EFFECTIVENESS OF TRIAZOLE SOIL TREATMENT AGAINST *PHAEOMONIELLA CHLAMYDOSPORA* ON GRAPEVINE PROPAGATION MATERIAL

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Introduction

In all grape growing countries the importance of trunk fungal diseases is increasingly being recognized. In different areas of Europe and the Mediterranean countries different pathogens prevail, such as *Eutypa lata*, *Botryosphaeria* species (Niekerk *et al.,* 2006), or pathogens more closely linked to collar and root infections such as *Cylindrocarpon* spp. (Halleen *et al*., 2006). But among these pathogenic fungi those causing esca of grapevine are surely the most widespread, and also those for which efficient control methods are most lacking (Di Marco, 2000).

One of the peculiarities of the main esca fungi, such as the tracheomycotic fungi *Phaeomoniella chlamydospora* (Pch) and *Phaeoacremonium aleophilum* (Pal), is that their spores can move from the wood vessels of colonized trunks to new, one-year-old canes, where those fungi produce latent, asymptomatic infections (Edwards and Pascoe, 2004; Edwards *et al*., 2004; Ridgway *et al*., 2003; Retief *et al*., 2005a), and where the wood is not colonized until later, when the vines are planted in the nursery soil for rooting. Colonization is often enhanced when the defence activity of the new vine is lowered by stress factors of various origin. Those early, asymptomatic infections can turn into Petri disease or young esca.

Ensuring that propagation material is free from infection is of basic importance when planning a control protocol for diseases of the esca complex. Latent infections can be substantially reduced by hot water treatment of cuttings or grafted cuttings, but this technique is not currently applied in most Mediterranean countries.

The present paper reports preliminary trials on the effectiveness with which a systemic fungicide applied to the soil in a slow-release granular formulation is able to keep the vessels free from spores carried by the sap flow in infected mother vines, and to prevent latent colonization of the wood in the grafted or non-grafted cuttings from those mother vines. Triazolic fungicides have a very efficient systemic movement through the plant with a wide spectrum of action, and they have already shown themselves to be fairly effective against the hyphomycetes causing esca (in particular Pch) (Di Marco *et al*., 2000; Jaspers, 2001; Groenwald *et al*., 2000). The triazoles have already been tested against esca in the field by soil application, but results were contradictory as at the time the aetiology and epidemiology of the disease were much less well understood. The vines tested in the earlier study were adult vines with a high incidence of wood colonization by a variety of pathogens, and they showed large areas of decayed and necrotic wood. Furthermore the formulations used were intended as foliar sprays (Di Marco *et al*., 2000).

This paper reports a preliminary test on the effectiveness of a new granular formulation of flutriafol, a triazolic fungicide (Atout® 0.5%), when applied in the soil to control Pch in artificially inoculated grapevine cuttings.

Materials and Methods

Fungicide tested

In vitro and *in vivo* trials were carried out to test flutriafol, a triazolic fungicide in a slow-release granular formulation, determining its systemic availability in relation to root system growth, and the absorption of the active ingredient by the vine.

In vitro tests

The growth inhibition of flutriafol was tested on Pch (CBS 229.95). The fungicide was added to potato dextrose agar (PDA) in 9-cm diam. Petri dishes, at concentrations of 0.001, 0.01, 0.1 and 1 μg ml^{-1}. The dishes were kept in growth chambers at 28±2°C in the dark and checked at 2-3-day intervals. Radial colony growth was recorded and mycelial growth inhibition determined as a percentage of the growth achieved with no fungicide, calculating the minimal inhibitory concentration (MIC) at which no mycelial growth was recorded.

In vivo trials

In April 2005, 150 40-cm-long, non-rooted rootstock cuttings SO4, clone 120, were taken from refrigerated storage rooms. Any wounds on the apical part had been protected with wax in the nursery to prevent desiccation. The basal end of 125 of the cuttings was dipped (after removing the first 2 cm), for 30 minutes in a 10^7 ml^{-1} Pch conidial suspension. Twenty-five of the cuttings were used as controls and dipped in water.

Cuttings were planted (2 per pot) in square pots (15 x 15), half filled with a mixture of peat:soil:sand (1:1:1), which was covered with a layer of sand that had been dried in an oven and mixed thoroughly with flutriafol so as to supply 0.17 g of active ingredient per pot (corresponding to 750 g of a.i. ha^{-1} previously used with other crops. The pots were then filled completely with the peat-soil-sand mixture. Vines were kept in an unheated glass-house and watered regularly.

Foliar symptoms were visually checked at weekly intervals. Isolations from the woody tissue were carried out soon after inoculation on 3 treated and 3 control vines. After 7 months, isolations were carried out on 12 treated and 5 control vines. Vines were debarked, surface-disinfected with 1% sodium hypochlorite, and cut into 10-cm segments. These segments were cut lengthwise and 5-6 wood fragments were taken in the 2 cm at the base of the stem and at 5-cm intervals from the base up to the top, and plated in PDA plus streptomycin.

Results and Discussion

In vitro tests

Mycelial growth of Pch was slightly reduced at a flutriafol concentration of 0.01 µg ml^{-1}; it was reduced to 45% at 0.1 µg a.i. ml^{-1}, while the MIC was determined to be 1 µg ml^{-1} (Fig. 1).

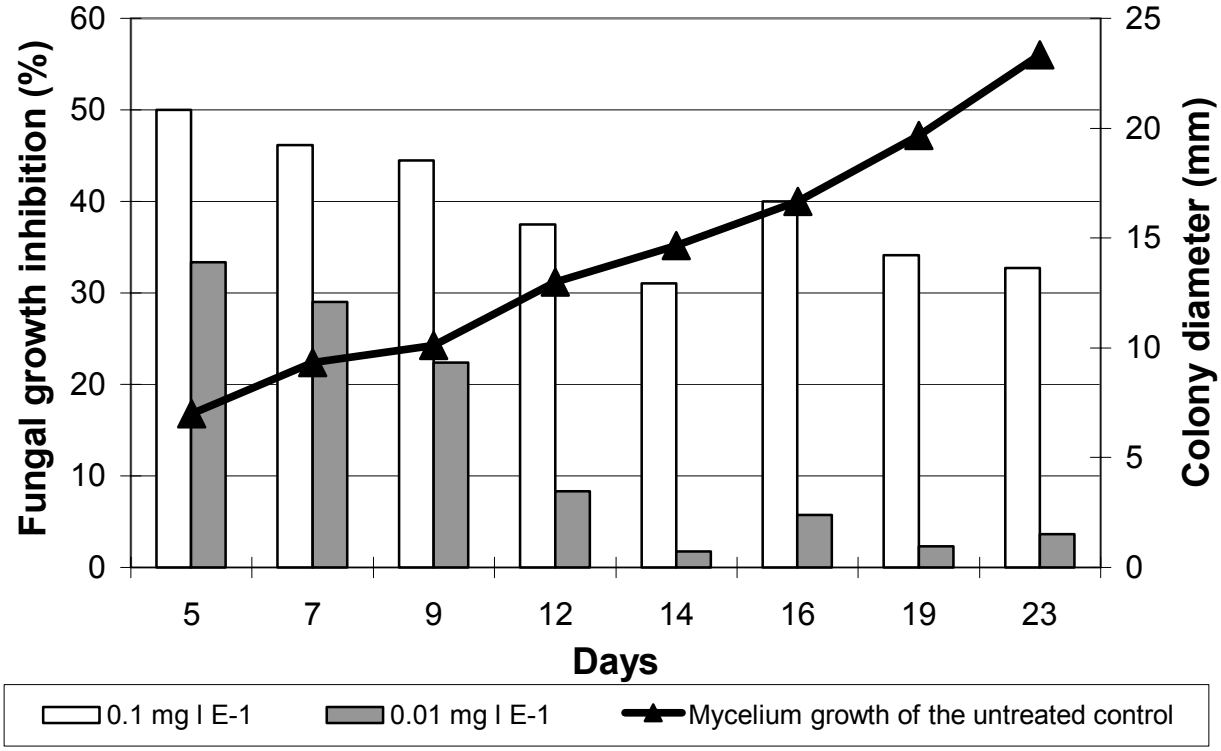

Fig. 1. Effectiveness *in vitro* of two concentrations of flutriafol (Atout® 0.5%) against *Phaeomoniella chlamydospora* mycelial growth.

In vivo trials

No difference was found between treated and untreated vines at sprouting (85-90%), nor were there any differences in foliar symptoms or growth throughout the trial. Moreover no toxic effects were recorded other than some chlorotic (or sometimes necrotic) spotting on treated vines at the very end of the season.

Blackish streaking in the wood was recorded after 7 months in both treated and control vines, always extending 5-6 cm beyond the highest area of successful isolation, both in control and in treated vines. Pch was successfully reisolated from 64-75**%** of the wood fragments in the first 15 cm above the inoculation point (base of the stem) in the control vines, and it was still present, though at a much lower frequency, at 20 cm (13.3% isolation). Treated vines showed a greatly reduced colonization in vines grown in soil with flutriafol; here Pch was reisolated infrequently (4-10%), and only up to 10 cm away from the stem base (Fig. 2).

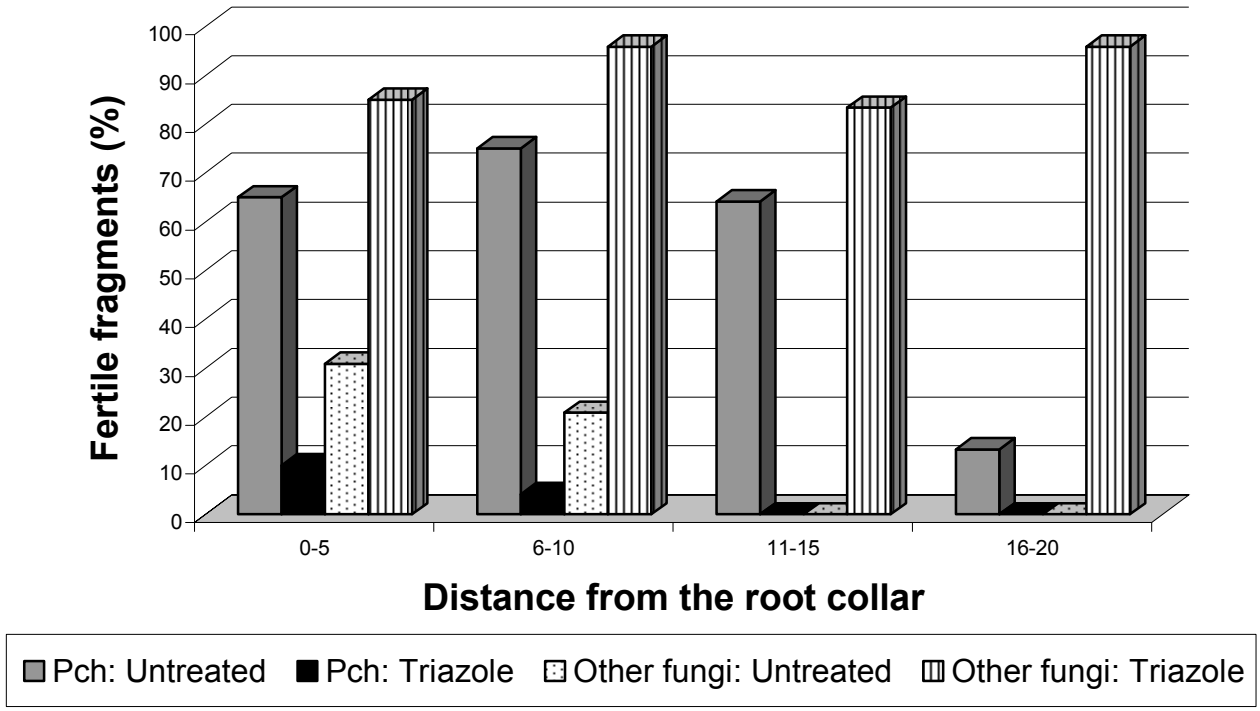

Fig. 2. Fungal isolation from the woody tissue of cuttings artificially inoculated with *Phaeomoniella chlamydospora* after application of 750 g a.i. ha^{-1} flutriafol to the soil.

The strong reduction in wood colonization obtained in *in vivo* trials despite the high inoculum concentration used seems to be a promising first step for further trials on the possible applicability of the granular formulation in the field. Flutriafol in the special slow-release formulation once the product has been applied to the soil will be further tested both for its effectiveness to prevent colonization of the canes of infected rootstock and scion mother vines, and to eliminate latent infections in newly grafted cuttings when they are planted in the nursery soil for rooting. Similar positive results have already been obtained with flutriafol protecting mother vines against coffee rust, *Hemileia vastatrix* (see http://www.cheminova.com/en/fungicides/impact/crops_and_diseases/coffee.htm). The frequent endophytic presence of Pch and Pal in propagating material (Edwards and Pascoe, 2004; Ridgway *et al.*, 2002; Retief *et al.*, 2005), and the many infection courts that the tracheomycotic esca fungi found during the preparation of grafted material in the nursery suggest that flutriafol in its granular slow-release form can be highly effective in soil treatments as part of a wider control programme which is necessary for esca disease, and which starts with the production of infection-free plant material.

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