Detection of Band Canker Pathogens in Young Almond Trees in Nurseries and Orchards and Disease Management

Project No.: PATH16

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A. Summary *(In laymen's terms – emphasize key findings and recommendations)*

In the last decade or so, almond band canker has become very severe in very young orchards, significantly impacting production in California. Various fungal species in the Botryosphaeriaceae fungal family have been reported as the causal pathogens of band canker. Some of these pathogens were found to occur in plant tissues collected from young orchards as latent infections before the appearance of disease symptoms in the field. The definition of latent infection according to the American Phytopathological Society is a close parasitic relationship between the pathogen and the host showing no symptoms or signs of disease and eventually producing disease. We also found significant levels of latent infection by various pathogen groups in samples from almond potted trees from nurseries. To check whether latent infections occur in budwood of different almond varieties, young almond shoots (budwood) were collected from budwood-source trees from two nurseries (Nursery A1 and Nursery B1). Our published *q*PCR system was applied to quantify the incidence of latent infection of six canker-causing pathogen groups. Three pathogen groups, species of *Cytospora*, *Neofusicoccum* and *Phomopsis* were detected in budwood from Nursery A1, and those of *Cytospora*, *Neofusicoccum* and *Lasiodiplodia* were detected in budwood from Nursery B1. Latent infection levels varied among the almond varieties. In a fungicide trial to control band canker disease in a 1st- and a 2nd-leaf almond orchards, the fungicides Topsin-M and Topsin-M + Rally were used to spray once on trunks of these young trees on 7 February and 19 March, 2019, respectively. Evaluations of disease symptoms were done on 21 November 2019. In the 1st-leaf orchard no band canker symptoms were found in any of the trees. However, in the 2nd -leaf orchard, the incidences of the trees showing band canker symptoms treated with Topsin-M and Topsin-M + Rally were significantly lower than those of untreated control, and there was no difference in canker incidence between Topsin-M and Topsin-M + Rally treatments, indicating no additive effects of Rally to the efficacy of Topsin-M against the band canker disease.

B. Objectives *(300 words max.)*

To determine (1) the existence of canker-causing pathogens in budwood from budwood trees in nurseries; and (2) the efficacy of a fungicide application on trunks of young trees early in season to protect trees from band canker disease development.

C. Annual Results and Discussion *(This is the core function of this report)*

Objective 1:

Two nurseries, Nursery A1 and B1, were identified. In Nursery A1, three pathogen groups, *Cytospora*, spp. *Neofusicoccum* spp. and *Phomopsis* spp. were detected in budwood shoots from three almond varieties, Nonpareil, Padre and Wood Colony (Figure 1). For *Cytospora* spp., the incidences (I) of latent infection on Nonpareil and Padre were significantly higher than that of Wood Colony. For *Neofusicoccum* spp., the incidence of latent infection on Nonpareil was significantly higher than that on Wood Colony (Figure 1A), while for *Phomopsis* spp., the incidence of latent infection on Padre was significantly lower than those on the other varieties (Figure 1A). The incidence of latent infection for *Neofusicoccum* spp. was much lower than those for other pathogen groups on all the varieties (Figure 1A).

For *Cytospora* spp., the molecular severity (MS) on Nonpareil and Padre were significantly higher than that on Wood Colony. MS is molecular severity (see section E for definition), and the results indicated that there was no difference in MS among the three varieties for *Neofusicoccum* spp., while the MS of Padre was significantly higher than those of other varieties for *Phomopsis* spp. (Figure 1B). The range of MS was from 4 to 5.

There was no significant difference in latent infection index (LII) (see section E for definition) among the three varieties for each of the three pathogen groups, indicating similarity in risk of overall latent infection in all three almond varieties for each pathogen (Figure 1C).

However in Nursery B1, *Phomopsis* spp. was not detected in any of the samples, while *Lasiodiplodia* spp. (a very aggressive species of Botryosphaeriaceae) was found in budwood shoots as latent infections (Figure 2). Compared with Nursery A1, the incidences of *Cytospora* spp. were lower in B1, and there was no significance among the four almond varieties, Monterey, Nonpareil, Padre and Wood colony (Figure 2A). *Lasiodiplodia* spp, was not detected from Monterey and Nonpareil, and there was no significant difference in incidence of *Lasiodiplodia* spp. between Padre and Wood Colony (Figure 2A). The range of incidence of *Neofusicoccum* spp. was from 5 to about 20% among these four varieties, but no significant differences were detected.

The results demonstrated that MS of *Cytospora* spp. was significantly higher than those of Monterey and Nonpareil, while there was no difference in MS among Monterey, Nonpareil and Wood Colony (Figure 2B). For *Lasiodiplodia* spp. the MS of Wood Colony was significantly higher than that of Padre, while for *Neofusicoccum* spp. there was no difference in MS among all the four varieties (Figure 2B).

The overall latent infection level described as index showed no difference among all four varieties for *Cytospora* spp. and *Neofusicoccum* spp. (Figure 2C), while the latent infection index of Wood Colony was significantly higher than that of Padre for *Lasiodiplodia* spp. (Figure 2C).

In general, *Cytospora* spp, and *Neofusicoccum* spp. were detected in budwood shoots from 32 samples in each of the two nurseries with various levels of incidence and severity, while the latent infection index levels varied between these two nurseries. The result demonstrated that these two pathogen groups are common among the studied nurseries. However, *Lasiodiplodia* spp. and *Phomopsis* spp. occurred differently in different nurseries in budwood. The results implied the possible risk of budwood carrying pathogens during the grafting process. Next step with these samples is to find out whether the same pathogens as those detected as DNA in the latent infections by using qPCR can be isolated from the symptomless tissues conventionally on agar media.

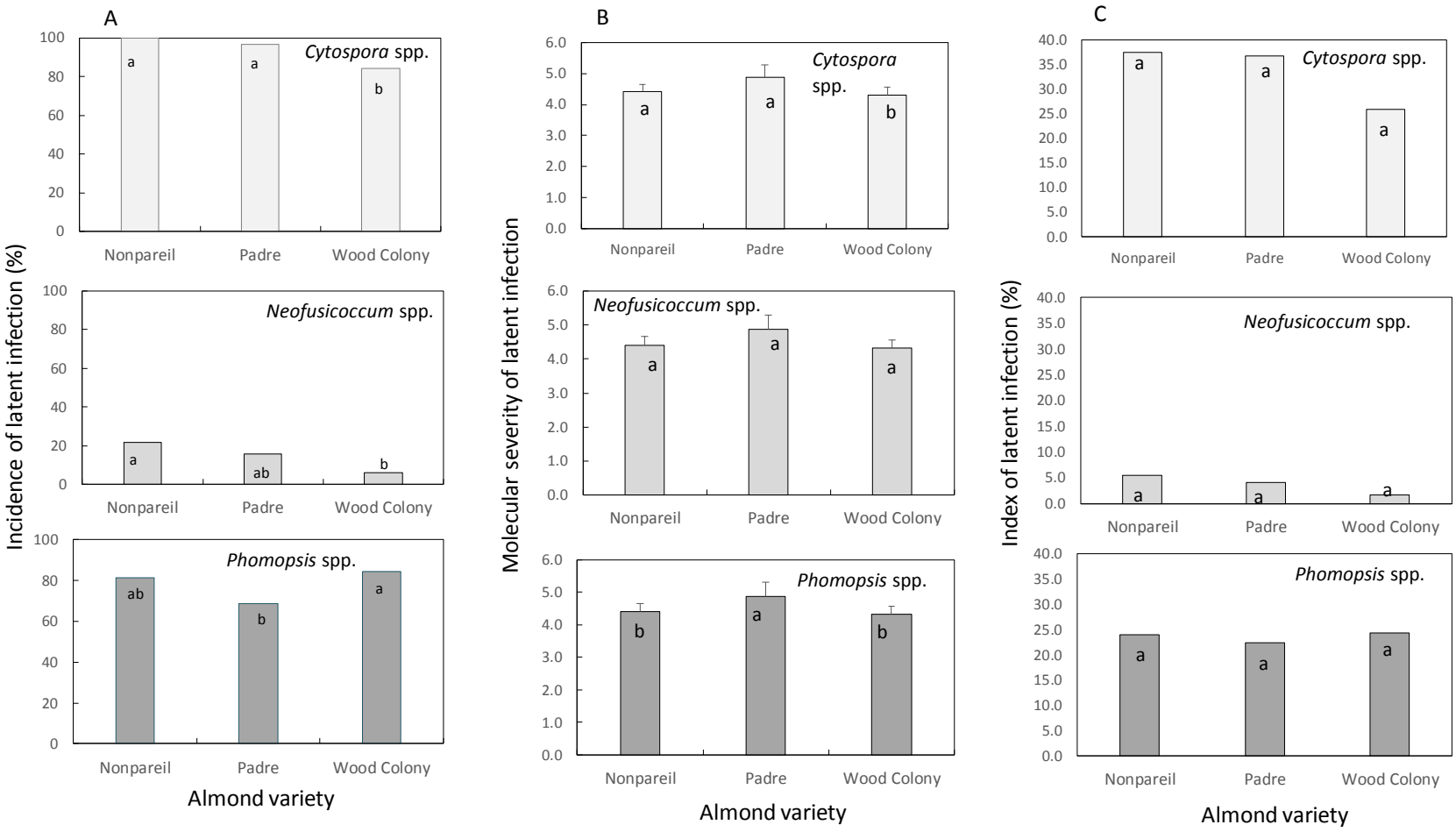

Figure 1. Comparison in incidence (**A**), molecular severity (MS) (**B**), and latent infection index (LII) (**C**) of canker-causing pathogens from budwood collected directly from almond bud wood-source trees of three different varieties. Each mean value was from 32 shoot samples collected from nursery A1.

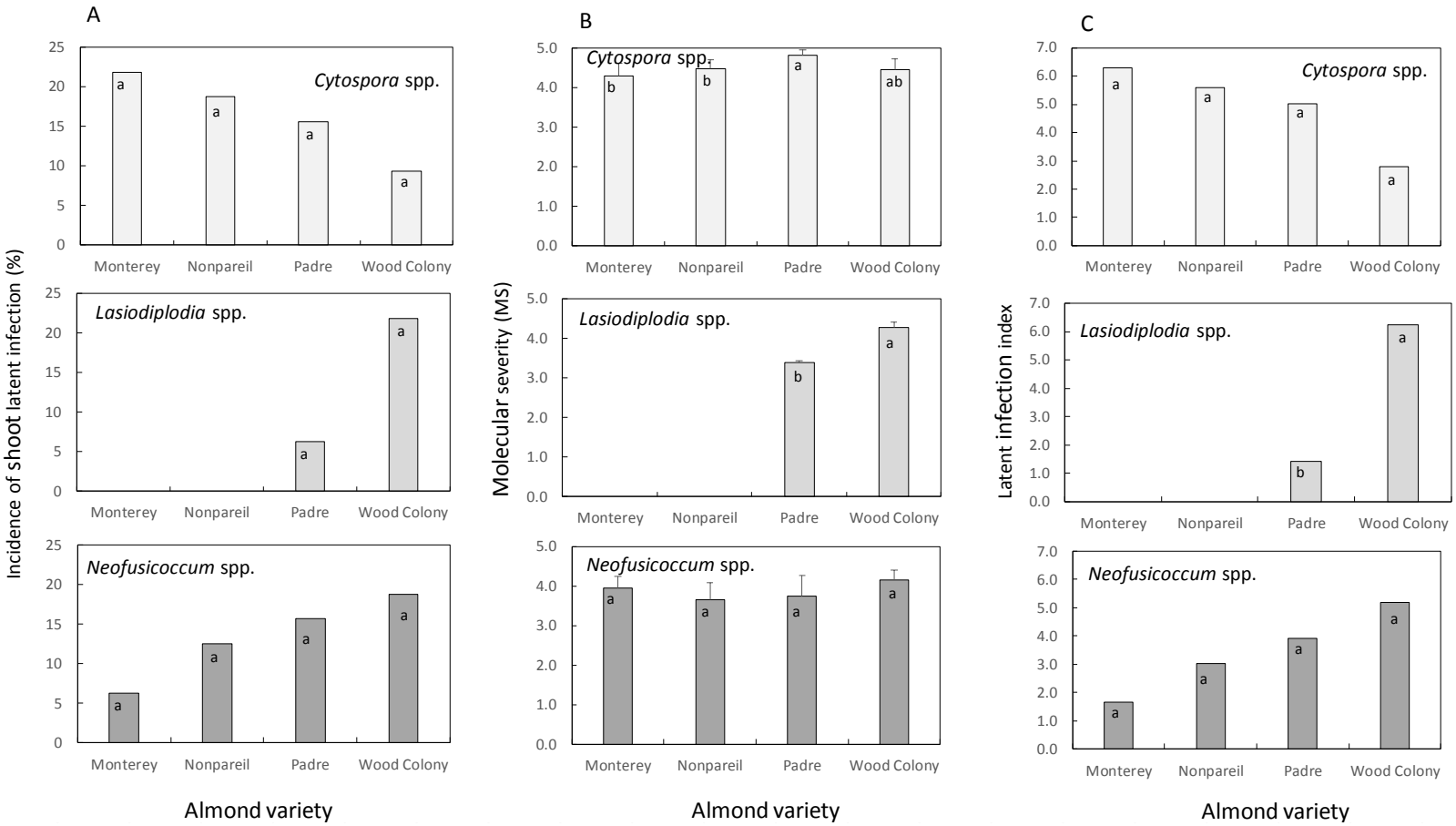

Figure 2. Comparison in incidence (**A**), molecular severity (MS) (**B**), and latent infection index (LII) (**C**) of canker-causing pathogens from budwood collected directly from almond budwood-source trees of three different varieties. Each mean value was from 32 shoot samples collected from nursery B1.

Objective 2:

No disease symptoms of band canker were developed in any of the trees in the 1st-leaf orchard by 21 November 2019. These trees will be evaluated again in spring of 2020. However, in the 2nd-leaf orchard, the incidence of trees showing canker symptoms treated with Topsin-M and Topsin-M + Rally was significantly lower than the incidence of disease in the untreated control for all the three replicates of this trial (Figure 3). The range of the incidence of trees showing canker symptoms was from 50% to 60% for untreated control, while those for the fungicide treated trees were less or up to 20% (Figure 3).

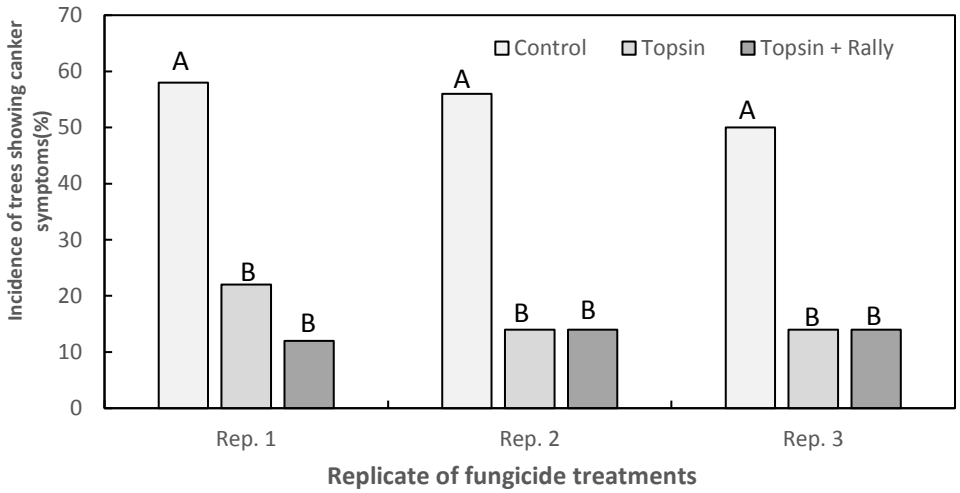

Figure 3. Efficacy of one fungicide spray applied in February 2019 on almond trees in a 2nd-leaf orchard in Glenn Co., California. Evaluation of the disease was done on 21 November 2019 and each value represents the mean incidence from three 50-tree replicates.

D. Outreach Activities

(Please describe outreach activities including the event description (date, location, topic of the presentation, aprox number of participants and type of audience)

1. Michailides, T. J. Band canker of almond becoming a Treat to new plantings. Invited Speaker. 30 minute talk at Grape, Nut & Tree Fruit Expo Seminars, Malcolm Media-Ag Publishing, Fresno, November 14, 2017. (120 attendees)

2. Michailides, T. J. Band canker early detection. Invited Speaker. 45th

Almond Conference, Almond Board of California, Sacramento, December 7, 2017. (320 attendees).

3. Michailides, T. J. Management of Botryosphaeria in trees and vines. Invited Speaker. Plant Food Systems Meeting. Bakersfield, January 20, 2017. (28 attendees).

4. Michailides, T. J. Band canker and Botryosphaeria diseases in almond. Invited Speaker. Butte/Glenn Almond Institute. UC Cooperative Extension. February 16, 2016. (200 attendees).

5. Michailides, T. J. (2017). Band canker detection in young almond trees before planting or just after planting in the field before disease symptoms appear. 45th Almond Conference (Poster), Sacramento, CA. (20 visitors).

6. Michailides, T. J. (2018). Detection of band canker pathogens in almond trees in nurseries and young orchards and disease management. 46th Almond Conference, Sacramento, CA. (22 visitors).

7. Luo, Y., Lake, J., Lightle, D., and Michailides, T. J. (2019). Detection of band canker pathogens in almond mother trees and disease management. 47th Almond Conference, Sacramento, CA. (28 visitors).

E. Materials and Methods *(500 word max.)*:

Objective 1.

Two commercial nurseries (assigned as Nursery A1 and Nursery B1) were selected where budwood shoots of different almond varieties were collected from mother trees. In Nursery A1, samples were collected from three varieties, Nonpareil, Padre, and Wood Colony on 22 May 2019. In Nursery B1, samples were collected from four varieties, including Monterey, Nonpareil, Padre, and Wood Colony on 10 October 2019.

For each variety from each nursery, 32 shoot samples were used. These shoot samples were processed with the *q*PCR technique (Luo et al, 2017) to quantify the fungal DNA and obtain the parameters describing “latent infection levels” including incidence of latent infections (I, %), average molecular severity (MS), and latent infection index (LII) for each treatment. MS was calculated as $MS= Log_{10}(P/H)$, where P is the weight of the pathogen’s DNA (femtograms, fg), which is calculated by using the equation of the standard curve for the corresponding pathogen based on the Ct (cycle number of threshold) value from its reaction with the corresponding primers (Luo et al., 2017), and where H is the shoot/bud weight (grams, g). Thus, the range of MS value is 0 to 15. The MS values show the amounts of “latent infections”. The incidence (I) was

calculated as I = N / T × 100, where N is the number of shoots showing positive in PCR, and T is total number (32) of shoot samples. The latent infection index (LII) was calculated as LII = I × MS / 15 (Luo et al., 2019). The LII indicates the magnitude of risk for "latent infections" by these fungi. Comparisons in each of above parameters among varieties for each detected canker-causing pathogen were conducted with SAS (Statistical Analysis System, Cary, NC).

Objective 2.

A 1st-leaf and a 2nd-leaf orchard each was selected in Glenn Co., California. In each orchard, three fungicide treatments were conducted: 1) Topsin-M WP 70 at 1.5 lbs /acre; 2) Topsin-M at 1.5 lbs/acre + Rally at 8.0 oz/acre; and 3) non fungicide control. Each treatment used a block of 50 trees, and three blocks as replicates per treatment were used in each orchard. The fungicides were sprayed onto the main trunk and crotch area of each tree on 7 Feb. in the 1st-leaf orchard and on 19 Mar in the 2nd-leaf orchard, using a hand gun. The cultural practices for the fungicide-treated trees and untreated control trees were the same throughout the growing season in each orchard. The incidence of trees showing typical canker symptoms was determined on 21 November 2019 for each treatment in each orchard. Comparison in canker incidence among the fungicide treatments was conducted.

F. Publications that emerged from this work

1. *List peer review publications in preparation, accepted or published*
2. *Other publications (e.g. outreach materials)*
3. *Please provide copies of publications.*

1) Luo, Y., Gu, S., Felts, D., Puckett, R. D., Morgan, D. P. and Michailides, T. J. 2017. Development of qPCR systems to quantify shoot infections by canker-causing pathogens in stone fruits and nut crops. Journal of Applied Microbiology 122: 416-428.

2) Luo, Y., Lichtemberg, P. S. F., Niederholzer, F. J. A, Lightle, D. M., Felts, D. G., and Michailides, T. J. 2019. Understanding the process of latent infection of canker-causing pathogens in stone fruit and nut crops in California. Plant Dis. 103: 2374-2384.

3) Luo, Y., Niederholzer, F. J. A, Felts, D., Puckett, R. D., and Michailides, T. J. (202x). Inoculum quantification of canker-causing pathogens in prune and walnut orchards using real-time PCR. (In preparation).

4) Luo, Y., Niederholzer, F. J. A., Lightle, D. M., Felts, D., Lake, J., and Michailides, T. J. (202x). Development of latent infections by canker-causing pathogens in young shoots of stone fruit and nut crops in California. (In preparation).

5) Luo, Y., Niederholzer, F., Lightle, D. M., Lake, J., and Michailides, T. J. (202x). Investigation of latent infections of young trees of prune and almond by canker-causing pathogens in nurseries of California. (In preparation).