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Control Strategies for Trunk Diseases of Grapevine (*Vitis vinifera* L.)

By

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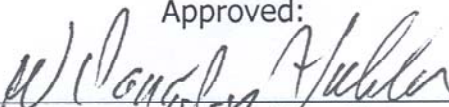
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Chapter 1

Control Strategies for Trunk Diseases of Grapevine (*Vitis vinifera* L.)

ABSTRACT

There are no therapeutic treatments for canker diseases of grapevine. These diseases can be prevented, however, by protecting dormant pruning wounds from airborne inoculum. A single fungicide application after pruning reduced canker incidence in 159/160 cases and canker severity in 16/16 cases. Thiophanate-methyl was most effective against *Lasiodiplodia theobromae* and myclobutanil was effective against *Eutypa lata*. Both fungicides were effective against *Phaeoacremonium aleophilum* and *Phaeoconiella chlamydospora*. Pruning wounds were found to be most susceptible to canker pathogens in December and least susceptible in March, and late or double pruning was shown to augment fungicide effectiveness in reducing canker disease incidence. Pruning wound susceptibility to canker pathogens was found to decrease significantly three weeks after pruning. The practical implications of these results are discussed.

INTRODUCTION

Although many fungi play a role in grapevine canker diseases, *Eutypa lata* (Pers:Fr.) Tul. & C. Tul. (= *Eutypa armeniacae* Hansf. & Carter), *Phaeoacremonium aleophilum* (W. Gams, Crous, M.J. Wingf. & Mugnai), *Phaeomoniella chlamydospora* (W. Gams, Crous & M.J. Wingf.), and fungal species in the family Botryosphaeriaceae are considered to represent the most prominent etiological agents of this disease complex (63,67,87). These ascomycete fungi represent the primary etiological agents of grapevine trunk disease worldwide (42).

Canker diseases reduce production quality and quantity, kill spur positions and diminish the life expectancy of the grapevine. Vineyard damage by members of the Botryosphaeriaceae is estimated to range from 30-50% loss of productivity (44,50). Net income losses from canker diseases in California winegrape vineyards have been estimated at over \$260 million per year (75). *Eutypa dieback* causes major reductions in grape yield (8) and losses in California production of 30-62% have been reported in affected areas (60). In South Australia, 47% of the Shiraz grape producing area is affected by *Eutypa dieback*, and losses in Australia are estimated to be Aus\$2800 per hectare (92). *Esca* reduces berry quality (6) and reduces vineyard longevity (10,57). *Esca* is a threat to developing economies in countries such as Slovakia (32,35). Control of grapevine canker diseases would help to protect the value of the vineyard, wine

and tourism industries which are valued at over \$51.8 billion in California alone (52).

Moller and Kasimatis (53) made the first report of *E. lata* as a causal agent of canker disease in grapevines. The pathogen causes Eutypa dieback of grapevine, which is manifest as cankers and small, chlorotic leaves adjacent to the infected area.

Symptoms of diseases caused by members of the Botryosphaeriaceae include delayed budbreak, bud mortality, cane and shoot dieback, stunted growth, leaf chlorosis, wood cankers and bunch rot (82,88,90). In California, spur death is the only reported symptom of Botryosphaeriaceae infection (87). Thirteen species in the Botryosphaeriaceae have been reported to cause canker diseases of grapevine (43,66,67,84,85,86,88,90). There is great variability reported in the virulence of these species. This can be attributed to diverse environmental influences across the cosmopolitan distribution of this group of fungi (66,82,84,88). Although various species have been found to be more virulent in various parts of the world (88), *Lasiodiplodia theobromae* (Berk. & M.A. Curtis) Arx is reported to be the most virulent species in California, based on pathogenicity assays on grapevine (87).

Phaeoacremonium aleophilum (*Pa*) and *Phaeoconiella chlamydospora* (*Pch*) are the causal agents of esca. Esca is usually manifest as foliar interveinal chlorosis or wilt of entire branches, a phenomenon known as apoplexy (63). These fungi also cause wood cankers and dark streaking of the xylem tissue

(56). Eleven species of *Phaeoacremonium* have been isolated from grapevine, but *Pal* is the most prevalent and has the most cosmopolitan distribution. *Pal* is more virulent than *Pch* (37), but *Pch* is the most frequently encountered species isolated from esca-affected grapevines in South Africa (56). Because of their wide distribution and high virulence, *Pal* and *Pch* appear to be the esca pathogens.

Pathogen life cycles and disease epidemiology are very similar among trunk disease fungi. Pathogen entry is facilitated by mechanical injury or pruning of the host followed by symptom development that takes months to years to develop and expression may be discontinuous in the case of esca (48). These fungi subsequently colonize host wood, causing structural decay and ultimately death of infected vines. Symptom development may be facilitated by the release of phytotoxins including naphthalenone pentaketides from *Pal* and *Pch*, and lipophilic low-molecular weight compounds from *E. lata* (20,21,39,46,81). Many members of the Botryosphaeriaceae are known to produce hydrophilic high-molecular weight compounds with phytotoxic properties, and some species produce lipophilic low-molecular weight phytotoxins (49).

Fruiting bodies form on dead wood, and spore release occurs during precipitation events. Spores are wind distributed to uninfected wounds. The canker pathogens have optimal dispersal and infectivity following dormant pruning (72). There is strong evidence that *Pal* and *Pch* infections occur primarily during the winter pruning season. Ascospores and conidia of *Togninia minima*

(*Pal*) and *Pch*, respectively, are aeri ally dispersed and have been isolated in the field from petrolatum-coated glass slides (17,19,40). In addition, several species in the *Botryosphaeriaceae*, as well as the diatrypaceous *E. lata* and *E. leptoplaca* have been shown to release conidia during winter rains in CA (30,83).

Pruning wounds are considered to be the key infection site for canker pathogens of grapevine. Pruning creates wounds which enable these pathogens to enter the grapevine (53). Workers in France found that *Pch* was isolated more frequently from pruned canes than unpruned canes. Transmission of *Pal* and *Pch* is thought to occur primarily through pruning wounds (55,56). Root infection with *Pal* is possible but rarely successful and is not considered to be of significant agronomic importance (18). *Pch* is more frequently isolated from grapevine rootstocks than *Pal* (73).

Eskalen and Gubler found airborne inoculum of *Pal* and *Pch* in the winter and spring (17). Rainfall stimulates spore release and appears to be a prerequisite for natural infection. South African workers correlated the incidence of *Phaeo moniella* spp. in mature grapevine cordons with rainfall patterns and found that both *Phaeo moniella* spp. predominated in winter rainfall regions (89). Pathogenicity studies have indicated that *Pal* and *Pch* effectively colonize pruning wounds following the application of an aqueous conidial suspension (37,41). Rainfall promotes release of conidia and ascospores in *Botryosphaeriaceae* (30), and pruning wounds are a demonstrated route of pathogen entry into the

grapevine (84). Xylem penetration by *E. lata* following pruning is the principal mode of infection for this pathogen (53,83).

Management of grapevine canker diseases is extremely difficult. Information on control measures is limited (90) and varies between geographical regions (50). Eradication is impossible, so control is focused on disease prevention and mitigation (12,15). Substantial efforts to identify resistant rootstocks have been ineffective in the case of *Pal* (18,56). Unless a resistance factor can be translocated upward from the resistant rootstock, such an approach would likely fail to inhibit fungal growth in the scion, which is the vulnerable component of the canker disease system.

Although the technical capacity for transgenic grapevine development has been well-established (69), there is significant public resistance to genetic modification of grapes, which would hinder successful adoption of transgenic grapes (33,62,68). There is little or no literature available on the development of transgenic grapevines resistant to canker diseases.

Efforts at biological control have yielded inconsistent results. Although *Fusarium lateritium* Nees, *Cladosporium herbarum* (Pers.) Link, *Trichoderma harzanium*, and *Bacillus subtilis* have been shown to independently reduce Botryosphaeriaceae or *Eutypa* mycelial growth in vitro (7,9,22,23,34,36,58), field results with these organisms have yielded erratic control (76,90). There may be insufficient residual protection or too narrow a list of target pathogens to justify commercialization of these biological control agents (76,94).

There is a consensus that canker disease transmission can be prevented by protecting pruning wounds. Several workers have noted that early pruning wounds are more susceptible to canker pathogen infection than late pruning wounds (65,80,91). This is thought to occur because pruning wounds heal faster in spring time and precipitation depletes initial inoculum load (53,72). Other workers indicated that reduced infection of late-season pruning wounds is due to host factors, such as enhanced healing by deposition of polymerized phenolic compounds in opened wood vessels. Improved resistance to infection could also be explained by the more rapid growth of symbiotic bacterial epiphytes on late-season pruning wounds, a form of ecological resource sequestration (61).

Weber et al (91) described a control method for *E. lata* on spur-pruned vineyards called "double pruning." Double pruning requires two pruning passes through the vineyard. The vines are first prepruned by uniformly cutting canes to a height of approximately 30 to 45 cm above spur positions using a tractor-mounted saw blade. The vines are then pruned to two-bud spurs in late winter when infection events were less likely. This method is particularly useful in large vineyards, where a single round of late pruning is too time-consuming to be logistically feasible. Double pruning can reduce the number of infected spurs if *E. lata* infections on early pruning wounds do not develop beyond the point where final pruning will occur. It is an attractive cultural control method because it incurs few additional costs beyond standard viticultural practices. It is cost effective and highly efficient against canker pathogens.

There is agreement among investigators that canker disease control measures should be integrated for maximum effectiveness (4,24). Double pruning ensures that final wounds are made when pathogens are least likely to successfully colonize exposed tissue. Wound protection complements double pruning or late pruning by ensuring that inoculum that presents on vulnerable sites fails to colonize the host.

Given the inadequacy of biological control agents, commercial efforts at pruning wound protection have centered on chemical control. The ancient Romans utilized various mixtures and potions of questionable effectiveness. Roman farmers applied macerated herbs (*Momordica elaterium* L., a species of *Equisetum*), honey or human urine at the base of infected vines (78). Other early control methods included brushing infected plants with boiled water and oil, water and wine, saltwater, or soaking ashes in water and pouring the mixture around symptomatic vines. Dormant applications of sodium arsenite were used in California and Europe until the compound was banned for use on grapes in 1977 (79). Sodium arsenite is an effective treatment for esca, but it poses a significant phytotoxicity risk to treated vines and also poses significant health risks to end users, consumers, and can bioaccumulate in the environment (38,45).

Moller and Kasimatis (54) demonstrated that grapevine pruning wounds painted with benomyl were significantly protected from infection by *E. lata*. Rolshausen et al (72) extended this work to show that boric acid paint was effective against *E. lata*, but the high labor cost of paint application has hindered

large-scale adoption of this control measure. In a different crop system, fungicide spray applications to pruning wounds were shown to reduce the incidence of Botryosphaeriaceae-induced cankers on apple trees (3). Several workers confirmed the effectiveness of benomyl as a sprayable pruning wound protectant against *E. lata* (11,59). However, use of benomyl was discontinued in 2001 (72).

Various workers have assayed the effectiveness of various hand-applied fungicide sprays against grapevine canker pathogens. Munkvold and Marois (59) found that flusilazole could protect pruning wounds artificially inoculated with *E. lata*. Carbendazim and pyrimethanil were found to reduce infection in pruning wounds inoculated with *E. lata* up to 14 and 7 days after inoculation, respectively (77). In a notable longitudinal study, airblast sprays of benomyl were found to reduce *E. lata* infection by 48.5% reduction over a five year period (70).

The sterol biosynthesis inhibiting fungicides (FRAC: G1) and the demethylation inhibiting fungicides (FRAC: B1) have shown promise against various canker pathogens (2,5,25,26,54). Thiophanate-methyl, fenbuconazole and myclobutanil are good representatives of the modes of action (FRAC: B1 and G1) observed to have in vitro effectiveness on canker diseases. These fungicides have established tolerances on grapes by the Environmental Protection Agency (EPA) in the United States, and are thus suitable candidates for field trials against these diseases.

Although myclobutanil and fenbuconazole are both G1 fungicides, several workers have found variable sensitivity to compounds with this mode of action in heterologous and wildtype ascomycetes (29,93). The mechanisms of this phenomenon are not fully understood. Some workers postulate that myclobutanil has a weaker binding potential to 14 α -demethylase than fenbuconazole; others suggest that the pathogen cell membrane may exhibit different permeability to these fungicides (27,28,74).

Other approaches to chemical control have been problematic. Some workers have postulated that the use of the melanin biosynthesis inhibiting fungicides could exacerbate esca disease symptoms because intermediates in the pentaketide biosynthetic pathway of these organisms are phytotoxic (1,49,64). Tests of systemic acquired resistance (SAR) activators have not been effective against esca (13). Trunk injections of fosetyl Al, cyproconazole and tetraconazole have demonstrated temporary curative activity against esca but such treatments are cost prohibitive for commercial use (5).

The first objective of this research was to evaluate the influence of pruning time on canker disease incidence. Previous studies focused primarily on the effect of pruning time on infection by *E. lata*. An important gap in the literature is the influence of pruning time across the four major trunk disease pathogens, so a comprehensive time course for pruning and pathogen inoculations was undertaken.

A second objective was to characterize the effect of pruning wound age on susceptibility to canker pathogens. It is important to know how long pruning wounds remain susceptible to specific pathogens in order to implement pruning wound protection regimes of sufficient duration.

Trunk diseases exact a large and ongoing economic toll on grape producers and allied industries, so the third objective of this project was to develop and implement chemical and culturally acceptable control methods for grapevine trunk diseases. Important work had been done on control of these pathogens *in vitro* and in nursery settings. Field studies on some individual pathogens had been carried out, but comprehensive field research on control methods remained to be done.

MATERIALS AND METHODS

Inoculum source. Two isolates of each pathogen except *E. lata*, obtained were obtained from different geographic areas in California. Because *E. lata* does not produce ascospores in vitro, inoculum was harvested directly from native stroma in sterile distilled water (SDW). *E. lata* stromas were obtained from various locations in northern California grape production areas. Isolates of *Phaeomoniella aleophilum*, *Phaeomoniella chlamydospora* and *Lasiodiplodia theobromae* were recovered from grapevine wood that had typical dark-brown to black vascular streaks for *Pal* or *Pch* or wedge-shaped cankers for *L. theobromae*. The isolates were *Pal* 33b, *Pal* a78a, *Pch* c40a, *Pch* c63b, *L. theobromae* 196 and *L. theobromae* 206. Sections were cut from infected areas of cordons and surface-sterilized. Small segments of discolored tissue were plated on potato dextrose agar (PDA) amended with 0.1 g l⁻¹ of tetracycline (PDA-tet). Plates were incubated for 10-15 days at 25 °C. Single-spore isolates of each were prepared according to the serial dilution method (14). Isolates were preserved on filter paper at -20 °C in 2 ml micro tubes.

Inoculum preparation. Ten to fifteen day-old cultures were harvested in SDW and passed through a double layer of cheesecloth to remove mycelial fragments. Inoculum was retained for up to a month at 4 C and tested within 7 days of field use by plating on PDA to ensure viability. A spore germination rate above 50% was considered viable. The final concentration of inoculum was

adjusted to 10^6 spores ml^{-1} using a haemocytometer, and 25 μl of inoculum was topically applied to each wound within 48 hours of pruning.

Isolations. One-year old spur positions were removed up to 10 cm below each pruning wound, and kept at 4 C until they could be processed. Isolations from spur wood were made by surface sterilizing samples, dissecting one-year old wood pieces and plating fragments from the margin of discolored vascular tissue on Potato Dextrose Agar +tetracycline (PDA-tet). Samples were incubated in light for up to 10 days at 20-25C to enable morphological identification according to Péros (63).

Fungicides. All fungicides were applied as a single directed spray to dormant pruning wounds in a water volume of 467 liters/hectare. Hi-Light spray pattern indicator (Becker Underwood, Ames, IA) was used to assure proper coverage and equipment calibration. Pentra-Bark (Quest Products Inc., Louisburg, KS) or Freeway (Loveland Products Inc., Greeley, CO) were included as penetrating organosilicone surfactants at label rates in each fungicide treatment. Fenbuconazole was applied as Enable 2F (Dow Agrosiences LLC, Indianapolis, IN 46268), thiophanate-methyl was applied as Topsin M (United Phosphorus Inc., King of Prussia, PA), and myclobutanil was applied as Rally 40WSP (Dow Agrosiences LLC, Indianapolis, IN, 46268). Treatments and application rates are listed in Table 1.

Pathogen incidence. Five field assays (designated Trials 1 through 5) were performed at two sites each to measure the effectiveness of different

fungicide treatments on pathogen recovery from inoculated pruning wounds. The plot size for Trials 1-5 was four vines, and each vine was a subplot. A randomized split-plot design with four replicates per treatment-pathogen combination was used in each trial with pathogen as the within factor and fungicide the between factor as described by Rao (71). Sites were located at Cabernet Sauvignon, Chardonnay, and Thompson vineyards located in Napa and Yolo counties. Vines were pruned to 4 buds and treatments were applied within 12 hours. All treatments were applied with motorized backpack sprayers at a rate of 50 gallons/acre. The protocol schedule for each trial is listed in Table 2.

Extent of Pathogen Development. A sixth assay (Trial 6) was performed at two Pinot Noir vineyards in Napa and Sonoma counties to measure the effectiveness of different fungicide treatments on pathogen infection severity on inoculated pruning wounds. Vines were pruned to 4 buds and treatments were applied within 12 hours. All treatments were applied with commercial tractor equipment as a directed spray on pruning wounds, and inoculations were performed within 24 hours. Infection severity was determined by longitudinally dissecting each inoculated cane, measuring lesion length, and confirming pathogen presence by isolation on PDA-tet. The plot size for Trial 6 was one vine, and each inoculated cane was a subplot. A partially randomized split-plot design with ten replicates per treatment-pathogen combination was used in each trial with pathogen as the within factor and fungicide the between factor as described by Rao (71). Experimental design at the subplot level was randomized;

however, arrangement at the plot level was not randomized since contiguous sets of vines were selected for the design. This experimental array was chosen to make the experiment logistically feasible, allowing tractor application as a more accurate approximation of actual use patterns.

Pruning and inoculation time. A randomized split-plot design with four replicates per treatment-pathogen combination was used in the seventh set of assays (Trial 7) with pathogen as the within factor and fungicide and pruning time or inoculation time as the between factors as described by Rao (71). Inoculation assays were conducted at two sites to measure the effect of pruning time and inoculation time (i.e. pruning wound age) on incidence of infection with each pathogen. Vines were pruned in November, December, January, February and March and fungicide was applied within 12 hours of each pruning time. Grapevines were inoculated at 12 hours, 7, 14 and 21 days after pruning in early December with inoculum of *L. theobromae*, *E. lata*, *Pal* and *Pch*.

Phytotoxicity. Phytotoxicity on new shoot growth was quantified by manually counting the incidence of bud failure in all trials in April of each trial year. General morphological observations were made to look for signs of tissue damage such as scorching or etiolation.

Statistical analyses. Once isolations were completed, incidence of infection was determined by calculating the proportion of infected canes out of the total inoculated canes ($n \geq 8$) on each vine. Data were analyzed with SAS V. 9.1 (SAS Institute, Cary, NC).. Residuals were examined to determine if

transformation could improve heteroskedasticity, and the original data were found to have the most normal distribution. Infection incidence and severity were analyzed by repeated measures ANOVA to evaluate the dependence of the biological response on each independent variable (fungicide, pruning time, inoculation). Significance was set at $\alpha = 0.05$ for these analyses.

Whereas the objective of ANOVA is to determine whether or not experimental factors have a significant effect on the response variable, the objective of the phytotoxicity observations was to determine if the subjects were significantly similar. For this reason, 95% confidence intervals were calculated for bud failure incidence with each treatment. Rao's (1998) method was used to find 95% confidence intervals, where $\text{mean} - t(n-1, \alpha/2) * SE \leq \mu \leq \text{mean} + t(n-1, \alpha/2) * SE$. The value of t is dependent on sample size, and was found for each trial using R V. 2.7.2 (The R Project for Statistical Computing, University of Auckland, New Zealand).

RESULTS

Pathogen Incidence. Among stand alone fungicides, Topsin M was most effective against *L. theobromae* and Rally 40WSP was most effective against *E. lata*. There was no apparent antagonism between these fungicides, and the tankmix of Enable 2F + Rally 40WSP + Topsin M (ERT) statistically performed as well or better than the best fungicide alone in each case. Incidence of all canker pathogens was higher in canes inoculated in January (Trials 2-3) compared with canes inoculated in February (Trials 4-5). Fungicides reduced *L. theobromae* and *Pa*/incidence significantly on Chardonnay grapevines in Trial 1 (Figure 1). Rally 40WSP was the most effective treatment on *L. theobromae* and *Pa*/in Trial 1.

Fungicides significantly reduced the incidence of all canker pathogens in Trials 2 and 3 on Chardonnay grapevines (Figure 2). Similar results were found on Cabernet Sauvignon grapevines (Figure 3). Results from 2008 and 2009 were comparable. The combination of Enable 2F+ Rally 40WSP + Topsin M (ERT) was the most effective treatment for all pathogens, although Rally 40WSP alone was as effective against *E. lata* as ERT. Topsin performed better than the FRAC: G1 fungicides against *L. theobromae*.

Trials 4 and 5 had generally lower levels of infection than Trials 2 and 3, which is consistent with the disease suppressive attributes of late pruning. There was no canker pathogen infection observed in Trial 4 Cabernet Sauvignon grapevines, so no differences were observed between fungicides and the untreated control. There were still significant differences in disease incidence

between fungicide-treated and control vines in the rest of this trial cohort. All fungicide treatments significantly reduced *L. theobromae* and *E. lata* incidence on Chardonnay grapevines in Trial 4 (Figure 4).

Topsin M and ERT were the most effective treatments against *L. theobromae* in Trial 5. Rally 40WSP and ERT were the most effective treatments against *E. lata*. All of the fungicides reduced *Pal* and *Pch* incidence to zero (Figure 5). Results from 2008 and 2009 were comparable across grapevine varieties tested in Trials 4 and 5.

Disease Severity. Fungicides reduced pathogen development significantly on both Pinot Noir sites in Trial 6. Overall infection pressure was lower at the Napa county site, and all fungicides reduced disease severity relative to the control. Rally 40 WSP + Topsin M was significantly more effective than Rally 40WSP alone against *L. theobromae*. Similar results were observed at the Sonoma site, but the differences were more significant as a result of higher disease pressure. In all cases, Rally 40WSP + Topsin M provided nearly complete control of disease severity (Figure 6).

Pruning and inoculation time. For all canker pathogens in Trial 7, disease incidence peaked on pruning wounds made in December, then decreased significantly for pruning wounds made each successive month through March. Wounds made in November and January had the second highest disease incidences, and fungicides reduced disease incidence to zero in each month for which isolations were made in Trial 7 (Figures 7-10).

For all canker pathogens in Trial 7, incidence decreased significantly from inoculations 1 to 21 days after pruning (Figures 11-14). ERT reduced *Pal* and *Pch* incidence to zero. The point of greatest pruning wound susceptibility to *L. theobromae* and *E. lata* was 7 days after pruning for fungicide-treated wounds, and 1 day after pruning for untreated wounds.

Phytotoxicity. Incidence of bud failure was significantly similar for all treatments in Trials 1-7 (Figures 15-17). There was overlap among all 95% confidence intervals for the mean bud failure incidence for each treatment within each trial. No general morphological signs of tissue damage were observed. This indicates that the fungicides used in this study did not cause injury to the grapevines.

DISCUSSION

This study showed that fungicides sprayed on dormant pruning wounds were an effective control measure for the primary canker pathogens of grapevine. Fungicide application reduced canker pathogen incidence in 159 out of 160 cases (Figures 1-5; 7-14) and reduced the extent of pathogen growth in all 16 cases (Figure 6). This expands on the work of Moller and Kasimatis (54), who found that hand-painted benomyl was effective against *E. lata*.

There were differences in pathogen susceptibility to each mode of action. Topsin M reduced the incidence of *L. theobromae* significantly more than a FRAC G1 fungicide alone in 9/14 cases. A FRAC G1 fungicide alone reduced the incidence of *E. lata* significantly more than Topsin alone in 8/12 cases, but Rally reduced *E. lata* incidence significantly more than Enable in 4/4 cases. Rally reduced *E. lata* incidence by an average of 24.3% more than Enable.

Both FRAC G1 and FRAC B1 modes of action were comparably effective against *Pal* and *Pch*, which is consistent with other reports that show representatives of both modes of action to be effective in other use patterns against these pathogens (5,26).

The Topsin + FRAC G1 fungicide mix significantly reduced pathogen incidence more than either fungicide alone in 41/88 cases, and the mix significantly reduced pathogen severity more than Rally alone in 3/8 cases. This indicates that in many cases, optimum control will be achieved by mixing these fungicides rather than using them alone.

Mixing fungicides has been shown to reduce the risk of pathogen resistance (31). This is important because pathogen populations sometimes retain fungicide resistance even in the absence of selection pressure. For example, in grapes, resistance to triadimefon remains prevalent in *Erysiphe necator* although use of this fungicide was limited in 1992 (51). This example reinforces the importance of anticipating the evolution of fungicide resistance and implementing appropriate prevention strategies.

Pruning wounds were most susceptible to canker pathogen infection in December and least susceptible in March, which supports the findings of previous investigators regarding *E. lata* on grapevine (61,65). These findings were consistent across all four canker pathogens considered in this study, and indicate that late or double-pruning reduced the incidence of *L. theobromae*, *Pal* and *Pch* as well as *E. lata*. This work extends the evidence of double-pruning effectiveness on *E. lata* to include the other three principal canker pathogens. Incidence of infection was higher for all pathogens in December than November, which could be explained by faster suberin and lignin accumulation at warmer temperatures (16). Reduced infection in spring also has been linked to faster growth of non-pathogenic microorganisms on wound surfaces (61).

In all cases, fungicide sprays conferred additive disease protection with late pruning on inoculated pruning wounds. These chemical and cultural control methods were compatible, consistent with the suggestion of Buckley and Gould (4) that canker control measures should be integrated for maximum

effectiveness. These results clearly support the case for dormant fungicide sprays with late or double-pruning as an integrated disease management strategy for grapevine canker diseases.

Wound susceptibility declined significantly during the 21 days following pruning. Susceptibility was initially high for pruning wounds made in December, but dropped to nearly zero in three weeks across all key canker pathogens. This is consistent with the findings of Petzoldt et al (65) on *E. lata*. The point of greatest disease susceptibility on fungicide-treated pruning wounds was 7 days after pruning, which is likely due to the additive pathogen resistance determinants of fungicide residues and degree of wound healing. Fungicide residues degrade as time passes, but wound healing (i.e. lignin and suberin deposition) simultaneously increases. Fungicide-treated wounds had zero disease incidence 14 and 21 days after pruning, indicating that wound healing was sufficient to resist further infection pressure even at reduced concentrations of fungicide. Previous work indicated that pruning wound susceptibility to *E. lata* decreased significantly up to 28 days after pruning, which is consistent with these findings (61). There is consensus that pruning wounds are most vulnerable to infection in the two weeks after pruning, and protection regimes should optimize coverage of this susceptible period. The lack of observed phytotoxicity strengthens the case for fungicide application in this use pattern.

Data from this work supported the registration of Rally 40WSP by tractor-applied directed spray for control of grapevine canker diseases (EPA 24(c)

Special Local Need Registration SLN-CA09002 R182-036). The cost savings of tractor application in place of hand application improves the feasibility of implementing this control method. These results support the implementation of an integrated program of double pruning and dormant fungicide application to pruning wounds. This will reduce the incidence of key grapevine canker pathogens. Future research should build on these findings to develop novel modes of canker pathogen protection for grapevine pruning wounds that incur reasonable economic costs and minimal environmental impact.

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Table 1. Chemicals used in canker disease fungicide trials.

Trade Name	Active Ingredient	Application rate	Manufacturer
Enable 2F	Fenbuconazole, 23.5%	877 ml/hectare 12 oz/acre	Dow Agrosiences LLC 9330 Zionsville Road Indianapolis, IN 46268
Freeway	Alcohol ethoxylates, silicon polyether copolymer, propylene glycol and dimethylpolysiloxane, 100%	3507 ml/hectare 64 oz/acre	Loveland Products Inc. P.O. Box 1286 Greeley, CO 80632
Pentra-Bark	Alkylphenol ethoxylate, polysiloxane polyether copolymer, propylene glycol, 99.8%	877 ml/hectare ^a 12 oz/acre ^a	Quest Products Inc. 601 Countryside Drive Louisburg, KS 66053
Rally 40WSP	Myclobutanil, 40%	420 g/hectare 6 oz/acre	Dow Agrosiences LLC 9330 Zionsville Road Indianapolis, IN 46268
Topsin M	Thiophanate-methyl, 70%	1681 g/hectare ^b 1.5 lb/acre ^b	United Phosphorus, Inc. 630 Freedom Business Ctr Suite 402 King of Prussia, PA 19406

^a In Trials 1-3, Pentra-Bark was applied at 3507 ml/hectare to test for phytotoxicity. In subsequent trials, the application rate was reduced to 877 ml/hectare.

^b In Trials 1-3, Topsin M was applied at 2242 g/hectare. In subsequent trials, the application rate was reduced to 1681 g/hectare to comply with new Federal regulations.

Table 2. Schedule of activities for canker disease fungicide trials.

Trial	Counties ^a	Varietals	Date	Activity		
1	Yolo	Cabernet Sauvignon	3-3-07	Prune and spray		
			Yolo	3-5-07	Inoculate 2 DAP ^b	
				Yolo	3-12-07	Inoculate 9 DAP
					4-2-07	Rate bud failure
					6-13-07	Remove wood for isolations
2	Napa	Cabernet Sauvignon	1-15-08	Prune, spray and inoculate		
			Napa	3-6-08	Remove Chardonnay wood	
				3-12-08	Remove Cabernet Sauv wood	
				4-12-08	Rate bud failure	
3	Napa	Cabernet Sauvignon	1-19-09	Prune, spray and inoculate		
			Napa	3-12-09	Remove Cabernet Sauv wood	
				3-18-09	Remove Chardonnay wood	
				4-22-09	Rate Cabernet Sauv bud failure	
				4-23-09	Rate Chardonnay bud failure	
4	Napa	Cabernet Sauvignon	2-29-08	Prune, spray and inoculate		
			Napa	3-6-08	Remove Chardonnay wood	
				3-12-08	Remove Cabernet Sauv wood	
				4-12-08	Rate bud failure	
5	Napa	Cabernet Sauvignon	2-24-09	Prune, spray and inoculate		
			Napa	3-12-09	Remove Cabernet Sauv wood	
				3-18-09	Remove Chardonnay wood	
				4-22-09	Rate Cabernet Sauv bud failure	
				4-23-09	Rate Chardonnay bud failure	
6	Napa	Pinot Noir	3-5-09	Prune, spray and inoculate		
			Sonoma	4-21-09	Rate bud failure	
				5-6-09	Remove wood for isolations	
7	Napa	Cabernet Sauvignon	11-6-08	Prune, spray and inoculate		
			Napa	12-6-08	Prune and spray	
				12-9-08	Inoculate 1 DAP	
				12-15-08	Inoculate 7 DAP	
				12-22-08	Inoculate 14 DAP	
				12-28-08	Inoculate 21 DAP	
				1-6-09	Prune, spray and inoculate	
				2-4-09	Prune, spray and inoculate	
				3-5-09	Prune, spray and inoculate	
				3-12-09	Remove Cabernet Sauv wood	
				3-18-09	Remove Chardonnay wood	
4-22-09	Rate Cabernet Sauv bud failure					
4-23-09	Rate Chardonnay bud failure					

^a California, USA

^b Days after pruning is abbreviated DAP

Table 3. The results of analysis of variance to test for effects of fungicide, pruning time and wound age on canker pathogen incidence and severity.

Trial	Counties ^a	Varietals	Variable	F value	p value
1	Yolo	Chardonnay	Fungicide	5.12	0.0671
2	Napa	Cabernet Sauvignon	Fungicide	18.20	<0.0001
	Napa	Chardonnay	Fungicide	30.33	<0.0001
3	Napa	Cabernet Sauvignon	Fungicide	459.49	<0.0001
	Napa	Chardonnay	Fungicide	590.92	<0.0001
4	Napa	Cabernet Sauvignon	Fungicide	--	--
	Napa	Chardonnay	Fungicide	5.68	0.0117
5	Napa	Cabernet Sauvignon	Fungicide	13.91	<0.0003
	Napa	Chardonnay	Fungicide	18.02	<0.0001
6	Napa	Pinot Noir	Fungicide	261.96	<0.0001
	Sonoma	Pinot Noir	Fungicide	450.85	<0.0001
7	Napa	Cabernet Sauvignon	Fungicide	69.91	<0.0001
	Napa	Cabernet Sauvignon	Pruning time	5.87	<0.0001
	Napa	Cabernet Sauvignon	Wound age	494.6	<0.0001
	Napa	Chardonnay	Fungicide	6199.01	<0.0001
	Napa	Chardonnay	Pruning time	606.28	<0.0001
	Napa	Chardonnay	Wound age	547.40	<0.0001

^a California, USA

^b Days after pruning is abbreviated DAP

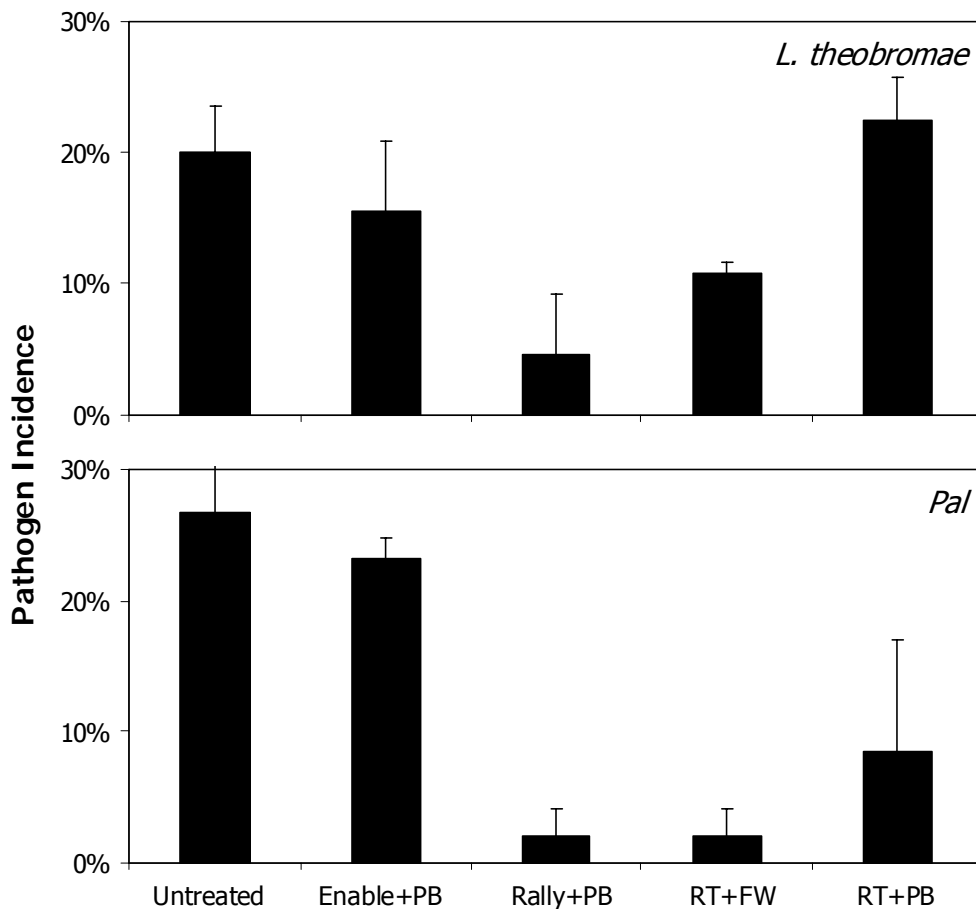


Figure 1: The effects of fungicide on canker pathogen incidence on Chardonnay grapevines in Trial 1. Incidence is the percentage reisolation of each pathogen from inoculated canes with different fungicide treatments. No significant difference was found between inoculation times 2 and 9 days after treatment ($p=0.0919$), so data were combined to add statistical power. Rally + Topsin is abbreviated RT, and surfactants are abbreviated Pentra-Bark (PB) and Freeway (FW). $N=8$. *L. theobromae* controls averaged 19.9% incidence and *Pal* controls averaged 26.7% incidence. Error bars = \pm SE.

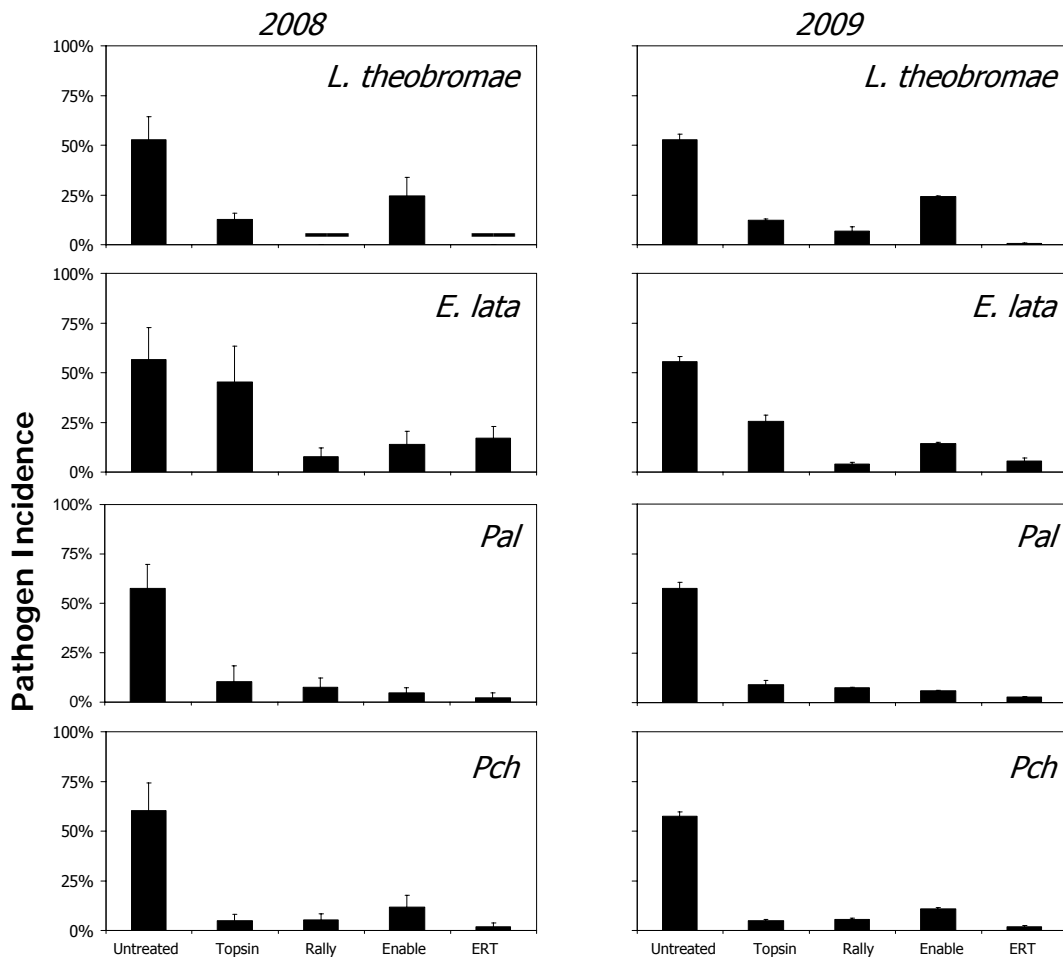


Figure 2: The effects of fungicide on canker pathogen incidence on Chardonnay grapevines in Trials 2 and 3. Incidence is the percentage reisolation of each pathogen from inoculated canes with different fungicide treatments. Treatments were made in mid-January. Results from 2008 are shown on the left, and results from 2009 are shown on the right. Enable + Rally + Topsin is abbreviated ERT. N=4. *L. theobromae* controls averaged 52.9%, *E. lata* controls averaged 56.0%, *Pal* controls averaged 57.4%, and *Pch* controls averaged 58.8% incidence. Error bars = ± SE.

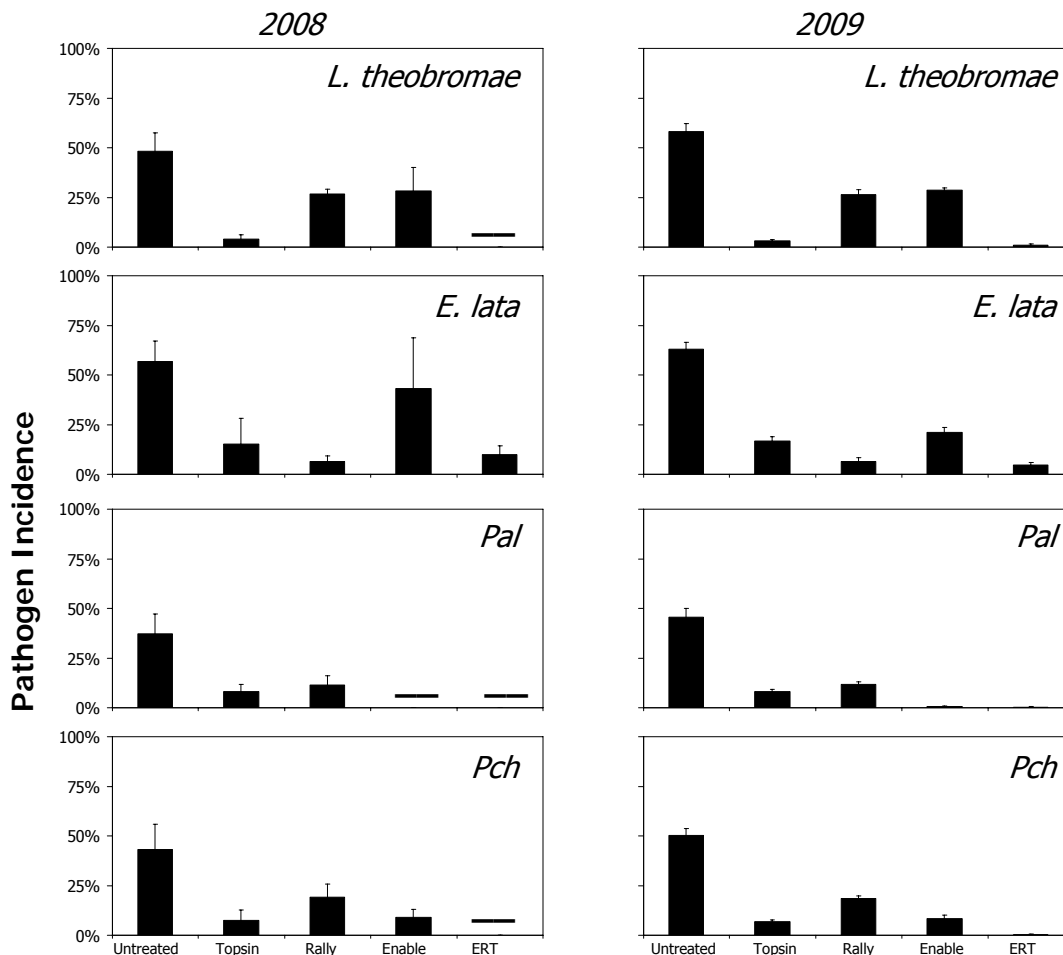


Figure 3: The effects of fungicide on canker pathogen incidence on Cabernet Sauvignon grapevines in Trials 2 and 3. Incidence is the percentage reisolation of each pathogen from inoculated canes with different fungicide treatments. Treatments were made in mid-January. Results from 2008 are shown on the left, and results from 2009 are shown on the right. Enable + Rally + Topsin is abbreviated ERT. N=4. *L. theobromae* controls averaged 50.5%, *E. lata* controls averaged 60.0%, *Pal* controls averaged 41.4%, and *Pch* controls averaged 46.6% incidence. Error bars = \pm SE.

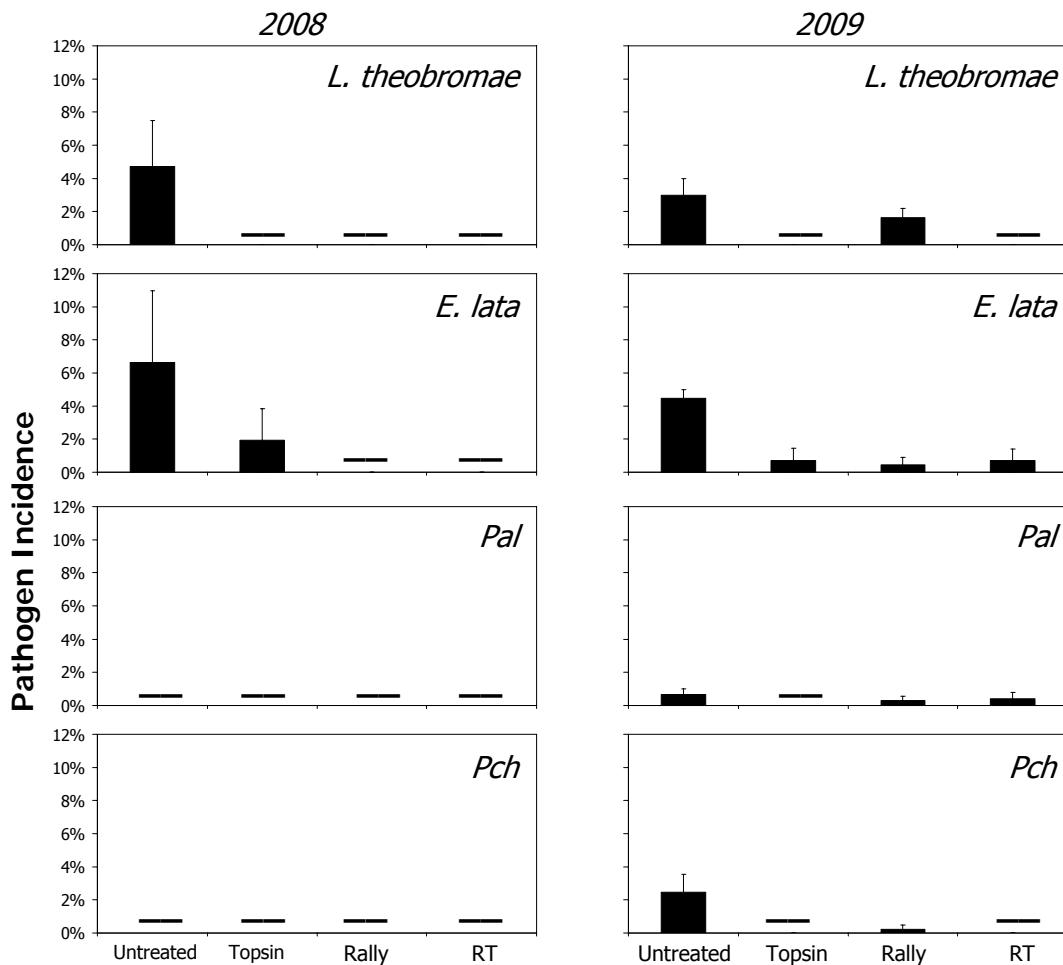


Figure 4: The effects of fungicide on canker pathogen incidence on Chardonnay grapevines in Trials 4 and 5. Incidence is the percentage reisolation of each pathogen from inoculated canes with different fungicide treatments. Treatments were made in late February. Results from 2008 are shown on the left, and results from 2009 are shown on the right. Rally + Topsin is abbreviated RT. N=4. *L. theobromae* controls averaged 7.7%, *E. lata* controls averaged 5.6%, *Pal* controls averaged 0.0%, and *Pch* controls averaged 0.0% incidence. Error bars = \pm SE.

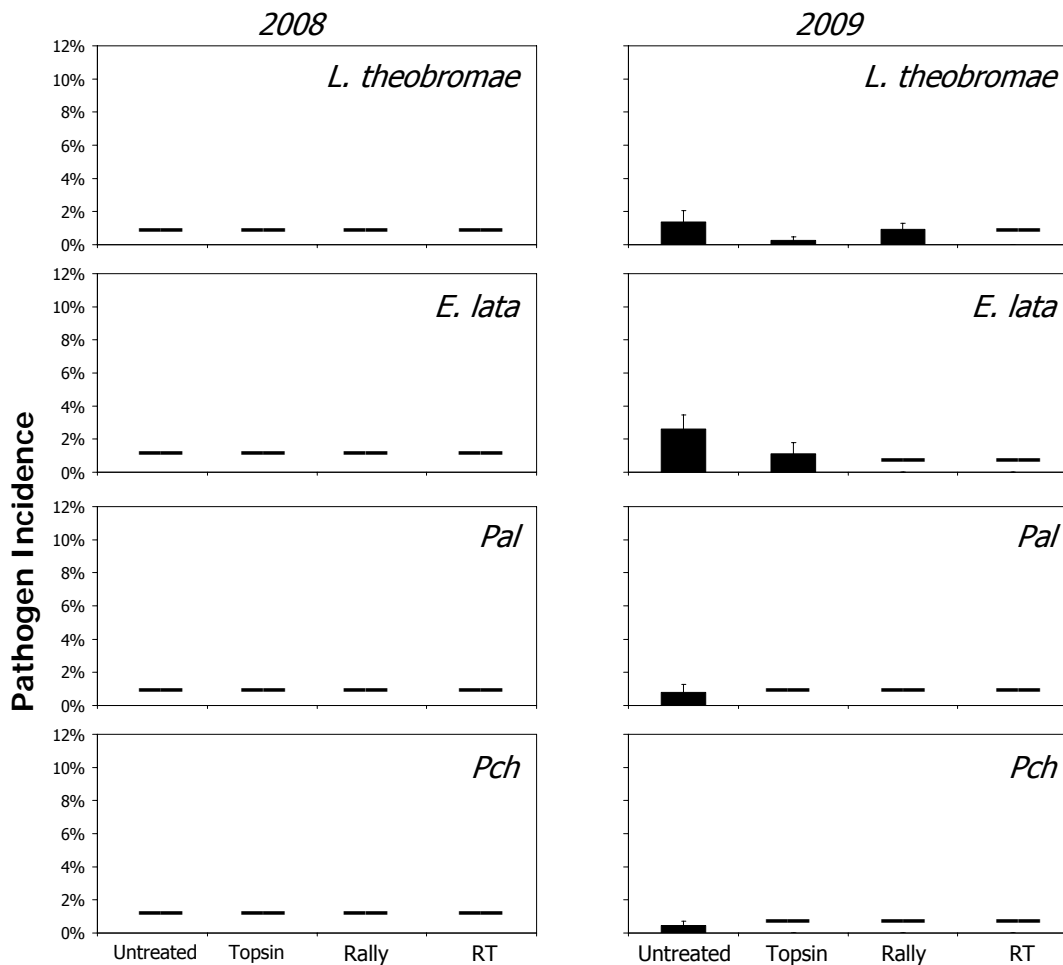


Figure 5: The effects of fungicide on canker pathogen incidence on Cabernet Sauvignon grapevines in Trials 4 and 5. Incidence is the percentage reisolation of each pathogen from inoculated canes with different fungicide treatments. Treatments were made in late February. Results from 2008 are shown on the left, and results from 2009 are shown on the right. Rally + Topsin is abbreviated RT. N=4. *L. theobromae* controls averaged 0.0%, *E. lata* controls averaged 0.0%, *Pal* controls averaged 0.0%, and *Pch* controls averaged 0.0% incidence. Error bars = \pm SE.

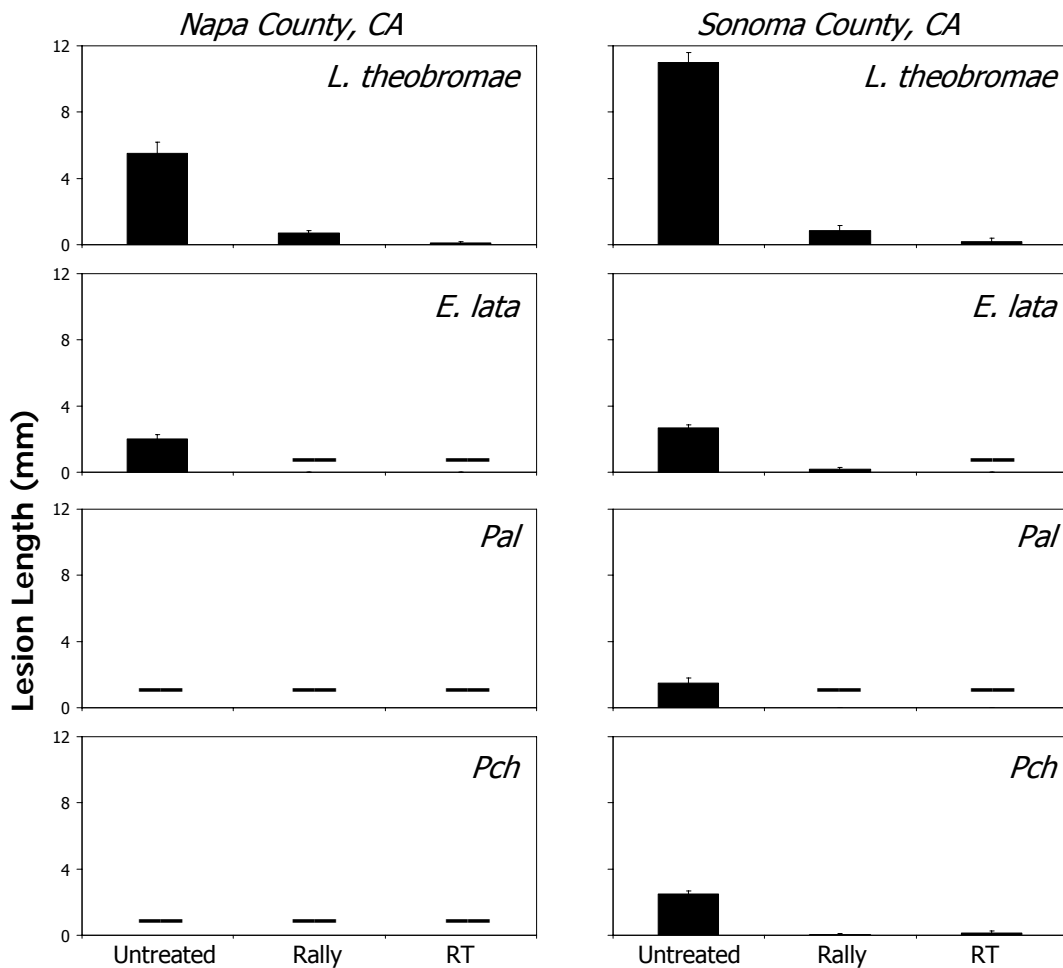


Figure 6: The effects of fungicide on the mean lesion length for each pathogen on inoculated canes of Pinot Noir grapevines in Trial 6. Fungicide applications were made in mid-February. Results from Napa County are shown on the left, and results from Sonoma County are shown on the right. Rally + Topsin is abbreviated RT. N=10. *L. theobromae* controls averaged 8.3 mm, *E. lata* controls averaged 2.4 mm, *Pal* controls averaged 0.8 mm, and *Pch* controls averaged 1.3 mm lesion length. Error bars = \pm SE.

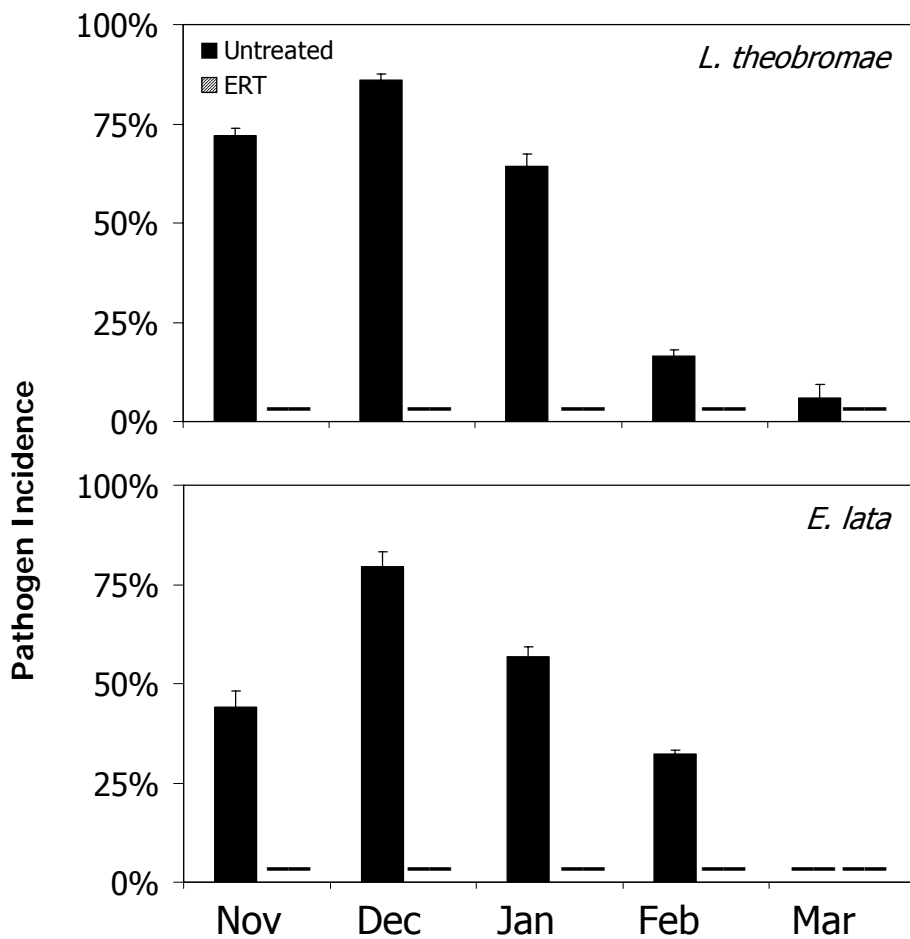


Figure 7: The effects of pruning time and fungicide on *L. theobromae* and *E. lata* incidence on Chardonnay grapevines in Trial 7. Incidence is the percentage reisolation of each pathogen from inoculated canes with different pruning times and fungicide treatments. N=4. Overall pathogen incidence by month averaged 49.3% in November, 68.2% in December, 49.7% in January, 20.7% in February, and 1.5% in March. Error bars = \pm SE.

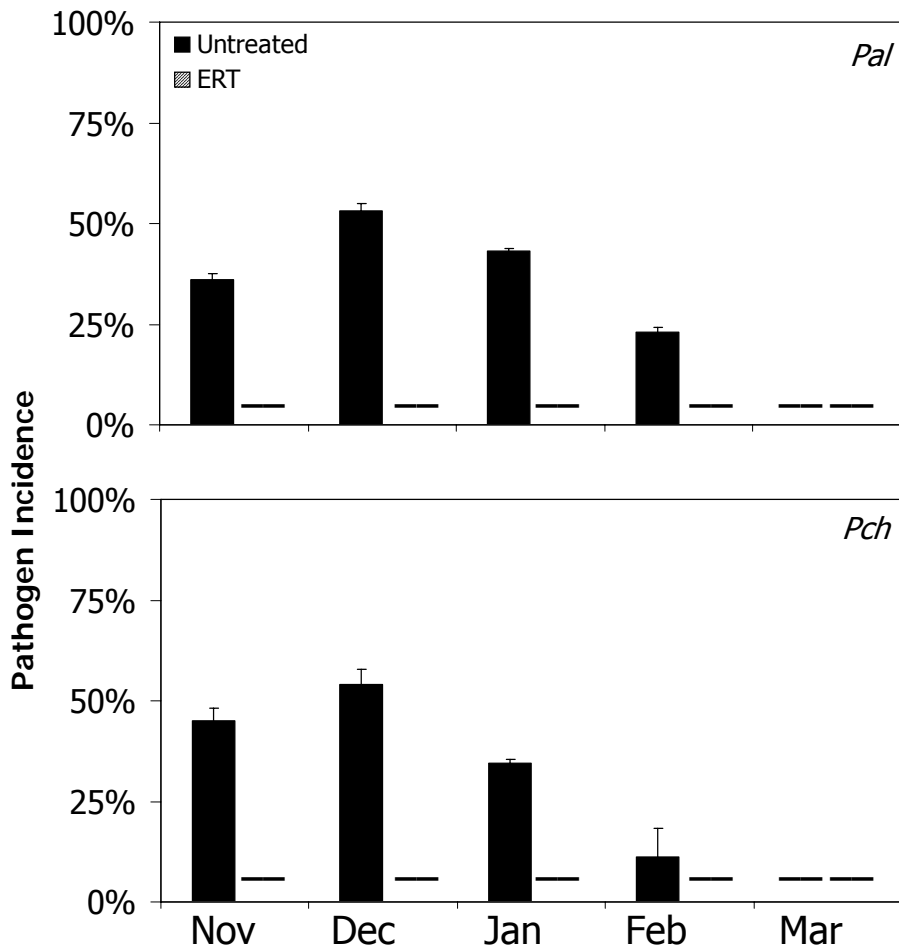


Figure 8: The effects of pruning time and fungicide on *Pal* and *Pch* incidence on Chardonnay grapevines in Trial 7. Incidence is the percentage reisolation of each pathogen from inoculated canes with different pruning times and fungicide treatments. N=4. Overall pathogen incidence by month averaged 49.3% in November, 68.2% in December, 49.7% in January, 20.7% in February, and 1.5% in March. Error bars = \pm SE.

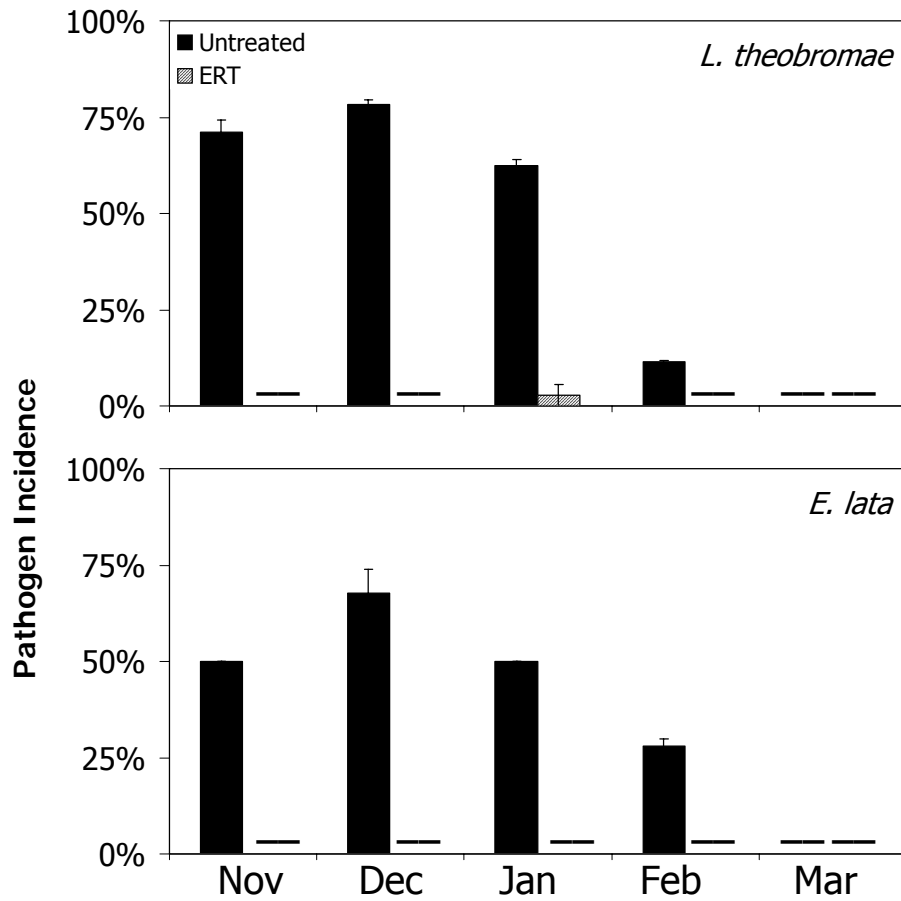


Figure 9: The effects of pruning time and fungicide on *L. theobromae* and *E. lata* incidence on Cabernet Sauvignon grapevines in Trial 7. Incidence is the percentage reisolation of each pathogen from inoculated canes with different pruning times and fungicide treatments. N=4. Overall pathogen incidence by month averaged 54.7% in November, 66.1% in December, 52.0% in January, 23.0% in February, and 0.0% in March. Error bars = \pm SE.

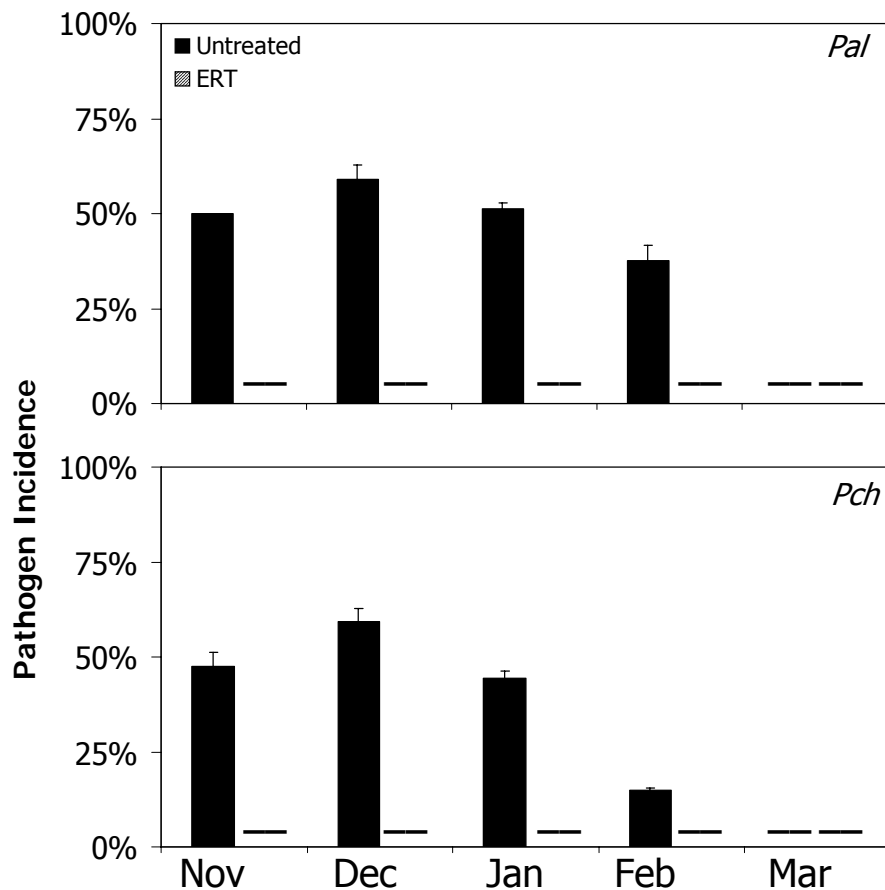


Figure 10: The effects of pruning time and fungicide on *L. theobromae* and *E. lata* incidence on Cabernet Sauvignon grapevines in Trial 7. Incidence is the percentage reisolation of each pathogen from inoculated canes with different pruning times and fungicide treatments. N=4. Overall pathogen incidence by month averaged 54.7% in November, 66.1% in December, 52.0% in January, 23.0% in February, and 0.0% in March. Error bars = \pm SE.

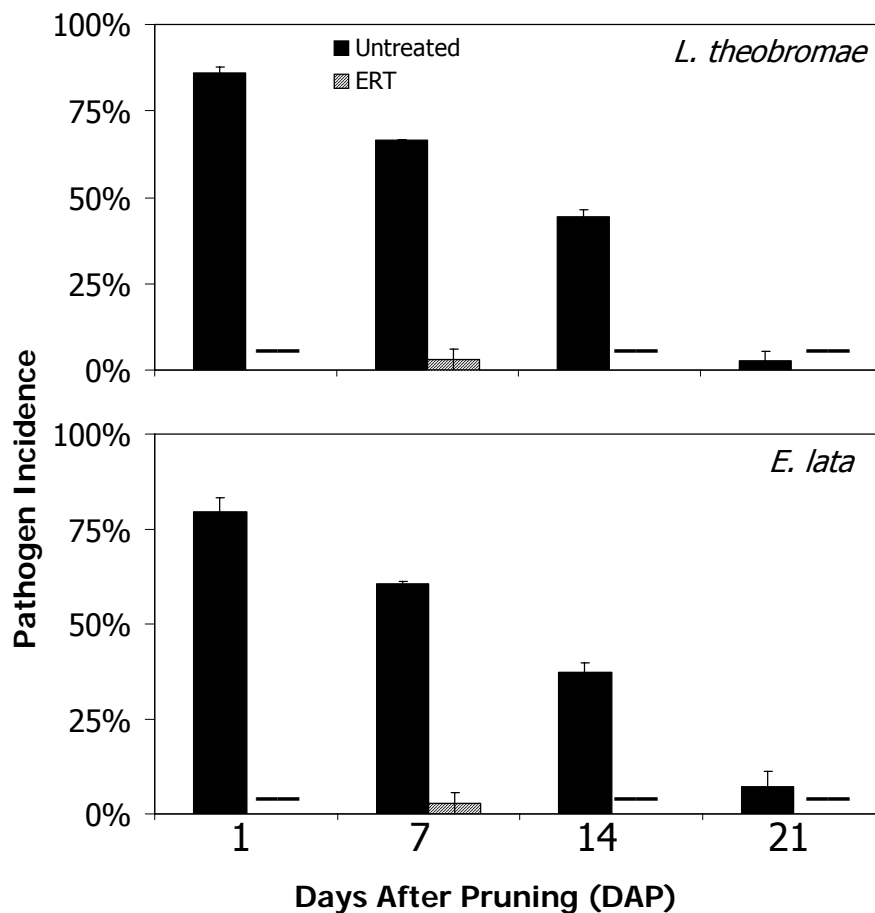


Figure 11: The effects of pruning wound age at inoculation on canker pathogen incidence on Chardonnay grapevines in Trial 7. Incidence is the percentage reisolation of each pathogen from inoculated canes with different pruning times and fungicide treatments. N=4. Overall pathogen incidence by inoculation time averaged 68.2% 1 day after pruning (DAP), 53.2% 7 DAP, 29.3% 14 DAP, and 2.5% 21 DAP. Error bars = \pm SE.

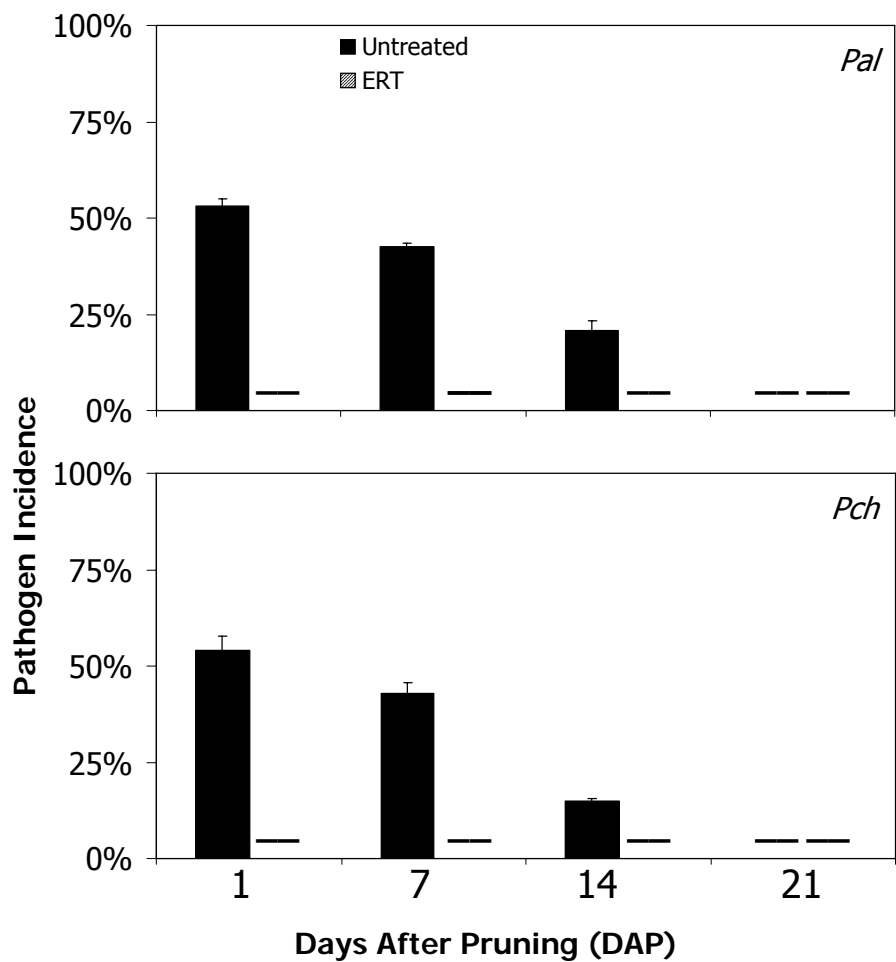


Figure 12: The effects of pruning wound age at inoculation on canker pathogen incidence on Chardonnay grapevines in Trial 7. Incidence is the percentage reisolation of each pathogen from inoculated canes with different pruning times and fungicide treatments. N=4. Overall pathogen incidence by inoculation time averaged 68.2% 1 day after pruning (DAP), 53.2% 7 DAP, 29.3% 14 DAP, and 2.5% 21 DAP. Error bars = \pm SE.

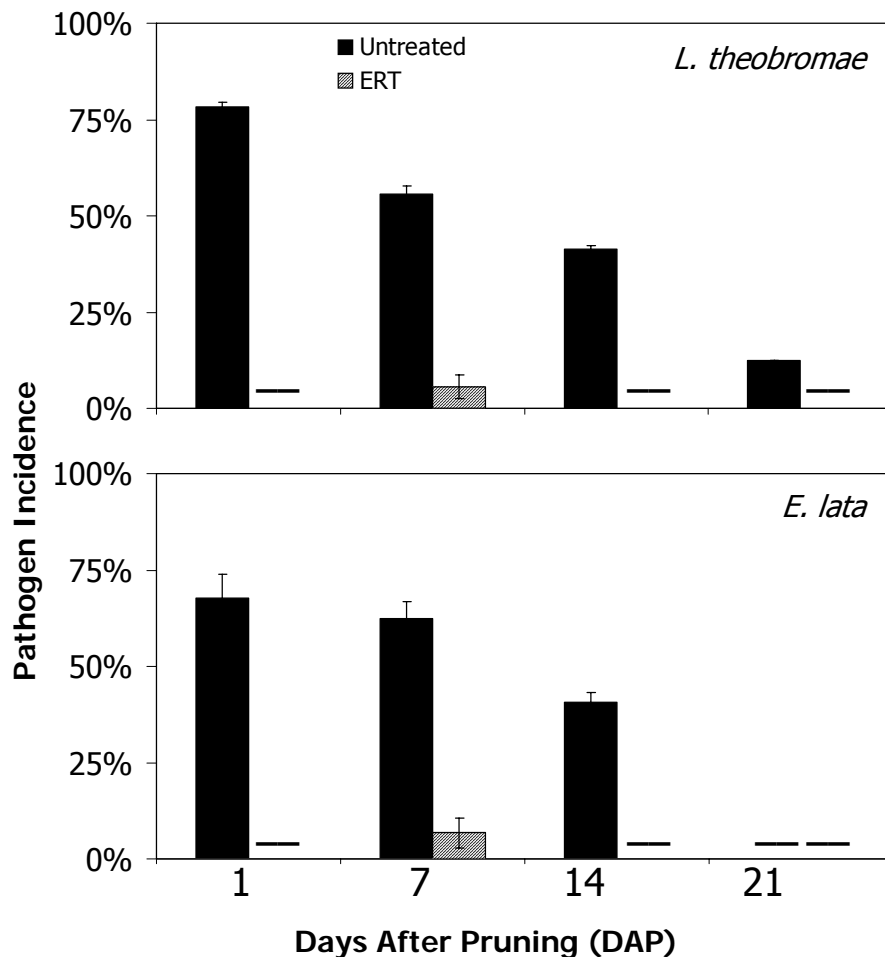


Figure 13: The effects of pruning wound age at inoculation on canker pathogen incidence on Cabernet Sauvignon grapevines in Trial 7. Incidence is the percentage reisolation of each pathogen from inoculated canes with different pruning times and fungicide treatments. N=4. Overall pathogen incidence by inoculation time averaged 66.1% 1 day after pruning (DAP), 46.9% 7 DAP, 31.7% 14 DAP, and 3.1% 21 DAP. Error bars = \pm SE.

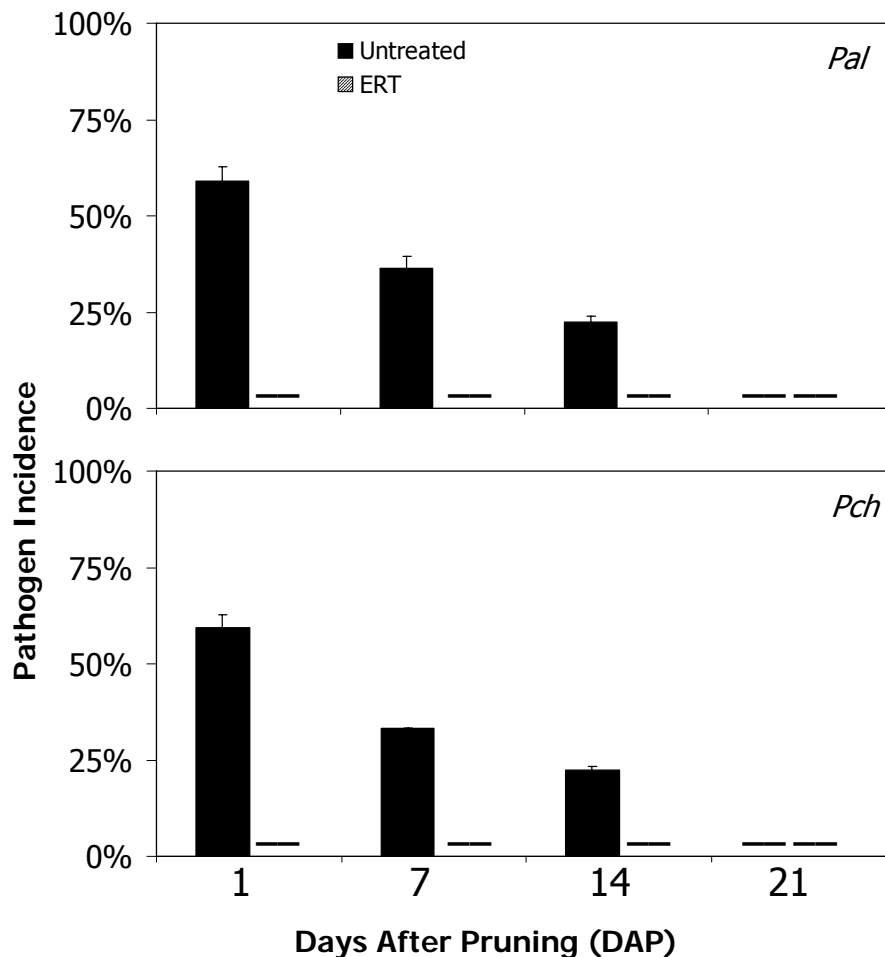


Figure 14: The effects of pruning wound age at inoculation on canker pathogen incidence on Cabernet Sauvignon grapevines in Trial 7. Incidence is the percentage reisolation of each pathogen from inoculated canes with different pruning times and fungicide treatments. N=4. Overall pathogen incidence by inoculation time averaged 66.1% 1 day after pruning (DAP), 46.9% 7 DAP, 31.7% 14 DAP, and 3.1% 21 DAP. Error bars = \pm SE.

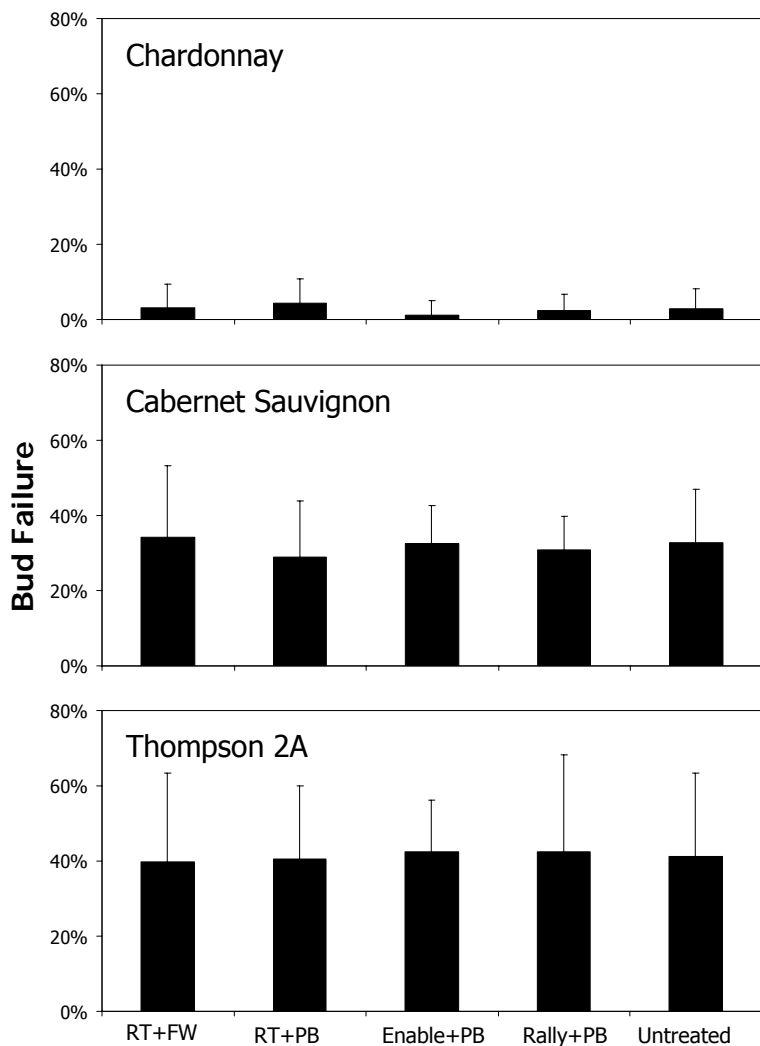


Figure 15: The effects of fungicide on incidence of bud failure on grapevines in Trial 1. Incidence of bud failure is the percentage of buds that have failed to emerge by mid-April. Rally + Topsin is abbreviated RT, and surfactants are abbreviated Pentra-Bark (PB) and Freeway (FW). N=16. Error bars = \pm 95% confidence interval.

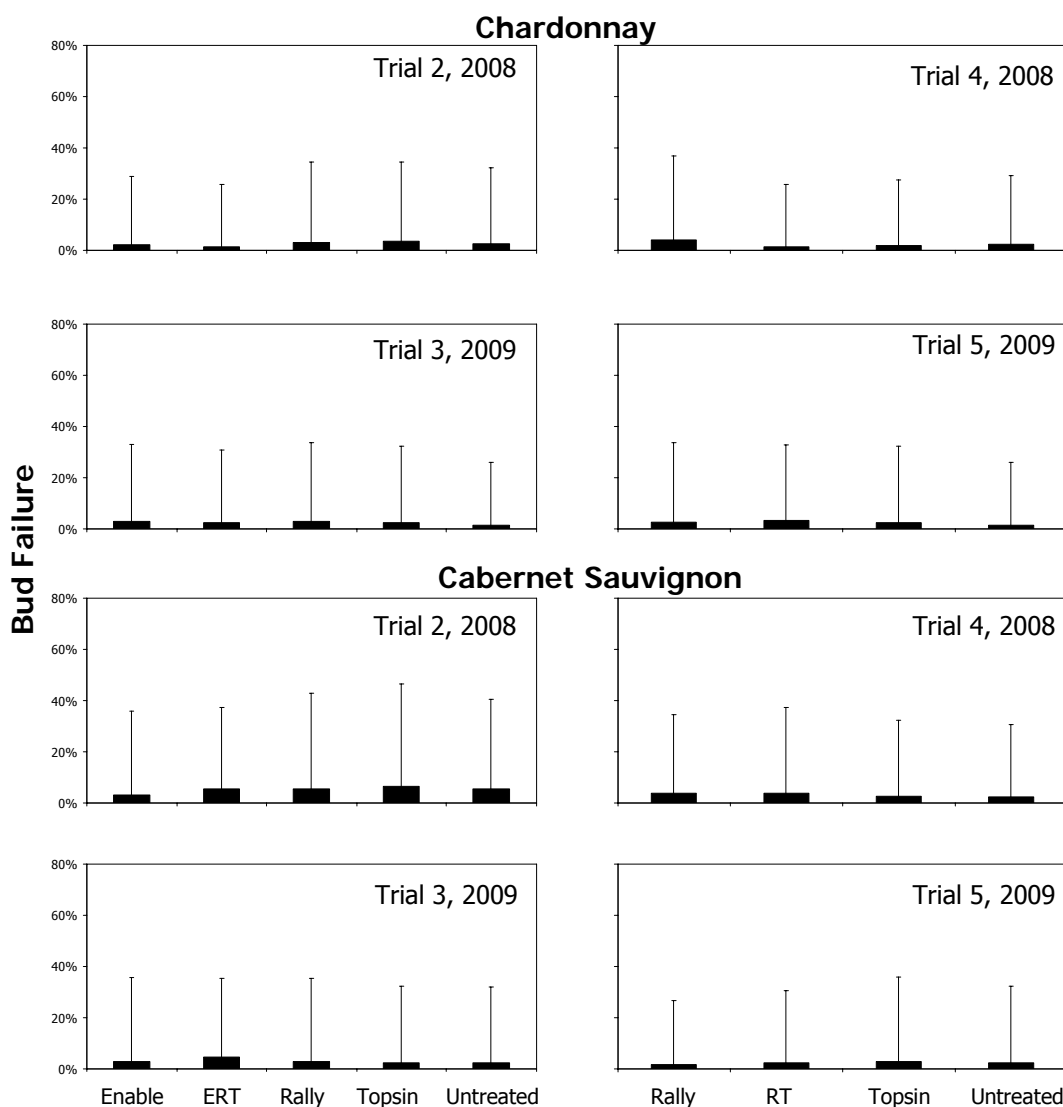


Figure 16: The effects of fungicide on incidence of bud failure on grapevines in Trials 2, 3, 4 and 5. Incidence of bud failure is the percentage of buds that have failed to emerge by mid-April. Enable + Rally + Topsin is abbreviated ERT and Rally + Topsin is abbreviated RT. N=16. Error bars = \pm 95% confidence interval.

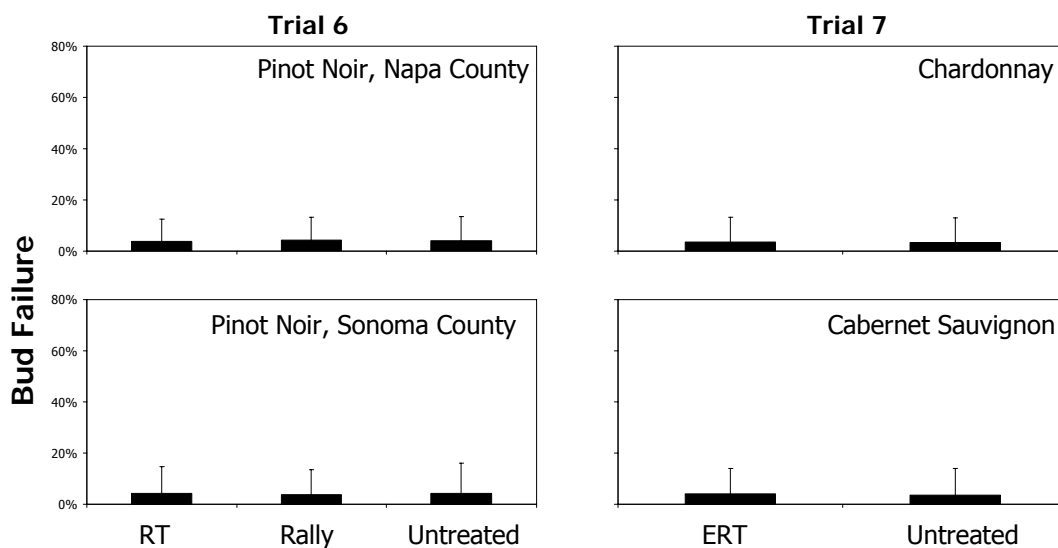


Figure 17: The effects of fungicide on incidence of bud failure on grapevines in Trials 6 and 7. Trial 6 results are shown on the left and Trial 7 results are shown on the right. Incidence of bud failure is the percentage of buds that have failed to emerge by mid-April. Enable + Rally + Topsin is abbreviated ERT and Rally + Topsin is abbreviated RT. N=20 in Trial 6; N=16 in Trial 7. Error bars = \pm 95% confidence interval.