Strategic use of sulphur in disease and pest management programs for dried vine fruit production

Dr Bob Emmett
VIC Department of Primary Industries

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Strategic Use of Sulphur in Disease and Pest Management Programs for Dried Vine Fruit Production

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Strategic Use of Sulphur in Disease and Pest Management Programs for Dried Vine Fruit Production

FINAL REPORT

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Final Report

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Strategic Use of Sulphur in Disease and Pest Management Programs for Dried Vine Fruit Production

Media Summary

- Sulphur is widely used to control powdery mildew and mites in Australian vineyards. Continued use of sulphur in spray programs is important to prevent the development of resistance to newer fungicides and ensure optimum control of powdery mildew.
- A research and development (R&D) project was established to address industry concerns about the indiscriminate use of sulphur, the inconsistent control of powdery mildew with sulphur and the need to develop strategies for the optimum use of sulphur in vineyards for dried grape production.
- Field studies showed that the addition of adjuvants to spray solutions of wettable sulphur did not improve the control of powdery mildew on bunches when sprays were thoroughly applied to grapevines.
- Other studies indicated that sulphur from a single, thoroughly applied spray of wettable sulphur at the recommended rate, persisted on leaves for more than 40 days. At three times the recommended rate, sulphur persisted and provided good control of powdery mildew for more than 50 days.
- Strategies for the optimum use of sulphur in disease and pest management programs were developed based on the outcomes of studies in the project and those of a similar, but more extensive, complementary project related to wine grape production.
- Industry recommendations from the projects cover the use of sulphur and other fungicides, sulphur application rates and adjuvants in spray programs for powdery mildew control. Recommendations for the control of rust and bud mites, and management of the effects of sulphur on the biological control of lightbrown apple moth are also presented.
- Recommendations for future R&D include further research on the degradation of sulphur on grape berries, sulphur phytotoxicity, and the biology and management of bud mites on grapevines.
- Information about sulphur and its optimal use for the control of powdery mildew in vineyards was communicated to industry and scientific audiences through presentations at industry and scientific conferences, seminars, workshops, discussion group meetings and field days in Victoria, New South Wales, South Australia, France, California USA and New Zealand. Publications relating to work on the project included project reports, articles in industry journals and papers in the proceedings of scientific and industry conferences and workshops.

Technical Summary

Industry issues

- Sulphur is a low cost, multi-site fungicide that is widely used to control powdery mildew (Ucinula necator) and mites in Australian vineyards. Continuation of the use of sulphur in spray programs is important to prevent the development of resistance to newer single-site fungicides and ensure optimum control of powdery mildew in vineyards.
- However, many grape growers apply sulphur frequently and indiscriminately because of its low cost. Potentially this over-use can lead to unnecessary residues in produce and adverse effects on beneficial insects and organisms, and the environment.
Furthermore, in recent years, some grape growers have reported inconsistent control of powdery mildew with sulphur. This may have resulted from a variation in efficacy of formulations, the use of wetting agents or adjuvants, spray application (rate and coverage) and/or effects of weather conditions.

Many grape growers have also moved away from the use of lime sulphur at vine woolly bud stage for mite control in recent years because the spray is expensive, undesirable to use and adversely affects predatory mite populations. Most growers now rely on wettable sulphur sprays applied early in the season and the activity of predatory mites for the control of pest mites (eg. bud, blister, bunch and rust mites).

Nevertheless, mite problems are still common in some vineyards. This may be a result of the ineffectiveness of some sulphur formulations, the reduced use of sulphur and/or the adverse effects of newer fungicides or pesticides on naturally occurring populations of predatory mites.

In view of the above, research was required to:
- re-examine the efficacy of sulphur for powdery mildew and mite control,
- clarify the effects of factors influencing the performance of sulphur, and
- develop strategies for the optimum use of sulphur in IPM programs that also minimise the potential for residues in grape products.

Research and development (R&D) strategies

Two complementary projects were established to address the required research and development (R&D). These projects were:
(1) Grape and Wine Research and Development Corporation (GWRDC) Project DAV 98/1 – Strategic use of sulphur in integrated pest and disease management (IPM) programs for grapevines, and
(2) Horticulture Australia (HA) Project DG 01002 – Strategic use of sulphur in disease and pest management programs for dried vine fruit production.

Objectives of GWRDC Project DAV 98/1 were to:
- Develop and evaluate strategies for the optimal use of sulphur in IPM programs for grapevines (with reference to monitoring, timing of sprays, rates, regional variation, and resistance).
- Examine the interaction between wettable sulphur and grapevine powdery mildew (with reference to particle size, temperature, humidity and residues).
- Examine the biology of bud and rust mites and develop diagnostic and monitoring techniques for IPM.
- Examine the effects of sulphur on the efficacy of Trichogramma wasps as a biological control agent for lightbrown apple moth.

Objectives of HA Project DG 01002 were to:
- Develop and promote strategies for the optimal use of sulphur in disease and pest management programs for dried vine fruit production,
- Examine the effects of sulphur with and without wetting agents or adjuvants on the development of grapevine powdery mildew, and
- Study the degradation of sulphur on grapevine foliage.

This report covers outcomes of HA Project DG 01002 with some reference to the outcomes of GWRDC Project DAV 98/1, especially in relation to the strategic use of sulphur in vineyard IPM programs.

Sulphur formulations, particle size and activity

Documented information and commercial knowledge of sulphur and its use for the control of powdery mildew on grapevines was reviewed, especially in relation to sulphur fungicide formulations, mode of activity, phytotoxicity, degradation, efficacy and the use of sulphur in spray programs to reduce the development of fungicide resistance.
• Sulphur formulations include dusts, wettable powders, dry flowable wettable granules (micronised sulphurs) and liquid flowables. Types of formulation differ with respect to particle size, which can affect persistence (degradation) and efficacy.

• It is generally accepted that the efficacy of sulphur for powdery mildew control is related to contact and vapour activity. Contact activity appears to have a minor role in the efficacy of sulphur products. Vapour activity has a major role but is temperature dependent. Below 15°C, sulphur activity is confined to contact activity because vapour activity is negligible.

• The degradation of sulphur on plant surfaces is related to temperature and particle size. Rainfastness is mostly related to particle size.

• Phytotoxicity can be related to sulphur particle size, temperature and relative humidity.

Effects of adjuvants on the efficacy of sulphur for powdery mildew control

• Various types of adjuvants can be used with fungicide sprays. These include wetter/spreaders (eg. Citowett®), oils (eg. Synertrol® Oil) and sticker/extenders (eg. Bond®).

• Field studies were conducted to examine the efficacy of spray programs with sulphur (Thiovit®) alone or in combination with adjuvants (Citowett®, Synertrol® or Bond®) for the control of powdery mildew on grapevines.

• In some trials, the efficacy of these programs was compared with that of programs with sulphur (Thiovit®) and a DMI fungicide (Bayfidan®).

• The addition of Citowett®, Synertrol® Oil or Bond® to spray solutions of wettable sulphur (Thiovit®) did not improve the control of powdery mildew on bunches, when sprays were thoroughly applied. However, spray programs with Thiovit® and Bond® reduced disease severity on leaves more than programs with Thiovit®, Thiovit® and Citowett®, and Thiovit® and Synertrol® Oil.

• In field trials conducted in commercial vineyards, spray programs with Thiovit® and Bayfidan® reduced disease severity on bunches more than spray programs with Thiovit® or Thiovit® and Synertrol® Oil.

Degradation of sulphur and the control of powdery mildew on grapevine foliage

• Field trials were conducted over three seasons to assess the rate of degradation and the efficacy of wettable sulphur (Thiovit®) on grapevine foliage.

• Single sprays of Thiovit® at 200g/100L (the recommended rate, Thiovit 1R) and/or Thiovit® at 600g/100L (Thiovit 3R) were applied to grapevine foliage with a commercial air-mist sprayer (in the first season) or with a hand-held sprayer (in the second and third seasons). Each season, treated and untreated leaves were sampled at 1-7 day intervals and analysed to determine percent weight for weight of total sulphur (% w/w S). In the third season, leaves were also assessed for severity of powdery mildew at 7-14 day intervals.

• Linear regression models were used to describe the change in sulphur levels and disease severity on leaves with time for each treatment.

• Estimated increments of sulphur beyond the background level on leaves just after spray application were lower when leaves on vines were sprayed with Thiovit 1R using a commercial air-mist sprayer (0.018 % w/w S) than when both sides of leaves were thoroughly hand-sprayed (0.064-0.082 % w/w S).

• When leaves were sprayed with the commercial air-mist sprayer, the estimated time for the sulphur level to return to the average background level on leaves treated with Thiovit 1R was 27 days.

• When leaves were hand-sprayed, the estimated times for sulphur levels to return to average background levels on leaves treated with Thiovit 1R and Thiovit 3R were 44-48 and 50-66 days, respectively.

• Hand-sprayed treatments with Thiovit 3R reduced powdery mildew more effectively than treatments with Thiovit 1R for at least 53 days.
The addition of a wetting agent (Citowett®) at 10mL/100L (the highest recommended rate) to Thiovit 1R did not affect the level of sulphur on leaves after spray application and subsequent sulphur degradation.

- Sulphur degradation on leaves was not affected by simulated rainfall of 10mm applied 24 hours after treatment with Thiovit 1R.
- Although sulphur levels just after treatment with Thiovit 3R were higher on older leaves than on young leaves, the rate of sulphur degradation on each group of leaves was similar.
- These field studies indicated that single sulphur treatments with good spray coverage will provide long-term control of powdery mildew on sprayed surfaces and that control can be improved by increasing application rates of wettable sulphur (Thiovit®) up to three times the recommended rate.

Outcomes of R&D in GWRDC Project DAV 98/1

- Summaries cover outcomes of the following studies.
  - The effects of spray programs containing fungicides with different chemistry on the development of powdery mildew on grapevines.
  - The effects of different sulphur formulations on the development of grape powdery mildew.
  - The effects of temperature and application rates on the degradation, efficacy and phytotoxicity of sulphur.
  - Sampling of mites in vineyards: Movement, distribution and the effects of rust mite on grapevines.
  - The effects of sulphur and other fungicides on populations of pest and predatory mites.
  - The development of species specific markers for Eriophyoid mites in grapevines.
  - The effects of sulphur on parasitism of lightbrown apple moth by Trichogramma carverae.

Technology transfer

- Information about sulphur and its optimal use for the control of powdery mildew in vineyards was communicated to scientific and industry audiences through a series of presentations and publications.
- Presentations included those given to dried vine fruit producers and chemical industry representatives at industry seminars, workshops, discussion group meetings and field days in Victoria, New South Wales and South Australia. Oral and poster presentations were also given at international scientific and industry meetings, workshops and conferences in France, California USA and New Zealand.
- Publications relating to work on the project included reports for project stakeholders and industry, articles in industry journals and papers in proceedings of scientific and industry conferences and workshops.

Recommendations for industry

- Recommendations for the strategic use of sulphur for powdery mildew control in vineyards were as follows.
  - Sprays of sulphur or alternative fungicides for powdery mildew control should be applied at 2 weeks after bud burst (when shoots are around 10cm with 5-6 separated leaves), at 4 weeks after bud burst (when shoots are around 20-30cm with 8-10 separated leaves), at 6-7 weeks after bud burst (at pre-flowering when shoots have 12-14 leaves) and/or at 8-10 weeks after bud burst (at berry set or post flowering, when berries are around 2mm).
  - Further sprays at 2-3 week intervals from berry set to berry softening may be needed, especially in disease-prone vineyards.
• Use of wettable sulphur at the recently introduced, highest recommended rate of application for powdery mildew control (i.e. 600g/100L) should increase the persistence of sulphur on vine foliage and increase disease control.
• Adjuvants such as wetter/spreaders or oils do not need to be tank mixed with sulphur sprays.
• Sulphur fungicides can cause phytotoxic effects of vine foliage and bunches in some circumstances and this should be considered when sprays are applied in vineyards.
• The application of 1-3 correctly timed demethylation inhibiting (DMI) fungicide sprays in sulphur spray programs should improve the control of powdery mildew, especially when sulphur is applied at 200g/100L.
• The use of spray programs with sulphur and fungicides with at least two other types of chemistry may also provide better disease control than sulphur programs, especially when sulphur is applied at 200g/100L.
• In sulphur and DMI fungicide spray programs, DMI sprays should be applied before and after flowering for optimum control of powdery mildew in most seasons.
• In the absence of information on the effects of higher sulphur application rates on the persistence of sulphur on grape berries and residues in dried grapes, growers could follow wine industry recommendations for sulphur spray withholding periods where required.
• Post harvest sulphur sprays may be worthwhile in some vineyards to maintain the health of vine foliage between harvest and leaf fall.

• Recommendations for the strategic use of sulphur for mite control in vineyards were as follows.
  • For the control of rust mites, a high volume spray of wettable sulphur (0.66% or 660g/100L Thiovit®) and canola oil (2% or 2L/100L Supastik®) should be applied at mid-late woolly bud. The spray should be applied to thoroughly wet the bark of vine cords and crowns when temperatures are at least 15°C.
  • Where possible, sprays of dithiocarbamate fungicides, benzimidazole fungicides, lime sulphur, chlorpyrifos and pyrethroid insecticides should be minimised or avoided because they can reduce natural predation of rust mites.
  • For the control of bud mites, it is suggested that a high volume spray of wettable sulphur at the label rate recommended for mite control after bud burst (e.g. 200g/100L Thiovit®), should be applied between 100% bud burst and 1 week after bud burst. Sprays should be applied to provide thorough coverage of young shoots.

• Recommendations for the use of sulphur in relation to the biological control of lightbrown apple moth were as follows.
  • If Trichogramma parasitoids (T. carverae) are released in vineyards to control lightbrown apple moth (LBAM), releases should be conducted at least six days after the application of wettable sulphur (at 200g/100L) to minimise the effects of the sulphur spray on the released organisms.
  • To avoid the effects of sulphur sprays on resident Trichogramma parasitoids (i.e. T. carverae and/or T. funiculatum) in vineyards, other chemicals should be used.

Recommendations for future R&D
Further R&D in the following areas would provide benefits for industry.
• Degradation of sulphur on grape berries.
  • With the introduction of higher application rates, sulphur residues on fresh and dried grapes are likely to be higher if sulphur sprays at the higher rate are applied later in the season and before harvest.
• Potential sulphur residue problems could be addressed by restricting the use of sulphur at higher application rates to earlier in the season, but further R&D is needed to support this strategy.

• Sulphur phytotoxicity
  • Further R&D is needed to increase understanding of the interaction between factors such as temperature, humidity and/or sulphur application rates that promote phytotoxicity.
  • Increased knowledge of conditions associated with phytotoxicity would be the basis for providing more definite advice on how to consistently prevent sulphur damage to vines.

• Biology and management of bud mites on grapevines
  • Further R&D on the biology and management of bud mites is needed to consolidate preliminary recommendations.

**Key project outcomes**

• Increased knowledge of:
  • Degradation and phytotoxicity of sulphur on grapevine foliage.
  • Use of sulphur for the management of grapevine powdery mildew in vineyards.
  • Introduction of higher application rates of sulphur formulations (eg. Thiovit® at 600g/100L) for the control of powdery mildew.
  • More effective spray programs for the control of powdery mildew in vineyards
  • Increased knowledge of:
    • Biology and management of grapevine mites.
    • Effects of sulphur on the parasitism of lightbrown apple moth by *Trichogramma* parasitoids.
  • Strategies for the optimal use of sulphur in programs for the control of grapevine powdery mildew and mites.
  • Increased efficiency and effectiveness of IPM programs in Australian dried grape vineyards when strategies for the optimal use of sulphur are applied.
1. Introduction

Strategic use of sulphur in disease and pest management programs for
dried vine fruit production

Bob Emmett

1.1 Background

Industry issues
- In Australia, sulphur is a low cost, multi-site fungicide that is widely used to control grapevine powdery mildew (Uncinula necator) and mites.
- The multi-site action of sulphur on the powdery mildew fungus inhibits the development of strains resistant to newer fungicides.
- Continuation of the use of sulphur in spray programs is important to prevent the development of fungicide resistant strains and ensure optimum chemical control of powdery mildew in vineyards.
- However, many grape growers apply sulphur indiscriminately because of its low cost.
- As a result, sulphur is often over-used. Potentially this can lead to unnecessary residues in produce and adverse effects on beneficial insects and organisms, and the environment.
- The inconsistent performance of sulphur against powdery mildew in some vineyards over recent years may have resulted from a variation in efficacy of formulations, the use of wetting agents, spray application (rate and coverage) and/or effects of weather conditions.
- Sulphur sprays also affect mite populations in vineyards. Although sulphur appears to have no effect on the predatory mite, Typhlodromus doreenae, it reduces populations of most pest mites (e.g. blister, bunch and rust mites).
- In recent years, many grape growers have moved away from the use of lime sulphur at vine woolly bud stage for mite control because the spray is expensive, undesirable to use and adversely affects predatory mite populations.
- Most growers now rely on wettable sulphur sprays applied early in the season for powdery mildew control and the activity of predatory mites for the control of pest mites.
- Nevertheless, mite problems are still common in many vineyards.
- This may be a result of the ineffectiveness of some sulphur formulations, the reduced use of sulphur and/or the adverse effects of newer fungicides or pesticides on naturally occurring populations of predatory mites.
- In view of the above, further studies were required to
  - re-examine the efficacy of sulphur for powdery mildew and mite control,
  - clarify the effects of factors influencing the performance of sulphur, and
  - develop strategies for the optimum use of sulphur in IPM programs that also minimise the potential for residues in grape products.

1.2 Research and Development (R&D) Strategies

- Funding proposals for research on the optimal use of sulphur in grapevine IPM programs were accepted by the Grape and Wine Research and Development Corporation and Horticulture Australia Ltd.
- Two complementary projects were established to address the required R&D. These projects were:
  - GWRDC Project DAV 98/1 – Strategic use of sulphur in integrated pest and disease management (IPM) programs for grapevines, and
HA Project DG 01002 – Strategic use of sulphur in disease and pest management programs for dried vine fruit production.

Funding support for these projects was also provided by the following R&D providers:
- Department of Primary Industries in Victoria (DPI),
- Primary Industries and Resources South Australia (PIRSA), and
- La Trobe University (LTU).

In addition, support for these projects through in-kind contributions was provided by various companies and industry groups including
- Syngenta Crop Protection, Australia and Syngenta Crop Protection AG, France
- Dow AgroSciences Australia Ltd
- Independent Horticultural Distributors Pty Ltd
- IPM Technologies Pty Ltd, and
- Wingara Wine Group

1.3 Project Objectives

Project objectives for GWRDC Project DAV 98/1
- Objectives of this project were to
  1. Develop and evaluate strategies for the optimal use of sulphur in IPM programs for grapevines (with reference to monitoring, timing of sprays, rates, regional variation, and resistance).
  2. Examine the interaction between wettable sulphur and grapevine powdery mildew (with reference to particle size, temperature, humidity and residues).
  3. Examine the biology of bud and rust mites and develop diagnostic and monitoring techniques for IPM.
  4. Examine the effects of sulphur on the efficacy of Trichogramma wasps as a biological control agent for lightbrown apple moth.

Project objectives for HA Project DG 01002
- Objectives of this project were to
  1. Develop strategies for the optimal use of sulphur in disease and pest management programs for dried vine fruit production, especially in relation to the use of wetting agents or adjuvants through the following sub-objectives:
  2. Examine the effects of sulphur with and without wetting agents or adjuvants on the development of grapevine powdery mildew,
  3. Study the degradation of sulphur on grapevine foliage, and
  4. Promote strategies that encourage the optimal use of sulphur in disease and pest management programs for dried vine fruit production.

1.4 Key Elements of the R&D

- Key elements of the R&D in relation to each project were
  - Effects of spray programs containing fungicides with different chemistry on the development of powdery mildew on grapevines (GWRDC)
  - Review of sulphur formulations and particle size (GWRDC and HA)
  - Effects of sulphur formulations on the development of grape powdery mildew (GWRDC)
  - Effects of adjuvants on the efficacy of sulphur for powdery mildew control (HA)
  - Effects of temperature and application rates on the degradation, efficacy and phytotoxicity of sulphur (GWRDC)
  - Degradation of sulphur and the control of powdery mildew on grapevine foliage (HA and GWRDC)
  - Sampling mites in vineyards: Movement, distribution and effect of rust mite on grapevines (GWRDC)
• Effects of sulphur and other fungicides on populations of pest and predatory mites (GWRDC)
• Development of species specific markers for Eriophyoid mites in grapevines (GWRDC)
• Effects of sulphur on parasitism of lightbrown apple moth by *Trichogramma carverae* (GWRDC)
• Strategies for the optimal use of sulphur in IPM programs for grapevines (GWRDC and HA)
• Communication of research (GWRDC and HA)
• Sections of the final report for each project address each of these key elements above.

1.5 Project reports

• This report covers R&D in HA Project DG 01002.
• Some reference is also made to outcomes of R&D in GWRDC Project DAV 98/1, which is covered by a separate report (Emmett, 2003).

1.6 References

2. Sulphur formulations, particle size and activity – a review

Bob Emmett, Trevor Wicks and Peter Magarey

2.1 Abstract

Documented information and commercial knowledge of sulphur and its use for the control of powdery mildew on grapevines was reviewed, especially in relation to sulphur fungicide formulations, mode of activity, phytotoxicity, degradation, efficacy and the use of sulphur in spray programs to reduce the development of fungicide resistance. Sulphur formulations include dusts, wettable powders, dry flowable wettable granules (micronised sulphurs) and liquid flowables. Formulations differ substantially with respect to particle size. Particle size can affect persistence (degradation) and efficacy. Larger particles persist and vaporise over longer periods, degrade at a lower rate and have lower efficacy. Smaller particles persist and vaporise over shorter periods, degrade at a higher rate and have higher efficacy. Sulphur efficacy for powdery mildew control is related to contact and vapour activity. Contact activity appears to have a minor role in the efficacy of sulphur products. Vapour activity is temperature dependent. Below 15°C, sulphur activity is confined to contact activity because vapour activity is negligible. Phytotoxicity can be related to sulphur particle size, temperature and relative humidity. The degradation of sulphur on plant surfaces is related to temperature and particle size. Rainfastness is mostly related to particle size. The effects of particle size and temperature can, to some extent, be offset by changes to application rates. In some countries, high application rates are used early in the season to increase efficacy during periods of low temperature. Product application rates have been established in each country on the basis of efficacy trials conducted by chemical companies. In Australia, most of these trials were conducted many years ago. Sulphur is a multi-site fungicide that is used in spray programs to minimise the selection of strains of powdery mildew that are resistant to newer single-site fungicides.


2.2 Introduction

Elemental sulphur is, undoubtedly, the oldest known pesticide. Ancient Greeks were aware of its pesticide properties as early as 1000 B.C. In the published literature, Forsyth (1802) was the first to suggest the application of sulphur for disease control (Tweedy, 1969). Since then, various forms of sulphur have been used for disease and pest control, especially for the control of powdery mildew and mites on plants. Although sulphur has been used as a fungicide for centuries, there is an apparent lack of knowledge on some aspects of its mode of activity and use in disease control programs. In view of this, a review of current knowledge of sulphur and its use for grapevine powdery mildew control was needed as a basis for further research.

2.3 Research objectives

This chapter reviews current knowledge of sulphur and its use for powdery mildew control, especially in relation to sulphur fungicide formulation, mode of activity, phytotoxicity, degradation, efficacy and the use of sulphur in spray programs to reduce the development of fungicide resistance.

2.4 Methods

Information on sulphur was compiled from published literature and reports obtained from researchers and chemical industry representatives. Sulphur fungicides, their properties and their mode of action were discussed with researchers and chemical industry representatives in Australia, Europe and the United States.
2.5 Review

*Sulphur formulations and particle size* (Thatcher and Streeter, 1925; Burchfield 1967; Somers, 1967; Hartley 1996; Mametz and Raoul 2001)

- Sulphur formulations include dusts, wettable powders, dry flowable wettable granules (micronised sulphurs) and liquid flowables.
- Micronised sulphurs are produced from sulphur residues (with 99.9% purity) which are by-products from oil refineries. Sulphur residues are infused with water and dispersants at 130°C to produce an emulsion. This is then placed in a reactor and centrifuged at high speed to produce very small droplets (1-8µm). Micronisation (in the liquid phase) is achieved by cooling to 40°C.
- Formulations differ substantially with respect to particle size. For example, the diameter of particles in dusts (D) is around 25µm; in wettable powders (WP) it is 0.1-25µm (average 7.6µm); in improved dry flowable (DF) wettable granule formulations it is mostly 1-8µm; in liquid flowables (LF) it is approximately 3.5µm (average).
- Particle size can affect persistence (degradation) and efficacy. Larger particles persist and vapourise over longer periods, degrade at a lower rate and have lower efficacy. Smaller particles persist and vapourise over shorter periods, degrade at a higher rate and have higher efficacy.

*Mode of action of sulphur fungicides* (Yarwood 1949; Tweedy, 1969; Mametz and Raoul 2001)

- Sulphur efficacy (eg. on powdery mildew) is related to contact and vapour activity.
- Contact activity usually has a minor role in the efficacy of sulphur products. Contact activity (eg. against powdery mildew conidia) declines as particle size increases. Particles of 12µm or more have less than 20% efficacy. Contact activity is not temperature dependent (providing particles have not vapourised).
- Vapour activity is temperature dependent. Below 15°C, sulphur activity is confined to contact activity because vapour activity is negligible. Vapour activity is optimal at 18-22°C.
- Sulphur inhibits germination of spores of the powdery mildew fungus although the mode of action is uncertain. Sulphur vapour may be absorbed into lipids in spores and metabolism by the fungus may lead to hydrogen sulphide (H₂S) production and blocking of respiration. The reduction of sulphur to H₂S also requires a large amount of energy (cf. Krebs cycle) which is drawn from the fungus. Sulphur has less efficacy against established mycelia of powdery mildew. Demethylation inhibiting (DMI) fungicides are superior to sulphur in this regard.
- Studies have indicated that when micronised sulphur (eg. Thiovit®) is applied to leaf surfaces:
  - 60% of sulphur is bound to lipids on leaves within 2 hours of application. Of this 60%, up to 10% is used in plant metabolism and incorporated into amino acids and other compounds;
  - 10% of sulphur is vapourised within 2 hours;
  - 10% remains on the leaf surface but is not fixed to lipids and is readily removed by washing;
  - 20% does not usually reach the leaf surface and is deposited on the ground or elsewhere.
- The effect of sulphur on mites appears to be predominantly associated with vapour activity on mite juveniles.

*Sulphur phytotoxicity* (Mametz and Raoul 2001)

- Phytotoxicity can be related to sulphur particle size. Particles less than 1µm readily vapourise (when temperatures exceed 15°C) and, at high temperatures, are absorbed into stomata where they can cause phytotoxicity. Products are more likely to cause phytotoxicity when more than 15% of particles are less than 1µm.
• Phytotoxicity occurs when sulphur (S) is oxidised to produce SO₂, which is then converted to SO₃ in humid air in stomata. The SO₃ absorbed into water to produce sulphuric acid (H₂SO₄) which burns tissues.
• Phytotoxicity is also affected by temperature and relative humidity. Sulphur phytotoxicity usually occurs within 2 hours of application when temperatures exceed 28°C. Phytotoxicity is more likely to occur when plant surfaces are dry and prevailing humidity is high. If humidity is high, phytotoxicity is more likely to occur at lower temperatures. Because of this, cut-off temperatures for potential phytotoxicity vary in different countries and regions (eg. 28°C in France, 32°C in Australia and 40°C or more in California USA). However, these cut-off temperatures may also be related to the rates of sulphur used in each country. Concentrate spraying may increase the risk of phytotoxicity.
• Phytotoxicity is also more likely to occur on foliage exposed to sunshine when temperatures exceed 28°C. In France, sprays can be applied in the early morning of a hot day, providing humidity is low.

Degradation and efficacy of sulphur (Thomas et al. 1993A, 1993B; Warren and Murphy 1998; Mametz and Raoul 2001)
• The degradation of sulphur on plant surfaces is related to temperature and particle size. At higher temperatures, efficacy is higher because particles vaporise at a higher rate. However, degradation is faster and persistence is less. At high temperatures (eg. 28-30°C or more), sulphur is not likely to persist on foliage and retain efficacy for more than 10 days. This is also likely to depend on the initial rate of application and the type of leaf surface.
• Washing effects of rain or overhead irrigation are mostly on the upper surface of leaves and on the outer parts of vine canopies. If rain occurs within 2 hours of application, micronised sulphur sprays should be re-applied. After 2 hours, micronised sulphur sprays are relatively washable and may only need to be re-applied if rainfall exceeds 25mm. This may not apply to wettable powders, which are likely to be less washable, or dusts, which are not rain-fast. Rainfastness is mostly related to particle size. Particles of 25µm are not rainfast

Application rates (Mametz and Raoul 2001)
• The effects of particle size and temperature can, to some extent, be offset by changes to application rates. In some countries (eg. France, Italy and Switzerland), high application rates are used early in the season to increase efficacy during periods of low temperature.
• Application rates vary between countries (eg. 1-1.2 kg/100L or 10-12 kg/ha in France, Italy, and Switzerland; 600g/100L or 6 kg/ha in the USA and most other countries, and 200-300g/100L or 2-3 kg/ha in Australia and South Africa).
• Product application rates have been established in each country on the basis of efficacy trials conducted by chemical companies. In Australia, most of these trials were conducted many years ago.

Effects of adjuvants (Mametz and Raoul 2001)
• Sulphur wettable powders are affected by some dispersants. Dispersants in emulsifiable concentrate formulations mixed with wettable powders in the spray tank can lead to flocculation (clumping) of sulphur particles which are then likely to rapidly settle on the floor of the tank and block nozzles. After flocculation, the dose applied to foliage and subsequent efficacy may be reduced substantially.
• Dry flowable micronised sulphur formulations (eg. Thiovit®) are not prone to immediate flocculation and can be mixed with a wider range of commonly used dispersants.
• Non-ionic wetting agents (eg. Citowett®) reduce the surface tension of water and appear to improve dispersion of particles and coverage. However, experience indicates that the addition of wetters to sprays may increase run-off and limit the amount of spray (and chemical) retained on canopies.
Use of sulphur to reduce the development of powdery mildew fungicide resistance (Steva 1994, 1995; Gubler et al. 1996; Ypema et al. 1997; Steva 2001)

- The excessive use of fungicides with single-site activity (eg. DMI fungicides) can increase the selection of strains of powdery mildew that are resistant to these fungicides.
- Sulphur is a multi-site fungicide that is used in spray programs to minimise the selection of strains of powdery mildew that are resistant to DMI fungicides.
- The aim of anti-resistance spraying strategies is to minimise or eliminate the selection of strains with DMI resistance. Increases in populations of DMI resistant strains can result in reduced control of powdery mildew in vineyards.
- Potential strategies include:
  1. changing the rate or dose of the DMI fungicide;
  2. using fungicide mixtures (eg. tank mixes of a DMI fungicide and sulphur);
  3. alternating single or groups of sprays of DMI fungicides with those of sulphur.
- Steva conducted large replicated field experiments in vineyards in Portugal and France over four consecutive years to determine the efficacy and shifts in fungicide resistance associated with spray programs containing different combinations of sulphur (Thiovit®) and DMI fungicide (Bayfidan®) sprays. Each year, six or seven sprays were applied at two-week intervals. Usually, three sprays were applied before flowering, one spray was applied at flowering and three sprays were applied after flowering.
- In Experiment 1, the dose rate of Bayfidan® was reduced to half of the recommended rate. Bayfidan® resistance increased and the efficacy of Bayfidan® decreased.
- In Experiment 2, spray programs with Bayfidan® (B) were compared with spray programs where tank mixes of Bayfidan® and sulphur (B+S) were applied. With B and B+S spray programs, Bayfidan® resistance increased to 47% and 25%, respectively.
- In Experiment 3, spray programs with the following fungicide treatments were compared (where B = Bayfidan®, S = sulphur and f = flowering):
  1. Bayfidan® only (ie. B, B, B, f, B, B, B);
  2. Bayfidan® followed by sulphur six hours later (ie. B/S, B/S, B/S, f, B/S, B/S, B/S);
  4. Sulphur then Bayfidan® treatments alternated (ie. S, B, S, f, B, S, B).
- Studies with C14 Bayfidan® showed that the uptake of Bayfidan® into grapevine foliage was reduced from 70% to 10% when Bayfidan® was tank-mixed with sulphur. Hence, the efficacy of Bayfidan® for powdery mildew control was reduced.
- The application of Bayfidan® and then sulphur six hours later resulted in the additive effects of both fungicides (in relation to powdery mildew control). The uptake and efficacy of Bayfidan® was not reduced by sulphur.
- When sulphur sprays were alternated with Bayfidan® sprays, the increase in Bayfidan® resistance was substantially less than when Bayfidan® alone was sprayed repeatedly.
- In Experiment 4, eight different sulphur and DMI fungicide spray programs were applied over three seasons. The programs, along with comments on their efficacy and the development of Bayfidan® resistance, are summarised in Table 1.

Table 1. The effects of Bayfidan® and sulphur spray programs on the control of powdery mildew and shifts in powdery mildew resistance to Bayfidan®.

<table>
<thead>
<tr>
<th>Spray Program</th>
<th>Efficacy (Powdery mildew control)</th>
<th>Shift in Bayfidan® resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) B, B, B, f, S, S, S</td>
<td>Good in all years</td>
<td>Little or no shift</td>
</tr>
<tr>
<td>(2) S, S, S, f, B, B, B</td>
<td>Poor in 2 out of 3 years</td>
<td>Higher shift in resistance than (1)</td>
</tr>
<tr>
<td>(3) B, B, B, f, B, B, B</td>
<td>Poor in all years</td>
<td>Highest shift in resistance</td>
</tr>
<tr>
<td>(4) S, S, f, S, S, S</td>
<td>Poor in some years</td>
<td>No shift in resistance</td>
</tr>
<tr>
<td>Spray Program</td>
<td>Efficacy (Powdery mildew control)</td>
<td>Shift in Bayfidan® resistance</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>(5) B, B, S, f, S, S</td>
<td>Good in most years</td>
<td>Higher shift than with (6) &amp; (7)</td>
</tr>
<tr>
<td>(6) S, B, B, f, B, S</td>
<td>Good in all years</td>
<td>Lowest shift in resistance</td>
</tr>
<tr>
<td>(7) S, S, B, f, B, B, S</td>
<td>Good in all years</td>
<td>Lowest shift in resistance</td>
</tr>
<tr>
<td>(8) S, S, f, S, B, B</td>
<td>Poor in some years</td>
<td>Higher shift in resistance than (5) but less than (3)</td>
</tr>
</tbody>
</table>

- These results support conclusions from studies in Australia (Chapter 5.1) that showed that two or three sprays of a DMI fungicide in a sulphur spray program should be applied around flowering for optimum control of powdery mildew and management of fungicide resistance.

*Use of sulphur for Phomopsis control* (Bugaret 2001)

- Field studies in France showed that high rates of wettable sulphur (1000g/100L) applied at or just after bud-burst provided good control of *Phomopsis*. The level of control was similar to that provided by mancozeb. The latter is widely used for *Phomopsis* control in Australian vineyards.

### 2.6 Summary

Sulphur formulations include dusts, wettable powders, dry flowable wettable granules (micronised sulphurs) and liquid flowables. Formulations differ with respect to particle size, which can affect persistence (degradation) and efficacy. Sulphur efficacy for powdery mildew control is related to contact and vapour activity. Contact activity appears to have a minor role in the efficacy of sulphur products. Vapour activity is temperature dependent. Phytotoxicity can be related to sulphur particle size, temperature and relative humidity. The degradation of sulphur on plant surfaces is related to temperature and particle size. Rainfastness is mostly related to particle size. Sulphur is a multi-site fungicide that is used in spray programs for powdery mildew control and to minimise the selection of strains of powdery mildew that are resistant to newer single-site fungicides.

### 2.7 Acknowledgements

The author thanks Graeme Hardwick and Scott Mathew, Syngenta Crop Protection (Australia) and Raymond Mametz and Thierry Roaul, Syngenta/Novartis Agro S.A. (France) for assistance and comments, Syngenta Crop Protection (Australia) and Syngenta/Novartis Agro S.A. (France) for financial support during a European study tour in 2001, and the Grape and Wine Research and Development Corporation, Horticulture Australia and the Department of Primary Industries in Victoria for financial support.

### 2.8 References


Steva, H. (2001). Personal communication. Meeting at Biorizon, Martillac, France


3. Effects of some adjuvants on the efficacy of sulphur for powdery mildew control

Bob Emmett, Shelley Rozario and Julie Hawtin

3.1 Abstract

Field studies were conducted to examine the efficacy of spray programs with sulphur (Thiovit®) alone, or in combination with adjuvants (Citowett®, Synertrol® or Bond®), for the control of powdery mildew on Sultana and Chardonnay grapevines. In some trials, the efficacy of these programs was compared with that of programs with sulphur (Thiovit®) and a DMI fungicide (Bayfidan®). When sprays were thoroughly applied, the addition of Citowett®, Synertrol® or Bond® to spray solutions of Thiovit® did not improve the control of powdery mildew on bunches. However, spray programs with Thiovit® and Bond® reduced disease severity on leaves more than programs with Thiovit®, Thiovit® and Citowett®, and Thiovit® and Synertrol®. In field trials conducted in commercial vineyards, spray programs with Thiovit® and Bayfidan® reduced disease severity on bunches more than spray programs with Thiovit® or Thiovit® and Synertrol®.

3.2 Introduction

Various types of adjuvants are available for use with fungicides, pesticides and weedicide sprays in Australian viticulture (Dawson, 2002). These include wetter/spreaders (eg. Citowett®), super wetter/penetrants (eg. Brushwet®), oils (eg. Synertrol® Oil), water softeners (eg. Liquid Boost®), drift reductants (eg. DrifteX®), acidifiers (eg. Companion®) and sticker/extenders (eg. Bond®).

Wetter/spreaders are used to lower the surface tension of water and increase the spread of chemicals over plant surfaces. This can be important with fungicides or pesticides that rely on contact with the fungus or pest for efficacy. Wetter/spreaders, however, can make droplets smaller and increase spray drift during spray application.

Super wetter/spreaders have similar properties to wetter/spreaders except that they are able to spread droplets up to 15 times further than wetter/spreaders. Super wetter/spreaders also have ‘penetrant’ qualities that make them superior to wetter/spreaders.

Oils are also used to lower the surface tension of water and improve spray coverage. However, as oils increase the average size of droplets, they can be used to reduce spray drift and reduce the evaporation of droplets. Some oils also dissolve the waxy cuticle of leaves and assist the penetration of pesticides or weedicides.

Water softeners are used as water conditioners to remove excess calcium and magnesium ions from ‘hard water’. With some products, excess calcium and magnesium ions can bind with pesticides and reduce efficacy.

Drift retardants are designed to create medium sized droplets that are heavy enough not to drift but are light enough to be retained on leaf surfaces. Some drift retardants also have ‘penetrant’ qualities.

Acidifiers are specifically designed to lower the pH of water and prevent the hydrolysis of some pesticides that can also reduce efficacy. Acidifiers are not wetting agents.
Stickers/extenders are designed to stick pesticides onto plant surfaces, improve rain fastness and extend the life of pesticide residues by protecting them from breakdown by ultra violet light. Stickers/extenders also are not wetting agents.

Historically, some dried grape growers have used wetters in fungicide sprays, including those with sulphur wettable powders, to improve spray coverage. In recent years, however, most labels of sulphur fungicides, especially those of improved formulations, do not indicate that there is a need to tank mix products with adjuvants. Most manufacturers of sulphur fungicides claim that their formulations contain sufficient spreaders to negate the need for tank mixing additional adjuvants. Nevertheless, some dried grape growers have reported that the use of wetters or oils has improved the control of powdery mildew in their vineyards. Other growers have occasionally observed increased phytotoxicity on grapevine foliage when vegetable oils [eg. canola oil (Syntrol® Oil)] have been used with sulphur fungicides.

In view of the above, field studies were needed to determine whether strategies for the use of sulphur fungicides in spray programs should include the use of adjuvants, especially non-ionic wetters or vegetable oils.

### 3.3 Research objectives

This chapter reports studies conducted to determine the efficacy of spray programs with sulphur (Thiovit®) alone, or in combination with selected adjuvants, for the control of powdery mildew on grapevines.

### 3.4 Methods

Field trials to determine the efficacy of programs with sprays of sulphur or sulphur tank mixed with selected adjuvants and DMI fungicides for powdery mildew control were conducted at Irymple Victoria (Vic.) (Field Trials 1 and 2) and Iraak Vic. (Field Trial 3) in 1999/2000. Vines at all trial sites had a recent history of powdery mildew disease. Irymple and Iraak are located approximately 5km east and 35km south of Mildura Vic., respectively, in the Sunraysia district.

**Field Trial 1. (Irymple Vic.)**

*Trial design.* Details of the design of Field Trial 1 are summarised in Table 1. Four rows of vines were used in the trial. Each ‘treated’ row was separated by an untreated buffer row. Each ‘treated’ row contained seven plots. Plots within each ‘treated’ row were separated by two or more untreated vines. A plot with each treatment appeared in each row at least once, but not more than twice. There were six replicate plots for treatment programs 3, 4 and 5, and five replicate plots for treatment programs 1 and 2.

*Treatments.* The five treatment programs evaluated in Field Trial 1 are summarised in Table 2. Fungicides used in the spray programs were wettable sulphur (Thiovit®, 800g/kg sulphur, Syngenta Crop Protection, Adelaide SA) applied at 200g/100L (the recommended application rate), and a widely used DMI fungicide, triadimenol (Bayfidan®, 250g/L triadimenol, Bayer Australia, Pymble NSW) applied at 10mL/100L (the recommended application rate). Adjuvants tank mixed with wettable sulphur (Thiovit®) were Citowett® Spreader-sticker (a non-ionic surfactant, 1000g/L alkylphenol ethoxylate, BASF Australia, Noble Park Vic.) at 6mL/100L (the lower recommended rate), Synertrol® Oil (a vegetable oil based adjuvant, 600mL/L canola oil and 100mL/L polyethoxylated oil, Organic Crop Protectants, Lilyfield NSW) at 200mL/100L (the recommended rate) or Bond® (a dispersant, 450g/L synthetic latex, Nufarm, Laverton North Vic.) at 140mL/100L (the recommended rate).
Table 1. Summary of the design of Field Trial 1 at Irymple Vic. in 1999/2000.

<table>
<thead>
<tr>
<th>Grapevine variety</th>
<th>Vitis vinifera cv. Sultana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design</td>
<td>Randomised block</td>
</tr>
<tr>
<td>Replicates (plots)</td>
<td>4-7</td>
</tr>
<tr>
<td>Plot size</td>
<td>3 vines</td>
</tr>
<tr>
<td>Age of vines</td>
<td>20+ years</td>
</tr>
<tr>
<td>Vines spacing</td>
<td>3.4m between rows</td>
</tr>
<tr>
<td></td>
<td>2.5m between vines</td>
</tr>
<tr>
<td></td>
<td>(1176 vines/ha)</td>
</tr>
<tr>
<td>Pruning</td>
<td>Cane pruned. Four cordons, each cordon with 2-4 canes and each cane with 12-16 buds</td>
</tr>
<tr>
<td>Trellising</td>
<td>T-trellis, 0.9m wide with foliage wires at 1.3m.</td>
</tr>
</tbody>
</table>

In the spray programs, the first three sprays were applied when shoots were approximately 10 cm (2 weeks after bud burst), 20-30 cm (4 weeks after bud burst) and 30-40 cm (6 weeks after bud burst). The last three sprays were applied at 2-3 week intervals from just after flowering (berry set) to berry softening (Table 2). Treatments were applied using a trailer-mounted sprayer with a hand wand operated at 200-300psi (1400-2000kPa). All vines were thoroughly sprayed to the point of run-off.

Table 2. Treatment programs applied to Sultana grapevines in Field Trial 1 at Irymple Vic. in 1999/2000.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth stage number</td>
<td>12-14</td>
<td>16-17</td>
<td>18</td>
<td>27</td>
<td>29</td>
<td>31-32</td>
<td>34</td>
</tr>
<tr>
<td>Growth stage</td>
<td>5-7 leaves, shoots 10-20cm</td>
<td>10-12 leaves, shoots 30-40cm</td>
<td>Pre-flowering (14 leaves)</td>
<td>Berry set, berries 2-3 mm</td>
<td>Berries 4-5mm</td>
<td>Berries pea-size (7-8mm)</td>
<td>Berry softening</td>
</tr>
<tr>
<td>Program 1 (untreated)</td>
<td>S²</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Program 2</td>
<td>S³</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Program 3</td>
<td>S+C⁴</td>
<td>S+C</td>
<td>S+C</td>
<td>S+C</td>
<td>S+C</td>
<td>S+C</td>
<td>-</td>
</tr>
<tr>
<td>Program 4</td>
<td>S+S⁵</td>
<td>S+S</td>
<td>S+S</td>
<td>S+S</td>
<td>S+S</td>
<td>S+S</td>
<td>-</td>
</tr>
</tbody>
</table>
Assessments of disease. Incidence of powdery mildew (percentage of leaves, berries and bunch stems diseased) and severity of powdery mildew (percentage of the area of leaves, berries and bunch stems diseased) were assessed on 24 bunches and 24 leaves per plot. The assessments were conducted before harvest on 24 January 2000, using standardised diagrammatic assessment keys (Emmett, unpublished). Twelve bunches were selected at random from the bunch zone along the length of each side of vines in the centre of each plot. Leaves were sampled from four typical shoots with bunches, evenly spaced along each side of vines in the centre of each plot. Three primary leaves, equidistant along the length of each shoot, were sampled (ie. one close to the bunch, one half way along the shoot and one about five nodes from the shoot apex). Leaves that were young, not fully expanded (ie. aged less than 10 days) and had not had sufficient time to develop disease were excluded from the assessments.

Field Trial 2. (Irymple Vic.)

Trial Design. The design of Field Trial 2 is summarised in Table 3. The trial was conducted in a vineyard established for commercial production, where plots with untreated vines were unacceptable because of the risk of substantial crop loss. Plots with each treatment program were randomised within each of four blocks.

Table 3. Summary of the design of Field Trial 2 at Irymple Vic. in 1999/2000.

<table>
<thead>
<tr>
<th>Grapevine variety</th>
<th>Vitis vinifera cv. Chardonnay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design</td>
<td>Randomised block</td>
</tr>
<tr>
<td>Replicates (plots)</td>
<td>4</td>
</tr>
<tr>
<td>Plot size</td>
<td>4 rows, each row with 24 vines</td>
</tr>
<tr>
<td>Age of vines</td>
<td>5 years</td>
</tr>
<tr>
<td>Vines spacing</td>
<td>3.0m between rows</td>
</tr>
<tr>
<td></td>
<td>2.5m between vines</td>
</tr>
<tr>
<td>Pruning</td>
<td>Minimally pruned. Four cordons, two cordons on each foliage wire</td>
</tr>
<tr>
<td>Trellising</td>
<td>Two wire vertical trellis, with foliage wires at 1.2m and 1.7m.</td>
</tr>
</tbody>
</table>

Treatments. The two treatment programs evaluated in Field Trial 2 are summarised in Table 4. Fungicides used in the spray programs were wettable sulphur (Thiovit®, 800g/kg sulphur, Syngenta
Crop Protection, Adelaide SA) applied at 200g/100L (the recommended application rate), and a widely used DMI fungicide, triadimenol (Bayfidan®, 250g/L triadimenol, Bayer Australia, Pymble NSW) applied at 10mL/100L (the recommended application rate). In Program 2, an adjuvant, Synertrol® Oil (a vegetable oil based adjuvant, 600mL/L canola oil and 100mL/L polyethoxylated oil, Organic Crop Protectants, Lilyfield NSW) at 200mL/100L (the recommended rate) was tank mixed with Thiovit®. All treatments were applied with a Charlie Agromaster® air blast sprayer (model: AT 2000).

Table 4. Treatment programs applied to Chardonnay grapevines in Field Trial 2 at Irymple Vic. in 1999/2000.

<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth stage number</td>
<td>12-14</td>
<td>16-17</td>
<td>18</td>
<td>27</td>
<td>29</td>
<td>31-32</td>
<td>34</td>
</tr>
<tr>
<td>Growth stage</td>
<td>5-7 leaves, shoots 10-20cm</td>
<td>10-12 leaves, shoots 30-40cm</td>
<td>Pre-flowering (14 leaves)</td>
<td>Berry set, berries 2-3 mm</td>
<td>Berries 4-5mm</td>
<td>Berries pea-size, pre-bunch closure</td>
<td>Berry softening</td>
</tr>
<tr>
<td>Program 1</td>
<td>S²</td>
<td>B³</td>
<td>B</td>
<td>B</td>
<td>S</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Program 2</td>
<td>S+S⁴</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>S+S</td>
<td>S+S</td>
<td>-</td>
</tr>
</tbody>
</table>

¹Growth stage number according to Eichhorn and Lorenz modified by Coome (1995).
²S = Thiovit® applied at 200g/100L.
³B = Bayfidan® applied at 10mL/100L.
⁴S+S = Thiovit® applied at 200g/100L tank mixed with Synertrol® at 200mL/100L.

Assessments of disease. Incidence of powdery mildew (percentage of leaves, berries and bunch stems diseased) and severity of powdery mildew (percentage of the area of leaves, berries and bunch stems diseased) were assessed on 24 bunches and 24 leaves per plot. The assessments were conducted before harvest on 17 February 2000, using standardised diagrammatic assessment keys (Emmett, unpublished). Twelve bunches were selected at random from the bunch zone along the length of each side of vines in each of the two centre rows of each plot. Leaves that were young, not fully expanded (ie. aged less than 10 days) and had not had sufficient time to develop disease were excluded from the assessments.

Field Trial 3. (Iraak Vic.)

Trial Design. The design of Field Trial 3 is summarised in Table 5. The trial was conducted in a commercial vineyard where plots with untreated vines were unacceptable because of the risk of substantial crop loss.

Treatments. The three treatment programs evaluated in Field Trial 3 are summarised in Table 6. Fungicides used in the spray programs were the same as in Field Trial 2. All treatments were applied with a low volume, Hardi® air mist sprayer.

Assessments of disease. Incidence of powdery mildew (percentage of leaves, berries and bunch stems diseased) and severity of powdery mildew (percentage of the area of leaves, berries and bunch stems diseased) were assessed on 64 leaves and 64 bunches per plot. The assessments were conducted before harvest on 8 February 2000, using standardised diagrammatic assessment keys (Emmett,
unpublished). Thirty-two leaves and 32 bunches were selected at random for assessment along the length of each side of vines in each of the two centre rows of each plot. Leaves that were young, not fully expanded (ie. aged less than 10 days) and had not had sufficient time to develop disease were not sampled and were excluded from the assessments.

Table 5. Summary of the design of Field Trial 3 at Iraak Vic. in 1999/2000.

<table>
<thead>
<tr>
<th>Grapevine variety</th>
<th>Vitis vinifera cv. Chardonnay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design</td>
<td>Randomised block</td>
</tr>
<tr>
<td>Replicates (plots)</td>
<td>4</td>
</tr>
<tr>
<td>Plot size</td>
<td>4 rows, each row with 106 vines</td>
</tr>
<tr>
<td>Age of vines</td>
<td>7 years</td>
</tr>
<tr>
<td>Vines spacing</td>
<td>3.0m between rows</td>
</tr>
<tr>
<td></td>
<td>2.5m between vines</td>
</tr>
<tr>
<td>Pruning</td>
<td>Minimally pruned. Four cordons, two cordons on each foliage wire</td>
</tr>
<tr>
<td>Trellising</td>
<td>Two wire vertical trellis, with foliage wires at 1.2m and 1.7m.</td>
</tr>
</tbody>
</table>

Table 6. Treatment programs applied to Chardonnay grapevines in Field Trial 3 at Iraak Vic. in 1999/2000.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth stage number</td>
<td>12-14</td>
<td>15-16</td>
<td>17-18</td>
<td>19</td>
<td>27</td>
<td>31-32</td>
<td>34</td>
</tr>
<tr>
<td>Growth stage</td>
<td>5-6 leaves, shoots 10-20cm</td>
<td>10 leaves, shoots 20-30cm</td>
<td>12-14 leaves, shoots 30-40cm</td>
<td>Early flowering</td>
<td>Berry set, berries 2-3 mm</td>
<td>Berries pea-size, pre-bunch closure</td>
<td>Berry softening</td>
</tr>
<tr>
<td>Program 1</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>-</td>
</tr>
<tr>
<td>Program 2</td>
<td>S+S</td>
<td>S+S</td>
<td>S+S</td>
<td>S+S</td>
<td>S+S</td>
<td>S+S</td>
<td>-</td>
</tr>
<tr>
<td>Program 3</td>
<td>S</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>S</td>
<td>S</td>
<td>-</td>
</tr>
</tbody>
</table>

1Growth stage number according to Eichhorn and Lorenz modified by Coombe (1995).
2S = Thiovit® applied at 200g/100L.
3S+S = Thiovit® applied at 200g/100L tank mixed with Synertrol® at 200mL/100L.
4B = Bayfidan® applied at 10ml/100L.

Monitoring of environmental conditions. Maximum and minimum daily temperatures (°C) and rainfall (mm) were recorded from September to April in 1999/2000 using automatic electronic weather stations located in or close to vineyards where the field studies were conducted.
Assessments of fungicide phytotoxicity. Foliage and bunches on vines in each plot were inspected regularly, usually one to seven days after each treatment, and any damage associated with fungicide treatments was assessed using standardised diagrammatic assessment keys (Emmett, unpublished). Occurrence of phytotoxicity was recorded in relation to environmental conditions.

Statistical analyses
The Genstat statistical package (Genstat 5 Committee, 1993) was used to analyse the assessment data. Incidence data, i.e. the percentage of bunches and leaves with powdery mildew, were transformed before analysis using the angular transformation (i.e. arcsine of the square root of the percentage) when required. Severity scores for powdery mildew on leaves and bunches were log transformed when required. The method of residual maximum likelihood (REML) was used to determine if there were effects of spray treatments on disease incidence and severity. Least significant difference (lsd) tests were used to investigate differences between particular treatments.

Values presented in Figures 1-12 are back transformed REML adjusted means of the angular transformed data for disease incidence and the log-transformed data for disease severity.

3.5 Results

Field Trial 1. (Irymple Vic.)
All spray programs did not reduce powdery mildew incidence on the upper and lower surface of leaves (Figures 1 and 2). An exception was the program with sulphur (Thiovit®) and Bond®. This program slightly reduced disease incidence on the upper surface of leaves (Figure 1). All spray programs substantially reduced disease severity on both leaf surfaces. On each surface, the Thiovit® and Bond® program provided slightly better disease control than most of the other programs.

On berries and bunch stems, all spray programs reduced disease severity to a similar level (Figures 3 and 4). However, none of the spray programs reduced powdery mildew incidence.

Field Trial 2. (Irymple Vic.)
Spray programs with sulphur (Thiovit®) with or without Synertrol® and a DMI fungicide (Bayfidan®) provided a similar level of powdery mildew control on leaves, berries and bunch stems (Figures 5-8). With both programs, less disease developed on the upper surface of leaves than on the lower surface. (Figures 5 and 6). More disease developed on bunch stems than on berries (Figures 7 and 8).

Field Trial 3. (Iraak Vic.)
The use of Synertrol® with sulphur (Thiovit®) did not improve the control of powdery mildew. Spray programs with sulphur (Thiovit®) or sulphur with Synertrol® provided a similar level of powdery mildew control on leaves, berries and bunch stems (Figures 9-12). On the lower surface of leaves, however, the spray program with sulphur and Synertrol® did not reduce disease severity as much as the sulphur program (Figure 10). The sulphur and DMI fungicide (Bayfidan®) program reduced severity of disease on berries and bunch stems more than the sulphur or sulphur with Synertrol® programs (Figures 11 and 12).

Phytotoxicity
No phytotoxic effects were observed on vine foliage and bunches after treatments in all trials, with the exception of the first treatment with sulphur and Synertrol® in Field Trials 1 and 2. With the latter, some burning was observed on the tips of young actively growing shoots three days after the treatments were applied.
3.6 Discussion

In these studies, sprays were thoroughly applied with a hand wand or a typical commercial air blast sprayer. Under these circumstances, the addition of Citowett\textsuperscript{®}, Synertrol\textsuperscript{®} or Bond\textsuperscript{®} to spray solutions of Thiovit\textsuperscript{®} did not improve the control of powdery mildew on bunches. Spray programs with Thiovit\textsuperscript{®} and Bond\textsuperscript{®}, however, reduced disease severity on leaves slightly more than programs with Thiovit\textsuperscript{®}, Thiovit\textsuperscript{®} and Citowett\textsuperscript{®}, and Thiovit\textsuperscript{®} and Synertrol\textsuperscript{®}. These results were obtained from one set of tests on selected adjuvants at recommended application rates. More extensive tests, covering a wider range of adjuvants, application rates (dose rates per 100L and per ha) and trial situations, are needed to fully evaluate the effects of adjuvants. These extensive tests were beyond the scope of this project. If these tests are conducted, it is likely that some spray adjuvants will improve the efficiency of disease control in some situations [eg. when there are lower rates of application (spray volumes per ha) or lower doses of fungicide on the surface of foliage or bunches] as reported by some authors (Gaskin and Manktelow, 2002).

In studies of the degradation of sulphur on grapevine leaves (Chapter 7), the addition of Citowett\textsuperscript{®} to spray solutions of Thiovit\textsuperscript{®} at the recommended rate (200g/100L) did not affect the rate of sulphur degradation. This lack of effect of Citowett\textsuperscript{®} is consistent with the absence of its effect on spray efficacy reported here.

3.7 Summary

The addition of Citowett\textsuperscript{®}, Synertrol\textsuperscript{®} or Bond\textsuperscript{®} to spray solutions of Thiovit\textsuperscript{®} did not improve the control of powdery mildew on bunches, when sprays were thoroughly applied. However, spray programs with Thiovit\textsuperscript{®} and Bond\textsuperscript{®} reduced disease severity on leaves more than programs with Thiovit\textsuperscript{®}, Thiovit\textsuperscript{®} and Citowett\textsuperscript{®}, and Thiovit\textsuperscript{®} and Synertrol\textsuperscript{®}. In field trials conducted in commercial vineyards, spray programs with Thiovit\textsuperscript{®} and Bayfidan\textsuperscript{®} reduced disease severity on bunches more than spray programs with Thiovit\textsuperscript{®} or Thiovit\textsuperscript{®} and Synertrol\textsuperscript{®}.

3.8 Acknowledgements

The authors thank Horticulture Australia, the Grape and Wine Research and Development Corporation, and the Department of Primary Industries in Victoria for financial assistance. They also thank John Reynolds for assistance with statistical analyses, David Lia, Christiane Jaeger and Lisa Mitchell for skilled technical assistance, farm staff at the Sunraysia Horticultural Centre at Irrymple Vic., and the Wingara Wine Group at Iraak Vic. for maintenance of the grapevine plantings used for the field studies and assistance with the application of fungicide treatments in Field Trials 2 and 3.

3.9 References


Figure 1. Effects of spray programs with sulphur (Thiovit\textsuperscript{®}), and sulphur tank mixed with different adjuvants (Thiovit\textsuperscript{®} + Citowett\textsuperscript{®}, Thiovit\textsuperscript{®} + Synertrol\textsuperscript{®} or Thiovit\textsuperscript{®} + Bond\textsuperscript{®}) on the incidence and severity of powdery mildew on the upper surface of leaves on Sultana grapevines in Field Trial 1 at Irymple Vic. on 24 January 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].

Figure 2. Effects of spray programs with sulphur (Thiovit\textsuperscript{®}), and sulphur tank mixed with different adjuvants (Thiovit\textsuperscript{®} + Citowett\textsuperscript{®}, Thiovit\textsuperscript{®} + Synertrol\textsuperscript{®} or Thiovit\textsuperscript{®} + Bond\textsuperscript{®}) on the incidence and severity of powdery mildew on the lower surface of leaves on Sultana grapevines in Field Trial 1 at Irymple Vic. on 24 January 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].
Figure 3. Effects of spray programs with sulphur (Thiovit®), and sulphur tank mixed with different adjuvants (Thiovit® + Citowett®, Thiovit® + Synertrol® or Thiovit® + Bond®) on the incidence and severity of powdery mildew on berries on Sultana grapevines in Field Trial 1 at Irymple Vic. on 24 January 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].

Figure 4. Effects of spray programs with sulphur (Thiovit®), and sulphur tank mixed with different adjuvants (Thiovit® + Citowett®, Thiovit® + Synertrol® or Thiovit® + Bond®) on the incidence and severity of powdery mildew on stems of bunches on Sultana grapevines in Field Trial 1 at Irymple Vic. on 24 January 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].
Figure 5. Effects of spray programs with sulphur (S, Thiovit®) or sulphur (Thiovit®) tank mixed with Synertrol (S+S) and a DMI fungicide (B, Bayfidan®) on the incidence and severity of powdery mildew on upper surface of leaves on Chardonnay grapevines in Field Trial 2 at Irymple Vic. on 17 February 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].

Figure 6. Effects of spray programs with sulphur (S, Thiovit®) or sulphur (Thiovit®) tank mixed with Synertrol (S+S), and a DMI fungicide (B, Bayfidan®) on the incidence and severity of powdery mildew on lower surface of leaves on Chardonnay grapevines in Field Trial 2 at Irymple Vic. on 17 February 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].
Figure 7. Effects of spray programs with sulphur (S, Thiovit®) or sulphur (Thiovit®) tank mixed with Synertrol (S+S), and a DMI fungicide (B, Bayfidan®) on the incidence and severity of powdery mildew on berries on Chardonnay grapevines in Field Trial 2 at Irymple Vic. on 17 February 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].

Figure 8. Effects of spray programs with sulphur (S, Thiovit®) or sulphur (Thiovit®) tank mixed with Synertrol (S+S), and a DMI fungicide (B, Bayfidan®) on the incidence and severity of powdery mildew on stems of bunches on Chardonnay grapevines in Field Trial 2 at Irymple Vic. on 17 February 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].
Figure 9. Effects of spray programs with sulphur (S, Thiovit®) or sulphur (Thiovit®) tank mixed with Synertrol (S+S) alone or in combination with a DMI fungicide (B, Bayfidan®) on the incidence and severity of powdery mildew on the upper surface of leaves on Chardonnay grapevines in Field Trial 3 at Iraak Vic. on 8 February 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].

Figure 10. Effects of spray programs with sulphur (S, Thiovit®) or sulphur (Thiovit®) tank mixed with Synertrol (S+S) alone or in combination with a DMI fungicide (B, Bayfidan®) on the incidence and severity of powdery mildew on the lower surface of leaves on Chardonnay grapevines in Field Trial 3 at Iraak Vic. on 8 February 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].
Figure 11. Effects of spray programs with sulphur (S, Thiovit®) or sulphur (Thiovit®) tank mixed with Synertrol (S+S) alone or in combination with a DMI fungicide (B, Bayfidan®) on the incidence and severity of powdery mildew on berries on Chardonnay grapevines in Field Trial 3 at Iraak Vic. on 8 February 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].

Figure 12. Effects of spray programs with sulphur (S, Thiovit®) or sulphur (Thiovit®) tank mixed with Synertrol (S+S) alone or in combination with a DMI fungicide (B, Bayfidan®) on the incidence and severity of powdery mildew on the stems of bunches on Chardonnay grapevines in Field Trial 3 at Iraak Vic. on 8 February 2000. [For each type of assessment, treatments with a lower case letter in common were not significantly different (P>0.05)].
4. Degradation of sulphur and the control of powdery mildew on grapevine foliage

Bob Emmett, Peter Magarey, John Reynolds, Carla Magarey and Kathy Clarke

4.1 Abstract

In Australia, wettable sulphur is widely used to control grapevine powdery mildew (Uncinula necator). Field trials during 1999, 2000 and 2001 at Loxton, SA, were conducted to assess the rate of degradation and efficacy of wettable sulphur. In 1999, a single spray of wettable sulphur (Thiovit®) at 200g/100L (recommended rate, Thiovit 1R) was applied to the foliage of grapevines with a commercial air-mist sprayer. In 2000 and 2001, single sprays of Thiovit 1R or Thiovit® at 600g/100L (Thiovit 3R) were hand-sprayed onto recently expanded leaves of similar size and age on unsprayed grapevines infected with powdery mildew. In all years, a selection of treated leaves was taken at 1-7 day intervals from Day –1 up to Day 49 after treatment for analysis to determine percent weight for weight sulphur (% w/w S). In 2001, remaining treated leaves were assessed for severity of powdery mildew at 7-14 day intervals from Day 0-53 after treatment.

Linear regression models described the change in sulphur levels and disease severity with time for each treatment. In 1999, estimated levels of sulphur on leaves that were untreated or treated with Thiovit 1R, just after spray application on Day 0, were 0.046 and 0.064 % w/w S, respectively. The estimated time for the sulphur level to return to the average background level (0.044 % w/w S) on leaves treated with Thiovit 1R was 27 days. In 2000, estimated levels of sulphur on leaves that were untreated, treated with Thiovit 1R and treated with Thiovit 3R, just after spray application on Day 0, were 0.060, 0.142 and 0.330 % w/w S, respectively. The estimated times for sulphur levels to return to average background levels (0.060 % w/w S) on leaves treated with Thiovit 1R and Thiovit 3R were 48 and 66 days, respectively. In 2001, estimated levels of sulphur on leaves that were untreated, treated with Thiovit 1R or treated with Thiovit 3R, just after spray application on Day 0, were 0.051, 0.115 and 0.229 % w/w S, respectively. Sulphur levels on leaves treated with Thiovit 1R or Thiovit 3R returned to the background level (0.044 % w/w S) in 44 and 50 days, respectively. Treatments with Thiovit 3R reduced powdery mildew more effectively than Thiovit 1R until Day 53 after treatment.

The field studies indicated that single sulphur treatments with good spray coverage will provide long-term control of powdery mildew on sprayed surfaces and that control can be improved by increasing application rates of wettable sulphur (Thiovit®) up to three times the recommended rate. The persistence and efficacy of Thiovit 1R also appeared to be related to the method of application and spray coverage, viz. levels of sulphur on leaves just after treatment. Estimated increments of sulphur beyond the background level on leaves just after spray application, were lower when leaves on vines were sprayed with Thiovit 1R using a commercial air-mist sprayer (0.018 % w/w S) than when both sides of leaves were thoroughly hand-sprayed (0.064-0.082 % w/w S). The implications of this in relation to the control of powdery mildew in vineyards are discussed.

The addition of a wetting agent (Citowett®) at 10mL/100L (the highest recommended rate) to Thiovit 1R did not affect the level of sulphur on leaves after spray application and subsequent sulphur degradation. Sulphur degradation on leaves was not affected by simulated rainfall of 10mm applied 24 hours after treatment with Thiovit 1R. Although sulphur levels just after treatment with Thiovit 3R were higher on older leaves than on young leaves, the rate of sulphur degradation on each group of leaves was similar.

Publications on this R&D: Emmett et al. 2002.
4.2 Introduction

In Australia, wettable sulphur is widely used in spray programs for the control of grape powdery mildew (Uncinula necator). Recommended rates of application range from 100g to 300g per 100L water, depending on the sulphur formulation and time of the season. Spray intervals usually range from 10 to 14 days.

4.3 Background and previous research

Recent studies by Wicks et al. (2002) indicated that control of grapevine powdery mildew in Australia could be improved by the application of higher rates of wettable sulphur. This applied particularly to Thiovit®, a formulation of wettable sulphur that is widely used in Australian viticulture. However, more studies were needed to increase understanding of the degradation and bio-efficacy of wettable sulphur on grapevine foliage after each spray application in Australian vineyards.

Previous studies of the application of some wettable sulphur formulations to other plants have shown that portions of the sulphur applied were either vapourised, attached to waxes on the plant surface and slowly vapourised, or absorbed and added to levels of sulphur already present in plant tissues (R. Mametz, personal communication; Chapter 3).

4.4 Research objectives

This chapter reports field studies of the degradation of sulphur on grapevine leaves and its effects on the development of powdery mildew after the application of single sprays of wettable sulphur. The studies also examined the effects of simulated rainfall, leaf age and the use of a wetting agent with sulphur sprays on the degradation of sulphur on grapevine foliage.

4.5 Methods

Trial sites.
Field experiments were conducted during March-April 1999, February-March 2000 and March-May 2001 in a vineyard at Loxton in the Riverland district of South Australia (SA). Treatments were applied to mature, minimally pruned grapevines (Vitis vinifera cv. Sultana) infected with U. necator.

Weather conditions.
During the experiments, weather data were collected with a Campbell Scientific CR 10 electronic weather station in an adjacent vineyard.

Experimental design.
Treated vines were arranged in four adjacent blocks with and without overhead irrigation, and with and without spray application. Each block comprised three rows of eight vines within the vineyard and all blocks were separated by two barrier rows. Each block contained four randomised plots, each with six vines. Vines in each plot had large canopies averaging 1.2 m high and 2 m wide with 3.05 m between rows.

Each fungicide treatment was applied to two recently expanded leaves of similar age and size on each of four shoots in close proximity that had not been sprayed previously. In 2001, some fungicide treatments were also applied to the two older leaves immediately below the two recently expanded leaves on each shoot to study the effects of leaf age. The sets of four adjacent shoots with each
treatment were replicated three times and arranged in a randomised block design with blocks deployed sequentially along six rows in the vineyard. Treated recently expanded leaves were fully flat, averaged 60-70 mm from tip to petiolar sinus and had emerged 7-10 days previously. Treated older leaves averaged 85-90 mm from tip to petiolar sinus and had emerged at least 14 days previously. Four recently expanded leaves and four older leaves on two of the shoots in each set were sampled for sulphur analyses and, in 2001, four recently expanded leaves and four older leaves on the two remaining shoots were used to study the development of powdery mildew.

Treatments in 1999.
Effects of sulphur treatments.  
Fungicide treated vines were sprayed with wettable sulphur (Thiovit®, 80% a.i. sulphur, Syngenta Crop Protection, Adelaide SA) at 200g/100L (the recommended application rate, Thiovit 1R) using an Orchard Aid® air-mist sprayer that applied 36.7L/minute at 300kpa while travelling at 5.26 km/hour (h). The sprayer delivered 1,373L/ha or 41.2L/100m of vine row, which was equivalent to standard grower practice for minimally pruned vines at the time of the year when the studies were conducted. This application rate was less than that recommended under the unit canopy row method (Furness et al. 1998) for best spray coverage of this canopy, viz. 72L/100m of vine row or 2,399L/ha. To optimise the uniformity of spray application in relation to the vine canopy, the sprayer travelled up and down each row so that the spray was applied only from the right side of the fan, which spun anti-clockwise.

Effects of simulated rainfall (overhead irrigation).  
Leaves on vines in irrigated plots that were untreated or treated with Thiovit 1R were exposed to overhead irrigation equivalent to 10mm of simulated rainfall. This irrigation was applied 24h after spray application to examine the effects of rain on sulphur levels.

Effects of sulphur treatments. Upper and lower surfaces of all treated leaves were hand sprayed with Thiovit 1R or Thiovit® at 600g/100L (Thiovit 3R). All leaves were sprayed lightly so that each surface was thoroughly covered without run-off. Between 4.0 and 4.7mL of spray was applied to each leaf.

Effects of sulphur with a wetting agent. In addition, the upper and lower surfaces of some sets of leaves were sprayed with a solution of Thiovit® at 200g/100L and a non-ionic wetting agent (Citowett®, 1000g/L alkylarylpolyglycol ether, AgrEvo, Glen Iris Vic.) at 10mL/100L (Thiovit 1R + C) to study the effects of including a wetting agent with the sulphur treatment.

Effects of simulated rainfall (overhead irrigation). In 2000, additional sets of leaves that were untreated or treated with Thiovit 1R were exposed to overhead irrigation equivalent to 10mm of simulated rainfall 24h after spray application to examine the effects of rain on sulphur levels.

Effects of leaf age. In 2001, Thiovit 3R was applied to older leaves (described above) as well as to recently expanded young leaves.

Sulphur analyses.  
In 1999, leaves were sampled for sulphur analysis on the day prior to treatment (Day –1), on the day of treatment (Day 0) and on Days 1, 3, 5, 7, 14 and 28 after treatment. At each sampling time, sets of 15 leaves of similar age and size, selected at random from the upper, middle and lower sectors (5 leaves from each sector) of the canopy of vines in each plot with each treatment, were collected (with minimal contact and residue disturbance) and successively stored at –20°C.

In 2000 and 2001, leaves were sampled for sulphur analysis on the day prior to treatment (Day –1), on the day of treatment (Day 0) and on Days 1, 3, 5, 7, 14, 28, 35, 42 and 49 after treatment. At each
sampling time, sets of four leaves with each treatment were collected (with minimal contact and residue disturbance) and successively stored at –20°C.

After all of the samples had been collected and stored, each sample was macerated, digested with nitric acid and analysed using an inductively coupled plasma-emission spectrometer at 182.034nm to determine percent weight for weight sulphur (% w/w S).

Disease development.
In 2001, the percent area with active powdery mildew on the upper and lower surfaces of recently expanded leaves and older leaves was assessed just before treatment on Day 0, 14 days later, and again every 7-10 days to Day 53 after treatment, using a standardised assessment key (Emmett, unpublished). At the time of treatment, up to 11 percent of the surface area of leaves had powdery mildew.

Statistical analyses.
Linear regression with a grouping factor, as implemented in the GenStat statistical package (GenStat Committee, 2000) was used to investigate the change in sulphur levels and disease severity with time for each treatment. Variance ratio (or F) tests and pair-wise t-tests of the regression coefficients were used to indicate models of best fit. A method like Fieller’s method, (Seber, 1977) was used to obtain 95% confidence intervals (CI) for the “inverse prediction” of the time for sulphur levels to return to background levels.

4.6 Results

Weather conditions.
Average median temperatures for the periods of study in 1999, 2000 and 2001 were 17.5°C, 22.6°C and 15.5°C, respectively.

Sulphur degradation in 1999.
Effects of simulated rainfall (overhead irrigation). A linear regression model was used to describe changes in the levels of sulphur on leaves from grapevines under the four treatment regimes [ie. untreated, with or without rain, and treated with Thiovit 1R, with or without rain]. Results of variance ratio (or F) tests and pair-wise t-tests of the regression coefficients indicated that there were no significant differences between untreated leaves with and without rain and between Thiovit 1R treated leaves with and without rain, in relation to their intercepts and slopes. Hence, simulated rainfall or overhead irrigation of 10mm did not affect the degradation of sulphur on leaves.

Effects of sulphur treatments. The results of variance ratio (or F) tests and pair-wise t-tests of the regression coefficients of the model above indicated that there were significant differences between the untreated and Thiovit 1R treated leaves in their intercepts and slopes. As there were no significant differences between leaves “with rain” and “without rain” in their intercepts and slopes, a model with separate linear degradation trends for the two groups of treatments (untreated and Thiovit 1R) provided a good fit to the sulphur levels through time (Figure 1). The adjusted R² for this model was 74.3%. The estimated regression coefficients, as well as estimates of the time taken for sulphur levels to return to the average background sulphur level (0.044 % w/w), are presented in Table 1.

Estimated levels of sulphur on leaves that were untreated or treated with Thiovit 1R, just after spray application on Day 0 (ie. intercepts of the linear regression model in Table 1), were 0.046 and 0.064 % w/w S, respectively. Hence, by deduction, the estimated increment in sulphur level on leaves after a single spray application was 0.018 % w/w S. Total sulphur on untreated leaves stayed very close to the background level. As a result, there was a wide 95% CI for the time taken to reach the background level. Total sulphur levels on leaves treated with Thiovit 1R were estimated to take 27 days to return to the background level.
Table 1. Estimates of regression coefficients and the predicted time for sulphur levels to return to the average background level (0.044 % w/w S) on leaves on Sultana grapevines at Loxton SA in 1999.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Intercept¹</th>
<th>Slope¹</th>
<th>Predicted time (days) taken to reach 0.044 % w/w sulphur</th>
<th>Lower 95% CI² for time taken (days)</th>
<th>Upper 95% CI² for time taken (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.0460 a</td>
<td>-0.000221 b</td>
<td>9.2</td>
<td>-35.7</td>
<td>53.1</td>
</tr>
<tr>
<td>Thiovit 1R</td>
<td>0.0637 b</td>
<td>-0.000734 a</td>
<td>26.8</td>
<td>14.5</td>
<td>39.5</td>
</tr>
</tbody>
</table>

¹ Within a column, estimates with a lower case letter in common were not significantly different (P>0.05).
² CI = confidence interval

Figure 1. Graph of the fitted linear model for the degradation of sulphur on leaves of Sultana grapevines treated with Thiovit at Loxton SA in 1999.

Sulphur degradation in 2000.
Effects of simulated rainfall (overhead irrigation). A linear regression model was used to investigate changes in the levels of sulphur on leaves in all of the treatment regimes. These regimes included untreated, with or without rain, and treated with Thiovit 1R, with or without rain. Variance ratio (or F) tests and pair-wise t-tests of the regression coefficients indicated that there were no significant differences between untreated leaves with and without rain and between Thiovit 1R treated leaves with and without rain in relation to their intercepts and slopes. Consequently, simulated rainfall or overhead irrigation of 10mm did not affect the degradation of sulphur on leaves.

Effects of sulphur treatments. A model with separate linear degradation trends for three groups of treatments (untreated, Thiovit 1R and Thiovit 3R) provided a good fit to the sulphur residue levels
through time in 2000 (Figure 2). The adjusted $R^2$ for this model was 95.5%. The estimated regression coefficients as well as estimates of the time taken for sulphur levels to return to the average background sulphur level (0.060 % w/w S) are presented in Table 2.

Table 2. Estimates of regression coefficients and the predicted time for sulphur levels to return to the average background level (0.060 % w/w S) on leaves on Sultana grapevines at Loxton SA in 2000.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Intercept¹</th>
<th>Slope¹</th>
<th>Predicted time (days) taken to reach 0.060% w/w sulphur</th>
<th>Lower 95% CI² for time taken (days)</th>
<th>Upper 95% CI² for time taken (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.0597 a</td>
<td>0.000398 c</td>
<td>0.733</td>
<td>-120.4</td>
<td>106.1</td>
</tr>
<tr>
<td>Thiovit 1R</td>
<td>0.142 b</td>
<td>-0.00171 b</td>
<td>47.5</td>
<td>28.2</td>
<td>69.1</td>
</tr>
<tr>
<td>Thiovit 3R</td>
<td>0.330 c</td>
<td>-0.00413 a</td>
<td>65.5</td>
<td>56.8</td>
<td>74.4</td>
</tr>
</tbody>
</table>

¹ Within a column, estimates with a lowercase letter in common were not significantly different (P>0.05).
² CI = confidence interval

Figure 2. Graph of the fitted linear model for the degradation of sulphur on leaves of Sultana grapevines treated with Thiovit at Loxton SA in 2000.
Estimated levels of sulphur on leaves that were untreated, treated with Thiovit 1R or treated with Thiovit 3R, just after spray application on Day 0 (ie. intercepts of the linear regression model in Table 2), were 0.060, 0.142 and 0.330 % w/w S, respectively. Hence, by deduction, the estimated increments in sulphur level on leaves after a single spray of Thiovit 1R and a single spray of Thiovit 3R were 0.082 and 0.270 % w/w S, respectively. Total sulphur on untreated vines stayed very close to the background level. As a result, there was a wide 95% CI for the time taken to reach the background level. Total sulphur levels on leaves treated with Thiovit 1R or Thiovit 3R were estimated to take 48 and 66 days, respectively to return to the background level.

Effects of sulphur with a wetting agent.
When separate linear degradation trends were included in a regression model for the six treatment groups (ie. untreated with and without rain, Thiovit 1R with and without rain, Thiovit 1R + C without rain, and Thiovit 3R without rain), the adjusted R² was 95.6%. This was nearly the same as the R² of the simpler model (ie. 95.5%, Figure 2) that ignored “rain” and the presence or absence of Citowett®. The estimated regression coefficients for this more detailed model are presented in Table 3.

Table 3. Estimates of coefficients for the linear regression model for six treatment groups including Thiovit® at the recommended rate plus Citowett® (Thiovit 1R + C), used to investigate the effects of the wetting agent, Citowett® on the degradation of sulphur on leaves on Sultana grapevines at Loxton SA in 2000.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Intercept¹</th>
<th>Slope¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.0588 a</td>
<td>0.000366 c</td>
</tr>
<tr>
<td>Untreated + rain</td>
<td>0.0616 a</td>
<td>0.000462 c</td>
</tr>
<tr>
<td>Thiovit 1R</td>
<td>0.1498 c</td>
<td>-0.001798 b</td>
</tr>
<tr>
<td>Thiovit 1R + rain</td>
<td>0.1415 bc</td>
<td>-0.001855 b</td>
</tr>
<tr>
<td>Thiovit 1R + C</td>
<td>0.1334 b</td>
<td>-0.001524 b</td>
</tr>
<tr>
<td>Thiovit 3R</td>
<td>0.3303 d</td>
<td>-0.004128 a</td>
</tr>
</tbody>
</table>

¹ Within a column, estimates with a lowercase letter in common were not significantly different (P>0.05).

The addition of Citowett® to a solution containing Thiovit 1R had no effect on degradation of sulphur after spray application. However, the estimated amount of sulphur “delivered” at Day 0 was less with the addition of Citowett®. The similarity in degradation was also reflected in the estimates of the time taken for the sulphur levels on leaves to return to background levels (0.060 %w/w S) which were nearly identical, namely 50 days with a 95% CI of 31 to 70 days for Thiovit 1R without Citowett®, and 48 days with a 95% CI of 26 to 72 days for Thiovit 1R with Citowett®.

Effects of sulphur treatments. In 2001, separate linear degradation trends were also required for the three groups of treatments, untreated (control), Thiovit 1R and Thiovit 3R (Figure 3). The adjusted R² for this model was 75.4%. The estimated regression coefficients for this model as well as estimates of the time taken for sulphur levels to return to the average background level (0.044 % w/w S) are presented in Table 4. Estimated levels of sulphur on leaves that were untreated, treated with Thiovit 1R or treated with Thiovit 3R, just after spray application on Day 0 (ie. intercepts of the linear regression model in Table 4), were 0.051, 0.115 and 0.229 % w/w S, respectively. Hence, by deduction, the estimated increments in sulphur level on leaves after a single spray of Thiovit 1R and a single spray of Thiovit 3R were 0.064 and 0.178 % w/w S, respectively. As with the previous models, in this model total sulphur on untreated vines stayed very close to the background level. As a result, there was a very wide 95% CI for the time taken to reach the background level. Total sulphur levels on leaves treated with Thiovit 1R and Thiovit 3R were estimated to take 44 and 50 days, respectively to return to the background level.
Table 4. Estimates of coefficients for the linear regression model and the predicted time for sulphur levels to return to the average background level (0.044 % w/w S) on leaves on Sultana grapevines at Loxton SA in 2001.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Intercept¹</th>
<th>Slope¹</th>
<th>Predicted time (days) taken to reach 0.044% w/w sulphur</th>
<th>Lower 95% CI² for time taken (days)</th>
<th>Upper 95% CI² for time taken (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.0513 a</td>
<td>-0.000251 c</td>
<td>29.0</td>
<td>-∞</td>
<td>+∞</td>
</tr>
<tr>
<td>Thiovit 1R</td>
<td>0.115 b</td>
<td>-0.00162 b</td>
<td>43.6</td>
<td>6.8</td>
<td>82.0</td>
</tr>
<tr>
<td>Thiovit 3R</td>
<td>0.229 c</td>
<td>-0.00374 a</td>
<td>49.6</td>
<td>33.6</td>
<td>65.9</td>
</tr>
</tbody>
</table>

¹Within a column, estimates with a lower case letter in common were not significantly different (P>0.05).
²CI = confidence interval

As well as the comparatively low adjusted R² value, this fitted model had some deficiencies. The fitted values for the Thiovit® treatments systematically under-estimated sulphur levels at Days 0 and 1, then over-estimated levels between Days 5 and 21, and then under-estimated levels at Days 42 and 49 in relation to the observed values.

Figure 3. Graph of the fitted linear model for the degradation of sulphur on leaves of Sultana grapevines treated with Thiovit® at Loxton SA in 2001.

Some of this curvature could be accommodated by fitting exponential curves, namely,

\[ y = a + br^x \]

where \( y \) = total sulphur, \( x \) = elapsed time (days) since application, \( r \) = the rate of exponential decrease, \( a \) = the lower asymptote for the total sulphur and \( b \) = the range of the decay curve between the initial amount of sulphur at Day 0 and the asymptotic value.
Separate exponential curves (Figure 4) were required for the three groups of treatments (untreated, Thiovit 1R and Thiovit 3R). The adjusted R² for this model was 85.3%. The estimated regression coefficients for this model as well as estimates of the time taken for sulphur levels to return to the average background level (0.044 % w/w S) are presented in Table 5.

**Table 5. Estimates of coefficients for the exponential regression model and the predicted time for sulphur levels to return to the average background level (0.044 % w/w S) on leaves on Sultana grapevines at Loxton SA in 2001.**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Asymptote¹ (a)</th>
<th>Range¹ (b)</th>
<th>Rate¹ (r)</th>
<th>Predicted time (days) taken to reach 0.044% w/w sulphur</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.0420 a</td>
<td>0.0155 a</td>
<td>0.842 a</td>
<td>11.8</td>
</tr>
<tr>
<td>Thiovit 1R</td>
<td>0.0549 a</td>
<td>0.0831 b</td>
<td>0.889 a</td>
<td>+∞</td>
</tr>
<tr>
<td>Thiovit 3R</td>
<td>0.0968 b</td>
<td>0.208 c</td>
<td>0.845 a</td>
<td>+∞</td>
</tr>
</tbody>
</table>

¹Within a column, estimates with a lower case letter in common were not significantly different (P > 0.05).

According to this fitted model, sulphur levels on the Thiovit® treated leaves will never return to the background level of 0.044% w/w sulphur because the estimated asymptotic levels of sulphur on leaves treated with Thiovit 1R and Thiovit 3R were 0.055% and 0.097% w/w sulphur, respectively. This fitted model also overshot the observed values for the Thiovit treatments at the final assessment time (Day 49).

**Figure 4.** Graph of the fitted exponential model for the degradation of sulphur on leaves of Sultana grapevines treated with Thiovit® at Loxton SA in 2001.

A simpler linear degradation model (Figure 5), restricted to fewer observations (ie. those from 12 days post application), provided a better estimate of the decay in total sulphur in the later stages of the study but did not fit as well because it was based on fewer observations. The adjusted R² for this
model was 72.3%. The estimated regression coefficients for this model as well as estimates of the time taken for sulphur levels to return to the average background level are presented in Table 6.

Table 6. Estimates of coefficients for the linear regression model for observations from Day 12 to Day 49 and the predicted time for sulphur levels to return to the average background level (0.044 % w/w sulphur) on leaves on Sultana grapevines at Loxton SA in 2001.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Intercept¹</th>
<th>Slope¹</th>
<th>Predicted time (days) taken to reach 0.044% w/w sulphur</th>
<th>Lower 95% CI² for time taken (days)</th>
<th>Upper 95% CI² for time taken (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.0422 a</td>
<td>0.0000054 b</td>
<td>327.5</td>
<td>-∞</td>
<td>+∞</td>
</tr>
<tr>
<td>Thiovit 1R</td>
<td>0.0796 b</td>
<td>-0.000632 a</td>
<td>56.3</td>
<td>10.7</td>
<td>111.1</td>
</tr>
<tr>
<td>Thiovit 3R</td>
<td>0.137 c</td>
<td>-0.00113 a</td>
<td>82.8</td>
<td>57.0</td>
<td>113.8</td>
</tr>
</tbody>
</table>

¹ Within a column, estimates with a lower case letter in common were not significantly different (P>0.05).
² CI = confidence interval

This model provided more conservative estimates of the time for sulphur levels to return to background levels for Thiovit 1R and Thiovit 3R (ie. 56 and 83 days, respectively).

Effects of sulphur with a wetting agent.

When separate linear degradation trends were included in a regression model for the four treatment groups (ie. untreated, Thiovit 1R, Thiovit 1R + C and Thiovit 3R), the adjusted R² was 75.5%. This was nearly the same as the R² of the simpler model (ie. 75.4, Figure 3) that ignored the presence or absence of Citowet®. The estimated regression coefficients for the full model are presented in Table 7.
Table 7. Estimates of coefficients for the linear regression model for four treatment groups including Thiovit® at the recommended rate plus Citowett® (Thiovit 1R + C), used to investigate the effects of the wetting agent, Citowett® on the degradation of sulphur on leaves on Sultana grapevines at Loxton SA in 2001.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Intercept¹</th>
<th>Slope¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.0513 a</td>
<td>-0.000251 c</td>
</tr>
<tr>
<td>Thiovit 1R</td>
<td>0.1239 b</td>
<td>-0.001833 b</td>
</tr>
<tr>
<td>Thiovit 1R + C</td>
<td>0.1057 b</td>
<td>-0.001410 b</td>
</tr>
<tr>
<td>Thiovit 3R</td>
<td>0.2293 c</td>
<td>-0.003737 a</td>
</tr>
</tbody>
</table>

¹ Within a column, estimates with a lowercase letter in common were not significantly different (P>0.05).

The addition of Citowett® to a solution containing Thiovit 1R had no effect on degradation of sulphur after spray application. This was also reflected by estimates of the time taken for the sulphur levels on leaves to return to background levels (0.044 %w/w S). These estimates were nearly identical, namely, 43.6 days with a 95% CI of 11 to 77 days for Thiovit 1R without Citowett®, and 43.7 days with a 95% CI of 1 to 88 days for Thiovit 1R with Citowett®.

**Effects of leaf age.**
A regression model with separate linear trends for the four combinations of Thiovit® (untreated and Thiovit 3R) and leaf age (young leaves and older leaves) was used to study the effects of leaf age on sulphur degradation. The adjusted R² for this model was 83.9%. The estimated coefficients for this fitted model are presented in Table 8.

Table 8. Estimates of coefficients for the linear regression model for the four treatment combinations of Thiovit® and leaf age, used to investigate the effects of leaf age on the degradation of sulphur on leaves on Sultana grapevines at Loxton SA in 2001.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Intercept¹</th>
<th>Slope¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated, Young Leaves</td>
<td>0.0523 a</td>
<td>-0.000282 b</td>
</tr>
<tr>
<td>Thiovit 3R, Young Leaves</td>
<td>0.2148 b</td>
<td>-0.003306 a</td>
</tr>
<tr>
<td>Untreated, Older Leaves</td>
<td>0.0463 a</td>
<td>-0.000126 b</td>
</tr>
<tr>
<td>Thiovit 3R, Older Leaves</td>
<td>0.2593 c</td>
<td>-0.003913 a</td>
</tr>
</tbody>
</table>

¹ Within a column, estimates with a lowercase letter in common were not significantly different (P>0.05).

After treatment with Thiovit 3R, the older leaves had significantly higher initial levels of sulphur than the young leaves. However, the rate of decline from these initial levels was the same for leaves of each age. Correspondingly, given the different intercepts but the same slopes, estimates of the time taken for the levels of sulphur to return to background levels (0.044 %w/w S), were slightly shorter for the young leaves, i.e. 51.7 days with a 95% CI of 34.4 to 69.4 days for the young leaves compared to 55.0 days with a 95% CI of 40.4 to 70.0 days for the older leaves.

**Disease development.**
**Effects of sulphur treatments on disease development.** A regression model with separate linear trends for three groups of treatments (untreated, Thiovit 1R and Thiovit 3R) provided a good fit to the levels of powdery mildew on the upper and lower surfaces of leaves during the period from 14 to 53 days after treatment in 2001.
**Disease on the upper leaf surface.** The adjusted $R^2$ for the model fitted to disease levels on the upper surface of leaves (Figure 6) was 91.3%. Because the percentage of leaf area covered by powdery mildew on Day 0 ranged from 5.8%-11.0%, increases in levels of powdery mildew from Days 0-14 were non-linear, especially on untreated leaves. In 2001, Thiovit 1R and Thiovit 3R reduced disease development until Day 53. The estimated regression coefficients for this model are presented in Table 9.


<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Intercept¹</th>
<th>Slope¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>28.6 b</td>
<td>1.12 c</td>
</tr>
<tr>
<td>Thiovit 1R</td>
<td>1.32 a</td>
<td>0.781 b</td>
</tr>
<tr>
<td>Thiovit 3R</td>
<td>4.65 a</td>
<td>0.121 a</td>
</tr>
</tbody>
</table>

¹ Within a column, estimates with a lowercase letter in common were not significantly different ($P>0.05$).

**Figure 6.** Linear regression model for the increase in grapevine powdery mildew on the upper surface of Sultana leaves treated with Thiovit® at Loxton, South Australia in 2001.

**Disease on the lower leaf surface.** The adjusted $R^2$ for the model fitted to disease levels on the lower surface of leaves (Figure 7) was 91.7%. Again, because the percentage of leaf area covered by powdery mildew on Day 0 ranged from 5.8%-11.0%, increases in levels of powdery mildew from Days 0-14 were non-linear, especially on untreated leaves. In 2001, Thiovit 1R and Thiovit 3R reduced disease development until Day 53. The estimated regression coefficients for this model are presented in Table 10.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Intercept¹</th>
<th>Slope¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>47.7 b</td>
<td>0.672 b</td>
</tr>
<tr>
<td>Thiovit 1R</td>
<td>18.5 a</td>
<td>0.362 a</td>
</tr>
<tr>
<td>Thiovit 3R</td>
<td>13.8 a</td>
<td>0.090 a</td>
</tr>
</tbody>
</table>

¹ Within a column, estimates with a lowercase letter in common were not significantly different (P>0.05).

Figure 7. Linear regression model for the increase in grapevine powdery mildew on the lower surface of Sultana leaves treated with Thiovit® at Loxton, South Australia in 2001.

Effects of sulphur with a wetting agent on disease development.

Disease on the upper leaf surface. A regression model with separate linear degradation trends for the four treatment groups in Table 11 was used to examine the effects of sulphur with the wetting agent, Citowett® (Thiovit 1R + C) on disease development on the upper surface of leaves. The adjusted R² for this model was 92.2%. The estimated regression coefficients for this model are presented in Table 10.

The addition of Citowett® to a solution containing Thiovit 1R significantly increased the rate of disease development on the upper leaf surface. That is, the rate, or slope for leaves treated with Thiovit 1R with Citowett®, was significantly greater than that for Thiovit 1R without Citowett® and not significantly different from the slope for the untreated leaves.
Table 11. Estimates of coefficients for the linear regression model for four treatment groups, including Thiovit\textsuperscript{®} at the recommended rate plus Citowett\textsuperscript{®} (Thiovit 1R + C), used to investigate the effects of sulphur with the wetting agent, Citowett\textsuperscript{®} on the development of powdery mildew on the upper surface of leaves on Sultana grapevines at Loxton SA in 2001.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intercept\textsuperscript{1}</th>
<th>Slope\textsuperscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>28.61 b</td>
<td>1.1218 c</td>
</tr>
<tr>
<td>Thiovit 1R</td>
<td>8.14 a</td>
<td>0.5080 b</td>
</tr>
<tr>
<td>Thiovit 1R + C</td>
<td>-5.51 a</td>
<td>1.0532 c</td>
</tr>
<tr>
<td>Thiovit 3R</td>
<td>4.65 a</td>
<td>0.1209 a</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Within a column, estimates with a lowercase letter in common were not significantly different (P>0.05).

*Disease on the lower leaf surface.* A regression model with separate linear degradation trends for the four treatment groups in Table 12 was also used to examine the effects of Thiovit 1R + C on disease development on the lower surface of leaves. The adjusted $R^2$ for this model was 92.1%. The estimated regression coefficients for this model are presented in Table 12.

Table 12. Estimates of coefficients for the linear regression model for four treatment groups, including Thiovit 1R + C, used to investigate the effects of sulphur with the wetting agent, Citowett\textsuperscript{®} on the development of powdery mildew on the lower surface of leaves on Sultana grapevines at Loxton SA in 2001.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intercept\textsuperscript{1}</th>
<th>Slope\textsuperscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>47.73 b</td>
<td>0.6716 c</td>
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<tr>
<td>Thiovit 1R</td>
<td>24.10 a</td>
<td>0.2775 ab</td>
</tr>
<tr>
<td>Thiovit 1R + C</td>
<td>12.94 a</td>
<td>0.4469 bc</td>
</tr>
<tr>
<td>Thiovit 3R</td>
<td>13.75 a</td>
<td>0.0898 a</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Within a column, estimates with a lowercase letter in common were not significantly different (P>0.05).

The addition of Citowett\textsuperscript{®} to a solution containing Thiovit 1R, did not significantly affect the development of the disease on the lower surface of leaves.

*Effects of leaf age on disease development.*

*Disease on the upper leaf surface.* A regression model with separate linear trends for the four combinations of Thiovit\textsuperscript{®} (untreated and Thiovit 3R) and leaf age (young leaves and older leaves) was used to study the effects of leaf age on the development of powdery mildew on the upper surface of leaves. The adjusted $R^2$ for this model was 96.2%. The estimated coefficients for this fitted model are presented in Table 13.

Table 13. Estimates of coefficients for the linear regression model for the four treatment combinations of Thiovit\textsuperscript{®} and leaf age, used to investigate the effects of leaf age on the development of powdery mildew on the upper surface of leaves on Sultana grapevines at Loxton SA in 2001.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intercept\textsuperscript{1}</th>
<th>Slope\textsuperscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated, Young leaves</td>
<td>28.92 b</td>
<td>1.1178 b</td>
</tr>
<tr>
<td>Thiovit 3R, Young leaves</td>
<td>4.91 a</td>
<td>0.1088 a</td>
</tr>
<tr>
<td>Untreated, Older leaves</td>
<td>43.39 c</td>
<td>1.0019 b</td>
</tr>
<tr>
<td>Thiovit 3R, Older leaves</td>
<td>12.71 c</td>
<td>-0.0586 a</td>
</tr>
</tbody>
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\textsuperscript{1} Within a column, estimates with a lowercase letter in common were not significantly different (P>0.05).
There was no significant difference in the development of powdery mildew on the upper surface of the young and older leaves that were sprayed with Thiovit 3R.

*Discease on the lower leaf surface.* A regression model with separate linear trends for the four combinations of Thiovit® (untreated and Thiovit 3R) and leaf age (young leaves and older leaves) was also used to study the effects of leaf age on the development of powdery mildew on the lower surface of leaves. The adjusted $R^2$ for this model was 94.5%. The estimated coefficients for this fitted model are presented in Table 14.

### Table 14. Estimates of coefficients for the linear regression model for the four treatment combinations of Thiovit® and leaf age, used to investigate the effects of leaf age on the development of powdery mildew on the lower surface of leaves on Sultana grapevines at Loxton SA in 2001.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intercept</th>
<th>Slope</th>
</tr>
</thead>
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<tr>
<td>Untreated, Young leaves</td>
<td>48.04 a</td>
<td>0.6693 b</td>
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<tr>
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<td>13.71 a</td>
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<td>Thiovit 3R, Older leaves</td>
<td>22.44 a</td>
<td>-0.0893 a</td>
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1 Within a column, estimates with a lowercase letter in common were not significantly different (P>0.05).

There was no significant difference in the development of powdery mildew on the lower surface of the young and older leaves that were sprayed with Thiovit 3R.

### 4.7 Discussion

The field studies in 2000 and 2001 indicated that levels of sulphur on grapevine leaves thoroughly treated once with the recommended rate of Thiovit® using a hand sprayer persisted and reduced the development of powdery mildew for at least 44 days. Significantly, this persistence and the level of control of disease increased with Thiovit® at three times the recommended rate. Furthermore, even when a wettable sulphur treatment was applied at the recommended rate (Thiovit 1R) with a spray machine in 1999, sulphur persisted on leaves for 27 days. These results indicate that the re-application of wettable sulphur (Thiovit®) in spray programs for powdery mildew control is more likely to be required because of the development of new unsprayed foliage that requires treatment to prevent infection than because of the lack of persistence of sulphur on foliage that was treated previously.

In comparing the rates of sulphur degradation over the three seasons, the decline was slightly slower at lower temperatures in 2001, especially for Thiovit 3R, while the rates of degradation in the three seasons in this study were much slower than those reported by Leavitt and Martin-Duvall (1998). The rates of sulphur degradation in the study reported here were also higher for Thiovit 3R than for Thiovit 1R in 2000 and 2001. Higher sulphur degradation rates when Thiovit® was applied at higher application rates were also observed in the studies reported in Chapter 6.1.

The studies reported here also support the hypothesis that the efficacy of wettable sulphur for the control of powdery mildew on grapevine foliage is related to spray coverage and the amount of sulphur deposited on leaves, which, in turn, is related to the method of spray application. When wettable sulphur (Thiovit®) was thoroughly applied at three times the recommended rate (Thiovit
3R), the estimated increment in sulphur level on grapevine foliage just after application (Thiovit 3R, 0.178-0.270% w/w S) was higher than when Thiovit® was applied at the recommended rate (Thiovit 1R, 0.064-0.082 % w/w S). The persistence of sulphur on grapevine foliage was also increased. This was reflected by improved control of powdery mildew with Thiovit 3R. Conversely, when spray application to leaves was not as thorough, such as when leaves were sprayed using a commercial air-mist sprayer in 1999, the increment (0.018 % w/w S) and persistence of sulphur on leaves and the potential for powdery mildew control was lower. The latter, coupled with poorly performing spray machinery, may explain why some grape growers have not achieved satisfactory control of powdery mildew in vineyards after treatments with wettable sulphur.

4.8 Summary

These studies highlighted the relatively slow degradation of sulphur on grapevine foliage and showed the value of wettable sulphur as a fungicide in Australian spray programs. Field trials over two seasons indicated that sulphur on leaves from a single, thoroughly applied spray of wettable sulphur at the recommended rate, persisted for more than 40 days. At three times the recommended rate, sulphur persisted for more than 50 days. Furthermore, a single spray of wettable sulphur at three times the recommended rate gave good control of grapevine powdery mildew for up to 50 days.

4.9 Acknowledgements

The authors thank the Grape and Wine Research and Development Corporation and Horticulture Australia for financial assistance, Sally Thiele, Katie Magarey, Sarah Magarey and Lisa Mitchell for assistance with the field studies and compilation of data, and George Croatto and staff at the State Chemistry Laboratories, Werribee Vic. for sulphur analyses.

4.10 References


5. Summary of related research in GWRDC Project DAV 98/1: Strategic use of sulphur in integrated pest and disease management (IPM) programs for grapevines

Bob Emmett

Related research in GWRDC Project DAV 98/1 (Emmett 2003) is summarised below.

5.1. Effects of spray programs containing fungicides with different chemistry on the development of powdery mildew on grapevines. (Bob Emmett, Shelley Rozario, Trevor Wicks, Catherine Hitch, Christiane Jaeger and John Reynolds).

- Spray programs for powdery mildew control with fungicides of different chemistry were applied to cool and warm climate vineyards with Sultana, Chardonnay, Crouchen and/or Shiraz grapevines.
- The products used were Thiovit® (800g/kg sulphur), Topas® (100g/L penconazole), Amistar® (500g/kg azoxystrobin) and/or Legend® (EF-1295, 250g/L quinoxyfen).
- When disease pressure was high, spray programs with sulphur (Thiovit®) and a demethylation inhibiting (DMI) fungicide (Topas®) provided better disease control than programs with sulphur.
- In sulphur and DMI spray programs, the application of the DMI fungicide just before and just after flowering provided optimum powdery mildew control.
- Spray programs with fungicides with three different types of chemistry (ie. Thiovit®, Topas® and Amistar® or Legend®) reduced powdery mildew incidence and severity on bunches more than programs with fungicides with one or two types of chemistry (ie. Thiovit®, or Thiovit® and Topas®, respectively).
- Despite high disease pressure, spray programs with Thiovit®, Topas® and Amistar®, or Thiovit®, Topas® and Legend® reduced disease severity to less than 2% berry area diseased and to less than 5% leaf area diseased.

5.2. Effects of sulphur formulations on the development of grape powdery mildew. (Trevor Wicks, Catherine Hitch and Bob Emmett).

- Five field trials were conducted to determine the effects of type of formulation on the efficacy of sulphur for the control of grapevine powdery mildew.
- Types of formulation that were compared included a wettable powder (Sulfine®), dry flowable, micronised wettable granules (Thiovit®) and a flowable liquid (Headland Flowable Sulphur®).
- In all trials the efficacy of spray programs with each sulphur formulation applied at an equivalent rate of active sulphur was similar.
- In most trials the efficacy of Sulfine® or Thiovit® was improved when the recommended application rate was increased by two or three times.
- In one trial, Sulphine® or Thiovit® applied at two or three times the recommended application rate increased necrosis on leaves of Sultana grapevines.
- When fungicides were applied at their recommended application rates, spray programs with Sulfine® or Thiovit® were not as effective as programs with a demethylation inhibiting (DMI) fungicide (Topas®).
5.3. Effects of temperature and application rates on the degradation, efficacy and phytotoxicity of sulphur

5.3.1 Effects of temperature and application rates on the degradation of sulphur on grapevine leaves. (*Trevor Wicks, Kent Davies, Bob Emmett and John Reynolds*).
- Studies of the degradation of sulphur (Thiovit 1.5R, 3R and 6R) were conducted on grapevine leaves in controlled environment rooms at temperatures of 15°C, 20°C, 25°C and 30°C.
- Samples of treated leaves were collected from Day 0 to Day 28 after treatment and analysed for total sulphur (% w/w S).
- Sulphur degradation was slow over 28 days, except at the highest application rate.
- The rate of degradation on leaves was similar at all temperatures for each application rate. The rate of wettable sulphur application influenced the total amount of sulphur on leaves and the rate of sulphur degradation.
- Rates of sulphur degradation were higher at higher application rates.
- When wettable sulphur is applied to leaves at up to three times the recommended rate, sulphur deposits may attach to cuticular waxes on the leaf surface and slowly degrade.
- At higher application rates, some sulphur deposits may attach to other sulphur deposits instead of to waxes on the plant surface and may degrade or become dislodged at a higher rate.
- On leaves of mature vines in vineyards, other factors such as sunlight, wind, physical abrasion and differences in leaf surface characteristics may also affect sulphur degradation.
- Profiles of sulphur degradation may also be different after multiple applications of wettable sulphur.

5.3.2 Effects of temperature and application rates on the efficacy of sulphur for powdery mildew control. (*Trevor Wicks, Catherine Hitch and Bob Emmett*).
- The efficacy of Thiovit 1R was slightly lower at 15°C than at 20°C and 30°C.
- Increasing the application rate from Thiovit 1R to Thiovit 2R improved efficacy at 15°C and 20°C.
- Increasing the application rate from Thiovit 1.5R to Thiovit 3R did not improve efficacy at 20°C.
- The efficacy of sulphur was slightly lower at 15°C when Thiovit 1.5R was applied up to 14 days before or after inoculation with powdery mildew.
- Good control of powdery mildew was achieved when Thiovit 1R and Thiovit 2R or Flosul 1R and Flosul 2R was applied up to 14 days after inoculation at 20°C.
- Almost complete control of established powdery mildew was achieved when sulphur treatments were applied to provide even and complete coverage of the leaf surface.
- Poor control of powdery mildew in some vineyards with Thiovit 1R may be due to poor spray coverage, instead of the low efficacy of sulphur or the reduced efficacy of sulphur at low temperatures (ie. 15°C).
- When spray coverage is poor, powdery mildew control may be increased by increasing the application rate from Thiovit 1R to Thiovit 2R.
- In cool and warm climate vineyards, the efficacy of spray programs with Thiovit® for powdery mildew control increased when application rates were increased.
- In most trials, treatment programs with Thiovit 3R and Thiovit 6R reduced disease incidence on leaves more than those with the standard Thiovit®/Topas® spray program.
- There was a trend towards lower disease severities on leaves, and sometimes, on bunches treated with Thiovit 3R and Thiovit 6R compared with those treated with Thiovit 1.5R.
5.3.3 Effects of temperature and application rates on the phytotoxicity of sulphur on grapevines.  (*Peter Magarey, Trevor Wicks, Catherine Hitch and Bob Emmett*).

- The use of sulphur is limited by the risk of phytotoxicity when applied at >32°C.
- Wettable sulphur (Thiovit 1R, 3R and 7.5R) was sprayed onto Sultana, Chardonnay, Shiraz or Sauvignon Blanc grapevines in cool and warm climate vineyards when temperatures were 33-44°C and relative humidity (RH) was 42–80%.
- Most foliage dried within 3-5 minutes (min) and all foliage dried within 50 min.
- No phytotoxicity was observed in eight field experiments, except on three occasions.
- Slight burning occurred on <2% on very young Chardonnay leaves with Thiovit 1R and Thiovit 3R at 40°C in one experiment, and with Thiovit 7.5R at 42°C in another experiment.
- Some minor burning occurred on very young Shiraz leaves after treatment with Thiovit® when temperatures exceeded 40°C in a further experiment.
- Phytotoxicity occurred with Thiovit 1R and Thiovit 3R only at RH >75%, despite temperatures >40°C.
- Wettable sulphur was phytotoxic at lower RH (viz 44°C, RH 63%) only with Thiovit 7.5R.
- Further tests are needed to determine threshold conditions for phytotoxicity.
- Phytotoxicity on vine foliage was influenced by an interaction between temperature, the rate of wettable sulphur applied and the duration of leaf wetness after spraying.
- These results suggest that wettable sulphur (Thiovit®) can be used safely, even at high temperatures under conditions of low RH (<70%), ie. in conditions that are common in inland Australian vineyards.

5.4. Sampling mites in vineyards: - Movement, distribution and effects of rust mites on grapevines.  (*Martina Bernard, Ary Hoffmann, Paul Horne and DeAnn Glenn*).

- The movement of rust mites (*Calepitrimerus vitis*) from winter shelters to grapevine leaves in spring and the movement back to winter shelters in autumn was studied.
- Most mites over-wintered in bark instead of in buds, as previously assumed.
- The spring movement of mites coincided with Chardonnay woolly bud stage and the mites were most vulnerable to spray treatments at this time.
- Autumn movement occurred over a more extended period.
- Rust mites caused restricted spring growth (RSG) and reduced yield when they were not adequately controlled.
- High mite infestations on leaves in late summer that caused typical leaf bronzing did not influence the rate of ripening of grapes.

5.5. Effects of sulphur and other fungicides on populations of pest and predatory mites.  (*Martina Bernard, Ary Hoffmann, Paul Horne, Pam Hurst and DeAnn Glenn*).

- Rust mite migration patterns, over-wintering habits, and the onset of feeding and egg lay in spring were studied alongside spray application trials over two years.
- Single high volume sprays (≥ 900-1000 L/ha) of wettable sulphur (0.66% or 660g/100L Thiovit®) and canola oil (2% or 20L/100L Supastik®), wettable sulphur, or canola oil applied just before bud burst (mid-late woolly bud stage) reduced rust mite damage to shoots in spring.
- For successful rust mite control, sprays of wettable sulphur and canola oil should be applied as above to thoroughly wet the bark of vine cordon and crowns when temperatures are at least 15°C.
• Some commonly used vineyard sprays promote rust mite outbreaks because their unintended secondary action decimated natural predators of rust mite.
• These sprays include dithiocarbamate fungicides [mancozeb (eg. Mancozeb®, Dithane®, Acrobat®, Bryzeb®, Manzate®, Penncozeb®, Recoil®, Galben®)], benzimidazole fungicides [benomyl and carbendazim (eg. Benlate®, Bavistin®, Spin®)], lime sulphur, chlorpyrifos (Lorsban®), and pyrethroid insecticides.
• Removing these sprays from seasonal spray schedules may, over time, eliminate the need for sprays for rust mite control without sustaining rust mite damage (‘RSG’ and yield losses).

For the control of bud mites, it is suggested that a high volume spray of wettable sulphur at the label rate recommended for mite control after bud burst (eg. 200g/100L Thiovit®), should be applied between 100% bud burst and 1 week after bud burst. Sprays should be applied to provide thorough coverage of young shoots.
• Lime sulphur applications at advanced woolly bud stage did not control bud mite populations in buds, highlighting the need for alternative control strategies.

5.6. Development of species specific markers against Eriophyoid mites in grapevines (Melissa Carew and Ary Hoffmann).
• Bud mite and blister mite (currently considered to be two forms of Colomerus vitis) and rust mite (Calepitrimerus vitis) were collected from sites in south eastern Australia.
• Genetic differentiation of mite populations was investigated though the use of Restriction Fragment Length Polymorphism (RFLP) of the Internal Transcribed Spacer (ITS) 1.
• ITS 1 genotypes revealed that bud mite and blister mite represent two closely related but distinct species.
• Micro-satellite markers were developed with the aim of undertaking intensive genetic studies of bud mite and blister mite populations.
• Preliminary analysis of the micro-satellite data indicated that blister mite populations may have haplo-diploid (possessing diploid females and haploid males).
• Genetic differentiation was high between populations and low within populations.
• These findings indicate that separate control strategies are needed for bud and blister mites because they appear to be separate species.
• As dispersal in these mites is limited, localised control strategies are likely to be effective.

5.7. Effects of sulphur on parasitism of lightbrown apple moth by Trichogramma carverae (Linda Thompson, DeAnn Glenn and Ary Hoffmann).
• Trichogramma carverae is released to control lightbrown apple moth in vineyards in south eastern Australia.
• Experiments were conducted to assess the potential impact of the use of sulphur on released and resident Trichogramma species (T. carverae, T. funiculatum), to develop a protocol to maximise the potential of Trichogramma parasitoids and to optimally integrate the use of chemicals with biocontrol strategies.
• Laboratory and field studies indicated that sulphur is harmful to adult Trichogramma wasps as well as to immature stages contained within hosts because it increased mortality and reduced fitness of the emerged wasps.
• Persistence trials showed that release of Trichogramma 6 days after sulphur has been sprayed will reduce the harmful effects of sprays on the released organisms.
• However, in order to avoid the impact of sulphur sprays on resident Trichogramma parasitoids, other chemicals should be used.
5.8 References

6. Technology transfer

Bob Emmett

6.1 Communication of R&D

Information about sulphur and its optimal use for the control of powdery mildew in vineyards was communicated to scientific and industry audiences through a series of presentations and publications.

Presentations

Presentations relating to work on the project are listed in Table 1. Information on the use of sulphur was presented in messages delivered to grape growers through the Horticulture Hotline service at Mildura, at dried fruits and chemical industry seminars and discussion group meetings at Mildura, Irymple, Merbein, Sunnyciffs, Red Cliffs, Robinvale and Geelong Vic., at Pomona and Albury NSW and at Adelaide SA, at field days at Mildura, Irymple, Tresco and Lake Boga Vic., and in Research to Practice® and chemical industry workshops in Victoria and South Australia. Oral and poster presentations were also made at international scientific and industry meetings, workshops and conferences in France, California USA and New Zealand.

Publications

Publications relating to work on the project include those listed below. Information on the project was incorporated into reports for project stakeholders and industry, into articles in industry journals and into papers in proceedings of scientific and industry conferences and workshops. After the project final report is accepted by Horticulture Australia, additional articles on the outcomes of the project will be submitted for publication in industry journals such as Australian Dried Fruit News.


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<td>IPM Viticulture Research to Practice Training Workshop Third Day</td>
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<td>GWRDC Project DAV 98/1 and DFRDC Project DAV 89D Sub-group Meeting</td>
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<td>Novartis Crop Protection Australia Viticulture Workshop</td>
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2001
### Table 1. List of Presentations

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<td>The phytotoxicity of sulphur applied to grapevines at high temperature</td>
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<td>Biology and management of powdery mildew of grapevines: strategic use of fungicides</td>
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7. Recommendations and project outcomes

Bob Emmett

7.1 Recommendations for industry

Powdery mildew management

• Recommendations for the strategic use of sulphur for powdery mildew control in vineyards for dried grape production were developed on the basis of results from studies in this project (HA Project DG 01002) and in GWRDC Project DAV 98/1 (Emmett 2003).

• Grape growers should carefully follow label recommendations when they use the fungicides discussed below.

• Sprays of sulphur or alternative fungicides for powdery mildew control should be applied at 2 weeks after bud burst (when shoots are around 10cm with 5-6 separated leaves), at 4 weeks after bud burst (when shoots are around 20-30cm with 8-10 separated leaves), at 6-7 weeks after bud burst (at pre-flowering when shoots have 12-14 leaves) and/or at 8-10 weeks after bud burst (at berry set or post flowering, when berries are around 2mm).
  • These sprays are required to prevent infection by spores from primary sources of inoculum [ie. cleistothecia or diseased (flag) shoots that have developed from infected buds].
  • These sprays are also required to prevent spread of disease early in the season on young vine foliage and bunches.

• Further sprays at 2-3 week intervals from berry set to berry softening may be needed, especially in disease-prone vineyards.
  • These sprays are often required to protect young berries, bunch stems and foliage from mid season secondary infection.

• Use of wettable sulphur at the recently introduced, highest recommended rate of application for powdery mildew control (ie. 600g/100L) should increase the persistence of sulphur on vine foliage and increase disease control.
  • This is important early in the season (from 2 weeks after bud burst until after flowering), especially in vineyards with a recent history of disease.
  • When this project commenced, the recommended rate for wettable sulphur (eg. Thiovit®) was 200g/100L for dilute spray application.
  • Research on the degradation and efficacy of sulphur in this project and GWRDC Project DAV 98/1 showed that sulphur efficacy could be improved by increasing application rates.
  • Subsequently, some chemical companies conducted further trials and registered a higher rate of application (600g/100L) for their formulations (eg. Thiovit® Jet).
  • Grape growers now have the option of using sulphur at two rates of application for powdery mildew control (ie. 200g/100L or 600g/100L).
  • Dried grape growers, however, should check product labels before they use sulphur fungicides. Higher rates of some products are only registered for use on wine grapes at this stage.

• Adjuvants such as wetter/spreaders or oils do not need to be tank mixed with sulphur sprays.
  • In studies in this project, the addition of these adjuvants did not improve disease control when sprays were thoroughly applied to vines.
  • There were also indications that the addition of oils to sulphur spray solutions may increase incidence of phytotoxicity on young vine growth in some circumstances.
  • On labels, most suppliers of sulphur fungicides do not recommend the use of adjuvants with their products.
• Sulphur fungicides can cause phytotoxic effects on vine foliage and bunches in some circumstances and this should be considered when sprays are applied in vineyards.
• Factors that can affect the incidence of phytotoxicity include interactions between temperature, humidity and the duration of leaf wetness, and the rate of sulphur application.
• Observations in field studies indicate that some formulations of wettable sulphur (eg. Thiovit®) can be applied without causing phytotoxicity, even at high temperatures when humidity is low (RH <70%).
• Potential for sulphur phytotoxicity appears to be increased when some formulations with very small sulphur particles (<1µm) are used, high rates of sulphur are applied, some adjuvants (eg oils) are used with sprays, and/or when temperature and humidity is high during or just after spray application.
• Although some sulphur products can be applied at high temperatures when humidity is low, applications are not recommended by some manufacturers when temperatures above 35°C because deposition of spray droplets on vine surfaces is impaired at these temperatures.

• The application of 1-3 correctly timed DMI fungicide sprays in sulphur spray programs should improve the control of powdery mildew, especially when sulphur is applied at 200g/100L.
• When sulphur was applied at 200g/100L, spray programs with sulphur and DMI fungicides provided better control of powdery mildew than programs with sulphur when disease potential was high.
• However, when wettable sulphur was applied at 600g/100L, the efficacy of sulphur spray programs was similar to sulphur and DMI programs, where sulphur was applied at 200g/100L.

• The use of spray programs with sulphur and fungicides with at least two other types of chemistry may also provide better disease control than sulphur programs, especially when sulphur is applied at 200g/100L.
• Apart from DMI fungicides, other fungicides with chemistry that is different to sulphur and can be used for powdery mildew control include strobilurin and quinoline fungicides.

• In sulphur and DMI fungicide spray programs, DMI sprays should be applied before and after flowering for optimum control of powdery mildew in most seasons.
• In these spray programs, optimum disease control was achieved when DMI sprays were applied to protect vines over periods of most rapid disease increase or highest disease potential.
• In dried grape vineyards in the Sunraysia and Riverland districts, the period of highest disease potential is around flowering in most seasons.

• In the absence of information on the effects of higher sulphur application rates on the persistence of sulphur on grape berries and residues in dried grapes, growers could follow wine industry recommendations for sulphur spray withholding periods where required.
• Withholding periods for pesticide treatments, including sulphur fungicides, are specified annually by the Australian Wine Research Institute (AWRI), Adelaide SA.

• Post harvest sulphur sprays may be worthwhile in some vineyards to maintain the health of vine foliage between harvest and leaf fall.
• These sprays may also reduce the development of cleistothecia on vines in the post harvest period.
• However, this may not significantly affect levels of over-wintering powdery mildew inoculum (cleistothecia) and subsequent disease development in spring, especially if substantial numbers of cleistothecia have developed before the post harvest sprays and/or significant levels of other forms of over-wintering inoculum (eg. bud infections) are present on vines.
Management of mites

- Recommendations for the strategic use of sulphur for mite control in vineyards were developed on the basis of results from studies in GWRDC Project DAV 98/1 (Emmett 2003).

- For the control of rust mites, a high volume spray of wettable sulphur (0.66% Thiovit®) and canola oil (2% Supastik®) should be applied at mid-late woolly bud stage. The spray should be applied to thoroughly wet the bark of vine cordons and crowns when temperatures are at least 15°C.
  - In field studies, single high volume sprays (≥ 900-1000 L/ha) of wettable sulphur (0.66% or 660g/100L Thiovit®) and canola oil (2% or 2L/100L Supastik®), wettable sulphur, or canola oil applied just before bud burst (mid-late woolly bud) reduced rust mite damage to shoots in spring.
  - Where possible, sprays of dithiocarbamate fungicides [mancozeb (eg. Mancozeb®, Dithane®, Acrobat®, Bryzeb®, Manzate®, Penncozeb®, Recoil®, Galben®)], benomyl and carbendazim (eg. Benlate®, Bavistin®, Spin®), lime sulphur, chlorpyrifos (Lorsban®) and pyrethroid insecticides should be minimised or avoided because they can reduce natural predation of rust mites.
  - Excessive use of these chemical is likely to promote outbreaks of rust mites.
  - Removing these sprays from seasonal spray schedules may, over time, eliminate the need for sprays for rust mite control without sustaining rust mite damage (ie. restricted spring growth and yield losses).

- For the control of bud mites, it is suggested that a high volume spray of wettable sulphur at the label rate recommended for mite control after bud burst (eg. 200g/100L Thiovit®), should be applied between 100% bud burst and 1 week after bud burst. Sprays should be applied to provide thorough coverage of young shoots.
  - These preliminary recommendations for the control of bud mite have been developed on the basis of current knowledge. Further R&D on the biology and management of bud mites is needed to consolidate these recommendations.
  - Sprays of lime sulphur (10%) at advanced woolly bud did not control bud mite populations in buds, highlighting the need for alternative control strategies.

Biological control of lightbrown apple moth

- If Trichogramma parasitoids (T. carverae) are released in vineyards to control lightbrown apple moth (LBAM), releases should be conducted at least six days after the application of wettable sulphur to minimise the effects of the sulphur spray on the released organisms.
  - Laboratory and field studies indicated that sulphur (Thiovit® applied at 200g/100L) is harmful to adult Trichogramma wasps as well as to immature stages contained within LBAM hosts because it increased mortality and reduced fitness of the emerged wasps.
  - Persistence trials showed that release of Trichogramma wasps six days after sulphur has been sprayed will reduce the harmful effects of sprays on the released organisms.

- To avoid the effects of sulphur sprays on resident Trichogramma parasitoids (ie. T. carverae and/or T. funiculatum) in vineyards, other chemicals should be used.

7.2 Recommendations for future R&D

Degradation of sulphur on grape berries

- The degradation of sulphur on grapevine leaves was studied in this project. Residues on leaves after a single spray were persistent and their rate of degradation was influenced by application
rate. Similar degradation studies are required for sulphur on grape berries. The deposition and
degradation of sulphur on the surface of grape berries is likely to be different to that on leaves.

- With the introduction of higher application rates, sulphur residues on fresh and dried grapes are
likely to be higher if sulphur sprays at the higher rate are applied later in the season and before
harvest. The implications of this for grape product quality should be clarified.

- Potential sulphur residue problems could be addressed by restricting the use of sulphur at higher
application rates to earlier in the season, but further R&D is needed to support this strategy.

Sulphur phytoxicity

- Studies in this project provided some information about factors associated with sulphur
phytoxicity.

- Further R&D is needed, however, to increase understanding of the interaction between factors
such as temperature, humidity and/or sulphur application rates that promote phytoxicity. The
condition of vines when sprays are applied may also warrant consideration.

- Increased knowledge of conditions associated with phytoxicity would be the basis for providing
more definite advice on how to consistently prevent sulphur damage to vines.

Biology and management of bud mites on grapevines

- Although preliminary recommendations for the control of bud mite have been developed on the
basis of current knowledge, further R&D on the biology and management of bud mites is needed
to consolidate these recommendations. Rates of sulphur for bud mite control just after bud burst,
for example, require further definition.

7.3 Key project outcomes

Some key outcomes of the R&D on sulphur in this project and GWRDC Project DAV 98/1
(Emmett 2003) include the following.

- Increased knowledge of:
  - Degradation and phytoxicity of sulphur on grapevine foliage.
  - Use of sulphur for the management of grapevine powdery mildew in vineyards.
  - Introduction of higher application rates of some sulphur formulations (eg. Thiovit®) for the
    control of powdery mildew.
  - More effective spray programs for the control of powdery mildew in vineyards.

- Increased knowledge of:
  - Biology and management of grapevine mites, especially rust and bud mites.
  - Effects of sulphur on the parasitism of lightbrown apple moth by Trichogramma
    parasitoids.

- Strategies for the optimal use of sulphur in programs for the control of grapevine powdery
  mildew and mites.

- Increased efficiency and effectiveness of IPM programs in Australian dried grape and wine grape
  vineyards when strategies for the optimal use of sulphur are applied.

7.4 References

programs for grapevines. Final Project Report for the Grape and Wine Research and Development
Corporation. Department of Primary Industries, Mildura Vic.