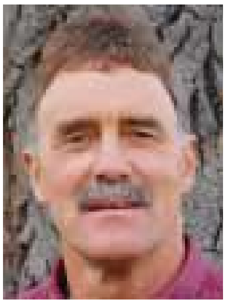
Trichoderma and trunk disease fungi: prospects for new protective management options



John S Hunt PhD
Technical director, Agrimm Technologies Ltd
PO Box 13-245, Christchurch, New Zealand

Introduction

Cordon and trunk dieback have been recognised for more than 60 years as a major disease affecting grapevines although demonstration that the *Eutypa lata* fungus is the causal agent is more recent. Eutypa dieback can affect a high percentage of vines in some old premium vineyards in South Australia (see right) causing significant losses to the industry. Losses in Shiraz alone are estimated to cost Australian growers $20million annually. Other trunk disease-causing fungi include *Botryosphaeria spp.* causing black dead arm, *Cylindrocarpon spp.* causing black root rot, *Phaeoacremonium* and *Phaeomoniella spp.* causing vine decline often associated with "black goo" symptoms. Esca is a more-complex disease involving a number of trunk disease-causing fungi. Although common in the Northern Hemisphere vineyards of France and Italy it has not yet been clearly identified in Australia and New Zealand. All these fungal diseases are generally spread by spores released from infected vines or other host plants during wet conditions being dispersed by wind currents and landing on fresh pruning wounds. Their economic impact on the vineyard may be quite significant but is as yet not well defined in Australasia [1].

Grapevine showing severe symptoms of Eutypa dieback in a South Australian vineyard.

With no chemical pesticides available for treatment of grapevine trunk diseases, management options have been largely preventive and limited to minimising infection risk by pruning in dry weather whenever possible. Also the use of wound sealants, often containing a fungicide such as benomyl for treatment of pruning cuts has been common practice. A relatively new option available for protective vineyard management is the application of beneficial microorganisms such as Trichoderma species. This natural, safe, soil-derived fungus can be applied to the soil as an amendment to improve root growth and health or to pruning wounds as a protectant. Commercial formulations containing this active ingredient have been available in New Zealand for over a decade and have recently been registered for use on grapevines in Australia.

Vaccinating the vine

New research has shown how this beneficial fungus can act as an immunising commensal (the term given to an organism that becomes established in a host and thereafter remains in a symbiotic/beneficial relationship with the host) to protect the vine with a "vaccination effect" once vines are inoculated so that the fungus becomes established inside the woody tissue of the trunk and cordon. The way in which this relationship works has been the subject of debate within the scientific community over the years as Trichoderma, being a saprophyte, normally lives by utilising dead organic matter as a food source. How then can it apparently live in amongst the healthy living tissue of a plant such as a grapevine? Previous research[2] has shown that Trichoderma could be re-isolated from vines and trees up to five years after inoculation with Trichodowel(R) or Trichoject(R) treatment. These plants had been treated when showing symptoms of disease and included kiwifruit vines with Armillaria root rot, grapevines with Eutypa dieback and fruit trees with silver leaf. Therefore, the Trichoderma that had been isolated could have been associated with the diseased part of the plant and not necessarily living in healthy tissue.

New research results from the work of Sharmini John, a student at Adelaide University, have shown how Trichoderma can exist within healthy grapevine tissue. Sharmini has just completed a PhD study[3] on the interactions of Trichoderma-inoculated grapevines, with and without co-inoculation with *Eutypa lata,* the fungus that causes dieback disease once vines become infected. Results from experiments with pruning wounds treated with Vinevax™ (formerly Trichoseal) wound dressing showed that the Trichoderma active ingredient was effective in protecting the wound against inoculation by *E. lata* (see Figure 1A). These results

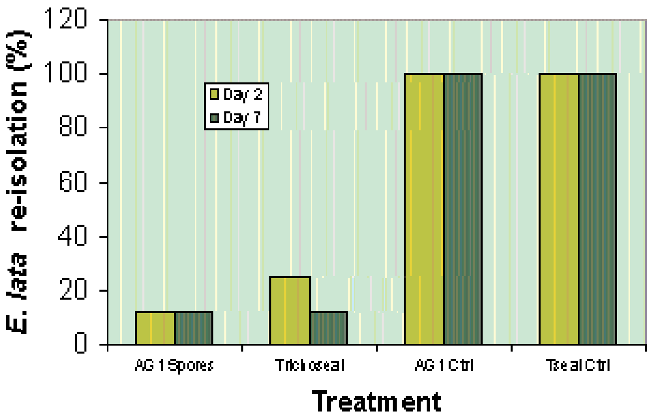


Fig. 1A. Protection of Eutypa-challenged pruning wounds by Trichoderma spores and Trichoseal. Glasshouse-treated canes (data courtesy of Sharmini John): pruning wounds made on glasshouse grown Shiraz canes (8 per treatment) were first treated with *T. harzianum* strain AG1 spore suspension or Trichoseal before being challenged with agar plugs of *E. lata* after 2 or 7 days. 12 weeks after treatment laboratory re-isolation was made on sections taken from below the wound. Controls were treated with gamma-irradiated material to destroy the bio-activity.

have been confirmed in a subsequent field trial conducted in California by Philippe Rolshausen, a PhD student at UC Davis, USA, where experimental wounds were treated and then also challenged with *E. lata* spores (see Figure 1B). In both sets of experiments the protection efficacy improved if the Trichoderma was allowed time to become established in the wound by treatment applied a few days prior to the challenge with pathogen.

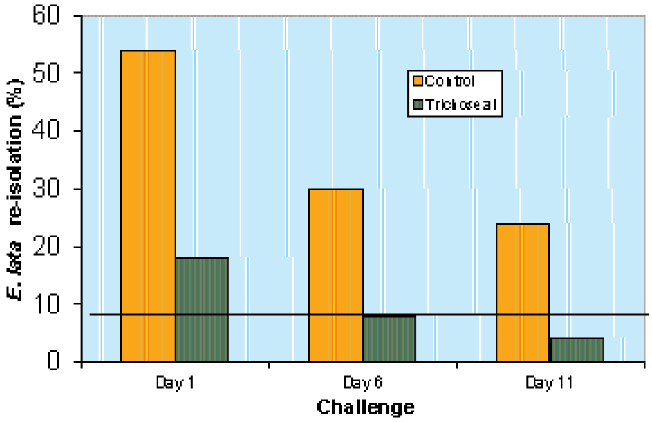


Fig. 1B. Vineyard treated vines (data courtesy of Philippe Rolshausen). Spur pruning wounds (50 per treatment) made on vineyard vines were first treated with Trichoseal before being challenged with *E. lata* spores (1000 spores per wound) 1, 6 or 11 days after the wound treatment. The following season laboratory re-isolation of *E. lata* was performed on sections made from below the wounded area. Dotted line represents the background *E. lata* in the vineyard isolated from unchallenged, untreated control wounds.

In other experiments John took scanning electron microscope (SEM) pictures from glasshouse-grown vines inoculated with the proprietary strain *Trichoderma harzianum* AG1 from Vinevax that showed this fungus growing through healthy vessels and in between the pith parenchyma cells[4]. When these same vines were co-inoculated with *E. lata* the SEM pictures showed evidence of fungal mycelium damage on *E. lata* (see Figure 2), indicative of a fungicidal effect that this *T. harzianum* strain had been shown capable of having *in vitro*[5].

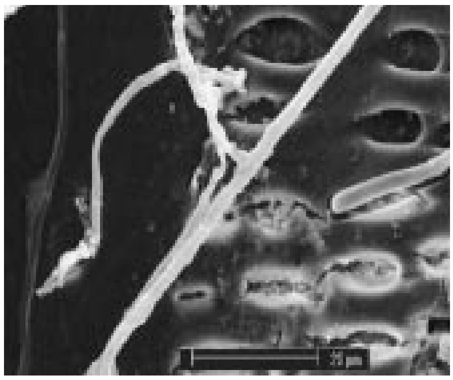


Fig. 2. Scanning electron micrograph of section obtained from glasshouse-grown cutting co-inoculated with *E. lata* and *T. harzianum.* Note the presence of collapsed hyphae centre left compared with healthy looking turgid hyphae top right. Photo courtesy of Sharmini John, Adelaide University.

These results show for the first time that Trichoderma can live in healthy grapevine tissues in association with the pith parenchyma cells. Furthermore, other experiments performed by John have shown that this particular *T. harzianum* strain, when inoculated into the healthy vine actively grows through the tissue over time. It has been isolated up to 18cm from the site of inoculation 18 months later[6]. These observations suggest Trichoderma has the potential to be applied as a treatment to vines for a prolonged protective effect. The concept of "vaccinating" grapevines in this manner has wide-reaching implications for the winegrowing industry.

The research project conducted in Adelaide has focused on Eutypa dieback, a particular problem causing significant economic loss in South Australian vineyards[7]. In New Zealand vineyards Eutypa dieback, although present, is not as prevalent as it is in Australia. Dr Ian Harvey of Plantwise Diagnostic

Services, Lincoln, has recently reviewed a considerable number of grapevine pathology samples over the past two seasons from New Zealand vines showing general decline symptoms. He has isolated *Cylindrocarpon destructans, Phaeomoniella chlamydospora, Phomopsis viticola* and various species of *Botryosphaeria* from these samples[8]. A great proportion of samples showed the presence of *Botryosphaeria spp.* suggesting that this pathogen may have a significant impact on vine decline in the New Zealand vineyard.

Recent *in vitro* analysis of Agrimm Technologies' proprietary strains of *T. harzianum* has shown evidence of both lysis (cell death) and parasitic activity on various species of *Botryosphaeria* (see Table 1). The myco-parasitic activity

	AGRIMM TRICHODERMA STRAIN													
PATHOGEN	AG 2		AG 3		AG 5		AG 8		AG 11		AG 15		AGS 28	
	L	P	L	P	L	P	L	P	L	P	L	P	L	P
Botryosphaeria stevensii	+++		+++		+++		+++	+	++	+	+++		++	+++
B. dothidea	++	+	+++		+++	++	+++		+++	++	+++		++	++
B. parva	++		+++		+++	+	+	+	++	+	+++		+	
B. lutea			+								+		+	+

L = lysis; P = parasitism

Table 1. *In vitro* activity of Agrimm *T. harzianum* strains against *Botryosphaeria spp.* trunk disease fungi. Dual inoculum cultures were established on malt agar by placing the pathogen on one side of the petri plate, grown for 24 – 96 hours and then the relevant strain of *T. harzianum* was placed on the other side. Plates were incubated for 5 days at 20ºC in the dark. Slides stained with lactophenol blue were observed under a light microscope and morphological changes to the target mycelium recorded by Dr Ian Harvey, Plantwise Services, Lincoln.

shown by *T. harzianum* on *B. stevensii* (see Figure 3) suggests a different mechanism of action from that on *E. lata,* as no evidence of myco-parasitism was observed in the Adelaide research study[5]. However, it is quite clear that the Trichoderma strains used as active ingredient in the Vinevax formulations have activity against many of the fungi-causing trunk disease and decline symptoms including the "black goo fungus", *P. chlamydospora* (data not shown).

In South Africa, pathology studies performed on field samples from nursery-grown grapevines near Stellenbosch, treated with Trichoderma formulations (Trichoflow at grafting and Trichopel at planting), by Dr Paul Fourie of ARC Infruitec-Nietvoorbj have confirmed the benefits from Trichoderma application[9]. Trichoderma-treated plants showed 42% better root growth than a standard treatment incorporating chemical fungicides. These treated plants also had 46% less pathogenic fungi associated with their roots with the rootstock showing 40% less incidence of *P. chlamydospora.*

Fig. 3. Light microscope image of interaction between *T. harzianum* and *B. stevensii.* Blue-stained strands of *T. harzianum* AGS28 show parasitic coiling around an unstained strand of *B. stevensii* (diagonal and bottom right). Photograph courtesy of Dr Ian Harvey, Plantwise, Lincoln.

While the focus of this research has been on disease protection or treatment there may be another equally important opportunity for application of the beneficial properties of Trichoderma. It seems that there is the potential for this remarkable fungus to be utilised as a treatment for improving grape yields and possibly also quality. To test this concept a number of 0.5ha field trials have been established in South

Australia on under-performing blocks of vines treated with Vinevax bio-dowel inoculation. The first set of results from the 2003 harvest of fruit from vines at a Clare Valley trial established in April 2002 showed a significant increase in the bunch numbers and yields from the treated flag vines (see Figure 4). When compared with untreated controls these 21-year-old Cabernet Sauvignon vines showed 19% more bunches and had a 46% increase in mean fruit weight per vine one season after treatment with 1 Vinevax bio-dowel. Pruning weights recorded in winter 2003 also showed a significant increase in mean weight per vine.

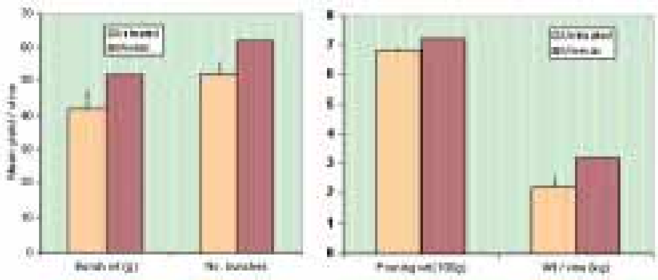


Fig. 4. April 2003 vintage yields from South Australia trial site treated in April 2002. Mean values obtained from April 2003 harvest from vines treated with 1 Vinevax bio-dowel per vine (n = 176) or untreated controls (n = 88). Statistical analysis of data was performed by ANOVA and least significant difference calculated at p <0.05. Bar represents LSD at p <0.05.

Discussion

What is the explanation for these surprising results? There may be a number of factors worthy of consideration. First, although there were no vines in the block showing symptoms of disease at the time of treatment (autumn 2002), the block was surrounded by vines with Eutypa dieback symptoms. Consequently this block was likely to be already under severe disease pressure. Secondly, it is known that in vines infected with *E. lata* expression of foliar symptoms may take some years to become apparent. Therefore, it seems that the treatment could have stimulated a response in infected, asymptomatic vines, that has lifted their productivity. Such a response may be the result of the immunising commensal benefit of the Trichoderma treatment imparting a "vaccination" effect on the vines.

Trichoderma has been shown to be capable of eliciting a systemic acquired resistance (SAR) response in plants, which can raise the levels of resistance to disease by stimulating phytoalexins, natural plant defence chemicals[10]. If this SAR mechanism is being stimulated in these Clare Valley trial vines to produce a better crop then the implications for the winegrowing industry are very exciting. It remains to be seen, however, if the increase in productivity is concomitant with an increase in fruit quality. Fruit quality indices will be tested from this trial site as well as the other sites where similar treatments have occurred between spring 2002 and autumn 2003.

The stimulation of a natural protective effect in the grapevine by "vaccination" has considerable potential in establishing new "high health" vineyards from grafted vines incorporating beneficials such as Trichoderma during the nursery propagation stage. "Vaccination" also has great potential in established vines where treatment could have the obvious benefits gained from improved health that is likely to lead to greater longevity with better fruit quality and yield expected to be associated with these healthier vines.

Acknowledgements

This work has been conducted in collaboration with a number of researchers in New Zealand and overseas who have kindly provided access to results. Contributions from the following are gratefully acknowledged; Sharmini John, University of Adelaide, Waite Campus, South Australia; Philippe Rolshausen, University of California, Davis Campus, USA; Dr Ian Harvey, Plantwise Services, Lincoln, New Zealand; Dr Paul Fourie, University of Stellenbosch, South Africa; Kate Strachan, Taylors Wines Pty. Ltd., Auburn, South Australia.

References

1. Edwards, J. & Pascoe, I. (2003) Incidence of *Phaeomoniella chlamydospore* infection in symptomless young vines. *The Australian Grapegrower & Winemaker*. 473a:90-92.

2. Hunt, J.S. (1998) Armillaria in Kiwifruit - a long-term management problem. NZ Kiwifruit J. 126:32-34.

3. John, S., Lardner, R., Scott, E., Stummer, B. & Wicks, T. (2001) Eutypa dieback: research on biological control and diagnostics. *The Australian Grapegrower & Winemaker*. 449a:73-75.

4. John, S., Scott, E.S., Wicks, T.J. & Hunt, J.S. (2003) Studies of interactions between *Eutypa lata* and *Trichoderma harzianum*. Phytopathologia Mediterranea "In press".

5. John, S., Scott, E.S., Wicks, T. & Hunt, J. (2001) Interactions between *Eutypa lata* and *Trichoderma spp*. Proceedings 13th Biennial Australasian Plant Pathology Society Conference, Cairns, Australia:p.338.

6. John, S., Scott, E.S., Wicks, T.J. & Hunt, J.S. (2003) Towards biological control of eutypa dieback of grapevines. Proceedings of 8th International Congress of Plant Pathology, Christchurch, New Zealand Abstracts: p. 43.

7. Wicks, T. & Davies, K. (1999) The effect of Eutypa on grapevine yield. *The Australian Grapegrower & Winemaker*. 406a:15-16.

8. Dr Ian Harvey (2003). Personal communication.

9. Fourie, P.H., Halleen, F., van der Vyver, J. & Schrueder, W. (2001) Effect of Trichoderma treatments on the occurrence of decline pathogens in the roots and rootstocks of nursery grapevines. Phytopathologia Mediterranea. 40: S473-S478.

10. Calderon, A.A., Zapata, J.M. & Barcelo, A.R. (1994) Peroxidase-mediated formation of resveratrol oxidation products during the hypersensitive-like reaction of grapevine cells to an elicitor from Trichoderma viride. Physiological and Molecular Plant Pathology. 44:289-299.

(R) TM Trademarks of Agrimm Technologies Ltd, Christchurch, New Zealand