2019 Final Report   **Project duration:** 3 years

Project Title: BIOLOGY AND CONTROL OF NEOFABRAEA LEAF AND TWIG LESIONS OF OIL OLIVES IN CALIFORNIA

Project leader: Florent Trouillas, Assistant C.E. Specialist, Dept. of Plant Pathology, UC Davis, flotrouillas@ucanr.edu

Location: Kearney Agricultural Research and Extension Center, Parlier, CA 93648

Cooperating personnel:
Renaud Travadon, Project Scientist, Dept. of Plant Pathology, UC Davis, rtravadon@ucdavis.edu
Mohamed Nouri, Farm advisor, UCCE San Joaquin County, mnouri@ucanr.edu
Daniel Lawrence, Project Scientist, Dept. of Plant Pathology, UC Davis, dlawrence@ucdavis.edu
Brenna Aegerter, Farm advisor, UCCE San Joaquin County, bjaegerter@ucanr.edu
Dani Lightle, Farm advisor, UCCE Glenn, Butte, Tehama Cos, dmlightle@ucanr.edu
Jim Adaskaveg, Professor of Plant Pathology, UC Riverside jim.adaskaveg@ucr.edu

Interpretive summary:
In the spring of 2016, the new disease of olive Neofabraea leaf and twig lesions was discovered in super-high-density (SDH) oil olive orchards in Glenn, San Joaquin, and Stanislaus Counties in California. The new disease was detected mainly in the Arbosana cultivar and to a lesser extent in the Arbequina cultivar. Arbosana was highly affected by the disease, whereas it was only sporadic in Arbequina and not found in Koroneiki. Two species, namely *Neofabraea kienholzii* and *Phlyctema vagabunda*, were found to be consistently associated with the disease. Species identity was confirmed by morphology and molecular data. Koch's postulates to determine the pathogenicity of these species in olive were completed. Pathogenicity studies determined also that wounds caused by mechanical harvesters are required for infection of leaves and twigs by *Neofabraea* and *Phlyctema* pathogens. Five field experiments were conducted during the 2016-17 and 2017-18 fall-winter seasons to evaluate the efficacy of various fungicides to control Neofabraea leaf and twig lesions. Field trials were conducted in the highly susceptible cultivar Arbosana in a commercial orchard in San Joaquin County and compared the efficacy of up to 10 fungicidal products and different application regimes (single versus two applications) to control this new disease. Results showed that several products were effective in reducing infection by the pathogens and limiting disease incidence. Overall, best disease control was achieved by Topsin M, Vanguard, Inspire Super, Bravo and Ziram fungicides, which provided up to 75% reduction in disease incidence. Copper fungicides did not control the disease. In 2018, Inspire Super (difenoconazole/cyprodinil – group 3+9) and Ziram (ziram – group M3) received support by the registrant and were included to IR-4 projects. Thus, during the fall-winter 2018-2019, we evaluated different programs and application regimes using Ziram and Inspire Super. Results showed that both products provided significant disease reduction although no difference in efficacy was found between these two products. Also, both products performed equally using one or two

applications (at two-weeks interval) after harvest. Several wound susceptibility trials were conducted also to determine the duration (0, 1, 2, 3, 4 or 5 weeks) after harvest when wounds on leaves remain susceptible to infection, and thus determine the number and timing of fungicide applications required to control Neofabraea and Phlyctema diseases. Results showed that leaves inoculated immediately after wounding and up to three weeks after wounding were most susceptible to infection. Overall, leaf wound susceptibility declined substantially after 4 weeks following wounding. This suggested that wounds had healed after 4 weeks following wounding. Overall, results suggested that one fungicide application after harvest followed by a second application 2 to 3 weeks later should suffice to protect olive trees from infection. The first fungicide application should be made immediately after harvest for best protection of olive trees. A second fungicide application two weeks after harvest may be required when rain persists or during years of heavy rainfall resulting in higher disease pressure. Finally, this work indicated that risks of infection of wounded leaves and twigs after four weeks following harvest (wounding) is low and that fungicide treatments are no longer required after this time period. Inspire Super and Ziram fungicides are currently being considered for registration as part of the IR-4 program. The availability of these two fungicides in olive will improve control of Neofabraea and Phlyctema leaf and shoot lesions and will allow for management of fungicide resistance by rotating modes of action.

1- Symptoms and geographic distribution:

Neofabraea leaf and twig lesions were found mainly in the 'Arbosana' cultivar and to a lesser extent in the 'Arbequina' cultivar in Super High Density (SHD) olive orchards in Glenn, San Joaquin, and Stanislaus Counties. Symptoms were most visible during the early spring in California (March/April). Lesions on leaves were necrotic, circular to elongated and usually occurred singly, ranging from 0.5 to 1 cm in diameter (**Fig. 1A, top row,** and **B**). Leaf lesions occurred at sites of injuries caused by mechanical harvesters and included abrasion sites where leaves rub against each other. Leaf lesions caused by *N. kienholzii* and *P. vagabunda* were similar but differed clearly from peacock spot lesions, as the former were necrotic, lacked the dark green halo typically seen around peacock spot (**Fig. 1A, bottom row**), and in general did not number more than one lesion per leaf (**Fig. 1A,** top row). Reddish-brown lesions on shoots and twigs and occasionally cankers in branches developed at wounds caused by mechanical harvesters (**Fig. 1C, D** and **E**). Cankers in branches appeared as sunken lesions in the bark that elongated from the site of injury. The disease occasionally caused fruit spots in the 'Arbequina' cultivar that were visible in December on unharvested fruits remaining on trees (**Fig. 1F**). In severely affected orchards, 'Arbosana' trees showed defoliation (**Fig. 1G**). Surveys to determine the host range of *Neofabraea kienholzii* and *P. vagabunda* failed to identify additional host plants for these fungi in California. Several studies have suggested that *P. vagabunda* can survive on dead bark tissues of apple, pear, and olive trees serving as inoculum for new infections (Verkley 1999). In Michigan, this fungus has been reported to cause the coin canker disease of ash (Rossman et al. 2002). Ash trees in riparian areas as well as apple trees near olive orchards were surveyed for this study, but the olive pathogens were not detected from these host plants.

Fig. 1. Symptoms on olive caused by *Neofabraea kienholzii* and *Phlyctema vagabunda* in California. **A,** Leaf lesions of *N. kienholzii and P. vagabunda* (top row) versus peacock spot symptoms on leaves (bottom row), caused by *Fusicladium oleagineum*. **B,** Leaf lesion of *N. kienholzii.* **C, D** and **E**, Shoot lesions of *N. kienholzii* and *P. vagabunda*. F, Fruit spots in 'Arbequina' olives associated with *P. vagabunda*. **G,** Tree defoliation in a row of 'Arbosana' olives (left) heavily affected by *N. kienholzii* and *P. vagabunda* leaf and shoot lesions in comparison with a row of healthy 'Koroneiki' cultivar (right).

2- Disease causal agents:

The identity of this new disease's causal agents was determine using PCR amplification of the ITS region, LSU, and TUB2 genes and subsequent phylogenetic analyses. Analyses identified *Neofabraea keinholzii* and *Phlyctema vagabunda* as the main two fungal pathogens associated with leaf and shoot lesions of olive. Analyses of the combined three-locus dataset revealed that 67 Californian isolates clustered strongly (100%/100%) with the ex-type of *N. kienholzii* (CBS 126461) and 42 other Californian isolates clustered strongly (100%/100%) with the ex-type of *P. vagabunda* (CBS 304.62) **(Fig. 2).**
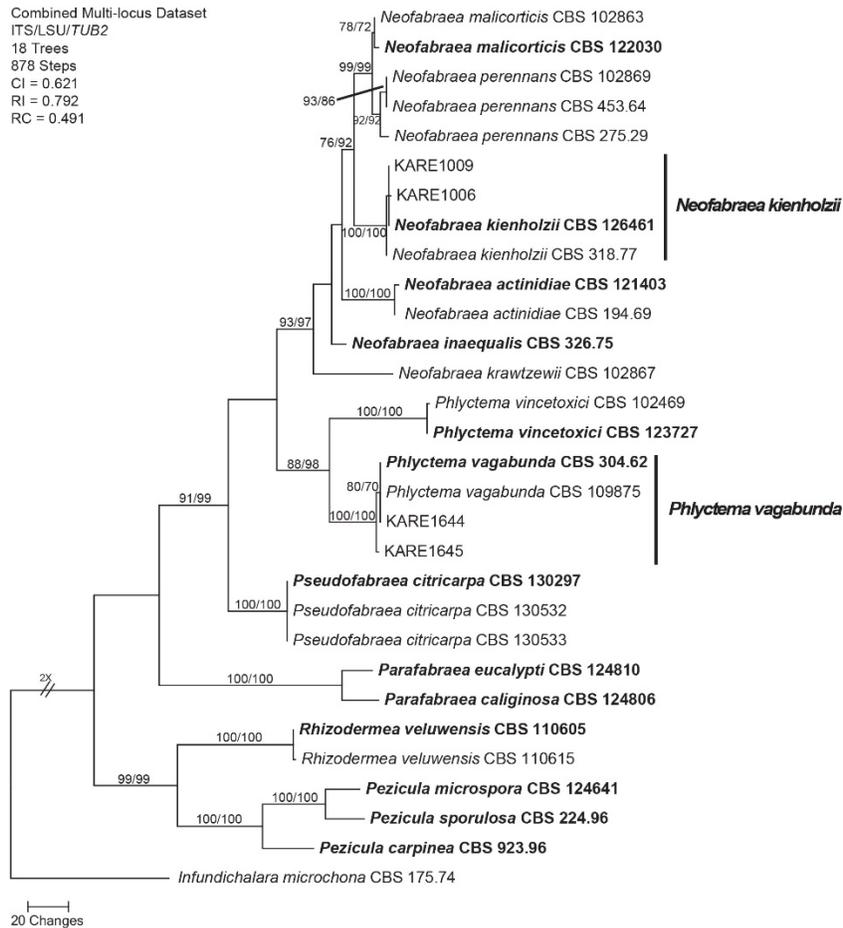
Fig. 2. One of 18 equally most parsimonious trees generated from maximum parsimony analysis of the three-gene (ITS+LSU+*TUB2*) combined dataset. Ex-type isolates are indicated in bold.

Morphological characterization and examination of these fungal pathogens fit the description of *N. kienholzii* and *P. vagabunda* based on morphological characteristics. On PDA medium, *P. vagabunda* isolates were characterized by mostly round, even colonies with radial furrows and scarce aerial mycelium reaching 38 mm in 23 days. *Phlyctema vagabunda* colonies were white initially, turning pinkish in their center and brown in their periphery with time, and with compact, hyaline, and septate mycelium **(Fig. 3A).** Conidiomata acervuloid to sporodochial producing conidia that were unicellular, hyaline, aseptate, fusiform to allantoid, with rounded and curved ends and averaged 21.6 ± 0.4 μm 3.7 ± 0.1 μm with a length-width ratio of 5.82 **(Fig. 3B).** *Phlyctema vagabunda* conidiomata also occurred in naturally infected leaves in the field **(Fig. 3C).**

Neofabraea kienholzii isolates produced 35 mm diameter colonies with white to brownish mycelium after incubation on PDA medium after 23 days. Colonies on PDA expanded unevenly giving the colony a slightly torn appearance with submerged, brownish peripheral hyphae and copious white aerial hyphae near the center **(Fig. 3D).** Conidia were aseptate, ellipsoidal, slightly asymmetrical to slightly curved with sizes averaging 10.4 ±

0.1 μm × 3.6 ± 0.1 μm and a length-width ratio of 2.85 **(Fig. 3E and F).** Naturally infected olive leaves produced many small, acervuloid to sporodochial conidiomata **(Fig. 3F)**.

Fig. 3. Asexual morphologies of *Phlyctema vagabunda* (A, B, C) and *Neofabraea kienholzii* (D, E, F). **A,** Colony of *P. vagabunda* cultured on PDA medium for 24 days at the laboratory's ambient conditions (24°C). **B,** Conidia of *P. vagabunda*. **C,** Conidiomata of *P. vagabunda* produced on a necrotic leaf lesion. **D,** Colony of *N. kienholzii* cultured on PDA medium for 24 days at the laboratory's ambient conditions (24°C). **E,** Conidia of *N. kienholzii*. **F,** Conidiomata of *N. kienholzii* produced on a necrotic leaf lesion. Scale bar in B and E = 10 μm; in C and F = 1 mm.

The optimal growth temperature was around 20°C for all three isolates of *N. kienholzii* tested and around 15°C for those of *P. vagabunda* **(Fig. 4A and 4B).** Mycelial growth rate averaged 2.53 mm per day for *N. kienholzii* and 3.02 mm per day for *P. vagabunda* cultured on TA medium for 21 days.
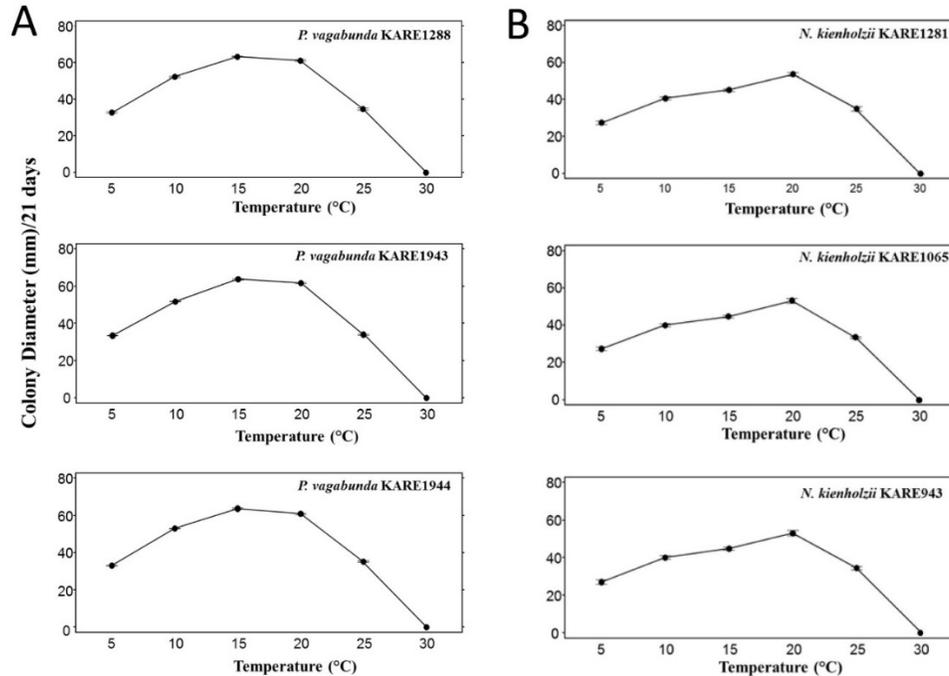
Fig. 4. Effect of temperature on the mycelial growth of three isolates of *Phlyctema vagabunda* (KARE1288, KARE1943, and KARE1944) (left) and *Neofabraea kienholzii* (KARE943, KARE1065, and KARE1281) (right) cultured on Tomato Agar for 21 days in the dark.

3- Pathogenicity of *Neofabraea kienholzii* and *Phlyctema vagabunda* in the main oil olive cultivars:

Pathogenicity assays were conducted on olive leaves, shoots and fruits. The inoculum consisted of a spore suspension (conidia) of *Neofabraea kienholzii* and *Phlyctema vagabunda.* Pathogenicity studies on olive leaves were conducted in November 2016 and 2017, respectively, to determine the ability of the newly reported fungi to cause lesions. The experiments were conducted in a lath-house at the University of California, Davis on 2 to 3-year-old potted olive saplings of the Arbosana, Arbequina and Koroneiki cultivars were used for these assays. Leaves were inoculated with a conidial suspension of *N. kienholzii* and *P. vagabunda* following artificial wounding that mimicked wounds caused by mechanical harvesters. Experiments were harvested after 3 months in February 2017 and February 2018, respectively. Leaves were inspected for the development of necrotic lesions and taken to the laboratory for lesion measurement as well as attempts to recover the inoculated fungal pathogens from the symptomatic tissues.

The ability of the various fungi to produce lesions in olive shoots was determined also in mature olive trees growing in the field. The Arbosana, Arbequina and Koroneiki cultivars were used for this experiment. Pathogenicity of various isolates was tested following inoculations of shoots with mycelium of *N. kienholzii* and *P. vagabunda* in November 2016 and 2017, respectively. Inoculations of shoots were conducted following artificial wounding that mimicked wounds caused by mechanical harvesters. The 2017 assay included shoots that were wounded and inoculated with a conidial suspension of *N. kienholzii* and *P. vagabunda*. Both assays used 1, 1- to 2-year-old shoots on each of 10, 9-year-old olive trees. Inoculated and mock-inoculated branches were collected after three

months and the length of lesions was measured above and below the point of inoculation for each shoot.

Pathogenicity studies were conducted to determine the ability of *N. kienholzii* and *P. vagabunda* to cause disease in fruits of 'Arbequina', 'Arbosana' and 'Koroneiki' cultivars. Olive fruit pathogenicity studies were conducted in the laboratory using detached fruits obtained from the field. Apparently healthy fruits were submerged for 3 min in 0.5% sodium hypochlorite solution, then rinsed twice in sterile distilled water, and air-dried on sterile paper towels. The experiment was conducted on fruits wounded (via puncture) with a needle and inoculated with a 10 μl drop of a conidial suspension of *N. kienholzii* and *P. vagabunda*. Inoculated olives were placed in moist chambers and incubated at 24°C. Inoculated and control fruits were assessed for fruit rot development after 14 days. The experiment was conducted twice using two different isolates for each fungal species.

In the <u>pathogenicity assays on leaves</u>, all cultivars tested appeared susceptible to both pathogens. In 2016, all isolates were pathogenic on leaves of the three cultivars and caused leaf lesions identical to the ones observed in the field. There were no differences in lesion sizes on the leaves of the 3 olive cultivars inoculated with two isolates of *N. kienholzii* ($P = 0.43$). In contrast, there were differences in cultivar susceptibility to isolates of *P. vagabunda*, with 'Arbosana' being more resistant than 'Arbequina' and 'Koroneiki'; the latter two showed similar susceptibility to the pathogen (**Fig. 5**).

In 2017, all isolates tested caused necrotic lesions on leaves. No symptoms were produced in the mock-inoculated control plants. Lesion size produced by isolates belonging to the same species did not differ significantly between isolates ($P < 0.05$). Accordingly, lesion diameter data were combined for isolates of the same species. Average lesion size after inoculations was 6.93 mm for *P. vagabunda* isolates and 6.66 mm for *N. kienholzii* isolates, respectively. According to ANOVA, there was a significant effect of the cultivar ($P < 0.00001$) on lesion diameter but no significant difference in aggressiveness was detected between the two fungal species ($P = 0.6383$). Arbosana was the most susceptible cultivar, with lesions averaging 7.2 mm in diameter, while Arbequina (4.9 mm) was the most resistant cultivar (**Fig. 6**). Inoculation studies confirmed also that wounds were required for the pathogens to successfully infect and cause lesions in leaves of olive.

In the <u>pathogenicity assays on shoots</u>, both 2016 and 2017 inoculations produced reddish-brown lesions in shoots that extended from the inoculation point and that were similar to those occurring in naturally infected twigs. In 2016, average lesion size produced across all cultivars and fungal pathogens was 8.5 ± 0.9 mm. There was significant ($P = 0.0203$) differences in susceptibility to the pathogens among olive cultivars with Arbosana being the most susceptible cultivar (10.83 mm average lesion size) and Koroneiki the most resistant (4.66 mm average lesion size). Arbequina showed an intermediate tolerance resistance level (5.6 mm average lesion size). In 2017, average lesion size produced across all cultivars and fungal isolates was 36 ± 5.4 mm. There were significant effects of the olive cultivar ($P <= 0.00108$) and fungal treatments ($P < 0.0001$) but no effect was detected for the interaction cultivar-isolate ($P = 0.3165$). According to these results, Koroneiki (39.66 mm average lesion size) was significantly ($P < 0.05$) more susceptible than Arbosana (35.16 mm average lesion size) and Arbequina (34.62 mm average lesion size), which did not differ significantly between them (**Fig. 7**).

In the pathogenicity assays on fruits, all fungal isolates tested produced circular rot lesions extending from the inoculation points. Control fruits (mock-inoculated) did not show disease symptoms, i.e. we did not detect latent infection of the pathogen. There were significant effects on the lesion size of the fungal species ($P = 0.00103$) and olive cultivar ($P = 0.0395$) but no interaction was found between species-cultivar ($P = 0.42548$). In this assay, *N. kienholzii* was significantly more virulent than *P. vagabunda* in the three evaluated olive cultivars. Also, the cultivar Koroneiki (6.9 mm average lesion size) was significantly more susceptible than Arbequina (5.8 mm average lesion size), while Arbosana (6.3 mm average lesion size) showed an intermediate susceptibility **(Fig. 8)**.
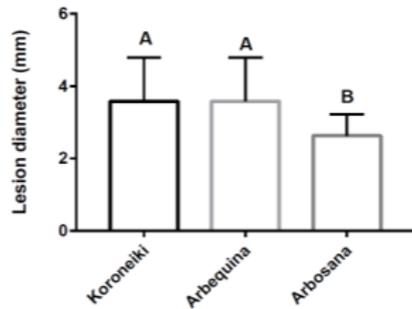
Figure 5. Lesion diameters caused by isolates of *Phlyctema vagabunda* inoculated onto leaves of three olive cultivars in 2016 in the lath-house. Data are means and standard errors (10 replicates). Data denoted by distinct letters are significantly different (Tukey's test; P < 0.05).

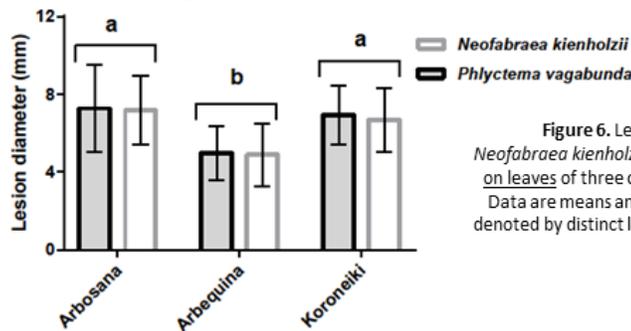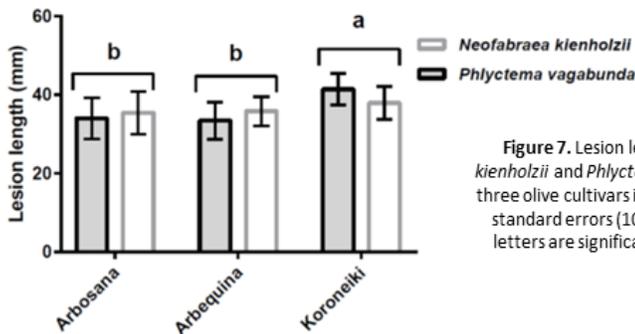**Figure 6.** Lesion diameters caused by isolates of *Neofabraea kienholzii* and *Phlyctema vagabunda* inoculated on leaves of three olive cultivars in 2017 in the lath-house. Data are means and standard errors (10 replicates). Data denoted by distinct letters are significantly different (Tukey's test; P < 0.05).
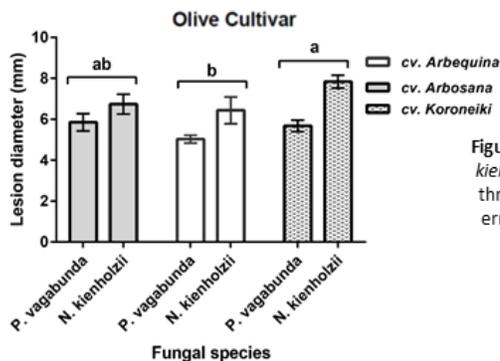
Figure 7. Lesion lengths caused by isolates of *Neofabraea kienholzii* and *Phlyctema vagabunda* inoculated on shoots of three olive cultivars in 2017 in the field. Data are means and standard errors (10 replicates). Data denoted by distinct letters are significantly different (Tukey's test; P < 0.05).

Figure 8. Lesion diameters caused by isolates of *Neofabraea kienholzii* and *Phlyctema vagabunda* inoculated on fruits of three olive cultivars in 2017. Data are means and standard errors (10 replicates). Data denoted by distinct letters are significantly different (Tukey's test; P < 0.05).

4- Management of Neofabraea and Phlyctema leaf and shoot lesions.

Fungicide efficacy

Fungicides in different FRAC groups (different modes of action) were screened in the field to determine their efficacy against *Neofabraea* and *Phlyctema* pathogens. Two trials were initiated each year during the fall of 2016, 2017 and 2018 in a super-high-density olive orchard near Walnut Grove, CA. Experimental units consisted of two adjacent trees arranged in each of four block replicates using a randomized complete block design. In 2016 and 2017, Trial 1 tested a single spray application after harvest (T1). Trial 2 tested two spray applications: one after harvest (T1) and a second application approximately 3 to 5 weeks later (T2). Eight products were tested during the fall and winter **2016-2017 and 2017-2018**:

- Topsin M (thiophanate-methyl – group 1) - **1.5 lbs/A**
- Inspire Super (difenoconazole/cyprodinil – group 3+9) - **20 fl oz/A**
- Kocide 3000 (copper hydroxide) - **7 lbs/A**
- Tebucon (tebuconazole – group 3) - **8 fl oz/A**
- Rhyme (flutriafol – group M3) - **7 fl oz/A**
- Vanguard WG (cyprodinil – group 9) **- 10 oz/A**
- Ziram (ziram – group M3) - **6 lbs/A**
- Bravo (chlorothalonil – group M5) - **6 lbs/A**

Following the 2016-2017 and 2017-2018 field trials, Inspire Super (difenoconazole/cyprodinil – group 3+9) and Ziram (ziram – group M3) received support by the registrant and were included to IR-4 projects. Thus, during the fall-winter 2018-2019, we further evaluated the efficacy of Inspire Super and Ziram in comparison to Kocide 3000 (copper hydroxide) and a water-only treated control. Combinations of 1 (**T1** = after harvest) and 2 (**T1** = after harvest + **T2** = 2-weeks after harvest) applications of these fungicides were compared. Ziram was used at 6 lbs/A, Inspire Super at 20 fl oz/A and Kocide 3000 at 7 lbs/A. The various treatment combinations were as follows:

Trt. #	Treatment	Assigned Flag
1	Ziram T1	Black (B)
2	Inspire Super T1	Pink (P)
3	Kocide 3000 T1	Yellow (Y)
4	Inspire Super T1 + T2	Orange (O)
5	Ziram T1 + T2	White Red Dots (RD)
6	Inspire Super T1 + Ziram T2	White Blue Stripe (BS)
7	Ziram T1 + Inspire Super T2	Green (G)
8	Control	White (W)

Two trials were conducted and in all experiments, infection relied on natural inoculum. The amount of leaf infections (number of leaf spots) was evaluated 4 months after fungicidal applications for each fungicide tested. Treatments that resulted in the lowest amount of leaf spots were considered as most effective against Neofabraea and Phlyctema diseases.

Results of 2017-2018 field experiments are presented in **Figs. 9** and **10.** Trial 1 (single application at harvest) confirmed the efficacy of most products tested in reducing leaf lesion development, except for Kocide 3000. Topsin M and Vanguard were the most efficient products providing up to 70% disease reduction compared to the water-only treated control **(Fig. 9)**. Results of Trial 2 (two spray applications, 6 weeks apart) confirmed Topsin M and Vanguard as the most efficient products **(Fig. 10)**. Ziram and Inspire Super also provided significant disease reduction. Vanguard and Bravo provided satisfactory disease control.

In 2018-2019 field experiments (Trials 1 and 2), Ziram and Inspire Super provided 50% disease reduction on average when compared to the water-only treated control. Kocide 3000 did not reduce disease incidence. Also, no significant difference of efficacy was found between Ziram and Inspire Super fungicides **(Figs. 11 and 12)**. Similarly, no significant difference was found between one (**T1** = after harvest) and two applications (**T1** = after harvest + **T2** = 2-weeks after harvest) of these fungicides as well as between the different combination of fungicide application (**T1** = Ziram + **T2** = Inspire Super and **T1** = Inspire Super and **T2** = Ziram).

Fungicide registration

Submission of ziram (Ziram 76WDG) and difenoconazole/cyprodinil (Inspire Super) fungicides to the IR-4 program were initiated in 2018 in coordination with Dr. Jim Adaskaveg's work on the management of peacock spot (cf. Peacock Spot Research Annual Report 2019). These fungicides have different modes of action, have low resistance potential, and are efficacious against Neofabraea and Phlyctema twig and leaf lesions as well as peacock spot. Ziram is a FRAC Code M3 whereas Inspire Super is a FRAC Code 3/9. Thus, integration of multi-site modes of action for both products was also established as an effective anti-resistance strategy. Additionally, registrants of each fungicide were contacted for approval for the proposed usage on olive and proposed labels were prepared. Subsequently, an IR-4 nomination was made based on the proposed usage (rates, timing, etc.) and IPM compatibility. Dr. Jim Adaskaveg traveled to the IR-4 Food Use Workshop in St. Louis, MO, in September 2018 to defend theses nominations. Ziram and Inspire Super were approved for residue trials at the National Food Use Workshop. Strong support was provided based on the after-harvest and winter season usage with expected zero to limit-of-detection residues on the crop in the following harvest season. In 2018, olive fruits from our fungicide trials were submitted for pesticide residue analysis to Environmental Micro Analysis, Inc., Woodland, California. Results revealed that ziram and difenoconazole/cyprodinil residues were non-detectable from olive fruits collected in July 2018 from trees previously treated with one application (Nov 2017 = after harvest) and two application (Nov 2017 and Dec 2017) of these fungicides (Trial 1 and 2).
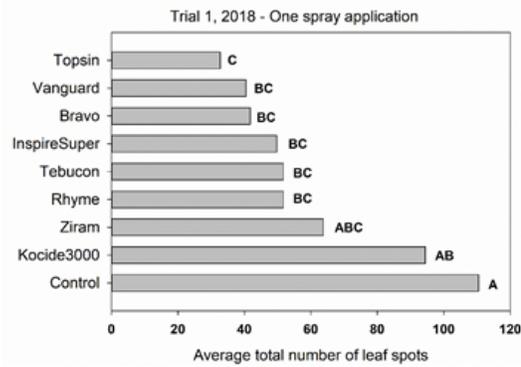
Figure 9. 2017/2018 - Trial 1, single spray application: Average number of leaf lesions per olive tree according to various fungicide treatments and compared to the water treatment a copper treatment (Kocide 3000).
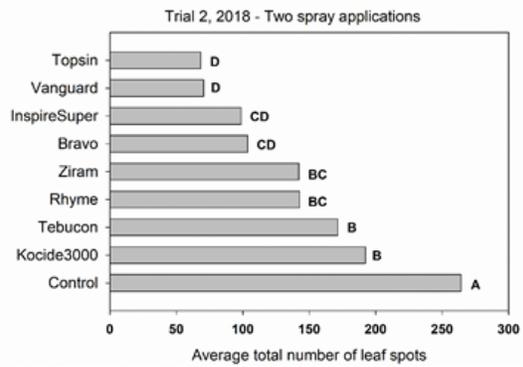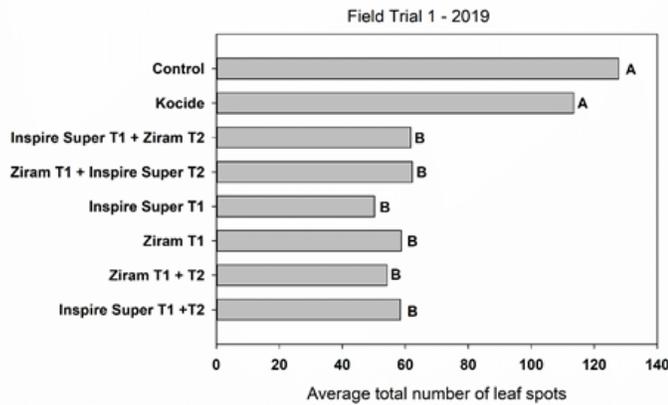
Figure 10. 2017/2018 - Trial 2, two spray applications: Average number of leaf lesions per olive tree according to various fungicide treatments and compared to the water treatment a copper treatment (Kocide 3000).

Figure 11. 2018/2019 - Trial 1, single spray and two-spray application comparison using Inspire Super, Ziram and Kocide fungicides, and a water treatment (control): Columns represent the average number of leaf lesions per olive tree for each treatment.
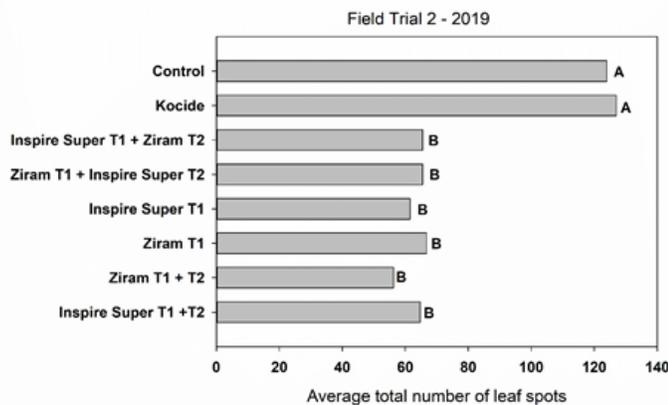

Figure 12. 2018/2019 - Trial 2, single spray and two-spray application comparison using Inspire Super, Ziram and Kocide fungicides, and a water treatment (control): Columns represent the average number of leaf lesions per olive tree for each treatment.

Duration of wound susceptibility and timing of fungicide applications

Trials were set up in November 2016-17 and 2017-18 to estimate the duration of wound susceptibility in leaves wounded during mechanical harvest. Knowledge of the duration of wound susceptibility (host susceptibility) to infection by *Neofabraea* and *Phlyctema* pathogens should help determine the number of fungicide applications required to protect olive trees following harvest. Trials included one assay conducted in a shade house in

Davis, CA (2016-2017), a field assay conducted in an orchard near Walnut Grove, CA (2017-2018) as well as an additional assay conducted in a in Davis, CA (2018-2019). For these studies, olive leaves were manually wounded with scissors in November to mimic wounds caused by mechanical harvesters. Wounded leaf subsets were then inoculated either directly after wounding or after 1, 2, 3, 4, or 5 weeks following wounding. Individual wounds on leaves were inoculated with 20 µL of a 1×10^5 spores mL-1 spore suspension of *Neofabraea kienholzii,* the most common pathogen of this complex found in olive. Each treatment was replicated 10 times. At the end of the experiment, leaves were collected and brought to the laboratory to proceed with fungal isolations, assess the percent fungal recovery and determine the susceptibility of wounded leaves according to the timing of infection following wounding in November (at harvest).

Results of the 2017/2018 leaf wound susceptibility study revealed that wounded leaves were most susceptible to infection by *Neofabraea kienholzii* during the first three weeks following wounding (**Figs. 13** and **14**). At four weeks following wounding, wound susceptibility in leaves substantially decline, suggesting leaves had healed after four weeks (**Figs. 13** and **14**).
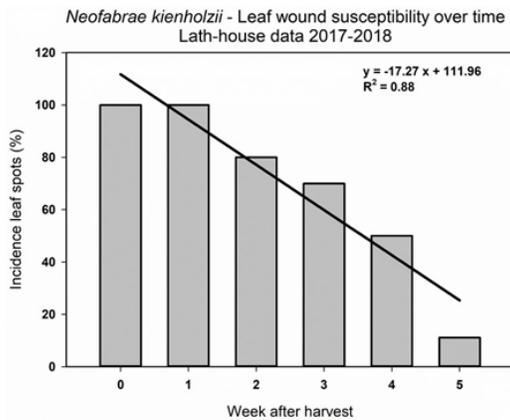

Figure 13. 2017/2018 – Lath house experiment, Davis, CA. Leaf wound susceptibility over time following a unique wounding event in November. Leaf wound susceptibility decreases with wound age, with 4 and 5-week-old wounds being less susceptible to infection by *Neofabraea* pathogen.
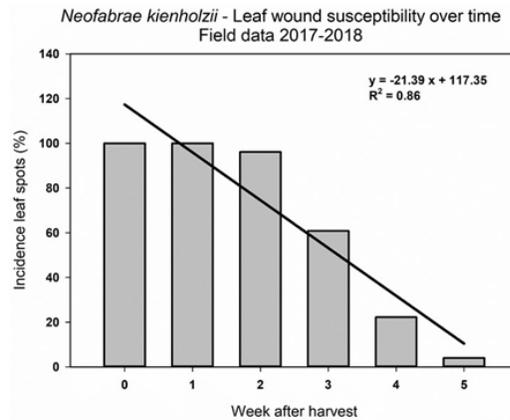
Figure 14. 2017/2018 – Field experiment, Walnut Grove, CA. Leaf wound susceptibility over time following a unique wounding event during harvest in November. Leaf wound susceptibility decreases with wound age, with 4 and 5-week-old wounds being less susceptible to infection by *Neofabraea* pathogen.

Trials conducted in a shad house at Davis during the fall-winter (2018/2019) indicated reduced levels of wound susceptibility in leaves and shoots to *Neofabraea* and *Phlyctema* pathogens as early as after two weeks following wounding (**Fig. 15).**

Overall, results suggested that one fungicide application after harvest followed by a second application 2 to 3 weeks later should suffice to protect olive trees from infection. A first fungicide application should be made immediately after harvest for best protection of olive trees. Neofabraea leaf and shoot lesions only developed at wounds caused by mechanical harvesters and an immediate protection is required to avoid risk of infections. A second fungicide application two weeks after harvest may be required when rain persists or during years of heavy rainfall resulting in higher disease pressure. Finally, this work indicated that risks of infection of wounded leaves and twigs after four weeks following harvest (wounding) is low and that fungicide treatments are no longer required after this time period.
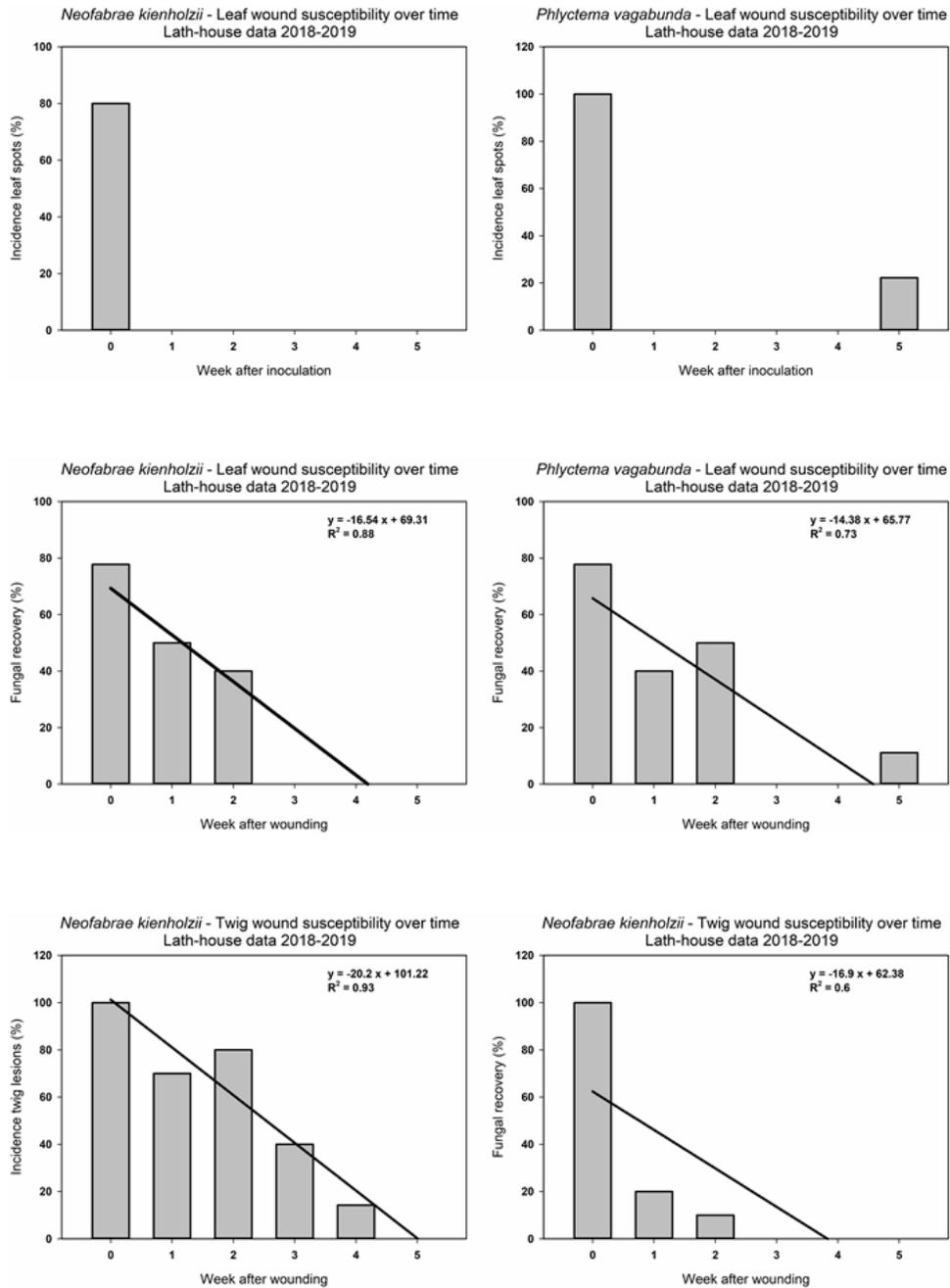
Figure 15. 2018/2019 – Lath house experiments, Davis, CA. Leaf and twig wound susceptibility over time following a unique wounding event in November. Leaf and twig wound susceptibility decreases with wound age, with 3, 4 and 5-week-old wounds being less susceptible to infection by *Neofabraea* and *Phlyctema* pathogens.

Acknowledgments