Developing an Ecotoxicological Testing Standard for Predatory Mites in Australia: Acute and Sublethal Effects of Fungicides on *Euseius victoriensis* and *Galendromus occidentalis* (Acarina: Phytoseiidae)

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ABSTRACT Laboratory bioassays for testing the effect of agrochemicals on *Euseius victoriensis* (Womersley) and *Galendromus occidentalis* (Nesbitt) on detached leaves of *Glycine max* (L.) (soybean) and *Phaseolus vulgaris* L. (French bean) were developed. The tests allowed standardized comparisons between mite species and leaf substrates, under "worst-case scenario" exposure, comparable with commercial pesticide application. Young juveniles, along with their initial food and the entire water supply, were sprayed to the point of runoff by using a Potter spray tower. The highest registered field rate concentration used on French bean was adjusted to deliver the same pesticide dose per higher runoff point spray volume on soybean. Cumulative mortality was assessed at 48 h, 4 d, and 7 d after spray application. Fecundity was assessed for 7 d from the onset of egg lay. Boscalid (Filan 500 WG), dithianon (Delan 700 WG), and kresoxim-methyl (Stroby 500 WG) caused no significant 7-d mortality or fecundity reduction to *G. occidentalis* or *E. victoriensis* compared with controls, and are classified as harmless to both species. Mancozeb (Mancozeb 750 WG) was highly toxic to both species, resulting in severe mortality and fecundity reduction and is considered incompatible with integrated pest management programs that use these species. Metiram (Polyram 700 WG) was highly toxic to *E. victoriensis* but only moderately toxic to *G. occidentalis*. Analyses of mortality proportions, including, and excluding unaccounted escapees, produced the same results. Test standardization on leaf substrates provides an alternative approach to standardization via residue on glass used by International Organisation for Biological and Integrated Control or Noxious Animals and Plants/West Palaearctic Regional Section regulatory testing in the European Union.

KEY WORDS *Euseius victoriensis*, *Galendromus occidentalis*, ecotoxicology, sublethal effects, biological control

MANY LABORATORY METHODS have been developed for testing pesticide effects on phytoseiid predators: acute topical and residue assays by dipping or spraying mites taped to slides; exposing mites on treated leaf discs, glass substrate, excised, or attached plant leaves (Overmeer 1985); and tests that measure both direct mortality and predator performance (van Zon and Wysoki 1978, Overmeer and van Zon 1982). Testing standards were developed for *Phytoseiulus persimilis* Athias-Henriot (Samsoe-Petersen 1983, Oomen 1988, Bakker et al. 1992) and for *Typhlodromus pyri* Scheuten (Overmeer 1988, Bluemel et al. 2000b). In these standardized tests, cumulative 7-d mortality and reproduction from 7 to 14 d after juvenile exposure to fresh dry residue are used to predict pesticide safety in the field. Direct measurements of predation are considered unnecessary, because reproduction is considered a good indicator of predation given gravid females consume far more prey than other life stages (Sabelis 1985), and adverse physiological effects manifest eventually as reduced fertility (Samsoe-Petersen 1990).

The standard tests are comprehensive, yet they may not be a sufficiently accurate predictor of pesticide safety. They have an inherent potential to deliver type II error, because they only test the effect of a limited dry residue dose picked up via tarsal contact, and via limited diet contamination. Toxicity due to direct overspray of animals, their food, and water is not measured at all. Yet, in standard commercial field applications of pesticides, such exposure of at least a portion of the predator population repeatedly occurs. Moreover, the toxic effect of a formulation where the active constituent has low toxicity may go undetected in residue assays, such as in EC formulations of some fungicides. There is evidence that topical and residue test results can vary significantly, for example, in the ovicidal effect of endosulfan on *P. persimilis* (Bluemel et al. 1993), and for twospotted spider mite, *Tetranychus urticae* Koch, where for the same population,

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direct exposure by slide dipping resulted in 5.7-fold resistance compared with 544-fold resistance in residue assays (Dennehy et al. 1983). Furthermore, some field results contradicted pesticide safety predictions based on laboratory residue assays; (Bakker et al. 1992), insect growth regulator (IGR) effects on coccinellids (Hattingh and Tate 1995, Hattingh 1996), and indoxacarb effects on transverse ladybird beetle, *Coccinella transversalis* Fabricius, the lacewing *Micromus tasmaniae*, and the damsel bug *Nabis kinbergii* Reuter (P.A.H., unpublished data).

The standard tests use inert substrate because it is considered to allow greater standardization (Overmeer 1985, Samsoe-Petersen 1990), and the toxic effect of a residue of only 1–2 mg/cm^2 wet spray deposit is measured. Yet it is recognized that this does not represent "worst case scenario" exposure (Samsoe-Petersen 1990, Bluemel et al. 2000a) required to designate pesticides as safe. A 2 mg/cm^2 deposit does not reflect the dose delivered to a leaf surface at or near runoff point in a commercial field application at 1000 liter/ha spray volume assumed by most pesticide labels. Whereas field deposits vary due to many spray application factors, wet spray deposits >2 mg/cm^2 are generally achieved at run-off point; Aliniazee and Cranham (1980) reported 4 mg/cm^2 deposit on plum, and 40% of product applied per ha was estimated to reach plant surface in three dimensional crop canopies (Barrett et al. 1994).

In Australia, there are no standard protocols for testing beneficial arthropods apart from honey bees, despite increasing awareness among growers of advantages in conserving beneficials. Few protocols have been proposed, such as for *Trichogramma* spp. Westwood (Thomson et al. 2000) and *M. tasmaniae* (Rumpf et al. 1998), and relatively few results published (Llewellyn 2002). Procedures are not standardized, and pesticides were classed safe based only on 48 h or 4 d mortality tests (Goodwin 1984, James and Rayner 1995), disregarding internationally established sub-lethal and worst-case exposure methodology. Claims about safety to beneficials are made in pesticide promotional material with no reference to testing methods, despite the fact that different results can be obtained depending on concentration, dose, species, strain, length of test, developmental stadium, residue versus topical, and acute versus sub-lethal testing. By contrast, overseas commercial insectaries do extensive testing, and in the European Union standardized fairly comprehensive testing is required for pesticide registration. Selected species are tested as representatives of beneficial fauna, *T. pyri* for agricultural mite fauna (Candolfi et al. 2000).

In Australia, the selection of representative test species is complicated by limited data. Historically, the introduction of exotic predators, such as *Anystis* spp. von Heyden, *T. pyri*, *Galendromus occidentalis* (Nesbitt), and *P. persimilis*, moved research away from indigenous species. Here we selected two commercially significant phytoseiids for developing testing procedures: a widespread indigenous generalist predator *Euseius victoriensis* (Womersley), and naturalized organophosphate resistant *G. occidentalis; T. urticae* predator feeding also on eriophyoids, tydeids and tarsonemids (McMurtry and Croft 1997), introduced in 1972 (Readshaw 1975, Field 1978, Schicha 1987, James 1988, Whitney and James 1996), adapted to hot, dry Australian conditions, and an excellent control agent in pome and stone fruit, grapes and outdoor vegetables (Llewellyn 2002). *E. victoriensis* is widespread in inland and sub-coastal citrus crops, controlling citrus eriophyoids, as well as *Polyphagotarsonemus latus* (Banks) (broad mite) and *Eutetranychus orientalis* (Klein) (oriental spider mite) (Schicha 1987, Smith and Papacek 1991, James and Rayner 1995, Smith et al. 1997, Walter 1999). It is reported in inland stone fruit and grapes, in association with *T. urticae*, and eriophyoids *Aculus cornutus* (Banks), *Calepitrimerus vitis* (Nalepa), and *Colomerus vitis* (Pagenstecher) (James 1989b, 1990, James and Whitney 1993, James et al. 1995).

The aim of this research was to develop a simple, economically viable laboratory test for routine evaluation of pesticide safety to phytoseiid biological control agents in Australia, to allow standardized comparison between species, and to modify existing methods to better approximate "worst-case scenario" and commercial pesticide application. A topical bioassay exposing young *E. victoriensis* and *G. occidentalis* juveniles on detached leaves, evaluating direct mortality and sublethal effects, was used to investigate the effect of five fungicides for control of *Plasmopara viticola* (Berkley & Curtis) Berlese & De Toni (downy mildew) and *Phomopsis viticola* Saccardo in grapevines, and fungal diseases in pome fruit, citrus, and other crops.

Materials and Methods

Cultures. *E. victoriensis* was collected in autumn 2002 from a commercial citrus orchard near Loxton, South Australia (140.57° E, 34.46° S), unsprayed for 8 yr and thus likely nonresistant, improving the predictive value for pesticide safety (Samsoe-Petersen 1990). Citrus leaves with predators were cut and transported in a cooler. The laboratory culture was initiated from 34 females and seven males, reared on black polyurethane plastic arenas (15 by 8 cm) edged by wet paper towels and nonmelting multipurpose hydrocarbon grease (556-446; RS Components, Northants, UK). Arenas were rested on wet foam in a water container, supplied with cotton wool strands and inverted V-shaped transparent plastic shelters (McMurtry and Scriven 1965, Overmeer et al. 1982), at 24 ± 1°C, 70–90% RH, a photoperiod of 16:8 (L:D) h, 400-1000 lux, and dusted twice weekly with *Typha orientalis* Presl (cumbungi) pollen (James 1993). Pollen collected the same season was stored at −1°C, batches for dispensing at 4°C. Eggs were removed to new arenas daily for 12 d (165 eggs in total). Field-collected animals cleared, slide-mounted, and identified (McMurtry and Schicha 1987, Schicha 1987, Walter 1999), and progeny combined to a single rearing arena. Contrary to James (1993), it proved difficult

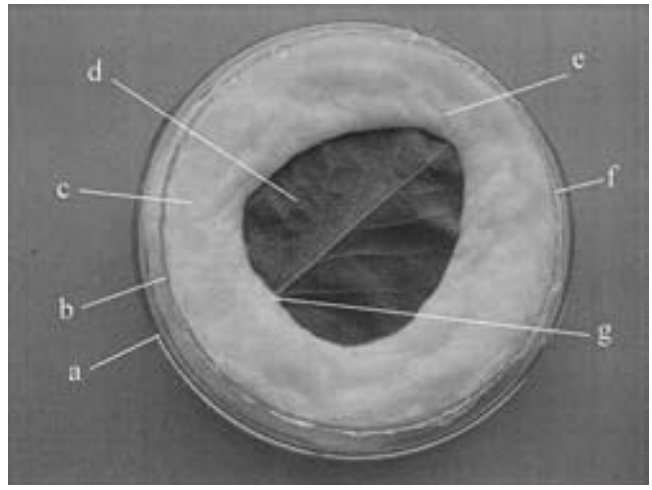

Fig. 1. *E. victoriensis* bioassay: detached leaf test unit (soybean). (a) Glass petri dish (100-mm diameter). (b) Plastic petri dish (90-mm diameter), with a base drilled centrally (10-mm diameter) and 10-mm-diameter cotton dental wick inserted (not visible). (c) Cotton wool lining (d) Upturned leaf embedded in cotton wool (petiole intact). (e) Cotton wool barrier around each leaf (2–4 mm high). (f) Sticky barrier. (g) Raised point at base of petiole to prevent water pooling.

to rear *E. victoriensis* on arenas; egg production declined over three generations. Half the remaining culture was transferred to *Glycine max* (L.) (soybean) per method devised by J.A. Altmann (Biological Services Ltd., Loxton, South Australia) and kept in the same cabinet as arenas. *E. victoriensis* eggs were laid almost exclusively at tips of leaf hairs, a phenomenon noted in other phytoseiids (Sanderson and McMurtry 1984, Sabelis 1985). The culture was moved under growth lights Son-T Agro 400W (Philips Lighting, North Ryde, Australia), 25 ± 1°C, 90 ± 10% RH, a photoperiod of 16:8 (L:D) h, 750–1,050 lux (measured under top-most single layer of leaves), and maintained at a census size >1000 mites.

G. occidentalis and *T. urticae* cultures were set up from mites obtained from Biological Services Ltd. Both were reared on dwarf hairless *Phaseolus vulgaris* L. (French bean). *G. occidentalis* was reared at 25 ± 1°C, 80 ± 10% RH L:D cycle, and lux as per *E. victoriensis* (Croft and Jeppson 1970, Tanigoshi et al. 1975), *T. urticae* at 23 ± 2°C, 60 ± 10% RH, 16:8 (L:D) h (Helle 1962). Bean plants were inoculated every 5–6 d by laying *T. urticae* infested leaves over plants, 4–6 d 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, under growth lights during winter, fertilized weekly, and transferred to mite cultures when first true leaves were fully expanded.

Test Units and Environment. Each test unit replicate (Fig. 1), was constructed from two open petri dishes placed one inside the other. A glass dish (100-mm diameter), and a plastic dish (90-mm diameter) with base drilled centrally to allow insertion of a 5-mm-long by 10-mm-wide cotton dental wick, connecting plastic dish to additional water supply in glass dish. During handling only the unsprayed glass dish was touched, reducing chemical hazard. Both dishes were lined with cotton wool and wetted. The plastic dish held an upturned bean leaf embedded in cotton wool. Fully expanded first true leaves, 10–14 d after plant germination were used. Dark green French bean leaves (≈5.5 cm at the widest point near base, tips removed) were used, as nutritional quality of leaves influences *T. urticae* oviposition (Laing and Huffaker 1969, Samsoe-Petersen 1983). French bean leaves and plastic arenas were tested for suitability to *E. victoriensis*, but few eggs were laid on these substrates. Leaves of both plants were excised with half petiole intact and placed upside down onto wet cotton wool, the petiole immediately embedded in moist cotton for root initiation. Care was taken to raise the leaf at the point of petiole insertion with extra cotton wool to prevent mites aggregated in this domatia from drowning. The leaf edge was tightly packed with cotton wool, creating a 2–4-mm-high barrier around each leaf that extended to the dish perimeter. Cotton wool in both dishes was kept moist by adding tap water via a syringe; 0.5–3 ml was added to the plastic dish twice a day. The leaf/cotton wool junction was not allowed to pool water to prevent mite drowning. The integrity of the barrier was checked and repaired daily at 10× magnification. Because both cotton wool and leaf were sprayed, twice daily live mites that strayed into the cotton wool were returned to the leaf surface rather than designated as escapees. This was necessary as *E. victoriensis* tended to disperse in all treatments after maturity, especially in 2 d after mating. Sticky barrier (Tac-Gel Formula 3, Rentokil Initial Ltd., Chatswood, Australia) was applied via a syringe to the rim of plastic dish 1 d after spraying.

Test units were kept in a controlled temperature cabinet; *G. occidentalis* 25 ± 1°C, 80 ± 10% RH, photoperiod of 16:8 (L:D) h, lux 750–1,050 and *E. victoriensis* in the same conditions, except for increased

Table 1. Fungicide common name, formulation, concentration, and dose used

Common name active constituent (chemical class)	Product formulation (manufacturer)	Product concentration (*G. occidentalis* assay) g/liter	Product concentration (*E. victoriensis* assay) g/liter	Label rate g/100 liter
Boscalid (anilide)	Filan 500 WG*	1.2	0.712	120
Dithianon (quinone)	Delan 700 WG*	1	0.594	100
Kresoxim-methyl (strobilurin)	Stroby 500 WG*	0.1	0.059	10
Mancozeb (ethylene (bis) dithiocarbamate)	Mancozeb 750 WG (Rohm & Haas, Philadelphia, PA)	2	1.187	200
Metiram (ethylene (bis) dithiocarbamate)	Polyram 700 WG*	2	1.187	200

G. occidentalis assay, 4.46 ± 0.06 mg/cm^2; *E. victoriensis* assay, 7.51 ± 0.09 mg/cm^2 wet spray deposit.
* BASF Australia, Baulkham Hills, NSW.

humidity, 90 ± 5% RH. The cabinet was fitted with two fluorescent light tubes (L 36 W/11–860 Lumilux Plus Eco Daylight, Osram, Munich, Germany), with an ultrasonic humidifier KT-100A (Humidaire Ltd., Surrey Hills, Australia) and was externally ducted exchanging air at a rate of one cabinet volume (550 liter) per hour, to prevent pesticide fume accumulation. Excised leaves of both bean species initiated roots and lasted in excess of 14 d without a nutrient solution, even with *T. urticae* feeding. Modifications to counter *E. victoriensis* tendency to disperse were tested. Plastic shelters (Overmeer et al. 1982) did not reduce dispersal, mites did not stay or lay eggs under shelters, >95% eggs were laid on trichomes, and mites preferred domatia along the main leaf vein. Lowering mite density from 22 to 15 failed to reduce dispersal.

Diet and Animal Handling. *T. orientalis* pollen was added to each *E. victoriensis* test unit before 22–25 predator eggs (0–48 h old). For *G. occidentalis*, 25 adult females and five adult males *T. urticae* were added per each test unit and established overnight before adding 22 predator eggs (0–48 h old). Predator eggs hatched within 48 h, by then an abundant food supply of *T. urticae* eggs was available. A further 20–40 live *T. urticae* adult females were brushed onto each test unit every 1–2 d. The females and their progeny provided the required optimal diet of all live stages with an abundance of eggs (Samsoe-Petersen 1983, Sabelis 1985). Prey supply far exceeded daily feeding requirements of *G. occidentalis* adult female at 24°C (Lee and Davis 1968). Predator sex ratio was adjusted for both species at the onset of maturity to ≥1 male: two female (Tanigoshi et al. 1975) to prevent poor fertilization.

Spraying. A Potter spray tower (Burkhard Manufacturing Ltd., Rickmansworth, United Kingdom) was calibrated before each assay to determine the spray volume required to achieve runoff point spray deposit. Runoff point (distilled water) was defined as the first point of droplets merging along leaf veins. The evenness of spray deposit was checked with water sensitive paper. *T. urticae* webbing was removed from test units before spraying to ensure predators received uniform topical exposure (van Zon and Wysoki 1978). Fungicide suspensions of highest registered field concentrations were made with distilled water. No adjuvants were used. All treatments were sprayed at 40-kPa, controls with distilled water. Mancozeb was the toxic reference. Test units were dried for 10–15 min after spraying at ambient temperature. The mean wet spray deposit (distilled water) at runoff point was 4.46 ± 0.062 mg/cm^2 (271 ± 3.8 mg/replicate) on hairless French bean (*G. occidentalis*), and 7.51 ± 0.09 mg/cm^2 (456 ± 5.39 mg/replicate) on hairy soybean (*E. victoriensis*). Thus, the pesticide concentration in *G. occidentalis* assays was lowered for *E. victoriensis* by a factor of 0.594 (271/456) to deliver the same pesticide dose (Table 1). The resulting spray concentrations were very similar to those obtained by an alternative calculation (Bluemel et al. 2000a), based on the IOBC Predicted Initial Environmental Concentration (Barrett et al. 1994). Treatments were replicated four or five times, (three times in repeated tests) and fully randomized after spraying.

Assessment. Juveniles were counted before spray. Mortality was scored 48 h, 4 d, and 7 d after spray; all dead and live mites were counted, and dead mites removed. Mites were considered dead when they failed to move after repeated gentle prodding with a brush. Predator eggs and larvae were counted and removed daily (*E. victoriensis*), every 2 d (*G. occidentalis*), from 3 to 9 d after spraying (3–7 d in one *G. occidentalis* assay), excluding a 24-h preoviposition period. All assessments were made with a binocular at 12–18× magnification under a cold light, alternating between treatments. Experiments were done with two sets of juveniles for each species that differed somewhat in their age. *E. victoriensis* juveniles were 10–58 or 22–70 h old and *G. occidentalis* 32–80 or 41–89 h old. The age groups were treated separately in all analyses and are referred to as comparisons 1 and 2 (Table 2).

Analysis. Cumulative mortality was calculated summing all dead mites (on leaf, in cotton wool, in sticky barrier) and unaccounted escapees at each 48-h, 4-d, and 7-d assessment. Totals were divided by mite numbers present before spraying (Bluemel et al. 2000b). This measure can overestimate mortality because it treats escapees as dead mites. Thus, a second mortality estimate was obtained by summing dead mites (on leaf, in cotton wool, in sticky barrier), and dividing this number by the total number of live and dead mites at each mortality assessment, excluding unaccounted escapees (Bluemel et al. 1993). Live mites found in sticky barrier during the twice-daily checks were ex-

Table 2. Cumulative mean mortality (95% CI), and fecundity (mean ± SE), after juveniles, their initial food, and their entire water supply were sprayed to runoff point on a leaf substrate

Treatment	% Mortality 48 h (95% CI)	% Mortality 4 d (95% CI)	% mortality 7 d (95% CI)	Reproduction (R) per female ± SE for 3–9 d after spraying	% Fecundity reduction
			G. occidentalis—Comparison 1		
Control	5.44^a (0.04, 10.7)	14.8^a (9.67, 19.9)	26.9^a (20.6, 33.3)	19.5 ± 0.65^a	
Dithianon	1.82^a (0.00, 3.53)	6.62^a (3.87, 9.38)	22.0^a (15.4, 28.7)	20.6 ± 0.48^a	0
Metiram	4.47^a (0.82, 8.00)	6.17^a (1.17, 11.0)	76.9^b (66.3, 87.7)	9.19 ± 1.36^b	52.9
Mancozeb	15.6^a (1.26, 29.6)	29.5^b (19.5, 39.4)	82.1^b (79.1, 85.2)	2.16 ± 0.34^c	88.9
			G. occidentalis—Comparison 2**		
Control	1.74^a (0.00, 3.11)	3.74^a (0.06, 7.29)*	18.6^a (8.63, 28.5)	13.6 ± 0.37^a	
Boscalid	2.57^a (0.00, 5.00)	4.65^a (0.08, 9.04)*	9.96^a (1.58, 18.0)	13.7 ± 0.51^a	0
Kresoxim-methyl	1.82^a (0.00, 3.25)	4.73^a (0.04, 9.29)*	12.7^a (2.23, 22.7)	12.4 ± 0.53^a	9.06
Metiram	1.67^a (0.00, 4.87)	13.9^a (12.5, 15.4)*	77.8^b (65.2, 89.1)	8.61 ± 0.67^a	36.8
Mancozeb	8.25^a (0.00, 22.2)	20.7^b (4.94, 39.8)*	93.8^b (91.4, 96.0)	0.95 ± 0.78^c	93.0
			E. victoriensis—Comparison 1		
Control	2.27^a (0.00, 5.15)	5.48^a (0.00, 12.6)	12.6^a (0.48, 26.5)	10.8 ± 0.41^a	
Metiram	9.05^a (3.60, 14.9)	15.2^a (3.51, 28.1)	20.8^a (5.84, 36.8)	0.71 ± 0.23^b	93.4
Mancozeb	82.3^b (74.3, 89.9)	97.7^b (95.0, 99.5)	98.9^b (97.5, 99.7)		
			E. victoriensis—Comparison 2		
Control	3.47^a (0.00, 7.06)	7.16^a (0.00, 11.4)	14.4^a (6.53, 22.8)	13.1 ± 0.30^a	
Boscalid	1.39^a (0.00, 3.07)	11.7^a (0.45, 24.7)	22.0^a (15.0, 29.2)	10.8 ± 1.18^a	17.7
Dithianon	1.19^a (0.00, 2.63)	10.0^a (0.63, 20.6)	14.0^a (1.16, 28.1)	12.4 ± 1.42^a	5.91
Kresoxim-methyl	1.04^a (0.00, 2.30)	6.24^a (4.62, 7.90)	17.5^a (9.78, 25.5)	11.4 ± 0.57^a	13.3
Mancozeb	50.9^b (41.0–76.6)	75.0^b (56.6, 91.4)	92.7^b (81.1, 100)	0.33 ± 0.27^b	97.5

Means are based on three to five replicates.

Means within the same comparison and column, followed by a different letter, are significantly different ($P < 0.001$; * $P < 0.05$; Tukey b test). ** *G. occidentalis*— comparison 2: Reproduction (R) per female ± SE for three to seven d after spraying.

cluded from both estimates. Results from the second estimate are reported as the two approaches led to the same analysis. Mortality proportions were angle (arcsine) transformed. Analyses of variance (ANOVAs) were used to compare treatments, Tukey b post hoc tests (Sokal and Rohlf 1995, SPSS Science 2002) to determine which treatments differed. Means and 95% confidence intervals were backtransformed and tabulated. For fecundity, mean numbers of eggs per female per replicate were calculated after Bluemel et al. (2000b), the formula slightly modified as follows:

$$R_x = [(nE_{3d} + nE_{4d})/nF_{4d}] + [(nE_{5d} + nE_{6d} + nE_{7d})/(nF_{4d} + nF_{7d})/2] + [(nE_{8d} + nE_{9d})/nF_{7d}]$$

where R_x is reproduction per female in a treatment, replicate x; nE_{yd} is eggs and larvae on y day after spraying; and nF_{yd} is live females on y day after spraying. Fecundity means were analyzed by ANOVA and Tukey b post hoc tests. Percentage of fecundity reduction relative to control was calculated as $(1 - R_{treatment}/R_{control}) * 100$.

Results

G. occidentalis. There was a significant difference in 7-d cumulative mortality effects of fungicides on *G. occidentalis* (comparison 1 [32–80 h old], $F = 60.40$; df = 3, 16; $P < 0.001$; comparison 2 [41–89 h old], $F = 32.09$; df = 4, 16; $P < 0.001$). Mortality was highest after exposure to mancozeb and metiram. Post hoc tests indicate that controls differed from mancozeb and metiram but not from dithianon, boscalid, or kresoxim-methyl (Table 2). Sublethal effects followed a similar pattern. Fungicides differed significantly in their effect on female fecundity (comparison 1, $F = 117.43$; df = 3, 16; $P < 0.001$; comparison 2, $F = 79.93$; df = 4, 16; $P < 0.001$). The greatest reduction in fecundity was due to mancozeb, which caused an almost complete cessation of egg lay. Post hoc tests on fecundity indicate that controls differed from mancozeb and metiram, but not from dithianon, boscalid, or kresoxim-methyl and that fecundity in metiram treatments was significantly higher than in mancozeb treatments (Table 2). Toxicity of metiram in the two comparison tests was similar. Toxicity of mancozeb in the two comparison tests was also similar (Table 2), suggesting that minor juvenile age differences did not influence the results. Metiram and mancozeb toxicity was not evident immediately after spraying. In both comparisons, mortalities for metiram and mancozeb were not significantly different from controls or other treatments at 48 h (comparison 1, $F = 1.79$; df = 3, 16; $P = 0.19$; comparison 2, $F = 0.72$, df = 4, 16: $P = 0.59$). At 4 d, mortalities for metiram were not significantly different from controls or other treatments, but mortalities for mancozeb were significantly higher (Table 2), (comparison 1, $F = 11.16$; df = 3, 16; $P < 0.001$; comparison 2, $F = 3.15$; df = 4, 16; $P < 0.05$). Cumulative 7-d mortality in the control treatment of one *G. occidentalis* assay (Table 2) exceeded the 20% maximum, suggested necessary to detect 25% treatment effect at 5% significance (Bluemel et al. 2000b), but

the result obtained was significant at $P < 0.001$. Mortality was <25% in all other *G. occidentalis* and all *E. victoriensis* controls.

E. victoriensis. There was a significant difference in 7-d cumulative mortality of fungicides to *E. victoriensis* (comparison 1 [10–58 h old], $F = 46.80$; df = 2, 9; $P < 0.001$; comparison 2 [22–70 h old], $F = 23.49$; df = 4, 14; $P < 0.001$). In both comparisons, mancozeb exposure caused the highest mortality. Post hoc tests indicate control mortality differed from mancozeb but not from metiram, dithianon, boscalid, or kresoxim-methyl (Table 2). Fungicides also differed in their sublethal effect on fecundity (comparison 1, $F = 447.75$; df = 1, 6; $P < 0.001$; comparison 2, $F = 17.63$; df = 4, 13; $P < 0.001$). Post hoc tests indicate that controls differed from metiram and from mancozeb, but not from dithianon, boscalid, or kresoxim-methyl (Table 2). Mancozeb and metiram substantially reduced fecundity. Fecundity in adult females exposed to mancozeb in comparison 1 was not analyzed as 4-d mortality had reached 97.7%. Unlike for *G. occidentalis*, mancozeb toxicity to *E. victoriensis* was evident early on. Mortality for mancozeb was significantly higher than for controls at 48 h and 4 d after spraying (comparison 1, $F = 97.39$; df = 2, 9; $P < 0.001$; $F = 61.17$; df = 2, 9; $P < 0.001$; comparison 2, $F = 28.80$; df = 4, 14; $P < 0.001$, $F = 14.88$; df = 4, 14; $P < 0.001$). Metiram toxicity to *E. victoriensis* is therefore due to a sublethal effect on fecundity, whereas the effect of mancozeb on *E. victoriensis* is due to both direct mortality and a sublethal effect. Observations on dispersal suggest that both these chemicals may also have a repellant effect.

Discussion

Comparison with Other Methods. A bioassay for *E. victoriensis* on detached soybean leaves was developed to test direct mortality and sublethal effects of pesticides, because the species could not be tested on glass or French bean due to poor oviposition on these substrates. The assay allowed for comparison with *G. occidentalis* assay on detached French bean leaves and was related to testing standards for *T. pyri* on glass and *P. persimilis* on detached leaves (Samsoe-Petersen 1983, Bakker et al. 1992, Bluemel et al. 2000b). We measured mortality due to direct overspray of mites, their initial food supply, and their entire water supply for 7 d, and fecundity from 3 to 9 d after spraying, by using 0–48-h-old juveniles. By comparison, Bluemel et al. (2000b) and Samsoe-Petersen (1983) measured mortality due to 7-d dry residue exposure, and fecundity from 7 to 14 d after spraying, by using 0–24-h-old juveniles. Our method differed by better representing both a commercial field application and the worst-case scenario exposure, 4.46 ± 0.062 mg/cm^2 wet spray deposit (French bean) and 7.51 ± 0.09 mg/cm^2 (soybean) were used, instead of a dry residue equivalent to only 1–2 mg/cm^2 wet spray. The IOBC requirement "to imitate the worst case imaginable" (Samsoe-Petersen 1990) is jeopardized by standardizing via residue on glass. Bluemel et al. (2000a) adjusted the spray concentration per 2 mg/cm^2 spray deposit upwards to reflect the field dose. We standardized for a comparison between two phytoseiid species on different leaf substrates by spraying French bean and soybean leaves to runoff point and delivering the same pesticide dose, to the two substrates at their respective runoff point spray deposits, by altering the concentration (Table 1).

Interpreting Results. Fungicides boscalid, dithianon, and kresoxim-methyl were harmless to *G. occidentalis* and *E. victoriensis.* They are nontoxic substitutes to mancozeb in integrated pest management (IPM) programs. Mancozeb was highly toxic to both *G. occidentalis* and *E. victoriensis*, resulting in severe reduction in their predatory capacity, suggesting incompatibility with IPM programs using these species. Our results are consistent with results reported for dithianon and mancozeb (Walker et al. 1988, Samsoe-Petersen 1990, Tuovinen 1990, Smith and Papacek 1991, Zacharda and Hluchy 1991, Ioriatti et al. 1992, Ingram and Nimmo 1993, Ioriatti et al. 1993, Angeli and Ioriatti 1994, Cross and Berrie 1996, Papaioannou Souliotis et al. 1998, Shaw et al. 1999). We showed that mancozeb is highly toxic to a strain of *G. occidentalis* resistant to organophosphates, suggesting no cross-resistance, even though resistance to mancozeb has recently been reported in some phytoseiids (Angeli and Ioriatti 1994). Metiram was more toxic to *E. victoriensis* than to *G. occidentalis*, and less toxic to both than mancozeb. It can be substituted for mancozeb with less detrimental consequences, but overuse is not recommended. The 7-d mortality of *G. occidentalis* due to metiram approached that of mancozeb, but fecundity of surviving females was significantly greater. *E. victoriensis* fecundity after metiram exposure was near zero, but 7-d mortality was not significantly different from the controls, in contrast to high mortality due to mancozeb already evident at 48 h. Sequential testing of metiram is recommended. The delay in expression of toxic effects of metiram and mancozeb (Table 2) highlights the need for sublethal testing and 7-d mortality assessments when assessing pesticide safety. It concurs with research on dithiocarbamate metabolite, ethylenethiourea, found to suppress ovarian development in house fly, *Musca domestica* L., and in phytoseiids (Mitlin and Baroody 1958, Baynon and Penman 1987).

Predicting Field Effects. Laboratory bioassays do not indicate the exact field effect of pesticides. They predict which pesticides are likely to be safe in the field and provide relative toxicity ratings. Extrapolating laboratory results to the field is the subject of ongoing research and debate (Duso 1994). New European Union proposals for regulatory ecotoxicological risk assessment favor dose-response tests for two sensitive indicator species, and sequential testing triggered by their dose-response toxicity quotients, to predict effects on other beneficials (Candolfi et al. 1999, Campbell et al. 2000, Vogt and Heimbach 2000). However, at present, progressively less toxic scenarios are tested and more beneficials are used (Samsoe-Petersen 1990), because field exposure is assumed less

severe and more variable than laboratory exposure due to e.g., canopy morphology, spray application, recolonization, pesticide degradation.

Yet, high-toxicity results of a single laboratory exposure may be directly indicative of the field effect on phytoseiids, because phytoseiids have no life stage substantially protected from exposure (Oomen 1988, Bakker et al. 1992), and repeated exposure to many pesticides and to mixtures occurs in the field. Research on pesticide mixtures is rare, but additive and synergistic toxic effects have already been reported in phytoseiids (Cross and Berrie 1996, Sengonca and Block 1997, Papaioannou Souliotis et al. 1998). For IPM implementation, sequential testing (field-weathered or laboratory-aged residues, semifield and field tests) to relate medium toxicity laboratory data to the field is thus more pertinent. However, field trials present problems of limited reproducibility, difficult interpretation (Samsoe-Petersen 1990), and less power to detect toxic effects due to inherently greater variability. Field trials often do not report concurrent pest numbers, evaluating number of survivors, but not their capacity to maintain pest control. They may be skewed by recolonization where small test plots are evaluated within an IPM block (Bluemel et al. 2000a), given phytoseiid capacity for some dispersal (Hoy et al. 1985, Dunley and Croft 1990). Field observations from IPM implementation in Australian table grapes indicate that where entire regions repeatedly spray, "a scale factor" occurs. Beneficial recolonization potential becomes minimal for an entire region and the resulting pesticide effect can be far more pronounced and lasting than that obtained in small plot trials within IPM environments (P.A.H., unpublished data), in citrus (Hattingh and Tate 1995, Hattingh 1996).

Selecting Test Species. We selected *E. victoriensis* for testing because of its wide distribution, presence on a wide range of plants (James 1989a,c; Smith and Papacek 1991), and generalist feeding on pollen of wind-pollinated plants (Smith and Papacek 1991, Smith et al. 1997) that allows early season pollen sustained releases and build up when prey density is low, enhancing biological control potential. *E. victoriensis* role in cool climate and subcoastal viticulture remains an open question. There are few studies, but *Typhlodromus* (*Anthoseius*) *doreenae* (Schicha) and *Typhlodromus* (*Anthoseius*) *dosseii* (Schicha) may be abundant (James and Whitney 1993, James et al. 1995). Other testing candidates may be generalist feeders: *Phytoseius fotheringhamiae* Denmark & Schicha, restricted to hairy domatia, but reproducing on *T. urticae* and abundant on apple in New South Wales and native vegetation along the southeastern seaboard, Tasmania, and Western Australia (Schicha 1975, Walter 1992, Walter and Beard 1997); *Euseius elinae* (Schicha), abundant in the coastal belt from western Australia to Queensland (Schicha 1987; Walter 1992; Walter and O'Dowd 1992a,b; Smith et al. 1997); *Typhlodromus helenae* Schicha & Dosse, found on apple in New South Wales, superficially similar to *T. pyri* (Schicha and Dosse 1973); *T. pyri* naturalized in Australia since 1977, controlling European red mite, *Panonychus ulmi* (Koch), in pome fruit (Readshaw et al. 1982); or *Typhlodromips montdorensis* (Schicha) a thrips predator (Llewellyn 2002).

In conclusion, the findings presented here provide a starting point for standardized ecotoxicological testing of phytoseiids in Australia. Testing could be extended to more species, particularly those resistant to the widely used wettable sulfur. Modifications may include testing juveniles of a more narrow age range.

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