
Trunk and Scaffold Canker Diseases of Almond in California

Project No.: PATH12.Trouillas

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A. Summary

Trunk and scaffold canker diseases (TSCD) caused by fungal pathogens are destructive diseases of almond trees. Surveys conducted by our laboratory revealed the occurrence of four major canker diseases of almond in California: band canker, Ceratocystis canker and the newly identified Cytospora and Eutypa cankers. Nonetheless, up to 27 fungal species were found associated with almond canker diseases. Pruning wounds made for primary and secondary scaffold selections as well as maintenance pruning were common entry sites for canker pathogens. Pruning wound protection and susceptibility studies were conducted to identify best products for preventing infection of almond pruning wounds and determine best pruning timing to reduce risks of infection with canker pathogens. Experiments indicated the superior efficacy of the fungicide Topsin M (Thiophanate-methyl) for which a 2(ee) recommendation was granted on almond for the protection of pruning wound against several canker pathogens. Additionally, Vintec, a biocontrol product formulated with the fungus *Trichoderma atroviride* SC1 provided excellent pruning wound protection thus allowing sustainable control solutions against canker diseases. Vintec is currently in the registration phase in California for use against canker diseases of almond. Additional studies were conducted in orchards to investigate the seasonal susceptibility of pruning wound and duration of pruning wound susceptibility according to the time (month) of pruning. Results of experiments confirmed that the duration of pruning wounds

susceptibility is lowest when pruning is done in January. Overall pruning wound susceptibility declined substantially after 1 to 2 weeks following pruning and continue to decrease overtime. In conclusion, this work indicates that one application of a pruning wound protectant such as Topsin M (conventional fungicide) or Vintec (biological control agent) following late pruning in January can significantly reduce risks of infection of pruning wounds by canker pathogens.

B. Objectives

1. Goal and objectives

Objective 1: Identify the main pathogens associated with trunk and scaffold canker diseases.

Objective 3: Determine the pathogenicity in almond of the main fungi associated with cankers.

Objective 4: Identify best fungicidal products including biocontrol agents for the protection of pruning wounds.

Objective 5: Determine the seasonal susceptibility of pruning wound and duration of pruning wound susceptibility according to the time (month) of pruning.

2. Annual outputs and milestones for each of the objectives

Objective 1: A survey of almond orchards expressing TSCD was conducted between 2015 and 2018. The survey revealed a broad diversity of fungal pathogens associated with cankers including Botryosphaeriaceae spp., *Ceratocystis destructans*, *Eutypa lata*, *Cytospora* spp., *Collophora* spp., *Phomopsis/Diaporthe* spp., *Phytophthora* and *Basidiomycetes* spp. A total of 90 almond orchards were visited and 400 fungal isolates were isolated from cankers and identified in this study.

Objective 2: Pathogenicity tests conducted in 2015, 2016 and 2017 revealed that isolates from the Botryosphaeriaceae were the most aggressive pathogens on almond, namely *Neofusicoccum arbuti*, *N. parvum* and *N. mediterraneum*. *Ceratocystis destructans* 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.) also were found to be pathogenic on almond causing vascular discoloration.

Objective 3: Pruning wound protection trials were conducted during the dormant season 2016-2017, 2017-2018 and 2018-2019 in Colusa and Kern Counties. Experiments revealed that the fungicide Topsin M (Thiophanate Methyl) and Vintec (a *Trichoderma* biocontrol product) provided the best pruning wound protection against several TSCD pathogens.

Objective 4: Pruning wound susceptibility trials were conducted during the dormant season 2017-2018 and 2018-2019 in Colusa and Kern Counties. Results 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.

C. Results and Discussion

Objective 1: Between 2015 and 2018, 90 almond orchards were visited throughout the Central Valley of California upon requests by farm advisors, PCAs or growers for canker disease diagnosis. Approximately 300 samples including bark and wood cankers in trunks and branches were collected from orchards in the main almond

producing counties. Two major canker diseases were found to be widespread in California, including Botryosphaeriaceae cankers and Ceratocystis canker. In addition, five, less prevalent canker diseases were identified including, Cytospora canker, Eutypa canker, Diaporthe canker, as well as Collophorina and Pallidophorina cankers. Cankers in almond were primarily associated with pruning wounds made during primary and secondary scaffold selections that provide the general structure of trees. Shaker injury, cracks on trunk or at the tree crotch also commonly lead to canker diseases. Among the 90 orchards with confirmed cases of canker diseases, 400 isolates were identified for this study. A subset of 74 fungal isolates in the Ascomycota were selected for multi-locus phylogenetic analyses, which identified 27 species. These included 12 Botryosphaeriaceae species, Ceratocystis destructans, 5 Cytospora species, Collophorina hispanica, Pallidophorina paarla, Eutypa lata, 2 Diatrype species, and 4 Diaporthe species. The most frequently isolated species were Ceratocystis destructans, Neoscytalidium dimidiatum and Neofusicoccum parvum.

Objective 2: Pathogenicity tests revealed that isolates from the Botryosphaeriaceae were the most aggressive pathogens on almond, namely Neofusicoccum arbuti, N. parvum and N. mediterraneum. In pathogenicity trials, N. parvum and N. arbuti killed 20 to 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.) were found to be pathogenic on almond causing vascular discoloration.

Objective 3: Topsin M (Thiophanate-methyl, FRAC 1) and the Trichoderma biocontrol product Vintec (Trichoderma atroviride SC1) demonstrated excellent disease control (80 to 100% disease control) when applied to fresh wounds immediately after pruning. Fungicides such as Quash (Metconazole, FRAC 3), Quilt Xcel (Propiconazole/Azoxystrobin, FRAC 3 & 11), Merivon (Pyraclostrobin/Fluxapyroxad, FRAC 7 & 11) and Rhyme (Flutriafol, FRAC 3) provided moderate disease control, with efficacy ranging from 40% to 70%. Acrylic paint was shown as being poorly effective overall as a pruning wound protectant against all the various canker pathogens, achieving only 20-40%. Recently, Topsin M has received a 2(ee) label recommendation for use in almonds as a pruning wound protectant against canker pathogens. Similarly, the biological control product Vintec is currently in the registration phase in California for use against canker diseases in almonds. This research indicates that effective protection of pruning wounds from infection by almond canker pathogens can be achieved with a one-time spray application of thiophanate-methyl or the biocontrol Trichoderma atroviride SC1 (1 g/liter) after pruning.

Objective 4: This objective aimed to identify lowest risk period for pruning of almond trees to reduce the risk of infection by canker pathogens and determine the duration of pruning wound susceptibility to fungal infection. During two consecutive dormant seasons, pruning wounds were made in September, October, November, December and January and inoculated with Botryosphaeria dothidea, Cytospora sorbicola, Eutypa lata, Neofusicoccum parvum or Neoscytalidium dimidiatum, for up to 0, 1, 2, 3, 5 and 8 weeks after pruning. Pruning wound susceptibility to all pathogens decreased significantly two weeks after pruning and continued to decrease with time. Pruning wounds inoculated immediately after pruning were the most susceptible to infection by all the canker pathogens tested. Overall infection rates following pruning in January were the lowest, and highest following pruning in September.

D. Outreach Activities

1. Canker diseases in stone fruits, Invited Speaker, Guest speaker for Roger Duncan (UCCE Farm Advisor Stanislaus County) for Pest Control Advisors and Almond Growers meeting, Modesto, California, 4/1/2015, 40 Attendees, Growers, PCAs, Industry members.
2. Canker diseases of almonds, Invited Speaker, Southern San Joaquin Valley Almond Symposium–2015, CE advisors, Kerman, CA, 05/28/2015, 250 Attendees. Growers, PCAs, Industry members.
3. Canker diseases of almond, Invited Speaker, Almond Conference, Sacramento, CA, 12/10/2015, 150 Attendees, Growers, PCAs, Industry members.
4. Update on almond canker disease research, Invited Speaker, Integrated Pest Management for Almonds meeting, UCCE Kern County, 01/26/2016, 50 Attendees, Growers, PCAs, Industry members.
5. Canker diseases of almond, Invited Speaker, Colusa/Sutter/Yuba Winter Almond Meeting, Colusa, CA, 02/03/2016, 70 Attendees, Growers, PCAs, Industry members.
6. Canker diseases of almond, Invited Speaker, Yolo Solano Sacramento Annual Almond Production Meeting, Woodland, CA, 02/03/2016, 70 Attendees, Growers, PCAs, Industry members.
7. Diagnosis and management of Wood Canker Diseases, Invited Speaker, 2016 North San Joaquin Valley Almond Day, Modesto, CA, 03/04/2016, 500 Attendees, Growers, PCAs, Industry members.
8. How to spot and control canker in almonds and cherries, Invited Speaker, Fresno-Madera CAPCA Spring CE 2016, Fresno, CA, 03/17/2016, 150 Attendees, Growers, PCAs, Industry members.
9. Limb, trunk and root diseases of almond, Invited Speaker, Almond short course, Modesto, CA, 11/10/2016, 500 Attendees, Growers, PCAs, Industry members.
10. Update on almond canker disease research, Invited Speaker, Almond Workgroup Meeting, UC Davis, CA, 12/09/2016, 40 Attendees, Growers, PCAs, Industry members.
11. Understanding new and emerging diseases in the fruit and nut crops in California, Invited Speaker, World Ag Expo, Tulare, CA, 02/14/2017, 20 Attendees.
12. Identification and management of wood canker pathogens in almonds, Invited Speaker, San Joaquin Sustainable Farming Project, Chowchilla, CA, 03/28/2017, 70 Attendees, Growers, PCAs, Industry members.
13. Almond canker diseases, biology and management, Invited Speaker, Kern County Almond Day, UCCE, 03/30/2017, 150 Attendees, Growers, PCAs, Industry members.
14. Canker diseases of almond, Invited Speaker, Almond Conference, Sacramento, CA, 12/07/2017, 500 Attendees, Growers, PCAs, Industry members.
15. Update on almond canker disease research, Invited Speaker, Almond Workgroup Meeting, Davis, CA, 12/08/2017, 40 Attendees, Researchers, ABC members.
16. Diagnosis and Management of Canker Diseases of Almond, Invited Speaker, Butte/Glenn/ Tehama Almond and Walnut Day 2018. Chico, CA, 01/31/2018, 400 Attendees, Growers, PCAs, Industry members.
17. Diseases of fruit and nut crops, Invited Speaker, 2018 California Plant and Soil Conference, Fresno, CA, 02/6-7/2018.

18. Management of Trunk and Scaffold Canker Diseases of Almond in California, Invited Speaker, 2018 South Valley Nut Show, Tulare, CA, 10/26/2018, 200 Attendees, Growers, PCAs, Industry members.
19. Canker diseases of almond, Invited Speaker, Almond Conference, Sacramento, CA, 12/06/2018, 500 Attendees, Growers, PCAs, Industry members.
20. Prevention and Management of Canker Diseases in Tree Crops, Invited Speaker, Growers and Pest Control Advisers, Madera, Dec 12, 2018, 60 Attendees.
21. Update on almond canker disease research, Invited Speaker, 2018 Almond Workgroup Meeting, Davis, CA, 12/07/2018, 40 Attendees, Researchers.
22. Diagnosis and Management of Almond Canker Diseases, Invited Speaker, 2019 Kern County Almond Day - UCCE Kern County, CA, 04/11/2019, 80 Attendees, Growers, PCAs, Industry members.
23. Limb, Trunk and Root Diseases. 2019 Almond Short Course. Visalia, CA, 11/7/2019, 300 attendees, Growers, PCAs, Industry members.
24. Yolo County Annual Almond Production Meeting. Woodland, CA, 2/05/2020, 40 Attendees, Growers, PCAs, Industry members.
25. Canker diseases of almond. 2021 UCCE Sacramento Valley Almond Grower Meeting, 1/20/2020, Growers, PCAs, Industry members.

E. Materials and Methods:

1. Surveys of trunk and scaffold canker diseases in California almond orchards and identification of pathogens:

Survey of diseased orchards were conducted in collaboration with farm advisors, PCAs and growers. Between 2015 and 2018, approximately 90 orchards with symptoms of almond TSCD were visited and sampled throughout the Central Valley, spanning 11 counties. Symptoms of TSCD included cankers, dieback, gummosis, and vascular discoloration on almond branches, scaffolds or trunks. Diseased wood samples were plated on 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*. Identification of pathogens associated with almond TSCD was carried out using morphology and DNA phylogeny using the internal transcribed spacer (ITS) region of the rDNA, translation elongation factor (TEF1), beta-tubulin (BT), and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

2. Pathogenicity of TSCD fungi on almond.

To determine pathogenicity of fungal isolates, pathogenicity studies were conducted in the field using 18 isolates representing the main 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 (2-year-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. Length of vascular discolorations were assessed and compared among all treatments including a non-inoculated control 3 to 6 months after inoculation.

3. Pruning wound protection

Field trials were established to test a wide variety of fungicide products (21 products total) against the main TSCD pathogens. Products tested included fungicides from different FRAC groups, biofungicides, biological control agents and wound sealants. Field trials were conducted in experimental almond orchards in Colusa and Kern counties. For each trial, 2 to 3 year-old branches were pruned to roughly 6-to-12 inches in length in December 2018. Following pruning, the various fungicidal products were applied to fresh wounds with a hand-held spray bottle until runoff. Twenty-four hours after treatment, wounds were sprayed with sterilized water to imitate rain and inoculated with the respective fungal pathogens *Eutypa lata*, *Ceratocystis destructans*, *Cytospora sorbicola*, *Neofusicoccum parvum* and *Botryosphaeria dothidea* (1,000 spores/wound). Treatment branches were collected three months after inoculation and brought back to the laboratory to assess the percent of fungal recovery. Presence (1) or absence (0) of the inoculated fungal species was recorded and averaged for each treatment. Low rates of fungal recovery correlated with high product efficacy. Infection rate was calculated as the percentage of pruning wounds from which the pathogen was recovered, out of the total number of inoculated pruning wounds.

4. Pruning wound susceptibility trials

Four independent field trials were conducted to determine the month (Sept, Oct, Nov, Dec, Jan) and duration (+0, 1, 2, 3, 5, 8 weeks following pruning) for which pruning wounds are most/less susceptible to infection by canker pathogens. Nonpareil trees in two orchards (Fresno and Colusa Counties) were pruned either in September 2018, October 2018, November 2018, December 2018, or January 2019, respectively. For each pruning time, subsets of branches were inoculated immediately after pruning (0), or after 1, 2, 3, 5 and 8 weeks following pruning. After wetting wounds with a spray bottle application of sterile water to mimic rain, spore suspensions (5×10^3 spores per wound) of canker pathogens *Cytospora sorbicola*, *Eutypa lata*, *Neofusicoccum parvum*, *Botryosphaeria dothidea*, and *Neoscytalidium dimidiatum* were applied separately to individual pruning wounds in all the various treatments. After 4 months, branches were processed in the laboratory to assess percent fungal recovery for each treatment and determine pruning wounds susceptibility according to the time (month) of pruning and timing of infection following pruning. High or low recovery of fungal pathogens corresponded to high or low susceptibility of pruning wound in each treatment, respectively.

F. Publications that emerged from this work

1. Peer review publications

1. Holland L.A., Travadon R., Lawrence D.P., Nouri M.T., and Trouillas F.P.* 2020. Effects of pruning month and wound age on the susceptibility of almond pruning wounds to fungal canker pathogen infection in California. *Plant Disease*, In preparation.
2. Holland L.A., Travadon R., Lawrence D.P., Nouri M.T., and Trouillas F.P.* 2020. Evaluation of pruning wound protection products for the management of almond canker diseases in California. *Plant Disease*, In press.
3. Holland L.A., Trouillas F.P.*, Nouri M.T., Lawrence D.P. et al. Fungal Pathogens Associated with Canker Diseases of Almond in California. *Plant Disease* <https://doi.org/10.1094/PDIS-10-19-2128-RE>

4. Holland L.A., Lawrence D.P., Nouri M.T., Travadon R., Harrington T.C., and Trouillas F. P.*. 2019. Taxonomic revision and systematics of the North American clade of *Ceratocystis fimbriata*. *Fungal Systematics and Evolution* 3: 135-156.
5. Nouri M.T., Lawrence D.P., Yaghmour M.A., Michailides T.J. and F.P. Trouillas*. 2018. *Neoscytalidium dimidiatum* causing Canker, Shoot Blight and Fruit Rot of Almond caused by in California. *Plant Disease* 102:1638-1647.
6. Lawrence D.P., Holland L.A., Nouri M.T., Travadon R., Abramians A., Michailides T.J., and Trouillas F.P. *. 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 journal* 9:333-378.
7. Holland L.A., Nouri M.T., Crespo M., Holtz B.A., Yaghmour M., Doll D.A., and Trouillas F.P.* 2017. First report of *Collophora hispanica* and *Collophora paarla* causing branch cankers of almond in California. *Plant disease*, <https://doi.org/10.1094/PDIS-09-17-1518-PDN>
8. Holland L.A., Nouri M., Lawrence, D.P., Travadon, R. and Trouillas F.P.* 2018. A re-examination of *Ceratocystis* canker in California almond orchards. *Acta Hort.* 1219: 311-317.
9. Holland L.A., Nouri M., Crespo, M. and Trouillas F.P.* 2018. Etiology and management of trunk and scaffold canker diseases of almond in California. *Acta Hort.* 1219: 319-324.
10. Nouri M.T., Holland L. A., Yaghmour M.A., Doll D.A., Browne G.T., and Trouillas F.P.* 2017. First report of *Phytophthora cinnamomi* causing trunk canker of almond in California. *Plant Disease*, doi.org/10.1094/PDIS-06-17-0872-PDN.

2. Other publications (outreach materials)

1. How to Control Fungal Canker Diseases. Industry News, Almond Board of California, Nov 2020.
2. Main Fungal Canker Diseases Affecting California Almonds. Industry News, Almond Board of California, Jan 2020.
3. Main Fungal Canker Diseases Affecting California Almonds. West Coast Nut Magazine, Nov 2020.
4. Canker Disease in Almond Orchards. West Coast Nut Magazine, August 2020.
5. Canker diseases with Florent Trouillas. Growing the Valley Podcast, 08/19/2019, UC ANR.
6. Pruning wound protection: How to reduce the impact of canker diseases. Sacramento Valley Orchard Source, Dec 2019.
7. Early Detection Key to Managing *Ceratocystis* Canker in Almonds, June 22, 2018, Growing Produce.
8. Pruning wounds can lead to cankers, UC specialist warns, July 03, 2018, Western Farm Press.
9. Wood canker pathogens in almonds, Youtube Video, 3/28/17.
10. Diagnosis and management of canker diseases in almond. West Coast Nut, Magazine Article, Sept 2017, West Coast Nut.