Warmer temperatures reduce chemical tolerance in the redlegged earth mite (Halotydeus destructor), an invasive winter-active pest

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Abstract

BACKGROUND: Quantifying how chemical tolerance of pest arthropods varies with temperature is important for understanding the outcomes of chemical control, for measuring and monitoring resistance, and for predicting how pesticide resistance will evolve under future climate change. We studied the redlegged earth mite, Halotydeus destructor (Tucker), a winter-active invasive agricultural pest in Australia. Using a replicated block experiment, we tested the effect of different thermal conditions on the expression of chemical tolerance to a pyrethroid and two organophosphates. Our chemical bioassays were conducted on two redlegged earth mite populations: one possessed organophosphate resistance, whilst the other was susceptible to pesticides. Mites were first acclimated at cool (4 °C) and warm (14 °C) conditions and then exposed to pesticides in both cool (11 °C) and warm (18 °C) test conditions.

RESULTS: Warm test conditions generally reduced chemical tolerance to all pesticides relative to cool test conditions. Median lethal dose (LD50) values of mites tested under cool conditions were 1.12–3.57-fold greater than of mites tested under warm conditions. Acclimation had a variable and small impact on chemical responses. Thermal factors (ratio between test temperatures) were similar between populations for each active ingredient. Despite reduced chemical tolerances under warm test conditions for individual mite populations, resistance factors (ratio between resistant and susceptible mite populations) were relatively consistent.

CONCLUSION: Our data provides context for prior theoretical work demonstrating climatically constrained pesticide resistances in Australian redlegged earth mites. Estimates of temperature dependent toxicity measured in this study may be useful in parameterizing models of redlegged earth mite control under an increasingly warm and more variable climate.

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Keywords: temperature dependence; organophosphates; pesticide resistance; pyrethroids; redlegged earth mites; Australia

1 INTRODUCTION

The Metabolic Theory of Ecology has argued for the importance of thermodynamic principles on metabolic rate, asserting that there are constraints imposed by temperature on all physiological and ecological processes. 1,2 In support of this, the expression of diverse functional traits is strongly dependent on temperature across different taxonomic groups and ecosystems. 3,4 Organismal fitness can therefore be tightly tied to temperature, particularly for ectotherms that are reliant on ambient conditions. 5,6 Moreover, temperature can reshape underlying additive genetic variation for fitness traits, such that the response to selection to other environmental conditions might depend on their interaction with temperature. 7–9

Chemical stress is one such environmental condition where temperature can significantly influence the expression of relevant functional traits and organismal fitness. At the level of an individual organism, chemical tolerance is a threshold trait, 10 in that the phenotype is binary: dead versus alive at a given dose. However, at the population level, chemical tolerance is continuously distributed because individuals vary in their maximally tolerable dose. 11 The population median chemical tolerance, measured as the median lethal dose (LD50), 12 can shift under different conditions.
environmental temperatures. Such shifts may be contingent on several factors, including: (1) the thermal physiology of individuals within the population; (2) the toxicokinetics of a chemical, including its rate of absorption, metabolism, and interaction with an organism’s physiology, at a given temperature; and (3) trade-offs between traits that promote thermal tolerance versus those that promote chemical tolerance.

Understanding thermal variation in chemical stress responses is relevant to the outcomes of pest control strategies. Chemical control is a major management tool for agricultural and disease-bearing arthropod pests, which may lead to the evolution of pesticide resistance, an important fitness trait in pest populations. Temperature-dependent pesticide toxicity not only affects the success of chemical control under different conditions, but also affects inferences of the magnitude of resistance in pest populations. Additionally, it is possible that climate change will affect the efficacy of chemical management and the evolution of pesticide resistance.

Thermal variation in pesticide tolerance has been demonstrated in a diverse range of arthropod species, including Coleoptera,14,26 Diptera,21,27,28 Ephemeroptera,17 Hemiptera,29 and Lepidoptera.30,31 Determining how chemical tolerance varies with temperature for different pesticide compounds is important for devising efficacious control strategies and for forecasting shifts in chemical tolerance under climate change.

Our study focuses on the redlegged earth mite, Halotydeus destructor (Tucker), a major pest of Australian grain crops and pastures. Redlegged earth mites are winter-active pests. During the Australian summer, eggs persist in a state of diapause with larvae emerging in autumn (April–May) when temperatures drop below critical thresholds and ambient moisture increases.32–34 In their active phase (April–November), redlegged earth mites undergo three generations before entering a summer diapause (December–March) as temperatures increase and humidity decreases.35 Redlegged earth mites were introduced into Australia from South Africa in the 1920s and chemical control with pyrethroid and organophosphate pesticides has been important in their management for many decades.37–38 Chemical tolerance in redlegged earth mites was first reported in the late 1990s and early 2000s,39,40 with pesticide resistance first detected in field populations in 2006.41 Since then, pyrethroid resistance has become relatively common in southern Australia, and organophosphate resistance, although locally constrained, has evolved and is increasing in frequency.37,42–44

Since invading Australia, redlegged earth mites have undergone an adaptive shift to hotter and drier conditions, relative to populations in their native South African range.45,46 Adult Australian redlegged earth mites have critical minimum temperature thresholds (CT_min) of ~30 to 40 °C.46 But across their Australian distribution,46 redlegged earth mites typically experience average field temperatures ≤ 15 °C during the Southern Hemisphere winter months of June to August.47 Recent predictive modelling has indicated that the distribution of pyrethroid resistance in Australia is associated with environmental temperature.48 There are few studies investigating the thermal physiology of redlegged earth mites,32,49,50 and to our knowledge, there are no studies that have examined how chemical tolerance of this species varies with temperature. This information is important when establishing methodologies to characterize chemical tolerance phenotypes and assessing the magnitude of pesticide resistance.

The purpose of this study was to understand how temperature affects the tolerance of redlegged earth mites to pesticides commonly used in their control. We performed a four-way factorial experiment involving two acclimation temperatures (4 °C and 14 °C), two test temperatures (11 °C and 18 °C), two mite populations (one to organophosphates and one susceptible to pesticide), and three chemical active ingredients (one pyrethroid and two organophosphates). We had two main aims. Firstly, we aimed to test whether temperature in chemical bioassays affects chemical tolerance. We predicted that mites exposed to pesticide doses at warmer temperatures would express lower chemical tolerance than those exposed to cooler temperatures, because warmer temperatures are more stressful. Secondly, we aimed to test the effects of acclimation temperatures prior to chemical bioassays on the level of chemical tolerance. We predicted that mites acclimated at temperatures different to the test temperatures would exhibit greater mortality. We expected such an effect of acclimation to be due to thermal shock associated with transitioning across a larger temperature differential from holding to testing conditions.

2 MATERIALS AND METHODS

2.1 Terminology

We use ‘chemical tolerance’ here for a population’s mean phenotype; that is, the LD50 estimate for a population to a specific active ingredient. We use ‘resistance factor’ (RF) in the canonical sense as being the ratio of a focal resistant population’s LD50 value relative to that of a susceptible population’s LD50 value.51,52 The RF is therefore a measure of the magnitude of difference in chemical responses between populations. Additionally, we use ‘thermal factor’ (TF) to describe the ratio of LD50 values between different test temperatures for a single population, which other studies have reported as the ‘temperature coefficient’.13,15,27,30

2.2 Field populations and collections

Two populations of redlegged earth mites were used in this study and were sourced near the township of Colbinabbin in rural Victoria, Australia. The resistant population, herein referred to as ‘OP-Res’, came from a farm where the first case of organophosphate resistance was documented in Victoria.37 The paddock where OP-Res was collected is irrigated and contains a mixture of subclover (Trifolium subterranaeum) and pasture grasses (predominantly annual rye grass, Lolium multiforme). The paddock has experienced ~15 years of chemical control, involving rotations and (or) combinations of the organophosphates, omethoate and chlorpyrifos, and the pyrethroid, alpha-cypermethrin. The susceptible population, herein referred to as ‘OP-Sus’, came from roadside vegetation containing a mixture of grasses, capeweed (Arctotheca calendula), medic (Medicago sp.), and clover (Trifolium sp.). This site was situated ~5 km from the OP-Res site and has previously been shown to contain mites that are susceptible to organophosphate and pyrethroid pesticides (J. Thia and X. Cheng, unpublished data). Winter temperatures (June–August) in the Colbinabbin region have a mean maximum of ~14 °C, a mean minimum of ~3 °C, and a daily mean of ~11 °C.

Our study involved three collections between June and August 2021. Our collections followed a spray event in early June 2021 that used a mixture of chlorpyrifos and alpha-cypermethrin. Mites were collected via suction using a blower vacuum with a fine gauze mesh placed over the vacuum tube. Mites were placed into plastic containers with paper towel and vegetation and transported back to the laboratory in cooler boxes with ice packs.
2.3 Experimental design

We performed a fully crossed four-way factorial experiment, measuring the responses of the two mite populations under different test and acclimation temperatures for three active ingredients. We replicated our experiment across three blocks, each constituting a field collection. These blocks were undertaken on 17 June, 24 June, and 12 August 2021. These days were chosen to ensure all collections occurred during the winter months when red-legged earth mites are at their peak abundance and to minimize seasonal variation.

Mites collected from the field were returned to the laboratory and stored at 4 °C (cool) or 14 °C (warm) for 42 to 50 h prior to testing. The cool 4 °C acclimation treatment was chosen to reflect standard practices, where mites are held at low temperatures to prolong their lifespan in the laboratory. The warm 14 °C acclimation treatment was chosen as a midpoint temperature between our two test temperatures (see later) and approximates the mean maximum temperature experienced by red-legged earth mites in Colbinabbin during winter. On the day of bioassays, adult mites from each acclimation treatment were placed into pesticide-coated vials (see later) and incubated at 11 °C (cool) or 18 °C (warm). The warm 18 °C test temperature is the standard that has been used for many years for red-legged earth mite chemical phenotyping. Our cool 11 °C test treatment was chosen to approximate the winter mean daily temperature at our study site. After 24 h exposure, mites were scored as alive or dead. Surviving mites were able to move freely when disturbed with a paintbrush. Dead mites were those that were unresponsive for 5 s when disturbed with a paintbrush, or that exhibited inhibited movement (incapacitated). For each pesticide concentration, we aimed for three replicate vials per block and ten mites per vial. Mites were at low abundance during some of our field collections, which limited the number of mites we could use for bioassays (see Results section). Notwithstanding, each dose was represented by ≥ 3 replicate vials, with an average of 5.1 mites, in our final analysed dataset. Sample sizes are shown in Supporting Information Table S1.

The coated vial method used in this study was similar to that used elsewhere. Pesticide dilutions were made using distilled water with 0.01% polysorbate 80 (Tween™), which acted as a surfactant. For each pesticide concentration tested, approximately 20 mL of solution was poured into a 30 mL plastic vial and swirled to ensure a complete coating, with excess liquid removed. Vials were then left to dry upside down overnight. We prepared serial dilutions of each pesticide as proportions relative to the recommended field rate: 10×, 1×, 0.01×, 0.005×, 0.001×, 1× to 4×, 1× to 5×, and 1× to 6×. Distilled water with 0.01% polysorbate was used as the control. Our active ingredients included one pyrethroid, bifenthrin, and two organophosphates, chlorpyrifos and omethoate. The field rates for these active ingredients are as follows: 100 mg L⁻¹ for bifenthrin (Astral® 250EC, Nufarm™), 700 mg L⁻¹ for chlorpyrifos (Lorsban® 500EC, Corteva Agriscience™), and 290 mg L⁻¹ for omethoate (Le-Mat®, Arysta LifeScience™). On the day of mite introduction, a moist wedge of filter paper (circular 50 mm diameter filter papers cut into wedges of ~20 mm²) was added to each vial to provide mites with moisture.

2.4 Statistical analyses

We performed three sets of analyses, all of which were executed in R version 4.1.2. Firstly, we examined background mortality in our controls among experimental blocks and temperature treatments. Secondly, we modelled dose–response relationships and partitioned the variance with respect to our temperature treatments within populations for each active ingredient. These dose–response models allowed us to determine how temperature treatments affected the expressed chemical tolerance in the pesticide-exposed mites. Thirdly, we performed post hoc tests of LD₅₀ values to assess how temperature might affect measures of chemical tolerance and pesticide resistance.

2.4.1 Control mortality

We tested whether red-legged earth mite populations varied in their mortality under different thermal conditions, and in different blocks, in our water controls. This analysis allowed us to assess levels of background mortality and account for this background mortality in downstream analyses of dose–response relationships. For each population, we pooled control mites across active ingredients and fitted the binomial generalized linear model (GLM):

\[ \text{logit}(P) = \text{BLOCK} + \text{ACCLIM} + \text{TEST} + \text{BLOCK.ACCLIM} + \text{BLOCK.TEST} + \text{ACCLIM.TEST} + \text{BLOCK.ACCLIM.TEST} \]

Here, \( P \) is the proportion of dead mites per vial. \( \text{BLOCK} \) is a categorical predictor for the experimental block, \( \text{ACCLIM} \) is a categorical predictor for cool or warm acclimation temperature, and \( \text{TEST} \) is a categorical predictor for cool or warm test temperature. Observations were weighted by the number of mites per vial, that is, vials with more mites made a greater contribution to the model fit. Control mortality models were fitted using R’s glm function from the ‘stats’ package and significance of model terms was assessed with Type II sums of squares using the ‘car’ package.

Our analysis of control mortality within each population revealed significant differences among experimental blocks and temperature treatments. There was one block for each mite population where control mortality was anomalously high. For the OP-Res population, this was Block2, whereas for OP-Sus population, this was Block3. Herein, we make the distinction between ‘acceptable’ and ‘anomalous’ blocks to refer those blocks with mean mortality < 10% and > 10%, respectively. The anomalous experimental blocks coincided with field collections where adult abundances were low but nymph and juvenile abundances were high. We suspect that adults in these collections were at later life stages, contributing to higher control mortality. Because of this high mortality, and because sample sizes were low, anomalous blocks were excluded from downstream analysis. Therefore, we proceeded with two acceptable blocks for each population: Block1 and Block3 for the OP-Res population, and Block1 and Block2 for the OP-Sus population.

We used our model of control mortality to correct for background mortality of the pesticide-exposed mites in each population’s acceptable blocks. We used Abbott’s correction:

\[ Y_{\text{Abbott}} = (Y_{\text{Exposed}} - Y_{\text{Control}}) / (1 - Y_{\text{Control}}) \]

Here, \( Y_{\text{Abbott}} \) is the corrected mortality for mites in a single vial, \( Y_{\text{Exposed}} \) is the observed mortality of mites in a single vial exposed to pesticide and, \( Y_{\text{Control}} \) is the estimated control mortality for a block–acclimation–test treatment combination.

2.4.2 Variance partitioning of temperature effects

Mites from acceptable blocks were used to quantify how different thermal conditions affected dose–response relationships. We fitted binomial GLMs for each population, for each active ingredient, containing a full three-way interaction among all our predictors:

\[ \text{logit}(P) = \text{ACCLIM} + \text{TEST} + \text{DOSE} + \text{ACCLIM.TEST} + \text{ACCLIM.DOSE} + \text{TEST.DOSE} + \text{ACCLIM.TEST.DOSE} + \text{BLOCK.VIAL} \]
Here, \( P \) is the Abbott’s-corrected mortality proportion for each vial. ACCLIM and TEST are categorical predictors, modelling the fixed effects of acclimation and test temperature, respectively, and DOSE is a continuous predictor (the log\(_{10}\)-transformed mg L\(^{-1}\) of active ingredient), modelled as a fixed effect. To account for the nesting of replicate vials in blocks, we fitted a random effect structure of VIAL nested in BLOCK, except where this led to an overfit model, where we instead fitted just the effect of BLOCK. Observations were weighted by the number of mites per vial. The models were fit with the GLMER function from the ‘lme4’ package. We performed model selection to see if reduced models provided better, more parsimonious fits to our data. These reduced GLMs included a two-way interaction model (no three-way interaction), a main effects model (no interactions), and a null model (no temperature effects). Model comparisons were made with R’s ANOVA function using likelihood-ratio tests, and performed sequentially, from most complex to most simple, stopping when the more complex model provided a better fit. Prior to fitting dose–response relationships, mortality data was truncated to only retain doses within the accelerating region of mortality necessary for \( LD_{50} \) estimation, and to remove concentrations in the uninformative regions of the curve.

### 2.4.3 \( LD_{50} \) values, thermal factors, and resistance factors

To simplify estimation of \( LD_{50} \) values, we fitted separate binomial GLMs for each acclimation–test temperature combination. Modified code from the ‘MASS’ package’s DOSE, P function was used to calculate \( LD_{50} \) values and their standard errors using the intercept and slope coefficients estimated from these GLMs. Post hoc contrasts were used to further examine differences in test temperatures (within populations) and between populations (within test temperatures). Differences between \( LD_{50} \) values observed under cool and warm test temperatures were performed for each population, active ingredient, and acclimation temperature. Differences between \( LD_{50} \) values between the OP-Res and OP-Sus populations were considered for each acclimation and test temperature. We tested the null hypothesis that the difference in \( LD_{50} \) values was 0 using Z-tests. We calculated TFs and RFs as summary statistics of the magnitude of differences between test temperatures and populations, respectively. TFs were calculated as described elsewhere. As per convention, TFs are the ratio of the test temperature with the higher \( LD_{50} \) relative to the test temperature with the lower \( LD_{50} \). When cool tested > warm tested, the TF is assigned a positive symbol (and vice versa), so that the sign of a TF indicates whether chemical tolerance is increasing (+TF) or decreasing (−TF) down a temperature gradient. RFs were calculated as the ratio of the OP-Res \( LD_{50} \) value over the OP-Sus \( LD_{50} \) value.

### 3 RESULTS

#### 3.1 Summary of sample sizes

In total, we phenotyped 13 602 mites (Table S1). After removing mites from anomalous blocks and concentrations unnecessary for fitting the dose–response curve, we were left with 5766 mites with an average of 9.89 per vial. For the OP-Res population, dose–response relationships were estimated with 1478 mites from Block1 and 1652 from Block3. For the OP-Sus population, 1196 mites from Block1 and 1440 mites from Block2 were used to estimate dose–response relationships.

#### 3.2 Mortality of control mites

We fitted a GLM of control mortality to understand how levels of background mortality varied among redlegged earth mite populations, blocks, acclimation temperatures, and test temperatures. For the OP-Res population, the effects of block (\( \chi^2_2 = 47.94, P < 0.001 \)), test temperature (\( \chi^2_1 = 20.47, P < 0.001 \)), and a block–test temperature interaction (\( \chi^2_2 = 6.56, P = 0.038 \)) were significant. For the OP-Sus population, the effects of block (\( \chi^2_2 = 31.72, P < 0.001 \)), test temperature (\( \chi^2_1 = 10.63, P = 0.001 \)), and an acclimation–test temperature interaction were significant (\( \chi^2_2 = 7.06, P = 0.008 \)). Full GLM model effects are reported in Table S2.

The observed mean control mortality for the OP-Res population was 5.4%, 18.9%, and 3.9%, and for the OP-Sus population was 2.1%, 1.7%, and 11.2%, for Block1, Block2, and Block3, respectively. Moreover, the mean control mortality for the OP-Res and OP-Sus population was 5.2% and 2.5%, respectively, under the cool test temperatures, and 13.4% and 7.5%, respectively, under warm test temperatures. The tendency for warmer temperatures to elevate the control mortality (Supporting Information Fig. S1) suggests these thermal conditions were more stressful (mortality related to thermal stress) or promoted more rapid senescence (natural mortality via elevated metabolic rate), or both. Because we were interested in contrasting different temperature treatments for their effect on pesticide dose–response relationships, our model of control mortality was used to correct the mortality of pesticide-exposed mites.

#### 3.3 Temperature effects on chemical tolerance

GLMs of dose–response relationships including temperature effects explained significantly more variation in mortality relative to GLMs excluding temperature effects (Table S3). We found support for our first hypothesis that warmer test temperatures reduce chemical tolerances of redlegged earth mites (Fig. 1; Tables 1, 2 and S4). In contrast to our second hypothesis that cool acclimation would reduce chemical tolerance, there was some evidence that warm acclimation might reduce chemical tolerance (Fig. 1; Tables 1, 2 and S4). However, the effect of acclimation temperature was weak and inconsistent (Table 1). For the response of the OP-Res population to bifenthrin (Fig. S2), the main effects model provided the best fit (Table 1). The main effect of test temperature was significant (\( P = 0.003 \); Table 1) and \( LD_{50} \) values were consistently higher in the cool test condition (Fig. 1(a); Table 2). Relative to warm test conditions, cool tested mites exhibited 1.72-fold and 2.48-fold greater tolerance to bifenthrin (as measured by TFs) under cool and warm acclimation conditions, respectively (Table 3). For the OP-Sus population (Fig. S2), the two-way interaction model provided the best fit (Table 1). The test–dose interaction was significant (\( P = 0.002 \); Table 1), but not the main effect of test temperature (\( P = 0.064 \); Table 1). Acclimation temperature had a marginally significant effect (\( P = 0.014 \); Table 1). \( LD_{50} \) values were slightly higher under cool test conditions (Fig. 1(a); Table 2). Relative to warm test conditions, mites in cool test conditions exhibited TFs of 1.17-fold and 1.23-fold greater bifenthrin tolerance under cool and warm acclimation conditions, respectively (Table 3).

For the response of the OP-Res population to chlorpyrifos (Fig. S3), the main effects model provided the best fit (Table 1). The main effect of test temperature was only marginally significant (\( P = 0.026 \); Table 1), but \( LD_{50} \) values were greater in the cool test treatment (Fig. 1(b); Table 2). Relative to warm test conditions, cool tested mites exhibited 2.98-fold and 3.05-fold greater
tolerance to chlorpyrifos (as measured by TFs) under cool and warm acclimation conditions, respectively (Table 3). For the OP-Sus population (Fig. S3), the two-way interaction model provided the best fit (Table 1). The main effect of test temperature was significant ($P < 0.001$; Table 1) and there was a significant acclimation–dose interaction ($P = 0.002$; Table 1). LD$_{50}$ values were higher under cool test conditions (Fig. 1(b); Table 2). Relative to the warm test conditions, cool tested mites exhibited TFs of 3.57-fold and 2.93-fold greater chlorpyrifos tolerance under cool and warm acclimation conditions, respectively (Table 3).

For the OP-Res population tested against omethoate (Fig. S4), the three-way interactive model provided the best fit (Table 1). The main effect of test temperature was significant ($P = 0.007$; Table 1) and the main effect of acclimation temperature was marginally significant ($P = 0.025$; Table 1). There was also a marginally significant acclimation–test–dose interaction ($P = 0.042$; Table 1). LD$_{50}$ values were greater under cool test temperatures (Fig. 1(c); Table 2). Relative to warm test conditions, mites tested at cool temperatures exhibited 1.40-fold and 2.93-fold greater tolerance to omethoate (as measured by TFs) under cool and warm acclimation conditions, respectively (Table 3). For the OP-Sus population (Fig. S4), the main effects model provided the best fit (Table 1). There was a significant main effect of test temperature ($P = 0.007$; Table 1) and LD$_{50}$ values were greater under cool test temperatures (Fig. 1(c); Table 2). Relative to warm test temperatures, cool tested mites exhibited TFs of 1.12-fold and 2.60-fold greater omethoate tolerance after cool and warm acclimation, respectively (Table 3).

To assess how characterization of resistance might be dependent on temperature, we calculated RFs between the OP-Res and OP-Sus population (Table 4). The OP-Res population was not resistant to pyrethroids, with RFs relative to the OP-Sus population ranging from 0.82-fold to 1.65-fold for bifenthrin. For chlorpyrifos, the OP-Res population had RFs between 139.11-fold and 307.19-fold relative to the OP-Sus population, depending on the acclimation ranging from 0.82-fold to 1.65-fold for bifenthrin. For chlorpyrifos, the OP-Res population had RFs between 139.11-fold and 307.19-fold relative to the OP-Sus population, depending on the acclimation and test conditions. For omethoate, RFs for the OP-Res population tested against omethoate (Fig. S4), the main effects model provided the best fit (Table 1). The main effect of test temperature was significant ($P = 0.007$; Table 1) and LD$_{50}$ values were greater under cool test temperatures (Fig. 1(c); Table 2). Relative to warm test temperatures, cool tested mites exhibited TFs of 1.12-fold and 2.60-fold greater omethoate tolerance after cool and warm acclimation, respectively (Table 3).

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Because many arthropod pest species are con-
In our study, both organophos-
and for pyrethroids to be more toxic
However, there
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Res population were lower, ranging from 5.07-fold to 9.68-fold. Collectively, RFs calculated between populations of redlegged earth mites did not appear to be influenced by temperature.

### 4 DISCUSSION

Temperature not only affects fitness directly from thermal stress but also indirectly by modulating the expression of other functional traits. Because many arthropod pest species are controlled with pesticides, chemical tolerance is an important fitness trait. In our study, we examined how chemical tolerance to different pesticides varied with temperature in the redlegged earth mite, an important agricultural pest in Australia. We found general support for greater chemical tolerance at cool (11 °C) versus warm (18 °C) test temperatures. The effect of acclimation temperature prior to pesticide exposure was weaker and less consistent, although in some cases, warm accli-

### Table 2. Dose–response summary statistics

| Population | Active ingredient | Acclimation treatment | Test treatment | Intercept (SE) | Slope (SE) | LD50 (95% CI) | χ² (DF) | p* |
|------------|-------------------|----------------------|---------------|---------------|------------|--------------|--------|----|
| Bifenthrin | OP-Res            | Acclim cool          | Test cool     | 2.08 ±0.97    | 4.48 ±0.85  | 0.34 (0.14–0.82) | 2.31 (3) | 0.510 |
|            |                   |                      | Test warm     | 3.08 ±0.42    | 4.4 ±0.55   | 0.2 (0.16–0.25)  | 2.21 (2) | 0.331 |
|            |                   | Acclim warm          | Test cool     | 1.59 ±0.44    | 3.76 ±0.55  | 0.38 (0.24–0.66) | 4.85 (4) | 0.303 |
|            |                   |                      | Test warm     | 2.1 ±0.31     | 2.56 ±0.36  | 0.15 (0.01–0.22) | 9.25 (3) | 0.026 |
| OP-Sus     | Acclim cool       | Test cool            |               | 3.11 ±0.91    | 5.67 ±1.08  | 0.28 (0.16–0.51) | 1.25 (3) | 0.742 |
|            |                   |                      | Test warm     | 5.23 ±1.16    | 8.47 ±1.81  | 0.24 (0.18–0.33) | 0.93 (2) | 0.628 |
|            | Acclim warm       | Test cool            |               | 4.91 ±1.23    | 7.66 ±1.82  | 0.23 (0.16–0.33) | 1.81 (3) | 0.613 |
| Chlorpyrifos| OP-Res            | Acclim cool          | Test cool     | −2.09 ±0.45   | 1.18 ±0.19   | 59.85 (17.88–200.41) | 3.38 (3) | 0.336 |
|            |                   |                      | Test warm     | −1.53 ±0.22   | 1.18 ±0.13   | 20.05 (10.71–37.57) | 3.54 (3) | 0.315 |
|            |                   | Acclim warm          | Test cool     | −2.22 ±0.44   | 1.2 ±0.16    | 70.74 (20.01–250.09) | 1.23 (3) | 0.745 |
|            |                   |                      | Test warm     | −1.4 ±0.21    | 1.02 ±0.11   | 23.21 (11.85–45.47) | 3.81 (3) | 0.283 |
| Chlorpyrifos| OP-Sus            | Acclim cool          | Test cool     | 1.89 ±1.95    | 4.46 ±1.35   | 0.38 (0.06–2.35)  | 71.8 (3) | <0.001 * |
|            |                   |                      | Test warm     | 3.28 ±2.34    | 3.35 ±0.81   | 0.11 (0.01–2.11)  | 52.03 (3) | 0.001 * |
|            |                   | Acclim warm          | Test cool     | 1.98 ±1.92    | 5.72 ±0.98   | 0.45 (0.1–1.96)   | 3.09 (3) | 0.379 |
|            |                   |                      | Test warm     | 2.8 ±1.29     | 3.61 ±0.67   | 0.17 (0.04–0.74)  | 4.85 (3) | 0.183 |
| Chlorpyrifos| OP-Res            | Acclim cool          | Test cool     | −1.53 ±0.25   | 2.65 ±0.44   | 3.79 (2.47–5.81)  | 0.13 (3) | 0.988 |
|            |                   |                      | Test warm     | −0.86 ±0.59   | 2 ±0.34      | 2.7 (0.74–9.83)   | 3.06 (4) | 0.548 |
|            |                   | Acclim warm          | Test cool     | −0.92 ±0.21   | 2.04 ±0.31   | 2.83 (1.79–4.94)  | 1.32 (3) | 0.726 |
|            |                   |                      | Test warm     | 0.04 ±0.32    | 2.88 ±0.47   | 0.97 (0.58–1.61)  | 0.12 (3) | 0.990 |
| Chlorpyrifos| OP-Sus            | Acclim cool          | Test cool     | 1.85 ±0.81    | 4.55 ±0.83   | 0.39 (0.19–0.81)  | 1.88 (2) | 0.391 |
|            |                   |                      | Test warm     | 1.62 ±0.33    | 3.53 ±0.51   | 0.35 (0.25–0.48)  | 0.57 (2) | 0.753 |
|            |                   | Acclim warm          | Test cool     | 1.41 ±0.32    | 4.64 ±0.64   | 0.5 (0.39–0.63)   | 0.36 (2) | 0.834 |
|            |                   |                      | Test warm     | 4.68 ±1.69    | 6.5 ±1.06    | 0.19 (0.07–0.54)  | 1.68 (2) | 0.431 |

* Log₁₀ (mg L⁻¹).b In mg L⁻¹. c Goodness-of-fit test statistic and degrees of freedom. d Goodness-of-fit P-value, with significance at α = 0.05 marked with an asterisk.

### Table 3. Post hoc tests for differences in median lethal dose (LD₅₀)

| Active ingredient | Population | Acclimation treatment | Z | P * | TF b |
|-------------------|------------|----------------------|---|-----|-----|
| Bifenthrin        | OP-Res     | Acclim cool          | 1.17 | 0.121 | 1.72 |
|                   |            | Acclim warm          | 2.95 | 0.002 * | 2.48 |
|                   | OP-Sus     | Acclim cool          | 0.47 | 0.321 | 1.17 |
|                   |            | Acclim warm          | 0.92 | 0.178 | 1.23 |
| Chlorpyrifos      | OP-Res     | Acclim cool          | 1.57 | 0.058 ~ 2.98 |
|                   |            | Acclim warm          | 1.53 | 0.063 ~ 3.05 |
|                   | OP-Sus     | Acclim cool          | 0.71 | 0.239 | 3.57 |
|                   |            | Acclim warm          | 0.93 | 0.176 | 2.70 |
| Omethoate         | OP-Res     | Acclim cool          | 0.49 | 0.313 | 1.40 |
|                   |            | Acclim warm          | 3.09 | 0.001 * | 2.93 |
|                   | OP-Sus     | Acclim cool          | 0.29 | 0.387 | 1.12 |
|                   |            | Acclim warm          | 1.75 | 0.040 ~ 2.60 |

* Significance following a Bonferroni correction for 12 independent tests is marked with an asterisk, that is, α < 0.004 (< 0.05/12). Signifi-
cance at α < 0.05 is marked with a tilde.

bThermal factors were calculated as the ratio of larger LD₅₀ to the smaller LD₅₀ estimates for the cool and warm test treatment. The posi-
tive sign (+) indicates a larger LD₅₀ in the cool test treatment relative to the warm test treatment.
mites to tolerate temperatures > 30 °C and evidence of thermal adaptation since their introduction to Australia, this species is adapted to cool winter conditions. Increasing temperatures might therefore exacerbate the effects of chemical stress on redlegged earth mites. It is possible that greater absorption and translocation rates of chemicals might also contribute to increasing toxicity under warmer conditions.

Our experimental work on thermal variation in chemical tolerances provides new context for previously demonstrated climatic constraints of pesticide resistance in redlegged earth mites. Assuming our laboratory observations are relevant to dynamics in the field, pesticides might be more toxic to redlegged earth mites in warmer climatic regions, which could impose greater selection for chemical resistance by increasing the fitness differential relative to cool conditions. But two mechanisms might also work against greater selection pressure under warm conditions. Initially, there may be direct trade-offs between chemical resistance and other fitness traits. Such trade-offs are often the major considerations when studying the constraints of resistance evolution and its spread. However, the underlying genetic variance of traits can be transformed by temperature. We are not aware of any studies that directly consider changes in the heritability of pesticide resistance at different temperatures. But decreasing heritability with increasing temperature would limit the evolution and spread of resistance under warmer conditions.

Our study, like others examining thermal variation in chemical tolerance, was limited to a few key temperatures due to the logistics of measuring dose–response at many temperatures. Experiments spanning much wider temperature gradients provide a more complete picture of the physiological landscape of chemical tolerance, but are challenging for redlegged earth mites due to difficulties in culturing this species. Additionally, due to the scale of our experiments, we only included two mite populations in our studies. Assaying more populations across a climatic gradient would provide further insight into how thermal variation in chemical tolerance covaries with environment. Our study included an organophosphate resistant and susceptible population. These populations exhibited similar TFs for chlorpyrifos and omethoate tolerance, suggesting that the organophosphate resistance may not impact the temperature dependence of organophosphate toxicity in redlegged earth mites. However, we did not consider a pyrethroid resistant population for parallel comparison. Whilst the mechanism of organophosphate resistance in redlegged earth mites is currently undescribed, pyrethroid resistance is largely monogenic and attributed to a kdr (knockdown) target-site mutation in the para gene. Such kdr mutations are known to affect thermal tolerance in other arthropods and can impact the temperature dependence of pyrethroid toxicity.

Previous studies have shown that acclimation conditions prior to pesticide exposure can have negligible, positive, or negative effects on the expression of chemical tolerance. Such variation depends on how a pest’s underlying physiology of thermal and chemical tolerance responds to acclimation and subsequent performance under a given test condition. Expression of thermal tolerance is affected by prior acclimation conditions in redlegged earth mites. Therefore, we initially hypothesized that acclimation at a cool temperature (4 °C) far from the test temperatures would reduce chemical tolerance due to a negative heat shock response, relative to acclimation at a warm temperature (14 °C) that was roughly equidistant from the test temperatures. In contrast, our data provided some support for a negative effect of warm acclimation. Based on our GLM results, warm acclimated increased toxicity in the OP-Sus population exposed to bifenthrin, and the OP-Res population exposed to omethoate. There was also some evidence of an interaction between acclimation temperature and test temperature in mites exposed to omethoate, suggesting the effect of warm testing might be exacerbated following warm acclimation. However, overall, observed acclimation effects were weak and not as consistent as those observed for test temperature.

One important caveat of our study is that despite consistent significant effects of test temperature detected in our GLMs, pairwise comparisons between cool tested LD50 and warm tested LD50 values were not all statistically significant. These pairwise tests on point estimates of the LD50 were underpowered as effect sizes are smaller and (or) variances around estimates are larger in these comparisons. We note that our GLMs had greater power to detect the global effect of temperature treatment on chemical tolerances and these represent our primary test of hypotheses for temperature dependent toxicity. Indeed, the greater mortality we observed under warm test conditions for both populations results in the significant effect of temperature detected in our GLMs.

### Table 4. Post hoc tests for differences in median lethal dose (LD50) values between the OP-Res and OP-Sus populations

| Active ingredient | Acclimation treatment | Test treatment | Z     | P a  | RF b |
|-------------------|----------------------|---------------|-------|------|------|
| Bifenthrin        | Acclim cool          | Test cool     | 0.35  | 0.363| 1.21 |
|                   |                      | Test warm     | −0.97 | 0.834| 0.82 |
|                   | Acclim warm          | Test cool     | 1.64  | 0.051| 1.65 |
|                   |                      | Test warm     | −0.91 | 0.819| 0.82 |
| Chlorpyrifos      | Acclim cool          | Test cool     | 4.53  | <0.001*| 158.99 |
|                   |                      | Test warm     | 3.36  | <0.001*| 190.21 |
|                   | Acclim warm          | Test cool     | 5.11  | <0.001*| 156.92 |
|                   |                      | Test warm     | 5.92  | <0.001*| 139.11 |
| Omethoate         | Acclim cool          | Test cool     | 5.24  | <0.001*| 9.68  |
|                   |                      | Test warm     | 3.01  | 0.001*| 7.75  |
|                   | Acclim warm          | Test cool     | 6.58  | <0.001*| 5.71  |
|                   |                      | Test warm     | 2.74  | 0.003*| 5.07  |

aSignificance following a Bonferroni correction for 12 independent tests is marked with an asterisk, that is, α < 0.004 (<0.05/12).
bResistance factors were calculated as the ratio of the resistant OP-Res population relative to the susceptible OP-Sus population.
exposed to all active ingredients, suggests a general negative effect of warming temperatures on chemical tolerance in red-legged earth mites.

5 CONCLUSION

Our work has practical implications for resistance monitoring programmes involving red-legged earth mites. Standard conditions for chemical sensitivity bioassays for red-legged earth mites have typically used a test temperature of 18 °C and holding conditions of 4 °C prior to testing. A test temperature of 18 °C is expected to be higher than average conditions experienced by red-legged earth mites over the winter months. At our study site, Colbinabbin, the mean winter daily temperature is 11 °C. Our work suggests that storage of mites at 4 °C prior to testing may not impose a heat shock that might reduce chemical tolerance. However, standard 18 °C testing temperatures may provide deflated estimates of chemical tolerance phenotypes for mite populations, although this will not necessarily impact the estimated RFs. In addition to informing bioassay methodologies, our results may also be useful for parameterizing models of chemical control under increasingly warming and more variable climates.

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CONFLICT OF INTEREST

The authors declare no conflict of interest associated with the work presented in this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in FigShare at https://figshare.com/articles/dataset/DATASET_Thia_2022_Pest_Man_Sci_Halotydeus_destructor_thermal_variation_in_chemical_tolerance_to_pesticide/19127210.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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