Annual Report: 2017

Project Title: BIOLOGY AND CONTROL OF NEOFABRAEA LEAF SPOT, BRANCH CANKER AND TWIG DIEBACK OF OIL OLIVES IN CALIFORNIA

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Interpretive summary:
In the spring of 2016, a new disease of olive, namely Neofabraea leaf and fruit spot, branch canker and twig lesion was discovered in super-high-density oil olive orchards in San Joaquin and Glenn Counties, California. Symptoms were characterized by large necrotic spots on leaves, reddish lesions on twigs and cankers on branches developing at wounds. Infections resulted in severe defoliation and fruit loss mainly in the Arbosana cultivar, which appeared highly susceptible. Fruit spots were detected occasionally in Arbequina olives near harvest time. This disease, also known as lepra fruit rot of olive, has been of increasing concern in recent years in Italy, Spain and Portugal. The emergence of this new disease in California olives has been a major concern to oil olive farmers and represents a serious threat to the industry. The present project was initiated to investigate various aspects of this new disease biology, including disease etiology and epidemiology, pathogen biology, host susceptibility as well as strategies to control the disease and limit its spread.

During 2016 and 2017, we conducted surveys of olive orchards in California. Two hundred fungal isolates were isolated from symptomatic olive trees and characterized for this study. Fungal pathogens associated with the disease were identified as *Neofabraea kienholzii* and *Phlyctema vagabunda* (syn: *Neofabraea vagabunda*) using morphological and molecular studies. The pathogenicity of *N. kienholzii* and *P. vagabunda* in olives was confirmed through pathogenicity studies. Both species produced lesions in leaves of Arbosana, Arbequina and Koroneiki cultivars following wounding and inoculation of spore suspensions. Wounding appeared critical for infection of leaves. Both species were shown to cause lesions also in wounded twigs of Arbosana whereas twigs of Arbequina and Koroneiki cultivars appeared resistant to the disease.

The disease was shown to be highly correlated with mechanical harvest, where wounds produced by harvesters on leaves, twigs and branches serve as the main infection sites for the pathogens. Infection occurs during rain events following harvest where spores of the pathogens are released and disseminated to fresh wounds. Main inoculum sources include infected leaves and rotten fruits within the olive orchards. Defoliation of affected trees initiates in the late winter and occur until the first spring months. New leaves produced on trees in the spring are not susceptible to infection in the absence of wounding events.

Last year, we conducted three fungicide trials to evaluate the efficacy of various fungicides to control Neofabraea leaf spot and twig lesion. Trials were conducted in the highly susceptible cultivar Arbosana in a commercial orchard in San Joaquin County. Experiments compared 10 fungicides and 2 spray programs. Results showed that several products were effective in reducing infection by the pathogens and limiting disease incidence. Best products included Luna Experience, Luna Sensation, Ziram, Topsin M and Tebucon. One fungicide application following harvest may suffice to control the disease. Copper (Kocide 3000) did not control the disease.

Objective 1: Establish the current distribution of Neofabraea disease in California

Surveys of table and oil olive orchards were conducted in Kings, Contra Costa, San Joaquin, Glenn and Colusa Counties. Olive trees were inspected for the presence of Neofabraea leaf and fruit spots, twig dieback and branch cankers. Diseased plant parts were taken to the laboratory to proceed with isolation and determine what pathogens might be present. To date, the disease was identified mainly from the Arbosana cultivar in orchards located in San Joaquin and Glenn Counties. The disease appeared to be limited to super-high-density oil olive orchards although a previous study in California reported the pathogens from fruit spots in table olive in Sonoma County (Rooney-Latham et al., 2012). Neofabraea fruit spots were detected sporadically in Arbequina olives near harvest time in an orchard in San Joaquin County **(Fig. 1)**. Main symptoms associated with the disease included spots on leaves, reddish lesions on twigs and cankers on branches developing at wounds **(Fig. 1)**. Lesions on leaves were circular to elongate and usually occurred as a single spot per leaf ranging from 0.5 to 1 cm in diameter **(Fig. 1)**. Twigs lesions and branches cankers developed at wounds caused by mechanical harvesters. Cankers in branches appeared as sunken lesions on the bark, reddish-brown in color and usually circular or elongate **(Fig. 1)**. Severe defoliation and fruit loss occurred eventually in this highly susceptible Arbosana cultivar.

Figure 1: Symptoms of Neofabraea leaf and fruit spot, branch canker and twig lesion

Objective 2: Identify and characterize the pathogens

Following isolation work, fungal colonies developing consistently from the culture medium were isolated into pure cultures. Identification of fungal pathogens was carried out using morphology and complemented using DNA based techniques including the polymerase chain reaction (PCR), amplification and sequencing of the internal transcribed spacer region (ITS) of the rDNA. Species identification was completed using the nucleotide query algorithms BLAST in GenBank and by mean of phylogenetic analyses. Fungal pathogens associated with the Neofabraea disease of oil olive were identified as *Neofabraea kienholzii* and *Phlyctema vagabunda* (syn: *Neofabraea vagabunda*) using morphological and molecular studies **(Fig. 2)**.

The genus *Neofabraea* includes several important fungal pathogens of various woody crops worldwide. *Neofabraea* species are responsible for the bull's eye rot disease of apple and pear fruits in the Pacific Northwest (Henriquez et al., 2004) as well as the coin canker of ash in the northern United States and Canada (Rossman et al., 2002). A recent study in California reported *Neofabraea alba* causing a fruit spot on Coratina and Picholine olives in two commercial orchards in Sonoma County (Rooney-Latham et al., 2012). *Phlyctema vagabunda* has been identified as the causal agent of lepra fruit rot of olive in earlier reports from Italy and Spain (Petri, 1915; Roca et al., 2004). Additional reports on olive have included *Neofabraea vagabunda* causing branch cankers and twig dieback in Arbequina and Picual cultivars in Spain (Romero et al., 2016).

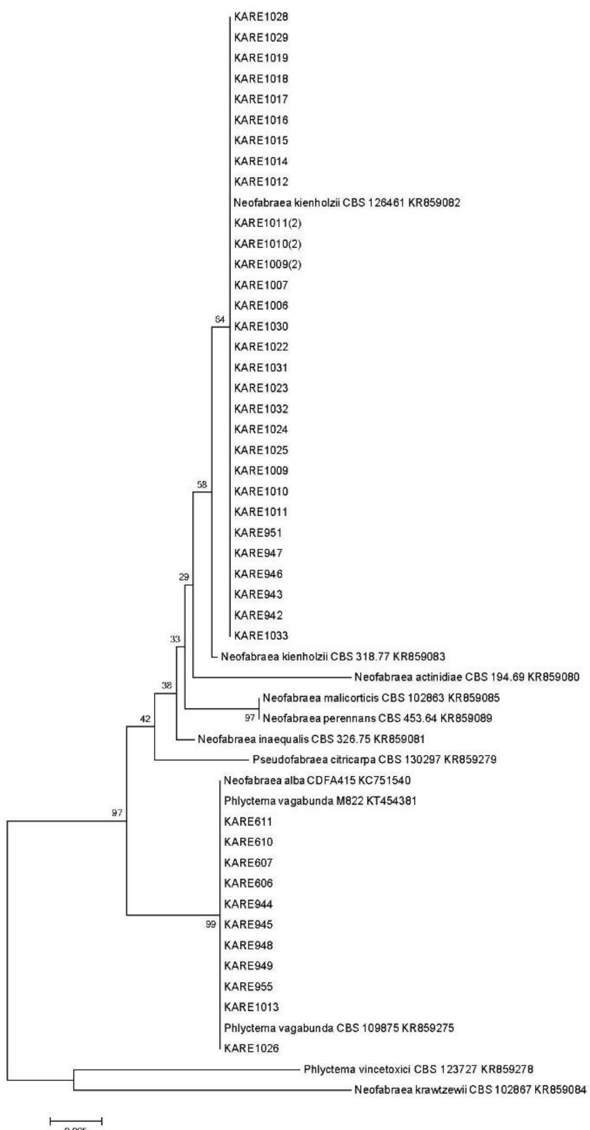

Figure 2: Phylogenetic analysis of the ITS rDNA region revealing 2 fungal species associated with Neofabraea disease of oil olive: *Neofabraea kienholzii* and *Phlyctema vagabunda*.

Objective 3: Determine the pathogenicity of *Neofabraea* fungi in the main oil olive cultivars

Pathogenicity studies were conducted in a lath house at Davis and in the field to determine the pathogenicity (ability to cause disease) of the various fungal species identified from diseased olive trees. Pathogenicity of various isolates was tested in twigs as well as on leaves in November 2017 in an attempt to reproduce the disease symptoms. Inoculation of twigs and branches was conducted following artificial wounding that mimicked wounds caused by mechanical harvesters. Pathogenicity studies on leaves were conducted following wounding of leaves using a sterile needle as well as without wounding. Inoculum of the various pathogens was produced in the laboratory on petri dishes filled with oatmeal agar, inoculated with the pathogens and incubated for 30-40 days at 24° C in the dark. Spore suspensions were adjusted to a final concentration of 100 spores per microliter of sterile water and 20 µl of the spore suspension was inoculated onto freshly wounded or unwounded tree parts (leaves, twigs and branches). The main cultivars tested against *N. kienholzii* and *P. vagabunda* included Arbosana, Arbequina and Koroneiki. All experiments were conducted using 10 replicates per treatment in a randomized complete block design.

In the leaves pathogenicity assay, all cultivars tested appeared susceptible to both pathogens **(Figs. 3a, 3b, 3c)**, however, wounding was required for infection to occur and for symptoms to develop. Leaves that did not received the needle wounding did not develop lesions. In the pathogenicity assay in twigs, only the Arbosana cultivar was susceptible to the disease and wounds were required for infection and disease to occur **(Figs. 4a, 4b)**.

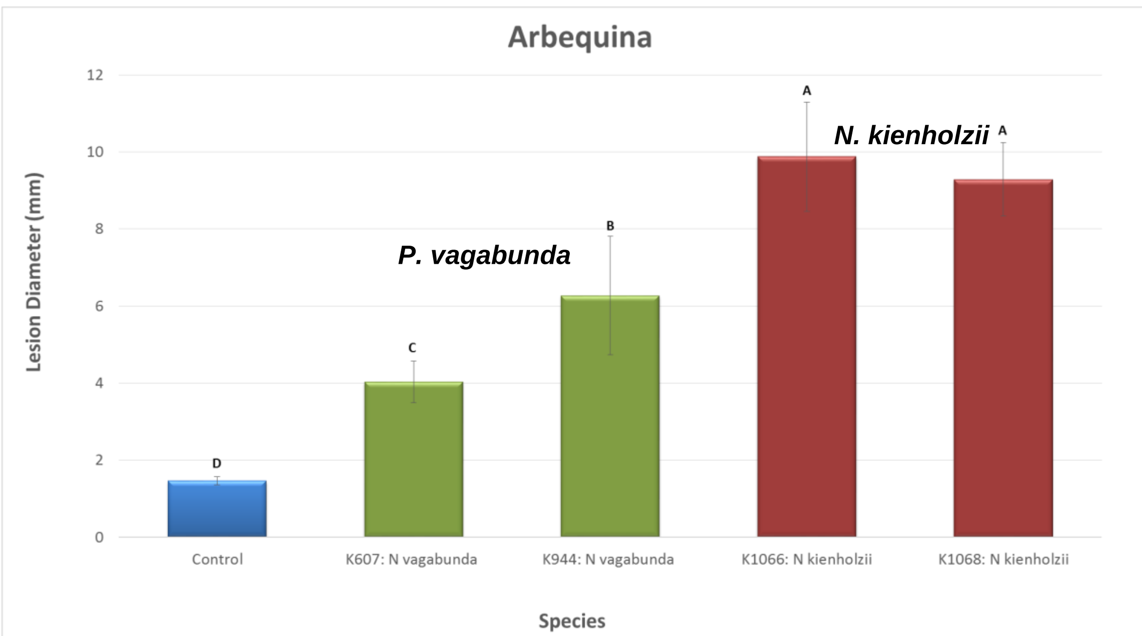

Figure 3a: Lesion size (mm) produced in leaves of Arbequina cultivar following wounding and inoculation of spore suspensions of *Neofabraea kienholzii* and *Phlyctema vagabunda*, respectively.

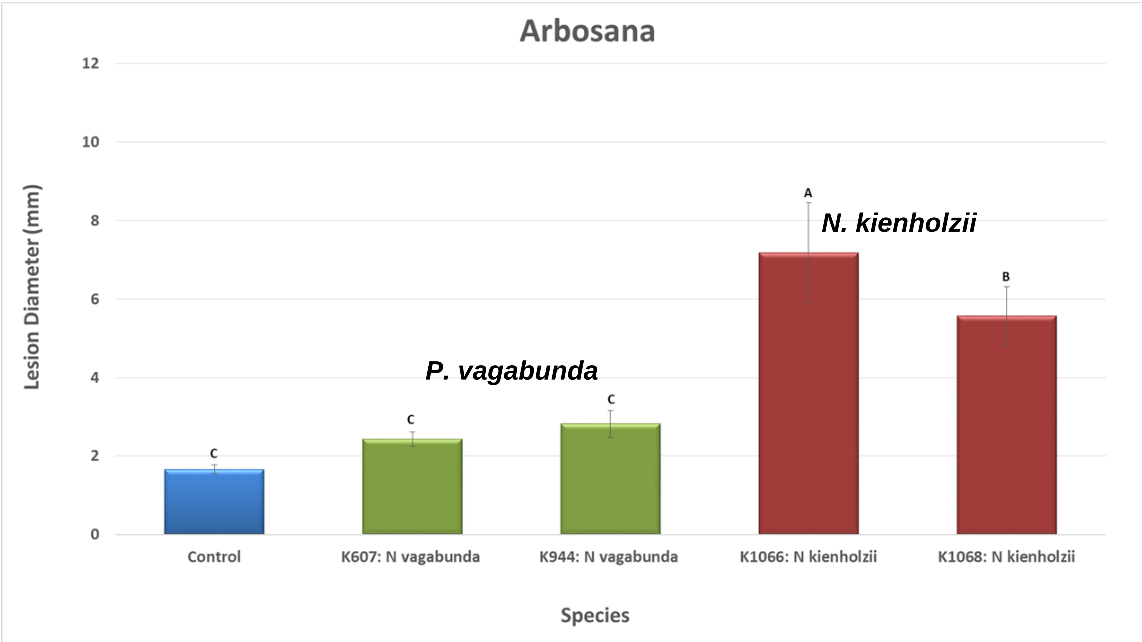

Figure 3b: Lesion size (mm) produced in leaves of Arbosana cultivar following wounding and inoculation of spore suspensions of *Neofabraea kienholzii* and *Phlyctema vagabunda*, respectively.

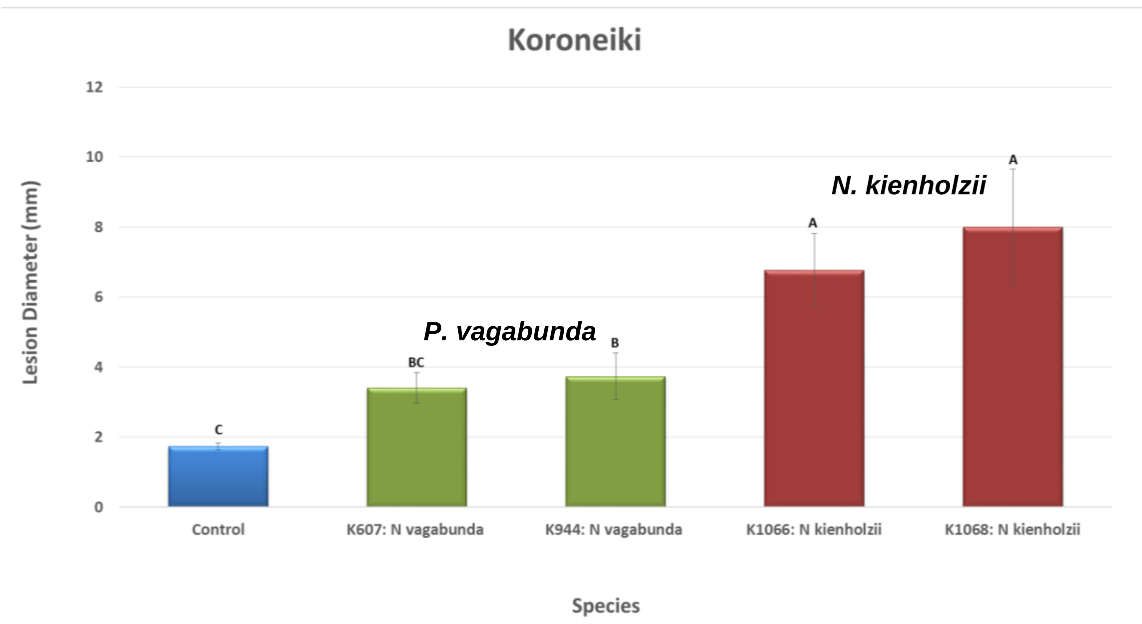

Figure 3c: Lesion size (mm) produced in leaves of Koroneiki cultivar following wounding and inoculation of spore suspensions of *Neofabraea kienholzii* and *Phlyctema vagabunda*, respectively.

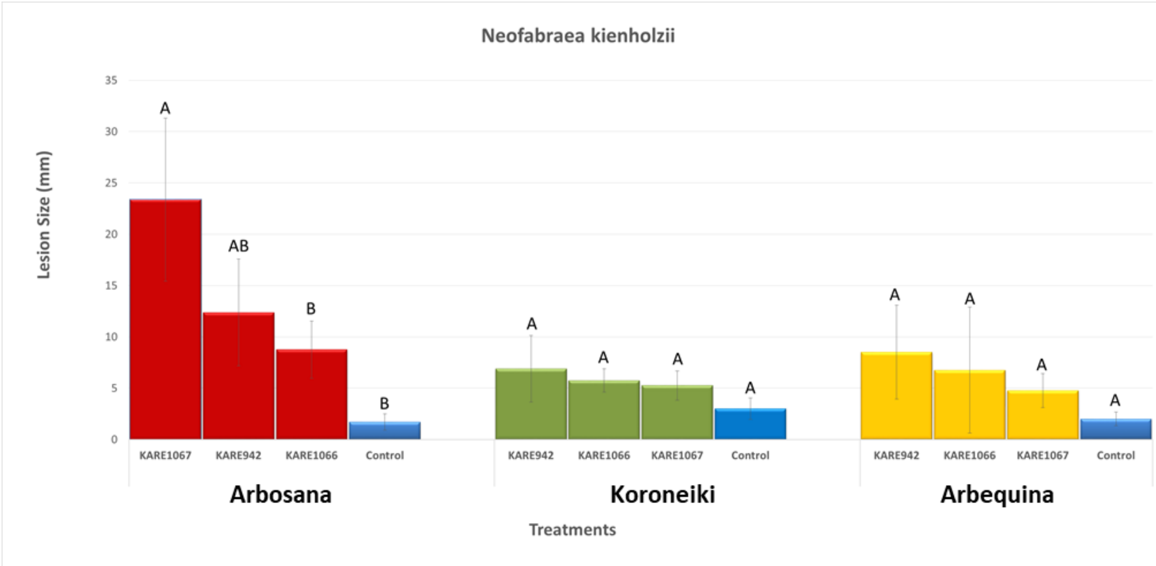

Figure 4a: Lesion size (mm) produced in twigs of Arbosana, Koroneiki and Arbequina cultivars following wounding and inoculation of spore suspensions of *Neofabraea kienholzii*.

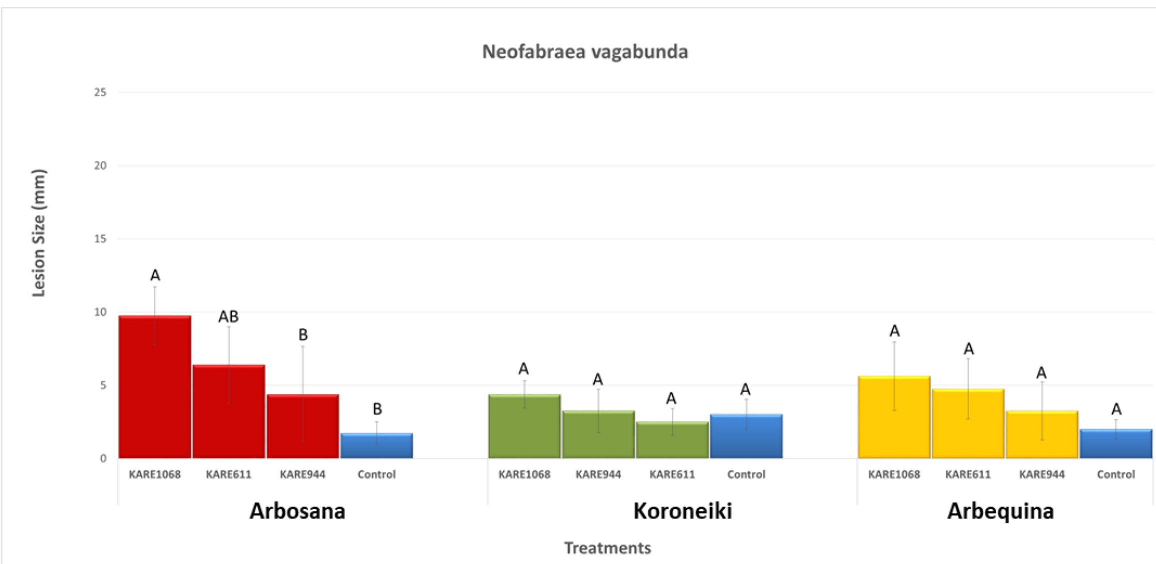

Figure 4b: Lesion size (mm) produced in twigs of Arbosana, Koroneiki and Arbequina cultivars following wounding and inoculation of spore suspensions of *Phlyctema (Neofabraea) vagabunda.*

Objective 4: Identify inoculum sources, determine the pathogen host range and disease progress over time

Different tree parts including dead portions were collected from diseased orchards and examined in the laboratory in order to identify reproductive structures of the pathogens and determine the main sources of inoculum for Neofabraea disease. Other plant species growing in the vicinity of oil olive orchards including pear and apple, as well as ornamentals and native plant species were examined to determine the host range of the pathogens.

Main sources of inoculum for the disease included fruiting bodies developing within lesions of infected leaves. Additional inoculum included spore masses produced on rotten olive fruits on the ground or that remained on trees after harvest. We also surveyed a few apple orchards in California to detect additional inoculum sources but none of the Neofabraea pathogens were found from apple. However, a recent study in California has reported the occurrence of *P. vagabunda* from branch cankers in container-grown apple trees (Rooney-Latham and Soriano, 2016). These findings indicate that apple trees may serve as possible sources of inoculum for Neofabreae disease of oil olives in California.

Objective 5: Develop and implement efficient control strategies

Fungicides in different FRAC groups (different modes of action) were screened in the laboratory as well as in the field to determine their efficacy against *Neofabraea* and *Phlyctema* pathogens. In vitro sensitivities were determined using an Autoplate Spiral Plating System as described by Forster et al. (2003). Fungicide solutions were amended to 15-cm Potato Dextrose Agar plates with the spiral plater using the exponential deposition mode. Spore inoculum was striped radially along the fungicide concentration gradient. Effective fungicide concentrations where 50% growth inhibition is observed was determined after 3 days of incubation using a computer program. Compounds with the best in vitro efficacy were selected for field studies.

Products tested in the field last year (fall winter 2016-2017) included Topsin M (thiophanate-methyl – group 1), Inspire Super (difenoconazole/cyprodinil – group 3+9), Luna Experience (fluopyram/tebuconazole – group 3+7), Luna Sensation (fluopyram/trifloxystrobin – group 7+11), Mertect (thiabendazole – group 1), Kocide 3000 (Copper Hydroxide), Rhyme (flutriafol – group M3), Vangard WG (Cyprodinil 75% – group 9), Ziram (ziram – group M3) and Bravo (Chlorothalonil – group M5). Product testing was organized in two field trials established in a super-high-density orchard located near Walnut grove. Experiments aimed to determine the ability of the various fungicides to provide protection against both twig and foliar infections. Experimental units consisted of two adjacent trees arranged in each of four blocks using a randomized complete block design. In trial 1, Topsin M, Inspire Super, Luna Experience, Luna Sensation, Mertect, Kocide 3000, Rhyme, Ziram, Tebucon and Vangard WG were applied once after harvest on harvest day (T1). Fungicides were applied using a backpack sprayer to insure a complete coverage of wounds and tree canopy. In trial 2, Luna Experience, Mertect, Kocide 3000, Ziram, Bravo and Tebucon were applied after harvest, then once in December and once in January. For these experiments, foliage infection relied on natural inoculum while twigs were wounded and artificially inoculated with spore suspensions. All fungicide treatments were evaluated for their ability to protect olive trees against Neofabraea twig cankers and leaf spots and were compared with a water treated control. Trials were rated 4 months after harvest. The amount of leaf and twig infections was evaluated for each fungicide tested and treatments that resulted in the lowest amount of leaf spots and twig lesions were considered as most effective against Neofabraea disease.

Trial 1A showed that all fungicides tested including Topsin M, Inspire Super, Luna Experience, Luna Sensation, Vangard WG and Ziram provided good protection against Neofabraea leaf spots and twig lesions (Figs. 5a and 5b). Trial 1B showed the best efficacy for Rhyme and Tebucon while Bravo, Mertect and Kocide 3000 had zero to little efficacy (Figs. 5c and 5d). Trial 2 confirmed that Luna Experience, Ziram and Tebucon were the best products overall to control Neofabraea leaf spots and twig lesions (Figs. 5e and 5f). The Copper compound Kocide 3000 show no efficacy in controlling the disease.

We are currently working to identify chemical companies that would be willing to work with the OOCC to engage in section 18 for pesticide emergency exemption. So far, Bayer expressed no interest following up with section 18 for Luna Experience. We are currently discussing with UPI to investigate their interest pursuing with section 18 for Ziram.

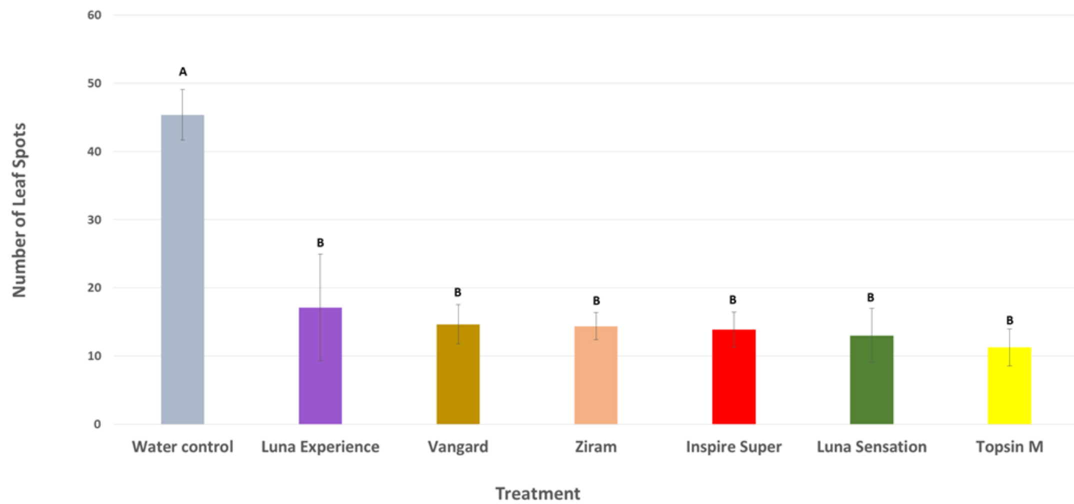

Figure 5a. Trial 1A: Average number of leaf spots per olive tree according to various fungicide treatments and compared to the water treatment.

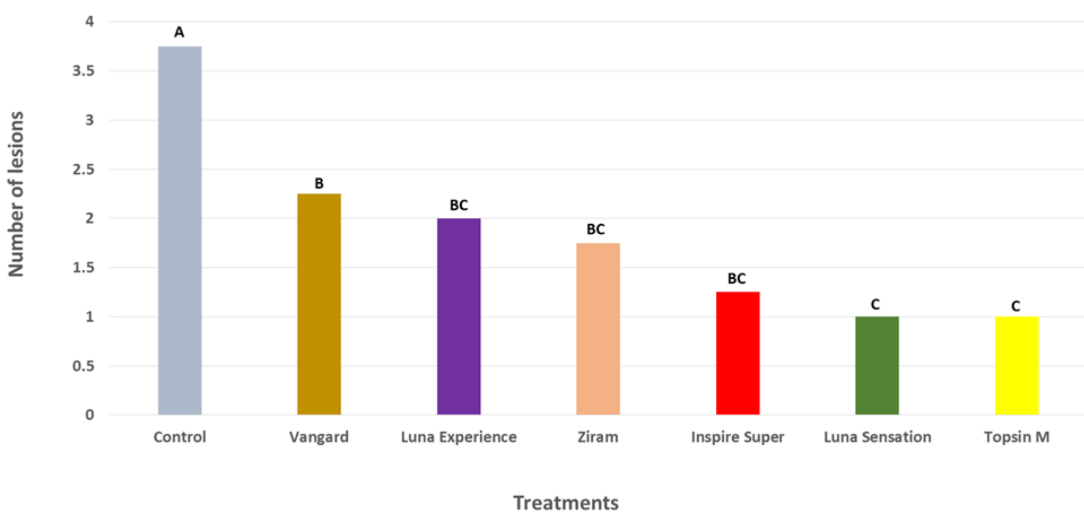

Figure 5b. Trial 1A: Average number of twig lesions per olive tree according to various fungicide treatments and compared to the water treatment.

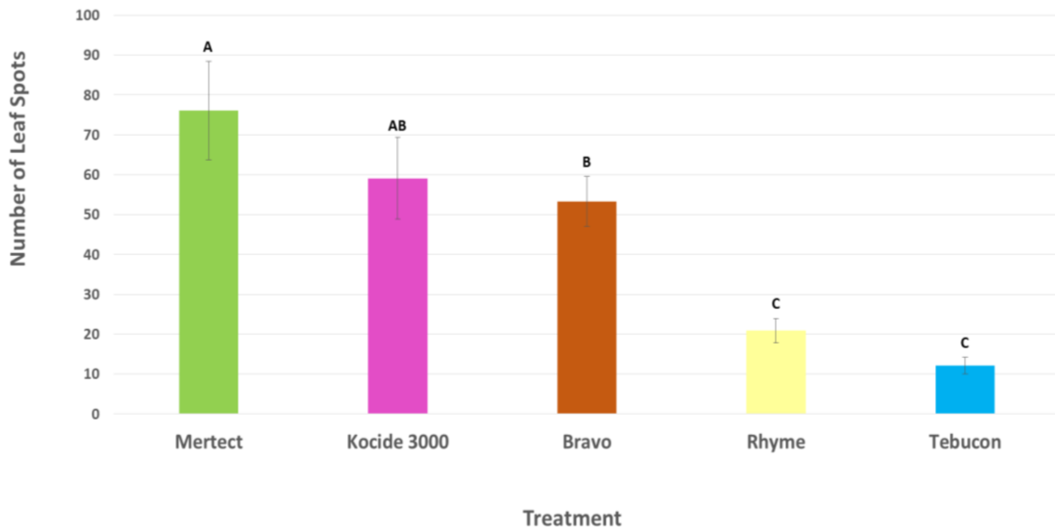

Figure 5c. Trial 1B: Average number of leaf spots per olive tree according to various fungicide treatments and compared to a copper treatment (Kocide 3000).

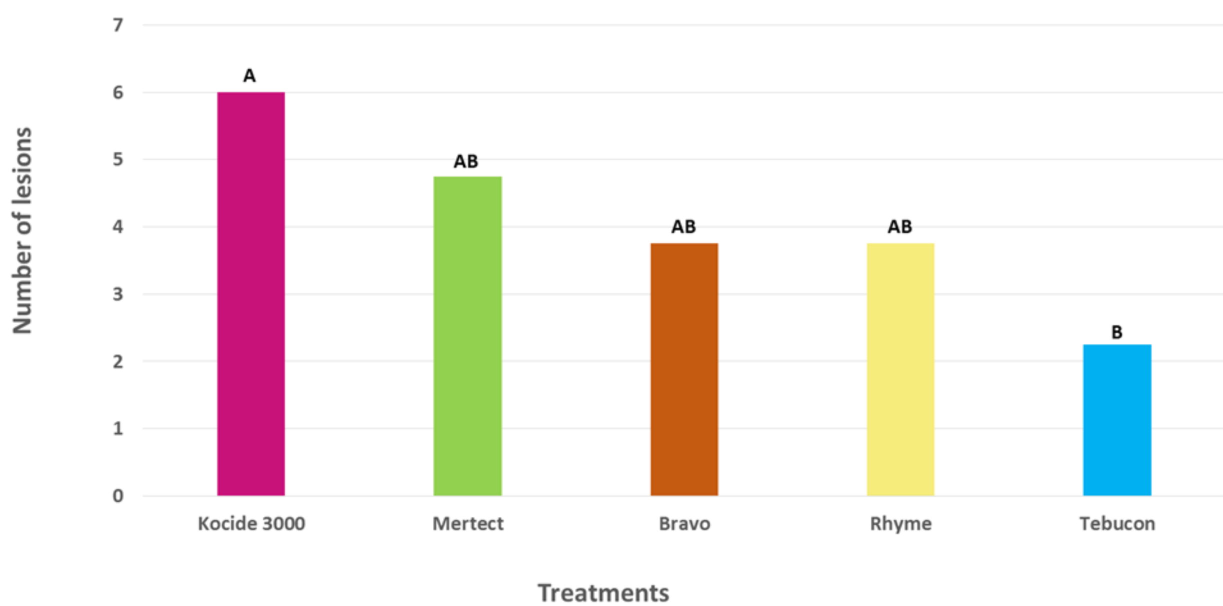

Figure 5d. Trial 1B: Average number of twig lesions per olive tree according to various fungicide treatments and compared to a copper treatment (Kocide 3000).

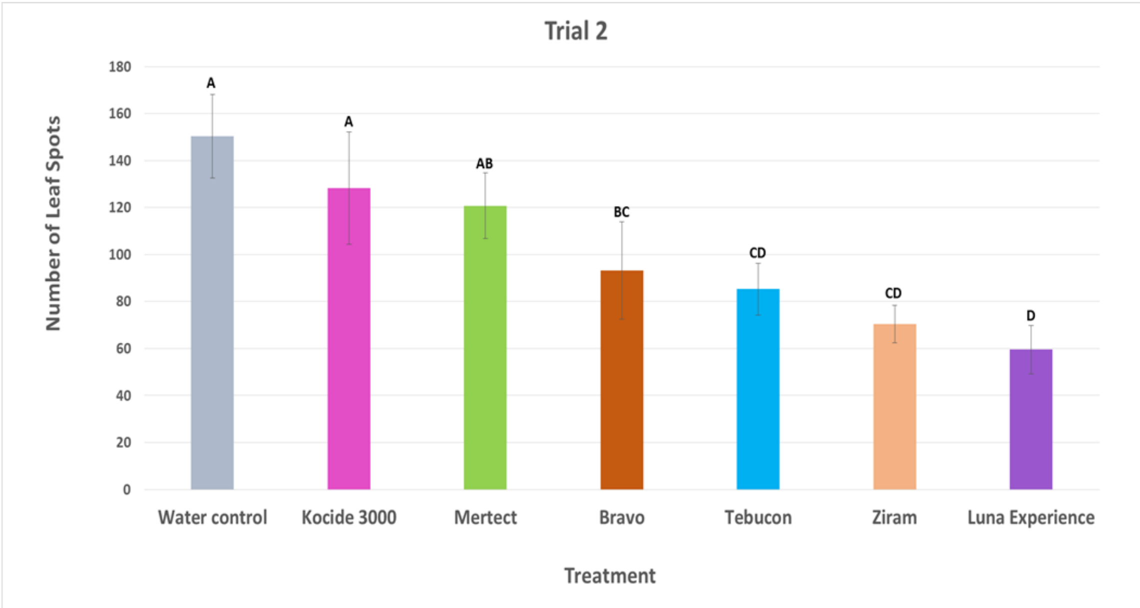

Figure 5e. Trial 2: Average number of leaf spots per olive tree according to various fungicide treatments and compared to the water treatment a copper treatment (Kocide 3000).

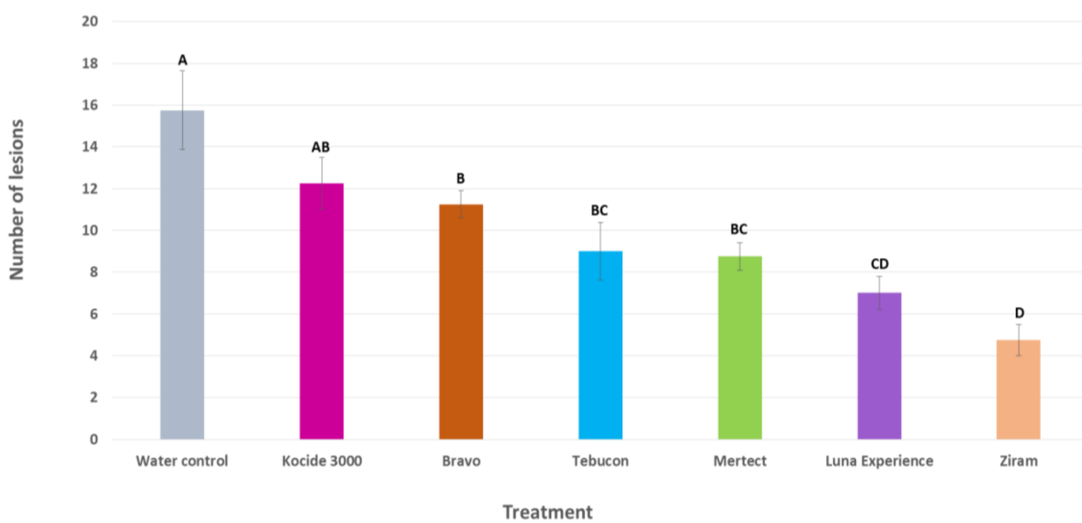

Figure 5f. Trial 2: Average number of twig lesions per olive tree according to various fungicide treatments and compared to the water treatment a copper treatment (Kocide 3000).

Objective 6: Outreach and Education:
Information from this work was presented at the Sacramento Valley Olive Day organized on June 21, 2016 by farm advisor Dani Lightle, and at the OOCC meeting at California Farm Bureau Federation on June 7, 2017. Information obtained from this work was shared among all farm advisors working with olives and was used to train various orchards manager including staff at Corto olive and California Olive Ranch. Information about this new disease was also distributed through various farm advisor and UCCE networks. This research was also presented at the 2017 annual meeting of the American Phytopathological Society, Pacific Division in Riverside, CA. Findings from this research has been covered also by various online journal articles and interviews, including the *Olive Oil Times and AGFAX*.

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