SENSITIVITY OF *PHAEOMONIELLA CHLAMYDOSPORA* TO FUNGICIDES IN VITRO

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ABSTRACT

Phaeomoniella chlamydospora has recently been shown to cause Petri vine decline in young grapevines. No recommendations are available for management of the disease using fungicides. In this study, 22 fungicides were tested for their effect on the radial growth of three field isolates of *P. chlamydospora*. Systemic fungicides inhibited mycelial growth of the fungus at similar concentrations to those reported for other fungi. The DMI fungicides, cyproconazole, bitertanol, tebuconazole, fenarimol, myclobutanil and prochloraz, gave EC₅₀ values for reduction of mycelial growth of less than 0.3 mg/litre, whereas triforine gave an EC₅₀ value of 26 mg/litre. For the benzimidazole fungicides, benomyl, carbendazim and thiophanate methyl, the EC₅₀ values were less than 0.4 mg/litre. For the anilopyrimidines, pyrimethanil and cyprodinil/fludioxonil (Switch), EC₅₀ values were less than 0.02 mg/litre. For the contact fungicides tested, EC₅₀ values were higher than those reported for similar fungi. The potential role of the fungicides in disease management is discussed.

Keywords: *Phaeomoniella*, grapevine decline, fungicides, *in vitro* assessment.

INTRODUCTION

Petri vine decline ('black goo' disease) in young grapevines (Mugnai et al. 1999) is currently of concern to grape growers, throughout the world. Affected young vines are frequently stunted with chlorotic, sparse and stunted leaves, and may break bud late in spring. Older vines often fail to thrive, produce poor fruit crops, and decline over a number of years. Internal examination of trunks and cordons shows longitudinal brown to black streaks. In cross-section, these lesions appear as dark spots which ooze tiny pinheads of shiny, black, tarry substances from cut surfaces. Several species of *Phaeoacremonium* are associated with these symptoms, however *Phaeoacremonium chlamydosporum* was identified as the fungus most commonly isolated from affected vines. It was initially identified by Crous et al. (1996) but later renamed *Phaeomoniella chlamydospora* Crous & Gams, after further molecular (Dupont et al. 2000), pathological and morphological studies (Crous et al. 2000). Although *P. chlamydospora* is not known to cause vine death, infected vines are thought to be more susceptible to other diseases since dead vines often show symptoms of other, often fatal diseases, such as black foot caused by *Cylindrocarpon* spp., or esca caused by *Fomitiporia punctata* (Mugnai et al. 1999).

The development of control strategies for prevention of Petri vine decline has been restricted because little is known of how the pathogen infects and spreads. The pathogen has been found in apparently healthy grape propagation material (Bertelli et al. 1998) which has led to speculation about possible infection routes for *P. chlamydospora* in young and mature vines. Cuttings may become infected either directly from infected source vines or by contamination of their cut surfaces by conidia of *P. chlamydospora*. Conidia are known to be produced on the surface of deep fissures in vine trunks and cordons (I. Pascoe, pers. comm.) and are able to infect through wounds in grapevine

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wood (Larignon & Dubos 2000). Either route provides for potential control by fungicides, either as wound protectants or by applying systemic compounds to propagation material in vineyards, during grafting or in nurseries. In this study, fungicides were screened for effects on mycelial growth of *P. chlamydospora*, to identify chemicals for further evaluation in the control of Petri vine decline.

METHODS

In vitro tests were conducted using three isolates of *P. chlamydospora*, which originated from samples of three infected vines, taken from New Zealand rootstock blocks. The 22 fungicides selected for study were either registered for use on grapes in New Zealand or were known to be effective against fungi related to *P. chlamydospora*. Stock solutions of some of the fungicides were made by suspending the commercial products directly in 1000 ml of water, while technical grade chemicals (chlorothalonil, fenarimol, orthocide, thiram and thiophanate methyl) were first dissolved in 10 ml of acetone, before addition of 990 ml of water as recommended by Tomlin (1994). At least six concentrations were made of each fungicide (Table 1), the most appropriate concentrations being selected from similar studies reported in the literature. For all the fungicides, except Switch (contains cyprodinil), final concentrations were made in molten (50°C) potato dextrose agar (Difco) and 20 ml aliquots were poured into Petri dishes. Within 2–4 h after pouring, the plates were each inoculated with three 5 mm diameter discs cut from the actively growing front of 2 week old colonies of the isolates. For cyprodinil, Czapek-Dox agar (Oxoid) was used because fungicide activity was reported to be reduced within complex media. In addition, the 5 mm inoculum discs were cut from plates on which conidia of *P. Chlamydospora* had been spread for germination 48 h earlier, because older mycelia were reported to be resistant to this fungicide (Hilber & Schuepp 1996). For all fungicides, three replicate plates were inoculated for each fungicide concentration, for each of the three *P. chlamydospora* isolates. Plates were incubated at room temperature (16–24°C) in natural light (day length 14–15 h).

After 4 weeks, when colony diameters of control plates were approximately 40 mm, the diameters of all colonies were measured and the mean diameter of the three colonies on each plate recorded. Mean diameters were used to calculate growth rate inhibition for each plate. Because data from the three isolates were very similar, all data were analysed together. Means were plotted against $\log_{10}$ values of the fungicide concentrations. Different regression functions were fitted for different fungicides, depending upon the shape of the dose/response curves. For the 14 fungicides with sigmoidal curves, probit analysis was used to fit the curves, and to calculate the EC_{50} values (concentrations of the fungicides which reduced mycelial growth by 50%) and their 95% confidence limits. For the remaining eight fungicides, the dose/response curves were not sufficiently sigmoidal for probit analysis and the EC_{50} values were 'read' directly from regression lines fitted to the data. However, their 95% confidence limits for the EC_{50} values could not be calculated (Table 1).

RESULTS AND DISCUSSION

The systemic fungicides tested inhibited mycelial growth of *P. chlamydospora* at similar concentrations to those described in the literature for other Hyphomycetes (Table 1). For the anilopyrimidines, the EC_{50} values for mycelial growth of *P. chlamydospora* were within the sensitive range listed for *Botrytis cinerea* (Hilber & Schuepp 1996). For the benzimidazoles, the EC_{50} values were similar to those described for *Sclerotinia homeocarpa* and *B. cinerea* (Burpee 1997; Grindle 1981). Similarly, the EC_{50} values of the DMI fungicides were all within the ranges reported for sensitive fungi by other authors (Burpee 1997; Grindle 1981; Liggit et al. 1997; Herman & Gisi 1994; Parker & Sutton 1993). They all inhibited mycelial growth at low concentrations, except triforine which gave an EC_{50} value of 26 mg/litre. The only other systemic compound which was relatively ineffective against *P. chlamydospora* was the dicarboximide, iprodione, with an EC_{50} value of 10 mg/litre, which is considerably more than the 0.3–0.5 mg/litre reported for *S. homeocarpa* and *B. cinerea* (Burpee 1997; Grindle 1981).

TABLE 1: Details of fungicides and their efficacy against *Phaeomoniella chlamydospora*.

Chemical group	Fungicide name	Trade name	Concentration tested (mg ai /litre)	EC ₅₀ (mg ai/litre)	95% C.L. (+/-) ²
Anilopyrimidines	cyprodinil/fludioxonil (60:40)	Switch	0, 0.01, 0.033, 0.1, 0.33, 1.0, 10	0.019	0.214
	pyrimethanil	Scala	0, 0.001, 0.003, 0.01, 0.033, 0.1, 0.33, 1.0	0.011	0.006
Benzimidazole	benomyl	Benlate	0, 0.01, 0.033, 0.1, 0.33, 1.0, 10	0.079	0.118
	carbendazim	Bavistin	0, 0.001, 0.01, 0.033, 0.067, 0.1, 0.33, 1.0	0.078	0.053
	thiophanate methyl	tech grade ¹	0, 0.01, 0.033, 0.1, 0.33, 0.67, 1.0	0.312	0.16
Copper hydroxide		Kocide DF	0, 0.1, 0.33, 1.0, 3.3, 10, 100	11.5	22.68
Cyclic imides	folpet	Phaltan 50W	0, 0.1, 1.0, 10, 33, 100, 1000	33.1	
	orthocide	tech grade	0, 1.0, 3.3, 10, 33, 100, 1000	27.5	
Dicarboximide	iprodione	Rovral WG	0, 0.1, 0.33, 1.0, 3.3, 10, 100	10.09	18.71
Disulphide	thiram	tech grade	0, 0.1, 1.0, 3.3, 10, 33, 100	17.4	
DMI-imidazole	prochloraz	Sportak EW	0, 0.001, 0.003, 0.01, 0.033, 0.1, 0.33, 1.0, 10	0.019	0.037
- piperazine	triforine	Saprol 190EC	0, 0.1, 1.0, 3.3, 10, 33, 100	26.3	
- pyrimidine	fenarimol	tech grade	0, 0.01, 0.033, 0.1, 0.33, 1.0, 10	0.176	0.14
- triazole	bitertanol	Baycor 300EC	0, 0.01, 0.1, 0.33, 1.0, 3.3, 10	0.188	0.277
	cyproconazole	Alto 100SL	0.01, 0.033, 0.1, 0.33, 10, 10	0.148	0.174
	flusilazol	Nustar	0.003, 0.01, 0.033, 0.1, 0.33, 1.0	0.031	0.044
	myclobutanil	Systhane 40W	0.01, 0.033, 0.1, 0.33, 1.0, 10	0.25	
	tebuconazole	Folicur 430SC	0.01, 0.033, 0.1, 0.33, 1.0, 3.33	0.06	0.146
Nitrile	chlorothalonil	tech grade	0.1, 0.33, 1.0, 3.3, 10, 100	9.12	
Phosphorous acid	potassium phosphate salts	Foli-R-Fos 400	1.0, 10, 33, 100, 333, 1000	>1000	
Quinolinic	hydroxyquinoline sulphate	Chinosol W	0.1, 0.33, 1.0, 3.33, 10, 100	9.12	
Strobilurin	kresoxim-methyl	Stroby WG	0.001, 0.003, 0.01, 0.033, 0.1, 1.0	0.086	0.153

¹Technical grade.²Some data did not fit probit analysis, so 95% confidence intervals were not calculated.

For the contact fungicides tested, EC₅₀ values for mycelial growth of *P. chlamydospora* were greater than those for other similar fungi (Smith & Black 1993). This indicated that the pathogen is much less sensitive to contact fungicides than other fungi. Since these fungicides prevent infection largely by inhibition of spore germination and germ tube elongation, germination studies need to be conducted to investigate these aspects. Such assays will indicate which fungicides may be effective as dressings to prevent infection through wounds, or as disinfectants of dormant cuttings, which is a common use of hydroxyquinoline.

These results clearly indicate that systemic fungicides could be used to inhibit development of *P. chlamydospora* mycelium within grape wood. Further greenhouse studies are needed to investigate the effects of different application strategies on incidence and severity of Petri vine decline in young vines. In addition, some products which are known to have greater effect in *in vivo* studies, such as phosphorous acid which is thought to induce plant resistance responses, can only be effectively tested on live plants. Such trials will give valuable information about the potential for using fungicides within an integrated disease management programme for control of Petri vine decline.

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