Managing new fungicides for the control of powdery mildew and other grape diseases

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: SAR 99/3

Principal Investigator: Catherine Hitch

Project Coordinator: Dr Trevor Wicks

Research Organisation: South Australian Research and Development Institute

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CONTENTS

1. Abstract

This project was undertaken to develop and evaluate management strategies for the efficient use of the new fungicide group, the strobilurins, for the control of powdery mildew, downy mildew and Botrytis bunch rot of grapes. Laboratory, greenhouse and field experiments were carried out to determine the protectant and curative limitation of the strobilurin fungicides Amistar and Flint. These studies showed that they were most effective when applied before infection, and while they were effective against both powdery and downy mildew they were less effective on Botrytis bunch rot.

Field trials over 3 seasons evaluating various sprays regimes were focused mainly on powdery mildew and these showed that excellent control of powdery mildew was obtained when 4 to 6 sprays per season were used and included either wettable sulphur or DMI before and after 2 to 3 applications of a strobilurin around flowering. Where Flint and Amistar were directly compared Flint was the most effective fungicide for controlling powdery mildew whereas Amistar was more effective than Flint in controlling downy mildew.

2. Executive Summary

Laboratory, greenhouse and field studies were carried out over 3 years to develop and evaluate management strategies for the efficient use of strobilurins the new fungicide group for the control of powdery mildew, downy mildew and Botrytis bunch rot of grapes.

In the first 2 years, work focused on the strobilurins Amistar and Flint and in the third year Cabrio, a new as yet unregistered strobilurin, was included in the trials. Emphasis was placed on the control of powdery mildew as the studies showed the strobilurins were most effective on this group of fungi. The fungicides also controlled downy mildew, but had minimal effect on *Botrytis*. Two new fungicides, Legend and Prosper with new modes of activity difference to the strobilurins were also evaluated in direct comparison to Flint and Amistar. Both gave good control of powdery mildew but more work needs to be undertaken to refine their most effective place in a spray regime including strobilurins.

Amistar and Flint were evaluated in numerous spray programs that included various combinations with Thiovit (wettable sulphur) and Topas (DMI). In most experiments this included 6 sprays per season that incorporated Thiovit followed by various combinations of Topas, Amistar and Flint. The strobilurins were applied between flowering to just after fruit set and the order in which various fungicides were applied during this period had little influence on the level of powdery mildew control. The results for the first season showed that Flint was more effective than Amistar in the control of powdery mildew so emphasis was placed on Flint in the second season. However the results showed that Amistar was more effective than Flint in the control of downy mildew. In the final season (2001/02 which was one of the worst years on record for powdery mildew) programs that included as few as 4 sprays per season provided excellent control of powdery mildew on the bunches. In commercial vineyards however, further sprays of wettable sulphur or a DMI would be required to protect new shoot growth. In the final season the strobilurins were generally more effective when applied during or just after flowering compared to just before flowering. However these differences may not be obvious in commercial vineyards where the level of inoculum is likely to be much lower than that of the experimental plantings where unsprayed plots are maintained.

In addition this project had demonstrated the importance of spray timing in the control of powdery mildew. The work showed that excellent control of powdery mildew on the bunches can be achieved when spray programs that include early application of either wettable sulphur or a DMI followed by 2 or 3 consecutive strobilurin sprays around flowering.

The results of this work have been communicated to industry through more than 14 grower meetings, Research to Practice workshops and personal discussions with industry. In addition numerous articles on the project have been published in industry magazines as well as two publications in refereed scientific journals.

Nevertheless further work on the strobilurin fungicides is required to fine tune their use in vineyards. Potential areas of research are to evaluate (1) the efficacy of strobilurins when applied in low and ultra low (100 L/ha and less) spray regimes, (2) their efficacy when used in tank mixes with other fungicides, (3) their use in programs with extended spray intervals ie greater than 14 days between sprays. Further work also needs to be done to develop base line levels of sensitivity of Australian isolates of powdery mildew and downy mildew to the strobilurin fungicides to provide early warning to the development of resistance.

3. Background

Powdery mildew is the most important fungal disease of grapes in Australia. Although sulphur and the DMI (demethylation inhibiting) fungicides control the disease there is concern with sulphur phytotoxicity, residues and decreased sensitivity of populations of powdery mildew to the DMI fungicides. Resistant strains of powdery mildew are now common in Europe (Steden, 1998) and the USA (Ericson et al, 1997, Gubler at al, 1996) and have recently been detected in Australia (Savocchia et al, 1998). In many parts of Europe and the USA, the DMI fungicides are no longer effective. Because of these reasons, alternatives to DMI's and sulphur have been evaluated (Wicks and Hitch, 1998).

New fungicides known as strobilurins based on naturally occurring products found in mushrooms have recently been developed overseas. These materials are unique in that they have a wide spectrum of activity and are one of a few fungicides that control both downy and powdery mildews. These products also have low mammalian toxicity, are reportedly non persistent and are likely to dominate the future fungicide market. However, there is concern that the widespread use of these products could give rise to fungicide resistant population. In the USA where one of the strobilurins has recently been registered for use on grapes, studies have been undertaken to determine the baseline sensitivity amongst isolates of downy mildew (Wong and Wilcox, 1998).

Their effect on other grape diseases, e.g. *Botrytis* is not well known. Although some commercial product has recently been registered for use on grapes in California, little work has been down with these products in Australia. There is concern that widespread use of the strobilurins may give rise to populations of fungi resistant to this group of chemicals and it is important that baseline sensitivities be determined. The aim of this project was to evaluate and develop the best use strategies for the strobilurins and other new fungicides in Australia to ensure that these products are used appropriately to achieve the most efficient control of diseases and to reduce the likelihood of resistance developing. These fungicides are likely to have a significant impact worldwide on disease control in vines and it is essential that Australian expertise in the use of these products is developed to ensure that the Australian industry competes to world standards.

4. Project Aims and Performance Targets

?? To develop and evaluate management strategies for the efficient use of new fungicides for the control of powdery and downy mildew and other grape diseases.

?? To determine the protective and curative limitations of new fungicides for the control of grape diseases.

?? To evaluate management strategies that reduce the likelihood of resistance developing to the new fungicide chemistry.

?? To reduce fungicide use on grapevines.

?? To establish baseline sensitivities of new fungicides and to develop early warnings of fungicide resistance.

5. Methods

Products and formulations

Syngenta Crop Protection Australasia

Amistar WG (500 g/kg azoxystrobin)

Ciba-Geigy Phosphorous Acid (200 ml/L phosphonic acid)

Ridomil Gold MZ (640 g/kg mancozeb plus 40 g/kg metalaxyl-M)

Ridomil Plus (614 g/kg copper oxychloride plus 150 g/L metalaxyl)

Switch (375 g/kg cyprodinil plus 250 g/kg fludioxonil)

Thiovit (800 g/kg sulphur)

Topas (100 ml/L penconazole)

Bayer – Australasia

Flint (500 g/kg trifloxystrobin)

Prosper (500 ml/L spiroxamine)

Teldor (500 ml/L fenhexamid)

Aventis CropSciences

Rovral (250 ml/L iprodione)

Scala (400 ml/L pyrimethanil)

Dow Agroscience Australasia

Legend (250 ml/L quinoxyfen)

BASF

Cabrio (250 ml/L pyraclostrobin)

LABORATORY AND GREENHOUSE EXPERIMENTS

Powdery mildew

This experiment was designed to evaluate the curative activity of Flint and Amistar using 8 to 10 week old Chardonnay seedlings. Treatments were artificially inoculated by brushing powdery mildew spores from infected leaves onto five disease free seedlings per treatment. Flint (0.15 g/L) and Amistar (0.5 g/L) were applied either 3, 5 or 7 days after inoculation using a hand held atomiser. Leaves were tagged to ensure treated and inoculated leaves were assessed 12 days after inoculation. A 0 to 10 rating scale developed by Dr. R.W. Emmett – Sunraysia Horticultural Centre, Mildura, Victoria (pers. comm.) was used to score the incidence and severity of powdery mildew using a 0 to 10 rating scale where 0 = 0%, 1 = 0.8%, 2 = 2.3%, 3 = 4.7%, 4 = 9.4%, 5 = 18.8%, 6 = 37.5%, 7 = 62.5%, 8 = 81.3%, 9 = 89.5% and 10 = 100% of the leaf area covered with mildew.

Downy mildew

A laboratory experiment was conducted to determine the effect of the strobilurins on spore production of downy mildew (*Plasmopara viticola*). In this experiment 1 cm diameter leaf discs of Chardonnay leaves were floated upside down in petri dishes containing suspensions of either Amistar (0.375 g/L), Flint (0.2 g/L) or Ridomil Gold MZ (2.5 g/L) or on demineralised water. Each disc was inoculated with 1 or 2 drops of a downy mildew spore suspension and incubated for a further 10 days before the discs were assessed for sporulation and then removed and shaken in water to dislodge the conidia. Spore numbers were counted with a haemocytometer.

A greenhouse experiment was undertaken on potted Chardonnay vines to determine the curative effect of Flint and Amistar. Downy mildew was artificially inoculated on Chardonnay shoots by spraying then with a spore suspension containing 10^6 downy mildew spores/ml. Shoots were then enclosed in plastic bags for 24 hours. Flint (0.2 g/L) and Amistar (0.375 g/L) were applied to shoots 3 and 5 days after inoculation, and Ridomil Plus (1.5 g/L) was applied at 3 days as a standard fungicide for comparison.

Leaves were assessed 14 days later for the number of oil spots per leaf on a 0 – 4 scale where 0 = 0 %, 1 = 1-5%, 2 = 6-25%, 3 = 26-50% and 4 = 51-100% of the leaf area covered with oil spots.

Botrytis bunch rot

Preliminary studies were carried out to determine the *in vitro* activity of Amistar and Flint against several strains of *Botrytis*. Seven isolates of *Botrytis* were tested to determine whether Flint or Amistar at 0, 0.05, 0.1, 1,5,10 and 100 ppm inhibited the growth of *Botrytis*. Each treatment consisted of five, 5mm plugs of hyphae plated onto the fungicide amended media and the hyphal growth measured 2 and 7 days later.

FIELD TRIALS

Sites

Twenty field trials were undertaken over the 3 year project to evaluate the effect of the strobilurins on powdery mildew, downy mildew and Botrytis bunch rot. These field trials were undertaken on Research Centres located at Lenswood, Nuriootpa and Loxton situated approximately 30 km east, 100 km NE and 300 km NE of Adelaide respectively.

Details of the grapevines used, planting architecture, and management methods are shown in Appendix 5.

Spray application

All back-pack sprayed trials were undertaken using "Solo" motorised back pack sprayers, applying up to 500 L/ha of spray in early spring up to 1000 L/ha when vines were in full foliage in mid summer. In trials where fungicides were applied by machine, a Hardi "mini-SPV" mist blower was used at Nuriootpa and an Air mist sprayer used at Loxton.

All inoculation suspensions and downy mildew fungicides were applied using a hand held atomiser to ensure complete coverage.

Data Analysis

Data from the final assessments were analysed with the analytical software package "Statistix for Window V2", using general analysis of variance to generate values for least significant differences (*LSD, P* = 0.05).

POWDERY MILDEW

Field trials were undertaken at the 3 sites over the duration of the project evaluating different combinations of fungicides applied at various growth stages for the control of powdery mildew. Grapevine varieties included Chardonnay, Verdelho, Shiraz and sites incorporated cool to hot grape growing areas in South Australia.

Timing

Fungicide combinations were applied from bud burst through to bunch closure applying as little as 3 and up to 7 sprays per season. Fungicides, spray timing, growth stages, rates and grape variety are all shown in Tables 3 to 15 (Appendix 5).

Leaf and bunch sampling and assessment methods

Fifty leaves and bunches were selected at random from the middle two vines of each plot at various times and assessed for disease incidence and severity. Leaves were assessed in the laboratory by examining the upper and lower leaf surface. All other bunch assessments were done *in situ* except for the final bunch assessment at harvest when bunches were picked and then examined.

At all sampling times leaves and bunches were assessed using a 0 to 10 rating scale as previously described (Appendix 4).

DOWNY MILDEW

Downy mildew field experiments were undertaken on Chardonnay grapevines located at the Lenswood Research Centre. Experiments were undertaken on shoots either artificially inoculated or naturally infected with downy mildew. Natural infection occurred in the Chardonnay planting in all 3 years of the project.

Timing

Fungicides, spray timing, growth stages, rates and grape variety are all shown in Tables 16 to 18 (Appendix 5).

Trial 1.

The natural infection of downy mildew in 1999/2000 was utilised as an inoculum source for this experiment. Late developing shoots that had not received any fungicide sprays and were located within 20 cm of a shoot with downy mildew lesions, were either left unsprayed or treated with Amistar, Flint or Ridomil Plus. Fungicides were applied to 25 shoots per treatment 1 day after a rain event that provided conditions suitable for sporulation and the spread of downy mildew (Table 16).

Trial 2.

The natural infection of downy mildew which occurred in 1999/2000 was used to compare the Flint and Amistar spray regimes that were initially applied for powdery mildew control in this season. Treatments are shown in Table 17.

Trial 3.

Chardonnay shoots were artificially inoculated with downy mildew as previously described and treated with either Amistar, Flint, Ridomil Plus or Phosphorous Acid either 3 or 7 days after inoculation (Table 18). Twenty one days after inoculation leaves were removed and incubated overnight to induce sporulation. Leaf disks of sporulating leaf tissue were also removed and shaken in distilled water before spore levels were measured using a haemocytometer.

Leaf and bunch sampling and assessment methods

The method used for leaf and bunch assessments on artificially inoculated shoots involved removing shoots from the vines and returning them to the laboratory where they were incubated in a moistened plastic bags in the dark to induce sporulation. The severity of the downy mildew was rated on each of the 6 to 10 leaves per shoot using a 0 to 4 rating scale where 0 = 0%, 1 = 1 to 5%, 2 = 6 to 25%, 3 = 26 to 50% and 4 = >50% of the leaf area covered with sporangia.

The assessment carried out on leaves in trial 2 were assessed using the same 0 to 10 rating scale as that used for powdery mildew assessment as previously described (Appendix 4).

BOTRYTIS BUNCH ROT

Field experiments were conducted on Pinot Noir grapevines to evaluate the efficacy of the strobilurins on the control of *Botrytis* infection at flowering and veraison. Because of the unreliability of natural infections, flower clusters and bunches were artificially inoculated by spraying them with approximately 0.5 to 1ml of a *Botrytis* spore suspension containing 10^6 spores/ml. Bunches or flower clusters were inoculated in the late afternoon and then enclosed in a plastic bag to ensure surface moisture was maintained to enhance infection. Bags were removed the following morning.

Timing

The efficacy of strobilurins were compared against standard fungicides when applied either before or after flowering or at veraison. All fungicide treatments, chemical rates, and timing in relation to inoculations are shown in Table 20 and 23 (Appendix 5).

Trial 1.

In this trial Flint and Rovral were compared when applied either 3 days before or 3 days after infection at flowering. The two fungicides were also compared at veraison when applied 8 days before infection (Table 20). The incidence of *Botrytis* at flowering and veraison was assessed on berries 7 days after inoculation. Bunches were harvest 24 days after inoculation at veraison.

Trial 2.

This trial was designed to determine the curative effect of fungicides in relation to veraison. Fungicides were applied at veraison or 10, 14, 17 and 24 days after veraison and inoculated 7 days before harvest (Table 23). The final application at 24 hours was applied 3 days after inoculation. All chemical treatments in this experiment were applied by dipping individual bunches into fungicide solutions to ensure complete coverage. All bunches were harvested for assessment 28 days after veraison.

Trial 3

The efficacy of Flint, Amistar, Rovral and Scala was evaluated applying them either 7 and 4 days before or 4 days after inoculation at flowering (Table 25). Berries were harvested 10-14 days after inoculation and brought back to the laboratory. Levels of latent infection was assessed on half of the berries in each treatment, by surface sterilisation. Berries were immersed in 0.4% hypochlorite for 2 minutes and rinsed three times in clean tap water. All berries were then plated onto *Botrytis* selective media and assessed for presence of *Botrytis* one week later.

Leaf and bunch sampling and assessment methods

The percent of infection at flowering and veraison was determine by collecting berries seven days after inoculation, 4 berries per treated bunch were removed and plated onto Botrytis selective media (Kerssies, A: Neth J.Pl. Path 96, 1990 pp 247-50) and incubated at room temperature for 7 days. After this time berries were assessed for the presence or absence of sporulating Botrytis.

At harvest all bunches were sampled and incubated at 100% relative humidity for 7 days in humidity trays. The bunches were then assessed for the incidence and severity of Botrytis bunch rot.

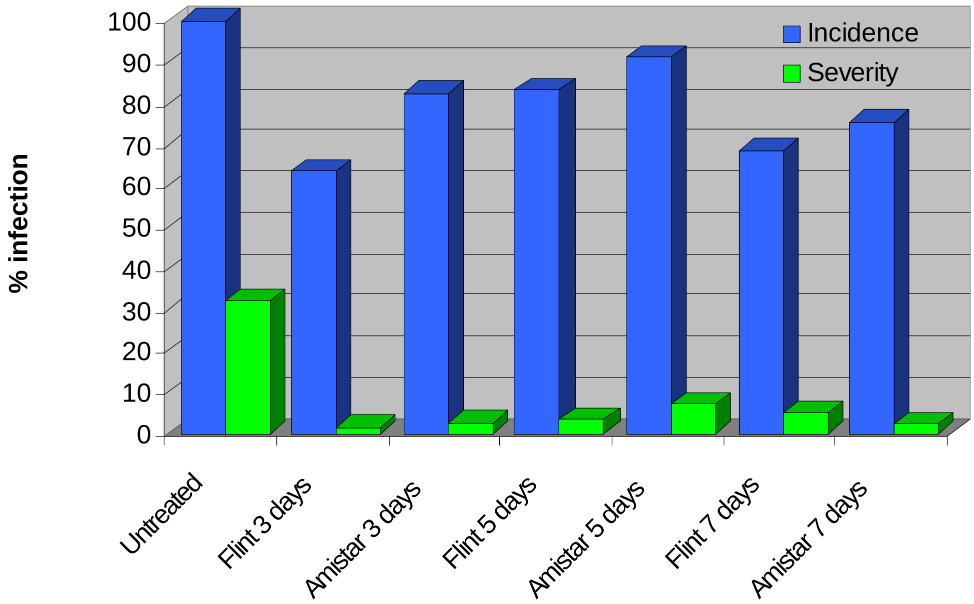
6. Results /Discussion

GREENHOUSE AND LABORATORY EXPERIMENTS

Powdery mildew

This experiment showed that the application of strobilurins either 3, 5 or 7 days after infection reduced the level powdery mildew on leaves compared to the untreated. The incidence of powdery mildew was significantly different in all strobilurin treatments compared to the untreated except for the Flint and Amistar treatments applied 5 days after inoculation. Disease severity was significantly less in all fungicide treatments with the greatest level of control achieved with the application of Flint 3 days after infection (Figure 1).

Figure 1. Incidence and severity of powdery mildew on Chardonnay leaves treated 3, 5, and 7 days after inoculation, July 2001.



Downy mildew

The laboratory experiment indicated that no fungicide inhibited sporulation completely (Table 1). Compared to the untreated, all fungicides inhibited the production of sporangia with the most inhibitory effect being produced by the Ridomil Gold treatment.

Table 1. Sporulation of *Plasmopara viticola* on Chardonnay leaf disks floating on fungicide suspensions.

Fungicide	**Rate/L**	**% disks sporulating**	**Area of disk sporulating**	**No. spores/cm^2 x 10^4**
Untreated	-	100	99 a	23 a
Amistar	0.375 g	100	98 a	16 b
Flint	0.2 g	100	89 a	13 b
Ridomil Gold MZ	2.5 g	100	70 b	5 c
LSD (*P* = 0.05)			16.5	4.6

Treatments with the same letter are not significantly different from one another (LSD *P*=0.05)

The artificial inoculation of downy mildew on Chardonnay vines in the greenhouse showed that all treatments significantly reduced the development of chlorotic lesions compared to the untreated with 100% of the leaves showing symptoms. The severity of downy mildew infection was significantly less in all fungicide treatments, with the greatest control achieved with Amistar applied 7 days after and Ridomil applied 3 days after inoculation, where no disease developed (Table 2).

Table 2. Incidence and severity of downy mildew on Chardonnay leaves treated with fungicides after inoculation, January 2000.

Fungicide	**Rate/L**	**Timing**	**Incidence**	**Mean % severity/leaf**
Untreated	-	-	100 a	24.8 a
Amistar	0.375 g	3 days	17.1 c	1.7 b
Amistar	0.375 g	7 days	0 d	0 b
Flint	0.2 g	3 days	5.6 cd	0.8 b
Flint	0.2 g	7 days	37.3 b	1.4 b
Ridomil Plus	1.5 g	3 days	0 d	0 b
LSD (*P* = 0.05)			16.5	3.7

Treatments with the same letter are not significantly different from one another (LSD *P*=0.05)

Botrytis bunch rot

These studies showed that even at the highest level of 100 ppm the strobilurins had little inhibitory effect on *Botrytis* when they were incorporated in agar (Figure 2 and 3). However since this work was done, reports from overseas (Olaya - personal communication) suggests that this technique may have been inappropriate and should have included the addition of salicylhydroxamic acid to inhibit the alternative oxidase respiratory pathway.

Figure 2. Percent inhibition of *Botrytis* hyphal growth on PDA amended with various rates of Flint.

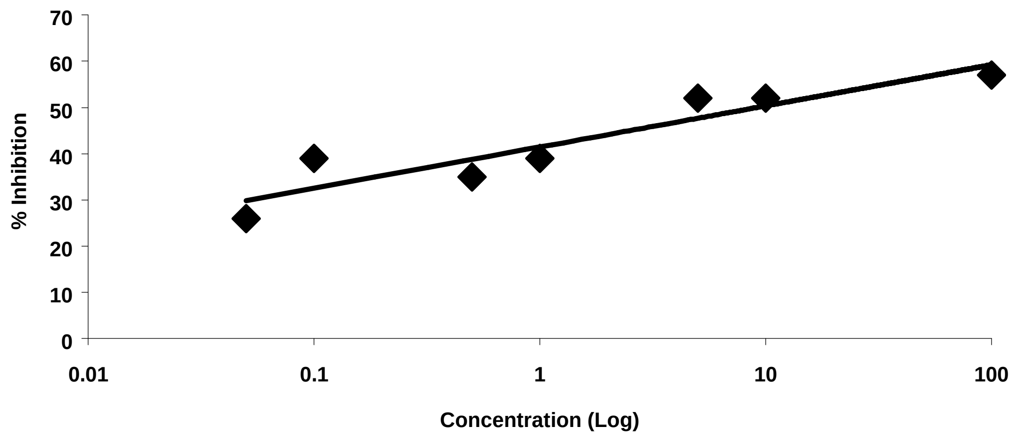
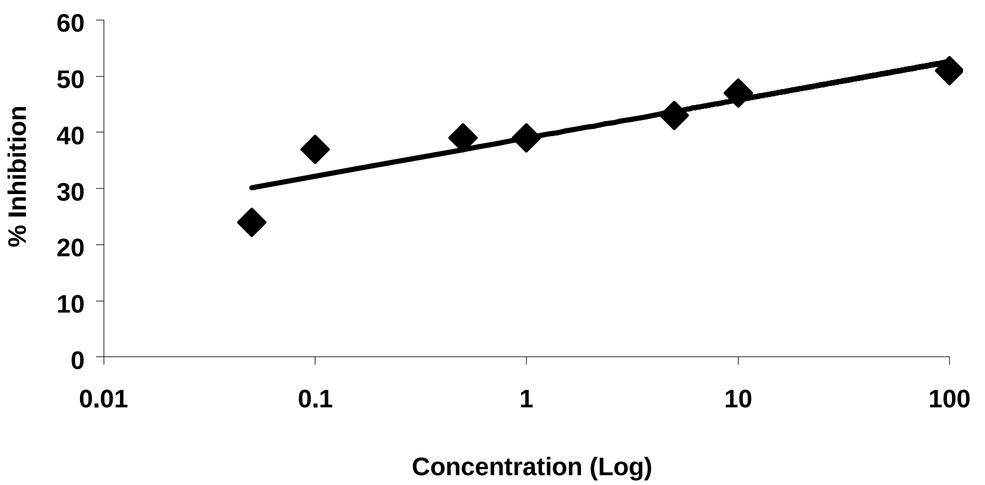


Figure 3. Percent inhibition of *Botrytis* hyphal growth of on PDA amended with various rates of Amistar.



FIELD TRIALS

Powdery mildew

The results of many of these trials have been written up and published in the Australian Journal of Grape and Wine Research (2002) 8:132-139 and in the Proceedings of the Brighton Crop Protection – Pests and Diseases (2002) copies of which are included in Appendix 1. Further details and results of all the trials carried out in this project are shown in Figures 4 to 48 (appendix 5).

Overall these trials have shown that the strobilurin group of fungicides provide excellent control of powdery mildew when applied around flowering and incorporated in spray programs with other materials such as wettable sulphur and DMI's.

In conditions and seasons of high disease pressure, programs that included 2 or 3 applications of the strobilurins Amistar, or Flint around flowering produced exceptional control of powdery mildew. Where the three different strobilurins were compared in different programs Flint and Cabrio were more effective than Amistar although these differences may not be obvious in commercial situations.

One of the major findings from this project was that the use of strobilurins will significantly reduce overall fungicide use in vines without any reduction in powdery mildew control.

In our studies, excellent control of powdery mildew was achieved with a 4 spray program where at least 2 of the sprays were strobilurins applied around flowering.

The new fungicides Legend and Prosper were also effective and should provide good alternatives to the other groups of fungicides currently registered.

DOWNY MILDEW

Trial 1.

The spread of natural downy mildew infection was significantly reduced with one application of Flint, Amistar or Ridomil Plus 24 hours after infection (Fig. 49). The incidence and severity of downy mildew was significantly less on all fungicide treatments compared to the untreated. Amistar and Ridomil Plus provided the best control of disease compared to the untreated and Flint treatment.

Trial 2.

Although these treatments were applied in a regime to control powdery mildew not downy mildew the assessment of leaves indicated that 2 applications of Amistar reduced the level of downy mildew on leaves at harvest (Fig. 50). The level of downy mildew found at harvest on untreated leaves was low, as the majority of viable leaves had already defoliated from the vines due to severe powdery and downy mildew infections throughout the season. The application of Flint in spray programs did not prevent the spread of disease compared to the level of control achieved with Amistar.

Trial 3.

When standard downy mildew fungicides like Ridomil Plus and Phosphorous Acid were compared to the standard downy mildew fungicides, neither Amistar or Flint had a marked effect on the development of oil spots (Fig. 51). Both Ridomil Plus and Phosphorous Acid completely inhibited the development of downy mildew when applied at either 3 or 7 days after inoculation whereas Amistar had little effect when applied at these times. Flint had some inhibiting effect, however while it was more inhibitory than Amistar its effect was not complete. Measurements of spore numbers showed that the application of either Amistar or Flint at 3, but not 7 days after inoculation inhibited spore production (Table 19).

Overall Amistar was more effective than Flint when used to control downy mildew. In situations where downy mildew protection is required around flowering, Amistar may be the preferred fungicide to use at that time. It appears that the strobilurin fungicides will be useful materials to be used in the management of grape diseases. The only draw back is that already strobilurin resistant strains of the downy mildew fungus have been detected overseas. Careful stewardship of these materials will be needed to prevent resistant strains developing in Australia.

BOTRYTIS

Trial 1

This work showed that more than 51% of berries were infected following inoculation at flowering. Neither of the Flint applications reduced the level of flower infection whereas Rovral applied 3 days either before or after inoculation reduced infection. At harvest 90% of inoculated clusters developed Botrytis bunch rot (Table 21). Levels were less in the treatments where fungicides were applied at flowering with the lowest level (43%) where Rovral was applied 3 days after inoculation.

At veraison, Rovral applied 8 days before infection was the only treatment which reduced the level of infection on berries (Table 22).

Trial 2

The season when this experiment was carried out was very hot and dry resulting in very poor bunch quality at harvest. The majority of bunches used in this experiment had dried up and berries had shrivelled from bird and bee damage. Only bunches that were suitable for assessment were harvested, resulting in the number of bunches picked ranging from 0 – 31. The results in Table 24 show that no conclusions can be drawn from this data due to the uneven number of bunches assessed and the low incidence of disease on the untreated bunches.

Trial 3

This experiment was analysed in two ways, firstly the differences between treatments at each fungicide application time was compared. Table 26 shows that none of the fungicides applied 7 or 4 days before inoculation significantly reduced the incidence of *Botrytis* at flowering. Rovral and Flint were the only fungicides that significantly reduced the level of *Botrytis* when applied 4 days after inoculation.

Although numerical differences were found the level of latent *Botrytis* infection between treatments applied 7 days before inoculation non of these differences were significant. Scala and Flint were the only two fungicides that significantly reduced the level of latent *Botrytis* when applied 4 days before infection. However when fungicides were applied 4 days after infection all treatments significantly reduced the level of latent *Botrytis* infection at flowering.

When all fungicide treatments and timings were compared in the second analysis, Rovral and Flint applied 4 days after infection at flowering were the most effective treatments. However when berries were surface sterilised and incubated to allow latent infection to develop, all fungicides applied 4 days after infection significantly reduced the level of infection (Table 26).

Overall these studies showed that neither Amistar of Flint controlled *Botrytis* when applied before infection however in one experiment Flint showed activity equivalent to Rovral when applied 4 days after infection at flowering.

7. Outcomes/Conclusion

This project has achieved most of its aims in developing and evaluating different strategies for efficient use of the strobilurins fungicides. These strategies will result in reduced fungicide use in vines, and improved control of powdery mildew. In addition the use of these strategies developed in this project will reduce the likelihood of resistant strains developing to these new materials.

The only objective not fully developed in this project was the establishment of baseline levels of sensitivity to the strobilurins. On reflection this was an over ambitious objective as it required considerable labour and input sufficient to warrant a separate 3 year project. However since this project commenced, data in this area has been published (Wong and Wilcox 2002). Overall this project has demonstrated that improved control of powdery mildew bunch infection can be obtained with as few as 4 correctly timed sprays. As a result of this work improvements in grape quality should eventuate as well as overall environmental benefits from reduced pesticide use.

8. Recommendations

Further research needs to be undertaken to fine tune the use of the strobilurin fungicides and in particular to ensure that they are retained as effective and useful products in Australian viticulture.

This includes:

(a) Developing base line levels of sensitivity to the strobilurins for a range of Australian isolates of downy mildew and powdery mildew.

(b) Evaluating strobilurin efficacy when applied in spray volumes of 100 L or less per Ha.

(c) Evaluating strobilurins when used in reduced spray regimes achieved by either extending the interval between sprays or using reduced rates in tank mixes with other fungicides.

(d) Extending the work to the new generation of strobilurin fungicides.

9. Appendix 1: Communication

The results of this work have been presented to a wide sector of industry through grower meetings, meeting with technical personnel of the major corporations and chemical companies, key industry personnel, scientific meetings and publication in industry journals.

Grape grower meetings and others where the results of this project have been presented were held in:-

1. Meetings of grape growers held in :-

Griffith, NSW	August 2000
Barossa Valley, SA	October 2000 and August 2001
Coonawarra, SA	March 2001 and May 2002
McLaren Vale, SA	June 2001 and May 2002
Barmera, SA	August 2001 and August 2002
Margaret River, WA	October 2001
Hunter Valley, NSW	November 2001
Mudgee, NSW	November 2001
Mildura, Vic	November 2001
Manjimup, WA	June 2002
Albany, WA	June 2002

2. Research to Practice Workshops held in:-

   Barossa Valley, McLaren Vale, Coonawarra, Robe, Padthaway, Adelaide Hills, Clare and Langhorne Creek, SA

3. Meetings with Industry and Agrochemical industry personnel in:-

   Barossa Valley and various areas in the Adelaide metropolitan area.

4. Australasian Plant Pathology Conference - Canberra, ACT in 1999

Publications

?? Australian Grape Grower and Wine Maker Magazine – Annual Technical Issue – 2001, pp 147-149.

?? Australian Grape Grower and Wine Maker Magazine – September 2001 Issue, pp 27-29.

?? Australasian Plant Pathology Society – 12th Biennial Conference Proceedings, 1999, pp 369.

?? Australian Journal of Grape and Wine Research (2002) Vol 8: 132-139.

?? Brighton Crop Protection Conference (2002) (in press).

?? Australian and New Zealand Grape Grower and Winemaker – Pests and Diseases Issue – September 2002.

?? Australian Viticulture – August (2002).

Integration of strobilurins and other fungicides for the control of powdery mildew on grapes

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The strobilurin fungicides Amistar (azoxystrobin) and Flint (trifloxystrobin) were evaluated in 1999/00 and 2000/01 in programs that included various combinations with Thiovit (sulphur) and Topas (penconazole) for the control of powdery mildew on grapes. In most experiments the application of 6 sprays per season that included Thiovit (3 g/L) followed by various combinations of Topas (0.125 mL/L), Amistar (0.5 g/L), Flint (0.15 g/L) and Thiovit (3 g/L) controlled powdery mildew. Although strobilurins were applied from flowering to after fruit set, the order in which various fungicides were applied had little influence on the level of powdery mildew control. In experiments where the bunch or leaf area diseased was 60% or higher in unsprayed plots, the severity was less than 5% where combinations of Thiovit and Topas with either Amistar or Flint were applied. In one experiment, 3 applications of either Legend (quinoxyfen) or Prosper (spiroxamine) following 2 applications of Thiovit also provided excellent control of powdery mildew. In programs where the application of Amistar and Flint were directly compared, Flint was the most effective fungicide for controlling powdery mildew. In the experiments where downy mildew also developed, Amistar was more effective than Flint in controlling this disease.

Keywords: *grapes,* Vitis vinifera L.*, powdery mildew, fungicides, strobilurins, downy mildew*

Introduction

Strobilurins are a unique group of crop protection compounds that possess a wide spectrum of activity against different pathogens. In grapes for example, strobilurins control both powdery mildew (*Uncinula necator*) and downy mildew (*Plasmopara viticola*). Two strobilurins, namely azoxystrobin (Amistar) and trifloxystrobin (Flint), have been evaluated overseas (Brunelli, *et al* 1996, Laird *et al* 1998, Wilcox and Riegel, 2000) and in Australian vineyards since the mid 1990's (Hitch *et al*, 1999, Tucker *et al*, 1997) and both are now registered for use on grapevines. The initial testing was based on complete programs of single products to evaluate the efficacy of different rates. Recent results from overseas indicate that continued spraying of the strobilurins was not an appropriate use for these products due to the development of resistant strains of fungi in some crops (Gullino *et al*, 2000 and Heaney *et al*, 2000). As an anti-resistance strategy, manufacturers now recommend that no more than 3 applications of strobilurins be applied to grapevines per season. However there is little published data on the efficacy of the strobilurins used with other fungicides and applied at different stages of plant growth or disease development. Because of this, experiments were undertaken to determine the most appropriate stage of vine growth to apply strobilurins and to evaluate the efficacy when applied in combined programs with other types of fungicides.

Methods

Products and formulations

Fungicides used were Amistar WG (500 g/kg azoxystrobin) – Crop Care Australasia, Legend (250 mL/L quinoxyfen) – Dow Agroscience Australasia, Prosper (500 mL/L spiroxamine), Flint (500 g/kg trifloxystrobin), Bayer – Australasia, Ridomil Plus (614 g/kg copper oxychloride plus 150 g/L metalaxyl), Thiovit (800 g/kg sulphur) and Topas (100 mL/L penconazole) – Novartis Crop Protection Australasia.

Sites

Six experiments were conducted at either Lenswood or Nuriootpa Research Centres situated approximately 30 km east and 100 km north-east of Adelaide, respectively. At Lenswood, treatments were applied to 9-year-old Chardonnay vines planted 1.5 metres apart at 3 metre row spacings. Rows contained 40 vines, spur-pruned and trained to a single cordon vertical foliage system. Plots consisted of 4 vines, each replicated 5 times in 1999/2000 (Experiment 2) and 7 times in 2000/2001 (Experiment 4) and arranged in a random block design. Another experiment (Experiment 5) was conducted on 3-year-old vines at Lenswood trained similarly to those described above. In this experiment, plots consisted of 8 vines with each treatment replicated four times and arranged in a randomised block. Experiments at Nuriootpa were carried out on young Chardonnay vines planted 2.25 metres apart and at 3.5 metre row spacings or mature Crouchen vines planted 1.2 metres apart and at 3.7 metre row spacings. All vines were spur-pruned and trained to a single cordon. Trials undertaken in the Chardonnay vines consisted of 8 vines with 5 replicates per treatments (Experiment 1) while Crouchen plots consisted of 4 vines with 4 replicates per treatment (Experiment 3) and both arranged in a randomised block.

Spray application

All sprays were applied with ‘Solo’ motorised back-pack sprayers. Up to 500 L/ha of spray were applied in early spring and 1,000 L/ha when vines were in full foliage in mid summer.

Timing – powdery mildew Experiments 1 – 5

Different combinations of fungicides were applied at various stages of plant growth. The main aim was to apply the strobilurins between the stages from flowering to after setting when berries were around 7 mm in diameter (Coombe 1995). The application dates and growth stage at the time of each application are shown in Tables 1 to5.

Table 1. Experiment 1 - application times, vine growth stages and fungicides used on Chardonnay vines at Nuriootpa 1999/2000.

Treatment	Application date and vine growth stage*						**Total no. applications**
	12/10/99	28/10/99	12/11/99	26/11/99	10/12/99	21/12/99	
	16	22	25	27	31	32	
1	-	-	-	-	-	-	-
2	S	T	F	F	T	S	6
3	S	T	A	A	T	S	6
4	S	T	T	F	F	S	6
5	S	F	F	T	T	S	6
6	S	T	T	A	A	S	6
7	S	S	T	T	F	F	6
8	S	S	A	T	A	T	6
9	S	S	T	T	S	S	6

* Coombe (1995)
Where: - = No fungicide, S = Thiovit (3 g/L), T = Topas (0.125 ml/L), A = Amistar (0.5 g/L), F = Flint (0.15 g/L)

Table 2. Experiment 2 - application times, vine growth stages and fungicides used on mature Chardonnay vines at Lenswood 1999/2000.

Treatment	Application date and vine growth stage*						**Total no. applications**
	14/10/99	29/10/99	24/11/99	10/12/99	20/12/99	6/1/00	
	15	17	22	25	27	31	
1	-	-	-	-	-	-	-
2	S	S	T	T	S	S	6
3	S	T	F	F	T	S	6
4	S	T	T	F	F	S	6
5	S	F	F	T	T	S	6
6	S	S	T	T	F	F	6
7	T	F	F	T	S	S	6
8	S	T	A	A	T	S	6
9	S	T	T	A	A	S	6
10	S	S	A	T	A	T	6
11	S	A	A	T	T	S	6
12	T	A	A	T	S	S	6

* Coombe (1995)
Where: - = No fungicide, S = Thiovit (3 g/L), T = Topas (0.125 ml/L), A = Amistar (0.5 g/L), F = Flint (0.15 g/L)

Table 3. Experiment 3 - application times, vine growth stages and fungicides used on Crouchen vines at Nuriootpa 2000/2001.

Treatment	Application date and vine growth stage*					**Total no. applications**
	16/10/00	3/11/00	17/11/00	30/11/00	15/12/00	
	13	16	25	29	31	
1	-	-	-	-	-	-
2	S	S	P	P	P	5
3	S	S	L	L	L	5
4	S	S	T	T	T	5
5	S	S	F	F	F	5

* Coombe (1995)
Where: **-** = No fungicide, S = Thiovit (3 g/L), T = Topas (0.125 ml/L) P = Prosper (0.6 ml/L), F = Flint (0.15 g/L) L = Legend (0.2 ml/L)

Table 4. Experiments 4 - application times, vine growth stage and fungicides used on young Chardonnay vines at Lenswood 2000/2001.

Treatment	Application date and vine growth stage*					**Total no. applications**
	23/10/00	13/11/00	29/11/00	13/12/00	27/12/00	
	14	17	23	27	31	
1	-	-	-	-	-	-
2	S	S	T	T	S	5
3	S	S	T	T	F	5
4	F	F	F	T	T	5
5	A	T	A	A	T	5
6	S	T	A	A	T	5

* Coombe (1995)
Where: - = No treatment, S = Thiovit (3 g/L), T = Topas (0.125 ml/L) A = Amistar (0.5 g/L), F = Flint (0.15 g/L)

Table 5. Experiments 5 - application times, vine growth stage and fungicides used on mature Chardonnay vines at Lenswood 2000/2001.

Treatment	Application date and vine growth stage*						Total no. applications
	23/10/00	13/11/00	29/11/00	13/12/00	27/12/00	12/1/01	
	14	17	23	27	31	32	
1	-	-	-	-	-	-	-
2	S	S	T	T	S	S	6
3	S	S	T	T	F	F	6
4	F	F	F	T	T	S	6
5	S	S	F	F	S	S	6
6	T	T	F	F	S	S	6
7	S	S	F	F	F	S	6
8	S	S	S	S	S	S	6

* Coombe (1995)
Where: - = No treatment, S = Thiovit (3 g/L), T = Topas (0.125 ml/L) A = Amistar (0.5 g/L), F = Flint (0.15 g/L)

Leaf and bunch sampling and assessment methods

Fifty leaves and fifty bunches were selected at random from the middle 2 vines of each plot at various times and assessed for disease incidence and severity. Leaves were assessed in the laboratory by examining the upper and lower leaf surface. All other bunch assessments were done *in situ* except for the final bunch assessment at harvest, when bunches were picked and then examined.

A 0 to 10 rating scale developed by Dr. R.W. Emmett (Sunraysia Horticultural Centre, Mildura, Victoria; pers. comm.) was used to score both leaves and bunches. In this scale 1 = <1%, 2 = 2%, 3 = 5%, 4 = 10%, 5 = 20%, 6 = 40%, 7 = 60%, 8 = 80%, 9 = 90% and 10 = 100% of bunch or leaf area infected with powdery mildew.

Data from the final assessments were analysed with the analytical software package Statistix for Window V2, using general analysis of variance to generate values for least significant differences (LSD, P = 0.05).

Downy mildew - Experiment 6

At the Lenswood site in 1999/2000 downy mildew developed throughout the trial area as a result of several infection periods in spring and early summer. This provided a further opportunity to compare Amistar and Flint. Late developing shoots that had not received any fungicide sprays and were within 20 cm of a shoot with downy mildew lesions were tagged and either left unsprayed or sprayed with Amistar at 0.375 g/L, Flint at 0.2 g/L or Ridomil Plus at 1.5 g/L. Fungicides were applied separately to 25 shoots per treatment 1 day after a rain event that provided conditions suitable for sporulation and infection of downy mildew. Applications were made with a hand-held atomiser and care was taken to ensure that all leaves were covered with fungicide. The treatments were randomised among several rows of vines. Shoots were removed from the vines 14 days after the fungicides were applied, brought back to the laboratory and incubated in plastic bags in the dark at high humidity to induce sporulation. The severity of the downy mildew was rated on each of the 6 to 10 leaves per shoot using a 0 to 4 rating scale where 0 = 0%, 1 = 1 to 5%, 2 = 6 to 25%, 3 = 26 to 50% and 4 = >50% of the leaf area covered with sporangia.

The extensive development of downy mildew in this vineyard also provided an opportunity for the level of infection to be assessed at harvest. Mature leaves from vines previously sprayed for powdery mildew (Table 2) were also assessed for downy mildew using the rating scale described above.

Results

1999/2000 POWDERY MILDEW EXPERIMENTS

Experiment 1. *Nuriootpa - Chardonnay*

In this planting the incidence of powdery mildew at harvest was extensive, with 40% of leaves and 80% of bunches infected in untreated plots. However, the severity was low with infection developing on less than 1% leaf area and 5% bunch area respectively. No disease was detected on the upper leaf surface.

All fungicide programs controlled powdery mildew on leaves and bunches and there were no significant differences (P = 0.05) between any of the treatments. In these plots less than 2% of bunches and 7% of the leaves were infected, which was significantly less than that in the unsprayed plots.

Experiment 2. *Lenswood - Chardonnay*

Powdery mildew was well established in the unsprayed plots by 22 November when over 14% of the leaves were infected mainly on the lower surface. At harvest 50% of the leaves in these plots were infected with a severity of 3% of leaf area infected. Disease levels were less on leaves of the sprayed plots with least developing in treatments3 to 7 which were those treated with the Topas and Flint combinations (Figure 1).

The development of powdery mildew on bunches differed to that on the leaves in that all unsprayed bunches were infected by early January. The incidence data showed that all sprayed bunches were infected but there was considerable variation between treatments. Those that had received 2 consecutive sprays of Flint had in most cases significantly less disease than other treatments (Figure 2). In the unsprayed plots the severity of bunch infection rose from 30% in January to 60% of bunch area infected at harvest in March.

By contrast, analysis of the severity data showed that all fungicide programs controlled powdery mildew on the bunches. There were no significant differences between treatments with the severity of bunch infection being less than 3% in all treatments. This was also reflected in the bunch weights where bunches in the unsprayed plots were in most cases less than half that of the sprayed treatments (Figure 3).

2000/2001 POWDERY MILDEW EXPERIMENTS

Experiment 3. *Nuriootpa* **-** *Crouchen*

At harvest, all unsprayed bunches were severely infected with powdery mildew (Figure 4). All treatments reduced the incidence and severity of mildew and there were no significant differences between the fungicide treatments. However the program that included Flint was the only one where disease was not detected.

Experiment 4. *Lenswood* **-** *Young Chardonnay*

Powdery mildew developed late in this planting and as a result bunch infection did not develop extensively compared to the level of leaf infection. Leaf assessments in 28 February showed that all unsprayed leaves were infected and that infection was similar on both leaf surfaces. On the unsprayed leaves 19% of the upper leaf area was diseased (Figure 5). Analysis of the severity data showed that all fungicide treatments controlled powdery mildew. In the most effective program, Flint was applied as a final application.

Experiment 5. *Lenswood* **-** *Mature Chardonnay*

At harvest, the untreated plots were severely diseased with 83% of leaves and all bunches infected (Figures 6 and 7). Flint was the only strobilurin evaluated in this experiment and analysis of the severity data on the upper leaf surface and bunches showed there was little difference in the efficacy of this material when 2 or 3 sprays were positioned at various times in a 6 spray program with other combinations of Topas and Thiovit (Figure 6 and 7). However all programs that included either two or three applications of Flint had significantly less disease than the Thiovit only or Thiovit and Topas program.

Downy Mildew - Experiment 6

Severe downy mildew infection developed on more than 90% of unsprayed shoots. Significantly less disease developed on the shoots sprayed with fungicides with the least occurring on the Amistar and Ridomil Plus treatments (Figure 8). Both Amistar and Ridomil Plus were most inhibitory to sporulation compared to that developing on the Flint and control treatments. With the downy mildew assessment made at harvest, both the incidence and the severity of downy mildew was least in the treatments where one or more applications of Amistar were included in the last three sprays (Figure 9). Overall these results showed that Amistar was more effective than Flint for downy mildew control.

Discussion

In these experiments where a number of different fungicide programs were applied, the relative importance of each fungicide or spray timing in the overall control of powdery mildew was difficult to determine. Nevertheless these results show that excellent control of powdery mildew can be achieved by utilising spray programs that include a number of fungicides with different modes of activity. In Australia, most spray programs for powdery mildew are based on sulphur applied alone or integrated in programs that include a DMI (demethylation inhibiting) fungicide (Wicks *et al*, 1997). With the strobilurins and the imminent registration of quinoxyfen and spiroxamine as well as sulphur and the DMI's, five different chemical groups will be available for use in spray programs on grapes in Australia.

The development of fungal populations with reduced sensitivity to fungicides is a disease management problem that must be considered in crops such as vines that are sprayed regularly. Poor disease control following the use of DMI's has been associated with populations of *U. necator* with reduced sensitivity to the DMI fungicides overseas (Ericson and Wilcox, 1997, Gubler *et al*, 1996, Stenden *et al*, 1994) and more recently in Australia (Savoccia *et al*, 1999). While resistance to the strobilurins has not been reported in *U. necator*, powdery mildew populations in cereals have been reported as resistant as well as populations of grape downy mildew (Heaney *et al*, 2000). The rapid development of *P. viticola* populations resistant to the strobilurins overseas is of concern and suggests that this group of fungicides should be used judiciously.

Our results showed that in programs where either 2 or 3 strobilurins were used, the extra application of a strobilurin did not improve control. Because populations of fungi resistant to the strobilurins has developed within a few years after their commercial release, limiting the strobilurins to 2 applications per season may be an appropriate disease management strategy.

Control failures and the widespread occurrence of fungicide resistant strains of grape pathogens reported overseas suggests that fungicide resistance management strategies should be implemented and promoted in Australia.

Our results showed that strategies such as the use of 3 or more different fungicide groups and no more than 2 applications of the same product in the season's program provided effective control of powdery mildew. The use of similar programs should limit selection pressure against the fungicide groups presently used on grapes and reduce the likelihood of further fungicide resistant strains developing in Australia vineyards. The use of a range of new compounds and five different chemical groups should provide vineyard managers with sufficient options to keep fungicide resistance under control. Nevertheless, before the new fungicides are widely used in Australia, studies need to be done to determine baseline levels of sensitivity to these fungicides so the efficacy of anti resistance strategies can be monitored.

In the program where applications of Amistar and Flint were directly compared, Flint generally provided better control of powdery mildew than that achieved by Amistar. On the other hand, Amistar was more effective than Flint in the control of downy mildew which suggests that Amistar may be the most appropriate strobilurin to apply at flowering if downy mildew infection events seem imminent during this period. Our results also confirmed recent studies by Wong and Wilcox (2001) showing that azoxystrobin (Amistar) significantly inhibits sporulation of *P. viticola* when applied 1 day after inoculation. The longevity and efficacy of the strobilurin leaf deposits was also demonstrated in these experiment as the single downy mildew infection period that gave rise to the natural infection occurred 6 weeks after the strobilurins were applied. furthermore, in one experiment at Lenswood, leaves remained disease-free for 6 weeks after the final application of Flint.

Differences in efficacy between Amistar and Flint for powdery mildew control were mainly detected in experiments where high levels of disease had developed in the unsprayed plots and where the disease pressure was high. In these cases differences were obvious in disease incidence rather than severity. Where disease incidence and inoculum pressure was low, significant differences between the two strobilurins were not detected. This suggests that in commercial vineyards where disease incidence and severity is likely to be less than that experienced in our experimental sites, differences in efficacy between Amistar and Flint are not likely to be recognised.

In Australia sulphur is traditionally applied as the first spray in most programs as it is generally considered effective against powdery mildew and an appropriate spray to control mites early in the season. Whether this early application had a significant role in suppressing disease development in our experiments is uncertain. Similarly, fungicides applied weeks after berry set may not have made a large contribution to disease control particularly on bunches as these would have been naturally resistant to powdery mildew (Gadoury *et al*, 1999).

Overall our results showed that the strobilurins were effective when applied during the period from just before flowering to just after fruit set. Applications during this time provided protection of newly developing berries for several weeks until they were naturally resistant to powdery mildew infection (Gadoury *et al*, 1999). However our results in 2000/2001 experiments showed that there was no marked difference in the degree of powdery mildew control with strobilurins applied either just before or after flowering. This may be a reflection of the persistence of the strobilurin deposit. Further studies need to be done to determine the limits of the protective activity as there may be potential for extending intervals between sprays.

Despite the excellent fungicidal activity of the strobilurins and the activity of Amistar against both downy and powdery mildews of grapevines, the widespread use of these chemicals on grapes in Australia may be limited by their cost. At present, the most cost effective program is a standard sulphur and DMI program consisting of 4 to 6 applications with sulphur applied before or after 2 to 3 consecutive DMI sprays applied around flowering. Such a program was shown in these experiments and others (Wicks et al, 1997) to provide good control of powdery mildew.

One area where the strobilurins may be particularly useful is in young vineyards where leaf retention and continued vine growth is encouraged. In these situations the late summer and autumn applications of strobilurins may provide extended protection of leaves and new shoot growth necessary for vine establishment. On the other hand this may not be a good practice from the perspective of fungicide resistance management as it may result in exceeding the minimum of 3 applications per season and limit their long term use.

In summary, our results showed that good control of powdery mildew can be achieved with 6 fungicide applications per season with programs that include 2 applications each of sulphur, a DMI and a strobilurin fungicide. The level of control was not influenced by the order in which the fungicides were applied. Despite this, Amistar is the preferred fungicide to apply during the flowering period because of its activity against powdery mildew and downy mildew.

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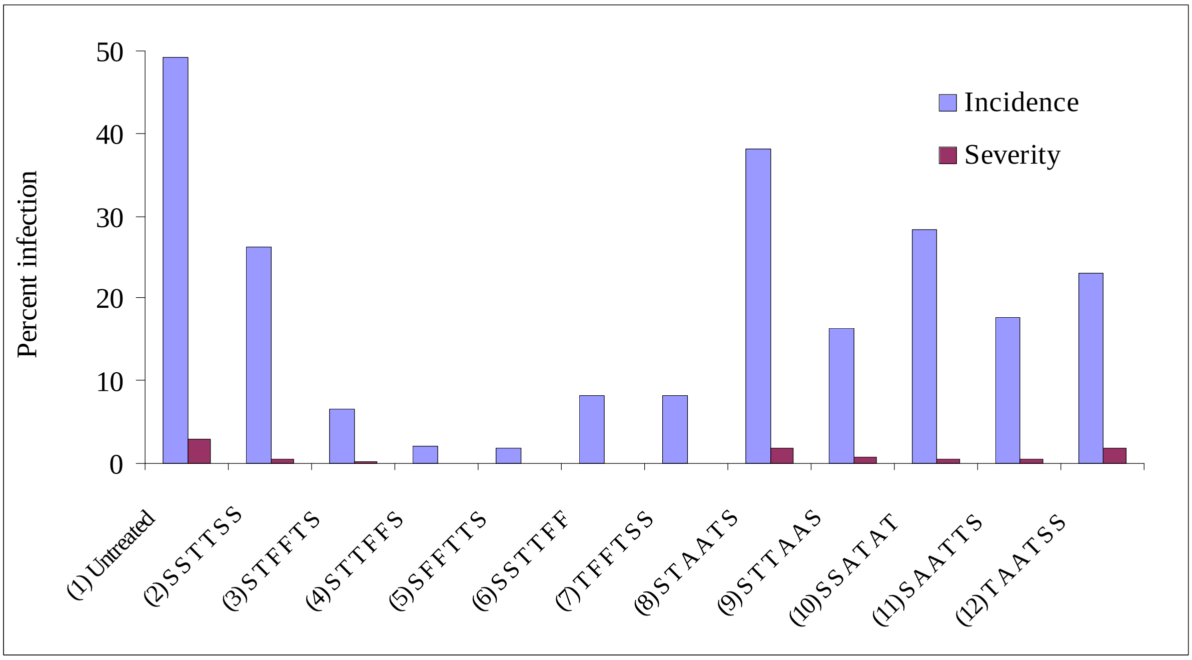
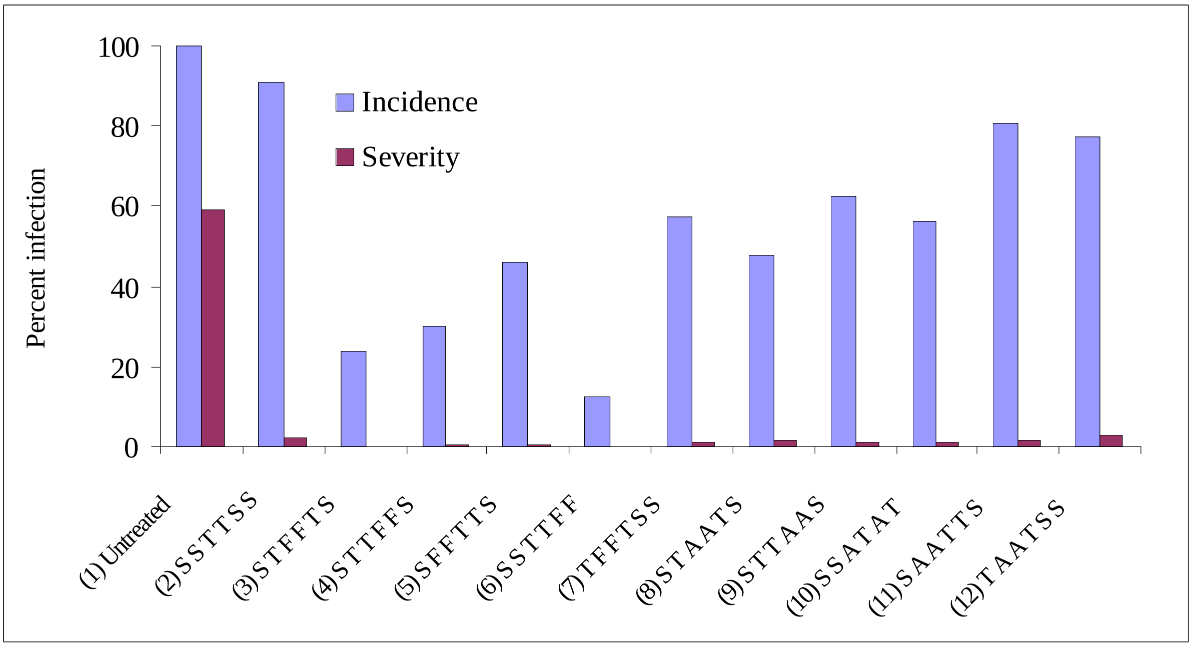
Figure 1 - Experiment 2. Incidence and severity of powdery mildew on the lower surface of mature Chardonnay leaves, Lenswood, 15/3/00.



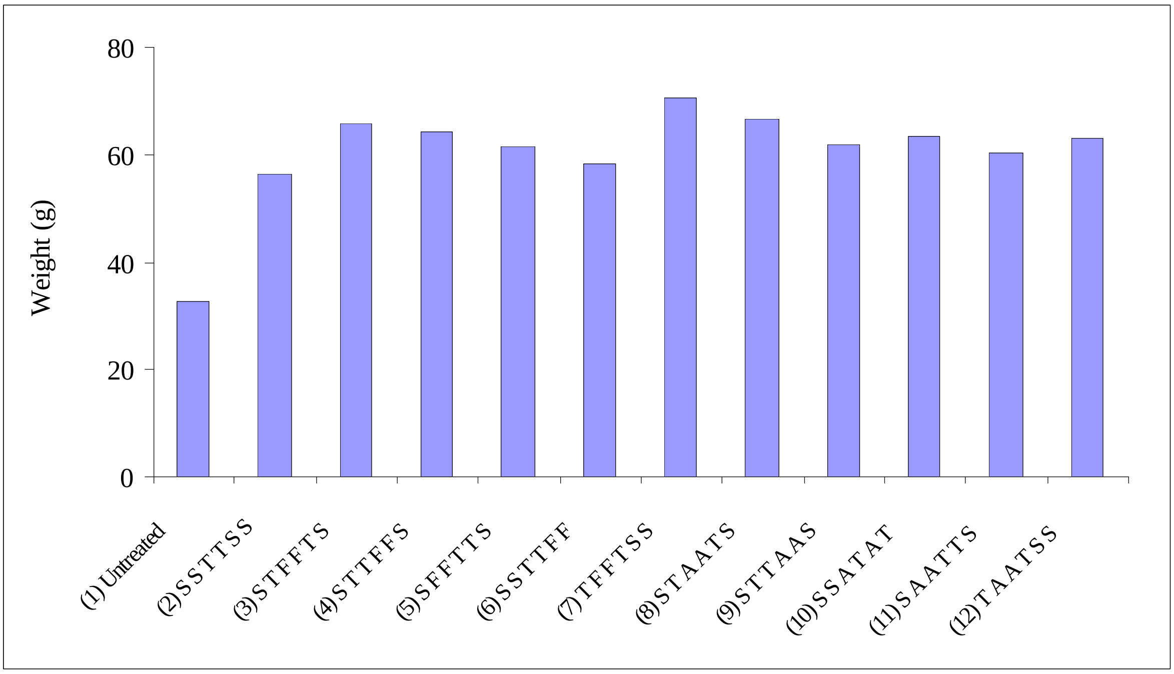
Where: - = No fungicide, S = Thiovit (3g/L), T = Topas (0.125ml/L), A = Amistar (0.5g/L), F = Flint (0.15g/L) applied at the times shown in table 2.

Figure 2 - Experiment 2. Incidence and severity of powdery mildew on mature Chardonnay bunches, Lenswood, 15/3/00.



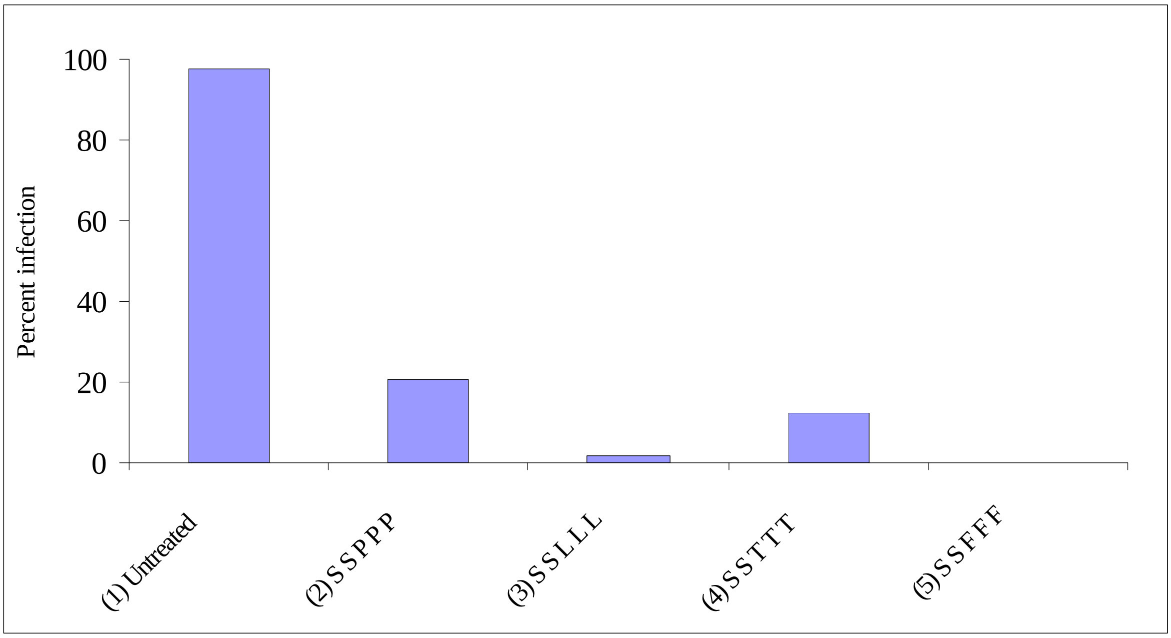
Where: - = No fungicide, S = Thiovit (3g/L), T = Topas (0.125ml/L), A = Amistar (0.5g/L), F = Flint (0.15g/L) applied at the times shown in table 2.

Figure 3 - Experiment 2. Average weight of mature Chardonnay bunches at harvest, Lenswood, 15/3/00.



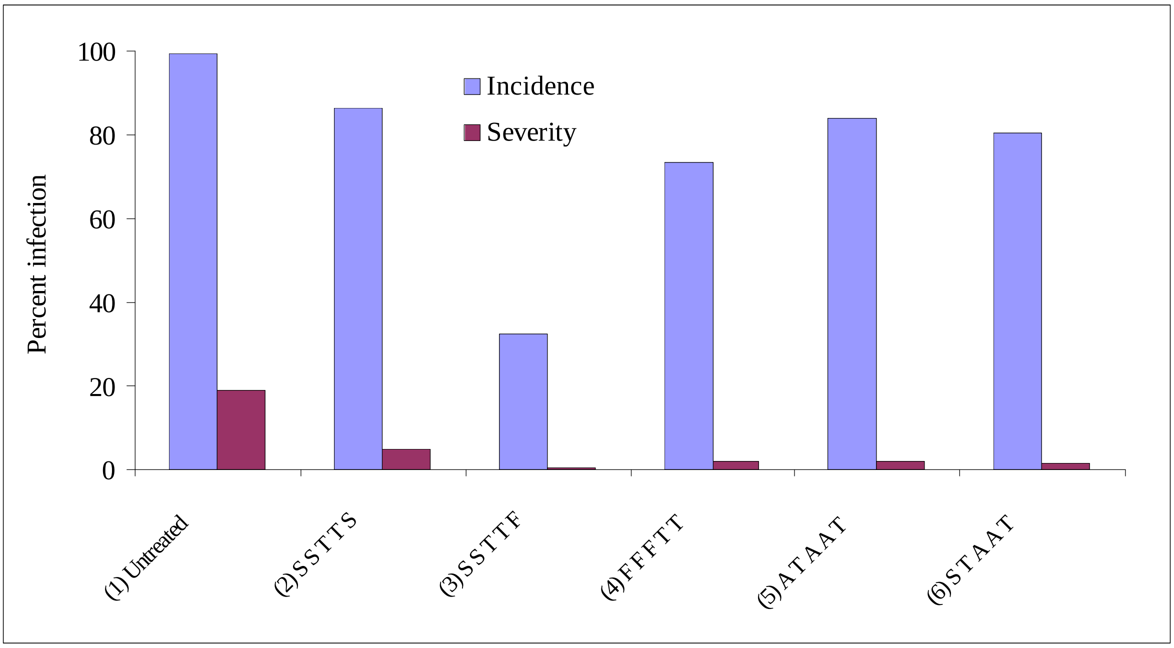
Where: - = No fungicide, S = Thiovit (3g/L), T = Topas (0.125ml/L), A = Amistar (0.5g/L), F = Flint (0.15g/L) applied at the times shown in table 2.

Figure 4 - Experiment 3. Incidence of powdery mildew on Crouchen bunches, Nuriootpa, 22/2/01.



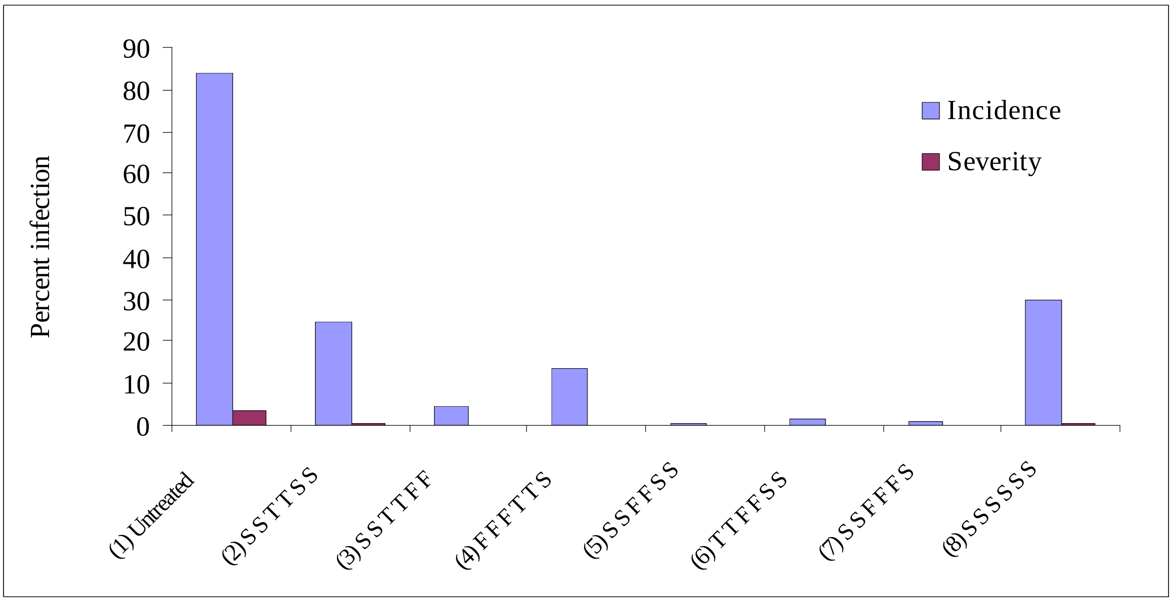
Where: - = No fungicide, S = Thiovit (3g/L), T = Topas (0.125ml/L) P = Prosper (0.6ml/L), F = Flint (0.15g/L) L = Legend (0.2ml/L) applied at the times shown in table 3.

Figure 5 - Experiment 4. Incidence and severity of powdery mildew on the upper surface of young Chardonnay leaves, Lenswood, 28/2/01.



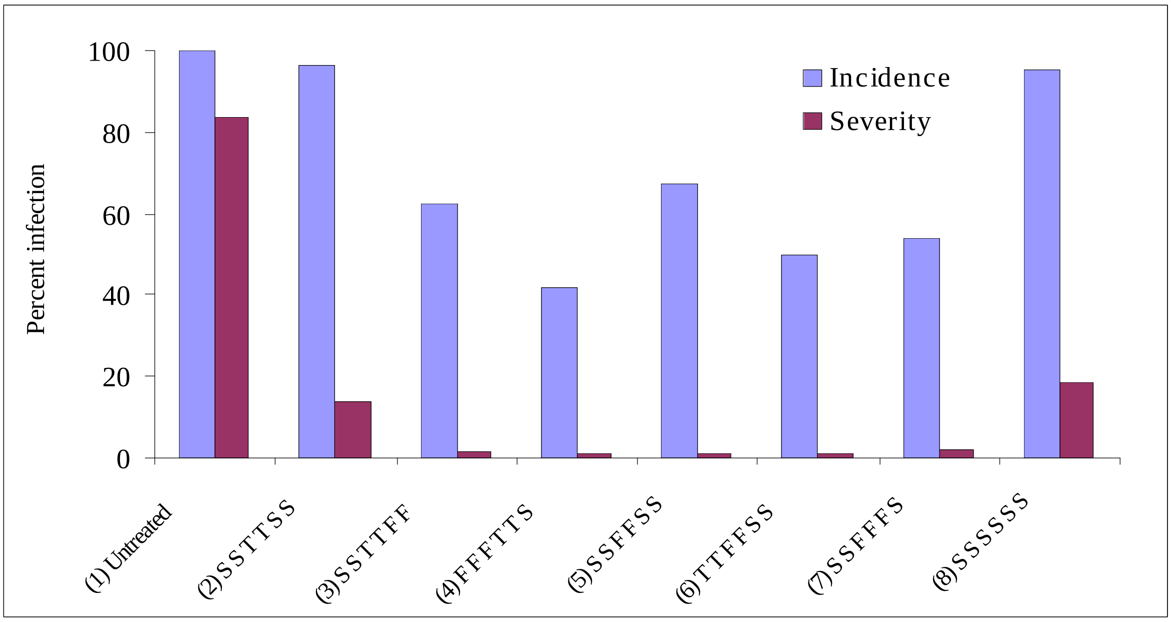
Where: - = No fungicide, S = Thiovit (3g/L), T = Topas (0.125ml/L), A = Amistar (0.5g/L), F = Flint (0.15g/L) applied at the times shown in table 4.

Figure 6 -Experiment 5. Incidence and severity of powdery mildew on the upper surface of mature Chardonnay leaves, Lenswood, 28/2/01.



Where: - = No fungicide, S = Thiovit (3g/L), T = Topas (0.125ml/L), F = Flint (0.15g/L) applied at the times shown in table 5.

Figure 7 - Experiment 5. Incidence and severity of powdery mildew on mature Chardonnay bunches, Lenswood, 28/2/01.



Where: - = No fungicide, S = Thiovit (3g/L), T = Topas (0.125ml/L), F = Flint (0.15g/L) applied at the times shown in table 5.

Figure 8 - Experiment 6. Incidence and severity of downy mildew on mature Chardonnay leaves, Lenswood, 2000.

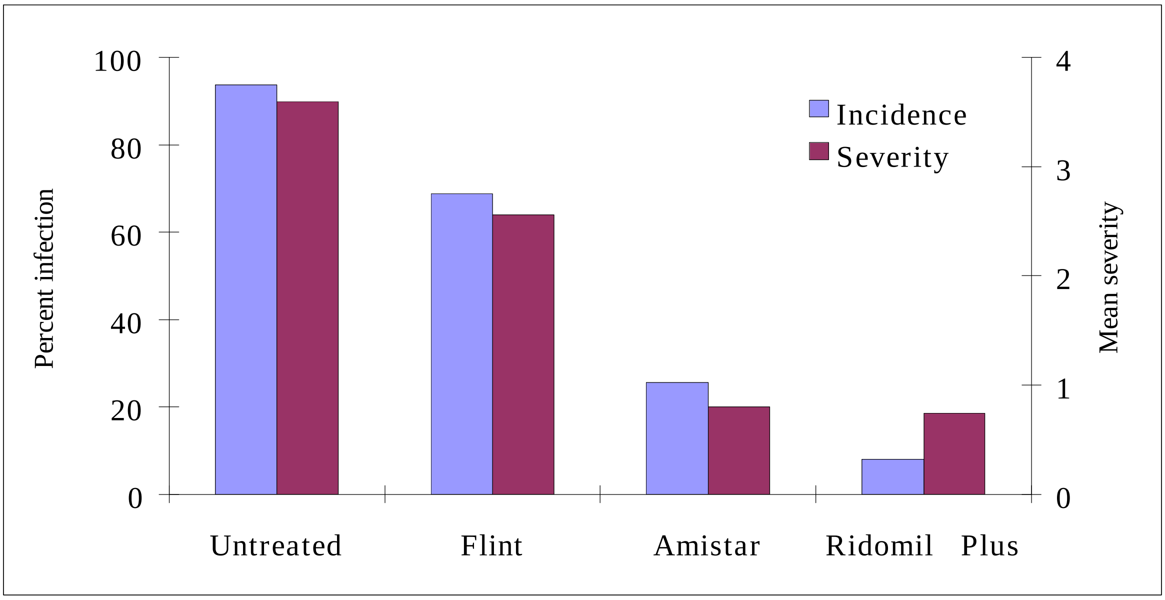
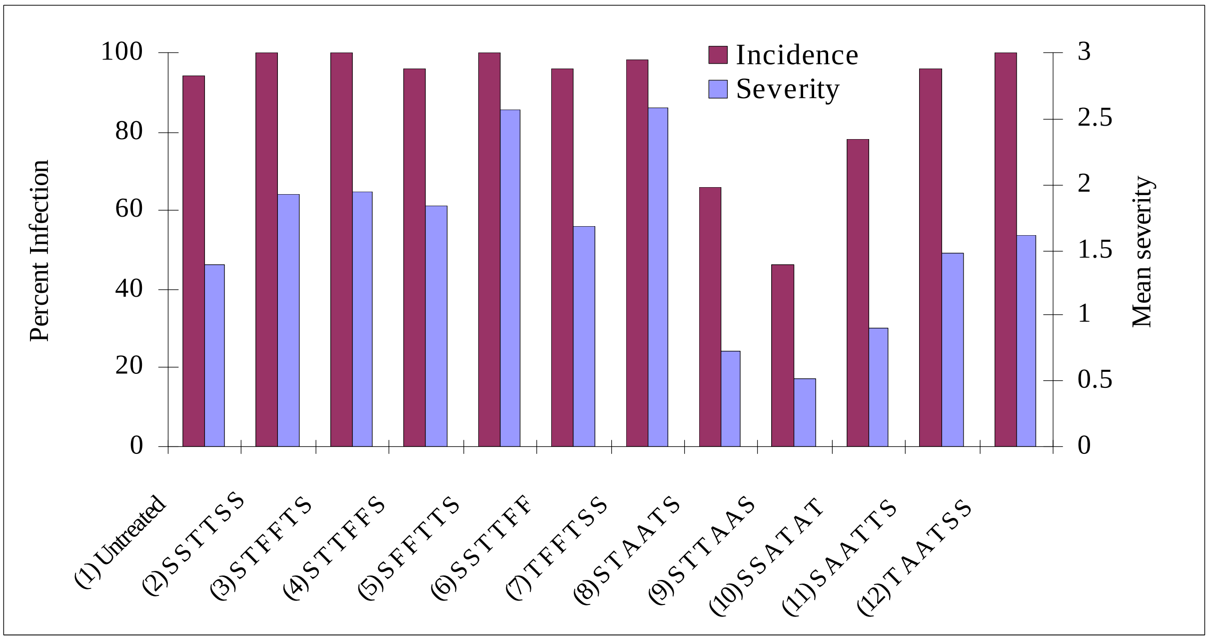


Figure 9 - Experiment 2. Incidence and severity of downy mildew on mature Chardonnay leaves, Lenswood, 15/3/00.



Where: - = No fungicide, S = Thiovit (3g/L), T = Topas (0.125ml/L), A = Amistar (0.5g/L), F = Flint (0.15g/L) applied at the times shown in table 2.

Integration of different fungicide groups in spray programs for the control of powdery mildew in grapevines

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ABSTRACT

Applications of either wettable sulphur, penconazole, azoxystrobin or trifloxystrobin at various grapevine growth stages were evaluated in five field experiments in 2001/2002. In 4 of the 5 unsprayed plots, all bunches were more than 50% covered with mildew and unmarketable. Most programs (except those with only wettable sulphur and penconazole) with 4 to 5 sprays between the growth stages of 5 leaves separated and just after fruit set provided good control. The most effective programs were those where early applications of either wettable sulphur or penconazole were followed by trifloxystrobin applied in 2 or 3 consecutive sprays around flowering.

INTRODUCTION

Powdery mildew (*Uncinula necator)* is the major fungal disease of grapes costing the Australian grape industry over 30 million dollars annually.

Fungicide programs based on wettable sulphur alone or in combination with DMI's (demethylation inhibiting fungicides) are widely used to control the disease in Australia. While these programs have provided good control in the past there are concerns with phytotoxicity when sulphur is applied at temperatures >35^{o}C and the development of DMI resistant strains of powdery mildew that have recently been detected in Australia (Savocchia *et al*. 1998).

New fungicides with activity against powdery mildew have been developed (Margal *et al.* 1998, Dacol *et al.* 1998), but few critical studies have been done in Australia to determine where they best fit in spray programs used on grapes. This paper reports on studies where the efficacy of strobilurin fungicides was evaluated when applied in programs with other types of fungicides.

MATERIALS AND METHODS

Products and formulations

Fungicides and rates per litre used were 0.5 g Amistar WG (500 g/kg azoxystrobin), 0.15 g Flint (500 g/kg trifloxystrobin), 6 g Thiovit (800 g/kg sulphur) and 0.125 ml Topas (100 ml/L penconazole).

Sites

Experiments were conducted at the Lenswood and Nuriootpa experimental stations, situated approximately 30 km east and 100 km north east of Adelaide respectively. At Lenswood, treatments were applied to Chardonnay vines planted 1.5 m apart with 3 m row spacings. At Nuriootpa, experiments were carried out on Chardonnay vines planted 2.25 m apart with 3.5 m row spacings, Crouchen vines planted 1.2 m apart with 3.7 m row spacings and Verdelho vines planted 2 m apart with 3.5 m row spacings. Plots varied from 3 to 8 vines each, replicated 5 times and arranged in a random block design.

Spray application and timing

Fungicides were applied with a "Solo" motorised back pack sprayer utilising up to 500 L/ha in early spring to 1000 L/ha when vines were in full canopy in mid summer. The combinations of different spray timings is shown in Tables 1a-e.

Assessment and analysis

At harvest, fifty bunches were selected at random from the middle 1 or 2 vines of each plot and assessed for disease incidence and severity. A 0 to 10 rating scale (Emmett, unpublished) was used to rate each bunch where 1=<1%, 2=2%, 3=5%, 4=10%, 5=20%, 6=40%, 7=60%, 8=80%, 9=90% and 10=100% of the bunch infected with powdery mildew. Data was analysed with the analytical software package "STATISTIX for Windows V2" using general analysis of variance to generate values for least significant differences (LSD, $P < 0.05$).

RESULTS

Powdery mildew developed extensively in all plantings, and by harvest most bunches in the unsprayed plots were severely diseased (Tables 1a-e).

In experiment 1, the lowest level of bunch infection was found in treatments where trifloxystrobin was applied in December. On the other hand the highest levels of disease in the sprayed treatments developed when penconazole was applied in December (Table 1a). In programs where penconazole and trifloxystrobin were applied, additional applications of wettable sulphur in early November and January did not improve the control of bunch infection.

In experiment 2, all programs controlled powdery mildew and although there was no significant difference between the spray programs, most disease developed in the wettable sulphur/penconazole program (Table 1b).

Table 1(a-e). Incidence and severity of powdery mildew on bunches treated with various fungicide regimes applied at different vine growth stages, 2001/2002.

Treatment, application date and vine growth stage[1]						Bunches diseased (%)[2]	Bunch area diseased (%)[2]
(a) Experiment 1 – Chardonnay – Lenswood							
Nov 1 GS 14	Nov 15 GS 15	Nov 28 GS 18	Dec 12 GS 23	Dec 27 GS 27	Jan 1 GS 29		
-	-	-	-		-	100 a	98.1 a
-	F	F	T	T	-	98.5 a	10 d
-	T	T	F	F	-	47.5 b	0.8 d
-	S	S	F	F	-	44.1 b	0.7 d
-	F	F	S	S	-	96.7 a	7.2 d
-	S	T	T	S	-	100 a	49.3 b
S	T	T	F	F	S	45.2 b	1.0 d
S	S	T	T	S	S	99.6 a	28.5 c
(b) Experiment 2 – Chardonnay – Lenswood							
Nov 1 GS 14	Nov 8 GS 15	Nov 22 GS 18	Dec 8 GS 23				
-	-	-	-			99.5 a	55.4 a
S	F	F	F			50.6 b	1.0 b
S	T	T	T			97.5 a	14.9 b
(c) Experiment 3 – Verdelho – Nuriootpa							
Nov 1 GS 15	Nov 14 GS 17	Nov 28 GS 23	Dec 12 GS 26				
-	-	-	-			100 a	83.3 a
S	S	S	S			92.5 ab	4.6 bc
S	T	T	S			95.4 a	14 b
T	T	F	F			41.8 c	0.6 c
F	F	T	T			78 b	6.6 bc
(d) Experiment 4 – Chardonnay – Nuriootpa							
Oct 16 GS 17	Nov 1 GS 18	Nov 14 GS 21	Nov 28 GS 27				
-	-	-	-			95.7 a	20.5 a
S	S	S	S			58.5 b	1.0 b
S	T	T	S			64.9 b	1.7 b
T	T	F	F			9.6 c	0.2 b
F	F	T	T			23.2 c	0.3 b
(e) Experiment 5 – Crouchen – Nuriootpa							
Oct 16 GS 12	Nov 1 GS 15	Nov 14 GS 17	Nov 28 GS 26	Dec 12 GS 29			
-	-	-	-			100 a	97.6 a
S	S	T	T	T		100 a	33.8 b
S	S	A	A	A		100 a	21.9 b
S	S	F	F	F		45 b	0.6 c

[1] GS= Growth stage (Coombe 1995),
[2] Treatments with the same letter are not significantly different from one another
Treatments: S = Wettable sulphur 6g/L, T = penconazole 0.125 ml/L, F = trifloxystrobin 0.15g/L, A = azoxystrobin 0.5g/L, - = no treatment.

At Nuriootpa, various programs of 4 sprays were applied to both the Verdelho (Experiment 3) and Chardonnay (Experiment 4) vines. In both plantings the lowest incidence of bunch infection developed in the vines where the initial two applications of penconazole were followed by trifloxystrobin (Table 1c and 1d). Similarly the highest level of bunch infection in the sprayed plots developed in the wettable sulphur/penconazole programs. In the Crouchen vines (Experiment 5) the lowest levels of disease developed in vines where trifloxystrobin was applied on three occasions following two applications of wettable sulphur (Table 1e). This treatment was significantly better than similar programs of either azoxystrobin or penconazole.

DISCUSSION

These results show that powdery mildew infection of grape bunches can be controlled with as few as four applications of fungicides, even in conditions of severe disease pressure. In these experiments the application of fungicides between the growth stages of 5 leaves separated and just after fruit set provided good control with most programs. Strobilurins were generally more effective when applied during or just after flowering compared to before flowering. In programs where the efficacy of different strobilurins was compared, trifloxystrobin was more effective than azoxystrobin.

These results confirm other studies showing that grape berries become resistant to powdery mildew 4 weeks or more after fruit set (Gadoury *et al.*, 1999). In our experiments, only low levels of disease were detected in bunches that remained unsprayed for 12 weeks or more after fruit set. During that time they were subjected to high levels of inoculum from adjacent unsprayed vines, and new shoot growth that developed after fruit set became heavily infected with powdery mildew. In commercial vineyards further applications of wettable sulphur would normally be used to protect this growth.

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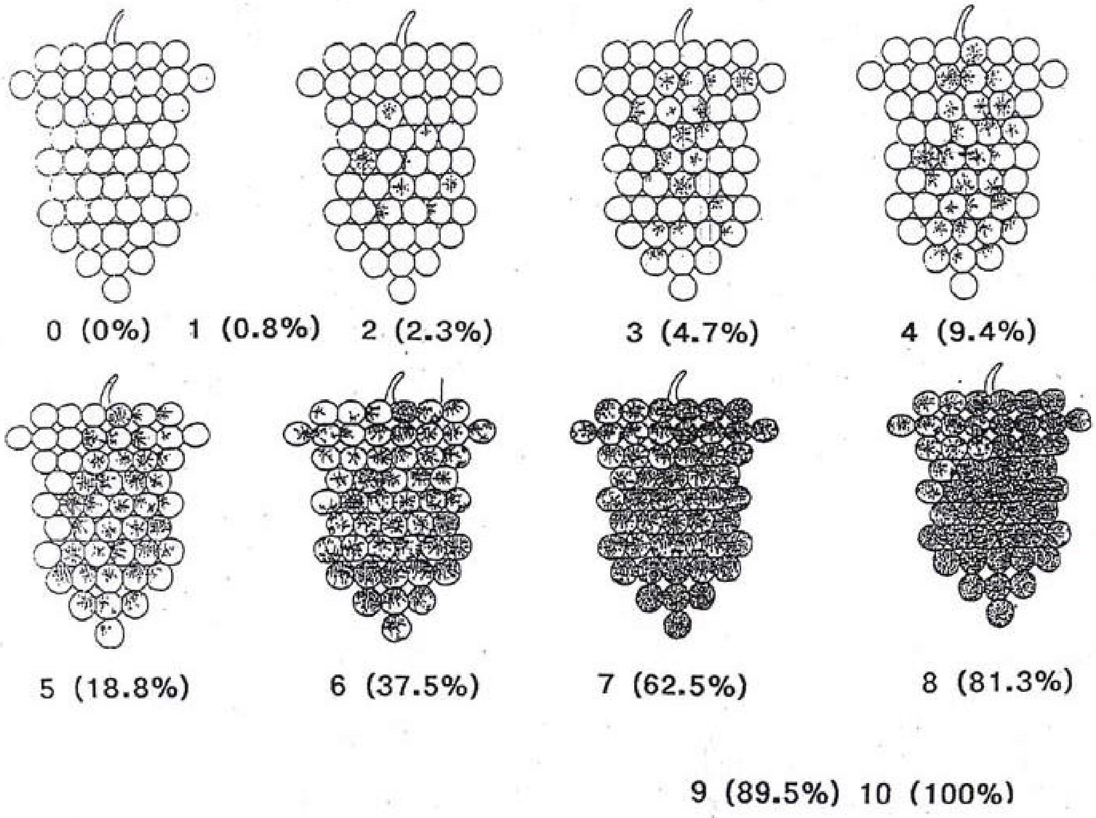
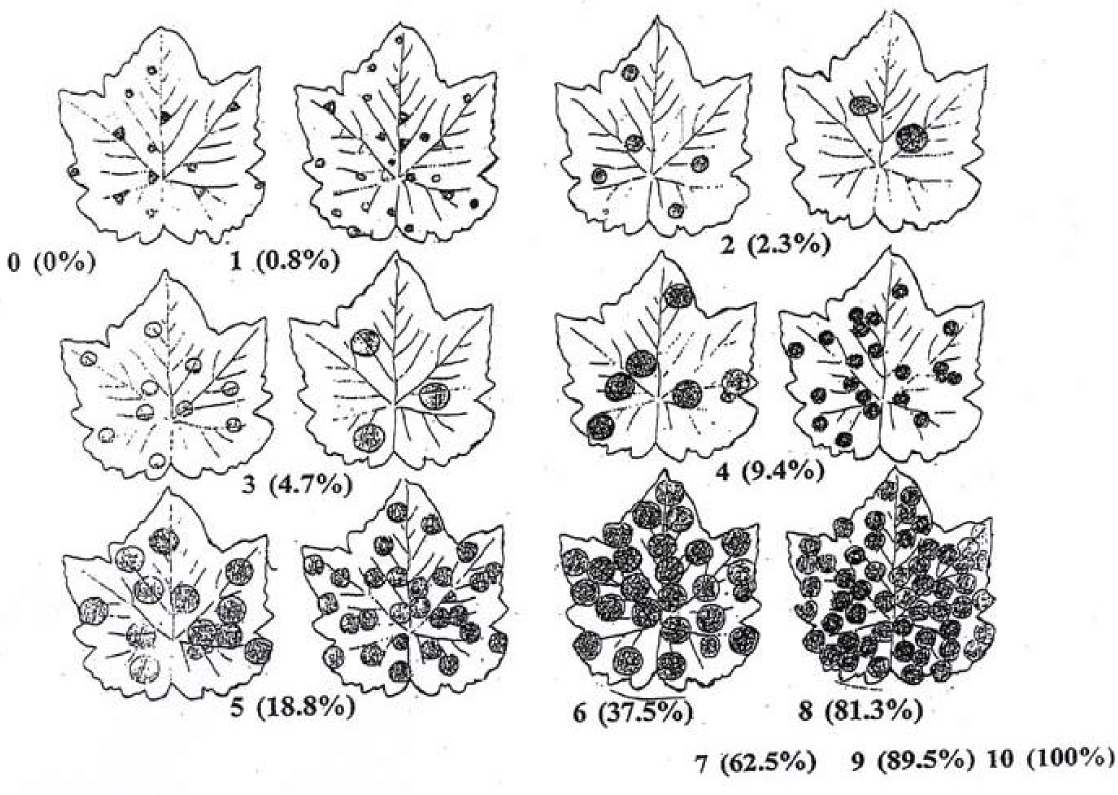
Appendix 4: Disease Assessment Keys

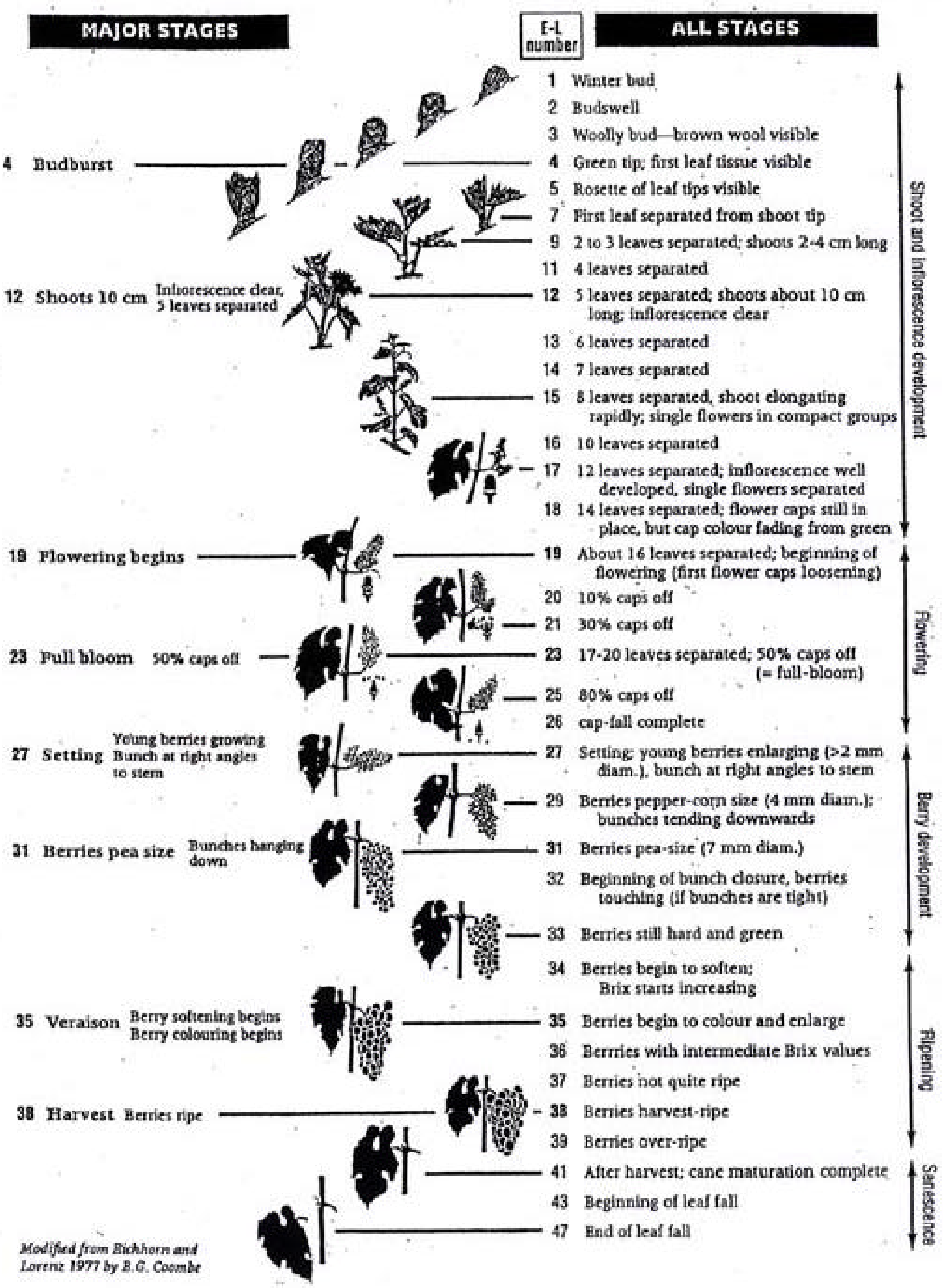
Disease assessment key for powdery mildew on grapevines bunches.



Disease assessment key for powdery mildew on grapevine leaves.



Grapevine growth stages – the modified E-L system



Appendix 5: Field Trial Details and Results

Powdery mildew Field Trial 1.

Table 3. Application times, vine growth stages and fungicides used on Chardonnay vines at Nuriootpa 1999/2000.

Treatment	Application date and vine growth stage*						Total no. applications
	12/10/99	28/10/99	12/11/99	26/11/99	10/12/99	21/12/99	
	16	22	25	27	31	32	
1	-	-	-	-	-	-	-
2	S	T	F	F	T	S	6
3	S	T	A	A	T	S	6
4	S	T	T	F	F	S	6
5	S	F	F	T	T	S	6
6	S	T	T	A	A	S	6
7	S	S	T	T	F	F	6
8	S	S	A	T	A	T	6
9	S	S	T	T	S	S	6

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (2 g/L), T = Topas (0.125 ml/L), A = Amistar (0.375 g/L), F = Flint (0.15 g/L)

Figure 4. Incidence and severity of powdery mildew on Chardonnay bunches at harvest, Nuriootpa February 10, 2000.

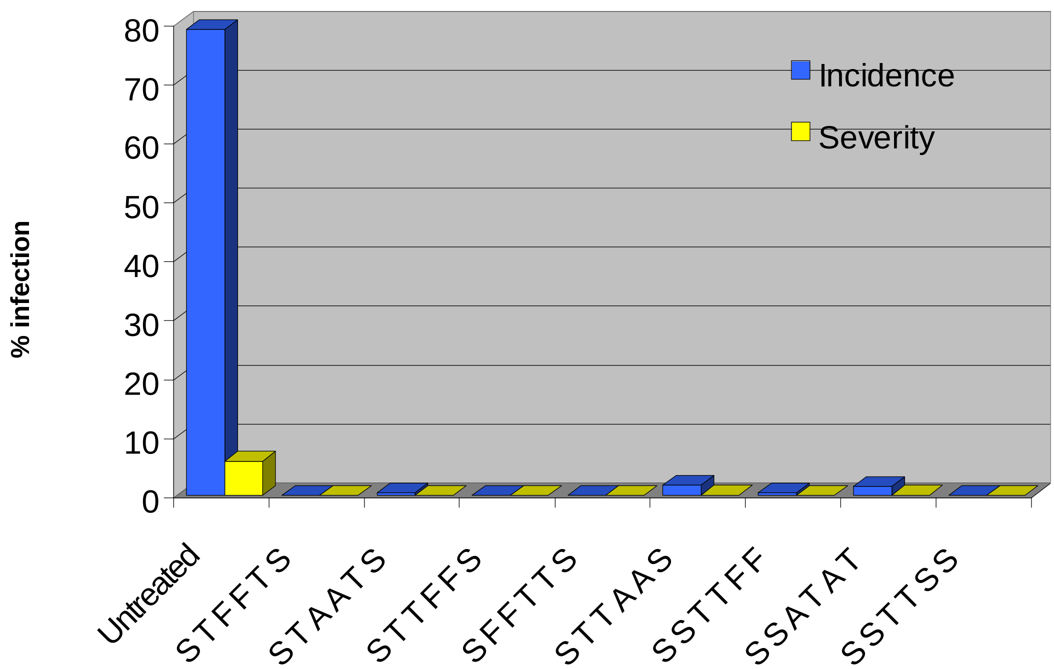


Figure 5. Incidence and severity of powdery mildew on Chardonnay leaves, Nuriootpa March 1, 2000.

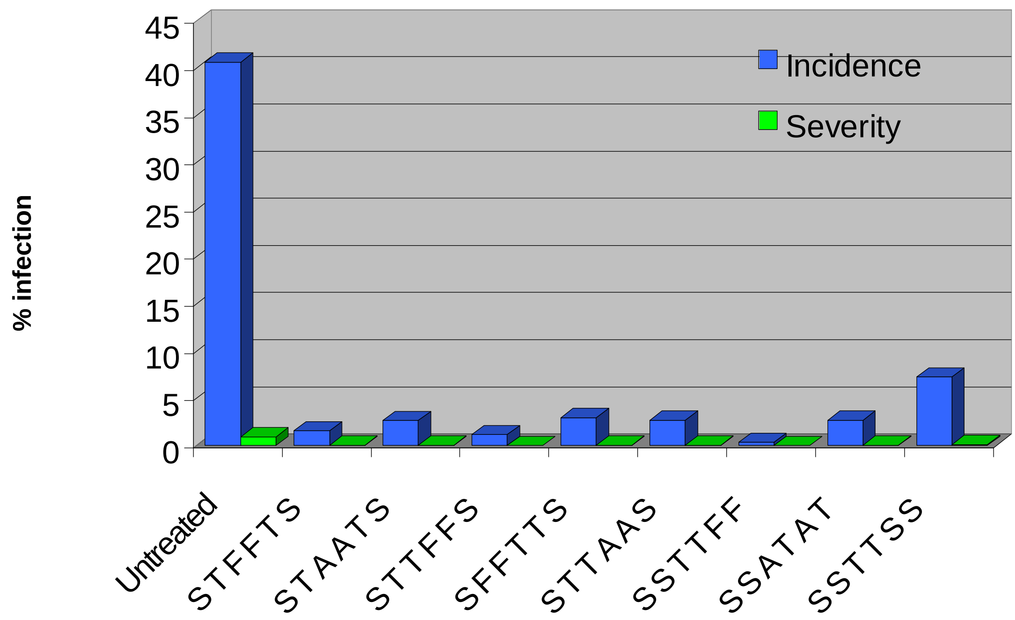
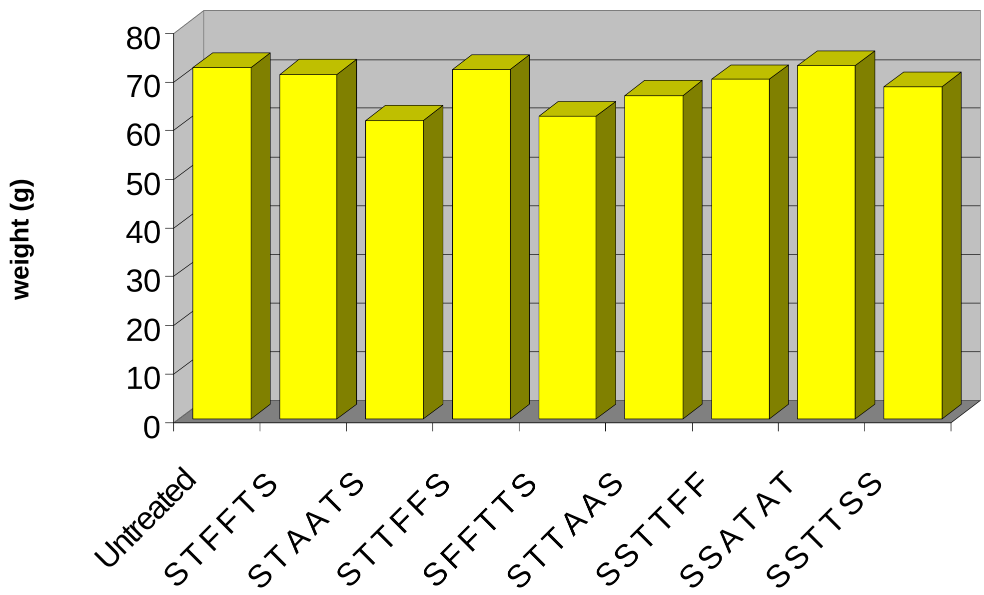


Figure 6. Average weight of Chardonnay bunches at harvest, Nuriootpa February 10, 2000.



Powdery mildew Field Trial 2.

Table 4. Application times, vine growth stages and fungicides used on Chardonnay vines at Lenswood 1999/2000.

Treatment	Application date and vine growth stage*						Total no. applications
	14/10/99 15	29/10/99 17	24/11/99 22	10/12/99 25	20/12/99 27	6/1/00 31	
1	-	-	-	-	-	-	-
2	S	S	T	T	S	S	6
3	S	T	F	F	T	S	6
4	S	T	T	F	F	S	6
5	S	F	F	T	T	S	6
6	S	S	T	T	F	F	6
7	T	F	F	T	S	S	6
8	S	T	A	A	T	S	6
9	S	T	T	A	A	S	6
10	S	S	A	T	A	T	6
11	S	A	A	T	T	S	6
12	T	A	A	T	S	S	6

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (2 g/L), T = Topas (0.125 ml/L), A = Amistar (0.5 g/L), F = Flint (0.15 g/L)

Figure 7. Incidence of powdery mildew on Chardonnay bunches, Lenswood 1999/2000.

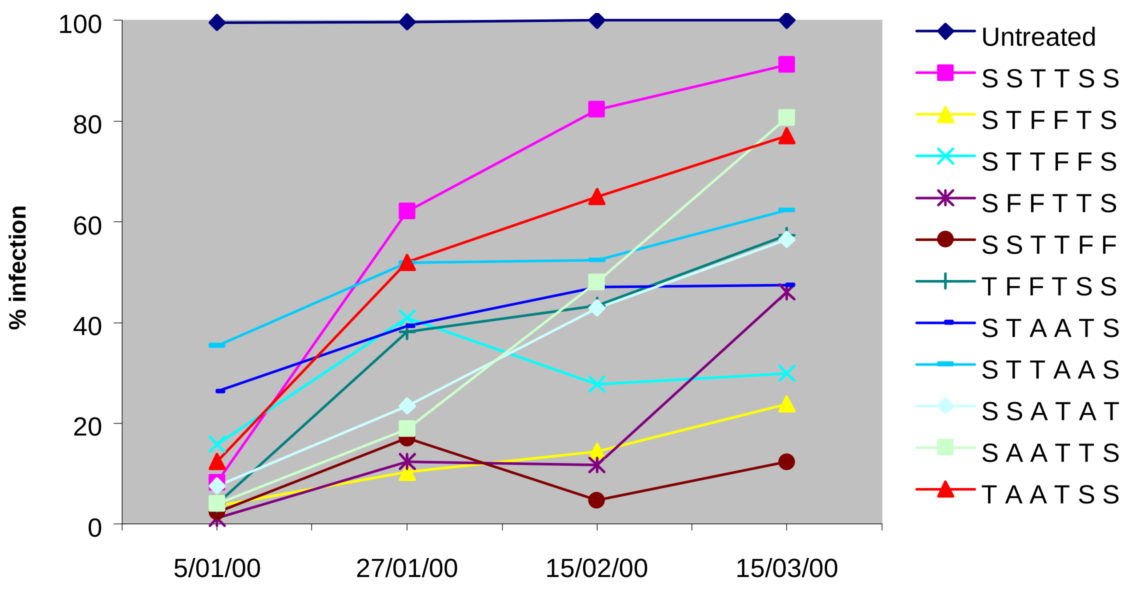


Figure 8. Severity of powdery mildew on Chardonnay bunches, Lenswood 1999/2000.

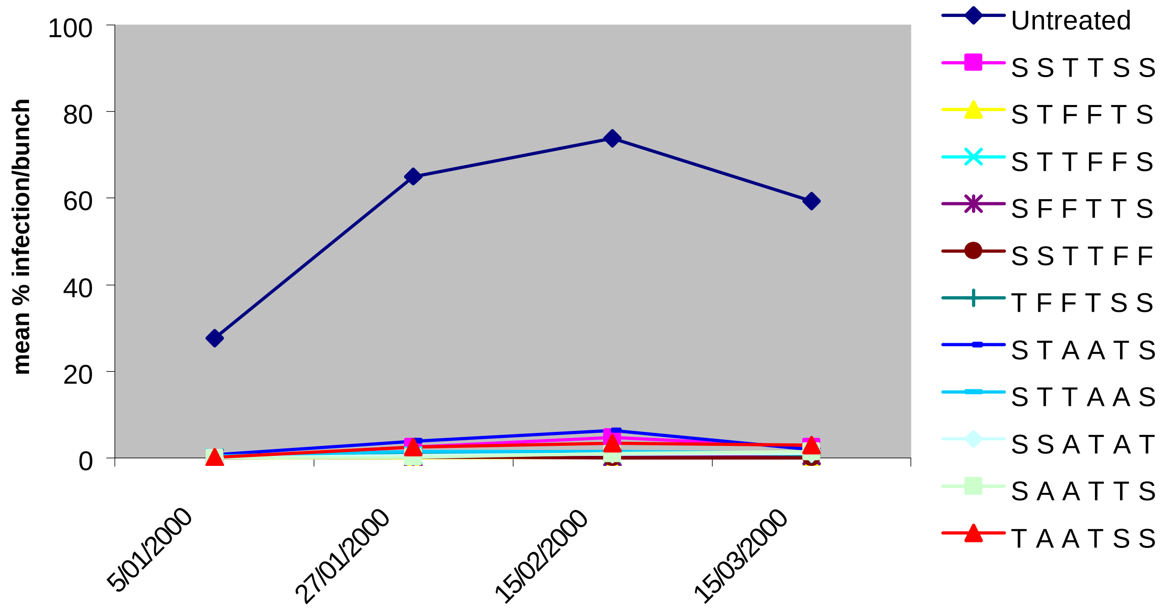


Figure 9. Severity of powdery mildew on Chardonnay bunches at harvest, Lenswood March 15, 2000.

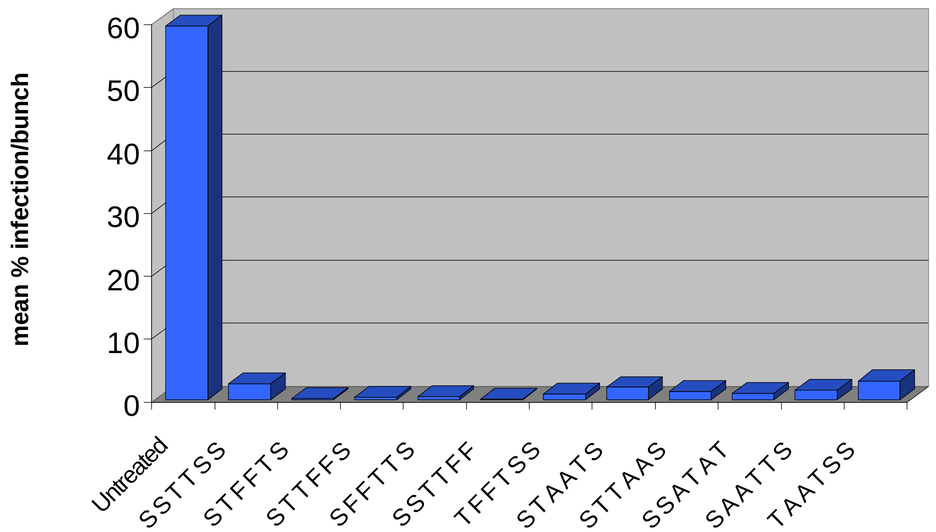


Figure 10. Incidence and severity of powdery mildew on Chardonnay leaves, Lenswood March 15, 2000.

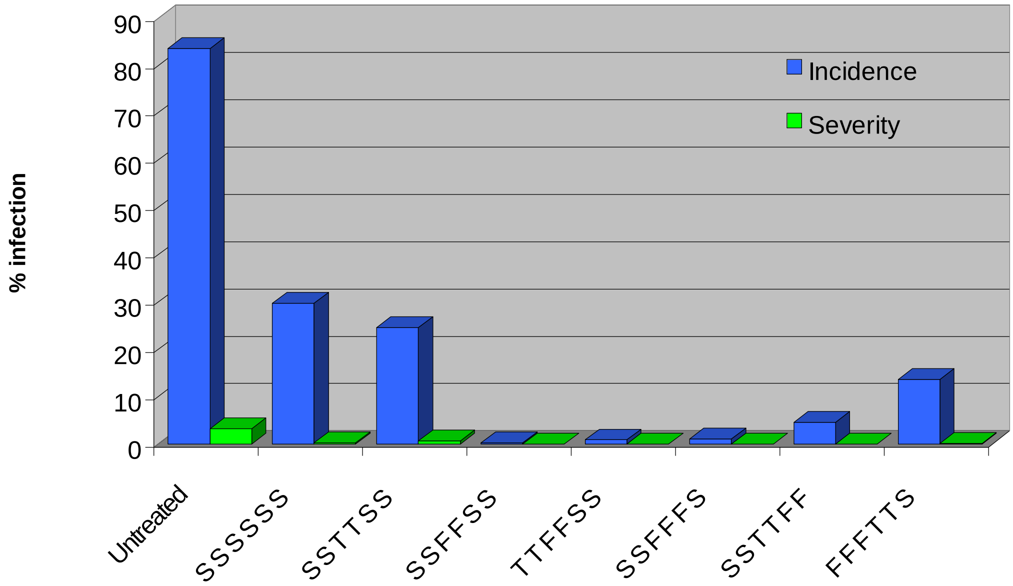
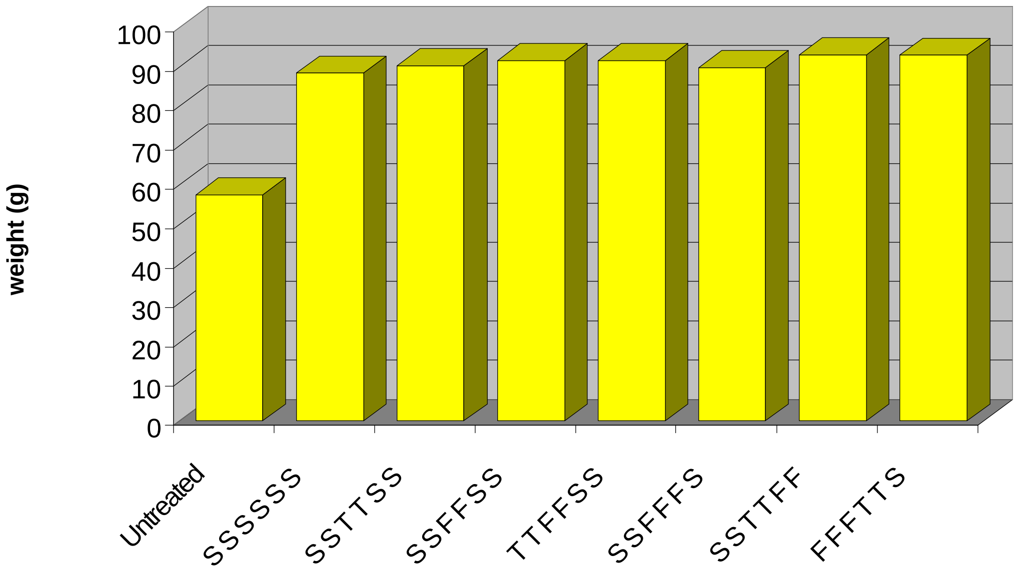


Figure 11. Average weight of Chardonnay bunches at harvest, Lenswood March 15, 2000.



Powdery mildew Field Trial 3.

Table 5. Application times, vine growth stages and fungicides used on Chardonnay vines at Nuriootpa 2000/2001.

Treatment	Application date and vine growth stage*						Total no. applications
	28/9/00	16/10/00	3/11/00	17/11/00	5/12/00	20/12/00	
	12	15	17	27	31	33	
Untreated	-	-	-	-	-	-	-
Topas	S	S	T	T	T	S	6
Flint	S	S	F	F	F	S	6
Prosper	S	S	P	P	P	S	6
Legend	S	S	L	L	L	S	6

* Coombe (1995)

Where: **-** = No fungicide, S = Thiovit (3 g/L), T = Topas (0.125 ml/L), F = Flint (0.15g/L) L = Legend (0.2 ml/L) and P = Prosper (0.6 ml/L)

Figure 12. Incidence and severity of powdery mildew on Chardonnay bunches at harvest, Nuriootpa February 22, 2001.

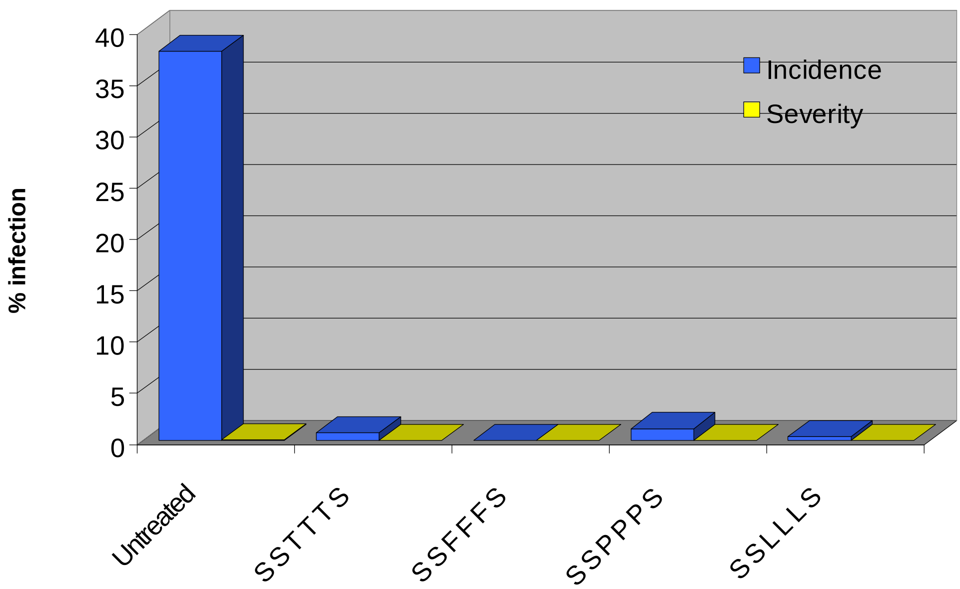


Figure 13. Incidence and severity of powdery mildew on Chardonnay leaves, Nuriootpa February 22, 2001.

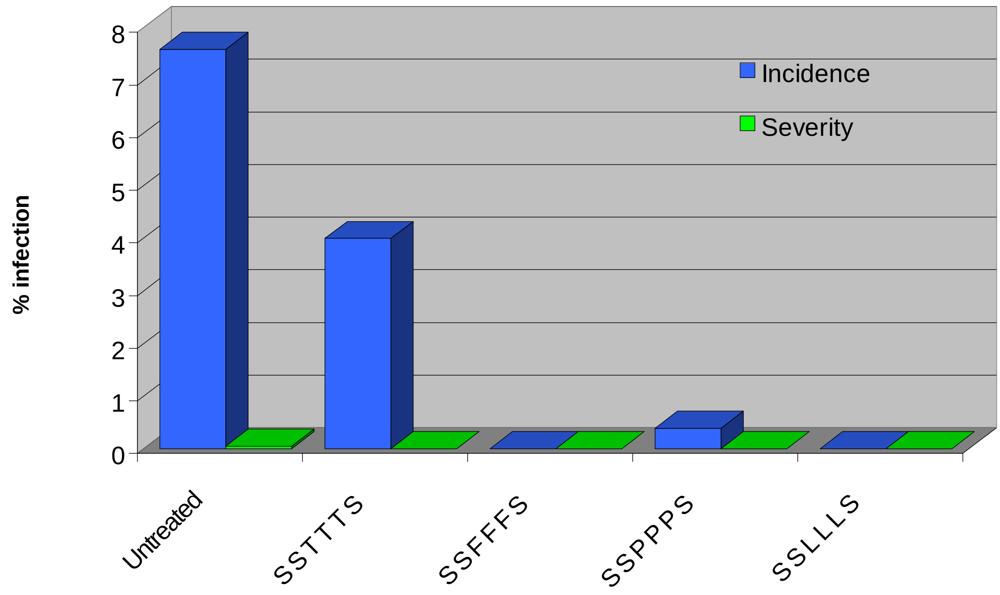
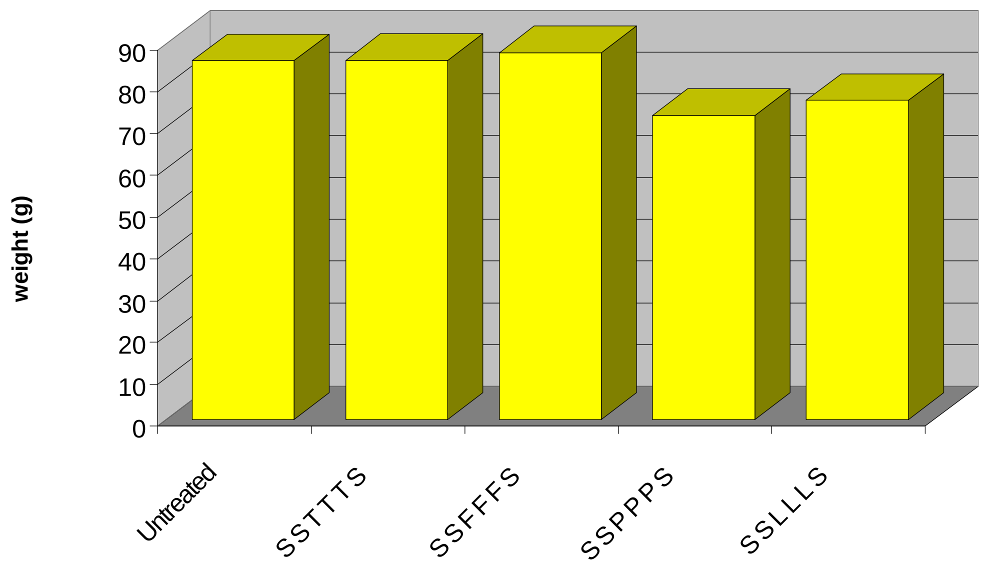


Figure 14. Average weight of Chardonnay bunches at harvest, Nuriootpa February 22, 2001.



Powdery mildew Field Trial 4.

Table 6. Application times, vine growth stages and fungicides used on Chardonnay vines at Nuriootpa 2000/2001.

Treatment	Application date and vine growth stage*					Total no. applications
	6/10/00 12	26/10/00 17	17/11/00 27	30/11/00 31	15/12/00 33	
1	-	-	-	-	-	-
2	S	S	S	S	S	5
3	S	S	T	T	S	5
4	S	S	F	F	S	5
5	F	F	F	T	T	5
6	T	T	F	F	F	5

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (3 g/L), T = Topas (0.125 ml/L), F = Flint (0.15 g/L)

Figure 15. Incidence and severity of powdery mildew on Chardonnay bunches at harvest, Nuriootpa February 22, 2001.

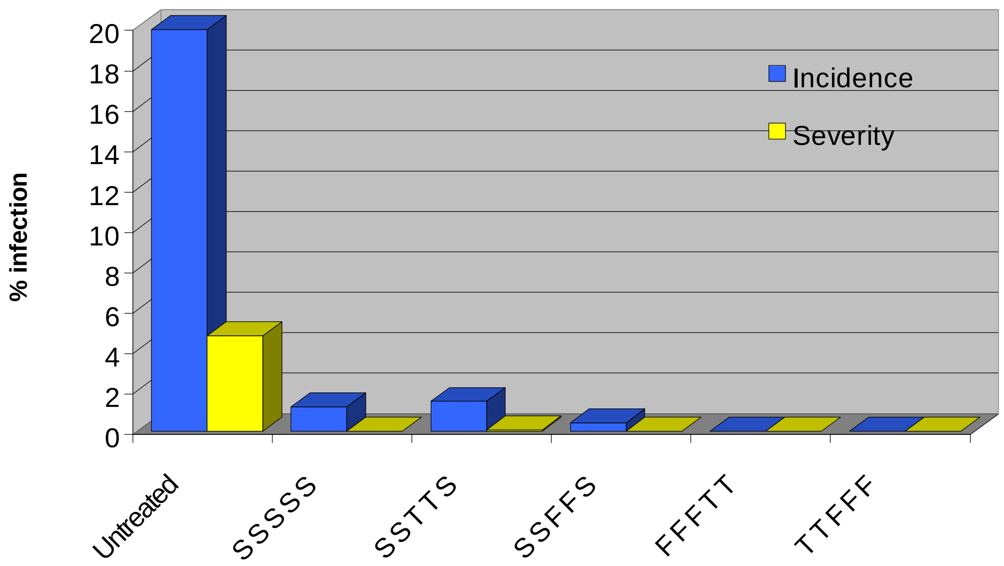


Figure 16. Incidence and severity of powdery mildew on Chardonnay leaves, Nuriootpa February 22, 2001.

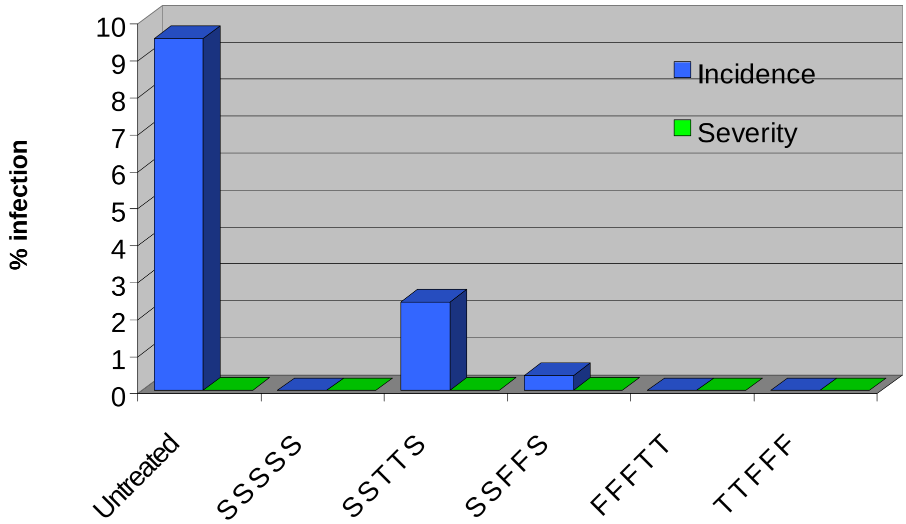
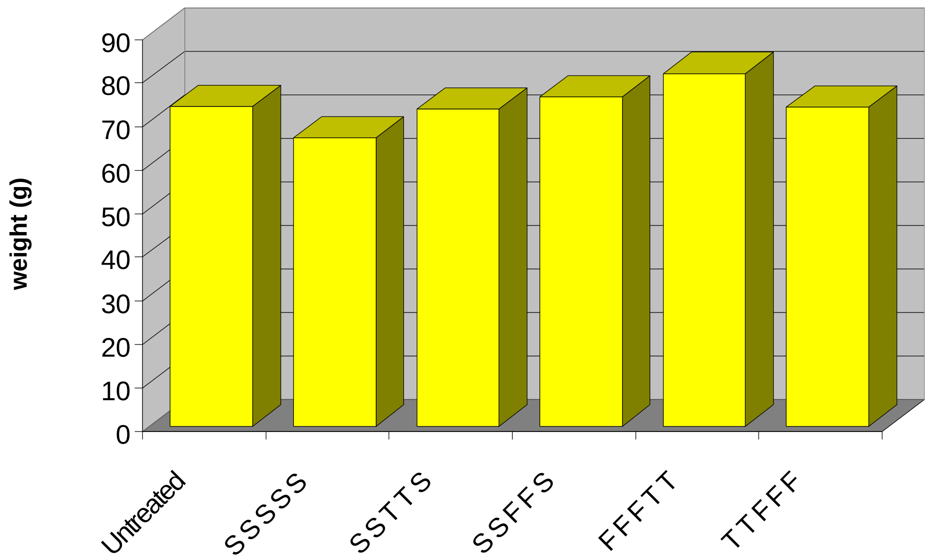


Figure 17. Average weight of Chardonnay bunches at harvest, Nuriootpa February 22, 2001.



__Powdery mildew Field Trial 5.__

Table 7. Application times, vine growth stages and fungicides used on Crouchen vines at Nuriootpa 2000/2001.

Treatment	Application date and vine growth stage*					Total no. applications
	16/10/00 13	3/11/00 16	17/11/00 25	30/11/00 29	15/12/00 31	
Untreated	-	-	-	-	-	-
Topas	S	S	T	T	T	5
Flint	S	S	F	F	F	5
Prosper	S	S	P	P	P	5
Legend	S	S	L	L	L	5

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (3 g/L), T = Topas (0.125 ml/L), F = Flint (0.15g/L) L = Legend (0.2 ml/L) and P = Prosper (0.6 ml/L).

Figure 18. Incidence and severity of powdery mildew on Crouchen bunches at harvest, Nuriootpa February 22, 2001.

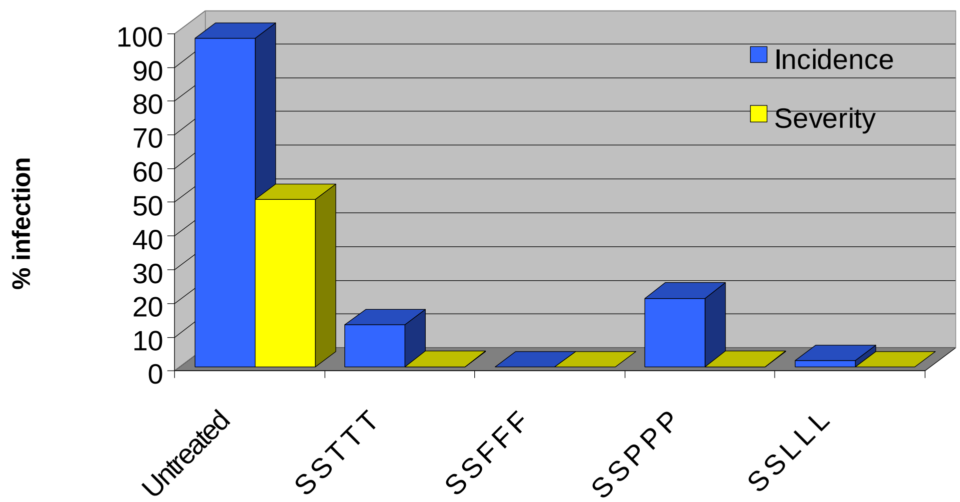


Figure 19. Incidence and severity of powdery mildew on Crouchen leaves, Nuriootpa February 22, 2001.

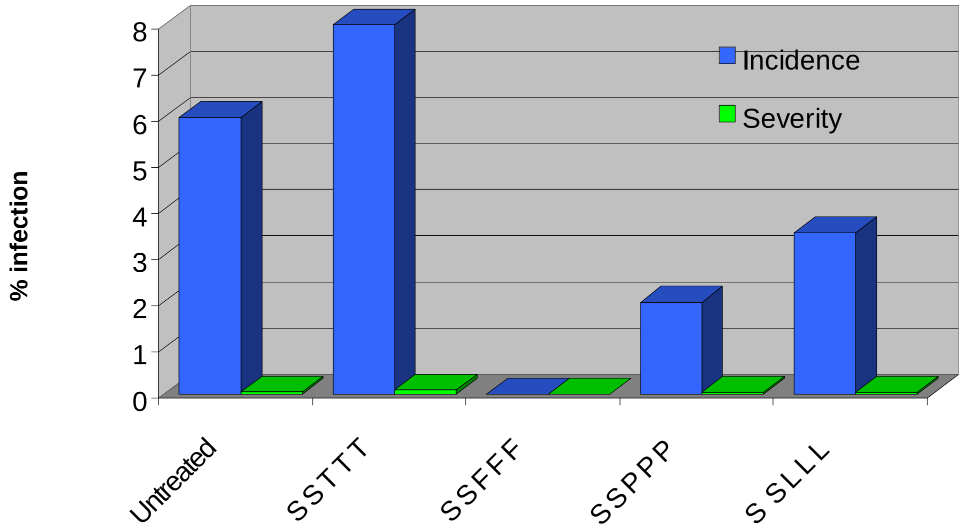
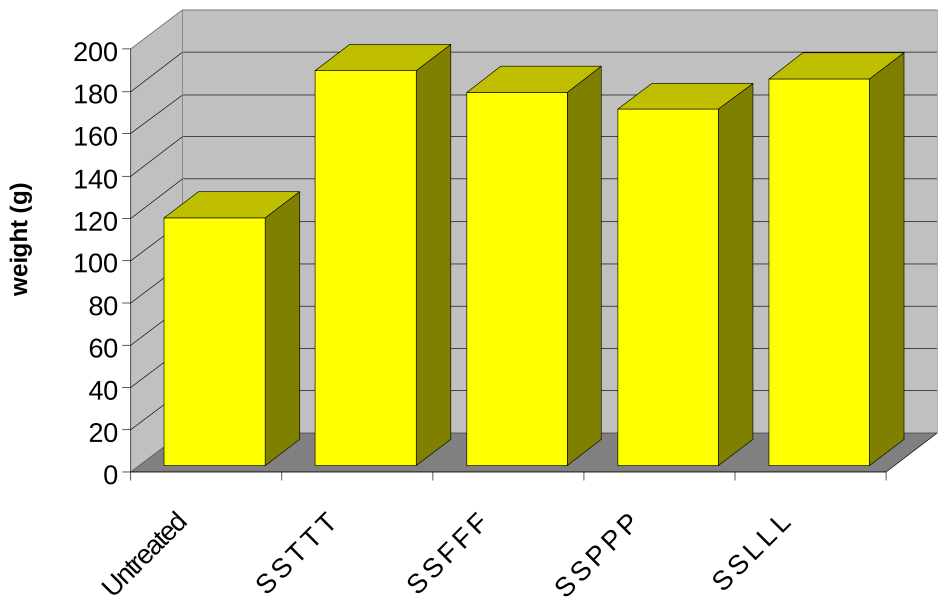


Figure 20. Average weight of Crouchen bunches at harvest, Nuriootpa February 22, 2001.



Powdery mildew Field Trial 6.

Table 8. Application times, vine growth stages and fungicides used on Chardonnay vines at Lenswood 2000/2001.

Treatment	Application date and vine growth stage*					Total no. applications
	23/10/00 14	13/11/00 17	29/11/00 27	13/12/00 31	27/12/00 32	
1	-	-	-	-	-	-
2	A	T	A	A	T	5
3	F	F	F	T	T	5
4	S	S	T	T	F	5
5	S	T	A	A	T	5
6	S	S	T	T	S	5
7	S	S	S+T	S+T	S	5
8	S+A	T	S+A	S+A	T	5
9	S+F	S+F	S+F	T	T	5

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (3 g/L), T = Topas (0.125 ml/L), A = Amistar (0.5 g/L), F = Flint (0.15 g/L)

Figure 21. Incidence and severity of powdery mildew on Chardonnay bunches at harvest, Lenswood February 28, 2001.

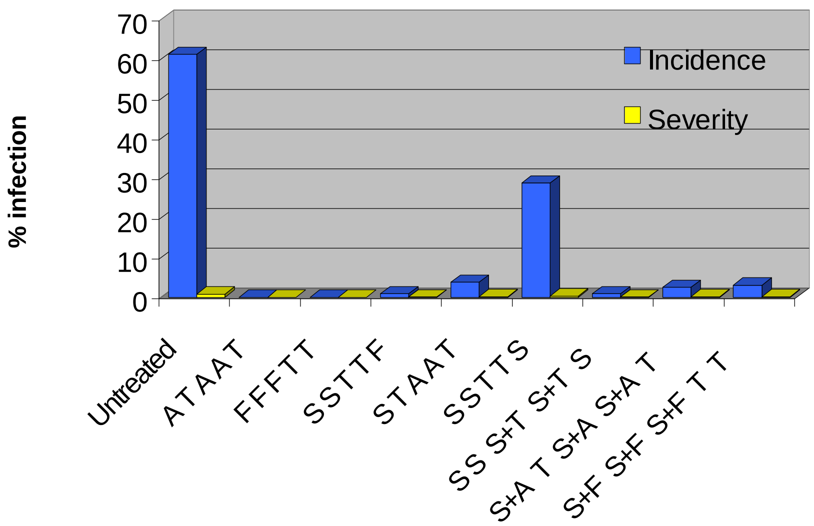


Figure 22. Incidence and severity of powdery mildew on the lower surface of Chardonnay leaves, Lenswood February 28, 2001.

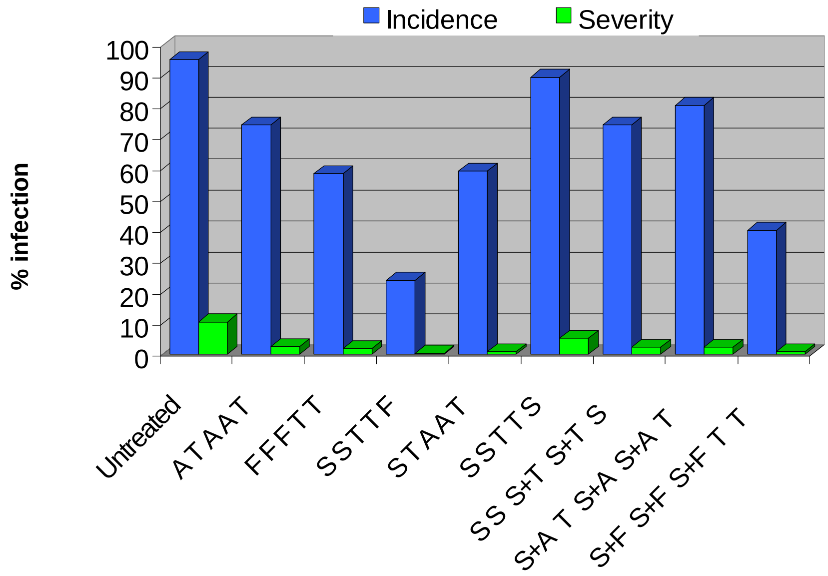


Figure 23. Incidence and severity of powdery mildew on the upper surface of Chardonnay leaves, Lenswood February 28, 2001

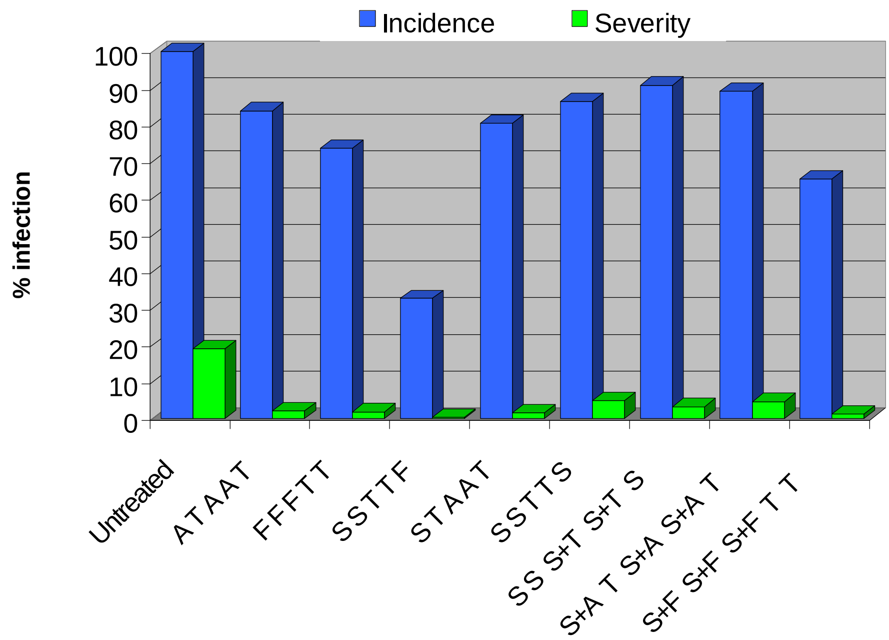
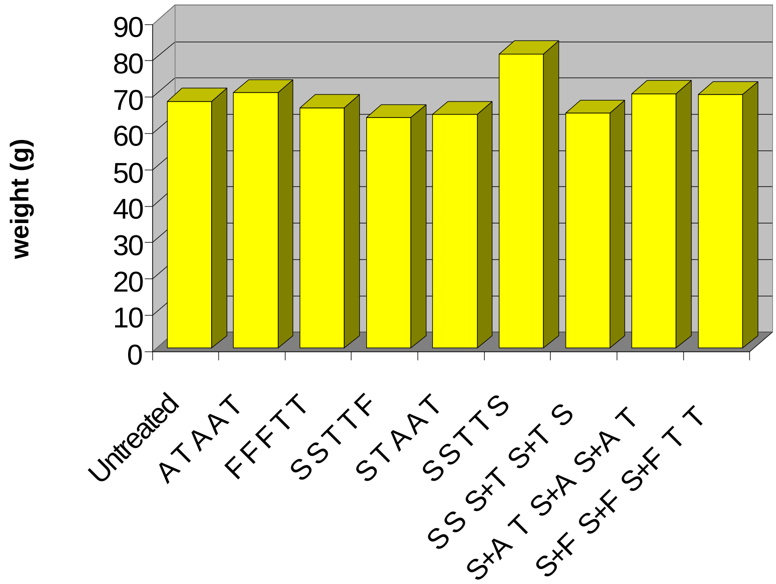


Figure 24. Average weight of Chardonnay bunches at harvest, Lenswood February 28, 2001.



Powdery mildew Field Trial 7.

Table 9. Application times, vine growth stages and fungicides used on Chardonnay vines at Lenswood 2000/2001.

Treatment	Application date and vine growth stage*						Total no. applications
	23/10/00 14	13/11/00 17	29/11/00 23	13/12/00 27	27/12/00 31	12/1/01 32	
1	-	-	-	-	-	-	-
2	S	S	T	T	S	S	6
3	S	S	F	F	S	S	6
4	T	T	F	F	S	S	6
5	S	S	F	F	F	S	6
6	S	S	T	T	F	F	6
7	F	F	F	T	T	S	6

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (3 g/L), T = Topas (0.125 ml/L), F = Flint (0.15 g/L)

Figure 25. Incidence of powdery mildew on Chardonnay bunches, Lenswood 2000/2001.

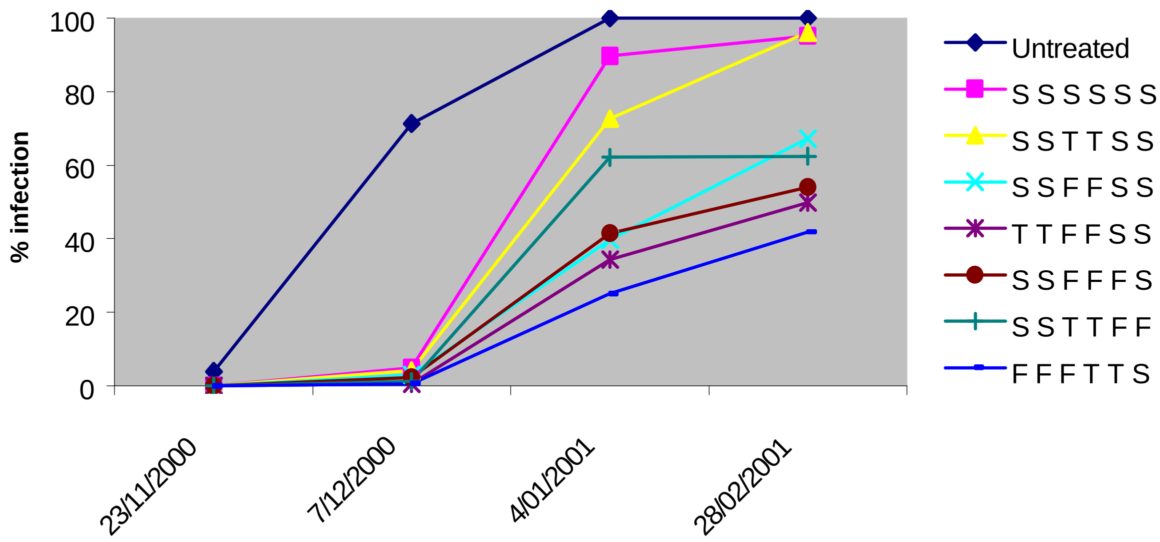


Figure 26. Severity of powdery mildew on Chardonnay bunches, Lenswood 2000/2001.

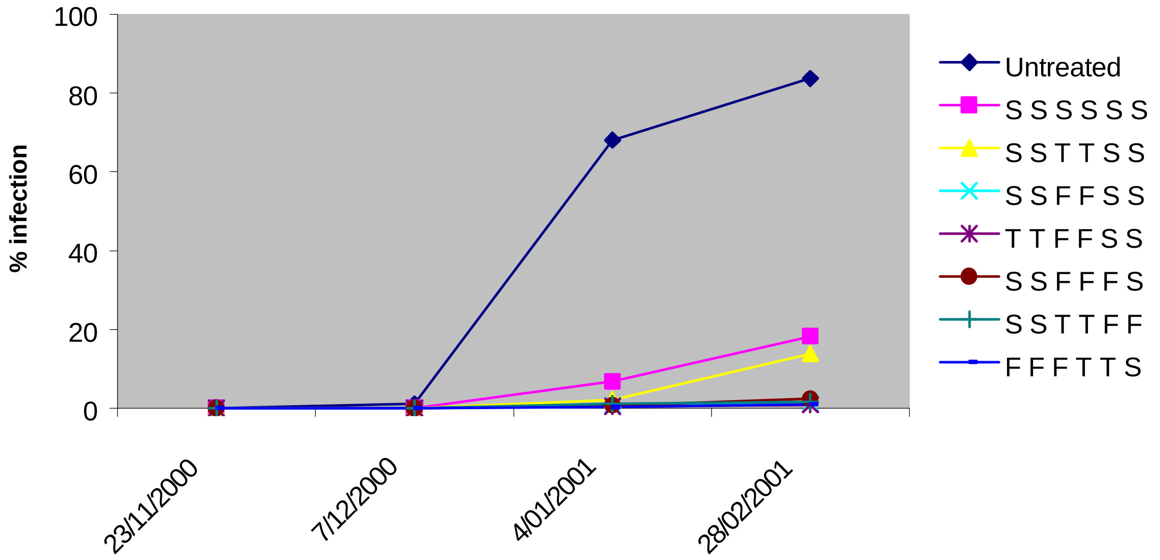


Figure 27. Incidence and severity of powdery mildew on the lower surface of Chardonnay leaves, Lenswood February 28, 2001.

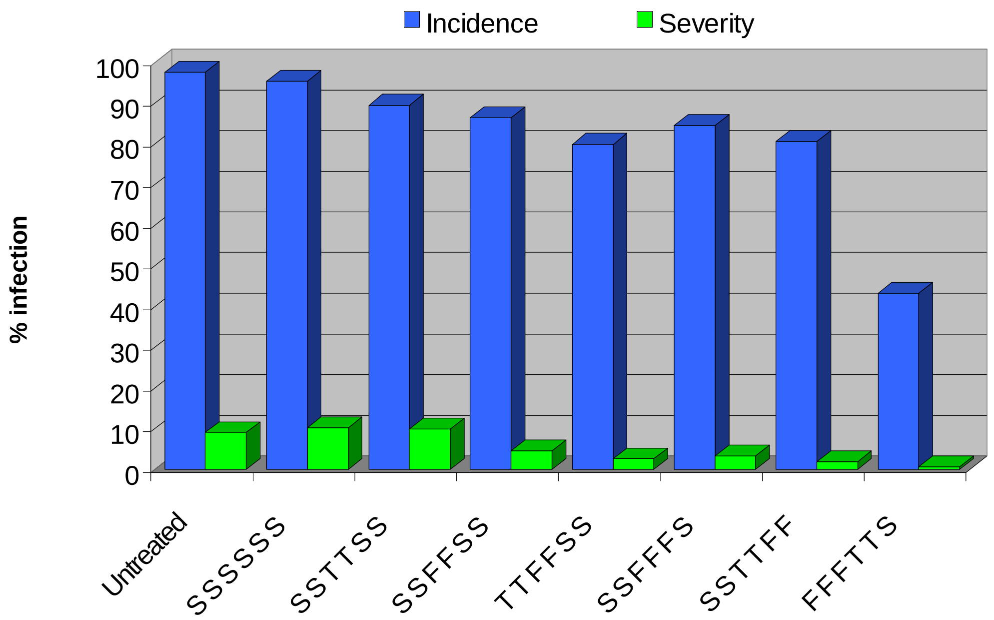


Figure 28. Incidence and severity of powdery mildew on the upper leaf surface of Chardonnay leaves, Lenswood February 28, 2001.

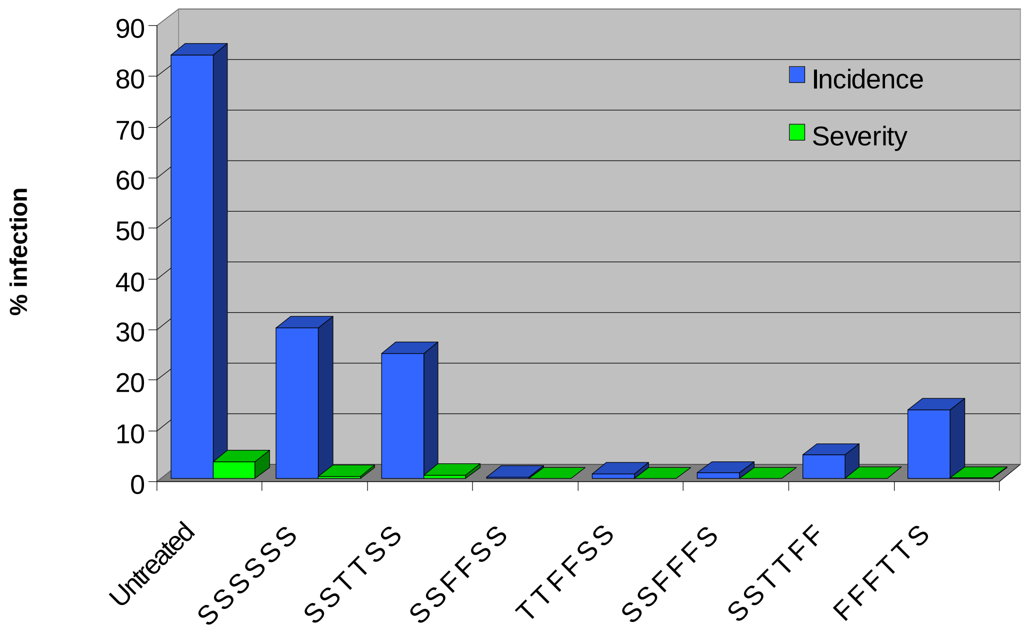
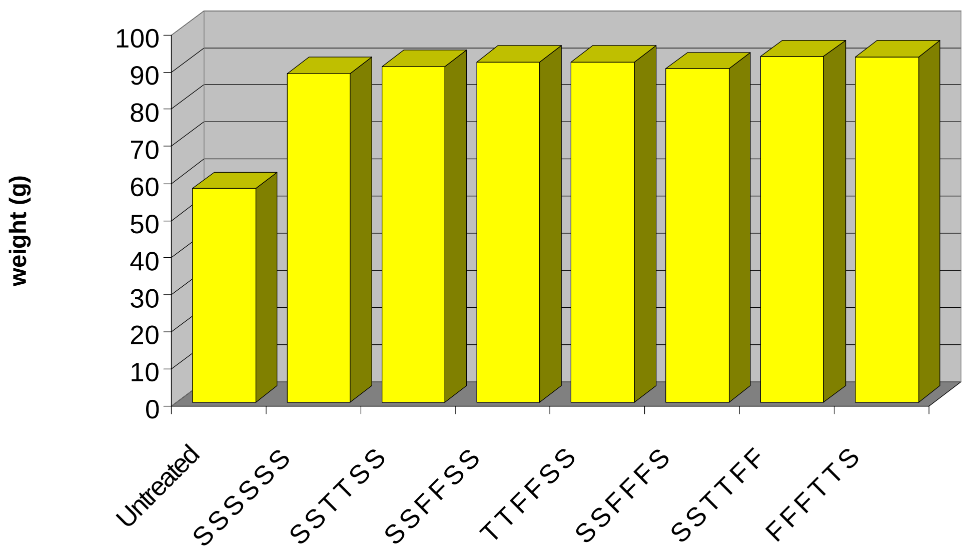


Figure 29. Average weight of Chardonnay bunches at harvest, Lenswood February 28, 2001.



Powdery mildew Field Trial 8.

Table 10. Application times, vine growth stages and fungicides used on Shiraz vines at Loxton 2000/2001.

Treatment	Application date and vine growth stage*							Total no. applications
	26/9/00	9/10/00	24/10/00	6/11/00	20/11/00	4/12/00	18/12/00	
	7	13	14	19	26	30	33	
1	-	-	-	-	-	-	-	-
2	F	F	F	T	T	S	S	7
3	S	T	T	F	F	F	S	7
4	S	S	T	T	S	S	S	7
5	S	S	F	F	S	S	S	7

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (2 g/L), T = Topas (0.125 ml/L), F = Flint (0.15 g/L)

Figure 30. Incidence and severity of powdery mildew on Shiraz bunches at harvest, Loxton February 13, 2001.

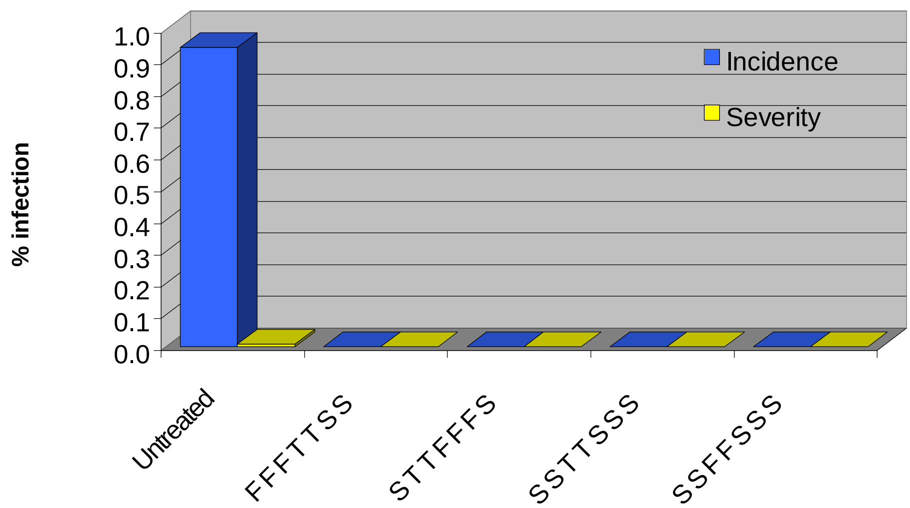
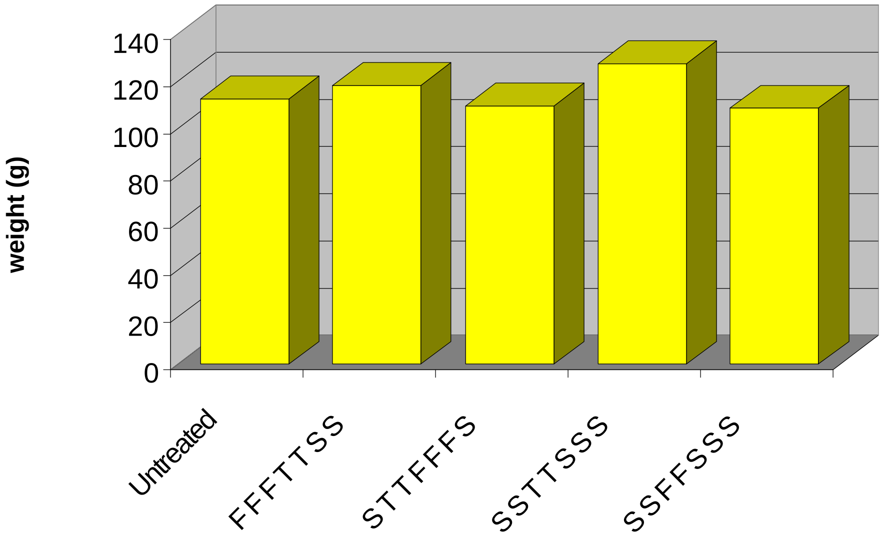


Figure 31. Average weight of Shiraz bunches at harvest, Loxton February 13, 2001.



Powdery mildew Field Trial 9.

Table 11. Application times, vine growth stages and fungicides used on Chardonnay vines at Nuriootpa 2001/2002.

Treatment	Application date and vine growth stage*			Total no. applications
	20/11/01	4/12/01	13/12/01	
	23	27	29	
1	-	-	-	-
2	A	A	S	3
3	F	F	S	3
4	C	C	S	3

* Coombe (1995)

Where: - = No fungicide, F = Flint (0.15 g/L), A = Amistar (0.5g/L), C = Cabrio (0.4ml/L), S = Thiovit (3g/L).

Figure 32. Incidence and severity of powdery mildew on Chardonnay bunches at harvest, Nuriootpa March 11, 2002.

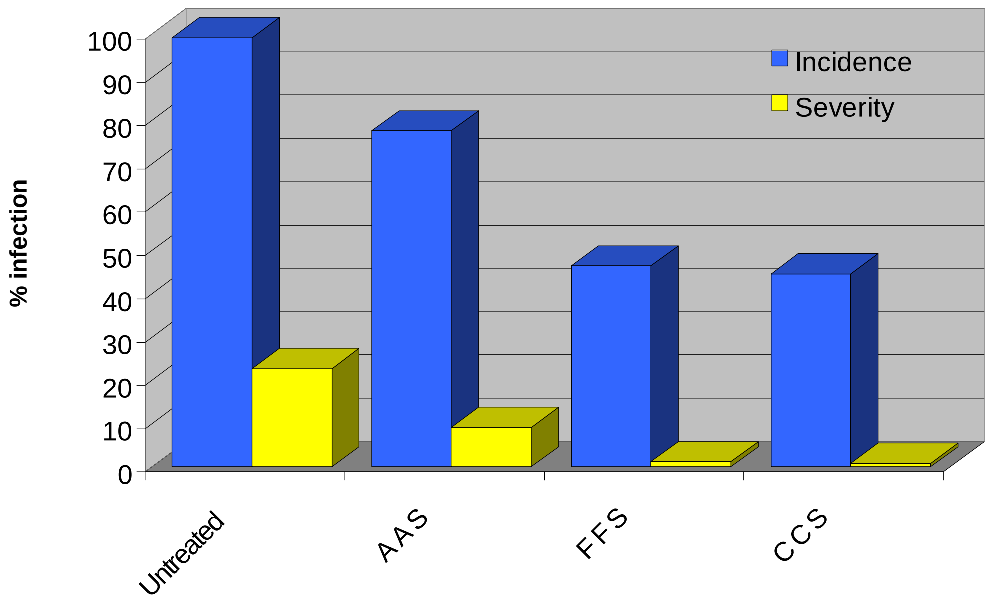


Figure 33. Incidence and severity of powdery mildew on the lower surface of Chardonnay leaves, Nuriootpa March 11, 2002.

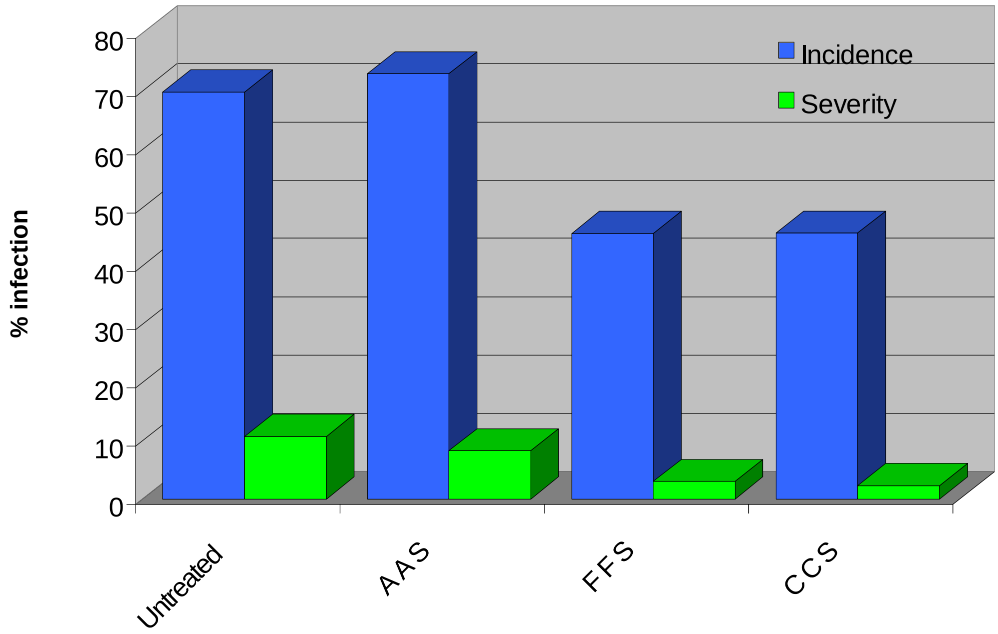
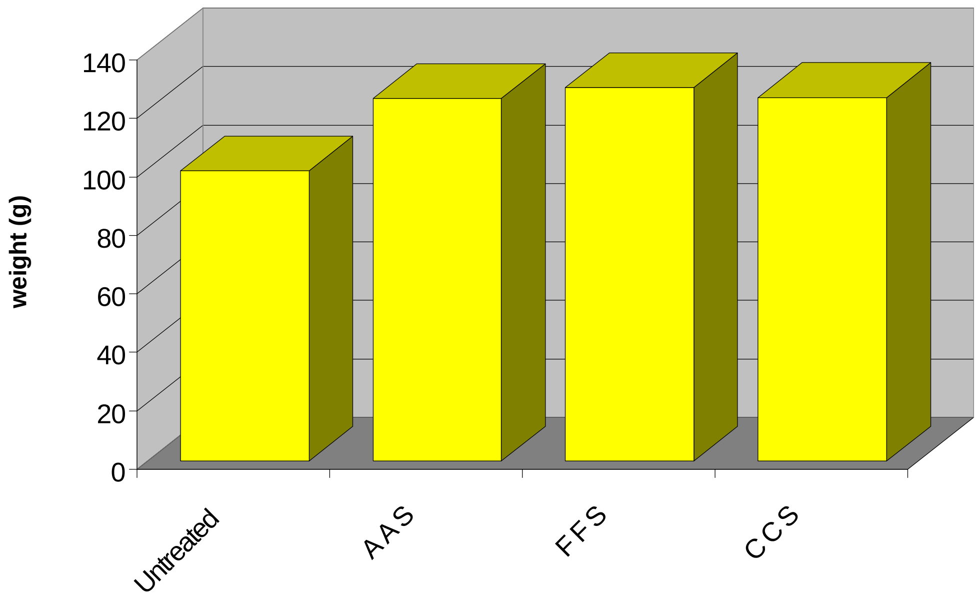


Figure 34. Average weight of Chardonnay bunches at harvest, Nuriootpa March 11, 2002.



Powdery mildew Field Trial 10.

Table 12. Application times, vine growth stages and fungicides used on Chardonnay vines at Nuriootpa 2001/2002.

Treatment	Application date and vine growth stage*				Total no. applications
	16/10/01 17	1/11/01 18	14/11/01 21	28/11/01 26	
1	-	-	-	-	-
2	S	S	S	S	4
3	S	T	T	S	4
4	T	T	F	F	4
5	T	T	C	C	4
6	F	F	T	T	4
7	C	C	T	T	4

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (6 g/L), T= Topas (0.125 ml/L), F = Flint (0.15 g/L), C = Cabrio (0.4ml/L)

Figure 35. Incidence and severity of powdery mildew on Chardonnay bunches at harvest, Nuriootpa March 11, 2002.

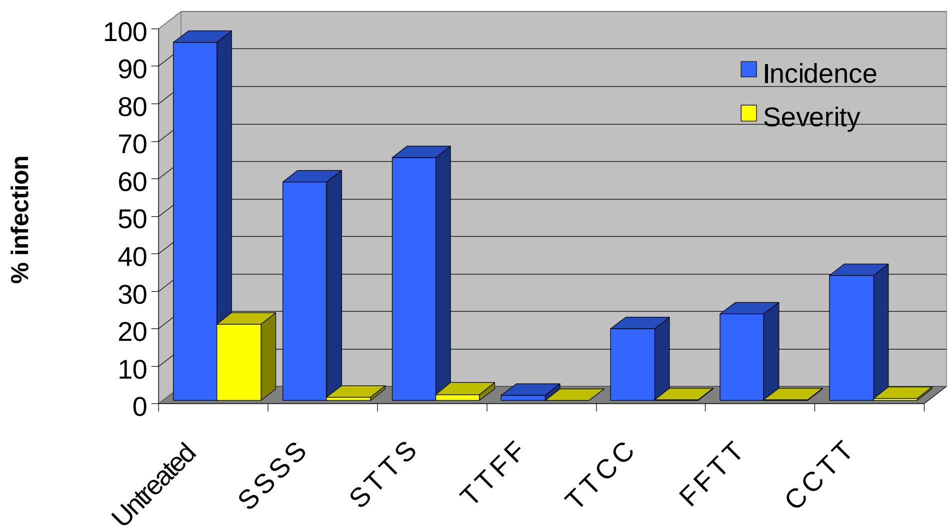
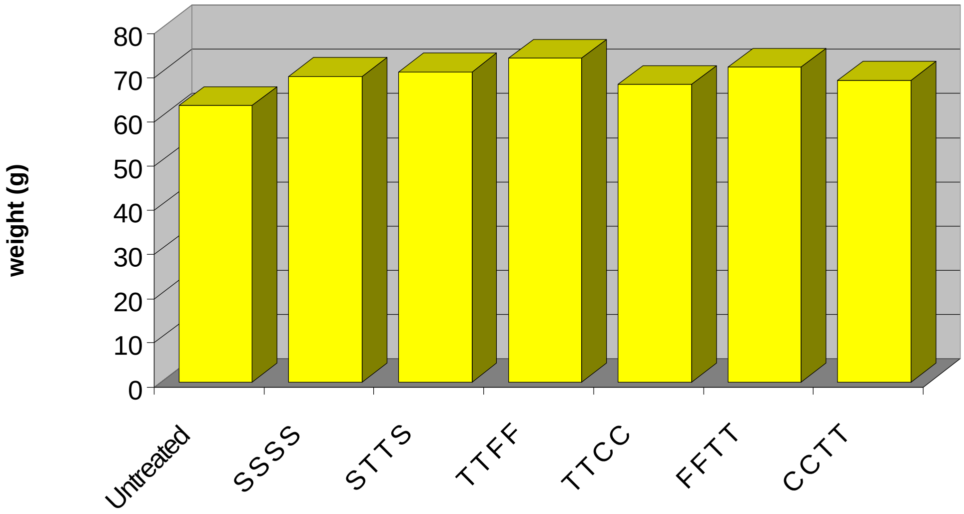


Figure 36. Average weight of Chardonnay bunches at harvest, Nuriootpa March 11, 2002.



Powdery mildew Field Trial 11.

Table 13. Application times, vine growth stages and fungicides used on Crouchen vines at Nuriootpa 2001/2002.

Treatment	Application date and vine growth stage*					Total no. applications
	16/10/01	1/11/01	14/11/01	28/11/01	12/12/01	
	12	15	17	26	29	
1	-	-	-	-	-	-
2	S	S	T	T	T	5
3	S	S	A	A	A	5
4	S	S	F	F	F	5
5	S	S	C	C	C	5

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (6 g/L), T= Topas (0.125 ml/L), F = Flint (0.15 g/L), A = Amistar (0.5 g/L), C = Cabrio (0.4ml/L)

Figure 37. Incidence of powdery mildew on Crouchen bunches, Nuriootpa 2001/2002.

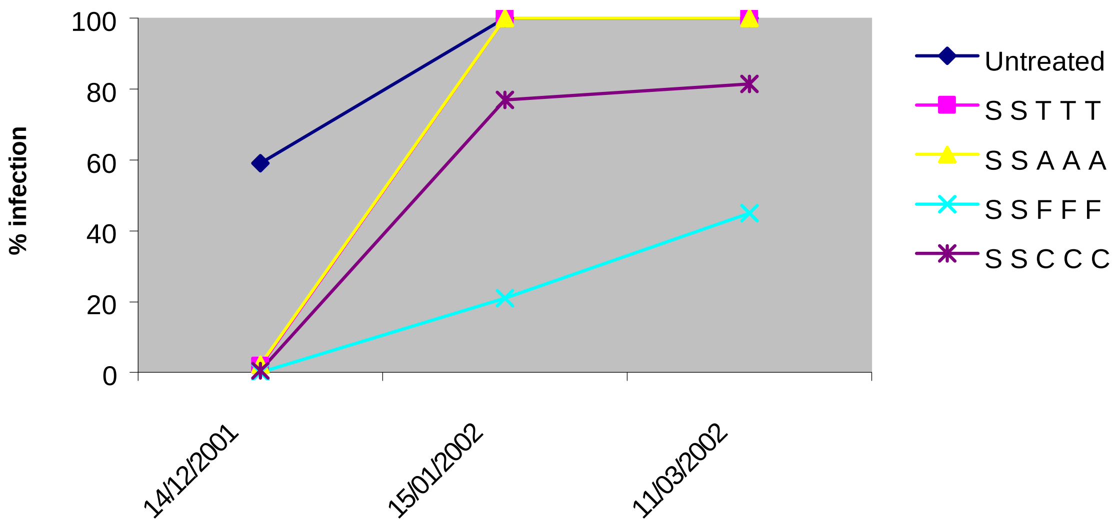


Figure 38. Severity of powdery mildew on Crouchen bunches, Nuriootpa 2001/2002.

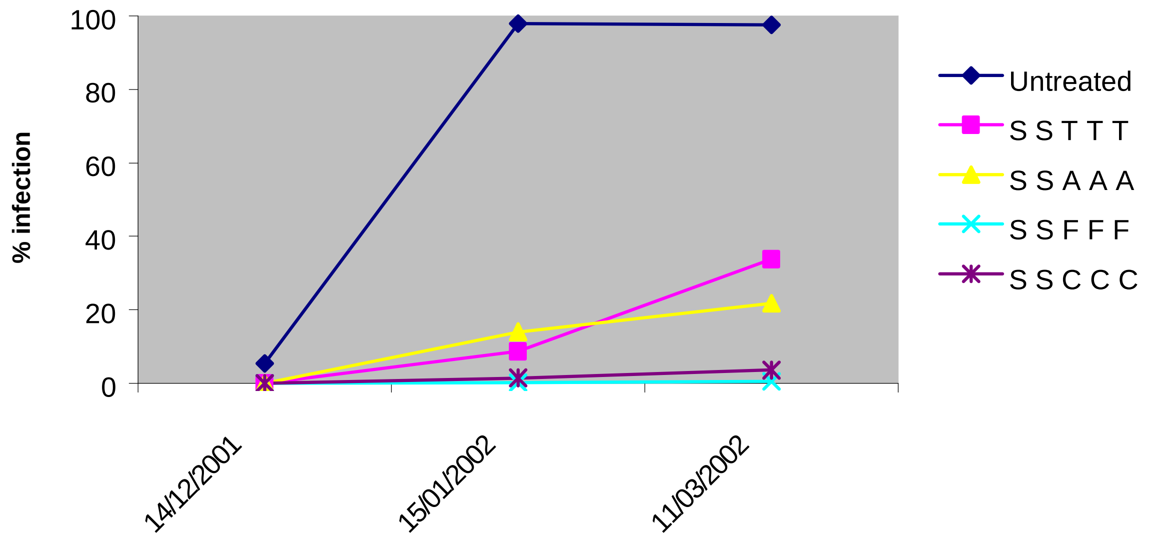


Figure 39. Incidence and severity of powdery mildew on Crouchen leaves, Nuriootpa March 11, 2002.

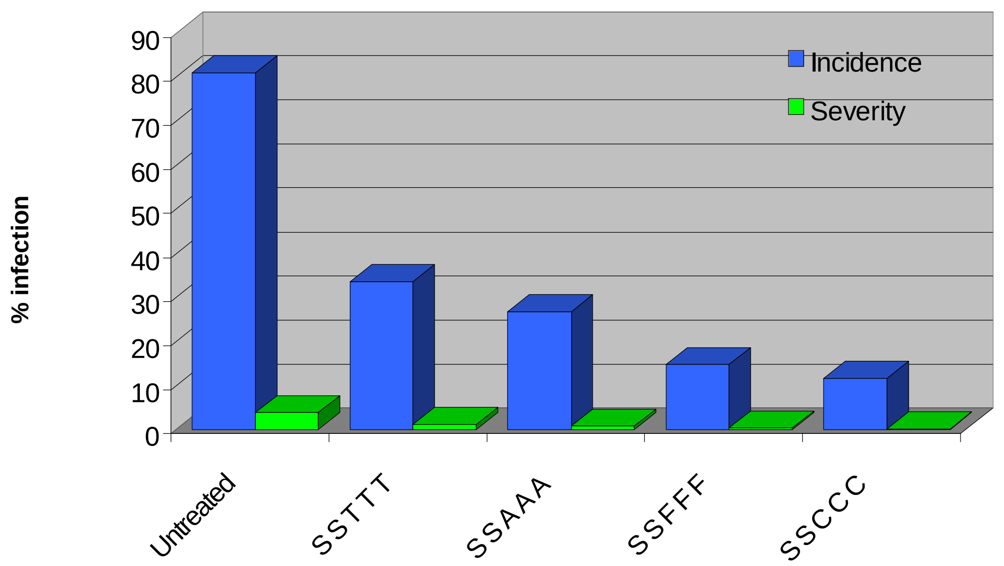
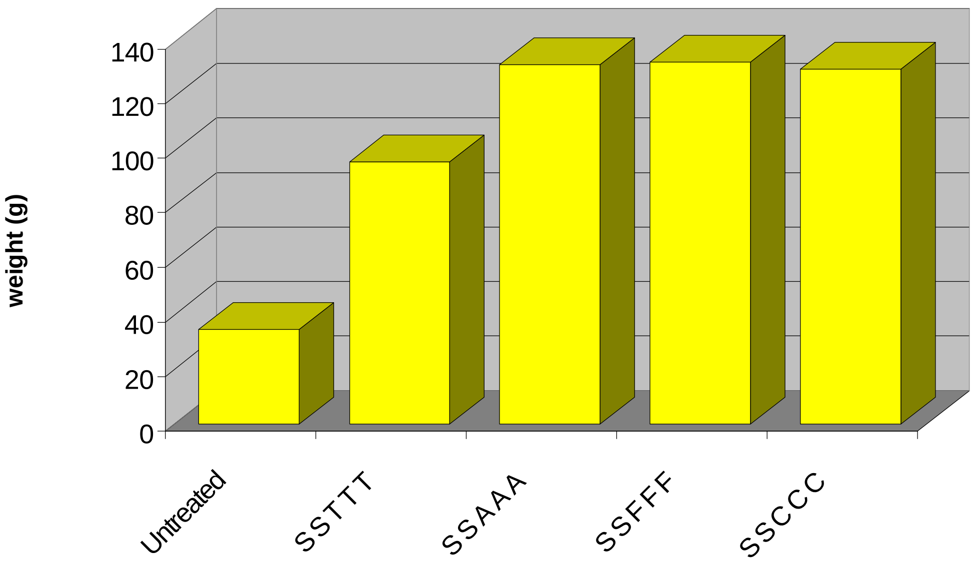


Figure 40. Average weight of Crouchen bunches at harvest, Nuriootpa March 11, 2002.



Powdery mildew Field Trial 12.

Table 14. Application times, vine growth stages and fungicides used on Verdelho vines at Nuriootpa 2001/2002.

Treatment	Application date and vine growth stage*				Total no. applications
	16/10/01	1/11/01	14/11/01	28/11/01	
	15	17	23	27	
1	-	-	-	-	-
2	S	S	S	S	4
3	S	T	T	S	4
4	T	T	F	F	4
5	T	T	C	C	4
6	F	F	T	T	4
7	C	C	T	T	4

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (6 g/L), T= Topas (0.125 ml/L), F = Flint (0.15 g/L), C = Cabrio (0.4ml/L)

Figure 41. Incidence of powdery mildew on Verdelho bunches, Nuriootpa 2001/2002.

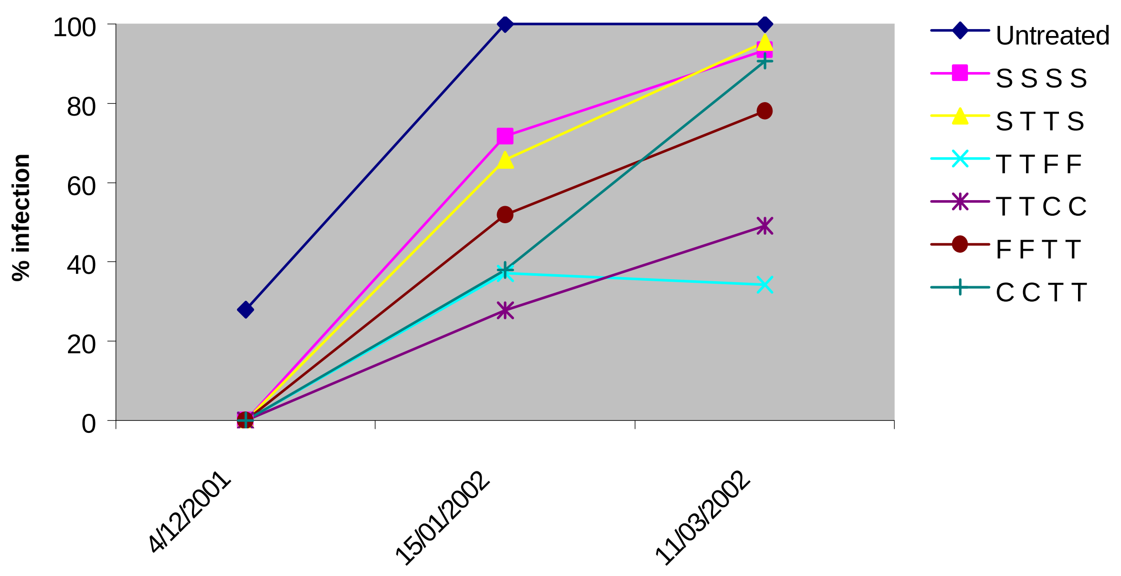


Figure 42. Severity of powdery mildew on Verdelho bunches, Nuriootpa 2001/2002.

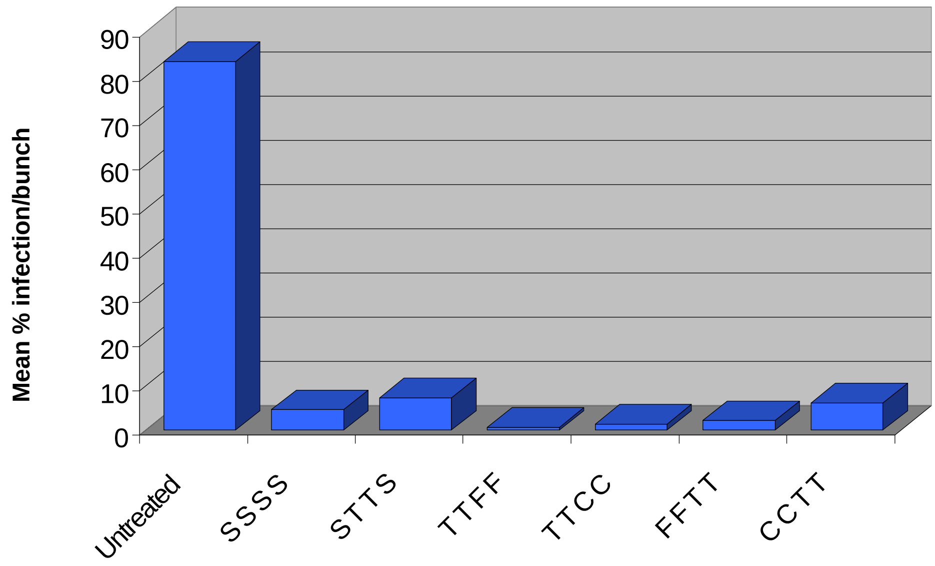
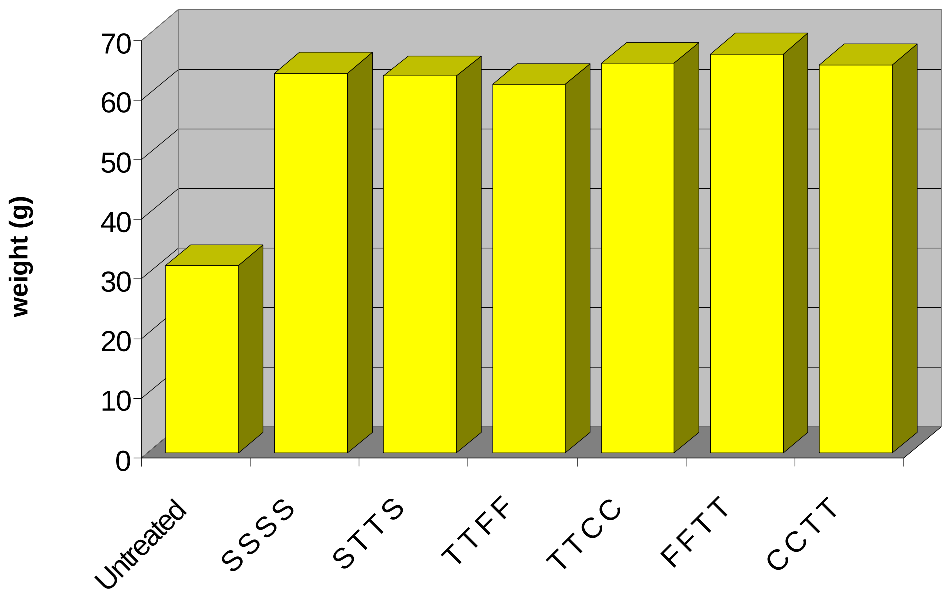


Figure 43. Average weight of Verdelho bunches at harvest, March 11, 2002.



Powdery mildew Field Trial 13.

Table 15. Application times, vine growth stages and fungicides used on Chardonnay vines at Lenswood 2001/2002.

Treatment	Application date and vine growth stage*						Total no. applications
	1/11/01	15/11/01	28/11/01	12/12/01	27/12/01	9/1/02	
	14	15	18	24	27	29	
1	-	-	-	-	-	-	-
2	-	T	T	F	F	-	4
3	-	F	F	T	T	-	4
4	-	S	S	F	F	-	4
5	-	F	F	S	S	-	4
6	-	S	T	T	S	-	4
7	-	T	T	C	C	-	4
8	-	C	C	T	T	-	4
9	S	T	T	F	F	S	6
10	S	T	T	C	C	S	6
11	S	S	T	T	S	S	6

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (6 g/L), T = Topas (0.125 ml/L), F = Flint (0.15 g/L), C = Cabrio (0.4 ml/L)

Figure 44. Incidence of powdery mildew on Chardonnay bunches, Lenswood March 19, 2001/2002.

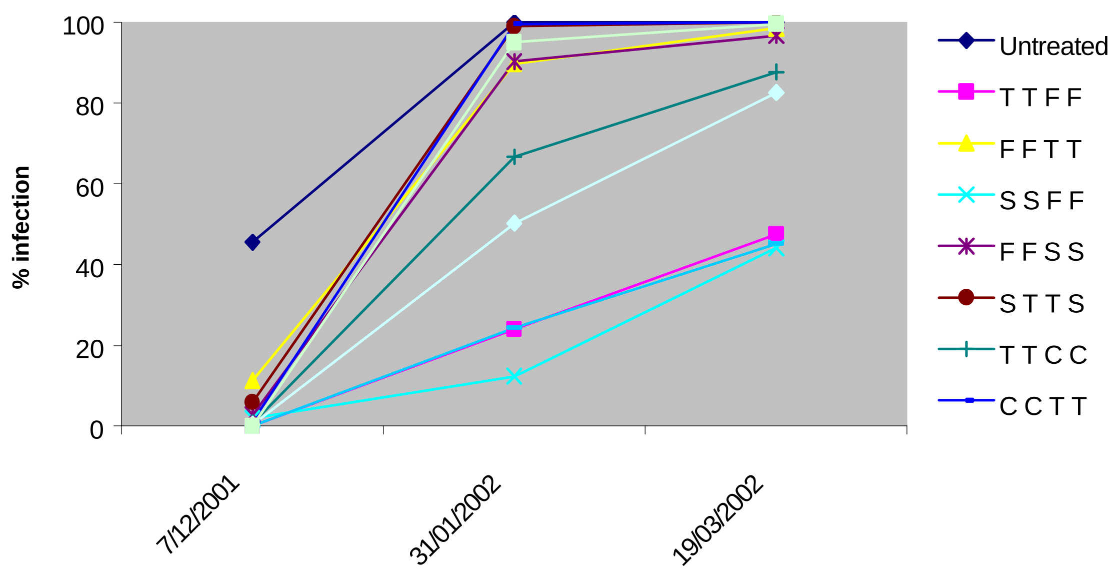


Figure 45. Severity of powdery mildew on Chardonnay bunches, Lenswood 2001/2002.

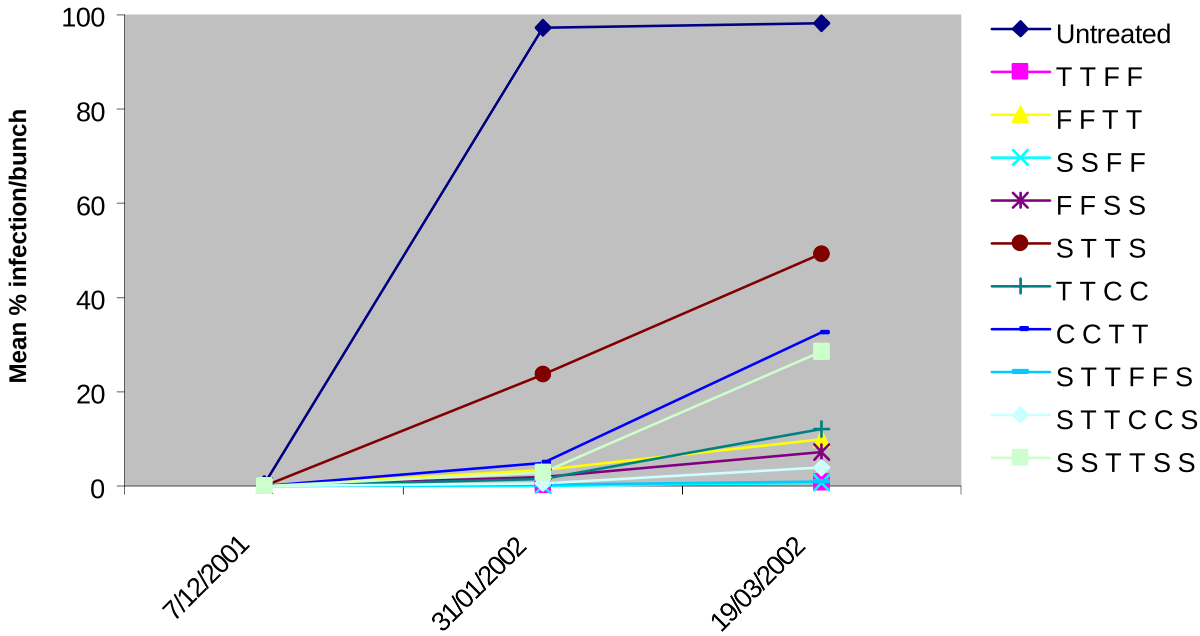


Figure 46. Incidence and severity of powdery mildew on the lower surface of Chardonnay leaves, Lenswood March 19, 2002.

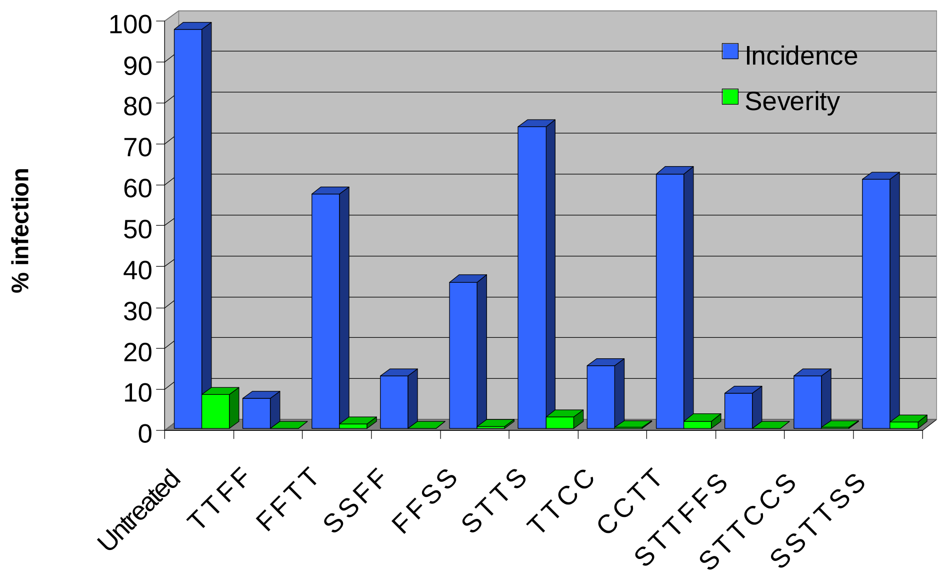


Figure 47. Incidence and severity of powdery mildew on the upper surface of Chardonnay leaves, Lenswood March 19, 2002.

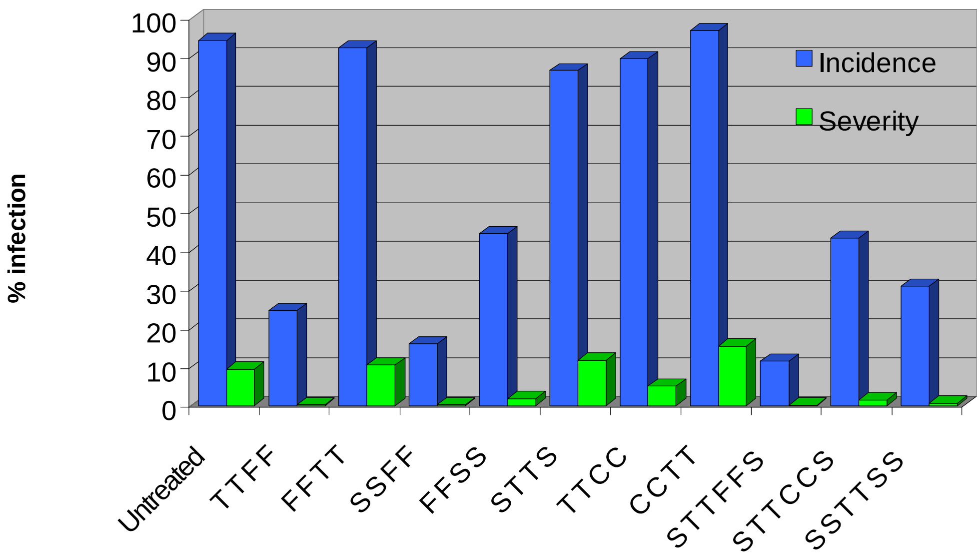
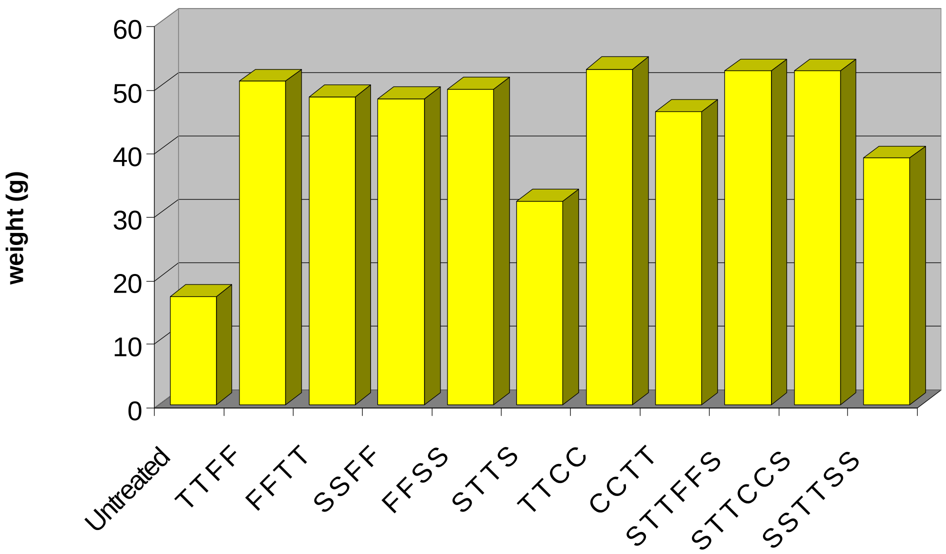


Figure 48. Average weight of Chardonnay bunches at harvest, Lenswood March 19, 2002.

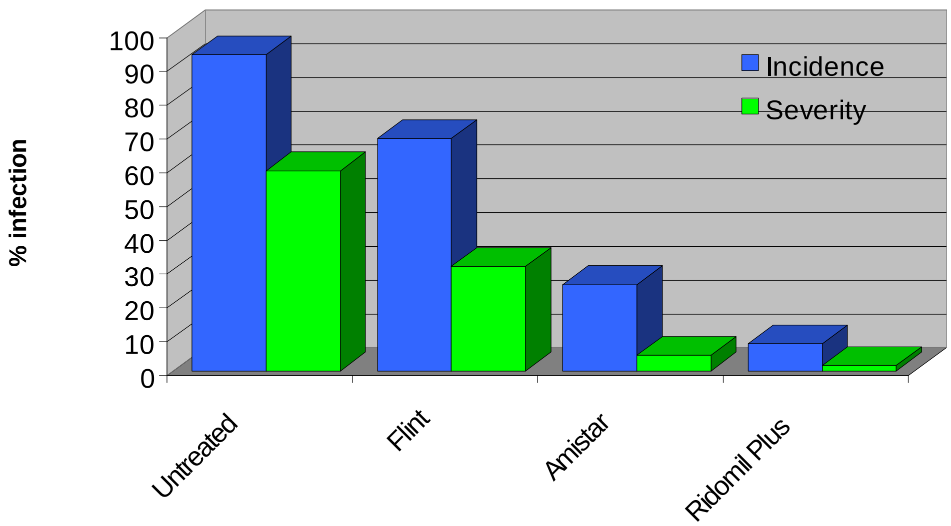


Downy mildew Field Trial 1.

Table 16. Application times, vine growth stages and fungicides used on Chardonnay vines at Lenswood February 2000.

Treatment	Rate / Litre	Number of applications
Untreated	-	-
Flint	0.2 g	1
Amistar	0.375 g	1
Ridomil Plus	1.5 g	1

Figure 49. Incidence and severity of downy mildew on Chardonnay leaves, Lenswood February 2000.



Downy mildew Field Trial 2.

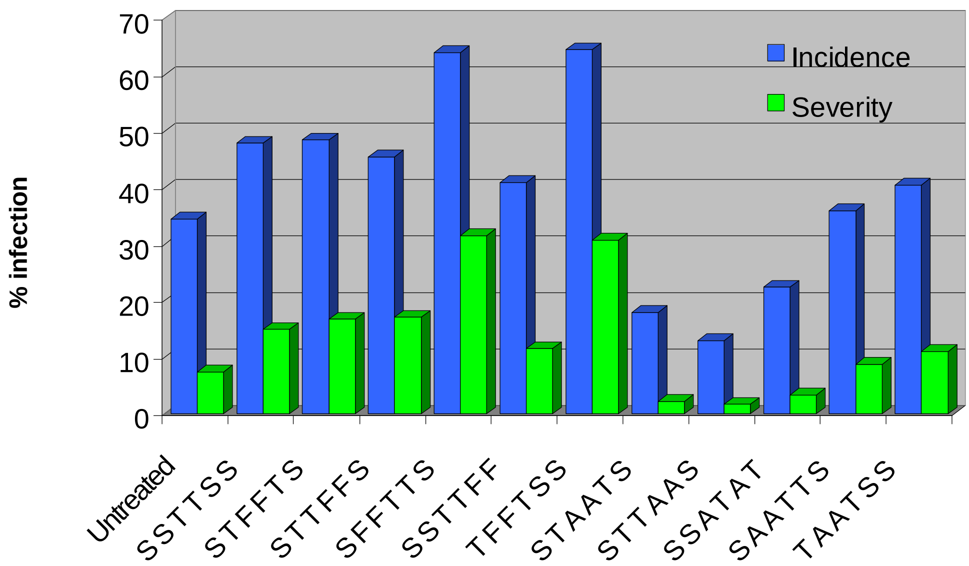
Table 17. Application times, vine growth stages and fungicides used on Chardonnay vines at Lenswood March 2000 (applied for powdery mildew control).

Treatment	Application date and vine growth stage*						Total no. applications
	14/10/99 15	29/10/99 17	24/11/99 22	10/12/99 25	20/12/99 27	6/1/00 31	
1	-	-	-	-	-	-	-
2	S	S	T	T	S	S	6
3	S	T	F	F	T	S	6
4	S	T	T	F	F	S	6
5	S	F	F	T	T	S	6
6	S	S	T	T	F	F	6
7	T	F	F	T	S	S	6
8	S	T	A	A	T	S	6
9	S	T	T	A	A	S	6
10	S	S	A	T	A	T	6
11	S	A	A	T	T	S	6
12	T	A	A	T	S	S	6

* Coombe (1995)

Where: - = No fungicide, S = Thiovit (2 g/L), T = Topas (0.125 ml/L), A = Amistar (0.5 g/L), F = Flint (0.15 g/L)

Figure 50. Incidence and severity of downy mildew on Chardonnay leaves at harvest, Lenswood March 15, 2000.



Downy mildew Field Trial 3.

Table 18. Application time and fungicides used on artificially inoculated Chardonnay vines at Lenswood February 22, 2000.

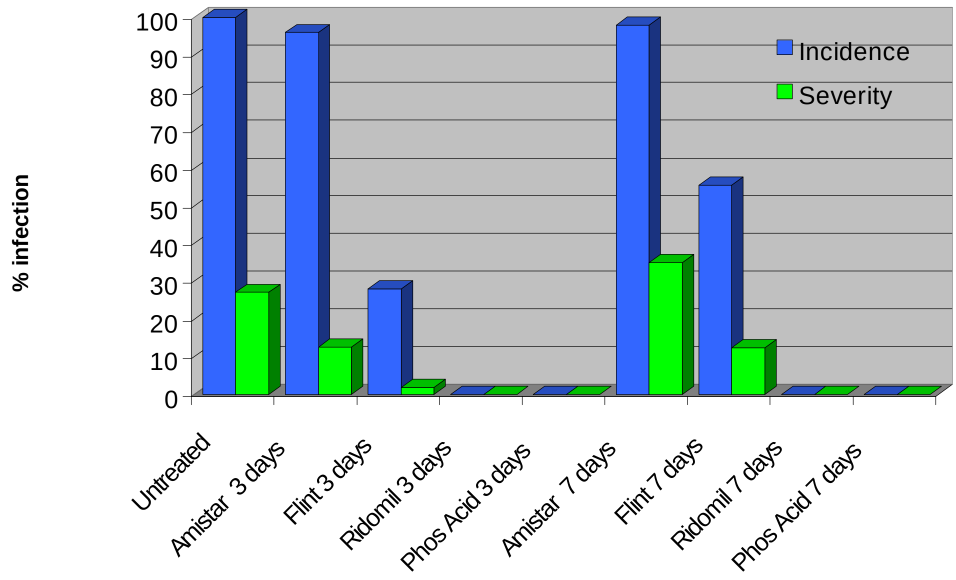
Treatment	Inoculation date	Application timing after inoculation	Rate / Litre	Number of applications
Untreated	-	-	-	-
Flint	14/12/99	3 days	0.2 g	1
Amistar	14/12/99	3 days	0.375 g	1
Ridomil Plus	14/12/99	3 days	1.5 g	1
Phosphorous Acid	14/12/99	3 days	10 ml	1
Flint	14/12/99	7 days	0.2 g	1
Amistar	14/12/99	7 days	0.375 g	1
Ridomil Plus	14/12/99	7 days	1.5 g	1
Phosphorous Acid	14/12/99	7 days	10 ml	1

Table 19. The effect of fungicides applied 3 or 7 days after the development of lesions and sporulation of *Plasmopara viticola* on Chardonnay vine leaves.

Treatment	Rate/L	Time of application (days after inoculation)	Spore production (conidia/cm^2 x 10^4)
Untreated		-	6.4
Amistar	0.375 g		1.7
Flint	0.2 g	3	0.4
Ridomil	1.5 g	3	0
Phosphorous Acid	10 ml	3	0
Amistar	0.375 g	7	5.4
Flint	0.2 g	7	2.1
Ridomil	1.5 g	7	0
Phosphorous Acid	10 ml	7	0
LSD (*P*=0.05)			1.9

* = % of leaf area sporulating

Figure 51. Incidence and severity of downy mildew on artificially inoculated Chardonnay leaves February 2000.



Botrytis bunch rot Field Trial 1.

Table 20. Fungicide and inoculation spray timings on Pinot Noir vines, Lenswood 1999.

Treatment	Rate / Litre	Time of fungicide application in relation to inoculation	Inoculation timing
Untreated	-	-	Flowering
Inoculation only	-	-	Flowering
Flint	0.25 g	3 days before	Flowering
Rovral	1 ml	3 days before	Flowering
Flint	0.25 g	3 days after	Flowering
Rovral	1 ml	3 days after	Flowering
Untreated	-	-	Veraison
Inoculation only	-	-	Veraison
Flint	0.25 g	8 days before	Veraison
Rovral	1 ml	8 days before	Veraison

Table 21. Effect of fungicides applied to Pinot Noir bunches before or after inoculation at flowering on the incidence on Botrytis bunch rot.

Treatment	Time of fungicide application	% berries infected	% bunches infected at harvest
Untreated	-	1.5	14
Inoculation only	-	51.6	90
Flint	3 days before	81	66
Rovral	3 days before	23	61.6
Flint	3 days after	48.5	54.4
Rovral	3 days after	25	42.7

Table 22. Effect of fungicides applied to Pinot Noir bunches before or after inoculation at flowering and veraison on the incidence on Botrytis bunch rot.

Treatment	Time of fungicide application	% berries infected	% bunches infected at harvest
Untreated	-	0	14
Inoculation only	-	49	68
Flint	8 days before	47	75.8
Rovral	8 days before	2	51.4

Botrytis bunch rot Field Trial 2.

Table 23. Application times, vine growth stages and fungicides used at veraison on Pinot Noir vines at Lenswood 2000.

Treatment	Rate / Litre	Time of fungicide application in relation to veraison (days after)
Untreated	-	0
Inoculation only	-	0
Rovral	1 ml	0
Scala	2 ml	0
Switch	0.6 g	0
Teldor	1 ml	0
Flint	0.15 g	0
Rovral	1 ml	10
Scala	2 ml	10
Switch	0.6 g	10
Teldor	1 ml	10
Flint	0.15 g	10
Rovral	1 ml	14
Scala	2 ml	14
Switch	0.6 g	14
Teldor	1 ml	14
Flint	0.15 g	14
Rovral	1 ml	17
Scala	2 ml	17
Switch	0.6 g	17
Teldor	1 ml	17
Flint	0.15 g	17
Rovral	1 ml	24
Scala	2 ml	24
Switch	0.6 g	24
Teldor	1 ml	24
Flint	0.15 g	24

Table 24. Incidence of *Botrytis* on bunches at harvest after being dipped with different chemical treatments at various times after Veraison and inoculated 7 days before harvest.

	% infected bunches				
Treatment	0 days	10 days	14 days	17 days	24 days
Control	4 (21)	-	-	-	-
Control -inoc	22 (23)	-	-	-	-
Rovral	0 (30)	0 (0)	0 (10)	0 (30)	3 (31)
Scala	0 (30)	0 (30)	0 (10)	0 (20)	9 (22)
Switch	0 (20)	0 (10)	100 (5)	0 (30)	9 (11)
Teldor	0 (10)	0 (20)	0 (30)	0 (20)	0 (10)
Flint	-	-	-	0 (10)	0 (10)

* Fungicide applied 3 days after inoculation.

Due to poor quality of bunches at harvest only those that were pick able were collected – hence number of bunches collected at harvest varied from 0 to 31. So the number in the () in the table show how many bunches were harvested form each treatment.

Botrytis bunch rot Field Trial 3.

Table 25. Fungicides, application timing, and rates used on Pinot Noir vines at flowering, Lenswood 2000.

Treatment	Rate / Litre	Time of fungicide application
Untreated	-	-
Inoculation only	-	-
Flint	0.25 g	7 day before
Amistar	0.5 g	7 day before
Rovral	1 ml	7 day before
Scala	2 ml	7 day before
Flint	0.25 g	4 days before
Amistar	0.5 g	4 days before
Rovral	1 ml	4 days before
Scala	2 ml	4 days before
Flint	0.25 g	4 days after
Amistar	0.5 g	4 days after
Rovral	1 ml	4 days after
Scala	2 ml	4 days after

Table 26. Effect of fungicides applied to Pinot Noir bunches before or after inoculation at flowering on the incidence of Botrytis bunch rot.

Treatment	Time of fungicide application	Non surface sterilise			Surface sterilise		
Untreated	7 days before	9.2	b[1]	d[2]	0		g
Inoculation only	7 days before	99.4	a	a	43.4		abcd
Rovral	7 days before	96.7	a	a	32.1		cdef
Scala	7 days before	92	a	ab	24.7		defg
Flint	7 days before	96.9	a	a	24.4		defg
Amistar	7 days before	97	a	a	47.7		abcd
LSD (*P*=0.05)		7.5			ns		
Untreated	4 days before	0	b	d	0	c	g
Inoculation only	4 days before	97.5	a	a	65	a	a
Rovral	4 days before	93.3	a	ab	53.6	ab	abc
Scala	4 days before	76.7	a	abc	40.2	b	abcd
Flint	4 days before	70	a	bc	35.2	b	bcde
Amistar	4 days before	76.7	a	abc	61.5	a	ab
LSD (*P*=0.05)		36.7			21.7		
Untreated	4 days after	2.5	b	d	0	c	g
Inoculation only	4 days after	86.7	a	abc	65.3	a	a
Rovral	4 days after	19.2	b	d	2.5	b	g
Scala	4 days after	87.5	a	abc	5	b	fg
Flint	4 days after	17.5	b	d	1.5	b	g
Amistar	4 days after	65	a	c	9.7	b	efg
LSD (*P*=0.05)		34			12.7		
LSD – all treatments (*P*=0.05)				26			28.2

Treatments with the same letter are not significantly different from one another

[1] Analysis of fungicide timing.

[2] Analysis of all fungicides and timing.

Appendix 6: Budget reconciliation