SOAK TREATMENT OF GRAPEVINE PROPAGATION MATERIAL AGAINST PETRI DISEASE

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Phenolic compounds were assessed for their ability to suppress development of causal agent of Petri disease Phaeomoniella chlamydospora. Phenol, cresol and thymol used in in vitro assays effectively suppressed the mycelial growth of this fungus in Petri dishes and also in grapevine wood segments artificially inoculated with P. chlamydospora. Consequently, these compounds were used for a soak treatment of grapevine propagation material and their phytotoxicity was evaluated. Results of this study suggest that phenol and cresol could be potentially used for control of P. chlamydospora.

Key words: grapevine, propagation material, treatment, phenolic compounds, control, Phaeomoniella chlamydospora

Phaeomoniella chlamydospora was recently described as an important trunk disease causing pathogen (Mostert et al. 2005), and the main causal agent of Petri disease in Slovakia (Kakalíková et al. 2006) resulting in premature decline and dieback of grapevines. Blocked xylem vessels as the host response to the fungal growth lead to insufficient water and nutrient supply to the vegetative plant parts. This consequently leads to symptom expression, which usually occurs during periods of high water demand (Ferreira et al. 1999).

A major means of spread is believed to be via infected propagation material, specifically rootstock material (Fourie & Halleen 2002; Halleen et al. 2003). P. chlamydospora can infect rootstock mother vines and disseminate from these infections by means of conidia or hyphal fragments via xylem vessels into the rootstock canes (Ridgway et al. 2002; Edwards et al. 2004; Fourie & Halleen 2002, 2004a). Unprotected wounds might also be infected at the various nursery stages. DNA of P. chlamydospora was detected at various stages during the grapevine propagation process (hydration tanks, drench water, grafting tools, callusing media) in nurseries (Whiteman et al. 2003; Retief et al. 2006) indicating that these should be considered as potential inoculum sources for this pathogen.

On the basis of recent research, various proactive management strategies have been recommended for prevention of propagation material infestation by trunk disease pathogens during the grafting and nursery stages. Prior to grafting, rootstock cuttings can be soak-treated in various chemical or biological formulations, practice that resulted in significantly reduced fungal infection levels in the nursery plants (Fourie & Halleen 2004b, 2006).

The aim of this study was to investigate the effect of some chemical soak treatments (basic phenols) to prevent natural infection of grapevine propagation material by trunk disease pathogens, P. chlamydospora.

The sensitivity of P. chlamydospora was determined by comparing the radial growth on phenolic compound amended medium to growth in non-amended medium.
Phenol, cresol and thymol were dissolved in sterile distilled water to obtain stock solution of 10 mg/ml. Each compound was incorporated into media after the media was autoclaved at the final concentration 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 mg/ml of compound. A 7 mm in diameter mycelial plug, from the margin of a fifteen day old actively growing culture of fungus, was inverted and transferred to the centre of Petri dishes (90 mm) with the amended media and the control. Sensitivity was assessed by measuring colony diameter of mycelial growth after 15 days of incubation at 25°C in the dark. Two perpendicular measurements of colony diameter, excluding the original plug diameter (7 mm), were obtained from each plate. Within each trial, isolates were replicated three times at each fungicide concentration. The diameter of each colony on fungicide amended medium relative to the diameter of the colony on non-amended medium was recorded. EC$_{50}$ values of each isolates were calculated by determining the effective fungicide concentration that inhibited mycelial growth by 50%.

Effect of phenolic compounds on fungal survival in grapevine tissues was assessed in grapevine wood segments artificially inoculated with _P. chlamydospora_. One-year-old dormant grape canes cv. Frankovka modrá were pruned, cut to segments of 40 mm in length, bark tissue was removed. A wound was created in each wood segment of cane by cutting to the pith with 2 mm diameter metal cork borer and then sterilized by autoclaving. Wood segments were immersed in sterile water for 20 min and then dipped in phenolic compounds solutions for another 20 min. Phenolic compounds solutions were prepared by dissolving phenolic compounds in sterile distilled water at final concentrations of 0, 0.2, 0.5, 1.0, 1.5 and 2 mg/ml). Control wood segments were immersed in water only. Wood blocks were then blotted on a sterile paper towel to remove excess liquid. Each wood segment was inoculated with _P. chlamydospora_ by inserting a 2 mm diameter inoculum plug from the margin of a five day old actively growing culture into each hole. The segments were covered with Parafilm. Each segment was placed into a separate, sterile Petri plate lined with sterile paper towel, moistened with sterile distilled water. The experiment consisted of 4 replicates. Plates were sealed with Parafilm and incubated for 30 days in the dark at 25°C. After 30 days, the concentration where there was no fungal growth at the ends of segments was recorded.

Figure 1. Comparision of EC$_{50}$ of phenolic compounds used _in vitro_ against _Phaeomoniella chlamydospora_ (less is better)
Evaluation of phenolic compounds phytotoxicity was conducted on rooted grapevine one-bud cuttings immersed for 24 hour in phenolic compounds solution (0.20, 0.50, 0.75, 1 mg/ml) and then left to grow in dark flasks with sterile tap water for 30 days in laboratory conditions. Control cuttings were immersed only in sterile tap water. Ten plants were used for each concentration. The percentage of bursting buds was recorded.

Several chemical, biological, and physical strategies are practiced and/or have been studied for hygiene and wound protection during the grapevine propagation process. Current practices in nurseries worldwide, aimed at limiting fungal infections on woody tissues, include from chemical strategies mainly drenches or dips of propagation material at various stages in a variety of broad-spectrum fungicides, such as 8-hydroxy-

Figure 2. Comparison of concentrations of phenolic compounds needed to full inhibition of *Phaeomoniella chlamydospora* in grapevine wood segments (less is better)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Phenol</th>
<th>Cresol</th>
<th>Thymol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration [mg/ml]</td>
<td>0</td>
<td>0.20</td>
<td>0.25</td>
</tr>
<tr>
<td>bursting buds [%]</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Phenol</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cresol</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Thymol</td>
<td>100</td>
<td>100</td>
<td>80</td>
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phenolic compounds against Petri disease pathogens – they inhibited enzyme activities of \( P. \) chlamydospora and have direct effect on fungal growth and sporulation (Río et al. 2004). Martin et al. (2009) concluded that the infection of young grapevine plants with \( P. \) chlamydospora induced an upregulation of plant defence mechanisms. This resulted in increased and localised accumulation of antifungal phenolic compounds (mainly resveratrol and viniferin), which are known to inhibit fungal growth. Di Marco et al. (2000) demonstrated activity of resveratrol, a common polyphenolic phytoalexin produced by grapevines, resulting in inhibition of this fungus. Extracts of the grapevine leaves, stems and berries of vines treated with Brotomax, which increases the biosynthesis of phenolic compounds, inhibited mycelial growth of the fungus. When vines affected by Petri disease were treated with Brotomax, plants showed an increase in growth and a reduction in Petri disease symptoms (Río et al. 2001). Our results are in coincidence with facts that phenolics inhibit fungal growth and indicate these compounds can be used as a preventive treatment for grapevine propagation material.

In conclusion, this study showed that all of the used phenolic compounds have direct antifungal activity against causal agent of Petri disease. Phenol and cresol could be potentially used for control of \( P. \) chlamydospora as a soak treatment of grapevine propagation material; thymol appears to be rather phytotoxic. It should be noted that the environment of the field is different than laboratory condition. Therefore, field trials need to be done for confirmation and adequate dosage regimen will have to be chosen to prolong usefulness of used compounds. However, because it is apparent that grapevine trunk pathogens cannot be eradicated from diseased plants, to suppress these diseases, whole complex of preventive treatments is needed so that nursery managers could ensure the production of high-quality grapevines with low level of infection.

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