PENETRATION OF *TRICHODERMA HARZIANUM* INTO GRAPEVINE WOOD FROM TREATED PRUNING WOUNDS

I.C. HARVEY[1] and J.S. HUNT[2]

[1]*PLANTwise Services Ltd, PO Box 181 Lincoln, New Zealand*
[2]*Agrimm Technologies Ltd, PO Box 35 Lincoln, New Zealand*

Corresponding author: harveyi@plantwise.co.nz

ABSTRACT

Vinevax™ (containing a mixture of strains of the fungus *Trichoderma harzianum*) is normally applied as soon as possible after grapevine pruning. However, using a range of six timings after pruning from immediate to 4 days later, it was shown that the best penetration of the fungi into the wood was with treatment at approximately 5 h after pruning. A novel method of measuring penetration and vigour of the growth of *Trichoderma* spp. into grape wood is described. A second trial using a range of concentrations of Vinevax™ (at and below label rates) was applied as a general cover spray over dormant, pruned vines. The percentage incidence and vigour of penetration of the *T. harzianum* into canes was concentration dependent and lower than the targeted hand-applied treatment earlier in the season. Therefore targeted, timely applications of the product appear to be best for good wood penetration of *T. harzianum*.

Keywords: *Trichoderma,* grapevines, pruning, wood-invading fungal pathogens.

INTRODUCTION

The treatment of pruning and trunk reconstruction wounds of grapevines has become important with the finding that these are often the portals for entry of many debilitating trunk diseases (Adalat et al. 2000; Larignon & Dubos 2000). The main pathogens involved are *Eutypa lata, Botryosphaeria* spp., *Phomopsis* spp. and the *Phaeomoniella/ Phaeoacremonium/Acremonium/Phialophora* complex (Hunt et al. 2001; Halleen et al. 2005). Hunt et al. (2001) also showed that *Trichoderma*-based treatments gave an 85% decrease in isolation of many of these fungi 8 months after pruning, while John et al. (2004) in an *in vitro* study showed that *Trichoderma*-treated grape wood gave a 90% decrease in colonisation by *E. lata*. Di Marco et al. (2004) showed that when *Trichoderma* was applied immediately after pruning in late winter, around 90% of wounds yielded the applied fungus 5 days after application and from 60–70% after 60 days.

Present label instructions for the *Trichoderma harzianum*-containing Vinevax™ recommends application of the product immediately after making pruning cuts, but the practicality of this timing in large vineyards and when using mechanical pruning techniques becomes problematic. The purpose of the present study was to determine the optimal time after pruning that a *Trichoderma* product should be applied and at what dilution to still obtain a substantial colonisation of the wood using spur and cane pruning techniques.

METHODS

In Trial 1, Vinevax™ (*Trichoderma harzianum* formulated for pruning wound application in vineyards) was applied at label rates (10 g/litre) at the following intervals after pruning: 15, 30 and 60 min; end of day (3-4 h); next day (24 h); and 4 days later

(96 h). Untreated controls were sprayed with water 15 min after pruning. There was also an additional treatment of the fungicide propiconazole (0.625 ml active ingredient/litre) applied 15 min after pruning. Treatments began on 30 June 2005 with all treatments on the day of pruning being applied with a hand-held trigger spray bottle while treatments on the following day and 4 days later were applied with a knapsack sprayer. Each treatment consisted of two vines spur pruned to 4 buds and two vines cane pruned. The treatments were randomised through a 15-year-old block of Chardonnay at Lincoln University experimental vineyard.

Thirty-three days after pruning, 8 spurs and 4 cane samples were harvested per treatment by trimming approximately 10 cm off the spur (leaving a 2 bud spur) and cane from the pruned and treated portions of the vines. The new pruning cuts were re-treated with Vinevax™ following collection of the samples. The cuttings were then cool-stored for 7 days until processing. From each vine four straight spurs and two cane-ends were selected and cut to fit inside a 9 cm plastic Petri dish. The distal (inoculated) end of the cane or spur was marked on the wood. A strip of bark was removed from two sides of each sample and then sprayed with alcohol and flamed in a laminar flow cabinet. Using flamed secateurs, each cane or spur was split longitudinally along the peeled surfaces by cutting into the proximal (non-treated) end and pulling the two sides of the cane apart. One half of each cane/spur was placed flat, cut side down onto the surface of a malt extract agar (MEA) plate. The cut surface was laid as flat as possible on the agar surface so that the entire cut area had good contact with the agar medium. Each plate was then marked to indicate the spray inoculated (or distal) end of the sample. The plates were incubated for 3 days at 25°C in the dark. The patterns of mycelium growing out from the portions of split cane into the agar were marked with felt pen on the base of the agar plates. The plates were then incubated for a further 3 days under near ultraviolet to stimulate sporulation of the colonies of *Trichoderma* that grew out from the tissue. Plates that demonstrated typical *Trichoderma* sporulation were then set aside and the original markings denoting the 3-day old growth were measured for distance down the cane where the colony originated and the radius of growth out from the source (Fig. 1).

In Trial 2, Vinevax™ was applied at the following three application rates to cane-pruned vines in late August: 10 g/litre (label rate), 2 g/litre and 1 g/litre. Each treatment was applied to one 15 m row containing 14-16 vines. Each vine had two canes and thus two treated wounds for evaluation. The trial was located in non-adjacent Chardonnay rows at the same location as Trial 1. Vines were pruned at 10 am and Vinevax™ at the above rates applied at end of day (6 pm), 8 hours after pruning. The treatments were applied using a Solo 475 knapsack with an E04-80 nozzle. This gave a consistent spray pressure of 40 psi. The application rate was 1300 ml/min at a walking speed of 1.8 km/h. This rate was required in order to apply sufficient spray to the vines and adequate wetting of the wounds. From the above parameters, the spray rate was calculated at 173 litres/ha.

After 4 weeks, a 10 cm portion was cut from the treated end of each cane on the vines in each row. Again, the pruning wounds were re-treated. These portions were assessed for colonisation by the *Trichoderma* sp. as described in Trial 1.

Data obtained from the trials were subjected to ANOVA where appropriate.

RESULTS

The results from Trial 1 (Table 1) showed that pruned vines treated with Vinevax™ either 4 h (end of the day) or 24 h (next day) after pruning gave the greatest incidence of inoculation, distance down the cane and vigour of growth of the *Trichoderma* sp. compared to those treated within 30 min or after 4 days.

The poor results from vines treated either 15 or 30 min after pruning may have been due to vascular "bleeding" or leakage following cutting. This flow may have impeded the growth or penetration of spores of *Trichoderma* into the wood. Once this had begun to diminish, then penetration may have been more readily achieved by the applied *Trichoderma* formulation.

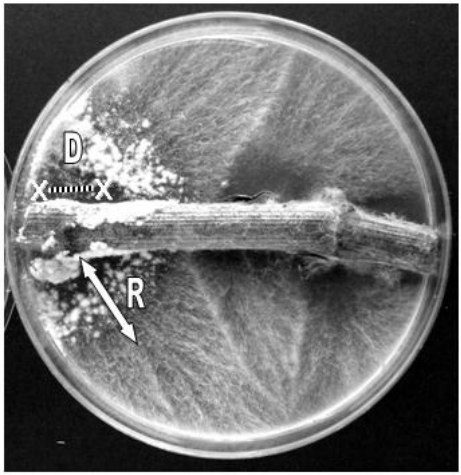

FIGURE 1: Typical pattern of growth of *Trichoderma harzianum* from a split spur portion from a Vinevax™-treated vine after 3 days incubation in the dark and 3 days under near UV light. D = approximate distance down the cane that was the focus of growth out into the agar of the *T. harzianum*. R = the radius of growth of *T. harzianum* out from the cane at 3 days.

TABLE 1: *Trichoderma* incidence (%), penetration (distance down cane from the pruning wound, mm) and vigour (growth radius out from cane into culture, mm) after treatment of pruned spurs and canes of grapevines with Vinevax™ at varying times after pruning in June.

Time of application	Incidence (%)		Penetration (mm)		Vigour (mm)	
	Canes	Spurs	Canes	Spurs	Canes	Spurs
15 min	50.0	50.0	6.8	7.0	2.0	3.9
30 min	25.0	37.5	2.8	5.8	2.0	3.7
1 h	75.0	87.5	10.5	12.8	7.3	6.4
4 h	100.0	87.5	13.6	16.7	10.3	14.8
24 h	100.0	87.5	13.4	11.5	15.8	13.4
4 days	75.0	62.5	23.4	8.3	14.6	8.2
nil	25.0	12.5	0.5	3.4	0.8	2.8
propiconazole	0.0	0.0	0.0	0.0	0.0	0.0
LSD (P<0.05)	–	–	15.2	8.7	7.9	6.3

The low scores when the Vinevax™ was applied after 4 days may have been due to initiation of the healing process of the cut-surfaces at this time. This appeared to have been greatest in the spur-pruned treatments compared to the cane-pruned. The difference may have been due to the orientation of the cut surfaces. The horizontal cut surfaces of the spur-pruned vines may have dried and "cured" faster than the vertically orientated surfaces of the cane-pruned vines.

It was noted that the *Trichoderma*-yielding cane/spur portions in culture grew almost no saprophytes or wood-invading pathogens. It is unclear if this was a reflection of competition in the cane by the applied *Trichoderma* or a case of competition in the culture plates. This observation may require further investigation.

The *Trichoderma* isolates yielded by the untreated controls (25% from canes and 12.5% from spurs) is probably a reflection of the background levels of this fungus in the environment. They were also located less deep in the wood, probably reflecting less vigour and penetration ability.

Note that the above results are only applicable to spurs and cane-end pruning wounds. The treatment may perform somewhat differently on large wounds created when re-working cane architecture or top-grafting. This requires further investigation, necessitating partial, or complete destruction of vines in the trial.

In Trial 2, *Trichoderma* spp. were isolated from only 35% of the cane portions at the lower rates of application (one-fifth and one-tenth of label rate) and only 15% at the highest rate (label rate) of application (Table 2). The incidence of recovery of the *Trichoderma* sp. in August was low at all application rates compared to equivalent treatments applied in late June. Measurements of penetration of the *Trichoderma* sp. into the canes and vigour of subsequent fungal growth out into culture were not significantly different between the three concentrations of the treatments applied. The penetration distances down the canes of the fungus were generally similar to those assessed in the previous trial, as were the vigour scores for mycelial growth out of the canes.

TABLE 2: ***Trichoderma*** **incidence (%), penetration (distance down cane from the pruning wound, mm) and vigour (growth radius out from cane into culture, mm) after treatment of pruned grapevine canes with different concentrations of Vinevax™ in August.**

Vinevax™ (g/litre)	Incidence (%)	Penetration (mm)	Vigour (mm)
10	15	5.4	3.4
2	35	10.0	8.9
1	35	4.8	10.2
LSD (P<0.05)	–	8.2	7.8

DISCUSSION

This is the first report of the efficacy of penetration of *Trichoderma* spp. into grape wood through wounds at different times after pruning. All other studies (Di Marco et al. 2004; John et al. 2004) have applied the treatments immediately after cutting. Moreover, Munkvold & Marois (1994) showed that pruning wounds remain susceptible to infection from *E. lata* for 4 weeks and European experiences on wound susceptibility suggests that vine pruning be delayed as late a possible in the winter (Di Marco et al. 2004).

The two major differences between these present trials were the time of the season when the treatments were applied and the concentration of Vinevax™ applied to the cut surfaces. The ability to apply sufficient inoculum on to the cut surfaces may be compromised by ground speed at application as well as the application of a blanket spray treatment to the whole vine compared to targeted, hand-applied treatments.

With a balance required between maximising the colonisation of the pruned vines and protection against air-borne and possibly even water-borne vine pathogen inoculum, it would appear from the results in this study that treating wounds with Vinevax™ should be carried out at the end of the day. A balance between the completion of pruning and the application of the wound protection on the same day must be worked out by operators within the vineyard, especially when considering the limited daylight hours available at this time of the year. The difference in time of application during the season

of the two trials may have been a contributing factor to the variation in results. Thus, it is planned to repeat the trails both early and late in the season using both spur and cane-pruned vines.

ACKNOWLEDGMENTS

We thank Penny Turpin and Winna Harvey for their valuable assistance in carrying out this work.

REFERENCES

Adalat K, Whiting C, Rooney S, Gubler WD 2000. Pathogenicity of three species of *Phaeoacremonium* spp. on grapevines in California. Phytopathologia Mediterranea 39 (1): 92–99.

Di Marco S, Osti F, Cesari A 2004. Experiments on the control of esca by *Trichoderma*. Phytopathologia Mediterranea 43 (1): 108-115.

Halleen F, van Niekerk J, Mostert L, Fourie P, Crous P 2005. Trunk disease pathogens associated with apparently healthy nursery grapevines. Wineland (Wynboer) Technical Yearbook. Stellenbosch, South Africa. Pp. 12-14.

Hunt JS, Gale DSJ, Harvey IC 2001. Evaluation of *Trichoderma* as bio-control for protection against wood-invading fungi implicated in grapevine trunk diseases. Phytopathologia Mediterranea 40 (Supplement): S485-486.

John S, Scott ES, Wicks TJ, Hunt JS 2004. Interactions between *Eutypa lata* and *Trichoderma harzianum*. Phytopathologia Mediterranea 43: 95-104.

Larignon P, Dubos B 2000. Preliminary studies on the biology of *Phaeoacremonium*. Phytopathologia Mediterranea 39 (1): 184-189.

Munkvold GP, Marois JJ 1994. Factors associated with the variation in susceptibility of grapevine pruning wounds for biological control of eutypa dieback. Phytopathology 83: 624-629.