

Grape & Wine Research
& Development
Corporation

PETROLEUM SPRAY OIL AND BIOLOGICAL PESTICIDE BASED IPDM FOR HORTICULTURE

FINAL REPORT to

GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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1. ABSTRACT

Tests carried out on grape (cv. Chardonnay) leaves naturally infected with powdery mildew and grown in glasshouse showed that BP Summer Spray Oil was effective as pre- and post-infection sprays in significantly reducing incidence of the disease. Applications of calcium at the end of flowering and at pre-bunch closure significantly reduced the incidence of *Botrytis* bunch rot of grapes in the vineyard. The spray oil was not suitable for dormant treatments in the management of cane and leaf blight [*Phomopsis viticola* (Type 2)] and bunch rot (*Botryis cinerea*) diseases. Applications of the spray oil reduced the populations of two-spotted mites (*Tetranychus urticae*) and blister mites (*Colomerus vitis*) on grapevines in the vineyard. The oil did not disrupt the natural populations of predators and parasitoids (*Phytoseiulus persimilis*, *Typhlodromus occidentalis, Cryptolaemus montrouzierei*) in the crop.

2. EXECUTIVE SUMMARY

The Grape and Wine Research & Development Corporation (GWRDC) and BP Australia jointly funded the project. The University of Western Sydney provided research facilities.

Modern petroleum spray oils are ideally suited to sustainable integrated pest and disease management (IPDM) programs in horticulture (Beattie *et al* 2002). They are environmentally acceptable and safe to use. Field residues do not pose a threat to the environment (Cornish *et al*. 1993). A wide-range of pests and diseases can be controlled simultaneously with minimal or negligible disruption of beneficial arthropods, including natural enemies of pests (Beattie and Smith 1997, Mensah 1997, Weiguang Liang, University of Western Sydney, pers. comm. 1999). There has been no empirically demonstrated case of resistance by pests or diseases in 100 years (Beattie and Smith 1997). They are approved under the National Standards for Organic and Bio-dynamic Produce as materials suitable for controlling plant pests and diseases (Anonymous 1992).

Although the effectiveness of petroleum spray oils on powdery mildew of grapevines was demonstrated (Wicks and Hitch 1998), their full potential for integrated pest and disease management (IPDM) programs was underestimated. For instance, the broader issue of simultaneous and sustainable control of diseases, insects and mites were not considered. Recent overseas studies (Northover and Schneider 1996, Davidson *et al*. 1991) have shown satisfactory and acceptable use of petroleum spray oils in grape pest management.

This project represents a major progression in the use of petroleum sprays oils in viticulture. It looks at the possibility of using spray oil (BP Summer Spray Oil) in an IPDM program involving powdery mildew, cane and leaf blight, and bunch rot diseases and arthropod pests such as two spotted mites and blister mites. Whereas petroleum spray oils were once used largely to drown pests they can now be used to control pests and diseases simultaneously through a range of insect behaviour such as mortality, settling, feeding, oviposition etc.

Investigations carried out in this project on leaves of cv. Chardonnay naturally infected with powdery mildew and grown in glasshouse showed that BP Summer Spray Oil was effective as pre- and post-infection sprays in significantly reducing incidence of the disease. Applications of calcium at the end of flowering and at pre-bunch closure significantly reduced the incidence of *Botrytis* bunch rot in the vineyard. The spray oil was not efficacious for dormant treatments in the management of *Phomopsis viticola* (Type 2) and *Botryis cinerea*. Four in season applications of the spray oil reduced the population of two spotted mites. However, the oil did not disrupt the natural populations of predators in the crop.

The two outcomes from the project of most benefit to grape growers in terms of economic returns are the potential for BP Summer Spray Oil to control powdery mildew and for calcium to control *Botrytis* bunch rot. Another practical implication is the key role that oil could play in a resistance management strategy for the control of powdery mildew. The use of spray oil applied in combination with fungicides to

which the powdery mildew fungus develops reduced sensitivity is likely to help in a resistance management strategy for the control of the disease. Where arthropod pests such as two spotted mites and blister mites are important, such as in the Hunter Valley, the spray oil also has the potential to reduce their effect on crop loss without disrupting populations of beneficial insects and mites.

An extension activity arising from this project could be to conduct vineyard demonstration trials on the effect of BP Summer Spray Oil to control powdery mildew on different cultivars of grapevines. The trial should be designed to demonstrate a resistance management strategy by incorporating 'at risk' fungicides (eg.DMI) in combination with spray oil for the control of powdery mildew.

The use of spray oil as part of an overall integrated pest and disease management program is recommended.

The effect of calcium in reducing incidence of *Botrytis* bunch rot should be investigated in further vineyard trials. Different timings of applications of calcium is required to determine the maximum impact of calcium on increasing the resistance of maturing grape berries to *Botrytis* infection.

3. BACKGROUND

Introduction to Petroleum Spray Oils

Petroleum oils of various types have been used in horticulture for over a century. The nature and use of spray oils are described in the proceedings of an international conference on petroleum spray oil (Beattie *et* al 2002). Modern products fall into two categories generally referred to as dormant, and white or summer oils. Dormant oils are usually applied as sprays of 3% oil in water emulsions to deciduous plants in winter and early spring. They contain significant levels of phytotoxic impurities and are not suitable for use on verdant plants. White oils are usually applied as 1-2% emulsions to verdant plants from spring to autumn. They are highly refined, colourless (white) oils. They are also called paraffin, mineral, narrow-range, stylet, summer, verdant, horticultural, superior, and petroleum spray oils. White oil and petroleum spray oil are the most common terms used in Australia.

Oils are not poisons. Historically they have been used to drown (suffocate) mites and small soft-bodied insects such as scales (Davidson *et al*. 1991). To a lesser extent they have been used to control diseases - mostly powdery mildews, banana Sigatoka, citrus greasy spot and viruses transmitted by aphids and thrips (hence 'stylet' oil; Allen *et al*. 1993, Wang and Pirone 1996, Powell *et al*. 1998). In most instances, sprays have been applied at high-volume. Emphasis on 'low-volume' application of pesticides (particularly synthetic pesticides) in recent decades (Mathews 1979) has tended to overshadow the importance of oils in sustainable cost-effective pest management. Exceptions include the use of banana misting oils for control of Sigatoka and the use of oils to enhance the effectiveness of synthetic and biological pesticides through better deposition, or translaminar and systemic activity.

The potential for using petroleum spray oils as biorational products in horticulture has been subject to increasing interest over the last decade as demand for sustainable and environmentally acceptable pest and disease management programs has grown. The most successful use of petroleum spray oils has been in citrus (Hely *et al*. 1982, Davidson *et al*. 1991) and in Australia they are a very important component of citrus integrated pest management programs (Smith *et al*. 1997). Since the late 1980s research in Australia and the United States of America has focused on their use in non-citrus horticultural crops such as grapes, apples, ornamentals and vegetables.

Modern petroleum spray oils are ideally suited to sustainable integrated pest and disease management (IPDM) programs in horticulture. They are environmentally acceptable and safe to use. Field residues do not pose a threat to the environment (Cornish *et al*. 1993). A wide-range of pests and diseases can be controlled simultaneously with minimal or negligible disruption of beneficial arthropods, including natural enemies of pests (Beattie and Smith 1997, Mensah 1997, Weiguang Liang, University of Western Sydney, pers. comm. 1999). There has been no empirically demonstrated case of resistance by pests or diseases in 100 years (Beattie and Smith 1997). They are approved under the National Standards for Organic and Bio-dynamic Produce as materials suitable for controlling plant pests and diseases (Anonymous 1992).

This project represents a major progression in the use of petroleum sprays oils in viticulture. Whereas they were once used largely to drown pests, they can now be used to control pests and diseases simultaneously through a range of effects such as increasing rate of mortality and decreasing oviposition. Drowning is still important but is now being overshadowed by behavioural effects on pests (many of which are too large to drown). Use with biopesticides and naturally derived products will greatly enhance the potential use of biorational products in IPDM programs and enhance the success of these programs. Their effects on plant pathogenic fungi and transmission of viruses mean that control of some economically important plant diseases can be achieved when sprays are used to control pests or *vice versa*.

Impact on Arthropod Behaviour. Significant effects of oil spray deposits on the behaviour of pests were first observed in the early decades of this century (Beattie *et al*. 1995). It is now clear that oil deposits can be used to significantly reduce settling, feeding or oviposition by a wide-range of pests (Ebeling 1936, Zhongmin Liu, unpublished data, HRDC project HG/96/011, Larew and Locke 1990, Beattie *et al*. 1995, Liu and Stansly 1995, Rae *et al*. 1997, Mensah 1996). Studies by Ebeling (1936) and work carried out by us in HRDC project (HG/96/011) have shown that some of these effects are related to distillation properties of oils. The implications of these findings for IPDM programs in viticulture are significant.

Two spotted mite (TSM), *Tetranychus urticae*, is a serious pest of a wide range of crops including grapevines in the Hunter Valley. Current control measures rely heavily on the use of synthetic pesticides. Many of these chemicals are toxic to mite predators and the natural enemies of other pests. Resistance to these chemicals is also widespread. Previous work with mineral oils has indicated that adult TSM are less susceptible to oils than other pests for which oils have traditionally been used. However, there is increasing evidence of significant effects of oil sprays on arthropod behaviour.

Grape leaf blister mite (*Colomerus vitis*) is a minor pest of grapes in Australia (Hely *et al* 1982). However, it has the potential to cause serious crop loss. There are two recognised forms, one that infests buds and another that infests leaves causing erinose blisters on the abaxial surface (Jeppson *et al* 1975, Hely *et al* 1982). The erinose form occasionally requires chemical control in winter with lime-sulphur in the absence of effective predators (Hely *et al* 1982, James and Whitney 1991). There appears to be no record of the effect of spray oil on their control.

Although the effectiveness of petroleum spray oils on powdery mildew of grapevine was clearly demonstrated and considered suitable for IPDM programs (Wicks and Hitch 1998), their full potential to control the disease was underestimated, since the conclusions were primarily based on a direct comparison with Topaz. No mention was made of a number of recent overseas studies (e.g. Northover and Schneider 1996) or of current satisfactory and acceptable uses of petroleum spray oils in grape pest management in North America (e.g. Davidson *et al*. 1991). It also did not consider the broader issue of simultaneous and sustainable control of diseases, insects and mites.

The use of spray oils applied alone or in combination with fungicide against fungal diseases has been reported (Steurbaut 1992). Mineral oils applied to banana provided good control of *Mycosphaerella musicola* especially when used in combination with benzimidazoles (Stover and Simmonds 1987). Mineral oils combined with benomyl, thiophanate or copper controlled *M. citri* in Cuba (Saenz and Lewis 1985). A paraffin-based narrow-range mineral oil controlled a benzimidazole and dicarboximide-resistant strain of *Botryis cinerea* on cucumber (Bourbos *et al* 1994).

Introduction to the use of calcium in the management of *Botrytis* bunch rot of grapevines

Calcium has a potentially important role to play during pathogenesis. For instance, Ca^{2+} may be translocated out of the berry during ripening and this contributes to the loss of resistance to infection (Kaile *et al* 1992). The largest concentration of Ca^{2+} in plants is associated with the cell wall (Demarty *et al* 1986), the vacuole and various organelles containing less of the cation (Kauss 1987). *Botrytis cinerea* secretes cell wall degrading enzymes during plant pathogenesis with the host middle lamella being initially broken down (Kaile *et al* 1991).

Several studies have reported that calcium treatment of plant tissues induces an increase in tissue Ca^{2+} content (Conway *et al* 1991) by cross-linking polygalacturonate polymers (Conway and Sams 1983, Conway *et al* 1987) resulting in strengthening the plant cell wall and reducing decay caused by *B cinerea* (Conway and Sams 1983, Chardonnet and Doneche 1995). In addition to the effect of Ca^{2+} in strengthening plant cell walls, fungal polygalacturonase, thought to be a key enzyme in infection process, is inhibited by Ca^{2+} (Biggs *et al* 1997). All the studies on the effect of calcium on *Botrytis* have shown that it has an inhibitory effect on the growth of the fungus at high concentrations (Kaile *et al* 1992, Wisniewski *et al* 1995).

Resistance to organic (Nair *et al* 1997, Sergeeva *et al* 2002) and inorganic (Parry and Wood 1958, Biggs *et al* 1997) fungicides has been shown in *B cinerea*. Calcium is not fungicidal and no resistance to high concentrations of Ca^{2+} has been shown (Chardonnet *et al* 2000). Due to the emergence of resistance to fungicides, alternative strategies in the control of *B cinerea* have been investigated. One alternative involves the use of biocontrol agents including bacteria (Ketterer *et al* 1992), yeasts (Droby *et al* 1997) or fungi (Tronsmo and Dennis 1974, Eden *et al* 1996). Calcium is known to improve the efficiency of biological control presumably by directly affecting spore germination and metabolism of the pathogen and indirectly by not altering the normal metabolism of the biocontrol organism when Ca is present in high concentrations (Wisniewski *et al* 1995).

The mode of action of Ca can be summarised as follows:

- Ca reduces the potential of the fungus to infect the host by limiting fungal growth;
- Ca inhibits polygalacturonase activity; and,
- Ca decreases the susceptibility of plant tissue to decay caused by *Botrytis cinerea*.

4. PROJECT AIMS AND PERFORMACE TARGETS

Project aims

The project aims in relation to GWRDC are:

- Study the effect of petroleum spray oil (BP Summer Spray Oil) on the control of powdery mildew (*Uncinula necator*) of grapevine.
- Determine the effect of calcium on the control of bunch rot (*Botrytis cinerea*) of grapevine.

The project aims in relation to BP (Australia) are:

- Study the effects of the spray oil on the control of powdery mildew (*U. necator*) of grapevine and on dormant structures of *B. cinerea* and *Phomopsis viticola* (Type 2).
- Determine the effect of the spray oil on arthropod pests of grapevine.

Performance targets

The effect of the spray oil on the control of powdery mildew (*U. necator*) of grapevine:

Determine the number and timing of applications of oil on the control of the pathogen in glasshouse and vineyard.

The effect of calcium on the control of bunch rot (*B cinerea*) of grapevine:

Determine the effect of two applications of calcium on the control of the pathogen in vineyard.

The effect of the spray oil on dormant structures of *B cinerea* and *P. viticola*:

Determine the viability of pycnidia of *P.viticola* (Type 2) and myceliogenic germination of sclerotia of *B. cinerea in vitro*.

The effect of the spray oil on arthropod pests of grapevine:

Determine the effect of spray oil on mortality and oviposition of two-spotted mite (*Tetranychus urticae*) and mortality of blister mite (*Colomerus vitis*) in laboratory bioassay.

Determine the effect of oil on beneficial insects in the vineyard.

Determine the effect of four in season applications of spray oil on the control of two-spotted mite and blister mite in the vineyard.

5. METHODS

Laboratory bioassays
(carried out during the first and second years – 2000/2002)

Spray oil

Two formulations of BP Summer Spray Oil were used. These were:

1. The standard BP Summer Spray Oil (referred to as "old formulation") based on ethoxylated alcohol surfactant.
2. An experimental grade of BP Summer Spray Oil (referred to as "new formulation") based on Yubase3 mineral oil.

The old and the new formulations were used in bioassays on powdery mildew and arthropod pests. The old formulation was used in bioassays on *B. cinerea* and *P viticola*.

Mycelial growth of *B. cinerea* Pers. and *P. viticola* (Sacc.) Sacc.(Type 2)

B.cinerea and *P.viticola* (Type 2) were isolated from naturally infected berries and dormant canes of grapevines (cv. Chardonnay) growing at Cessnock (Hunter Valley), New South Wales.

Axenic cultures of *B.cinerea* and *P.viticola* (Type 2) were used in all *in vitro* bioassays. Mycelial discs (7 mm in diameter) were cut from 7 days old cultures and placed in the centre of Petri dishes (80 mm) containing potato dextrose agar (PDA) amended with different concentrations (0, 0.5 and 1.0%) of BP Summer oil. The inoculated plates were incubated at a constant temperature of 20^0C. Radial growth of the mycelium was measured at the end of 6 days. Three replicates were used in all treatments.

Exudations of pycnidia of *P.viticola* (Type 2) and myceliogenic germination of sclerotia of *B.cinerea*

Pycnidia and sclerotia from axenic cultures of *P.viticola* (Type 2) and *B.cinerea* respectively were used in all the *in vitro* bioassays. Pycnidia and sclerotia from 14 day old cultures of the pathogens were collected. These dormant structures were placed separately on PDA amended with different concentrations (0, 0.5 and 1.0%) of BP Summer oil. Three replicate Petri dishes containing 10 sclerotia and pycnidia each were used.

The number of pycnidia showing exudations of conidia was counted and percent exudation calculated. The results were also expressed as per cent reduction in exudation of pycnidia. Myceliogenic germination of sclerotia was measured as mycelial growth and per cent reduction in germination was calculated.

U necator, Powdery mildew

Discs (9 mm) were cut from grape (cv. Chardonnay) leaves naturally infected with powdery mildew and grown in the glasshouse and sprayed with different concentrations (0, 0.5 and 1.0%) of the oil. The sprayed discs were then transferred to healthy leaves on the vines. Similarly, healthy, surface-sterilised (1% sodium hypochlorite for 1 minute, rinsed three times in sterile

water), non-symptomatic leaves were sprayed with the oil first and then inoculated with infected leaf discs. Infected leaves sprayed with sterile water were used as control. Ten replicates were used for each treatment.

T. urticae Koch [Acari: Tetranychidae], Two Spotted mite (TSM)

Oviposition: Bioassays were carried out on oviposition of adult TSM using French bean leaf discs. Five adult female TSM were transferred to the abaxial surface of French bean leaf discs (19 mm diameter) placed on moist cotton wool in Petri dish (90 mm). Four discs were placed in each Petri dish. The leaf discs were sprayed with different concentrations (0, 0.25, 0.5 and 1.0 %) of the oil using a Potter spray tower immediately after transferring the mites on the discs. The protocol of Herron *et* al (1995) was used for the spray application. Eight replicates were maintained for each treatment. The number of eggs laid was assessed 24, 48 and 72 hours after spray treatment.

Hatchability of eggs: Five adult female TSM were placed on French bean leaf discs as described above. They were left overnight for oviposition to occur. The adult female TSM were removed from the discs, and the discs containing the eggs were sprayed with the oil at different concentrations (0, 0.25, 0.5, 1.0, and 2.0%). The method of spray application was the same as used previously. Thirty replicates were used in the bioassay. Percentage hatchability was recorded from 3 to 7 days. The results are expressed as per cent TSM eggs hatched.

Mortality: Twenty adult female TSM were placed on French bean leaf discs as described before. The discs were sprayed with the oil (0, 0.25, 0.5, 1.0, and 2.0%). The method of spray application was the same as before. Eight replicates were maintained for each treatment. Per cent mortality was assessed 24 and 48 hours after treatment.

C. vitis Pagenstecher [Acarina: Eriphyidae], grape leaf blister mite

Mortality: Leaves of cv. Shiraz naturally infested with blister mite were collected from the University of Western Sydney vineyard, Richmond, New South Wales. Leaf discs (19 mm) were cut from erinose areas containing a variable number of mites and placed in Petri dishes as described earlier. The discs were sprayed with the oil (0, 0.25, 0.5, 1.0, and 2.0%) in a Potter tower as before. Three replicates were maintained for each treatment. Per cent mortality was assessed 24 and 48 hours after treatment.

Statistical analysis

Analysis of variance was carried out on the data to determine the level of significance between the treatments.

Vineyard trial at Mudgee, NSW
(First year – 2000/2001)

Cultivar

Chardonnay

The vines were > 16 years old, spur pruned and trained on a vertical shoot positioning trellis; number of vines per row = 100; number of vines per panel = 4; vine spacing = 2 m.

Treatments

Treatment 1 = BP Summer Spray Oil (old formulation).
(Targets: powdery mildew, *B. cinerea*, *P. viticola* (Type 2) and arthropod pests)
Treatment 2 = Untreated control
Treatment 3 = Calcium
(Target: *B. cinerea*)
The treatments were arranged in a randomised block design and five replicates per treatments were used.

Concentrations of chemicals

BP Summer Spray Oil = 3.0% (dormant spray) and 0.5% (in season sprays) (vol./vol.)
Calcium = 1.5 Kg hectare^{-1}

Timing of application of chemicals

BP Summer Spray Oil
Powdery mildew = At budburst + 2 weeks, budburst + 4 weeks, budburst + 6 weeks and budburst + 8 weeks.
B. cinerea = 80% budburst, 30% capfall, 100% capfall and pre-bunch closure.
P. viticola = 2 weeks before budburst, 20% budburst and 80% budburst (further in-season applications were terminated due to the appearance of phytotoxic symptoms on the treated vines).
Arthropod pests = 80% budburst, 30% capfall, 100% capfall and pre-bunch closure.

Calcium
B. cinerea = At the end of flowering and at pre-bunch closure.
No other chemicals were applied to the trial vines for the control of pests and diseases except one application of Ridomil® 8 weeks before harvest to control downy mildew (petroleum spray oils are not known to be effective against downy mildew).

Spray application

All treatments were applied using pump-action 20L backpack sprayers with hollow cone nozzles. The vines were sprayed to run off from both sides of the vine canopy. The untreated plots were sprayed with water.

Pest and disease assessments

The incidence of disease caused by *P. viticola* (Type 2) was assessed on the leaves at first 6 nodes and on the first 5 internodes of stem (15 shoots per

replicate) at the beginning of flowering, 30% cap fall, pre-bunch closure, bunch closure and 3weeks before harvest. The incidence of *B. cinerea* was assessed at 100% cap fall, pre-bunch closure, bunch closure and 3 weeks before harvest. Six bunches per replicate (ie. total of 30 bunches per treatment) were collected and incubated for one week at laboratory temperature (20-22^{0}C). The occurrence of *B. cinerea* was checked using a binocular microscope. Fifteen shoots per replicate were examined to assess the incidence of *U. necator*.

Arthropod pests: Arthropods were assessed in the field by, labeling 10 shoots of the middle vine in each panel per replicate. Both sides of all leaves and all bunches on each shoot were examined with a 10x hand lens for the presence of arthropods. The monitoring was carried out at fortnightly intervals.

Statistical analysis

Analysis of variance was carried out on the data to determine the level of significance between the treatments.

Vineyard trial at Muswellbrook, NSW
(Second year – 2001/2002)

Treatments

Treatment 1 = Experimental grade of BP Summer Spray Oil (new formulation)
(Targets: powdery mildew and arthropod pests)
Treatment 2 = Untreated control
Treatment 3 = Calcium
(Target:*B. cinerea*)

Description of Chardonnay trial block

Number of vines in each row = 276; number of vines per panel = 5; vine spacing = 1.2 m

Spray application

Ten panels (50 vines) in each replicate of treatments 1 and 3 were sprayed.
Buffer rows were maintained between each treatment row.
Green Tech sprayer was used.
Routine spray applications were carried out in the trial rows unless otherwise stated (e.g. exclude powdery mildew routine sprays in powdery mildew treatment rows).

Concentrations of chemicals

Spray Oil = 0.5% (vol./vol.)
Calcium = 1.5 Kg hectare^{-1}

Timing of application of chemicals

Spray Oil

Arthropod pests = 80% budburst, 30% capfall, 100% capfall and pre-bunch closure.

Powdery mildew = At budburst + 2 weeks, budburst + 4 weeks, budburst + 6 weeks and budburst + 8 weeks.

Calcium

B. cinerea = At the end of flowering and at pre-bunch closure.

Pest and disease assessments

Panels 2, 5 and 10 in each replicate of treatments 1 and 2 were monitored for pests (two spotted mites, blister mites, predatory mites and vinescale) and diseases (powdery mildew and *Botrytis* bunch rot). Panels 2, 5 and 10 in each replicate of treatment 3 were monitored for *Botrytis* bunch rot. The monitoring was carried out once every two weeks.

Statistical analysis

Analysis of variance was carried out on the data to determine the level of significance between the treatments.

Fluorochrome bioassay

A bioassay using fluorochromes developed by Sergeeva *et al* (2002) to quantify the survival of fungal pathogens on plants following fungicide applications was used to determine the effect of spray oil on *U. necator* on grape leaves.

Discs (10 mm) were cut from grape leaves (cv. Chardonnay) naturally infected with *U. necator* in the glasshouse. These were sprayed separately with 2 ml of the spray oil in groups of 10 discs using an atomiser, each group receiving different concentrations (0, 0.5 and 1.0%) of the oil. Discs sprayed with sterile water served as controls. The sprayed discs were allowed to dry for 5 min and then were mounted on glass slides with the aid of double-sided tapes. One drop (30 μml) of each fluorochrome [5,6 carboxyfluorescein diacetate (CFDA) and propidium iodide (PI)] was placed on the treated and untreated infected leaf discs, and allowed to stand for 5 minutes. Cover slips were placed on the leaf discs before examination. The specimens were viewed with blue excitation (460-490 nm), dichroic mirror 505 and barrier filter BA515IF at magnifications x 250 and x 1000. Counting 100 conidia each on 10 treated and untreated leaf discs assessed the survival of *U. necator*.

6. RESULTS AND DISCUSSION

Laboratory bioassays

Bioassay on mycelial growth of *P viticola* (Type 2) and *B cinerea in vitro*
Mycelial growth of *B cinerea* was reduced significantly and *P viticola* (Type 2) totally inhibited at all the concentrations of oil tested *in vitro* (Table 1). Therefore, in-season applications of oil at 0.5% are likely to reduce the levels of these pathogens.

Table 1. – Mycelial growth (mm)* of *Phomopsis viticola* (Type 2) and *Botrytis cinerea in vitro* on potato dextrose agar amended with BP Summer Spray Oil[1]

Concentration of oil (%)	*B. cinerea*	*P. viticola* (Type 2)
Unamended	> 85 a	60 a
0.5	40 b	0 b
1.0	40 b	0 b
2.0	41 b	0 b

* Incubation period = 6 days LSD (P = 0.05) [1] old formulation

Bioassay on dormant structures *P viticola* (Type 2) and *B cinerea in vitro*
Percentage exudation of conidia from pycnidia of *P. viticola* was reduced by 11.5 % when they were treated with the oil at a concentration of 3% (Table 2). However, at this concentration, phytotoxicity was observed on the treated grapevines. It appears therefore that application of the oil at dormancy to reduce the carry over of *P. viticola* into the new season is not suitable due to its phytotoxic effects on grapevines, although application at 4 weeks before bud burst may overcome this problem. There were no significant differences in myceliogenic germination of sclerotia of *B. cinerea*. Percentage reduction in germination of sclerotia was 2%. Therefore, application of the oil at dormancy may not reduce the carry over of *Botrytis* inoculum significantly.

Table 2. – Effect of BP Summer Spray Oil[1] on the dormant structures of *Phomopsis viticola* (Type 2) and *Botrytis cinerea in vitro* on potato dextrose agar

Level of oil (%)	Exudation of pycnidia of *P.viticola** (%)	Reduction in exudation (%)	Myceliogenic germination of sclerotia of *B. cinerea** (mm)	Reduction in germination (%)
0	95 a	-	50 a	-
0.5	-	-	48 a	4
1.0	94 a	1	52 a	-
2.0	93 a	2.1	53 a	-
3.0	84 b	11.5	49 a	2

LSD (P = 0.05) [1] old formulation

Bioassay on leaves naturally infected with *U. necator*

When grape leaves naturally infected with *U. necator* were sprayed with the old and new formulations of the oil and placed on healthy leaves, they did not transmit the infection to the healthy leaves indicating that the oil inhibited the growth of the pathogen (Tables 3 and 4). However, when grape leaves naturally infected with *U. necator* were sprayed with water, they transmitted the disease to healthy leaves. Similarly, when non-symptomatic, healthy leaves were sprayed with the oils first and then inoculated with *U. necator* –infected leaf discs, no infection occurred on the sprayed leaves. These results show that BP Summer oil was effective as pre- and post-infection sprays in preventing infection of grape leaves by powdery mildew (*U. necator*).

Table. 3 – Effect of BP Summer Spray Oil[1] on pre- and post-infection of grapevine (cv. Chardonnay) leaves by *Uncinula necator*

Treatment of infected leaves (post-infection)	Transmission of infection to healthy leaves (%)	Treatment of healthy leaves (pre-infection)	Infection after inoculation of treated leaves (%)
Untreated (water)	100	Untreated (water)	100
Oil 0.5%	0	Oil 0.5%	0
Oil 1.0%	0	Oil 1.0%	0

[1] old formulation

Table. 4 – Effect of BP Summer Spray Oil[1] on pre- and post-infection of grapevine (cv. Chardonnay) leaves by *Uncinula necator*

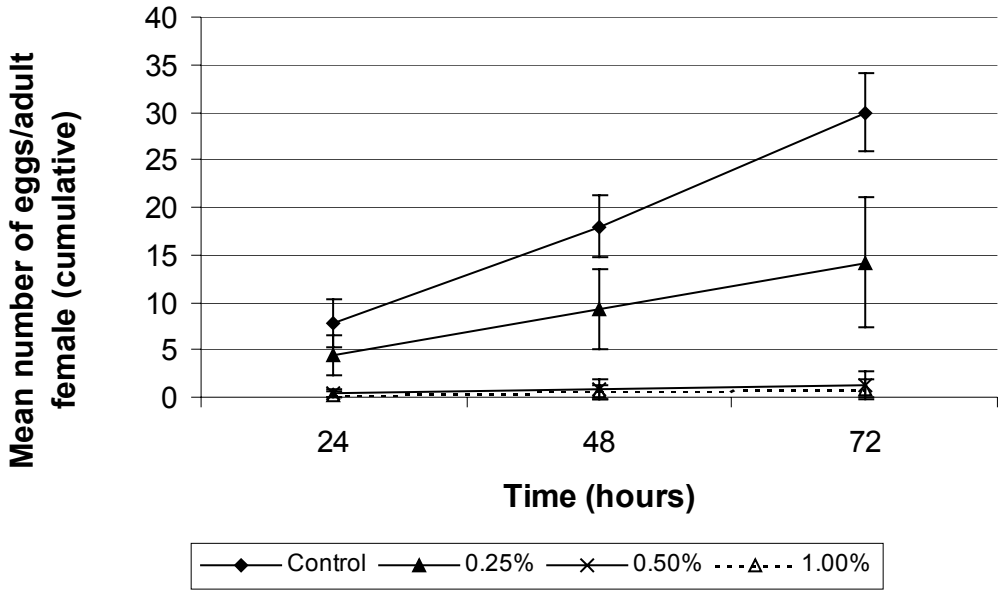
Treatment of infected leaves (post-infection)	Transmission of infection to healthy leaves (%)	Treatment of healthy leaves (pre-infection)	Infection after inoculation of treated leaves (%)
Untreated (water)	90	Untreated (water)	95
Oil 0.5%	0	Oil 0.5%	0
Oil 1.0%	0	Oil 1.0%	0

[1] new formulation

Bioassay on oviposition of *T. urticae*

Adult females of *T. urticae* (two spotted mite) laid significantly less eggs when treated with the spray oil at concentrations of 0.25, 0.5 and 1.0 % over a period of 72 hours (Fig. 1). Minimum number of eggs was laid at 1% oil.

Fig. 1. – Effect of sub-lethal doses of BP Summer Spray Oil[1] on ovisposition by *Tetranychus urticae*

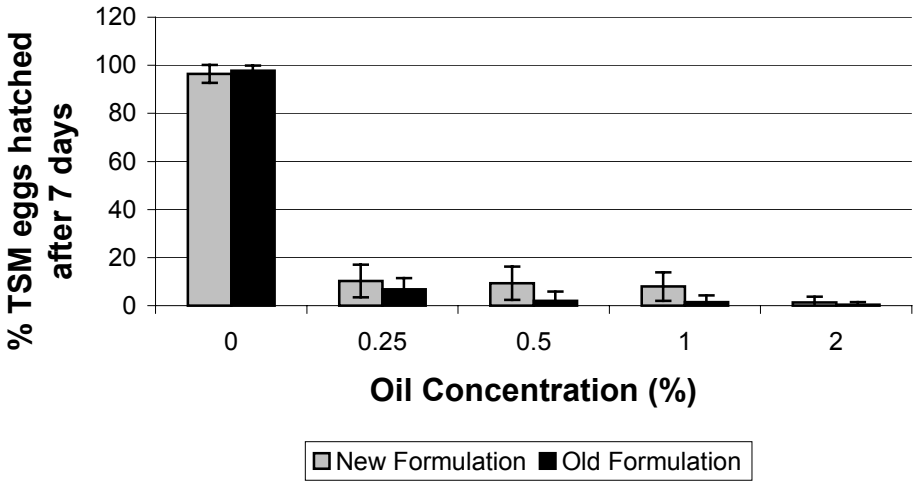


[1] old formulation

Bioassay on hatchability of eggs of *T. urticae*

Percent egg hatch after seven days was significantly less in all oil treatments (Fig. 2.) However, there were no significant differences in egg hatch between the levels of oil treatments. No significant difference in efficacy was observed between the old and new formulations of the oil.

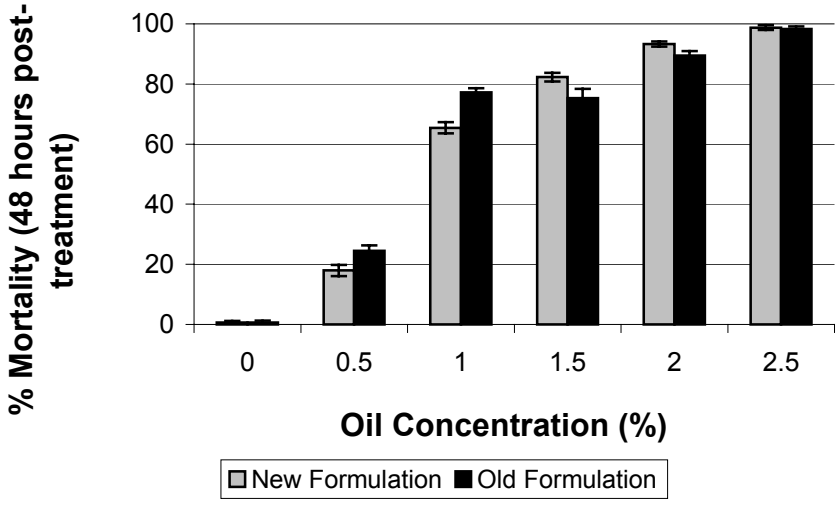
Fig. 2. – Hatchability of eggs of *T. urticae* treated with BP Summer Spray Oil



Bioassay on mortality of *T. urticae*

Mortality of adult female *T. urticae* was observed to be less than 100% at the highest concentration (2.5%) of the oil used (Fig. 3). However, it is likely to be phytotoxic at this concentration. Therefore, the oil has limited potential for direct control of *T. urticae*. However, combining its significant reduction in oviposition of adult female TSM with its significant reduction in hatchability of the eggs indicates that the oil can be used in the management of *T. urticae*. Significant differences were not observed between the old and new formulation of the oil.

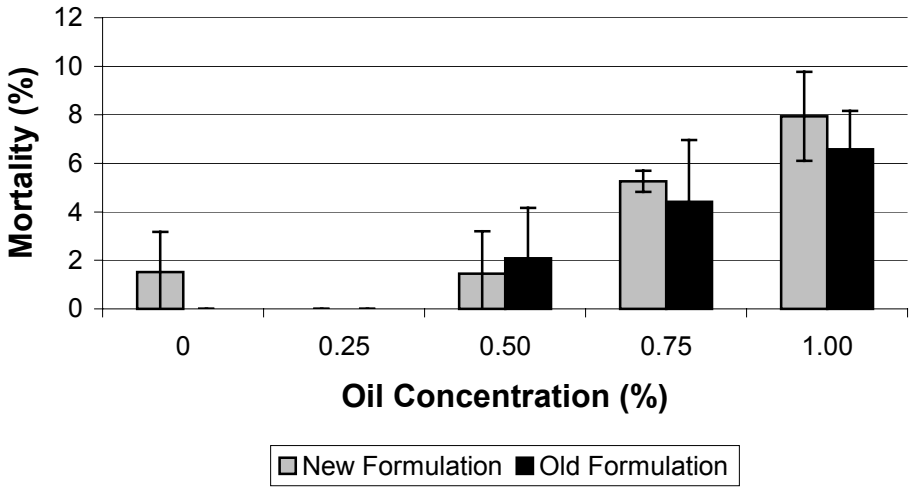
Fig. 3 – Effect of BP Summer Spray Oil on mortality of *Tetranychus urticae*



Bioassay on mortality of *C. vitis*

Blister mite mortality was found to be low after a single application at all levels of the oil tested (Fig. 4). A high level of mortality within erinose blisters on foliage was not expected as the plant hairs within the blisters protect the mite from desiccation and spray applications. However, Kallianpur *et al* (2000) found that in laboratory bioassays mortality was high when a second application of horticultural mineral oil was applied to *C. vitis* within erinose blisters. Our results from spray application trials on cv. Chardonnay at the University of Western Sydney vineyard also showed that multiple in season applications of BP Summer oil reduced the number of blisters formed on leaves (unpublished data). Repeated applications of the oil may have changed the surface tension of the leaves resulting in greater penetration of the blisters by the oil. Horticultural mineral oils have also been shown to have an effect on insect behaviour, such as feeding and oviposition.

Fig.4 - Effect of BP Summer Spray Oil on mortality of *Colomerus vitis*



Vineyard trial at Mudgee, New South Wales
(First year 2000/2001)

P. viticola (Type 2)

The incidence of *P. viticola* (Type 2) was lower in the dormant and in season treatments compared with that in the untreated control (Table 5). However, there were no consistent differences between the treated and the untreated grapevines during the growth of the crop. The incidence of *P. viticola* at 3 weeks before harvest was not significantly different between the treated and the untreated vines. It appears therefore that one application of the oil at dormancy may not be sufficient to lower the level of inoculum carry over to the new season.

Table 5. - Percent incidence of *Phomopsis viticola* (Type 2) on cv. Chardonnay in relation to spray applications of BP Summer Spray Oil[1] (Mudgee, NSW, 2000/2001)

Treatment	Beginning of flowering		30% cap fall		3 weeks before harvest
	Leaves	Internodes	Leaves	Internodes	Internodes
Dormant 3%	15 a	0 a	30 a	16 a	-
In season 0.5%	34 b	2 a	42 b	16 a	44 a
Unsprayed	50 c	34 b	51 c	34 b	40 a

LSD (P = 0.05) [1] old formulation

B. cinerea

There were no consistent differences in the incidence of *B. cinerea* between the untreated and the in season treatments at the different phenological stages monitored (Table 6). It appears therefore that oil is unlikely to control *Botrytis* in the field.

Table 6. - Percent incidence of *Botrytis cinerea* on cv. Chardonnay in relation to spray applications of BP Summer Spray Oil[1] (Mudgee, NSW, 2000/2001)

Treatment	100 %cap fall	Pre-bunch closure	Bunch closure	3 weeks before harvest
Dormant 3%	-	-	-	
In season 0.5%	4 a	26 a	13 a	26 a
Unsprayed	4 a	33 a	13 a	20 a

LSD (P = 0.01) [1] old formulation

U. necator

Incidence of *U. necator* (powdery mildew) was not recorded either in the oil-treated or untreated grapevines. This was due to the relatively high temperatures (weekly average $>30^0$C) recorded from fruit set to harvest coupled with little or no rainfall (weekly average <2mm). High incidence of *Plasmopara viticola* (downy mildew) was observed after veraison in all the treatments in spite of attempts to keep the disease under control by repeated spray applications of Ridomil®.

Arthropod pests

Arthropod pests were not present or present in very low numbers in the vineyard during the trial period. Low levels of light brown apple moth, two spotted mites and vine scale were observed; however, there were no significant differences in their numbers between the treatments, either before or after investigations.

Effect of calcium on incidence of *B. cinerea*

There were significant differences in the incidence of *Botrytis*, number of bunches and yield of grapes between the treated and the untreated vines (Table 7). Calcium appears to reduce the incidence of *Botrytis*. This supports what is previously known about the role of Ca^{2+} in maintaining cell membrane stability and thus increasing resistance to infection. It also appears that the application of calcium nitrate has compensated for the loss of Ca^{2+} that occurs normally through translocation out of the berries during ripening.

Table 7. - Incidence of *Botrytis cinerea* in cv. Chardonnay in relation to applications of calcium nitrate[1] at end of flowering and pre-bunch closure (Mudgee, NSW 2000/2001)

Treatment	Incidence of *Botrytis* (%)	Number of bunches	Yield of grapes (Kg)
Calcium	0 a	256 a	11.2 a
Untreated	20 b	236 b	7.3 b

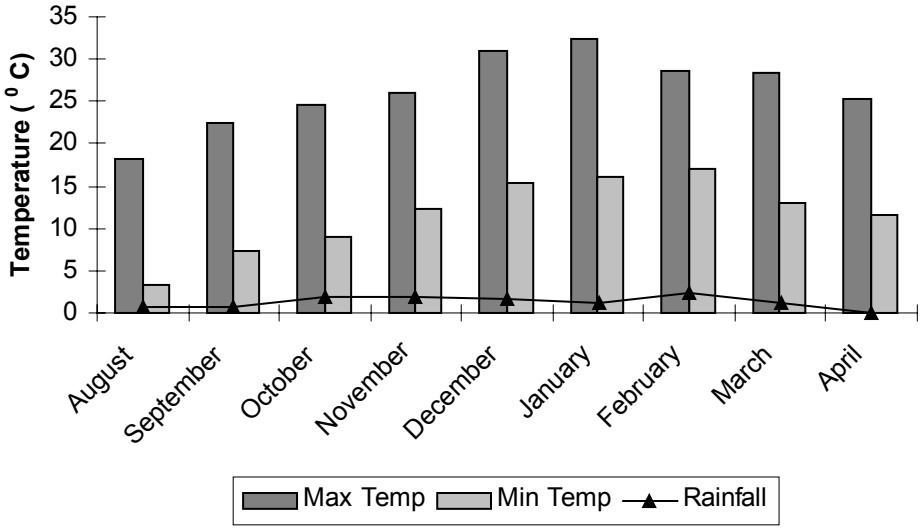
[1] rate of application = 1.5 Kg hectare^{-1} LSD (P = 0.01)

Vineyard trial at Muswellbrook, New South Wales
(First year 2001/2002)

U. necator

The incidence of *U. necator* (powdery mildew) was not observed in either the spray oil treated or the untreated vines. This was due to the vagaries of the weather prevailing during the trial. The monthly average maximum temperature during the trial was approximately 28.2^0C and the monthly average rainfall 1.3 mm (Fig. 5). It should be noted that grapevines in this trial block had been infected with powdery mildew during the previous season. Carry over of the disease was not observed.

Fig. 5 – Monthly maximum and minimum temperatures and rainfall during the trial at Muswellbrook, NSW, (2001-2002)



B. cinerea

The number of bunches per vine and weight of bunches per vine were significantly higher in the calcium-treated than in the untreated vines (Table 8, Figs. 6 and 7). It is not known whether the increase in weight of bunches in calcium treatments is due to the increase in their numbers due to the application of calcium. The application of calcium reduced significantly the incidence of *Botrytis* bunch rot. Although, in general, the incidence of *Botrytis* was relatively low, the application of calcium reduced bunch rot disease. These results confirm those obtained in the first trial (Mudgee, NSW, 2000/2001) of the project. These results also support once again what is previously known about the role of Ca^{2+} in maintaining cell membrane stability and thus increasing resistance to infection. It also appears that the application of calcium nitrate compensated for the loss of Ca^{2+} that occurs normally through translocation out of the berries during ripening.

Table 8. – Effect of applications of calcium nitrate[1] at end of flowering and at pre-bunch closure on incidence of *Botrytis cinerea* in cv. Chardonnay (Musswellbrook, NSW, 2001-2002)

Treatment	Incidence of *Botrytis* bunch rot (%)
Untreated	9 a
Calcium[1]	2 b

[1] rate of application = 1.5 Kg hectare^{-1} LSD (P = 0.05)

Fig. 6 - Effect of applications of calcium nitrate and BP Summer Spray Oil on the number of bunches in cv. Chardonnay (Muswellbrook, NSW, 2001-2002)

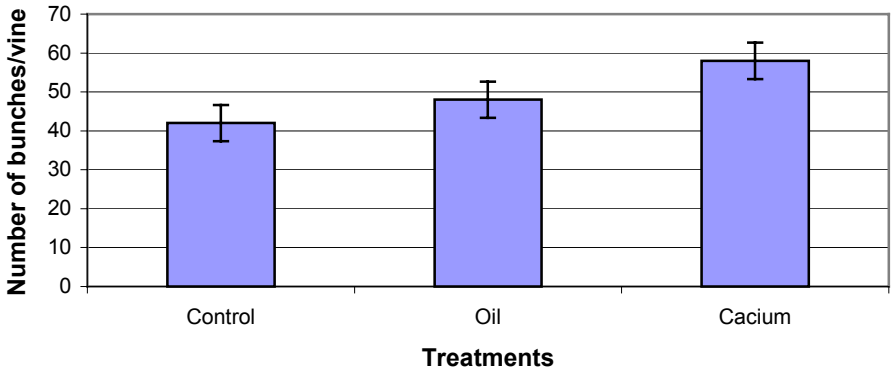
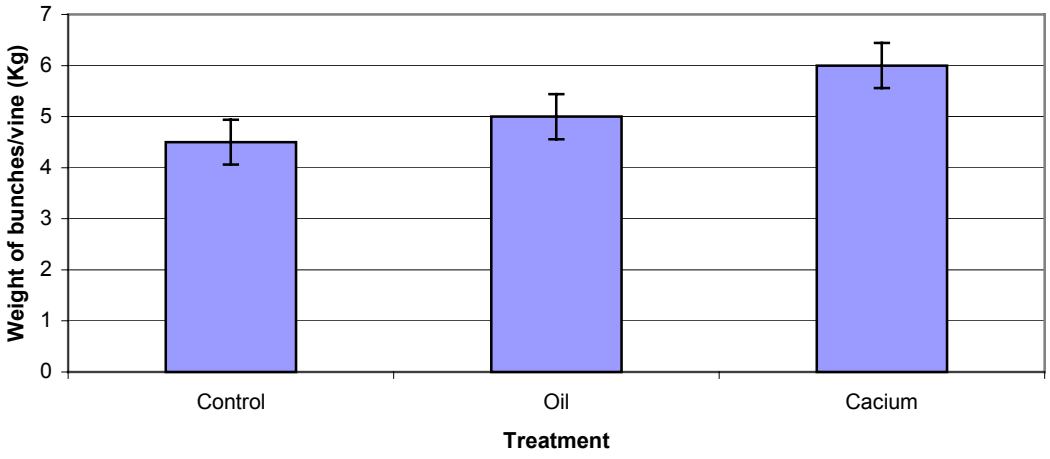


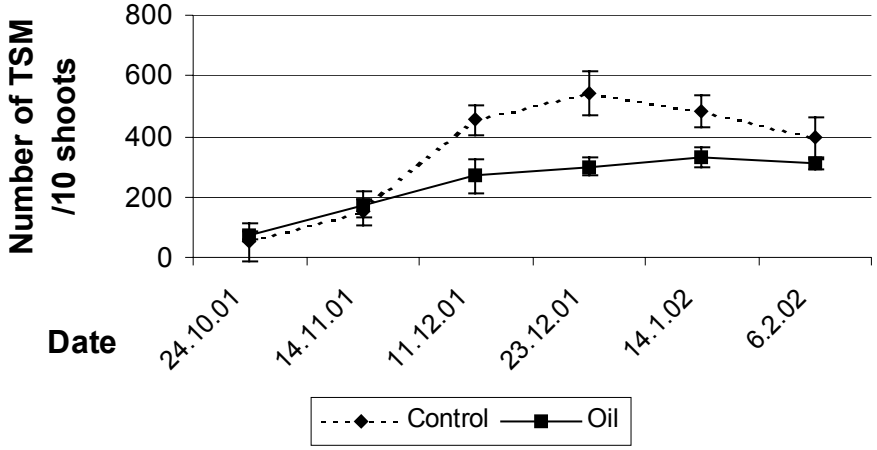
Fig. 7 - Effect of applications of calcium nitrate and BP Summer Spray Oil on the weight of bunches in cv. Chardonnay (Muswellbrook, NSW, 2001-2002)



T. urticae

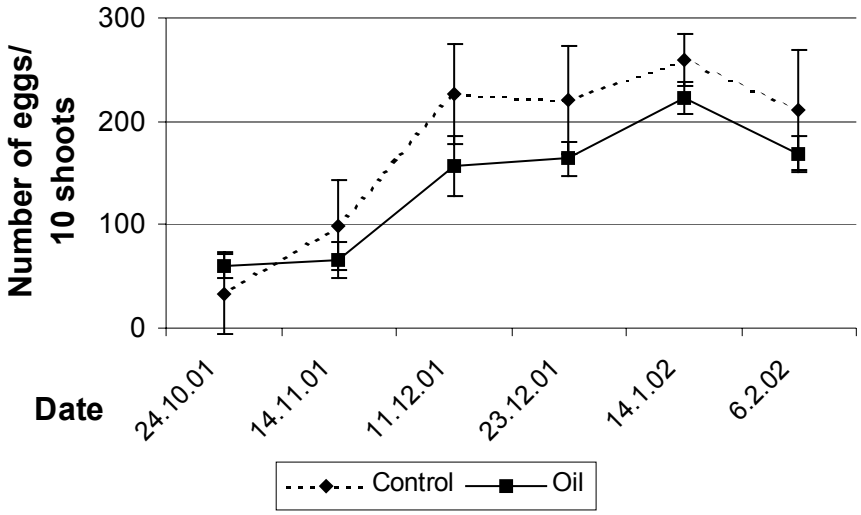
The population of two-spotted mites (motile and eggs) was not significantly different between the treatments at the start of the season. However, after four applications of the oil, the number of *T. urticae* motile was significantly lower in the oil treatment from fruit set to post-veraison (Fig. 8). It appears that adult females present on the vines treated with the oil lay fewer eggs (Fig. 9) that are unlikely to hatch resulting in low population. However, as *T. urticae* has a very short generation time, its population can only be kept low with repeated applications of the oil.

Fig. 8 – Numbers of *Tetranychus urticae* (two spotted mites) on cv. Chardonnay in relation to applications of BP Summer Spray Oil[1] (Muswellbrook, NSW, 2001-2002)



[1] new formulation

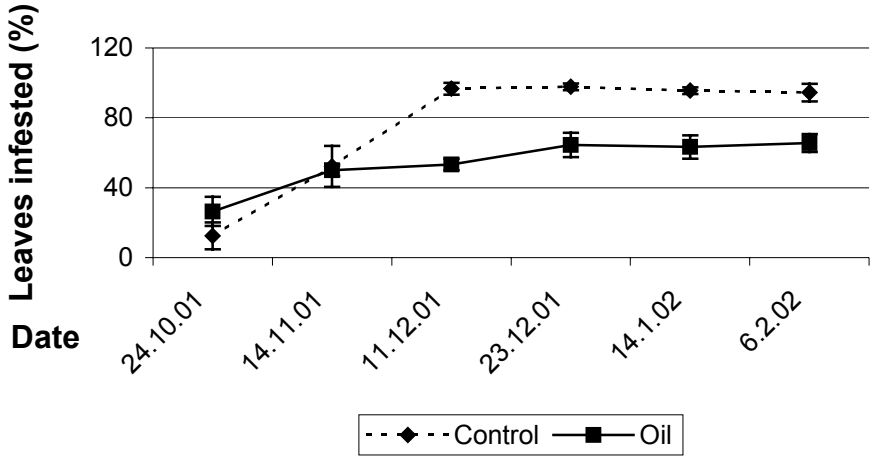
Fig. 9 – Numbers of eggs of *Tetranychus urticae* (two spotted mites) on cv. Chardonnay in relation to applications of BP Summer Spray Oil[1]
(Muswellbrook, NSW, 2001-2002)



[1] new formulation

Per cent grape leaves infested with two spotted mites and eggs was significantly lower in the oil treatment than in the untreated control from fruit set to pre-harvest (Fig. 10). This confirms the laboratory bioassay results which demonstrated lower oviposition and egg hatchability in oil treated leaves.

Fig.10 – Leaves of cv. Chardonnay infested with *Tetranychus urticae* (two spotted mites) and eggs in relation to applications of BP Summer Spray Oil[1] (Muswellbrook, NSW, 2001-2002)

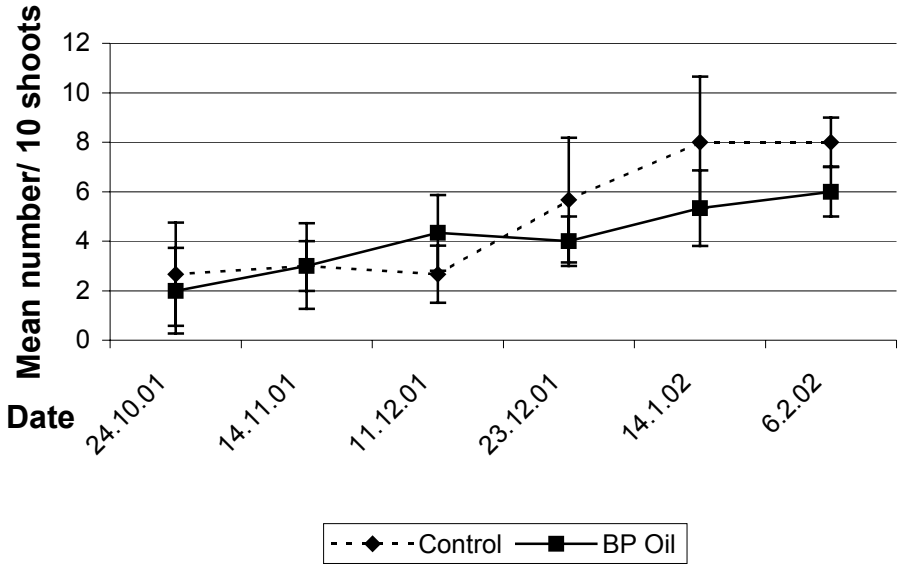


[1] new formulation

Predatory mites (*Phytoseiulus persimilis*, *Typhlodromus occidentalis*)

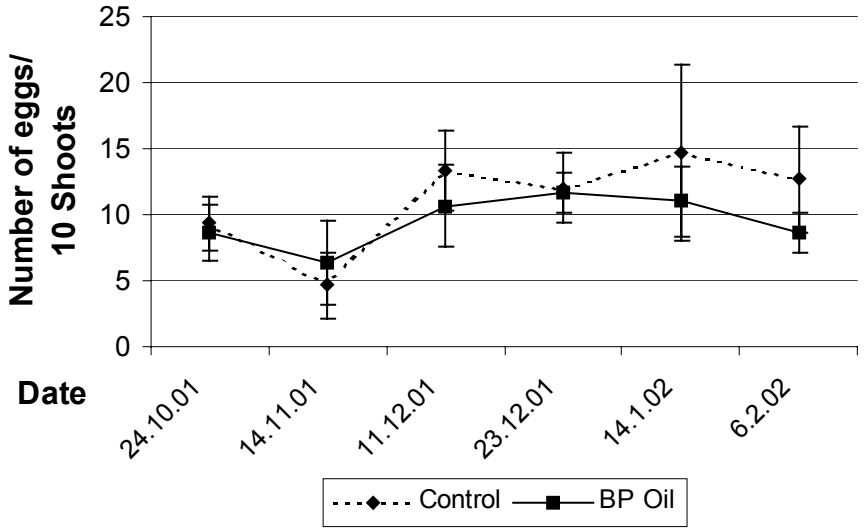
The numbers of predatory mites and eggs were not significantly different between the oil treatment and the untreated control (Figs. 11 and 12). Therefore, it appears that application of the oil does not influence the population of predatory mites in the vineyard.

Fig. 11 – Numbers of predatory mites on cv. Chardonnay in relation to application of BP Summer Spray Oil[1] (Muswellbrook, NSW, 2001-2002)



[1] new formulation

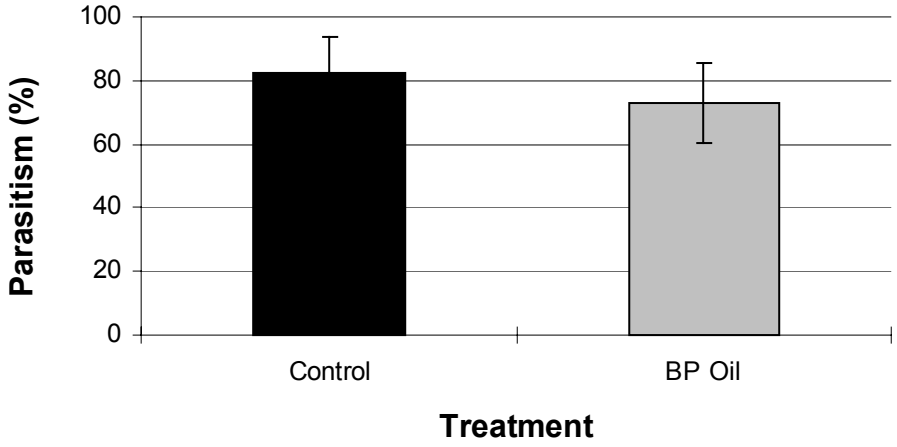
Fig. 12 - Numbers of predatory mite eggs on cv. Chardonnay in relation to application of BP Summer Spray Oil[1] (Muswellbrook, NSW, 2001-2002)



[1] new formulation

The numbers of adult scale parasitised during the season (number of adult scale present at the beginning of the season (September 2001) and those that had been parasitised in December (2001) were not significantly different between the oil treatment and the untreated control (Fig. 13).

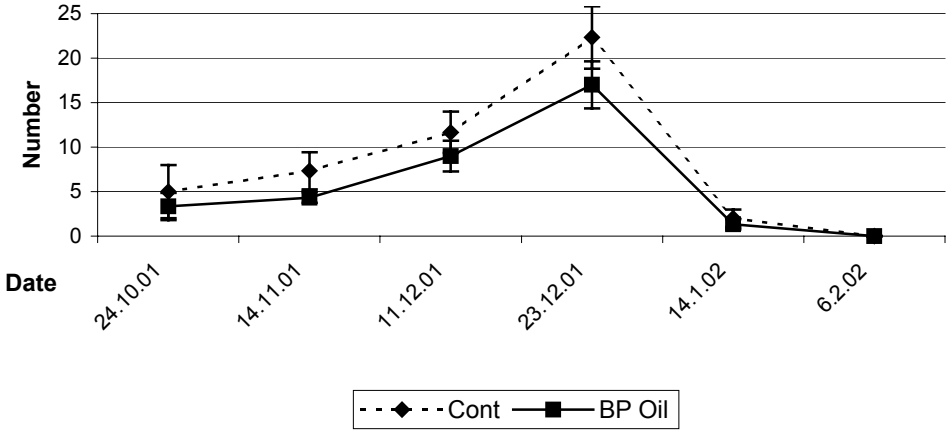
Fig. 13 – Parasitised adult scale (11.12.2001) on cv. Chardonnay in relation to application of BP Summer Spray Oil[1]
(Muswellbrook, NSW, 2001-2002)



[1] new formulation

Similarly, there were no significant differences between the numbers of ladybeetle predators from the Coccinellidae family in the oil-treated and untreated vines (Fig. 14). The indigenous ladybeetle *Cryptolaemus montrouzierei* was recorded feeding on grapevine scale crawlers in late November through to December 2001 on both treated and untreated rows. These results indicated that applications of the oil did not upset the natural balance of the predators in the crop.

Fig. 14 – Numbers of predatory ladybeetles (*Cryptolaemus*) on cv. Chardonnay
(Muswellbrook, NSW, 2001-2002)



Fluorochrome bioassay

Staining specificities and spectral properties of these fluorochromes are given in Table 9.

Table 9 Staining specificities and spectral properties of 5,6 carboxyfluorescein diacetate and propidium iodide

Stain	Specificity	Excitation (nm)	Barrier filter (nm)	Colour Live conidia	Dead conidia
CFDA[1]	esterases	490 – 510	515	green	none
PI[2]	DNA of damaged cells	460 - 490	515	none	red

[1] Carboxyfluorescein diacetate [2] Propidium iodide

Unstained conidia of *U. necator* did not show autofluorescence under the light sources used; however, the fluorochromes stained the conidia of *U. necator*. The staining combination of CFDA/PI produced intense green fluorescence of live conidia and red fluorescence of dead conidia of the fungal pathogen under blue excitation. The contrasting fluorescence of live (green) and dead (red) conidia of the fungus treated with different concentrations of the spray oil enabled us to estimate quantitatively the percentage survival of *U. necator* and thus, the efficacy of the oil.

The percentage of live conidia on the leaves decreased significantly with increase in the concentration of the oil as evidenced by the decreasing number of conidia emitting green fluorescence (Table 10). Percentages of live and dead conidia on leaves treated with 0.5% of the oil were 4 and 96% respectively. No conidia survived on leaves treated with 1.0% oil. Therefore application of spray oil significantly reduced the survival of the pathogen.

Table 10 Effect of BP Summer Spray Oil applied on grapevine (cv. Chardonnay) leaves naturally infected with *Uncinula necator* on survival of the pathogen

Concentration of spray oil [1] (%)	*Uncinula necator* Conidia (%)	
	Green fluorescence[2]	Red fluorescence[3]
0	95	5
0.5	4	96
1.0	0	100

[1] exposure time = 5 min [2] live conidia [3] dead conidia

These results confirm those obtained by Sergeeva *et al* (2002) on survival of *Plasmopara viticola* on grapevine following application of ClO_2 using the fluorochrome bioassay.

7. CONCLUSIONS

The effect of BP Summer Spray Oil on the control of powdery mildew (*Uncinula necator*) of grapevine:

Bioassay on leaves naturally infected with *Uncinula necator* showed that BP Summer Spray Oil was effective as pre- and post-infection sprays in preventing infection of grape leaves by powdery mildew (*U. necator*). These results could not be confirmed in the vineyard due to absence of the disease during two consecutive trials at Mudgee (2000/01) and at Muswellbrook (2001/02) New South Wales.

Effect of calcium nitrate on the incidence of *Botrytis cinerea* in cv. Chardonnay in the vineyard:

Applications of calcium at the end of flowering and at pre-bunch closure reduced the incidence of *Botrytis* bunch rot significantly in trials carried out during two consecutive seasons (2000/01 and 2001/02) in two different vineyards. These results also support what is previously known about the role of Ca^{2+} in maintaining cell membrane stability and thus increasing resistance to infection. The application of calcium nitrate may have compensated for the loss of Ca^{2+} that occurs normally through translocation out of the berries during ripening.

The effect of BP Summer Spray Oil on dormant structures of *Botrytis cinerea* and *Phomopsis viticola*:

When the oil was applied at a concentration of 3% as a dormant spray at two weeks before bud burst, exudation of pycnidia of *Phomopsis viticola* (Type 2) was reduced by 11.5 %; however, grapevines developed phytotoxic symptoms after bud burst. It is likely that application at 4 weeks before bud burst may overcome the problem of phytotoxicity. The oil did not affect germination of sclerotia of *Botrytis* and hence, may not be suitable for dormant application in the management of this pathogen.

The effect of BP Summer Spray Oil on arthropod pests:

The oil appears to have low potential in causing direct mortality in two spotted mites (*Tetranychus urticae*) (TSM). However, the significant reduction observed in oviposition of adult female TSM as well as in hatchability of the eggs indicates that the oil can be effective in the management of TSM. Significant differences were not observed between the old and new formulations of the oil.

The numbers of adult vinescale parasitised during the season (number of adult scale present at the beginning of the season (September 2001) and those that had been parasitised in December (2001) were not significantly different between the oil treatment and the untreated control. Similarly, there were no significant differences between the numbers of ladybeetle predators in the oil

and untreated vines. These results indicate that applications of the oil did not disrupt the natural balance of the predators and parasitoids (*Phytoseiulus persimilis*, *Typhlodromus occidentalis, Cryptolaemus montrouzierei*) in the crop.

The two outcomes from the project that could be of most benefit to grape growers in terms of economic returns are the potential for BP Summer Spray Oil to control powdery mildew and for calcium to control bunch rot. Another practical implication is the key role that oil could play in a resistance management strategy for the control of powdery mildew. Where pests such as two spotted mites and blister mites are important, such as in the Hunter Valley, the oil has the potential to reduce their effect on crop loss without disrupting the populations of beneficial arthropods.

8. RECOMMENDATIONS

An extension activity arising from this project could be to conduct vineyard demonstration trials on the effect of BP Summer Spray Oil to control powdery mildew on different cultivars of grapevines. The trial should be designed to demonstrate a strategy for the management of fungicide resistance by incorporating spray oil and 'at risk' fungicides such as DMIs.

The use of spray oil as part of an overall integrated pest and disease management program is recommended.

The effect of calcium in reducing incidence of *Botrytis* bunch rot should be further investigated in vineyard trials. Different timings of applications of calcium are required to determine the maximum impact of calcium on increasing the resistance of maturing grape berries to *Botrytis* infection.

9. APPENDIX 1: COMMUNICATION

Communication of the outcomes of the project to grape growers through workshops/seminar would enhance the uptake of the findings of this project.

An article will be prepared on the data collected in this project for publication in the Technical Issue of the Australian Grapegrower and Winemaker.

10 APPENDIX 2: INTELLECTUAL PROPERTY

There is no intellectual property associated with the outcomes of this project. The standard (old formulation) and the experimental grade (new formulation) of BP Summer Spray Oil are based on technologies developed by BP Australia.

11. APPENDIX 3: REFERENCES

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