Trunk and Scaffold Canker Diseases of Almond in California

18-PATH12-Trouillas

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Objectives:

Project No.:

- Diagnosis & Incidence Survey almond canker diseases in orchards in the Central Valley (*completed*)
- 2. Identification & Pathogenicity Identify the major pathogens associated with trunk and scaffold cankers using taxonomic methods and pathogenicity tests (*completed*)
- 3. Establish effective control strategies against canker pathogens of almond (on-going)

Interpretive Summary:

Trunk and scaffold canker diseases (TSCD) constitute the major cause of tree death in almond orchards in California. These diseases affect both young and mature orchards, sometimes causing significant tree losses soon after orchard establishment, and impacting also yield, the lifespan and profitability of orchards. Canker diseases have become an increasing concern to almond growers in recent years. The broad cultivation of almond combined with intensive production practices including mechanical harvest and repeated pruning have contributed to a recent increase in canker disease occurrence. TSCD are caused by many unrelated pathogens that infect trees mainly through pruning wounds, cracks and shaker injuries. Management of canker diseases has been challenging for growers and rely currently on cultural and prophylactic practices including remedial surgery and removal of trees. With pruning wounds acting as main entry sites for infection with canker pathogens, protection of pruning wounds following primary and secondary scaffold selections is recommended to prevent early infection and ensure almond tree longevity. Pruning wound protection trials were conducted during the dormant season 2016-2017 and 2017-2018 in Colusa and Kern

Counties, respectively. Experiments revealed that the fungicide Topsin M and a *Trichoderma* biocontrol product provided the greatest pruning wound protection against several TSCD pathogens. Studies were conducted also in two commercial orchards in Colusa and Fresno Counties, respectively, to investigate the duration of pruning wound susceptibility and the seasonal susceptibility of pruning wounds in order to limit risks of infection by canker pathogens according to the time of pruning. Results of the pruning wound susceptibility trials showed that the duration of pruning wounds susceptibility is lowest when pruning is done in January. Overall pruning wound susceptibility is significantly reduced after two weeks following pruning and susceptibility of wounds continue to decrease overtime. This suggests that one application of a pruning wound protectant following late pruning in January should significantly reduce risks of infection of pruning wounds by canker pathogens.

Materials and Methods:

Trunk and scaffold canker disease survey of almond orchards in the Central Valley (completed). Survey of diseased orchards continued throughout 2017 and 2018 as farm advisors, PCAs and growers continue to reach out to our laboratory for diagnosis of TSCD. Between 2015 and 2018, approximately 120 orchards with symptoms of almond TSCD were visited/sampled throughout the Central Valley, spanning 11 counties. Symptoms of TSCD included dieback, gummosis, girdling, resinosis and vascular discoloration on almond branches, scaffolds or trunks. Symptom differences were annotated for the different causal agents. From this sampling, approximately 400 isolates were isolated from cankers and discolored wood and characterized for this study. Trunks and scaffolds displaying symptoms were sampled by removing bark and branches to reveal the inner wood revealing the canker. Wood samples were plated on several artificial media in the laboratory. This included Potato Dextrose Agar (PDA) amended with 100-ppm tetracycline (PDA-tet) for the isolation of true fungi, PARP medium for the isolation of *Phytophthora* spp. and the use of humid crispers for the isolation of *Ceratocystis fimbriata*.

Identify the major pathogens associated with trunk and scaffold cankers using taxonomic methods and pathogenicity tests (completed). As survey and sampling of orchards continue through our extension mission, identification of fungal pathogens associated with canker diseases is routinely conducted in our laboratory. Identification of pathogens associated with almond TSCD was carried out using culture morphology and microscopic features and confirmed with DNA sequencing. Sequencing was first performed with the internal transcribed spacer (ITS) region of the rDNA to determine our isolates to species. In addition to the ITS region, additional loci were sequenced to enhance phylogenetic resolution and included: translation elongation factor (TEF1- α), beta-tubulin (BT), and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). All loci were amplified using PCR conditions previously published and DNA was sequenced using Sanger sequencing technologies. Sequences were viewed in Sequencher, manually edited in Mesquite and phylogenies were constructed in MEGA 6.

To determine pathogenicity, during the fall of 2016 field trials on almond were set up to test the aggressiveness of 18 isolates representing the various TSCD fungi isolated during the survey. Three trials were set up; the first on potted 1-year-old saplings in the Kearney Agricultural Research and Extension (KARE) Center lath house, the second in an almond orchard at

Nickel's Soil Laboratory, and the third in an almond orchard at KARE. Almond branches (2year-old) or the main stem of the saplings were inoculated with the fungal species. The central to distal portion of the branch was inoculated by placing a 5-mm-diameter mycelium plug from a 7- to 10-day-old PDA culture in a wound made by a 5-mm-diameter cork borer. Wounds were sealed with petroleum jelly to maintain moisture during the incubation period and protected with Parafilm. Fungal treatments were compared to control treatments inoculated with non-inoculated media plugs. Samples in trial 1 were collected after 6 weeks and after 3 months. Trial 2 and 3 were sampled after 6 months for evaluation of canker length.

Establish effective control strategies against canker pathogens of almond (on-going).

Pruning wound susceptibility and pruning wound protection trials were established to provide management guidelines to almond growers. These trials aimed to generate information about timing when wounds from pruning are less susceptible to infection and what fungicidal products can best protect pruning wounds from infection by canker pathogens.

Pruning wound susceptibility

Our work aims to determine wound susceptibility according to the time of pruning (pruning month) to identify lowest risk periods for pruning wound infection. This in turn will allow us to make recommendations to growers about when to prune their trees. The duration of wound susceptibility over time (weeks) will allow us to make recommendations about the number of fungicide applications that may be required to best protect pruning wounds when environmental conditions remain conducive for pathogen dispercal and infection.

Two independent field trials were set up in experimental orchards in Fresno (trial 1) and Colusa (trial 2) Counties. A set of branches on Nonpareil trees (7th and 8th leaf) were flagged and pruned in September 2017, October 2017, November 2017, December 2017, and January 2018, respectively. A subset of these branches for each month was then inoculated at six time points, respectively at 0, 1, 2, 3, 5 and 8 weeks after pruning to reflect different wound ages. Spore suspensions (5×10³ spores/wound) of four almond TSCD pathogens (*Eutypa lata, Neofusicoccum parvum, Botryosphaeria dothidea*, and *Neoscytalidium dimidiatum*) were applied to individual wounds following spray bottle application of sterile water to imitate rain (to mimic how these pathogens typically spread). A non-inoculated control was used to determine if natural infections would take place. Wounds were left uncovered following inoculation to mimic natural conditions and to allow for natural wound healing. At 3.5 months post-inoculation, branches were processed in the laboratory to assess the percent fungal recovery and determine the susceptibility of pruning wounds according to the time of pruning and timing of infection following pruning. Data will be analyzed in the statistical software R.

Pruning wound protection

During the 2016-2017 (year 1) and 2017-2018 (year 2) dormant period, five independent field trials were conducted in commercial almond orchards in Colusa and Kern counties. In year 1, three trials were established to test a wide variety of fungicide products (21 products total) against several of the TSCD pathogens including *Cytsopora leucostoma, Eutypa lata, Botryosphaeria dothidea, Neofusicoccum parvum, N. mediterraneum and Neoscytalidium dimidiatum.* In year 2, product selection was narrowed down based on the most efficacious products from year 1 and the addition of several biological control agents. Products tested

included fungicides from different FRAC groups, biofungicides, biological control agents and wound sealants. Fungal inoculum ranged from 1,000 to 10,000 spores.

For each trial, 2nd to 3rd year branches were pruned to roughly 6-to-12 inches in height, ensuring the resulting wound made a flat surface. Following pruning, the various products were applied to the wound with spray bottles until runoff. One product was used per tree. 1 or 24 hours after the product was applied, treated wounds were sprayed with sterilized water to imitate rain and then inoculated with the respective fungal pathogens. Each trial was set up in a randomized complete block design with 4 (year 1) or 6 (year 2) replicates per treatment (treatment = product + fungal pathogen). Treatment branches were collected three months after inoculation, brought back to the laboratory for assessment of fungal colonization, and wound protection. For each branch we measured the length of vascular discoloration. Isolations from the treated branches were performed and 10 wood pieces were plated onto a single plate of PDA-tet per branch. Plates were rated for fungal recovery at 3, 5 and 7 days. Fungal recovery was rated either a value of "0" (no fungal recovery) or "1" (fungal recovery from at least 1 wood piece). For plates rated "1" we also made note of the number of wood pieces that a fungal isolate was re-isolated from as a measure of severity.

Results and Discussion:

Survey and pathogen identification

Results from our survey (Objective 1) were provided in our 2015.2016 and 2016.2017 Annual Research Reports, and are summarized below:

Between 2015 and 2018, we visited/sampled approximately 120 almond orchards throughout the Central Valley with symptoms of almond TSCD, spanning 11 counties. From this sampling, approximately 400 isolates were isolated from cankers and were characterized using morphological and molecular methods. To date, Botryosphaeria canker (band canker or pruning wound-related canker) and Ceratocystis canker were the most commonly encountered canker diseases. Other prevalent canker diseases found in almond included Eutypa canker, Cytospora canker, and Phytophthora canker. Cankers caused by Diaporthe spp. and Collophora spp. were also identified in this survey, but do not appear to be widespread. Ceratocystis cankers were found in all counties surveyed and in both young and mature trees. Ceratocystis canker of almond has not been investigated since the emergence of modern techniques in molecular biology and the genetic diversity and population structure of Ceratocystis fimbriata need to be investigated. Questions such as the origin of the inoculum of Ceratocystis canker and the role played by mechanized harvesting equipment in spreading the disease remain elusive. Botryosphaeria cankers were associated with pruning wounds and prevalent in 3-4th leaf orchards. A total of 12 Botryosphaeriaceae species were identified in association with cankers on almond. An emerging Botryosphaeriaceae species, Neoscytalidium dimidiatum, was associated with pruning wounds and tree crotch infections in 3-4th leaf almond orchards in Madera, Merced, Fresno and Kern Counties. This fungus has been known as the causal agent of branch wilt in walnut and was recently reported in table grape, citrus and figs in California. We hypothesized that the emergence of this pathogen in the fruit and nut crops in California is related to the severe drought affecting California, with drought conditions and the rising of temperatures being conducive to the pathogens fitness in tree crops. Cytospora cankers were also detected near pruning wounds and found in 3-4th leaf

orchards and older in Merced, Fresno and Stanislaus counties. To date we have recognized at least 5 different species in almond. Little work has been conducted on Cytospora cankers in almond but studies in sweet cherry in CA have shown that *Cytospora* species constitute some of the most aggressive canker pathogens in this host (Trouillas et al. 2012). *Eutypa lata* was associated with pruning wounds and infections at the tree crotch in 3-4th leaf orchards in the Sacramento Valley. Infections at the crotch generally coincided with poor scaffold selection. *Collophora hispanica* and *C. paarla* were associated with reddish-colored, circular branch cankers. Additionally, *Phytophthora cinnamomi* was isolated from second leaf almond trees in an orchard in Kern Co. While *Phytophthora* spp. are known to cause cankers on almonds, this is the first report of *P. cinnamomi* on almond in California and it needs to be further investigated as a serious pathogen of almond.

Pathogen aggressiveness

Results from our pathogenicity tests (Objective 2) were provided in our 2016.2017 Annual Research Reports, and are summarized below:

Pathogenicity tests revealed that isolates from the Botryosphaeriaceae were the most aggressive pathogens on almond, namely *Neofusicoccum arbuti, N. parvum* and *N. mediterraneum.* In all three pathogenicity trials, *N. parvum* and *N. arbuti* killed 20-60% of inoculated branches. *Ceratocystis fimbriata* was also very aggressive causing large lesions and gumming at the point of inoculation. Other species (*Eutypa lata, Cytospora* spp., *Diaporthe* spp., and *Collophora* spp.) proved to be pathogenic on almond causing vascular discoloration, but not as aggressive compared to cankers caused by Botryosphaeriaceae spp. and *Ceratocystis fimbriata.* The results of the pathogenicity tests suggest that Botryosphaeria cankers can be the most devastating, especially on young trees.

Control strategies (pruning wound susceptibility and protection)

Pruning wound susceptibility

In the pruning wound susceptibility trials, all wounds inoculated right after pruning in September through January were the most susceptible to pathogen infection in comparison to wounds inoculated 1-8 weeks after pruning (Figures 1 and 2). Wounds susceptibility declined substantially after two weeks following pruning during all months of pruning. Freshly made wounds were most susceptible to infection, and susceptibility decreased over time as wounds age. A steady decrease in infection rates was observed during each month (September, October, November, December and January) from 0 to 8 weeks. September had the highest infection rate across all wound ages in both trials, while January had the lowest infection rate. Pruning wounds made in January (Figures 1 and 2) appeared less susceptible to fungal pathogen infection than pruning wounds made in the late summer, fall or winter (Nov-Dec). The different pathogens used in ours trials also varied greatly in their ability to infect pruning wounds of different ages. Namely, Eutypa lata and Neofusicoccum parvum, were able to infect up to 8 weeks after pruning in several pruning months. These results suggest that some almond TSCD pathogens pose a higher risk as canker pathogens infecting pruning wounds. Results in trial 1 and 2 revealed a similar trend overall in pruning wound susceptibility, however trial 1 located in Fresno County showed a higher rate of infection than observed in trial 2 in Colusa County. Temperature averages in Fresno County are higher than those in Colusa County during the fall and winter months when the trials took place. Differences in environmental conditions (temperature, relative humidity, etc.) between the different almond

growing regions in the Central Valley are likely to influence host response to infection. Future work will include a repetition of this study to support the first year's findings.

Pruning wound protection

In year 1 of the pruning wound protection trials, Topsin M (thiophanate-methyl) provided excellent control (**Tables 1** and **2**) across all the fungal pathogens tested, with disease control ranging from 70 to 100%. Other products that exhibited good pruning wound protection in at least one trial for several pathogens included Luna Experience (tebuconazole/fluopyram), Luna Sensation (fluopyram/trifloxystrobin), Quash (metconazole), Quilt Xcel (propiconazole/azoxystrobin), Inspire Super (difenoconazole/cyprodinil), Rally (myclobutanil), Merivon (fluxapyroxad/pyraclostrobin), Quadris Top (difenoconazole/azoxystrobin), and the *Trichoderma* biocontrol. The sealants (paint and CropSeal) were not widely effective across the pathogens but did show protection against *Eutypa lata*.

In year 2 of the pruning wound protection trials, Topsin M provided excellent control compared to the other fungicides tested (> 80% disease reduction, **Table 3**). It reduced the infection rate of *Eutypa, Cytospora* and *B. dothidea* by 83%, and reduced *N. parvum* infections by 50 to 83% (**Table 3**). Other products that exhibited good pruning wound protection in both trials included Quilt Xcel (79% reduction in infections) and Quadris Top (75% reduction in infections). Among the biocontrol products tested, the *Trichoderma* sp. applied at various rates had the lowest fungal recovery (13, 0 and 2 % recovery of the pathogen) and highest percent disease control which correlates with reduction of infection by 87 to 100%. In these trials, this biocontrol is the highest performing product. In the sealants category for pruning wound protection, acrylic paint had a low overall average recovery, reducing infection by 79%, however looking at the individual pathogens, acrylic paint worked only against some of the pathogens. Conversely, Topsin M and the *Trichoderma* biocontrol showed efficacy across a wide diversity of TSCD pathogens.

The second year of pruning wound protection trials supports our observations from year one; Topsin M, Quadris Top, Quilt Xcel and the *Trichoderma* biocontrol provide excellent protection of pruning wounds against canker pathogen entry. A final year of pruning wound protection trials will be initiated in December 2018 to test the top-performing products using an air blast sprayer to imitate grower practices and determine the coverage efficiency of this application method for pruning wounds.

Overall, these findings illustrate differences in sensitivity to fungicides among the various canker pathogens and emphasize the importance of accurate disease diagnosis to improve disease control. Nevertheless, the fungicide Topsin M can provide excellent control against the broad range of canker pathogens. Products that have the largest range of protection across TSCD pathogens will be recommended for management of canker diseases.

In conclusion, results of the pruning wound susceptibility trials showed that the duration of pruning wounds susceptibility is lowest when pruning is done in January. Overall pruning wound susceptibility is significantly reduced after two weeks following pruning and susceptibility of wounds continue to decrease overtime. This suggests that one application of a pruning wound protectant such as Topsin M following late pruning in January should significantly reduce risks of infection of pruning wounds by canker pathogens.

Research Effort Recent Publications:

Peer-reviewed publications

Nouri MT, Lawrence DP, Yaghmour MA, Michailides TJ and Trouillas FP. 2018. Neoscytalidium dimidiatum Causing Canker, Shoot Blight and Fruit Rot of Almond in California. Plant Disease 102(8): 1638-1647. <u>https://doi.org/10.1094/PDIS-12-17-1967-RE</u>

Lawrence DP, Holland LA, Nouri MT, Travadon R, Abramians A, Michailides TJ, Trouillas FP. 2018. Molecular phylogeny of *Cytospora* species associated with canker diseases of fruit and nut crops in California, with the descriptions of 10 new species and one new combination. IMA Fungus (*accepted*)

Holland LA, Nouri, MT, Crespo M, Holtz BA, Yaghmour MA, Doll DA and Trouillas FP. (2018) First report of *Collophora hispanica* and *Collophora paarla* causing branch cankers of almond in California. *Plant Disease* (102) 8: 1663. <u>https://doi.org/10.1094/PDIS-09-17-1518-PDN</u>

Nouri MT, Holland LA, Yaghmour MA, Doll DA, Browne GT and Trouillas FP. (2018) First Report of *Phytophthora cinnamomi* Causing Trunk Canker of Almond in California. *Plant Disease* (102) 1: 253. <u>https://doi.org/10.1094/PDIS-06-17-0872-PDN</u>

Conference proceedings

Holland LA, Nouri MT., and Trouillas FP. 2017. A re-examination of Ceratocystis canker in California almond orchards. *Acta Horticulturae*

Holland LA, Nouri MT, Crespo M and Trouillas FP. 2017. Etiology and management of trunk and scaffold canker diseases of almond in California. *Acta Horticulturae*

Abstracts

- Holland et al. 2018. International Congress of Plant Pathology, Concurrent Session: *Taxonomy* of *Plant Pathogenic Fungi* (Boston, MA) A taxonomic re-examination of *Ceratocystis* fimbriata, the causal agent of Ceratocystis canker of almond in California
- Holland et al. 2018. American Phytopathological Society Pacific Division Meeting (Portland, OR) An integrated approach towards management of canker diseases of almond in California.
- Holland et al. 2017. VII International Symposium on Almonds and Pistachio, International Society for Horticultural Science (Adelaide, South Australia) A re-examination of Ceratocystis canker in California almond orchards
- Holland et al. 2017. Plant Pathologists of the Future Showcase, American Phytopathological Society Annual Meeting (San Antonio, TX) Almond Trunk and Scaffold Canker Diseases in California: Diagnosis, Pathogenicity, and Management

Online articles

https://www.westernfarmpress.com/tree-nuts/pruning-wounds-can-lead-cankers-uc-specialistwarns

References Cited:

Trouillas F.P., Peduto F., Lorber J.D., Sosnowski M.R., Grant J., Coates W.W., Anderson K.K., Caprile J. and Gubler W.D. 2012. Calosphaeria canker of sweet cherry caused by *Calosphaeria pulchella* In California and Australia. *Plant Disease* 96:648-658.

Figures and Tables

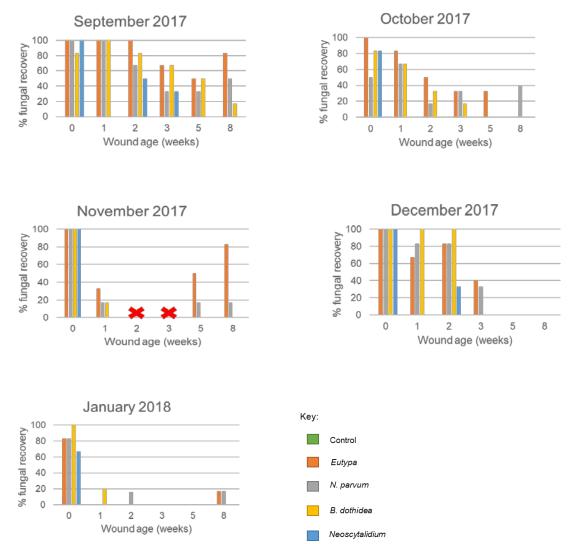


Figure 1: Trial 1 - Percent fungal recovery over an 8-week period in September, October, November, December and January, respectively.

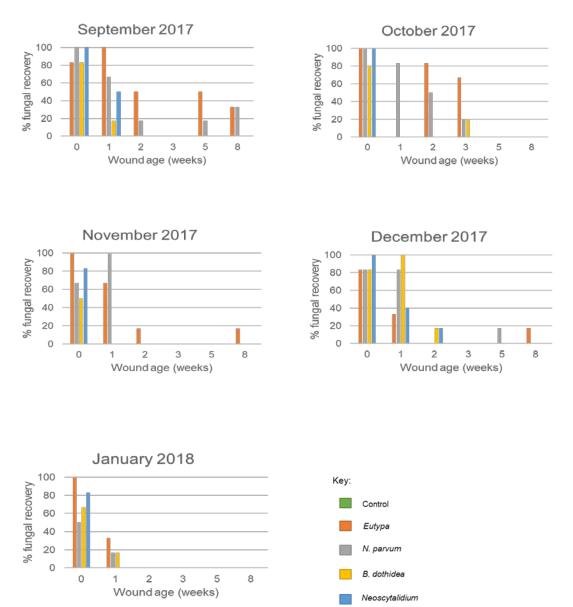


Figure 2: Trial 2 - Percent fungal recovery over an 8-week period in September, October, November, December and January, respectively.

Table 1: Year 1, Trial 1 - Percent fungal recovery for pruning wound protection field trials based on four replicates; seven fungal species tested against 10 products; dark gray-colored cells represent 0% recovery of the fungus from wounds treated with product (i.e. high performance product), light gray-colored cells represent 25-50% recovery of the fungus from wounds treated with product (i.e. moderate to good performance), white-colored cells represent 60-100% recovery of the fungus from wounds treated with product (i.e. limited performance). Averages for each product in red.

Product	Cytospora	Eutypa	Ceratocystis	B. dothidea	N. parvum	N. medit.	Neoscytalidium	Avg. recovery (%)
Control	25	75	50	50	100	50	50	57
Luna Experience	75	25	25	25	0	25	25	29
Merivon	50	25	25	0	25	50	50	32
Topsin M	0	0	0	0	0	0	0	0
Quash	25	50	0	0	25	50	50	29
Inspire Super	25	75	0	0	0	25	25	21
Quadris Top	100	0	0	0	0	0	100	29
Rally	50	25	0	0	25	0	50	21
Thyme oil #1	100	100	0	75	50	75	50	64
Thyme oil #2	75	25	0	50	100	75	100	61
Neem oil	100	100	0	100	100	100	100	86

Table 2: Year 1, Trial 2 & 3 - Percent fungal recovery for pruning wound protection field trials based on four replicates; seven fungal species tested against 21 products; dark gray-colored cells represent 0% recovery of the fungus from wounds treated with product (i.e. high performance product), light gray-colored cells represent 25-50% recovery of the fungus from wounds treated with product (i.e. moderate to good performance), white-colored cells represent 60-100% recovery of the fungus from wounds treated with product (i.e. limited performance). Averages for each product in red.

	Cytospora		Eutypa		B. dothidea		N. parvum		N. medit.		Neoscytalidium		D. mutila		Avg. recovery (%)
Product	T2	T3	T2	Т3	T2	т3	T2	т3	T2	Т3	T2	Т3	T2	Т3	T2 & T3 combined
Control	na	75	75	75	100	100	50	100	75	100	50	100	50	100	81
Neem oil	na	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Thyme oil #1	na	100	50	100	100	100	100	100	100	100	100	50	100	100	92
Trichoderma	na	0	50	0	50	75	75	0	100	25	50	0	75	50	42
Quash	na	100	25	25	50	33	50	0	75	75	75	75	75	100	58
Topsin M	na	50	25	0	50	50	50	25	0	25	25	50	0	25	29
Paint	na	75	0	50	25	75	75	100	75	100	25	100	25	100	63
CropSeal	na	100	0	25	25	100	25	100	75	50	0	75	75	75	56
Rally	na	100	25	75	25	75	75	75	0	100	75	100	50	100	67
Indar	na	100	75	75	100	100	75	100	100	75	75	100	50	100	87
Fontelis	na	100	67	50	100	75	50	100	75	100	100	75	75	100	82
Inspire Super	na	100	50	75	25	25	75	100	50	75	50	100	50	100	67
Luna Sensation	na	75	25	75	0	33	25	25	50	100	25	100	0	50	45
Quilt Xcel	na	75	25	25	0	50	0	25	0	75	75	100	50	100	46
Viathon	na	100	25	75	50	25	0	25	25	50	25	100	75	75	50
Luna Experience	na	75	0	75	67	100	0	25	100	100	0	100	25	75	57
Bravo	na	100	25	50	50	75	75	75	100	75	25	75	50	100	67
Quadris Top	na	75	25	50	25	50	50	0	100	50	75	50	67	100	55
Merivon	na	75	50	50	0	0	0	25	0	25	25	75	0	50	29
Pristine	na	75	50	75	25	25	0	75	100	75	50	100	0	75	56
Abound	na	75	33	50	67	75	50	100	100	100	50	75	100	100	75
Ziram	na	75	75	100	100	100	100	100	100	100	75	75	100	100	92

Table 3: Year 2, Trial 1 & 2 - Percent fungal recovery for pruning wound protection field trials based on six replicates; four fungal species tested against 15 products; dark gray-colored cells represent 0% recovery of the fungus from wounds treated with product (i.e. high performance product), light gray-colored cells represent 15-50% recovery of the fungus from wounds treated with product (i.e. moderate to good performance), white-colored cells represent 60-100% recovery of the fungus from wounds treated with product (i.e. limited performance). Averages for each product in red.

	Eutypa		Cyto	spora	B. do	thidea	N. pa	Avg.	
	Trial 1	Trial 2	recovery (%)						
Control (water)	67	50	83	50	100	67	67	83	71
Topsin M	17	17	0	17	17	17	17	50	19
Rally	67	17	67	50	0	17	0	33	31
Quadris Top	50	17	83	17	0	17	0	17	25
Inspire Super	67	50	67	83	0	17	50	83	52
Quilt Xcel	33	50	17	33	0	0	0	33	21
Luna Experience	67	0	50	83	0	0	0	83	35
Merivon	0	50	33	83	0	17	17	83	35
Quash	33	17	50	100	0	20	0	33	32
Luna Sensation	100	33	17	17	0	33	20	33	32
Trichoderma spp.	17	0	0	50	0	50	17	83	27
Trichoderma sp. (0.5g/L)	0	20	0	33	0	33	0	17	13
Trichoderma sp. (5.0g/L)	0	17	0	0	0	17	0	0	0
Trichoderma sp. (50g/L)	0	0	0	0	0	0	0	17	2
Acrylic paint	50	0	67	50	0	0	0	0	21
Sealant/barrier	100	17	83	67	50	33	100	83	67