

Preventative Management of Trunk Diseases in Table Grape *Vitis vinifera* Autumn King

Kendra Baumgartner ^{1,*} Gabriel Torres ² Alejandro I. Hernandez Rojas ³
and Renaud Travadon ³

Abstract

Background and goals

In California's southern San Joaquin Valley, applications of pruning-wound protectants may lower the incidence of grapevine trunk diseases. Rain is important for spore dispersal of the causal fungi, which infect pruning wounds. Despite low annual rainfall in this region, pruning wounds are at risk of infection, based on reports of symptoms and pathogen detection in vineyards, and from controlled inoculations demonstrating susceptibility of *Vitis vinifera* table grape cultivars. Therefore, our goal was to evaluate protectants for pruning wounds, testing two commercial fungicides, thiophanate-methyl (Topsin; UPL) and pyraclostrobin + boscalid (Pristine; BASF), for three years in an 8-yr-old Autumn King vineyard.

Methods and key findings

For three years, liquid formulations of fungicides were applied alone or with Pentra-bark (Quest Products Corp.), an adjuvant to enhance pesticide penetration of the bark of woody crops. After spray application, pruning wounds were inoculated with spores of the fungi that cause the trunk diseases Botryosphaeria dieback (*Neofusicoccum parvum*), Esca (*Phaeoacremonium minimum*), and Eutypa dieback (*Eutypa lata*). Both thiophanate-methyl and pyraclostrobin + boscalid were effective against *N. parvum*, with greater efficacy using Pentra-bark in two study years. Both fungicides were moderately effective against *E. lata*, with greater efficacy using Pentra-bark in one study year. Neither fungicide, alone or with Pentra-bark, was effective against *P. minimum*.

Conclusions and significance

One application of thiophanate-methyl or pyraclostrobin + boscalid to pruning wounds (after pruning and before rain) may prevent Botryosphaeria and Eutypa dieback, but not Esca. Greater fungicide efficacy with Pentra-bark against *E. lata* and *N. parvum* in some years warrants further testing, for example, at higher concentrations.

Key words: Botryosphaeria dieback, Esca, Eutypa dieback, fungicides, grapevine trunk diseases

Introduction

Preventing trunk diseases in California table grape vineyards when vines are young and asymptomatic is a cost-effective approach to manage these chronic diseases (Baumgartner et al. 2019). California table grapes were valued at \$1.7 billion in 2023 (NASS 2024). Approximately 30% of the bearing acreage of California table grape vineyards is planted with cultivars that were bred and released by the USDA (CDFA 2024), e.g., *Vitis vinifera* Autumn King (Ramming and Tarailo 2006a) and *V. vinifera* Scarlet Royal (Ramming and Tarailo 2006b). All of these cultivars share a common genetic background that includes Thompson Seedless (syn: Sultanina). Thompson Seedless, as a cultivar and parent material, is highly susceptible to Esca, Eutypa dieback, and Phomopsis dieback (Travadon et al. 2013, Travadon and Baumgartner 2023).

Prior research that used spore traps placed in young, healthy vineyards to detect the fungal pathogens that cause Botryosphaeria dieback, Eutypa dieback, and Phomopsis dieback (Fujiyoshi et al. 2021b) suggests that the spores can spread to young vines by wind and/or rain. Such spores may originate from outside the vineyard or from asymptomatic but infected vines within the vineyard. Once an infection is established, there is a long incubation period ranging from several months (Phomopsis-dieback pathogen *Diaporthe ampelina* [Anco et al. 2012]) to several years (Eutypa-dieback pathogen *Eutypa lata* [Travadon et al. 2024]) before leaf symptoms appear.

Contaminated nursery stock may serve as a source of inoculum in young vineyards, so preventing trunk diseases is an important phytosanitary measure. California grapevine nurseries

¹United States Department of Agriculture - Agricultural Research Service, Davis, CA 95616; ²University of California Cooperative Extension, 4437 S. Laspina Street, Tulare, CA 93274; and ³Department of Plant Pathology, University of California, One Shields Avenue, Davis, CA 95616.

*Corresponding author (kbaumgartner@ucdavis.edu, tel: 530-754-7461, fax: 530-754-7195)

Manuscript submitted April 2025, accepted June 2025, published Aug 2025

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that participate in the California Department of Food and Agriculture's 'clean nursery stock' certification program must establish their 'mother vines' with certified virus-free plants (CDFA 2016). Mother vines are the source of vegetative cuttings that nurseries use each year to propagate grapevines. An additional requirement of the certification program is that mother vines are regularly tested for over a dozen viruses, including Grapevine leafroll-associated viruses, Grapevine fanleaf virus, and Grapevine red blotch-associated virus. These viruses, as well as trunk diseases, cause chronic, incurable diseases. Furthermore, examination of nursery stock detected the fungal pathogens that cause *Botryosphaeria* dieback, Esca, *Eutypa* dieback, and *Phomopsis* dieback from both California (Garcia et al. 2025) and worldwide (Gramaje et al. 2018, Carbone et al. 2022, Akgül et al. 2023, Hrycan et al. 2023). It is not only viruses that infect nursery stock. To ensure that nursery stock is truly clean, disease diagnostics specifically designed for nursery production should reflect the variety of pathogens that infect the vegetative cuttings from which grapevines are propagated.

An important factor in the epidemiology of trunk diseases is rain, which stimulates spore production and dispersal for some of the causal fungi, including *Botryosphaeria*-dieback pathogen *Neofusicoccum parvum* (Úrbez-Torres et al. 2010), Esca pathogen *Phaeoacremonium chlamydospora* (González-Domínguez et al. 2020), *Eutypa*-dieback pathogen *E. lata* (Ramos et al. 1975), and *Phomopsis*-dieback pathogen *D. ampelina* (Gonzalez-Dominguez et al. 2022). Pruning wounds are important infection courts for the spores of most of these pathogens (Petzoldt et al. 1981, Eskalen et al. 2007, Úrbez-Torres and Gubler 2011, Luque et al. 2014, Henderson et al. 2021). Treating pruning wounds with pruning-wound protectants such as fungicides (Rolshausen et al. 2010, Brown et al. 2021) or biocontrol agents (Travadon et al. 2023b, Leal et al. 2024) prior to rain can minimize the risk of infection.

In a previous field trial, we tested four protectants against *D. ampelina*, *E. lata*, *N. parvum*, and *P. chlamydospora* in a Scarlet Royal table grape vineyard located in the southern San Joaquin Valley of California (Brown et al. 2021). The fungicides thiophanate-methyl (Topsin M WSB; UPL, Inc.) mixed with myclobutanil (Rally 40 WSP; Dow AgroSciences LLC), and pyraclostrobin + boscalid (Pristine; BASF) were most effective, particularly against *D. ampelina* and *N. parvum*. However, none of the protectants were effective against *P. chlamydospora*. Since this field trial was completed, research findings have revealed the importance of Esca as a trunk disease in California. In a survey of table grape vineyards with symptoms of trunk diseases (leaf and wood symptoms of Esca, and general symptoms of the dieback-type trunk diseases), *P. chlamydospora* and another Esca pathogen, *Phaeoacremonium minimum*, were detected in a surprisingly high proportion of the samples (~40%) (Travadon et al. 2022). Examination of California nursery stock and soil and water samples collected during nursery propagation revealed a greater incidence of *P. minimum* than *P.*

chlamydospora (Garcia et al. 2025). As nursery stock may be a source of *P. minimum*, it is important to evaluate protectants that can be applied both to commercial vineyards and to mother vines in nurseries.

The few studies testing protectants against *P. minimum* showed poor results (e.g., Rolshausen et al. 2010). To fill a gap in the knowledge of Esca management, we designed a new field trial to include *P. minimum*, which was not previously tested; *E. lata* and *N. parvum*, pathogens we previously examined (Brown et al. 2021), were also included. To improve the efficacy of thiophanate-methyl and pyraclostrobin + boscalid for preventing infection by *E. lata*, *N. parvum*, and *P. minimum*, the adjuvant Pentra-bark (Quest Products Corp.) was added. Pentra-bark is a non-ionic, silicone-based adjuvant used as a wetting agent to increase penetration of pesticides through the bark of woody, perennial crops. We tested the hypothesis that Pentra-bark, when added to the fungicides, would be associated with lower recovery of the pathogens (i.e., greater fungicide efficacy) than the fungicides alone.

Materials and Methods

Study vineyard in the southern San Joaquin Valley

A replicated field trial was conducted for three years, from 2020 to 2022, in a commercial vineyard in Earlimart, California (35°53.6412'N; 119°08.1042'W). The vineyard was planted in 2012 with Autumn King on Freedom rootstock. Spacing is 4 m between rows and 2 m between vines. Vines are trained to a dual-trunk bilateral cordon and are spur pruned, and the canopy is trained to an open-gable 'Y' trellis system. The vineyard is drip irrigated and the irrigation season runs from approximately April to October. The climate is Mediterranean, characterized by hot, dry summers and mild winters (USDA Cold Hardiness Zone 9a). Most of the average 22 cm of annual precipitation falls between November and March.

Our experimental approach included the following steps: 1) just before the entire vineyard was to be pruned, prune all canes on all vines in data rows and buffer rows to ~30 cm long spurs; 2) apply experimental treatments (fungicides, water-treated control) to pruning wounds on data vines, up to 6 days after pruning; 3) inoculate pruning wounds (including a set of non-inoculated pruning wounds) on data vines, up to 6 days after experimental treatments; and 4) collect the distal 15 cm of the spurs for pathogen detection, before budbreak (Table 1). For step 1, pruning cuts were made horizontally to prevent the droplet of inoculum from running off. The relatively long length of the spurs (30 cm) after step 1 provided a sufficient length of woody cane tissue to minimize the risk of an infection progressing from an inoculated pruning wound into the cordon. It also allowed us to collect inoculated tissue at step 4 while still leaving a spur of sufficient length for normal shoot growth after budbreak. The short incubation period between steps 3 and 4 (ranging from 18 to 32 days; Table 1) was necessary to minimize the

risk of disease spread from the inoculated spurs to the rest of the vineyard, which did not have a history of trunk diseases.

Fungicide applications to pruning wounds

Experimental treatments included a water-treated control, the fungicide pyraclostrobin + boscalid (Pristine; BASF), the fungicide thiophanate-methyl (Topsin M WSB; UPL), and both fungicides mixed with the adjuvant Pentra-bark (Quest Products Corp.; Table 2). Treatments were applied to pruning wounds at a spray volume equivalent of 187 L/ha (20 gal/acre), using a CO₂ backpack sprayer (Bell-spray, Inc.) with an air induction spray nozzle (TeeJet AITXA 8002). The application rate of thiophanate-methyl was recommended by the manufacturer for management of ‘canker diseases’, and the application rate of pyraclostrobin + boscalid was the maximum recommended by the manufacturer for management of fungal diseases of grape (although not specifically for trunk diseases). During application, the backpack sprayer was agitated regularly by physically shaking the tank to keep the fungicides in suspension and to evenly disperse Pentra-bark, which was more viscous than the diluent (water). The spray nozzle was directed at each pruning wound and the treatments were applied to runoff. Thorough coverage of spray applications was confirmed as 100% each year, using two, 2-cm² pieces of water- and oil-sensitive paper (TeeJet) attached to the pruned spurs on one data vine per treatment per block. Pentra-bark has shown no efficacy against *E. lata* when used alone as a protectant (Sosnowski et al. 2013), so we did not include a Pentra-bark-only treatment.

Experimental treatments (including the water-treated control) were applied in a randomized complete block design

with three blocks, each of which consisted of one treated row separated by five buffer rows. Within each treated row (i.e., block), the five treatments (pyraclostrobin + boscalid, pyraclostrobin + boscalid + Pentra-bark, thiophanate-methyl, thiophanate-methyl + Pentra-bark, water-treated control) were distributed among five-vine sets, and these five treatments were randomized within the three blocks. For each five-vine set, all five vines were treated on both sides of the row, with the three central vines as the data vines (where the spurs were inoculated). On each data vine, there were five spurs inoculated with each pathogen and five non-inoculated spurs randomized among all spurs on all four cordons. The same five-vine sets within the three blocks received the same experimental treatments each year. As spurs are renewed annually, however, different spurs were inoculated or non-inoculated from year to year.

Inoculation of pruning wounds

N. parvum isolate KARE1463 was originally isolated from almond in Sacramento County, CA (Travadon et al. 2023a). Inoculum consisted of asexual spores (conidia), which were harvested from a mature pycnidium that developed on a 1-mo culture on potato dextrose agar (PDA), with an overlay of an autoclaved pistachio leaf, and incubated under UV light (Holland et al. 2021). Conidial masses were removed from the pycnidium with a flame-sterilized needle, placed in 1-mL sterile water, and gently mixed to release the conidia into suspension. The concentration of conidia was estimated with a hemacytometer (Reichert) and adjusted with sterile water to 1 × 10⁵ spores/mL. *P. minimum* isolate Kern725 was originally isolated from grape in Kern County, CA (Travadon et al. 2022). Inoculum consisted of conidia,

Table 1 Dates of pruning, experimental treatments (applications of fungicides and a water-treated control), inoculations (spurs inoculated with three pathogens and non-inoculated spurs), and collection of inoculated and non-inoculated spurs. All steps were carried out during the dormant season.

Practice	2020	2021	2022
Step 1: Pruning	12 Feb	8 Feb	27 Jan
Step 2: Experimental treatments	18 Feb	9 Feb	31 Jan
Step 3: Spur inoculations	19 Feb	15 Feb	3 Feb
Step 4: Collection of spurs for pathogen detection	9 March	16 March	7 March
Incubation period of pathogens in inoculated spurs	18 Days	29 Days	32 Days
Total study period	25 Days	36 Days	39 Days

Table 2 Materials spray-applied to pruning wounds after pruning and before inoculation. a.i., active ingredient.

Trade name ^a	Active ingredient(s)	Fungicide group (FRAC number)	Application rate (mL or g of a.i./L)	Manufacturer
Pentra-bark (adjuvant)	Alkylphenol ethoxylate, polysiloxane polyether copolymer, propylene glycol	Non-ionic, organosilicone, wetting agent	2% (v/v) (20 mL/L)	Quest Products Corp.
Pristine (fungicide)	Pyraclostrobin + boscalid	Quinone outside inhibitor (QoI) (FRAC 11) + succinate-dehydrogenase inhibitors (SDHI) (FRAC 7)	1.7 L/ha (0.98 g a.i./L)	BASF
Topsin M WSB (fungicide)	Thiophanate-methyl	Methyl benzimidazole carbamates (MBC) (FRAC 1)	1.7 kg/ha (1.8 g a.i./L)	UPL

^aBoth fungicides formulated as dry solids; Pristine is a water-dispersible granule, Topsin M WSB is a wettable powder in a water-soluble bag. The adjuvant Pentra-bark is a liquid.

which were harvested from a 1-mo culture on PDA by pipetting 2 mL of sterile water onto the agar surface, filtering the suspension through two layers of sterile cheesecloth, then adjusting the conidial concentration with sterile water to 1×10^5 spores/mL.

Ascospores of *E. lata* were harvested each year from fruiting bodies (perithecia) that were collected in the field from the infected wood (visibly covered in stromata, within which perithecia were embedded) of a *Nerium oleander* L. in Yolo County, CA. Perithecia were present on dead wood of a branch on the tree. Their morphology corresponded to that of *E. lata*, based on identification of fungal species from the Diatrypeaceae family (Trouillas et al. 2010). To collect ascospores from perithecia, stromata were sliced with a sterile razor blade to reveal and cut open the perithecial cavities, and a drop of sterile water was placed on the perithecia. Masses of ascospores were then collected with a sterile probe and transferred to 1-mL sterile water, then the concentration of ascospores was adjusted with sterile water to 1×10^5 spores/mL.

Each year on the day before inoculations, inoculum (i.e., the spore suspensions described above) was prepared in the lab in Davis, CA and stored overnight at 4°C. On the day of inoculation, spore suspensions were kept on ice during transport to the field site. For each pathogen, inoculum was pipetted onto the cut surface of the pruning wounds on the spurs of data vines (50 µL or 5000 spores per spur). For non-inoculated (water-inoculated, control) spurs, 50 µL of sterile water was pipetted onto the cut surface of the pruning wound. After all inoculations were done and we returned to the lab, viability of the unused portion of inoculum was tested by plating 50 µL of inoculum per pathogen on PDA.

Detection of pathogens to measure fungicide efficacy

Inoculated and non-inoculated spurs (the distal 15 cm) were collected each year before budbreak. From spurs inoculated with *N. parvum*, pieces of wood were used for recovery attempts in culture to determine whether the spur was infected (i.e., the fungicide did not prevent infection) or not infected (i.e., the fungicide prevented infection). The bark was first scraped off the surface of each spur with a flame-sterilized knife to reveal discolored wood, also known as wood 'lesions'. The distal section of ~0.5 cm of dried wood at the cut surface of the pruning wound was cut away. From the 2-cm section of wood below this discarded 0.5-cm end, and specifically from the margin of the discolored wood, we cut 16 small (each ~5 mm³) pieces of wood (if present). The pieces of wood were surface-sterilized in 0.6% sodium hypochlorite (pH 7.2) for 15 sec, rinsed twice in sterile distilled water for 1 min, then plated onto two PDA plates amended with tetracycline (1 mg/L; Sigma-Aldrich) with eight wood pieces per plate. PDA plates were incubated at 22°C in the dark for 10 days. Plates were examined daily for up to 7 days to evaluate *N. parvum* recovery, based on colony morphology. Subcultures were hyphal-tip purified to PDA plates for identification.

From spurs inoculated with either *E. lata* or *P. minimum*, separate sets of quantitative PCR (qPCR) reactions were used to detect their DNA, with species-specific *E. lata* primers (Brown et al. 2021, Fujiyoshi et al. 2021a) and *P. minimum* primers (Pouzoulet et al. 2013), as both species are difficult to isolate in culture. From the distal 15 cm of wood from each inoculated spur, the bark was first scraped off the surface with a flame-sterilized knife and the ~0.5 cm of dried wood at the cut surface of the pruning wound was cut away. A 2.5-cm section of wood from below this discarded 0.5-cm end was sealed in a pre-labeled glass vial and stored at -80°C. Wood samples were ground to a powder in chilled containers (Grinder MM400, Retsch) and stored at -80°C in 2-mL microcentrifuge tubes. For DNA extraction, 1 mL of extraction buffer (100 mM Tris-HCl, 20 mM ethylenediaminetetraacetic acid [EDTA], 1.4 M sodium chloride, 2% cetyltrimethylammonium bromide [CTAB], 2% polyvinylpyrrolidone [PVPP], 0.5% β-mercaptoethanol, and 0.4% v/v RNase A; Qiagen) was added to 100 mg of wood powder in the 2-mL tube. Tubes were briefly vortexed, 500 µL of chloroform-isoamyl-alcohol (24:1) was added, and tubes were incubated on ice for 5 min then centrifuged (2300 g, 10 min, 4°C). The supernatant was transferred to a new tube and mixed with AP2 buffer and the rest of the manufacturer's protocol for the DNeasy plant mini kit (Qiagen) was followed.

For qPCR, 1 µL of 1x DNA extraction was used as template in a 25-µL reaction volume consisting of 1x Brilliant SYBR Green q-PCR Master Mix (Stratagene), 150 nM for each primer (Operon Biotechnologies), 30 nM ROX Reference Dye (Invitrogen), and sterile molecular biology-grade water (GIBCO). All reactions were performed in 200-µL tubes in 96-well plates using an Mx3000p Real-time PCR Thermal Cycler (Stratagene). The PCR program was as follows: initial denaturation step at 95°C for 3 min, 50 cycles of 20 sec at 94°C, followed by 20 sec at 65°C for both annealing and extension, and additional melting analysis. After the amplifications were completed, dissociation curves were obtained based on a standard protocol from manufacturer instructions, and the temperature of the peak of the curve was checked to confirm the correct PCR product. The threshold level for fluorescence was set arbitrarily within the log-linear phase of increase. Genomic DNA from pure cultures was used for positive controls. Amplification of target DNA was based on the dissociation temperature (79.0 to 79.5°C for *E. lata*, 75.9°C for *P. minimum*). Positive detection was defined as samples crossing the threshold level by 40 cycles. Detection (%) for each data vine was the average percentage of spurs positive for the inoculated pathogen, out of five inoculated.

There were no obvious symptoms of trunk diseases in the vineyard. Nonetheless, our experimental design included non-inoculated (water-inoculated, control) spurs on the data vines, to determine whether spores of the causal fungi of trunk diseases were present in the vineyard. Possible sources of such spores, which could infect spurs on the data vines between the time of pruning and collection of the spurs, could include infected vines within the vineyard,

infected vines outside the vineyard, or alternate hosts outside the vineyard. Groups of pathogens we were particularly interested in isolating from non-inoculated spurs were the three species inoculated as part of the experimental design (*E. lata*, *N. parvum*, and *P. minimum*), the natural presence of which would be expected to contribute to the detection rates we measured; the causal fungi of Botryosphaeria dieback, Eutypa dieback, and Esca, other than the three species we inoculated to spurs; and the causal fungi of other trunk diseases, namely Cytospora dieback and Phomopsis dieback. To check this 'background level' of 'local' pathogens, we attempted to culture fungi from all non-inoculated spurs, which included both spurs treated with fungicides and those treated with water. Spur processing was the same as described above for *N. parvum*, except that PDA plates were examined every other day for 2 wk. Within 2 wk of incubation of the PDA plates, fungal colonies with morphological characteristics of the species listed above were subcultured onto new PDA plates and further hyphal-tip purified for identification, based on DNA sequencing of the nuclear, ribosomal internal transgenic spacer (ITS) region (Lawrence et al. 2017).

Statistical analyses

For each pathogen, separate analyses of variance (ANOVAs) were used to determine the main effects of year (2020, 2021, 2022), experimental treatment (a water-treated control, pyraclostrobin + boscalid, pyraclostrobin + boscalid + Pentra-bark, thiophanate-methyl, thiophanate-methyl + Pentra-bark), and their interaction on detection (%). Normality and homogeneity of variances were evaluated and confirmed using normal probability plots and Levene's test, respectively. ANOVAs were performed using the MIXED procedure in SAS ver. 9.4 (SAS Institute, Inc.), with year and experimental treatment as fixed effects and block and block interactions as random effects. Year was considered a repeated measure. For significant main or interactive effects ($p < 0.05$), means were compared by Tukey's tests. When interactive effects were not significant, means for significant main effects were compared by Tukey's tests. To aid in the presentation and interpretation of the detection rates, we also calculated efficacy (%) as $100 \times [1 - (\text{Detection}_{\text{Fungicide}} / \text{Detection}_{\text{Control}})]$, where $\text{Detection}_{\text{Fungicide}}$ is detection (%) of the pathogen from fungicide-treated spurs and $\text{Detection}_{\text{Control}}$ is detection (%) of the pathogen from water-treated spurs. In this way, a high detection rate corresponds to a low efficacy. Efficacies of the fungicides against the pathogens were calculated from detection rates, but efficacies were not analyzed statistically.

Results

Differences in detection among study years

To ensure that our experimental approach evaluated fungicide efficacy consistently among fungicide treatments and over time, we used the same procedures to prepare inoculum each year and we evaluated inoculum viability on

the day of inoculation. In 2020 (year 1), no colonies grew from the inoculum of *E. lata* (ascospores), which was plated on the day of inoculation. This finding of non-viable *E. lata* inoculum in 2020 was consistent with no detection of *E. lata* (via the DNA-based method) from the water-treated, inoculated control spurs which were collected 18 days later (Table 3). However, *E. lata* inoculum was viable (and inoculations were successful) in two of three study years. *E. lata* was detected in 100% and 64% of control spurs in 2021 and 2022, respectively. The inoculum of *N. parvum* (conidia) was viable in all three study years. Detection of *N. parvum* from control spurs ranged from a low of 33% in 2022 to a high of 47% in 2020. The incubation period between the day of inoculation and collection of inoculated spurs increased each year (18, 29, and 32 days in 2020, 2021, and 2022, respectively; Table 1). The inoculum of *P. minimum* (conidia) was viable in all three study years. There were incremental increases in detection with each study year found for *P. minimum* only, with detection in 41, 79, and 90% of control spurs in 2020, 2021, and 2022, respectively (Table 3).

Fungicide efficacy

In 2021, detection percentages of *E. lata* from all fungicide-treated spurs were significantly lower than that of the water-treated, inoculated control (significant main effect of experimental treatment, $p = 0.0001$; Table 3). Detection percentages of *E. lata* from spurs treated with fungicide either alone or with Pentra-bark were approximately half that of the control in 2021. This corresponded to efficacy levels ranging from 42% for thiophanate-methyl + Pentra-bark to 55% for pyraclostrobin + boscalid. Detection was significantly higher in 2021 for all treatments (especially the control) than in 2022 (significant main effect of year, $p = 0.04$). In 2022, the combination of Pentra-bark with pyraclostrobin + boscalid was associated with significantly lower detection of *E. lata* (42%), and this was the only treatment with a level of detection significantly lower than that of the control. In contrast, detection of *E. lata* from spurs treated with pyraclostrobin + boscalid alone was not significantly different from that of the control (52% versus 64%, respectively).

In 2020, 2021, and 2022, detection percentages of *N. parvum* from spurs treated with thiophanate-methyl, both alone and with Pentra-bark, were significantly lower than that of the control (significant main effect of experimental treatment, $p < 0.0001$; Table 3), illustrating the stable efficacy over time of thiophanate-methyl against *N. parvum*. In 2020 and 2021, the combination of Pentra-bark with pyraclostrobin + boscalid was associated with significantly lower detection of *N. parvum*, whereas detection from pyraclostrobin + boscalid alone was not significantly different from that of the control. Other fungicide treatments that were associated in some years with significantly lower detection of *N. parvum*, relative to that of the control, included thiophanate-methyl (2021 and 2022) and pyraclostrobin + boscalid + Pentra-bark (2020 and 2021). Detection of *N. parvum* was higher for all experimental treatments

(including the control) in 2020 than in 2021 and 2022 (significant main effect of year, $p < 0.0001$). Fungicide efficacy against *N. parvum* was greater than 50% for all fungicides alone and with Pentra-bark in 2021 and 2022, but in 2020, only the fungicides with Pentra-bark had efficacies greater than 50%.

Detection percentages of *P. minimum* from spurs treated with fungicides alone or with Pentra-bark were not significantly lower than that of the control in any study year (main effect of experimental treatment not significant, $p = 0.1424$; Table 3). In 2021 and 2022, there was a trend of lower detection of *P. minimum* from spurs treated with thiophanate-methyl alone or with Pentra-bark, relative to that of the control. Detection of *P. minimum* was lower for all experimental treatments (including the control) in 2020 than in 2021 and 2022 (significant main effect of year, $p < 0.0001$).

Pathogens and other fungi isolated from non-inoculated spurs

Across the three years, isolations from non-inoculated spurs on data vines in the different experimental treatments yielded many isolations of *Botrytis cinerea*, as identified based on colony morphology. The presence of this generalist fungus, the causal agent of grapevine bunch rot and grey mold, may have obscured the isolation of other fungi of

interest. Nonetheless, based on culture attempts from 675 total non-inoculated spurs (45 water-inoculated spurs per experimental treatment $\times$ five experimental treatments $\times$ three years), we cultured eight isolates with colonies on PDA resembling that of *E. lata*. These eight isolates were further identified (based on DNA sequencing of the ITS gene region) as *E. lata* (one isolate) and *Eutypella microtheca* (seven isolates). The estimated natural infection rate of non-inoculated spurs with these two species was thus ~1% (eight spurs out of 675 total).

Discussion

Thiophanate-methyl and pyraclostrobin + boscalid were both effective against *Botryosphaeria*-dieback pathogen *N. parvum*, a common pathogen in the southern San Joaquin Valley (Úrbez-Torres et al. 2006). Lower detection percentages of *N. parvum* from spurs treated with fungicide alone and with Pentra-bark, compared to those of *E. lata* and especially *P. minimum*, suggests that of the three pathogens we examined, *N. parvum* was managed most effectively. Compared to fungicides alone, the addition of Pentra-bark to pyraclostrobin + boscalid and to a lesser extent, to thiophanate-methyl, was associated with lower detection percentages (i.e., greater efficacy

Table 3 Detection of pathogens inoculated onto pruning wounds after application of experimental treatments, compared to that of a water-treated, inoculated control. CL, confidence limits.

Year	Experimental treatment	<i>Eutypa lata</i>		<i>Neofusicoccum parvum</i>		<i>Phaeoacremonium minimum</i>	
		Detection (%) (95% CL) ^a	Efficacy (%) ^b	Detection (%) (95% CL)	Efficacy (%)	Detection (%) (95% CL)	Efficacy (%)
2020	Water-treated, inoculated control	0	-	47 a (37 to 56)	-	41 a (26 to 55)	-
	Pyraclostrobin + boscalid	0	-	27 ab (18 to 37)	42	38 a (23 to 52)	7
	Pyraclostrobin + boscalid + Pentra-bark	0	-	17 b (7 to 28)	63	50 a (34 to 65)	0
	Thiophanate-methyl	0	-	39 ab (30 to 48)	17	52 a (37 to 66)	0
	Thiophanate-methyl + Pentra-bark	0	-	17 b (7 to 26)	64	48 a (32 to 63)	0
2021	Water-treated, inoculated control	100 a (83 to 100)	-	24 a (15 to 34)	-	79 a (60 to 98)	-
	Pyraclostrobin + boscalid	45 b (29 to 61)	55	9 ab (0 to 18)	64	87 a (72 to 100)	0
	Pyraclostrobin + boscalid + Pentra-bark	51 b (36 to 66)	49	0 b (0 to 10)	100	87 a (72 to 100)	0
	Thiophanate-methyl	56 b (41 to 71)	44	0 b (0 to 10)	100	60 a (46 to 74)	24
	Thiophanate-methyl + Pentra-bark	58 b (43 to 73)	42	0 b (0 to 10)	100	72 a (58 to 86)	9
2022	Water-treated, inoculated control	64 a (49 to 79)	-	33 a (24 to 43)	-	90 a (75 to 100)	-
	Pyraclostrobin + boscalid	51 ab (34 to 68)	20	10 ab (0 to 20)	70	80 a (66 to 94)	11
	Pyraclostrobin + boscalid + Pentra-bark	42 b (27 to 57)	35	13 ab (4 to 23)	60	80 a (61 to 99)	11
	Thiophanate-methyl	56 ab (41 to 71)	14	7 b (0 to 16)	80	62 a (48 to 77)	31
	Thiophanate-methyl + Pentra-bark	44 ab (29 to 59)	31	0 b (0 to 10)	100	66 a (52 to 80)	27

^aDetection is the percentage of spurs from which the pathogen was detected, out of the total number of inoculated spurs (95% CL are shown in parentheses). Each value is the mean of 39 to 45 observations, summed across three replicate blocks. For each pathogen, detection rates within a year with different letters are significantly different (Tukey's test, $p < 0.05$).

^bEfficacy is $100 \times [1 - (\text{Detection}_{\text{Fungicide}} / \text{Detection}_{\text{Control}})]$ and represents the percentage of spurs protected from infection. Fungicide-treated spurs with detection percentages greater than that of the water-treated control were completely ineffective and given an efficacy of 0, rather than the calculated negative value.

against) of *N. parvum* and to a lesser extent, of *E. lata*. However, greater efficacy with Pentra-bark was not consistent over time or among pathogens. When sprayed on the bark surface of oak trees, Pentra-bark mixed with phosphites is reportedly effective against the Sudden oak death pathogen *Phytophthora ramorum* (Garbelotto et al. 2007). Fungicides mixed with Pentra-bark also show promise against *Harringtonia lauricola* (syn. *Raffaelea lauricola*), the causal fungus of Laurel wilt of avocado (Ploetz et al. 2012). It is possible that a higher concentration of Pentra-bark would have been associated with more consistent improvements in fungicide efficacy over time and/or among pathogens. As all materials we tested required constant agitation during application, we made a point of shaking the tank of the backpack sprayer frequently. However, spray application using a tractor, with a highly effective agitator, may have yielded more consistent results among experimental treatments and years.

Thiophanate-methyl is a grower standard as a pruning-wound protectant against trunk diseases in California, where its use was primarily adopted based on reports of its efficacy against *E. lata* (Rolshausen et al. 2010). It was later found to be effective against *N. parvum* in table grapes Scarlet Royal (Brown et al. 2021) and Thompson Seedless (Travadon et al. 2023a). Other *Botryosphaeria*-dieback pathogens against which thiophanate-methyl provides some protection (relative to water-treated, inoculated controls) include *Botryosphaeria dothidea*, *Diplodia seriata*, *Dothiorella viticola*, and *Lasiodiplodia theobromae* (Rolshausen et al. 2010), *D. seriata* (Diaz and Latorre 2013, Martinez-Diz et al. 2021), and *Neofusicoccum luteum* (Amponsah et al. 2012). When tested against Esca pathogens, thiophanate-methyl is reported to have moderate-to-high efficacy against *P. chlamydospora* (Rolshausen et al. 2010, Diaz and Latorre 2013) and null-to-moderate efficacy against *P. minimum* (Rolshausen et al. 2010). Empirical data from our field trial suggest that thiophanate-methyl is not effective against *P. minimum*.

Thiophanate-methyl is now labeled for dormant-season application against 'canker diseases' across the United States. In California, and later in other states, thiophanate-methyl applications were allowed only through supplemental labels [special local need FIFRA 24(c), special 2(ee) recommendation]. Thiophanate-methyl belongs to the methyl-benzimidazole carbamate group of fungicides and has a single-site mode of action. Thiophanate-methyl is thus considered at high risk for development of fungicide resistance. It is not recommended for successive applications within a single season for fungal pathogens with high evolutionary potential, due to their capacity for sexual reproduction, dispersal over large distances (i.e., high gene flow), and large effective population sizes (i.e., millions of spores per generation). Fungal pathogens that fit into this category include *B. cinerea*, for which resistance to thiophanate-methyl is documented in one report (Avenot et al. 2020). To date, there are no reports of resistance to thiophanate-methyl among the pathogens that cause trunk diseases.

Various formulations of pyraclostrobin have been tested for management of trunk diseases in vineyards around

the world. Depending on the study, pyraclostrobin is reportedly weakly-to-moderately effective against *E. lata* (e.g., in California [Rolshausen et al. 2010, Brown et al. 2021], Washington [Baumgartner et al. 2023], and Australia [Sosnowski et al. 2008, 2013, Ayres et al. 2017, 2022]), moderately-to-highly effective against *Botryosphaeria*-dieback pathogens (e.g., *B. dothidea*, *D. seriata*, *D. viticola*, and *L. theobromae* in California [Rolshausen et al. 2010], *N. parvum* in California [Brown et al. 2021], and *N. luteum* in Australia [Ayres et al. 2022]), and weakly-to-highly effective against Esca pathogen *P. chlamydospora* (Rolshausen et al. 2010, Diaz and Latorre 2013, Baumgartner et al. 2023). Empirical data from our field trial suggest that pyraclostrobin + boscalid is not effective against *P. minimum*. Pyraclostrobin is typically applied during the growing season in California vineyards to manage fruit and foliar diseases (albeit with annual restrictions on maximum dosage per hectare). Based on our findings of greater efficacy of the combination of pyraclostrobin + boscalid and Pentra-bark against *N. parvum* and to a lesser extent, *E. lata*, pyraclostrobin + boscalid may be a means of utilizing this fungicide against trunk diseases during the dormant season.

Year-to-year variation in the efficacy of thiophanate-methyl against *E. lata* was observed previously in pruning-wound protection trials in Chardonnay and Thompson Seedless vineyards (Baumgartner et al. 2023, Travadon et al. 2023b). Because we rely on *E. lata* inoculum (ascospores) from environmental samples each year, variation in spore viability affects the success of the inoculations. Indeed, in 2020 there was 0% detection of *E. lata* from spurs of all experimental treatments, including the water-inoculated (control) spurs. Further, our routine quality-control check of inoculum viability, which involves plating the spore suspension on PDA plates on the day of inoculation and incubating them for a week (to determine if *E. lata* colonies grow from the spores), was negative.

Detection of the three pathogens varied significantly from year to year, although not consistently; there was no single year in which all three pathogens were detected most or least frequently. Ideal environmental conditions associated with successful infection are not known for these pathogens, which makes it difficult to speculate on a relationship between variable environmental conditions and variable detection. Longer incubation periods with each successive year were associated with higher detections of *P. minimum* over time, suggesting that detection of this pathogen benefitted from longer incubation periods in 2021 and 2022 and/or that pruning wounds were more susceptible to infection in these years. Nonetheless, our similar experiences of variable detection rates from year to year in this and other studies (Brown et al. 2021, Baumgartner et al. 2023) highlight the importance of multiyear field trials for fungicide evaluations.

Our isolations from the non-inoculated spurs, which were randomized along with inoculated spurs among data vines within each experimental treatment, revealed that *E. microtheca* was the only 'local' pathogen able to infect the pruning wounds at the time we conducted our experiments each year in this vineyard. *E. microtheca* is reported from table

grape vineyards in the southern San Joaquin Valley (Travadon et al. 2022) and the Coachella Valley (Úrbez-Torres et al. 2020). Identification of pathogens from naturally infected pruning wounds is a relatively rare approach to characterizing which pathogens are present in a vineyard (e.g., Leal et al. 2024) during a field trial. This is an important quality-control measure to determine whether the pathogens you are inoculating into vines are naturally present in the vineyard during the study, which can bias results. We did not find evidence that *E. lata*, *N. parvum*, or *P. minimum* were present at our study site, which is consistent with the absence of symptoms of the trunk diseases they cause.

Conclusion

In contrast to winegrapes, new cultivars of seedless table grapes are continually being bred and planted, often by grafting over older cultivars. As the trunks of these vines are long-lived and may support more than one cultivar grafted to the head of the trunk, it is thus important to prevent chronic wood infections from becoming established in the vineyard. The tested fungicides were applied at the typical time when final pruning is performed, in a commercial table grape vineyard in the southern San Joaquin Valley. Such field testing of fungicides, with the timing and methods of application reflective of what is used by growers (rather than timing selected by the schedules of researchers and/or at a smaller, experimental scale), is required to validate fungicide efficacies against trunk diseases. The results of field trials are an important source of information for growers and pest-control advisers. As such, our research helps inform crop protection of table grapes against trunk diseases.

Acknowledgements

This research was funded by grant 21-2621 to K. Baumgartner from the California Table Grape Commission. For inoculations in the field and processing samples in the lab, we thank Tian Tian, Paula Eschen, and Israel Luna. For carrying out the fungicide applications, we thank Walter Martinez. Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

ORCID

Kendra Baumgartner  0000-0002-5682-9016

Gabriel Torres  0009-0008-2053-7637

Alejandro I. Hernandez Rojas  0009-0009-3680-9374

Renaud Travadon  0000-0001-6062-1755

Citation

Baumgartner K, Torres G, Hernandez Rojas AI and Travadon R. 2025. Preventative management of trunk diseases in table grape *Vitis vinifera* Autumn King. *Am J Enol Vitic* 76:0760022. DOI: 10.5344/ajev.2025.25018

Data Availability

The data underlying this study are available on request from the corresponding author.

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