IN VITRO EFFECTS OF FUNGICIDES ON CONIDIUM GERMINATION OF *SPILOCAEA OLEAGINA*, THE CAUSE OF OLIVE LEAF SPOT

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ABSTRACT

Twenty fungicides were tested *in vitro* for their effects on the germination of conidia of *Spilocaea oleagina*, the fungus that causes olive leaf spot. Conidia used in this evaluation were obtained from naturally infected olive leaves in Canterbury. Of the fungicides tested, kresoxim-methyl and captan were the most effective in preventing conidium germination at low concentrations, with EC_{50} values of 0.002 and 0.003 μg/ml, respectively. The newer fungicides, boscalid and boscalid/pyraclostrobin, were also effective (EC_{50}=0.031 and 0.006 μg/ml, respectively). Of the benzimidazole fungicides tested, carbendazim was effective (EC_{50}=0.005 μg/ml), but thiophanate-methyl was not (EC_{50}=2.6 μg/ml). None of the demethylation inhibitor fungicides tested were very effective (EC_{50} values > 1 μg/ml), except flusilazol (EC_{50}=0.075 μg/ml). Two copper-containing fungicides, copper hydroxide and copper sulphate, were ineffective for preventing conidium germination (EC_{50}=3.0 and 44.3 μg/ml, respectively). This study has identified candidate fungicides for further evaluation as tools for management of olive leaf spot.

Keywords: Conidium germination, peacock spot, fungicides.

INTRODUCTION

The New Zealand olive industry is a new, fast-growing industry with potential for high returns from premium oil. One of the major problems threatening this industry is the disease olive leaf spot (OLS), also called peacock spot. This disease, caused by the fungus *Spilocaea oleagina* Castagne (Hughes) (syn. *Cycloconium oleagina*), is widespread in all olive growing regions of the world, and has been recognised in Mediterranean areas for over a century (Bernès 1923). In warm, dry climates the disease is not usually a significant problem because cool, moist weather is required for epidemic development.

A preliminary survey of the prevalence of OLS during the summer of 1999/2000 revealed that the disease is widespread throughout New Zealand, with all regions and cultivars affected (MacDonald et al. 2000). Forty percent of all olive trees assessed were infected with OLS, suggesting that it is a serious disease in New Zealand olive groves and may play a major role in the low productivity of olives. Losses in olive yield due to OLS have been estimated to be as high as 20% in California (Wilson & Ogawa 1979).

The principal method used to control OLS throughout olive-growing regions of the world is chemical fungicides (Teviotdale et al. 1989; Graniti 1993). The most commonly used fungicides contain copper, and these have included Bordeaux mixture, copper hydroxide, copper oxide and copper oxychlorides, although some long-persisting preventative fungicides (e.g. chlorothalonil and dodine) have also been used to control the disease. These fungicides are usually applied before or at the beginning of the main infection periods, which often coincide with the main shoot-growth seasons (spring and/or autumn) (Prota 1995). Teviotdale et al. (1989) reported that in Californian olive groves, one annual application of a copper-containing fungicide, in autumn before rain began, effectively controlled OLS under low disease pressure, irrespective of the rate or type of fungicide. However, there have been no reports on the control of OLS using copper-containing fungicides under high disease pressure, as may occur in wet or humid conditions. In New Zealand, the cool, moist climate favours OLS development causing higher levels of disease, which are unlikely to be controlled with single applications of copper-containing fungicides. In addition, new fungicides have been developed during the last few decades with very effective and sustainable control of many other diseases, and these may provide more effective control of OLS.

Systemic fungicides, such as difenoconazole, myclobutanil, fenarimol and tebuconazole, have the potential to replace copper-containing fungicides to effectively control OLS under high disease risk. Apple scab, which is caused by the closely related pathogen *Venturia inaequalis,* and other fungal diseases of tree crops have been successfully controlled with systemic fungicides (Jones 1981). Because the modes of infection of *S. oleagina* and *V. inaequalis* are similar (Graniti 1993), it is likely that these fungicides could be effective in controlling OLS.

In the present study, 20 fungicides were screened for their effects on germination of *S. oleagina* conidia, to identify chemicals that have potential for control of OLS and that may be suitable for further evaluation in growth chamber and field studies.

MATERIALS AND METHODS

The fungicides selected for the study were from several chemical classes, representing different modes of action, and included those with contact and systemic activity as well as some known to be effective against *V. inaequalis*. The formulations of the 20 fungicides tested, their chemical classes and concentrations used are listed in Table 1. The selected fungicide concentrations were based on the range of activity for each product. For all fungicides, 10-fold stock solutions were prepared in water with at least five different concentrations of each chemical.

Olive leaf extract (OLE) was prepared according to Saad & Masri (1978) and was enriched with 40 g/litre of potato dextrose broth (PDB; Difco Laboratories, USA) (OLE+PDB). Conidia of *S. oleagina* for the evaluation were from naturally infected olive leaves (cv. Barnea) picked from a commercial olive grove in Canterbury. Conidium suspensions were obtained from the leaves by agitating them in distilled water and filtering the suspension through a double layer of cheesecloth to remove leaf debris. The conidium concentrations were adjusted to 5 x 10^5 conidia/ml using a microscope and haemacytometer to determine numbers of conidia in the suspension. The conidium suspension (100 μl) was then mixed with 100 μl of each fungicide stock solution and 800 μl of OLE+PDB in Eppendorf tubes. Three 20 μl droplets of the suspension were placed separately onto three replicate glass slides. The slides were then placed on the lid of a Petri dish containing approximately 30 ml water agar to provide high humidity (>95%) and the dish was then incubated upside down at 20°C for 24 h. Germination of 100 randomly selected conidia in each droplet was evaluated with a compound microscope at x200 magnification, and the mean percent germination, relative to nil fungicide controls, was calculated for each fungicide. A conidium was considered germinated if the length of the germ tube exceeded half the length of the conidium. The experiment was repeated three times.

All data were analysed using the command GLM Probit analysis (Genstat 7.2) to determine the EC_{50} values (effective concentrations of the fungicides that reduced conidium germination by 50%) and their confidence intervals.

RESULTS AND DISCUSSION

In this study, five of the fungicides tested markedly reduced the germination of *S. oleagina* conidia (Table 1). The cyclic imide and strobilurin chemicals, captan and kresoxim-methyl, respectively, were the most effective in preventing conidium germination with EC_{50} values of less than 0.003 μg/ml. A similar effect by kresoxim-methyl on spore germination has also been reported for *V. inaequalis* (Ypema & Gold

1999). Kresoxim-methyl is characterized by a novel mode of action (inhibition of electron transport at the bc1-complex in mitochondria) and by having a broad spectrum of activity. It has been used for many scab diseases of fruit trees as a protectant fungicide, and acts by building a stable deposit on the leaf surface and in the epicuticular wax layers. Besides having a direct preventative effect on spore germination, kresoxim-methyl prevents the formation of conidiophores and conidia from active scab lesions 7 days after antisporulant application (Politi 1997).

TABLE 1: EC_{50} values for different fungicides tested at different concentrations for inhibition of germination of *Spilocaea oleagina* conidia. Values are the mean from the three experiments.

Chemical class/ fungicide common name	Trade name	Conc. range (µg ai[2]/ml)	EC_{50} (µg ai/ml)	95% CI[3] (±)
Anilopyrimidine				
Cyprodinil/fludioxonil	Switch	0.01–1	0.011	0.005
Pyrimethanil	Scala	10–300	15.16	3.098
Benzimidazole				
Carbendazim	Bavistin	0.005–0.5	0.005	0.001
Thiophanate-methyl	Topsin M-4A	1–100	2.599	1.593
Copper				
Copper hydroxide	Kocide DF	10–2000	2.991	4.980
Bordeaux mixture	Cuprofix Disperss	10–2000	4.420	1.550
Carboxamide				
Boscalid	Endura	0.005–0.5	0.031	0.013
Carboxamide/strobilurin				
Boscalid/Pyraclostrobin	Pristine	0.005–0.5	0.006	0.002
Cyclic imide				
Captan	CropCare Captan WG	0.001–0.5	0.003	0.003
Dithiocarbamate				
Mancozeb	Penncozeb DF	1–50	1.090	0.223
DMI[1]-piperazine				
Triforine	Saprol 190EC	0.1– 0	1.236	1.615
DMI-pyrimidine				
Fenarimol	Rubigan Flo	0.1–15	0.218	0.194
DMI-triazole				
Difenoconazole	Score WG	1–50	3.601	0.901
Flusilazol	Nustar	0.1–40	0.075	0.015
Penconazole	Topas 200EW	0.1–25	0.289	0.192
Tebuconazole	Folicur SC	1–200	3.459	2.331
Myclobutanil	Systhane 125	1–100	5.972	4.348
Guanidine				
Dodine	Dodine 400	0.01–5	0.359	0.459
Nitrile				
Chlorothalonil	Bravo 720SC	0.1–10	0.029	0.012
Strobilurin				
Kresoxim-methyl	Stroby WG	0.001–0.05	0.002	0.002

[1]Demethylation inhibitor.
[2]ai=active ingredient.
[3]CI=Confidence interval.

The contact fungicide, captan was reported to be consistently effective in inhibiting spore germination in *Rhizopus oryzae*, which causes storage rot of potato (Amadioha 1996). However, a high EC_{50} value of 34.1 mg/litre for this chemical was also reported for conidial germination of *Phaeomoniella chlamydospora* (Jaspers 2001). In New Zealand, the fungicide is widely used to control many crop diseases such as apple scab and OLS.

The new-generation fungicides, boscalid and boscalid/pyraclostrobin, were effective in preventing conidium germination, with EC_{50} values of 0.031 and 0.006 μg/ml, respectively. These fungicides have been registered in the USA for use on apples and grapes in the control of scab and botrytis rot (Babadoost 2004), respectively. However, they are not yet registered in New Zealand for use to protect any crop from fungal diseases. Of the anilopyrimidine fungicides, only cyprodinil/fludioxonil was effective at low concentrations, with an EC_{50} value of 0.011 μg/ml.

Two benzimidazole fungicides were tested, but only carbendazim was effective in inhibiting conidium germination with an EC_{50} value of 0.005 μg/ml. A similar inhibitory effect has also been reported for chickpea blight pathogen, *Ascochyta rabiei* (Demirci et al. 2003). Carbendazim is a systemic fungicide with both protective and curative action. The fungicide is absorbed through roots and green tissues of treated plants and acts by inhibiting development of the fungal germ tubes, formation of appressoria and mycelia growth. This fungicide has been used both in New Zealand and overseas in the control of fungal diseases of a range of crops, including stone and pome fruits, vines and vegetables. However, the chemical is not currently being used for the control of OLS in New Zealand.

All the DMI fungicides tested were ineffective in inhibiting conidium germination of *S. oleagina*, except flusilazol, for which the EC_{50} value was 0.075 μg/ml. Although inhibition of spore germination by triazole fungicides has been reported for other fungal species (Clarkson et al. 1997), their ineffectiveness in preventing germination of *S. oleagina* conidia was not surprising because they are generally known to inhibit hyphal growth rather than spore germination (Sisler & Ragsdale 1984).

The two copper-containing fungicides, copper hydroxide and Bordeaux mixture, were ineffective at reducing germination of *S. oleagina* conidia, having EC_{50} values of 3.0 and 4.43 μg/ml, respectively. However, the inhibitory effect of copper on conidium germination has been demonstrated for other plant pathogenic fungi. For example, Franich (1988) reported that exposure of *Mycosphaerella pini* conidia to 20 μg/ml Cu^{2+} for 1.5 h was sufficient to kill the spores. In addition, at lower concentrations the rate of conidium germination was not greatly reduced but the germ tube length was significantly reduced. Copper fungicides are the chemicals most commonly used to control OLS in New Zealand olive groves and have been showed to be effective in controlling OLS in California olive groves (Teviotdale et al. 1989). Their ineffectiveness in inhibiting *S. oleagina* conidium germination at low concentrations suggests that their mode of action may be through means other than conidium mortality. Although several copper-containing fungicides are used on olives in New Zealand, only Bordeaux mixture (Cuprofix; recommended field rate of 500 g/100 litres) is registered for this use, and its efficacy in controlling OLS has not been established. The advantages of systemic fungicides over contact fungicides are well known, but contact fungicides are generally less expensive than systemics and are suitable as rotation chemicals in fungicide resistance management programmes (Fry 1982).

This work has identified several fungicides as potent inhibitors of *S. oleagina* conidium germination and these may have potential to protect olive trees from OLS. They include kresoxim-methyl, captan, boscalid, cyprodinil/fludioxonil, and boscalid/pyraclostrobin. *In vivo* screening with whole plants is considered to be the most accurate approach for predicting fungicide performance in the field (Knight et al. 1997). The selected fungicides are currently being tested with potted plants and in the field, to further validate their efficacy in controlling OLS.

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