Susceptibility to Diazinon in Populations of the Horn Fly, *Haematobia irritans* (Diptera: Muscidae), in Central Brazil

Antonio Thadeu M Barros/+, Alberto Gomes*, Ana Paula K Ismael, Wilson W Koller*

Embrapa Pantanal, Rua 21 de Setembro 1880, 79320-900 Corumbá, MS, Brasil *Embrapa Gado de Corte, Campo Grande, MS, Brasil

From October 2000 to April 2001, insecticide bioassays were conducted in 18 ranches from 10 counties in the states of Mato Grosso and Mato Grosso do Sul, in Central Brazil. Horn flies from wild populations were exposed to diazinon-impregnated filter papers immediately after collection on cattle, and mortality was recorded after 2 h. A high susceptibility to diazinon was observed in all tested populations. The LC_{50}s ranged from 0.15 to 0.64 µg/cm^2, and resistance ratios were always lower than one (ranging 0.1-0.6). Pyrethroid products, most applied by back-pack sprayers, have been used since the horn fly entered the region, about 10 years ago. The high susceptibility observed to diazinon indicates that this insecticide (as probably other organophosphate insecticides) represents an useful tool for horn fly control and resistance management, particularly in pyrethroid-resistant populations.

Key words: horn flies - insecticide - organophosphate - resistance - Centtral Brazil

Horn fly resistance to synthetic pyrethroids is currently widespread in North America (Sparks et al. 1985, Mwangala & Galloway 1993, Kunz et al. 1995) as far South, in Argentina (Guglielmone et al. 1998). However, reports of resistance to organophosphate (OP) insecticides have been relatively rare events.

Horn fly resistance to OPs has been reported to fenchlorphos (Burns & Wilson 1963), tetrachlorvinphos (Sheppard 1983, Harvey et al. 1984), pirimiphos-methyl (Cilek et al. 1991, Steelman et al. 1994, Barros et al. 1999, 2001), ethion, fenthion (Barros et al. 2001), and diazinon. Resistance to diazinon was initially detected in bioassays (Cilek et al. 1991, Steelman et al. 1994) and later correlated with product failure (Barros et al. 2001). Diazinon resistance developed in four years due to yearly ear tag use, followed by cross-resistance to ethion, fenthion, pirimiphos-methyl, and tetrachlorvinphos (Barros et al. 2001). Currently, there are no reports of horn fly resistance to diazinon outside the USA. Rather, high susceptibility to diazinon has recently been detected in several populations from Argentina and Southern Brazil (Guglielmone et al. 2000).

The OP insecticides are considered important tools in the management of pyrethroid-resistant populations. Diazinon is particularly important because enhanced susceptibility of the pyrethroid-resistant flies to this OP (Sheppard & Marchiondo 1987, Cilek et al. 1995) providing adequate control under field conditions (Byford et al. 1988).

This study was part of a comprehensive survey on horn fly resistance to insecticides conducted in several regions of the country and reports some initial information on the susceptibility of horn fly populations to diazinon from two states in Central Brazil.

+Corresponding author. Fax: +55-67-233.1011. E-mail: thadeu@cpap.embrapa.br
Received 19 December 2001
Accepted 4 April 2002

MATERIALS AND METHODS

From October 2000 to April 2001, susceptibility of wild horn fly populations to diazinon was measured at 18 cattle ranches from 10 counties, in the states of Mato Grosso and Mato Grosso do Sul, Central Brazil.

Susceptibility of horn fly populations was assessed by using impregnated filter paper bioassays adapted from Sheppard and Hinkle (1987). Insecticide kits for the bioassays were produced at the Laboratory of Entomology of the Embrapa Pantanal by diluting technical diazinon (97.3% purity, Novartis) in acetone p.a. (Merck) to obtain eight concentrations (0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.2, and 1.6 µg/cm^2) of the insecticide on labeled filter papers (Whatman #1). Three replicates were made for each concentration. Impregnated filter papers were wrapped in aluminum foil after at least 3 h drying and then stored in refrigerator (6ºC) until use. Bioassay kits were set up by placing the impregnated papers on the inverted top (top facing down) of disposable plastic petri dishes (Cral, 90 x 15 mm). Papers were used four times, twice each paper side. A single hole (about Ø1 cm) was made at the center of the bottom (facing up) of the dishes to permit fly loading. Filter papers treated with acetone only were used as controls.

Flies were tested immediately after capture with hand nets on cattle. About 100 flies were distributed in three petri dishes per concentration. Mortality was assessed immediately after dishes were loaded, to check for any early mortality caused by fly manipulation, and after 2 h of exposure to the insecticide. Flies unable to walk were considered dead. Mortality data were corrected by Abbott's formula (Abbott 1925) when necessary and analyzed by POLO-PC (LeOra Software 1987). Bioassays with fly mortality at the control dishes higher than 10% were excluded.

A bioassay with horn flies from an insecticide-susceptible colony maintained at the Knipling-Bushland US

Livestock Insects Research Laboratory, USDA-ARS (Kerrville, TX) was conducted in November 2000, using the same insecticide kit, to provide a susceptible reference LC_{50} for resistance ratio (RR) calculations (LC_{50} from field population/LC_{50} from susceptible colony).

In addition, wild fly mortality at the highest concentration (1.6 µg/cm^2) was checked after 4 h exposure for the presence of diazinon-resistant individuals as suggested by Barros et al. (2001).

RESULTS AND DISCUSSION

The LC_{50}s from wild populations ranged from 0.15 to 0.64 µg/cm^2 and were always lower compared with the LC_{50} (1.09 µg/cm^2) for the susceptible reference population (Table). Consequently, all tested populations expressed a high level of susceptibility to diazinon. Similar findings have been previously reported from pyrethroid-resistant horn flies (Sheppard & Marchiondo 1987, Crosby et al. 1991). The mechanism behind this negative cross-resistance was suggested to be increased metabolism (activation) of diazinon by increased activity of mixed function oxidases present in resistant flies (Cilek et al. 1995).

Two factors may explain the high susceptibility to diazinon observed in this study: low OP use and the presence of pyrethroid resistance. History of insecticide use for the last 10 years where horn flies were sampled in this study included pyrethroid applications (variable frequency) by using backpack sprayers. Only three sites (Estância Lagoa Vermelha, Fazenda Flor da Serra, and Sítio Cruzeiro do Sul) recorded some OP use (coumaphos, in mixture with pyrethroids) but in low frequency during the last years. Pyretroid resistance suspected by most producers was later confirmed by bioassays (authors information, unpublished data) which also may explain the high susceptibility found to diazinon (Sheppard & Marchiondo 1987, Cilek et al. 1995). It should be noted that one of the lowest susceptibility to diazinon was found at "Fazenda Nhumirim", an experimental station of Embrapa where horn flies on cattle have been maintained without pesticide exposure and fly population is highly susceptible to pyrethroids.

A mortality of 96.7% was observed in flies from the susceptible reference colony after a 4h-exposure to the top concentration (1.6 µg/cm^2). This finding seems to be in agreement with the discriminating concentration (1.72 µg/cm^2) proposed by Barros et al. (2001), in which a 100% mortality was observed in flies from the same colony exposed to a similar period. In this study, not a single fly from the wild populations survived the 1.6 µg/cm^2 concentration after a 4 h-exposure, strongly suggesting the absence of resistant individuals in all tested populations. This finding confirms the high susceptibility to diazinon measured by the 2 h-bioassays in those populations. Despite methodological differences, this high susceptibility to diazinon found in horn fly populations in Central Brazil was also observed in Southern Brazil and Argentina (Guglielmone et al. 2000).

The history of insecticide use and bioassay results indicate that diazinon, as well as other OPs, can be an useful tool for horn fly control and resistance management, particularly in pyrethroid-resistant populations. Diazinon ear tags and a pour-on formulation were recently

TABLE

Susceptibility to diazinon (LC_{50} expressed as µg/cm^2) and resistance ratios of horn flies, in filter paper bioassays conducted from October 2000 to April 2001, in the states of Mato Grosso and Mato Grosso do Sul, Brazil

Site	County	LC_{50}	RR
Mato Grosso do Sul			
Fazenda Bocaiúva	Corumbá (19º00'S, 57º39'W)	0,15	0,1
Fazenda São José do Japorá	Corumbá (19º00'S, 57º39'W)	0,15	0,1
Fazenda Laguna	Três Lagoas (20º45'S, 51º40'W)	0,19	0,2
Fazenda Cabeceira Limpa	Rochedo (19º57'S, 54º53'W)	0,22	0,2
Sítio Cruzeiro do Sul	Mundo Novo (23º56'S, 54º16'W)	0,23	0,2
Sítio Lilocris	Três Lagoas (20º45'S, 51º40'W)	0,23	0,2
Fazenda Garimpo	Rio Negro (19º26'S, 54º59'W)	0,27	0,2
Fazenda Angico	Corumbá (19º00'S, 57º39'W)	0,30	0,3
Estância Lagoa Vermelha	Mundo Novo (23º56'S, 54º16'W)	0,47	0,4
Fazenda Santa Maria	Corumbá (19º00'S, 57º39'W)	0,56	0,5
Estância Rincão doVale	Corumbá (19º00'S, 57º39'W)	0,57	0,5
Fazenda Nhumirim	Corumbá (19º00'S, 57º39'W)	0,63	0,6
Mato Grosso			
Fazenda Flor da Serra	Sto. Antonio do Leverger (15º51'S, 56º04'W)	0,19	0,2
Fazenda Montana	Barra do Garças (15º53'S, 52º15'W)	0,23	0,2
Fazenda Brasil	Barra do Garças (15º53'S, 52º15'W)	0,25	0,2
Fazenda Monjólo	Cuiabá (15º35'S, 56º05'W)	0,45	0,4
Fazenda São Sebastião	Pontal do Araguaia (15º53'S, 52º15'W)	0,49	0,5
Fazenda Boi Branco	Cáceres (16º04'S, 57º40'W)	0,64	0,6
Susceptible colony		1,09	-

Fazenda (ranch); LC: lethal concentration; Resistance ratio (RR): LC_{50} from field population/LC_{50} from susceptible colony

introduced into the market for horn flies in Brazil. Care should be exercised to avoid early development of resistance to diazinon, as reported somewhere else after four years of ear tag use, and which may lead to cross-resistance to several OPs (Barros et al. 2001). Thus, it is paramount to avoid the indiscriminate use of diazinon to preserve not just the insecticide itself but the high efficacy of the entire OP class.

ACKNOWLEDGEMENTS

To Matthew Waldon (Knipling-Bushland US Livestock Insects Research Laboratory) for supplying insecticide-susceptible flies, and Lane Foil (Louisiana State University) for conducting the bioassay with the susceptible population and review the manuscript. To Ernande Ravaglia and Dalsiza Candal Gomes for technical assistance, and Joe Kellerby (Y-Tex Corp.) for providing the insecticide. To the ranch owners and staff for their logistic support.

REFERENCES

Abbott WS 1925. A method of computing the effectiveness of an insecticide. *J Econ Entomol 18*: 256-257.

Barros ATM, Alison Jr MW, Foil, LD 1999. Evaluation of a yearly insecticidal ear tag rotation for control of pyrethroid-resistant horn flies (Diptera: Muscidae). *Vet Parasitol 82*: 317-325.

Barros ATM, Ottea J, Sanson D, Foil LD 2001. Horn fly (Diptera: Muscidae) resistance to organophosphate insecticides. *Vet Parasitol 96*: 243-256.

Burns, EC, Wilson BH 1963. Field resistance of horn flies to the organic phosphate insecticide Ronnel. *J Econ Entomol 56*: 718.

Byford RL, Sparks TC, Green B, Knox J, Wyatt W 1988. Organophosphorus insecticides for the control of pyrethroid-resistant horn flies (Diptera: Muscidae). *J Econ Entomol 81*: 1562-1566.

Cilek JE, Dahlman DL, Knapp FW 1995. Possible mechanism of diazinon negative cross-resistance in pyrethroid-resistant horn flies (Diptera: Muscidae). *J Econ Entomol 88*: 520-524.

Cilek JE, Steelman CD, Knapp FW 1991. Horn fly (Diptera: Muscidae) insecticide resistance in Kentucky and Arkansas. *J Econ Entomol 84*: 756-762.

Crosby BL, Byford RL, Kinzer HG 1991. Insecticide resistance in the horn fly, *Haematobia irritans* (L.), in New Mexico: survey and control. *Southwestern Entomol 16*: 301-309.

Guglielmone AA, Kunz SE, Castelli ME, Volpogni MM, Kammalah D, Martins JR, Mattos C, Aguirre DH, Suárez VR, Anziani OS, Mangold AJ 2000. Susceptibilidad al diazinón de la *Haematobia irritans* (Diptera: Muscidade) de diferentes localidades argentinas y del sur de Brasil. *Rev Med Vet 91*: 184-186.

Guglielmone AA, Kunz SE, Volpogni MM, Anziani OS, Flores SG 1998. Diagnóstico de poblaciones de la *Haematobia irritans* (Diptera: Muscidade) resistentes a la cipermetrina en Santa Fe, Argentina. *Rev Med Vet 79*: 353-356.

Harvey TL, Brethour JR, Broce AB 1984. Loss in effectiveness of insecticide ear tags for horn fly (Diptera: Muscidae) control. *J Kansas Entomol Soc 57*: 715-717.

Kunz SE, Estrada MO, Sanchez HF 1995. Status of *Haematobia irritans* (Diptera: Muscidae) insecticide resistance in Northeastern Mexico. *J Med Entomol 32*: 726-729.

LeOra Software. 1987. *POLO-PC a User's Guide to Probit or Logit Analysis*, LeOra Software, Berkeley, 22 pp.

Mwangala FS, Galloway TD 1993. Susceptibility of horn flies, *Haematobia irritans* (L.) (Diptera: Muscidae), to pyrethroids in Manitoba. *Can Entomol 125*: 47-53.

Sheppard C 1983. Stirofos resistance in a population of horn flies. *J Ga Entomol Soc 18*: 370-376.

Sheppard DC, Hinkle NC 1987. A field procedure using disposable materials to evaluate horn fly insecticide resistance. *J Agric Entomol 4*: 87-89.

Sheppard DC, Marchiondo AA 1987. Toxicity of diazinon to pyrethroid resistant and susceptible horn flies, *Haematobia irritans* (L.): laboratory studies and field trials. *J Agric Entomol 4*: 262-270.

Sparks TC, Quisenberry SS, Lockwood JA, Byford RL, Roush RT 1985. Insecticide resistance in the horn fly, *Haematobia irritans*. *J Agric Entomol 2*: 217-233.

Steelman CD, McNew RW, Brown MA, Tolley G, Phillips JM 1994. Efficacy of Brahman breeding in the management of insecticide-resistant horn flies (Diptera: Muscidae) on beef cattle. *J Econ Entomol 87*: 7-14.