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Title: Evaluation of new fungicides for control of olive leaf spot (peacock spot)

***Introduction*.** Olive leaf spot or peacock spot, caused by the fungus *Fusicladium oleagineum* (syn. *Spilocea oleaginea, Venturia oleaginea*), occurs sporadically on olive trees in California. In years with favorable environmental conditions, an orchard may lose 9 to 15% of its leaves and 10 to 20% of fruiting twigs if the disease is not managed. Excessive leaf loss can also result in more olive knot because leaf scars are sites for bacterial infection. Symptoms most commonly develop on the leaf blade but are also found on petioles, fruit, and fruit peduncles (stems). At first, lesions are inconspicuous, superficial, sooty blotches. Later, they become dark green to black and circular, and form conidia. Spots are surrounded by yellow halos. Lesions resemble the spots on the tail of a peacock, and hence the name peacock spot. With numerous lesions, the leaf becomes chlorotic and falls.

Leaves in the lower canopy where the humidity is higher are more severely affected, resulting in greater defoliation. Defoliated twigs often die later in the summer. Leaf infections occur on the upper surface and seldom expand beyond the epidermal layer. Once the leaf drops, however, the fungus colonizes the internal tissues forming a dense mass of stromatic tissue. The sexual state of the pathogen has not been observed. Olive cultivars vary in their susceptibility. Mission is the most susceptible followed by Manzanillo and to a lesser extent Sevillano, but all cultivars including oil cultivars such as Arbequina and Arbosana may be affected by peacock spot.

Leaf drop occurs mostly in late spring and summer. Infected leaves remaining on the tree start sporulating along the margins of lesions in the fall. Rainfall and wind-driven rain are the main dissemination methods; whereas wind alone is not effective in detaching and disseminating conidia. In California, lesions start forming in the fall and winter, but most disease develops in the spring. Rainfall is essential for infections to occur regardless of the season. Temperature is important but often is not limiting the development of the pathogen. High temperatures are more limiting to spore germination and mycelial growth than low temperatures. The optimum temperature for growth of the fungus is 21ºC, but growth can occur at 6 to 28ºC. The minimum duration of leaf wetness for spore germination is 48 h at 16ºC, 24 h at 20ºC, or 36 h at 24ºC. The incubation period for symptom development is 12 to 19 days over a temperature range of 10ºC to 25ºC.

Currently available chemicals for managing the disease are copper and lime sulfur. Copper is commonly used as Bordeaux mixtures or as fixed coppers to prevent phytotoxicity. Lime sulfur can also eradicate the fungus in leaf tissue. Other fungicides such as zineb are effective but no longer available in the United States. With more regulations concerning the use of copper (new copper limits for agricultural uses for all registered crops) and lime sulfur, alternative fungicides are needed that are highly efficacious and persist for extended time periods to prevent infections over the winter and spring when rainfall results in infection periods. Therefore, we initiated field studies to evaluate alternatives including older and newer fungicides. Currently, timing of fungicide treatments in California includes a postharvest fall application before winter rains begin, and again in early spring if wet conditions continue. These timings coincide with copper treatments for olive knot management. Some researchers have indicated that spring treatments are less effective, but this needs to be further substantiated.

OBJECTIVES

1. Evaluate the performance of new and older fungicides in field trials.
   a) Dithicarbamates (ziram), chlorinated hydrocarbons (chlorothalonil), and phthalimides (captan) (FRAC codes M3, M4, M5), DMIs (FRAC 3), SDHIs (FRAC 7), QoIs (FRAC 11), dodine (FRAC U12), polyoxins (FRAC 19), or mixtures such as FRAC 3/11, FRAC 3/7, FRAC 7/11, and FRAC 3/19.
2. Evaluate application timing of selected treatments.
   a) Fall, spring, or fall <u>and</u> spring.
3. Evaluate new fungicides for their in vitro activity.
   a) Determine the in vitro activity of selected fungicides that are effective in field trials.

MATERIALS AND METHODS

Evaluate the performance of new and older fungicides in field trials. In studies in two commercial Ascolano or Manzanillo olive orchards where the disease is known to occur, fungicides including ziram (FRAC M3), copper (Champ, FRAC M1), captan (FRAC M4), chlorothalonil (Bravo, FRAC M5), difenoconazole (Inspire, FRAC 3), polyoxin-D (Ph-D, FRAC 19), and dodine (Syllit, FRAC U12) were applied in combination with 2% Omni Oil (in the first two applications) using an air-blast sprayer (100 gal/A) on Dec. 20, 2017, Feb. 7, 2018, and Oct. 19, 2018. There were four replications for each treatment in a randomized complete block design. Disease incidence was evaluated on 5-1-18. For this, eight 15- to 25-cm-long terminal twigs were collected from two sides of the tree, and the number of diseased and healthy leaves was counted. Data were analyzed statistically using ANOVA and mean separation procedures of SAS 9.4.

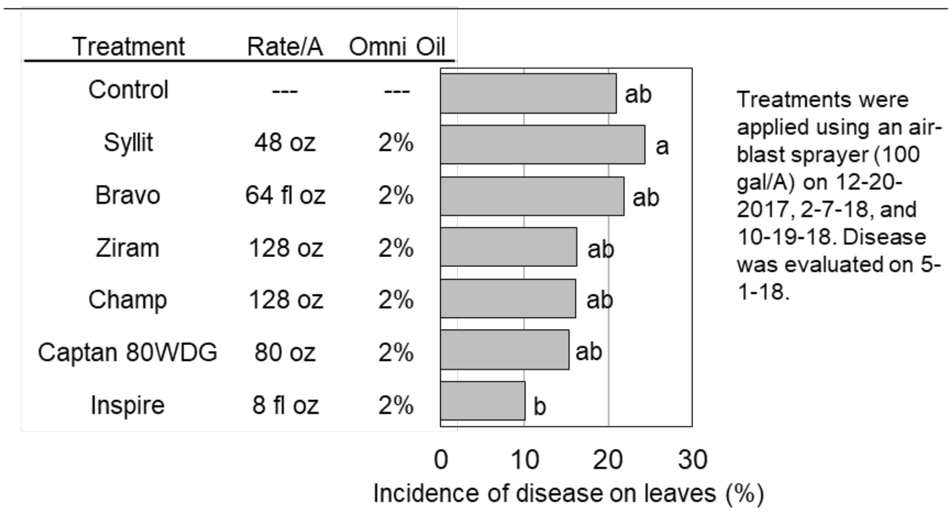
Evaluate application timing of selected treatments. A field trial on Arbequina olive was established with applications of selected fungicides on 2-8-18 (in combination with 2% Omni Oil) or 10-19-18. Disease will be evaluated in the spring of 2019.

Submission of fungicides to the IR-4 program. Based on the results of our efficacy trials on peacock spot (see below) and in coordination with the research project on the management of the newly described Neofabraea and Phlyctema diseases of olive in California (project leader is F. Trouillas, funded by OOCC), fungicides were identified that are potentially registerable, have different modes of action, have low resistance potential, and are efficacious against the three diseases. 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. An emergency registration was also requested and coordinated with OOCC. The PI of this COC project traveled to the IR-4 Food Use Workshop in St. Louis, MO, in September 2018 to defend the nominations.

RESULTS AND DISCUSSION

Evaluate the performance of new and older fungicides in field trials. Among the two trial sites where fungicides were applied for evaluation of their efficacy against peacock spot, the disease only developed in the orchard with cv. Manzanillo that is considered more susceptible to the disease. Typical symptoms were present on leaves. On untreated control trees, 21% of the leaves were diseased. Among treatments evaluated in this trial, only Inspire significantly reduced the disease (10.1% incidence) compared to the control (Fig. 1). A numerical reduction in incidence was observed for Ziram, Captan, and Champ. These results are encouraging because application timings in the first year of the study were done late (Dec. 20, 2017) due to the approval of this project in early December of 2017. At this time, major infection periods likely had already occurred, and protective treatments no longer could inhibit disease development. The efficacy of Inspire in these late applications can be attributed to its local systemic and post-infection activity. Because most infections of the peacock spot pathogen occur in the fall, the late-winter applications in early February 2018 may not have been as effective. Applications at these trial sites are being continued, and in 2018, a fall application was done in mid-October. Disease will be evaluated again in the spring of 2019. Thus, in these 1.5- to 2-year-long trials, the pathogen will go through its complete disease cycle, and with two annual applications, the efficacy of these treatments can be most accurately established.

Efficacy of fungicide treatments for management of peacock spot of Manzanillo olive 2017-18



Evaluate application timing of selected treatments. A field trial was established with spring and fall timings, and results are pending in spring of 2019.

Evaluate new fungicides for their in vitro activity. Fungal isolations from diseased olive leaves were attempted but were not successful when done in the springtime. The pathogen is difficult to isolate because it is very slow growing and easily overgrown by contaminating micro-organisms. We will repeat our isolations in the fall of 2018 when sporulation typically occurs on leaves. Isolates will then be evaluated for their in vitro sensitivities to selected fungicides.

Submission of fungicides to the IR-4 program. Three fungicides were nominated to the IR-4 program in 2018: ziram (Ziram 76WDG), difenoconazole/cyprodinil (Inspire Super), and thiophanate-methyl (Topsin-M). Ziram and Inspire Super were approved for residue trials at the

National Food Use Workshop in Sept. for registration on olives. 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. 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. Topsin-M was not accepted due to a low probability of registration because of the EPA Re-registration Eligibility Decision concerning its human safety and the potential for selection of resistance in the *F. oleaginum* pathogen population.