ANNUAL RESEARCH REPORT
California Olive Board and California Olive Oil Commission
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Project Title: Evaluation of new fungicides for control of olive leaf spot

Keywords: Fungicides, timing of applications, in vitro toxicity_

JUSTIFICATION/ BACKGROUND

Olive leaf spot or peacock spot, caused by the fungus *Fusicladium oleagineum* (syn. *Spilocea oleaginea, Venturia oleaginea*), is a sporadic disease of 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 the 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 circular spots containing mycelium and conidia (Fig. 1), and spots are surrounded by yellow halos. These lesions resemble the spot 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 penetrate beyond the epidermal layer. Once the leaf drops, however, the fungus colonizes the internal leaf 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 are generally susceptible.

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; 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 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. Bordeaux mixtures or fixed coppers are commonly used to prevent copper injury. Lime sulfur can also eradicate the fungus in leaf tissue, but lime sulfur is difficult to work with and requires extra protective equipment for workers. Other fungicides such as zineb are effective but no longer available. Timing of fungicide treatments in California include a postharvest application and an early spring application. Others, however, have indicated that spring treatments are less effective. Use of copper treatments at these time periods corresponds with olive knot management timings. With more regulations concerning the use of copper (new copper limits for agricultural uses) 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. Based on our results in field studies, the multisite mode of action (MOA) fungicide Ziram and a pre-mixture of two single-site MOA fungicides, Inspire Super, were approved for residue trials at the IR-4 National Food Use Workshop in September for registration on olives in 2018,. 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 fungicide. Thus, integration of multi-site MOA for both products was also established as an effective anti-resistance strategy. Residue trials were conducted in 2019, and laboratory analyses will be done in 2020. Research on these and other fungicides needs to continue to identify other potential products and optimal use strategies (e.g., timing, adjuvants). Thus, in 2019, we continued our field studies on the identification of new fungicides for the management of peacock spot.

Fig. 2. Range of symptoms of Peacock spot caused by *Fusicladium oleagineum.*

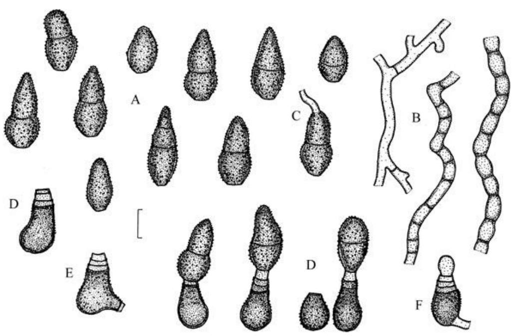

Fig. 2. *Fusicladium oleagineum.*
A - conidia. B - superficial septate hyphae, C - germinating conidium, D - conidiogenous cells with several conspicuous annellations, E – percurrent proliferating conidiogenous cell, F - conidiogenous cell arising from a hypha. Scale = 10 µm.

OBJECTIVES

1. Evaluate the performance of new and older fungicides in field trials.
    a) Dithiocarbamates (ziram), chlorinated hydrocarbons (chlorothalonil), and phthalimides (captan) (FRAC Groups – M3, M4, M5), DMIs (FG 3), polyoxins (FG 19), or mixtures such as FG 3/9, and FG 3 + 19.
    b) Evaluate proprietary fatty acids to improve performance of fungicides (pesticides).
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.

PLANS AND PROCEDURES

1. *a,b. Evaluate the performance of new and older fungicides in field trials.* In studies in two commercial olive Arbequina or Manzanillo orchards where the disease is known to occur, fungicides including ziram (FC M3), chlorothalonil (FC M5), difenoconazole (FC 3), and polyoxin-D (FC 19), or mixtures such as difenoconazole/cyprodinil (FC 3/9) and difenoconazole+polyoxin D (FC 3+19) were applied using an air-blast sprayer. There were four replications for each treatment in a randomized complete block design. Disease was evaluated in late spring. For this, the number of diseased leaves was counted for 30 sec on each tree. Data were analyzed statistically using ANOVA procedures and mean separation procedures of SAS 9.4.

2. *Evaluate application timing of selected treatments*. The standard program of two applications was done in 2018-2019 to compare the fungicides. Applications in winter only or fall and winter will be evaluated in the 2019-2020 season.

3. *Evaluate new fungicides for their in vitro activity*. Isolates of the pathogen were obtained from several locations. To evaluate the in vitro toxicity of selected new fungicides with efficacy in field trials, the SGE method will be used. Agar media will be amended with fungicides in radial concentration gradients using a spiral plater. Suspensions of spores or mycelial strips will be placed radially onto the amended media. This will allow the determination of EC_{50} values for each fungicide and isolate using a computer program.

RESULTS AND DISCUSSION

1. a,b. Evaluate the performance of new and older fungicides in field trials. Dithiocarbamates (ziram - FRAC Code M3), chlorinated hydrocarbons (chlorothalonil - FC M5), polyoxins (polyoxin-D - FC 19), and mixtures such as DMIs/ anilinopyrimidines (difenoconazole/cyprodinil - FC 3/9) were evaluated in two field trials in Glenn and Sutter Co. Treatments were applied in the fall (Oct.) and winter (Jan.) and trees were evaluated in April/May 2019 for peacock spot.

Table 1. Efficacy of fungicide treatments for management of peacock spot of Arbequina olive, Yuba Co. 2018-2019

No.	Treatments*	Product rate/A	Applications 10-18-18	1-17-19	No. leaves diseased**	LSD^
1	Control	---	---	---	28.3	a
2	Champ	128 oz	@	@	14.5	b
3	Syllit	48 oz	@	@	11.3	bc
4	Ziram	128 oz	@	@	11.0	bcd
5	Inspire Super	20 fl oz	@	@	8.5	cd
6	Bravo	64 fl oz	@	@	7.3	cd
7	Ph-D***	6.2 oz	@	@	6.8	d

*Treatments were applied using an air-blast sprayer at 100 gal/A. Treatments on 1-17-19 were applied with NuFilm-P (8 fl oz).
** Disease was evaluated on 5-1-2019. For this, the number of leaves with typical disease symptoms was counted on each tree for 30 sec.

Table 2. Efficacy of fungicide treatments for management of peacock spot of Manzanillo olive, Glenn Co. 2018-2019

No.	Treatments*	Product rate/A	Applications 10-18-18	1-17-19	No. leaves diseased**	LSD
1	Control	---	---	---	70.9	a
2	Bravo	64 fl oz	@	@	45.0	b
3	Syllit	48 oz	@	@	42.5	b
4	Ziram	128 oz	@	@	37.0	b
5	Ph-D	6.2 oz	@	@	35.8	b
6	Inspire Super	20 fl oz	@	@	33.5	b
7	Champ	128 oz	@	@	27.5	b

*Treatments were applied using an air-blast sprayer at 100 gal/A. Treatments on 1-17-19 were applied with NuFilm-P (8 fl oz).
** Disease was evaluated on 5-1-2019. For this, the number of leaves with typical disease symptoms was counted on each tree for 30 sec.

In the first trial on Arbequina olive, peacock spot incidence was moderate with 28 infected leaves counted in a 30-sec evaluation period for each tree (Table. 1). All fungicides evaluated were effective and significantly reduced the disease as compared to the non-treated controls. Polyoxin-D (Ph-D) was highly effective in our trials, and the registrant UPL has agreed to add olive to the fungicide label. Label amendments to add olive are expected in 2020. Because Ph-D is a biopesticide, it is exempt from tolerance and thus, no residue studies are needed. We are planning to evaluate proprietary fatty acids to improve performance of fungicides.

In the second trial on Manzanillo olive, disease pressure was higher with over 70 leaves with peacock spot counted in a 30-sec period (Table 2). All fungicides significantly reduced the disease as compared to the non-treated control. Champ, Inspire Super, Ph-D and Ziram had numerically the lowest levels of disease following a fall and winter application. Registration of Ph-D as described above, as well as Inspire Super and Ziram are being pursued as described below under IR-4 GLP Studies.

Proprietary fatty acids were not provided for field applications in these trials. However, a mixture of capric and caprylic acids (i.e., Dart) is now available and will be evaluated in future trials; this is included in our 2020 research proposal.

2. Evaluate application timing of selected treatments. In the above trials, fall and spring applications were done for all treatments. This is because none of the treatments reduced the disease to very low levels. In our studies in 2019-2020, fall/winter and winter-only applications will be done for some treatments (e.g., Inspire Super) because most infections are thought to occur during the rainy period of the year.

3. Evaluate new fungicides for their in vitro activity. We are attempting to determine the in vitro activity of selected fungicides that are effective in field trials. This is very challenging because of the difficulty in getting isolates to grow on laboratory media, and because the fungus has an extremely slow growth rate and is easily overgrown by fungal environmental contaminants. These factors prevent us from using the spiral gradient assay, and other assays will be needed.

4. IR-4 GLP Studies. IR-4 projects are ongoing for ziram and difenoconazole/ cyprodinil (Inspire Super). Registrations of these fungicides are considered of low risk 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 (Table 3). We have reviewed protocols and we are assisting in ongoing field studies. Ziram is a FRAC Code M3, whereas Inspire Super is a FRAC Code 3/9. Inspire FRAC Code 3 was considered best registered in the pre-mixture Inspire Super. Thus, integration of multiple modes of action for both products represented as FRAC Codes M3 and 3/9 was established as an effective anti-resistance strategy for disease control. These fungicides are also highly effective against newly described Neofabraea and Phlyctema diseases of olive in California. The parent registrant for Topsin-M has contacted us seeking use on olive. Topsin-M, however, was not accepted into the IR-4 program. It has a low probability for registration because of the EPA Re-registration Eligibility Decision concerning its human safety and the potential for selection of plant pathogen resistance. Currently, the risk cup is nearly full, and EPA has placed a “red light” label for no further development with IR-4 for Topsin-M. This was discussed at the Food Use Workshop in September 2019. Thus, registration of Topsin-M will not be pursued for olive or any other crop based on EPA concerns (Table 3).

Table 3. Summary of new fungicides accepted into the IR-4 Program at the Food Use Workshop in September 2018 and ongoing in 2019

Fungicide	Active Incredient(s)	FRAC	Registrant	IR-4/EPA	Approved for Residue Trials
Ziram	ziram	M3	Supported	Supported	In 2018/19 season
Inspire	difenoconazole	3	Not supported	Supported	
Inspire Super	difenoconazole + cyprodinil	3/9	Supported	Supported	In 2018/19 season
Topsin-M	thiophanate-methyl	1	Supported	Not supported	