Use of Predatory Mites for Long Term Prevention of Rust Mite, Bud Mite and Other Pest Mite Outbreaks in Australian Vineyards

FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Umbrella Project - Project Numbers:
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ABSTRACT

This study on Integrated Pest Management (IPM) of rust mite, bud mite, and two spotted mite in Australian viticulture concentrated on: **(i)** identifying predatory mite species (Family: Phytoseiidae) responsible for natural biological control of these vineyard mite pests in previously un-studied regions; **(ii)** laboratory investigation of wettable sulphur effects on these economically important predators; **(iii)** investigation of field effects of pesticides on these predators (naturally present and field released), with emphasis on wettable sulphur; **(iv)** evaluation of field releases of predatory mites **(v)** active involvement of wine regions in the project (via RITA), assisting growers to gain further knowledge in sustainable pest management, and how to minimise pesticide use; **(vi)** extensive research extension, including extension of overseas cutting-edge research; **(vii)** development and fine tuning of predatory mite rearing and selection for wettable sulphur tolerance; **(viii)** investigation of previously un-studied abundant predatory mites in the King Valley (Family: Anystidae).

We concentrated on effects of wettable sulphur (i.e. new high label rates against powdery mildew). These new high rates were identified as a key new disruptor of biological control of rust and bud mite in Australian vineyards. Wettable sulphur residues and direct oversprays were tested across a key range of concentrations in the laboratory on the predatory mites *Euseius victoriensis* and *Galendromus occidentalis,* followed by an investigation of field effects via predatory mite release, and assessments of released and naturally present predatory mite species. Concurrently run with this project was a laboratory pesticide-testing project (MU LTU 04-01- PART B), designed to test other pesticides registered for use in viticulture since previous testing (PART A). Analysis of a very large data set from all three projects, was used here to comprehensively determine the role of wettable sulphur, and other new pesticides in disrupting biological control of pest mites in vineyards, by predator suppression. Laboratory results on wettable sulphur (MU 05-01), and results on all other key wine industry pesticides (MU LTU 04-01- PART A, B) are reported in a combined form in large scientific and technical publications; a total of 36 pesticides was tested in comprehensive 14-day duration tests (evaluating 2, 4, 7-day mortality effects and effects on predatory mite egg lay). Several pesticides other than high rates of wettable sulphur were identified as disruptors of rust and bud mite biological control, via predator suppression; for complete analysis see Appendix 7: (Bernard et al. 2010a, Bernard et al. 2010b); Bernard M., Final Report to GWRDC MU LTU 04-01-PART B, June 2010). Wettable sulphur laboratory data in this report are extracted from these publications. Due to the tiny size of predatory mites and their prey, all laboratory and field assessments were done under the microscope (species determinations under compound microscope with oil immersion at 1000x magnification), resulting in a highly labour intensive project.

EXECUTIVE SUMMARY

- **Designating pesticides 'safe' to predatory mites.** Laboratory tests on detached soybean leaves embedded in cotton wool were used to test effects of wettable sulphur on *Euseius victoriensis*. Tests on detached French beans were used to test effects on *Galendromus occidentalis*. Tests were standardised across these two substrates as per (Bernard et al 2004) to provide direct comparison of results across test species. Tests were performed under "worst-case scenario" conditions. Worst-case pesticide exposure is exposure: **(i)** at highest registered field rate; **(ii)** to direct pesticide overspray/not just dry residue; **(iii)** of the youngest most susceptible life stage (freshly hatched 0-48 hours-old predatory mite juveniles); **(iv)** that mimics real conditions, spraying the initial food and water supply together with mites. **(v)** The length of the test is also important; 7-day mortality (not just 48-hour tests), and study of effects on female egg lay are essential for predatory mite testing by internationally established protocols for rigorous pesticide testing. This level of rigour is required to designate pesticides 'safe' to predatory mites. This project delivered such a high standard of testing, and could thus confidently assess wettable sulphur safety.

- **How laboratory data predict pesticide safety in the field.** When a test is performed in the laboratory under "worst case exposure" and the result shows a pesticide safe (i.e. no different in causing predator mortality than water), there is a high likelihood the pesticide will be safe under less intensive, but multiple field use. There is also a high likelihood the pesticide will impact even less on older less sensitive predatory mite life stages (most importantly, on reproducing adult females), and also impact less on other more pesticide-tolerant species within the vineyard predatory mite species complex. Information on the type and rigor of laboratory test is thus crucial when evaluating pesticide toxicity. This information should always be sought by growers and vignerons, when they are presented with pesticide safety claims by manufacturers or resellers of pesticides; without such information pesticide safety claims are meaningless. Yet in Australia, information on type and rigor of testing is seldom provided by commercial proponents of pesticide safety, and no compulsory testing standards on beneficials other than honey bees are legally required.

- **2nd tier testing.** Direct oversprays of wettable sulphur ≥ 600g/100L in the laboratory did not pass the 'worst case scenario' safety standard. Further testing of lower than maximum label rates, and fresh dry residues, was thus undertaken (maintaining all other worst-case conditions), to elucidate the level of wettable sulphur use safe to predatory mites. This testing is known as 2nd tier testing and is used to evaluate pesticide toxicity, by progressively reducing the severity of the test, and build a picture of likely pesticide field toxicity profile.

- **High wettable sulphur rates – toxic to predatory mites.** Concentrations of ≥ 600g/100L were highly toxic to *E. victoriensis* predatory mite. In fact wettable sulphur direct oversprays and freshly dry residues ≥ 400g/100L, equivalent to single use dilute spray at spray volume of 1000L/ha, were highly toxic to *E. victoriensis* already within 48-hours of exposure. The level of toxicity of 600g/100L was comparable to that of mancozeb, which completely suppressed female fecundity, and is highly toxic to a large number of predatory mite species world-wide. Highly concentrated low volume wettable sulphur sprays were identified as a key disruptor of biological control of grapevine rust mite and bud mite in Australian vineyards, through predator suppression. Besides the most toxic pesticide mancozeb (no longer widely used in Australian viticulture), high

label rates of wettable sulphur suppress predatory mite populations in vineyards, due to high single-application toxicity to some predatory mite species, combined with very high frequency of use, and high early season use. However, wettable sulphur overall is not as highly toxic to predatory mites in Australian vineyards as mancozeb, and does not induce rust mite and bud mite outbreaks as readily as mancozeb. This is because a variable degree of wettable sulphur tolerance exists among vineyard predatory mite species, and because some growers moderate sulphur use by applying rates at the lower range of the pesticide label.

- **Wettable sulphur tolerance - in some predatory mite species**. Young *E. victoriensis* juveniles were relatively tolerant of direct oversprays of 200g/100L (81% 7-day mortality; 52% overall fecundity reduction), allowing for some recruitment of the next predator generation after knockdown, and indicating a higher tolerance in more robust, adult female life stage of the predator. By contrast, (400g/100L; at 1000L/ha) as direct overspray, and even as residue caused effects (90% 7-day mortality; 95% overall fecundity reduction) to *E. victoriensis* juveniles. Young juveniles of the predatory mite *G. occidentalis* were considerably more tolerant of wettable sulphur than *E. victoriensis*. Wettable sulphur (400g/100L at 1000 L/ha spray volume; 4kg/ha) applied as direct overspray, was moderately toxic to *G. occidentalis* (59% 7-day mortality), and had no effect on fecundity of surviving females. Wettable sulphur (300g/100L at 1000L/ha; 3kg/ha), and the same spray at half spray volume (600g/100L at 500 L/ha; 3kg/ha) had a similarly low toxic effect, and there was no significant difference in between these two treatments on *G. occidentalis*. Results indicate differences in wettable sulphur tolerance in the predatory mite species complex, and strongly suggest that:

- **(1)** Traditional use of wettable sulphur at (200-400g/100L; 2kg/ha) is basically safe to most key phytoseiid predatory mites in the vineyard species complex, and has relatively low toxicity to even the most wettable sulphur-sensitive species in the complex (*E. victoriensis).*

- **(2)** Moderate increase in wettable sulphur use to 3kg/ha (concentrating up 2x to 600g/100L at 500 L/ha spray volume), or using a maximum of 400g/100L as dilute spray at 1000 L/ha (4kg/ha; no concentrating), is still relatively safe to some key predatory mite species. However, given some significant toxicity of these sprays **(1-2)**, and given synergistic toxic effects with repeated use of multiple pesticides, wettable sulphur sprays at even these rates need be well moderated.

- **(3)** The above wettable sulphur use still represent a more traditional, restrained practices. Wettable sulphur use beyond this level is detrimental to predatory mite populations, and to rust mite, and bud mite outbreak prevention. This detrimental effect further intensifies with repeated use, and with the use of high rates particularly early in the season.

- **Wettable sulphur tolerance limit.** Field data indicate the most wettable sulphur tolerant species is *Amblydromella doreenae*. *A. doreenae* displaced other phytoseiid predatory mite species in vineyards previously more species diverse; notably *E. victoriensis* predatory mites in some Riverland vineyards. However, even *A. doreenae* has a wettable sulphur tolerance limit. Extremely few or no *A. doreenae* predators were recovered from vineyards where repeated applications of wettable sulphur at 6 kg/ha in low volume 2-3x concentrated sprays were made in the same growing season and the preceding year. Such vineyards are highly susceptible to rust and bud mite outbreaks due to predator

suppression. Bud mite surveys in 11 vineyards (Riverland, McLaren Vale) revealed such bud mite infestations; results and damage photos (Table 8).

Given this high level of use is now a common industry practice in Australia, the wine industry has a problem in poor harmonising of powdery mildew control and bud and rust mite outbreak prevention. In some vineyards, predatory mites and their ecosystem service (effective free-of-charge rust mite and bud mite control) have become the casualty of powdery mildew management. Yet, effective control of powdery mildew has been reported for many years by growers who use low (2kg/ha) wettable sulphur rates.

Powdery mildew management compatible with predatory mites. The fungicides wettable sulphur (2kg/ha) azoxystrobin, fenarimol, hexaconazole, myclobutanil, penconazole, potassium bicarbonate, pyraclostrobin, quinoxyfen, spiroxamine, triadimenol, and trifloxystrobin have no or very minimal toxic effect on predatory mites (Bernard et al 2010 ab). Substitution for high rates of wettable sulphur would harmonise powdery mildew control with predatory mite conservation and rust mite and bud mite outbreak prevention.

- **Other pesticides toxic to predatory mites.** Pesticides other than high rates of wettable sulphur can contribute to rust mite and bud mite outbreaks. These are: botrytis fungicides benomyl, carbendazim, and pyrimethanil, and the insecticides spinosad and emamectin benzoate (M. Bernard, Final Report to GWRDC MU LTU 04-01 PART-B; Bernard et al. 2010a, b). When devising vineyard spray programs, it is important to minimise use of sprays that contribute to predatory mite kill. Carefully devised wettable sulphur programs are likely to have lower detrimental effects on predatory mites, when other pesticides toxic to predatory mites are not used; reduced synergistic toxic effects.

- **Predatory mites naturally present in vineyards (South Australia 2005; Victoria 2000-2001).** Survey of 26 vineyards (21 in South Australia; 5 in Victoria) revealed the predatory mite species complex (Family: Phytoseiidae) associated with rust mite and bud mite. Vineyards varied greatly in their predatory mite numbers. The average number, at flowering/ or mid-summer per 25 leaves (based on four replicates) varied from 0.25 ± 0.25 to 37.8 ± 6.05. Differences in abundance are attributed to differences in spray programs. All species collected, except *Gallendromus occidentalis,* were indigenous to Australia.

- **Predatory mite species (Riverland, McLaren Vale).** *Amblydromella doreenae* was the most abundant phytoseiid predatory mite species in all Riverland vineyards (6), and all McLaren Vale vineyards (7). It was the only phytoseiid predatory mite species (100%) collected in McLaren Vale vineyards, and the only species (97.9%) collected from Riverland, save two individual specimens of other species (*Neoseiulus dieteri*; *Neoseiulus loxtoni*); species abundance per individual vineyards are presented (Table 7).

- **Predatory mite species (Limestone Coast).** In Limestone Coast vineyards (7), the most abundant phytoseiid predatory mite species was *Amblydromella dossei*, and occurred in all vineyards surveyed. It was the most abundant species in six of these vineyards, and the only species collected from four vineyards. *Amblydromella doreenae* was most abundant in one of seven Limestone Coast vineyards, and co-occurred with *Amblydromella dossei* in three vineyards. Besides *A. dossei* and *A. doreenae*, only one other species was

collected (*Neoseiulus tareensis),* comprising 0.24% of all mites collected. Species abundance per individual vineyards are presented (Table 7).

- **Predatory mite species (Yarra Valley, King Valley; 2000-2001).** A total of five phytoseiid species were collected from five vineyards in Victoria, three were common: *Amblydromella doreenae, Amblydromella dossei, Galendromus occidentalis.* The most abundant was *A. doreenae*, and occurred in all five vineyards. *A. dossei* commonly occurred in high numbers alongside *A. doreenae* in two sustainably-managed vineyards in the Yarra Valley, and *G. occidentalis* alongside *A. doreenae* in one sustainably-managed vineyard in Yarra Valley, and two in King Valley. Species abundance per region: Yarra Valley (N = 113) - *A. doreenae* (53.1%), *A. dossei* (33.6%), *G. occidentalis* (12.4%), *Amblydromella recki* (0.9%); King Valley (N = 238) - *A. doreenae* (68.9%), *A. dossei* (0%), *G. occidentalis* (29.4%), *Neoseiulus helmi* (1.68%). Species abundance per individual vineyards are presented(Table 7).

- **Predatory mite releases.** *Euseius victoriensis* established in three of 10 vineyards where it was released; all had low (2kg/ha) wettable sulphur use. Predatory mites were naturally present in vineyards regardless of *E. victoriensis* releases, in all vineyards these were species other than *E. victoriensis*, notably *Amblydromella doreenae.* This project concludes that *A. doreenae* is the most wettable sulphur/ pesticide tolerant predatory mite species in Australian vineyards. This project also concludes that predatory mite releases in vineyards with traditional wettable sulphur use are not necessary. Releases of *A. doreenae* species in vineyards where predatory mites have been wiped-out by sprays, for initial re-establishment could be of great benefit. Such releases would only be successful if subsequent spray programs were predatory-mite-compatible. Predatory mite species per individual vineyards are presented (Tables 9-10).

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3. BACKGROUND

INTERNATIONAL CONTEXT: Integrated Pest Management (IPM) of rust mites, bud mites and two-spotted mites in vineyards and orchards world-wide is successfully achieved by biological control, provided mainly by phytoseiid predatory mites (Gambaro and Ivancich Gambaro 1975, Field 1978, Collyer 1980, Genini and Baillod 1987, Smith and Papacek 1991, Hardman et al. 1997, Hardman et al. 2000). The value of this biological control ecosystem service (ES) is clearly indicated by the rather astonishing fact that rust mites (including grapevine rust mite, citrus rust mites, stone and pome fruit rust mites) were not major pests in any crop prior to the widespread use of insecticides after World War II (Easterbrook 1996). The pest status of these rust mite species has increased world-wide since the 1960's, alongside major increases in the use of pyrethroid insecticides and synthetic acaricides (Perring and McMurtry 1996). In viticulture, outbreaks of grapevine rust mite in Europe (Baillod and Guignard 1986, Duso and De Lillo 1996, Perez Moreno and Moraza Zorrilla 1998, Duffner 1999).

AUSTRALIAN CONTEXT: Grapevine rust mite outbreaks in the 1990's in Australia (Bernard 2005), and the rising pest status of grapevine bud mite in Australia and South Africa (Dennill 1986, 1991, Bernard et al. 2005), indicate the same trend, of diminishing biological control provided by phytoseiid predatory mites and other natural enemies. This ecosystem service (ES) provided by predatory mites is crucial to the control and prevention of outbreaks of grapevine rust mite, grapevine bud mite, and two spotted mite in Australian vineyards.

In the Australian wine industry, typical damage symptoms caused by grapevine rust mite - vine leaf and shoot distortions, retarded early spring growth, and leaf bronzing in summer - were widespread and severe in the 1990's across all wine regions. These symptoms were subject to investigation by a PhD research project (part of GWRDC DAV 98/1). As a result, symptoms were prevented by a single spray of wettable sulphur/canola oil, precisely timed to rust mite emergence from winter shelters (Bernard 2005, Bernard et al. 2005), and by limiting the use of mancozeb (widely used at the time, and toxic to predatory mites). This large scale, cross-regional successful removal of grapevine rust mite symptoms unmasked underlying patchy bud mite infestations and damage (Bernard et al. 2005), that was in turn successfully treated by wettable sulphur timed to the narrow window of bud mite spring emergence Dennill (1986). While these sprays were effective in killing large rust and bud mite populations, field experience in the Australian wine industry over the subsequent ten years repeatedly showed that long term prevention of rust mite damage (without a need for these annual curative sprays) requires the conservation of phytoseiid predatory mites.

A recent resurgence (since *c.* 2005/06) of rust mite damage symptoms in some Australian vineyards indicated predatory mite conservation was again jeopardized, through natural enemy suppression, potentially by some of the many newly registered fungicides and insecticides, and by the tripling of wettable sulphur label rates (from 200g/100L to 600g/100L @ 1000L/ha), and further concentrated (up to 3x) in low volume sprays. The pesticide mancozeb, highly toxic to predatory mites (Bernard et al 2004) and widely used prior to 2003, was no longer widely used due to adoption into vineyard practice of previous research (M. Bernard; DAV 98/1). Newly registered pesticides contributing to the new outbreaks were investigated in MU LTU 04/01 PART-B, and studies on wettable sulphur were undertaken in this current project (MU 05-01/ RT 05/02-3a,b,c,d).

PREVIOUS RESEARCH PROJECTS. Prior to this project, Integrated Management of vineyard mite pests was studied in Australia in three small GWRDC projects: **(i)** PhD scholarship (David James 1992-1996) study of predatory mite species in warm climate vineyards of Murrimbigee irrigation district, and cool climate Canberra region; **(ii)** PhD scholarship (M. Bernard 2000-2004) funded as part of (DAV 98/1); **(iii)** testing of predatory mites as part of a vineyard insect ecology project (M. Bernard, MU LTU 04-01-PART A). The current project (MU 05-01//RT 05/02-3 abcd) thus aimed to build on knowledge gained by these previous studies, filling in information gaps crucial to sustaining economic benefits gained from industry-wide breakthroughs on successful rust and bud mite control gained by M. Bernard et al. (DAV 98/1). The current project was an umbrella project, comprising direct GWRDC funds (MU 05-01) for laboratory research and extension, and for field research the funding involvement of wine industry regions via RITA: King Valley in Victoria (RT 05/02-3a), Riverland (RT 05/02-3b), McLaren Vale (RT 05/02-3c), and Limestone Coast (RT 05/02-3d) in South Australia. We studied predatory mite species in hitherto unexplored wine regions and effects specifically of wettable sulphur on predatory mites. In two regions (Riverland, King Valley), further related studies were undertaken, to answer practical questions of grower interest unique to each region. In Riverland, a survey of grapevine bud mite (*Colomerus vitis)*, a pest issue in the region, was undertaken. In the King Valley, a study of an abundant, but almost unstudied, predatory mite *Anystis* sp. (whirligig mite) was undertaken. Identification to species of previously collected predatory mite specimens from the King Valley and Yarra Valley was also carried out.

WETTABLE SULPHUR USE - AUSTRALIAN VINEYARDS. Wettable sulphur is arguably the key most widely used wine industry pesticide. Traditional use (1-2 kg/ha; spray tank concentrations 200 - 400g/100L) has been replaced since *c.* 2004 by much higher label rates (2-6 kg/ha). Wettable sulphur is now thus commonly used in a wide range of concentrations; at the lower limit are dilute high volume sprays (200 - 400g/100L), delivering a 2kg/ha mass of pesticide and representing traditional restrained use, at *c.* 3 - 5 sprays per season. At the high-use range are 9-12 applications per season of concentrated low volume sprays (600g/100L -1800g/100L) at the highest allowable mass of pesticide per hectare (6 kg), delivering an extremely concentrated micro-dose at the point of spray contact with predatory mites and other beneficial invertebrates. Compared to other pesticides registered for use in Australian viticulture, a label rate that allows 300% range in the mass of pesticide per hectare, and 900% range in spray tank concentrations is unprecedented. It reflects a general confusion about what rates of wettable sulphur actually deliver effective powdery mildew control, and it has created the expectation that high use is best. Given this rage of use, an elucidation of wettable sulphur effects on predatory mites was a complex task.

Field research in this project (RT 05/02-3 abcd) thus carried out grower-driven predatory mite releases, and as add-on research value, attempted to elucidate wettable sulphur field effects on <u>both</u> released and naturally present predatory mite species.

PROJECT AIMS

1. ✓ *Euseius victoriensis* predatory mite selection for wettable sulphur tolerance
2. ✓ Testing wettable sulphur tolerance of a selected *E. victoriensis* strain
3. ✓ Mass rearing and release of *E. victoriensis* in 3 sites where rust mite is still reaching outbreaks conditions, and possibly where RSG due to bud mite occurs
4. ✓ Investigating *E. victoriensis* establishment in vineyards following inoculative releases
5. ✓ Determining predatory mite species feeding on rust mite in SA and Victoria
6. ✓ Survey of unburst buds in Riverland for bud mite and predators of bud mite
7. ✓ Determining predatory mite species found feeding on bud mite in Riverland
8. ✓ Survey of other key predatory mite species other than Phytoseiidae: Anystidae (King Valley) and species determination
9. ✓ Extension of results to growers in participating regions
10. ✓ Comprehensive publications in trade and scientific journals and final report

PERFORMANCE TARGETS AND DATES

From 'Final project application'; approved variations as per annual and biannual progress reports to GWRDC.

Year I: 2005/2006 - July 2005 – June 2006

Output	**Performance target** **Performance target date**		**Completion report**
1A. (Approved variation 1) Pre- release assessment of naturally present predatory mite fauna: baseline against which to evaluate releases	Field: Enlist growers for project, visit all sites to discuss project requirements with growers, decide and mark release/control blocks, collect pre-release samples. Laboratory: Assess pre-release samples for number, and species of predatory mites.		Field: site selection and set up; 21 vineyards sampled in pre-release assessments - **Completed Dec 2005** Laboratory: assessment of mite numbers - **Completed Dec 2005;** Mounting of specimens onto slides - **Completed Jan 2006;** Species ID of all specimens from 21 vineyards - **Completed March 06**
1. E. *victoriensis* strain for mass release established	Laboratory mass rearing techniques fine-tuned; Mass rearing for releases		**Completed Oct 2005** **Completed Nov 05 -Jan 06**
2. E. victoriensis releases	3 release sites (McLaren Vale, Riverland, Limestone Coast)		Releases in McLaren Vale (4 sites), Riverland (4 sites), 8 sites in total. **Completed for 2005/06**, **target exceeded by 5 vineyards!** Limestone releases delayed to November 2006.
3. Bioassays - *E. victoriensis* wettable sulphur tolerance in the laboratory	Preparation of bioassays Bioassays completed to international IOBC standard; Data analysed/ publication prepared by Dec 2006	Bioassays planned by Dec 2007. New completion date June 2008 (Approved variation 2; due to building refurbishment/pesticide testing facility delays associated with lab relocation from La Trobe University to The University of Melbourne.) Bioassays **Completed February - June 2008;** Manuscript **Completed/Submitted Oct 2009;** Scientific paper published **December 2010.**	
4. Extension workshops in all participating regions	Completed by September 2006		Six grower seminars **- Completed August- October 2006; target exceeded by 2 seminars**; details see 'Communication' p. 48

Year II: 2006/2007 - July 2006 - June 2007

November 2006 - July 2007: Part time employment (0.6 EFT) due to CI's medical condition (medical certificate supplied to GWRDC)

OUTPUTS	PERFORMANCE TARGETS		TARGET DATE	Completion report
1. *E.victoriensis* releases (McLarenVale, Riverland, Limestone Coast)	Three release sites (McLaren Vale, Riverland, Limestone Coast)	2006-07 season	**Completed for 2006/2007 Novemer 2006, target exceeded by 6 vineyards;** (Approved Variation 3: increased number of vineyards participating in project, due to grower demand; see Output 4 2007-08)	
2. Evaluation of releases (McLaren Vale, Riverland, Limestone Coast)	Quantitative assessments of releases and identification to species in three above release sites		2006-07 season	Postponed until 2007-08 Field work July - Dec 2006 postponed to July- Dec 2007 (Approved variation 4; postponing field research component of project, due to CI's part time employment/ due to a medical condition)
3. Predatory mite species feeding on rust mite determined	Quantitative assessment of stored samples from DAV 98/01 samples from sites with extremely high, known rust mite numbers, identification to species (Yarra Valley, King Valley) by July 2007		July 2007	**Completed January 2007, seven months ahead of time;** in lieu of variation 4.
* 4. Prevalence of bud mite in PBN (Primary bud necrosis) and poor bud burst symptoms determined (Riverland)	Survey of unburst buds in 5-15 sites, bud dissections and bud mite numbers assessed, and predatory mites feeding on bud mite determined to species (Riverland)	Octo-ber 2007	Survey commenced Spring 2007, but was subsequently postponed on growers' request due to extremely severe drought conditions in Riverland, interfering with normal bud burst, and confounding investigation of PBN due bud mite; **Completed October 2008; Laboatory assessments completed November 2008** (Approved variation 5)	
5. Literature review	Anystidae literature review, and all other literature reviews completed		October 2006	**Completed October 2006**
6. Predatory mite surveys: Anystidae (King Valley)	Survey of Anystidae and other predatory mites, and identification to species continued (King Valley)	2006-07 season	**Completed November 2006 -** survey **Completed January 2007 -** quantitative assessments, mounting, identification to Family level	
7. Extension workshops Winter 2006 (all regions)	7. Extension workshops Winter 2007	Oct 2007	13 grower seminars - **completed Aug 2007; exceeding project target by 9 seminars (!)** details see 'Communication' p. 49-50	

*** Survey of unburst buds (PBN) for prevalence of bud mite, Riverland, SA**

Survey of unburst buds in Riverland commenced in spring 2007, but was then postponed on growers' request, due to severe drought. The survey was advertised in the Riverland Wine Industry Council (RWIC) online newsletter in early spring 2007, 9 growers, with vineyards that had a history of poor budburst and bud burst delay were, interviewed by phone, and bud mite/ PBN assessments dates set. But within a few weeks, vines showing delayed bud-burst finally grew, growers attributed delayed bud-burst to drought, and suggested no bud mite/ PBN assessments for such an anomalous year. The drought-related, late patchy bud burst, masked bud mite/ PBN symptoms, making these difficult to separate from similar symptoms due to drought. The survey was then set for October 2008 in Riverland and if needed, would have been completed in regions where drought conditions have abated. The survey was completed in Riverland in October 2008.

Year III: 2007/2008 - July 2007 - June 2008

OUTPUTS	PERFORMANCE TARGETS	TARGET DATE	Completion report
1. *E. victoriensis* releases (Limestone Coast, McLaren Vale, Riverland)	Three release sites (McLaren Vale,Riverland, Limestone Coast	2007-08 season	*E. victoriensis* culture mass-reared in spring 2007; for releases in 9 vineyards in spring, and some consecutive releases in January (Riverland, Limestone Coast, McLaren Vale). **Completed Nov- Dec 2007, Jan - Feb 2008, target exceeded by 6 vineyards.**
2. Output 2006/07 Evaluation of releases (Limestone Coast, McLaren Vale, Riverland)	Quantitative assessments and identification to species in 3 release sites	New target 2007-08 season	Harvest assessments of *E. victoriensis* releases, and of naturally present predatory mite species in release sites. **Completed in 9 vineyards, Match 2008, target exceeded by six vineyards.** (Approved Variation 4)
2. Output 2007/08 Evaluation of releases (Limestone Coast, McLaren Vale, Riverland)	Final evaluation of releases-assessments and identification to species in 3 sites	New target 2008-09 season	Final assessments of *E. victoriensis* releases, and of naturally present predatory mite species in release sites. Due 6 months later than listed in Final Project Application, due to variation 4 extending total length of project field work by 6 months.(Approved Variation 4)
3. Predatory mite surveys: Anystidae (King Valley)	Survey of Anystidae and other predatory mites, and identifica-tion to species - continued	2007-08 season	Detailed annual seasonality survey of Anystidae and associated predatory mites in two vineyards King Valley (every 3-4 wks, March 2007 - March 2008); sampling vine canopy, mid-rows, and trapping mites moving between the two strata **Completed March 2008;** Identifications: Specimen samples mounted and identified to Family, Sub-Family/Genus, most commonly found (*Anystis baccarum*) identified to species **Completed June 2008;** Species ID of less common species associated with *A. baccarum* - **half completed August 2008**; completion on schedule for publication Feb 2012. (Approved Variation 6 - Maternity leave)
4. Honours project	Honours student project investigating suitability of pollens of typical inter-row grasses as diet for *E. victoriensis*; pollen diet evaluated, data processed and analysed, publication prepared by student by Aug 2008	Season 2007-08	Substituted output 4 below due to grower demand
* 4. Substitute output: Investigation of field effects of wettable sulphur on predatory mites (Approved Variation 3)	**Substitute Outputs 1 – 2**: 3x increased size of Output I 2006/ 07, 2007/08, 2008/09 seasons, and Output II in 2007/08, 2008/09, due to: **(i)** grower demand for direct participation in releases; **(ii)** gain site numbers high enough to investigate field effects of wettable sulphur on predatory mites in study sites; a key industry priority.		See Year II, III, VI, Outputs 1 and 2.
5. Extension workshops	Extension workshops winter 2008	August 2008	Five grower seminars, **exceeding project target by one seminar - Completed September 2008;** for details see 'Communication' p. 50
6. Publications	two technical; two scientific publications	February 2011	**Completed papers:** (Approved Variation 6) **(1)** Technical - Australian Viticulture **Sept/Oct 2010** **(2)** Scientific - J of Economic Entomology **Dec 2010**; **(3)** Technical - June 2011 (see Appendix 7) (Variation 7 -Maternity leave) **(4)** Scientific - due date February 2012

***Substitute Output 4:** Nine release/assessment vineyards instead of three. In order to: **(i)** accommodate more growers in the regions who wished to take part in predatory mite releases; **(ii)** The question of wettable sulphur use compatible with predatory mites, proved a far more topical industry question, then suitability of pollen diet for predatory mites; even an average wettable sulphur use of new high label rates can kill predatory mites, rendering provision of pollen resources secondary; **(iii)** Increase in release/assessment sites from 3 to 9 allowed for investigation of *E. victoriensis* establishment and of survival of naturally present predatory mite species in vineyards under varying wettable sulphur use; **(iv)** Laboratory evaluations of wettable sulphur tolerance were thus matched by field evaluations; representing substantial value adding to project outcomes.

Year IV: 2008/2009

July 2008- December 2008: Employment 6 months full time

January 2010 - 13 July 2009: Maternity leave - 6 months full time

OUTPUTS	PERFORMANCE TARGETS	TARGET DATE	Completion details and date
1. Final E. victoriensis releases 2008-09	Releases in new and existing sites	June 2009	*E. victoriensis culture* was mass-reared in early spring for final October- November 2008 releases, Releases were completed on schedule; **target of 3 release sites exceed by 6 vineyards.**
2. Final evaluation of releases	Final evaluation in Mclaren Vale, River-land, Limestone Coast; samples collected, quantitative assessment and ID to species	June 2009	Final quantitative assessments of *E. victoriensis* releases, and of naturally present predatory mite species in release sites - **Completed December 2008**; Final qualitative assessment - mounting of specimens onto glass slides and identification to species - **Completed October 2010, target of 3 assessment sites exceed by 6 vineyards.**
(Approved variation 5) Output 4. 2007/08 Prevalence of bud mite in PBN and poor bud burst symptoms determined (Riverland)	Survey of unburst buds in 5-15 sites, bud dissections and bud mite numbers assessed, and predatory mites feeding on bud mite determined to species (Riverland)	October 2008	Survey and assessment of 8 Riverland sites, and additional, above target, 4 McLaren Vale sites (on grower request) - **Completed October 2008** Laboatory disections of buds and assessments of bud mite damage and presence - **Completed November 2008**
(Approved variation 2) Output 3. 2005/06 Bioassays - *E. victoriensis* wettable sulphur tolerance in the laboratory	Preparation of bioassays, assays completed to international IOBC standard; Data analysed/ publication prepared	Bioassays planned by Dec 2007, completed June 2008	Bioassays - **Completed Feb - June 2008;** Data analysis **- Completed December 2008** Scientific manuscript - **Submitted Oct 2009** Scientific paper - **Published December 2010** (Approved variation 6- Maternity leave) **Wettable sulphur laboratory data were also reported to GWRDC in the format of Final Report MU LTU 04-01-PART B, June 2010, 6 months ahead of due date February 2011. Reported again Final Report MU 05-01, 1 June 2011.**
3. Final report	4th February 2011		**1st June 2011** (Variation 7 - Maternity leave)

Year V: 2009/2010
July 2009 - September 2010: Employment (0.2 EFT), Marternity leave (0.8 EFT)
Completion of GWRDC outputs/ performance targets during 0.8 EFT maternity leave

OUTPUTS	PERFORMANCE TARGETS	Completion details and date
Publications	two technical; two scientific publications	**(1)** Technical I- Published Australian Viticulture Sept/Oct 2010 **(2)** Scientific I- Published Journal of Economic Entomology - Dec 2010 **(3)** Scientific manuscript II - substantial progress to completion made
Final Reports to GWRDC		**(4)** * Final report to GWRDC MU LTU 04-01- PART B, 30 June 2010
Progress Reports to GWRDC		**(5)** Progress Report to GWRDC MU 05-01 February 2009 **(6)** Progress Report to GWRDC MU 05-01 February 2010 **(7)** Progress Report to GWRDC MU 05-01 July 2010 **(8)** Progress report to GWRDC MU LTU 04-01-PART B February 2009 **(9)** Progress report to GWRDC MU LTU 04-01-PART B February 2010

* Final Report to GWRDC MU LTU 04-01 PART B also included all MU 05-01 wettable sulphur laboratory data, because MU 05/01 wettable sulphur testing and MU LTU 04-01- PART B testing of other pesticides was undertaken concurrently in large-size combined bioassays, in order to: **(i)** provide direct (within bioassay) comparisons of same-target-pesticides for maximum practical value to vignerons; and **(ii)** to make the best, most time-efficient, use of mite colonies in block testing, reducing facilities fees payable to DPI Victoria for the use of Knoxfield Pesticide Testing Laboratory.

Year VI: 2010/2011
1 October 2010 – 11 April 2011: Maternity leave - full time
12 April - to date: Casual employment completing project

5.1 METHODS
LABORATORY TESTING

5.1 Wettable sulphur tests on predatory mites

Three laboratory bioassays on wettable sulphur were carried out on two predatory mite species as part of this project; eight wettable sulphur concentrations were tested in direct oversprays and residue tests (Bioassays I-III; Table 1). Results are direct oversprays unless otherwise stated.

Wettable sulphur testing in this project was carried out in large combined bioassays jointly with project (MU LTU 04-01- PART B), which tested other same-target fungicides. This allowed for direct comparisons of same-target pesticides, providing the most practical value to grape growers. Testing was combined also for efficiency reasons, to make best use of mite colonies in block testing and of labour in very highly labour-intensive experiments. Prior to experiments four separate mite cultures were reared in the laboratory, from which experimental predatory mite material and live mite diet for predatory mites was derived. Moreover, due to the microscopic size of predatory mites and their prey, all testing, including daily feeding, daily test-unit edge integrity checks, mortality, egg and juveniles counts, and mite colony assessments were carried out under 9-40x magnification, with a light microscope. Results from both projects were published in combined comprehensive publications (Bernard et al 2010 ab; M. Bernard, Report to GWRDC MU- LTU 04-01- PART B). Only wettable sulphur results are reported here.

Test species. Wettable sulphur was tested on the predatory mites *Euseius victoriensis* and *Galendromus occidentalis* (Family: Phytoseiidae). *E. victoriensis* is the most sensitive species from Australian vineyards tested to date (James and Rayner 1995, Bernard et al. 2004); it was thus used as the key species for designating 'safe' pesticides, with the highest accuracy (see 'worst-case-scenario'- p. 4). *E. victoriensis* indicated likely effects on the more sensitive range of predatory mite species within the vineyard predatory mite species complex. *G. occidentalis*, the key most naturalized abundant predator of two-spotted-mite, tolerant of dry Australian conditions and mass-released in commercial orchards in Australia in the 1970's, is resistant to the organophosphate insecticide azynphos-methyl. It was thus used here to indicate likely wettable sulphur effects on moderately tolerant species within the vineyard predatory mite species complex.

Mite cultures and rearing. *E. victoriensis* was collected from a commercial orchard near Loxton, South Australia (140.57 E, 34.46 S) and mass-reared by Biological Services Pty. Ltd. Loxton, South Australia. A laboratory colony culture was initiated from this predatory mite culture at DPI Knoxfield Pesticide Testing Laboratory, and was reared on *Glycine max* (L.) (soybean) in a controlled temperature room at 24 ± 1 °C, 90% ± 10 RH, and dusted twice weekly with (cumbungi) pollen (James 1993). An additional diet of tomato russet mite was provided to supplement *E. victoriensis* pollen diet. The tomato russet mite colony was reared separately outdoors at DPI Knoxfield, in a glass house. Plant leaves highly infested with russet mite were collected twice weekly and were brought into the predatory mite culture, and placed over soybean plants in the predatory mite colony. Soybean plants used in mite colonies and experiments were grown in a glasshouse at a separate location (Burnley College, University of Melbourne), under conditions preventing insect entry, to minimise mite colony contamination.

G. occidentalis culture and a culture of its prey (two-spotted-mite) were set up at DPI Knoxfield Pesticide Testing Laboratory from mites obtained from Biological Services Ltd. The cultures were reared in two separate controlled-temperature rooms on dwarf French bean plants, maintaining strict quarantine conditions between CT rooms, to prevent predator entry into the prey colony. *G. occidentalis* was reared at 25 ± 1 °C, 80 ±10% RH, 16:8 L.D. cycle, lux as per *E. victoriensis* (Bernard et al. 2004). The two spotted mite culture was reared at 23 ± 2 °C, 60 ± 10% RH, 16:8 (L:D) (Bernard et al. 2004). Bean plants were inoculated every 5 – 6 days by laying two- spotted-mite-infested leaves over bean plants. 4-6 days later they were transferred to *G. occidentalis* culture and overlaid with leaves containing *G. occidentalis.* Predator eggs were collected for bioassays 10-14 d later. Beans were grown at 20 °C, 16:8 (L:D) h in a glasshouse at Burnley College, fertilised weekly, and transferred to mite cultures at DPI Knoxfield, when first true leaves were fully expanded.

5.1.1 Wettable sulphur testing - *Euseius victoriensis*

Test units. Each test unit replicate was constructed from two open Petri-dishes lined with cotton wool. The inner dish had an upturned bean leaf embedded in cotton wool, and a sticky barrier applied to its rim; Figure 1, p. 893 (Bernard et al. 2004). The integrity of the cotton wool barrier was checked and repaired daily, and scanned twice daily for escapees from the leaf. Both the cotton wool and the leaf surface were sprayed. Mites straying into cotton wool thus received the same pesticide exposure as those on the leaf. Test units were held at 24.0± 1°C, 80 ± 10% RH, 16:8 L:D, lux 750-1050 in an externally vented CT cabinet.

Predatory mite diet and mite handling. Cumbungi pollen (*Typha orientalis*) and 22-25 predator eggs were added to each test unit. Old pollen was removed and fresh added every 2 days. Predatory mites in Assays III-V received an additional diet of laboratory reared tomato russet mite (*Aculops lycopersici*). Plant leaves heavily infested with tomato russet mite were brushed over test units immediately after adding predator eggs, then every 1-2 days. Predator eggs hatched within 48 h. Juveniles were 0-48 h old when sprayed with pesticides, and when exposed to pesticide residues. Predator sex ratio in each replicate was adjusted, within each treatment from maturity, to ≥ 1 male : 2 female to prevent poor fertilization.

Spraying. Bioassays were sprayed at 40 kPa with pesticide suspensions made in distilled water (controls with distilled water), using a Potter Spray Tower (Burkhard Manufacturing Ltd., Rickmansworth, UK). Pesticide residues (spinosad and wettable sulphur) were tested by transferring juveniles onto test units containing fresh dry residues. Mancozeb and carbendazim were used as toxic reference. Treatments were replicated 4x, except for the toxic reference mancozeb, adjuvants, and spinosad residue (replicated 3x). Surfactants were not used, except with potassium bicarbonate treatment. Test units were dried for 10-20 min at ambient temperature after spraying and placed in randomized positions in the CT cabinet. The Potter tower was calibrated before spraying to determine the spray volume required to achieve runoff point spray deposit. Runoff point was defined as the first point of droplets merging along leaf veins. To achieve adequate spray exposure of predators, assays on hairy soybean leaf substrate required *c.* double the runoff point spray volume needed for French bean assays (Bernard et al. 2004); lower spray volumes reached only the tips of soy leaf hairs, and mites were untouched beneath. To standardize tests on soy bean and French bean substrates and deliver a comparable pesticide dose as per Bernard et al.(2004), the highest registered pesticide field concentrations (at 1000 L/ha) were diluted for soybean assays by a

factor of 0.48-0.55. The resulting spray concentrations were similar to those obtained by an alternative calculation (Bluemel et al. 2000), based on the IOBC Predicted Initial Environmental Concentration (PIEC) (Barrett et al. 1994).

Assessment. Predatory mite juveniles were counted, and dead larvae removed, just before spraying. Mortality was then scored at 48 hours, 4 days and 7 days after spraying; all dead and live mites were counted, and dead mites removed. Mites were considered dead when they failed to move after gentle prodding with a brush. Predator eggs/larvae were counted and removed daily for 7-8 days, from 5 -12 days after spraying, excluding a 24-hour preoviposition period from analysis. Assessments were made with a dissecting microscope at 12-18x magnification, under a cold light.

Analysis. Cumulative mortality was calculated after Bluemel et al.(1993), by summing dead mites (on leaf and cotton wool) at each assessment time; 48-hour, 4-days, and 7-days. Totals were divided by the total number of live and dead mites at each assessment; unaccounted escapees, and dead mites found in the sticky barrier, were excluded. Mortality proportions were angle transformed, and analysed by ANOVA, and Tukey b post hoc tests for unequal sample sizes applied. Means and 95% confidence intervals were back-transformed and tabulated. Fecundity means were analyzed by ANOVA and Tukey b post hoc tests. Two fecundity measures were calculated: **(i)** Mean fecundity per surviving female - mean number of eggs per female per replicate over 7-days was calculated as per Bernard et al.(2004). **(ii)** Percent overall fecundity reduction per pesticide treatment relative to control, was calculated as per Bernard et al.(2004). The second measure gives an estimate of fecundity reduction based on both, mean fecundity of surviving females and mortality indices of each pesticide.

5.1.2 Wettable sulphur testing - *Galendromus occidentalis*

Bioassays on *G. occidentalis* (Table 1) were carried out as per methods above, except for differences in spray application and predatory mite diet. Two-spotted-mite (*Tetranychus urticae*) adult females were added to test units prior to juvenile predators, and then every 2 days, to maintain an oversupply of food. Once on test units, two-spotted-mite adult females laid an abundant supply of eggs; an ideal diet for *G. occidentalis* juveniles. 0-4 day old *G. occidentalis* juveniles were tested, and fecundity of surviving females was assessed from 5-11 days after spray application; these indices varied slightly from *E. victoriensis* testing.

No pesticide dilutions were used in *G. occidentalis* testing, as testing substrate did not require high spray volume to achieve run-off point spray deposits. To achieve adequate spray exposure of predators, bioassays on hairless French bean required *c.* half the runoff point spray volume required for *E. victoriensis* bioassays on hairy soy bean leaf substrate. Spray application was thus adjusted between *G. occidentalis* and *E. victoriensis* tests, to deliver standardised spray deposits across the two different leaf substrates (Bernard et al.(2004), and allow meaningful comparisons of pesticide results across the two test species. Mean wet spray deposit in *G. occidentalis* assays was 275 ± 4.8 mg/ replicate. The highest registered pesticide field concentrations (at 1000 L/ha) were used, except where concentrated slow volume sprays (half spray volume) were deliberately tested, or when lower rates of wettable sulphur were tested in 2nd tier testing (Table 1).

Table 1. List of bioassays and wettable sulphur concentrations and rates tested (highlighted in blue)

Pesticide common name of active constituent	Pesticide product name	Product concentration applied g/L or ml/L	Equivalent label rate g/100L or ml/100L @ 1000L/ha spray volume
Bioassay I - Powdery mildew fungicides **Test species: *Euseius victoriensis***			
azoxystrobin (500g/kg)	**Amistar® WG**	0.239	50 g
benomyl (500g/kg)	**Marvel®**	0.478	100 g
hexaconazole (50g/L)	**Anvil®**	0.191	40 ml
myclobutanil (125g/lL)	**Mycloss®**	0.120	25 ml
penconazole (100g/L)	**Topas® 100EC**	0.060	12.5 ml
wettable sulphur (800g/kg elemental sulphur)	**Thiovit Jet**	0.957	200 g
wettable sulphur (800g/kg elemental sulphur)	**Thiovit Jet**	2.870	600 g
wettable sulphur (800g/kg elemental sulphur)	**Thiovit Jet**	5.739	1200 g
toxic reference: carbendazim (500g/L)	**Bavistin® FL**	0.478	100 ml
Bioassay II - Sulphur residues tests and tests on botrytis fungicides **Test species: *Euseius victoriensis***			
iprodione (500g/L)	**Chief® Aquaflo**	0.539	100 ml
residue test - wettable sulphur (800g/kg elemental sulphur)	**Thiovit® Jet**	2.155	400g
residue test - wettable sulphur (800g/kg elemental sulphur)	**Thiovit® Jet**	3.233	600 g
toxic ref: mancozeb (750g/kg)	**Mancozeb 750WP**	1.078	200 g
Bioassay III **Test species: *Galendromus occidentalis***			
wettable sulphur (800g/kg elemental sulphur)	**Thiovit® Jet**	3.00	300g
concentrated low volume spray wettable sulphur (800g/kg elemental sulphur)	**Thiovit® Jet**	6.00	300g/100L applied 2x concentrated at 500 L/ha spray volume
wettable sulphur (800g/kg elemental sulphur)	**Thiovit® Jet**	4.00	400g
fenhexamid(500g/L)	**Teldor 500 EC**	1.00	100 ml
hydrogen peroxide (250g/L)/peroxy acetic acid (50g/L)	**Peratec®**	20.0	2 000 ml
buprofezin 440g/L	**Applaud®**	0.60	60 ml
toxic ref: mancozeb (750g/kg)	**Mancozeb 750WP**	2.00	200 g

5. 2 METHODS
FIELD RESEARCH

5.2.1 Survey of phytoseiid predatory mite species in Victoria (1999-2001): Predatory mite species feeding on rust mite (Yarra Valley, King Valley)

Studies on *Calepitrimerus vitis* (grapevine rust mite) were carried out in five commercial vineyards in Victoria 1999-2001, and phytoseiid predatory mite surveys were carried out in unsprayed control plots (GWRDC Project DAV 98/1). Predatory mite survey of vineyards severely infested with rust mite directly indicated predatory mite species associated with and feeding on rust mite. All vineyards had uniform severe leaf bronzing due to *C. vitis* in the summer preceding experiments. Severe leaf bronzing was defined as: 80-100% vine canes bronzed, 80-100% leaves per cane bronzed, 100% leaf surface area at each node bronzed. Vines in experimental sites were hanging-cane, or vertical-shoot-position trained and pruned to 2-3 buds, spaced at 1.8 m, and rows were spaced at 3.4 m. No broad spectrum insecticides or mancozeb were used during experimentation, but mancozeb was repeatedly used prior to 1998/1999. Wettable sulphur use during experimentation did not exceed rates of 1-2 kg/ha, except for a single experimental application (against rust mite at woolly bud) of wettable sulphur (600g/100L)/ canola oil mixture (Bernard et al 2005). Site I -Yarra Valley was a 29 ha vineyard near Yarra Glen (-37.653244, 145.379411); predatory mite sampling a 5.4 ha block of six-year-old Chardonnay. Site II- Yarra Valley was a 37 ha vineyard near Yarra Glen (-37.658494, 145.374347); sampling a 4 ha block of four-year-old Chardonnay. In Sites III - Yarra Valley - Dixons Creek (37.609753, 145.415244); IV- King Valley: Myrrhee (-36.73295, 146.320538); and Site V - King Valley: Whitfield (-36.700141, 146.410709), Chardonnay and Cabernet Sauvignon were sampled.

Mites were collected from dormant winter buds prior to experimentation (Site I), and from vine leaves in control plots in summer (January), collecting four replicates of 25 leaves per site; (Sites I-V). Plant material was brought to the laboratory and stored up to seven days at 4°C during assessments. Dissections of dormant winter buds were carried out as per Bernard (2005). Mites (all Families) and predatory thrips found inside winter buds and on canes around leaf petiole scars were assessed (Site I). Only Phytoseiidae predatory mites were collected from vine leaves in summer (Sites I-V). All assessments were done under a dissecting microscope at 6-24x magnification. Specimens were stored in 70% ethanol until species identification carried out in this project (GWRDC MU 05-01).

All individual specimens stored in ethanol were mounted onto glass slides in preparation for species identification. Specimens were cleared in Nesbitt solution. Cleared specimens were crush-mounted onto glass slides as per method devised by D. E. Walter, to expose taxonomic characters required for species identification (Walter and Proctor 2001), and mounted and sealed in Hoyers medium. Slides were dried in a slide oven for 6-8 weeks, allowed to cool, and sealed with a varnish resin, to prevent Hoyers medium from imbibing atmospheric moisture and spoiling specimens over time Walter and Proctor (2001). Mites were then identified to species by the CI. Voucher specimens were identified by Phytoseiidae taxonomy specialist H. A. Denmark (USDA, Florida). Voucher specimens were deposited in the Florida State Collection of Arthropods (FSCA), U.S. National Museum of Natural History, and at DPI Knoxfield. Tydeiidae voucher specimens were identified by M. Malipathil (DPI Knoxfield) and deposited in the Collections of Department of Primary Industries, Knoxfield, Victoria.

5.2.2 Survey of Phytoseiid predatory mite species in South Australia (2005): McLaren Vale, Limestone Coast, and Riverland

Phytoseiidae predatory mite species surveys (pre-release assessments) were carried out in November to December 2005, across 21 vineyards in South Australia (Table 2). Seven vineyards were sampled in each region: McLaren Vale, Limestone Coast, and Riverland. Three vineyards in each region were sampled once in 2005. Further four vineyards in each region were earmarked for predatory mite releases the following growing season, and predatory mites were sampled from both control and release blocks in 2005. These blocks were also used for subsequent predatory mite releases in 2006 - 2008. Four replicate samples of 25 randomly selected leaves were collected from each of 21 vineyards. Leave samples were stored inside ventilated plastic bags in a cooler packed with insulated ice packs, air-lifted to Melbourne, and stored in the laboratory at 8°C within 48 hrs of collection. Assessments were carried out immediately, and were completed within 7 days of collection.

Samples from within each region were assessed in a random order. For each sample, every individual vine leaf was examined under a dissecting microscope, at 6 - 24x magnification, and all phytoseiid predatory mites (eggs, juveniles, adults) were counted, and collected. Juveniles and adult mites from leaves were collected live directly into 70% ethanol and stored for several weeks prior to mounting onto glass slides in preparation for species identification. Plastic bags from samples were carefully rinsed (2x) and rinse water was sieved through a 90 micron mash sieve, as per standard phytoseiid washing methods (Bernard 2005), to recover any mites dislodged from leaves during storage; no mites were recovered from washing and sieving of sample bags.

Mounting onto glass slides of all individual specimens of phytoseiid mites stored in ethanol was carried out in preparation for species identification as per methods above. Species determinations were undertaken with a compound microscope at 1000x magnification and oil immersion. Phytoseiidae specimens from South Australia (including most of Riverland specimens) were identified by Phytoseiidae taxonomy specialist Dr J. J. Beard (Queensland Museum). These specimens were then used as voucher specimens for all other identifications carried out by the CI, Dr M. Bernard. In this way, the complex specialist task of accurate mite species identification was accomplished. Selected voucher specimens from South Australia were deposited in the Taxonomy Collection of Department of Primary Industries, Knoxfield, Victoria.

Table 2: Survey of Phytoseiidae predatory mites at flowering, South Australia (November - December 2005)

Site Number	Name	Geographic location
	Limestone Coast	
I	Stonehaven Padthaway Vineyards - Blk 218	37°39.454' S, 140°31.405' E
II	Stonehaven Padthaway Vineyards - Blk 104, 112	37°39.222' S, 140°31.817' E
III	Bold Vineyards	37°00.293' S, 140°52.255' E
IV	Koonara Management	37°14.730' S, 140°52.716' E

V	Chapoutier Vineyards - organic	37º01.415' S, 139º47.970' E
VI	Crawford, Hugh & Sara	37º00.762' S, 140º48.066' E
VII	Parker Estate Vineyard	37º21.668' S, 140º50.167' E

	McLaren Vale	
I	Wirra Wirra	35º13.658' S, 138º32.816' E
II	Paxton Vineyard - organic	35º11.910 S, 138º32.691' E
III	Paxton Vineyard - Chapple Hill Cabernet	35º15.818' S, 138º30.119' E
IV	Tinlins Wines - Lot 18	35º17.175' E, 138º28.432' E
V	Tinlins Wines - Barytes Rd.	35º11.151' S, 138º30.935' E
VI	Gemtree Vineyards	35º11.151' S, 138º30.935' E
VII	The Terraces Vineyard - Yangarra Management	35º09.428' S, 138º37.838' E

	Riverland	
I	Clark-Taylor	34º20.071' S, 140º35.627' E
II	Coldridge Vineyards	34º22.666' S, 140º37.047' E
III	Ridge Hill Vineyard	34º26.295' S, 140º38.490' E
IV	Lory	34º28.321' S, 140º34.976' E
V	Coldridge Vineyards - Blk 38,41	34º22.180' S, 140º39.503' E
VI	Munchenburg Vineyard - organic	34º24.538' S, 140º36.979' E
VII	Hill	34º25.169' S, 140º39.243' E

5.2.3 Bud mite survey Riverland and McLaren Vale (October 2008), and predatory mite species feeding on bud mite

Eight vineyards in Riverland and four (above target) vineyards in McLaren Vale were surveyed for grapevine bud mite. Growers self selected for the survey, based on presence of significant symptoms of poor bud burst and primary bud necrosis (PBN) in their vineyards; symptoms typically associated with bud mite. Bud mite damage and bud mite presence were evaluated. Direct association of phytoseiid predatory mite species with bud mite was also recorded. Predatory mites found inside buds, around outer bud scales, and around petiole scars during bud dissections of heavily bud mite infested plant material were collected, mounted, and identified to species, as per methods (Section 5.2.1 - 5.2.2).

Buds that failed to burst but appeared as normal buds at bud swell (E-L 2), and severely shortened twin shoots, with zigzag growth from one inter-node to the next were collected. Buds were bisected longitudinally, and bud scales removed one at a time from the centre outwards. Both sides of scales were examined, hairs removed as required, and bud mite counted using a dissecting microscope, at 35-60x magnification and cold light. Shoot bases were similarly examined removing base scales one at a time. Bud mite presence was scored into categories: 0, 1-50, 51-100, >100 per bud or shoot base. Bud damage and shoot base damage were scored into categories previously designated by Bernard et al. (2005). Bud mite and bud mite damage were photographed (Figure 1 a – e; page 40-41). A total of 219 dissections of 163 buds and 56 shoot bases were made. Buds containing no living tissue, too disintegrated to determine the cause of damage, were excluded from assessments.

5.2.4 Mass rearing of predatory mites for field release, and selection for wettable sulphur tolerance by Biological Services, Loxton, SA

Euseius victoriensis predatory mites (Family: Phytoseiidae) were mass-reared for field releases, by Biological Services Pty. Ltd., Loxton, South Australia (www.biologicalservices.com.au; Figure 2). Selection of *E. victoriensis* for wettable sulphur tolerance, and repeated releases in 12 vineyards in South Australia over three consecutive years, were also carried out by Biological Services as part of the project. Moreover, Biological Services investigated alternative diet systems and rearing-substrates for Phytoseiidae predatory mites, to substitute for plant substrates currently used in rearing, and attempted to rear another key vineyard predatory mite species *Anystis baccarum* (Family: Anystidae). Project laboratory cultures of *E. victoriensis* and *Galendromus occidentalis* predatory mites used in pesticide testing were initiated from commercial cultures owned by Biological Services.

Biological Services is the oldest commercial insectary in Australia, supplying 12 biological control agents (predatory mites, and predatory and parasitic insects) and consultancy to commercial sustainably-produced horticultural crops. A sister company, Fruit Doctors Ltd, supplies Integrated Pest Management consultancy and monitoring for viticulture based on sustainable systems, and works predominantly in the Riverland/ Sunraysia districts but has experience through all South Australian viticultural districts and the MIA.

Figure 2: Biological Services, Loxton, SA; A photo of *E. victoriensis*/ *G. occidentalis* predatory mite at base of picture, second from left.



Predatory mite colony instigation and rearing

The *G. occidentalis* project colony was initiated at DPI Knoxfield Pesticide Testing Laboratory from a commercial culture produced by Biological Services. This culture originated from *G. occidentalis* collected from pome fruit orchards in Orange, NSW, comprising a *G. occidentalis* strain resistant to organosphosphate and carbamate insecticides. Releases of *G. occidentalis* into pome fruit orchards in Orange were originally carried out in 1980's, releasing a strain, which originated in California, and was imported into Australia via New Zealand in the 1970's, where it acquired its insecticide resistance.

The *E. victoriensis* project colony for field releases was initiated at Biological Services in Loxon, in Autumn 2006, from predators collected in a vineyard at Bookpurnong, South Australia. The source vineyard has had a history of over 10 years of regular sulphur use at 200g/100L at a maximum 2kg/ha, and copper oxychloride use at 150g/100L at a maximum 1.5kg/ha. Very few other fungicides, and no insecticides or miticides were applied to this vineyard. The *E. victoriensis* project colony was maintained for the duration of the project at a lower level during winter, and scaled up each year in late winter/ early spring to large scale mass-rearing, for field release in 12 vineyards across South Australia (McLaren Vale, Limestone Coast, and Riverland; Table 3). *E. victoriensis* predatory mites were predominantly reared on a diet of cumbungi pollen, utilising soy bean plants a host plant for the colony. E. *victoriensis* diet was supplemented by tomato russet mite prey *(Aculops lycopersici)*. A colony of tomato russet mite prey was produced in a separate glass house on black berry nightshade plants. The same *E. victoriensis* colony was used to initiate a laboratory colony of *E. victoriensis* at the Pesticide Testing Facility at DPI Victoria, Knoxfield in 2007-2008, for wettable sulphur testing in this project, and concurrent pesticide testing in project MU LTU 04-01 PART B.

***E. victoriensis* selection for wettable sulphur tolerance.** Each project year, during the period May to August, the *E. victoriensis* project colony at Biological Services was exposed to wettable sulphur sprays equivalent to 200g/ 100L concentration. Two sprays, six weeks apart were applied. Survivors were allowed to multiply, in an attempt to select for and to maintain field tolerance to wettable sulphur.

Alternative rearing methods and new species. Both the predatory mites *G. occidentalis* and *E. victoriensis* were reared in this project on bean plants (Figure 3). The two colonies of prey mites, supplying predator diet, were also reared on plants. Predatory mite rearing on plant substrate is a costly exercise, due to the need to maintain a constant production of substrate plants of varying stages of development, in 15 cm pots, to circulate through the rearing systems. As part of this project, a method of rearing an alternative prey for predatory mites away from plants on a vermiculite substrate was investigated. It is envisaged that in the future, mass rearing of the most wettable sulphur tolerant phytoseiid predatory mite species identified by this project, *Amblydromella doreenae*, could be tested and developed on this alterative prey/ vermiculite system.

Field collections of live adult females of the predatory mite *Anystis baccarum* (Family: Anystidae) were also made, and attempts were made to rear this species in the insectary. Field-collected *A. baccarum* was able to survive in the insectary for long periods of time on an alternative prey system. However, this vine canopy/mid-row-dwelling predatory mite failed to reproduce in culture, due to complex soil-based egg laying requirements, as yet difficult to replicate in culture, and the colony eventually died out. Both predators are worthy

of further studies and would be valuable biological control organisms for release on demand, if future mass-rearing proves successful.

Figure 3. *E. victoriensis* rearing on soy been plants, dusted with cumbungi pollen diet. At field release, each bean leave hosts *c.* 20-50 predators.



5. 2.5 Predatory mite releases

Field releases of predatory mites. *E. victoriensis* predatory mites were mass-released consecutively over three years in 10 vineyards (Table 3). In each vineyard the release rate was 7,000 - 10,000 predators per ha per release; aiming for an overall average of *c.* 1 predator/ m², and concentrating release in release assessment plots of each target block. Release rates varied slightly depending on the volume of mass-rearing production at the time. Predators on bean leaves (Figure 3) were packed in polystyrene boxes with insulated cool packs by Biological Services, and sent by express courier to growers, participating in regional RITA projects. Growers carried out releases in each target vineyard in the Limestone Coast, and McLaren Vale within 24 hours of delivery; if delays occurred, replacement predatory mites were provided by Biological Services. In Riverland, predatory mite releases were carried out on growers' behalf by Biological Services. Releases were carried out in established, ≥ six years old vines; Shiraz in McLaren Vale, Cabernet Sauvignon in Limestone Coast, and Cabernet Sauvignon and Chardonnay (Blair) in Riverland. A total of six consecutive mass-releases were made in each of 10 vineyards, three releases in 2006/2007 growing season, two releases in 2007/2008, and one release in 2008/09. Releases were made in November/ December, and follow-up releases were made January/ February, or March each year.

In each vineyard, bean leaves containing predators were placed in the crowns of vines in three central vine rows of each release assessment plot. Release assessment plots were spaced 3 rows apart, and replicated 5x. The rest of the predatory mites were released in adjacent vine

rows, by placing predators in three vines in each row radiating outwards from release assessment plots across one hectare of the release block. Control plots were set up in the same block upwind from prevailing winds at a distance of at least 100m (30 rows) from release plots, to prevent *E. victoriensis* spread from release to control plots. In vineyards where release block size did not allow for this design, a paired control block of the same vine variety, upwind from release block, was chosen at a distance of at least 100m. Inter-row management practices in release and control blocks were identical, and matching of soil types (based on information provided by growers) was also done.

Table 3: Predatory mite (*E. victoriensis)* release sites

Site Nº.	Name	Geographic location	Releases		
			2006/07	2007/08	2008/09
Limestone Coast					
II	Stonehaven Padthaway Vineyards	37º39.222' S, 140º31.817' E	✓	✓	✓
III	Bold Vineyards	37º00.293' S, 140º52.255' E	✓	✓	✓
IV	Koonara Management	37º14.730' S, 140º52.716' E	✓	✓	✓
McLaren Vale					
I	Wirra- Wirra	35º13.658' S, 138º816' E	✓		
I	Glenchera			✓	✓
IV	Tinlins Wines - Lot 18	35º17.175' E, 138º28.432' E	✓	✓	✓
VII	Terraces - Yangarra Management	35º09.428' S, 138º37.838' E	✓	✓	✓
Riverland					
I	Clark-Taylor	34º20.071' S, 140º35.627' E	✓	✓	✓
III	Ridge Hill Vineyard	34º26.295' S, 140º38.490' E	✓	✓	✓
IV	Lory	34º28.321' S, 140º34.976' E	✓	✓	✓
extra	Altmann - Blair		✓	✓	✓

Releases assessments. Two consecutive release assessments were carried out in each of the 10 vineyards. One assessment was carried out at the end of the growing season in March 2008. A follow up assessment was carried out in late spring the following season, in late November 2008. 35 vine leaves were collected from each release plot; 10 leaves were collected at random from the lower vine canopy, 15 leaves at random from the middle vine canopy, and 10 leaves at random from the upper middle vine canopy. Very old and very young leaves were avoided (Smith and Papacek 1991). Assessment leaf samples in Riverland were collected by Biological Services. Assessment leaf samples in other regions were collected by the CI. Leave samples were stored inside ventilated plastic bags in a cooler packed with insulated ice packs, air-lifted to Melbourne, and stored in the laboratory at 8°C within 48 hrs of collection. Assessments were carried out immediately, and were completed within 7 days of collection.

Pesticide use in release sites. Release and control plots/ blocks received an identical spray program and application in each vineyard. Spray programs, including the use of wettable sulphur, varied between vineyards, but key pesticides (other than wettable sulphur) of known high toxicity to predatory mites were avoided in release and control blocks in all 10 vineyards, for the duration of the project. A list of pesticides to be avoided and ok to use in

release and control blocks was provided to growers at the start of the project (Appendix 10). Only growers who were committed to avoiding the use of some pesticides in release and control plots/ blocks, and willing to share information about their spray use participated in the project's predatory mite release program.

Spray diaries from all 10 vineyards for growing seasons 2006/2007, 2007/2008, and 2008/2009, were collected. Spray diaries for growing seasons 2004/2005 and 2005/2006 from 21 vineyards surveyed in 2005 were also collected. Statistical analysis (Principal Component Analysis) of spray use and predatory mite numbers (released and naturally present) across all predatory mite data collected will be provided by the February 2012 publication deadline. The data analysis could not be completed within the time frame of 1 June 2011, however all data pertaining to this analysis are provided in this report.

5.2.7 Seasonal abundance of Anystidae predatory mites (King Valley)

Survey of Anystidae predatory mites was carried out in seven vineyards in the King Valley in spring of 2006/2007 growing season. Two vineyards with high Anystidae abundance were selected for a year long study of Ansytidae population dynamics in the 2007/2008 growing season. Anystidae predatory mites and Bdellidae predatory mites in the vine canopy were sampled every two weeks over a year. Movement between vine canopy and mid-row was also studied. *Anystis baccarum* (Family: Anystidae) was the key species present. This striking, bright red, furry, and fast running mite, is known to many observant Australian vine growers, because of its large adult female size, and bright colour. Adult females moving in high numbers on vine cordons are just visible with the naked eye. *A. baccarum* is reported world-wide from sustainably-managed agricultural and horticultural crops, but its role in agricultural eco-systems, its seasonality, biology and ecology are almost completely unknown. This project thus aimed to study *A. baccarum* seasonal population dynamics in the vine canopy, and to identify the predatory mite complex associated with *Anystis baccarum* in the King Valley. For full reporting of methods, results and discussion see Appendix 9.

6.1 RESULTS AND DISCUSSION
LABORATORY TESTING

Bioassay I - *Euseius victoriensis*

There was a significant difference ($P < 0.001$; Anova, Tukey b) in the effects treatments on 48-hours, 4-days and 7-days cumulative mortality of *E. victoriensis* juveniles compared with the control. Post hoc tests indicate wettable sulfur (all concentrations tested: 200g/100L; 600g/100L; 1200g/100L), benomyl, and carbendazim, resulted in significantly higher 7-day mortality compared with the control (Table 4). Wettable sulfur (200g/100L) was significantly less toxic than its higher concentrations, and caused a significant, moderate (51.7%) fecundity reduction (Table 2). Wettable sulfur (600g/100L), benomyl, and carbendazim completely suppressed fecundity (Table 4). Azoxystrobin, hexaconazole, myclobutanil and penconazole did not differ significantly from the control or each other in mortality and fecundity effects (Tukey b; Table 4). Toxic effects of wettable sulfur, benomyl, and carbendazim were not fully expressed 48 hours or 4 days after pesticide exposure, indicating a need for extended mortality testing to evaluate pesticide safety to phytoseiid predatory mites.

Bioassay II - *Euseius victoriensis*

There was a significant difference ($P< 0.001$; Anova, Tukey b) in the effects of treatments on 48-hour, 4-day, and 7-day cumulative mortality of *E. victoriensis* juveniles compared with the control. Post hoc tests indicate wettable sulfur residues (400g/100L and 600g/100L) and mancozeb resulted in significantly higher mortality compared with the control; wettable sulfur residues and mancozeb were equally toxic, whereas iprodione had only a minor significant mortality effect at 4 days, and no significant effect at 48 hours and 7 days (Tukey b; Table 4). Post hoc tests indicate a significant difference between treatments in fecundity effects; wettable sulfur residues and mancozeb resulted in a significant fecundity reduction compared with the control, and iprodione in a minor fecundity reduction (Tukey b; Table 4).

Bioassay III – *Galendromus occidentalis*

There was a significant difference ($P<0.001$; Anova, Tukey b) in the effects of wettable sulphur (all three treatments), fenhexamid, and mancozeb on 48- hour, 4-day, and 7-day mortality of *G. occidentalis* juveniles compared with the control. Post hoc test indicate 7-day mortality effects due to mancozeb were significantly higher compared with all other treatments, and no eggs were laid by females surviving mancozeb application (Table 5). Wettable sulphur treatments 300g/100L (1000L/ha volume spray), 600g/100L (500L/ha spray volume), and 400g/100L (1000L/ha spray volume) did not differ significantly from each other in 48-hour, 4-day, or 7-day mortality effects, indicating there was no additional toxic effect on mite survival when wettable sulphur 300g/100L at 1000L/ha was 2x concentrated to 600g/100L and applied at half-spray volume (500L/ha), and also indicating there was no additional toxic effect due to applying wettable sulphur at 400g/100L. Post hoc tests indicate wettable sulfur 600g/100L applied at 500 L/ha resulted in a minor (tending towards significance) reduction in predator female fecundity compared with the control, and other wettable sulphur treatments.

Differences in *E. victoriensis* and *G. occidentalis* response to wettable sulphur

The two predatory mite species varied in their response to wettable sulphur, indicating differences in wettable sulphur tolerance in the vineyard predatory mite species complex. *G. occidentalis* was considerably more wettable sulphur tolerant than *E. victoriensis*. Wettable sulphur toxic effects on *G. occidentalis* were moderate compared with effects on *E. victoriensis*. The same concentration (400g/100L) applied as direct overspray, was moderately toxic to *G. occidentalis* (59% 7-day mortality; no fecundity effect on surviving females; Table 5), compared with toxicity to *E. victoriensis* of fresh dry residue of the same concentration (90% 7-day mortality; 95% overall fecundity reduction; Table 4). Direct overspray of wettable sulphur 200g/100L was more toxic to *E. victoriensis* (81% 7-day mortality; 52% overall fecundity reduction; Table 4) than was a direct overspray of double the concentration to *G. occidentalis* 59% 7-day mortality; no fecundity effect on surviving females; Table 5).

Sub-lethal fecundity effects of pesticides are studied chiefly to elucidate the level of recruitment of the immediate next predator generation after pesticide exposure. They indicate the capacity of a predatory mite population to recover after a single pesticide application. Results show that a single spray of wettable sulphur of 300g/100L (at 1000L/ha), 400g/100L (at 1000L/ha) and 600g/100L (at 500L/ha i.e. 3kg/ha 2x concentrated) had only a minor effect on the recruitment of the next *G. occidentalis* generation, and a single spray of 200g/100L (at 1000L/ha) had a moderate effect (52% reduction) on recruitment of the next generation of *E. victoriensis.* These sprays, represent more traditional, restrained use of wettable sulphur, and are recommended in place of higher wettable sulphur rates, in vineyards where conservation of predatory mites, and biological control of rust mite and bud mite are a priority.

Suppression of predatory mites

By contrast, the commonly used upper limit of the wettable sulphur label rate delivering a mass of 6kg/ha, applied even as a dilute spray (at 1000L/ha) at spray tank concentration of 600g/100L, resulted in 100% 7-day *E. victoriensis* mortality and no recruitment of the next generation (Bioassay I; Table 4). The effect of wettable sulphur 600g/100L on *E. victoriensis* fecundity was in fact equal to that of mancozeb, highly toxic to predatory mites (Bernard et al. 2004), the toxic reference used in the testing. Laboratory data predict repeated use of wettable sulphur at 6kg/ha rate and concentrations $\geq$ 600g/100L will displace *Euseius victoriensis* from vineyards. *E. victoriensis* release data, and naturally present predatory mite species data from release vineyards in Riverland confirm this displacement (Section 6.2.4).

The upper limit of wettable sulphur concentrations toxic to *G. occidentalis* may be higher than 400g/100L; it was not determined in laboratory testing of this project. However, field data indicate the most wettable sulphur tolerant species in Australian vineyards is *Amblydromella doreenae*. *A. doreenae* was the only phytoseiid predatory mite species present in many vineyards sampled for predatory mites in 2005 and 2008, and *A. doreenae* displaced *A. dosseii* in some Limestone coast vineyard by 2008 (Tables 7, 9 - 10). Field data also indicate *A. doreenae* upper tolerance limit was exceeded by many vineyards. None or extremely few *A. doreenae*, and no other phytoseiid species, were recovered from vineyards where repeated applications of wettable sulphur at 6 kg/ha (2-3x concentrated in low volume sprays) were made in the same growing season and the preceding year (Section 6.2.4).

Table 4. Mean cumulative mortality and fecundity (mean ± S.E.) of predatory mite *Euseius victoriensis* following exposure to pesticides; results are direct oversprays unless residue stated.

Treatment	% Mortality 48 hours	% Mortality 4 days	% Mortality 7 days	Mean reproduction per surviving female ± SE 6 - 12 days after spraying	Percent overall treatment fecundity reduction relative to control
Bioassay I - Powdery mildew fungicides Test species: *Euseius victoriensis* (0- 48 h old juveniles)					
control	0.00[a]	1.32[a]	2.95[a]	*11.5 ± 0.70[a]	
azoxystrobin	0.00[a]	3.03[a]	6.37[a]	*11.3 ± 0.67[a]	2.32
hexaconazole	2.78[ab]	4.25[a]	4.90[a]	*9.93 ± 0.76[a]	14.0
myclobutanil	0.00[a]	0.00[a]	2.94[a]	*10.8 ± 0.89[a]	6.37
penconazole	1.39[ab]	2.78[a]	4.51[a]	*7.85 ± 1.00[ab]	32.0
wettable sulphur 200 g/100L	**49.7[cd]**	**72.2[b]**	**81.7[b]**	***5.58 ± 1.52[b]**	**51.7**
wettable sulphur 600 g/100L	**61.2[d]**	**98.7[c]**	**100.0[c]**		
wettable sulphur 1200 g/100L	**81.8[e]**	**100.0[c]**	**100.0[c]**		
benomyl	28.8[c]	58.7[b]	78.6[b]	0.00 ± 0.00[c]	100
toxic reference: carbendazim	12.7[b]	57.0[b]	97.4[c]	0.00 ± 0.00[c]	100
Bioassay II - Sulphur residues tests and tests on botrytis fungicides Test species: *Euseius victoriensis* (0- 48 h old juveniles)					
control	3.00[a]	5.08[a]	22.2[a]	8.04 ± 1.13[a]	
iprodione	11.8[a]	19.3[b]	37.0[a]	5.70 ± 0.12[ab]	29.1
wettable sulphur 400 g/100L residue	**48.7[b]**	**69.6[c]**	**90.4[b]**	**0.43 ± 0.43[c]**	**94.7**
wettable sulphur 600 g/100L residue	**59.6[b]**	**76.9[c]**	**89.0[b]**	**2.43 ± 1.36[bc]**	**69.7**
toxic reference: mancozeb	51.3[b]	71.4[c]	83.7[b]	0.9 ± 0.49[c]	91.6

Means are based on four replicates, except for three replicates for toxic reference. Means within the same bioassay and column followed by a different letter are significantly different (P< 0.001; *P< 0.01; Tukey b test).
Reproduction (*R*) per female measured for 7 d from maturity, excluding the preoviposition period.

Table 5. Mean cumulative mortality and fecundity (mean ± S.E.) of predatory mite *Galendromus occidentalis*, following exposure to pesticides; results are direct oversprays unless residue is stated.

Treatment	% Mortality 48 hours	% Mortality 4 days	% Mortality 7 days	Mean reproduction per surviving female ± SE 5 - 11 days after spraying	Percent overall treatment fecundity reduction relative to control
Bioassay III **Test species: *Galendromus occidentalis* (2- 4 days old juveniles)**					
control	6.52[a]	10.5[a]	14.3[a]	19.3 ± 0.99 [a]	
wettable sulphur 300 g/100L	**39.4 [b]**	**47.6 [b]**	**62.9 [c]**	**16.2 ± 1.91 [a]**	
wettable sulphur 600g/100L @ 500L/ ha	**29.7 [b]**	**40.4 [b]**	**61.9 [c]**	**13.7 ± 1.27 [ab]**	
wettable sulphur 400 g/100L	**38.3 [b]**	**48.6 [b]**	**58.7 [c]**	**18.6 ± 1.47 [a]**	
fenhexamid	29.0[b]	33.6[b]	38.7[b]	18.1± 0.22 [a]	
hydrogen peroxide/ peroxy acetic acid	17.8[a]	27.7[ab]	33.1[ab]	17.3 ± 0.71[a]	
buprofezin	9.56 [a]	19.0[a]	26.6 [a]	17.5 ± 0.55 [a]	
toxic ref: mancozeb	23.1[b]	34.7 [b]	81.6 [d]	0.00 ± 0.00 [c]	100

Means are based on four replicates, except for three replicates for toxic reference. Means within the same bioassay and column followed by a different letter are significantly different (P< 0.001; Tukey b test).
Reproduction (*R*) per female measured for 7 d from maturity, excluding the preoviposition period.

Predicting pesticide field effects

Laboratory tests on predatory mites (Phytoseiidae) can reasonably predict pesticide field effects, because no predatory mite life stages are substantially protected from sprays. This is because of the rapid development from egg to adult, because there is no pupae resting stage, naturally protected from sprays, and because mites have no capacity to fly away from spray exposure (Bakker et al. 1992). Field effects are generally expected to be lower, due to imperfect spray application and pesticide weathering, but repeated use, use of pesticide mixtures, and the use of concentrated low volume sprays go against such a field toxicity-lowering trend (Ingram and Nimmo 1993, Cross and Berrie 1996, Papaioannou Souliotis et al. 1998). The common Australian wine industry practice of concentrating pesticide label rates and lowering spray volumes, delivers the same mass of pesticide per ha, but up to 3x more concentrated dose at the micro-scale of predatory mites and other beneficials. The toxicity of some low volume sprays can thus be significantly higher than the equivalent 'dilute' applications. Results here were obtained per concentrations equivalent to label rates at 1000 liter/ha spray volume; a very limited investigation of 2x concentrated low volume sprays was also made.

Powdery mildew fungicides - safe to predatory mites

Pesticide tests under a different project (MU/LTU 04-01- PART B; Bernard et al. 2010 ab) indicate there are many fungicides registered for use in viticulture that have no significant single-use toxic effect on *E. victoriensis* predatory mite at label rates applied at 1000 liter/ha. Given *E. victoriensis* is the most sensitive phytoseiid predatory mite from Australian vineyards tested to date (James and Rayner 1995, Bernard et al. 2004), by inference, no toxicity to the whole phytoseiid predatory mite species complex in Australian vineyards is likely. Substitution of highly concentrated wettable sulphur sprays with some of the many non-toxic-to-predatory-mites fungicides registered in Australian viticulture for control of powdery mildew is thus possible. However, it appears to be seldom used, possibly due to the additional expense compared with relatively low cost of wettable sulphur.

Powdery mildew fungicides azoxystrobin, fenarimol, hexaconazole, myclobutanil, penconazole, potassium bicarbonate, pyraclostrobin, quinoxyfen, spiroxamine, triadimenol, and trifloxystrobin were harmless to *E. victoriensis* predatory mite (Bernard et al 2010 ab: Appendix 8), except for a slight (32%) fecundity reduction due to penconazole (Bioassay I). Wettable sulphur (200g/100L) was moderately toxic (82% mortality; 52% fecundity reduction) to *E. victoriensis*, but allowed for recruitment of the next generation of predatory mites (Bioassay I). All the above powdery mildew fungicides, including wettable sulphur (200g/100L), have no or very minimal toxic effect on predatory mites. Results suggest these pesticides should be used in preference to high rates of wettable sulphur, to concurrently achieve effective control of powdery mildew and conservation of naturally present phytoseiid predatory mites. Studies on other predatory mites confirm low toxicity for these pesticide (Walker et al. 1988, Hassan et al. 1994, Sterk et al. 1999, Bluemel and Hausdorf 2002, Metzger and Pfeiffer 2002), http://www.biobest.be, including penconazole toxicity (Camporese et al. 1993).

Wettable sulphur sprays against rust mite and bud mite

Wettable sulphur (600g/100L)/canola oil (2L/100L) spray used as high volume sprays, or either compound used alone, timed to woolly bud, were very effective against large populations of overwintered rust mite, migrating from winter shelters, in vines where severe leaf bronzing occurred the previous summer (Bernard 2005, Bernard et al. 2005). However, this spray is so effective, and has been so enthusiastically adopted across the Australian wine industry, it is now often used on a calendar basis, even where no bronzing occurred. Our results on wettable sulphur suggest such misuse is likely to actually contribute to rust mite outbreaks due to predator suppression. Canola oil was less toxic to *E. victoriensis* (Bernard et al. 2010 ab: Appendix 8) than wettable sulphur, and is a safer, if more expensive, choice for preserving predatory mites. However, field experience in the Australian wine industry has repeatedly shown that where the above woolly bud wettable sulphur spray is used based on bronzing threshold of previous summer, NOT on a calendar basis, (and other sprays highly damaging to predatory mites such as mancozeb are not used), there is no lasting disruption to predatory mites. This means leaf bronzing due to rust mite at the end of the growing season in which the spray is used is prevented. This was confirmed by this project; high predatory mite numbers were collected from vineyards in January 2000/2001 in Yarra Valley and King Valley (Section 6.2.1).

6. 2 RESULTS AND DISCUSSION

FIELD RESEARCH

6.2.1 Survey of predatory mite species (Family: Phytoseiidae) in Victoria: predatory mite species feeding on rust mite (Yarra Valley, King Valley)

Predatory and other taxa wintering in association with rust mite - *Cal. vitis*
Dissection of dormant winter buds (Site I - Yarra Valley). Dormant vine canes 6 canes; 90 buds, nodes 1-15 dissected) severely bronzed the previous autumn (i.e. from a vineyard under severe rust mite outbreak), had 12 adult female phytoseiid predatory mites over-wintering inside bark fissures around petiole scars (1-3 per cane), and 10 predatory thrips (*Haplothrips victoriensis*) eggs/ or instars (0-3 per cane). Intact dormant buds with fully enclosed outer bud scales contained *H. victoriensis* eggs/or 1st instar larvae, and also tydeiid mites (non-predatory), indicating that intact bud scales were not a barrier to egg laying by *Haplothrips victoriensis* predator, or to tydeiid mite entry. Tydeiidae were the most numerous mites inside dormant buds; an average of 7.3 ± 0.74 bud $^{-1}$ and 101 ± 22.3 cane $^{-1}$ were collected, and tydeiid mites were present in 94.4% of buds sampled. Tydeiid mites were reported to feed on powdery mildew spores, and a potential spread of its inoculum by these mites was suggested (Crisp P, PhD Thesis, University of Adelaide). This may have further detrimental effects in a vineyard already experiencing rust mite outbreak.

When a further 20 dormant canes (180 buds, nodes 3-11) from the same vineyard were dissected, 18 predatory thirps (*H. victoriensis)* were found wintering in buds and bark fissures, and only 11 phytoseiid predatory mites (Figure 2). Phytoseiidae species from all canes (23) were: *Amblydromella doreenae* (Schicha) 16 (69.6%), and *Galendromus occidentalis* (Nesbitt) 4 (17.4%), *Amblydromella recki* 3 (13.0%). Cane data underestimated phytoseiid predatory mites overwintering on vines, as these also over-winter in the deep-fissured bark of vine cordons. Repeated annual use of mancozeb (highly toxic to predatory mites; Bernard et al. 2004) in the season immediately prior to sampling, also likely contributed to low predatory mite numbers.

Predatory mites associated with rust mite *(Cal. vitis)* - during the growing season
Assessment of January leaf samples (Yarra Valley, King Valley). Predatory mites (F: Phytoseiidae) are well known predators of various rust mite and bud mite species occurring across many horticultural crops, including predators in vineyards of grapevine rust mite and bud mite. Species survey in 5 vineyards in Victoria (January 2000) revealed the predatory mite species complex associated with, and feeding on these pests.

Vineyards varied in the average number of predatory mites per 25 leaves. Yarra Valley - Site - II had a history of repeated mancozeb use in the season of predatory mite sampling, and 0.50 ± 0.29 predatory mites per 25 leaves (based on four replicates). High numbers of predatory mites, 15.3 ± 3.30 to 37.3 ± 6.10 per 25 leaves, were found in sustainably managed vineyards (Yarra Valley Sites I, III ; King Valley -VI, V; Table 6). These vineyards applied a single spray of wettable sulphur (600g/100L)/ canola oil mixture against rust mite at woolly bud in the same growing season as sampling, but wettable sulphur use against powdery mildew was otherwise low (100-200g/100L at 2 kg/ha), and no mancozeb or broad spectrum insecticides were used.

Sites had a history of repeated mancozeb use and rust mite outbreaks the previous growing season, and were selected to participate in rust mite research due to a high level of rust mite-leaf bronzing the previous summer. Predatory mite numbers collected in these sites January 2000, indicate, once sustainably managed, vineyards fully recovered their predatory mite populations in 1-2 growing seasons. Field data are based on low wettable sulphur use against powdery mildew. These rates have since tippled, and may have substantially altered predatory mite numbers and species abundance, compared to that reported here.

Species abundance (Yarra Valley and King Valley). A total of five phytoseiid species was collected across five vineyards: *Amblydromella doreenae, Amblydromella dossei, Galendromus occidentalis* were common, and *Amblydromella recki* and *Neoseiulus helmi* were rare. The most abundant species, which was *A. doreenae*, occurred in all five vineyards. *A. dossei* commonly occurred in high numbers alongside *A. doreenae* in two sustainably managed vineyards in Yarra Valley, and *G. occidentalis* alongside *A. doreenae* in one sustainably managed vineyard in Yarra Valley and two sustainably managed vineyards in King Valley; species abundance per individual vineyards (Table 6). When data were pooled per region, species abundance was as follows: Yarra Valley (N = 113) - *A. doreenae* (53.1%), *A. dossei* (33.6%), *G. occidentalis* (12.4%), *A. recki* (0.9%); King Valley (N = 238) - *A. doreenae* (68.9%), *A. dossei* (0%), *G. occidentalis* (29.4%), *N. helmi* (1.68%).

All species, except *G. occidentalis,* were indigenous to Australia. *G. occidentalis* occurred only in Victoria; (not South Australia; see 6.2.2). *G. occidentalis* is a North American species naturalised in Australia. An organophosphate-resistant strain of *G. occidentalis* was introduced to Australia, via New Zealand, in the 1960's and mass-released in very large-scale releases throughout commercial orchards in the 1970's, for biological control of pesticide-resistant two-spotted mite, devastating orchards at the time. *G. occidentalis* presence in the Yarra and King Valley suggests spread to vineyards from past and present orchard use in the area, and may reflect historical use of actual vineyard sites.

Table 6. Species abundance and mean number mites (eggs, juveniles, adults) per 25 leaves ± S.E., based on four replicates (January 2000)

Site Number	**Mean predatory mites per 25 leaves ± S.E. (adults, nymphs, larvae, eggs)**	**Total number live mites per 100 leaves (eggs, larvae, nymphs, adults)**	**Species ID: Number mites assessed (adults, nymphs)***	**Species abundance:** *Amblydromella doreenae* *Amblydromella dossei* *Galendromus occidentalis* *Amblydromella recki* *Neoseiulus helmi*
I -Yarra Valley	15.3 ± 3.30	61	55	*A. doreenae* (24) 43.6% *A. dossei* (16) 29.1% *G. occidentalis* (14) 25.5%, *A. recki* (1) 1.81%
II -Yarra Valley	0.50 ± 0.29	2	2	*A. doreenae* 100%
III -Yarra Valley Dixons Creek	16.8 ± 6.03	67	56	*A. doreenae* (34) 60.7% *A. dossei* (22) 39.3%
IV – King Valley Myrrhee	29.3 ± 8.37	117	111	*G. occidentalis* (63) 56.8% *A. doreenae* (45) 40.5% *N. helmi* (3) 2.70%
V – King Valley Whitfield	37.3 ± 6.10	149	127	*A. doreenae* (119) 93.7% *G. occidentalis* (7) 5.51% *N. helmi* (1) 0.79%

* Nymphs were identified to genus and associated with the most numerous species of the same genus per site.

6.2.2 Survey of Phytoseiidae predatory mite species in South Australia: Limestone Coast, McLaren Vale, and Riverland

Survey of predatory mite numbers and species at flowering in a total of 21 vineyards in South Australia in 2005 (seven vineyards per each region) revealed the predatory mite species complex associated with, and feeding on rust mite and bud mite. Vineyards varied greatly in the average number of predatory mites per 25 leaves (based on four replicates); ranging from 0.25 ± 0.25 to 37.8 ± 6.05 (Table 7; Figure 4). Differences in predatory mite numbers are attributed to differences in spray programs. Further species identification assessments from 20 of these vineyards revealed very low species diversity in the phytoseiid predatory mite complex across all the vineyards surveyed. All species collected in South Australian vineyards were species indigenous to Australia.

Riverland and McLaren Vale. *Amblydromella doreenae* was the most abundant phytoseiid predatory mite in Riverland and McLaren Vale. This species was the most abundant species in all six Riverland vineyards, and all seven McLaren Vale vineyards. It was, in fact, the only phytoseiid species (100%; N = 182) collected from McLaren Vale, and the only phytoseiid species (97.9%; N = 94) collected from Riverland, except for two individual specimens (*Neoseiulus dieteri* and *Neoseiulus loxtoni*) collected from two Riverland vineyards. *Amblydromella dossei* and *Euseius victoriensis* were not found in McLaren Vale or Riverland vineyards surveyed. Species abundance per individual vineyards is reported (Table 7).

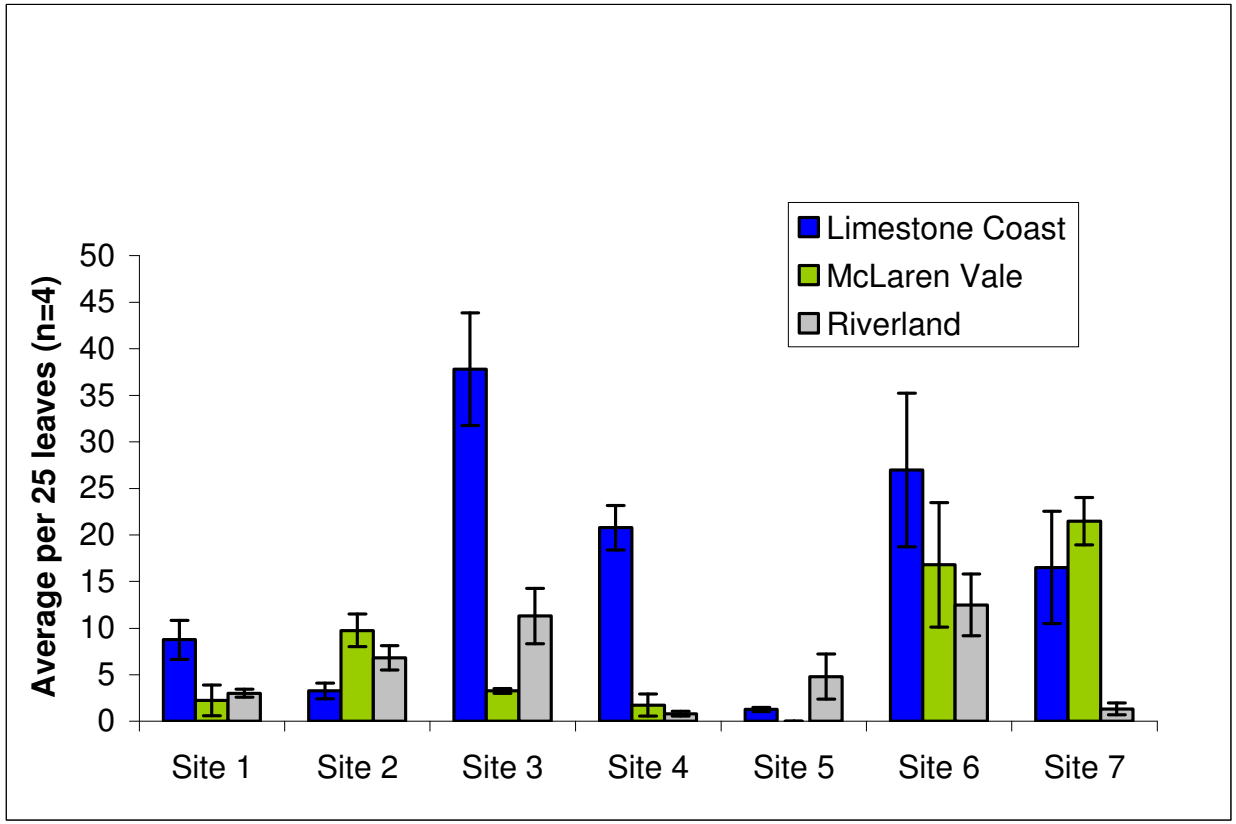
Limestone Coast. *Amblydromella doreenae* was the dominant and most abundant phytoseiid species in one of seven vineyards surveyed in Limestone Coast wine region; overall abundance per all Limestone Coast vineyards (24.6%; N = 382). It co-occurred with *Amblydromella dossei* in three vineyards of the seven surveyed at Limestone Coast (Table 7).

The dominant and most abundant species in Limestone Coast vineyards overall, however, was *Amblydromella dossei* (75.1%, N = 382). It occurred in all seven vineyards surveyed, it was the most abundant species in six of seven vineyards surveyed, and it was the only species collected from four vineyards (Table 7). Only one other phytoseiid species besides *A. dossei* and *A. doreenae* was collected from Limestone Coast vineyards, *Neoseiulus tareensis;* comprising (0.26%, N= 382) of all phytoseiids collected in Limestone Coast. *Euseius victoriensis* was not found in the Limestone Coast vineyards surveyed. Species abundance per individual vineyards is reported (Table 7).

Table 7: Predatory mites (Family: Phytoseiidae) in South Australian vineyards at flowering : Species abundance and mean number mites (eggs, juveniles, adults) per 25 leaves ± S.E., based on four replicates (Pre-release assessments)

Site N°.		Name of vineyard	Mean predatory mites per 25 leaves ± S.E. (adults, nymphs, larvae, eggs)	Number live mites per 100 leaves (adults nymphs, larvae eggs)	Species ID: *Number mites assessed (adults, nymphs)	Species abundance: *Amblydromella doreenae* *Amblydromella dossei* *Neoseiulus dieteri* *Neoseiulus loxtoni* *Neoseiulus tareensis*
Limestone Coast						
I		Stonehaven Padthaway Vineyards - Blk 218	8.75 ± 2.10	35	31	*A. dossei* 100%
II	✓	Stonehaven Padthaway Vineyards - Blk 104, 112	3.25 ± 0.85	13	11	*A. dossei* 100%
III	✓	Bold Vineyards	37.8 ± 6.05	151	130	*A. dossei* (129) 99.2%, *A doreenae* (1) 0.8%
IV	✓	Koonara Management	20.8 ± 2.39	83	50	*A. dossei* (48) 96.0%, *A doreenae* (1) 2.0%, *N. tareensis* (1) 2.0%
V		Chapoutier Vineyards - organic	1.25 ± 0.25	5	5	*A. dossei* 100%
VI		Crawford, Hugh & Sara	27.0 ± 8.50	108	95	*A. doreenae* (92) 96.8%, *A. dossei* (3) 3.2%
VII		Parker Estate Vineyard	16.5 ± 6.03	66	60	*A. dossei* 100%
McLaren Vale						
I	✓	Wirra Wirra	2.25 ± 1.65	9	6	*A. doreenae* 100%
II		Paxton Vineyard - organic	9.75 ± 1.75	39	31	*A. doreenae* 100%
III		Paxton Vineyard - Chapple Hill Cabernet	3.25 ± 0.25	13	10	*A. doreenae* 100%
IV	✓	Tinlins Wines - Lot 18	1.75 ± 1.18	7	5	*A. doreenae* 100%
V		Tinlins Wines - Barytes Rd.	0.25 ± 0.25	1	1	*A. doreenae* 100%
VI		Gemtree Vineyards	16.8 ± 6.69	67	58	*A. doreenae* 100%
VII	✓	Yangarra -The Terraces Vineyard Mgmt.	21.5 ± 2.53	86	71	*A. doreenae* 100%
Riverland						
I	✓	Clark-Taylor	3.0 ± 0.41	15	15	*A. doreenae* 100%
II		Coldridge Vineyards Blocks 4,7	6.8 ± 1.31	27	16	*A. doreenae* 100%
III	✓	Ridge Hill Vineyard	11.3 ± 2.95	45	19	*A. doreenae* (18) 95.0%, *N. dieteri*- (1) 5.0%
IV	✓	Lory	0.8 ± 0.25	3	0	
V		Coldridge Vineyards - Blk 38,41	4.8 ± 2.43	19	9	*A. doreenae* (8) 88.9% *N. loxtoni* (1) 11.1%
VI		Munchenburg Vineyard - organic	12.5 ± 3.30	50	31	*A. doreenae* 100%
VII		Hill	1.3 ± 0.63	5	2	*A. doreenae* 100%

Figure 4. Density of predatory mites (Phytoseiidae) per 25 leaves ± S.E. (based on four replicates), naturally present in 21 vineyards in South Australia (November 2005)



6.2.3 Bud mite survey Riverland and McLaren Vale (October 2008)/ Phytoseiidae predatory mite species feeding on bud mite

Bud damage due to bud mite

Bud mite was found in 11 of 12 vineyards surveyed, and a high level of bud mite damage (bud axis necrosis and polyps) were found in all 12 vineyards surveyed; total number of dissections (n = 216; buds = 163; shoot bases = 56). Bud mite (Figure 1a) was present inside 42.3% of unburst buds that superficially appeared as healthy buds in bud swell (n = 163). No bud mite was found at shoot bases (n = 56). Bud mite infestation and damage symptoms per individual vineyards are recorded (Table 8). In three vineyards, symptoms of both bud mite and early spring rust mite damage were recorded.

Bud scale blistering, also known as 'polyps' (Figure 1c), caused by hypertrophy and division of epidermal cells due to bud mite feeding (Kido and Stafford 1955, Smith and Schuster 1963, Bernard et al. 2005), was found on inner surfaces of bud scales and at shoot bases in 68.5% of buds/shoot bases (n=216), and buds/shoot bases from all 12 vineyards had 'polyps'. Polyp symptoms ranged from 29.6% of buds/shoot bases in one sample where no bud mite was collected, to 48.1%-100% of buds/shoots bases in 11 samples where bud mite presence was confirmed. Primary bud axis necrosis (PBN) (Figure 1b) and secondary bud necrosis: Buds with primary bud axis necrosis (PBN), and necrotic secondary buds (developed in place of dead primary buds), were found in samples from all 12 vineyards, and comprised a high proportion of buds collected from each site (Table 8). Overall, the incidence of PBN in dissected buds was 34.4% (n = 163); i.e. 34.4% of buds dissected had secondary buds developed in place of a dead primary bud (Figure 1b). Overall 68.7% of buds dissected (primary and secondary buds combined) from the 12 vineyards had necrotic hbud axis. Most dissected buds also had necrotic spotting (Figure 1b) regardless of the presence or absence of bud axis necrosis.

Bud mite damage symptoms were found in some buds where no bud mite were present at the time of bud collection, and in the sample from the one vineyard (Riverland VII) from which no bud mite were collected (Table 8). This is because sampling took place approximately 6-8 weeks after bud mite migration into next season's bud primordia took place, in the narrow time-window soon after bud burst (Dennill 1986). Despite this, sampling for bud mite well after bud burst, once all buds capable of bud burst have opened, is recommended, to direct sampling effort to un-burst buds alone, given bud mite presence in vineyards (unlike that of rust mite) is not randomly distributed, but it clustered, due to unique aspects of bud mite biology (Bernard et al. 2005).

Shoot damage due to bud mite

Distinct shoot distortions were present in association with unburst buds. In order of increasing severity, these shoot distortions were: (i) a high incidence of 'twin shoots' due to secondary bud burst in place of dead primary buds, (ii) shoots of apparently normal vigour but with compressed internodes 1-4, absent fruit clusters, and with blistering/polyps at bases of shoots, (iii) thin shoots with zigzagged angular growth from one inter-node to the next, (iv) short 1-4 internode shoots terminated by dead apical meristems, and with absent fruit clusters; for more photographs see (Bernard et al. 2005; Bernard et al 2002) Shoot injury associated with bud mite is due to a lower bud mite infestations in developing bud the previous season, than infestations leading to bud burst failure (Smith and Stafford 1948, Kido and Stafford 1955, Smith and Schuster 1963, Bernard et al. 2005)

Predatory mites feeding on bud mite. Only 10 phytoseiid predatory mites (8 adults, 2 eggs) were found on plant material in extensive bud and shoot base dissections (N= 220), from vineyards substantially infested with bud mite (Table 8). This suggests a history of spray programs incompatible with predatory mites for these sites; particularly given 9 of 10 of these predatory mites were collected from just two vineyards (CDD, North Renmark; Harnett, McLaren Flat). Adult mites were identified as *Amblydromella doreenae*. *A. doreenae* was also collected from heavily bud mite-infested vines in Lower Hermitage, South Australia; a bud mite-damaged vineyard studied in (Bernard et al 2005).

Economic damage due to bud mite. Economic damage in vineyards due to bud mite can over time spread from clustered infestations, and become extensive (Dennill 1986, 1991). Quality of next season's buds, shoots, and bunch clusters is effected, progressively over time declining to a point where yields can be severely affected. This occurs because bud mite damage is inflicted during the growing season, deep inside developing next season's bud primordia. Feeding of high numbers of bud mite inside these developing buds damages bunch primordia in a very early stage of their development, and can lead to complete absence of bunch clusters, even on relatively 'normal' vine shoots. Vegetative growth of the vine will often superficially mask the absence and damage to bunch clusters. Bud mite damage can be confused with cold damage, or other early spring restricted growth symptoms such as Australian Grapevine Yellows (AGY) (Bernard et al. 2005).

Successful bud mite control

Growers who participated in this survey were advised how to achieve successful bud mite management via an effective single wettable sulphur spray against bud mite precisely timed to the narrow window of bud mite migration to next season's bud primordia in early spring (Dennill 1986, Bernard et al. 2002, Bernard et al. 2005). When curatively and sparingly applied, in no more than 1-2 spray over two growing seasons in conjunction with predatory mite conservation, vineyards can be successfully protected from bud mite damage. Because bud mite bud and shoot injury is due to infestations in the previous growing season, the effect of wettable sulphur spray against bud mite applied in early spring of a current season will thus not be apparent, until the next spring when healthy vine shoots emerge.

However, prevention of bud mite damage in vineyards is not achieved by the curative sprays alone. Bud mite is, in fact, extremely difficult to control by sprays alone. Given its unique lifecycle inside vine buds, it is only exposed to sprays very briefly, during a very narrow (*c*. 2 week) window soon after bud burst, when it migrates from burst buds to the underside of stipular scales of new season's shoots, entering next season's bud initials, newly forming under stipular scales (Dennill 1986, Bernard et al. 2005). Due to their extremely small size (130-150 micron), bud mite are able to enter next season's bud primorida as these begin to form. In order to achieve successful control of bud mite in vineyards and minimise economic damage due to bud mite, the capacity of vineyards to sustain phytoseiid predatory mites and the ecosystem service (ES) they provide, natural biological control of bud mite and rust mite, is essential. Conservation of these economically important predatory mites, by minimising use of pesticides harmful to them, is therefore very important.

Current wine industry powdery mildew control practice, of using repeated applications of high rates (6kg/ha) of wettable sulphur (further concentrated 2-3x, in low volume sprays of up to 1,800 g/100L), in place of traditional sulphur use (200-400g/100L), is jeopardising bud

mite control in vineyards. Harmonising powdery mildew control practices with predatory mite conservation is urgently needed to prevent bud mite damage increases in vineyards.

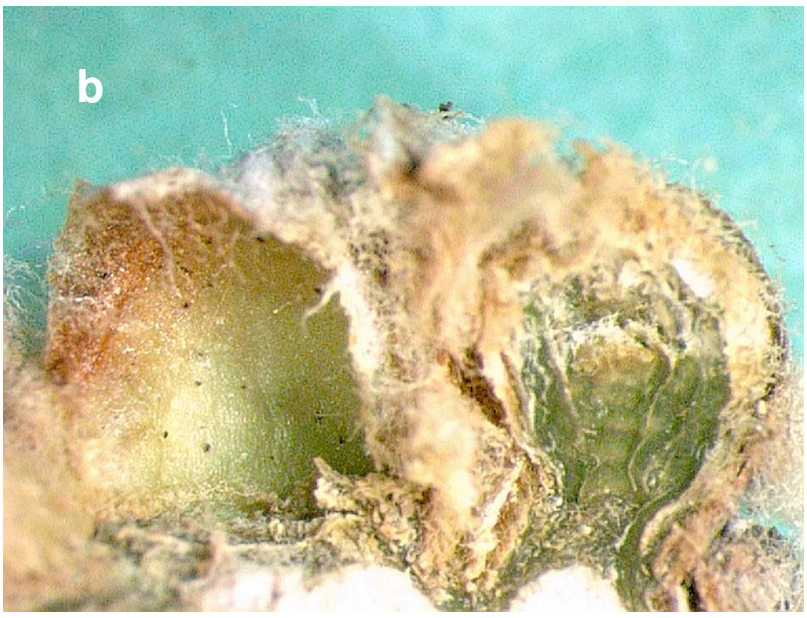
Table 8. Bud mite presence and damage due to bud mite, in un-burst buds and bases of vine shoots from 12 vineyards, in Riverland and McLaren Vale (October 2008)

Site Sampling date	Vine variety (No. of buds; No. shoot bases dissected)	Live bud mite (all life stages) and dead bud mite	No. of buds/ shoot bases per bud mite infestation		Buds/ shoot bases with polyps	Primary bud necrosis (PBN)	Buds with necrosis (1° & 2° buds combined)
Riverland I-II Beswick, Loxton 8 Oct 2008	Shiraz (27; 0)	0	18		13 (48.1%)	6	14
		1-50	9	33.3%			
		51-100	0				
		>100	0				
	Gordo (15; 0)	0	6		11 (73.3%)	5	8
		1-50	8	60.0%			
		51-100	0				
		>100	1				
Riverland III, Kingston Estate 8 Oct 2008	Petit verdot/ Chardonnay (11; 0)	0	2		8 (72.7 %)	5	3
		1-50	9	81.8%			
		51-100	0				
		>100	0				
Riverland IV Wutke, Combdogla 9 Oct 2008	Chardonnay (6; 3)	0	3		7 (77.0%)	1	4
		1-50	4	66.7%			
		51-100	2				
		>100	0				
Riverland V-VI CDD, North Renmark 8 Oct 2008	Chardonnay (23; 7)	0	18		22 (73.0%)	9	17
		1-50	12	40.0%			
		51-100	0				
		>100	0				
	Gordo (10; 2)	0	6		12 (100%)	4	8
		1-50	6	50.0%			
		51-100	0				
		>100	0				
Riverland VII-VIII Ludas, Renmark 9 Oct 2008	Chardonnay (25; 2)	0	27		8 (29.6%)	5	20
		1-50	0	0.0%			
		51-100	0				
		>100	0				
	Shiraz (20; 0)	0	15		10 (50.0%)	10	14
		1-50	4	25.0%			
		51-100	1				
		>100	0				
McLaren Vale I Collett 21 Oct 2008	Shiraz (8; 13)	0	17		18 (85.7%)	3	5
		1-50	3	19.0%			
		51-100	1				
		>100	0				
McLaren Vale II Harnett 21 Oct 2008	Logas Legacy Shiraz (8; 7)	0	13		13 (86.7%)	4	8
		1-50	1	13.3%			
		51-100	1				
		>100	0				
McLaren Vale III Darenberg 21 Oct 2008	Shiraz (8; 8)	0	13		14 (87.5%)	3	7
		1-50	3	18.8%			
		51-100	0				
		>100	0				
McLaren Vale IV Legard 21 Oct 2008	Shiraz (4; 12)	0	11		12 (75.0%)	1	4
		1-50	4	25.0%			
		51-100	0				
		>100	0				

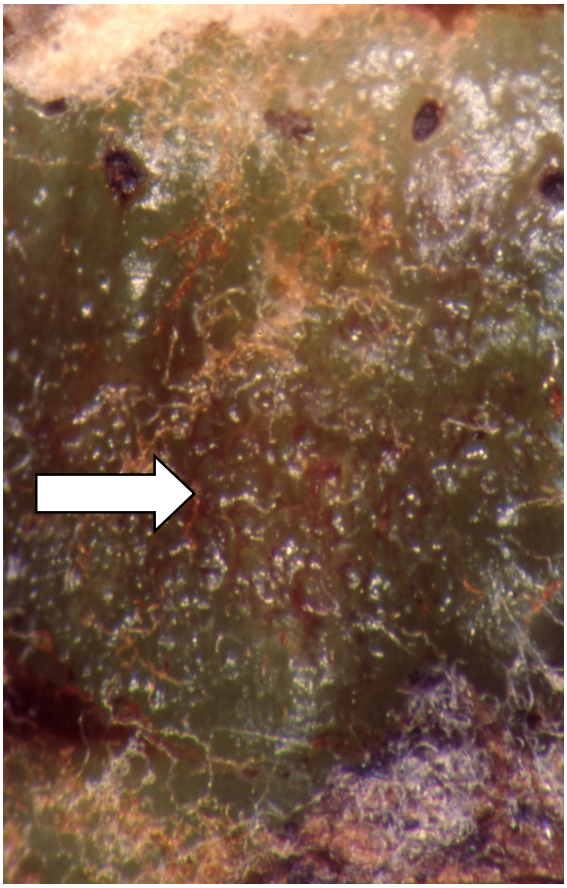
Figure 1. Bud mite (*Col vitis*) damage to vine buds:
(a) Bud mite inside a bud with primary bud necrosis (PBN). PBN is typically found in buds with > 100 bud mite



(b) Secondary buds developed in place of dead primary bud; Left - bud centre removed to show necrotic spotting; Right - shoot primordia extended and apical meristem discoloured and necrotic, preventing bud burst



(c) Blistering/‘polyps’ and purple coloration of scales, typically found in buds with > 50-100 bud mite. Necrotic spotting on scales (at the top of picture) is also visible.



6.2.4 Predatory mite releases and wettable sulphur field effects on released and naturally present predatory mites

Predatory mites were naturally present in vineyards regardless of *E. victoriensis* releases, in all vineyards these were species other than *E. victoriensis*, notably *Amblydromella doreenae* (Tables 9-10). Results show the most abundant, i.e. most wettable sulphur/ pesticide tolerant, naturally present species in vineyards was *A. doreenae* (Tables 7, 9 - 10). In many vineyards *A. doreenae* was the only phytoseiid predatory mite species present, and it displaced *A. dosseii* in some Limestone coast vineyards between 2005 and 2008. It also displaced *E. victoriensis* in Riverland, where it was previously common (James and Whitney 1993). Predatory mite numbers in both assessments, March and November 2008, varied greatly between vineyards (Tables 7, 9 - 10). Vineyards with high predatory mite populations in March had lower population numbers recorded the following spring. This is likely to be due to normal seasonal variation in numbers. Out of 10 vineyards, *E. victoriensis* releases were only successful in three vineyards, all in Riverland. All three vineyards were traditional low users of wettable sulphur (2 kg/ha).

Comparison of predatory mite numbers and spray diaries from 10 vineyards for growing seasons 2006/2007, 2007/2008, and 2008/2009, indicate the level of wettable sulphur use incompatible with predatory mites, in vineyards with extremely low or no predatory mite numbers (Table 9 - 10). This needs to be confirmed by further analysis of data on predatory mite abundance against vineyard spray programs. Principal Component Analysis on sprays applied and predatory mite numbers (released and naturally present) across all predatory mite data collected, including spray use in 2004/2005 and 2005/2006 from 21 vineyards surveyed in 2005, will be provided by the February 2012 publication deadline. Collation of spray programs for the analysis could not be completed within the 1 June 2011 time provided for this report. However all data pertaining to the analysis are provided in this report (Tables 7, 9-10).

When spray programs were examined, against high and low predatory mite numbers, some trends are apparent. Repeated use of highly concentrated wettable sulphur sprays (≥600g/100L) delivering mass of pesticide > 4 kg/ha only occurred only in vineyards where predatory mite numbers were extremely low. Very few *A. doreenae*, and no other phytoseiid species, were recovered from vineyards where repeated applications of wettable sulphur at 6 kg/ha (2-3x concentrated in low volume sprays) were made in the same growing season and the preceding year.

Conversely, vineyards with the highest predatory mite numbers at flowering, in summer January (Table 9), or at harvest (Table 10) all had fewer applications of wettable sulphur, and at far lower rates (200-400g/100L at 2kg/ha). In the three vineyards where *E. victoriensis* releases resulted in *E. victoriensis* establishment, wettable sulphur use was also low (200-400g/100L at 2kg/ha).

Table 9. Predatory Mite Release Assessment (March 2008): Mean predatory mites of all species (eggs, juveniles, adults) per 35 leaves ± S.E. and species abundance in release and control plots (based on five replicates)*

Site N°.	Name of vineyard	Treat-ment	Mean per 35 leaves ± S.E.	Total number collected (eggs, larvae, nymphs, adults)	Species ID Total number assessed (deuto-nymphs, adults)	Percent released species: *Euseius victoriensis*	Percent naturally present species: *Amblydromella doreenae ,A. dossei, Neoseiulus dieteri, N. loxtoni, N. tareensis*
Limestone Coast							
II	Stonehaven Padthaway Vineyards	release	2.40 ± 0.68	12	10	0	*A. doreenae* (10) 100%
		control	2.75 ± 1.12	11	6	0	*A. doreenae* (6) 100%
III	Bold Vineyards	release	0.60 ±0.40	3	3	0	*A. dossei* (3) 100%
		control	0.25 ± 0.25	1	1	0	*A. doreenae* (1) 100%
IV	Koonara Management	release	1.60 ±0.68	8	7	0	*A. doreenae* (7) 100%
		control	0.25 ± 0.25	1	0	0	0
McLaren Vale							
I	Glenchera	release	0.60 ± 0.40	3	3	0	*A. doreenae* (3) 100%
		control	0.60 ± 0.60	3	2	0	*A. doreenae* (2) 100%
IV	Tinlins - Lot 18	release	0.60 ± 0.40	3	3	0	*A. doreenae* (3) 100%
		control	0.50 ± 0.50	2	1	0	*A. doreenae* (1) 100%
VII	The Terraces-Yangarra	release	33.4 ± 2.62	167	76	0	*A. doreenae* (76) 100%
		control	14.2 ± 2.78	71	39	0	*A. doreenae* (39) 100%
Riverland							
I	CT Farms - Clark Taylor	release	3.20 ± 1.39	16	10	*E. victoriensis* (5) 50.0%	*A. doreenae* (5) 50.0%
		control	6.00 ± 2.17	30	22	0	*A. doreenae* (22) 100%
III	Ridge Hill Vineyard	release	6.60 ± 1.54	33	20	*E. victoriensis* (2) 10.0%	*A. doreenae* (18) 90.0%
		control	8.00 ± 1.92	40	34	0	*A. doreenae* (31) 91.2%, other (3) 8.8%
IV	Lory	release	0.40 ± 0.25	2	1	0	*A. doreenae* (1) 100%
		control	0.00	0	0	0	0
extra	Altmann- Blair	release	9.00 ± 3.00	45	27	*E. victoriensis* (23) 88.9%	*A. doreenae* (3) 11.1%

* Except for four replicates: Stonehaven Padthaway Vineyards - control; Bold vineyards- control; Koonara Management -control; Tinlins – control.

** Larvae and protonymphs were identified to genus and associated with the same genus adults.

Table 10. Predatory Mite Release Assessment (November 2008): Mean predatory mites of all species (eggs, juveniles, adults) per 35 leaves ± S.E. and species abundance in release and control plots, (based on five replicates)*

Site N°.	Name of vineyard	Treat-ment	Mean per 35 leaves ± S.E.	Total number collected (eggs, larvae, nymphs, adults)	** Species ID Total number assessed (deuto-nymphs, adults)	Percent released species: *Euseius victoriensis*	Percent naturally present species: *Amblydromella doreenae ,A. dosseii, Neoseiulus dieteri, N. loxtoni, N. tareensis*
Limestone Coast							
II	Stonehaven	release	4.40 ±1.86	22	20	0	*A. doreenae* (20) 100%
	Padthaway Vineyards	control	3.00 ±0.89	15	11	0	*A. doreenae* (11) 100%
III	Bold Vineyards	release	3.20 ± 0.58	16	14	0	*A. dosseii* (12) 85.7%, *A. doreenae* (2) 14.3%
		control	3.60 ± 1.08	18	15	0	*A. dosseii* (11) 73.3%, *A. doreenae* (4) 26.7%
IV	Koonara Management	release	2.00 ± 0.71	10	8	0	*A. doreenae* (7) 87.5%, *A. dosseii* (1) 12.5%
		control	1.20 ± 0.58	6	6	0	*A. doreenae* (6) 100%
McLaren Vale							
I	Glenchera	release	13.60 ± 2.40	68	61	0	*A. doreenae* (61) 100%
		control	9.75 ± 1.11	39	27	0	*A. doreenae* (27) 100%
IV	Tinlins - Lot 18	release	1.00 ± 0.32	5	4	0	*A. doreenae* (4) 100%
		control	1.60 ± 0.68	8	7	0	*A. doreenae* (7) 100%
VII	The Terraces-Yangarra	release	2.20 ± 0.58	11	8	0	*A. doreenae* (8) 100%
		control	3.80 ± 1.43	19	16	0	*A. doreenae* (16) 100%
Riverland							
I	CT Farms - Clark Taylor	release	6.60 ± 2.20	33	27	*E. victoriensis* (16) 59.3%	*A. doreenae* (11) 40.7%
		control	1.20 ± 0.73	6	6	0	*A. doreenae* (6) 100%
III	Ridge Hill Vineyard	release	7.00 ± 2.74	35	30	*E. victoriensis* (13) 43.3%	*A. doreenae* (17) 56.7%
		control	1.80 ± 0.49	9	6	0	*A. doreenae* (6) 100%
IV	Lory	release	0.20 ±0.20	1	1	0	*A. doreenae* (1) 100%
		control	0.20 ±0.20	1	1	0	*A. doreenae* (1) 100%
extra	Altmann - Blair	release	4.20 ± 0.97	21	14	*E. victoriensis* (12) 85.7%	*A. doreenae* (2) 14.3%

*Except for four replicates - Glenchera control.
** Larvae and protonymphs were identified to genus and associated with the same genus adults.

Discussion

Field results concur with laboratory data on wettable sulphur (Section 6. 1), collected in testing of 11-12 days testing duration. A previous, limited duration laboratory study (48 hour tests) of wettable sulphur residues also concur with field data (James and Rayner 1995). Based on these short tests, James and Rayner (1995) suggested *Amblydromella doreenae* adult females were tolerant, for the first 48 hours of exposure, to wettable sulphur residues of 600g/100L. Field results also concur with recommendations for low-moderate wettable sulphur use in sustainable viticulture in Europe (Malavolta and Boller 1999), and complement research on wettable sulphur toxicity to the lightbrown apple moth (LBAM) egg parasitoid *Trichogramma* sp. (Thomson et al. 2000).

Wettable sulphur is the key most widely used wine industry pesticide. Traditional label rate use (1-2 kg/ha) at spray tank concentrations (200-400g/100L) was largely replaced since *c.* 2004 by much higher label rates. Wettable sulphur is now commonly used in a very wide range of concentrations; at the lower limit are dilute high volume sprays (200-400g/100L) delivering a mass of 2kg/ha, representing traditional, restrained use at *c.* 3 - 5 sprays per season. At the high-use range are 9-12 applications per season of concentrated low volume sprays (600g/100L -1800g/100L) at the highest allowable mass of pesticide (6 kg) per ha, delivering an extremely concentrated micro-dose at the point of spray contact with predatory mites and other beneficials. Compared to other pesticides registered for use in Australian viticulture, a label rate that allows 300% range in the mass of pesticide per hectare (2-6 kg/ha), and 900% range in spray tank concentrations (200g/100L - 1800g/100L) is unprecedented. It reflects a general confusion about what rates of wettable sulphur actually deliver effective powdery mildew control, and it has created the expectation that high use is best.

In the face of such confusion, predatory mites and their ecosystem service - effective free-of-charge rust mite and bud mite control- have in many vineyards become a casualty of powdery mildew management. Use of highly concentrated low volume sprays for control of powdery mildew, especially of highly concentrated early season sprays, leads to predator knockdown before over-wintered predatory mite populations have a chance to build up. This is likely to have a greater knockdown effect than late season sprays. This is because a well-developed vine canopy can afford some protection from sprays, and a larger predatory mite population has a greater potential to seed the next generation after a partial knockdown.

Pesticides other than high rates of wettable sulphur can contribute to low predatory mite numbers and to rust mite and bud mite outbreaks overtime. These are: botrytis fungicides benomyl, carbendazim, and pyrimethanil, and the insecticides spinosad and emamectin benzoate (M. Bernard, Final Report MU LTU 04-01 PART-B; Bernard et al. 2010a, b). Data analysis on spray programs and predatory mite numbers, will include analysis of use of these pesticides alongside that of wettable sulphur.

When devising vineyard spray programs, it is important to minimise use of all sprays that contribute to predatory mite kill. Carefully devised wettable sulphur programs are likely to have lower detrimental effects on predatory mites, when other pesticides toxic to predatory mites are not used. This is due to reduced synergistic toxic effects.

RECOMMENDATIONS

- Further extension of results is highly recommended to the wine industry extension team at Wagga-Wagga, for maximum benefit of project findings to the wine industry. Pesticide effects on beneficial invertebrates are a highly topical, sought-after information by many growers and vignerons in Australia. Pesticide results in this project (MU 05-01), and in another project (MU LTU 04-01 PART-B), and publications (Bernard et al. 2010ab) represent the first comprehensive rendering of this topic in viticulture world-wide.

- The Australian wine industry needs to determine in future research on powdery mildew, minimal wettable sulphur programs necessary to deliver successful powdery mildew control. This would provide the confidence needed for growers to use wettable sulphur concentrations at the lower end of the pesticide label, and harmonise powdery mildew management with predatory mite conservation and rust mite and bud mite outbreak prevention. The issue of wettable sulphur overuse and induced rust and bud mite outbreaks will not be solved until powdery mildew control approaches become more sophisticated, then simply applying high rates of a pesticide.

- Given that botrytis fungicides can also disrupt predatory mites (Bernard et al. 2010ab), there is a need to further extend in Australia research results on biological control of botrytis bunch rot conducted in New Zealand (Jacometti et al. 2007b, 2007c, Jacometti et al. 2007a). Research on biological control of over-wintering powdery mildew inoculum along the lines of the botrytis research is also needed.

APPENDIX 1: COMMUNICATION

Published papers

Refereed scientific paper

MARTINA B. BERNARD, PETER COLE, AMANDA KOBELT, PAUL A. HORNE, JAMES ALTMANN, STEPHEN D. WRATTEN, ALAN L. YEN, 2010. Reducing the impact of pesticides on biological control in Australian vineyards: pesticide mortality and fecundity effects on an indicator species, the predatory mite *Euseius victoriensis* (Acari: Phytoseiidae), Journal of Economic Entomology, 103(6): 2061-2071

Entomological Society of America. DOI: 10.1603/EC09357.
http://www.bioone.org/doi/full/10.1603/EC09357

ABSTRACT Laboratory bioassays on detached *Glycine max* (L.) (soybean) leaves were used to test 23 fungicides, 5 insecticides, 2 acaricides, 1 herbicide, and 2 adjuvants on a key Australian predatory mite species *Euseius victoriensis* (Womersley) in "worst-case scenario" direct overspray assays. 0-48 h old juveniles, their initial food and water supply, were sprayed to runoff with a Potter tower; spinosad and wettable sulphur residues were also tested. Tests were standardized to deliver a pesticide dose comparable to commercial application of highest label rates at 1000 liter/ha. Cumulative mortality was assessed 48 h, 4 d and 7 d after spraying. Fecundity was assessed for 7 d from start of oviposition. No significant mortality or fecundity effects were detected for the following compounds at single-use application at 1000 liter/ha: azoxystrobin, *Bacillus thuringiensis* subsp. *kurstaki*, captan, chlorothalonil, copper hydroxide, fenarimol, glyphosate, hexaconazole, indoxacarb, metalaxyl/copper hydroxide, myclobutanil, nonyl phenol ethylene oxide, phosphorous acid, potassium bicarbonate, pyraclostrobin, quinoxyfen, spiroxamine, synthetic latex, tebufenozide, triadimenol, and trifloxystrobin. Iprodione and penconazole had some detrimental effect on fecundity. Canola oil as acaricide (2 liter/100 liter) and wettable sulphur (200g/100 liter) had some detrimental effect on survival and fecundity, and cyprodinil/fludioxonil on survivor. The following compounds were highly toxic (high 48 h mortality): benomyl, carbendazim, emamectin benzoate, mancozeb, spinosad (direct overspray and residue), wettable sulphur (≥ 400g/100 liter), and pyrimethanil; pyrimethanil had no significant effect on fecundity of surviving females. Indoxacarb safety to *E. victoriensis* contrasts with its toxicity to key parasitoids and chrysopid predators. Potential impact of findings is discussed.

KEY WORDS: *Euseius victoriensis*, Phytoseiidae, Restricted-Spring-Growth, biological control, Integrated Pest Management in viticulture

Technical wine industry paper

Martina Bernard, P. Cole, A. Kobelt, P. Horne, J. Altmann, S. Wratten, and A. Yen, 2010. Predatory mites in vineyards - Conserving biological control and preventing mite outbreaks in Australian vineyards: The effect of pesticides on a predatory mite indicator species, Australian Viticulture, 14 (5): 20-28

ABSTRACT: Effects of direct oversprays of 33 pesticides including wettable sulphur, concentrations 200g/100L - 1,200g/100L on two predatory mite indicator species from Australian vineyards (*Euseius victoriensis* and *Galendromus occidentalis)* were studied in the laboratory. Bioassays on predatory mites were comprehensive 13-15 day duration tests, investigating both mortality, and sub-lethal effects on fecundity, following pesticide exposure of young juveniles; the most susceptible life stage of the predatory mite. Tests were carried out to international IOBC (International Organisation for Biological Control) standards for designating pesticides safe to beneficials. The pesticide window within which sustainable viticulture can be practiced is comprehensively determined here, providing commercial growers and vignerons with information to enable successful conservation of beneficial predatory mites, and reaping of economic benefits from the Ecosystem Service (ES) predatory mites provide. The publication is a grower-friendly rendering of a scientific paper (Bernard et al. 2010), with additional testing over and above the scientific publication included. Both publications represent the most comprehensive publications of pesticide effects on beneficial invertebrates in vineyards, ever published in Australia, and are definitive world-wide references on the topic. Given pesticide tested here are registered for use in many other crops, and given that phytoseiid predatory mites are predators in all horticultural and most agricultural crops, both publications have far-reaching application outside viticulture in Australia and world-wide.

Wine Industry Seminars

Four annual grower seminars, one per each region, were carried out as per project targets, in participating RITA wine regions (King Valley, Limestone Coast, McLaren Vale, Riverland); these seminars were fully funded by the RITA funding stream of this project. Over and above this target, additional 12 above-target seminars were carried out as part of this project due to grower demand; (2 in 2006, 9 in 2007, 1 in 2008) in wine regions other than the participating regions.

Travel costs of additional seminars were funded by the requesting regions, and CI salary costs were fully funded by (MU 05-01). In 2007, for 9 additional seminars, CI salary costs were fully funded by MU 05-01, salary costs for all other staff and speakers were met by their home organisations, and all travel and operating costs were funded by RITA project (GWRDC RT 06/05-1). This project was jointly initiated by Limestone Coast Wine Industry Regional Council, Dr Martina Bernard, Prof Rick Roush, (Dean, School of Land and Environment, University of Melbourne), and DPI Victoria. For full details see (Bernard et al., Final Report to GWRDC RITA- RT0605-1, 15 Nov 2007; Appendix 7). The CI organised and coordinated the 13th AWITC Regional Forum as an add-on value to this project, and brought to Australia two international speakers, to present new, cutting edge research in sustainable pest management in viticulture, alongside Australian speakers at the forum. The seminar cycle was extremely successful, and allowed the 13th Australian Wine Industry Conference Workshop (W35) to travel to regional Australia. Workshop (W35) was voted in the top 10 most subscribed workshops at 13th Australian Wine Industry Conference.

2006

In winter 2006, the following six seminars were conducted, exceeding project target by two. The seminars were conducted on invitation of Grape Industry and Phylloxera Board of SA), and presented (in RITA participating regions) new data for project MU 05-01. Seminars extended: up to date information on pesticides safe to beneficial invertebrates in vineyards, that constitute natural vineyard defences against mite pests, mealybugs, and other pests; latest New Zealand research and its practical application in vineyards on how to supply nectar to parasitoid wasps of lightbrown apple moth (LBAM) via cover crops was also covered.

1. August 2006, Limestone Coast Grower Seminar, SA: attendance 50+
2. September 2006 – Riverland Grower Seminar, SA: attendance 150+
http://www.phylloxera.com.au/board/meetings/view.asp?region=Riverland
3**. September 2006 - Adelaide Hills Grower Seminar, SA**: attendance 25+
http://www.phylloxera.com.au/board/meetings/view.asp?region=Adelaide%20Hills
4. September 2006- Barossa Valley Grower Seminar, SA: attendance 100+
(also attended by then GWRDC executive director, Jim Fortune)
http://www.phylloxera.com.au/board/meetings/view.asp?region=Barossa%20Valley
5. September 2006 – McLaren Vale: attendance 50+
6. October 2006 Barossa, SA: attendance 25+ ; Sustainable wine grape production half-day seminar jointly with other researchers (Adelaide University, Lincoln University, New Zealand) on IPM and biological control to key wine industry leaders in SA.

2007

1) 13th AWITC, Workshop W 35 , 31 July 2007
55 participants; Convener: Dr M Bernard
Speakers: Prof S. Wratten, Dr C. Ohmart, Dr C. Paull (LBAM parasitoid wasp species on Limestone Coast), Dr M. Bernard (pesticide effects on pest natural enemies commonly found in vineyards). The workshop was voted in the top 10 workshops most subscribed at the conference.

2-12) 13th AWITC Regional Innovation and Technology Transfer Forum 2007
Martina Bernard was the overall manager and co-ordinator of the technology transfer forum. The forum was a joint project between Martina Bernard, Prof Richard Roush, Dean, Melbourne School of Land and Environment, many regional wine industry councils, and the Victorian Department of Primary Industries. The Forum delivered 10 regional seminars to growers in August 2007, and brought two international speakers, Prof Steve Wratten (National Centre For Advanced Bio-protection, Lincoln University, New Zealand), and Dr Cliff Ohmart (IPM Director Lodi Wine Commission, California, USA) to Australia, to speak on key most up to date topical issues in sustainable pest management in viticulture. Martina Bernard made presentations at four of these seminars alongside overseas speakers, on the topic of this project: pesticide effects on predatory mites in vineyards and vineyard beneficials generally. The forum delivered extension targets of MU LTU 04-01- PART B and MU 05-01, and international speakers to wine regions. For full details, please see final report (GWRDC RT 06/05-1; Appendix 7). The forum was a very successful, highly subscribed event, which brought the 13th AWITC Workshop 35 to regional Australia, and allowed participation of growers who could not attend the 13th AWITC conference in Adelaide.

(2) 1/8/07 CropWatch Wine industry IPM Dinner, Adelaide
Convener: James Wright; Speaker: C. Ohmart: Lodi data base presentation and discussion

(3) 3/8/07 Barossa Seminar: c. 30 participants;
Conveners: M. Bernard and B. Hannaford
Speakers: S. Wratten, C. Ohmart, T. Ayers

4) 3/8/07 McLaren Vale/Adelaide Hills/Langhorne Ck/Fleurieu Seminar
c. 50 participants; Convener: James Hook; Speakers: S. Wratten, C. Ohmart, T. Ayers
A grower dinner was also attended by speakers, giving growers an opportunity to further talk to speakers.

(5) 6/8/07 Limestone Coast Seminar, c. 40 participants
Convener: Kerry DeGaris; Speakers: S. Wratten, C. Ohmart, T. Ayers

(6) 7/8/07 Yarra Valley/Mornington/Gippsland/Macedon Ranges/Sunbury Seminar
c. 40 participants; Conveners: Dr M. Bernard, Kieran Murphy (DPI Victoria Grapecheque)
Speakers: S. Wratten, C. Ohmart, T. Ayers, D. Braybrook

(7) 8/8/07 Ararat/Geelong Seminar c. 65 participants
Convener: K Murphy, Speakers: C. Ohmart, M Bernard (presenting T Ayers', S Wratten's work); D. Braybrook (presenting a report on GWRDC IPM survey conducted in the region)

(8) 9/8/07 Lower Hunter Valley Seminar: c. 50 participants;
Convener: Tony Somers; Speaker C. Ohmart

(9) 10/8/07 Mudgee Seminar: c. 50 participants;
Convener: Tony Somers; Speaker C. Ohmart

(10) 13/8/07 N-East Victoria Joint Seminar – hosted by King Valley Vignerons:
c. 40 participants; Convener: K Murphy; Speakers: C. Ohmart, M. Bernard (presenting T Ayers' and S Wratten's work)

(11) 7/8/07- University of Melbourne Faculty of Land and Food Resources Seminar:
Topic: Successful knowledge transfer in agriculture and horticulture: The Lodi experience
Convener: Rick Roush, Speaker: C. Ohmart

12) 13th AWITC Workshop on Organic Viticulture 2 August 2007
Langhorne Creek, Temple Breuer Wines. Martina presented at this workshop by invitation from the workshop convenors.

2008

1) King Valley Seminar - 26 August 2008
2) McLaren Vale and Adelaide Hills Combined Seminar - 29 August 2008
3) Yarra Valley Vignerons Seminar - September 2008
4) Mornington Peninsula Vignerons Seminar- September 2008
5) Riverland Seminar – October 2008

APPENDIX 2: INTELLECTUAL PROPERTY

The intellectually property sharing arrangement between GWRDC and the University of Melbourne is arranged to be 50% ea, as is appropriate for a PhD scholarship. However, this arrangement was not appropriate for this post- doctoral level research project. Data from this project are not expected to be commercialised, and are already in the public domain.

APPENDIX 3: STAFF

Chief Investigator: Dr Martina B. Bernard

Project Supervisors: Prof Ary A. Hoffmann, Dr Martina B. Bernard

Expertise Mass- Rearing predatory mite colonies/selection for wettable sulphur tolerance: James Altmann , Rob Weppler, Biological Services, Ltd., Loxton, SA

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Laboratory field assessments: Alen Rako, Hoffmann Lab, University of Melbourne
Field work - King Valley: Gina Martinelli

Senior academic advisors:
Dr Alan Yen, Head of Entomology, Department of Primary Industries, Victoria
Prof Steve Wratten, National Centre for Advanced Bio-Protection Technologies, Lincoln University, New Zealand
Dr Jennifer J. Beard, Queensland Museum
James Altmann - Biological Services, Ltd., Loxton, SA
Dr Paul Horne, Director, IPM Technologies Ltd., Hurstbridge, Victoria

Other scientific collaborators:
Peter Cole - IPM Technologies Ltd., Hurstbridge, Victoria
Dr Amanda Kobelt - Primary Industries Research Victoria, Knoxfield, Victoria

Department of Primary Industries, Victoria, Knoxfield Centre provided the use of its Pesticide Testing Laboratory to the project, per contractual agreement between Hoffmann Laboratory/ University of Melbourne, and DPI Knoxfield.

APPENDIX 4: Results- statistical reporting

Bioassay I. There was a significant difference ($P<0.001$) in the effects of treatments on 48-h, 4-d and 7-d cumulative mortality of *E. victoriensis* juveniles compared with the control ($F = 45.18$, 86.50, and 90.05 respectively; $df = 9, 28$ in each case). Post hoc tests indicate wettable sulphur (all concentrations), benomyl, and carbendazim, resulted in significantly higher 7 d mortality compared with the control (Table 2). Wettable sulphur (200g/100 liter) was significantly less toxic than its higher concentrations, and caused a significant, moderate (51.7%) fecundity reduction ($F = 5.856$; $df = 5, 18$; $P= 0.002$; Tukey b; Table 2); wettable sulphur ($\geq$ 600g/100 liter), benomyl, and carbendazim completely suppressed fecundity (Table 2). Azoxystrobin, hexaconazole, myclobutanil and penconazole did not differ significantly from the control or each other in mortality and fecundity effects (Tukey b; Table 2). However a minor fecundity reduction was recorded for penconazole. Toxic effects of wettable sulphur, benomyl, and carbendazim were not fully expressed at 48 h and 4 d, indicating a need for extended mortality testing to evaluate pesticide safety to Phytoseiidae.

Bioassay II. There was a significant difference ($P<0.001$) in the effects of treatments on 48-h, 4-d and 7-d cumulative mortality of *E. victoriensis* juveniles compared with the control ($F = 13.96$, 41.01, and 15.65 respectively; $df = 4, 14$ in each case). Post hoc tests indicate wettable sulphur residues (400g and 600g/100 liter) and mancozeb resulted in significantly higher mortality compared with the control; wettable sulphur residues and mancozeb were equally toxic, while iprodione had only a minor significant mortality effect at 4 d, and no significant effect at 48 h and 7 d (Tukey b; Table 2). Post hoc tests indicate a significant difference between treatments in fecundity effects ($F= 13.90$, $df= 4,14$, $P<0.001$); wettable sulphur residues and mancozeb resulted in a significant fecundity reduction compared with the control, and iprodione in a minor fecundity reduction (Tukey b; Table 2).

Bioassay III. There was a significant difference ($P<0.001$) in the effect of treatments on 48-h, 4-d, and 7-d cumulative mortality of *E. victoriensis* juveniles, compared with the control ($F = 29.98$; $df = 4, 12$), ($F = 31.55$; $df = 4, 12$), and ($F = 28.07$; $df = 3, 10$) respectively. Post hoc tests indicate surfactants nonyl phenol ethylene oxide and synthetic latex were not significantly toxic and caused no significant fecundity effects compared with the control, but mancozeb and spinosad residue were significantly more toxic at all assessment times (Tukey b, Table 3), and surviving females reproduced at a significantly lower rate compared with the control; 83% fecundity reduction ($F = 25.99$; $df = 3, 10$; $P<0.001$; Table 3).

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