CROP PROTECTION

Strategies for Controlling House Fly Populations Resistant to Cyromazine

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Estratégias Para o Controle de Populações de Mosca Doméstica Resistentes à Ciromazina

RESUMO – Os objetivos deste estudo foram comparar, dos pontos de vista biológico e econômico, o impacto de várias estratégias de controle e avaliar os seus efeitos sobre a *Musca domestica* L., resistente à ciromazina, e sobre parasitóides benéficos da pupas de mosca doméstica em aviários (240.000 frangos) na Argentina. As estratégias avaliadas foram: controle químico, controle químico + cultural, e químico + cultural + biológico (manejo integrado de pragas). Os produtos utilizados foram: ciromazina 1% e 50%, DDVP, azametifós com e sem z-9-tricoseno, calcário, e os parasitóides *Spalangia endius* Walker e *Muscidifurax raptor* Girault & Sanders. No caso de ausência de medidas de controle, a densidade populacional da mosca aumentou rapidamente e a média de parasitismo foi de 12%. Quando somente o controle químico foi utilizado, as populações de moscas foram reduzidas para 40 por grupo e a média de parasitismo foi de 2%. Quando a ciromazina tópica foi utilizada em conjunto com o controle cultural (calcário), a população de moscas foi reduzida mais rapidamente que nos tratamentos com moscas alimentadas com ciromazina 1%. Com o uso subseqüente de vespas parasitas, altos índices de parasitismo foram observados e a população de mosca doméstica foi reduzida aos níveis de tolerância em tempo menor que em todos os outros tratamentos. Dos pontos de vista biológico e econômico, o melhor tratamento para controle de mosca doméstica resistente à ciromazina foi o controle biológico + cultural + químico com aplicações localizadas de ciromazina tópica.

PALAVRAS-CHAVE: *Musca domestica*, resistência a inseticidas, controle biológico, *Spalangia endius*, *Muscidifurax raptor*, aviário.

ABSTRACT – The objectives of this study were to compare, from both biological and economic viewpoints, the impact of various control strategies and evaluate their effect on cyromazine-resistant *Musca domestica* L., and beneficial house fly pupal parasitoids on caged-layer farms (240,000 hens) in Argentina. The strategies evaluated were: chemical, chemical + cultural, and chemical + cultural + biological (integrated management). The products used were: cyromazine 1% and 50%, DDVP, azamethiphos with and without *z*-9-tricosene, lime, and the parasitoids *Spalangia endius* Walker and *Muscidifurax raptor* Girault & Sanders. In the absence of control measures, fly density increased quickly and the average parasitism rate was 12%. When only chemical control was used, fly populations were reduced to ca. 40/grid and parasitism averaged 2%. When topical cyromazine was used in conjunction with cultural control (lime), fly populations were reduced more rapidly than those treated with cyromazine 1% feed-through. With the subsequent use of parasitic wasps, high parasitism levels were observed and house flies were reduced to tolerance levels in the shortest time. From an economic and biological point of view, the best treatment for house flies resistant to cyromazine was biological + cultural + chemical with localized applications of topical cyromazine.

KEY WORDS: *Musca domestica*, insecticide resistance, biological control, *Spalangia endius*, *Muscidifurax raptor*, poultry house.

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The house fly, *Musca domestica* L., is a vector of many metaxenic pathogens and can cause serious sanitary problems because of its high reproductive potential, feeding habits and ability to disperse (Del Ponte 1958, Aberg-Cobo *et al*. 1959). Organic wastes from intensive animal production (e.g. poultry, swine, dairy farms) provide excellent habitats for the growth and development of this insect (Thomas & Skoda 1993). Certain biotic (parasitoids, predators, and pathogens) and abiotic (temperature, humidity, quality and quantity of available food, etc.) factors naturally regulate house fly populations. In Argentina, studies made in the field allowed to identify several natural enemies species of *M. domestica*, that do not constitute an efficient natural control to the pest. For this reason inundatives releases are used in IPM, as for example pupae parasitoids (Axtell & Rutz 1986, Crespo & Lecuona 1996). In relation to abiotic conditions, improper management of organic wastes and high temperatures (spring and summer) generates favorable conditions for populations of this pest to increase.

Normally, chemical larvicides and adulticides are the primary means of nuisance fly control employed by poultry producers. Improper use of those products combined with the housefly's short life cycle (<seven days) (Larsen & Thomsen 1940) and high biotic potential, produce conditions conducive to the development of resistance to insecticides.

Cyromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine) is an insect growth regulator commonly used to control immature houseflies on poultry farms (Hogsette 1979, Miller & Corley 1980, Miller *et al.* 1981, Awad & Mulla 1984). Cyromazine is formulated as a pre-mix (1%), which is added to poultry food; it is also formulated as a water-soluble granule and a soluble powder (50%) for topical application to manure containing fly larvae.

Cyromazine produces irreversible morphophysiological changes, which culminate in the death of the insects (Hogsette 1979, Awad & Mulla 1984). The effect varies according to the developmental stage of the insects. When housefly larvae are exposed to cyromazine, deformations may be observed in the pupal stage, which result from interference with chitin digestion and synthesis. When applied at the prepupal stage, cyromazine produces morphogenic aberrations in the adults, like absence of wings and underdevelopment of the genitalia in both males and females (Cerf & Georghiou 1974).

Resistance to Cyromazine, resulting from the overuse and improper use of the product, was first reported in Florida about the same time the product was registered in the U.S. (Bloomcamp *et al.* 1987). Subsequent reports indicate that resistance or increased tolerance is widespread in the U.S. and Europe (Sheppard *et al.* 1989, Geden *et al.* 1992, Sheppard *et al.* 1992). Without Cyromazine, producers must rely on alternative strategies to attain the degree of housefly control they previously expected from Cyromazine alone. The objectives of our work were to compare, from both biological and economic viewpoints, the efficacy of various alternative strategies to control populations of Cyromazine-resistant houseflies on poultry farms, and evaluate their effects on beneficial housefly pupal parasitoids.

Materials and Methods

The experiments were conducted in 1994 in La Matanza, Buenos Aires, Argentina, on four caged-layer poultry farms with a combined total of 240,000 hens. Houses were similar in design to California-style houses, i.e., hens in cages suspended ca. 1 m above the ground under metal roof, side walls opened to the air or closed with curtains in winter. Each house on each farm contained 10,000 birds. Housefly populations on these farms were known to have a 12-fold resistance level to Cyromazine (Dr. Carlos Hereus, pers. comm.).

A different treatment was assigned to each farm with the exception of farm 4 (Table 1). On farm 4 there were three blocks of four houses, each block separated by 400 m. A different treatment, 3b, 4a and 4b, was assigned to each block of houses, respectively. The six treatments, including the amounts used and application rates of the chemical and non-chemical components, are shown in Table 2. Treatments were designed to show the additive effect of the various chemical and non-chemical components tested. Application of treatments began in January and ended in May, 1994.

Cyromazine was used in two forms: Larvadex® (1% [AI] premix, Novartis Buenos Aires, 500 g/per ton of poultry feed) as a feed-through, and Neporex® (50% [AI], Novartis Buenos Aires, 1 g/m^2) applied topically to manure. As per company recommendations, cyromazine (1%) was fed continuously for a maximum of 5 wk. then removed from the feed for a

Table 1. Description of poultry farms in La Matanza, Argentina, and treatments and components assigned to each.

Farm number	Number of houses	Treatment (number and type)	Components of treatments[1]
1	four houses	1 Control	none
2	four houses	2 Chemical	Larvadex® + Nuvan® + Alfacron® + Snip®
3	four houses	3a Chemical and cultural	Larvadex® + Nuvan® + Alfacron® + Snip® + lime
4	12 houses total:		
	four houses	3b Chemical and cultural	Neporex® + Nuvan® + Alfacron® + Snip® + lime
	four houses	4a Chemical, cultural and biological	Treatment 3a + parasitic wasps
	four houses	4b Chemical, cultural and biological	Treatment 3b + parasitic wasps

[1] Larvadex®: Cyromazine 1% premix; Neporex®: Cyromazine 50% topical; Nuvan®: DDVP 100% AI; Alfacron®: Azamethiphos 10% paint; Snip®: Azamethiphos 1% AI (with z-9-tricosene) granular bait; parasitic wasps: *Spalangia endius* and *Muscidifurax raptor*

Table 2. Treatments, treatment components, application rates and amounts used for each treatment group (four houses per treatment) for management of housefly populations in poultry houses, during a 20-week study in La Matanza, Argentina.

Treatment[1]	Cyromazine[2]	DDVP[3]	Azamethiphos[4] (10% paint)	Azamethiphos[5] bait (Snip®)	Lime	Parasitic[6] wasps
1	No	No	No	No	No	No
2	(1%) 91 kg on wk. 1 and on wk. 10	20 L used in 17 applications in wk. 1-20	20 kg in 20 wk.	20 kg in 20 wk.	No	No
3a	(1%) 91 kg on wk. 1 and on wk. 8	20 L used in 12 applications in wk. 1-17	20 kg in 20 wk.	20 kg in 20 wk.	20 ton. in seven applications in wk. 1-17	No
3b	(50%) 2 kg on wk. 1 and on wk. 7	10 L used in three applications in wk. 1-7	10 kg in 20 wk.	18.5kg in 20 wk.	20 ton. in seven applications in wk. 1-11	No
4a	(1%) 91 kg on wk. 1	10 L used in four applications in wk. 2-6	10 kg in 20 wk.	8.5 kg in 20 wk.	18 ton. in six applications in wk. 1-13	Yes
4b	(50%) 1.5 kg on wk. 1	5 L used during wk. 1	3 kg in 20 wk.	6 kg in 20 wk.	12 ton. in six applications in wk. 1-15	Yes

[1]Treatments: 1 = Control, 2 = Chemical, 3a = Chemical and cultural, 3b = Chemical and cultural, 4a = Chemical, cultural and biological, 4b = Chemical, cultural and biological.
[2] Cyromazine, 1% = 500 g/Tn, 50% = 1 g/m^2
[3] DDVP 100% AI, 250 cc/liter applied *ad libitum.*
[4] Azamethiphos 10% paint, 250 g/250 cc.
[5] Snip® = Azamethiphos 1% AI (with z-9-tricosene) granular bait and lime applied *ad libitum.*
[6] Parasitic wasps: *Spalangia endius* and *Muscidifurax raptor* - six insects of each species.

minimum of 2 wk. before it could be fed again. DDVP (Nuvan® 100 EC [100 g/liter AI], Novartis Buenos Aires, 250 cc/20 liter of water) was applied as an adulticide (fog) outside the houses. Azamethiphos was also used in two forms: Alfacron® (10% [AI], Novartis Buenos Aires, 250 g/250 cc of water, applied ad libitum) as a paint on adult resting sites, and Snip® (1% [AI] with *z*-9-tricosene [Muscalure], Novartis Buenos Aires, applied at the rate of 2 kg/house/month) as a granular bait. Lime (calcium oxide) was applied ad libitum directly to wet areas of manure as needed as a desiccant.

The parasitic wasps, *Spalangia endius* Walker and *Muscidifurax raptor* Girault & Sanders, were reared in our laboratory in Buenos Aires and released weekly at the rate of six wasps per bird (Crespo *et al.* 1998). Parasitized housefly pupae were placed in paper bags and wasps were allowed to emerge. Bags were suspended in the poultry houses under the cages and a hole was made to facilitate the escape of the parasitoids. When parasitized pupae were placed in the poultry houses, parasitoids were already emerging inside the closed bags.

Fly density was estimated weekly with a Scudder-grid (45 x 45 cm) using the method of Murvosh & Thaggard (1966). The grid was placed at sites on manure where high concentrations of adult flies were present. After 10 seconds, flies were counted for 1 min. An average grid count was computed for each house using the five highest counts. Variation between average grid counts per house was minimal during each sampling period (Crespo *et al.* 1998) and for this reason, standard error values, which were very small, are not presented with average grid count values. Fly densities were considered to be acceptable when the average grid count in the houses was 10 flies/grid.

Housefly puparia were collected at random and separated from the manure. The first 500 intact puparia from each house were considered to be a sample. Puparia were kept at 27 ± 1ºC and 70% relative humidity for 30 days to await the emergence of flies and/or parasitoids. Unclosed puparia were dissected to determine if they had been parasitized. Puparia containing dead (aborted) parasitoids were considered as parasitized. Percentage parasitism, estimated weekly in each house using the method of Petersen (1986), was calculated in the following manner: % parasitism = [(emerged + aborted parasitoids)/ intact puparia] x 100.

The curves of flies N°/grid and parasitism percentage of each treatment were compared, as well as to the control, using the Chi square test ($\alpha = 0.05$).

Results

In the control (Fig. 1A), population density averaged ca.

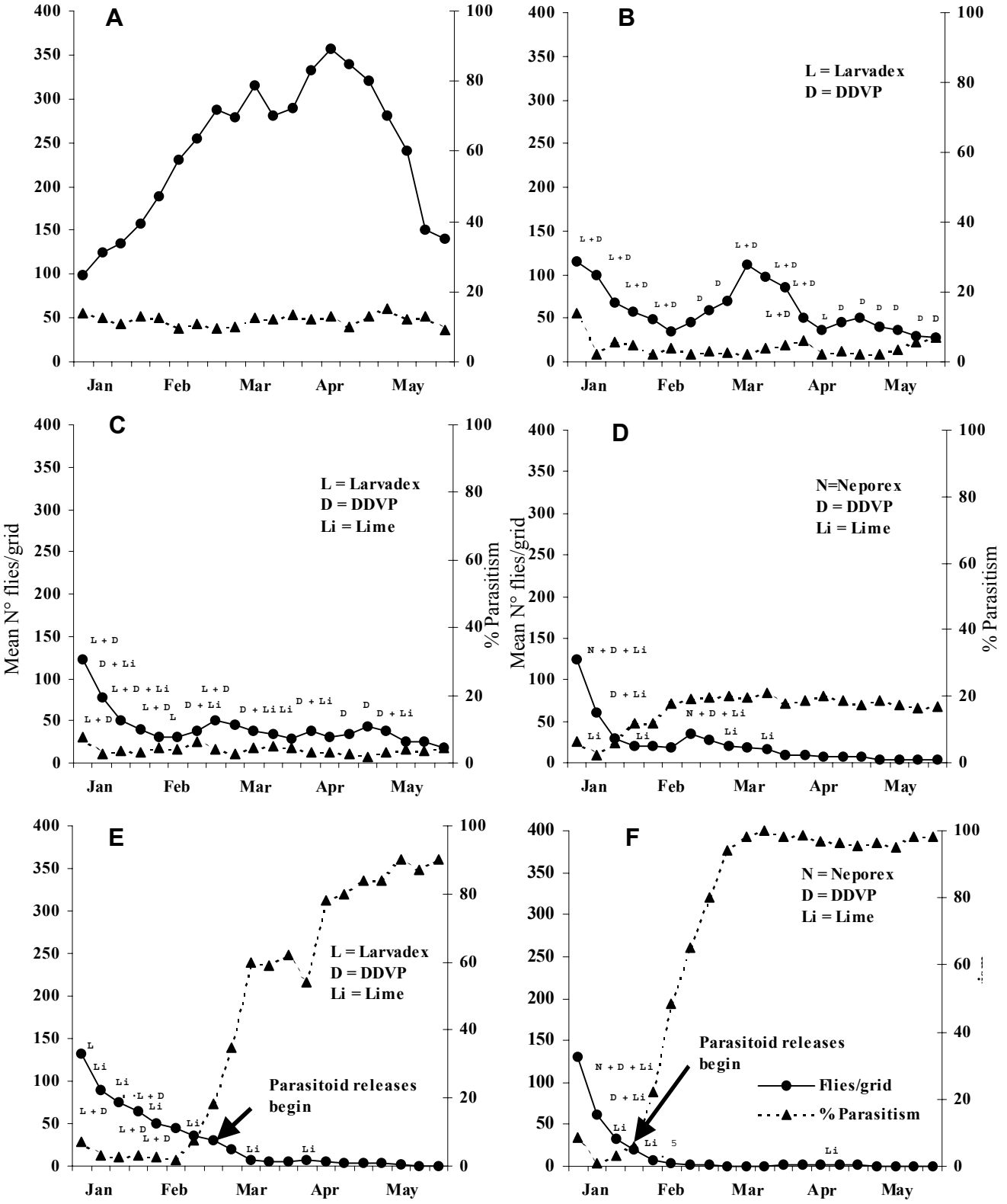

Figure 1. Adult fly density and percentage parasitism. Buenos Aires, 1994. **A**. Control treatment (1). **B**. Cyromazine 1% + chemical treatment (2). **C**. Cyromazine 1% + chemical + cultural treatment (3a). **D**. Cyromazine 50% + chemical + cultural treatment (3b). **E**. Cyromazine 1% + chemical + cultural + biological treatment (4a). **F**. Cyromazine 50% + chemical + cultural + biological treatment (4b). *n* = four houses, five grids per house per week.

100 flies/grid in January, reached a maximum of 350 flies/grid in April, and decreased to ca. 135 flies/grid by the end of the study, because of the beginning of low temperature periods (autumn-winter). The average natural parasitism rate was 12%, caused by the presence of *M. domestica* pupae parasitoids on the field.

In treatment 2 (cyromazine 1% and chemical control, Fig. 1B), initial counts were 114 flies/grid, but these decreased after 5 wk. to 35 flies/grid. We attributed this to the combined action of cyromazine 1% and the adulticides, DDVP, azamethiphos paint-on, and azamethiphos granular bait with sexual lure. Average grid values 4 wk. later, after cyromazine 1% use was discontinued, again approached the initial fly density value. It was necessary to feed cyromazine 1% for a second period to effect a second major reduction in fly density. Natural parasitism was reduced from 14% to 2% in just one wk. and averaged < 3% throughout the test because of the DDVP applications. Although these chemical components reduced the fly density level compared with the untreated control (Fig. 1A and B), minimum density values still exceeded the acceptable tolerance threshold (10 flies/grid).

In treatment 3a (cyromazine 1%, chemical and cultural control, Fig. 1C), the initial density value of 124 flies/grid was slightly higher than that in Treatment 2. Cyromazine 1% and the adulticides reduced this to 30 flies/grid in 5 wk. However, after the obligatory discontinuation of cyromazine 1%, fly density increased to 45/grid 3 wk. later. House fly populations failed to reach the initial density level because lime was used for drying wet areas in the manure. This reduced the number of larval foci and created conditions less favorable for oviposition by the flies. As with treatment 2, the average parasitism rate was low (< 4%) most likely because of the frequency and volume of DDVP fogging. Fly density was not reduced within the tolerance level using this treatment.

The initial grid value for treatment 3b (cyromazine 50%, chemical and cultural control, Fig. 1D) was similar to that of Treatment 3a. In 5 wk, the density was reduced from 125 to 18 flies/grid. Changing to cyromazine 50%, a more concentrated formulation applied exclusively to larval foci, gave a satisfactory level of control which treatments 2 and 3a failed to achieve with cyromazine 1%. A second application of cyromazine 50% was made when the house fly density increased to 35 flies/grid. This reduced flies below the tolerance threshold after 4 wk. The parasitism rate was higher in treatment 3b than in treatment 3a, which we attributed to the decreased fogging with DDVP.

In treatment 4a (cyromazine 1%, chemical, cultural and biological control, Fig. 1E) initial counts averaged 132 flies/grid, then declined to 30 flies/grid 7 wk. later. The decrease in fly density and the initial reduction in parasitism (< 4%) were similar to those in treatment 3a, probably because the same active ingredients were used. Parasitoids were released weekly when counts averaged 19 flies/grid, and average fly counts were reduced to seven after 1 wk. This was maintained for 11 wk. with an average of 4 flies/grid, which was below to the tolerance level. Average rate of parasitism during this period was 84%.

In treatment 4b (cyromazine 50%, chemical, cultural and biological control, Fig. 1F), initial counts averaged 130 flies/grid, then decreased to 19/grid in 3 wk. Weekly parasitoid releases began at the end of January, attaining a level of parasitism > 80% just 4 wk. later. Average density values were < 2 flies/grid, and the parasitism rate was 95%.

The curves of flies N°/grid and parasitism percentage of all treatments were different, as well as those compared to the control. Treatments 4a (Fig. 1E) and 4b (Fig. 1F) were excelled due to the higher values of Chi square.

The only two parasitoid species recovered were *S. endius* and *M. raptor* and these were recovered at a ratio similar to the one at which they were released. These results are similar to previous study (Crespo *et al.* 1998). Although many other parasitoid species are present in Argentina (De Santis & De Sureda 1988), *S. endius* and *M. raptor* appear to be the most commonly encountered around poultry facilities.

It can be seen that the most efficient fly control program was treatment 4b (Table 3), with costs for chemical products averaging ca. $0.05 per bird; this was a savings of 2.1-3.5x compared to the other treatments tested. Also, treatment 4b was more effective in decreasing the fly population, requiring only 4 wk. to reach the accepted tolerance level (10 flies/grid, Fig. 1F).

Table 3. Cost comparison of treatments tested to control houseflies in poultry houses in La Matanza, Argentina.

Treatment[1]	Cost per bird ($US)[2]	Cost ratio compared with treatment 4b
2	0.12	2.5x
3a	0.17	3.5x
3b	0.17	3.5x
4a	0.10	2.5x
4b	0.05	-

[1]Treatments: 2 = Chemical, 3a = Chemical and cultural, 3b = Chemical and cultural, 4a = Chemical, cultural and biological, 4b = Chemical, cultural and biological.
[2]Costs calculated October, 1994

Discussion

For facilities with high housefly populations and problems with insecticide resistance, integrated management provides the best fly control, both economically and biologically (Table 2, Fig. 1F). In all treatments where lime was used, it appeared to help dry the manure, thereby reducing the presence of larval foci and adult oviposition sites. In establishments with large numbers of flies with resistance to cyromazine 1%, the program for application of chemical products should be modified. One strategy is the early topical application of cyromazine 50% (Fig. 1D and F) to larval foci. This can be a satisfactory alternative if complemented by immediate and continuous management of manure moisture by minimizing water leaks, ventilation problems, and wet droppings resulting from feed, disease or temperature fluctuations. However,

continued reliance on cyromazine 50% or any other chemical control could result in the development of resistance.

With rapid destruction of larval foci and control of manure moisture, concentrated larvicide applications can be minimized and the swift generation of resistance to insecticides delayed. At the same time, decreasing the adult fly population with efficacious products reduces the possibility that new larval foci will be generated.

It was not possible to reduce fly populations to the tolerance level using chemical control exclusively (treatment 2, Fig. 1B). We attributed this to the flies resistance to cyromazine 1%. When the chemical control was complimented with cultural control (Fig. 1C), a greater reduction in fly population was observed, and the large population increase in March (treatment 1, Fig. 1A) was avoided. However, fly populations were still not reduced below the tolerance level. Finally, when the above two strategies were coupled with biological control (treatment 4a, Fig. 1E), it was possible to reduce and maintain fly populations below the tolerance level.

When cyromazine 50%, the more concentrated topical formulation of cyromazine, was used with cultural control (treatment 3b, Fig. 1D), there was a more rapid reduction in fly populations than observed with treatment 3a (Fig. 1C); and flies were reduced and maintained below the accepted tolerance level. This phenomenon was more pronounced with the addition of biological control, which resulted in a high percentage of parasitized house fly pupae (treatment 4b, Fig. 1F).

The deleterious effect of DDVP on parasitic wasps was graphically illustrated, particularly in treatments 2 and 3a where fogging applications were made throughout the study (Fig. 1B and C). We found similar results in a previous study, which indicated that parasitic wasps were more effective if released after the cessation of DDVP applications (Crespo *et al.* 1998). In some studies cyromazine 1% was also shown to be detrimental to parasitic wasps (Klunker, 1991), but in our study as well as others (Mandeville *et al.* 1990) it caused no adverse effects. In treatment 3b, parasitism plateaued after a single application of DDVP, and was not adversely affected by the continued feeding of cyromazine 1% (Fig. 1E).

A major cost in our fly control programs was the cost of the adulticides, especially cyromazine 1 and 50%, and DDVP. The cost of adulticide products in 3b was 50% less than those of treatments 2 and 3a because immature fly populations were effectively eliminated with cyromazine 50% and only 4 kg were used (Table 3). The sequential reduction of pesticide use in treatment 4a and again in treatment 4b further reduced costs despite the added cost of parasitic wasps. The cost of purchasing parasitic wasps from commercial insectaries may increase costs slightly because our production costs are slightly lower and our percentage parasitism is generally higher.

In conclusion, the control of house fly populations resistant to cyromazine 1% is possible through an integrated management scheme that includes cultural and biological strategies plus adulticides and concentrated larvicides used in a rational manner. This results in a reduction in costs and a more effective program for decreasing the pest population.

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