INSECTICIDE SUSCEPTIBILITY OF HORN FLIES, *Haematobia irritans* (DIPTERA: MUSCIDAE), IN THE STATE OF MATO GROSSO DO SUL, BRAZIL*

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ABSTRACT:- BARROS, A.T.M.; GOMES, A.; KOLLER, W.W. **Insecticide susceptibility of horn flies, *Haematobia Irritans* (Diptera: Muscidae), in the State of Mato Grosso do Sul, Brazil.** [Suscetibilidade da mosca-dos-chifres, *Haematobia irritans* (Diptera: Muscidae), a inseticidas no Estado de Mato Grosso do Sul, Brasil]. *Revista Brasileira de Parasitologia Veterinária*, v. 16, n. 3, p. 145-151, 2007. Embrapa Pantanal, Rua 21 de Setembro, 1880. Corumbá, 79320-900 MS, Brasil. E-mail: thadeu@cpap.embrapa.br

Horn fly susceptibility to insecticides was evaluated in the state of Mato Grosso do Sul, Brazil, from October 2000 to September 2002. Insecticide bioassays (n=57) were conducted in 38 ranches from 14 municipalities throughout the state. Horn flies from wild populations were collected on cattle and exposed to filter papers impregnated with cypermethrin, permethrin, or diazinon and mortality was assessed after two hours. Resistance to cypermethrin was detected in all populations, with resistance ratios (RR) ranging from 27.6 to 91.3-fold. Permethrin bioassays provided apparently low levels of resistance (RR<5), however, resistant flies were found in 96.9% of the populations based on diagnostic concentrations. From both pyrethroid bioassays, resistance was detected in 97.4% of the populations. On the other hand, a high susceptibility to diazinon (RR ≤ 1.1) was detected in all populations. Pyrethroid products, most cypermethrin (92.3%) and deltamethrin (66.7%), were used in all ranches controlling horn flies (97.5%). Insecticide treatments, usually incorrectly applied, were routinely delivered by manual backpack sprayers in most ranches (84.5%). This profile of insecticide use helps to explain the widespread resistance of horn flies to pyrethroids in the state as well as their high susceptibility to the organophosphate. Inadequate control practices contribute to aggravate the resistance problem and its consequences.

KEY WORDS: *Haematobia irritans*, insecticide resistance, pyrethroid resistance, organophosphate, cypermethrin.

RESUMO

A suscetibilidade da mosca-dos-chifres a inseticidas foi avaliada no estado de Mato Grosso do Sul de outubro/2000 a setembro/2002 com a realização dos ensaios biológicos (n=57) em 38 propriedades, de 14 municípios. Moscas coletadas em bovinos foram expostas a papéis de filtro impregnados com cipermetrina, permetrina, ou diazinon, registrando-se a mortalidade após duas horas. Resistência à cipermetrina foi detectada em todas as populações, com fatores de resistência (FR) entre 27,6 e 91,3. Ensaios biológicos com permetrina resultaram em níveis de resistência aparentemente baixos (FR<5), entretanto, o uso de concentrações diagnósticas evidenciaram a ocorrência de moscas resistentes em 96,9% das populações. Considerando ambos os bioensaios com piretróides, 97,4% das populações apresentaram resistência. De outro modo, todas as populações demonstraram elevada suscetibilidade ao diazinon (FR ≤ 1.1). Todas as propriedades onde o controle da mosca-dos-chifres era realizado (97,5%) utilizavam produtos piretróides, principalmente à base de cipermetrina (92,3%) e deltametrina (66,7%). Tratamentos inseticidas utilizando bombas costais manuais eram realizados em 84,5% das propriedades, geralmente de forma inadequada. O perfil de uso de inseticidas nas propriedades contribui para explicar a ampla ocorrência de resistência da mosca-dos-chifres aos piretróides, assim como a elevada suscetibilidade aos organofosforados, no Estado. Práticas inadequadas de controle contribuem para agravar o problema da resistência e suas conseqüências.

PALAVRAS-CHAVE: *Haematobia irritans***,** resistência, inseticida, resistência a piretróides, organofosforado.

INTRODUCTION

The horn fly, *Haematobia irritans irritans*, is considered a major livestock pest in Brazil. Estimates of yearly economical

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losses caused by this parasite to the national cattle industry varied from US$ 150 million (GRISI et al., 2002) to US$ 865 million (BIANCHIN et al., 2006). After its entry in the north of the country, horn fly dispersion was facilitated by cattle transporting and eventually reached the state of Mato Grosso do Sul in 1990.

Historically, horn fly control has been largely dependent on chemical products, leading to development of resistance as reported to organochlorines, organophosphates, carbamates, and pyrethroids (BYFORD et al., 1985; SPARKS et al., 1985). Since resistance to distinct insecticide classes may develop in just two to four years in a horn fly population (QUISENBERRY et al., 1984; BARROS et al., 2001), it was not a surprise that complaints regarding reduced product efficacy and control failure had arisen all over the country following several years of chemical control of this livestock pest.

Resistance of horn flies in Brazil has been previously reported to pyrethroids in Southern (GUGLIELMONE et al., 2001) and Northern (GIRÃO et al., 2002; OLIVEIRA et al., 2006) states. As part of a comprehensive survey on the status of horn fly susceptibility to insecticides carried out in several regions of the country, the present study focused on the particular status of resistance in the state of Mato Grosso do Sul, Brazil.

MATERIAL AND METHODS

From October 2000 to September 2002, susceptibility of wild horn fly populations to pyrethroid and organophosphate (OP) insecticides was assessed at cattle ranches from distinct municipalities in the State of Mato Grosso do Sul (MS) (Figure 1) by using impregnated filter paper bioassays (SHEPPARD; HINKLE, 1987).

Insecticide kits for bioassays were produced at the Laboratory of Entomology of the Embrapa Pantanal by using technical diazinon (>93.5% purity, Novartis and Chem Service), permethrin (>97% purity, FMC do Brasil and Chem Service), and cypermethrin (92%, Minerthal) diluted in acetone. Each insecticide kit contained three replicates of eight to ten concentrations of permethrin (0.4-51.2μg/cm^2), cypermethrin (1.6-819.2μg/cm^2) or diazinon (0.1-3.2μg/cm^2). Control papers were treated with acetone only.

Impregnated filter papers were maintained under refrigeration and placed in plastic disposable Petri dishes just before field trips. In the field, dishes were immediately loaded after an adequate fly sample was collected on cattle by using hand nets. Mortality was assessed immediately after dishes were loaded, to check for early mortality due to fly collection and manipulation, and also after two hours of exposure to the insecticide. Flies unable to walk were considered dead. Additional information regarding bioassays can be found in Barros et al. (2002). Bioassays with fly mortality >10% in control dishes or without showing fiducial limit at the probit analysis were not considered for the purpose of this publication.

Mortality data were analyzed by POLO-PC (LEORA SOFTWARE, 1987) to obtain lethal concentration (LC_{50}) for each population. Resistance ratios (RR) were calculated by dividing the LC_{50} from field populations by the LC_{50} from an insecticide-susceptible colony maintained at the USDA Knipling-Bushland US Livestock Insects Research Laboratory (Kerrville, TX, USA). Yearly insecticide kits were sent to this research laboratory and bioassays were performed using the susceptible reference population. Differences in LC_{50} were considered significant when their 95% fiducial limits did not overlap.

Bioassays with colonized susceptible flies also provided a diagnostic concentration (DC) for permethrin, which was considered as the lowest concentration in each insecticide kit that resulted in 100% mortality of the susceptible individuals after exposure for two hours. Interpretation of permethrin DC bioassays was based on frequency of resistant individuals. Wild populations up to 1% of flies surviving the DC in field bioassays were assumed to be susceptible (DAVIDSON; ZAHAR, 1973; MAY; DOBSON, 1986). Populations above 1% surviving at the DC were considered as resistant and the survival frequency was considered as the resistance frequency (RF) of the population. Correlation analysis was performed between permethrin RR and RF.

A standard questionnaire regarding parasite control was applied in several ranches, particularly where bioassays were conducted. The questions focused mainly on chemical control of horn flies and the cattle tick (*Boophilus microplus*) in order to get background information about insecticide use and cattle pest control practices at each site.

RESULTS AND DISCUSSION

Bioassay results

A total of 57 bioassays with permethrin (32), cypermethrin (15), and diazinon (10) were conducted on 38 ranches from 14 municipalities in the state of Mato Grosso do Sul (Figure 1). As observed in other long-term studies, the LC_{50} from the susceptible reference population varied among years, probably due to differences in insecticide kits (SHEPPARD; JOYCE, 1992), fly gender and age (PRUETT et al., 2000), and/or mortality assessors. To avoid such problems, comparisons between yearly results from different kits used RR instead of LC_{50} and a permethrin DC was established for each kit.

Permethrin LC_{50} varied from 1.61 to 9.25μg/cm^2 and RR ranged from 0.6 to 4.2 (Table 1). Relatively low RR may suggest either susceptibility or an initial development of resistance (low frequency of resistant individuals) in tested populations. However, failure of horn fly control has been previously associated with resistance approaching three fold (KUNZ; SCHMIDT, 1985), which suggests that the resistance level showed by some populations in this study may affect efficacy of pyrethroid products in the field.

Permethrin LC_{50} from field populations was significantly higher than the susceptible colony, thus indicating resistance, in 50% of these populations. However, even quite high

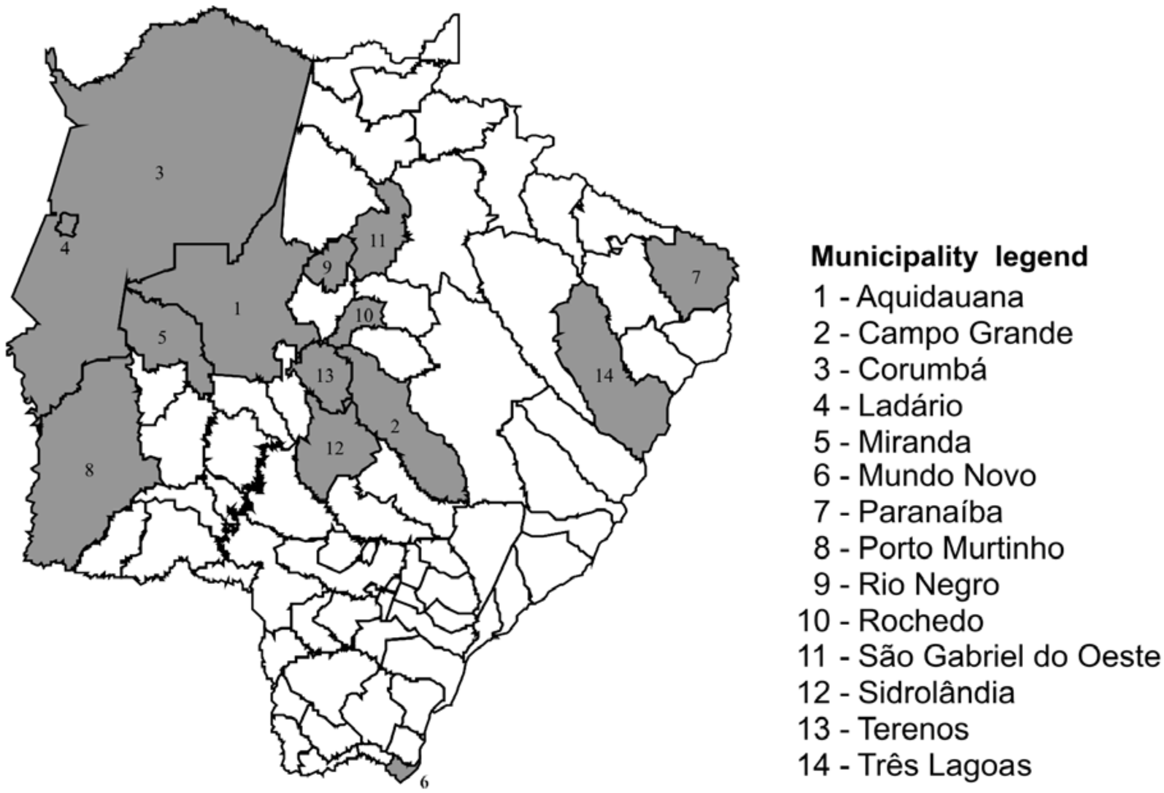

Figure 1. Survey on horn fly resistance in the State of Mato Grosso do Sul, Brazil, from October 2000 to September 2002.

frequencies of resistant individuals can result in small changes in the LC_{50} making resistance undetectable, particularly in its early stages, and the use of discriminatory concentrations is a more suitable approach (DAVIDSON; ZAHAR, 1973). The DC analysis detected resistant flies in 96.9% of the permethrin bioassays (Table 1). In all populations with a RF>1% there were flies able not just to survive twice the DC for the standard exposure period of two hours but, in most cases, flies were able to survive the DC and even twice the DC for a period of four hours. On the other hand, in the population with RF<1% no fly survived higher concentrations of the insecticide or longer periods of exposure, confirming the suitability of DC bioassays as well as the criteria for interpreting their results. Furthermore, low frequencies of horn flies with the *kdr* genotype were detected by molecular analysis in some of these populations (G. Sabatini, personal information) confirming the presence of pyrethroid-resistant flies in populations with low (as low as 1.5-fold) RR to permethrin.

The finding of permethrin-resistant flies in most populations can not be directly associated with control problems. Actually, it is probable that some of the populations with lower RR and RF were still potentially susceptible in the field. Barros et al. (2001) observed reduction of OP ear tags efficacy associated with horn fly RF of at least 5%, measured in pre-season bioassays. Considering this RF=5% as a criterion for potential control risks, 89.5% of the populations bioassayed with permethrin in this study would show control problems in the field, as evidenced by complaints from most ranchers.

Additional information from field questionnaires helped to a better understanding and interpretation of permethrin bioassays results. No selective pressure by permethrin took place, since none of the populations has been previously exposed to products containing such insecticide. On the other hand, a widespread use of cypermethrin products has been a common routine for horn fly control in the studied sites for several years. Thus, resistance detected to permethrin was secondary (a cross-resistance or a multiple resistance) to the primary resistance developed to cypermethrin. Cross-resistance is a well known feature among pyrethroids and has been reported between cypermethrin and permethrin in horn flies (BYFORD et al., 1985).

Once cypermethrin was detected as the major insecticide behind the development of pyrethroid resistance, it was used in bioassays in the second and third years of the study. Cypermethrin LC_{50}'s ranged from 15.84 to 39.27μg/cm^2, with RR between 27.6 and 91.3 (Table 1). These results characterized all tested populations as resistant to this pyrethroid and confirmed initial suspects of resistance by producers. Guglielmone et al. (1998a) observed that cypermethrin pour-on treatments failed to control horn fly populations with smaller RR's to cypermethrin than the reported in the present study.

Bioassay results from both pyrethroids showed that resistance to this class was present in 97.4% of the studied populations. High frequencies of resistance to pyrethroids also have been found in surveys conducted in Northern states

Table 1. Pyrethroid susceptibility of horn fly populations, from October 2000 to September 2002, in the State of Mato Grosso do Sul, Brazil.

Municipality	Site (ranch)	Permethrin				Cypermethrin		
		$LC_{50\text{-}2h}$ (95% FL)	Slope (SE)	RR	RF (%)	$LC_{50\text{-}2h}$ (95% FL)	Slope (SE)	RR
Aquidauana	Santa Cruz	-	-	-	-	21.69 (14.66 - 30.93)	2.84 (0.20)	50.4
	São Salvador	2.57 (2.31 - 2.83)	3.61 (0.33)	3.5	56.27	23.51 (17.79 - 30.60)	2.31 (0.19)	54.7
	Taboco	1.89 (1.65 - 2.18)	3.50 (0.23)	2.6	40.23	38.17 (32.85 - 43.93)	2.49 (0.22)	88.8
	Vitória	1.64 (1.50 - 1.80)	3.15 (0.24)	2.2	32.11	33.17 (20.76 - 44.50)	2.77 (0.36)	77.1
Campo Grande	Matel	4.16 (3.29 - 5.15)	1.92 (0.11)	1.6	37.57	-	-	-
	Pouso Alegre	3.67 (2.44 - 5.18)	2.37 (0.14)	1.4	22.33	-	-	-
Corumbá	Alegria	1.61 (1.44 - 1.79)	3.55 (0.30)	0.6	2.50	-	-	-
	Angico	3.28 (2.64 - 3.82)	3.30 (0.47)	1.3	10.63	-	-	-
	Band'Alta	2.99 (2.35 - 3.71)	4.32 (0.60)	4.1	66.14	-	-	-
	Bocaiúva	2.20 (0.96 – 3.67)	2.77 (0.28)	0.8	3.08	-	-	-
	Figueirinha	2.40 (2.23 - 2.58)	5.09 (0.47)	3.3	56.22	21.07 (12.08 - 28.66)	3.45 (0.30)	49.0
	Itacupê	-	-	-	-	25.37 (12.99 - 38.97)	2.89 (0.27)	27.6
	Nhumirim	3.98 (3.09 - 4.92)	5.20 (0.53)	1.5	11.31	-	-	-
	Novo Norte	2.38 (2.15 - 2.62)	4.96 (0.53)	0.9	2.37	-	-	-
	Santa Maria	4.11 (3.77 - 4.47)	4.40 (0.34)	1.6	21.09	-	-	-
	São Bernardo	2.40 (1.66 - 3.49)	4.71 (0.39)	0.9	0.86	-	-	-
	São José do Japorá	-	-	-	-	28.24 (23.46 - 33.18)	2.20 (0.26)	30.7
Ladário	Baía Branca	-	-	-	-	39.27 (20.96 - 68.03)	1.86 (0.12)	91.3
	Machado de Ouro	3.03 (2.79 - 3.27)	4.22 (0.39)	4.2	62.50	34.32 (25.84 - 44.94)	1.74 (0.11)	79.8
Miranda	Caiman	4.28 (3.89 - 4.75)	4.37 (0.45)	1.7	23.66	23.19 (17.32 - 30.71)	2.67 (0.19)	53.9
	Cristo	2.05 (1.64 - 2.58)	4.11 (0.35)	0.8	1.32	38.84 (33.23 - 47.30)	2.53 (0.29)	90.3
	Guaicurus	2.09 (1.89 - 2.32)	4.15 (0.34)	0.8	3.30	15.84 (9.05 - 27.04)	3.42 (0.27)	36.8
	Novo Horizonte	3.67 (3.33 - 4.05)	4.77 (0.52)	1.4	11.14	24.47 (21.56 - 27.70)	2.77 (0.22)	55.9
Mundo Novo	Cruzeiro do Sul	2.37 (1.44 – 3.84)	4.47 (0.37)	0.9	7.95	-	-	-
	Lagoa Vermelha	5.44 (3.82 - 7.86)	4.20 (0.37)	2.1	45.95	-	-	-
Paranaíba	Carminho	4.72 (3.56 – 6.14)	3.50 (0.27)	1.8	40.78	-	-	-
	Irara II	9.25 (8.32 - 10.21)	3.87 (0.30)	3.6	74.16	-	-	-
	Saltador	6.28 (5.51 - 7.09)	1.91 (0.11)	2.4	48.73	-	-	-
Porto Murtinho	Brasília	-	-	-	-	18.15 (11.71 - 24.84)	3.16 (0.31)	42.2
	Sta. Izabel	-	-	-	-	23.74 (20.12 - 27.51)	2.84 (0.29)	55.2
Rio Negro	Garimpo	3.10 (1.53 – 4.68)	3.05 (0.37)	1.2	23.44	-	-	-
Rochedo	Cabeceira Limpa	3.13 (2.21 - 4.19)	3.21 (0.30)	1.2	14.02	-	-	-
São Gabriel do Oeste	Museu	2.62 (2.17 - 3.19)	3.55 (0.20)	1.0	7.17	-	-	-
Sidrolândia	Sossego	6.01 (4.04 - 8.13)	2.56 (0.26)	2.3	46.63	-	-	-
Terenos	Cabeceira do Indaiá	2.81 (2.06 - 3.89)	3.79 (0.25)	1.1	6.79	-	-	-
Três Lagoas	Laguna	3.57 (2.67 - 4.73)	2.64 (0.23)	1.4	19.75	-	-	-
	Lilocris	7.17 (6.21 - 8.29)	1.87 (0.12)	2.8	50.89	-	-	-
	Renascença	4.31 (2.20 - 6.91)	3.64 (0.31)	1.7	36.27	-	-	-

LC_{50} = lethal concentration to 50% of the population (expressed as $\mu g/cm^2$); resistance ratio (RR) - LC_{50} from field population/LC_{50} from susceptible colony

Resistance frequency (RF) - percentage of horn flies surviving a diagnostic concentration of permethrin that killed all flies from Kerrville susceptible colony.

Kerrville strain LC_{50} and DC for permethrin in 2000: 2.59 (2.42-2.79) and 6.4$\mu g/cm^2$; and 2001: 0.73 (0.46-0.99) and 2.4$\mu g/cm^2$, respectively.

Kerrville strain LC_{50} for cypermethrin in 2001: 0.43 (0.29-0.70) $\mu g/cm^2$ and 2002: 0.94 (0.64-2.11) $\mu g/cm^2$.

(GIRÃO et al., 2002; OLIVEIRA et al., 2006) as well as in neighbor countries such as Argentina (GUGLIELMONE et al., 2001) and Uruguay (MARQUES et al., 1997).

Relatively low RR may be difficult to be interpreted conclusively as a real susceptibility or even as a low level of resistance and caution should be exercised in their interpretation. Such results can be somewhat puzzling and lead to unsuitable conclusions unless additional information is available. Background information regarding history of pesticide use tends to be useful and bioassays with insecticides to which populations have been previously most exposed tend to be the best choice for surveying for susceptibility. However, it is important to stress that the ultimate confirmation regarding resistance should be provided by molecular analysis and/or product efficacy studies.

A general pattern with much higher RR to cypermethrin than to permethrin was observed in all populations. Although this trend would suggest the development of higher resistance levels to the primary selective agent (cypermethrin), a similar pattern has been observed also in permethrin-resistant horn flies exposed to cypermethrin (BYFORD et al., 1985). Since no correlation was found between permethrin and cypermethrin LC_{50} and RR, a higher resistance to cyanopyrethroids compared to noncyano pyrethroids may suggest the

Table 2. Susceptibility and resistance ratios of horn fly populations to diazinon, from October 2000 to September 2002, in the State of Mato Grosso do Sul, Brazil.

Municipality	Site (ranch)	$LC_{50\text{-}2h}$ (95% FL)	Slope (SE)	RR
Aquidauana	Santa Cruz	0.69 (0.56 - 0.85)	8.11 (0.70)	0.3
	São Salvador	0.46 (0.41 - 0.52)	6.39 (0.55)	0.2
	Taboco	0.48 (0.45 - 0.51)	6.89 (0.64)	0.2
Corumbá	Angico	0.55 (0.44 - 0.62)	8.42 (1.05)	0.5
	Novo Norte	1.10 (0.87 - 1.51)	7.19 (0.62)	1.0
	São Bernardo	1.17 (0.90 - 1.54)	6.40 (0.49)	1.1
Miranda	Cristo	0.39 (0.35 - 0.45)	6.65 (0.53)	0.4
	Novo Horizonte	0.78 (0.61 - 0.96)	7.22 (0.71)	0.7
Porto Murtinho	Sta. Izabel	0.82 (0.62 - 1.59)	2.63 (0.64)	0.4
Rochedo	Cabeceira Limpa	0.32 (0.30 – 0.33)	14.41 (2.11)	0.3

LC_{50} = lethal concentration to 50% of the population; expressed as µg/cm²
Resistance ratio (RR) = LC_{50} from field population/LC_{50} from Kerrville susceptible colony.
Kerrville strain LC_{50} for diazinon in 2000: 1.09 (1.00-1.21) µg/cm² and 2001: 2.29 (2.19-2.40) µg/cm².

participation of an oxidative metabolic mechanism (SHEPARD, 1995), despite the fact that target site insensitivity (*kdr*) and esterase-mediated metabolism have been recently detected in horn fly populations in the state (GUERRERO; BARROS, 2006).

Diazinon bioassays showed LC_{50} varying from 0.39 to 1.17µg/cm² (Table 2). The diazinon RR did not exceed 1.1-fold (most below 0.7), which characterized all populations as highly susceptible to this OP and confirmed previous reports from the same region (BARROS et al., 2002). A higher susceptibility to diazinon has been previously observed in pyrethroid resistant populations (SHEPPARD; MARCHIONDO, 1987) associated to an increased activation of diazinon by oxidative metabolism (CILEK et al., 1995).

Information on pesticide use and pest control practices

Once the susceptibility profile of horn fly populations was obtained it became useful to fit it in the management system adopted in the region, so a broader understanding of the resistance situation could be achieved.

Chemical control of horn flies was considered an important part of the cattle operation, being a routine in 97.5% out of the 40 ranches were the questionnaire was applied. Decisions about insecticide treatment were mostly based on arbitrary unacceptable infestation levels, animal behavior, owner convenience, and/or taking advantage of other management practices, such as vaccination.

Pyrethroid products, mainly cypermethrin (92.3%) and deltamethrin (66.7%), were routinely used in all ranches controlling horn flies, reflecting the market domain of such products. A similar situation was found also in Northern states of Brazil (OLIVEIRA et al., 2006). On the other hand, dependence on pyrethroids for horn fly control has been under reduction along the years in Argentina due to resistance (GUGLIELMONE et al., 2001). Insecticide products from other classes were relatively poorly used for horn fly control. Macrocyclic lactone pour-on formulation, recommended for horn fly control, was used in just 2.6% of the ranches; however, its injectable formulation (primarily indicated for helminth control) was eventually used in 30.8% of the ranches and may play some role in fly control (GUGLIELMONE et al., 1998b). Although most mixture products contained pyrethroids, their use in 25.6% of the ranches was generally associated with previous control failure and suspect of resistance to pyrethroids in those sites.

Because lower costs and operational convenience, spraying became the most common (86.5%) method of insecticide application in the region. Similar situations have been found elsewhere in Brazil (OLIVEIRA et al., 2006) as well as in other Latin American countries (MALDONADO et al., 2005). In most ranches (86.2%) where spraying was used to control horn flies, insecticide treatments relied on backpack manual sprayers, resulting in much lower volumes (sometimes less than 10%) than the technically recommended. Although the use of pour-on products was commonly mentioned (45.9%), they were not routinely used or applied on all animals of the herd. A random rotation of insecticide products is a common practice, in most cases triggered mainly by a previous product failure and lacking technical information on what should be used next. In general, this profile of the horn fly chemical control coincided with a previous study made in 216 ranches by Rodrigues et al. (2004) and seems to represent adequately the situation in the state of Mato Grosso do Sul.

Considering such control practices, the observed widespread resistance of horn fly populations to pyrethroid insecticides may be explained by the inadequate chemical control routinely performed in the ranches as well as the higher selective pressure by pyrethroid products due to their commercial success. However, despite the cypermethrin RR were high enough to impair fly control, resistance levels tended to be relatively lower than commonly reported in the literature. This situation may result from the extensive cattle raising system and the management practices adopted, in which relatively few insecticide treatments per year and insufficient insecticide volumes are common.

Acceptable and sustainable levels of horn fly control will depend on the adoption of strategies focusing not just on improvement of the fly control itself but also on resistance management. The high susceptibility observed to diazinon as well as the low use of insecticides from other classes suggested that non-pyrethroid insecticides remain as useful alternatives for horn fly control and resistance management in the region.

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