GRAPE AND WINE RESEARCH COUNCIL

FINAL REPORT

Project DAS 4V: Sensitivity and tolerance to fungicides.

OBJECTIVES

This project was designed to determine the sensitivity of Australian isolates of Botrytis cinerea to commonly use fungicides and to monitor any change in sensitivity. Monitoring the downy mildew fungus for resistance to metalaxyl was also undertaken.

RESULTS

Botrytis

Over two seasons, 234 isolates of B.cinerea were recovered from 570 samples collected from the main grape growing areas of Australia. Samples were collected from rotted bunches near harvest, infected leaf or stem tissue in spring, or mummified berries and peduncles in spring. Other samples were collected at harvest by exposing agar plates 1 to 5 m down wind from crushers.

Isolates were grown on P.D.A. (potato dextrose agar) either with or without Bavistin or Rovral incorporated at the rates of 1 or 10 ug/ml which had previously been shown to discriminate between sensitive and insensitive isolates.

To date resistance to Rovral and other dicarboximide fungicides (Ronilan and Sumisclex) has not been detected. However resistance to the benzimidazole fungicides (Benomyl, Bavistin) has been found in one vineyard in the South East of South Australia.

Downy Mildew

A floating leaf disc technique was used to monitor downy mildew isolates for resistance to metalaxyl (Ridomil) and related fungicides. The incidence of downy mildew was low in the 1987 to 1989 seasons and only 32 isolates were collected. No resistance was detected.

CONCLUSION

These studies have shown that populations of Botrytis and downy mildew resistant to commonly used fungicides do not occur in Australia, or if they do they exist at undetectable levels.

The reason that resistance is not a problem in Australian vineyards is probably due to the fact that fewer sprays are applied per season compared to that in Europe.

Base levels of sensitivity have been determined and will be used for testing samples submitted in the future.

TECHNICAL INFORMATION TRANSFER

Preliminary results of this work have been presented in the technical issue of the Australian Grape grower and Wine maker.

cc cB

24 JAN 2001 SARDI

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Developing South Australian Research and Innovation

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23 January 2001

⇒ Carole
for your
follow up
please
David H
31/1/01.

Mr. D. Hall
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31/1.

Dear David,

Further to our phone conversation on the final reports of some of my projects and their use on the GWRDC website.

[1] *DAS4V Sensitivity and tolerance to fungicides.*
I have checked with the Australian Journal of Experimental Agriculture and there are no problems using the abstract, tables and figures for the paper published in Aust. J. Exp. Agric. (1987), 27: 601-4. However, if the complete article was scanned, negotiations with the publishers of the journal would be required.

[2] *DAS3V Evaluation of phosphorous acid as an inexpensive downy mildew fungicide.*
This work was published in the scientific journal, Plant Disease (1991) 75:40-43.
The document is in the public domain and can be printed providing the publisher, American Phytopathology Society is credited as the source.

[3] *DAS92/1 Biological and non conventional control of powdery mildew.*
Details of this work were published in the 10th Biennial Australian Plant Pathology Society Conference, New Zealand, 1995, pg. 98 and can be reproduced on the web site.

I trust this is sufficient details.

Yours sincerely,

TJ Wicks

DR. T.J. WICKS
Senior Plant Pathologist

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Sensitivity and tolerance to fungicides

FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **DAS 4V**

Principal Investigator: **Dr T Wicks**

Research Organisation: **Department of Agriculture (SA)**
Date: **January 1987**

DAS 4V.

Sensitivity of Australian isolates of *Plasmopara viticola* to acylanaline fungicides

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Summary. A floating leaf disk technique was used to test the sensitivity of Australian isolates of *Plasmopara viticola* to 4 acylanaline fungicides, phosethyl-Al and phosphorous acid.

Sporulation of all 15 isolates was completely inhibited by 1 mg/L of metalaxyl. Benalaxyl, oxadixyl and ofurace at 10 mg/L and phosethyl-Al and phosphorous acid at 100 mg/L also completely inhibited sporulation of each of the isolates tested for each fungicide.

These results indicate that acylanaline insensitive isolates of *P. viticola* are either not present or are not widely distributed in grape growing areas of Australia.

Cultures of selected *P. viticola* isolates have been established on tissue-cultured grapevines. Sporangia from these cultures can be used as standards for future fungicide sensitivity testing.

Introduction

Grapevine downy mildew caused by *Plasmopara viticola* (Berk. et Curt. ex de Barry) Berl. et de Toni occurs in all Australian wine-producing areas except those of Western Australia.

The disease is most destructive during late spring and early summer, the period that fungicides are frequently applied to protect bunches and leaves. Although fungicides such as copper oxychloride and mancozeb are widely used and applied in protectant schedules, the most effective material for the control of downy mildew is a formulated mixture of metalaxyl and copper oxychloride, Ridomil Plus. Because of the relative high cost of this formulation and the excellent eradicant properties of metalaxyl (Wicks and Lee 1982), it is commonly used only during high risk periods.

Strains of *P. viticola* insensitive to recommended rates of metalaxyl have developed in several grape-growing areas in other countries (Clerjeau *et al.* 1984; Herzog and Schuepp 1985; Leroux and Clerjeau 1985) resulting in the recommendations for use of acylanaline fungicides such as metalaxyl to be temporarily suspended in some areas (Leroux and Clerjeau 1985).

In Australia, the formulated mixture has been widely used since the late 1970s. Because of the possible development and spread of insensitive isolates which could have a serious impact on Australian viticulture, we determined the sensitivity of isolates to metalaxyl and related fungicides.

We also determined levels of sensitivity to a range of fungicides and attempted to establish cultures of various isolates that could be used as standards for future comparisons with suspected insensitive isolates.

Materials and methods

Isolates of *P. viticola* were collected in summer and autumn of 1986 from the grape-growing areas shown in Table 1. With the South Australian samples at least 50 infected leaves were collected from each site. The production of sporangia was induced by moistening the leaves with tap water, sealing them in plastic bags and incubating them overnight at 20–25°C. Sporulating leaves were shaken vigorously in deionised water and the suspension adjusted to about 6×10^4 sporangia per mL.

Isolates from other States were imported as water suspensions of sporangia produced in the State of origin as described above and then transported within 24 h of production. These suspensions were sprayed onto the undersurface of leaves of potted vines to produce lesions from which a further crop of sporangia was subsequently induced.

A modification of the floating leaf disk technique described by Herzog and Schuepp (1985) was used to test the sensitivity of isolates. The fungicide dilutions tested ranged from 0·01 to 500 mg/mL of active ingredient. The acylanaline fungicides used were 25% wettable powder (W.P.) formulations of benalaxyl, metalaxyl, ofurace and

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Table 1. Origin of *Plasmopara viticola* isolates

Isolate No.	Area and month collected in 1986		Cultivar	Fungicide history 1985–86
			South Australia	
	Adelaide	January	Sultana	Nil
			Muscat Gordo	Nil
SBS 86	McLaren Vale	February	Cabernet Sauvignon	Nil
			Grenache	Nil
	Nuriootpa	February	Grenache	Metalaxyl form.
			Cabernet Sauvignon	Unknown
SS 86	Padthaway	January	Shiraz	Metalaxyl form.
			Rhine Riesling	Unknown
			Unknown	Unknown
SLB 86	Loxton	March	Unknown	Unknown
SLR 86			Sultana	Unknown
			Victoria	
VM 86	Mildura	March	Sultana	Nil
			Rhine Riesling	Nil
			New South Wales	
	Hunter Valley	April	Shiraz	Nil
			Queensland	
	Stanthorpe	May	Unknown	Nil

oxadixyl. Other fungicides tested were phosethyl-Al (74% W.P.) and phosphorous acid. High concentrations of phosphorous acid were initially phytotoxic to leaf disks due to the low pH of the solution, therefore subsequent tests were conducted with phosphorous acid neutralised to pH 6·5–7 with equal concentrations of KOH. Other fungicides were not phytotoxic. Disks (10 mm diameter) were cut from expanding grape leaves of cultivars of either Cardinal, Muscat Gordo, Shiraz or Sultana. Different cultivars were used in various experiments according to the availability of leaves. Leaves of the same cultivar were used for each experiment but varied between experiments.

Each floating disk was inoculated on the lower leaf surface with a 0·025 mL drop of sporangia suspended in deionised water. From 6 to 10 leaf disks were floated in a 10 cm diameter plastic Petri dish containing 20 mL of either deionised water or fungicide suspension. Each experiment was repeated once. Floating disks were incubated on the laboratory bench beneath a window where the temperature ranged from 15 to 25°C. Presence or absence of sporulation on each disk was recorded within 7–9 days after sporulation was detected on the control disks in each experiment. The area of leaf covered with sporangia was not assessed as in most experiments sporulation occurred over most of the inoculated surface.

Sporangia that developed on disks floating on the low concentrations of fungicide were removed with fine forceps and placed into droplets of water that were then used to inoculate leaf disks floating in higher concentrations of the same fungicide.

Slight modifications of the dual culture technique described by Lee and Wicks (1982) were used to maintain isolates of *P. viticola* recovered from various grape-growing districts. Aseptic sporangia of *P. viticola* were prepared by (i) inoculating the leaves of glasshouse-grown vines by spraying the undersurface of expanding leaves with a spore suspension and then enclosing the leaves in a plastic bag overnight; (ii) after incubation, excising chlorotic lesions and then immersing them for 30 s in a 1% solution of sodium hypochlorite; (iii) washing the lesions in 3–5 rinses of sterile deionised water before placing them onto moistened, sterilised filter paper and incubating them in a closed Petri dish for 2–3 days at 20°C. Sporangia produced on the excised lesions were then used to inoculate tissue-cultured grapevines of either cv. Cabernet Sauvignon or Sultana. Two methods were used: (i) plants were immersed in a suspension of sporangia prepared by washing the excised surface-sterilised leaf tissue in

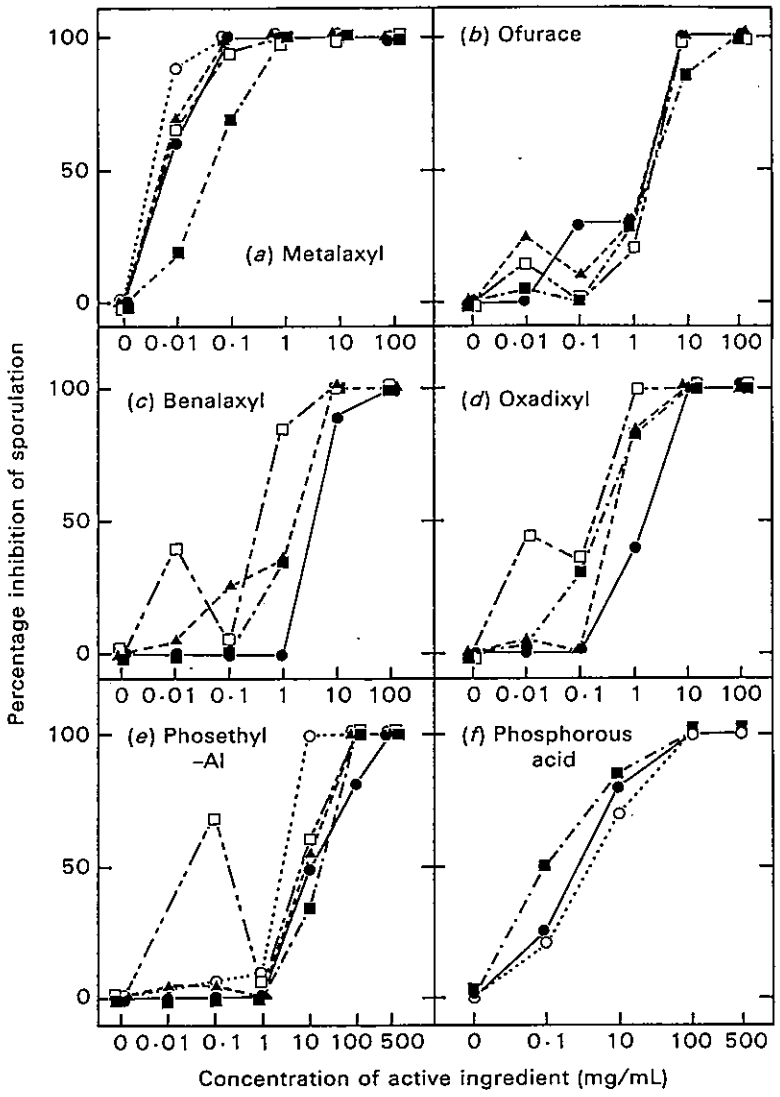


Fig. 1. Effect of fungicides on the sporulation of *Plasmopara viticola*. Isolates: ■, Hunter Valley; ●, Mildura; ▲, Loxton; □, McLaren Vale; ○, Padthaway.

deionised sterile water; (ii) fine tweezers were used to remove sporangia from sporulating leaf tissue and to place them into a drop of sterile deionised water. Drops of this suspension were picked up with a sterile loop and streaked across the undersurface of tissue-cultured grapevines. Inoculated tissue-cultured vines were returned to sealed tubes and incubated at 25°C (Lee and Wicks 1982). After 5–7 days; sporangia were produced on inoculated plants. Once dual cultures were established, cultures were maintained by regular inoculation of tissue-cultured plants.

Results

Metalaxyl at 1 mg/L or greater completely inhibited the production of sporangia in all 15 isolates tested. Since the response to metalaxyl was similar in all isolates, only 5 of these from widely different grape-growing areas are shown in Fig. 1*a*. Metalaxyl at 0·1 mg/L inhibited the production of sporangia significantly in all isolates, but at 0·01 mg/L, the degree of inhibition was more variable between isolates. Fewer isolates were tested against the other acylalanine fungicides and of these oxadixyl was the most inhibitory. Few sporangia were produced at 1·0 mg/L

oxadixyl whereas at the same concentration, benalaxyl and ofurace were less inhibitory in most of the isolates tested (Fig. 1*b*, *c* and *d*).

Benalaxyl, ofurace and oxadixyl at 10 mg/L or greater completely inhibited the production of sporangia in 90% of the isolates tested. Significant inhibition of sporulation occurred only at concentrations of 10 mg/L and above of phosethyl-Al and phosphorous acid. At 100 mg/L or greater of phosethyl-Al and phosphorous acid, sporulation was inhibited completely in all of the isolates tested (Fig. 1*e* and *f*).

When the sporangia that developed on leaf disks floated in the low fungicide concentrations were retested, all were sensitive to higher concentrations of the same fungicide (data not presented).

Discussion

Isolates of *P. viticola* insensitive to metalaxyl and related fungicides were not detected in these studies indicating that insensitive isolates do not occur in Australia or if they do, they exist at low levels and are not widespread in the main grape-growing areas.

Sporulation of all 15 isolates was completely inhibited by 1 mg/L of metalaxyl showing that the Australian isolates were similar to the sensitive isolates of *P. viticola* reported from Europe (Herzog and Schuepp 1985).

It was not possible to test large numbers of isolates in this study as in 1985–86 the disease was sporadic and rarely widespread in any grape-growing area. However, the isolates tested were in most cases obtained from large numbers of field samples from widely dispersed geographical areas and therefore represent typical Australian populations of *P. viticola*.

Because Australian vineyard managers rely heavily on metalaxyl for the control of *P. viticola* it is important that populations of the fungus be regularly monitored to detect any change in sensitivity to metalaxyl and related acylanaline fungicides. Our results have shown that metalaxyl between 1 and 10 mg/L and benalaxyl, ofurace and oxadixyl from 10 to 100 mg/L should be used to initially discriminate between sensitive and insensitive isolates.

The sensitivity of the Australian isolates to metalaxyl compared with the recent development of insensitivity in European vineyards is most likely related to the frequent use of metalaxyl in Europe compared with Australia and the consequently greater opportunity for resistant populations of the fungus to develop.

Another factor may be that metalaxyl has never been applied alone to grapes in Australia. The metalaxyl formulation registered for use on grapes in Australia contains 15% metalaxyl and 35% copper oxychloride. Thus the formulated mixture of fungicides with different modes of action may have prevented or retarded the development of insensitive strains as has been suggested for *Phytophthora infestans* in Europe (Carter *et al.* 1982).

The inhibition of sporulation with phosphorous acid suggests that further evaluation of this material is warranted as it may be a cheap and effective means of controlling *P. viticola*.

Maintaininig dual cultures of grapevines and *P. viticola* should enable fungicide sensitivity tests to be conducted throughout autumn and winter without the use of heated glasshouses or growth cabinet facilities. Several dual cultures of metalaxyl sensitive isolates from different grape-growing areas of Australia have been established and will be used as standards for testing future isolates for fungicide sensitivity.

Acknowledgments

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