GRAPE AND WINE RESEARCH AND DEVELOPMENT CORPORATION

Strategies to Reduce the Incidence of Cane and Leaf Blight Disease of Grapevines

Project DAN 94/1

Final Report

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1. Executive Summary

Cane and leaf blight disease of grapevines caused by *Phomopsis viticola* (Sacc.) Sacc. is a serious disease in several viticultural regions of the world. The disease is economically damaging in most of the grapevine growing areas in New South Wales, Victoria, South Australia, Tasmania and Western Australia. The first report of the disease in Australia was in 1911 in Mildura, Victoria, followed by a report of the disease in New South Wales in 1935. In recent years severe symptoms of the disease were found in a number of vineyards in New South Wales, Victoria and South Australia.

The previous Grape and Wine Research and Development Corporation project (DAN 8GW) on cane leaf blight disease of grapevines has shown for the first time that there are at least two distinct Types of *Phomopsis* on grapevines in Australia (Type 1 and Type 2). The separation of these types has been based on the size of alpha spores and *in vitro* growth characteristics, including pycnidial appearance, cirrhus colour and rate of mycelial growth and sporulation. The host response, pectic enzyme profiles and optimum temperatures for germination support the separation of these Types.

Type 1 occurs mainly in coastal or southern vineyards of South Australia and Victoria as well as in Western Australia and Tasmania, whereas Type 2 is mainly found in New South Wales and inland vineyards of Victoria as well as the areas surrounding Adelaide, South Australia. However, there are some areas of overlap in the Hunter Valley, New South Wales, Victoria and in South Australia.

The study carried out in the last GWRDC project (DAN 8GW) showed the need for further studies on the behaviour of Type 1 and Type 2 *in vitro* and *in vivo* in relation to their symptomatology, epidemiology and pathogenicity. The current

GWRDC project (DAN 94/1) has investigated these aspects of the cane and leaf blight disease and has developed a practical management strategy for the disease.

The main objectives of the project were to develop a practical management strategy for the cane and leaf blight disease of grapevines and increase the grower awareness of the importance of the disease. This was achieved by : defining the two Taxa or Types of *Phomopsis* (Type 1 and Type 2); describing disease symptoms in relation to these Types; testing their pathogenicity ; measuring yield losses; and, testing the effects of different fungicides and their timing of application on disease development.

Two different types of vineyard trials were carried out : (1) Yield trials, to test the effectiveness of an early bud burst spray (30 - 50% bud burst) followed by four further applications of mancozeb (dithane) and/or delan (dithianon) on disease incidence; and, (2) Dormant spray trials, to study the effect of reducing carry over of inoculum into the new season by applying relatively safe chemicals such as shirlan (fluazinam), mancozeb (dithane) and delan (dithianon). Several fungicides were screened in the laboratory for their ability to inhibit the germination of pycnidia (the resting structures of *Phomopsis*) on one-year-old canes prior to selecting fluazinam, dithane and dithianon for vineyards trials.

The yield trials showed, in general, that mancozeb (dithane) and delan (dithianon) were effective in reducing the incidence of *Phomopsis* symptoms when an application at 30 - 50% bud burst followed by further applications 10 days and 3,5 and 7 weeks after bud burst. Earlier work (GWRDC project DAN 8GW) also showed that two applications of delan (dithianon) during spring when there was high risk of infection (ie. at first leaf and at two-to-three leaf stages) effectively reduced the disease from 80% in the untreated grapevines to 25% in the treated ones.

Treatment of dormant canes carrying *Phomopsis* infection *in vitro* with benlate (benomyl), shirlan (fluazinam), mancozeb (dithane) and chinosol (8-hydroxyquinoline sulphate) inhibited the viability of pycnidia *in situ*. There is potential, therefore, to reduce the onset of cane and leaf blight disease at the beginning of each growth season by using chemicals that are less toxic than sodium arsenite. A winter treatment of dormant canes with chemicals such as shirlan (fluazinam) or mancozeb (dithane) should lower the carry over of the disease into the new season and reduce pressure on spray program applied in spring. The need to improve the efficiency of application of dormant spray was indicated in this work.

The current practice of preparing grapevines for propagation involves soaking cuttings in chinosol (8-hydroxyquinoline sulphate). Our study has shown that shirlan (fluazinam), benlate (benomyl) and mancozeb (dithane) inhibit the viability of pycnidia. Since *Phomopsis* can be readily transmitted as pycnidia, the dipping of plant materials in systemic fungicides such as benlate (benomyl) or protectant fungicides such as shirlan (fluazinam) and mancozeb (dithane) appears to be an efficient and safe method for reducing the spread of the pathogen.

The data on incidence of disease following dormant treatment showed, in general, that this approach to the management of *Phomopsis* was valid and could reduce the carry over of inoculum and, therefore, the occurrence of the disease. Further vineyard trials are required to develop a protocol for dormant spray technology.

This project has shed more light into the nature and distribution of Type 1 and Type 2 of *Phomopsis* occurring in Australia. For instance, it is now known that Types 1 and 2 can now occur together in some vineyards. This may explain why the symptoms of bleaching and scabbing occur together under certain conditions.

Although bleaching, a symptom traditionally associated with *Phomopsis* infection, occurred at relatively high levels in two vineyard trials in the Yarra Valley, Victoria, the pathogen occurred in only 14% of the bleached spurs.

The vineyards known to have *Phomopsis* Type 1 did not exhibit abnormal bud burst and there were no significant differences in bud burst between fungicide treated and untreated vines.

Tests on the pathogenicity of Type 1 and Type 2 carried out by artificially inoculating grapevines grown under glass house conditions showed that while Type 2 was a virulent pathogen, Type 1 did not appear to cause infection.

Microscopic examination of longitudinal and cross sections of the shoots infected with *Phomopsis* showed that the pathogen was present below the epidermal tissue. This observation will have important bearing not only on the selection of chemicals for disease management but also for eliminating the pathogen from propagating materials.

2. Background

Cane and leaf blight disease of grapevines caused by *Phomopsis viticola* (Sacc.) Sacc. is a serious disease in several viticultural regions of the world (Reddick 1909; Hiura 1924; du Plessis 1938; Taylor and Mabbitt 1961; Lal and Arya 1982; Bugaret 1984). The disease is economically damaging in most of the grapevine growing areas in New South Wales, Victoria, South Australia, Tasmania and Western Australia (Merrin *et al.* 1995a). Severe symptoms of the disease were found in a number of vineyards in New South Wales and Victoria. The disease has also been identified as a major problem in eastern Europe (Machowicz-Stefaniak *et al.* 1991).

Symptoms of the disease include dark-coloured lesions on canes, leaves, petioles, rachae, bunches and tendrils, and bleaching of canes (Pine 1959, Nair *et al.* 1995). Infection can lead to stunting of the grapevine, reduced bud burst and infection of mature berries (Bugaret 1990). Reduced bunch setting and subsequent losses in yield of 20-38% may also occur (Lal and Arya 1982; Pseicht and Pearson 1989). Success in striking cuttings and grafting can be reduced significantly (Simon *et al.* 1969).

The first report of the disease in Australia was by Brittlebank (1911) in Mildura, Victoria; and, it was later reported in New South Wales by Noble *et al.* (1935). However, it was only in the 1990's that work was carried out to determine the extent and nature of the disease in Australia (Nair *et al.* 1995). Moller and Kasimatis (1978) showed that the disease previously known as 'dead arm' was caused by two different fungi *Eutypa lata* (Pers. :Fr.) Tul. & C. Tul. (now known to cause the symptoms of 'dead arm') and *P. viticola* (now known to cause 'cane and leaf blight'). Previously, it was not known that *E. lata* was pathogenic on grapevine, and the two fungi may have been confused in some of the earlier descriptions of etiology and epidemiology.

It is not known whether the teliomorph of *P. viticola* (*Diaporthe*) plays any part in the development of the disease. The sexual stage has been found recently in Australian vineyards in association with *Phomopsis* Type 1 (Scheper *et.al.* 1997).

P. viticola produces pycnidia which mature during winter under the epidermal layer of the canes. At maturity, from late winter to late summer, when the weather is wet, the pycnidium releases conidia (spores) formed on conidiophores which line the pycnidial chamber (Cucuzza and Sall 1982). Two kinds of spores can be present : the alpha spores, which cause primary infection of green, susceptible tissue, and the beta spores, whose function is not known. The proportion of beta to alpha conidia can vary from 1-25% on canes and *in vitro*, increasing with the carbon:nitrogen ratio of the medium, temperatures above

30^0C, the CO_2 content of the atmosphere and the presence of *Bacillus subtilis* (Pine 1958; Pezet 1974).

The identification of isolates of *Phomopsis* to one of the 800 species or sub-species is difficult. It is based largely on morphological and cultural characteristics such as type of host, spore dimensions and growth *in vitro* (Wechtl 1990).

The previous Grape and Wine Research and Development Corporation project (DAN 8GW) on cane leaf blight disease of grapevines showed that the disease is widespread in Australia, with isolates of *Phomopsis* being found in all the major viticultural regions. It was shown for the first time that there were at least two distinct types of *Phomopsis* on grapevines in Australia (Type 1 and Type 2). The separation of these types had been based on the size of alpha spores and *in vitro* growth characteristics, including pycnidial appearance, cirrhus colour and rate of mycelial growth and sporulation. The host response, pectic enzyme profiles and optimum temperatures for germination supported the separation of these types.

Type 1 occurred mainly in coastal or southern vineyards of South Australia and Victoria as well as in Western Australia and Tasmania, whereas Type 2 was mainly found in New South Wales and inland vineyards of Victoria as well as the areas surrounding Adelaide, South Australia. However, there were some areas of overlap in the Hunter Valley, New South Wales and in South Australia.

The optimum temperature for mycelial growth and pycnidial formation *in vitro* for Type 1 was approximately 5^0C lower than that of Type 2. However, the data obtained so far indicate that the distribution of *Phomopsis* appears to be more closely related to grape cultivar than to a climatic pattern. This suggests that the presence of a particular taxa or type may be based on the type of vine planting material, rather on environmental factors, although there is a close relationship

between cultivar grown and climate. More work to compare detailed climatic data with the presence of each Type may clarify the situation.

The study carried out in the last GWRDC project (DAN 8GW) showed the need for further studies on the behaviour of Type 1 and Type 2 *in vitro* and *in vivo* in relation to their symptomatology, epidemiology and pathogenicity. The current GWRDC project (DAN 94/1) investigated these aspects of the cane and leaf blight disease and has developed a practical management strategy for the disease. The results obtained in these investigations are presented in this report.

3. Objectives

The main objectives of the project were to develop a practical management strategy for the cane and leaf blight disease of grapevines and increase grower awareness of the importance of the disease. This was achieved by :

- defining the two Taxa or Types of *Phomopsis* (Type 1 and Type 2);
- describing disease symptoms in relation to the *Phomopsis* Type;
- testing the pathogenicity of each Type;
- measuring yield losses caused by the Types; and,
- testing different timing of application of fungicides and its effect on disease development

4. Methods

Selected vineyards in New South Wales, Victoria and South Australia were used for the trials. The spore Types of *Phomopsis* at the different vineyard trial sites are given in Table A. Although there were minor changes to the trial sites during the project, 11 sites were generally used for the experimental trials as follows:

State	Site	Type of trial
New South Wales	Griffith	Dormant spray &
	Yenda and Koraleigh	Yield trials
		Dormant spray trials
Victoria	Vinifera and Robinvale	Yield trials
	Lancefield	Dormant spray & yield
	Yarra Valley	trials
		Dormant spray & yield
		trials
South Australia	Padthaway	Dormant spray & yield
	Summertown	trials
	Lenswood	Dormant spray & yield
	Hillstowe	trials
		Dormant spray & yield
		trials
		Yield trial

Yield trial

Yield trials were designed to study the effect of natural infection of grapevines by *Phomopsis* on the yield and vigour of grapevines. Various viticultural assessments (bud burst, cane bleaching, yield per vine, weight of prunings etc.), phenological details (shoot length, leaves per shoot etc.) and incidence of the pathogen (infection of internodes, leaves etc.) were made at different times during the season such as 0-4 weeks before budburst, 30-50% bud burst, 50% bud burst + 3 weeks, 50% bud burst + 5 weeks, 50% bud burst + 7 weeks, 50% bud burst + 10-11 weeks and 1 week before harvest. Chemical treatments involved application of delan (dithianon) or mancozeb (dithane) to treatment blocks at specified times (at 50% bud burst with one leaf showing and four further applications at fortnightly intervals).

Details of the protocol used in the yield trials are presented in Appendix 1 of this report.

Dormant spray trial

The effect of a dormant spray on the incidence of cane and leaf blight disease was studied by *in vitro* experiments in the laboratory and vineyard trials at selected sites in New South Wales, Victoria and South Australia.

In vitro studies :

Isolates of two different Types of *P. viticola* were used; an isolate of Type 1 from the cultivar Touriga at Whorouli South, Victoria, and an isolate of Type 2 from the cultivar Nyora at Griffith, New South Wales.

One-year-old canes of grapevines (cultivar Shiraz) infected with Type 2 collected from a vineyard at Yenda, New South Wales, were used in experiments on pycnidia *in situ*.

Production of pycnidia *in vitro* : A mycelial disk (4 mm) from 3-day-old cultures of *P. viticola* was placed in Petri dishes containing ¼ strength potato dextrose agar (PDA) amended with pieces (approximately 1 cm) of sterilised carnation leaves. The dishes were incubated for 7 days at 20^0 C and then for 7 days at 15^0 C to obtain pycnidia on the pieces of carnation leaves.

Assessment of viability of pycnidia : The viability of pycnidia was assessed in terms of mycelial growth (myceliogenic germination) and sporulation (sporogenic germination) with a binocular microscope Zeiss (Stemi V8) at 640x magnification.

Chemical treatment of pycnidia *in vitro* : Five pieces (approximately 1cm long) of carnation leaf carrying the pycnidia (CLP) were immersed in different chemicals at various concentrations. The chemicals used were 8-

hydroxyquinoline sulphate (Chinosol W 67.5%WP, AgrEvo), fluazinam (Shirlan 500 g/L SC, Crop Care Australasia Pty. Ltd.), fluazinam + mineral oil(Lovis 994 ml/L, Caltex), benomyl (Benlate 500 g/L WP, Du Pont), mancozeb (Dithane M45 800 g/kg, Crop Care Australasia Pty. Ltd.), iprodione (Rovral 250 g/L SC, Rhone Puolenc), dithianon (Delan 750 g/L, Shell), chlorothalonil (Bravo 500 g/L SC, Crop Care Australasia Pty. Ltd.) and cupric hydroxide (Kocide 500 g/kg SC, Shell) at 10, 50, 100, 200 and 500 ppm of active ingredient. These chemicals were also tested at the concentrations that were recommended by the manufacturers (fluazinam 500 ppm, dithane 500 ppm, iprodione 500 ppm, dithianon 750 ppm, cupric hydroxide 1250 ppm, chlorothalonil 1500 ppm and mineral oil 20 ppm). The CLP were immersed in the chemicals for 24 hours at 25^0 C in a constant temperature shaker at 200 rpm, with a series containing CLP immersed in sterile water as controls. The CLP were drained and transferred onto PDA in Petri dishes and incubated at 20^0 C. Sporogenic germination of the pycnidia was recorded after 72 hours.

Chemical treatment of infected canes of grapevine : One-year-old canes infected with Type 2 were cut into pieces 3 cm long, each piece containing several pycnidia. These pieces were immersed in sterile water for 1 min followed by further immersion in the chemicals (benomyl 500 ppm, fluazinam 500 ppm, fluazinam 500 ppm + mineral oil 20 ppm, 8-hydroxyquinoline sulphate 1500 ppm and dithane 500 ppm) for 3 minutes. Fifteen canes per treatment were incubated on moist blotting paper in plastic trays for 7 days at room temperature (18-25^0 C). Viability of the pycnidia was recorded after 7 days by examination under a binocular microscope.

Treatment of infected canes with a mixture of chemicals : One-year-old canes infected with Type 2 were soaked for 5 minutes in fluazinam (500 ppm) to which was added an adjuvant (Nu Film-17, 1 ppm, Amcor Australia). Treatments with fluazinam (500 ppm) and Nu Film-17 (1 ppm) separately served as controls. Canes immersed in sterile water served as a second series of controls. Two

pieces of cane, 5 cm long, were used per treatment. The treated canes were then incubated in trays as before. The number of viable pycnidia on the canes was recorded after 7 days.

Different methods of treating infected canes : One-year-old canes infected with Type 2 were cut into pieces 5 cm long and treated with fluazinam (500 ppm) plus mineral oil (20 ppm) or with 8-hydroxyquinoline sulphate (1500 ppm). Three different methods of treatments were used: (1) canes were soaked in the chemicals twice, with the second soaking 7 days after the first; (2) the canes were initially sprayed to run-off with sterile water followed by a second spray to run-off with the chemicals; and, (3) the canes were sprayed to run-off with the chemicals without any prior spraying with sterile water. Fifteen pieces of cane were used per treatment. Incubation and assessment were as before.

Vineyard trials :

Dormant spray trials were conducted in the selected vineyards listed above. Four treatments were carried out :

1. Untreated control
2. Dormant spray
3. Dormant spray + in season spray
4. In-season spray

Shirlan (fluazinam) and mancozeb (dithane) were used separately in consecutive years for the dormant treatments. Details of the protocol used in the dormant spray trials are given in Appendix 1 of this report. Cover sprays applied over the *Phomopsis* trial site during the period of the trials are also indicated in Appendix 1.

5. Results and Discussion

Yield trials

The Types of *Phomopsis* occurring in the trial sites are given in Table A. The main aim of yield trials was to test the effectiveness of an early bud burst spray (30 - 50% bud burst) followed by four further applications of mancozeb (dithane) and/or delan (dithianon). The application of dithianon in trials during1995/96 and 1996/97 at Vinifera, Victoria, reduced the incidence of disease significantly when assessed at ten and twelve weeks after bud burst respectively (Fig. 1). The disease incidence in the untreated controls were 64% and 87% whereas it was 2% and 3% in the dithianon treatments in 1995/96 and 1996/97 respectively. In a similar trial at Robinvale, Victoria, during 1995/96, disease incidence on internodes at bud burst + 10 weeks in the dithianon treatment was significantly less (0.7%) than that in the untreated control (12.6%) (Fig. 2). When the trial was repeated at Vinifera during the following season (1996/97), the grapevines treated with dithianon again showed significantly less disease (3%) than the untreated grapevines (86.8%) (Fig. 3). This was also the case with the disease incidence on leaves of the grapevines (Fig. 4). In the same trial, assessments of dormant canes showed that there were more bleached internodes (16.4%) in the untreated vines than in the treated ones (7.9%) (Fig. 5). There was also a significant difference in scarring of internodes between the treated grapevines (4.6%) and the untreated ones (22.2%) in the trial at Vinifera (Fig. 6). The significant reduction in bleaching and scarring of canes in the 1996/97 season could have been due to the dithianon treatment in the previous season (1995/96). Progressive assessments of disease incidence at dormancy, bud burst + 8 weeks and bud burst + 12 weeks showed that the treated grapevines had less disease incidence than the untreated control vines. The application of mancozeb (dithane) as in-season sprays applied at the same times as the dithianon sprays also resulted in effective management of the disease. In Vinifera trial in 1995/96, there was an average of 4.6 kg per vine yield difference

between untreated and treated vines; and a difference of 4.8 kg in the trial in 1996/97.

The yield trials showed, in general, that mancozeb (dithane) and delan (dithianon) substantially reduced the incidence of *Phomopsis* symptoms when an application at 30 - 50% bud burst was followed by further applications at 10 days and 3,5 and 7 weeks after bud burst. Earlier work (GWRDC project DAN 8GW) also showed that two applications of delan (dithianon) during spring when there was a high-risk of infection (ie. at first leaf and at two-to-three leaf stages) effectively reduced the disease from 80% in the untreated grapevines to 25% in the treated ones.

Dormant spray trials

The objective of fungicide application during dormancy in winter was to reduce inoculm potential and therefore carry over of the disease into the new season. Several fungicides were screened in the laboratory for their effectiveness to inhibit the germination of pycnidia (resting structures of *Phomopsis*) on one-year-old canes prior to selecting shirlan (fluazinam), mancozeb (dithane) and delan (dithianon) for vineyard trials.

Laboratory experiments :

Chemical treatment of pycnidia produced *in vitro* : Benomyl completely inhibited the viability of pycnidia of Types (Taxon) 1 and 2 (Tables 1 and 2). Fluazinam + mineral oil and fluazinam alone were also effective. This was also reflected in the LD_{50} values. Iprodione and dithianon were not as effective on Type 1 as on Type 2. In general, 8-hydroxyquinoline sulphate was more effective on Type 2 than on Type 1. Commercial concentrations of fluazinam (500 ppm) with and without mineral oil (20 ppm), 8-hydroxyquinoline sulphate (1500 ppm), benomyl (500 ppm) and dithane (500 ppm) reduced the viability of pycnidia by 96 - 100% (Table 3). Dithianon (750 ppm) inhibited pycnidial germination of Type 2 by 90%

but reduced the viability of Type 1 by only 52%. In addition, dithane reduced pycnidial viability of Types 1 and 2 at higher levels than dithianon (Tables 1 and 2).

Chemical treatment of infected grapevine canes : 8-hydroxyquinoline sulphate reduced the number of pycndia showing myceliogenic and sporogenic germination on canes *in situ* significantly more than the other chemicals and the untreated control (Table 4). Fluazinam + mineral oil and benomyl were less effective than 8-hydroxyquinoline sulphate.

Treatment of infected canes carrying viable pycnidia with mixtures of chemicals and adjuvant : Fluazinam treatments reduced the number of viable pycnidia (Table 5). Addition of adjuvant did not significantly affect the viability of pycnidia.

Different methods of treating infected canes : All methods of chemical treatments reduced the viability of pycnidia to the same extent (Table 6).

Treatment of dormant canes carrying *Phomopsis* infection with benomyl, fluazinam, dithane and 8-hydroxyquinoline sulphate inhibited the viability of pycnidia *in situ*. There is potential, therefore, to reduce the onset of cane and leaf blight disease at the beginning of each growth season by using chemicals that are less toxic than sodium arsenite. Inhibition of pycnidial viability will reduce inoculum carry over and consequently lower the disease risk. Pscheidt and Pearson (1989) found that the higher the amount of infected canes bearing pycnidia in the canopy at the beginning of the growing season, the higher the incidence of disease.

Although sprays of dormant canes with eradicant fungicides such as sodium arsenite (Malathrakis 1989; Merrin *et al.* 1995b), dinitrobutylphenol and dinitro-o-cresol (Malathrakis 1989) and phenyl mercuric chloride (Taylor and Mabbitt 1961) reduce disease, the use of these chemicals is now undesirable because of

their toxicity. Sodium arsenite has been removed from markets in Australia because of human toxicity. This project has shown that relatively safer chemicals such as shirlan (fluazinam), mancozeb (dithane) and benlate (benomyl) reduce the viability of pycnidia and potentially can be used to reduce the carry over of inoculum in vineyards. Use of benomyl should be restricted in some viticultural regions as this chemical has the capacity to develop resistance in certain pathogens such as *Botrytis cinerea*. Mancozeb (dithane) and delan (dithianon) are currently being used by grape growers to manage cane and leaf blight disease during the spring and summer growth periods of vines. Therefore, a winter treatment of dormant canes with dithane or fluazinam should significantly lower the carry over of the disease into each season and reduce pressure on spray programs applied in spring. This project has shown that these chemicals inhibit viability of pycnidia on canes *in situ* by 96 - 100%. The use of an adjuvant or a sticking agent such as mineral oil may enhance the effect of fluazinam. The addition of Nu Film-17 did not enhance the effect of fluazinam in our laboratory tests, although Ramsdell (1995) found increased efficacy of fluazinam in the presence of Nu Film-17 in field trials to control *Phomopsis* fruit rot. Our laboratory studies indicate that spraying is an efficient method of application of chemicals to inhibit the pycnidia.

The current practice of preparing grapevine material for propagation involves soaking cuttings in 8-hydroxyquinoline sulphate. Since this study has shown that shirlan (fluazinam), benlate (benomyl) and mancozeb (dithane) are effective in inhibiting the viability of pycnidia, dipping the cuttings in these chemicals appears to be an efficient and safer method to reduce the spread of the pathogen (Castillo-Pando *et al.* 1997).

Vineyard trials :

The trial carried out at Griffith and Yenda, New South Wales, during the 1995/96 season using fluazinam as the dormant spray and dithianon as the in-season

spray did not show any significant differences in grape bunch number and weight between the treatments (Tables 7 and 8). This may have been due to the absence of detectable disease at the two sites in Griffith during the season.

In a similar trial carried out at Robinvale, Victoria, during 1995/96, no significant differences were observed in disease incidence between dormant (fluazinam), dormant + in-season (dithianon) and in-season treatments (Fig. 7). However, there was a significant difference in disease incidence between the treated and the untreated vines.

Two dormant treatments using fluazinam in one and dithianon in the other carried out at Padthaway, South Australia, during 1995/96 showed that dormant application of fluazinam was significantly more effective in reducing the disease incidence than spring applications of dithianon (Table 9). Similar results were obtained when this treatment block was assessed thirty-nine days after the first assessment (Table 10).

In another trial at Padthaway (1995/96) using mancozeb (dithane) as the dormant spray and delan (dithianon) as the in-season spray, incidence of the disease was significantly lower in the grapevines that received the dormant spray than in those that received the in-season spray as well as those that received no spray (Figs. 8 and 9). However, similar treatments carried out with mancozeb (dithane) as the dormant spray and delan (dithianon) as the in-season spray at Griffith, significant differences in disease incidence was not observed between the control and dormant treatments and between the dormant + in-season and in-season treatments (Figs. 10,11,12 and 13). The reasons for these results are not clear.

Dormant application of dithane followed by in-season sprays of dithianon reduced the disease incidence to 67% from a level of 100% in the untreated grapevines at Koraleigh, New South Wales (Fig. 14) in incubated green shoots at

harvest; however, mancozeb (dithane) was not considered to be effective when used only as a dormant spray (Fig. 14a). When mancozeb (dithane) was used as a dormant spray followed by in-season spray of delan (dithianon), there was a significant reduction in disease (Fig. 14b).

The data on incidence of disease following dormant treatment showed, in general, that this approach to manage *Phomopsis* was valid and could reduce the carry over of inoculum and, therefore, the occurrence of the disease. However, further testing of fungicides mixed with different adjuvants, spreaders and oils is required for increasing the efficiency of dormant treatments for the management of *Phomopsis.*

Viticultural aspects and disease incidence

Data on the numbers of canes/vine, spurs/vine, nodes/vine, buds/vine, bud burst/vine, weight of canes/vine, shoot length and leaves/cane were not significantly different between the different treatments (dormant spray, in-season spray and untreated). These results are presented in Appendix 2.

In trials carried out in two vineyards in the Yarra Valley. Victoria, where Type 1 of *Phomopsis* were present, we did not observe any significant differences in bud burst between the untreated and fungicide treated vines (Figs. 15,16). This is in contrast to the observation made by Scheper *et.al.* (1997) who reported that Type 1 caused failure of bud burst.

Definition and distribution of the Types of *Phomopsis*

Earlier work carried out in GWRDC project DAN 8GW showed that the two Types of *Phomopsis* were geographically separated (Merrin, Nair and Tarran 1995). Type 1 occurred predominantly in coastal or southern vineyards of South

Australia and Victoria as well as in Western Australia and Tasmania whereas Type 2 was found mainly in New South Wales and inland vineyards of Victoria as well as the areas surrounding Adelaide, South Australia. However, there were some areas of overlap in Victoria, the Hunter Valley, New South Wales and in South Australia.

This project has shed more light into the nature and distribution of Type 1 and Type 2 of *Phomopsis* occurring in Australia. For instance, Types 1 and 2 have now been found to occur in the same vineyard. This may explain why the symptoms of bleaching and scabbing occur together under certain conditions. Cane bleaching is normally associated with Type 1 and scabbing with Type 2. The symptoms associated with the two Types of *Phomopsis* are given in Table 11. This will enable the growers to initially identify the Types of *Phomopsis*. This initial identification should be confirmed by further incubating the sample and examining the types of spores produced from the pycnidia.

Although bleaching, which is thought to be a symptom associated with *Phomopsis* infection, occurred in relatively high numbers in two vineyard trials at Yarra Valley, Victoria, the pathogen was found to occur in only 14% of the bleached spurs.

Tests on the pathogenicity of Type 1 and Type 2 conducted on grapevines grown under glass house conditions showed that Type 2 was a virulent pathogen. Artificial inoculations of the grapevines with spore suspensions of Type 2 resulted in necrotic lesions on green shoot. However, Type 1 failed to produce any symptoms on shoots after such inoculations.

The microscopic examination of longitudinal and cross sections of the shoots infected with *Phomopsis* showed that the pathogen was present below the epidermal tissue. The pathogen was also seen to be able to spread internally from lower internodes to the upper internodes. These observation will have

important bearing not only on the selection of chemicals for disease management but also for eliminating the pathogen from propagating material.

6. Recommendations

- A management strategy for the cane and leaf blight disease caused by *Phomopsis* should involve an initial fungicide application at 30-50 % budburst (or at first leaf stage after bud burst). This should be followed by further applications at 10 days and 3 and 5 weeks after bud burst. When there is a severe outbreak of the disease, an additional application 7 weeks after bud burst may be made.
- Currently mancozeb (dithane) and delan (dithianon) are commonly used by grape growers depending on appropriate regulations on chemical use in the different states. On the basis of data obtained in this project, attempts should be made to register fluazinam for use in managing *Phomopsis* on grapevines.
- The results obtained in this project have clearly shown the potential for increased efficiency in the management of the disease by the application of a dormant spray. Further vineyard trials are required to develop this approach of reducing the carry over of inoculum and thereby the disease.
- The demonstration of the presence of *Phomopsis* underneath the epidermis of the canes points to the need for eliminating the pathogen efficiently from propagating material.

7. Technology transfer

Oral presentations :

Progress achieved during the course of the project was presented orally by members of the project team to different regional organisations of grape growers at workshops and seminars in New South Wales, Victoria, South Australia and Western Australia. Additionally, lectures were given as part of GWRDC project on IPM in Viticulture - Research to Practice Training Workshops in different states in Australia.

Publications :

Scientific and extension papers were published in refereed scientific and industry publications. These are cited under the section "Literature Cited" in this report.

AusVit :

This project has contributed data required to build the *Phomopsis* module in AusVit

8. Literature cited

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9. Acknowledgments

We are grateful to the Grape and Wine Research and Development Corporation for financial assistance to carry out the project. We thank the grape growers who provided us with the vineyard trial sites and for their assistance during the course of the trials.

Table A - Spore Types of *Phomopsis* at the different vineyard trial sites used in the project.

Trial sites		*Phomopsis* spore types
New South Wales	Yenda	2
	Griffith	2
	Koraleigh	1 and 2
Victoria	Robinvale	2
	Vinifera	1 and 2
	Lancefield	2
	Yarra Valley	1
South Australia	Padthaway	1 and 2
	Lenswood	1
	Summertown	1 and 2
	Hillstowe	1

Table 1 - Effect of fungicides on the viability of pycnidia of *Phomopsis viticola* (Type 1) *in vitro* on carnation leaves

Effect of fungicides on the viability of pycnidia of *Phomopsis viticola* (Taxon 1) *in vitro* on carnation leaves

Chemical	Viability of pycnidia (%) Chemical concentration (ppm) 10	50	100	200	500	LD_{50} ^
Benomyl	0	0	0	0	0	–
Fluazinam+ mineral oil	5	10	0	0	0	0.1
Fluazinam	32	33	28	16	0	5.0
8–hydroxyquinoline sulphate	84	52	57	16	8	62.0
Mancozeb	98	74	19	16	4	70.0
Dithianon	100	100	100	95	55	>500
Cupric hydroxide	95	94	100	86	17	>500
Iprodione	100	100	75	83	81	>1000
Chlorothalonil	83	90	90	79	76	>1000

^Concentration at which pycnidial viability was inhibited by 50% of that of the control (probit analysis).

Table 2 - Effect of fungicides on the viability of pycnidia of *Phomopsis viticola* (Type 2) *in vitro* on carnation leaves

Effect of fungicides on the viability of pycnidia of *Phomopsis viticola* (Taxon 2) *in vitro* on carnation leaves

Chemical	Viability of pycnidia (%) Chemical concentration (ppm) 10	50	100	200	500	LD_{50} [A]
Benomyl	0	0	0	0	0	–
Fluazinam	32	30	5	4	0	0.8
Fluazinam + mineral oil	85	87	88	100	100	6.0
8–hydroxyquinoline sulphate	53	8	8	5	7	9.0
Mancozeb	93	67	7	0	2	47.0
Dithianon	100	44	56	23	24	93.0
Cupric hydroxide	100	60	28	36	68	>100
Iprodione	100	83	75	58	30	>200
Chlorothalonil	79	67	52	71	50	>600

[A]Concentration at which viability was inhibited by 50% of that of the control (probit analysis).

Table 3 - Effect of chemicals at commercial concentrations on the viability of pycnidia of *Phomopsis viticola* (Types 1 and 2) *in vitro* on carnation leaves

Effect of chemicals at commercial concentrations on viability of pycnidia of *Phomopsis viticola* (Taxa 1 and 2) *in vitro* on carnation leaves

Chemical	Concentration (ppm)	Viability of pycnidia (%)	
		Taxon 1	Taxon 2
Fluazinam + mineral oil	500+20	0	0
Fluazinam	500	0	0
Benomyl	500	0	0
8–hydroxyquinoline sulphate	1500	4	4
Mancozeb	500	4	2
Dithianon	750	48	10
Cupric hydroxide	1250	19	72
Chlorothalonil	1500	30	50
Iprodione	1500	81	30

Table 4 - Effect of chemical treatment of one-year-old canes of grapevines infected with *Phomopsis viticola* (Type 2) on the viability of pycnidia *in situ*

Effect of chemical treatment of one–year–old canes of grapevines infected with *Phomopsis viticola* (Taxon 2) on the viability of pycnidia *in situ*

Chemical	Concentration[A] (ppm)	Viability of pycnidia (%)	Number of viable pycnidia[B]
8–hydroxyquinoline sulphate	1500	0.1	0.8c
Fluazinam + mineral oil	500 + 20	19.1	8.9b
Benomyl	500	40.7	17.7a
Mancozeb	500	64.5	27.4a
Control	–	100.0	41.9d

[A]Commercial rates for general disease management.
[B]Predicted values when control has 41.9 viable pycnidia. Figures followed by the same letter are not significantly different ($P<0.01$).

Table 5 - Effect of addition of adjuvant to fungicide on viability of pycnidia of *Phomopsis viticola* (Type 2) on one-year-old canes of grapevines *in situ*

Effect of addition adjuvant to fungicide on viability of pycnidia of *Phomopsis viticola* (Taxon 2) on one–year–old canes of grapevine *in situ*

Treatment	Number of viable pycnidia^	
	With adjuvant (NU Film–17)	Without adjuvant
Fluazinam	2.9b (14.5)	4.0b (20.0)
Control	19.9a (99.5)	14.8a (74.0)

^Predicted values based on number of viable pycnidia in 20 replicates. Figures followed by different letters are significantly different ($P<0.01$). Figures in parentheses denote percent viable pycnidia.

Table 6 - Effect of different methods of chemical treatment of canes infected with *Phomopsis viticola* (Type 2) on viability of pycnidia *in situ*

Effect of different methods of chemical treatment of canes infected with *Phomopsis viticola* (Taxon 2) on viability of pycnidia *in situ*

Method of treatment of canes	Number of viable pycnidia[A]	
	Fluazinam + mineral oil[B]	8–hydroxyquinoline sulphate[C]
Untreated	37.4 (100)[D]	37.49 (100)
Chemical soaking (×2)	8.4 a (56)	6.9 a (46)
Chemical spray only	5.0 a (33)	4.8 a (32)
Water spray prior to chemical spray	5.2 a (35)	3.9 a (26)

[A]Predicted values when control has 37.4 viable pycnidia.
[B]500 ppm + 20 ppm.
[C]1500 ppm.
[D]Figures in parentheses denote percent viability of pycnidia. Figures followed by the same letter are not significantly different ($P<0.01$).

Table 7 - Effect of spray application on grapevines at Griffith, New South Wales (1995/96)

Treatment	mean Weight (kg)/vine	Bunch No.	Avg weight(g) /bunch
Untreated	14.4	163	11.3
Dormant[a]	14.2	163	11.5
Dormant/In season	15.4	185	12
In season[b]	13.7	170	12.4

[a] Fluazinam

[b] Dithianon

Table 8 - Effect of spray application on grapevines at Yenda, New South Wales (1995/96)

Treatment	mean Weight (kg)/vine	Bunch No.	Avg weight(g) /bunch
Untreated	19.4	190	102
Dormant[a]	17.4	182	96
Dormant/ In season	14.4	189	76
In season[b]	19.1	218	88

[a] Fluazinam

[b] Dithianon

Table 9 - Effect of dormant spray application of fluazinam on incidence of *Phomopsis viticola* on grapevines at Padthaway, South Australia (1995/96)

Disease assessment (canes) made on January 11, 1996

Treatment	% Area diseased		% Diseased	
Control	0.5	a	56.11	a
Fluazinam - dormant application	0.16	b	20	c
Dithianon - in season application	0.44	a	41.11	b
LSD (P=0.05)	0.1175		10.81	

Table 10 - Effect of dormant spray application of fluazinam on incidence of *Phomopsis viticola* on grapevines at Padthaway, South Australia (1995/96)

Disease assessment (canes) made on February 19, 1996 (compare with Table 9)

Treatment	% Area diseased		% Diseased	
Control	0.17	a	23.89	a
Fluazinam - dormant application	0.07	b	11.11	b
Dithianon - in season application	0.21	ab	27.78	a
LSD (P=0.06)	0.1212		10.81	

Table 11 - Comparison of symptoms caused by *Phomopsis* Type 1 and Type 2 on canes and leaves of grapevines and characteristics of spores produced by the two different Types in axenic cultures *in vitro*.

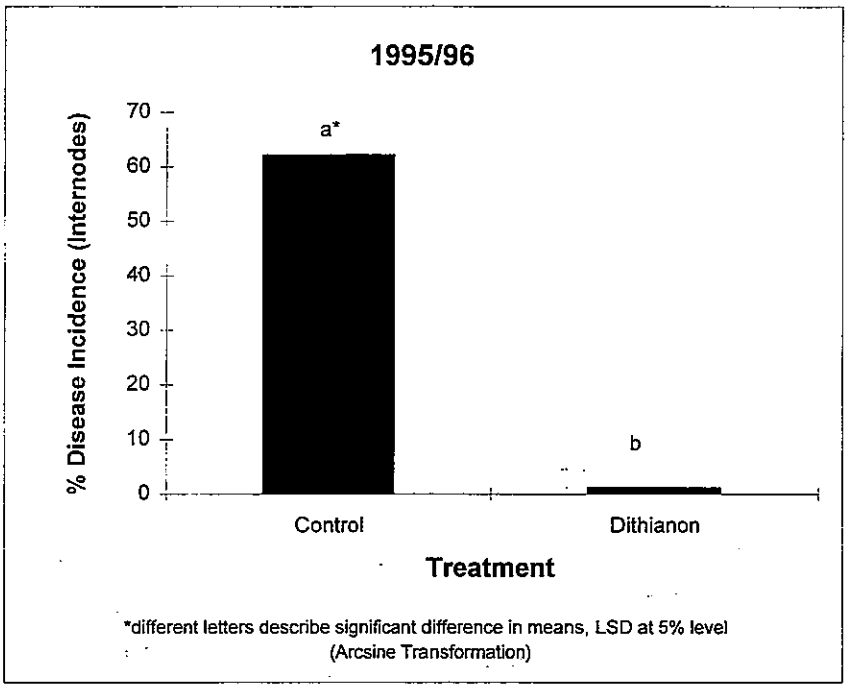
Grapevine tissue	*Phomopsis* Type 1	*Phomopsis* Type 2
Cane		
Scars	-	+
Scabs	-	+
Bleaching	+	+
Breakage	-	+
Leaf		
Brown spots & yellow halo	-	+
In vitro culture		
Cirrhus	White	Yellow
Spores	Small ~ 5.9 x 1.8 µm	Large ~ 9.8 x 2.5 µm

+ = present
- = absent

Fig. 1 - Incidence of *Phomopsis viticola* on grapevines at Vinifera, Victoria (1995/96 and 1996/97)

Budburst + 10 weeks

Treatment	% Disease Incidence on Internodes
Control	64
Dithianon	2



Budburst + 12 weeks

Treatment	% Disease Incidence on Internodes
Control	87
Dithianon	3

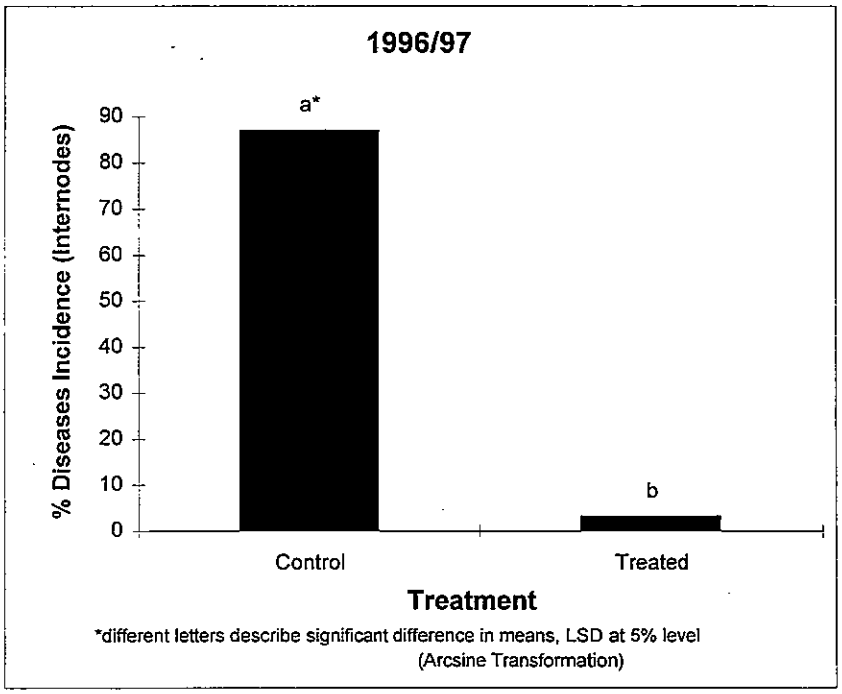
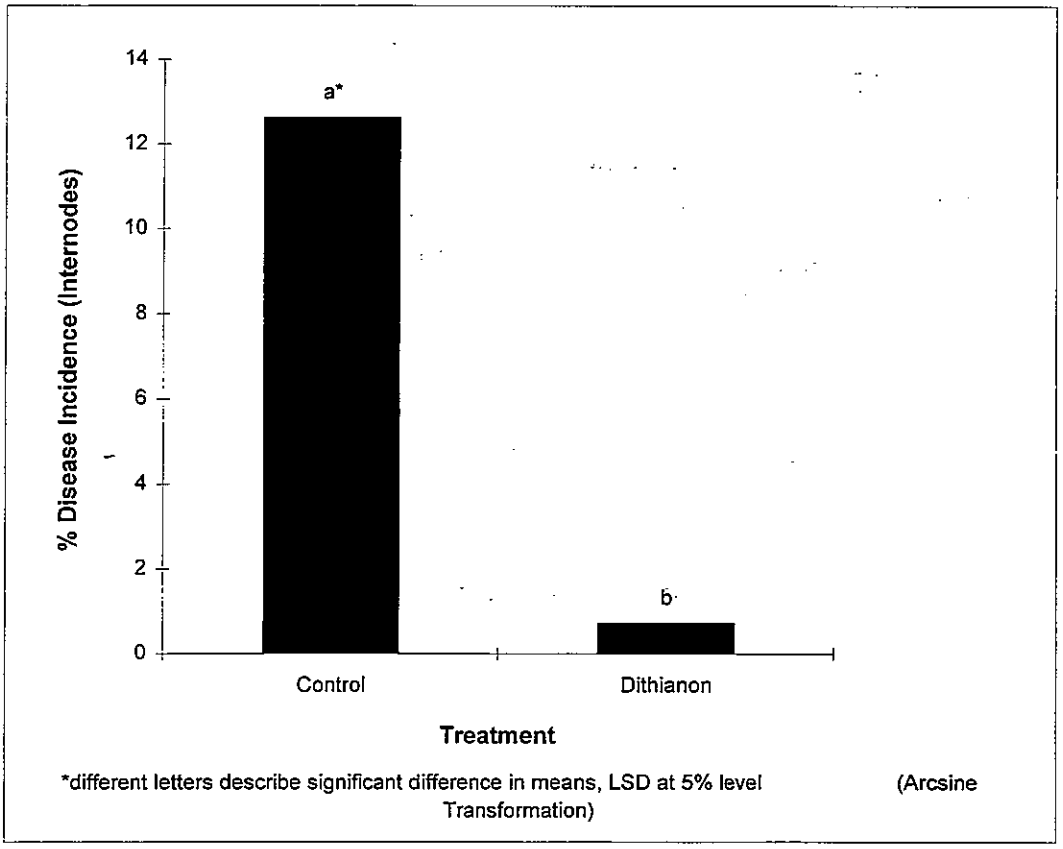


Fig. 2 - Incidence of *Phomopsis viticola* on grapevines at Robinvale, Victoria (1995/96)

Disease Assessment Budburst + 10 weeks

Treatment	% Disease Incidence on Internodes
Control	12.6
Dithianon	0.7



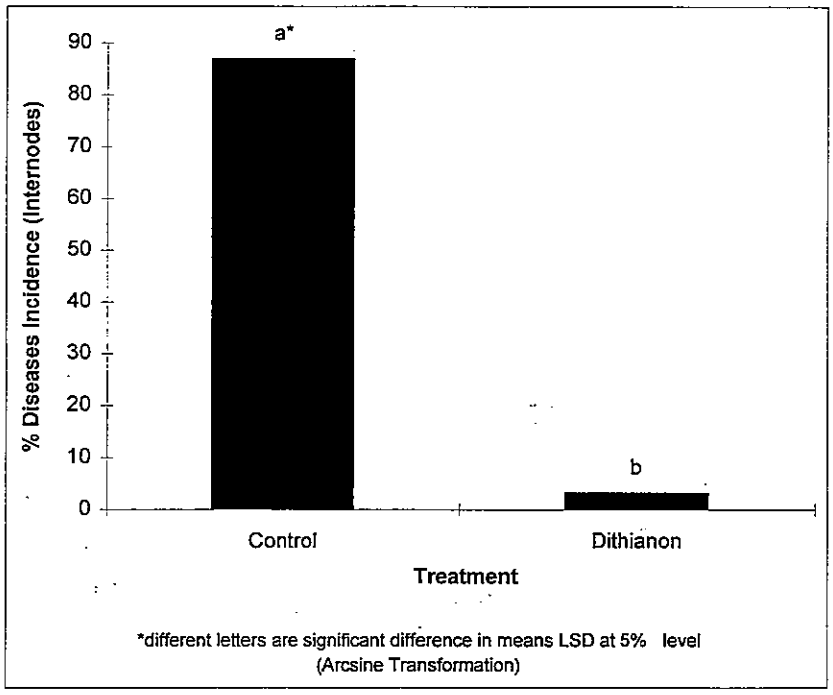
*different letters describe significant difference in means, LSD at 5% level (Arcsine Transformation)

Fig. 3 - Incidence of *Phomopsis viticola* on grapevines (internodes) at Vinifera, Victoria (1996/97)

Fig. 4 - Incidence of *Phomopsis viticola* on grapevines (leaves) at Vinifera, Victoria (1996/97)

Budburst +12 week

Treatment	% Disease Incidence on Internodes
Control	86.8
Dithianon	3



Budburst +12 week

Treatment	% Disease Incidence on Leaves
Control	87.3
Dithianon	23.6

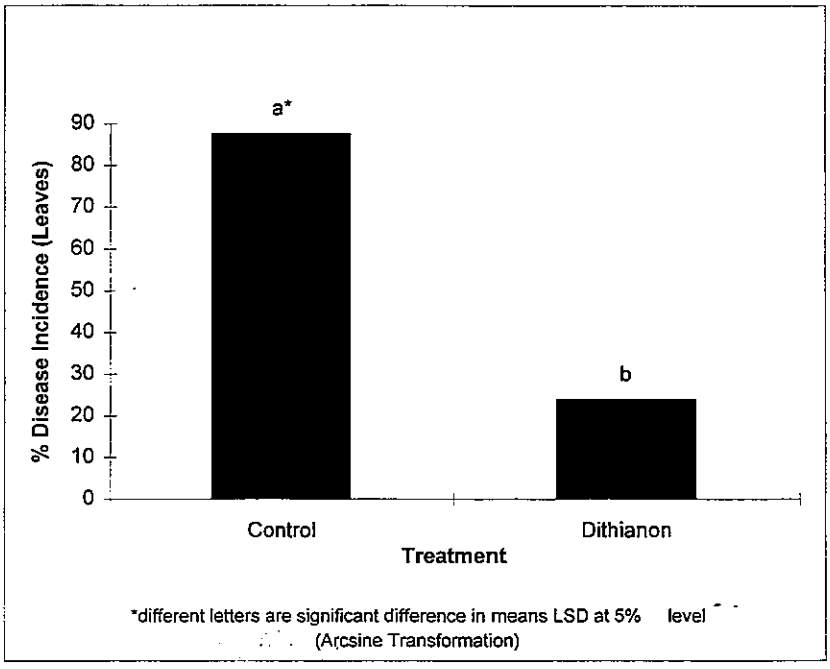
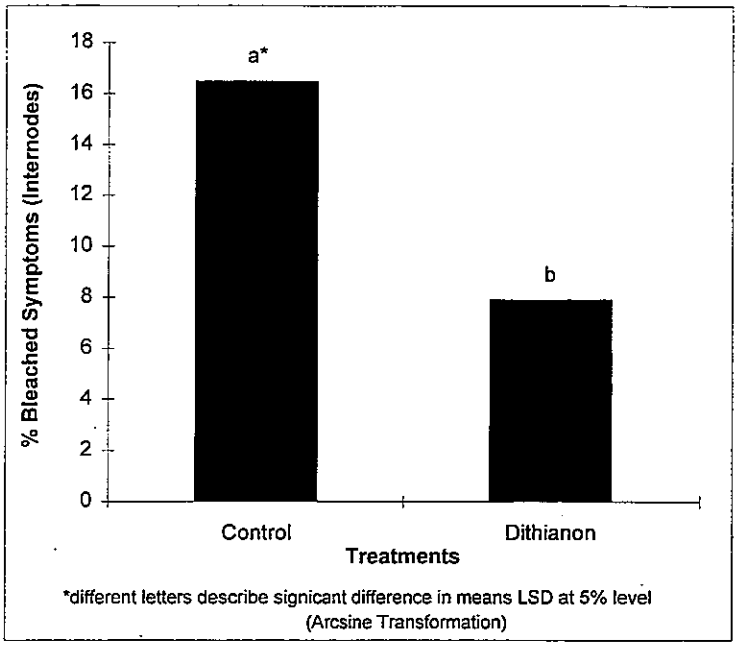


Fig. 5 - Bleached symptoms on grapevines at Vinifera, Victoria (1996)

Fig. 6 - Scarred symptoms on internodes of grapevines at Vinifera, Victoria (1996/97)

Dormant cane assessment

Treatment	% Bleached Symptoms on Internodes
Control	16.4
Dithianon	7.9



Dormant cane assessment

Treatment	%Scarred Symptoms on Internodes
Control	22.2
Dithianon	4.6

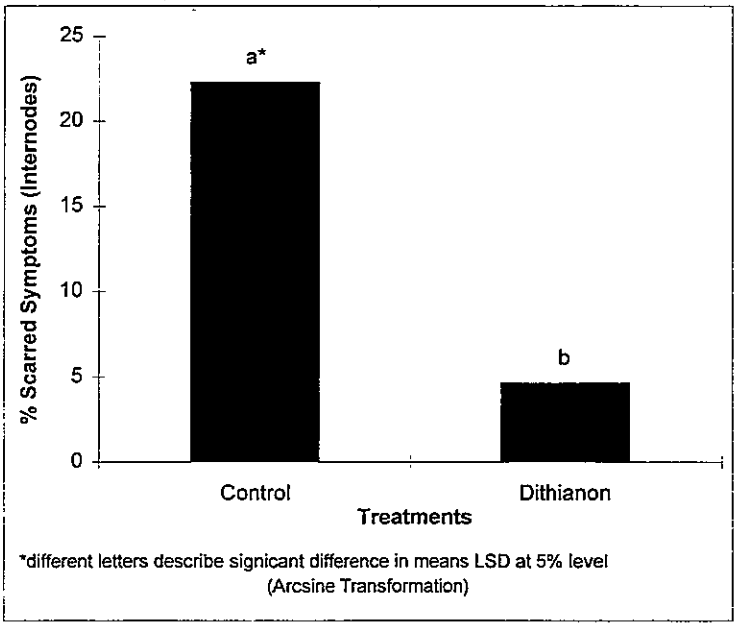
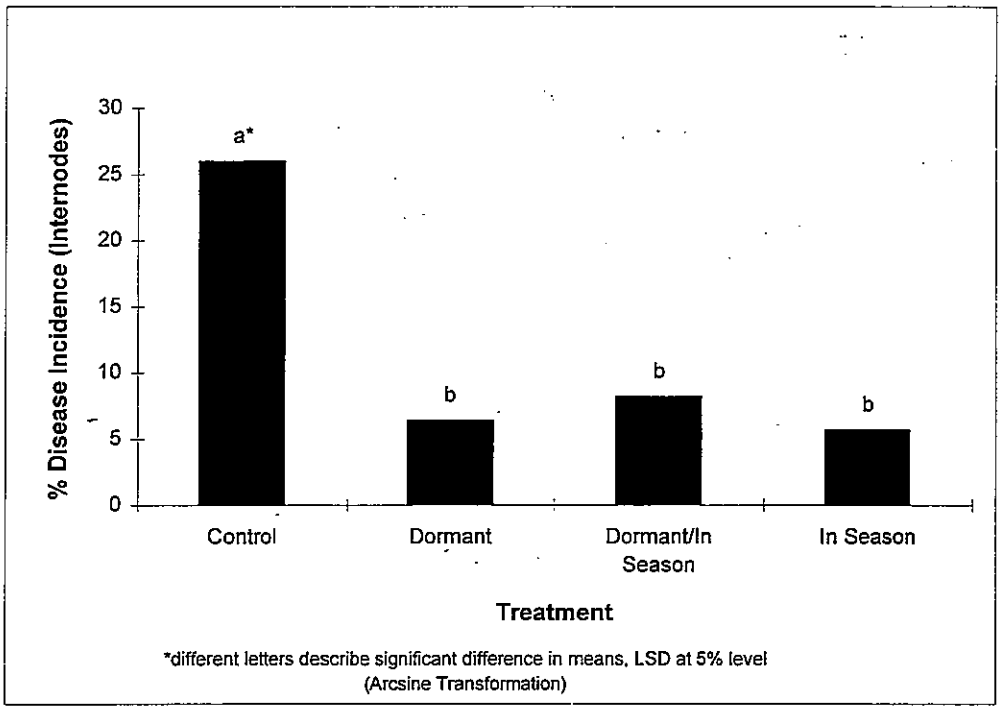


Fig. 7 - Effect of spray application on incidence of *Phomopsis viticola* on grapevines at Robinvale, Victoria (1995/96)

Harvest Disease Assessment

Fungicide	Treatment	% Diseases Incidence on Internodes
No spray	Control	25.9
Fluazinam	Dormant	6.3
Fluazinam/ Dithianon	Dormant/ In Season	8.1
Dithianon	In Season	5.6



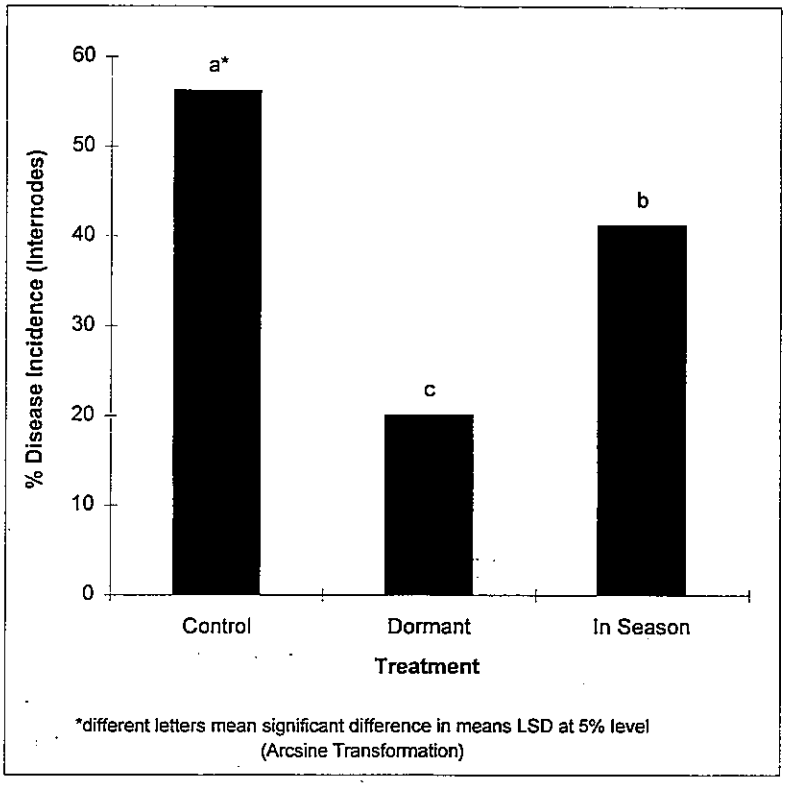
*different letters describe significant difference in means, LSD at 5% level (Arcsine Transformation)

Fig. 8 - Incidence of *Phomopsis viticola* on grapevines (internodes) at Padthaway, South Australia (1995/96)

Fig. 9 - Incidence of *Phomopsis viticola* on grapevines (leaves) at Padthaway, South Australia (1995/96)

Disease Assessment - Budburst + 12 weeks

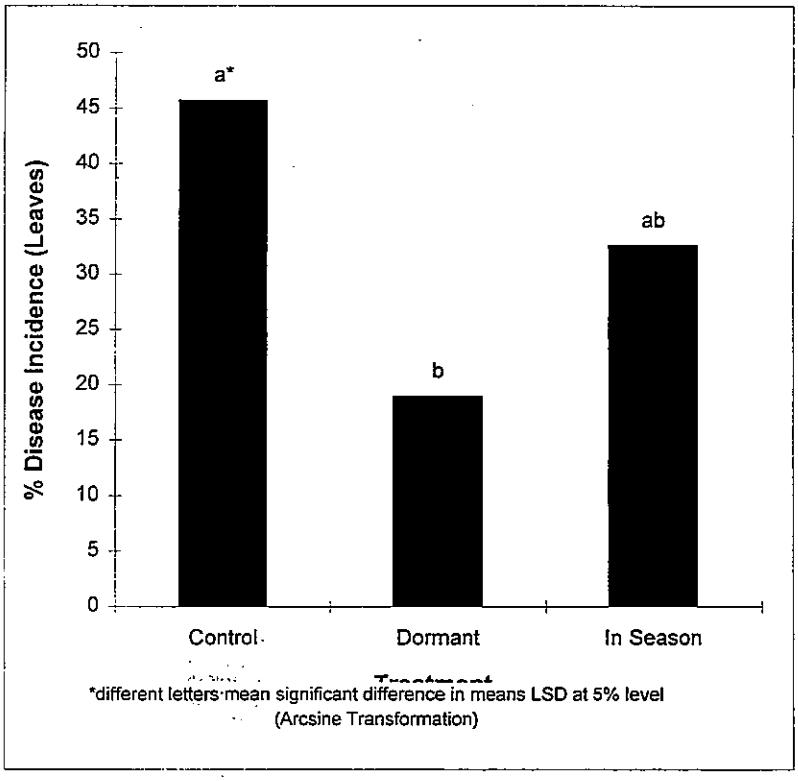
Funcigide	Treatments	% Disease Incidence on Internodes
No spray	Control	56.1
Dithane	Dormant	20
Dithianon	In Season	41.1



*different letters mean significant difference in means LSD at 5% level
(Arcsine Transformation)

Disease Assessment - Budburst + 12 weeks

Funcigide	Treatments	% Disease Incidence on Leaves
No spray	Control	45.6
Dithane	Dormant	18.9
Dithianon	In Season	32.5



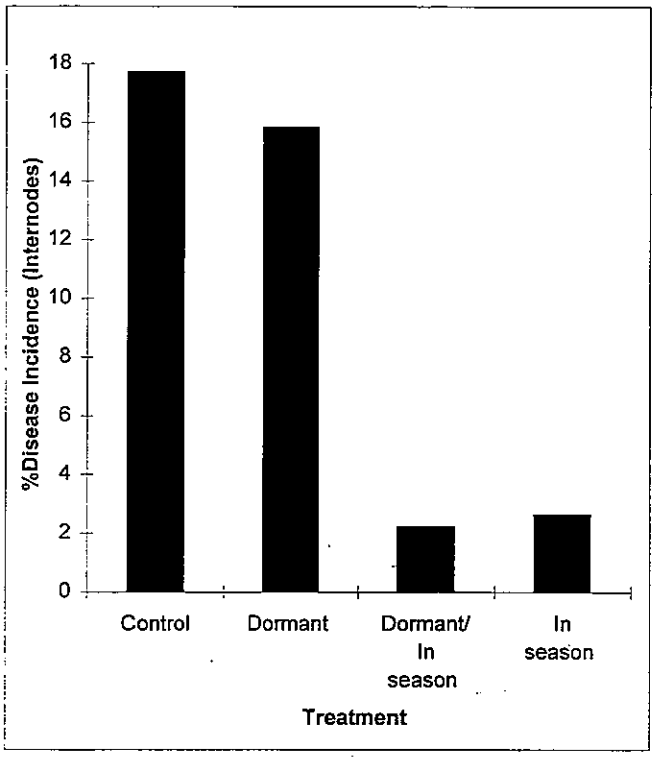
*different letters mean significant difference in means LSD at 5% level
(Arcsine Transformation)

Fig. 10 - Incidence of *Phomopsis viticola* on grapevines at Griffith, New South Wales (1996/97)

Fig. 11 - Area of internodes of grapevines infected with *Phomopsis viticola* at Griffith, New South Wales (1996/97)

Bud burst+ 11 weeks

Fungicide	Treatments	% Disease Incidence on Internodes
	Control	17.7
Dithane	Dormant	15.8
Dithane/ Dithianon	Dormant/ In season	2.2
Dithianon	In season	2.6



Bud burst+ 11 weeks

Fungicide	Treatments	% Area Affected on Internodes
	Control	0.74
Dithane	Dormant	0.74
Dithane/ Dithianon	Dormant/ In season	0.12
Dithianon	In season	0.05

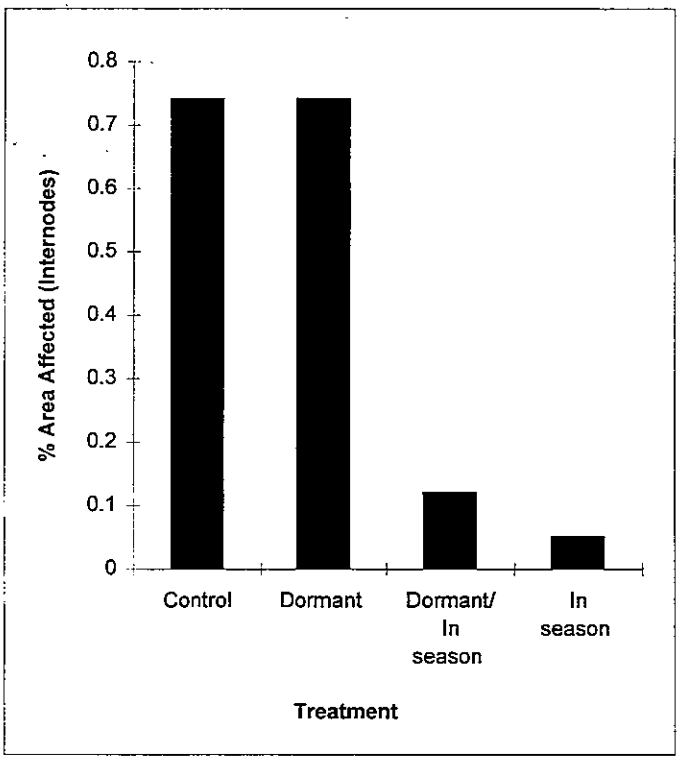
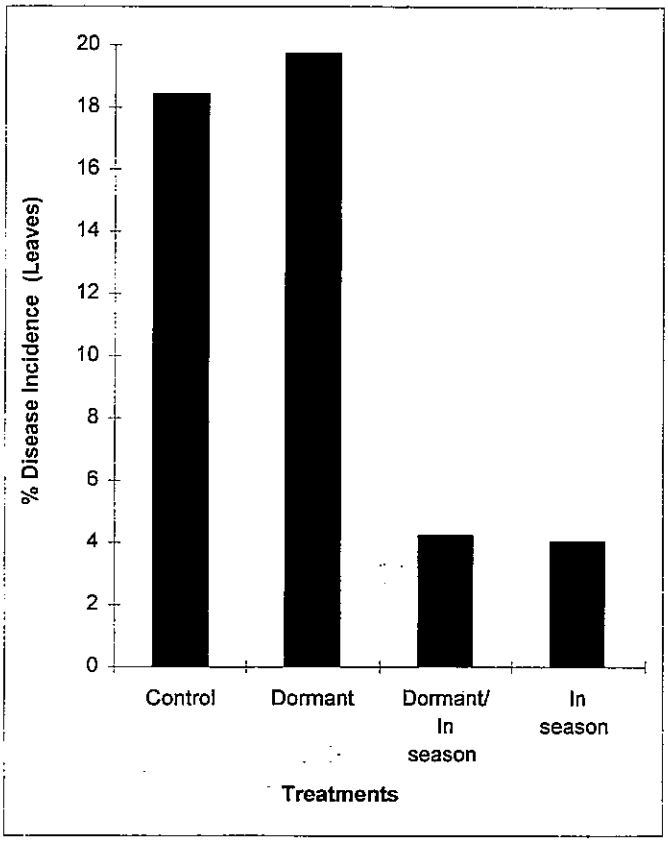


Fig. 12 - Incidence of *Phomopsis viticola* on grapevines at Griffith, New South Wales (1996/97)

Fig. 13 - Area of leaves of grapevines infected with *Phomopsis viticola* at Griffith, New South Wales (1996/97)

Budburst + 11 weeks

Fungicide	Treatments	% Disease Incidence on Leaves
	Control	18.4
Dithane	Dormant	19.7
Dithane/ Dithianon	Dormant/ In season	4.2
Dithianon	In season	4



Budburst + 11 weeks

Fungicide	Treatments	% Area Affected on Leaves
	Control	0.43
Dithane	Dormant	0.38
Dithane/ Dithianon	Dormant/ In season	0.11
Dithianon	In season	0.06

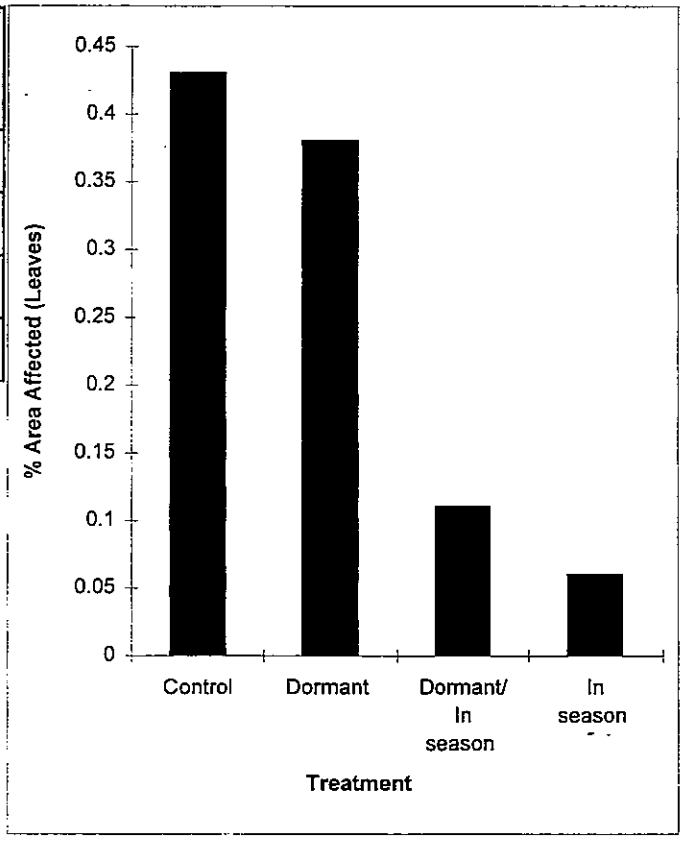
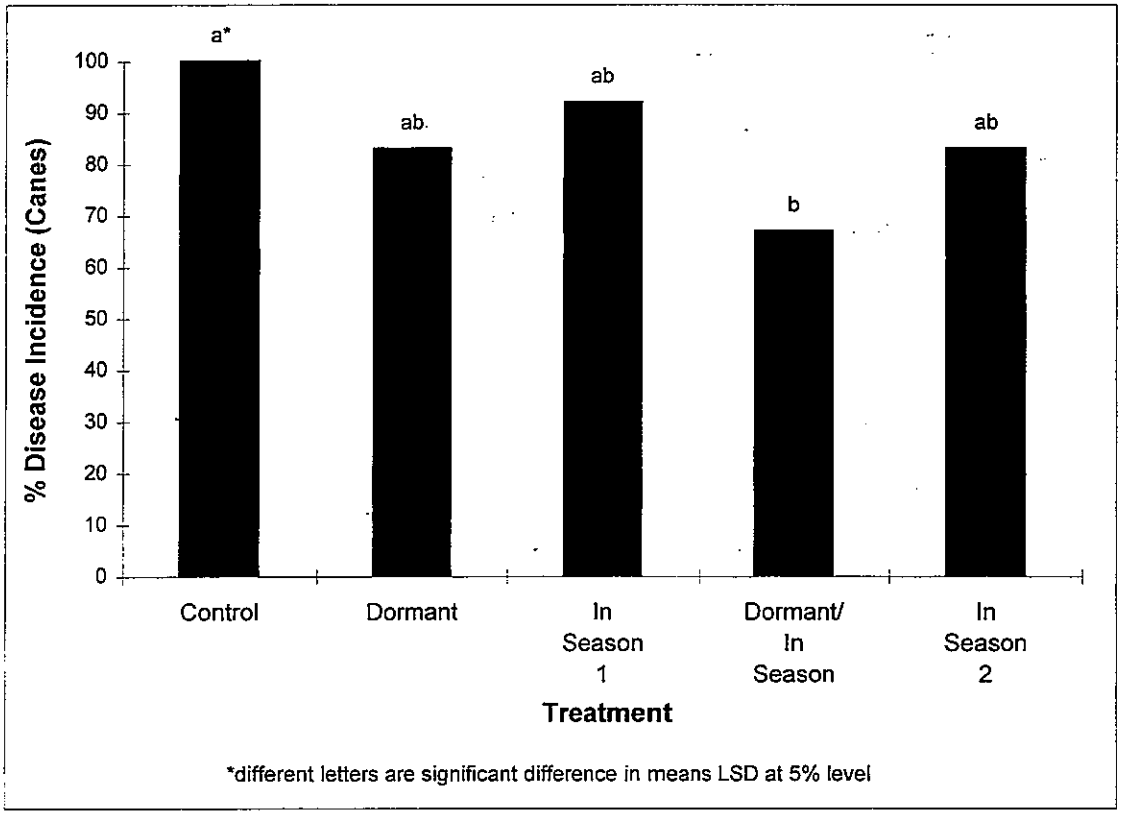


Fig. 14 - Effect of dormant spray application of dithane on incidence of *Phomopsis viticola* on pruned canes from Koraleigh, NSW. Pruned canes collected at harvest 26/02/97, (incubated in the laboratory).

Fungicides	Treatments	% Disease Incidence of Canes
	Control	100
Dithane	Dormant	83
Dithane	In Season 1	92
Dithane/ Dithianon	Dormant/ In Season 2	67
Dithianon	In Season 2	83



*different letters are significant difference in means LSD at 5% level

Fig. 14a - Effect of dormant spray application of dithane on in-season incidence of *Phomopsis viticola* on grapevines in the vineyard at Koraleigh, New South Wales

Disease Assessment Budburst + 12 weeks

Fungicides	Treatment	Mean % Diseased (Internodes)
	Control	83.1
Dithane	Dormant	87.4
Dithane	In Season 1	8.5
Dithane/ Dithianon	Dormant/ In Season	4.4
Dithianon	In Season 2	1.9

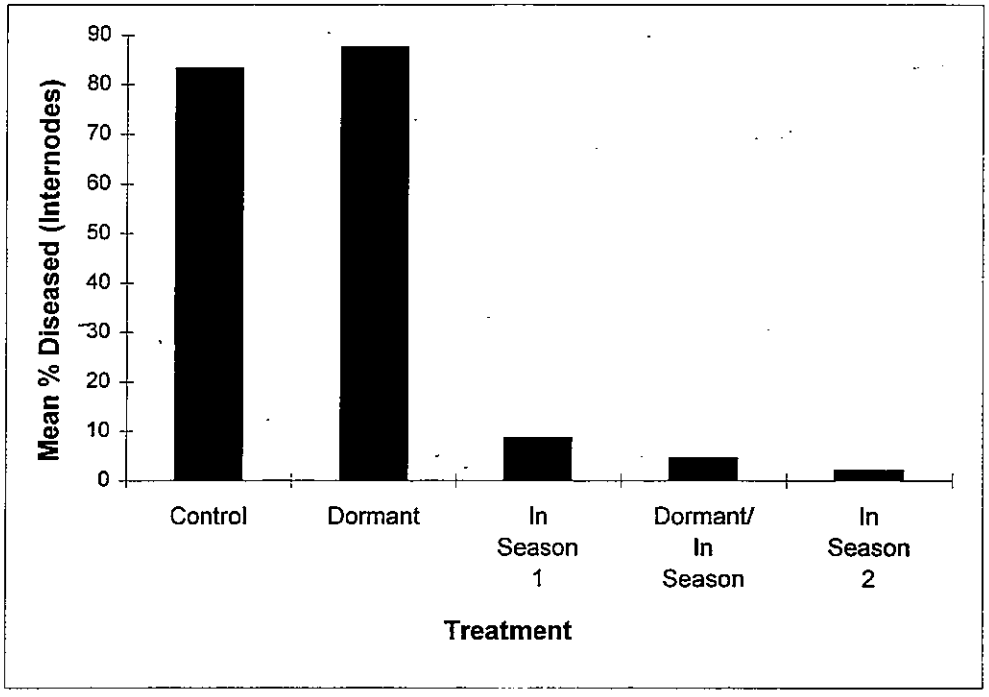


Fig 14b - Effect of dormant spray applications of dithane on carry over incidence of *Phomopsis viticola*, Koraleigh, NSW

Fungicides	Treatment	% Shoots Diseased				
		Dormant Cane 25/09/96	Budburst + 8 Weeks 08/11/96	Budburst + 12 weeks 04/12/96	Dormant Cane 21/08/97	Difference (between 25/09/96 & 21/08/97)
	Control	78	90	100	83	.+5%
Dithane	Dormant	77	95	100	88	.+11%
Dithane	In Season 1	72	3	17	32	-39%
Dithane/ Dithianon	Dormant/ In Season	74	16	31	35	-40%
Dithianon	In Season 2	84	8	9	25	-59%

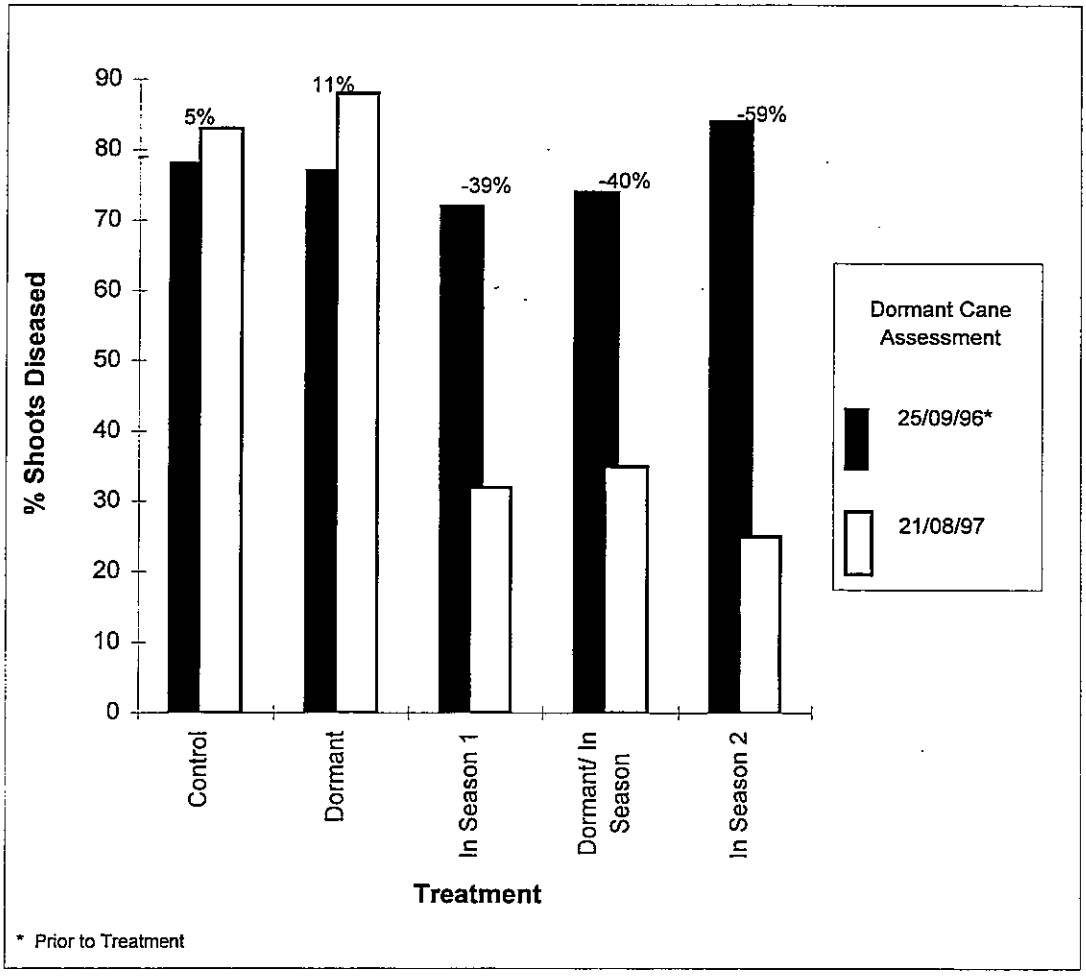
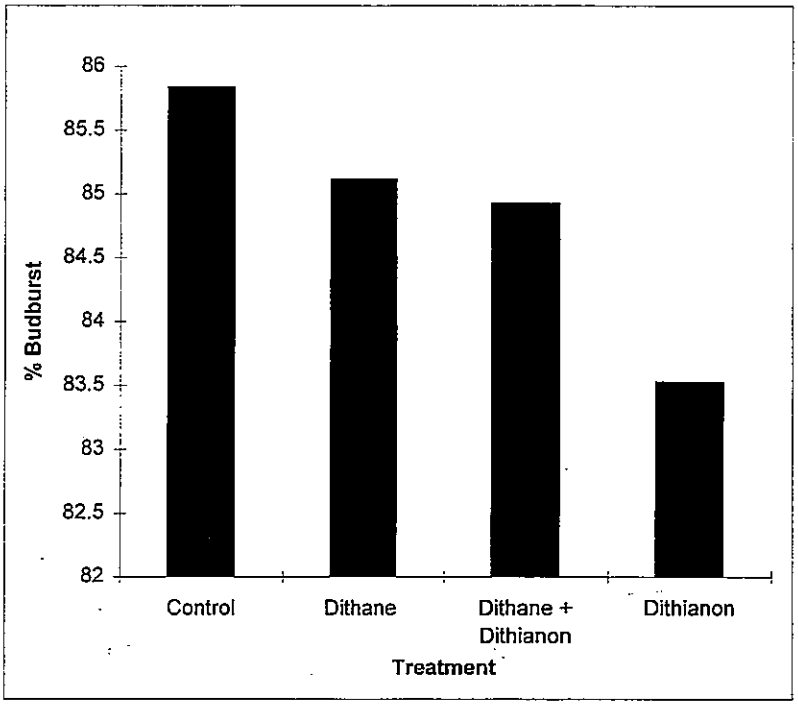


Fig. 15 - Effect of dormant spray applications of Dithane on *Phomopsis viticola* (Type 1) at Vineyard 1, Yarra Valley, Victoria (1996/97)

Treatment	% Budburst
Control	85.83
Dithane	85.11
Dithane + Dithianon	84.92
Dithianon	83.52



Treatment	Average Bunch Weight (Kg)
Control	0.77
Dithane	0.67
Dithane + Dithianon	0.98
Dithianon	0.85

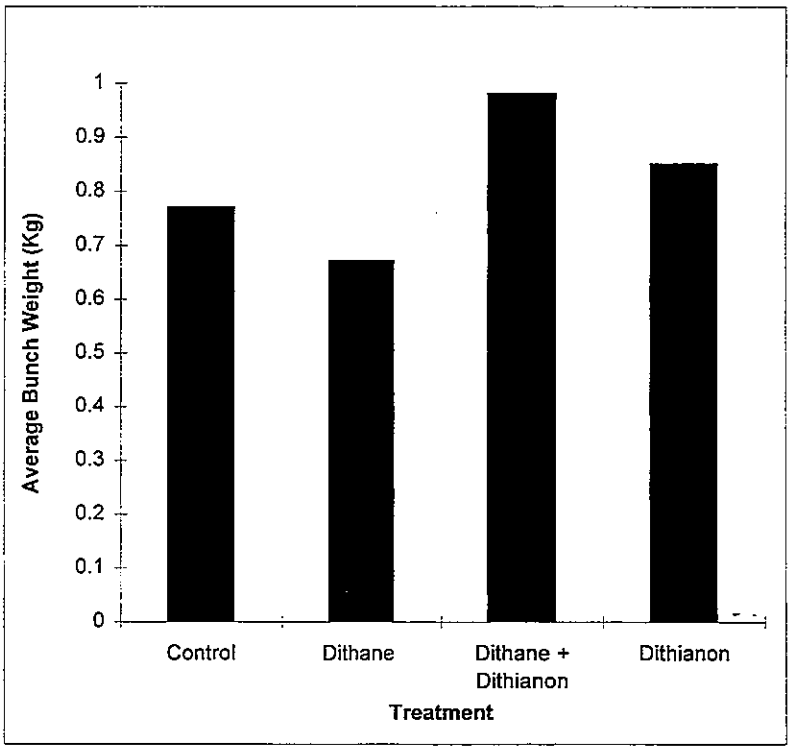
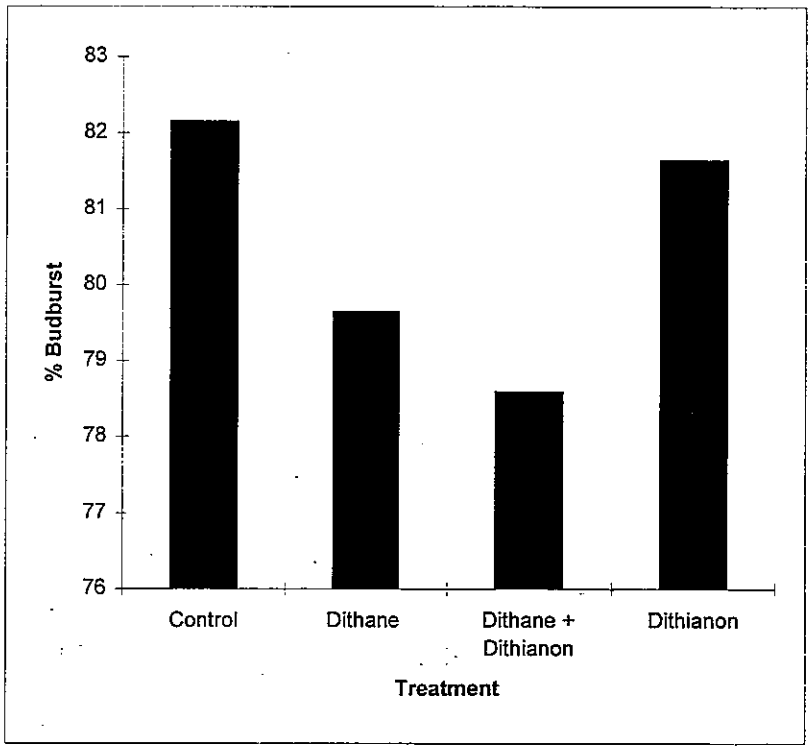
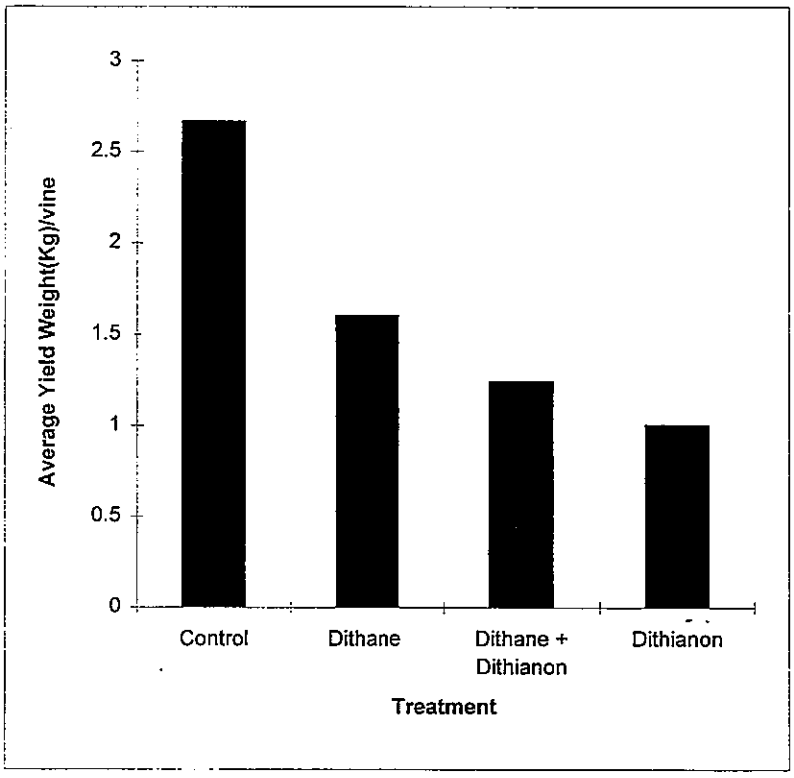


Fig. 16 - Effect of dormant spray applications of Dithane on *Phomopsis viticola* (Type 1) at Vineyard 2, Yarra Valley, Victoria (1996/97)

Treatment	% Budburst
Control	82.14
Dithane	79.63
Dithane + Dithianon	78.58
Dithianon	81.63



Treatment	Average Yield Wt(Kg)/vine
Control	2.66
Dithane	1.6
Dithane + Dithianon	1.24
Dithianon	1



APPENDIX 1

Protocols for yield and dormant trials

PROTOCOL FOR YIELD AND DORMANT TRIALS

YIELD TRIAL

Task:

Crop loss assessment of Phomopsis cane blight and leaf spot disease.

JOB SCHEDULE

0-4 Weeks before Budburst (Where applicable)

- Select trial sites in highly diseased vineyards.
  (Sunraysia, Mid-Murray - Taxon 2: SHC)
  (Yarra Valley, Southern Vic. - Taxon 1: IHD)
  (Griffith, NSW - Taxon 2)
  Ensure sites selected do not have a history of black spot.
- Map trial sites.
- Prepare a trial plan (see attached example)
- Mark out trial area and put up signs.
- Collect 20+ diseased canes or spurs and forward to Tan Nair for Phomopsis isolation and typing.
- Incubate some samples at SHC or IHD and check for Phomopsis spore types etc.
- Ask co-operating grower (vineyard manager) to record rainfall and provide a maximum-minimum thermometer, rain-gauge and rainfall record sheet if an automatic weather station is not present in the vineyard.
- Ensure vineyard manager signs a grower agreement allowing for experimentation on his property. (Optional in NSW)
- Provide the vineyard manager with spray chemicals if appropriate.
- Discuss the project work with the vineyard manager and ensure that there is an understanding of his or her responsibilities. If necessary provide a copy of the job schedule.
- Provide the vineyard manager with a list of cover sprays to be applied to the trial area, spray record sheets and irrigation record sheets.
- Obtain copies of diagrammatic keys and appropriate photographs (optional) of Phomopsis symptoms on dormant canes, leaves, shoots and berries.
- Prepare record sheets.
- Forward copies of the job schedule, diagrammatic keys, photos of symptoms, record sheets etc. to the following:
  (1) Kathy Clarke, SHC Irymple, VIC
  (2) Bob Emmett, SHC Irymple, VIC
  (3) Tan Nair, BCRI, Rydalmere, NSW
  NB. University of Western Sydney (from end of November)
  (4) Trevor Wicks / Kristen Campbell, Plant Research Centre, Adelaide, SA
  (5) Jan Strawhorn, IHD, Knoxfield, VIC
  (6) Andrew Watson, YAI Yanco, NSW

At 30-50% Budburst (50% buds with one leaf exposed)
(Approximately 5-9 September = Sunraysia, Griffith; 19-23 September = Yarra Valley)

- Record vine phenological details (range and average shoot length and leaves per shoot).
- **Examine** 1 year old canes or spurs on vines to be assessed (centre 1-3 vines) in each plot.•**Count** total number of canes or spurs per vine and for each, count the total number of internodes.

Where there are canes, record the number of internodes out of the first six internodes on each cane with:

(1) bleaching (as usually associated with Phomopsis)

(2) elongated scarring or scabbing (symptoms often associated with Phomopsis).

- **Calculate** % canes or spurs, % internodes and % internodes (for internodes 1-6 on canes) with each type of Phomopsis symptom.
- Describe (in detail where necessary) the symptoms being assessed; take appropriate photographs. (Optional)
- **Apply Delan WP** (100g/100L) (to the point of run-off) to plots (and buffer vines) that are to be sprayed for Phomopsis control. Ensure there is no spray drift onto untreated plots.•Record application date and volume of spray used.

At Budburst (30-50%) + 10 days
(Approximately 12-16 September = Sunraysia, Griffith; 26-30 September = Yarra Valley)

- Record vine phenological details (range and average shoot length, leaves per shoot).
- **Apply Delan WP** (100g/100L) (to the point of run-off) to plots (and buffer vines) that are to be sprayed for Phomopsis control. Ensure there is no spray drift onto untreated plots.•Record application date and volume of spray used.
- **Ensure** that vineyard manager will apply the first cover spray (at BB+ 2 weeks) to all plots for control of mites and powdery mildew (Micronised sulphur, 300g/100L) and lightbrown apple moth (chlorpyrifos at label rates) with approximately 600L/ha. if applied as a high volume spray.

At Budburst (50%) + 3 weeks
(Approximately 19-23 September = Sunraysia, Griffith; 3-7 October = Yarra Valley)

- Record vine phenological details (range and average shoot length, leaves per shoot, cluster length).
- **Count** the total number of buds per vine and number of buds burst per vine (on **centre** 1-3 vines per plot).•**Calculate** the percent buds burst per vine for each plot.
- Examine new shoot growth for disease symptoms. If symptoms are observed, write a detailed description and take photographs. (Optional)

- **If necessary**, assess the percentage of leaves and internodes diseased on **15** shoots per plot using diagrammatic keys. Otherwise leave this task until preflowering (i.e. Budburst + 7-8 weeks).
- **Apply Delan WP** (100g/100L) (to the point of run-off) to plots (and buffer vines) that are to be sprayed for Phomopsis control. Ensure there is no spray drift onto untreated plots.•Record application date and volume of spray used.
- **Ensure** that the vineyard manager will apply the second cover spray (at BB+ 4 weeks) to all plots for control of mites and powdery mildew (micronised sulphur, 300g/100L) and downy mildew (copper oxychloride at label rate) with 600-800L/ha. if applied as a high volume spray.

At Budburst + 5 weeks
(Approximately 3-7 October = Sunraysia, Griffith; 17-21 October =.Yarra Valley)

- Record vine phenological details (range and average shoot length, leaves per shoot, cluster length).
- Examine new shoot growth for disease symptoms. If symptoms are observed, write a detailed description and take photographs. (Optional)
- **If necessary,** assess the percentage of leaves and internodes diseased on 15 shoots per plot using diagrammatic keys. Otherwise leave this task until pre-flowering (i.e. Budburst + 7-8 weeks).
- **Apply Delan WP** (100g/100L) (to the point of run-off) to plots (and buffer vines) that are to be sprayed for Phomopsis control. Ensure that there is no spray drift onto untreated plots.•Record application date and volume of spray used etc.
- **Ensure** that the vineyard manager will apply the third cover spray (at BB+ 6 weeks) for control of mites and powdery mildew (micronised sulphur, 300g/100L), downy mildew (copper oxychloride or copper oxychloride plus potassium phosphite at label rates) and lightbrown apple moth (chlorpyrifos at label rates) with approximately 800L/ha. if applied as a high volume spray.

At Budburst + 7 weeks
(Approx. 17-21 October, = Sunraysia, Griffith; 31 October - 4 November, = Yarra Valley)

- Record vine phenological details (range and average shoot length, leaves per shoot, cluster length).
- Examine new shoot growth for disease symptoms. If symptoms are observed, write a detailed description and take photographs. (Optional)
- **If necessary,** assess the percentage of leaves and internodes diseased on **15** shoots per plot using diagrammatic keys. Otherwise leave this task until post-flowering (i.e. Budburst + 10-11 weeks).
- **Apply Delan WP** (100g/100L) (to the point of run-off) to plots (and buffer vines) that are to be sprayed for Phomopsis control. Ensure that there is no spray drift onto untreated plots.•Record application date and volume of spray used etc.
- **Ensure** that the vineyard manager will apply the fourth cover spray (at BB+ 8 weeks) for control of mites and powdery mildew (micronised sulphur, 300g/100L), downy mildew (copper oxychloride or copper oxychloride plus potassium phosphite at label

rates) and lightbrown apple moth (chlorpyrifos at label rates) with approximately 800L/ha. if applied as a high volume spray.

At Budburst + 10-11 weeks; Berries 2-5mm diam.
(Approx. 14-25 November = Sunraysia, Griffith; 28 November - 8 December = Yarra Valley)

- Record vine phenological details (range and average shoot length, leaves per shoot, cluster length).
- Carefully examine new shoot growth for disease symptoms. If symptoms are present write a detailed description and take photographs. (Optional)
- **Assess** the percentage area diseased per leaf for leaves on the first six nodes of each of **15** shoots per plot using diagrammatic keys. Record missing leaves on each shoot.
- Assess the percentage area diseased per internode on the first six internodes on each of **15** shoots per plot using diagrammatic keys.
- Assess the percentage area of bunch stems diseased and berries diseased per bunch for **20** bunches per plot using diagrammatic keys.(**NB**. RWE to supply diagrammatic key).
- **Calculate**
  (1) the mean percentage of leaves diseased per shoot for each plot.
  (2) the mean percentage area of each leaf diseased per shoot for each plot.
  (3) the mean percentage of leaves fallen (missing) per shoot for each plot.
  (4) the mean percentage of internodes diseased per shoot per plot.
  (5) the mean percentage area of each internodes diseased per shoot per plot.
  (6) the mean percentage of bunch stems diseased per plot.
  (7) the mean percentage area of bunch stems diseased per plot.
  (8) the mean percentage of berries per bunch diseased per plot.
  (9) the mean percentage area of berries diseased per bunch per plot.
- **Randomly sample 10** bunches per plot. Surface sterilise the bunches and incubate each bunch in a separate moistened clip-seal polyethylene bag (see Phomopsis isolation procedures). After 1-4 weeks, assess the percentage of berries per bunch and the percentage of bunches with sporulating Phomopsis. Note the dimensions and shape of Phomopsis conidia and record the Phomopsis taxa observed.
- **Ensure** that the vineyard manager will or has applied the fifth cover spray (at BB+ 10 weeks)for control of mites and powdery mildew (sulphur, 300g/100L), downy mildew (copper oxychloride or copper oxychloride plus potassium phosphite at label rates), Botrytis if necessary (iprodione at label rate) and lightbrown apple moth (chlorpyrifos at label rates) with approximately 1000L/ha. if applied as a high volume spray.
- Remind the vineyard manager to subsequently spray every 2-3 weeks until berry softening for the above diseases and pests and with the above chemicals where appropriate.

1 Week before Harvest
(Approximately 6-30 February = Sunraysia, Griffith; ? = Yarra Valley)

- Harvest the vines being assessed (centre 1-3 vines) in each plot.
- Count the number of bunches harvested from each vine
- Record the weight of fruit harvested per vine per plot.
- Calculate the mean bunch weight per vine per plot.

- Take berry subsamples per plot and extract berry juice (by manual crushing and sieving or by using a juice extractor).
- Estimate Brix of the berry juice using a refractometer (see Brix procedures).
- Randomly sample **3** shoots (or canes) per plot from untreated plots. Carefully cut shoots to include basal internodes and trim the shoots to the first 8 nodes. Wrap shoot stems with attached leaves in moist paper towel, place in a large plastic bag, label the bag with details of trial location, sample date etc. and forward to -

  Dr Tan Nair
  School of Horticulture
  University of Western Sydney
  Bourke Street
  Richmond NSW 2753

  The samples will be incubated and examined for presence of latent phomopsis.

At Pruning (Approximately mid-winter)

- Prune the vines to be assessed (centre 1-3 vines) per plot and record pruning weights per vine.

PHOMOPSIS ISOLATION PROCEDURES

1. One Year Old Canes

- Cut into pieces approximately 8cm long.
- Dip in 70% ethanol for 3-5 seconds.
- Then immediately dip in 1% sodium hypochlorite for 2 minutes.
- Rinse in sterile distilled water.
- Incubate the surface sterilised pieces in a humid chamber (or clip sealed polyethylene bag containing damp paper towel) at room temperature (20°C).
- After one week, examine pieces for sporulation of Phomopsis Taxon 2.
- After 2-4 weeks, examine pieces for sporulation of Phomopsis Taxa 1,3 and 4.
- Examine sporulating pycnidia under the microscope and record spore types and spore sizes etc.

2. Green Stems, Leaves and Berries

- Treat as for one year old canes but omit the ethanol treatment.

DORMANT SPRAY TRIAL

Task:

Assessment of incidence and severity of cane blight and leaf spot disease

Treatments:* same spray times as in Yield Trial protocol.

1. Untreated control
2. Winter dormant spray
3. Winter dormant spray + in - season Delan spray*
4. In season Delan spray*
5. In - season mancozeb spray* (Koraleigh only)

JOB SCHEDULE

4 weeks before budburst

- Mix thoroughly mancozeb (@ 300 g / 100 litres of water) and mineral oil (Caltex Lovis miscible summer oil @ 20 ml/litre of water).
- Spray vines thoroughly to run off. Ensure weather is dry, preferably for 24 hrs after spray application. Record liquid volume per hectare.

At 30-50% budburst (50% buds with one leaf exposed)
(Approximately 5-9 September = Sunraysia, Griffith; 19-23 September = Yarra Valley)

- Record vine phenological details (range and average shoot length and leaves per shoot).
- **Examine** 1 year old canes or spurs on vines to be assessed (centre 1-3 vines) in each plot.•**Count** total number of canes or spurs per vine and for each, count the total number of internodes.
  Where there are canes, record the number of internodes out of the first six internodes on each cane with:
  (1) bleaching (as usually associated with Phomopsis)
  (2) elongated scarring or scabbing (symptoms often associated with Phomopsis).
- Describe (in detail where necessary) the symptoms being assessed; take appropriate photographs. (Optional)
- Calculate % canes or spurs, % internodes and % internodes (for internodes 1-6 on canes) with each type of Phomopsis symptom.
- **Select buds from untreated plots**; tag **50** individual buds from nodes that are bleached and **50** buds from unbleached nodes at identical positions on the canes or spurs. (Record bud position to correlate with alternatively-pruned varieties)
  Record budburst date and rate of shoot growth (length and number of separated leaves) when applying the other 4 Delan sprays during spring. ie. at 30-50% Budburst,

BB+ 10 days, and BB+ 3, 5, and 7 weeks. Assess shoot developement using Brian Combe's growth stage assessment key. Record date when first symptoms appear on shoots, and later on bunches.

- **Apply Delan WP** (100g/100L) (to the point of run-off) to plots (and buffer vines) that are to be sprayed for Phomopsis control. Ensure there is no spray drift onto untreated plots.•Record application date and volume of spray used.

At budburst (30-50%) + 10 days
(Approximately 12-16 September = Sunraysia, Griffith; 26-30 September = Yarra Valley)

- Record vine phenological details (range and average shoot length, leaves per shoot).
- **Apply Delan WP** (100g/100L) (to the point of run-off) to plots (and buffer vines) that are to be sprayed for Phomopsis control. Ensure that there is no spray drift onto untreated plots.•Record application date and volume of spray used.
- **Ensure** that vineyard manager will apply the first cover spray (at BB+2 weeks)to all plots for control of mites and powdery mildew (Micronised sulphur, 300g/100L) and lightbrown apple moth (chlorpyrifos at label rates) with approximately 600L/ha. if applied as a high volume spray.

At budburst + 3 weeks
(Approximately 19-23 September = Sunraysia, Griffith; 3-7 October = Yarra Valley)

- Record vine phenological details (range and average shoot length, leaves per shoot).
- **Apply Delan WP** (100g/100L) (to the point of run-off) to plots (and buffer vines) that are to be sprayed for Phomopsis control. Ensure that there is no spray drift onto untreated plots.•Record application date and volume of spray used.
  Ensure that the vineyard manager will apply the second cover spray (at BB+4 weeks) to all plots for control of mites and powdery mildew (micronised sulphur, 300g/100L) and downy mildew (copper oxychloride at label rate) with 600-800L/ha. if applied as a high volume spray.

At budburst + 5 weeks
(Approximately 3-7 October = Sunraysia, Griffith; 17-21 October =.Yarra Valley)

- Record vine phenological details (range and average shoot length, leaves per shoot).
- **Apply Delan WP** (100g/100L) (to the point of run-off) to plots (and buffer vines) that are to be sprayed for Phomopsis control. Ensure that there is no spray drift onto untreated plots.•Record application date and volume of spray used.•
  Ensure that the vineyard manager will apply the third cover spray (at BB+6 weeks) for control of mites and powdery mildew (micronised sulphur, 300g/100L), downy mildew (copper oxychloride or copper oxychloride plus potassium phosphite at label rates) and lightbrown apple moth (chlorpyrifos at label rates) with approximately 800L/ha. if applied as a high volume spray.

At budburst + 7 weeks

(Approx. 17-21 October, = Sunraysia, Griffith; 31 October - 4 November, = Yarra Valley)

- Record vine phenological details (range and average shoot length, leaves per shoot, cluster length).
- Examine new shoot growth for disease symptoms. If symptoms are observed, write a detailed description and take photographs. (Optional)
- **If necessary**, assess the percentage of leaves and internodes diseased on 15 shoots per plot using diagrammatic keys. Otherwise leave this task until post-flowering (i.e. Budburst + 10-11 weeks).
- **Apply Delan 750 WP** (100g/100L) (to the point of run-off) to plots (and buffer vines) that are to be sprayed for Phomopsis control. Ensure that there is no spray drift onto untreated plots.•Record application date and volume of spray used etc.•
  Ensure that the vineyard manager will apply the fourth cover spray (at BB+8 weeks) for control of mites and powdery mildew (micronised sulphur, 300g/100L), downy mildew (copper oxychloride or copper oxychloride plus potassium phosphite at label rates) and light brown apple moth (chlorpyrifos at label rates) with approximately 800L/ha. if applied as a high volume spray.

At budburst + 10 - 11 weeks; berries 2-5mm diam.

(Approx. 14-25 November = Sunraysia, Griffith; 28 November - 8 December = Yarra Valley)

- Record vine phenological details (range and average shoot length, leaves per shoot, cluster length).
- Carefully examine new shoot growth for disease symptoms. If symptoms are present write a detailed description and take photographs. (Optional)
- **Assess** the percentage area diseased per leaf for leaves on the first six nodes of each of **15** shoots per plot using diagrammatic keys. Record missing leaves on each shoot.
- Assess the percentage area diseased per internode on the first six internodes on each of **15** shoots per plot using diagrammatic keys.
- Assess the percentage area of bunch stems diseased and berries diseased per bunch for **20** bunches per plot using diagrammatic keys.
- **Calculate**
  (1) the mean percentage of leaves diseased per shoot for each plot.
  (2) the mean percentage area of each leaf diseased per shoot for each plot.
  (3) the mean percentage of leaves fallen (missing) per shoot for each plot.
  (4) the mean percentage of internodes diseased per shoot per plot.
  (5) the mean percentage area of each internodes diseased per shoot per plot.
  (6) the mean percentage of bunch stems diseased per plot.
  (7) the mean percentage area of bunch stems diseased per plot.
  (8) the mean percentage of berries per bunch diseased per plot.
  (9) the mean percentage area of berries diseased per bunch per plot.
- **Randomly sample 10** bunches per plot. Surface sterilise the bunches and incubate each bunch in a separate moistened clip-seal polyethylene bag (see Phomopsis isolation procedures). After 1-4 weeks, assess the percentage of berries per bunch and the percentage of bunches with sporulating Phomopsis. Note the dimensions and shape of Phomopsis conidia and record the Phomopsis taxa observed.

- **Ensure** that the vineyard manager will or has applied the fifth cover spray (at BB+10 weeks) for control of mites and powdery mildew (sulphur, 300g/100L), downy mildew (copper oxychloride or copper oxychloride plus potassium phosphite at label rates), Botrytis if necessary (iprodione at label rate) and lightbrown apple moth (chlorpyrifos at label rates) with approximately 1000L/ha. if applied as a high volume spray.
- **Remind** the vineyard manager to subsequently spray every 2-3 weeks until berry softening for the above diseases and pests and with the above chemicals where appropriate.

1 week before harvest
(Approximately 6-30 February = Sunraysia, Griffith; ? = Yarra Valley)

- Record incidence and severity of disease (on the first 6 internodes of 10 randomly selected canes on each of the 2 centre vines per plot) using the incidence and severity chart.
- Count the number of bunches from each of the centre 1-3 vines per plot.
- Record the weight of fruit harvested per vine per plot.
- Randomly select **2 canes per plot**. Carefully cut shoots to include basal internodes and trim the shoots to the first 8 nodes. Wrap shoot stems with attached leaves in moist paper towel, place in a large plastic bag, label the bag with trial location, sample date etc. and forward to:

  Dr. Tan Nair
  School of Horticulture
  University of Western Sydney
  Bourke Street
  Richmond NSW 2753

  At Pruning (Approximately mid-winter)

- Prune the vines to be assessed (centre 1-3 vines) per plot and record pruning weights per vine.

COVER SPRAYS TO BE APPLIED OVER THE PHOMOPSIS TRIAL SITE BY THE VINEYARD MANAGER.

1. Fungicide/Insecticide to be used on trial site.

Downy mildew	Copper Oxychloride
	Potassium Phosphite (e.g. Foli-R-Fos)
Powdery mildew / mites	Sulphur (300g/100L)
Botrytis rot	iprodione (Rovral)
Lightbrown apple moth (LBAM)	chlorpyrifos (e.g.Lorsban)

2. Spray timing	**Spray for**
Budburst	Nil
Budburst + 2 weeks	powdery mildew / mites lightbrown apple moth?
Budburst + 4 weeks	powdery mildew / mites downy mildew
Budburst + 6 weeks	powdery mildew / mites downy mildew
Budburst + 8 weeks (preflowering)	powdery mildew / mites downy mildew lightbrown apple moth
80% capfall	Botrytis rot (if required)

Budburst + 10 weeks (Berries 2-3mm)	powdery mildew / mites downy mildew lightbrown apple moth
Berries pea-size	powdery mildew / mites downy mildew
1 week before bunch closure	powdery mildew / mites downy mildew lightbrown apple moth Botrytis
Berry softening mites	powdery mildew / downy mildew
After berry softening	Botrytis rot (only if it rains or the disease appears).

APPENDIX 2

Viticultural aspects and incidence of cane and leaf blight disease

PHOMOPSIS TRIAL - PADTHAWAY
22nd NOV. 1994.

No. of spurs and no. with symptoms.

Vine No.	No. Spurs	No. Nodes	Bleaching	Scarring	% Symptoms in Total
1	15	3.3	5	8	53
2	15	3.1	5	6	53
3	15	3	4	8	60
4	15	2.7	4	4	47
5	15	2.7	5	3	40

% Area Infected/Cane.

Vine No.	% Area Infected/Cane				
	Node 1	Node 2	Node 3	Node 4	Node 5
1	0.21	0.37	0.05	-	-
2	0.64	0.43	0.11	0.05	0.05
3	6.76	2.24	1.41	0.05	-
4	0.37	0.21	0.11	-	-
5	5.79	0.96	0.11	-	-

% Area Infected/Leaf.

Vine No.	% Area Infected/Leaf				
	Node 1	Node 2	Node 3	Node 4	Node 5
1	0.21	0.16	-	-	-
2	0.43	0.21	0.05	0.05	-
3	0.53	0.37	0.21	-	-
4	0.21	0.16	0.05	-	-
5	0.37	0.16	-	-	-

PHOMOPSIS TRIAL - PADTHAWAY
22nd NOV. 1994.

Buds per vine and buds burst per vine.

Vine No.	Canes/Vine	Buds/Vine	Buds Burst/Vine	%
1	10	2.9	2.5	86
2	10	2.6	2.1	81
3	10	2.2	2.0	91
4	10	2.9	2.4	83
5	10	2.2	1.8	82

Hargraves Vineyard, Summertown.
Phomopsis Trial - 1995/96.

21/8/95 - Pre-spray assessment of phomopsis symptoms on spurs.

Treatment	% vines with symptoms (bleaching or cracking)
Untreated	52.7
Delan	57.3
Shirlan	65.5
Delan + Shirlan	58.4
LSD (P=0.05)	n.s.

25/9/95 - Assessment of number of spurs/vine at 30-50% budburst.

Treatment	No. spurs/vine
Untreated	17.2
Delan	17.3
Shirlan	16.5
Delan + Shirlan	20.5

All spurs pruned to 2 nodes.

6/10/95 - Assessment of shoot length and leaf number at budburst plus 10 days.

Treatment	Shoot length (cm)	No. leaves/shoot
Untreated	5.6	2.8
Delan	6.3	2.9
Shirlan *	3	2.4
Delan + Shirlan *	6.2	2.4

* = very small sample size

Hargraves Vineyard, Summertown.
Phomopsis Trial - 1995/96.

16/10/95 - Assessment of shoot length and leaf number at budburst plus 3 weeks.

Treatment	Shoot length (cm)	No. leaves/shoot
Untreated	10.8	3.6
Delan	11.6	3.8
Shirlan *	6.4	3
Delan + Shirlan *	8	3.4

1/11/95 - Assessment of budburst.

Treatment	% Budburst
Untreated	90.3
Delan	83.4
Shirlan *	75
Delan + Shirlan *	50
LSD (P=0.05)	n.s.

* = very small sample size

1/4/96 - Number of bunches/vine and bunch weight at harvest.

Treatment	No. Bunches/vine#	Wt/Bunch (g)
Untreated	45.5	30.8
Delan	49.0	29.6
Shirlan *	43.7	31.9
Delan + Shirlan *	46.7	31.7
LSD (P=0.05)	n.s.	n.s.

= Includes bunches eaten by birds and still on the vine.
* = very small sample size

Hargraves Vineyard, Summertown.
Phomopsis Trial - 1995/96.

1/4/96 - Brix reading taken at harvest.

Treatment	Brix (°)
Untreated	19.19
Delan	19.41

30/8/96 - Weight of prunings.

Treatment	Weight (kg)	
Untreated	1.063	a
Delan	0.991	a
Shirlan *	0.956	ab
Delan + Shirlan *	0.696	b
LSD (P=0.05)	sig.	

Phomopsis Dormant Trial 1995/96

Harvest Data 1995/96

CIA, Griffith

Treatment	mean Weight (kg)/vine	Bunch No.	Avg weight (g)/bunch
Untreated	14.4	163	11.3
Dormant Spray[a]	14.2	163	11.5
Dormant spray and in season spray	15.4	185	12
In season spray[b]	13.7	170	12.4

McClearys,Yenda

Treatment	mean Weight (kg)/vine	Bunch No.	Avg wt(g) /bunch
Untreated	19.4	190	102
Dormant Spray[a]	17.4	182	96
Dormant spray and in season spray	14.4	189	76
In season spray[b]	19.1	218	88

Centre of Irrigated Agriculture, Griffith
Phomopsis trial 1996/97

Harvest Data

Sender: Andrew Watson

Mean Weight calculated from 5 replicates

Treatment	mean Weight (kg)/vine	Bunch No.	Avg wt(g) /bunch
Untreated	23	270	85
Dormant Spray[a]	20	261	77
Dormant spray and in season spray	23	248	92
In season spray[b]	23	283	81

[a]mancozeb
[b]diathanon

Hillstowe Vineyards, Carey Gully.
Phomopsis Trials - 1995/96.

21/8/95 - Pre-spray assessment of phomopsis symptoms on canes.

Treatment	% vines with symptoms (bleaching or cracking)
Untreated	26.89
Delan	34.86
Combined total	**29.19**
LSD (P=0.05)	n.s.

25/9/95 - Assessment of number of spurs per vine at 30-50% budburst.

Treatment	No. spurs/vine
Untreated	52
Delan	54

All spurs pruned to 2 nodes.

6/10/95 - Assessment of shoot length and leaf no. at budburst plus 10 days.

Treatment	Shoot length (cm)	No. leaves/shoot
Untreated	4.6	2.5
Delan	4.96	2.6

16/10/95 - Budburst plus 3 weeks

Treatment	Shoot length (cm)	No. leaves/shoot
Untreated	9.55	3.45
Delan	8.74	3.51

Phomopsis Trial - 1996/97
Cabernet Sauvignon - Padthaway

26/3/97 - Bunch weight

Treatment	Spray timimg	No. bunches	Weight of bunches (Kg)	Average weight/bunch (g)
Control	No sprays applied	361	12.68	35
Dithane	Winter dormant spray	425	19.04	45
Dithane / Delan	Winter dormant spray plus spring application	470	18.24	39
Delan	Spring application only	646	30.68	47

Vines were pruned before weights of pruned canes could be taken.