**Discovery and characterisation of field resistance to organophosphorus
chemicals in a major mite pest, *Halotydeus destructor***

Paul A. Umina^{1,2*}, Alan Lord³, Svetlana Micic⁴, Owain Edwards⁵

¹ School of BioSciences, Bio21 Institute, The University of Melbourne, Parkville,
Victoria 3010, Australia.

² **cesar**, 293 Royal Parade, Parkville, Victoria 3052, Australia.

³ Department of Agriculture and Food Western Australia, South Perth, WA 6151,
Australia.

⁴ Department of Agriculture and Food Western Australia, Albany, WA 6330,
Australia.

⁵ CSIRO Land & Water, Centre for Environment and Life Sciences, Floreat, WA
6014, Australia.

*Correspondence to: E: pumina@unimelb.edu.au; Ph: +61 3 9349 4723; Fax: +61 3
9349 4723

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Abstract

BACKGROUND: The redlegged earth mite (*Halotydeus destructor*) is an agricultural pest in Australia that attacks a wide variety of crops and pasture species. Chemicals remain an important part of control strategies for *H. destructor*, despite the existence of resistance to pyrethroid insecticides in this species. Recent chemical control failures involving a second insecticide class, organophosphates, were investigated using pesticide bioassays. RESULTS: We confirmed, for the first time, resistance to organophosphates in *H. destructor*, and show that resistance is not confined to a single property, or region. There was no evidence that resistance to organophosphorus chemicals has evolved in Australian States outside of Western Australia. CONCLUSION: These findings demonstrate the strong evolutionary capability of *H. destructor* and highlight the need for ongoing resistance surveillance within Australia.

Keywords

resistance, insecticide, redlegged earth mite, control failure

1. INTRODUCTION

Insecticides are important management tools in many agricultural systems, although their long-term sustainability is questionable. In addition to the harmful ecological impacts, organisms have the ability to evolve resistance to these chemicals. Pest control failures due to resistance reduce quality and yield expectations of farmers, while increasing input costs through ever-higher amounts of sprays required to achieve some level of pest control. Given the current trajectory of pesticide use globally, resistance issues will almost certainly continue to increase¹. Within Australia, insecticide resistance presents substantial economic losses and differing control challenges for several important pests, including aphids,^{2, 3} caterpillars,^{4, 5} flies⁶ and thrips.^{7, 8}

Chemical control difficulties also exist for several mite pests of Australian crops.^{9, 10} One species where this is particularly evident in the grains and pastoral industries is the redlegged earth mite (*Halotydeus destructor* Tucker).¹¹ *Halotydeus destructor* is an intractable pest causing damage to pastures and most crop species in southern Australia. They feed on all stages of plants, but particularly damage seedlings in autumn.¹² *Halotydeus destructor* are typically active from May to October, completing at least three generations a year.¹³ In the hotter, drier months of summer the mites survive as diapause eggs in the cadavers of adult females.¹² Farmers rely heavily on chemicals to control *H. destructor*, either as insecticide seed dressings or foliar spray applications.¹¹

In 2006, chemical control failures were reported from a population of *H. destructor* in Western Australia, and later confirmed as the first documented case of insecticide resistance in this species.¹⁴ Very high levels of resistance to two pyrethroid chemicals, bifenthrin and alpha-cypermethrin were discovered.¹⁴ Since the initial discovery, extensive surveillance programs have found pyrethroid resistance has spread considerably within the state of Western Australia, but not to other Australian states.¹⁵ This field surveillance conducted between 2007-2010, identified 15 individual properties in Western Australia, ranging almost 500 km, with resistance. Umina *et al.*¹⁵ showed that resistance in *H. destructor* was found across the entire pyrethroid group, but not to other chemical classes, including organophosphates, carbamates, phenylpyrazoles, avermectins and diafenthiuron.

In winter 2014, we received a field report of chemical control failures involving *H. destructor*. A pasture-seed paddock near Capel (Western Australia) was sprayed with several applications of organophosphates (and pyrethroid chemicals) prior to sowing and again during the seedling establishment phase, but failed to achieve adequate control of mites. Each spray was applied at a dose considerably higher than the registered field rate for *H. destructor*.

In this paper we establish toxicity baselines for *H. destructor* populations from different regions of Australia and use this to develop reliable discriminating doses for

use in the evaluation of field resistance to organophosphates. The response of *H. destructor* collected from Capel and elsewhere in Australia to organophosphates was then investigated using pesticide bioassays to understand the distribution of resistance. We also investigated the presence of resistance to pyrethroids in mites that were found to have evolved organophosphate resistance.

2. EXPERIMENTAL METHODS

2.1. Baseline sensitivity of *H. destructor* to an organophosphate chemical

Pesticide laboratory bioassays were undertaken to assess the baseline sensitivity of *H. destructor* populations to organophosphates and to calculate a discriminating dose to screen for resistance shifts. We collected three *H. destructor* populations from geographically distinct regions in Australia that are known to be susceptible to insecticides: Diamond Creek, Victoria (37°40'33" S, 145°07'45" E); South Perth, Western Australia (31°59'27" S, 115°53'14" E); and Albany, Western Australia (34°44'20" S, 117°39'36" E). Mites from each of these populations were tested against eight concentrations of omethoate (Le Mat® 290EC, Bayer Crop Science, Hawthorn, Victoria, Australia), ranging from 1×10^{-6} to 1×10^{-1} times the recommended field rate, and a control of water. The recommended field rate is 100 mL formulation diluted in 100 L of water (290 mg litre⁻¹).

The method employed was a glass vial technique used by numerous authors to examine the chemical responses of earth mites.^{10, 16} For each concentration tested, approximately 30 mL of solution was poured into a 40 mL glass vial and swirled to ensure a complete coating, with all excess liquid removed and vials left to dry overnight. Between 15-20 vials were used per concentration. A single adult mite was then placed into each vial along with a leaf of common vetch (*Vicia sativa*), which provides food and increases humidity. Vials were then sealed with Parafilm and placed at 18°C. After 8 h, and again at 24 h, mites were scored as alive (moving

freely), incapacitated (inhibited movement) or dead (no movement over a 5 s period). Incapacitated mites were pooled with dead individuals for analysis as they invariably die and therefore do not contribute to the next generation.¹⁶

For each population and each scoring period, LC₅₀, LC₉₀ and LC₉₉ values (along with 95% confidence intervals, CIs) were estimated following a logit analysis as outlined by Robertson and Preisler¹⁷ using SPSS (version 22.0). χ^2 tests indicated adequate fits in all cases.

2.2. Response of *H. destructor* from Capel to an organophosphate

In spring 2014, *H. destructor* were collected from a pasture-seed paddock located south-east of Capel, Western Australia. This paddock (herein referred to as ‘Capel1’) had a history of pesticide applications and experienced chemical control failures in winter 2014. Mites were collected by suction and stored at 4°C before testing their response to pesticides. Mites were also collected from Diamond Creek, and used as a control population. This site was used previously to generate baseline sensitivity data and has no known history of pesticide application for the previous 10 years. Mites from each population were tested for their response to omethoate (Le Mat® 290EC) using the pesticide bioassay approach outlined above. Ten concentrations were tested, ranging from 1×10^{-5} to 10 times the field rate, along with a control of water. 20 vials per concentration were coated in this manner and mites scored after 8 h.

Concentration–mortality curves were generated from the bioassay results. LC_{50} and LC_{90} values (and 95% CIs), along with regression coefficients were estimated following logit analyses. χ^2 tests indicated an adequate fit of the data in each case.

2.3. Response of *H. destructor* from Capel to other chemicals

To understand the nature of resistance among multiple organophosphates, three chemicals were tested against populations of *H. destructor* collected from neighboring paddocks to the original Capel population (herein referred to as ‘Capel2’ and ‘Capel3’). A third population (collected near to Capel) that had no known resistance issues, or recent history of pesticide application, was used as a control. We tested each of these populations against omethoate (Le Mat® 290EC), and two other organophosphates, chlorpyrifos 500 g litre⁻¹ (Surefire Fortune 500, PCT Rural, Acacia Ridge, Queensland, Australia) and malathion 500 g litre⁻¹ (Maldison 500, NuFarm, Laverton, Victoria, Australia). The recommended label rate of Surefire Fortune 500 is 140 mL diluted in 100 L of water (700 mg litre⁻¹). The recommended label rate of Maldison is 100 mL diluted in 100 L of water (500 mg litre⁻¹). For all three chemicals, we tested 11 concentrations ranging from 1×10^{-5} to 10 times the recommended field rate, and a control of water. The same methodology was employed as previously outlined, except 8 vials were used per concentration with 5 mites placed into each vial (i.e. 40 mites tested per concentration), plastic vials were used, and a surfactant (0.03% v/v Triton) was included in all treatments. LC_{50} values (along with 95% CIs), and regression coefficients, were estimated for each bioassay.

Q2 tests indicated adequate fits in all cases. In cases where resistance was detected, regression coefficients were compared using the GT-2 method, following Sokal and Rohlf.¹⁸

In addition to screening organophosphates, we investigated the response of *H. destructor* populations to synthetic pyrethroids. This was performed through a bioassay against bifenthrin (Talstar 100EC®; CropCare, Muarrie, Queensland, Australia) using mites from Capell. The recommended label rate of Talstar 100EC® is 100 mL diluted in 100 L of water (100 mg litre⁻¹). We tested 10 concentrations ranging from 1 x 10⁻⁵ to 100 times the recommended field rate, and a control of water. Mites from Diamond Creek were used as the control population. Six concentrations were tested, ranging from 1 x 10⁻⁵ to 1 times the recommended field rate, and a control of water. For both populations 15-20 vials were treated per chemical concentration (one mite added per vial) and mites scored after 8 h.

2.4. Screening field populations of *H. destructor* for resistance

During the 2015 winter growing season, *H. destructor* samples were collected from across Australia, with 75 populations from Western Australia and 13 populations from other States where *H. destructor* are known to occur (3 paddocks in South Australia, 7 in Victoria and 3 in New South Wales) (Supplementary Table 1). Samples were taken from paddocks with a relatively high pesticide load and intensive cropping history, and in the case of Western Australia, collections were from

paddocks with reported chemical control failures involving *H. destructor*. Mites were collected from each of these paddocks via suction and transported back to the laboratory. Mites were screened for resistance to organophosphates using a discriminating dose of omethoate (see below) and a control of water. Mite mortality was assessed after 24 h and if any mites were found surviving, the population was deemed as having insecticide resistance. A control population (either South Perth or Diamond Creek, depending on where the bioassays were conducted) was included as a comparison in all bioassays.

3. RESULTS

3.1. Baseline sensitivity of *H. destructor* to omethoate

Concentration-mortality lines for *H. destructor* from Diamond Creek, South Perth and Albany to omethoate were comparable to one another (Fig. 1), and analogous to earlier published work on this species.¹⁴ LC₅₀, LC₉₀ and LC₉₉ values were estimated and further show the three populations responded similarly after 8 h and 24 h exposure (Table 1). The Albany population consistently had the lowest LC₅₀, LC₉₀ and LC₉₉ estimates, but in all cases 95% confidence intervals overlapped with the other populations, except for the LC₅₀ value after 24 h exposure. Here, the LC₅₀ estimate was slightly lower than the corresponding LC₅₀ value for the Diamond Creek population. Mites from the Albany population were collected from the field a day earlier than mites from South Perth and Diamond Creek, and also spent considerably more time in transit prior to testing.

Using logit models on this baseline data, a discriminating dose for future screening of field resistance to omethoate was estimated. We averaged the LC₉₉ values for all 3 populations at 24 h exposure, and decided on a final dose that was slightly higher (0.87 mg litre⁻¹). This gave confidence that only those mites with resistance to omethoate would survive exposure at this dose.

3.2. Resistance in *H. destructor*

Concentration-mortality curves and LC_{50} values indicate a significant difference between the Capel1 and Diamond Creek populations (Fig. 2; Table 2). The Capel1 population had an LC_{50} estimate that was almost 30 times greater than the Diamond Creek population, demonstrating resistance to this chemical. Regression slopes also differed significantly between the two populations (Table 2).

Additionally, the responses of *H. destructor* from two populations collected the subsequent year on the same Capel property (Capel2 and Capel3) were tested against three organophosphorous chemicals. LC_{50} values were estimated from concentration-mortality curves for each chemical (Table 3). Resistance in Capel2 and Capel3 populations was found to omethoate, chlorpyrifos and malathion. The levels of resistance varied somewhat between Capel2 and Capel3 mites, particularly for omethoate. Capel2 showed a resistance ratio of 13, while Capel3 had a resistance ratio of 41. The regression slope for Capel3 was also significantly shallower than Capel2 (using the GT2-method). For chlorpyrifos, Capel2 had a slightly higher level of resistance and a significantly shallower regression slope than Capel3. For malathion, Capel2 and Capel3 showed high levels of resistance (resistance ratios of 415 and 387, respectively) and there were no differences in regression slopes between either populations, or with the control population (Table 3).

Concentration-mortality curves for bifenthrin indicated a significant difference between Capel1 and Diamond Creek populations (Fig. 2). The LC_{50} estimates were

31.77 mg litre⁻¹ (95% CIs 4.40, 128.26) for Capell and 0.007 mg litre⁻¹ (95% CIs 0.0003, 0.014) for the Diamond Creek population, showing a high level of resistance to pyrethroids. Regression slopes did not differ significantly between these two populations.

3.3. Distribution of organophosphate resistance

Halotydeus destructor were sampled from 75 paddocks in Western Australia and screened for resistance to omethoate. A single discriminating dose was tested for each population and compared with mites from a susceptible control population. In all cases, the mortality at the discriminating dose in the control populations was 100%. Sixteen populations showed evidence of resistance, with survivorship at the discriminating dose (Supplementary Table 1). Paddocks containing resistant mites were found across a reasonably wide geographical range in Western Australia (spanning more than 400km), with resistance most prevalent in the great southern and south-west regions (Fig. 3).

In other parts of Australia, *H. destructor* were screened from 13 paddocks spanning three States (South Australia, Victoria and New South Wales) and more than 1,500 km. There were no mites that survived the discriminating dose, indicating resistance to omethoate was not present in these populations (Supplementary Table 1).

4. DISCUSSION & CONCLUSIONS

In 1997, Hoffmann *et al.*¹⁹ predicted *H. destructor* would evolve resistance to insecticides. This followed a series of chemical bioassays on mites collected from various paddocks with different histories of insecticide exposure. The authors provided some evidence of tolerance to omethoate in two field populations and suggested there was a heritable basis for this tolerance.¹⁹ Almost twenty years later, our work is the first demonstrated case of resistance to organophosphates in *H. destructor*. The development of this resistance is likely to be the direct consequence of previous paddock histories; multiple applications of insecticides were applied over an extended period of time at many of the paddocks identified with resistance. In the case of all three Capel populations, both organophosphate and pyrethroid chemicals were repeatedly applied. Although not all spray applications were targeted at *H. destructor*, the associated selection pressure for resistance development would have been experienced at these sites whenever mites were active at the time of spraying for other pests. This work is yet another example illustrating the evolutionary capacity of a genetically variable species when strong selection pressure is persistently applied.

We show that resistance in *H. destructor* exists across multiple organophosphorus chemicals, including omethoate, chlorpyrifos and malathion. Varying levels of resistance to structurally different compounds may indicate that a metabolic resistance mechanism is involved, although the role of target-site resistance in other mites is common²⁰²⁰ and cannot be discounted. Higher levels of resistance to malathion may

indicate the involvement of carboxylesterases, as the two carboxylic ester bonds in this compound make it much more sensitive to carboxylesterase activity²¹. Further bioassays (including synergist assays) and genetic investigations are needed to determine the mechanism of resistance and explore cross-resistance to other chemical classes, such as carbamates.

This work has shown some *H. destructor* populations possess dual insecticide resistances; to both organophosphate and pyrethroid chemicals. Additionally, we have identified some field populations that only have organophosphate resistance, while mites in other areas only have pyrethroid resistance (P. Umina, unpubl. data).

Although we did not specifically test for evidence of cross-resistance (when resistance to one insecticide confers resistance to another insecticide regardless of exposure to the latter chemical), our work indicates organophosphate resistance has evolved independently to pyrethroid resistance. A recent restriction site-associated DNA sequencing (RAD-Seq) analysis of *H. destructor* populations has indicated the strong likelihood of multiple independent evolution events conferring resistance to pyrethroids in mites within Western Australia (G. Racic and A. Hoffmann, pers. comm.), further demonstrating the evolutionary potential of this species. From a control perspective, the presence of mite populations with dual insecticide resistances is particularly concerning for farmers. *Halotydeus destructor* is one of the most economically damaging and widely distributed pests in broadacre farming systems in Australia¹¹ and there are approximately 200 insecticide products registered to control

this species in Australia.²² However, these chemicals only represent three unique Insecticide Resistance Action Committee (IRAC) Mode of Action groups – organophosphates, pyrethroids and neonicotinoids. Furthermore, neonicotinoids are only registered as seed treatments (they cannot legally be applied as foliar sprays). There are no chemical options available for those paddocks where mites possess resistance to both organophosphates and pyrethroids, and the crop has already been planted.

Organophosphate use precedes the introduction of pyrethroid chemicals by ~30 years,¹ yet in the case of *H. destructor*, resistance appears to have first evolved to the pyrethroids. This could reflect the differing biochemical mechanisms that confer resistance to these different chemical classes, the historical patterns of chemical applications targeting mites and/or the fitness costs of resistance.^{1, 23} Somewhat surprisingly, our investigation of *H. destructor* field populations in 2015 found organophosphate resistance is already distributed over several hundred kilometres and across multiple properties in Western Australia. Given the relatively low-levels of resistance that were identified in these populations, it is probable organophosphate resistance has been present for some time prior to 2014, but has gone undetected. Similar to the known distribution of pyrethroid resistance, it appears that resistance to organophosphates may be currently confined to Western Australia.¹⁵ This could point towards differences in the management practices among growers in Western Australia and other parts of Australia. Because gene flow occurs across Australia,²⁴ it is

possible that resistance alleles will spread throughout the range of *H. destructor*, including into eastern Australia. Coupled with pyrethroid resistance, this would result in significant financial, labor and yield losses.

This work demonstrates the importance of resistance surveillance of *H. destructor* populations, and has generated baseline sensitivity data and discriminating doses for omethoate whereby resistance testing can be undertaken at a relatively low cost. Sensitivity to pesticides in combination with the mechanistic basis and fitness cost(s) of resistance, have been identified as crucial factors modulating the evolution and spread of insecticide resistance in *H. destructor*.²⁵ Such data can be used in simulation studies to develop management strategies tailored to unique chemicals, regions and crops, which aim to reduce the likelihood of resistance evolving. Very recently, the Australian grains industry has commissioned the development of regionally specific resistance management strategies for *H. destructor* in Australian crops and pastures.²⁶ These strategies provide recommendations for the judicious use (and rotation) of insecticides, while combining non-chemical options such as targeted weed control, host plant resistance and crop rotations. The detection of organophosphate resistance in multiple localities highlights the importance that farmers implement these strategies.

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Figure 3. Distribution of *H. destructor* populations collected in 2015 from Western Australia with omethoate resistance.

Table 1. LC₅₀, LC₉₀ and LC₉₉ values (and 95% confidence intervals) and regression coefficients for *H. destructor* populations, computed from logit models for responses to omethoate.

Exposure	Population	LC ₅₀ (95% CIs) mg litre ⁻¹	LC ₉₀ (95% CIs) mg litre ⁻¹	LC ₉₉ (95% CIs) mg litre ⁻¹	Regression coefficient (<i>b</i>) ± se
8 h	Diamond Creek	0.378 (0.269–0.532)	1.012 (0.684–2.169)	2.965 (1.557–12.262)	5.137 (± 1.044)
	South Perth	0.332 (0.215–0.504)	1.460 (0.881–3.517)	7.339 (3.150–38.326)	3.419 (± 0.599)
	Albany	0.220 (0.147–0.325)	0.748 (0.474–1.717)	2.844 (1.342–13.394)	4.132 (± 0.779)
24 h	Diamond Creek	0.038 (0.020–0.062)	0.201 (0.117–0.541)	1.232 (0.478–9.812)	3.044 (± 0.618)
	South Perth	0.034 (0.015–0.056)	0.154 (0.088–0.485)	0.820 (0.312–9.863)	3.308 (± 0.791)
	Albany	0.008 (0.004–0.016)	0.038 (0.019–0.127)	0.192 (0.070–2.021)	3.379 (± 0.760)

Table 2. LC₅₀ and LC₉₀ values (and 95% confidence intervals), and regression coefficients for *H. destructor* populations from Diamond Creek and Capell, computed from logit models for responses to omethoate after 8 h exposure.

Population	LC₅₀ (95% CIs) mg litre⁻¹	LC₉₀ (95% CIs) mg litre⁻¹	Regression coefficient (b) ± se	Resistance ratio
Diamond Creek	0.226 (0.122–0.361)	1.126 (0.666–2.814)	3.154 (± 0.612)	-
Capell	6.531 (2.022–17.37)	225.05 (66.50–2631.5)	1.429 (± 0.205)	28.9

Table 3. LC₅₀ values (and 95% confidence intervals) and regression coefficients for *H. destructor* populations, computed from logit models for responses to omethoate, chlorpyrifos and malathion after 8 h exposure.

Pesticide	Population	No. mites tested	LC₅₀ (95% CIs) mg litre⁻¹	Regression coefficient (b) ± se	Resistance ratio
Omethoate	Control	481	1.58 (1.21-2.06)	3.96 ± 0.52	-
	Capel2	479	21.05 (15.7-28.91)	2.71 ± 0.32	13.3
	Capel3	478	64.29 (24.92-178.5)	1.57 ± 0.16	40.7
Chlorpyrifos	Control	482	2.75 (1.58-4.74)	1.43 ± 0.21	-
	Capel2	481	97.64 (61.72-153.5)	1.43 ± 0.15	35.5
	Capel3	481	69.43 (28.99-161.6)	2.28 ± 0.24	25.2
Malathion	Control	473	16.38 (4.16-48.98)	2.07 ± 0.23	-
	Capel2	438	6789 (2717-39095)	1.26 ± 0.25	415
	Capel3	438	6334 (2958-33509)	1.56 ± 0.39	387

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Author/s:

Umina, PA;Lord, A;Micic, S;Edwards, O

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