Identification and Management of Fungal Species Associated with Branch Canke and Dieback Disease on Pear in California

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ABSTRACT

Recently, branch canker and dieback disease of pear has occurred in major pear producing regions of Northern California. The disease was observed in multiple pear orchards in the fall of 2023 by our lab; however, the cause of the disease remained unknown. This study aimed to identify associated fungi, confirm pathogenicity on pear, characterize seasonal spore capture in orchards, and evaluate pruning wound protectants.. Artificial inoculations on 2 to 3-year-old dormant 'Bartlett' pear branches showed that multiple taxa associated with pear wood cankers, including *Diplodia* spp. and other canker-associated fungi (*Dothiorella Iberica*, *Eutypa lata, Phaeoacremonium minimum*, *Kalmusia variispora*), caused vascular necrosis relative to non-inoculated controls, and pathogens were re-isolated from lesion margins, supporting pathogenicity under field conditions. In spore-trapping assays using petroleum jelly-coated slides processed by dilution plating, fungal colonies were recovered, but target canker pathogens were not identified based on colony morphology. In pruning wound protection trials challenged with *Diplodia* spp., Luna Sensation and Topsin M had the lowest mean percent infection, while Bio-Tam provided moderate protection. These results support *Diplodia* spp. as important pathogens associated with pear branch canker and indicate that pruning wound protection can reduce infection risk.

INTRODUCTION

European pear (*Pyrus communis*) is an important agricultural commodity bearing 8,500 acres in California (USDA 2024). In 2023, 162,500 tons were produced with a market value of $117,325,000 (CDFA 2024). California is the nation's third leading producer of Bartlett pears, followed by Washington and Oregon, in both fresh and processed markets. The majority of 'Bartlett' pears are produced in the Northern region of California, specifically in the counties of Lake, Mendocino, and Sacramento.

Branch dieback and canker diseases are extensively destructive on various woody hosts and have been studied extensively in agricultural and non-agricultural fields (Mayorquin et al. 2016). Several members of Botryosphaeriaceae, Diaporthaceae, Diatrypaceae, and Togniniaceae are common wood-colonizing pathogens on various fruit and nut crops such as apple, pear, apricot, cherry, grapevine, etc., in California. Notably, species such as *Lasiodiplodia theobromae, Neofusicoccum parvum*, *Diplodia* spp., *Diaporthe* spp.,*Eutypa*

lata, and *Phaeoacremonium minimum* have been reported to cause branch canker and dieback on pear in major pear producing countries (Cloete et al. 2011; Lawrence et al. 2015; He et al. 2022; Shah et al. 2010).

While the two most common diseases affecting pear trees in California are fire blight and pear scab, a variety of fungal pathogens have also been associated with canker and dieback in pear trees. *Diplodia seriata* has been reported to cause branch canker dieback on pear (Choudhury et al. 2014). *Eutypa lata* has also been found associated with the disease, but confirmation as a fungal pathogen on pear is unknown (Trouillas and Gubler 2010). These fungi rapidly colonize and invade the inner layers of wood, causing damage and blockage of vascular systems. The blockage disrupts the translocation movements of water and solutes, leading to substantial economic losses (Downer et al. 2022; Mayorquin et al. 2016).

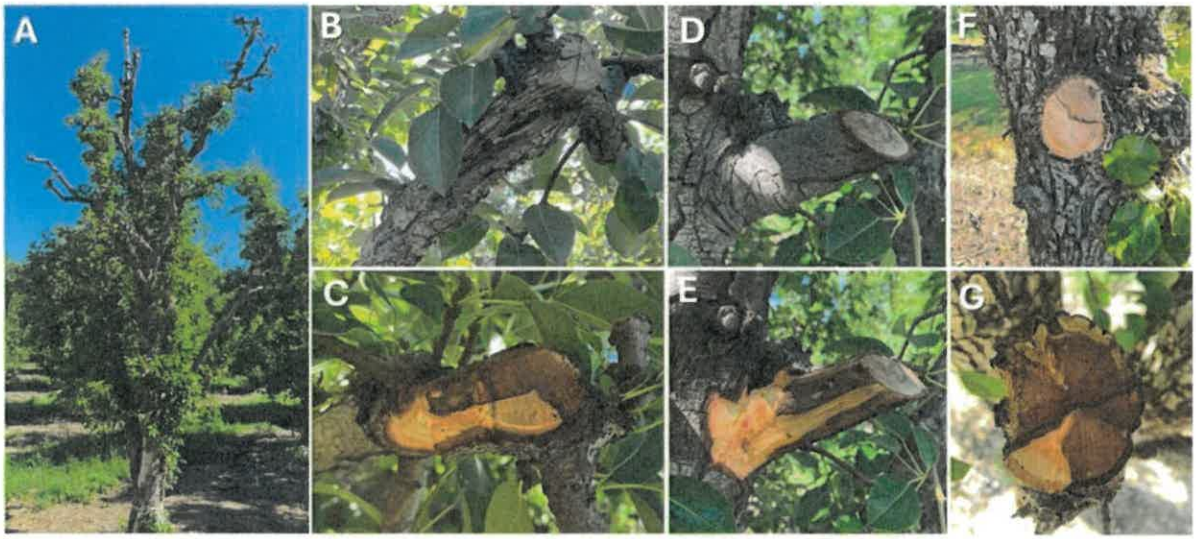

Figure 1. Pear Branch Canker and Dieback symptoms. **A,** dieback on pear tree. **B,** Sunken canker on pruning wound. **C,** Reddish-brown canker under the bark. **D** and **E,** Necrotic tissue on pruning wound. **F** and **G,** Canker on the main branches of pear trees.

Typical symptoms include branch dieback characterized through progressive death of affected branches, usually starting at the tips of twigs and branches and often localized on single or multiple branches. Cankers are found near old pruning wounds of branches. Externally, sunken discolored lesions appear oblong or ellipsoid shaped where underneath the bark of the lesion, reveals a vascular discoloration depicting canker tissue. Tissue is characterized by localized necrotic tissue shown reddish-brown in color with margins separated from healthy tissue. Some canker tissues are not limited to pruning wounds but observed extensively reaching into main branches and trunks of the tree (Figure 1). These symptoms were observed in multiple northern California pear orchards where our lab identified several fungal species, including *Diplodia malorum, D.*

seriata, *D. mutila, Dothiorella iberica, Neofusicoccum parvum, Eutypa lata, Phaeoacremonium minimum,* and *Kalmusia variispora* associated with the disease, but knowledge of these fungal species as pathogens on pear remains unknown.

Pruning cuts, broken branches, or other mechanical injuries are typical entry points for the fungal canker pathogens. They can also produce overwintering fruiting structures (pycnidia and perithecia) on infected and dead tissues and become a source of inoculum. Spore dissemination usually occurs during precipitation (rain, fog, etc.) and coincides with dormant pruning events (Eskalen and Gubler 2001). Long-distance spread of spores occurs by air movement. Once fungal spores come into contact to plant tissue and trees that are already stressed, they colonize and initiate a new infection, leading to the loss of branches and reduced fruit production.

Effective management practices in reducing infection by these fungal pathogens require pruning protectants after dormant pruning. Therefore, the purpose of this study was to determine the pathogenicity of fungal species associated with pear branch dieback, determine optimal environmental conditions for spore dissemination of wood canker pathogens released in California's pear orchard regions, and evaluate several synthetic and biological pruning wound protectants to control pear branch canker and dieback in California.

OBJECTIVES

Objective 1. Assess the pathogenicity of fungal species associated with pear branch dieback and canker in California.

Objective 2. Determine when and under what environmental conditions spores of wood canker pathogens are released in California's pear orchard regions.

Objective 3. Evaluate the effectiveness of various registered synthetic and biological pruning wound protectants in pear orchards.

PROCEDURES

Field Survey and Fungal Identification.

In the early summer of 2021 and 2023, our lab identified additional fungal species, including *Diplodia malorum, D. seriata*, *D. mutila, Dothiorella Iberica, Eutypa lata, Phaeoacremonium minimum,* and *Kalmusia variispora*. The pathogenicity of 16 fungal isolates representing eight fungal species were evaluated in two locations (Mendocino and Lake counties). Average age of the trees in each orchard was about 60 years old. 2-to-3-year-old healthy looking pear branches were surface disinfected with 70% ethanol

and wounded with a 2-mm-diameter sterile drill bit. The pathogen inoculum consisted of toothpick fragments (~ 5mm) which were soaked in Potato Dextrose Broth (PDB), then completely colonized with mycelium of each isolate on acidified potato dextrose agar (APDA) plates. Controls consisted of sterile toothpicks. Isolates were inoculated by inserting a colonized toothpick fragment onto the wound, allowing the fungus to contact the woody tissue of the branch, then wrapped with Parafilm (Bemis Co., Neenah, WI, USA) to prevent contamination (Bustamante et al. 2024). Inoculations were done on six separate branches from different trees in a completely randomized design. Four months after inoculation, treated branches were pruned and brought to the lab for further evaluation. Inoculated branches were evaluated by peeling the bark with a sterile scalpel and measuring the necrotic lesion in millimeters using a Mitutoyo Absolute Digimatic caliper around the inoculation region. Isolations of the resulting lesions (5 x 5 mm) were placed on APDA and incubated for 7 to 14 days. After incubation, obtained colonies were recorded and identified based on colony morphology and conidial characteristics. Results were subjected to a two-way analysis of variance (ANOVA). Means comparisons were assessed using Fisher's least significant difference (LSD) test ($P < 0.05$) on SigmaStat 3.1 software (Systat Software Inc.).

Spore Trapping Study

Spore traps comprised of petroleum jelly (Vaseline) coated microscope slides as described by Eskalen and Gubler (2001) were placed in three previously surveyed pear orchards in counties of Lake, Mendocino, and Sacramento, respectively. A total of 10 spore traps were hung within the canopy of randomly selected trees in each orchard and collected and replaced every two weeks and processed in the lab. This study was conducted from October to June to evaluate spore discharge under various climatic conditions. In the laboratory, each slide was rinsed with sterile distilled water and Tween 80 in 50 ml falcon tubes, and two 20-µl aliquots will spread on acidified potato dextrose agar (APDA) media and spread via a sterile glass rod. Fungal colonies were recorded after a one-to-two weeks of incubation at room temperature. Pure cultures were obtained resembling wood canker pathogens to confirm identification based on colony morphology. Weather data (temperature and rainfall) were collected from the nearest weather stations from each orchard to determine the correlation of spore dissemination.

Pruning Wound Protectant Field Trial

Field trials were conducted in two different pear orchards in two locations (Mendocino and Sacramento 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. Seven days later, spore suspensions (20µl

of 10^5 conidia/mL) 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 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

Pathogenicity Test

Four months after inoculation, all the tested isolates were pathogenic on 2-to-3-year-old pear branches in > 60-year-old trees under field conditions in both of Lake and Mendocino counties. Inoculated branches exhibited dark brown vascular discoloration extending from the inoculation point (Figure 2). Locations did not significantly affect lesion length ($P = 0.1431$), and no interaction was detected between isolates and location ($P = 0.5287$).

Virulence varied significantly among the fungal isolates ($P < 0.0001$). Mean lesion lengths ranged from 4.9 to 72.6 mm, which were significantly greater than those observed in the non-inoculated controls (mean = 2.4 mm) (Figure 3). Among *Diplodia* species, isolate-level variations were observed. One isolate of *D. mutila* (UCD 11584) produced shorter lesions (mean = 9.4 mm) than *D.mutila* isolate UCD 11583 (mean = 13.2 mm). *Diplodia seriata* isolates produced lesions ranging from 10.8 to 13.2 mm. *Diplodia malorum* was the most virulent species, producing mean lesions ranging from of 40.3 to 72.6 mm.

Re-isolations from the margin of necrotic lesions consistently recovered the inoculated fungi. Recovery frequencies ranged from 67 to 100% across species, while no fungal pathogens were recovered on the non-inoculated controls. These results are consistent with fulfilling Koch's postulates for the fungal species evaluated under field conditions.

Figure 2. Internal necrotic lesion presented by vascular discoloration caused by fungal species associated with pear branch canker and dieback disease

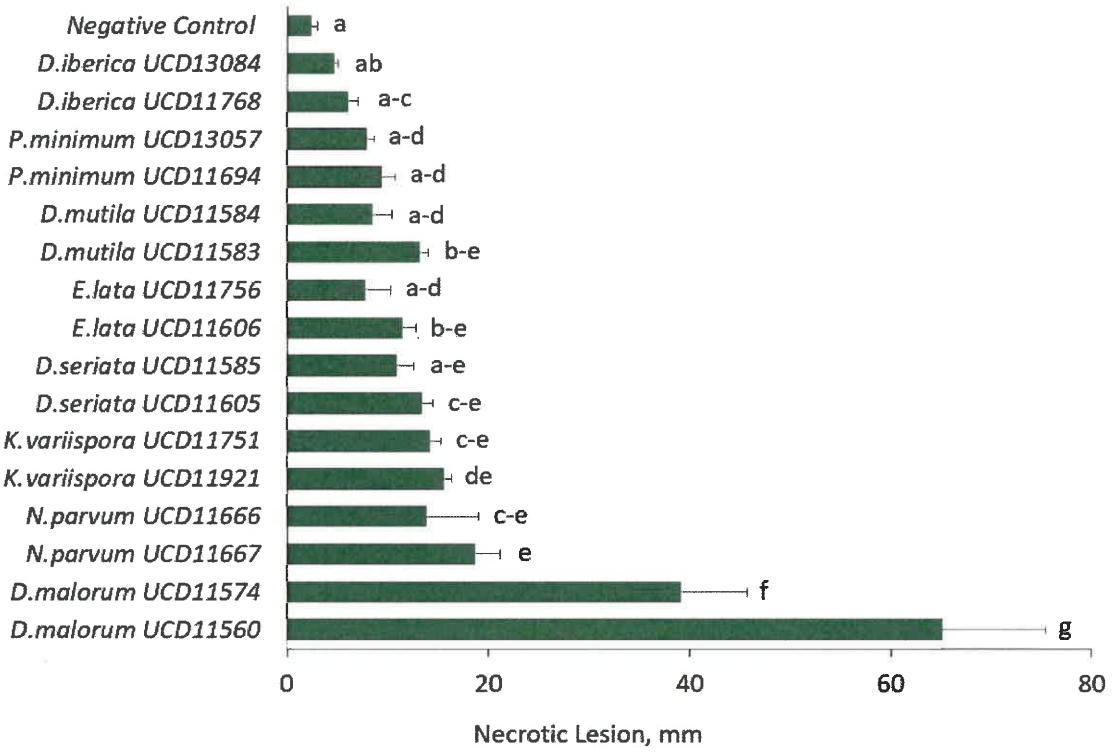

Figure 3. Internal necrotic lesions caused by fungal isolates inoculated on 2-to-3-year-old 'Bartlett' pear branches. Means (out of the six replicates) followed by the same letter in each column are not different according to Fisher's least significant difference (LSD) test ($P > 0.05$).

Spore Trapping Study

Fungal colonies were recovered from spore traps placed in surveyed orchards; however none of the recovered cultures were identified as target wood canker pathogens associated with pear branch canker dieback based on colony morphology. regardless of weather conditions and location from previously surveyed orchards. Colonies initially suspected to resemble members of the Botryosphaeriaceae or *Eutypa lata* were further examined and did not match the morphological characteristics of these taxa. White, sparse colonies recovered from multiple samples were morphologically consistent with Basidiomycetes, which are common wood-decaying fungi.

In Mendocino County, Basidiomycetes colonies were recovered during winter months, in December, January, and February with a total of 28 colonies recorded (Table 2). In Sacramento County, four Basidiomycetes colonies were recovered exclusively in February (Table 3). None of the colonies were recovered from Lake County spore traps during sampling period.

Monthly colony recovery did not correspond consistently with total monthly precipitation, as colonies were detected during months with both low and high precipitation totals, according to monthly weather data collected by the California Irrigation Management Information System (CIMIS) (Table 2 and 3). These results suggest that factors other than monthly rainfall totals, such as cooler temperatures and prolonged periods of moisture or high relative humidity, may influence spore capture, although event-level weather data were not evaluated in this study.

Table 2. Colony counts of Basidiomycetes recorded from spore traps and average monthly weather temperature (°F) and precipitation (in) October to June in Mendocino County.

Month	Basidiomycetes	Average Temperature (°F)	Average Precipitation (in)	Relative Humidity (%)
October	0	60.5	0.72	57
November	0	47.8	12.80	79
December	4	48.2	9.02	86
January	10	43.7	0.96	77
February	14	47.9	9.35	80
March	0	49.8	4.81	77
April	0	55.7	0.87	71
May	0	61.7	0.15	59
June	0	67.3	0.00	56

Table 3. Colony counts of Basidiomycetes recorded from spore traps and average monthly weather temperature (°F) and precipitation (in) October to June in Sacramento County.

Month	Basidiomycetes	Average Temperature (°F)	Average Precipitation (in)	Relative Humidity (%)
October	0	67.2	0.28	58
November	0	51.0	4.56	77
December	0	49.3	4.52	94
January	0	47.1	1.03	80
February	4	50.9	4.96	85
March	0	53.0	1.98	83
April	0	58.9	0.24	77
May	0	67.6	0.00	61
June	0	72.0	0.00	56

Pruning Wound Protectant Field Trial

There was no significant interaction between the three *Diplodia* species and pruning wound protectant treatments ($P = 0.3183$). However, both fungal species and treatments significantly affected the mean percent infection (MPI) ($P < 0.05$).

Among the three *Diplodia* species evaluated, *D. seriata* exhibited the lowest aggressiveness with a MPI of 43%, which was significantly lower than observed for *D. malorum* and *D.mutila* (Figure 4).

Among pruning wound protectants, the synthetic pruning wound protectants, Luna Sensation and Topsin M, provided the highest level of protection, with MPI values of 8% and 15%, respectively. Bio-tam provided moderate protection, resulting in a 50% of infection, while Botector resulted in higher infection levels (MPI = 68%).Lime sulfur Solutions and PureSpray Green were not significantly different from the inoculated untreated control (MPI= 78%), ans showed the highest infection levels, of 81% and 84%, respectively. (Figure 5).

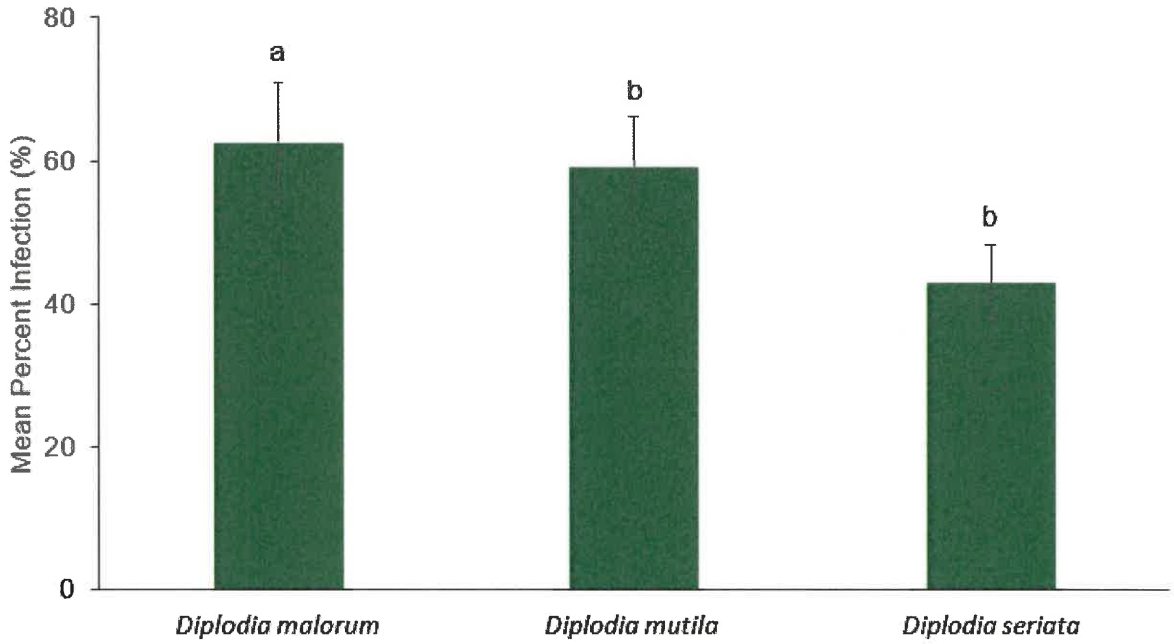

Figure 4. Evaluation of *Diplodia* spp. mean percent infection (MPI) rates. The bars represent the mean percent infection. Bars with different letters are different according to Fisher's least significant difference ($P < 0.05$).

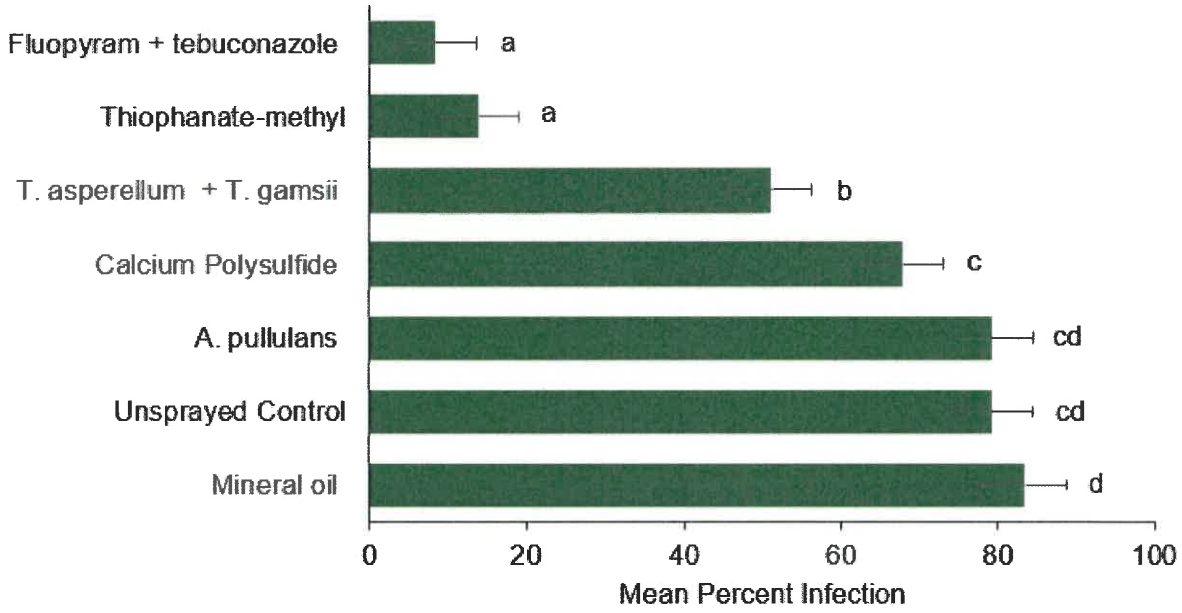

Figure 5. Evaluation of pruning wound protectant mean percent infection (MPI) rates with *Diplodia* spp. The bars represent the mean percent infection. Bars with different letters are different according to Fisher's least significant difference ($P < 0.05$).

DISCUSSION

This study confirms the pathogenicity of multiple fungal species associated with pear branch canker and dieback in Northern California, including *Diplodia* spp. (*D. malorum*, *D. seriata*, *D. mutila*) *Dothiorella Iberica, Neofusicoccum parvum*, *Kalmusia variispora, Phaeoacremonium minimum* and *Eutypa lata*. Several of these taxas have been previously reported on pear in other producing regions worldwide; however, their pathogenic role on pear under California field conditions had not been clearly established.

Phaeoacremonium. minimum and *D. seriata* have been reported causing dieback symptoms on pear in South Africa, while *N. parvum* has been identified as the causal agent of stem canker and twig dieback of pear in China (Cloete et al. 2011; He et al. 2022). In the present study, artificial inoculations demonstrated that these species are capable of causing vascular necrosis on pear under field conditions in California. *Diplodia malorum,* which produced the longest lesions in pathogenicity tests, has previously been reported infectingapple (*Malus pumila*) and Asian pear (*Pyrus pyrifolia*) trees in Santa Clara, County, CA (CDFA. 2020). To our knowledge, this study represents the first report of *Do.iberica, D.mutila, E. lata, K. variispora*, and *N. parvum* as primary causal agents of branch canker and dieback disease on pear in California.

The spore trapping study did not recover target wood canker pathogens using petroleum jelly-coated slides processed through dilution plating. Although fungal colonies were recovered, these were primarily identified as Basidiomycetes, which are commonly associated with wood decay rather than canker formation. Monthly colony recovery did not align consistently with total monthly precipitation, indicating that precipitation totals alone may not adequately predict spore capture. Previous studies have shown that spore release of Botryosphaeriaceae and other wood-infecting fungi is often associated with short-term wetness events, including rain splash, fog, and prolonged periods of high relative humidity (Úrbez-Torres et al. 2010; Eskalen and Gubler. 2001; Eskalen et al. 2013). The absence of target pathogens in this study may be influenced by trap placement, sampling frequency, or the use of morphology-based identification, and highlights the need for refined approaches to better characterize spore dispersal dynamics in pear orchards.

Pruning wound protection significantly reduced infection by *Diplodia* species in this study. Synthetic fungicides Luna Sensation and Topsin M were the most effective treatments, providing strong protection against artificial inoculation. Bio-Tam (*Trichoderma asperellum* and *T. gamsii*) provided moderate protection, consistent with previous studies demonstrating that *Trichoderma* spp. suppress wood-infecting pathogens through rapid colonization, competition, and induced host defenses (Blundell and Eskalen 2022). In contrast, Lime sulfur solution and PureSpray Green did not significantly reduce infection compared with the untreated control, indicating limited efficacy under the conditions evaluated.

Overall, identification of fungal pathogens and evaluation of pruning wound protectants provide critical information for managing pear branch canker and dieback in California. While pruning wound protection shows promise as a management strategy, additional studies are needed to refine spore trapping methodologies and better define periods of infection risk, which would allow growers to optimize pruning timing and wound protection practices.

Acknowledgements

We would like to thank to the California Pear Research Advisory Committee for their funding. Thanks to the various industry donors for providing testing materials. Thanks to farm advisor Clebson Gonçalves (Lakeport and Mendocino counties) and the pear growers for their collaboration and support for these projects.

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