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ORIGINAL PAPER

Insecticide resistance of house fly, *Musca domestica* (L.) from Argentina

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Abstract The status of resistance to cyromazine, 2,2-dichlorovinyl dimethyl phosphate (DDVP), and permethrin relative to field populations of the house fly, *Musca domestica* L. from Argentinean poultry farms was studied. All the three studied populations (SV, Q, and C) showed resistant ratios (RRs) to cyromazine of 3.9, 10.98, and 62.5, respectively. We observed high levels of resistance toward the organophosphate DDVP and permethrin. The RRs to DDVP ranged from 45.4 to 62.5. No significant differences were found among the studied populations. All the house fly populations were permethrin-resistant, in comparison with the susceptible strain. Two of the analyzed populations (SV and Q) differed significantly in toxicity to the population C. This is the first evidence that house flies from Argentina showed a multi-resistance pattern. The implementation of an insecticide monitoring program on poultry farms of Argentina is needed to prevent field control failures. Furthermore, integrated control strategies are needed to delay detrimental development of insecticide resistance.

Introduction

The house fly, *Musca domestica* (L.) is an important sanitary pest of humans and domesticated animals. They are mechanical carriers of more than 100 human and animal intestinal diseases and are responsible for protozoan, bacterial, helminthic, and viral infections (Greenberg 1971; Förster et al. 2007; Malik et al. 2007). Flies pick up disease-causing organisms via their mouthparts, feces, through vomits, and via their body surface. It has been shown that some bacteria could proliferate in the mouthparts (Kobayashi et al. 1999). Transmission takes place when the fly makes contact with people and/or the animals (Malik et al. 2007). In poultry farms, great quantities of manure exposed to high temperature and humidity levels provide an ideal environment for the development of house fly. High density of flies can cause stress to poultry workers and hens or affect the economic value of their products (Moon et al. 2001; Learmount et al. 2002). In poultry farms, the application of cyromazine and the neurotoxic 2,2-dichlorovinyl dimethyl phosphate (DDVP) and pyrethroids have been shown to be a successful control strategy worldwide (Kristensen et al. 2001). The annual cost of house fly control in poultry farms in the USA has been estimated to be over 1.6 millions of dollars (Crespo et al. 1998). Cyromazine is an insect growth regulator derived from azidotrazine herbicides (Shen and Plapp 1990) that affects the endocrine system of developing larvae causing abnormal growth, integument swelling, thinning of the cuticle, cuticular lesions, larviform puparia, and irregular muscle formation (Awad and Mulla 1984; Friedel et al. 1988; Tang et al. 2002). Either DDVP or pyrethroid-based products are neurotoxic insecticides applied as aerosols or space sprays for adult house fly control. However, the repetitive and inappropriate use of compounds in all these classes has led to resistance worldwide (Shen and Plapp 1990; Pinto and Prado 2001; Liu and Yue 2000; Tang et al. 2002; Marçon et al. 2003; White et al. 2007). In Argentina, cyromazine has been used in two ways: added to poultry food and sprayed

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over the manure. The most widely used insecticide against house fly is the organophosphate DDVP sprayed over the surfaces where flies rest. Even though pyrethroid products were registered to control house fly, their use in the farms is four to five times lesser than the organophosphates.

In 1998, the annual cost estimation in Argentina of house fly control in poultry farms was over 10,000 US dollars (Crespo et al. 1998). Nowadays, this estimation has increased considerably. Even though there are numerous reports of insecticide resistance of house fly populations worldwide, no previous work was reported to assess the susceptibility of *M. domestica* field populations from Argentina. The aim of the present work was to study the resistance spectrum of fly populations from poultry farms.

Materials and methods

Chemicals

The larvicide ciromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine) of technical grade (95.0% purity) was provided by CIBA-GEIGY Ltd, Basle, Switzerland. For topical application tests, technical samples of permethrin (95.4%, 52.4% cis) and DDVP (97.8 %) were provided by Chemotecnica S.A., Argentina.

House flies

House fly pupae were collected (≈400–600 per site) from three poultry farms located in Buenos Aires province (SV, S34.56848 W59.11743; Q, S34.32077 W59.00690; C, S34.92691 W58.94680).The farms were situated 70 km apart from one another and were not surrounded by another poultry farms. Thus, we expected that populations were not connected between them. In the laboratory, house fly pupae were held for eclosion in plastic jars (250 ml) with a small quantity of untreated wood shavings. Containers where placed into 28-×28-×28-cm plastic boxes that were screened on both sides and the top. Pupae were maintained at 25±1°C, 57–75% RH, and a photoperiod of 12:12 (L/D) for 2–6 days. During this eclosion period, emerged adult flies were fed by placing dry milk, sugar, and water inside the boxes. The medium to rear larvae consisted of dried yeast, whole dry milk, agar, and nipagine diluted in absolute ethanol (10%), diluted in water in a proportional amount of 1:1:0.2:0.1, respectively. The strain CIPEIN was laboratory insecticide-susceptible that had never been exposed to insecticides and originated from the Institute for Pesticide Research, Wageningen, The Netherlands in 1981. Colony rearing rooms were maintained at the conditions mentioned above.

Bioassay

Larvicide tests

Cyromazine was dissolved in the fresh water and added to the larval medium. Final concentrations ranged from 0.08 to 10 ppm. Treated medium was added into plastic pots (55-mm high×90-mm diameter), and first larvaes (≈20-100) were individually transferred using a fine paintbrush and covered with an autoclaved cloth. Each concentration was replicated three to six times. Control consisted of the medium without the addition of the larvicide. The number of emerging house flies was recorded 2 weeks after setting up the tests, and larval mortality was calculated. Tests were kept at 25±1°C, 57–75% RH, and a photoperiod of 12:12 (L/D).

Adulticide tests

Four- to 7-day-old adult houseflies were anesthetized with CO_2, and 0.2 µl of the insecticide diluted in acetone was applied on the ventral side of the abdomen using a 25-µl Hamilton syringe. F1 and F2 generations were used for topical bioassays. Final concentration ranged from 0.005 to 15 mg/ml for DDVP and from 0.0003 to 15 mg/ml for permethrin. Batches of ten to 20 house flies per concentration were replicated three to five times. Control groups received acetone alone. After topical application, house flies were kept in plastic jars (250 ml), covered with tulle cloth, and secured with rubber bands. Insects were kept at 25±1°C, 57–75% RH, and a photoperiod of 12:12 (L/D). A water-saturated piece of cotton was placed on the bottom of each jar. Mortality consisted of flies without any movement and was recorded 18 h after treatment.

Statistical analysis

Because some mortality occurred in some controls (<10%), data were separately corrected according to Abbott's formula (Abbott 1925). Mortality data were subjected to probit analysis (Litchfield and Wilcoxon 1949) to estimate the lethal concentration (parts per million) or the lethal dose (microgram per insect) required to kill 50% of treated insects (LC_{50}) or (LD_{50}), respectively. Resistance ratios (RRs) and 95% confidence limits were estimated by comparison with the susceptible strain CIPEIN, as reported by Robertson and Preisler (1992). Data were analyzed by using the Polo-PC v 2.0 (LeOra software, 2002).

Results

Larvicide analysis

The results of the concentration–mortality test of larvicidal effect are presented in Table 1. LC_{50} and LC_{90} values indicated that all the studied populations were resistant to cyromazine. There were significant differences among all the populations. The population SV showed the highest resistant ratio (RR=62.5), followed by Q and C, with RR levels of 10.9 and 3.9, respectively.

Adulticide analysis

Data on adults exposed to DDVP and permethrin are shown in Table 2. LD_{50} and LD_{90} values revealed that the individuals from the Q population were the most tolerant to DDVP. However, no significant differences in susceptibility to DDVP were found among the field populations. The RRs to DDVP ranged from 45.4 to 62.5. Permethrin LD_{50} and LD_{90} values revealed that all the field populations differed significantly from the reference strain. The population Q showed the highest RR (RR=117.3). Both Q and SV populations differed significantly from the C population, and were 1.7- and 1.4-fold more tolerant than the mentioned population.

Discussion

The results of the current study indicate that house fly populations from Argentina are highly resistant to the larvicide cyromazine and to the adulticides DDVP and permethrin. This is the first study reporting that house fly from Argentina are resistant to a variety of different insecticides. A multi-resistance pattern was found in the studied poultry farms, suggesting an intensive and continuous selective pressure against house fly populations. Scott et al. (2000) studied house fly populations from New York that were exposed to a wide variety of insecticides and found a strong correlation between insecticide use and control histories. However, different regional or local chemical control strategies would also affect the evolution of house flies.

Cyromazine was used in the three poultry farms since the last 20 years ago. The different resistance levels to cyromazine found in the studied populations would suggest that the application of this larvicide has been heterogeneous in every site. Information collected from the farmers indicated that this larvicide has been more frequently used than product label recommendations, suggesting possible field control failures. In the three studied sites, cyromazine was sprayed onto surfaces of manure and used as food additive. However, in the last 5 years in Argentina, the incorporation of this larvicide as food additive was limited and controlled due to the international food regulations. In the USA, cyromazine feed-through was commercially introduced in 1982, and 2 years later, house flies tolerant to this larvicide were found (Bloomcamp et al. 1987). Moreover, house flies collected from a population where cyromazine control failed had an average resistant factor of 4.2 (Sheppard et al. 1989). These suggest that field house fly with resistant ratios over 5 would be a useful value to predict control failures in the field. In Brazil, a study by Pinto and Prado (2001) revealed that three out of five house fly populations were cyromazine resistant, with RRs of 6.5 to 12.8. However, no correlation between history application and resistance levels was made. In Europe, cyromazine has also been used as a manure application in Denmark since 1984 and in the UK since 2000. A survey of the impact of house fly resistance strategies in intensive animal units in the UK revealed that, after 5 years of cyromazine application, all the 15 field populations analyzed were fully susceptible to this larvicide (Learmount et al. 2002). On the other hand, the monitoring program of cyromazine susceptibility developed at the Danish pest infestation laboratory indicated that, after >10 years of intensive use, tolerance or low-level resistance was found (Kristensen and Jespersen 2003). After 15 generations of cyromazine selection, a 4.5-fold resistant field strain was selected to a 70-fold resistance (Bloomcamp et al. 1987). The high resistance levels of cyromazine found in the field house fly populations from Argentina suggest that it was widely overused in Argentina and played an important role in the development of

Table 1 Responses to cyromazine of house fly larvae

Population	*n*	LC_{50} (ppm) (95%CI)	LC_{90} (ppm) (95%CI)	Slope ± SE	χ^2	RR (95%CI)
CIPEIN	1,652	0.09 (0.07–0.10)	0.24 (0.20–0.33)	2.91±0.4	3.75	–
SV	1,136	5.59 (3.67–7.01)	8.78 (6.93–15.38)	6.10±0.9	4.71	62.50 (52.63–76.92)
Q	489	0.97 (0.73–1.20)	3.17 (2.53–4.26)	2.49±0.3	0.68	10.98 (8.33–14.92)
C	2,410	0.34 (0.01–0.97)	2.56 (0.89–58.08)	1.47±0.3	6.02	3.92 (2.54–6.02)

Table 2 Toxicity of adult house flies to DDVP and permethrin

Insecticide	Population	*n*	LD_{50} (μg/insect) (95%CI)	LD_{90} (μg/insect) (95%CI)	Slope ± SE	χ^2	RR (95%CI)
DDVP	CIPEIN	493	0.007 (0.004–0.009)	0.019 (0.015–0.029)	3.01±0.4	5.07	–
	SV	608	0.36 (0.28–0.44)	0.74 (0.58–1.24)	4.12±0.4	12.32	50 (40.00–62.50)
	Q	581	0.46 (0.37–0.56)	1.15 (0.86–2.00)	3.19±0.3	7.18	62.50 (50.00–76.92)
	C	447	0.33 (0.24–0.41)	0.79 (0.61–1.30)	3.38±0.4	8.49	45.45 (35.71–58.82)
Permethrin	CIPEIN	222	0.014 (0.010–0.018)	0.034 (0.026–0.047)	3.45±0.5	7.34	–
	SV	223	1.36 (0.84–3.17)	6.91 (3.04–74.96)	1.81±0.3	6.00	94.38 (80.81–170.82)
	Q	251	1.69 (1.18–2.98)	9.83 (4.81–45.42)	1.68±0.3	2.12	117.34 (113.33–160.40)
	C	277	0.94 (0.70–1.39)	6.42 (3.42–22.73)	1.54±0.3	2.65	65.52 (58.33–75.04)

insecticide resistance populations. In addition, the exposure of house flies to the two treatments—food additive and direct sprayed onto the manure—would probably led to a very high selection pressure.

The organophosphate DDVP has been introduced in Argentina for the use on poultry farms two decades ago. Since then, it has been one of the products most employed against adult house flies. The three studied populations of house fly were highly resistant to the organophosphate DDVP. Direct sprayed actions to knock down high levels of house flies has led to an overuse of this insecticide in the poultry farms studied. This selective pressure could explain the elevated RRs reported in this study. Scott et al. (2000) found low to moderate resistant levels to organophosphates from several house fly strains collected from New York poultry farms. These authors found a correlation between the insecticide histories of organophosphates and the resistant levels. Moreover, Kristensen et al. (2000) studied the azamethiphos tolerance of house flies from Denmark, showing that after 15 years of intensive resistance monitoring, 10% of the population was highly resistant. However, this resistance pattern was highly labile, disappearing within 1 or 2 years.

All the studied populations were highly resistant to permethrin. No previous information about field control failures was available. A correlation of data topical application of permethrin and control failures in the field made by Farham et al. (1984) revealed that control failures usually occurs when RRs are over 15-fold. Marçon et al. (2003) found that two studied house fly populations from the USA had RRs less than fivefold, suggesting that permethrin should still be used against house fly. The number of generations required for a tenfold increase in LD_{50}s through different permethrin selection intensity varied from 9 to 21 (Zhu et al. 2002). Another experiment of permethrin selection showed that, after five generations, the level of resistance in the house flies could increase to 1,800-fold (Lui and Yue 2000). Similarly, Zhang et al. (2008) reported that, after 25 generations of selective pressure with the pyrethroid beta-cypermethrin, house fly resistance strains increased 1,700-fold. These indicate that resistance to pyrethroids in house fly could be developed rapidly.

The permethrin resistance found in the present work could be considered as part of a multi-resistance mechanism with incremented detoxification metabolism of xenobiotics.

This is the first report of a multi-resistance pattern of Argentinean house flies collected in the field. Considering that cyromazine and DDVP are the most sold products in the Argentinean market and that they had been used in the studied poultry farms with slightly different chemical control strategies; we can assumed that these products were responsible for the resistance pattern found in this work. Pospischil et al. (1996) reported that a field population of house fly had adults highly resistant to organophosphates and pyrethroids but tolerant to cyromazine. The reported multi-resistance profile could also be attributable in part to the movement of house flies between poultry houses and into appropriate breeding habitats. The study of Lysyk and Axtell (1986) indicated that house fly dispersal plays an important role in the movement of insects from one area to another. Moreover, the three poultry farms are surrounded by several crop fields where insecticide applications are frequent. The insecticide resistance profile showed in the present study could be associated with both the application exposure of larvae and adults at the poultry farms and to direct and indirect insecticide residues from surrounding fields. Further work is needed to understand the multi-resistance pattern found in this study. These studies should be focused at either biochemical or molecular level, since a lot of information is currently available worldwide. Actually, no insecticide monitoring program of house fly population is currently available in Argentina. An effective resistance management strategy would bring new insights into the level, extend, and degree of resistance in the studied sites. Thus, the implementation of regular surveys on poultry farms would be very informative in order to establish effective strategies against house flies. In addition, the implementation of successful guidelines implemented in Denmark and UK would prevent the detrimental effects of multi-resistance insects avoiding future field control failures.

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