2007 End of Year Report for Marlborough Grapevine Health Project

Mundy DC, Manning MA, Cocchi C

May 2007

Report to Marlborough Research Centre Trust, Project number 20957
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Manning MA
The Horticulture and Food Research Institute of New Zealand Ltd
HortResearch Mt Albert
Private Bag 92 169
Auckland Mail Centre
Auckland 1142
NEW ZEALAND
Tel: +64-9-815 4200
Fax: +64-9-815 4201

Mundy DC and Cocchi C
The Horticulture and Food Research Institute of New Zealand Ltd
HortResearch Marlborough
PO Box 845
Blenheim 7240
NEW ZEALAND
Tel: +64 3 577 2370
Fax: +64 3 578 0153

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This report has been prepared by The Horticulture and Food Research Institute of New Zealand Ltd (HortResearch), which has its Head Office at 120 Mt Albert Rd, Mt Albert, AUCKLAND. This report has been approved by:

_____	_____
Research Scientist	Group Leader, Bioprotection
Date: 29 May 2007	Date: 29 May 2007

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EXECUTIVE SUMMARY

2007 End of Year Report for Marlborough Grapevine Health Project

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Mundy DC, Manning MA, Cocchi C. May 2007

The aim of this project is to investigate grapevine trunk health in the Marlborough region, specifically to:

- Determine what impact this has on vineyard productivity and fruit quality
- Generate a better understanding of the economic impact of the problem.

The project identified the main fungal genera (*Botryosphaeria* and *Eutypa*) associated with three vineyards in Marlborough and investigated how these fungi affected the performance of the vines. Of greatest concern to grape growers will be the rate at which these diseases kill vines, as that will directly affect productivity.

Sick vines did not have a significantly reduced yield compared to healthy vines. However, the findings of this project show that indirect costs to the grower may occur as a result of reduced fruit quality. Monitoring of the vineyards that have visual symptoms of decline has shown that grapevine trunk diseases can affect the composition of fruit, by reducing soluble solids and berry nitrogen content, although this was not observed at all sites.

Additional work is required to provide the industry with the tools to manage trunk diseases actively under New Zealand conditions. This process has started with workshops, articles and information to growers.

A list of the milestones is provided in Appendix 1 and a selection of outputs in Appendix 2.

For further information, please contact:

Dion Mundy
The Horticulture and Food Research Institute of New Zealand Ltd
HortResearch Marlborough
PO Box 845
Blenheim 7240
NEW ZEALAND
Tel: +64 3 577 2370
Fax: +64 3 578 0153
Email: dmundy@hortresearch.co.nz

INTRODUCTION

Concerns about grapevine trunk diseases have increased in the last 10-15 years because they cause long-term effects that could threaten the sustainability of grape and wine production in affected orchards (Pascoe & Cottral 2000). Grapevine trunk diseases are responsible for significant economic losses to the wine industry worldwide. The causes of these trunk diseases are usually fungi and they colonise the vascular system and disrupt the flow of nutrients in the plant. Typical symptoms can include chlorotic leaves, stunted shoots, necrotic wood and death of the plant.

The impact of grapevine trunk diseases can be significant in older vineyards, and usually becomes more severe as vineyards become older. Infected vines do not normally become symptomatic until they are at least eight years old, which is a stage of vineyard production when most growers are looking for a return on the investment that they have made in establishment phase of vineyard development.

The Marlborough vineyard area has developed very quickly in the last 15 years and is still growing. New Zealand Winegrowers predict an increase of 4% for the next year. The Marlborough District Council has been mapping the area that is now involved in wine grape production and currently estimates the area planted to be 18,460 ha. In 1992, New Zealand Winegrowers reported only 1,900 ha of grapes in production. While the majority of the vines in the region are young at present, in the last few years, several trunk disease fungi that can cause the death of plants have been detected in some older plants. For this reason, during 2006 the Marlborough Research Centre funded a project to investigate the infection of older vines for fungal infection.

Grapevine trunk diseases are generally caused by fungal species such as *Eutypa lata*, *Botryosphaeria obtusa*, *Phomopsis vitcola*, and *Cylindrocarpon destuctans,* which affect the vascular system of the plant. There is also Petri disease, which is associated with a complex of fungi including *Phaeomoniella* sp., *Phaeoacremonium* sp. and *Formitoporia punctata*. There has been much debate on the importance of trunk diseases in the various wine growing regions of the world and many questions remain concerning the impact and control of these diseases.

Initial studies have shown that some vines can have more than one trunk disease fungus present. In the first study conducted by HortResearch for the Marlborough Research Centre, the most commonly isolated fungal species was *Botryosphaeria obtusa*. Many other species of *Botryosphaeria* are associated with disease of grapevine trunks. This fungus is very adaptable and contains a large number of species. However, the initial study was based on a single vineyard and may not represent the district as a whole. Other trunk disease fungi have also been reported in New Zealand and internationally (Pascoe & Cottral 2000). Sauvignon blanc is the flagship variety of Marlborough region. In California, *Botryosphaeria* spp. were often isolated from these vines.

The fungi associated with trunk disease have many different ways of infecting or colonising trunk wood. They can enter the vascular system of young plants through surface wounds of mother plants in the nursery or during subsequent pruning during establishment in the vineyard, and often symptoms do not appear until the vines are older. Nurseries can take a number of measures to reduce the risk of infection during propagation. However, the relative roles of other potential sources of inoculum, such as aerial spores and contamination during

propagation, have not been clarified (Whiteman et al. 2007). Vineyards can also reduce the chances of infection during the establishment and production phases of vineyard management.

Grapevine trunk diseases have the potential to increase costs of production, with the requirement to apply controls such as extra pruning to remove dead tissue or the total removal and replacement of infected vines. The costs of reduced wine quality from uneven berry maturity from infected vines as well as the costs associated with vine removal, replanting or reworking and the loss of productivity also need to be considered, in addition to the direct yield loss attributed to the disease. The Californian studies estimated that yield reductions ranged from 30% to 62% in vineyards, depending on the severity of the disease. Similar trends are shown with bunch numbers and wine quality. The data shows that in a vineyard with 30.8% *Eutypa* infection, a loss of at least 860 kg/ha can be expected.

We have very limited information on how any of the fungal trunk diseases react with the vines in the New Zealand climate to affect yield and fruit composition.

This project investigated the detrimental consequences that grapevine trunk diseases had on vine performance, by monitoring all phenological stages from veraison to harvest, using qualitative analysis on bunches collected. All results have been compared with the samples collected from healthy vines at sites adjacent to the infected ones. From this comparison, it was possible to measure the relative differences and calculate economic damage.

MATERIAL AND METHODS

SURVEY AND ISOLATION

Three sites in Marlborough were selected for intensive investigation of species of fungi present in the vines. Two of these were Riesling sites and the other was Sauvignon blanc. At these sites, vines were selected with physical symptoms consistent with trunk disease and a non symptomatic vine in the adjacent row was also selected for sampling. At each site and for each of the sample vines a 10mm wide core of wood was removed from the vine using a hand-coring device and placed in a sterile tube (Figures 1 and 2).

Figure 1. Removing a core of wood from the head of a grapevine using a hand coring device.

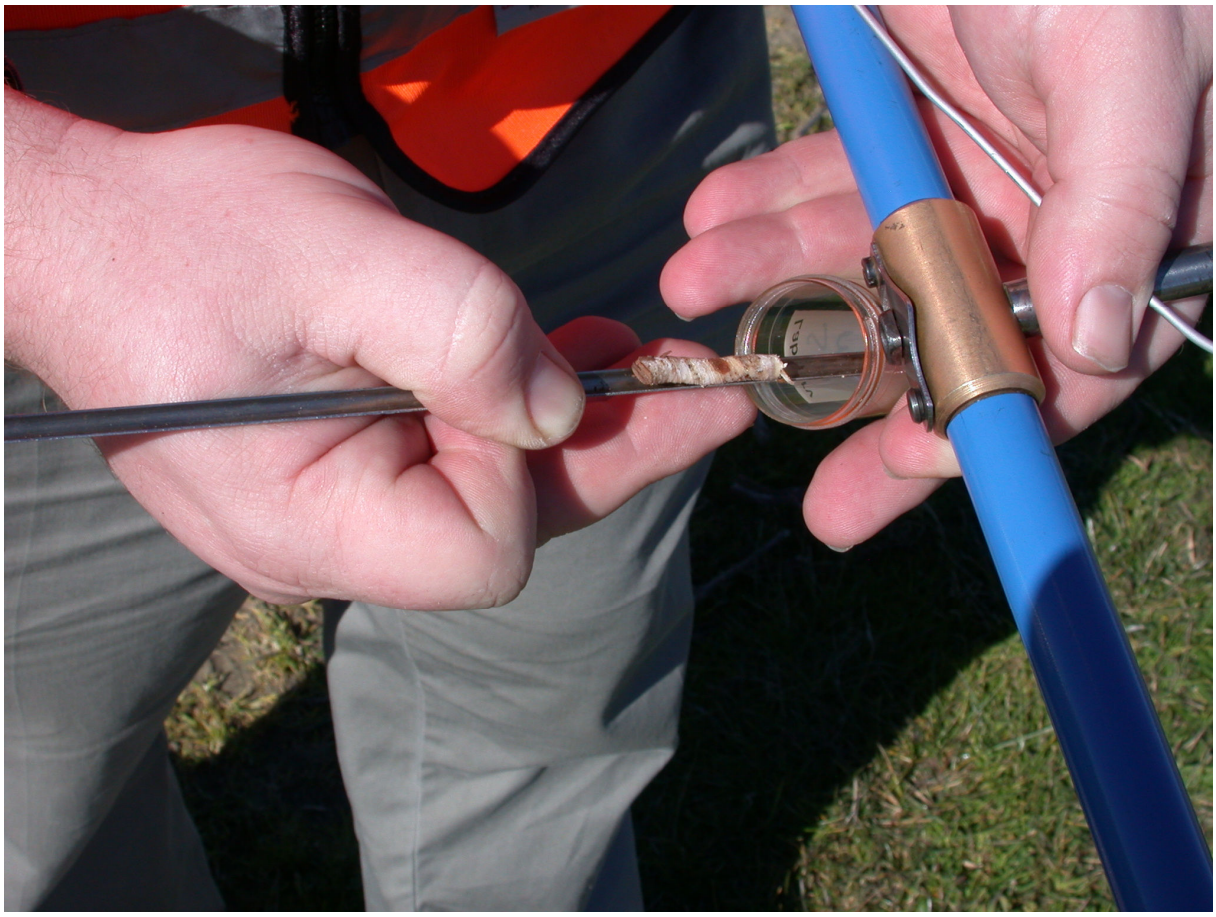


Figure 2. Transferring an aseptic core of grapevine head from a hand-coring device to a sterile tube.

The cores extracted from the symptomatic and non symptomatic vines were taken to the laboratory and cut into small sections under sterile conditions. From these cores, fungal isolates were grown (Figure 3) and then transferred onto new Potato Dextrose Agar (PDA) plates for systematic identification. Most of the identification was made using standard morphological methods, but a selected number of isolates were also identified using molecular methods.

Figure 3. Fungal isolates growing out of sections of a wood core taken from a symptomatic grapevine. Fungi on the plate are ready to be transfer on to new agar plates for systematic identification.

MONITORING VINE PERFORMANCE

The same vines that we sampled for the presence of fungi (both symptomatic and non symptomatic vines) were also monitored for vine performance during the 2006/2007 vintage. Originally, the vines were to be sampled at harvest and fruit composition and yield measured. However, as part of her eight week internship with HortResearch in Marlborough, Cristina Cocchi from Bologna University conducted an intensive pre-harvest sampling programme as well as the harvest assessment. Therefore, the monitoring and results for vine performance section of the project are more extensive than originally planned.

Samples consisting of 30 berries were collected weekly from pre-veraison until harvest. Early season samples were assessed for soluble solids (°Brix), berry weight and hardness. Veraison is the term used to describe the time when a grape berry undergoes a number of physiological changes. In red grapes, these changes include formation of pigments in the skin, resulting in colour change from green to red. In white grapes, the point of colour change is not easy to define but in all grapes a softening of the berry also takes place at veraison, so that an assessment of the number of hard berries can provide a proportion of the berries that have undergone veraison. When 50% or of the berries were assessed as having undergone veraison, pH, titrated acidity (TA) and yeast available nitrogen (YAN) sampling were also conducted (Table 1). All fruit composition sampling was made using standard industry tests.

Table 1. Dates and measurements made of berry quality for each of the experimental Marlborough grapevine sites in the trunk disease survey, 2007.

Date	° Brix	pH	TA[(1)]	YAN[(2)]	Yield[(3)]/vine	Note
22/02/2007	√					Only at Site 3
28/02/2007	√					Only at Site 3
1/03/2007	√					Only at Sites 1 and 2
5/03/2007	√	√	√	√		On all sites
12/03/2007	√	√	√	√		On all sites
19/03/2007	√	√	√	√		On all sites
26/03/2007	√	√	√			On all sites
2/04/2007	√	√	√	√	√	Harvest at Site 3 and analysis on all
10/04/2007	√	√	√	√	√	Harvest at Site 2 analysis on Sites 1 and 2
16/04/2007	√	√	√	√	√	Harvest and analysis only on Site 1

(1) YAN – Yeast available nitrogen
(2) TA - Titrated acidity
(3) Yield included the recording of bunch number, 32-berry sample weight, total weight of fruit per vine and calculating the average bunch weight per vine.

TECHNOLOGY TRANSFER

One of the key aims of this project was to increase awareness of the trunk diseases, trunk disease symptoms and to encourage growers to talk about the problem. Information has been transferred to the wine industry and to the wider community via a fact sheet, public presentations, newspaper stories, magazine articles, science publications, reports and the Marlborough Wine Research Centre website (Appendix 2).

RESULTS

SURVEY AND ISOLATION

When cores of wood from vines were plated, some cores resulted in multiple fungal isolations from the same vine and other cores did not give rise to any isolates. Table 3 provides a summary of the most commonly isolated pathogenic fungi for each of the three sites. *Botryosphaeria* spp. were isolated from all three sites from both vines with visual symptoms (Sick) and vines without visual symptoms (Healthy). At Site 1, all the sick vines had *Botryosphaeria* spp. isolated from them but so did three of the five healthy vines. These isolations were particularly interesting, as the wine company had considered that they had a problem at this site with *Eutypa lata,* which we did not isolate from these samples. *Eutypa* sp. was isolated from sick vines at the other two sites (2 and 3) as well as from healthy vines at Site 3. Basidiomycete fungi were isolated from all three sites and *Phomposis* spp. were isolated from both sick and healthy vines at Site 3. No simple pattern linking symptoms and isolations was observed.

Table 3. Summary of the number of fungi isolated (in 2006) and identified from each of the five main pathogenic groups associated with grapevine trunk diseases. Before isolation vines had been grouped into vines with visual symptoms (Sick) and vines without visual symptoms (Healthy).

		Botryosphaeria spp.	*Cylindrocarpon* sp.	*Eutypa lata*	*Phomposis* sp.	*Basidiomycete* spp.
Site 1	**Riesling**					
	Sick (n=5)	5				
	Healthy (n=5)	3				1
Site 2	**Riesling**					
	Sick (n=10)	3		4		1
	Healthy (n=10)	2				
Site3	**Sauvignon blanc**					
	Sick (n=10)	3		5	2	2
	Healthy (n=10)	5	2	2	3	1

MONITORING VINE PERFORMANCE

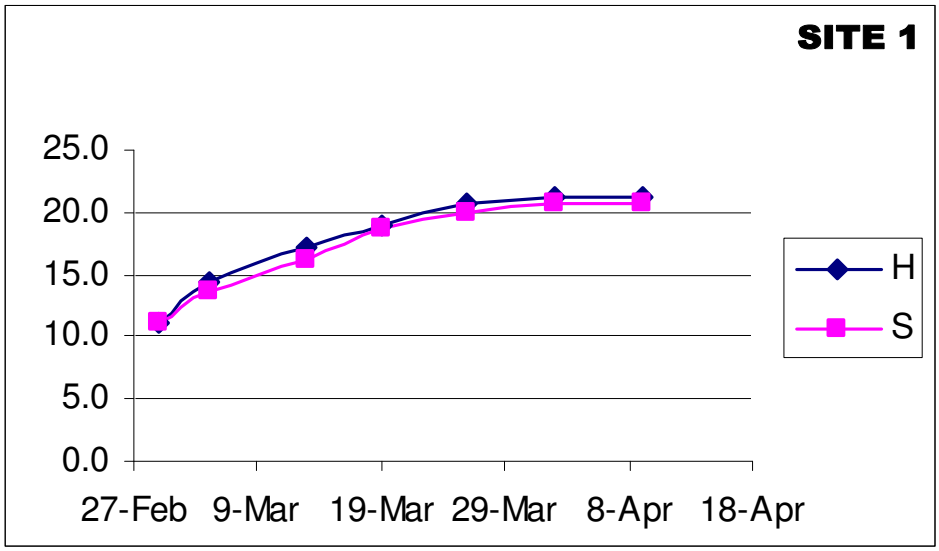
Differences in mean soluble solids at harvest at all three sites were small (Figure 4) and there were no consistent trends amongst sites. However, the trends at harvest were also observed during the pre-harvest period, with healthy vines at Site 1 having slightly higher soluble solid readings during pre-harvest (Figure 4). At Site 2, sick vines had slightly higher average soluble solids measurements during the season at harvest. At Site 3, which consistently had higher mean soluble solid values during the season for healthy fruit, harvest levels of soluble solids were also higher.

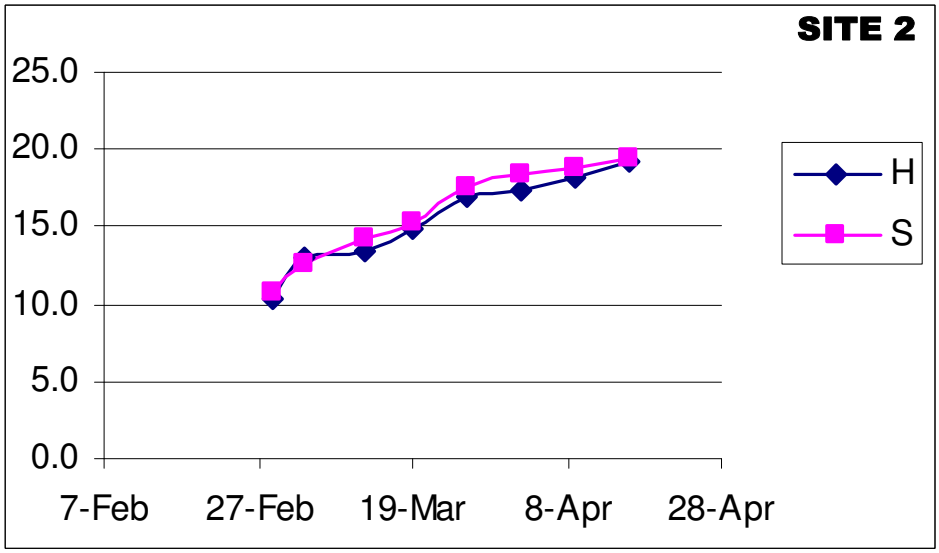
While the vines with and without visual symptoms might appear to be have differences in pH (Figure 5), the actual differences are very small (Table 3). The same trend of no real

difference between vines with and without symptoms can be seen with TA and ammonium concentration (Figures 6 and 7).

However at Sites 1 and 3, consistent differences in juice amino acid concentration and total available nitrogen (YAN) were observed (Figures 8 and 9). The vines that had been selected as Healthy (no visual symptoms) had higher amino acid levels than those that appeared sick.

At harvest, all the berry sampling was repeated and yield and bunch numbers were also compared between vines that appeared healthy and those that appeared sick (with symptoms). When the yields of the vines were compared statistically for each site, no significant differences were detected. The number of bunches per vine was also not significantly different at any of the three sites.





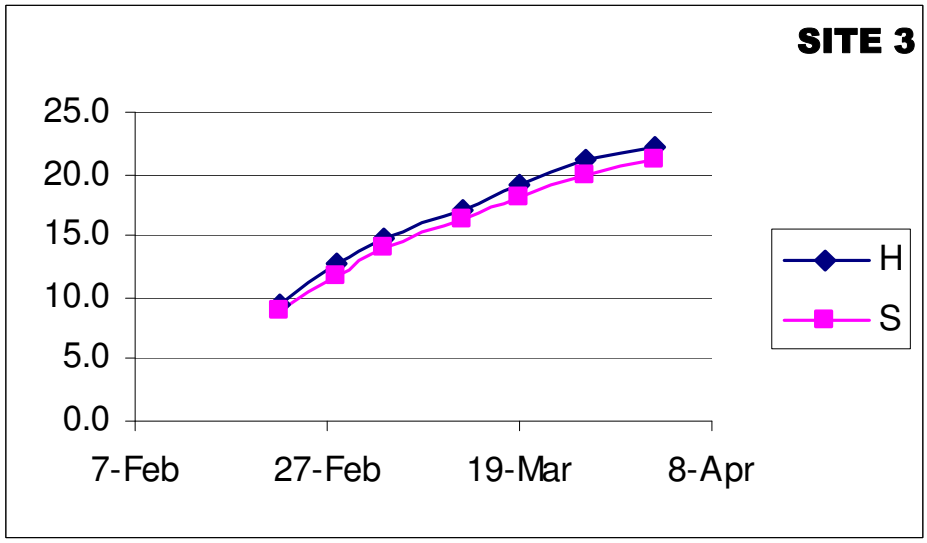


Figure 4. Soluble solids (°Brix) values for vines with (S) and without (H) grapevine trunk disease symptoms at the three Marlborough sites over time until harvest 2007. Measurements are the means of paired samples collected from Site 1 Riesling (n=10), Site 2 Riesling (n=20) and Site 3 Sauvignon blanc (n=20).

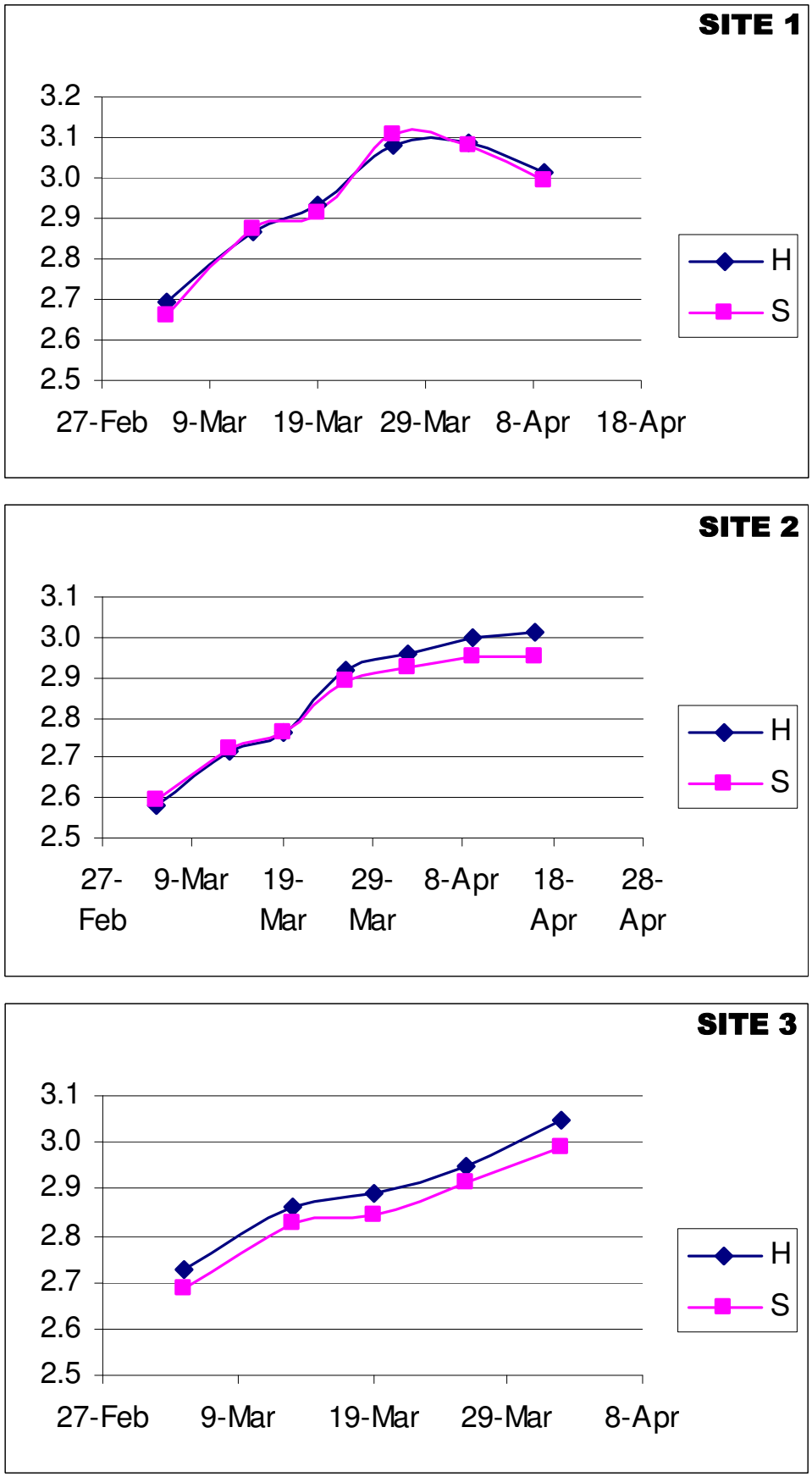


Figure 5. pH values for vines with (S) and without (H) grapevine trunk disease symptoms at the three Marlborough sites over time until harvest 2007. Measurements are the means of paired samples collected from Site 1 Riesling (n=10), Site 2 Riesling (n=20) and Site 3 Sauvignon blanc (n=20).

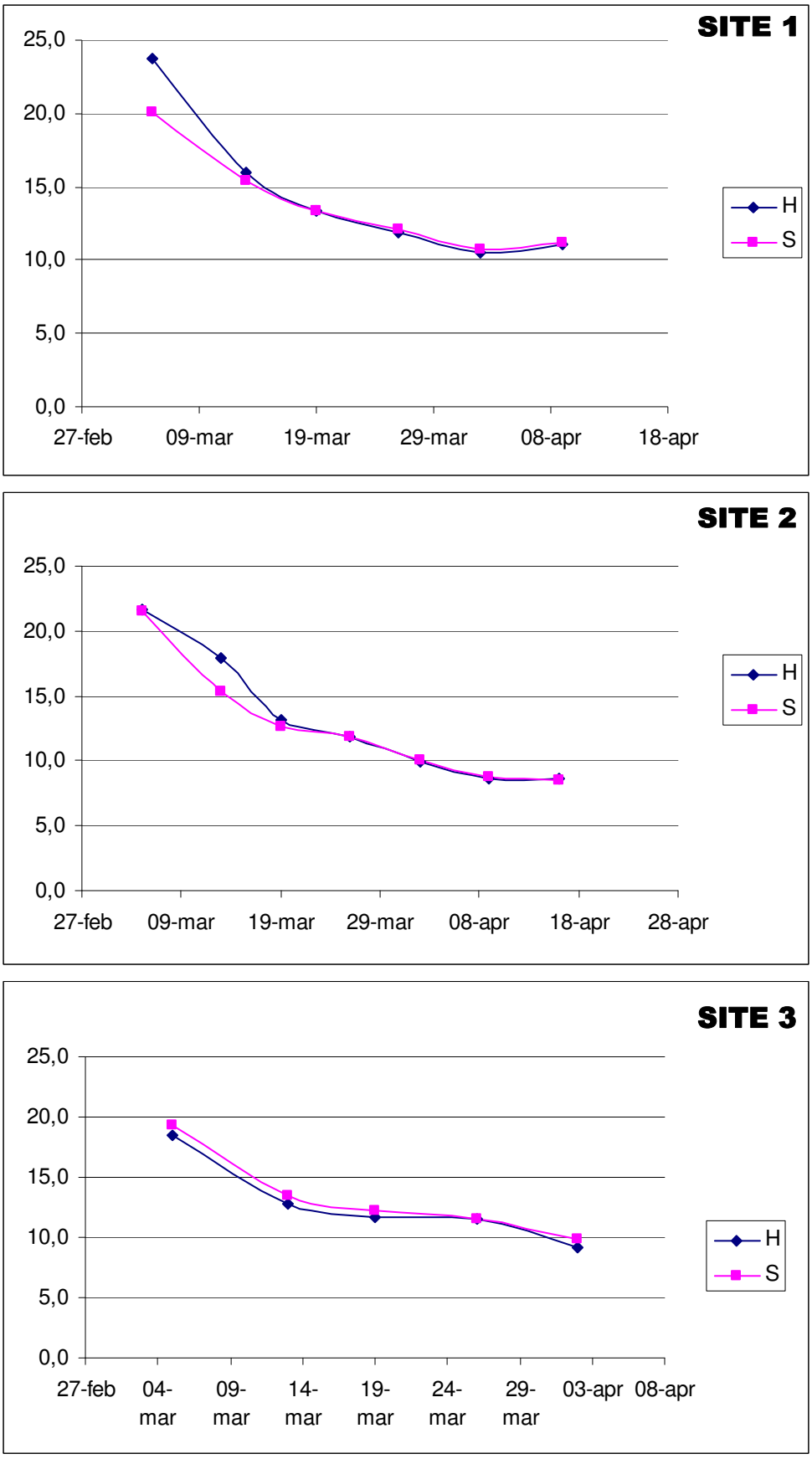
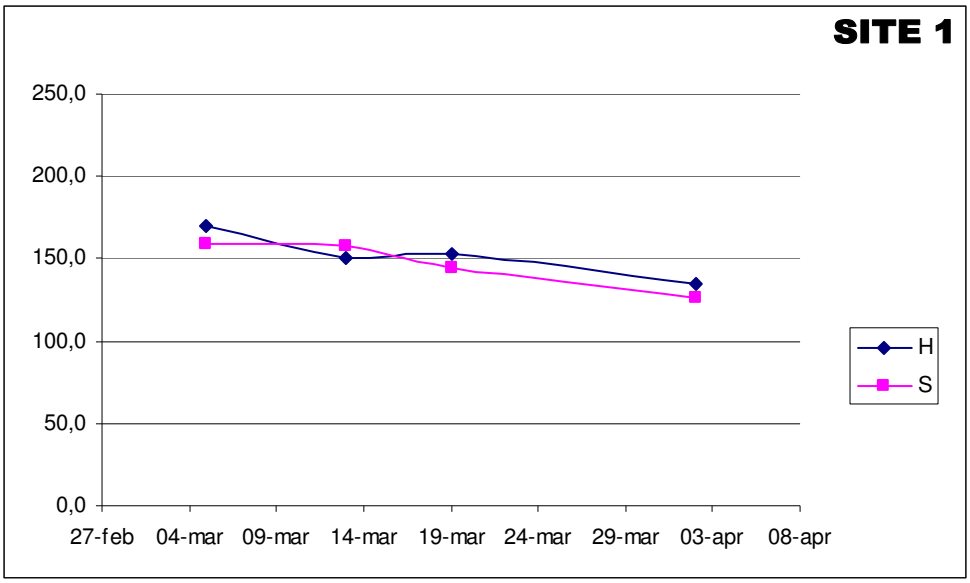
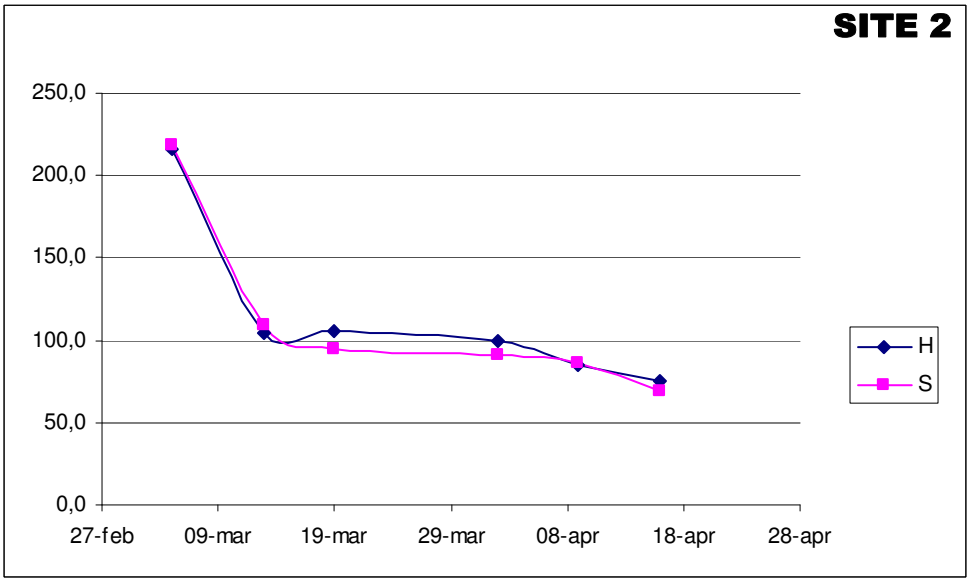


Figure 6. Titratable acidity values for vines with (S) and without (H) grapevine trunk disease symptoms at the three Marlborough sites over time until harvest 2007. Measurements are the means of paired samples collected from Site 1 Riesling (n=10), Site 2 Riesling (n=20) and Site 3 Sauvignon blanc (n=20).





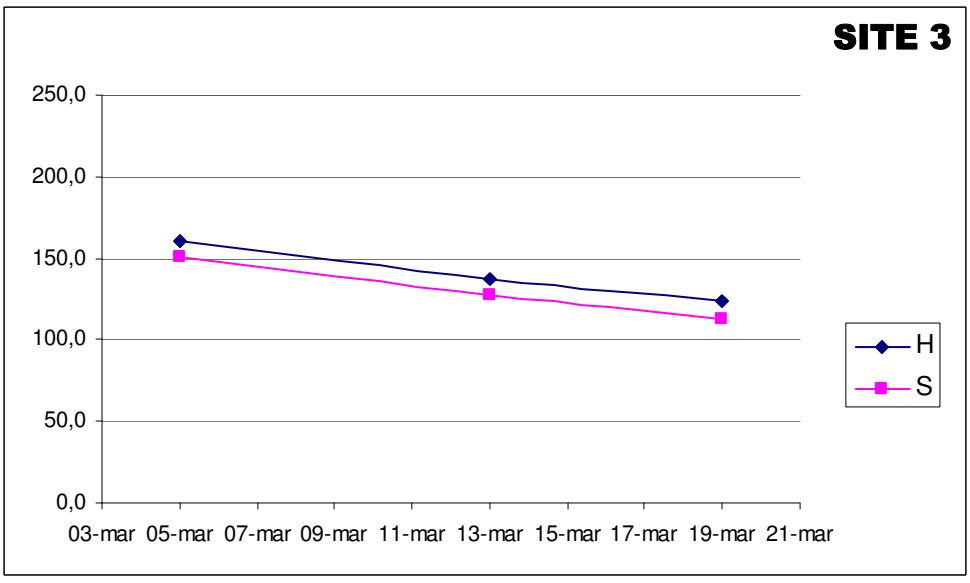


Figure 7. Ammonium values for vines with (S) and without (H) grapevine trunk disease symptoms at the three Marlborough sites over time until harvest 2007. Measurements are the means of paired samples collected from Site 1 Riesling (n=10), Site 2 Riesling (n=20) and Site 3 Sauvignon blanc (n=20).

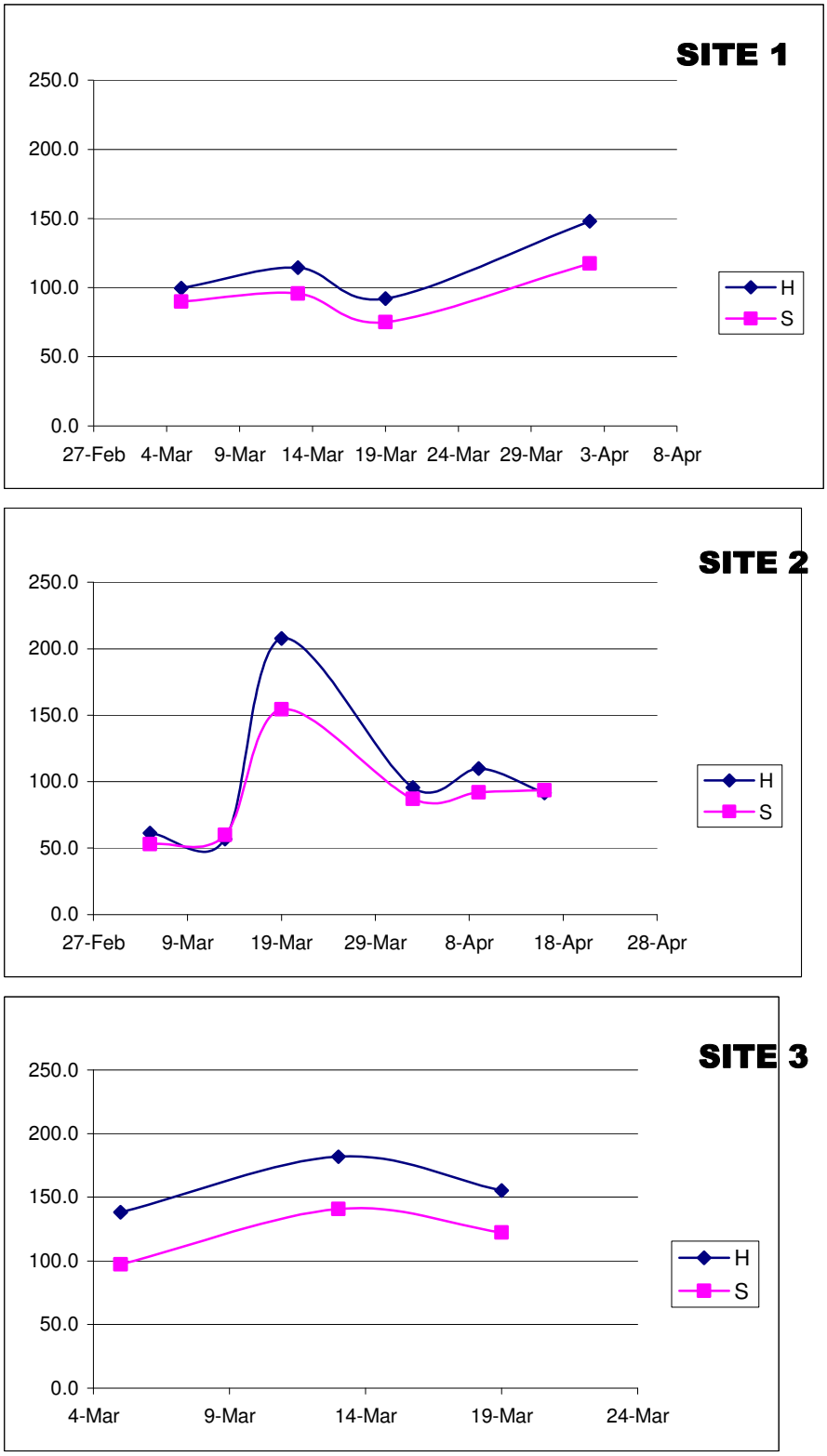


Figure 8. Primary amino acid values for vines with (S) and without (H) grapevine trunk disease symptoms at the three Marlborough sites over time until harvest 2007. Measurements are the means of paired samples collected from Site 1 Riesling (n=10), Site 2 Riesling (n=20) and Site 3 Sauvignon blanc (n=20).

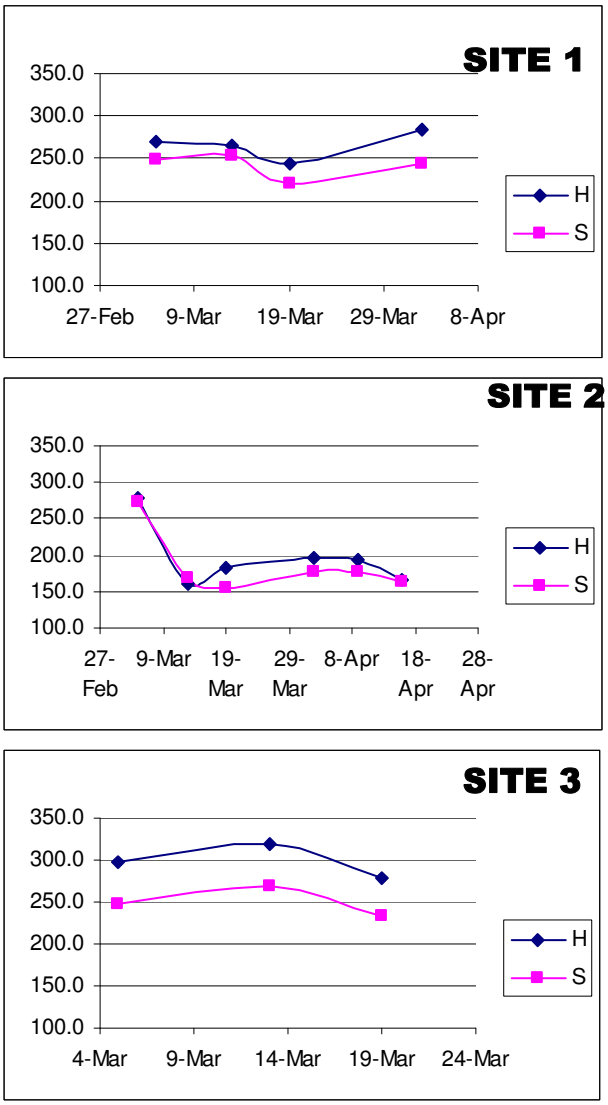


Figure 9. Total yeast available nitrogen (YAN) values for vines with (S) and without (H) grapevine trunk disease symptoms at the three Marlborough sites over time until harvest 2007. Measurements are the means of paired samples collected from Site 1 Riesling (n=10), Site 2 Riesling (n=20) and Site 3 Sauvignon blanc (n=20).

Table 3. Summary of physiological measurements at harvest 2007 of vines with (S) and without (H) grapevine trunk disease symptoms at the three Marlborough sites. Measurements are the means of paired samples collected from Site 1 Riesling (n=10), Site 2 Riesling (n=20) and Site 3 Sauvignon blanc (n=20).

	Sick vines			Healthy vines			Δ (Sick-Healthy)		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
YIELD AT HARVEST							n= 10	n=20	n=20
32-berry sample weight (g)	45.3	43.4	58.0	44.5	42.8	56.7	0.9	0.6	1.3
Bunch number	54.6	79.4	55.0	48.6	91.5	59.0	6.0	-12.1	-4.0
Total yield per vine (kg)	3.70	5.40	4.27	2.90	6.10	3.78	0.80	-0.70	0.60
HARVEST JUICE ANALYSIS									
Soluble solids (Brix°)	20.70	19.50	21.40	21.30	19.10	22.30	-0.60	0.40	**-0.90**
pH	2.99	2.95	2.99	3.01	3.01	3.05	-0.02	-0.06	-0.06
TA (g/L)	11.13	8.49	10.10	11.12	8.60	10.00	0.01	-0.11	0.10
HARVEST JUICE NITROGEN CONCENTRATION									
Ammonium (mg/L)	125.6	69.1	112.2*	135.0	75.5	123.4*	-9.4	-6.4	-11.2*
Amino acids (mg/L)	117.5	93.7	122.3*	148.0	91.6	155.2*	-30.6	2.1	**-32.9***
Total yeast available nitrogen (mg/L)	243.1	162.7	234.5*	282.8	167.1	278.6*	-39.8	-4.4	**-44.1***

* Harvest samples for juice nitrogen at Site 3 were not available, so the last pre-harvest samples are compared.
Delta values in bold are significantly different (P=0.05).

DISCUSSION

This project has focused on identifying which fungi are associated with grapevine trunk disease in Marlborough, how these fungi affect vine performance, and increasing growers' awareness of grapevine trunk diseases. Fungal isolations and monitoring of vines were conducted on Sauvignon blanc and Riesling grapevines to overcome concerns that a single variety may not provide a good representation of what was happening in the district. While from the scientific point of view, we might want to understand exactly how these diseases interact with the plant and the environment in which vines are growing, for the grower the questions are less complex. The grower wants to know, "What does having this fungus mean for my production system, and what can I do about it?"

The identification stage of the project has shown that *Botryosphaeria*, *Eutypa* and some Basidiomycete fungi are present in Marlborough vineyards. However, the relationship between symptoms and the ability to isolate the fungus from a core of wood is less clear. *Botryosphaeria* was isolated at all three sites but from vines both with and without symptoms. *Botryosphaeria* is certainly strongly associated with disease but is it primarily responsible for the symptoms? The vines without symptoms may develop symptoms over time because of the slow development of the disease.

At two of the sites (2 and 3) we were able to isolate *Eutypa,* but at Site 3 we also isolated the fungi from the healthy-looking vines without symptoms. Again, as the isolation was at a single time, we have only a snapshot of what was happening in the vineyard. We would have to monitor fungal presence over time to determine if the vines without symptoms develop these over time. The Basidiomycete fungi we isolated were always associated with the presence of at least one other fungus, suggesting that they are not the primary cause of symptoms. More isolations and observations over time will be required to determine the importance of the fungi isolated from trunk disease symptoms in Marlborough.

Regardless of which fungi are responsible for the symptoms, the grower wants to know how having infected vines will influence productivity and profitability of the vineyard. With no significant differences in yield at the three sites, growers would be interested to see if fruit composition is affected, as this may lower the price they are paid for the grapes.

The main composition factor upon which growers are paid is soluble solids. Significant differences in soluble solids were only observed at Site 3. The delay observed at this site might be a concern to the grower, as this could have financial implications in reducing payment if fruit were harvested at normal time, or increased disease risk if they had to harvest later in the season. The results from Site 1 also suggest that soluble solids accumulation may be delayed in vines with symptoms. However, the results at Site 2 did not show the same trend. Large vine-to-vine variation in the vines both with and without symptoms made it hard to demonstrate statistically that differences were due to the presence of symptoms.

Of interest to winemakers are the concentrations of primary amino acids and ammonium that are added together to determine total YAN. At Site 3, both primary amino acid and total YAN concentration were significantly lower on vines with trunk disease. Low total YAN can lead to problems in winemaking, as the yeast needs the primary amino acids and ammonium to grow and complete the wine making process. While winemakers are able to add ammonium to the juice to increase the total YAN, primary amino acids cannot easily be added, limiting

the options for the winemaker. The best way for a winemaker to increase juice amino acid levels is to have the viticulturist increase them in the vineyard, using good vineyard practices.

While a viticulturist will aim to provide a balanced nutrient budget, other factors such as disease can interfere with this. The viticulturist will try to provide a balance in the vineyard with the correct amount of nitrogen to provide the YAN the winemaker wants, without adding too much, that could lead to excessive vegetative growth or leaching into the soil. In the case of trunk diseases, the nitrogen that the roots are providing for the fruit may be intercepted by the fungus. Alternatively the fruit may not receive the nitrogen, as the whole process of nutrient uptake within the vine may be damaged by the blockages to the vascular system (the vine's plumbing). Regardless of why the nitrogen does not reach the fruit, the viticulturist has some options for treating the vines.

Options for treatment include removing stress, increasing nitrogen availability and removing damaged tissue. If other stresses on the vine such as water stress are removed, the ability of the vine to take up nutrients will be increased and the limited resources used to overcome the water stress can be reallocated to increasing fruit nitrogen content. Supplying more nitrogen to the roots is another possible way to overcome low YAN. By making nitrogen more available to the vine, less carbon and other reserves will be required to take up the nutrients, and the uptake of more nitrogen will be possible.

The third option, of surgery, may seem radical but it has some other potential benefits as well. Unlike humans, grapevines are able to have large sections removed and continue growing as long as a connection from the roots to shoots is maintained. If the trunk of the grapevine becomes damaged, a new shoot can be trained up and the old section of trunk removed. The new shoot will have undamaged plumbing to allow nutrients and water to flow to the leaves and fruit and should be free of the trunk disease. If done correctly, this surgery can remove the sections of the vine with the fungal infection and increase the productive life of the vine. However, this procedure is time consuming and most be carried out before the disease has spread extensively through the vine.

Providing growers with management options, including vine surgery or vine removal in terminal cases, is an area that we have identified while conducting this project. Attendance at the workshops and the questions asked during the discussion groups indicate that growers and viticulturists are looking for ways to identify these diseases, and to manage them before they reduce the productivity of the vineyard. The monitoring conducted this season indicates that even before the vine dies, the value of the fruit produced may be lowered because of lower soluble solids and nitrogen content.

CONCLUSIONS

Our work this season has shown that both *Botryosphaeria* and *Eutypa* fungi are present in the vineyards of Marlborough. Monitoring of the vineyards that have visual symptoms of decline has also shown that grapevine trunk diseases can affect the composition of fruit, by reducing soluble solids and berry nitrogen content. These reductions have the potential to result in additional losses for growers, prior to the losses incurred by the eventual death of the vine from the disease. Additional work is required to provide the industry with the tools to manage trunk diseases actively under New Zealand conditions.

RECOMMENDATIONS

Based on the finding of this project and the general lack of understanding of how grapevine trunk diseases affect the New Zealand wine industry, we recommend the following areas of activity be pursued:

1. A nationwide identification of which fungal pathogens are present in each of the major wine growing regions
2. A study of the rate of decline of vines with these pathogens and a model of how this decline will affect economic sustainability of vineyards
3. A study of the method of infection of these pathogens
4. Field testing of pruning wound dressings to find effective management options, as this vineyard activity has been shown to be important in other countries
5. Provision of workshops and fact sheets on vine surgery and other management options
6. More industry workshops promoting discussion about trunk diseases and awareness of management options
7. Underpinning science that allows researchers to continue to provide the industry with management options.

REFERENCES

Pascoe I & Cottral E 2000. Developments in grapevine trunk disease research in Australia. *Phytopathologia Mediterranea* 39 (1):68-75.

Whiteman SA, Stewart A, Ridgeway HJ, Jaspers MV 2007. Infection of mother-vines by *Phaeomoniella chlamydospora* results in infected young grapevines. *Australasian Plant Pathology*, 36, 198-203.