Susceptibility of the bird cherry-oat aphid, *Rhopalosiphum padi* (Hemiptera: Aphididae), to four insecticides

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Abstract

The bird-cherry oat aphid (*Rhopalosiphumpadi*) is a global pest, attacking most cereal crops including barley, wheat, oats and triticale. The aphids cause yield losses through direct feedingdamage and the transmission of plant viruses. In Australia, feeding injury can reduce cereal yields by 6%, with the damage caused by aphid-vectored viruses reducing the yield of cereal crops by up to 30%. Aphid control in these crops is achieved almost exclusively with insecticides, and there is growing concern surroundinginsecticide resistance evolution in multiple aphid species. In this study, nine field populations of *R. Padi* were collected from localities representing the major grain growing regions of Australia. Toxicity data against four insecticides(dimethoate, alpha-cypermethrin, pirimicarb, imidacloprid)was generatedfor each aphid population. Thisrevealedlittle differences in population responsesfor three of these insecticides. For alpha-cypermethrin, a widely used insecticide in Australia, there weresignificant differences between several populations. These data will be important for future monitoring of insecticide responses of *R. Padi* and highlight the impending pest management challenges growers could encounter in Australia.

Key words: toxicity data, oataphid, insecticides, resistance monitoring

Introduction

The bird cherry-oat aphid, *Rhopalosiphumpadi*(Linnaeus, 1758), is one of the most important cereal pests worldwide. While itcan be found feeding on~20 plant families, it is a pest mainly on Poaceae(particularly wheat, barley, corn, oats and triticale) and to a lesser extent Rosaceae crops(Blackman and Eastop 2000). In Australia, *R. Padi* is present throughout all major grain growing regionsand can cause damage through both direct feeding and by transmitting more than a dozen plant viruses, including Barley yellow dwarf virus (BYDV)(Lucio-Zavaleta*et al.* 2001; Bailey 2007;Edwards *et al*. 2008;Schliephake*et al*. 2013). Both types of damage significantly reduce the quality and yield of cereal crops.Direct feeding by *R. Padi* and other cereal aphids can reduce yield by 6%, while damage from aphid-vectored viruses can reduce yield by up to 30% in cereals crops such as barley, oats and wheat (Valenzuela & Hoffmann 2015).

In Australian cereals, insecticides are the most commonly used management tool to control *R. padi*, with growers largely relying on foliar applications of organophosphates, synthetic pyrethroids and carbamates, as well as neonicotinoids applied as a seed treatment prior to sowing (Edwards *et al.* 2008;GRDC 2010; Kirkland *et al.* 2018;APVMA 2020). Synthetic pyrethroids and organophosphatesare estimated to account for >85% of insecticide use in cereal crops in Australia (Umina *et al.* 2019). Pyrethroids in particular are widely used prophylactically in cereals to reduce the transmission of aphid-vectored diseases includingBYDV (Edwards*et al.* 2008).Neonicotinoids, predominantlyimidacloprid,have become commonplace in many cereal-growing regions, particularly inareas where virus risk is deemed high and/or Russian wheataphid,*Diuraphisnoxia*(Mordvilko, 1913),ispresent (Yazdani*et al.* 2018; Umina *et al.* 2019).Pirimicarb, a carbamate insecticide that is highly effective against aphids (Xiao *et al.* 2015), isalso registered and used (although to a lesser extent)against *R. padi* and other cereals aphids (APVMA 2020; Umina *et al.* 2019).

*Rhopalosiphumpadi*is thought to have been introduced to Australia more than 100 years ago and is now widespread and very abundant across the country (Valenzuela *et al.* 2010; Macfadyen&Kriticos, 2012). In many parts of the world, *R. padi* is heteroeciousholocyclic, whereby its lifecycle alternates between a sexual and asexual phase on a primary and a secondary host, respectively (Halkett*et al.* 2006). Although males are present in Australia, the primary host essential for mating is rare and population genetic studies have shown *R. padi* to be anholocyclic, with parthenogenetic females reproducing only by viviparity. There is also evidence of a very low proportion of androcyclic clones (where males and viviparous females are produced) (Hughes *et al.* 1964; Simon *et al.* 1996; Delmotte *et al.* 2003; Valenzuela *et al.* 2010). In addition to cereal crops, grasses play a critical role in the lifecycle and biology of *R. padi*. Perennial grass pastures in particular are thought to bean important refuge for *R. padi* in some parts of Australia. de Barro *et al.* (1992) demonstrated irrigated pastures are capable of producing very large numbers of alates during late autumn, placing nearby cereal crops at risk from infestation of migrating aphids; importantly this coincides with the early establishment stage of winter cereals. Annual grasses are also consideredimportant as they have the potential to link aphids in perennial grass pastures with cereal crops and/or by allowing low numbers of *R. padi* to persist between cropping seasons over summer (de Barro&Wallwork 1992). These grasses (and volunteer cereals) often persist during summer months interspersed within fields, in headlands and along roadside verges.

There is some conjecture regarding insecticide resistance in *R. padi*. There are currently no documented cases of field-evolved resistance captured in the Arthropod Pesticide Resistance Database,a global resource that captures insecticide resistance cases from 1914 to present (Mota-Sanchez & Wise 2020).However,there are multiple publications that claim low-level resistance is evolving in China (Cheng *et al.* 2004; Zhang *et al.* 2016; Zuo *et al.* 2016). There are also several studies that have selected for high-level resistance through repeated insecticide exposure in the laboratory (e.g. Chen *et al.* 2007; Wang *et al.*2016;Wang *et al.* 2018), demonstrating the evolutionary capacity for resistance to evolve in *R padi*. Furthermore, a recent analysis conducted on Australian grain pests which considered the evolutionary potential of resistance, targeted and off-target insecticide pressure, and the availability of pest refuges, identified *R. padi* as a species at risk of evolving resistancewithin Australia (McDonald *et al.* 2019).

An insecticide study conducted four years ago found that Australian *R. Padi* populations had relatively low sensitivity across a range of insecticides commonly applied to grain crops (Clouston *et al.* 2016). Importantly, Clouston *et al.* (2016) identified *R. padi* as one of two species (the other being *Aphis craccivora* Koch, 1854) that should be the focus of continued monitoring for insecticide resistance to strengthen future risk-mitigation tactics. The generation of regular toxicitydata for high risk pests is paramount ifgrowersare to implement proactive and long-term insecticide management strategies (Umina *et al.* 2019).Here, we conduct a comprehensive study to establish current insecticide toxicitydata for field populationsof *R. Padi* collected from all major grain growing regions of Australia.

Materials and Methods

Aphid collections and culturing

Between January and December 2017, *R.padi* were collected from agricultural regions of New South Wales (NSW), Victoria (Vic.), South Australia (SA) and Western Australia (WA). At each location, global positioning system (GPS) coordinates and plant host were recorded. Aphids at each location were sampled from multiple plants to increase the likelihood of collecting multiple clonal types. Aphids were collected from wheat, barley, oats orgrass weeds (Table 1).As with most invasive aphid populations (Figueroa *et al*. 2018), *R. padi* in Australia has limited clonal variability and populations tend to be characterised by a small number of dominant clonal lineages, which show no clear geographic or host-related distributions (Valenzuela *et al.* 2010).

Field populations were isolated in the laboratory to ensure the absence of parasitoid wasps, using the method described in Umina *et al.* (2014), except we used oat (*Avenasativa*) leaf sections instead of sprouting radish. Individual aphid populations were then maintained in 55mm petri dishes containing 10 g/L agar inserted with excised *A. sativa* leaf sections. Petri dishes were kept in a Controlled Temperature (CT) cabinet set at 10^{o}C, with a L14:D10h photoperiod; these conditions are ideal for maintaining healthy aphid populations but minimising the number of generations that occur in the laboratory. Aphids were transferred to new petri dishes every 14 days.

To produce sufficient numbers for bioassays, aphids were transferred to wheat plants (*Triticumaestivum*) grown in potting mix in 900mLpots. Both oat and wheat are recognised as highly suitable host plants for *R padi*, which is considered to be polyphagous on C3 grasses and crops. Both species are routinely used as *R. Padi* hosts in laboratory rearing and insecticide assays. Additionally, these hosts do not differentially affect insecticide efficacy (Araya & Foster 1987). Pots containing wheat plants were placed insideinsect-proof cages (45cm H, 28cm W, 38cm L) and kept under natural light at approximately 20^{o}C untilaphid numbers were sufficient (typically 21 days).Under these unconstrained growth conditions, there is unlikely to be strong selection against tolerant(or resistant) aphid lineages.We have not observed any phenotypic or genetic changes over time in colonies of other aphid species maintained this way and repeatedly assayed as known resistant or susceptible controls(e.g. de Little & Umina 2017; Umina *et al.* 2014).

Insecticides tested

Aphid populations were screened against insecticides representing four chemical modes of action:neonicotinoids (Group 4A) represented by imidacloprid, carbamates (Group1B) represented by pirimicarb, organophosphates (Group 1A) represented by dimethoate, and synthetic pyrethroids (Group 3A) represented by alpha-cypermethrin (Table 2). A stock solution of the recommended field rate of each chemical product was prepared in water, and serially diluted to represent fiveconcentrations ranging from 1 to 10^{-4} times the field rate. The recommended field rates were based on recommendations for aphid control, assuming an application in 100L water/ha. These equate to 525 mg a.i./L imidacloprid, 5000mg a.i./L pirimicarb, 2000mg a.i./L dimethoate and 125 mg a.i./L alpha-cypermethrin.

Bioassay procedures

The laboratory bioassays undertaken closely followed those described by Umina *et al.* (2014),Clouston*et al.* (2016) and the Insecticide Resistance Action Committee (IRAC) Susceptibility Test Method No. 019 (IRAC 2016). The plant host of *T.aestivum* was used to emulate insecticide contact in a leaf dip bioassayfor each chemical product.For imidacloprid, a single excised *T.aestivum* leaf section (~25mm in length) was submerged for 10s in 0.0525 mg a.i./L solution, placed abaxial side up on 10 g/L agar in a 35mm petri dish and air dried.This was performed a further four times, resulting in five replicate petri dishes,each containing a single treated leaf. This was then repeated for each of the remaining imidacloprid solutions (moving sequentially from the lowest concentrations to the field rate). Five untreated control replicates were established in the same manner, except water was used in place of the chemical solution. Once dry, apterous aphids from a singlepopulation were transferred to each petri dish using a fine-haired paintbrush. Betweeneightand10adult aphids were added to each dish. Lids were then placed on the petridish plates and the dishes inverted to simulate natural aphid feeding on the underside of leaves. These were placed at 20°C with a L14:D10h photoperiod for 48 h, after which aphids were scored visually as dead (not moving over 5s period), incapacitated (inhibited movement), or alive (moving freely).The above procedure was repeated for the remaining insecticides, with three populations tested simultaneously on the same day.In all cases, insecticide stock solutions were made fresh on each bioassay day, and these were then serially diluted to generate fresh chemical concentrations for each bioassay.

Bioassays were performed for pirimicarb, dimethoate and alpha-cypermethrin using the same procedure described above, expect excised *T. aestivum* leaf sections were submerged in each chemical solution for 1seach (not 10 s).

Statistical analysis

As incapacitated individuals invariably die and therefore do not contribute to the next generation,we pooled incapacitatedindividuals withdead individuals for data analysis across all bioassays. Aphid 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 aphids 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 a.i./L) anda_i and b_i are intercepts and slope coefficients respectively for each population i(Robertson *et al.* 2017; Venables & Ripley 2002). Variance was robustly estimated by allowing for dispersion (McCullagh&Nelder 1989).

Each chemical product was analysed separately, with differences in population effects (intercepts) assessed using an analysis of variance (ANOVA).They were 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 (Hsu 1996). For each bioassay, LC_{50} values (with 95% confidence intervals) and slope coefficient estimates of dose-response slopes were computed. To test differences in mortality responses between products, an additional model was fitted that includedchemicalproduct as a model factor, which was similarly contrasted using Tukey’s HSD method.

All analyses were performed using the R language for statistical computing (R Core Team 2018).

Results

χ^2 tests indicated the models adequately fitted the data at the 0.05 significance level across allpopulations for each insecticide tested. Importantly, the mortality of the untreated control aphids was very low across all bioassays; there was no mortality in 32of the bioassays, 2% mortality in threebioassays and 4% mortality in one bioassay.

The dose-responses of *R. padi* were significantly different between the four chemical products (χ^2= 74.7, df = 3, $p < 0.001$).Imidacloprid and alpha-cypermethrin werethe most toxic chemical productsto *R. padi*,but were not significantly differentto one another ($p = 0.97$), with average LC_{50} values(±s.e.) across all nine populations of 8.72 (± 1.86) mg a.i./L and 9.94 (± 2.56) mg a.i./L,respectively. Dimethoate (34.42 ± 3.04 mga.i./L) was significantly less toxic when compared with imidacloprid ($p < 0.001$) and alpha-cypermethrin ($p < 0.001$), while pirimicarb (103.99±14.71 mga.i./L) was significantly less toxic than imidacloprid ($p < 0.001$), alpha-cypermethrin ($p < 0.001$), and dimethoate ($p = 0.005$). When averaged across populations, the slopes for alpha-cypermethrin (0.94 ± 0.07) than slopes for imidacloprid (1.23 ± 0.09), dimethoate (1.52 ± 0.17) and pirimicarb(3.73 ± 1.19), however, only differences between alpha-cypermethrin and pirimicarb were statistically significant ($p = 0.019$).

Imidacloprid

There wasno significant difference in the response of the nine populations to imidacloprid (χ^2 = 13.33, df = 8, $p = 0.10$), indicating all populations have a similar sensitivity to this chemicalproduct. Further, there were no differenceswhen comparing LC_{50} values and 95% confidence intervals(Table 3). The estimated LC_{50} values for imidacloprid across the nine populations ranged from 3.77 to 21.81 mg a.i./L. Unique regression slopes for populationsexposed to imidacloprid did not significantly improve the model (χ^2 =2.87, df = 8, $p = 0.94$).

For eight of the nine populations, the imidacloprid field rate (525 mg a.i./L) was adequate in supressing 100% of aphid individuals. Five aphids (from a total of 44) from the ADEpopulation were still alive at the field rate after 48 h exposure.

Pirimicarb

There was no significantdifference in responsesof the nine populations to pirimicarb (χ^2 =1.17, df = 8, $p =0.99$), indicating all populations have a similar sensitivity to this chemical product.There were also no differenceswhen comparing LC_{50} values and 95% confidence intervals(Table 4). The estimated LC_{50} values across all populations ranged from 38.19 to 172.08 mg a.i./L.

Unique regression slopes forpopulations exposed to pirimicarb did notsignificantly improve the model (χ^2 =1.69, df = 8, p = 0.99). However, regression slope coefficientscould not be estimated for two populations:ADE and BKV, due tothe partial-separation of the response variable (e.g. 96% of individuals from ADE survived at 50 mga.i./Lwhereas0% survived at the subsequent dose of 500 mg a.i./L). This indicates that the biological response of *R. padi* to pirimicarb varies and the doses we tested were appropriate for some, but not all, populations. Regardless, the removal of ADE and BKV from the analysis did not affectthe conclusion for the remaining seven populations (data not shown).

For all nine populations, the highest rate of pirimicarb (5000 mg a.i./L) was adequate in supressing 100% of individuals after 48 h.

Dimethoate

There was no significant difference in the response of the nine populations to dimethoate (χ^2 =2.01, df = 8, p = 0.98)and there were also no differenceswhen comparing LC_{50} values and 95% confidence intervals (Table 5). Thisindicatesdimethoate sensitivity was similar across all populations. Similarly, unique regression slopes for each population exposed to dimethoate did not significantly improve the model (χ^2 = 2.15, df = 8, p = 0.97).

For all nine populations, the dimethoate field rate (2000 mg a.i./L) was more than adequate in supressing 100% of individuals after 48 h. LC_{50} values across all populations ranged from 14.68 to 48.20 mg a.i./L (Table 5).

Alpha-cypermethrin

Noticeable differences in dose-response curves were detected between populations exposed to alpha-cypermethrin,which were found to be statistically significant(χ^2 =23.92, df = 8, p =0.002). These differences arefurther supported by non-overlapping 95% confidence intervals of LC_{50} values (Table 6).Aphids from KATwere significantly more sensitive to alpha-cypermethrinthan aphids from TMW (p =0.006), MNJ (p =0.03) and PIC (p =0.02). Unique regression slopes for populations exposed to alpha-cypermethrindid not significantly improve the model (χ^2 = 3.16, df = 8, p = 0.92).

LC_{50} values across all nine populations ranged from 0.6 to 24.57 mg a.i./L (Table 6).The field rate of alpha-cypermethrin (125 mg a.i./L) controlled 98%, 93%and 91% of aphids from ADE, MNJ and TMW, respectively. For the remaining six populations, the field rate was adequate in supressing 100% of individuals after 48 h (Table 6).

Discussion

Our results providetoxicity data for nine populations of *R. padi* against four insecticides widely used for aphid control in cereal crops within Australia. It is worth noting that the mortality responses quantified in our study represent variation between individual aphids and populations taken at a point in time, while other sources of variation may exist, e.g. the same populations exposed to different seasonal conditions may behave differently (see Robertson *et al.* 2017). Monitoring insecticide susceptibility is an important part of pest management strategies, particularly for species with a history of insecticide resistance. Insecticide toxicity data not only provides an indication of the level of insecticide susceptibility in the field, but also allows researchers to accurately assess if population responses to insecticide products are increasing or decreasing through time (Roush & Miller 1986). Resistance to neonicotinoids, carbamates and organophosphates has been selected under laboratory conditions (Chen *et al.*2007;Wang *et al.* 2018), demonstrating*R. padi* could evolve field resistance if selection pressures were sufficiently strong, whichhighlightsthe valueof the toxicity data we have generated.

Recently, *R. Padi* sourced from populations across Australia were shown to have lower insecticide sensitivity compared withseveral other grain aphids (Clouston *et al.* 2016). Further, at two locations in Queensland and South Australia, some *R. padi* populations were observed to survive field rates of alpha-cypermethrin when tested using laboratory bioassays (Clouston *et al.* 2016). Our study provides a broader survey of insecticide responses among *R. padi* populations and identified differences in sensitivity levels to alpha-cypermethrin, but not to other insecticides. Interestingly, out of the four insecticides tested, the estimated slopes from the dose-response curves were lowest for alpha-cypermethrin. Typically, a lower slope value indicates a more heterogeneous response to the insecticide within a population (Chilcuit&Tabashnik 1995), and thus a greater likelihood that the population will evolve more quickly to insecticide selection pressure.

Clouston *et al.* (2016) estimated LC_{50} values for alpha-cypermethrin in the range of 15.36 – 72.10 mg a.i./L for *R. padi*, with an average LC_{50} of 33.83 (± 8.14) mg a.i./L across six populations. This is considerably higher than the average LC_{50} value calculated from the nine populations we tested of 9.94 (± 2.56) mg a.i./L, which followed the same bioassay methodology and tested the same formulated chemical product. At a finer level, two populations from our study (one each from WA and Vic.) were collected in close proximity (<20km) to populations from the Clouston *et al.* (2016) study, allowing a provisional comparison of how sensitivity levels may have shifted. In the four years since the Clouston *et al.* (2016) study, the LC_{50} value for alpha-cypermethrin has dropped from 24.88 to 9.90 mg a.i./L at the two Vic. populations (Pimpinio and Natimuk) and from 30.84 to 0.60 mg a.i./L for the two WA populations(Katanning and Woodanilling). These shifts in sensitivity through time may be a consequence of changes in insecticide selection pressure. Thiscould be linked to a reduced risk of early-season BYDV infections (vectored by *R. padi*), which is known to be influenced by changing rainfall patterns (Thackray *et al.* 2009). Like many other countries, Australia has also seen a considerable shift in the adoption of neonicotinoid seed treatments, which has reduced the reliance on foliar applications of synthetic pyrethroids and other broad-spectrum insecticides for early season aphid/virus control (Bass *et al.* 2015; Attwood & Paisley 2019; Umina *et al.* 2019).

In our study, the several*R. padi* populations were not fully controlled by alpha-cypermethrin applied at the recommended field rate of 125 mg a.i./L. However, insecticide rates applied in laboratory bioassays do not necessarily indicate that these populations are resistant under field conditions;laboratory conditions provide a standardised environment while field populations are typically under more stress, which can impact both survival and chemical responses (Cahill *et al.* 1996; Iwasaki *et al.* 2018;Heye*et al.* 2019). Furthermore, exposure to insecticides when applied in the field will be considerably differentto what aphidsexperience when tested through laboratory bioassays.For example, aphid mortality was assessed 48 h after chemical exposurein our experiments. However, products like alpha-cypermethrincan persist in the environment for many weeks and thus the exposure period for aphids is likely to be substantially longer in the field. For the remaining insecticides, our findings are similar to those reported by Clouston *et al.* (2016). We found little variation in *R. Padi* sensitivity to dimethoate, pirimicarb andimidacloprid among populations, and the overall LC_{50} values are comparable between studies. The imidacloprid results, in particular, were very similar between those we found (8.72 ± 1.86 mg a.i./L) and those estimated by Clouston *et al.* (2016) (9.56 ±1.73 mg a.i./L).

There was considerable variation in responses of *R. padi* to alpha-cypermethrin, with reduced sensitivityto this chemical product identified in three populations. Aphids from MNJ, PIC and TMW had LC_{50} values 26-, 27- and 41-fold (respectively) greater than aphids from the most sensitive population, KAT. Despite this, it appears there has been an overall increase in sensitivity to alpha-cypermethrin across Australia, which may reflect changes in insecticide use. For the other products, we found a relatively consistent level of insecticide toxicity across *R. padi* field populations. The toxicity data we have generated are now available for comparison to those obtained in any future monitoring activities. Importantly, they have been established before field resistance has evolved, allowing for insecticide resistance management strategies to be developed and implemented before resistance frequencies becometoo high (see Roush 1998; Sudo *et al.* 2018). Field surveillance can now be prioritised to focus on alpha-cypermethrin and other pyrethroid chemicals, and in regions of Australia where toxicity responses in aphid populationsare approaching levels that may impact the efficacy of chemical applications in the field.

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Table 1. Sampling information of Australian *R.padi* populations used in study.

Location	**State**	**Code**	**Date collected**	**Host plant**	**Latitude**	**Longitude**
Tamworth	NSW	TMW	January 2017	*Triticumaestivum*	-31.18	150.91
Picola	Vic	PIC	March 2017	*Triticumaestivum*	-35.99	145.09
Dookie	Vic	DOK	June 2017	*Triticumaestivum*	-36.39	145.69
Pimpinio	Vic	PIM	June 2017	*Hordeum vulgare*	-36.62	142.03
Back Valley	SA	BKV	September 2017	Unknown	-35.52	138.51
Adelaide	SA	ADE	December 2017	Grass weeds	-34.97	138.63
Manjimup	WA	MNJ	August 2017	*Avenasativa*	-34.31	116.17
Capel	WA	CAP	August 2017	Grass weeds	-33.58	115.66
Katanning	WA	KAT	August 2017	*Avenasativa*	-33.70	117.57

Table 2. Insecticides tested in this study against *R.padi*.

Trade Name	**Active ingredient**	**Chemical group**	**Formulation**	**Supplier**
Nuprid® 200SC insecticide	imidacloprid	Neonicotinoid	200 g/L SC	NuFarm Australia
Pirimor®WGaphicide	pirimicarb	Carbamate	500 g/L WG	Syngenta Australia
Dimethoate 400SC®	dimethoate	Organophosphate	400 g/L SC	NuFarm Australia
Astound® Duo insecticide	alpha-cypermethrin	Synthetic pyrethroid	100 g/L EC	NuFarm Australia

SC = suspension concentrate, EC = emulsifiable concentrate, WG = water-dispersible granule.

Table 3. Toxicity data for*R.padi* after exposure to imidacloprid for 48 h.

Population	**No. aphids tested**	**Regression slope coefficient (±SE)**	**LC_{50} (mg a.i./L)**	**Lower-upper 95% CIs (mg a.i./L)**
TMW[a]	259	1.39 (0.65)	3.77	1.22 – 11.65
PIC[a]	263	0.78 (0.29)	5.57	1.21 – 25.68
DOK[a]	258	1.31 (0.58)	8.06	2.46 – 26.37
PIM[a]	266	1.15 (0.47)	6.81	1.97 – 23.44
BKV[a]	266	1.54 (0.75)	7.40	2.65 – 20.68
ADE[a]	255	0.86 (0.34)	21.81	5.00 – 95.16
MNJ[a]	254	1.35 (0.63)	4.20	1.29 – 13.66
CAP[a]	259	1.56 (0.70)	12.83	4.19 – 39.31
KAT[a]	257	1.16 (0.49)	8.02	2.29 – 28.08

Different letters indicate significant differences between populations ($p<0.05$).

Table 4. Toxicity data for*R.padi* after exposure to pirimicarb for 48 h.

Population	**No. aphids tested**	**Regression slope coefficient (±SE)**	**LC_{50} (mg a.i./L)**	**Lower-upper 95% CIs (mg a.i./L)**
TMW[a]	266	1.42 (0.83)	134.50	27.78 – 651.26
PIC[a]	265	1.43 (0.87)	98.67	20.35 – 478.42
DOK[a]	266	2.55 (1.97)	113.01	25.33 – 504.14
PIM[a]	269	1.69 (1.03)	139.52	30.89 – 630.17
BKV[a]	261	9.66 (7702)	51.93	NC
ADE[a]	270	10.32 (4909)	67.59	NC
MNJ[a]	258	1.99 (1.80)	38.19	10.73 – 135.82
CAP [a]	266	1.93 (1.21)	172.08	39.10 – 757.16
KAT[a]	263	2.65 (2.02)	120.47	25.82 – 562.02

Different letters indicate significant differences between populations ($p<0.05$). NC indicates 95% confidence intervals could not be computed.

Table 5. Toxicity data for*R.padi* after exposure to dimethoate for 48 h.

Population	**No. aphids tested**	**Regression slope coefficient (±SE)**	**LC_{50} (mg a.i./L)**	**Lower-upper 95% CIs (mg a.i./L)**
TMW[a]	270	1.82 (1.52)	33.59	7.15 – 157.70
PIC[a]	272	1.26 (0.84)	40.36	6.00 – 271.12
DOK[a]	278	2.37 (2.17)	39.53	8.36 – 186.90
PIM[a]	268	1.54 (1.08)	48.20	8.31 – 279.45
BKV[a]	262	1.42 (1.05)	31.65	5.34 – 187.39
ADE[a]	261	2.09 (2.10)	30.77	6.86 – 137.93
MNJ[a]	258	1.36 (0.97)	36.49	5.96 – 223.29
CAP[a]	277	1.11 (0.70)	34.50	4.86 – 244.57
KAT[a]	266	0.73 (0.43)	14.68	1.24 – 173.70

Different letters indicate significant differences between populations ($p<0.05$).

Table 6. Toxicity data for*R.padi* after exposure to alpha-cypermethrin for 48 h.

Population[1]	**No. aphids tested**	**Regression slope coefficient (±SE)**	**LC_{50} (mg a.i./L)**	**Lower-upper 95% CIs (mg a.i./L)**	**Mortality at registered rate[2]**	**RF[3]**
TMW [a]	260	1.16 (0.50)	24.57	7.51 – 80.47	91%	41.0
PIC [a]	285	1.31 (0.54)	16.22	5.74 – 45.80	100%	27.0
DOK [ab]	273	1.06 (0.38)	3.84	1.16 – 12.68	100%	-
PIM [ab]	253	0.64 (0.24)	9.90	1.88 – 52.01	100%	-
BKV [ab]	257	0.92 (0.33)	2.34	0.63 – 8.61	100%	-
ADE [ab]	279	0.66 (0.23)	7.34	1.51 – 35.64	98%	-
MNJ [a]	265	0.95 (0.37)	15.34	4.08 – 57.68	93%	25.6
CAP [ab]	261	0.98 (0.37)	9.31	2.67 – 32.39	100%	-
KAT [b]	247	0.85 (0.31)	0.60	0.14 – 2.43	100%	-

[1] Different letters indicate significant differences between the populations (p<0.05).

[2] Registered field rate equates to 125 mg a.i./L alpha-cypermethrin.

[3] Resistance Factors (RF) were calculated by dividing the population LC_{50} value by 0.60 (the lowest LC_{50} value estimated from KAT).