Susceptibility of *Alphitobius diaperinus* (Panzer) (Coleoptera, Tenebrionidae) to cypermethrin, dichlorvos and triflumuron in southern Brazil

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ABSTRACT. Susceptibility of *Alphitobius diaperinus* (Panzer) (Coleoptera, Tenebrionidae) to cypermethrin, dichlorvos and triflumuron in southern Brazil. The lesser mealworm, *Alphitobius diaperinus* (Panzer), is an important insect pest in poultry houses in Brazil. Susceptibility of the lesser mealworm collected from eight poultry houses in Paraná state, southern Brazil, was evaluated for cypermethrin, dichlorvos and triflumuron. Adult *A. diaperinus* were tested in bioassays with cypermethrin and dichlorvos. Larvae were fed rabbit feed wetted with a triflumuron-water solution. Concentration-mortality regressions were estimated using Probit analysis and resistance ratios were calculated based on the susceptible population. Among the field populations evaluated, cypermethrin LC_{50} values for adults, ranged from 68.1 to 6,263 ng (AI)/cm^2. LC_{50} values for adults challenged with dichlorvos ranged from 10.3 to 1,385 ng (AI)/cm^2. One population from Pato Branco showed reduced susceptibility to triflumuron (LC_{50} = 272 µg (AI)/ml of solution) when compared to the most susceptible population (LC_{50} = 109.8 µg (AI)/ml). Application of cypermethrin and dichlorvos analogues should be managed with caution to minimize insecticide resistance problems.

KEYWORDS. Insect growth regulator; lesser mealworm; poultry house; pyrethroid; organophosphates.

RESUMO. Suscetibilidade de *Alphitobius diaperinus* (Panzer) (Coleoptera, Tenebrionidae) a cipermetrina, diclorvós e triflumurom no sul do Brasil. O cascudinho, *Alphitobius diaperinus* (Panzer), é uma importante praga em aviários no Brasil. A suscetibilidade do cascudinho à cipermetrina, diclorvós e triflumurom foi avaliada em oito aviários do Estado do Paraná, Brasil. Adultos de *A. diaperinus* foram testados mediante bioensaios com cipermetrina e diclorvós. As larvas foram alimentadas com ração para coelhos impregnada com suspensões de triflumurom. As regressões de concentração-mortalidade foram estimadas usando análise de Probit e as razões de resistência calculadas com base na população suscetível. Entre as populações de campo avaliadas, os valores da CL_{50} para adultos tratados com cipermetrina variaram entre 68,1 to 6.263 ng (IA)/cm^2. Os valores da CL_{50} para adultos tratados com diclorvós variaram de 10,3 to 1.385 ng (IA)/cm^2. Uma população de Pato Branco apresentou reduzida suscetibilidade ao triflumurom (CL_{50} = 272 µg (IA)/ml), quando comparada à população mais suscetível (CL_{50} = 109,8 µg (IA)/ml de solução). Cipermetrina, diclorvós e análogos devem ser manejados e aplicados com cautela para minimizar os problemas de resistência a inseticidas.

PALAVRAS-CHAVE. Aviário; cascudinho; inseticidas reguladores do crescimento; organofosforados; piretróides.

The lesser mealworm *Alphitobius diaperinus* (Panzer), is an important insect pest in poultry houses around the world and is one of the most common beetles inhabiting poultry litter and manure. This species serves as a vector of several poultry diseases (Chernaki-Leffer *et al.* 2002, 2010) and chickens feeding on larvae show poor weight gain and increased mortality. High beetle populations could lead to reductions in weight gain and feed conversion due to fewer rest periods caused by beetles biting resting birds at night (Hamm *et al.* 2006).

The first suspicion of insecticide resistance of *A. diaperinus* was described in turkey broiler houses in the UK (Cogan & Wakefield 1996). Lambkin & Cameron (1999) reported that failures to control this beetle in southeast Queensland, Australia, were due to fenitrothion resistance and the pest's ability to avoid contact with the insecticide. An Australian survey of the lesser mealworm population for insecticide resistance showed that beetle response to cyfluthrin and fenitrothion insecticides was heterogeneous with relatively low resistance: the mean resistance factors were 3.5 and 21 respectively (Lambkin 2001). Later, Lambkin (2005) reported strong resistance to fenitrothion, up to 79 times the LC_{50} observed, on susceptible populations of *A. diaperinus* from southeastern Queensland, where this insecticide has been continuously used for 20 years. More recently, resistance reports involving pyrethroids and organophosphates have been published in other countries (Hamm *et al.* 2006).

Beyond the pyrethroids, insect growth regulators (IGR), especially triflumuron, are commonly used in poultry houses in Brazil. However, studies show that IGR resistance develops if the selection pressure is sufficiently strong (Keiding 1999). While studies of the susceptibility of *A. diaperinus* to various IGRs has been reported (Weaver & Kondo 1987; Miller & Redfern 1988), no literature was found regarding IGR resistance.

There is no information regarding responses to the most commonly used insecticides for controlling lesser mealworm populations in Brazilian poultry houses. Therefore, we tested susceptibility of populations of *A. diaperinus* from Paraná State, Brazil to the insecticides cypermethrin, dichlorvos and the growth regulator triflumuron.

MATERIAL AND METHODS

Insects. Adults and larvae of *A. diaperinus* were collected from eight poultry houses in Paraná state: Araucária and Lapa (Metropolitan area), Cascavel and Corbélia (west), Londrina (north), Pato Branco (south-west) in January and March, 2003. Poultry houses had been subjected to variable application frequencies of pyrethroids, organophosphates and triflumuron. The population from Araucária had never been exposed to insecticides and was considered the most susceptible. The populations from Cascavel and Corbélia had been regularly exposed (every 40–50 days) to cypermethrin during the past 4 years. The Londrina population had been exposed to insecticide applications but no written documentation of this existed. One population from Lapa had been exposed only once to cypermethrin approximately one month before the sampling date. The second population collected from Lapa had been exposed to cypermethrin, cyfluthrin, azamethiphos, lambda-cyalothrin, permethrin, chlorpyriphos, and cypermethrin plus dichlorvos every 43–50 days for 6 years. Two populations collected in Pato Branco had been treated every 45–50 days over four years with cyfluthrin and triflumuron. All the insect colonies were maintained at 27 ± 2ºC, 60–70% RH and provided with rabbit food *ad libitum*.

Chemicals. Commercial grade cypermethrin 15% (Ciperpurina CE, Purina, SP, Brazil) and dichlorvos 50% (DDVP 500 CE, Bio Carb, SP, Brazil) were evaluated in vial bioassays, and triflumuron 48% (Staricide 480 SC, Bayer, SP, Brazil) was evaluated using a diet incorporation method.

Vial bioassays. Glass vials were treated with 500 µL of insecticide in acetone, or with acetone only for control treatments. Twenty adults were placed in each vial and exposed to the pesticides and moistened rabbit food was provided *ad libitum*. Insects were kept in environmental chambers at 0:24 L:D cycle, 26 ± 1°C, and 75% RH. All bioassays were replicated 3 to 4 times. The insect mortality was scored every day for 16 days (cypermethrin) and 7 days (dichlorvos). Insect were considered dead if they did not move after prodding.

Diet incorporation. Triflumuron was diluted with water. Rabbit chow was wetted with 6 ml of each dilution of the insecticide in concentrations of 10, 20, 40, 80, 160 and 320 ppm (µg/ml) or with water only for control treatments. Three groups of 10 larvae of 1.0–1.3 cm were placed in plastic vials at 28 ± 1°C, R.H. ⩾ 75%, in darkness, and counted daily for 15 days.

Statistical Analysis. Mortality data were analyzed by probit analysis (Finney 1971) by using the POLO-PC software (LeOra Software 1987) and SAS (2004) to obtain the lethal concentration, its 95% confidence intervals and the slopes of dose-mortality curves. The resistance ratios between geographical populations were considered significantly different when the confidence interval did not include the value of 1 (Robertson & Preisler 1992).

RESULTS

Susceptibility to cypermethrin, dichlorvos and triflumurom varied among populations Tables I, II and III). LC_{50}s of cypermethrin ranged from 68 ng (AI)/cm^2 for the most susceptible population collected in Araucária to 6,263 ng (AI)/cm^2 for the most resistant population from Corbélia. The slopes of the dose-mortality lines ranged from 0.6–1.9 and the LC_{90} from 6,263 ng (AI)/cm^2 in Araucária to 208,136 ng (AI)/cm^2 for Corbélia (Table I). The LC_{50} of cypermethrin in seven of the populations was significantly greater than the Araucária population. The resistance ratio of Corbélia was 92 times higher than the most susceptible population. The Lapa 1 and Londrina populations had the lowest resistance factors (RR = 4.2 and 7.5, respectively) and the difference between their LC_{50} of cypermethrin did not differ significantly (Table IV). Populations from Cascavel, Corbélia, Lapa-1, Lapa-2, Londrina, Pato Branco-1 and Pato Branco-2 were somewhat resistant to cypermethrin. Most of these populations had been frequently exposed to pyrethroids for more than four years. All slopes were higher than that from the susceptible population originally collected from Araucária.

Table I. Predicted LC_{50} and LC_{90} values, expressed as nanograms of AI per cm^2, from dose–mortality bioassays of *A. diaperinus* populations exposed to cypermethrin.

Population	n	Slope ± SE	LC50 (FL 95%)	LC90 (FL 95%)	χ^2 (df)	RR
Araucária	323	0.6 ± 0.1	68.10 (27.4–140.9)	6,711 (2,044–63,338)	6.6 (5)	1.0
Cascavel	159	0.9 ± 0.3	962.90 (463.9–5,302)	20,028 (4,136–5,168,777)	0.5 (3)	14.1
Corbélia	200	0.8 ± 0.1	6,263 (3,300–13,586)	208,136 (68,891–1,346,292)	1.6 (2)	92.0
Lapa-1	220	1.0 ± 0.2	284.10 (139.9–497.4)	4,714 (1,915–39,918)	8.5 (6)	4.2
Lapa-2	259	0.8 ± 0.2	1,246 (678.3–4,016)	50,493 (10,653–2,321,257)	10.4 (7)	18.3
Londrina	280	1.2 ± 0.1	509.4 (342.6–788.6)	5,866 (3,055–16,073)	7.2 (5)	7.5
Pato Branco-1	240	1.9 ± 0.3	1,184 (844.8–1,567)	5,791 (4,002–10,299)	4.1 (7)	17.4
Pato Branco-2	240	1.4 ± 0.3	2,481 (1,094–3,787)	19,926 (12,189–57,018)	8.1 (7)	36.4

LC = Lethal Concentration; Heterogeneity = χ^2; df = degrees of freedom; RR = Resistance Ratio (LC_{50} of the putative resistant population/LC_{50} of susceptible population).

Table II. Predicted LC_{50} and LC_{90} values, expressed as nanograms of AI per cm^2, from dose–mortality bioassays of *A. diaperinus* populations exposed to dichlorvos.

Population	n	Slope ± SE	LC_{50} (95% CL)	LC_{90} (95% CL)	χ^2 (df)	RR
Araucária	378	0.5 ± 0.1	10.30 (0.90–30.30)	2,916 (879.00–45,568)	5.0 (7)	1.0
Cascavel	360	0.9 ± 0.1	47.30 (24.70–77.60)	1,072 (580.20–2,705)	3.2 (6)	4.6
Corbélia	300	0.8 ± 0.1	479.40 (292.50–846.20)	16,651 (6,370–81,635)	7.7 (5)	46.5
Lapa-1	260	1.5 ± 0.2	67.93 (45.80–95.60)	479.80 (311.70–891.40)	7.6 (4)	6.6
Lapa-2	260	1.1 ± 0.2	220.10 (132.30–339.70)	3,071 (1,554–10,366)	2.1 (4)	21.4
Londrina	280	1.0 ± 0.2	423.40 (239.40–727.80)	7,231 (3,202–31,389)	3.5 (3)	41.1
Pato Branco-1	275	0.9 ± 0.2	448.60 (253.50–1,000)	9,966 (3,157–111,048)	0.2 (3)	43.5
Pato Branco-2	211	0.6 ± 0.2	1,385 (403.90–30,482)	179,366 (12,917–2,961,093)	1.9 (3)	134.4

LC = Lethal Concentration; Heterogeneity = χ^2; df = degrees of freedom; RR = Resistance Ratio (LC_{50} of the putative resistant population/LC_{50} of susceptible population).

Table III. Comparison of *A. diaperinus* populations exposed to triflumuron.

Population	n	Slope ± SE	LC_{50} (95% CL)	LC_{90} (95% CL)	χ^2 (df)
Corbélia	150	1.4 ± 0.4	109.8 (65.3–263.8)	959.5 (347.8–40,145)	2.4 (3)
Pato Branco-1	180	1.5 ± 0.4	205.9 (131.4–397.6)	1,534 (655.6–14,830)	1.0 (4)
Pato Branco-2	120	2.5 ± 1.1	272.4 (150.2–979.4)	878.4 (455.9–55,953)	0.3 (2)

LC = Lethal Concentration ppm (µg/ml); Heterogeneity = χ^2; d.f. = degrees of freedom.

The LC_{50} and LC_{90} of dichlorvos ranged from 10–1,385 ng (AI)/cm^2 and from 480–179,366 ng (AI)/cm^2, respectively (Table II). Susceptibility to dichlorvos of the Cascavel population (LC_{50} = 47.3 and LC_{90} = 1,072 ng (AI)/cm^2) was similar to that of the Araucária population (LC_{50} = 10.3 and LC_{90} = 2,916 ng (AI)/cm^2). The LC_{50} of dichlorvos was not significantly different between Lapa-1 and Cascavel. The highest LC_{50} values were observed in the bioassays from the Pato Branco-2 population (RR = 134; CL_{50} = 1,385 ng (AI)/cm^2) (Table II and IV) which had been exposed to organophosphate and cypermethrin applications for four years.

In bioassays using larvae fed with diet plus triflumuron, the LC_{50} was similar for the populations from Pato Branco-1 and Corbélia (never exposed to triflumuron). The LC_{50} of triflumurom for the Pato Branco-2 population was significantly higher than that for Corbélia (Table III and IV).

DISCUSSION

Based on the exposure history for cypermethrin and dichlorvos, the greatest susceptibility of the Araucária population to cypermethrin and the Araucária and Cascavel populations to dichlorvos is probably due to no insecticide being used over the last two years. Corbélia had the greatest LC_{50} and also received the greatest selection pressure from cypermethrin. The high LC_{50} and LC_{90} values for the Corbélia strain reflect cypermethrin overuse since this pyrethroid was applied at each cleaning of the facilities and repeatedly in the areas close to the chicken feeders. The Pato Branco populations were resistant, with Pato Branco-2 being the least susceptible to dichlorvos and the second least susceptible to

Table IV. Comparison of LC_{50} values among *A. diaperinus* populations from Paraná state, according to Robertson & Preisler (1992).

Insecticide	Location							
		Ar	Lp-1	Ld	Cc	Pb-1	Lp-2	Pb-2
Cypermethrin	Ar							
	Lp-1	S						
	Ld	S	NS					
	Cc	S	S	S				
	Pb-1	S	S	S	S			
	Lp-2	S	S	S	S	S		
	Pb-2	S	S	S	S	S	S	
	Cb	S	S	S	S	S	S	S
Dichlorvos		Ar	Cc	Lp-1	Lp-2	La	Pb-1	Cb
	Ar							
	Cc	NS						
	Lp-1	S	NS					
	Lp-2	S	S	S				
	Ld	S	S	S	S			
	Pb-1	S	S	S	S	S		
	Cb	S	S	S	S	S	S	
	Pb-2	S	S	S	S	S	S	S
Triflumuron		Cb	Pb-1					
	Cb							
	Pb-1	NS						
	Pb-2	S	NS					

Ar = Araucária; Lp-1 = Lapa-1; Ld = Londrina; Cc = Cascavel; Pb-1 = Pato Branco-1; Lp-2 Lapa-2; Pb-2 = Pato Branco-2; S = significant; NS = not significant.

cypermethrin whereas Pato Branco-1 was the third most resistant to dichlorvos. This suggests that cross-resistance between cyfluthrin and cypermethrin and multiple resistances between cypermethrin and dichlorvos has occurred in these populations. The use of insecticides with different modes of action could affect the frequency of individuals resistant to one pesticide which is declining during the application of an alternate insecticide (Georghiou 1983).

In the bioassays of larval susceptibility to triflumuron, the Pato Branco-2 population was less susceptible to triflumuron than the Corbélia population but the ratio between them was low (ca. 2.5). Susceptibility of populations to triflumuron can be attributed to the low frequency of resistant genotypes or natural geographic differences between populations, despite the selection pressure of repeated applications every 45–50 days for four years.

The dose/response method is suitable for documenting resistance that has reached high levels but is very inefficient for detecting an incipient resistance outbreak (Roush & Miller 1986). The screening dose can be more effective, making a precise distinction between resistant and susceptible individuals. We suggest screening doses for resistance monitoring of *A. diaperinus* in poultry houses: 6,711 ng (A.I.)/cm^2 for cypermethrin and 2,916 ng (A.I.)/cm^2 for dichlorvos since these concentrations can cause 90% mortality in susceptible populations.

The high resistance ratios of *A. diaperinus* is a consequence of the inappropriate management of their populations, and specifically of the overuse of insecticides. The low cost of cypermethrin is one of the most important reasons for this overuse. Tactics that promote the resistance management of *A. diaperinus* must be practiced (see Roush 1989) to preserve susceptible genotypes by avoiding unnecessary insecticide applications, rotating insecticides with different modes of action for each insect generation and using recommended application rates. Once a resistance problem is detected, it is necessary to begin resistance-avoiding measures together with a poultry industries awareness program.

CONCLUSION

Our study indicates that high resistance ratios to cypermethrin and to dichlorvos occurred in most of the poultry houses in Paraná State, Brazil. A population from Pato Branco shows resistance to triflumuron at an early stage of development.

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