Epidemiology and Control of Almond Scab and Alternaria Leaf Spot

Project No.: **PATH3-Adaskaveg**

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A. Summary

Scab caused by *Fusicladium carpophilum* and Alternaria leaf spot caused by *Alternaria alternata* and *A. arborescens* occurred at lower severity in 2019 following a general trend over the last few years. This is likely because of reduced irrigation and subsequent less favorable disease conditions. We continued to evaluate populations of the scab pathogen from different areas in the state. Molecular population methods indicated no evidence for sexual recombination. Thus, *Fusicladium* and *Alternaria* spp. appear to only reproduce asexually by conidia in California. Based on molecular analyses, however, two species of *Fusicladium* may exist sympatrically and occupy the same niche on almond tissues and may even be present in the same lesions.

Chlorothalonil-oil dormant treatments delay scab inoculum production on overwintering twig lesions and are an anti-resistance strategy (a smaller population is exposed to selection processes) that align in-season, springtime treatments for scab, Alternaria leaf spot, and rust. In-season fungicide timings for scab are initiated at twig lesion sporulation, whereas the Disease Severity (DSV) model (with positioning of sensors 10-16 ft in the outer canopy) optimizes timing for Alternaria management. Calendar-based timing starting in late spring with warm temperatures and dew formation can also be used for Alternaria leaf spot with 1-3 applications in 3-week intervals. In two trials on the management of scab, in-season treatments with Cevya, Luna Experience, Quadris Top, a mixture of Ph-D and Tebucon, as well as the experimentals pyraziflumid, V-10424, UC-2, EXP-AD, and EXP-AF resulted in the lowest disease levels. Based on previous trials, under high-disease conditions, a three-spray program should include dormant applications with chlorothalonil-oil (or the less effective copper-oil) and two petal-fall applications. Under lower disease pressure, a dormant treatment or in-season treatments alone should be considered.

In two trials on Alternaria leaf spot in 2019 with moderate levels of disease, the experimentals V-10424, EXP-AD, EXP-AF, and UC-2 were similarly highly effective as several registered fungicides (e.g., Cevya, Fervent, Fontelis + Teb, Luna Experience, Ph-D, and Quadris Top).

On almond, the FRAC code 7 (SDHI) boscalid, fluopyram, fluxapyroxad, isofetamid, and penthiopyrad are registered, and pydiflumetofen and pyraziflumid are in development. Genetic mutations have been identified in *Alternaria* spp. in the SDH target gene that correspond with resistance to selected SDHI fungicides. The highest incidence of resistance occurs against boscalid, fluxapyroxad, penthiopyrad, and pyraziflumid. Currently, only moderate resistance (EC_{50} <0.5 ppm) has been detected to fluopyram and in one isolate to the new pydiflumetofen. Because resistance to newer SDHI sub-groups is detected before commercial introduction, either less-

sensitive variants pre-exist, or resistance is an ongoing selection process related to fitness or non-detrimental changes in the pathogen. Knowledge of cross resistance patterns among SDHI fungicides will help in the design of rotation spray programs.

B. Objectives

I. Epidemiology

a. *Determine the effects of wetness at three temperatures and time periods on germination and growth of* F. carpophilum.

b. *Additionally, determine the effects of survival of germinated spores under subsequent levels of relative humidity during desiccation periods at different temperatures.*

c. *Evaluate the modified DSV model described for forecasting Alternaria leaf spot for also predicting scab*.

II. Management

a. *For scab management, evaluate the effect of dormant applications*.

b. *Evaluate new and registered fungicides for their efficacy as in-season treatments for managing scab and Alternaria leaf spot*.

c. *Establish and expand baseline sensitivities, monitor for shifts in sensitivity in populations of* Alternaria *and* Fusicladium *spp*., and c*ontinue to characterize molecular mechanisms for SDHI resistance*.

Table 1. Main goals, objective timelines, and milestones

Main Goal: Sustainable & effective management of scab and Alternaria leaf spot based on pathogen biology		
Objective(s)	**Date to be accomplished**	**Milestones and deliverables associated to the objective**
IA-Wetness effects	2020-2021	Min. temp. & wetness for growth
IB-Desiccation effects	2020-2021	Min. desiccation period for death
IC-DSV for Scab	2020-2021	Evaluate, modify, develop a wetness-temp. model for scab
IIA-Evaluate new modes of action	2020-2022	New FRAC code registered
IIB-Bravo-oil dormant air	2020-2021	New application strategy
IIC-Baselines and resistance mechanisms	2020-2022	Baseline as reference point for new FRAC code and strategy for using SDHI fungicides

C. Annual Results and Discussion

I. Epidemiology.

a. *Determine the effects of wetness at three temperatures and time periods on germination and growth of* F. carpophilum. These studies are ongoing and are dependent on the identification of the species/sub-populations involved. In our collaboration with USDA in Georgia studying scab pathogens of tree crops (e.g., peach, pecan, and almond), we have identified sympatric populations of slow and fast growing isolates of *Fusicladium* causing scab of amonds. These two populations probably represent two species occupying the same niche and even the same lesions. Additional isolates were collected from almond scab samples in 2019 and the two types of subpopulations were isolated. A genetic comparison is ongoing. We compared parital sequences of fast and slow growing isolates and found differences.

b. *Additionally, determine the effects of survival of germinated spores under subsequent levels of relative humidity during desiccation periods at different temperatures.* These studies are ongoing and are dependent on the identification of the two species/sub-populations involved. The slow growing isolates is very difficult to work with and we will focus on the fast growing isolate.

c. *Evaluate the modified DSV model described for forecasting Alternaria leaf spot for also predicting scab*. These studies are ongoing but appear to match for the two diseases when dormant applications of chlorothalonil and oil are applied for scab delaying the sporulation of twig lesions until late spring. Accumulation of wetness periods and warm temperatures can then be applied to forecast both diseases provided that scab lesions are present on 1-year-old twigs in the orchard. Alternaria is a ubiquitous aerophile, and apparently favorable environments (i.e., high density orchards with high humidity that are subjected to consistent dew periods) are essential to establish the disease in an orchard.

II. Management.

a. *For scab management, evaluate the effect of dormant applications*. Delayed dormant applications with Bravo-oil or Syllit as air applications to delay and reduce sporulation of twig lesions. We conducted air application trials for two years with a cooperator to determine if the efficacy is satisfactory but there was low disease incidence in the orchard preventing any conclusion. Studies need to be established in a new orchard with high disease pressure and a cooperator willing to do delayed dormant air applications of these fungicides.

b. *Evaluate new and registered fungicides for their efficacy as in-season treatments for managing scab and Alternaria leaf spot*. Two field trials were conducted for each of the two diseases that included in-season treatments with single-fungicide, pre-/mixture programs, and rotations of different fungicide chemistries.

i. Scab trials. Three applications of each treatment and a three-application rotation program were evaluated in two field trials in 2019. Disease incidence (40.9% on untreated control trees) and severity (4.5 lesions/fruit) on untreated control trees were moderate at the first trial site on cv. Monterey. All fungicide treatments significantly reduced the disease from the control, but Ecoswing (an extract of the plant *Swinglea glutinosa*) was not effective (Fig 1A). Several treatments were highly effective and reduced the disease to zero levels (i.e., Luna Experience, EXP-AF) or by at least 90% as compared to the control (i.e., Quadris Top, a mixture of Ph-D and Tebucon, and the experimentals pyraziflumid, V-10424, and EXP-AD).

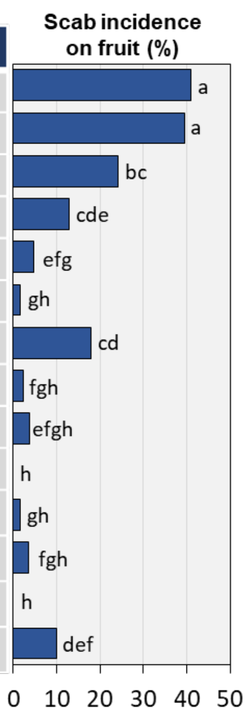
In the second trial on cv. Carmel, disease pressure was higher and the average scab incidence on untreated trees was 79.9% with 14.3 lesions per fruit. Treatments that reduced the incidence to between 10 and 20% included Quadris Top, mixtures of Fontelis with Teb or of Merivon with Serifel, a rotation of Fontelis + Teb with Quadris Top, the new FRAC 3 Cevya, and the experimental UC-2 (Fig. 1B). Merivon by itself was not very effective in this trial, thus the addition of the biocontrol Serifel (*Bacillus amyloliquefaciens* strain MBI600) seems to be beneficial.

These data indicate that very effective fungicides and programs are currently available to manage almond scab. Additional new highly effective treatments are in development. When disease was high in the previous season and many overwintering twig lesions are present in an orchard, a three-spray program should include dormant applications with chlorothalonil-oil (or copper-oil) and two late petal-fall (early cover timed in April and May) applications. Under lower disease pressure, a dormant treatment (i.e., when twig lesions are observed) or in-season treatments alone can be considered. If other summer diseases such as rust, Alternaria leaf spot, or hull rot also have to be managed at an orchard site, late spring and early summer applications with selected fungicides could also manage scab (because the onset of scab epidemics is delayed by the dormant application).

In-season treatments should start 2 to 3 weeks after petal fall (March or early April) or at the onset of twig lesion sporulation; we demonstrated previously that programs starting later in the season are not as effective. Effective fungicides are chlorothalonil, DMIs such as Quash, Inpire, or Inspire Super; Syllit, Ph-D, and compounds containing SDHIs or QoIs (at locations where the pathogen population has not developed resistance). Multi-site fungicides with low resistance potential (e.g., chlorothalonil, captan, ziram) applied at petal fall satisfy PHI requirements. Overuse of any FRAC code may lead to practical or field resistance. Thus, resistance management should be strongly followed by using all FRAC codes in rotations or mixtures. None of these materials should ever be used under pre-existing, high disease levels. Because many products are available, a FRAC code should only be applied once per season after twig sporulation begins (this would still allow applications of these materials during bloom and petal fall when the scab pathogen is not actively growing). If high disease levels exist, multi-site materials such as sulfur and captan can be used with short preharvest intervals.

A. cv. Monterey

Treatment	Rate(/A)
Control	---
EcoSwing	32 fl oz
Rhyme	5 fl oz
Quash	3 oz
Pyraziflumid	3.1 fl oz
Pyraziflumid	4.7 fl oz
Magister	36 fl oz
V-10424	3 fl oz
Ph-D + Tebucon	6.2 + 8 oz
Luna Experience	8 fl oz
Quadris Top	14 + 8 fl oz
EXP-AD	13.7 fl oz
EXP-AF	9.1 fl oz
F4406-3	6 fl oz



B. cv. Carmel

Treatment	Rate(/A)
Control	---
Super Tin	12 fl oz
Cevya	4 + 6 fl oz
Ph-D + Tebucon	6.2 + 8 oz
Fontelis + Tilt	20 fl oz + 8 fl oz
Fontelis + Teb	20 fl oz + 8 oz
Merivon + Serifel	5.5 fl oz + 8 oz
Luna Sensation	7.8 fl oz
Luna Experience	8 fl oz
Merivon	5.5 fl oz
Quadris Top	14 + 8 fl oz
UC-2	7 + 6 fl oz
Fontelis + Teb	20 fl oz + 8 oz
Quadris Top	14 + 8 fl oz

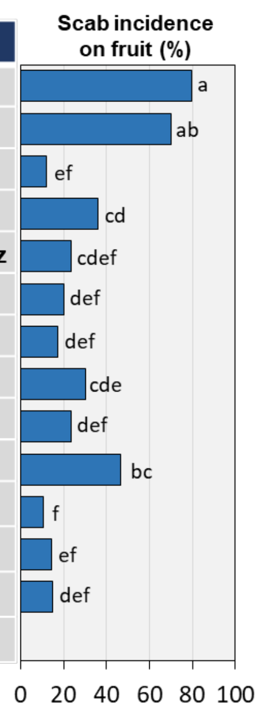


Fig. 1. Efficacy of new and registered fungicides for managing almond scab in orchards in Colusa Co. 2019

ii. Alternaria leaf spot trials.

Single-fungicides, mixtures, pre-mixtures, and rotation programs were used in two trials. The incidence of disease was high in both trials in the controls (98.3% and 90.2%) and disease severity was rated at 2.7 and 1.9 (on a scale from 0 to 4), respectively. In the first trial on cv. Monterey, all treatments significantly reduced the disease from the control (Fig. 2A). Numerically the lowest severity rating was obtained using Ph-D, Luna Experience, Fervent, and the experimentals EXP-AD and UC-2. These treatments all contain a FRAC code 3 DMI, except Ph-D. EcoSwing was among the least effective, but still significantly reduced disease severity from the control. In the second trial on cv. Carmel, again all fungicides were effective (Fig. 2B). The most effective treatments included Ph-D, Luna Experience, Fervent (similar as in the first trial), a mixture of Fontelis with Teb, the experimentals V-10424 and EXP-AF, as well as a rotation of Cevya, Merivon, and Ph-D.

Our data indicate that Alternaria leaf spot can be effectively managed with currently available fungicides in an integrated program with cultural practices. Additionally, we identified new highly effective fungicides and pre-mixtures that are being developed for use on almond. Rotation programs in our trials are excellent examples for resistance management programs using fungicide classes currently available. Resistance management is importance in the presence of widespread resistance against FRAC code 7 SDHI (see below) and FRAC code 11 QoI compounds.

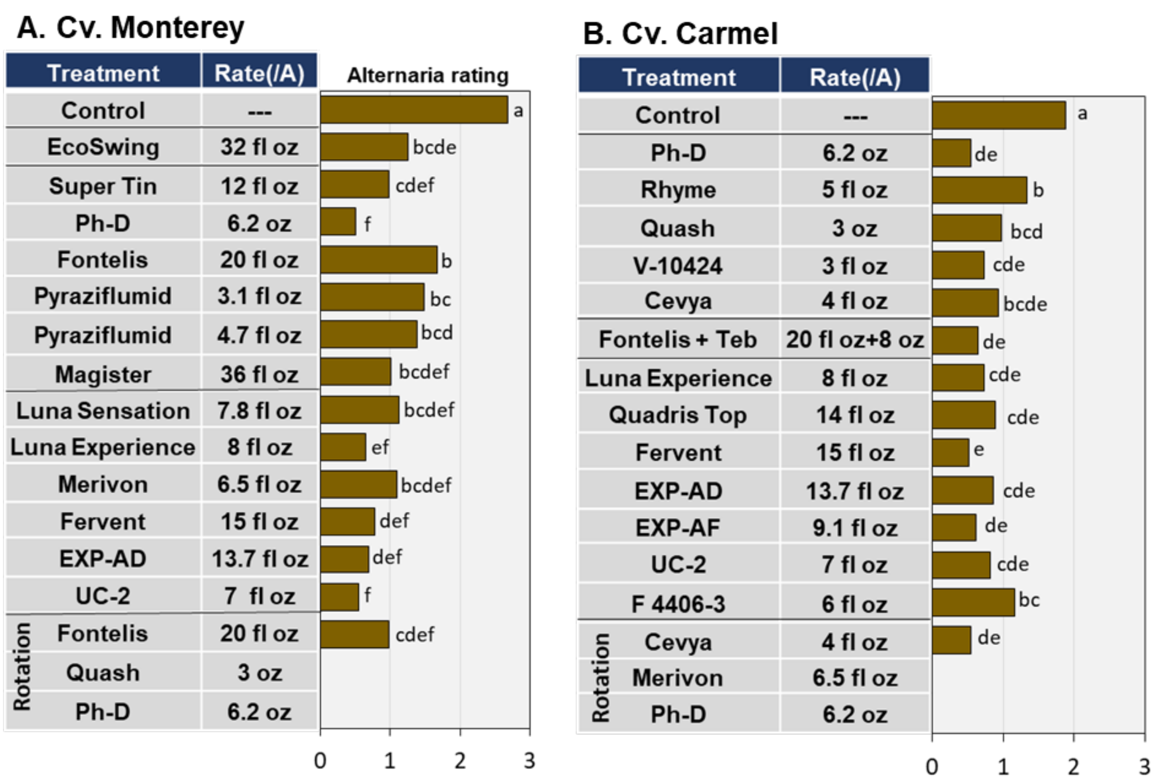
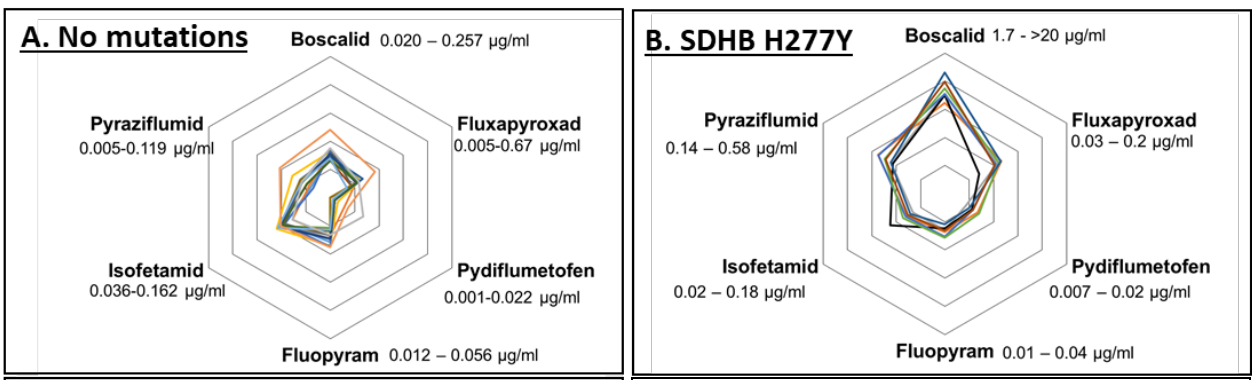


Fig. 2. Efficacy of new and registered fungicides for managing Alternaria leaf spot of almond in orchards in Colusa Co. 2019

a. *Continue to characterize molecular mechanisms for SDHI resistance in* Alternaria *spp. from almond*

We sampled additional almond orchards for isolates of *Alternaria* species to determine the extent of SDHI resistance in the state. Among 5 northern orchards (North of Sacramento) the incidence of resistance to boscalid (a first generation SDHI and a component of Pristine) ranged from 40 to 100% (average 80.9%), and among 6 orchards from southern growing regions (South of Fresno), the incidence ranged from 64.7 to 100% (average 81.2%). All isolates were also tested for sensitivity to fluopyram, fluxapyroxad, isofetamid, pydiflumetofen, and pyraziflumid, each belong to a different SDHI subgroup. The highest incidence of resistance occurs against boscalid, fluxapyroxad, penthiopyrad, and pyraziflumid. Currently, only moderate resistance (EC_{50} <0.5 ppm) has been detected to fluopyram and in one isolate to the new pydiflumetofen. To relate cross resistance patterns among SDHI fungicides to specific mutations in subunits of the SDH target gene, partial sequences were obtained for 23 isolates, in addition to the 44 isolates sequenced previously. We identified cross resistance patterns that correlated with mutations SDHB-H277Y, SDHB-277L, or SDHC-H134R. Most isolates carried the SDHC-H134R mutation.

Fig. 3 illustrates cross resistance patterns for mutations SDHB H277Y and SDHC H134R. Both mutations confer resistance to boscalid (EC_{50} values 1.7 to >40 ppm). SDHB-H277Y is mostly found in isolates collected before 2013, and these isolates also mostly show some reduced sensitivity to pyraziflumid (EC_{50} values 0.14 to 0.58 ppm) and fluxapyroxad (EC_{50} values 0.03 to 0.2 ppm), but they are mostly similar in sensitivity to isolates with no mutations for isofetamid, fluopyram, and pydiflumetofen. Mutation SDHC H134R is mostly found in isolates collected after 2012, and these are also less sensitive to pyraziflumid (EC_{50} values 3.1 to >40 ppm), fluxapyroxad (EC_{50} values 0.34 to 4 ppm), isofetamid (EC_{50} values 0.1 to 2.4 ppm), and fluopyram (EC_{50} values 0.1 to 0.45 ppm), but all except one isolate with an EC_{50} value of 0.45 ppm were highly sensitive to pydiflumetofen.



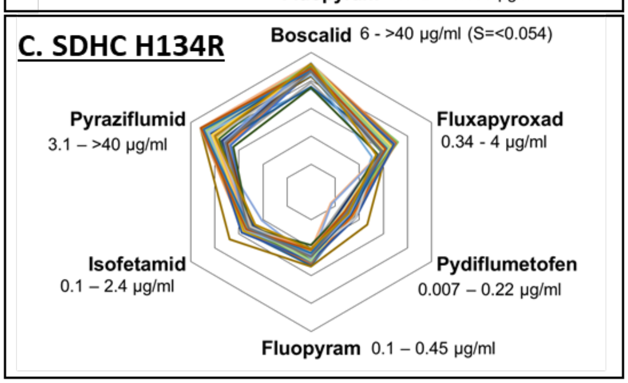


Fig. 3. Sensitivity phenotypes of 15 isolates with no mutation (**A)**, 7 isolates with mutation H277Y in SDHB (**B)**, and 46 isolates with mutation H134R in SDHC (**C)**. EC_{50} values for each fungicide are on a log_{10} scale with 50 ppm at the edge of each diagram. The range of EC_{50} values for isolates with each mutation is indicated.

These results indicate that resistance (i.e, pyraziflumid) or reduced sensitivity (i.e., isofetamid) to newer SDHI sub-groups is detected before commercial introduction. Thus, either less-sensitive variants pre-exist, or resistance is an ongoing selection process related to fitness or non-detrimental changes in the pathogen. These data also show that cross resistance among SDHI fungicides is incomplete and that resistance to boscalid could be managed using fluopyram, isofetamid, and pydiflumetofen (once registered). Still, rotation among SDHI fungicides is not recommened. From the information provided above, it appears that isolates resistant to boscalid collected before 2013 show less cross resistance to other SDHI subgroups than those collected later. Therefore, commercial use of additional subgroups as represented by fluxapyroxad (a component of Merivon) or penthiopyrad (the active ingredient of Fontelis) may be selecting for new mutations that drive resistance to higher levels and also affect other SDHI compounds. Thus, SDHI fungicides and pre-mixtures should be used in rotation with other FRAC classes such as DMIs (FRAC 3) or Ph-D (FRAC 19) or QoIs (FRAC 11) where resistance has not been detected. For a summary on the management of scab and Alternaria leaf spot with currently registered fungicides we refer to the "Fungicide Efficacy Tables" at http://www.ipm.ucdavis.edu).

D. Outreach Activities

1. Dr. Adaskaveg participated at several grower/PCA meetings at different locations in California over the year and gave presentations on almond diseases including scab and Alternaria leaf spot and their management. At each meeting, there were approximately 50-100 participants. Additionally, a presentation on SDHI resistance in *Alternaria* sp. isolates from almond was given at an international fungicide conference in Germany with approximately 120 participants.
    a. January 2019. Managing Diseases of Almond in California; Bayer Tree, Nut, and Vine Meeting; Organizer: Bayer CropScience; Universal Studios, Universal City, CA 91608
    b. January 2019. Almond Diseases: Key Economic Pests, ID, Biology, and Treatments in Almonds; 2019 Independent PCA Symposium; Organizer: Bayer CropScience; Monterey Plaza Hotel and Spa, Monterey, CA 93940
    c. January 2019. Almond Disease Management, Colusa Winter Almond Meeting; Organizer: UCCE; Granzella's Banquet Hall, Williams, CA
    d. Feb 5, 2019. Bloom and Foliar Diseases; Annual Almond Production Meeting; Organizer: UCCE; Norton Hall, Woodland, CA
    e. April 7-11, 2019. Characterization of Resistance to Five SDHI Sub-Groups in *Alternaria* Species Causing Leaf Spot of Almond in California; 19th International Reinhardsbrunn Symposium on Modern Fungicides and Antifungal Compounds; Friedrichroda, Germany
    f. Nov 8, 2019, Foliage, Blossom and Nut Diseases; Almond Short Course, Organizer UCCE; Visalia Convention Center, Visalia, CA

E. Materials and Methods:

a. **Fungicide evaluations for management of scab.** In-season treatments were inititated after petal fall (after the onset of twig sporulation) in a cvs. Monterey and Carmel orchards in Colusa Co. Fungicides were applied 5-8, 6-6, and 6-25-19 using an air-blast sprayer at 100 gal/A. For evaluation of disease on 8-20-19, 25-30 random fruit were collected from each tree and the number of lesions on each fruit was counted. Disease incidence was based on the number of fruit with scab lesions of total number of fruit evaluated.

b. **Fungicide evaluations for management of Alternaria leaf spot of almond.** The modified DSV model was used to determine initiation times of spray programs. Two trials were established in Colusa Co. on cvs. Carmel and Monterey. Treatments using an air-blast sprayer were done on 5-23, 6-12, and 7-3-19. For evaluation of disease on 8-27-19, a severity scale was used on 25-40 leaves of each single-tree replication: 0 (healthy), 1 (<33%), 2 (33-66%), and 4 (>66%) of leaf surface diseased.

c. **Molecular mechanisms of SDHI resistance in *Alternaria* spp. from almond.** Isolates of *Alternaria* spp. were obtained from infected almond leaves in six locations in northern and five locations in central California (5 to 20 isolates per location). Leaf tissues were plated using standard procedures. In vitro sensitivities were determined using the spiral gradient dilution (SGD) method as described previously (Forster et al., Phytopathology 94:163-70. 2004). Partial sequences of SDH-B, SDH-C, and SDH-D were obtained using primers previously published by others. For mutations identified, resistance phenotypes were graphically displayed in spider web diagrams with EC_{50} concentrations on a log_{10} scale using MS PowerPoint.

F. Publications that emerged from this work

1. Adaskaveg, J. E. , et al. 2017. UC IPM Pest Management Guidelines: Almond Diseases. UC ANR Publication 3431. https://www2.ipm.ucanr.edu/agriculture/almond/.
2. Adaskaveg, J. E., Gubler, D., and Michailides, T. 2017. Fungicides, bactericides, and biologicals for deciduous tree fruit, nut, strawberry, and vine crops. http://ipm.ucanr.edu/PDF/PMG/fungicideefficacytiming.pdf.
3. A peer-reviewed publication on the ‘Characterization of resistance to five SDHI sub-groups in *Alternaria* species causing leaf spot of almond in California’ based on the presention at the 19th International Reinhardsbrunn Symposium in Germany is in press.