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Susceptibility of the Lesser Mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), From Broiler Farms of Southern Brazil to Insecticides

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Abstract

The lesser mealworm, *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera: Tenebrionidae), is considered the primary insect pest in broiler farms in Brazil. In this study, we characterized the susceptibility of *A. diaperinus* populations from broiler farms of southern Brazil to cypermethrin and chlorpyrifos. Larvae and adults of *A. diaperinus* were exposed to these technical insecticides diluted in acetone in residual bioassays. A geographic variation in the susceptibility of larvae and adults of *A. diaperinus* to both insecticides was detected. The larval LC_{50} for cypermethrin ranged from 0.43 to 7.33 µg a.i./cm^2. Two populations from Santa Catarina state presented higher resistance ratios of 13.6- and 17-fold. When adults were exposed to cypermethrin, the LC_{50} ranged from 0.46 to 4.93 µg a.i./cm^2, with population SC-3 from Santa Catarina having lower susceptibility (resistance ratio of 10.7-fold). When exposed to chlorpyrifos, *A. diaperinus* larvae present LC_{50} values ranging from 0.21 to 4.30 µg a.i./cm^2. Larvae from Paraná and Santa Catarina (SC-1 population) presented the highest resistance ratios, ranging from 10- to 20-fold. In adults, the LC_{50} of chlorpyrifos ranged from 0.17 to 5.30 µg a.i./cm^2, showing a maximum resistance ratio of 31-fold in a population from Paraná state. Based on LC_{99} values, candidate diagnostic concentrations of 15 and 12 µg a.i./cm^2 of cypermethrin and chlorpyrifos, respectively, were also estimated for the resistance monitoring of *A. diaperinus* in Brazil. The implications of these results in Insect Resistance Management are discussed.

Key words: poultry, lesser mealworm, pyrethroid, organophosphate, insecticide resistance

The lesser mealworm, *Alphitobius diaperinus* (Panzer, 1797) (Coleoptera: Tenebrionidae), is a key pest species of the poultry industry worldwide (Axtell 1999, Lambkin 2011). In broiler farms, species is abundant where food and suitable temperature provide favorable environmental conditions for its development and rapid proliferation (Chernaki-Leffer et al. 2002). This species causes damage to the structure of poultry operations by boring through wood in flooring and building insulation, due to the darkling beetle behavior of building galleries (Despins et al. 1987). However, the main economic losses are the beetles' effects on the development of the poultry. Poultry ingest the beetles, which replaces the intake of the nutritionally balanced poultry feed, reducing their weight gain (Despins and Axtell 1995). The ingestion of beetles can also transmit diseases, such as avian sarcoma leukosis virus (Eidson et al. 1966), bursal disease virus (McAllister et al. 1995), and Coronavirus (Watson et al. 2000). Beetles can also transmit several bacteria, such as *Micrococcus* sp., *Streptococcus* sp., *Corynebacterium* sp., *Staphylococcus aureus*, *Proteus mirabilis*, *Paracolobactrum intermedium*, *Escherichia coli*, and *Salmonella typhimurium*, among others (De Las Casas et al. 1972; McAllister et al. 1994, 1996; Goodwin and Waltman 1996). It is estimated that this insect causes annual losses of more than $9 million to the poultry industry in Georgia alone, the largest broiler producing state in United States (Guillebeau et al. 2008).

The current control tactics used against *A. diaperinus* in broiler farms normally have limited efficacy. In an attempt to reduce population densities, poultry bedding removal and a period of poultry absence are recommended (Hamm et al. 2006). Another control tactic is to cover the poultry bedding with tarpaulin to promote fermentation, thus raising the temperature above levels critical to beetle survival (Salin et al. 1998). These methods have limited efficacy, since they do not reach all insects present, due to some insects surviving in galleries in the farm structure, favoring new infestations. Alternative control strategies, such as biopesticides and entomopathogenic fungi have some efficacy against *A. diaperinus* but are rarely used in broiler farms (Chernaki-Leffer et al. 2007, Rezende et al. 2009, Szczepanik et al. 2016). Though some of these management strategies reduce the infestation of this species, the use of insecticides remains the main control strategy (Oliveira et al. 2016). Currently, the insecticides registered for application in poultry facilities in Brazil include pyrethroids and organophosphates that contain the active ingredients cypermethrin and chlorpyrifos, respectively. These insecticides are widely used, isolated or in mixture, in commercial formulated products against *A. diaperinus* (Silva et al. 2007, Oliveira et al. 2016). This reduced number of insecticides registered for use against this species favors the evolution of resistance.

Resistance to insecticides is a result of the chemical control of *A. diaperinus* worldwide. Resistance of this species to the insecticides fenitrothion and permethrin has been reported in turkey farms in the United Kingdom (Cogan et al. 1996) and to fenitrothion, deltamethrin, cyfluthrin, and lambda-cyhalothrin in broiler farms in Australia (Lambkin 2005, Lambkin and Rice 2006, 2010; Lambkin and Furlong 2011). In the Americas, since 2006, resistance to carbaryl, methoxychlor, DDT, cyfluthrin, permethrin, cypermethrin, tetrachlorvinphos, and chlorpyrifos has been documented in broiler farms in Texas and Arkansas (Hamm et al. 2006, Steelman 2008, Singh and Johnson 2015). In Brazil, there have been no resistance cases reported; however, low susceptibility to cypermethrin and dichlorvos were detected in *A. diaperinus* in broiler farms of the Paraná state (Chernaki-Leffer et al. 2011). To test this report further, the objective of the current study was to characterize the susceptibility of *A. diaperinus* populations from southern Brazil to cypermethrin and chlorpyrifos and to estimate diagnostic concentrations for resistance monitoring programs.

Materials and Methods

Populations

To determine the susceptibility data to insecticides, populations of *A. diaperinus* were collected in poultry farms in southern Brazil, where there is the highest number of broiler farms in the country (Table 1; Fig. 1). Larvae and adults of *A. diaperinus* were collected and transported to the laboratory, where they were placed in plastic pots (24 cm × 18 cm × 8 cm [length by width by height]) containing wood shavings, commercial chicken feed, and a piece of apple (water source), according to the methodology proposed by Singh and Johnson (2015). Insects were maintained in a climatic room at 28 ± 1°C, relative humidity 70 ± 10% and a photophase of 14 h. In addition to the field populations, a susceptible reference population (Lab) was also tested. The Lab population has been kept in the laboratory for >3 yr, free of selection pressure by insecticides.

Bioassays

In the bioassays, technical-grade cypermethrin (92.6% purity) and chlorpyrifos (97.5% purity) provided by Ouro Fino Saúde Animal Ltda., Cravinhos, São Paulo, Brazil, were used. Both insecticides were diluted in acetone to prepare concentrations for testing in the residual contact bioassay, as suggested by Hamm et al. (2006). For each population, five to seven concentrations (0.016 to 16 μg a.i./cm^2) of each insecticide were applied in glass Petri dishes (9 cm × 1 cm [diameter by height]) using a repetition pipette (1 ml per dish). Petri dishes were rotated manually so that an even layer of insecticide dried on the inner surface. Control dishes were prepared using 1 ml of acetone only. Petri dishes were then placed for 2 h in a fume hood to dry. After this, each dish was infested with 10–20 larvae (8–10 mm length) or 10–20 adults (30–40 d in adult stage) of the F$_1$ or F$_2$ generations in the laboratory. The bioassays were repeated twice for each population and development stage, with each concentration being repeated twice per bioassay (a total of four replicates per concentration). After infestation, all plates were placed in a climatic chamber at 25 ± 1°C, relative humidity 70 ± 10%, and photophase of 12 h. Mortality was assessed after 48 h. Adults and larvae were considered dead if they were unable to move out of a 5-cm circle within 15 min, as proposed by Hamm et al. (2006).

Statistical Analyses

To estimate the LC$_{50}$ (LC—lethal concentration) and respective confidence intervals (CIs), the concentration-mortality data of each population were submitted to Probit analysis (PROC PROBIT, SAS Institute 2000). A likelihood ratio test was conducted to test the hypothesis that the LC$_p$ values (lethal concentration at which a percentage mortality P is attained) were equal. If the hypothesis was rejected, pairwise comparisons were performed and significance was declared if CIs did not overlap (Savin et al. 1977, Robertson et al. 2007). The significance of differences among slopes was determined by likelihood ratio test for parallelism and equality (Savin et al. 1977). Resistance ratios were calculated by dividing the LC$_{50}$ of the populations from broiler farms by the corresponding parameter for the susceptible reference population (Lab). To estimate diagnostic concentrations for insect resistance monitoring programs, a joint analysis was performed. In this analysis, mortality data were fitted with a binomial model using the log-log complement connection function (gompit) (PROC PROBIT, SAS Institute 2000). In this analysis, LC$_{99}$ values and respective CIs were estimated. Based on the LC$_{99}$ values, candidate diagnostic concentrations were designated for the resistance monitoring of *A. diaperinus* to cypermethrin and chlorpyrifos in Brazil.

Results

Susceptibility to Cypermethrin

There was significant variation in the biological activity of cypermethrin against *A. diaperinus* larvae (Table 2). For the populations

Table 1. Identification, location, date, and number of *A. diaperinus* (larvae and adults) collected in broilers farms from southern Brazil used to characterize the susceptibility to insecticides

Pop. Code	City, state	Farm	Latitude	Longitude	Date	Number
Lab	Santa Maria, RS	UFSM	–	–	–	–
RS-1	Santa Maria, RS	Avesui	29°41′03″S	53°48′25”W	October, 2016	500
RS-2	Miraguaí, RS	Canterle	27°29′39″S	53°44′51″W	October, 2016	800
RS-3	Vista Alegre do Prata, RS	Ramon	28°48′31″S	51°47′25″W	October, 2016	900
SC-1	Itapiranga, SC	Hickmann	27°10′12″S	53°42′39″W	October, 2016	2,500
SC-2	Sul Brasil, SC	Bida	26°44′10″S	52°57′53″W	October, 2016	5,000
SC-3	Biguaçú, SC	Vieira	27°29′38″S	48°39′21″W	October, 2016	2,000
PR-1	Verê, PR	Zanetti	25°52′51″S	52°54′28″W	October, 2016	800
PR-2	Imbituva, PR	Jesus	25°13′45″S	50°36′19″W	October, 2016	2,800

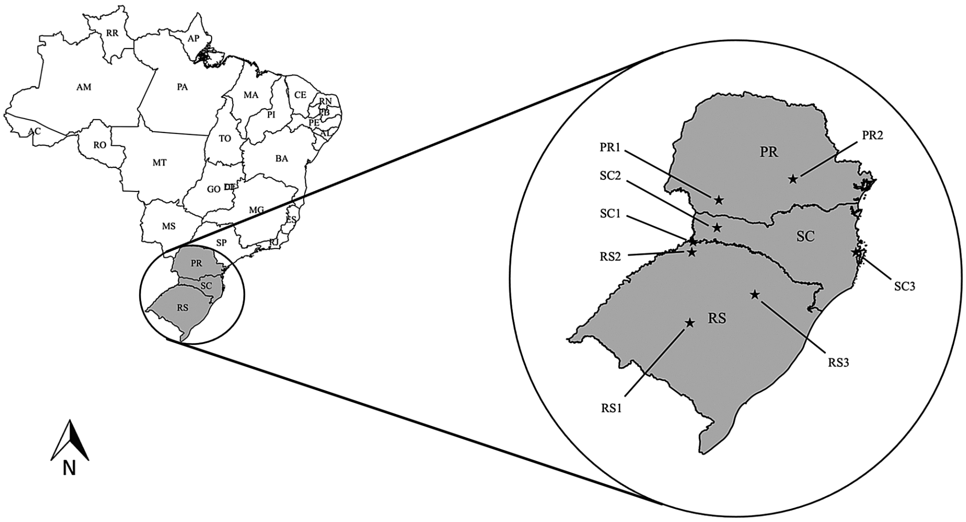

Fig. 1. Locations of collection of *A. diaperinus* populations in broiler farms from southern Brazil used to characterize the susceptibility to insecticides.

Table 2. Concentration-mortality response (LC; µg a.i./cm²) of larvae and adults of *A. diaperinus* from southern Brazil exposed to cypermethrin

Pop. code	*n*	Slope ± SE[a]	LC_{50} (95% CI)[a,b]	χ^2[c]	df[d]	Resistance ratio[e]
Larvae						
Lab	186	1.10 ± 0.24 a	0.43 (0.27–0.72) a	2.28	4	–
RS-1	182	1.36 ± 0.29 ab	0.50 (0.24–1.13) a	1.45	4	1.2
RS-2	210	1.06 ± 0.12 a	0.45 (0.21–0.79) a	6.86	4	1.0
RS-3	183	1.80 ± 0.19 b	1.70 (1.29–3.28) b	2.83	4	3.9
SC-1	290	1.11 ± 0.25 a	7.33 (4.13–11.87) c	5.43	5	17.0
SC-2	210	1.19 ± 0.19 a	2.22 (1.95–2.94) b	3.70	4	5.2
SC-3	187	1.09 ± 0.18 a	5.87 (4.81–8.71) c	3.67	4	13.6
PR-1	183	1.67 ± 0.17 ab	1.72 (1.20–2.32) b	1.01	4	4.0
PR-2	180	1.47 ± 0.12 ab	1.84 (1.14–2.45) b	4.07	4	4.3
Adults						
Lab	150	1.39 ± 0.42 abc	0.46 (0.27–0.88) a	5.25	4	–
RS-1	133	1.64 ± 0.22 b	1.23 (0.40–1.72) ab	1.05	4	2.7
RS-2	210	1.16 ± 0.16 a	0.77 (0.38–1.02) ab	3.59	4	1.7
RS-3	184	1.85 ± 0.19 c	0.71 (0.28–1.05) ab	2.50	4	1.6
SC-1	242	1.06 ± 0.17 a	2.09 (0.92–3.93) bc	2.72	4	4.5
SC-2	181	1.57 ± 0.50 abc	1.43 (0.99–1.64) b	6.05	4	3.1
SC-3	187	3.18 ± 0.68 d	4.93 (3.11–6.62) c	1.09	4	10.7
PR-1	185	1.54 ± 0.12 bc	3.99 (2.88–6.24) c	5.01	4	8.7
PR-2	214	1.02 ± 0.18 a	2.45 (1.78–3.60) bc	6.76	4	5.3

[a] LC_{50} values designated by different letters within a column are significantly different from each other through nonoverlap of 95% confidential intervals. Significance of differences among slopes determined by likelihood ratio test of equality followed by pairwise comparisons using nonoverlapping fiducial limits.

[b] LC_{50}: concentration of cypermethrin (µg a.i./cm²) required to kill 50% of insects in the observation period of 48 h.

[c] $P > 0.05$ in the goodness-of-fit test.

[d] Degrees of freedom.

[e] Resistance ratio = (LC_{50} of indicated population)/(LC_{50} of Lab population).

tested, cypermethrin concentrations from 0.16 to 16 µg a.i./cm² caused larval mortality ranging from 7 to 92%. For larvae from nine geographically distinct populations of *A. diaperinus*, the LC_{50} values ranged from 0.43 (Lab population) to 7.33 (SC-1 population) µg a.i./cm². A similar susceptibility was detected to cypermethrin in *A. diaperinus* larvae from the Lab population and some populations collected in the Rio Grande do Sul state, RS-1 and RS-2. In contrast, the RS-3 population and those populations from broiler farms of Santa Catarina (SC-1, SC-2 and SC-3) and Paraná state (PR-1 and PR-2) showed a lower susceptibility than previous populations (Table 2). However, the SC-1 and SC-3 populations had lower susceptibility to cypermethrin than the Lab population and all other field populations tested. These two populations presented the highest resistance ratios of 13.6- and 17-fold, respectively (Table 2).

In the bioassays with adult *A. diaperinus*, significant variation was also detected in the susceptibility to cypermethrin (Table 2). The concentrations from 0.025 to 10 µg a.i./cm² caused mortality ranging from 5 to 94%. In adults from nine distinct populations of *A. diaperinus*, the LC_{50} for cypermethrin ranged from 0.46 (Lab population) to 4.93 (SC-3 population) µg a.i./cm² (Table 2). Based on the LC_{50} values, similar susceptibility was detected between populations from Rio Grande do Sul state (RS-1, RS-2, and RS-3) and the susceptible reference population (Lab). Similar susceptibility was also observed among the populations RS-1, RS-2, RS-3, SC-1, SC-2, and PR-2, with LC_{50} values ranging from 0.71 to 2.45 μg a.i./cm^2 (Table 2). However, there was significantly lower susceptibility to cypermethrin in adults from the SC-3 and PR-1 populations, compared with Lab, SC-2, and those populations collected in the Rio Grande do Sul state (Table 2). The variation in susceptibility indicated by adult mortality in the PR-1 and SC-3 populations represents the highest resistance ratio observed, ranging from 8.7- to ~11-fold (Table 2).

For estimating LC_{99}, the mortality data of all populations tested were pooled and analyzed jointly. The LC_{99} of cypermethrin against *A. diaperinus* larvae was estimated to be 15.14 [CI 95% (9.33–21.08)] μg a.i./cm^2 (n = 1811; slope [± SE] = 2.02 [± 0.16]; χ^2 = 8.69; df = 4). For the adults, the LC_{99} was 14.30 [CI 95% (7.27–22.18)] μg a.i./cm^2 (n = 1686; slope [± SE] = 1.75 [± 0.12]; χ^2 = 10.06; df = 4). From the LC_{99} values, the candidate diagnostic concentration of 15 μg a.i./cm^2 is suggested for resistance monitoring programs of *A. diaperinus* (larvae and adults) to cypermethrin in Brazil.

Susceptibility to Chlorpyrifos

Significant variation was also detected in the susceptibility of *A. diaperinus* larvae to chlorpyrifos (Table 3). Concentrations from 0.016 to 30 μg a.i./cm^2 caused larval mortality ranging from 2 to 97%. In bioassays with larvae, the LC_{50} values ranged from 0.21 (Lab population) to 4.30 (PR-1 population) μg a.i./cm^2. A similar susceptibility to chlorpyrifos was observed in larvae from the Lab, RS-1, RS-2, and SC-2 populations, with LC_{50} values ranging from 0.21 to 1.26 μg a.i./cm^2. However, the RS-2 population do not differ in terms of susceptibility of the populations RS-3, SC-1, SC-3, and PR-2 (Table 3). In contrast, larvae from the PR-1 population showed significantly lower susceptibility than previous populations, with an LC_{50} of 4.30 μg a.i./cm^2. This population also had the highest resistance ratio to chlorpyrifos of more than 20-fold (Table 3).

There was also significant variation in the susceptibility to chlorpyrifos in adults from distinct Brazilian populations of *A. diaperinus* (Table 3). Concentrations from 0.16 to 30 μg a.i./cm^2 caused mortality ranging from 6 to 93%. The LC_{50} for adults from nine populations ranged from 0.17 (Lab population) to 5.30 (PR-1 population) μg a.i./cm^2 (Table 3). A similar susceptibility to chlorpyrifos was observed in adults from Lab, populations collected in broiler farms of Rio Grande do Sul (RS-1, RS-2, and RS-3) and Santa Catarina (SC-1, SC-2, and SC-3) states, with LC_{50} values ranging from 0.17 (population Lab) to 1.13 (population RS-2) μg a.i./cm^2 (Table 3). In contrast, adults from Paraná state showed significantly lower susceptibility than previous populations, with LC_{50} values ranging from 2.90 to 5.30 μg a.i./cm^2 (Table 3). This difference in susceptibility represents a resistance ratio of 17- and 31.2-fold, (Table 3).

The estimated LC_{99} of chlorpyrifos against *A. diaperinus* larvae was 11.03 [CI 95% (5.23–18.51)] μg a.i./cm^2 (n = 1613; slope [± SE] = 1.89 [± 0.14]; χ^2 = 5.33; df = 4). For the adults, the LC_{99} was 12.81 [CI 95% (6.08–19.09)] μg a.i./cm^2 (n = 1515; slope [SE]= 2.00 [± 0.14]; χ^2 = 7.56; df = 4). Based on these values, the candidate diagnostic concentration of 12 μg a.i./cm^2 can be considered for the resistance monitoring of *A. diaperinus* (larvae and adults) to chlorpyrifos in Brazil.

Discussion

The larvae and adults from southern Brazilian populations of *A. diaperinus* presented significant interpopulation variation in the observed susceptibility to cypermethrin and chlorpyrifos. The susceptibility of *A. diaperinus* larvae to these insecticides, based on LC_{50} values ranging from 0.43 to 7.33 μg a.i./cm^2 and 0.21 to 4.30 μg a.i./cm^2, represented resistance ratios of 17- and 20-fold, respectively. Lower variation in larval susceptibility was reported for

Table 3. Concentration-mortality response (LC; μg a.i./cm^2) of larvae and adults of *A. diaperinus* from southern Brazil exposed to chlorpyrifos

Pop. code	n	Slope ± SE[a]	LC_{50} (95% CI)[a,b]	χ^2[c]	df[d]	Resistance ratio[e]
Larvae						
Lab	181	2.12 ± 0.60 bc	0.21 (0.09–0.33) a	1.74	4	–
RS-1	182	1.19 ± 0.27 ab	0.52 (0.27–0.98) a	3.20	4	2.5
RS-2	150	1.16 ± 0.36 ab	1.26 (0.29–2.22) ab	1.58	4	6.0
RS-3	151	1.40 ± 0.34 ab	1.66 (1.23–2.26) b	3.62	4	7.9
SC-1	240	1.09 ± 0.20 a	2.28 (1.09–4.24) bc	1.32	4	10.8
SC-2	186	1.76 ± 0.24 bc	0.32 (0.25–0.79) a	9.84	4	1.5
SC-3	184	1.82 ± 0.28 c	1.30 (0.50–2.02) b	1.95	4	6.2
PR-1	154	1.42 ± 0.29 b	4.30 (3.22–4.85) c	2.49	4	20.5
PR-2	185	1.04 ± 0.40 a	2.36 (1.31–4.16) bc	1.10	4	11.2
Adults						
Lab	187	1.95 ± 0.24 bc	0.17 (0.07–0.34) a	4.51	4	–
RS-1	182	2.28 ± 0.55 c	0.29 (0.18–0.40) a	2.62	4	1.7
RS-2	156	1.87 ± 0.33 bc	1.13 (0.26–1.59) a	2.67	4	6.6
RS-3	180	1.01 ± 0.27 a	0.70 (0.23–1.12) a	5.18	4	4.1
SC-1	139	3.00 ± 0.66 c	0.52 (0.30–0.78) a	1.14	4	3.0
SC-2	182	1.24 ± 0.31 ab	0.26 (0.21–0.33) a	11.00	4	1.5
SC-3	177	1.50 ± 0.16 b	0.62 (0.20–0.90) a	1.32	4	3.6
PR-1	160	2.22 ± 0.65 c	5.30 (3.49–7.37) b	2.94	4	31.2
PR-2	152	1.83 ± 0.27 bc	2.90 (1.72–3.83) b	2.15	4	17.0

[a] LC_{50} values designated by different letters within a column are significantly different from each other through nonoverlap of 95% confidential intervals. Significance of differences among slopes determined by likelihood ratio test of equality followed by pairwise comparisons using nonoverlapping fiducial limits.

[b] LC_{50}: concentration of chlorpyrifos (μg a.i./cm^2) required to kill 50% of insects in the observation period of 48 h.

[c] $P > 0.05$ in the goodness-of-fit test.

[d] Degrees of freedom.

[e] Resistance ratio = (LC_{50} of indicated population)/(LC_{50} of Lab population).

cypermethrin, permethrin, and cyfluthrin in Arkansas, with a resistance ratio inferior to 15-fold (Steelman 2008). In another study, larvae also presented lower than 14-fold resistance to cyfluthrin in Arkansas (Singh and Johnson 2015) and to beta-cyfluthrin (3.5-fold resistance) in Texas (Lyons et al. 2017). In contrast, a higher resistance ratio up to 29-fold, was recorded for cyfluthrin in beetles' larvae from poultry farms in the eastern United States (Hamm et al. 2006). However, in these same populations, resistance to tetrachlorvinphos, an organophosphate with a similar mode of action of chlorpyrifos, was lower than ninefold. A lower resistance ratio, inferior to threefold, was also verified to chlorpyrifos and tetrachlorvinphos in larvae from broiler house facilities in Arkansas (Steelman 2008).

High variation in the susceptibility to cypermethrin and chlorpyrifos was also observed among adult populations of *A. diaperinus* from southern Brazil. In this development stage, the LC_{50} to cypermethrin and chlorpyrifos ranged from 0.46 to 4.93 µg a.i./cm^2 and 0.17 to 5.30 µg a.i./cm^2, respectively, showing 10- and 31-fold resistance. A similar resistance ratio was also reported to cypermethrin in adult beetles from north Paraná (up to 17-fold resistance) (Chernaki-Leffer et al. 2011). However, populations from western and southwestern Paraná had strong resistance to cypermethrin, ranging from 36- to 92-fold. A similar resistance ratio was also observed to cyfluthrin, of up to 10-fold, in adult beetles from the eastern United States (Hamm et al. 2006) and to beta-cyfluthrin, lambda-cyhalothrin, and deltamethrin, up to 16-fold, in Australia (Lambkin and Furlong 2011). However, a lower resistance ratio, inferior to fivefold, was reported to cypermethrin, permethrin, and cyfluthrin in beetles from broiler houses in Arkansas (Steelman 2008). In contrast, higher resistance ratios, from 19- to 36-fold, were reported for cyfluthrin in adult *A. diaperinus* from broiler farms in Australia (Lambkin and Rice 2006, Lambkin and Furlong 2011). To organophosphates, a similar resistance ratio, from 1- to 14-fold, was reported in populations of *A. diaperinus* from Paraná (Lapa, Araucária, and Cascavel); to dichlorvos, an insecticide that act as an acetylcholinesterase inhibitor, resistance ratios were similar to chlorpyrifos (Chernaki-Leffer et al. 2011). However, populations of the same state from Pato Branco, Londrina, and Corbélia showed higher resistance to dichlorvos, of up to 134-fold (Chernaki-Leffer et al. 2011). In another study, a higher resistance ratio up to 79-fold, was also reported to fenitrothion in adult beetles in Australia (Lambkin 2005). The high resistance ratio to chlorpyrifos and dichlorvos in *A. diaperinus* populations from Paraná reported here and in previous studies indicates a possible cross-resistance among these insecticides, as published in another Coleopteran species (Attia and Frecker 1984, Zettler and Cuperus 1990).

The interpopulation variation in the susceptibility to chemical insecticides is a common phenomenon when bioassays are repeated (Robertson et al. 2007). However, from an Insect Resistance Management (IRM) perspective, variation in susceptibility is also an indication of the ability of the beetles to adapt to the insecticides, especially in populations with the highest LC_{50} values. In other words, the relative high resistance ratio to cypermethrin and chlorpyrifos in some *A. diaperinus* populations from Santa Catarina and Paraná seems a probable response of high exposure to selection pressure. Based on this, IRM strategies should be adopted, by associating physical and chemical methods using insecticides with distinct modes of action against this species, to delay or prevent resistance evolution (Wolf et al. 2015).

Understanding the mode of action of each insecticide is also crucial in developing an effective IRM program. Cypermethrin is a sodium channel modulator that causes excitatory paralysis of the insect, whereas chlorpyrifos is an acetylcholinesterase inhibitor (Insecticide Resistance Action Committee [IRAC] 2017). Both active ingredients are widely used against *A. diaperinus* in broiler farms in Brazil and other countries (Steelman 2008, Tomberlin et al. 2008, Chernaki-Leffer et al. 2011, Oliveira et al. 2016). Specifically, in Brazil, the reduced number of active ingredients registered for use in broiler farms has contributed to the resistance evolution in *A. diaperinus*. Thus, insecticides from other chemical groups should be considered against this pest, such as spinosad and imidacloprid, which mimic the agonistic action of nicotinic acetylcholine receptors. Additionally, chlorfenapyr, which uncouples oxidative phosphorylation through the disruption of the proton gradient and interferes with metabolic processes and energy production in mitochondria (IRAC 2017). Spinosad, imidacloprid, and chlorfenapyr are not registered for use in broiler farms in Brazil, but they showed high efficacy against *A. diaperinus* in Australia and the United States (Lambkin and Rice 2007, Singh and Johnson 2015). Spinosad also increased the mortality of resistant beetles to cyfluthrin and fenitrothion, due to the absence of cross-resistance among these insecticides (Lambkin and Furlong 2014). Therefore, the Brazilian pesticide regulatory agencies and companies should register these and other chemical insecticides for use in broiler farms, to improve the management of *A. diaperinus*.

In the context of IRM, monitoring the susceptibility of *A. diaperinus* to insecticides is essential to subsidize their management. For susceptibility monitoring, the candidate diagnostic concentrations of 15 and 12 µg a.i./cm^2 of cypermethrin and chlorpyrifos, respectively, are suggested. The use of these diagnostic concentrations in residual contact bioassays make this procedure fast, practical, and compatible with large scale bioassays in IRM programs. Future efforts should be concentrated on collecting representative samples of this species, especially in those regions with the highest amount of broiler farms. Then, expose the beetles to the diagnostic concentrations defined here, to identify resistant levels in Brazilian populations of this species. In summary, the compilation of this susceptibility database provides an opportunity for poultry companies and producers to identify possible changes in the susceptibility of *A. diaperinus* to cypermethrin and chlorpyrifos due to resistance evolution, thus establishing effective IRM strategies.

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