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Curative treatments trialed on young grapevines infected with *Phaeomoniella chlamydospora*

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Summary. Internal symptoms of Petri disease include brown wood-streaking and an abnormally dark pith, caused by *Phaeomoniella chlamydospora* (*Pch*). This paper reports on the effect of eight fungicides and a hot water treatment on naturally infected one-year-old Pinot Noir rootlings in a glasshouse trial. Vines were destructively assessed 22 weeks after planting. All treatments significantly reduced the incidence of dark pith. Phosphonate treatment was the most effective and benzothiodiazole was the least effective treatment with respect to controlling *Pch*. These results will contribute to the development of an integrated management program for grapevine propagating material.

Key words: Petri grapevine decline, black goo, *Vitis vinifera*, fungicides, hot water treatment.

Introduction

Petri disease[(1)] is a disease of young vines resulting in stunted growth and poor vineyard establishment. The causal organism is the fungus *Phaeomoniella chlamydospora* (*Pch*) (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams (Crous and Gams, 2000). Internal symptoms include brown wood-streaking in longitudinal sections and black dots in transverse sections of the trunk, and an abnormally dark pith. *Pch* has been detected in apparently healthy one-year-old rootlings prior to planting, suggesting that the disease can be spread via infected propagation material (Bertelli *et al*., 1998). Preliminary *in vitro* studies in Italy (Di Marco *et al*., 1999), the United States (Khan and Gubler, 1999) and South Africa (Groenwald *et al*., 2000) tested fungicide sensitivity in *Pch* and identified a number of promising fungicides for further glasshouse evaluation. Phosphonate combined with resveratrol reduced mycelial growth of *Pch in vitro* (Di Marco *et al*., 1999) as did prochloraz manganese chloride and tebuconazole (Groenewald *et al*., 2000). *In vivo*, phosphonate (Di Marco *et al*., 2000), benomyl and fenarimol (Khan and Gubler, 1999) were successful in reducing symptoms of *Pch*.

At present, there are no recommendations to help nurseries ensure they provide *Pch*-free stock. Hot water treatment of cuttings is recommended as standard practice in Victoria, Australia (Waite

(1) At the general Assembly of the 2nd ICGTD meeting held in Lisbon 2001 it was unanimously decided that young grapevine decline, 'black goo', Petri vine decline will henceforth be called Petri disease.

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and May, 1999) but its effectiveness on *Pch* infection has not been verified. Systemic fungicides or those readily taken up by the root system are potential candidates for control. This paper reports on the effect of several treatments trialed on naturally infected one-year-old Pinot Noir rootlings.

Materials and methods

During 2000, testing of one-year-old Pinot Noir rootlings supplied by a Victorian nursery found that all of the test sample (five plants) had internal brown wood-streaking colonised by *Pch*. On the basis of this, the nursery rejected the plants, donating 200 for use in research. The rootlings were divided into groups of 20 and each group was subjected to one of ten treatments (Table 1). The treatments were chosen to represent a range of different modes of actions and, where possible, included those identified as promising by Di Marco *et al.* (1999; 2000), Khan and Gubler (1999) and Groenewald *et al.* (2000).

Prior to planting, the roots were trimmed to 15 cm and the canes were cut back to three buds (as per commercial practice). The vines were planted singly in 15 cm pots, placed in a randomized design in the glasshouse, and watered and fertilised as required. The hot water treatment was applied immediately prior to planting. All other treatments were applied as a foliar spray to run-off (40–50 ml/vine) combined with a soil drench to saturation (200–250 ml/pot) at two and 19 weeks after planting (growth stages eight leaves separated and pre-harvest respectively).

Vines were destructively assessed at the end of the growing season as leaves began to senesce (22 weeks from planting). Cane and root dry weights, stem fresh weight and diameter were recorded per vine. The stems were surface-sterilised by dipping in 100% ethanol and then flaming, then split longitudinally and examined for the incidence and intensity of brown wood-streaking and dark pith. Intensity was assessed using a scale from 0 to 3, where 0 indicated no discolour-

Table 1. Treatments applied to naturally *Pch*-infected Pinot Noir rootlings.

Treatment (a.i.)	Product[a]	Rate	Time of applications (growth stage)
Control	Water		1st: 8 leaves separated 2nd: pre-harvest
Hot water treatment		50°C for 30 min	pre-planting
Benomyl	Benlate DF® (Du Pont)	0.4 ml l^{-1}	1st: 8 leaves separated 2nd: pre-harvest
Benzothiodiazole	Bion 500WG® (Novartis)	0.08 g l^{-1}	1st: 8 leaves separated 2nd: pre-harvest
Fenarimol	Rubigan 120SC® (Dow Agriscences)	0.2 ml l^{-1}	1st: 8 leaves separated 2nd: pre-harvest
Kresoxim-methyl	Stroby WG® (Basf)	0.1 g l^{-1}	1st: 8 leaves separated 2nd: pre-harvest
Phosphonate	Agri-fos Supol 400® (Agrichem)	50 ml l^{-1}	1st: 8 leaves separated 2nd: pre-harvest
Prochloraz	Sportak® (Aventis)	0.2 ml^{-1}	1st: 8 leaves separated 2nd: pre-harvest
Tebuconazole	Folicur® 250 (Bayer)	2.5 ml l^{-1}	1st: 8 leaves separated 2nd: pre-harvest
Triadimenol	Bayfidan 250EC® (Bayer)	0.1 ml l^{-1}	1st: 8 leaves separated 2nd: pre-harvest

[a] All products were supplied by Australian companies.

ation and 3 indicated strong discolouration. Immediately after examination, the stem pieces were moist incubated for 4–6 weeks and re-examined at ×40 for the presence of fungal growth. Differences between treatments with respect to incidence of the above-mentioned symptoms and *Pch* were determined using the logistic regression. The group average method of cluster analysis (Gordon, 1981) was also used to classify the treatments with respect to their effectiveness against the above-mentioned symptoms and *Pch*.

Results

Visual assessments of the growth stages of the vines indicated that the growth of the hot water treated vines was retarded and that phosphonate was slightly phytotoxic, causing some leaf scorch. However, the fresh and dry weight measurements showed no significant differences between treatments (data not shown), indicating that the affected vines had recovered by the time assessments were made.

When the effects of the treatments on symptoms and presence of the fungus were analysed independently, all treatments significantly reduced the incidence and intensity of dark pith ($P<0.01$), but none significantly reduced either the brown wood-streaking or the incidence of *Pch*. No *Pch* was observed in the phosphonate treatment (Table 2).

Cluster analysis showed that the phosphonate treatment was different from the rest and the furthest removed from the control, indicating that it was the best treatment with respect to controlling *Pch*. Benzothiodiazole was the least effective (Fig. 1).

Discussion

The modes of action of the fungicides we tested included inhibition of ergosterol biosynthesis (fenarimol, prochloraz, tebuconazole and triadimenol) (Tomlin, 2000), induction of systemic acquired resistance (benzothiodiazole) (Heil *et al*., 2000), disruption of host plant metabolism (phosphonate) (Guest and Grant, 1991), inhibition of microtubulin biosynthesis (benomyl) (Hewitt, 1998), and inhibition of cellular respiration and interference with mitochondrial activity (kresoxim-methyl) (Vasciminno *et al*., 1997). Cluster analysis operating on a similarity matrix obtained from the proportions in Table 2 revealed three distinct groups. Group 1 contained the four most effective treatments: phosphonate, prochloraz, benomyl and fenarimol; Group 2 contained kresoxim-methyl, triadimenol, tebuconazole, hot water treatment and benzothiodiazole, and Group 3 contained only the control. All treatments successfully limited symptom development, particularly pith discolouration,

Table 2. Effect of treatments on symptom appearance (brown wood-streaking and dark pith) and presence of *Pch* (sporulation in wet chamber) in one-year-old Pinot Noir rootlings. Incidence of symptom was measured as the percentage of 20 vines per treatment showing discolouration; intensity of symptom was assessed using a scale of 0–3, where 0 = no discolouration, 1 = faint discolouration, 2 = moderate discolouration and 3 = strong discolouration. The values are expressed as the average over 20 vines per treatment.

Treatment	Wood-streaking		Dark pith		*Pch* (%)
	Incidence (%)	Intensity (0–3 scale)	Incidence (%)	Intensity (0–3 scale)	
Control	95	2.90	95	2.85	30
Hot water treatment	60	2.40	30	1.05	20
Benomyl	65	2.40	45	1.50	10
Benzothiodiazole	50	2.35	40	1.40	25
Fenarimol	70	2.50	30	1.10	10
Kresoxim-methyl	75	2.70	35	1.25	20
Phosphonate	70	2.65	15	0.75	0
Prochloraz	56	2.22	44	1.56	6
Tebuconazole	70	2.55	30	1.00	25
Triadiamenol	75	2.75	30	1.10	15

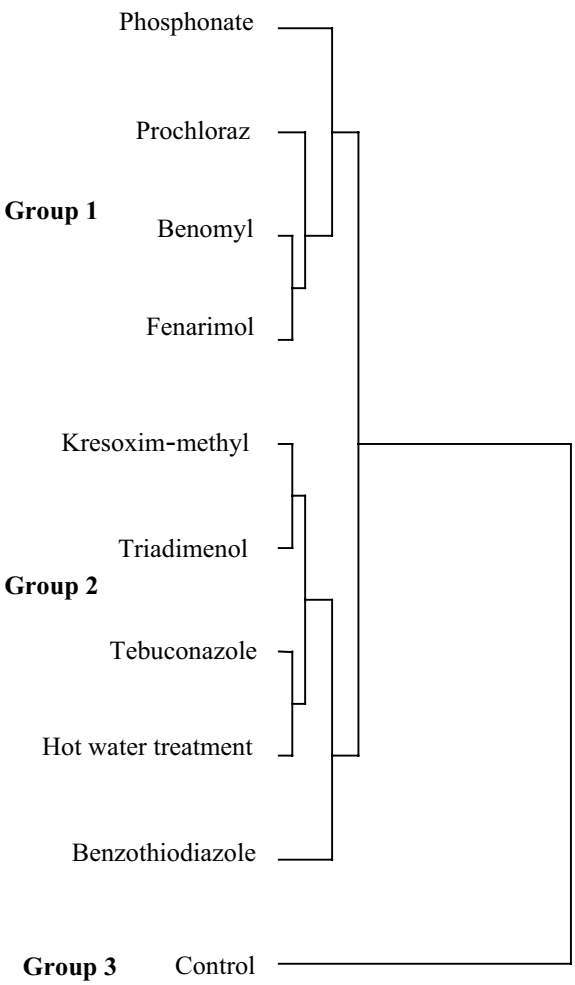

Fig. 1. Dendrogram of treatments with respect to their effectiveness against *Phaeomoniella chlamydospora* infection of one-year-old Pinot Noir rootlings constructed using cluster analysis determined by the group average method and Euclidean distance.

but due to the low level (30%) of *Pch* present in the untreated control plants, only phosphonate (0% *Pch*) can be identified from this study as a potentially useful curative treatment for young vines infected with *Pch*.

The four fungicides from Group 1 (phosphonate, prochloraz, benomyl and fenarimol) have all been reported as promising by other research groups. Groenewald *et al*. (2000) found that benomyl, fenarimol, kresoxim-methyl, prochloraz manganese chloride and tebuconazole all effectively inhibited *Pch* mycelial growth *in vitro*, although phosphonate did not. However, Di Marco reported that phosphonate acts synergistically with resveratrol, a host defence compound, and demonstrated that when the two were present together, phosphonate was effective against *Pch* both *in vitro* (Di Marco *et al*., 1999) and *in vivo* (Di Marco *et al*., 2000). Khan and Gubler (1999) showed that benomyl and fenarimol reduced symptom development in young vines inoculated with *Pch*.

In the present study the poorest compound was benzothiodiazole, which acts by eliciting systemic acquired resistance in the host. This mode of action is unlikely to be effective against existing infections. We also demonstrated that hot water treatment was not very effective as a curative treatment, in accord with the results of Rooney and Gubler (2001), who found that immersion of *Pch*-inoculated cuttings in a hot water bath (51°C) for 30 minutes was not effective against subsequent disease development. However, subjecting *Pch* spore suspensions to 51°C for 15 minutes prevented the conidia from germinating (Whiting *et al*., 2001), so in practice hot water treatment may not play a role as a curative treatment but is potentially useful as a disinfectant during the propagation process.

At present, the importance of using *Pch*-free material in vineyard establishment is recognised, but it is not possible for nurseries to ensure *Pch*-free stock. The results from this study will contribute to the development of an integrated management program for grapevine propagating material.

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