Resistance in House Flies (Diptera: Muscidae)
Selected with 5.0 PPM Feed-Through
Cyromazine

D. Craig Sheppard², Douglas M. Gaydon³, and Richard W. Miller⁴

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ABSTRACT House fly populations in Mississippi, Maryland, and New Jersey which were poorly controlled with a 5.0-ppm cyromazine feed-through treatment were bioassayed to determine their response to this chemical. Resistance factors (RF's) of 25.6 to 44.6 were found. These are significantly higher than RF's selected with an earlier 1.5-ppm cyromazine feed-through treatment. Resistance potential of a more concentrated sprayable cyromazine has proven to be less in a United States study and in Denmark because its higher intermittent dose is less selective for resistant individuals. The sprayable cyromazine's potential for resistance selection in these preselected populations is unclear.

KEY WORDS Resistance, cyromazine, Larvadex®, house fly, feed-through, Musca domestica, Diptera, Muscidae.

Cyromazine is a novel insect growth regulator (IGR) effective at very low levels for control of dipterous larvae, especially house flies, Musca domestica L. (Hall and Foehse 1980). This material was derived from azidotriazine herbicides. The mode of action may be to disrupt ecysis through the endocrine system (Friedel et al. 1988).

A brief commercial history of cyromazine was given by Sheppard et al. (1989). Cyromazine was first marketed in 1982 by Ciba-Geigy Corp. (Greensboro, North Carolina) as Larvadex® for house fly control in caged-layer hen manure. The formulation was 0.3% feed-through premix designed to be mixed in a ton of feed to produce 1.5 ppm cyromazine in the final mixture. In 1987 Ciba-Geigy introduced a 1.0% Larvadex premix to increase the cyromazine concentration to 5 ppm in the layer's feed.

Previous to, and during its first two years of commercial use, Larvadex was used successfully in the United States (Sheppard et al. 1989). House fly control at the 1.5-ppm rate or even ca. half of that was reported to be completely effective (Hall and Foehse 1980, Sheppard et al. 1989). However since 1985 there have been reports of control problems associated with cyromazine resistance in field-collected house flies in Texas (Shen and Plapp 1990), and in Georgia and Mississippi (Sheppard et al. 1989). Resistance factors (RF) ranged

1 Received for publication 29 June 1991; accepted 19 August 1991.
2 Dept. of Entomology, University of Georgia, Coastal Plain Experiment Station, Tifton, Georgia 31793.
3 Mississippi State University, P. O. Box 5446, Mississippi State, Mississippi 38762.
4 Livestock Insects Laboratory, ARS-USDA, Beltsville, Maryland 20705.
from 4.2 to 21.0 in these reports. Iseki and Georghiou (1986) reported on a laboratory strain of house flies from Pennsylvania that had an RF of 3.8. These had been selected with cyromazine in the field for 2 yr, but failure of control was not mentioned. Bloomcamp et al. (1987) reported on a lab selected colony which reached an RF of 69.8. The stock for this colony was collected from a Florida caged-layer facility where cyromazine had been used for two seasons.

This paper reports on three caged-layer operations in three states where house fly populations were not controlled with the newer 5.0-ppm cyromazine feed treatment and where high levels of cyromazine resistance were detected.

Methods and Materials

The house fly populations studied were collected from caged layer facilities in Hinds Co., Mississippi in 1988, Kent Co., Maryland in 1990, and Warren Co., New Jersey in June 1990 and January 1991. Cyromazine use was discontinued at the New Jersey site in mid-August 1990. Cyromazine feed-through has been used at these sites more or less continuously since 1982. The field collections were shipped to the laboratory in Tifton and held in 30- by 30-cm screen cages at 27°C and ambient humidity. Adults were fed granulated sucrose, dried non-fat milk, and water. Eggs were collected on cellulose pads soaked with sour milk. F_1 larvae from these eggs were reared in Purina fly larvae media (St. Louis, Missouri) for 48 h and tested on sequential dilutions of cyromazine on Purina house fly larva media. The test method was after Sheppard et al. (1989) but three cups of 35 larvae were used per dose level, and cyromazine levels up to 11.4 ppm were used to approach 100% kill. Mortality was regressed on cyromazine rate by a computer program for probit analysis (Daum 1970). LC_{50}’s with 95% confidence intervals (CI) were generated by this program. LC_{50}’s with non-overlapping CIs were considered significantly different. A laboratory colony of house flies never exposed to cyromazine was used as a check population, and was similarly tested with rates of cyromazine as high as 1.5 ppm to achieve 100% kill. The average LC_{50} for this susceptible population in 1990 was 0.19 ppm and was used in calculating RF’s in this study, dividing the LC_{50} for the resistant population by this average susceptible value.

Results and Discussion

The 1988 Mississippi population had an RF of 44.6 (Table 1). Its LC_{50} of 8.47 ppm was significantly higher than any LC_{50} of this same population when collected in 1986 (Sheppard 1989). The 1986 Mississippi population had been selected with the 1.5-ppm Larvadex feed-through and exhibited RF = 9 to 21 (\overline{x} = 14). The 1988 collection from the same farm had been selected with the 5.0-ppm Larvadex feed-through for 13 mo and had an RF about three times as high as in 1986. This is proportional to the increased level of cyromazine delivered by the newer feed-through formulation. The 1990 and 1991 New Jersey and the 1990 Maryland population RF’s were 25.6, 31.6, and 29.5 respectively. These values are higher than any reported RF’s selected with the 1.5-ppm treatment.

The January 1991 New Jersey sample exhibited a higher LC_{50} than the June 1990 sample from that same population despite no cyromazine usage after August
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Table 1. Cyromazine LC50’s and resistance factors of house flies where 5.0 ppm Larvadex® feed-through gave poor control.

<table>
<thead>
<tr>
<th>Population</th>
<th>LC50 (ppm)</th>
<th>95% CL</th>
<th>Resistance Factor</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988 Hinds Co.</td>
<td>8.47</td>
<td>12.37 - 6.14</td>
<td>44.6</td>
<td>5.20</td>
</tr>
<tr>
<td>Mississippi</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990 Warren Co.</td>
<td>4.86</td>
<td>5.25 - 4.47</td>
<td>25.6</td>
<td>4.58</td>
</tr>
<tr>
<td>New Jersey</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1991 Warren Co.</td>
<td>6.00</td>
<td>6.37 - 5.61</td>
<td>31.6</td>
<td>7.66</td>
</tr>
<tr>
<td>New Jersey</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1990 Kent Co.</td>
<td>5.61</td>
<td>7.02 - 4.52</td>
<td>29.5</td>
<td>3.52</td>
</tr>
<tr>
<td>Maryland</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

1990. The house fly generations that occurred in that 5-mo untreated interval were apparently insufficient for any abatement of resistance to occur. This unknown number of generations was probably small since this interval covers the fall of 1990 and winter of 1991. Also, at least the first generation during the untreated interval was exposed to cyromazine residue from the last August treatment.

These findings of elevated cyromazine resistance indicate a widespread ability of housefly populations to adapt to higher rates of cyromazine. In unselected house fly populations the sprayable cyromazine (Larvadex 2 SL, recently labelled in eight states) has been less selective for resistance than the feed-through formulations. Persistent (constant exposure) formulations, such as the cyromazine feed-through, select for resistance rapidly (Brown 1971, Georgiou and Taylor 1977). Also, the lower dose of the feed-through kills all fully susceptible individuals, but may allow survival of heterozygous resistant individuals. This causes resistance to be functionally dominant and selection is more rapid (Georghiou 1980). In field tests in 1986 and 1987, 0.1% cyromazine spray did not select for significant levels of resistance, while the 1.5-ppm feed through did (Sheppard 1989). In Denmark, sprayable cyromazine has been used since the early 1980’s and there is no indication of resistance in treated house fly populations there (Jespersen 1990).

The Danish Pest Infestation Laboratory uses a diagnostic dose of 1.1 ppm cyromazine, and the World Health Organization uses 0.92 ppm to test for resistant house fly larvae (Jespersen 1990). Before widespread cyromazine use, a diagnostic dose of 1.0 ppm gave 100% kill of house fly larvae in Georgia. Hall and Foehse (1980) reported 100% house fly larvae kill with 0.8 ppm cyromazine. In the three populations reported here, 1.0 ppm cyromazine gave 0 to 5% kill, indicating an 95 to 100% increase in the proportion of resistant individuals. Thus the frequency of resistant individuals in the U.S. has been greatly elevated by the gradual
increase in cyromazine rates used in the field. The new sprayable Larvadex formulation must be used carefully, treating as few generations as possible to maximize its useful life and minimize further selection of these preselected populations.

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