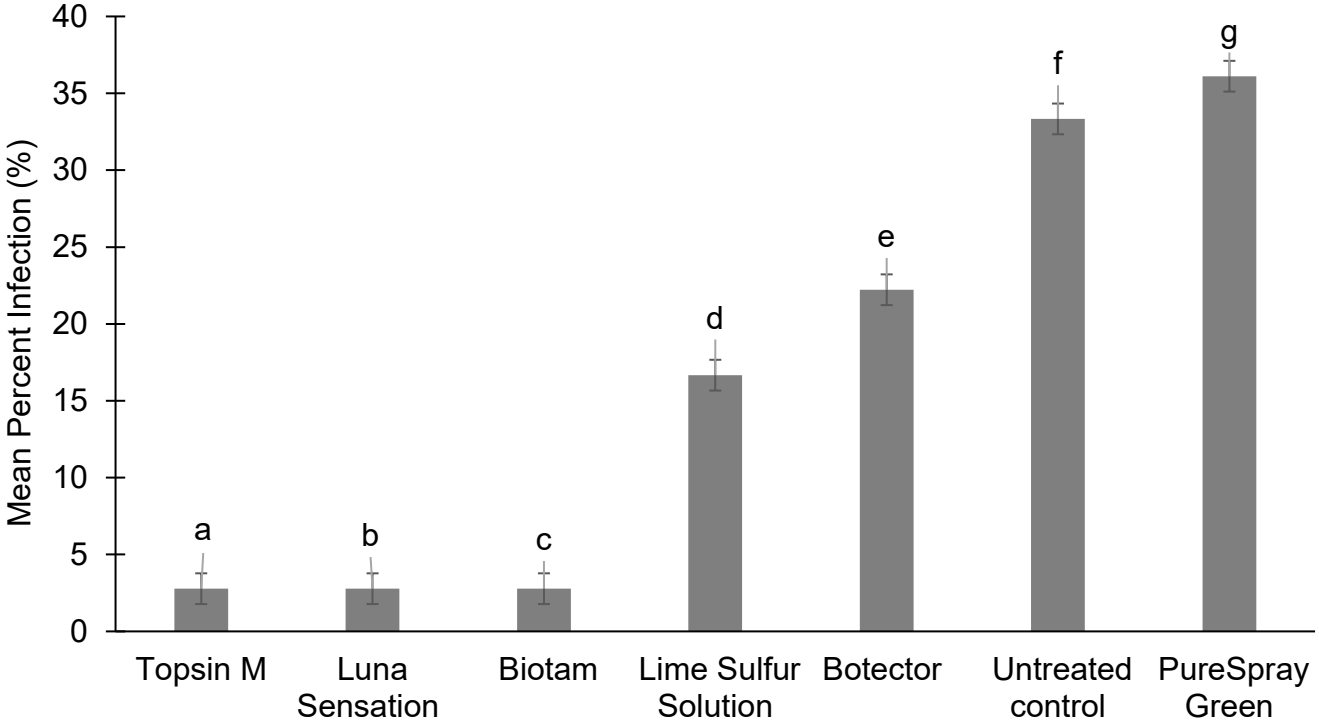

Figure 4. Evaluation of pruning wound protectant mean percent infection (MPI) rates with *Diplodia* spp. The bars represent the mean percent infection. Bars with a different letter are different according to Fisher's least significant difference (P < 0.05).

DISCUSSION

Isolations from symptomatic tissues revealed the occurrence of *Diplodia* species as the most prevalent, particularly *D. malorum, D. seriata*, and *D. mutila*, followed by *Kalmusia variispora, Phaeoacremonium minimum* and *Eutypa lata.* These fungal species associated with pear branch canker and dieback disease were observed on most of the surveyed orchards, suggesting that several species are present among all three major pear producing regions in Northern California.

Among the pruning wound protectants, most showed a reduction in fungal pathogens on pruning cuts. The following protectants, Biotam, Luna Sensation, and Topsin M showed the best as a preventative for the disease. The combination of *Trichoderma asperellum* (ICC 012) + *Trichoderma gamsii* (ICC 080), the active ingredients in Biotam, showed effective control against the disease. Depending on the strain and environmental conditions, *Trichoderma* spp. can provide long-term protection than synthetic various benefits: stimulate plant growth, suppress fungal pathogens through faster colonization, and induce systemic resistance (Blundell and Eskalen 2022). Identification of fungal pathogens is crucial for implementing effective management strategies. Despite these initial insights, there is a need to investigate biology and epidemiology and continue to evaluate the efficacy of biological and synthetical pruning wound protectants against this disease for multiple years. Currently, we are examining pathogenicity, epidemiology, and a second-year evaluation of several synthetic and biological pruning wound protectants to control pear branch canker and dieback in California.

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