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Does stress express vine trunk diseases?

Mundy D

June 2012

A report prepared for
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This report has been prepared by The New Zealand Institute for Plant & Food Research Limited (Plant & Food Research), which has its Head Office at 120 Mt Albert Rd, Mt Albert, Auckland.

This report has been approved by:

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Date: 14 June 2012

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Ministry for Primary Industries
Manatū Ahu Matua

Final Report Template

Project Title:	Does stress express vine trunk diseases?
Project Number:	L10/184
Date of Report:	June 30 2012

Note: The Final Report is due to your Project Adviser within two months after the project completion date.

If any material supplied in, or attached to, this report contains confidential information, or is otherwise unsuitable for wider dissemination, please clearly mark accordingly and highlight directly with your Project Adviser (including the reason for wishing to treat the material in this manner).

This information from Sections 2 – 5 and Section 11 will be published on the Ministry for Primary Industries (MPI) website unless you advise us otherwise.

The paper provided in Appendix 1, and referred to in Section 2, is to remain confidential and not published on the website until after the New Zealand Plant Protection Conference in August 2012.

1. Milestone Summary Table

Milestone Number	Milestone [As per SFF contract schedule]	Completion Date Original	Completion Date Actual	Percent Complete
1	Establishment of vines	September 2010	August 2010	100%
2	First set of Infection and stress of vines	November 2010	September 2010	100%
3	First set of Harvest and pathology of vines	March 2011	March 2011	100%
4	28 February Report - invoicing for work completed to date	February 2011	February 2011	100%
5	Annual Report (popular article)	June 2011	June 2011	100%
6	Second set of Infection and stress of vines	November 2011	December 2011	100%
7	Second set of Harvest and pathology of vines	March 2012	March 2012	100%
8	28 February Report - invoicing for work completed to date	February 2012	February 2012	100%
9	Popular article and seminar	June 2012	June 2012	100%
10	**Final Report**	June 2012	June 2012	100%

Summary of Key Performance Indicators

KPI Description	Overall Progress
This project will provide evidence of plant stress factors influencing grapevine trunk disease expression.	The review conducted in 2011 provides a strong indication that stress is a factor in trunk diseases of grapes. The experiments conducted and accepted for publication in 2012 have not been decisive, with some of the typical limitations of small potted plant experiments, but research by collaborators has shown that water stress and temperature stress are important for expression of disease caused by *Eutypa lata*.
This project will make a recommendation of whether larger field programme on grapevine trunk disease symptom expression should be undertaken.	We recommend that field experiments be conducted over at least three seasons to investigate questions about symptom expression and infection rate raised in the literature review.

2. Project Objectives

(Why did you do this project? What were your key objectives at the start of the project? Outline if any of these objectives changed during the course of the project.)

The project was established to show that stress is an important factor in symptoms expression for grapevine trunk diseases. the experiments were set up to investigate this and the results will reported in a paper to be presented at the 2012 New Zealand Plant Protection conference (Appendix 1). However, as part of the project a review was conducted of stress and trunk diseases of grapevines and that publication (2011) indicated that two further questions should have been asked: “Does stress lead to symptom expression in infected vines?” and “Does stress increase or decrease infection rates?” The review also allowed connections with other research groups internationally to be made following publication and the New Zealand project has benifited from knowledge of the most recent international studies.

3. Approach

(What did you do – how did you go about it?)

the project team conducted experiments to answer our questions but also reviewed international research that has been and is currently been conducted. As a result of this project and other related work on grapevine pathogens, the team established collaborations with scientists who have also conducted research on the effects of stress on expression of *Eutypa lata* infection on grapevines. These collaborations have allowed the team to achieve more work than otherwise would have been possible with the resources available for this project.

4. What were the main findings from this project?

- 1. It has been shown that stress can influence disease expression and may also be important in determining the success of wound infection.
- 2. A survey of growers showed that awareness of trunk diseases is increasing in the wine industry.

5. What difference has this project made to your group / community of interest / industry?

(Include intangible benefits where significant — e.g. “enabled us to develop a strong on-going working relationship with the scientists”).

We have evidence from our survey that the wine industry has changed practices in the last five years with regards to both the use of pruning wound dressings and not pruning in the rain, the two main management options we have been communicating as part of our ongoing research.

We have also had a number of wine companies and vineyard managers offer us the use of vineyards for large-scale trunk disease investigations.

6. If you did the project again, what would you do differently?

(i.e. what worked and what didn’t?).

We would conduct the research part of the project over a longer time frame. It was hard to conduct the number of experiments we wanted to and to have the results ready within the two years of the project, becausebecause of to the slow growth of the fungi in the wood of the vines. We also spent considerable time growing the vines to have them ready for experiments.

However, we are confident that we have achieved results that, when combined with our reviewed material, allow us to make sound recommendations.

7. Is there anything the SFF could have done differently?

No. SFF have been very good to deal with and proactive in reminding us ofdeadlines so that they could be achieved.

8. Is there anything that you have learnt that would be useful for new project teams?

From our expirence, as part of the almost any project, we would recommend a review of current knowledge and communication with international researchers working in the same area to be a very valuable exercise, for which budget should be allocated.

9. Where to from here – what are the next steps?

Plant & Food Research have a pilot project with New Zealand Wine investigating the management of grapevine trunk diseases, this project also includes working with SARDI (South Australian Research and Development Institute) scientists. The

research team is currently working with SARDI to develop a new project on grapevine trunk disease control which will also have linkages with GWRDC (Grape and wine research and development corporation, Australia). Ongoing underpinning research into grapevine trunk diseases is also being conducted by Plant & Food Research, with outcomes of the research reported to the industry so that any new options for management are communicated directly to industrythose who need to know and could benefit.

10. Financial summary

Provide a brief comment as to whether the project was completed on budget; whether there is any grant money left unspent. Please provide a financial statement to summarise the incomings/ outgoings over the life of the project – you can either attach a copy of your own financial statement or use the "final financial template" available at our website http://www.mpi.govt.nz/sff/

11. List and attach any major outputs from the project.

- Two scientific papers - Appendices 1 and 2
- Continued updating of the trunk disease website http://wineresearch.org.nz/publications/MarlboroughTrunkDiseaseWeb.htm
- Survey of growers and summary of responses - Appendix 3
- Popular article - Appendix 4.

If appropriate, we would like to publish a copy of the above on our website: please provide an electronic copy for this purpose preferably in Word format.

Report Confirmation

Name [Project Manager]	Confirmation	Date
	I hereby confirm the above information is true and correct:	

Submission Notes:

1. **Final Reports should be sent electronically** to the MPI SFF Fund Administrator **and** your Project Adviser (in the same e-mail as the final Request for Payment form and invoice). Also attach electronic versions of any resources developed.

   Please ensure you put your project number in the e-mail's subject line: e.g., 09/999 Final report 2011.

2. **Hardcopies of any project resources** developed should be **posted** to the Fund Administrator **and** your Project Adviser.

Appendix 1. New Zealand Plant Protection Paper 2012 accepted for publication Confidential until published in August 2012

Colonisation of grapevine pruning wounds under carbohydrate stress

Inoculation of one-year-old lignified grapevine pruning wounds on potted vines with fungi associated with trunk disease

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Abstract Current management options for grapevine trunk diseases are focused on ameliorating symptoms once they have occurred. In grapevines and other crops, plant stress has been observed to increase symptom expression for different pathogens, depending on timing and type of stress. This research is aimed to establish whether carbohydrate stress to vines at the time of wounding enhances colonisation by fungi associated with trunk disease. Potted plants were wounded and inoculated with two grapevine trunk fungi: *Eutypa lata*, and *Botryosphaeria lutea*. Stress treatments were applied to the vines immediately following inoculation. Lesion size at the wound site, when infection was confirmed by re-isolation of the pathogen, was used to indicated the extent of symptom development. In these experiments stress did not induce greater infection incidence or larger lesions at the site of wounding. Some difficulties in using potted plants to simulate responses of mature vines in the field are discussed.

Keywords trunk disease, carbohydrate stress, vine, *Eutypa lata*, *Botryosphaeria lutea.*

INTRODUCTION

Visible grapevine trunk disease symptoms often develop only 10 or more years after planting (Rolshausen et al. 2010). Investigation of grapevine trunk diseases often occurs only after symptom expression is observed. Because of the cryptic nature of these diseases, there may already be a large number of established vines infected by trunk disease pathogens in New Zealand that are not expressing symptoms. Studies in New Zealand (Whiteman et al. 2007; Graham et al. 2009) and overseas (Edwards & Pascoe 2004; Fourie & Halleen 2004; Aroca et al. 2006) have indicated that mother vines often carry a range of endophytic fungi. However, younger vines that are infected can express young grapevine decline which can lead to a decrease in productivity and vine death (Rolshausen et al. 2010). Currently nurseries cannot

economically test grafted plants for the presence of trunk disease fungi, as the diseases are not uniformly distributed and sampling is destructive.

During a recent survey of trunk diseases in New Zealand, only three of the 41 vineyard blocks sampled across eight regions had no detectable fungal trunk pathogens (Manning & Mundy 2009; Mundy et al. 2009). However, many of the vines sampled were not expressing any symptoms of trunk diseases even when the pathogens were present. The pathogens of interest have been identified as wound invaders (Mundy & Manning 2010) and stress on the vines at the time of wounding has been reported as one possible factor that could influence vine response to pathogen invasion (Rudelle et al. 2005; Desprez-Loustau et al. 2006; Petit et al. 2006; Mundy & Manning 2011). Carbohydrates are important both to the vine's defence mechanisms against wounding and to the wound invading process of many pathogens (Mundy & Manning 2011). If carbohydrate stress can influence pathogen invasion of the wound and trigger latent endophytic infections to become pathogenic, this has implications for the management of the vineyard in terms of risk of trunk diseases. Ongoing carbohydrate research has demonstrated that over-cropped grapevines, which have carried more fruit than the vine can ripen with the available canopy, have a higher soluble sugar concentration and lower starch in the trunk than lower cropped vines (Trought & Bennett 2009). Leaf removal and girdling have also been reported to change the availability of sugars within the vine so that different sinks such as roots and fruit have changed allocation (Caspari et al. 1998). Decreases in carbohydrate reserves (starch) or allocation have been hypothesised to reduce the rate of wound healing and also decreases fungal colonisation of wounds (Mundy & Manning 2011).

In long-term studies on older vines in Italy, stress has been shown to increase disease expression of esca, a grapevine trunk disease that is not present in New Zealand (Corino et al. 2004). As symptom expression for trunk diseases in the field can vary from year to year (Rolshausen et al. 2010) plant stress, environmental interactions with the pathogen may determine expression as well as initial infection rates of pathogens. Symptoms such as lesion formation require not only the pathogen to be present but also the plant to respond to the pathogen by accumulation of phytoalexins and other pigmented defence compounds at the site of invasion (Mundy & Manning 2011). Carbohydrates have been shown to be important in trunk disease response of vines even when symptoms are not observed (Petit et al. 2006). Carbohydrates are important both to the vine's defence mechanisms against wounding and to the wound invading process of many pathogens (Mundy & Manning 2011).

The present research aimed to determine whether stress is an important factor in wound invasion by trunk diseases under New Zealand conditions.

METHOD

Overall design

Potted 2-year-old grapevines were used to investigate the effect of stress and pathogen interactions on vines. Experimental treatments included leaf removal (to induce carbohydrate stress), girdling (wound response and carbohydrate stress) and a control where stress was not induced. Vines subjected to these stress regimes were inoculated with isolates of *E. lata* and *B. lutea.*

Experiment 1

Potato Dextrose Agar (PDA) plugs without or with one of the two fungal isolates were prepared and used to inoculate potted grapevines as described above. The two carbohydrate stress treatments with the addition of a non-stress control created a 3×3 factorial experiment. Each of the nine treatment combinations was repeated nine times, with 81 vines in total being used. Stress treatments were applied to vines prior to wounding and inoculation on 10 February 2010 and the wood samples from the vines were harvested in September 2010.

Experiment 2

This experiment was established at the same time as Experiment 1 to determine if a time period longer than 12 months was required for latent infections to express symptoms. The same treatments were applied using 14 replicates, and the vines were harvested in October 2011.

Experiment 3

This experiment compared methods of inoculation (following poor establishment of this fungus in Experiments 1 and 2). *Eutypa lata* plugs (which contained agar and mycelium) and ascospore suspensions (2.5×10^4/ml) were tested. This experiment was established in January 2012 and assessed in March 2012.

Inoculation

Inoculation with isolates of *E. lata* and *B. lutea* as mycelium/agar plugs (5 mm) cut from the edges of 8-day old cultures on PDA. Inoculation wounds were made to freshly cut end wounds on 1-year-old wood on the vines. The wound was made to the one year old wood above the point the main shoot branched from the lignified wood. The plugs were placed onto freshly cut end wounds and covered with Parafilm® for 3 days following inoculation with the fungal plugs or agar controls. The vines were incubated in a non temperature controlled vented plastic house for different periods of time depending on the experiment (Table 1). For Experiment 3, both a 10µl ascospore suspensions (2.5×10^4 viable ascospores/ml) and plugs of *E. lata* were used, following the discovery of fruiting bodies of the pathogen on dead vines in Marlborough (D.C. Mundy, unpublished data). The ascospore inoculations, using the methods of Sosnowski et al. (2008) involved wetting the end wound before applying spores but the wound was not covered with Parafilm® following application. This method was included to allow comparisons with the plug method often used in plastic house experiments and spore infection method used in field experiments on grapevines.

Vine preparation

Two-bud grafted Sauvignon blanc vines on Schwarzmann rootstock were chosen for uniformity from a larger pool of vines, re-potted into individual 5-litre black polythene bags and grown to break dormancy and establish a single healthy shoot in leaf (5-6 months). Plants were selected for uniformity of growth, and assigned randomly to the experimental treatments. Two treatments of leaf removal and girdling were used to simulate carbohydrate stress and an untreated control. For the leaf removal treatment half of the leaves were removed from the shoot. Girdling was applied to the vine by cutting the bark and phloem tissue around the trunk of the vine 15 cm from

ground level using two scalpels taped together to produce parallel cuts 5 mm apart. Wounding was applied to the treatments immediately following inoculation.

Measurements

At the end of each experiment, each vine was assessed for the presence of disease symptoms, including vine death, lesion formation and discoloration of internal tissue. Each vine was split length-wise and the length of the symptomatic tissue within the vine measured.

Each split vine was dipped into 70% ethanol for 1 min to kill any surface contamination. Inner tissue was taken from the lesion sites with a scalpel from 10 mm below the cut surface (Experiments 1 and 2) or from the edge of the lesion (Experiment 3) and plated onto PDA. These plates were then incubated at 20°C and isolates observed over a 2-week period. For Experiments 1 and 2, isolations were made 10 mm below the cut and treated surface. For Experiment 3, the isolations were taken from the edge of the discoloration zone distal to the treated wound. Once sufficient growth had occurred, the colonies were then sub-cultured to fresh PDA to obtain a pure culture that could be further identified.

Statistical methods

The effects of the stress and fungal treatments on lesion lengths were analysed using two-way ANOVA. The lengths were log-transformed before analysis to equalise variances, and means were back-transformed for presentation of results. The proportions of vines from which the pathogen was re-isolated were analysed using a binomial generalised linear model with a logit link. Analysis presented exclude vines that that died during the experiment. Contrasts were used to compare main effects means.

RESULTS

Visual assessment of internal symptoms

No significant effect of any of the treatments was detected on discoloration of internal tissues in any of the experiments (Table 1). In Experiment 1, the length of internal symptomatic tissue did not differ significantly with fungal treatment (P=0.97) or stress treatment (P=0.30) and there was no significant Stress×Fungi interaction (P=0.30). Similarly, in Experiment 2, the length of lesions did not differ significantly with fungal treatment (P=0.43) or stress treatment (P=0.67). There was a tendency for the combination of stress and fungal treatment to affect the lesion lengths (Stress×Fungi, P=0.083). For the *Botryosphaeria*-inoculated treatment, lesions tended to be longer on the vines that had leaves removed than on the girdled vines (5% least significant ratio = 1.63). In contrast, for the untreated control and *Eutypa*-inoculated treatments, the lesion lengths were not significantly different between the stress treatments. In Experiment 3, the length of discoloration did not differ significantly with inoculation method (P=0.28) or stress treatment (P=0.90) and there was no significant Stress×Inoculation interaction (P=0.84). In Experiments 1 and 3, the internal discoloured tissue was sometimes absent or indistinct.

Re-isolation of *E. lata*

The success of re-isolation was not consistent across the three experiments (Table 2). In Experiment 1, no significant differences were found however very low numbers

of re-isolations were achieved making the biological relevance of this result questionable. In Experiment 2, the percentage of vines from which *E. lata* was re-isolated differed significantly with respect to the inoculating fungal species (P<0.001) but not with the stress treatment (P=0.47), and there was no significant Stress×Fungi interaction (P=0.32). In Experiment 3, the percentage of vines that had *Eutypa* re-isolated from them differed significantly with the Inoculation treatment (P<0.001) but not with the stress treatment (P=0.99), and there was no significant Stress×Inoculation interaction (P=0.57). When the data from the three stress treatments in Experiment 3 were pooled, the vines that were inoculated with plugs had more *Eutypa* re-isolated from them (85%) than the vines that received the ascospores (54%), which in turn had more successful re-isolations than the vines that received no fungus (4.5%). The results also indicated that there may have been some pre-existing latent infections of *E. lata* in the test plants or that there had been some natural contamination during the periods of the experiments.

Re-isolation of *Botryosphaeria*

In Experiments 1 and 2, the percentage of vines that had *Botryosphaeria lutea* (identified by morphology on plates) re-isolated from them, differed significantly with the fungal treatment (P=0.005 and P<0.001, respectively) but not the stress treatment (P=0.34 and P=0.94, respectively) (Table 3). In Experiment 3 when *Botryosphaeria* was not used as inoculum, only a single isolate was recovered indicating that vines were not contaminated with this pathogen during the experiment.

Table 1 Lengths of discoloration of tissues (mm) from the cut ends of grapevines at the end of Experiments 1, 2 and 3.

	Stress treatment		
Fungal treatment	No stress	Leaf removal	Girdling
Experiment 1[1]			
None added	6.0	7.4	14.9
Botryosphaeria lutea	9.5	7.2	8.3
Eutypa lata	6.7	10.1	8.8
Experiment 2[2]			
None added	3.6	5.0	5.3
Botryosphaeria lutea	6.0	7.0	3.9
Eutypa lata	5.4	4.7	5.6
Experiment 3[1]			
None added	7.4	4.9	7.2
Eutypa plugs	3.8	4.4	4.3
Eutypa spores	8.0	7.4	4.9

[1]length of discoloration.

[2]length of lesion with distinct edges following lignifications.

Table 2 Percentages of successful re-isolations of *Eutypa lata* at the end of Experiments 1, 2 and 3.

Fungal treatment	Stress treatment		
	No stress	Leaf removal	Girdling
Experiment 1			
None added	0	10.0	20.0
Botryosphaeria lutea	0	0	0
Eutypa lata	10.0	10.0	10.0
Experiment 2			
None added	6.7	6.7	6.7
Botryosphaeria lutea	6.7	20.0	0
Eutypa lata	60.0	40.0	40.0
Experiment 3			
None added	14.3	0.0	0.0
Eutypa plugs	77.8	88.9	87.5
Eutypa spores	50.0	55.6	55.6

Table 3 Percentages of successful re-isolations of *Botryosphaeria lutea* at the end of Experiments 1 and 2

Fungal treatment	Stress treatment		
	No stress	Leaf removal	Girdling
Experiment 1			
None added	10.0	0	10.0
Botryosphaeria lutea	30.0	20.0	40.0
Eutypa lata	10.0	0	0
Experiment 2			
None added	20.0	13.3	0
Botryosphaeria lutea	46.7	53.3	66.7
Eutypa lata	0	6.7	6.7

DISCUSSION

In applying carbohydrate stress in the form of girding and leaf removal our experimental design may have contributed to our non detection of significant differences in our treatments. Field grown vines have also been shown to have different methods of wound healing in the xylem in winter and summer (Sun et al. 2008) when carbyhoyrate availaliblity will also differ in the the wood tissue. The current experiments were established during summer when carbohydrate stress could be induced and when a reduced availability of carbohydrates might interfere with the vines' normal defence mechanisms (Mundy & Manning 2011). It was hypothesised that the change in wood carbohydrate concentration may give the fungi a competitive advantage, allowing greater colonisation of the wood tissue. By manipulating the carbohydrate levels in the vine we wanted to induce increased colonisation and spread of the fungi within the lignified tissue. However this timing of the experiment was different to field inoculations of trunk disease pathogens that are generally conducted in winter and sampled 9–12 months later (Sosnowski et al. 2008; Rolshausen et al. 2010). By changing the timing of inoculation we may have reduced the rate of infection as the rate of wound healing may have been increased due to the higher temperatures and greater plant activity during summer compared to dormancy. Reported susceptibility of grapevine pruning wounds to infection by *E. lata* during this late pruning time was lower than that of vines pruned early in the dormant season (Munkvold & Marois 1995). The pathogens that we used in our experiments may not be adapted to invading wounds during the summer growth of grapes. Experiments conducted on young potted vines using plugs of *E. lata* (Sosnowski et al. 2011) were conducted in Summer but inserted into a hole drilled into the stem of the vine rather than a cut end. In experiments using *B. lutea* (Amponsah et al. 2011) inoculated with mixed isolate conidial suspension in spring resulting in 92% infection rate suggesting that this method of infection may be useful for further studies of this type.

Previous research on the effects of plant stress on response to pathogen challenge or disease symptom development suggested that stress (Ferreira et al. 1999; Treutter 2005; Desprez-Loustau et al. 2006), particularly carbohydrate stress (Rudelle et al. 2005), may be important for decreasing vine defence response but also can reduce the ability of the pathogen to invade and establish depending on the timing of the stress. Desprez-Loustau et al. (2006) reported that many of the positive drought–disease interactions involved increased severity or impact of the disease rather than increased incidence of infection and so the timing of stress is important.

The present experiments have failed to show a difference in either the extent of internal symptoms or the establishment (as determined by re-isolation rate) of trunk disease fungi used in this potted vine system following summer infections. In Experiment 3, inoculations made with *E. lata* plugs, which contained some nutrients in the media, had a consistently higher rate of re-isolation than that following inoculation with the ascospores, the preferred method of inoculation for winter infection experiments reported overseas.

In five field experiments at the Nuriootpa Research Centre in South Australia using *E. lata* ascospores, the mean percentage recovery from the inoculated controls ranged from 29 to 74% for vines inoculated within 1 day of wounding (Sosnowski et al. 2008). Other studies have also reported re-isolation rates ranging from 30% to 80% following inoculation of *E. lata* ascospores (John et al. 2005). The re-isolation rate of

50% for Experiment 3 is consistent with these reports. The relatively low rate of re-isolation in Experiment 3 for ascospores and the higher rate for plugs may indicate that the importance of nutrient availability for the fungus, and timing of the stress during the infection process should be further investigated. However, as the plug system was covered for 3 days and the spore method was not, differences in wound moisture levels may also have been a factor. The present experiments were also conducted on single isolates of *E. lata* obtained from vines in the local Marlborough region where the presence of *E. lata* in grapes has only recently been confirmed (Mundy et al. 2009). Differences have been shown in the rate of invasion and wood staining between different isolates of *E. lata* (Sosnowski et al. 2007). Studies of the virulence of isolates present in New Zealand on commonly grown grapevine rootstock and scion varieties of New Zealand isolates should be conducted before further studies of stress, vine and pathogen interactions are conducted.

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Appendix 2. New Zealand Plant Protection Paper 2011

Physiological response of grapevines to vascular pathogens: a review

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Abstract The successful infection of a grapevine vascular system by a plant pathogen and expression of disease symptoms occur only when the pathogen has overcome the wound response and other defences of the vine. Even when pathogens do successfully infect the vascular system of grapevines, symptom expression is not often observed in the first season. Symptoms may be observed in one year but the same vine can have reduced or no symptoms the following season. Information is presented on physiological stress in association with trunk diseases as one factor that may contribute to symptom expression in vines. A hypothesis of grapevine wound response is proposed as part of the discussion of vine physiological response. Information on individual trunk diseases and physiological interactions is also provided.

Keywords grape, wound response, symptoms, stress, trunk disease.

INTRODUCTION

A complex series of interactions between the causal agent and the grapevine has already occurred before trunk disease symptoms are observed on vines in the vineyard. Many of the steps before the expression of trunk disease symptoms occur in the wood, where they are not easily observed. The time delay between infection and symptom expression with diseases like eutypa dead-arm makes understanding the relationship between disease and symptom expression more difficult.

A successful trunk pathogen has contended with the vine's wound response and other defences. Toxins produced by the pathogen and other pathogenicity factors have had the opportunity to interact with the vine's normal physiological function. During infection and establishment, the pathogen can colonise other tissue as well as/before the vessels (Pascoe & Cottral 2000). In order to induce vascular disease symptoms, the xylem cell's function is often reduced (Hopkins 1989; Edwards et al. 2007). A vine responding to an infection will have a limited pool of resources to allocate to defence mechanisms. Abiotic stress may limit the vine's ability to allocate resources to infection, resulting in subsequent colonisation of tissues by the pathogen.

Often disease symptoms are both spatially and temporally removed from the initial infection of the vine (Rudelle et al. 2005; Sosnowski et al. 2007). When symptoms are observed in woody plants, the expression may not be the same from season to season (Octave et al. 2006; Sosnowski et

al. 2007), possibly because of changes in the vine reserves. Host response to vascular infection is a dynamic interaction between host and parasite in which resistance is the rule and susceptibility the exception (Beckman 1964).

PRUNING WOUND RESPONSE

Using a model system for wound response in flowering plants and investigations of grapevine pathogen interactions, a hypothesis of grapevine response for further investigation has been generated (see below). General wound response of flowering plants has received considerable study and review (Bloch 1941; Bostock & Stermer 1989). The structure and physiology of grapevines, which may be important to wound response, have also been investigated and summarised (Pratt 1974; Mullins et al. 1992). Understanding grapevine wound response is important, as several grapevine vascular pathogens are believed to enter the vine via mechanical injuries such as pruning wounds (*Eutypa lata*, *Botryosphaeria* species, *Phaeoacremonium* species) (Mundy & Manning 2010; Rolshausen et al. 2010; Úrbez-Torres & Gubler 2010). Recent studies of grapevine wounds (Pascoe & Cottral 2000; Harvey & Hunt 2006; Sun et al. 2006; Eskalen et al. 2007; Sun et al. 2007; Weber et al. 2007; Sun et al. 2008; Rolshausen et al. 2010; Úrbez-Torres & Gubler 2010) and the use of wound dressings (Moller & Kasimatis 1980; Jaspers 2001; John et al. 2005) in relation to grapevine trunk disease pathogens have provided observations for the model proposed.

Plants resist pathogen attack via multilayered constitutive and inducible defences. For example, high lignin production in cell walls has been reported to confer tolerance to *E. lata* (Rolshausen et al. 2008). When a plant is cut (or mechanically damaged), resulting in disruption to the vascular system, a multi-step wound response is activated (Bloch 1941; Bostock & Stermer 1989; Hawkins & Boudet 1996). Wound responses take place at cellular and tissue levels, with the degeneration or necrosis of cells at the wound site providing signals to surrounding healthy cells (Bloch 1941). The wound response signalling pathway shares common components (e.g. jasmonate signals) with those activated following insect and pathogen attack. The rate of response has implications for the disease resistance of the plant (Bostock & Stermer 1989).

Generalised cell response to wounding includes signalling pathways that are triggered by elicitors, including ion fluxes, oxidative burst and synthesis of signal compounds such as ethylene, salicylic acid and jasmonic acid (Belhadj et al. 2006). The pathogen may act on the elicitor signal pathways, producing toxin-induced symptoms remote from the site of production (Valtaud et al. 2009). These defence pathways include reinforcement of plant cell walls, accumulation of phytoalexins and other antimicrobial compounds, and proteins inhibitory or hydrolytically active towards microbes may be induced (Belhadj et al. 2006).

Bostock & Stermer (1989) described three wound response types in plants, with the most complex being observed in woody perennial stems as well as potato tubers, which have been used as a model system for understanding the response. The response process involves three key steps: (i) the cells adjacent to the wound dying from autolysis, (ii) existing parenchyma cells undergoing redifferentiation and lignosuberization to form a boundary zone with increased physical resistance and (iii) the formation of a suberized wound periderm below the boundary zone as a result of meristematic activity (Bostock & Stermer 1989). Biochemical changes in individual cells' induced responses to pathogen attack and/or mechanical wounding include the accumulation of phenols, phytoalexin production, synthesis of hydrolytic enzymes, and cell wall reinforcement with the phenolic polymers suberin and/or lignin (Hawkins & Boudet 1996).

Within the vascular tissue, the vine responds to wounding of the vessels by the production of tyloses. Tyloses form when the protoplasmic membrane of the parenchyma cells next to the xylem cells extends into the vessel via the pit to form a balloon-like structure that, in grapes, eventually becomes lignified (Pratt 1974). When

a large number of tyloses form, they can block the vessel. The formation of tyloses and other responses occur over time in response to the initial wounding event. Hawkins & Boudet (1996) developed a model system for studying the gene expression response to mechanical wounding in flowering plants and used it to investigate changes over time (1–7 days) in lignin and suberin deposition.

The formation of tissue impervious to water and microorganism penetration is a common feature of wound response of woody plants associated with resistance to pathogens (Bostock & Stermer 1989). When physical barriers are produced, this is often in association with production of anti-microbial phenolics (Del Rio et al. 2001; Treutter 2005) and a reduction in the availability of simple sugars (and other nutrients) at the wound site (Bostock & Stermer 1989).

Unique features of grapevines

Grapevine xylem has some distinct physiological and structural features that distinguish it from other flowering plants. In grapevines, the secondary xylem is described as diffuse-porous with ladder-like thickening surrounded by living xylem parenchyma (Mullins et al. 1992). Septate fibres with bordered pits are the predominant xylem elements (Mullins et al. 1992). The xylem cells have been observed to respond to wounding with seasonal differences in the mechanism for sealing grapevine trunk xylem, with gel (temporary) closers produced in winter and more permanent tyloses in summer (Sun et al. 2008). Most plants produce either gels or tyloses (Sun et al. 2008). In grapevines a single ring of xylem is produced each year, but the vessels can remain functional for up to 7 years (Mullins et al. 1992). Most grapevine xylem vessels become inactive because of tylose formation after 2–3 years (Pratt 1974), rather than after a single season as is observed in other woody plants.

Hypothesis of grapevine wound healing

When a grapevine is cut, the vine responds as a woody plant (described above), cells die next the wound, signals are sent to the rest of the tissue and undifferentiated cells start to form a periderm. Within the vessels, gels or tyloses form depending on the season. For a large cut to the vine, up to 7 years of xylem may need to be sealed to prevent water loss and microbial entry. Phenolic compounds are deposited at the wound, and over time the wound becomes less susceptible to infection by trunk pathogens. Investigations have indicated that the timing of the wounding event can determine the length of the wound susceptibility.

PHYSIOLOGICAL STRESS AND SYMPTOM EXPRESSION

Interactions between disease symptom expression and grapevine stress have been suggested as one possible explanation for inconsistent visual symptoms for some vascular diseases. In seasons when growth of the vine is not inhibited by abiotic factors, foliar symptoms of *E. lata* in grapes are not often observed or are reduced in severity (Sosnowski et al. 2007). In seasons when vines experience abiotic stresses, individual vine reserves may influence symptom expression.

If vine stress does have a role in symptom expression, then the mechanism of this reaction needs to be considered. Although grapevines are grown in a highly managed production system, they are still subject to the ambient climate of the region. Some of the management methods used, such as controlled irrigation or removal of leaf area, may produce stress within the vine, which can be further exacerbated by environmental conditions. A range of abiotic stresses may occur in vineyards; defoliation, freezing stress and nutrient stress are factors that have been reported to predispose forest trees to disease symptom expression comparable to water stress for canker diseases (Desprez-Loustau et al. 2006).

Drought/water stress

Desprez-Loustau et al. (2006) proposed four main types of drought-disease interactions that could be expected in forest trees. The summary below has been broadened to include all abiotic stresses:

1. Direct effects of abiotic stress on the pathogen.

2. Indirect effects of abiotic stress on the pathogen through other community interactions (such as an increase or decrease of naturally occurring biological control agents).
3. Host predisposition, i.e. the effect of abiotic stress on the host physiology leading to susceptibility.
4. Multiple stresses, i.e. the combined effects of infection and abiotic stress on tree physiology.

Drought-disease interactions in forest trees have been reviewed, with a majority of published studies referring to a positive association between drought and disease (Desprez-Loustau et al. 2006). When investigating drought and disease interactions for grapes, it is important to consider symptoms and timing of water stress. The symptoms of trunk diseases and water stress are often related, with both resulting in reduced movement of water in the xylem.

More than half the reviewed studies of forest tree drought-disease interactions were for canker/die-back pathogens such as *Botryosphaeria* (Desprez-Loustau et al. 2006), which correspond with the type of diseases that are commonly grouped as trunk diseases of grapes. In the case of *Xylella* spp. infection, the gene transcription response of vines is similar to responses to drought stress (Choi et al. 2010). As vascular diseases are found in the vessels and vine responses to the pathogen involve blocking infected vessels, aggregates and tyloses can physically block a sufficient number of vessels to prevent xylem flow (Martelli et al. 1986). For Pierce's disease, the observed water stress symptoms are the result of xylem occlusions (Hopkins 1989). Beckman (1964) noted that physiological changes in the plant, such as increased respiration and changes in water balance (reduced supply), result in wilting due to lack of water, rather than toxins produced by the trunk diseases.

However, water stress or drought may also predispose a vine to disease development (Ferreira et al. 1999) or lead to a more rapid exhaustion of the vine as a result of additive deleterious effects, as reported for forest trees (Desprez-Loustau et al. 2006). Extended drought conditions have been reported to exacerbate the development of Pierce's disease, as water-stressed vines in vineyard conditions appear more susceptible than well watered vines (Hopkins 1989).

When considering water stress, the timing of stress may also be important. For young grapevines already infected with *Phaeoacremonium chlamydosporum,* water stress significantly increased vine death (Ferreira et al. 1999). However, in contrast, vines that had been water stressed and then infected with *Neofusicoccum luteum* had a reduction in shoot die-back lesion length at 25% field capacity compared with 50 and 75% field capacity (Amponsah 2011). Desprez-Loustau et al. (2006) reported that many of the positive drought–disease interactions involved increased severity or impact of the disease rather than increased incidence of infection. For the *N. luteum* experiment (Amponsah 2011), existing stress may have reduced the susceptibility of tissues to infection via the blocking of vessels or other mechanisms, reducing the spread of the pathogen following infection.

Limited resources

For plants, a "trade-off" exists between growth and defence-related metabolism (Treutter 2005). When defence compounds are produced, they use plant resources including amino acids, carbohydrates and nutrients. The removal of these resources from the vine's pool of reserves can then have follow-on effects on plant growth. One of the typical symptoms associated with diseases such as eutypa dead-arm is reduced vine vigour. Reduced vigour may be the result of diversion of resources to a defence response or a knocking out of part of the vine's infrastructure.

The induction of flavonoid biosynthesis and accumulation as part of a defence response has been shown to be limited by the availability of carbon, energy or other resources (Treutter 2005). Amino acids within the vine may be used for the production of phenolic compounds in response to infection with botrytis bunch rot. However, the same amino acids are also used in a

number of plant biochemical pathways (Mundy 2008). If a vine has already experienced water stress and allocated other amino acids to proline production to maintain osmotic pressure (Keller 2005), then less raw material will be available to produce flavonoids as part of a plant defence.

Vines infected with *E. lata* have reduced starch storage in the xylem parenchyma cells and rays compared with healthy vines (Rudelle et al. 2005). Rudelle et al. (2005) have suggested high metabolic activity is associated with observed secretory defence responses involving well developed and more numerous mitochondria. A high metabolic activity could account for the reduced starch storage. *Eutypa lata* can produce a polypeptide fraction that induces changes in grape leaves, resulting in reduced assimilation of the products of photosynthesis and lower leaf respiration (Octave et al. 2006), which may result in further reduction in vine reserves.

Vines with *Phaeomoniella chlamydospora* had reduced carbohydrate reserves compared to the control vines during winter dormancy, even if symptoms were not expressed, and an overall loss of plant vigour (Petit et al. 2006), indicating changes in vine resources as a result of infection.

Nutrient supply may be important for disease response. In strawberries (Walter et al. 2008) and grapes (Elmer & Reglinski 2006), calcium has been associated with cell wall integrity and resistance to fungal penetration. Increases in vine uptake of calcium ions during Pierce's disease infection, as part of the plant's defence system, have been reported (Xu et al. 2003). Magnesium ions have been reported as a possible factor for detoxification of fungal toxins produced by *E. lata* (Colrat et al. 1999). Changes in nutrient reserves within tissues may also be related to symptom expression, with the suggestion of a link between toxic amounts of macronutrients (Ca^{++} and Mg^{++}) in the petioles and leaf symptom expression in Pierce's disease (Xu et al. 2003).

Temperature/seasonal differences

Increased rates for wound healing later in the pruning season (late winter to early spring) have been reported for grapevines. Reported susceptibility of grapevine pruning wounds to infection by *E. lata* during this late pruning time was lower than that of vines pruned early in the dormant season (Munkvold & Marois 1995). These observations may be linked to the reported seasonal differences in grapevine wound response of tyloses or gum formation (Sun et al. 2008). Tyloses and gums have different chemical and physical characteristics, and R genes that allow a pathogen to overcome one set of wound responses may not be effective against the other. Seasonal differences in infection and susceptibility have been reported for *E. lata* (Petzoldt et al. 1981; Munkvold & Marois 1995; Chapuis et al. 1998) and botryosphaeria canker (Úrbez-Torres & Gubler 2010). However, Chapuis et al. (1998) suggested that infection may be linked to temperature, with *E. lata* growing well at low temperatures and other microorganisms being suppressed. Temperature conditions can also influence the plant's growth, with climatic conditions that are conducive to vigorous vine growth in spring reported to reduce foliar symptoms of *E. lata* infection (Sosnowski et al. 2007).

TRUNK DISEASES AND POSSIBLE PATHOGENICTY FACTORS

The grapevine wound response is effective in stopping most pathogens from entering and establishing in the vine (non-host resistance (Agrios 2005)). In order to be effective pathogens of grapevines, the casual organisms need characteristics that allow them to succeed under conditions that are not conducive to other pathogens. The vessels of grapevines are a nutrient-poor environment for the growth of microorganisms (Valtaud et al. 2009; Ciraulo et al. 2010) but do provide a pathway for movement within the trunk. The successful pathogens of the grapevine vascular system require one or more virulence genes to operate in this grapevine tissue. Researchers have studied different vascular pathogens of grapes to obtain an understanding of the processes involved in successful infection. Histopathology of infections in vine wood has been reported for a range of vascular

pathogens including *Botryosphaeria stevensii* (Whitelaw-Weckert et al. 2006), *Lasiodiplodia theobromae* (Úrbez-Torres & Gubler 2010), *Neofusicoccum parvum* (Úrbez-Torres & Gubler 2010), *Phaeomoniella chlamydospora* (formerly *Phaeoacremonium chlamydosporum*) (Pascoe & Cottral 2000; Whiteman et al. 2002; Whiteman et al. 2007) and *Xylella fastidiosa* (Roper et al. 2007; Ciraulo et al. 2010).

Botryosphaeria canker

Pycnidiospores are dispersed by rain splash, spreading botryosphaeria canker via the infection of exposed xylem of pruning wounds (Úrbez-Torres & Gubler 2010). The importance of wound age for the infection of botryosphaeriaceous species has been reported, with no conidial infections of *Neofusicoccum luteum* 14 days after wounding (Amponsah et al. 2009). These results are consistent with studies of wound periderms (cork barriers) (Bostock & Stermer 1989), although the formation of a periderm was not investigated for *N. luteum*. Both duration of susceptibility of pruning wound and effect of pruning time on susceptibility of fresh wounds to infection by *Lasiodiplodia theobromae* and *Neofusicoccum parvum* have been studied in California (Úrbez-Torres & Gubler 2010). In the artificially-inoculated Californian vineyard studies, pruning vines in late, rather than early, winter resulted in reduced percentage of botryosphaeria canker infections. The mechanism for differences in infection rates is yet to be determined.

Eutypa dead-arm

The observations of Rudelle et al. (2005) suggest that vessel-associated cells are very important for the vine's defence against trunk diseases and these cells can be activated remotely from the site of infection by toxin/signalling compounds conducted in the xylem. Vessel-associated cells appear to slow the progress of *E. lata* even in susceptible cultivars. However, the anatomy of the vine prevents this mechanism from completely protecting the vessels, as vessel-associated cells do not form a complete ring around the conducting xylem (Rudelle et al. 2005). Vessel-associated cells have structural analogies to phloem companion cells, but have lignified walls, and no plasmodesmata interface with the vessel element (Fromard et al. 1995).

Sosnowski et al. (2007) hypothesised that climate is one factor that may influence the expression of foliar symptoms of *E. lata* in grapes. They developed a conceptual model that predicts expression of symptoms based on winter rainfall and spring temperatures in conjunction with initial observations of disease severity. Additional study is required to elucidate how the climate influences the fungal pathogen or the vine response, leading to symptom expression (Sosnowski et al. 2007). In forest trees it has been observed that, while drought conditions may be negatively correlated to successful new fungal infections, they can be positively related to existing endophytes and saprophytes becoming damaging pathogens in plants predisposed by stress (Desprez-Loustau et al. 2006). While there are models for the infection stage of many diseases, modelling of climate and other life cycle stages may allow better management of vascular diseases that are already present in vineyards.

Esca complex, including *Phaeomoniella chlamydospora*

Pascoe & Cottral (2000) investigated *Phaeomoniella chlamydospora* infection of tissue cultured Chardonnay plants and observed infection of the xylem parenchyma cells, which then moved into the adjacent vascular vessels. The pathogen hyphae were observed to enter the vessels at the sites of tylose formation. Hyphae travelled along vessels and phenolic compound accumulations in the trunk tissues were not always associated with heavy concentrations of hyphae. As "goo" and other phenolic compounds were often some distance from the site of infection, a toxin or some other signalling compound may be involved, possibility explaining why *P. chlamydospora* is not always isolated from the sites where "black goo" is observed (Pascoe & Cottral 2000). Later research from the same group showed that in vines infected with

P. chlamydospora, blockages of the xylem function could be significantly higher (16%) than the percentage of vessels with "goo" symptoms (1%) (Edwards et al. 2007).

Esca disease symptoms include distinctive tiger-striped leaves with orange inter-vein regions (Mundy & Manning 2010). The observed foliar symptoms are distant from the primary site of infection (Valtaud et al. 2009). The leaf symptoms of esca are probably the result of extensive cellular oxidation following a decrease in leaf glutathione content (Valtaud et al. 2009). Intracellular structural damage can be detected in leaf cells before visible symptoms appear, suggesting modification of plant metabolism in the early stages of symptom expression (Valtaud et al. 2009).

Pierce's disease

The bacterium *Xylella fastidiosa* is the causal agent of Pierce's disease. The physiological effects of this disease have been the focus of considerable study following the arrival of an insect vector (glassy winged sharpshooter – *Homalodisca vitripennis*) into Californian vineyards. As with many of the trunk diseases, infections by *X. fastidiosa* can occur remotely in time and space from the site of symptom expression. Investigations have shown that vines often harbour high titres of *X. fastidiosa* before visual symptom expression (Choi et al. 2010). The bacteria effectively bypass the vine wound response when injected directly into the xylem from the foregut of the glassy winged sharpshooter (Redak et al. 2004). When infections do occur, *X. fastidiosa* has been shown to change the nutrient content of leaves before and during symptom expression (Xu et al. 2003).

CONCLUSIONS

The successful trunk disease pathogens of grapevines have evolved with the host plant and have mechanisms to overcome at least some of the host defences. To develop better control of these diseases, the interactions between the plant pathogen and the environment must first be understood, so that conditions can be manipulated in favour of the vine. In summary:

1. Grapevines appear to have the same basic wound healing mechanism as most woody plants.
2. The speed of wound response depends on timing of wounding.
3. Wound susceptibility decreases as wounds age.
4. Disease symptom expression can be influenced by abiotic stress.
5. Vines have limited resources/reserves to respond to biotic or abiotic stress.
6. Physical structures such as vessel-associated cells or thickened lignin deposits may be important for varietal tolerance of disease.

Understanding how the physiology of vines influences pathogen infection and expression will allow the development of better management and control strategies for grapevine trunk diseases in New Zealand and internationally.

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Appendix 3. Questionnaire used to benchmark current management of trunk diseases and summary of answers

QUESTIONNAIRE
Version 1 : November 2011
Written by Dion Mundy[1]

Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

www.plantandfood.co.nz

Survey of current and past practices for managing grapevine trunk diseases

To help to provide a baseline of current management practices for grapevine trunk diseases, we would like you please to answer a few questions about your current and past grapevine trunk disease management practices. Thank you for taking the time to complete the survey.

General Questions

Which trunk or root disease are you most concerned about?

Do you feel you have access to the type of trunk disease information that you need to make good management decisions?

What additional information about trunk diseases would you like to access or find useful?

Current practices

For the vineyard that you are associated with, do you currently:

1. Prune in the rain or within 36 hours of rainfall?
2. Use pruning pastes or covering (if yes, what do you use)?
3. Remove all old wood from the vineyard (if yes, how)?
4. Cut out infected cordons or vines when they show symptoms?

Past practices

To allow us to track changes in industry practice, please answer the four questions below with regard to the practices you were following five years ago:

1. Pruned in the rain or within 36 hours of rainfall?

2. Used pruning pastes or covering (if yes, what do you use)?

3. Removed all old wood from the vineyard (if yes, how)?

4. Cut out infected cordons or vines when they showed symptoms?

Thank you again for completing this survey.

Contact details for returning the survey, or to make additional comments:

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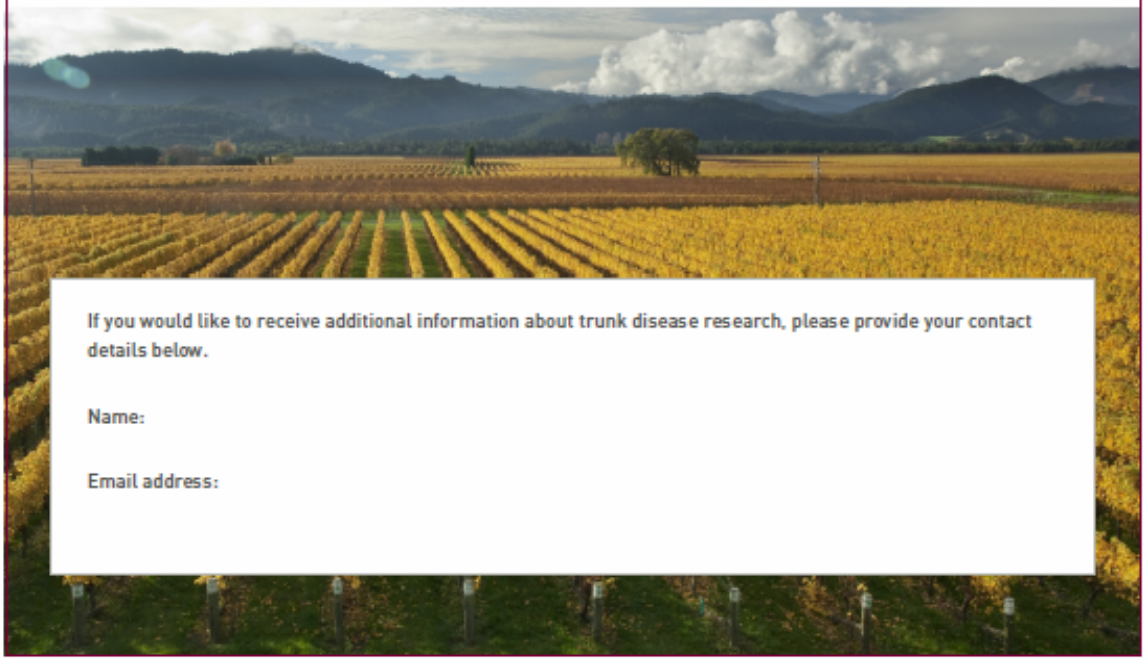
Plant & Food Research Limited



If you would like to receive additional information about trunk disease research, please provide your contact details below.

Name:

Email address:

Response to survey conducted in late 2011 and early 2012

The survey was made to determine how industry awareness and practices had changed during the last five years during the period thatwhich SFF-funded projects on grapevine trunk diseases have been operating.

Which trunk or root diseases/ pathogens are you most concerned about?

Disease	Number of respondents
Eutypa lata	14
Botryosphaeria spp.	11
Black foot	3
Black goo	4
Phomopsis sp.	1
Virus	1

34 respondents completed the survey; some listed more than one disease.

For the vineyard you are associated with, do you

	Currently		**5 years ago**	
	Yes	No	Yes	No
Prune in the rain or within 36 hours of rainfall	17	7	20	11
Use pruning pastes or covering	24	10	19	12
Remove all old wood from the vineyard	19	10	15	17
Cut out infected cordons or vines when they show symptoms	18	13	21	9

Not all respondents answered all questions.

If you use a pruning wound dressing, what do you use?

Product	**Currently**	**5 years ago**
Vinevax™/Trichoderma	8	3
Greenseal™	9	0
Bacseal®	16	8
Garrison™	1	0
paint	1	1
Silicone sealant	1	0

Some respondents have used more than one product.

Note that only Vinevax™ and Greenseal™ have a label claim for *Eutypa lata* and none is registered for the control of *Botryosphaeria* spp.

If you remove old wood from the vineyard, how is it removed?

How removed	Currently	5 years ago
Burning	9	9
Mulching	7	4

Some respondents used a combination of both methods.

Do you feel you have access to the type of trunk disease information that you need to make good management decisions?

17 said yes, 7 said no, and 10 gave another response, such as “today helped” or “factsheets would be useful”.

What other additional information about trunk diseases would you like to have access to?

Responses included:

Access to science papers
Photo cards
Best practice guide lines
More information on application methods, product evaluation
More workshops
Identification, treatment

Documented benefits of compost Potential soil drenches or foliar sprays
We need a spray that can go on daily after pruning and kill all spores on the new cuts.
Economic effects of the disease versus cost of prevention
Do pruning pastes work? what to do when vines are bleeding how long to wait after rain & how to persuade pruners to wait!
Mechanically sprayed pruning treatments
Rely on field days WinePress & emails from Winegrowers

Control options both cultural, chemical and biological (organic)
Identification, treatment

Appendix 4. Popular article for the end of the project (Objective 9).

Grapevine trunk diseases, what are we doing in 2012, and what current practice was 5 years ago

By Dion Mundy, Plant & Food Research, Marlborough

As the current Sustainable Farming Fund project on grapevine trunk diseases neared completion, the PFR team felt it was a good time to ask the industry what current practices are used to control trunk diseases. It was also an opportunity to ask what management practices were used 5 years ago when SFF-funded trunk disease research was just beginning. This allows us to measure what impact five years of investigation and communication have had on the industry.

While the results give only an indication of what is happening in the industry, they are consistent with the research team's observations over five years of presentations and answering industry questions. Our survey showed the following changes:

- The survey confirmed that *Eutypa lata* and *Botryosphaeria* species, which are the fungi under investigation in the SFF project, were the fungi of greatest concern to the respondents.
- Use of pruning paints and removal of old wood from the vineyard had increased over the five years.

Fewer respondents now prune in the rain than was the case five years ago.

When asked about the available information on grapevine trunk diseases, respondents were generally positive. A number of people made suggestions for additional research, or for information that would be useful to them. These ideas will be considered for future project work.

A full summary of the results and a copy of the questionnaire are posted on the Marlborough Wine Research Centre website in the grapevine trunk disease section: http://wineresearch.org.nz/publications/MarlboroughTrunkDiseaseWeb.htm.

ACKNOWLEDGEMENTS

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