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Original Article

Resistance to ß-cyfluthrin in populations of the pest lesser mealworm, *Alphitobius diaperinus*

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ABSTRACT

The lesser mealworm, *Alphitobius diaperinus*, is an invasive tenebrionid beetle infesting poultry facilities worldwide. As large population outbreaks occur regularly in broiler farms, insecticides are massively sprayed to control populations in poultry houses. In some countries, there has been evidence that *A. diaperinus* can develop resistance to several classes of insecticides. Here, we evaluated the insecticide susceptibility of several *A. diaperinus* populations collected in 2018 from various poultry farms in Northern Brittany (France). The adults were exposed to increasing doses of four different insecticides (technical-grade): two pyrethroids (ß-cyfluthrin, permethrin), and two organophosphates (azamethiphos and pirimiphos-methyl). Results revealed the existence of significant resistance to ß-cyfluthrin in three out of 11 populations. One population in particular was extremely resistant to ß-cyfluthrin. For this population the highest tested dose, equivalent to 500 times the recommended application rate (i.e. 10 g pure ß-cyfluthrin per m²), was not even sufficient to induce a low level of mortality. In contrast, for permethrin, azamethiphos, or pirimiphos-methyl, the results from dose-response bioassays did not suggest the occurrence of resistance. Hence, insecticides containing ß-cyfluthrin as active ingredient, which has a long use history in France, should be avoided in poultry facilities.

KEYWORDS: pest beetle, poultry farm, insecticide, bioassay.

INTRODUCTION

The lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) is a small tenebrionid beetle prospering in poultry facilities by forming massive populations in the poultry droppings and in the litter [1]. This invasive insect of agroecosystems, nowadays cosmopolitan, was originally distributed in sub-Saharan Africa, where it usually colonizes bird nests and stored products [2]. In Europe and USA, this gregarious beetle is a major pest of poultry facilities [3-6]. This insect is listed among the most undesirable insect pests of poultry farming in many countries [7]. As these beetles can feed on the carcasses of sick dead birds, they may become vector of many diseases and pathogens, such as viruses responsible for *e.g.* Marek's and Newcastle's diseases, avian influenza, or bacteria such as *Salmonella typhimurium* and *Escherichia coli* [8-10]. More importantly, mature larvae, while searching for pupation sites, climb the walls of poultry houses and dig galleries into insulation panels often made of polystyrene [11, 12]. The resulting structural deterioration can significantly reduce the thermal

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*Corresponding author: herve.colinet@univ-rennes.fr resistance of the insulation panels, hence, increasing heating bills and causing additional building repair costs for farmers. For instance, energy costs in beetle-damaged buildings can be 67% higher than in healthy buildings [13].

Export of insect-containing litter and chemical treatments of the litter, soil and walls with insecticides are the two main control methods to limit beetle populations in poultry farms. Many biological agents that may act against the lesser mealworm are documented, but none appear to have provided sufficient or satisfying control in poultry houses [14]. In this context, the misuse and repeated application of insecticides may have resulted in the selection of insecticide-resistant populations. Nowadays, it appears that none of the available insecticides can fully control *A. diaperinus* populations and prevent outbreaks [15, 16]. Resistant populations have been reported in several countries where organophosphates, carbamates, neonicotinoids and pyrethroids were intensively used [16-20].

In France, this pest was first observed in 1977 in Brittany where it quickly colonized the region. It is now abundantly found in various farms (broiler turkeys, laying hens, chickens, pigs), with frequent outbreaks, especially in broiler turkey farms [5]. The lesser mealworm usually colonizes livestock or food storage structures in its area of introduction, but it can also be observed outside the buildings, including in cultivated areas and in natural environments [21]. The development of resistant populations is therefore an ecological concern as the adults in the wasted litter can disperse into agricultural fields and nearby houses over several kilometres [22].

In 2014, a very first assessment of insecticide resistance was conducted in France using *A. diaperinus* populations collected in poultry farms in Southern Brittany. This monitoring program showed signs of resistance to commercial insecticides, especially towards those formulated with pyrethroids [23]. Yet, resistance is a dynamic phenomenon and the underlying mechanisms may evolve over time with changes in chemical treatment use and practices. Hence, continuous monitoring is crucial to determine whether management strategies remain effective. Control failure of an insecticide relies on the evaluation of a decrease in the efficacy of a commercial product not reaching an expected control level [24]. Thus in our previous monitoring study [23] we used commercial products to detect resistance at the recommended label rate (i.e. single dose). In the current work, we adopted another complementary approach, based on dose-response assays, with new populations collected in other farms from Northern Brittany (France) in 2018. The dose-response approach allows to quantify resistance level in populations. Here, the adults were exposed to increasing doses of four technical-grade insecticides: two pyrethroids (ß-cyflutrhrin, permethrin), and two organophosphates (azamethiphos and pirimiphos-methyl). The use of technical-grade active ingredients allows to avoid confusion with the potential effect(s) caused by other compounds of the formulation, and allows cross-comparison among studies [25]. Large population outbreaks regularly occur in poultry houses in Brittany France, despite regular insecticide applications [26]. Thus, we expected to find evidence of resistance in some populations, especially towards pyrethroids. Identification of highly resistant population(s) would provide an opportunity to explore the underlying mechanisms.

MATERIALS AND METHODS

Collection of insects

Insects were collected from eleven poultry farms (Pop1 to Pop11) in October 2018 from different locations in Northern Brittany (France) (Table 1). Adults of *A. diaperinus* were hand-collected from crevices and cracks, from the litter nearby the feeders, and along the walls of the buildings. A twelfth population, designated as PopS, was used. It was formerly described to be insecticide-sensitive [23]. Indeed, individuals from PopS have been maintained in an insecticide-free insect rearing cultures for many years. Following collection, populations from farms were maintained under controlled conditions (25 ± 1 °C, constant dark, and relative humidity ranging from 55 to 70%) in incubators (Thermostat cabinet TC 255 S,

Table 1. Location of the twelve populations of *Alphitobius diaperinus* whose susceptibility to insecticides was assayed. Populations 1 to 11 (Pop 1-11) were sampled from poultry farms in Northern Brittany (France), and the insecticide-sensitive population (PopS) was kept in insecticide-free rearing cultures.

Population Id	Locality	Long. coordinates	Lat. coordinates
Pop1	Meslin (building A)	-2°35'17.542"	48°26'35.246"
Pop2	Meslin (building B)	-2°35'17.542"	48°26'35.246"
Pop3	Andel	-2°33'18.018"	48°29'34.865"
Pop4	Broons (building A)	-2°14'30.52"	48°18'48.301"
Pop5	Broons (building B)	-2°14'30.52"	48°18'48.301"
Pop6	Lanrodec	-3°2'36.863"	48°29'51.835"
Pop7	Loudéac	-2°44'39.538"	48°10'25.025"
Pop8	Miniac sur Bécherel	-1°55'46.844"	48°16'44.18"
Pop9	Guer	-2°7'41.516"	47°54'18.058"
Pop10	Pommerit-le-Vicomte	-3°5'45.11"	48°37'5.606"
Pop11	Plaine-Haute	-2°51'40.216"	48°26'46.039"
PopS	Paimpont	-2°10'45.152"	48°1'57.173"

Lovibond). Each population was maintained isolated in a plastic box (27 × 28 × 8 cm, L × W × H), whose bottom was covered with a mix of sawdust and oat bran. The insects were supplied with dry dog food *ad libitum*, pieces of carrots, and Styrofoam™ to stimulate pupation. Water was supplied in pieces of cotton wetted with tap water. Only mature adults were used for the below-described experiments.

Bioassays

The technical-grade active ingredients (AI) were solubilized in acetone and the resulting highly concentrated stock solutions were stored at 4 °C. For each insecticide, a reference dose (RD) calculated to be equivalent to recommended label rate (RLR) of the corresponding formulated product was used (see Table 2). Bioassays were conducted with various doses, higher and lower than the RD, to obtain mortality levels ranging from 0 to >50%. Insects were deprived of water and food during all the bioassays. A 5-day continuous contact exposure was chosen based on preliminary assays, as this duration ensured that the insects died from the insecticide exposure, and not from starvation or thirst. In the preliminary tests (with only evaporated acetone), no mortality was observed for >10 days of observation of the insects without food and water.

Bioassays were conducted in a room maintained at 25 ± 1 °C. Circular glass Petri dishes (90x20mm) containing insecticide-treated filter papers (63.6 cm^2) were prepared as follows: a volume of 2 mL of the acetone-dissolved AI or of acetone only (for control) was deposited on the filter paper. For each insecticide, at least six different doses were assayed: these doses were multiples (concentrated or diluted) of the RD, plus the acetone control (see Table 3). The filter paper was let drying out for 20 minutes to ensure that the acetone had evaporated before the insects were introduced into the Petri dishes. Each assay consisted of placing 10 adults of each population on the treated filter paper of the Petri dish; mortality of the insects was scored after 5 days of contact exposure. At that time, the beetles were categorized into a binary classification: a) dead (*i.e.* no visible movement of any appendage even

Table 2. Description of the four insecticides assayed. The name of the active ingredient (AI), the chemical family, the mode of action, the CAS number, the Sigma reference as well as the brand names of formulated products are provided. The reference dose (RD) was calculated based on the recommended label rate (RLR) and the percent of AI of the corresponding formulated products.

Active ingredient (AI) assayed	**ß-Cyfluthrin**	**Azamethiphos**	**Pirimiphos-methyl**	**Permethrin**
Chemical family	Pyrethroid	Organophosphate	Organophosphate	Pyrethroid
Mode of action	Inhibits voltage-gated Na^+ channel	Inhibits acetylcholinesterase	Inhibits acetylcholinesterase	Inhibits voltage-gated Na^+ channel
CAS Number of the AI	1820573-27-0	35575-96-3	29232-93-7	52645-53-1
Sigma reference of the technical-grade AI	46003	45331	32058	45614
Commercial name of the formulated product[$]	SOLFAC®10	MOUXINE TWENTY ONE	MOSCA GRAINS - PIRIGRAIN 250	TOP KILL 10
Recommended label rate (RLR) of the formulated product	0.2 $g.m^{-2}$	5 $g.m^{-2}$	0.8 $g.m^{-2}$	2 $g.m^{-2}$
Percent of AI in the formulated product	10%	10%	24%	10
Reference dose (RD)*: the amount of AI in the RLR	20 mg $AI.m^{-2}$	500 mg $AI.m^{-2}$	192 mg $AI.m^{-2}$	200 mg $AI.m^{-2}$

[$] These formulated commercial products were used to deduce a “reference dose” of AI to apply in assays.
*This amount of technical-grade AI (per surface unit) was used as the “reference dose” in the bioassays, and the other tested doses were multiples of this reference dose.

Table 3. Active ingredient (AI) concentration ranges tested in bioassays.

Active Ingredient	**Reference Dose (RD)**	**Tested concentrations expressed as a multiple of the RD**						
ß-Cyfluthrin[$]	20 mg $AI.m^{-2}$	0	0.25	0.5	1	5	10	20
Azamethiphos	500 mg $AI.m^{-2}$	0	0.1	0.25	0.5	1	2	5
Pirimiphos-methyl	192 mg $AI.m^{-2}$	0	0.01	0.02	0.05	0.1	0.5	1
Permethrin	200 mg $AI.m^{-2}$	0	0.25	0.5	1	5	10	20

[$] For ß-cyfluthrin, some populations did not suffer high mortality even at 20-fold RD; hence in these few cases, doses of 100- or 500-fold RD had to be applied.

after mechanical stimulation) or b) alive (*i.e.* walking and fit or moribund but alive). For each insecticide assayed, a minimum of 140 individuals was required to test the six doses, plus the control, with two independent replicates for each dose. For ß-cyfluthrin and azamethiphos, all the populations (*i.e.* 11 from poultry farms, plus one sensitive) could be tested. For pirimiphos-methyl and permethrin, however, in some populations the number of insects was insufficient to perform all tests. For pirimiphos-methyl and permethrin, we thus tested eight and seven different farm populations, respectively.

Statistical analyses

Analyses were conducted and figures were designed using R-studio (R version 3.6.2). Changes in mortality were analyzed using generalized linear models (GLMs) with logit link function for binary outcome (dead/alive beetles). The models were applied for each AI separately with "population" and "dose" as explanative variables. The effects of the variables were assessed *via* an analysis of deviance ("Anova" function in 'car' R package) [27]. Then, for each population separately, the lethal concentration 50 (*i.e.* dose that kills 50%, LC50) was individually computed as follows: LC50 = (logit (0.5) – a) / b, where a and b correspond respectively to the intercept and the slope of GLM prediction [28]. The estimated LC50 values were obtained using the "dose.p" function (setting a 0.5 probability) in the 'MASS' R package. Model parameters such as estimated LC50, their fiducial limits (FL), slope and intercept were also confirmed using the "LC_Logit" function in the 'ecotox' R package. The resistance ratios (RR) were calculated as the ratio of the LC50 of the farm populations (Pop1 to 11) over the LC50 of the susceptible population (PopS) [29]. To compare LC values, ratio tests were performed using the 'ecotox' R package [30]. To show the resistance level in clearer biological meanings, the RRs were classified into five levels according to Ma *et al.* [29]: 1) susceptible: $RR \leq 1$, 2) tolerance/low resistance: $1 < RR < 10$, 3) moderate resistance: $10 \leq RR < 100$, 4) high resistance: $100 \leq RR < 1000$, 5) extremely high resistance: $RR \geq 1000$.

RESULTS

For ß-cyfluthrin, we observed large differences in mortality among populations (F=232.95, df=11, P < 0.001) (Figure 1). The dose effect was significant (F=37.347, df=1, P < 0.001) indicating increasing mortality with increasing doses. For each population, the LC50 value was estimated from logit models. A summary of estimated LC50 values, with their fiducial limits and corresponding RR is provided in Table 4. A comprehensive table with all model parameters, their statistical outputs and the ratio tests is available in Table S1. Among the 11 farm populations assayed with ß-cyfluthrin, three showed tangible signs of resistance: Pop7, Pop8 and Pop11. Beetles from these three populations had LC50 values much higher than the value obtained for the susceptible population (PopS), and thus, AI doses had to be increased to 100-fold the RD to generate a level of mortality that allowed computing an accurate estimate of LC50. Insects from Pop8 were surprisingly resistant. For this population, a dose of 500-fold the RD was even tested. Even at this extremely high AI concentration, we could not observe any significant mortality (10% only). Therefore, for this particular population, the estimated LC50 was extrapolated from the model because 50% mortality was not reached.

For azamethiphos, pirimiphos-methyl, and permethrin, we also observed significant differences among populations (azamethiphos: F=205.42, df=11, P < 0.001; pirimiphos-methyl: F=105.06, df=8, P < 0.001; permethrin: F=73.49, df=7, P < 0.001) (Figures 2 to 4). Likewise, the dose effect was significant in all cases, indicating increased mortality with increasing doses (azamethiphos: F=445.34, df=1, P < 0.001; pirimiphos-methyl: F=1046.77, df=1, P < 0.001; permethrin: F=163.36, df=1, P < 0.001). Despite differences among populations, and unlike ß-cyfluthrin, for azamethiphos, pirimiphos-methyl, and permethrin we did not observe marked deviations of LC50 values from that of PopS (Table 4; Table S1). This led to rather low RR values, at least not reaching 10-fold, and thus suggesting the tolerance or low resistance according to Ma *et al.* (2021).

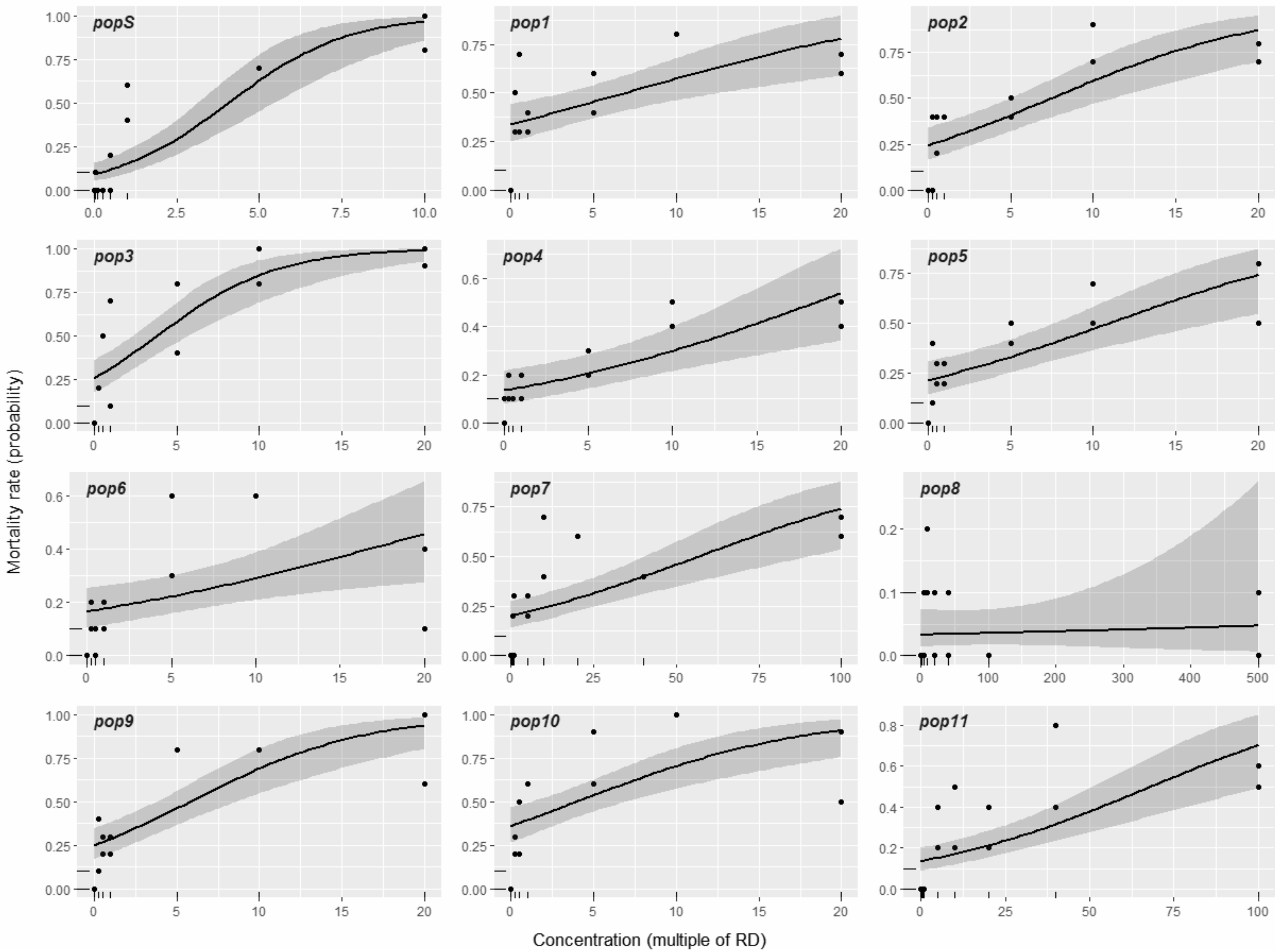

Figure 1. Probability of mortality of 12 different *A. diaperinus* populations exposed to increasing concentrations of the active ingredient ß-cyfluthrin. Populations 1 to 11 were hand-collected from poultry farms in Northern Brittany (France). The first population (PopS) is susceptible to insecticides. For each population, the solid line shows the prediction of logit model and the shaded areas indicate the 95% credible intervals. Tested concentrations of ß-cyfluthrin are expressed as a multiple of the reference dose (RD) (see Tables 2 and 3).

Table 4. Median lethal concentration (LC50) for each population and active ingredient expressed as multiple of the reference dose (RD) (see Table 2).

Active ingredient	Population	LC50	L-F[a]	U-F[b]	RR[c]
ß-Cyfluthrin	PopS	4.07	2.49	7.83	1.0
	Pop1	6.93	-0.66	34.00	1.7
	Pop2	7.46	3.73	14.90	1.8
	Pop3	3.80	0.80	9.82	0.9
	Pop4	18.50	14.00	28.60	4.5
	Pop5	10.90	6.81	20.80	2.7
	Pop6	22.40	11.10	-43.40	5.5
	Pop7	56.60	31.70	174.00	13.9
	Pop8	4595	630	-652	1129
	Pop9	5.80	1.19	18.40	1.4
	Pop10	3.99	1.30	6.93	1.0
	Pop11	68.20	41.70	175.00	16.8
Azamethiphos	PopS	1.30	1.06	1.67	1.0
	Pop1	1.64	0.67	4.53	1.3
	Pop2	3.47	2.58	5.18	2.7
	Pop3	3.67	2.64	5.90	2.8
	Pop4	1.16	0.34	2.63	0.9
	Pop5	2.05	0.91	5.37	1.6
	Pop6	2.03	1.13	4.52	1.6
	Pop7	4.06	3.21	5.47	3.1
	Pop8	2.97	2.27	4.18	2.3
	Pop9	2.82	1.77	5.72	2.2
	Pop10	0.30	0.20	0.44	0.2
	Pop11	0.79	0.52	1.26	0.6
Pirimiphos-methyl	PopS	0.02	0.01	0.04	1.0
	Pop1	0.06	0.04	0.08	3.2
	Pop2	0.09	0.07	0.20	5.0
	Pop4	0.09	0.09	0.10	5.0
	Pop6	0.11	0.09	0.16	5.9
	Pop7	0.16	0.10	0.79	8.7
	Pop8	0.09	0.08	0.10	4.9
	Pop10	0.06	0.04	0.09	3.2
	Pop11	0.04	0.03	0.04	2.0

Table 4 continued..

Permethrin	PopS	8.45	7.24	10.10	1.0
	Pop1	15.60	10.70	29.60	1.8
	Pop2	15.90	8.53	275.00	1.9
	Pop6	7.77	1.49	30.60	0.9
	Pop7	20.50	15.40	33.50	2.4
	Pop8	22.30	16.40	42.00	2.6
	Pop10	13.50	8.00	36.00	1.6
	Pop11	14.40	9.30	30.80	1.7

[a]L-FL: lower fiducial limit; [b]U-FL: upper fiducial limit; [c]RR: resistance ratio as in Ma *et al.*, 2021.

DISCUSSION

The use of synthetic insecticides, although highly restrictive and only partially effective, remains the primary means of controling population's outbreaks of the lesser mealworm in poultry facilities. Behavioral avoidance may limit the contact of insects with insecticides, as *A. diaperinus* adults and larvae may hide in the litter, cracks and crevices of the farm buildings, thus decreasing the efficacy of chemical control [31]. Moreover, the reduced effectiveness of the treatments suggests the development of insecticide resistance in some French populations [23, 26]. Resistance to fenitrothion and permethrin has been reported in populations from UK [32], and to fenitrothion, deltamethrin and cyfluthrin in Australia [15, 33, 34]. In USA, resistance to several pyrethroids (cyfluthrin, permethrin, cypermethrin), organochloride (DTT), and organophosphates (tetrachlorvinphos, and chlorpyrifos) has also been reported [16, 18, 35]. This increasing number of studies reporting insecticide resistance in *A. diaperinus* populations is alarming as this invasive pest, normally restricted to broiler facilities, may colonize natural environments in temperate regions with uncertain ecological and economic consequences. Previous studies support this assumption, with several field observations of the species in cultivated and forest environments in France and other countries [21, 36, 37].

In the present work, we evaluated the insecticide susceptibility of various populations of *A. diaperinus* collected in 2018 from several poultry farms of Northern Brittany by exposing them to increasing doses of four different AI. Results revealed the existence of significant moderate to high resistance to ß-cyfluthrin (RR > 10, see Ma *et al.*, [29]) in three out of 11 populations. One population (Pop8) appeared extremely resistant to this AI. Resistance to pyrethroid has previously been reported in populations from Australia and USA [33, 35]. There is a correlation between the number of cyfluthrin applications and the level of resistance [34]. A cyfluthrin-based formulation, for which SOLFAC®10 was granted a patent, was massively sprayed in poultry farms in Brittany since the 90's. The recommended label rate of SOLFAC®10 is 0.2 $g.m^{-2}$, and with 10% AI in the formulated product, the amount of AI corresponds to 20 $mg.m^{-2}$. For Pop8, application of 500 times this dose (*i.e.* 10 g pure ß-cyfluthrin per m^2) was not even sufficient to start inducing a low mortality (10%), evidencing a very high level of resistance. In a previous field survey conducted in 2014, we tested other populations with commercial formulations at their label rates, and we also detected signs of resistance to cyfluthrin [23]. Hence, based on a different experimental approach (i.e. dose-response with pure AI) and different populations, the present study, underscores the existence of various degrees of resistance to ß-cyfluthrin, with some populations being potentially extremely resistant. The reduced efficiency of ß-cyfluthrin in populations from Brittany is consistent with the general

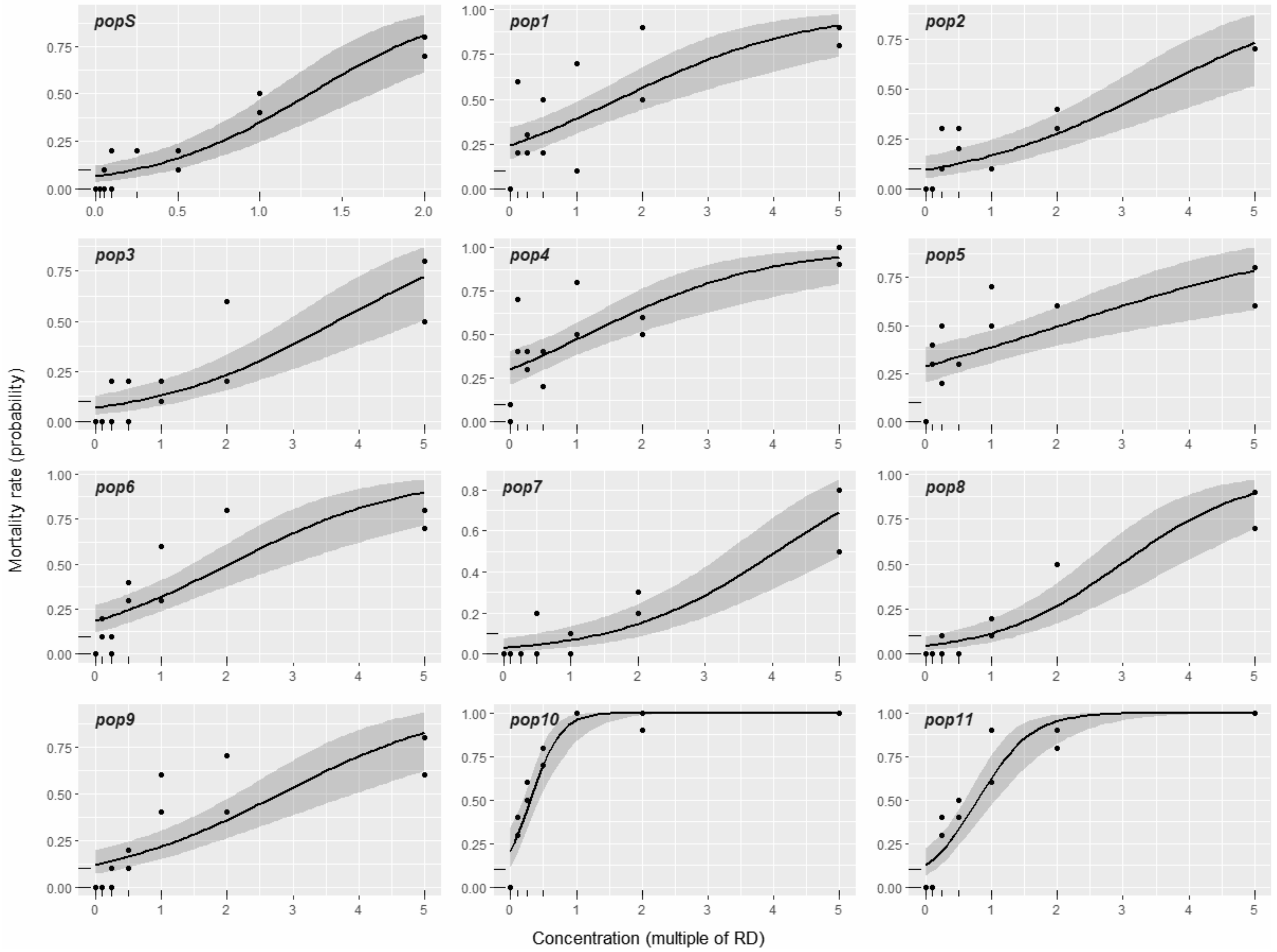

Figure 2. Probability of mortality of 12 different *A. diaperinus* populations exposed to increasing concentrations of the active ingredient azamethiphos. Populations 1 to 11 were hand-collected from poultry farms in Northern Brittany (France). The first population (PopS) is susceptible to insecticides. For each population, the solid line shows the prediction of logit model and the shaded areas indicate the 95% credible intervals. Tested concentrations of ß-cyfluthrin are expressed as a multiple of the reference dose (RD) (see Tables 2 and 3).

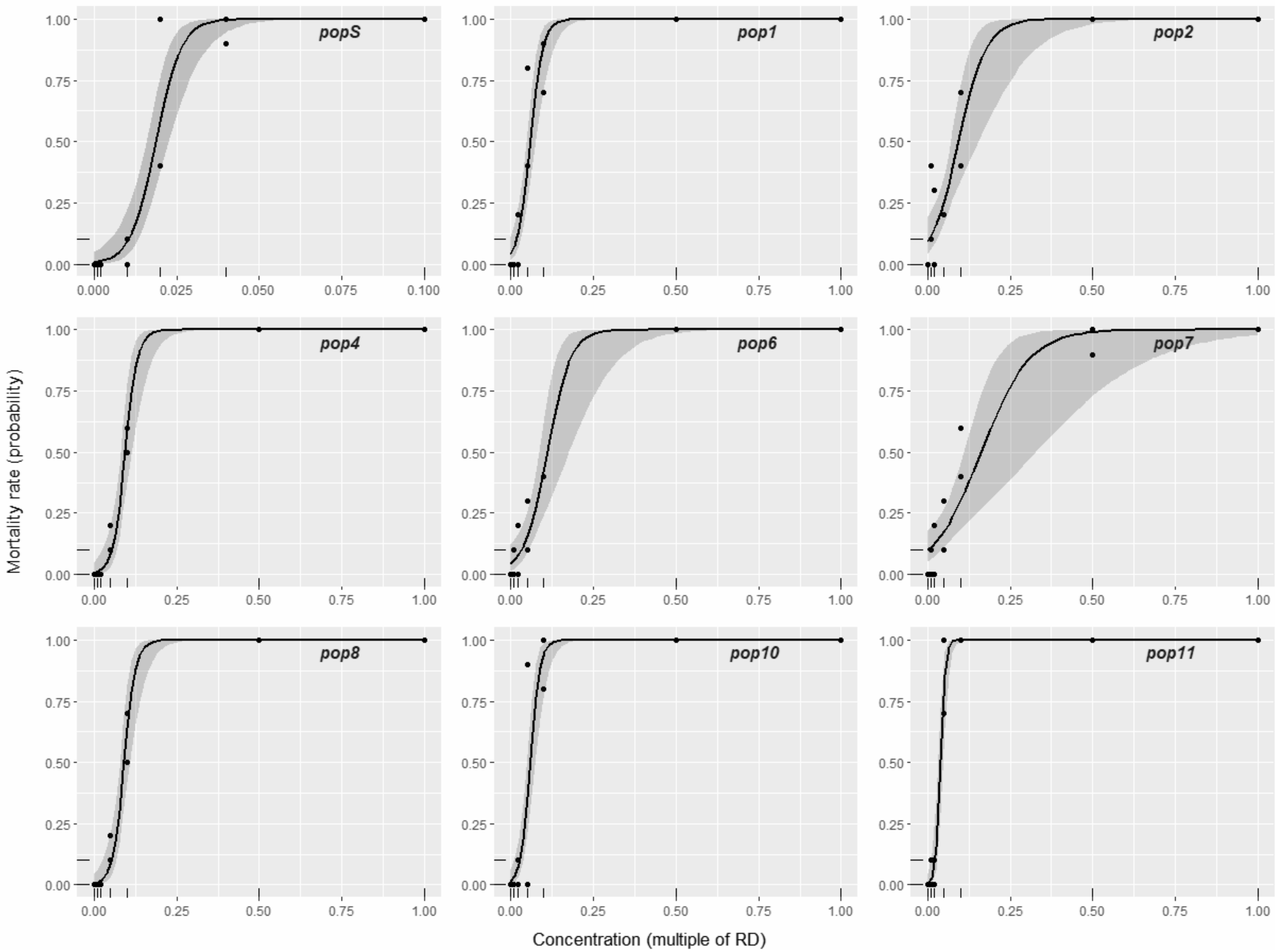

Figure 3. Probability of mortality of 9 different *A. diaperinus* populations exposed to increasing concentrations of the active ingredient pirimiphos-methyl. Populations 1 to 11 were hand-collected from poultry farms in Northern Brittany (France). The first population (PopS) is susceptible to insecticides. For each population, the solid line shows the prediction of logit model and the shaded areas indicate the 95% credible intervals. Tested concentrations of ß-cyfluthrin are expressed as a multiple of the reference dose (RD) (see Tables 2 and 3).

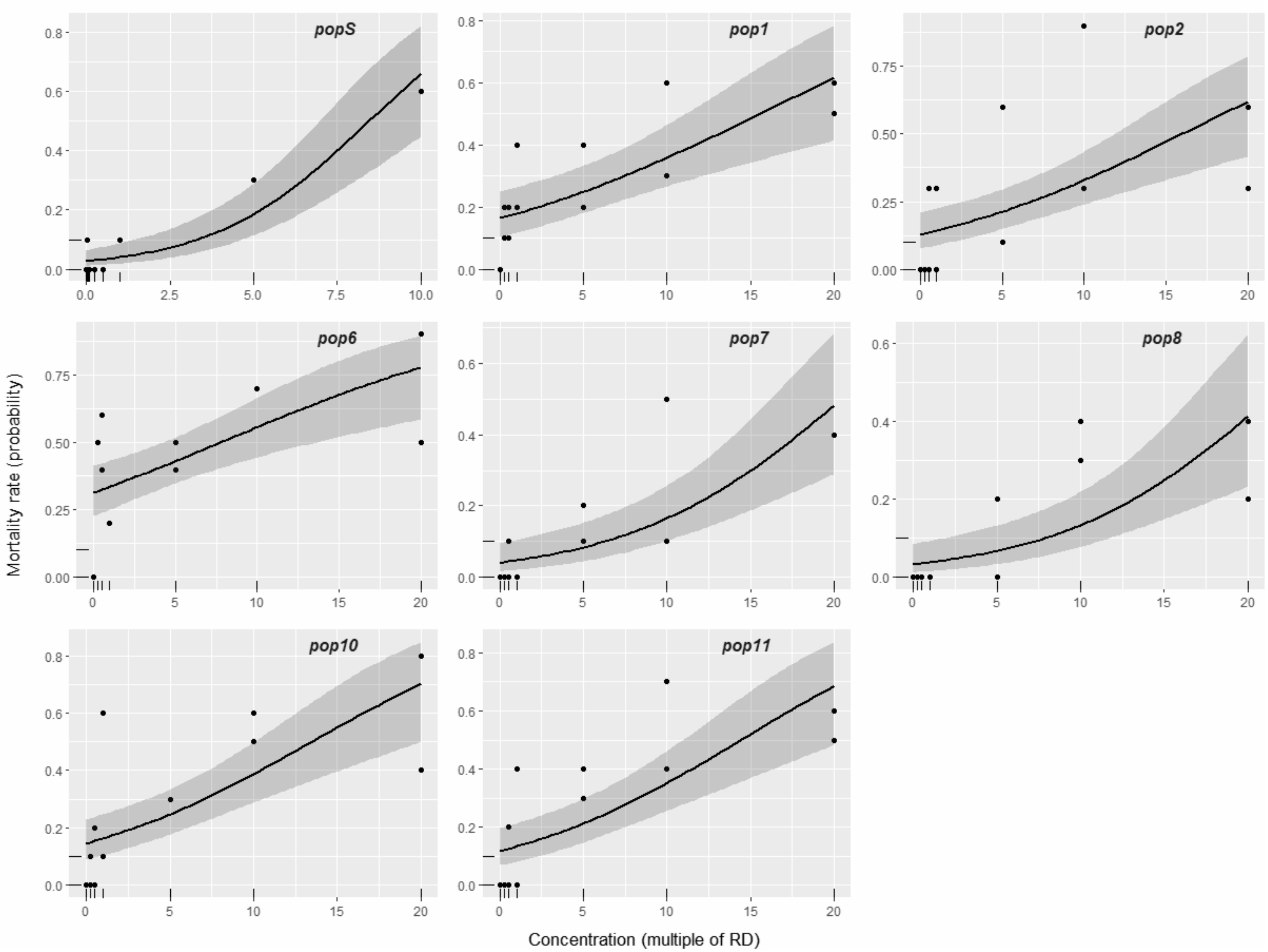

Figure 4. Probability of mortality of 8 different *A. diaperinus* populations exposed to increasing concentrations of the active ingredient permethrin. Populations 1 to 11 were hand-collected from poultry farms in Northern Brittany (France). The first population (PopS) is susceptible to insecticides. For each population, the solid line shows the prediction of logit model and the shaded areas indicate the 95% credible intervals. Tested concentrations of ß-cyfluthrin are expressed as a multiple of the reference dose (RD) (see Tables 2 and 3).

use of SOLFAC®10 in poultry farms since the 90's. In June 2020, the European commission decided the non-renewal of the approval of the active ingredient ß-cyfluthrin (Commission Implementing Regulation EU 2020/892 of 29 June 2020 [38]), but the presence of resistant populations is a concern as it may lead to cross-resistance with insecticides having the same – or similar – modes of action or targets [39]. Yet, in the current study, we did not detect sign of resistance to the other pyrethroid assayed: permethrin. Similarly, Lambkin and Furlong [33] did not evidenced cross-resistance in populations tested with four pyrethroids.

For the two organophosphates assayed here, azamethiphos and pirimiphos-methyl, we found that *A. diaperinus* died at doses close to, or even inferior to RD. This suggests high efficiency (toxicity) of both AIs even without adjuvant. The results of the bioassays with azamethiphos, pirimiphos-methyl did not suggest the occurrence of insecticide-resistance in the tested populations. These observations are consistent with our previous survey in which we did not identify signs of resistance to commercial products formulated with organophosphate [23]. There are numerous products on the market that claim to be efficient against lesser mealworm outbreaks. Many of these products may gradually lose their insecticidal properties after repeated uses, and decreased insecticide effectiveness can occur quite quickly. The first reported case of insecticide resistance in *A. diaperinus* was reported by Cogan *et al.* [36] in the UK. They reported that after a few applications of iodofenphos, fenitrothion, permethrin or azamethiphos, all chemicals but azamethiphos were found to be inefficient for suppressing *A. diaperinus* populations. So, despite organophosphates seem to remain efficient in populations assayed in France, continuous use should be avoided because resistance may also develop within this chemical family [16, 18, 35, 36].

CONCLUSION

Large population outbreaks regularly occur in poultry houses in Brittany France, despite regular insecticide applications [26]. Thus, we expected to find evidence of resistance. We identified resistant populations including one (Pop8) that was particularly resistant to ß-cyfluthrin. Comparisons of metrics such as LC50s or RR among populations provide a valuable way to highlight the degree of susceptibility to insecticides. However, these metrics do not provide any clue on the underlying genetic mechanisms [40]. Concerning the resistance to pyrethroids in the lesser mealworm, it seems that metabolic mechanisms partially explain resistance [33], but other underlying mechanisms are not yet known. Our next objective is now to precisely explore these mechanisms using RNA sequencing in that population (Pop8) extremely resistant to ß-cyfluthrin. The determination of the mechanisms underlying insecticides' resistance could greatly improve our ability to predict and thus manage the potential loss of the effectiveness of insecticides in poultry facilities. There is also a need to understand the ecological impact of moving litter containing resistant beetles when it is spread into agricultural fields and pasture.

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AUTHOR CONTRIBUTIONS

Insects' collection: NR; conceptualization: HC & DR; experimentations: HC, ADL, DR; data analyses & visualization: HC; draft preparation HC; draft revision and interpretation: DR, GG & HC; funding acquisition: HC & NR.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Table S1. Statistical outputs of all LC-Logit models used to estimate LC50 values in the ecotox R package.
Left part: The table provides predicted LC for p = 0.5, lower fiducial confidence limit (L-FL), upper fiducial confidence limit (U-FL), Pearson's chi square goodness-of-fit test (pgof), slope, intercept, slope and intercept significance (sig) and their standard errors (se).
Right part: The table gives the statistical output of ratio tests in the ecotox R package: it compares two LC values from two separate logit models with: dose_A (LC50 of popS) vs. dose_B (LC50 of farm pop x).

Active ingredient	Population	LC50	L-FL	U-FL	se	chi²	df	pgof_sig	slope	slope_se	slope_sig	intercept	intercept_se	intercept_sig	dose_A	dose_B	se	ratio_test	p_value
Cyfluthrin	popS	4.07	2.49	7.83	0.17	393.00	14	4.08E-75	0.56	0.03	1.55E-82	-2.29	0.10	1.37E-123	/	/	/	/	/
Cyfluthrin	pop1	6.93	-0.66	34.00	0.62	245.00	12	1.24E-45	0.10	0.01	1.42E-27	-0.67	0.07	2.92E-21	4.07	6.93	0.31	1.71	8.75E-02
Cyfluthrin	pop2	7.46	3.73	14.90	0.45	215.00	12	2.82E-39	0.15	0.01	5.53E-49	-1.13	0.08	6.31E-49	4.07	7.46	0.23	2.62	8.89E-03
Cyfluthrin	pop3	3.80	0.80	9.82	0.25	337.00	12	9.08E-65	0.28	0.02	3.36E-54	-1.06	0.08	4.70E-40	4.07	18.50	0.24	6.24	4.27E-10
Cyfluthrin	pop4	18.50	14.00	28.60	1.19	65.90	12	1.86E-09	0.10	0.01	2.48E-30	-1.86	0.09	1.23E-88	4.07	18.50	0.24	6.24	4.27E-10
Cyfluthrin	pop5	10.90	6.81	20.80	0.65	158.00	12	1.63E-27	0.12	0.01	5.68E-40	-1.29	0.08	8.81E-60	4.07	10.90	0.23	4.28	1.90E-05
Cyfluthrin	pop6	22.40	11.10	-43.40	2.04	305.00	12	5.24E-58	0.07	0.01	2.29E-17	-1.62	0.09	6.76E-77	4.07	22.40	0.32	5.36	8.43E-08
Cyfluthrin	pop7	56.60	31.70	174.00	3.37	394.00	16	5.81E-74	0.02	0.00	4.54E-44	-1.39	0.07	3.01E-94	4.07	56.60	0.23	11.40	4.09E-30
Cyfluthrin	pop8	4595.00	630.00	-652.00	4520	197.00	18	4.65E-32	0.00	0.00	3.18E-01	-3.37	0.14	1.04E-134	4.07	4595.00	3.11	2.26	2.39E-02
Cyfluthrin	pop9	5.80	1.19	18.40	0.35	400.00	12	4.72E-78	0.19	0.01	9.89E-54	-1.10	0.08	6.09E-46	4.07	5.80	0.23	1.52	1.29E-01
Cyfluthrin	pop10	3.99	1.30	6.93	0.41	525.00	12	1.15E-104	0.14	0.01	1.43E-38	-0.57	0.07	5.51E-16	4.07	3.99	0.35	0.06	9.54E-01
Cyfluthrin	pop11	68.20	41.70	175.00	3.53	411.00	16	1.61E-77	0.03	0.00	3.06E-53	-1.86	0.08	1.60E-128	4.07	68.20	0.21	13.30	2.11E-40

Table S1 continued..

Azamethiphos	popS	1.30	1.06	1.67	0.05	110.00	14	5.89E-17	2.07	0.11	4.30E-79	-2.69	0.11	9.84E-130	/	/	/	/	/
Azamethiphos	pop1	1.64	0.67	4.53	0.10	293.00	12	1.35E-55	0.70	0.05	1.03E-44	-1.14	0.08	4.24E-49	1.30	1.64	0.07	3.26	1.11E-03
Azamethiphos	pop2	3.47	2.58	5.18	0.16	129.00	12	1.04E-21	0.65	0.04	6.60E-59	-2.27	0.10	1.16E-108	1.30	3.47	0.06	17.00	4.03E-65
Azamethiphos	pop3	3.67	2.64	5.90	0.15	190.00	12	3.56E-34	0.71	0.04	1.03E-64	-2.61	0.12	1.62E-114	1.30	3.67	0.05	18.90	6.44E-80
Azamethiphos	pop4	1.16	0.34	2.63	0.09	214.00	12	3.72E-39	0.73	0.06	2.13E-39	-0.85	0.08	1.46E-29	1.30	1.16	0.08	1.40	1.61E-01
Azamethiphos	pop5	2.05	0.91	5.37	0.15	186.00	12	2.71E-33	0.44	0.04	8.67E-31	-0.90	0.07	3.84E-35	1.30	2.05	0.08	5.55	2.94E-08
Azamethiphos	pop6	2.03	1.13	4.52	0.11	266.00	12	7.75E-50	0.74	0.05	1.16E-51	-1.49	0.08	3.04E-72	1.30	2.03	0.06	6.92	4.66E-12
Azamethiphos	pop7	4.06	3.21	5.47	0.14	133.00	12	1.30E-22	0.86	0.05	1.18E-72	-3.48	0.16	1.21E-108	1.30	4.06	0.05	22.80	1.55E-115
Azamethiphos	pop8	2.97	2.27	4.18	0.11	143.00	12	1.24E-24	1.03	0.06	2.86E-75	-3.06	0.13	3.14E-119	1.30	2.97	0.05	15.80	6.45E-56
Azamethiphos	pop9	2.82	1.77	5.72	0.13	271.00	12	6.20E-51	0.71	0.04	9.66E-60	-1.99	0.09	3.98E-100	1.30	2.82	0.06	13.10	2.42E-39
Azamethiphos	pop10	0.30	0.20	0.44	0.17	70.50	12	2.60E-10	4.43	0.31	5.37E-47	-1.34	0.11	1.78E-33	1.30	0.30	0.07	21.50	4.55E-102
Azamethiphos	pop11	0.79	0.52	1.26	0.03	195.00	12	3.52E-35	2.46	0.15	8.49E-60	-1.94	0.11	3.53E-69	1.30	0.79	0.06	8.88	6.64E-19
Pirimiphos-methyl	popS	0.02	0.01	0.04	0.00	367.00	12	4.58E-71	265.00	18.30	2.03E-47	-4.96	0.32	5.26E-53	/	/	/	/	/
Pirimiphos-methyl	pop1	0.06	0.04	0.08	0.00	162.00	12	1.63E-28	53.70	3.18	8.06E-64	-3.16	0.17	1.00E-75	0.02	0.06	0.13	8.55	1.25E-17
Pirimiphos-methyl	pop2	0.09	0.07	0.20	0.01	157.00	12	2.63E-27	24.00	2.11	4.70E-30	-2.26	0.13	9.37E-64	0.02	0.09	0.19	8.51	1.73E-17
Pirimiphos-methyl	pop4	0.09	0.09	0.10	0.00	26.50	12	9.15E-03	53.30	3.89	8.58E-43	-5.02	0.33	3.66E-51	0.02	0.09	0.11	14.10	2.67E-45

Table S1 continued..

Pirimiphos-methyl	pop6	0.11	0.09	0.16	0.01	70.50	12	2.60E-10	28.50	2.52	1.60E-29	-3.13	0.18	1.26E-65	0.02	0.11	0.18	10.00	1.09E-23
Pirimiphos-methyl	pop7	0.16	0.10	0.79	0.01	202.00	12	1.34E-36	13.70	1.30	8.30E-26	-2.22	0.11	1.22E-86	0.02	0.16	0.25	8.71	2.98E-18
Pirimiphos-methyl	pop8	0.09	0.08	0.10	0.00	30.70	12	2.22E-03	56.30	3.96	4.81E-46	-5.12	0.34	5.94E-52	0.02	0.09	0.11	14.10	3.72E-45
Pirimiphos-methyl	pop10	0.06	0.04	0.09	0.00	255.00	12	1.05E-47	70.20	4.38	8.90E-58	-4.15	0.24	3.02E-69	0.02	0.06	0.13	9.17	4.72E-20
Pirimiphos-methyl	pop11	0.04	0.03	0.04	0.00	76.80	12	1.65E-11	138.00	8.70	7.84E-57	-5.22	0.34	7.87E-52	0.02	0.04	0.12	5.83	5.38E-09
permethrin	popS	8.45	7.24	10.10	0.27	91.40	16	1.35E-12	0.43	0.02	3.72E-82	-3.62	0.15	5.68E-128	/	/	/	/	/
permethrin	pop1	15.60	10.70	29.60	0.95	127.00	12	2.22E-21	0.11	0.01	5.61E-34	-1.63	0.09	3.20E-79	8.45	15.60	0.22	2.81	5.00E-03
permethrin	pop2	15.90	8.53	275.00	0.86	424.00	12	2.44E-83	0.12	0.01	6.30E-41	-1.91	0.09	1.12E-91	8.45	15.90	0.20	3.21	1.33E-03
permethrin	pop6	7.77	1.49	30.60	0.61	247.00	12	4.90E-46	0.10	0.01	1.81E-30	-0.79	0.07	3.01E-28	8.45	7.77	0.27	0.31	7.54E-01
permethrin	pop7	20.50	15.40	33.50	0.89	148.00	12	1.54E-25	0.16	0.01	2.96E-45	-3.18	0.15	1.95E-100	8.45	20.50	0.17	5.18	2.20E-07
permethrin	pop8	22.30	16.40	42.00	1.03	170.00	12	5.59E-30	0.15	0.01	4.13E-39	-3.41	0.17	1.52E-94	8.45	22.30	0.18	5.45	5.01E-08
permethrin	pop10	13.50	8.00	36.00	0.69	292.00	12	2.77E-55	0.13	0.01	4.70E-47	-1.77	0.09	3.10E-86	8.45	13.50	0.19	2.45	1.41E-02
permethrin	pop11	14.40	9.30	30.80	0.69	250.00	12	1.49E-46	0.14	0.01	1.11E-50	-2.00	0.10	9.72E-96	8.45	14.40	0.18	2.94	3.31E-03

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