Fungicide Sensitivity of *Phaeomoniella chlamydospora*, the Causal Organism of Petri Grapevine Decline

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Twelve fungicides, benomyl, chlorothalonil, fenarimol, fosetyl-Al, iprodione, kresoxim-methyl, mancozeb, metalaxyl, prochloraz manganese chloride, quintozene, tebuconazole and thiram were screened *in vitro* for their ability to inhibit mycelial growth of *Phaeomoniella chlamydospora*, the causal organism of Petri grapevine decline. Isolates of *Pa. chlamydospora* were obtained from different geographical areas in the Western Cape province. The effective concentration at which 50% of mycelial growth was inhibited (EC_{50}) was calculated for each fungicide. Benomyl, fenarimol, kresoxim-methyl, prochloraz manganese chloride and tebuconazole were the most effective in inhibiting mycelial growth of *Pa. chlamydospora* with EC_{50} values ranging from 0.01 to 0.05 µg/mL. Data obtained in this study represent the base-line sensitivity of local isolates to these fungicides, which is important for monitoring the development of pathogen resistance to fungicides.

Grape production is an important agricultural industry in South Africa that earned an estimated R1,4 billion in 1998 (Anon., 1998). Losses caused by pests or diseases are thus of great concern to the industry. It has only recently been proven that the condition in which young grapevines show stunted growth and slow die-back is a disease caused by fungal pathogens (Morton, 1995; Ferreira, 1998; Scheck *et al.*, 1998; Mugnai *et al.*, 1999). This disease is widespread and causes serious losses in South African vineyards.

Several fungi have been associated with slow die-back of vines worldwide, but the organism most commonly isolated from affected vines in South Africa was identified as *Phialophora parasitica* (Ferreira *et al.*, 1994). However, after an extensive study, Crous *et al.* (1996) established the genus *Phaeoacremonium* for a group of fungal pathogens associated with die-back diseases of woody hosts. Although several species of *Phaeoacremonium* have been associated with the disease, namely *Pm. aleophilum*, *Pm. chlamydosporum* and *Pm. inflatipes* (Morton, 1995; Larignon & Dubos, 1997; Scheck *et al.*, 1998; Mugnai *et al.*, 1999), *Pm. chlamydosporum* is the dominant fungus isolated from symptomatic young grapevines in South Africa (Groenewald *et al.*, unpublished data) and Italy (Sidoti *et al.*, 2000). A re-examination of numerous *Pm. chlamydosporum* isolates from diverse geographical regions showed several prominent morphological differences between this species and others accommodated in *Phaeoacremonium*. Based on molecular (Dupont *et al.*, 1998), morphological and pathological differences, a new genus *Phaeomoniella* Crous & W. Gams was therefore introduced, typified by *Phaeomoniella chlamydospora* (=*Phaeoacremonium chlamydosporum*) (Crous & Gams, 1999).

Much confusion has surrounded the name of the disease. Morton (1995) reported a mysterious disease from young vines in California in which diseased vines had shiny black streaks in their vascular vessels and showed poor vine growth, sometimes leading to a sudden collapse of the vine. In response to the disease, plants produced black gum and thus the name 'black goo' originated (Morton, 1995). In older vines the disease has been referred to as esca, apoplexy and black measles, but in young vines it is called Petri grapevine decline (Mugnai *et al.*, 1999).

In order to effectively implement integrated control of *Pa. chlamydospora*, it is important to know how infection becomes established. Recently it was discovered that *Pa. chlamydospora* was present in apparently healthy propagation material (cuttings and rootstock) in a latent or endophytic form (Bertelli et al., 1998; J.H.S. Ferreira, pers. comm.). This evidence supports the belief that the disease could have its origin in propagation material. Preplant treatment of nursery material to eradicate the pathogen would thus be of practical use to the industry.

One possibility of eradicating the pathogen from cuttings is to soak them in a solution of systemic or other fungicides so that the chemicals can be taken up and transported through the vascular tissue of the cuttings. Similarly, fungicide drenches could be taken up by the rooted cuttings and transported through the plant and this may also offer good control. The purpose of this experiment was to screen a number of fungicides against *Pa. chlamydospora* isolates to determine their effect on mycelial growth. Results of this screening test also provide base-line sensitivities of South African isolates of *Pa. chlamydospora*. Data obtained in these *in vitro* tests should thus lead to further testing in pot and field trials.

MATERIALS AND METHODS

***In vitro* tests on mycelial inhibition:** Twelve fungicides representing contact and systemic products were screened (Table 1). The screenings were carried out in a block repetition conducted in December 1998 and January 1999. All twelve fungicides were tested at the following concentrations: 0.005, 0.01, 0.05, 0.1, 0.5, 1 and 5 µg a.i./mL. Chlorothalonil, thiram and mancozeb were also tested at 10, 50 and 100 µg a.i./mL. One litre of stock solution was prepared for each fungicide and appropriate dilutions were added to 1000 mL 2% malt extract agar (MEA, Biolab) (50°C). Only MEA was present in the control medium. Within 24 h after they had been poured, plates were inoculated with a 5 mm

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TABLE 1

Fungicides screened against *Phaeomoniella chlamydospora.*

Active ingredient	Trade name	Fungicide group	Type	EC_{50} value (μg a.i./mL)	Registered on grapevines in South Africa[a]
benomyl	Benlate	Benzimidazoles	Systemic	0.206	+
chlorothalonil	Bravo	miscellaneous	Contact	1.379	−
fenarimol	Rubigan	DMI-pyrimidine	Systemic	0.254	+
fosetyl-Al	Aliette	Organic compounds	Systemic	−	+
iprodione	Rovral Flo	Dicarboximide	Contact	5.13	+
kresoxim-methyl	Stroby	Strobilurin	Locally-systemic	0.457	+
mancozeb	Dithane	Dithiocarbamates	Contact	10.891	+
metalaxyl	Ridomil	Acylanalines	Systemic	13.59	+
prochloraz manganese chloride	Octave	DMI-imidiazole	Systemic	0.015	−
quintozene	PCNB	Aromatic compounds	Contact	11.199	−
tebuconazole	Folicur	DMI-triazoles	Systemic	0.148	+
thiram	Thiram	Dithiocarbamates	Contact	1.677	+

[a] According to Nel *et al.* (1999).

diam. mycelial disc from the cultured *Pa. chlamydospora* isolates. Two mycelial discs were placed opposite each other on plates. Six isolates of *Pa. chlamydospora* from different geographical regions (STE-U 2863-2868) were used for the tests. These isolates are maintained at the Department of Plant Pathology, University of Stellenbosch (STE-U). Linear mycelial growth was recorded after 14 days incubation at 22°C in the dark by measuring the perpendicular diameters.

The effective concentration at which mycelial growth was inhibited by 50% relative to the control (EC_{50}) was calculated. Percentage inhibition of mycelial growth relative to the control was plotted against fungicide concentration for each fungicide/isolate combination. Different regression functions were fitted to the percentage inhibitions and the r^2 were recorded. A linear function $y = a+b(conc)$ was fitted for iprodione, metalaxyl and quintozene. For fungicides benomyl, chlorothanonil, fenarimol, kresoxim-methyl, mancozeb, prochloraz manganese chloride, tebuconazole and thiram the function $y = a(1-exp(bx))$ was fitted and the EC_{50} value calculated. An analysis of variance was conducted on the EC_{50} values to determine significant differences between the inhibitory effects of the various fungicides.

RESULTS

Differences between the means of the two repetitions (1998, 1999) were not significant ($P = 0.2538$). Therefore, the pooled data of the two repetitions were used to calculate the EC_{50} values. Since there were no significant differences between isolates ($P = 0.3354$) in response to the fungicides, each isolate was regarded as a replicate in the statistical analysis. Interaction between the isolates and fungicides ($P = 0.6110$) was not significant. However, the effects of the various fungicides on mycelial growth were significantly different ($P = 0.0001$). No EC_{50} values could be calculated for fosetyl-Al, due to the lack of inhibition of mycelial growth. EC_{50} values calculated for the remaining 11 fungicides are shown in Table 1.

DISCUSSION

Ferreira (1998) reported that fosetyl-Al and metalaxyl depressed *Phaeoacremonium* growth in the laboratory and in glasshouse

tests. Our results showed no inhibition of mycelial growth at effective concentrations by fosetyl-Al or metalaxyl. Fosetyl-Al is reported to have weak curative action and is known to stimulate host resistance (Schwinn & Staub, 1995) rather than being fungicidal. Since it has a broad spectrum of applications ranging from foliar sprays to trunk injections and dip treatment (Schwinn & Staub, 1995), and is also known to move symplastically in plant tissue, it may be able to stimulate host defence mechanisms which would prevent the spread of the pathogen. Although fosetyl-Al was found to be unsuccessful for the control of the esca disease complex (Mugnai *et al.,* 1999), Ferreira (1998) reported success in glasshouse trials and hence further testing to determine the exact effect of this chemical on disease control should be carried out.

Metalaxyl is known to target some chromistan fungi, specifically the Oomycetes (Kerkernaar & Kaars Sijpesteijn, 1981). It has been effectively used to control *Pythium* and *Phytophthora* in soil (Schwinn & Staub, 1995). Ferreira (1998), however, reported effective disease control of *Pa. chlamydospora* with metalaxyl in laboratory and glasshouse tests. Results from our study showed that metalaxyl had no inhibiting effect on the *in vitro* mycelial growth of this fungus. The unexpected effective control of *Pa. chlamydospora* by metalaxyl, as reported by Ferreira (1998), is therefore unexplained by this study.

An important management aspect of vascular diseases is prevention rather than the cure of the disease. The use of *Phaeomoniella*-free cuttings is of primary importance, and healthy pathogen-free propagation material should be planted in pathogen-free soil. However, it is difficult to ensure pathogen-free nursery stock, because the fungus could be latent or endophytic in rootstock cuttings (Bertelli *et al.,* 1998). Therefore, pre-plant treatments with fungicides might be useful in eradicating the pathogen from cuttings. Benomyl, fenarimol, kresoxim-methyl, prochloraz manganese chloride and tebuconazole showed mycelial inhibition of *Pa. chlamydospora* at low concentrations (0.01–0.5 ppm). This *in vitro* test gives a good indication of which fungicides can be selected for further studies in glasshouses and nurseries. These data could therefore contribute to inte-

grating an effective fungicide application programme. When used in combination with sanitation and stress relief, healthy, productive grapevines are an achievable goal.

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