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Learnings from over a decade of increasing pesticide resistance in the redlegged earth mite, *Halotydeus destructor* Tucker

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Running title: Pesticide resistance in the redlegged earth mite

Abstract

Background: The redlegged earth mite, *Halotydeus destructor* Tucker*,* is a destructive and economically important pest of winter grain crops and pastures in Australia. It is largely controlled by pesticides, but this mite has evolved resistance to pyrethroid and organophosphate chemicals. A national Resistance Management Strategy has been developed for pro-active management to delay further resistance evolution, though its success is reliant on a detailed understanding of the incidence, patterns of spread, current distribution and the nature of resistance in the field. Here, we report on a long-term resistance surveillance program undertaken between 2006 and 2019 informed by resistance risk forecasting.

Results: By mapping the Australian distribution of resistance through time, we show that resistance is present across three Australian states and covers more than 3000 km. This current range includes a recently identified population exhibiting organophosphate resistance representing the most easterly location of resistance in *H. destructor*. Using field history information, we identify associations for the first time between crop management practices employed by farmers and the presence of pyrethroid resistance. Management strategies that could minimise the risk of further resistance include limiting local spread of resistance through farm hygiene practices, crop rotations and reducing pesticide usage.

Conclusion: This study highlights the challenges of resistance in *H. destructor* but also indicates how quantitative resistance risk analysis can be developed to target field surveillance and delay further resistance. The management strategies highlighted in this study can help maintain the effectiveness of control options but will depend on farmer engagement and adoption.

Keywords: resistance surveillance, pesticide, mite, pest, model

1. Introduction

The redlegged earth mite, *Halotydeus destructor* Tucker*,* is one of the most destructive and economically important pests of winter grain crops and pastures in Australia. This pest is most damaging at the establishment phase of crops in autumn, where feeding can result in retarded plant growth and/or seedling death.[1,2] It is highly polyphagous, attacking a wide range of plants such as field crops, vegetables, pastures, flowers and broadleaf weeds.[1,3] *H. destructor* is found throughout areas of southern Australia with a Mediterranean-type climate and is active from April to November where it typically undergoes three to four generations.[4,5] The mites survive the hot dry summer months as diapausing eggs in the cadavers of adult females. Mites hatch the following autumn in response to cooling temperatures and adequate rainfall, often coinciding with the emergence of pasture and crop seedlings.[3,5,6]

Control of *H. destructor* is heavily reliant on the application of pesticides, through foliar sprays and seed treatments.[1] Of the five registered chemical modes of action for *H. destructor* control,[7] three (pyrethroids, organophosphates and neonicotinoids) are frequently used by Australian farmers.[8] These pesticides are often applied prophylactically as they are perceived as a cost effective means of protecting small gross margins and as a safeguard against infestations of this pest.[1,9] However, this creates significant selection pressure for mites to evolve resistance. This, along with the high densities of *H. destructor* populations,[1] has led to pesticide resistance issues. Populations of *H. destructor* that are resistant to organophosphates and pyrethroids are now present across Western Australia (WA) and parts of eastern Australia.[10–13]

Pesticide resistance to pyrethroids in *H. destructor* was first detected in WA in 2006.[13] High levels of resistance were found for two commonly used pesticides, bifenthrin and alpha-cypermethrin, and later extended to the entire pyrethroid group.[12] Resistance to a second chemical group, the organophosphates, was first discovered in 2014, once again in WA. Several field populations of *H. destructor* exhibit resistance to both pyrethroids and organophosphates.[10] Resistance to both chemical groups is now common across large areas of WA[10,12] and has recently been discovered in South Australia (SA) in a population in the Upper South East district, the first resistance detection in *H. destructor* outside of WA.[11]

Resistance ratios in *H. destruc*tor populations are much higher for pyrethroids (~200,000 times) than for organophosphates (4-415 times).[10–13] This is likely due to the underlying mechanisms conferring resistance. In *H. destructor* pyrethroid resistance is linked to a single-nucleotide polymorphism (*kdr*) in the voltage-gated *para* sodium channel[14] and is incompletely recessive.[15] Mutations within the sodium channel gene are linked to pyrethroid resistance in a large number of insects and mites.[16–18] The sodium channel contains four homologous domains (I-IV), each having six segments (S1-S6),[16] with genetic mutations contributing to cross-resistance to pyrethroids and DDT known as knockdown resistance (*kdr*).[19] In *H. destructor*, a novel amino acid change (L1024F) is associated with pyrethroid resistance, whereby phenylalanine (F) is substituted with leucine (L) in the II6 region[14]. While this mutation has not been linked previously to pyrethroid resistance, a different amino acid change at the same locus (L1024V) has been associated with pyrethroid resistance in the two spotted mite, *Tetranychus urticae* Koch.[16,18,20,21] Different amino acid changes at the same locus conferring resistance are not uncommon, occurring in different species and occasionally in the same species.[16] The mechanism(s) underlying organophosphate resistance in *H. destructor* remains unclear, although mutations within the acetylcholinesterase (AChE) gene seem to be involved (Weeks. A, 2020, pers. comm.). Organophosphates target AChE and insensitivity within this gene appears to be the main mechanism conferring organophosphate resistance in a number of insects and Acari.[18,22] Most insects and ticks have at least two AchE genes (*Ace* 1 and *Ace* 2), with point mutations in both genes found to be associated with organophosphate resistance.[17]

Umina *et al*.[12] predicted that resistance would spread to SA before other eastern Australian states given the closer proximity of SA to known resistant WA populations. However, a recent study by Yang *et al*.[23] found no indication of relatedness between resistant mites from a resistant mite population in SA to those from WA, suggesting that SA populations may have evolved resistance independently in response to local selection pressures. Indeed, Maino *et al.*[24] identified SA as being at high risk on the basis of chemical usage, as well as several climatic based variables linked to evolutionary processes of resistance in *H. destructor*. Furthermore, multiple independent evolutionary events appear responsible for pyrethroid resistance in a number of *H. destructor* populations within WA, although there is also evidence for long distance movement of pyrethroid resistance mutations between WA populations.[23] Early studies investigating the genetic structure of Australian *H. destructor* populations with microsatellite DNA markers suggested high levels of ongoing gene flow among Australian populations.[25,26] However, recent high resolution SNP based data shows a more complex pattern,[23] with *H. destructor* populations from eastern Australia having more similarity to each other and being distinct from WA populations. In SA, a complex gene flow pattern exists between WA and SA, with some SA populations being genomically similar to WA populations, but also mixing with those from other parts of eastern Australia.

Pro-active management will be necessary to delay the spread and evolution of further resistance in this species. An initial Resistance Management Strategy has been developed for *H. destructor* in Australian pastures and grain crops.[27] To build on this strategy, an understanding of the incidence, patterns of spread, current distribution and nature of resistance is needed. Since the first discovery of pyrethroid resistance in *H. destructo*r in 2006,[13] ongoing surveillance efforts across large portions of the known Australian distribution of *H. destructor* have been undertaken.[11,12] Here, we continue to monitor the distribution and levels of pesticide resistance over the last decade (2011 – 2019) and combine this with earlier surveillance data[10–13] to map the current distribution of resistance across southern Australia. Through this compiled dataset, we investigate associations between crop management practices employed by farmers and the presence of field resistance. Specifically, we investigate whether pyrethroid resistance is associated with pesticide usage, crop rotations, and proximity to other known resistant populations.

2. Materials and Methods

2.1 Mite collections

Resistance screening of *H. destructor* was undertaken in the winter growing seasons between 2011 and 2019 across Australia (Victoria, New South Wales, SA and WA). Targeted collections of *H. destructor* were undertaken from fields with reported control failures involving *H. destructor* and/or fields with a history of high pesticide and intensive cropping usage. Some indiscriminate collections were also taken from fields and roadside vegetation. Between 2017 and 2019, the majority of our collections focussed on geographical regions where recently-developed *H. destructor* models predicted resistance evolution to be highest.[24]

Mites were collected via suction using a Stihl SH55 blower vacuum with a fine gauze mesh placed over the end of the vacuum tube. Samples were placed in plastic containers with paper towelling and vegetation, and then transported back to the laboratory. Containers were stored in a fridge at 4°C until *H. destructor* were required for testing (up to 7 days). Between 2011-2019, more than 850 mite populations were collected from the field and screened for pesticide resistance using phenotypic laboratory bioassays and/or genetic markers targeting the known resistance mechanism to pyrethroids (see below). Field information was sought in all cases. Information collected included the location, grower details, crop details (plant type and growth stage) and pesticide usage (chemicals, rates and timing of application) in the year mites were sampled. We also collected detailed information on the cropping history (plant type) and pesticide usage from the previous six years.

2.2 Phenotypic laboratory bioassays to assess resistance

Mite populations were screened for resistance against pyrethroid and organophosphate pesticides via laboratory bioassays, following the methodology described by Hoffmann *et al*.[28] For all bioassays undertaken, a known pesticide-susceptible *H. destructor* population was included as a control and tested alongside the mite populations being screened for resistance across all doses tested (described below). These control populations were collected from field sites with no known history of pesticide applications within the last 5 years.

To screen for the presence of resistance to pyrethroids, two discriminating doses (1 mg L^{-1} and 100 mg L^{-1}) of bifenthrin 100 g L^{-1} (Talstar 100EC®, FMC Australasia Pty Ltd, North Ryde, NSW, Australia) were tested, along with a water control. If mite numbers were insufficient to allow both discriminating doses to be tested, the dose of 100 mg L^{-1} was used. This dose has previously been used as a reliable discriminating dose for pyrethroid resistance in *H. destructor.*[12] Populations were classed as resistant if there were survivors at 100 mg L^{-1} and no survivors in the susceptible control population tested simultaneously.

To screen for the presence of resistance to organophosphates, omethoate 290 g L^{-1} (Le Mat®, Cheminova, North Ryde, NSW, Australia) was tested along with a water control. Between 2011 and 2013, the omethoate doses tested ranged from 0.1 to 580 mg L^{-1}. No differences between any mite populations and the control population were observed at any of these doses. Following the discovery of organophosphate resistance in 2014, mite populations were initially tested against a single discriminating dose of 1.8 mg L^{-1}.[10] In some bioassays, only a single (or a few) individual(s) survived, making it difficult to confidently determine the resistance status of mites. In these cases, mites were re-collected and tested against a broad range of omethoate doses ranging from 0.0000087 mg L^{-1} to 290 mg L^{-1}. From 2015 onwards, two discriminating omethoate doses of 0.87 mg L^{-1} (which is approximately the LC_{99} value) and 5.8 mg L^{-1} (which was chosen because no known susceptible mites have survived this dose across multiple bioassays) were tested.

Populations were classed as resistant if there were survivors at 5.8 mg L^{-1} and no survivors in the susceptible control population tested simultaneously.

For each chemical and dose tested, the pesticide solution was poured into a plastic or glass vial (15 or 18 mL) and swirled to ensure complete coating, with excess liquid discarded. Between four and eight vials per dose were coated in this manner and left to dry upside down on a drying rack overnight. The control vials were treated in the same manner, except water was used. When plastic vials were used, the surfactant Tween at 0.01% (v/v) (Ecoteric 20®; Thermo Fisher Scientific; Scoresby, Vic, Australia) was added to the pesticide solution and water control to aid the spread of the pesticide and ensure even coating. Approximately eight *H. destructor* adults were placed into each vial, along with a leaf of common vetch (*Vicia sativa* L.), which provided food and humidity. Vials were then sealed with parafilm or screw lids and placed at 18-20°C for 24 hours. After this time, individuals were scored as alive (moving freely), incapacitated (inhibited movement) or dead (no movement over a 5 s period), following Hoffmann *et al.*[28]. After bioassays were undertaken, dead and alive mites from each dose in each population were separated and placed in 100% ethanol at -20°C.

In 2018, we received a field report of chemical control failures involving *H. destructor* near Wanalta, in Victoria. This is greater than 400 km from the nearest location where resistant populations had previously been found. Three adjacent fields had been sprayed with organophosphates but failed to achieve adequate control of mites. These fields (herein referred to as Vic 1, Vic 2 and Vic 3) have a long-term history of pesticide usage, particularly organophosphates. In August 2019, mites from each field were collected via suction, as described above, and transported back to the laboratory. Vic 1 and Vic 2 were pasture fields at that time, while Vic 3 was sown to canola. *H. destructor* collected near Elmore, an unsprayed site approximately 30 km from Vic 1, Vic 2 and Vic 3, acted as a control population. Mites from each population were tested for their response to omethoate 290 g L^{-1} using the bioassay methodology described above. Six doses ranging from 0.0029 mg L^{-1} to 290 mg L^{-1}, along with a water control, were tested. We additionally tested these populations against a second organophosphate, malathion 290 g L^{-1} (Maldison 500®, Nufarm Limited, Laverton North, VIC, Australia), to understand the nature of resistance across different organophosphates. Malathion can also provide greater dose separation between resistant and susceptible mite populations compared with other organophosphate chemicals.[10] Six doses ranging from 0.05 mg L^{-1} to 500 mg L^{-1}, along with a water control, were tested following the bioassay methodology described above. Six replicate vials were coated per chemical dose and mites were scored after 8 hours as alive, incapacitated or dead as described above.

As incapacitated mites invariably die and therefore do not contribute to the next generation, we pooled incapacitated and dead individuals for data analysis across all bioassays. Mite mortality in each bioassay Y was modelled as a binomial response variable $Y \sim \mathrm{Binomial}(p,\ n)$, where p is the probability of mortality, and n is the number of mites in each replicate. The probability of mortality was related to linear covariates with a logistic link function $\mathrm{logit}(p) = a_i + b_i \ln(x)$, where x is chemical dose (mg L^{-1}) and a_i and b_i are intercepts and slope coefficients respectively for each population i.[29,30] Variance was robustly estimated by allowing for dispersion.[31] Models were fit using the *glm* function available in the R programming language for statistical computing.[32]

Each pesticide product was analysed separately, with differences in population effects (intercepts) assessed using an analysis of variance (ANOVA) and then examined in pairwise comparisons using Tukey's Honest Significant Difference (HSD) method at the 0.05 significance level, which corrects for Type I errors when performing multiple hypotheses tests.[33] For bioassays where a broad range of doses were tested, LC_{50} values (with 95% confidence intervals), regression coefficient estimates and dose-response slopes were computed. To test differences in mortality responses between chemical groups, an additional model was fitted that included active ingredient as a model factor, which was similarly contrasted using Tukey's HSD method.

2.3 Molecular screening for pyrethroid resistance

Molecular screening was undertaken to assess pyrethroid resistance on more than 320 *H. destructor* populations by testing mites for *kdr* mutations known to confer pyrethroid resistance.[14] This was undertaken on populations where numbers were insufficient to undertake phenotypic laboratory bioassays. Additionally, we screened a subset of populations where phenotypic bioassays indicated pyrethroid resistance to confirm the utility of the molecular diagnostic. Mite populations that exhibited a *kdr* mutation frequency of more than 2% were classified as resistant, although frequencies in resistant populations were typically 50% or greater. Prior to molecular screening, mites were placed in 100% ethanol and frozen at -20°C.

DNA was extracted from pooled mite samples (10-50 mites) from each population using a Qiagen DNeasy Blood & Tissue Kit. Mites were placed into 1.5 mL tubes along with a 3 mm glass bead (Retsch GmbH, Haan, Germany) and ultrapure water (20 ul). Each tube was then shaken in a Mixer Mill (MM300, Retsch GmbH, Haan, Germany) at 30 oscillations per second for 1 min. After bead beating, buffer ATL (180 ul) and Proteinase K (20 ul) were added to each tube followed by an incubation step at 56°C for 1 hour. The Qiagen DNeasy Blood & Tissue Kit (manufactures protocol) was then followed for the remaining part of the DNA extraction, with a final elution step of AE Buffer (100 μl) for each sample. Samples were quantified using a Qubit (Invitrogen) and normalised to 1 ng/ul.

TaqMAN SNP genotyping assays were designed to detect two single nucleotide polymorphism resistance mutations in the *H. destructor* parasodium channel gene (TTG - > TTT) and (TTG - > TTC) which both produce a leucine (L) to phenylalanine (F) amino acid substitution (L1024F) as described in Edwards *et al.*[14] For L1024F (TTG - > TTT), detection was through primers (forward: GCAATCTGGTTGTGCTGAATCTTTT reverse: CGAGAGATTAGATGCGCCAAAACT) and probes (wild type allele: VIC-CTTGCCCTTTTGCTGTCA mutant type allele: FAM-CTTGCCCTTTTTCTGTCA). For L1024F (TTG - > TTC), detection was through primers (Forward: GCAATCTGGTTGTGCTGAATCTTTT reverse: CGAGAGATTAGATGCGCCAAAAC) and probes (wild type allele: VIC-CTTGCCCTTTTGCTGTCAA mutant type allele: FAM-CTTGCCCTTTTCCTGTCAA). The ddPCR mixtures, 20 μL in volume, contained DNA (4 ng), 1× ddPCR supermix for probes (no dUTP), 900 nM of each primer and 200 nM of each probe. Droplets were generated and analysed using the QX200 system (Bio-Rad). Amplification was performed as follows: 95°C for 10 mins (1 cycle), 94°C for 30 secs and 60°C for 1 mins (40 cycles), and 98°C for 10 mins (1 cycle) with a ramp rate of 2°C per sec; the reaction was then held at 4°C. The frequency of mutant alleles in mite populations was estimated by modelling as a Poisson distribution using QuantaSoft v1.6.6 analysis software (Bio-Rad).

2.4 Association between pyrethroid resistance in H. destructor *and crop management*

The influence of crop management factors on the evolution of pyrethroid resistance was explored using field histories collected (described above) during ongoing resistance surveillance programs from the time resistance was first discovered in 2006 until 2019.[10–13]

Pyrethroid resistance R (resistant = 1; susceptible = 0) was modelled as a Bernoulli response variable $R \sim \mathrm{Bernoulli}(p)$, where p is the probability of resistance. Models were fit using the *glm* function available in the R programming language for statistical computing.[32] While organophosphate resistance has also been documented, here we only analysed pyrethroid resistance due to the longer history of resistance (first recorded in 2006 for pyrethroid resistance compared with 2014 for organophosphate resistance) and the much greater number of resistance records of pyrethroid resistance. The probability of pyrethroid resistance was related to linear covariates with a logistic link function. A logit link function was used to model the effect of covariates on the evolution of resistance including pyrethroid usage, rotation of pesticide groups, distance to other known resistant populations, time since initial detection of resistance in 2006, and agricultural land use type. Initially, we considered a range of agricultural land use types; however, to simplify the model, we focussed on three variables representing the key crop types in rotation in southern Australia: pasture, canola, cereal. These account for over 80% of land uses surveyed. Further detail on the construction of model variables is provided in Table 1.

To assess the importance of model variables, the full model was compared with simpler models including only the variable of interest. All analyses were performed using the R language for statistical computing.[32]

3. Results

3.1 Organophosphate resistance in H. destructor *in Victoria*

Dose-mortality curves (Supplementary Figure 1) and LC_{50} values (Table 2) show significant differences between the control population and the three Vic populations (Vic 1, Vic 2, Vic 3). Low levels of resistance to both omethoate and malathion were found in the three Vic populations. For omethoate, the LC_{50} values were consistent across the three populations and ranged between 5.09 and 6.81-fold higher than the control population. For malathion, the LC_{50} values varied considerably between the three Vic populations and ranged between 6.57 and 70.97-fold higher than the control population. *H. destructor* from Vic 3 exhibited a significantly higher level of resistance to malathion than Vic 2 (Table 2). No differences in regressions slopes between populations were present for either omethoate or malathion (Table 2).

3.2 The distribution of pesticide resistance in H. destructor *across Australia*

Resistance surveillance data on H*. destructor* collected between 2006 and 2010[10–13] was combined with resistance data collected in this study (2011-2019) to map the current distribution of resistance across Australia. Since the first detection of pyrethroid resistance in *H. destructor* in 2006,[13] resistance surveillance screening of *H. destructor* has been undertaken on a yearly basis resulting in 1029 populations being tested over the last 13 years (Table 3; Supplementary Figure 2). One hundred and ninety-five mite populations were detected with pyrethroid resistance, 59 populations with organophosphate resistance and 24 populations were found to have resistance to both chemical groups (Table 3). Our surveillance program has covered a wide geographical range throughout western and eastern Australia (SA, Victoria and New South Wales), encompassing a large portion of the entire known Australian distribution of *H. destructor* (Figure 1). As of 2019, the current distribution of synthetic pyrethroid resistance (Figure 1a) is widespread across WA covering the south-west, great southern, south coastal and wheatbelt regions. It is also present in several areas in SA including Kangaroo Island, the Fleurieu Peninsula and the south east region (Figure 1a). The current distribution of organophosphate resistance covers the great southern, south west and wheatbelt regions of WA, Kangaroo Island, the Fleurieu Peninsula and south east regions of SA and the north central area of Victoria (Figure 1b).

Figures 2 and 3 show the distribution of resistance through time by mapping *H. destructor* populations sampled and screened for organophosphate and pyrethroid resistance separated into two-year periods from 2006 to 2019. There has been a consistent increase in resistance detections across time. Resistance to pyrethroids first appeared near Esperance, WA in 2006; by 2007 resistance was detected over 400 km away in several areas within the great southern region near Cranbrook, WA. Several more resistant populations were detected in 2008 - 2009 with known resistance detections spanning over 500 km. In 2010, resistance was detected for the first time in the south west region, north of Boyup Brook, WA, approximately 100 km from the closest previously recorded resistant site, with several more populations detected within the great southern region. In 2014-2015, resistance populations were detected in the south west and great southern regions of WA, with the furthest resistant populations over 700 km apart. In 2016, resistance was detected for the first time outside of WA in a population in the Upper South East district in SA over 2000 km from the closest resistant site in WA. Between 2017 and 2019, several more resistant populations were discovered in SA, with populations found on Kangaroo Island, the Fleurieu Peninsula and near Bordertown. During this period, further resistant populations were detected in WA spanning the south-west, great southern, south coastal and wheatbelt regions (Figure 2).

Resistance to organophosphates was first detected in 2014 near Capel, WA and by 2015 several more resistant populations were detected spanning a wide geographical range of > 400 km within the south-west, great southern and wheatbelt regions of WA. This corresponded with the first discovery of a population exhibiting dual resistance to both pyrethroids and organophosphates (Table 3). In 2016, organophosphate resistance was discovered for the first time outside of WA in the same SA population that pyrethroid resistance was discovered. From 2017 to 2019, several more organophosphate resistant populations were discovered in SA, with populations found on Kangaroo Island, the Fleurieu Peninsula and near Bordertown. During this period, several resistant populations were detected in WA spanning 500 km in the south-west, great southern and wheatbelt regions (Figure 3). As detailed above, organophosphate resistance was detected for the first time in Victoria in the north central region, which is > 400 km from the closest resistant site in SA. *H. destructor* from these Victorian fields were screened for the known *kdr* resistant mutations that confer resistance to pyrethroids; no resistant alleles were present in any mite tested (*n* = 129).

3.3 Association between pyrethroid resistance in H. destructor *and crop management*

The covariate with the largest effect on pyrethroid resistance in *H. destructor* was distance to nearest known resistant population, followed by crop type, and pesticide usage (Figure 4). While pesticide usage is important, there are clearly other important considerations in resistance management of this pest. The model of resistance probability that included all covariates performed better than several other candidate models (AIC weight = 0.22), including models that respectively excluded distance, pesticide usage, and crop type. However, it performed similarly well to models that excluded chemical rotation (AIC weight = 0.40), crop rotation (AIC weight = 0.30), and time (AIC weight = 0.08), suggesting the lower importance of these variables in predicting pyrethroid resistance in our data set. The predictability of resistance was lowest when the covariate for distance to nearest known resistance was removed, followed by crop type, and then pesticide usage (Table 4).

The coefficient for distance was negative, reflecting the decreasing probability of pyrethroid resistance as distance from known resistant populations increases (Table 1). The model coefficient (and 95% CIs) estimated for log distance (km) was -0.72 (-0.89; -0.57), suggesting the resistance odds decrease on average by 82.2% for each 10-fold increase in distance (1 - exp(-0.72 x log(10)) = 0.822). For example, a site 100 km from the nearest resistant population has the resistance-odds of 18.8% of a site only 10 km from the nearest resistant population, while a 1000 km site has 18.8% x 18.8% = 3.5% odds of resistance of a 10 km site. The effect of time was estimated as marginally positive, suggesting that the probability of detecting resistance tends to increase with time. Unsurprisingly, a significantly positive effect of pyrethroid usage was identified (Figure 4). Interestingly, there was no support for chemical rotations or crop rotations being associated with resistance evolution (Table 4), with the 95% confidence intervals of the coefficients encompassing zero (Table 1). The frequency at which cereals or pasture occurred in farmers' field histories was negatively associated with pyrethroid resistance, while no significant effect of canola cropping frequency was detected. The marginal effect of pyrethroid usage, distance, and crop type on resistance probability is shown in Figure 4.

4. Discussion

Since the first reported case of pyrethroid resistance in 2006,[13] ongoing surveillance shows that resistance in *H. destructor* has emerged over a wide area across southern Australia. Resistance to both pyrethroids and organophosphates is now common across large areas of WA and present in several areas within SA. The first case of resistance in *H. destructor* in Victoria has now been documented due to a population exhibiting low levels of organophosphate resistance, representing the most easterly location of resistance in this pest. Over 190 populations have now been confirmed with pyrethroid resistance, 59 populations with organophosphate resistance and 24 populations with resistance to both chemical groups, spanning three Australian states and over 3000 km.

The efficiency, sensitivity, and coverage of resistance surveillance has been aided by improved resistance monitoring tools. The development of a high throughput molecular pyrethroid resistance screening test in 2014[14] has allowed for intensive monitoring, while resistance surveillance efforts have improved by targeting "at risk" areas, identified through predictive modelling data.[24] Using previous resistance distributions and associated field history data as well as long-term climatic data, Maino *et al.*[24] identified chemical usage, as well as several climatic based variables linked to voltinism and abundance, to be associated with the spatial pattern of pyrethroid resistance in *H. destructor*. This data was incorporated into a model to identify areas where future pyrethroid resistance evolution was likely. These predictions were used to direct our resistance surveillance efforts and, importantly, resulted in several new resistance detections which might not have otherwise been identified. This included the south-west region in WA, and both Kangaroo Island and the Fleurieu Peninsula in SA. This spatial prediction model is novel in resistance management, although predictive models have more generally been used to direct monitoring efforts in other aspects of pest management,[34,35] and in surveillance programs for invasive pests.[36–38]

In Victoria in 2018, three adjacent fields were found with low resistance to organophosphates, with resistance ratios between 5 and 7-fold for omethoate and between 7 and 70-fold for malathion. Variation in levels of resistance between populations to different organophosphate chemicals has been shown previously in *H. destructor*[10] and may reflect the effectiveness of metabolic resistance mechanims in countering organophosphate susceptibility. Higher resistance levels to malathion could stem from two caboxylic ester bonds in malathion that make it more sensitive to carboxylesterase activity.[39] Congruent with our findings, relatively low resistance ratios for organophosphate resistance have been found in other *H. destructor* populations.[10,11] This is not the case for pyrethroids, where resistance ratios up to ~250,000 have been recorded.[10–13] High levels of resistance to pyrethroids have been identified for many other pests[40–43] and may relate to the effectiveness of resistance mediated through alteration of the target site.

Differences in responses between chemical groups will have important implications for ongoing resistance management in the field. Due to low resistance to organophosphates, current field rates of chemicals may still provide some control of *H. destructor* populations even when resistance has been detected, whereas treatments with pyrethroids will lead to rapid increases in the frequency of *kdr* resistant alleles which provide a high level phenotypic resistance.[14] Differences in field control with low levels of resistance have previously been noted for other pests such as the western corm rootworm (*Diabrotica virgifera virgifera* LeConte).[44] It is therefore important to define “practical resistance” for a chemical, the field-evolved resistance that reduces pesticide field efficacy to levels that have practical consequences for pest control.[45] This may not necessarily be reflected by resistance ratios which are not linearly connected to pesticide-based control failure risks. The relationship between resistance levels observed in dose-response bioassays and actual efficacy of products in the field against *H. destructor* requires further investigation; this is needed to better understand the practical implications of resistance.[44]

The rate at which resistance to pyrethroids and organophosphates will develop in the future will be influenced by a range of factors such as migration, proximity of resistant populations, crop rotations, and climate, as well as the rate, timing and frequency of pesticides applications.[8,46] These factors can vary in different environments, but ultimately work either by increasing the rate at which resistant mutations are selected or by increasing the rate at which resistant mutations enter a population.[11,24,47] The high densities of *H. destructor* populations, which can exceed 15 000 mites/m^2, [1] increase the likelihood of rare resistance mutations entering a population. Proximity to resistant populations may increase the likelihood of resistant mutants entering through dispersal of resistant mites, but close proximity to susceptible populations could decrease this likelihood by having a buffering effect.[11] In the present study we found that the distance to the nearest known pyrethroid resistant population was the strongest predictor of pyrethroid resistance in *H. destructor*, which could spread locally within an area after resistance has emerged.[23] Furthermore, this suggests that the simple positive association between time and resistance (Tables 3 and 4) can be explained by the changing distribution of resistance and, thus, the changing proximity to resistant populations. Adult mites are known to only move ~10 m in their lifetime, which will contribute to the spread of resistance,[48] but long distance dispersal can occur through the movement of diapausing eggs (e.g. by summer winds, soil on farm machinery or livestock, and the transportation of fodder).[1] Farm hygiene should form a part of resistance management practices in *H. destructor* in much the same way as it is used for weed and disease management. Such programs could help reduce local spread of resistance in *H. destructor*, by limiting movement of livestock, fodder and farm machinery (that may carry vegetation or soil contaminated with resistant mites or eggs) between known resistant and susceptible fields within and among farms. Maintaining reservoirs of susceptible mites along fence-lines, rather than removing these through pesticide applications, could also help maintain susceptibility in mite populations with a low incidence of resistance. Recent research has shown pyrethroid resistance is recessive[15] and there are likely fitness costs associated with the L1024F *kdr* mutation in *H. destructor.*[49] As such, fence-line reservoirs may enable mites (which in the absence of pesticides, have a higher level of fitness than resistant individuals) to build-up in numbers and disperse into adjacent fields, diluting the frequency of resistant alleles within a population.

Our analysis of crop management information collected between 2006-2019 unsurprisingly identified increasing pyrethroid chemical usage as associated with resistance evolution. Chemical usage has previously been identified as a key driver for pyrethroid resistance in *H. destructor,*[24] consistent with the long-held notion that repeated use of the same pesticide or with the same mode of action are main drivers for selection of pesticide resistance in invertebrate pests.[50] The dose and frequency of pesticide applications will continue to play an important role in resistance evolution in *H. destructor,* particularly in context of the current over-reliance on a small number of broad-spectrum pesticide groups. Interestingly, we found that the frequency of particular crops in rotation may minimize the likelihood of resistance in *H. destructor*, with a decrease in pyrethroid resistance under cereal and pasture management. Cereals and/or pastures are less commonly treated with pesticides than canola, which is more vulnerable to mite attack[3,9] and is generally associated with larger chemical inputs.[24] Cereals are considered to be unfavorable to *H. destructor* and have been recommended for use in crop rotation situations for protection against this pest.[51,52] In the same way, rotating corn with soybean and diversifying the type of *Bt* corn planted annually can mitigate the impacts of western corn rootworm resistance.[53] This information should be incorporated into future revisions of the national Resistance Management Strategy that has been developed for *H. destructor* as a strategy to minimise resistance evolution.[27]

Conclusion

Pesticides will continue to play an important part in *H. destructor* control, but the increasing emergence of resistance over a wide area of Australia raises concerns for the long-term viability of chemical control. Our findings highlight the importance of management strategies that could minimise the risk of further resistance by minimising chemical applications, farm biosecurity, utilising non-chemical controls such as crop rotations, and rotating between available registered chemistries. In addition, new options for *H. destructor* suppression should now shift towards biological molecules and extracts, entomopathogens and habitat manipulation to increase native predators.[54–57] By understanding the impact of crop management on resistance, we are now better able to predict resistance ‘hot spots’ and educate farmers of practical ways to minimise future selection pressures.[24] This increased understanding has been used to develop forecasting tools[24] with the goal of increasing farmer confidence in anticipated *H. destructor* risks and ultimately decreasing the reliance on pesticide ‘insurance sprays’.

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Conflict of Interest Declaration

The authors declare no conflict of interest relevant to this study.

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Table 1. Variables included in the binomial regression model used to test the association of crop management factors on pyrethroid resistance evolution in *H. destructor*.

Model variable name	**Description**	**Model coefficient (95% confidence interval)**
Resistance	A binary variable for resistance status (resistant = 1, susceptible = 0) for a population. Resistance was defined as >5% bioassay survival treated with 0.1 g L^{-1} of bifenthrin or molecular identification of a known resistance conferring mutation at the *kdr* site in > 1% of tested mites.	*NA (response variable)*
Intercept	Model intercept. The estimated log odds of resistance when all covariates are set to zero.	0.43 [-1.10. 1.94]
Distance	The log-transformed distance, in km, between a sample site and the nearest site that was coded as resistant during all previous surveillance years.	-0.72 [-0.89. -0.57]

Time	At the time of testing, the number of years after the first detected pyrethroid resistant population in 2006.	0.09 [0.00. 0.18]
Pyrethroid usage†	The average annual pyrethroid applications per year according to available paddock records	0.98 [0.41. 1.57]
Crop rotation†	The number of rotations between different crop types over the years covered by available paddock records, normalised by year	0.09 [-0.10. 0.28]
Crop type (pasture, canola, cereal)†	For each crop group of pasture, canola, and cereal, the proportion of occurrences in the available paddock records	-2.25 [-3.50. -0.99] -0.74 [-3.14. 1.61] -3.82 [-5.94. -1.80]
Chemical rotations†	The number of rotations between different pesticide groups (pyrethroids, organophosphates, and neonicotinoids) over the years covered by available paddock records, normalised by year	1.03 [-0.60. 2.69]

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† Records span between two and seven years across different fields, with most covering six years.

Table 2. LC_{50} values (with 95% confidence intervals) and regression coefficients (with standard errors) for *H. destructor* populations from Victoria when exposed to omethoate and malathion. Different letters within each pesticide represent significant differences ($P < 0.05$, Tukey's HSD tests).

Pesticide	**Population**	**LC_{50} (95% CI) mg L^{-1}**	**Regression coefficient (*b*) ± SE**	**Resistance ratio**
Omethoate	Elmore (control)	0.98 (0.56-1.71)^{a}	1.167 ± 0.205^{a}	---
	Vic 1	6.68 (3.53-12.62)^{b}	0.773 ± 0.116^{a}	6.81
	Vic 2	4.99 (2.74-9.09)^{b}	0.884 ± 0.141^{a}	5.09
	Vic 3	6.64 (3.60-12.25)^{b}	0.975 ± 0.161^{a}	6.77
Malathion	Elmore (control)	1.23 (0.63–2.42)^{a}	1.533 ± 0.571^{a}	---
	Vic 1	19.83 (4.03–97.46)^{bc}	0.501 ± 0.161^{a}	16.12
	Vic 2	8.08 (1.94–33.58)^{b}	0.548 ± 0.169^{a}	6.57
	Vic 3	87.31 (8.39–908.18)^{c}	0.384 ± 0.142^{a}	70.97

Table 3. Number of *H. destructor* populations collected between 2006 and 2019 from Western and eastern Australia screened for resistance to organophosphates and synthetic pyrethroids. Total number of populations with resistance to organophosphates (OP), to synthetic pyrethroids (SP) and to both chemical groups (dual) is documented.

	Populations from Western Australia				**Populations from eastern Australia**			
Year	**Sampled**	**With SP resistance**	**With OP resistance**	**Dual resistance**	**Sampled**	**With SP resistance**	**With OP resistance**	**Dual resistance**
2006	1	1	0	0	1	0	0	0
2007	33	12	0	0	5	0	0	0
2008	7	0	0	0	-	-	-	-
2009	46	12	0	0	3	0	0	0
2010	44	12	0	0	34	0	0	0
2011	108	14	0	0	19	0	0	0
2012	7	7	0	0	-	-	-	-
2013	8	6	0	0	-	-	-	-
2014	127	28	12	1	39	0	0	0
2015	95	10	6	0	24	0	0	0
2016	28	10	0	0	7	1	1	1
2017	119	22	8	4	47	6	1	1
2018	26	5	1	1	19	3	4	2

2019	117	37	17	10	65	9	9	4

Table 4. Comparison of the full model used to investigate the association between pyrethroid resistance in *H. destructor* and crop management, with models computed where one variable is omitted.

Model	K	AIC	ΔAIC	Model Likelihood	AIC weight	Log likelihood	Cumulative weight
No chemical rotation	8	412.65	0.00	1.00	0.40	-198.19	0.40
No crop rotation	8	413.24	0.59	0.75	0.30	-198.49	0.69
Full model	9	413.78	1.13	0.57	0.22	-197.72	0.92
No time	8	415.79	3.14	0.21	0.08	-199.76	1.00
No pesticide usage	8	423.19	10.54	0.01	0.00	-203.46	1.00
No crop	6	427.18	14.53	0.00	0.00	-207.51	1.00
No nearest resistance	8	532.41	119.76	0.00	0.00	-258.07	1.00

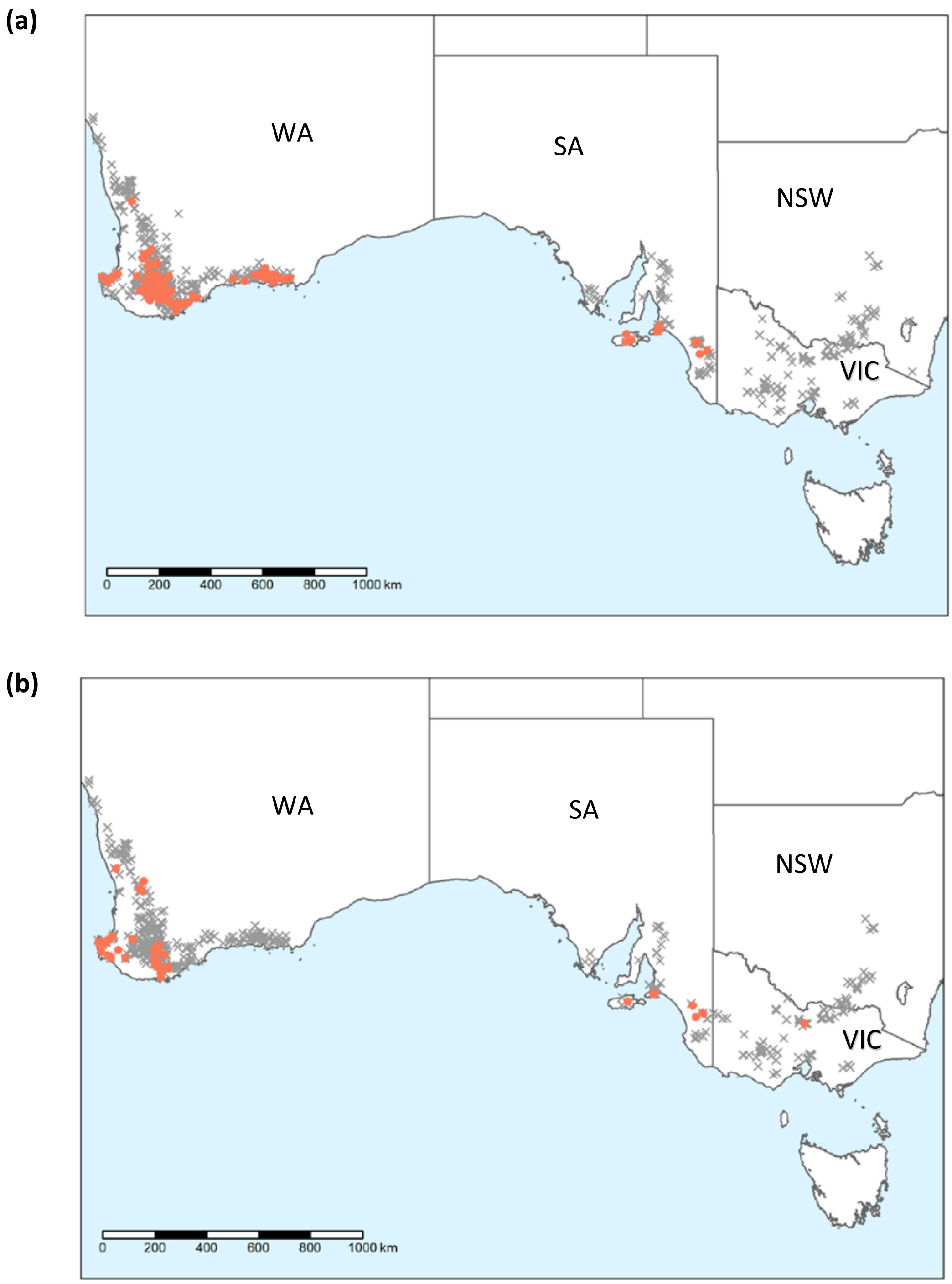


Figure 1. The distribution of *H. destructor* populations screened for (a) pyrethroid and (b) organophosphate resistance across Australia as of 2019. Red closed circles represent populations with resistance, and grey crosses indicate populations that are susceptible to pesticides.

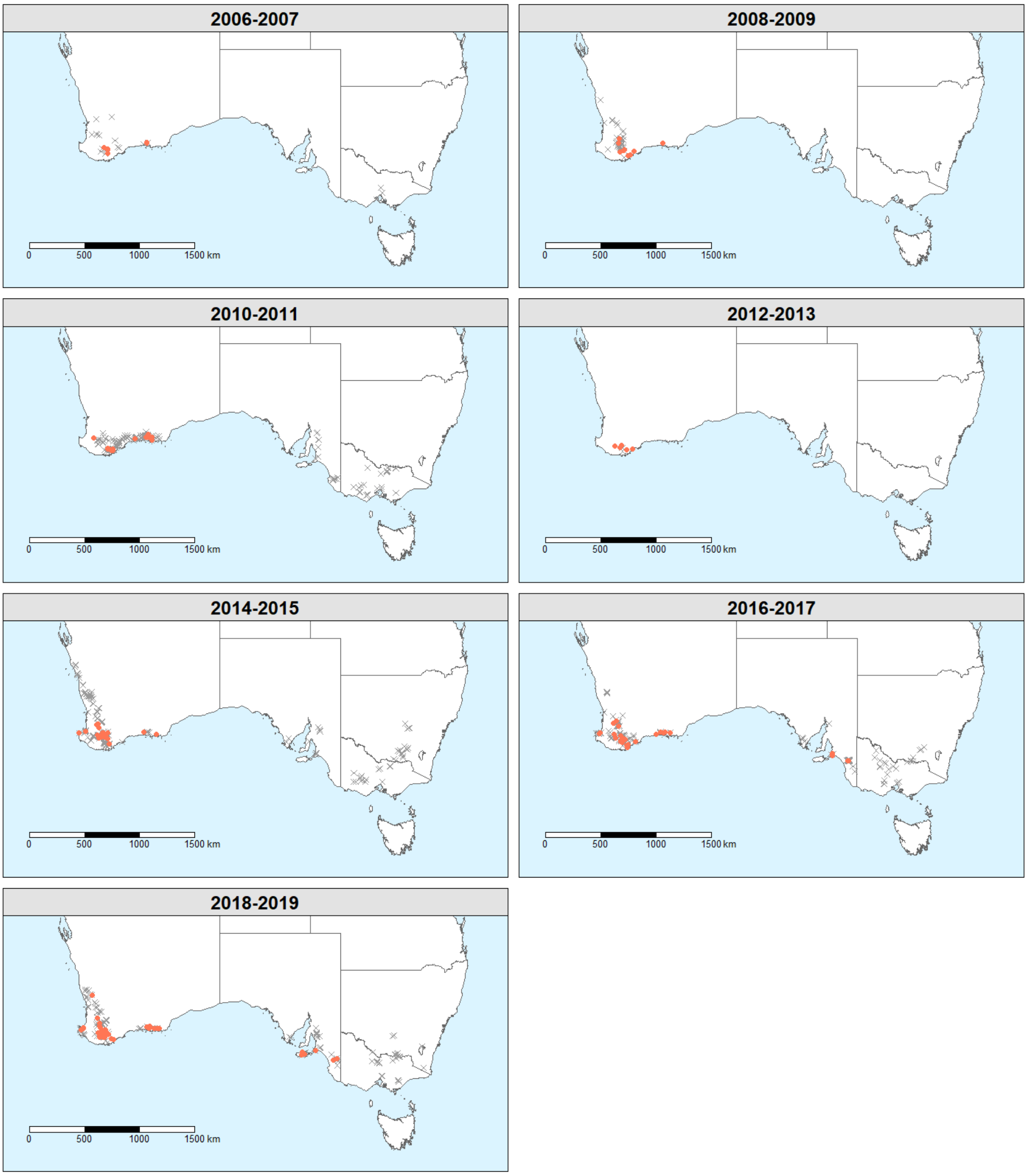


Figure 2. *Halotydeus destructor* populations collected and screened for pyrethroid resistance across Australia over two-year periods from 2006 to 2019. Red closed circles represent populations with resistance to pyrethroids, and grey crosses indicate populations that are susceptible.

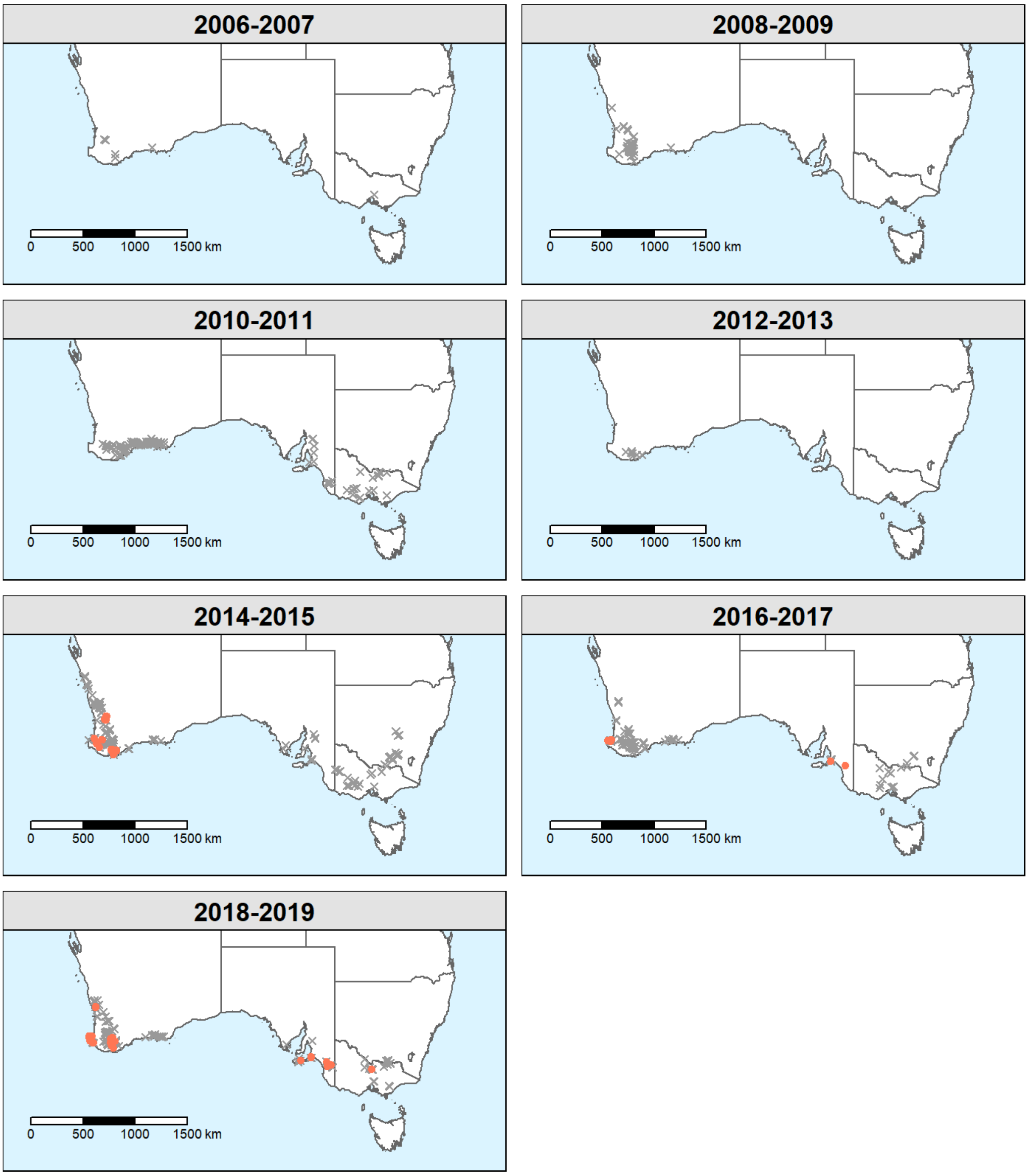


Figure 3. *Halotydeus destructor* populations collected and screened for organophosphate resistance across Australia over two-year periods from 2006 to 2019. Red closed circles represent populations with resistance to organophosphate, and grey crosses indicate populations that are susceptible.

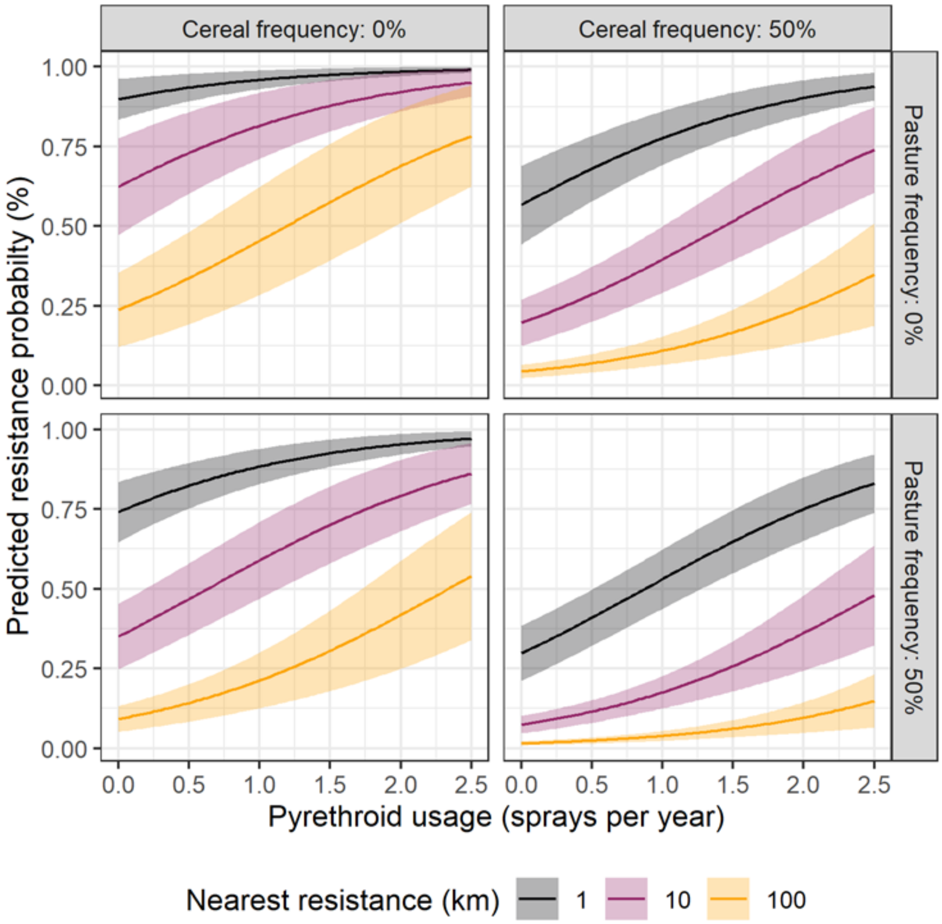


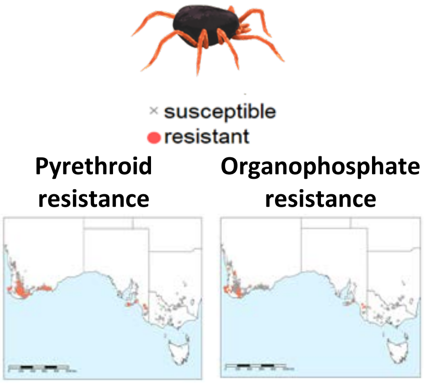
Figure 4. The marginal predicted effects of model covariates on resistance probability in *H. destructor*. The effect of pyrethroid usage is represented by annual pyrethroid applications spanning 0 to 2.5 mean applications per year. The effect of distance to nearest known resistance is predicted at three levels: 1, 10, and 100 km to the nearest known pyrethroid resistant population. The effect of crop is represented by the frequency of cereal and pasture in the cropping history, where 0% represents no occurrence in the known cropping history of a field while 50% represents half of all crops in the known cropping history. Shaded regions denote standard errors.

Learnings from over a decade of increasing pesticide resistance in the redlegged earth mite, *Halotydeus destructor* Tucker

Aston L Arthur*, James Maino, Ary A Hoffmann, Moshe Jasper, Alan Lord, Svetlana Micic, Owain Edwards, Anthony van Rooyan and Paul A Umina

Graphical Abstract

Pesticide resistance in *H. destructor* has emerged over a wide geographic area in Australia. Associations between crop management practices and resistance are identified, which will aid future resistance management efforts.


× susceptible
● resistant
Pyrethroid resistance
Organophosphate resistance