Insecticide resistance in house flies from caged-layer poultry facilities

Jeffrey G Scott,* Timothy G Alefantis, Phillip E Kaufman and Donald A Rutz
Department of Entomology, Cornell University, Ithaca, New York 14853-0999, USA

Abstract: The frequency of resistance of eight strains of house flies, *Musca domestica* L., collected from caged-layer poultry facilities across New York state, to nine insecticides (dimethoate, tetrachlorvinphos, permethrin, cyfluthrin, pyrethrins, methomyl, fipronil, spinosad and cyromazine) was measured relative to a laboratory susceptible strain. Percentage survival was evaluated at five diagnostic concentrations: susceptible strain LC_{99}, $3 \times LC_{99}$, $10 \times LC_{99}$, $30 \times LC_{99}$ and $100 \times LC_{99}$. The highest levels of resistance were noted for tetrachlorvinphos, permethrin and cyfluthrin. There was substantial variation in the levels of resistance to the different insecticides from one facility to another, independent of their geographical location. There was very little cross-resistance detected in these populations to either fipronil or spinosad. Overall, there was a good correlation between insecticide use histories and the levels of resistance. The apparent isolation of fly populations within poultry facilities suggests that there are good opportunities for the implementation of successful resistance management strategies at these facilities. Differences between these results and those of a resistance survey on New York dairy farms in 1987 are discussed.

Keywords: Insecta; resistance; insecticides; poultry; organophosphate; carbamate; pyrethroid; *Musca domestica*

1 INTRODUCTION

Resistance to pesticides is one of the most serious problems facing agriculture today. Resistance can lead to increased application rates, increased frequency of pesticide use, and ultimately loss of efficacy. We cannot assume there will be an unending supply of new insecticides to replace current compounds, due to the increased difficulty in discovering pesticides, the tremendous cost of their development,[1,2] and the loss of currently available materials due to implementation of federal laws, including the Food Quality Protection Act of 1996. Thus, we need to improve the manner in which the few remaining compounds are used in order to delay the onset of resistance. However, before we can implement resistance management strategies, we need to know the geographic distribution and frequency of resistant individuals. Although the number of resistant species has been well documented,[3] the geographical extent of resistance has received far less attention. For example, while diamondback moth has rapidly acquired resistance to virtually all insecticides used against it, there are significant differences in susceptibility, even at sites separated by only a few kilometers.[4] Thus, even for species with extensive resistance histories, and where resistance is perceived to be widespread, broad geographical surveys are needed to establish the full extent of resistance.

House flies, *Musca domestica* L, are major pests in poultry facilities.[5] Concerns about animal health, public health and potential litigation all result from house fly activity.[6] House flies have developed resistance to virtually every insecticide used against them[7,8] and insecticide resistance in house flies is a global problem.[8–11] Recently in New York state there have been reports of control failures with cyfluthrin, the most recently registered insecticide for fly control.

A 1987 survey of house fly resistance on New York dairy farms showed similar patterns of resistance among farms, independent of insecticide use. This observation led to the suggestion that the relatively open architecture of dairy farms permitted the dispersal of insecticide-resistant house flies. In this study we examined levels of resistance in house flies from New York caged-layer poultry facilities. These facilities differ from dairy farms in that the poultry facilities are closed, which would be expected to substantially limit the migration of house flies. Herein, we report the frequency of resistance to seven commonly used insecticides, and two new insecticides

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* Correspondence to: Jeffrey G Scott, Department of Entomology, Comstock Hall, Cornell University, Ithaca, New York 14853-0999, USA
E-mail: jgs5@CORNELL.edu

Contract/grant sponsor: Hatch Projects; contract/grant number: 139414; 139418.
Contract/grant sponsor: Regional Project; contract/grant number: S-274.
(*Received 29 March 1999; revised version received 6 August 1999; accepted 7 October 1999*)

with high potential for fly control, in house flies from caged-layer poultry facilities in seven different counties within New York state.

2 EXPERIMENTAL

2.1 Insects and chemicals

House flies were collected by sweep net from within caged-layer poultry facilities in seven different counties across New York state during the summer of 1998. House flies free of pathogens and ectoparasites were used to establish each strain. House fly larvae were reared on medium containing 2.3 liters of water, 0.5 kg calf manna (Manna Pro Corp, St. Louis, MO), 90 g bird and reptile litter wood chips (Northeastern Products Corp, Warnersburg, NY), 50 g dried active baker's yeast (ICN Biomedicals, Costa Mesa, CA), and 0.8 kg wheat bran (Agway; Ithaca, NY). Adults were reared using sugar + powdered milk (1 by 1 weight) and water *ad libitum*. Flies were bioassayed beginning with the first generation of adults produced by the field-collected flies.

Three standard laboratory strains were used: Cornell susceptible (CS),[12] OCR, having high levels of cyclodiene resistance (presumably due to *Rdl*),[13] and Cornell-R, which is highly resistant to organophosphates due to an altered acetylcholinesterase.[14]

Nine insecticides were tested: cyfluthrin (91%, *cis:trans* 40:60, Bayer, Kansas City, MO), cyromazine (96.2%, Novartis, Greensboro, NC), dimethoate (99.7%, American Cyanamid, Princeton, NJ), fipronil (96.3%, Rhône Poulenc, Research Triangle Park, NC), methomyl (99.8%, DuPont, Wilmington, DE), permethrin (94.7%, *cis:trans* 25:75, AgrEvo, Wilmington, DE), pyrethrins (Fairfield American, Frenchtown, NJ), spinosad (88%, Dow AgroSciences, Indianapolis, IN) and tetrachlorvinphos (99.5%, Chem Service, West Chester, PA).

2.2 Bioassays

A residual contact method was used for most insecticide bioassays. Ten to 25 two- to five-day-old adult female house flies were placed inside a 230-ml glass jar (internal surface area 180 cm^2) that had been treated with insecticide.[15] Methomyl is formulated as a bait for use in caged-layer poultry facilities, therefore, a feeding assay was used for this insecticide. Female flies (10–25) were placed in glass jars (as above) and were given two 2-cm pieces of cotton dental wick that had been soaked in 15% sugar/water containing different concentrations of methomyl. Mortality was assessed after 48 h, except for spinosad, which was assayed after 72 h due to the slower-acting nature of this insecticide.[16] For all insecticides, at all concentrations a minimum of 100 house flies were tested. All bioassays were done at 25 °C with a 12:12 h L:D photoperiod. Flies were considered dead if they were unable to right themselves.

To evaluate the levels of resistance to the larvicide cyromazine, we used a method based on that of Iseki and Georghiou.[17] House fly medium (10 g, see above) was placed into a 50-ml polystyrene vial (VWR, Bridgeport, NJ). Cyromazine in aqueous solution (water for the controls) was added and the medium was thoroughly mixed. Twenty two- to three-day-old house fly larvae were added to the medium, the vial was covered with muslin and held at 25 °C with a 12:12 h L:D photoperiod for two weeks. Flies were scored as 'survived' if they were able to emerge successfully from the puparium. The LC$_{99}$ value of 1.4 μg cyromazine g^{-1} medium for the susceptible strain was calculated based on the data of Iseki and Georghiou.[17] All tests were replicated a minimum of four times at each concentration for all strains.

The insecticide-susceptible CS strain was used to generate complete concentration–response lines. Bioassay data from a minimum of three replications were pooled and analyzed by standard probit analysis,[18] as adapted to personal computer use by Raymond[19] using Abbott's correction[20] for control mortality. The LC$_{99}$ for the CS strain was determined for each insecticide (except for cyromazine, see Section 3).

Bioassays of field-collected colonies were carried out using at least five diagnostic concentrations (susceptible strain LC$_{99}$, 3 × LC$_{99}$, 10 × LC$_{99}$, 30 × LC$_{99}$ and 100 × LC$_{99}$ (300 × LC$_{99}$ was also used for cyromazine bioassays)), as this method is considered best for the detection of resistant individuals.[21] Ultimately, such diagnostic tests are most useful in detecting resistance

Table 1. Toxicity of nine insecticides to the susceptible CS strain of house fly

Insecticide	*LC$_{99}$ (ng cm^{-2})^{b,c} (95% CI)*	*n*	*Slope (+ SE)*
Cyfluthrin[a]	8.3 (6.4–13)	355	3.5 (0.4)
Dimethoate[a]	31 (24–47)	380	4.1 (0.5)
Fipronil[a]	63 (43–110)	450	2.1 (0.2)
Tetrachlorvinphos[a]	67 (50–100)	520	3.1 (0.3)
Permethrin[a]	78 (55–130)	325	2.9 (0.3)
Spinosad[a]	160 (120–250)	430	3.1 (0.3)
Pyrethrins[a]	850 (780–940)	420	18 (2.2)
Methomyl[b]	14 (12–18)	690	4.3 (0.4)
Cyromazine[c]	1.4	–	–

[a] Adults were exposed to insecticide residue on glass.

[b] Adult feeding assay; μg methomyml^{-1} sugar water.

[c] Larval feeding assay data from Reference 17; μg cyromazine g^{-1} medium.

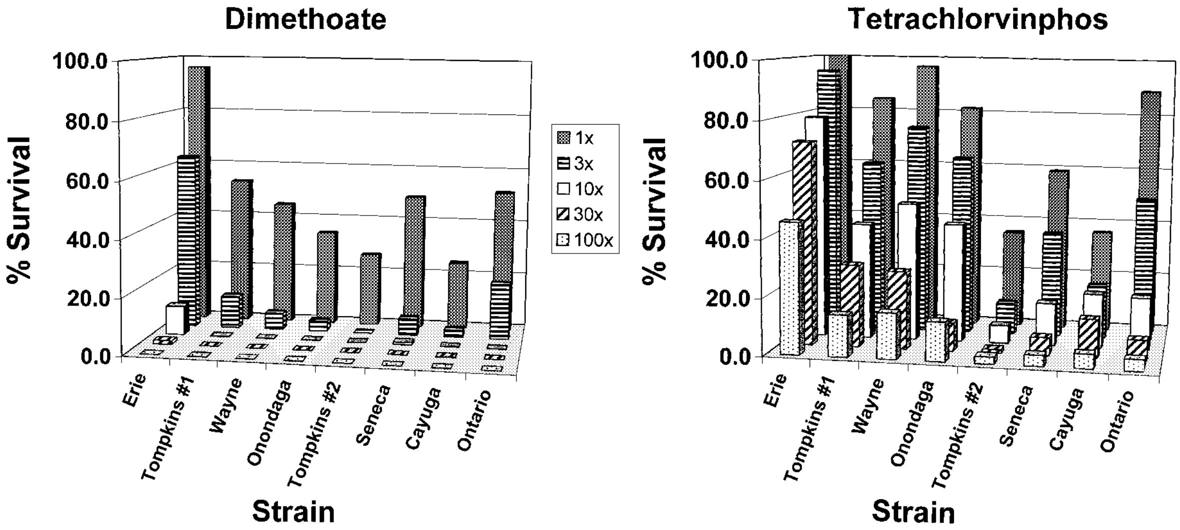

Figure 1. Percentage survival of house flies from eight poultry facilities across New York state exposed to two organophosphate insecticides at the susceptible strain LC_{99}, $3 \times LC_{99}$, $10 \times LC_{99}$, $30 \times LC_{99}$ and $100 \times LC_{99}$. SE (of the mean) was $<5\%$ for nearly all points.

where mortality at a diagnostic concentration can be correlated with control failure. However, such correlations have not been made for house flies and, as such, diagnostic concentrations have not been established. Therefore, we covered a 100-fold range of concentrations to avoid either over- or under-estimating the extent of the problem.

3 RESULTS AND DISCUSSION

The toxicity of the nine insecticides to the insecticide-susceptible CS strain of house fly is given in Table 1. Cyfluthrin was the most toxic of the seven insecticides tested against adult house flies by residue exposure. Given the high toxicity of this material by residual exposure it is not surprising that it became one of the most popular materials for house fly control at caged-layer poultry facilities and dairy farms in New York in the 1990s. Toxicity decreased in the order of cyfluthrin > dimethoate > fipronil ~ tetrachlorvinphos ~ permethrin > spinosad > pyrethrins. A previous study of the toxicity of tetrachlorvinphos, permethrin and dimethoate reported similar values.[15]

The results of bioassays on the field-collected house flies (Figs 1–4) are presented as percentage survival at the five diagnostic concentrations (ie frequency of flies resistant to a given concentration). Given the nature of these graphs, error bars could not be included. However, the SE of the means presented in the figures was $<5\%$ for nearly all determinations.

Two organophosphates, dimethoate and tetrachlorvinphos, were tested. Very low levels of resistance to dimethoate were detected (Fig 1). Only one poultry facility (Erie) had $>50\%$ survival at the $3 \times LC_{99}$ concentration and house flies at several facilities (eg Tompkins #2 and Cayuga) appeared quite susceptible. In contrast, resistance to tetrachlorvinphos was much higher and was present at many of the poultry facilities (Fig 1). The highest resistance ($>40\%$ survival at $100 \times LC_{99}$) was found at the Erie country

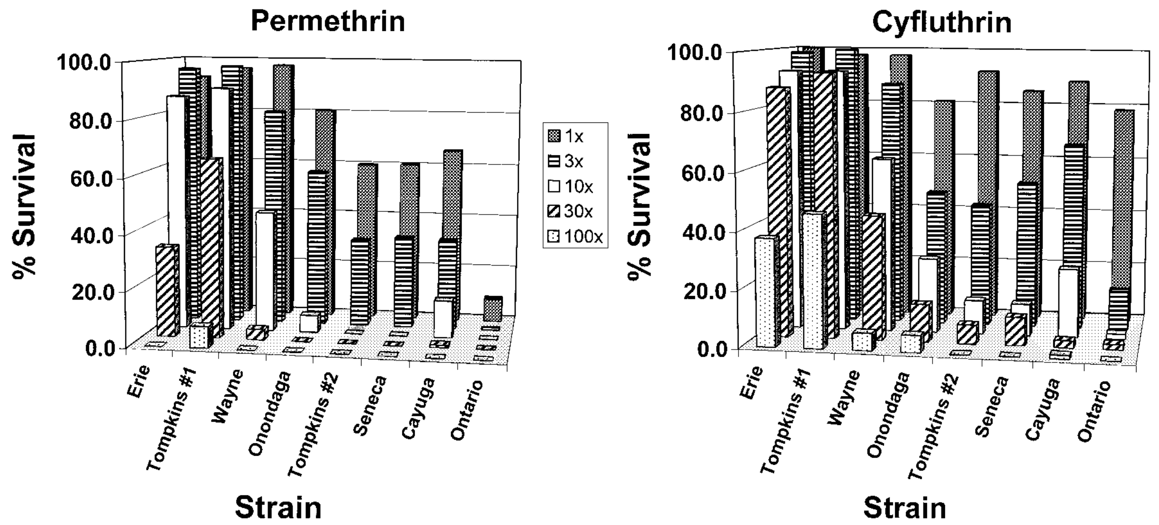

Figure 2. Percentage survival of house flies from eight poultry facilities across New York state exposed to two pyrethroid insecticides as described in Fig 1.

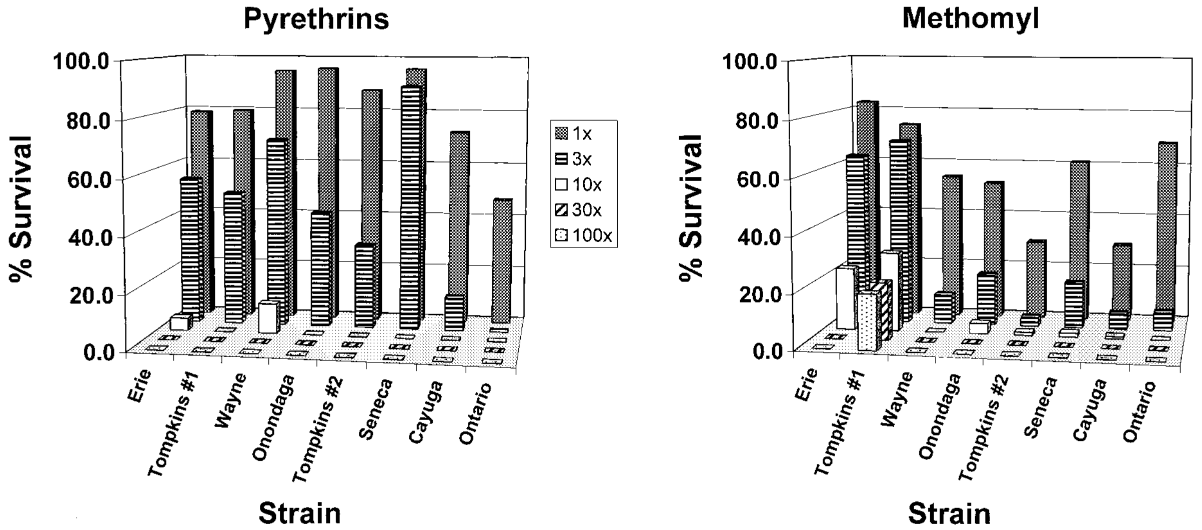

Figure 3. Percentage survival of house flies from eight poultry facilities across New York state exposed to pyrethrins and methomyl as described in Fig 1.

facility. More than half of the poultry facilities had >45% survivorship at the 3 × LC_{99}.

The levels of resistance to two pyrethroids (permethrin and cyfluthrin) and pyrethrins were examined. There were substantial levels of permethrin resistance, with more than half of the poultry facilities having >50% survival at 3 × LC_{99} (Fig 2). However, house flies at one poultry facility, Ontario, remained highly susceptible to permethrin. The levels of resistance to cyfluthrin were considerably higher, with two facilities (Erie and Tompkins #1) having >35% survival at the 100 × LC_{99} concentration (Fig 2). Resistance to pyrethrins was widespread at the LC_{99} and 3 × LC_{99} concentrations, but there was very little survival at the higher concentrations at any of the poultry facilities (Fig 3). These results suggest that permethrin, cyfluthrin and pyrethrins have become much less effective due to resistance. It is curious that resistance to pyrethrins does not extend to higher concentrations, as was found for permethrin and cyfluthrin. This implies that one or more mechanisms responsible for resistance to cyfluthrin (and probably permethrin) may not confer substantial cross-resistance to pyrethrins.

The only carbamate insecticide employed for fly control in New York is methomyl, which is formulated as a bait. Using our feeding assay the levels of resistance to methomyl varied from one poultry facility to another (Fig 3). High levels of resistance (20% survival at the 100 × LC_{99} concentration) were detected at only one facility (Tompkins #1) and populations at two facilities had only low levels of resistance (<30% survival at the LC_{99}, Tompkins #2 and Cayuga).

Fipronil and spinosad are two relatively new and highly promising insecticides that are effective against house flies and for which only low levels of cross-resistance have been detected.[13,16] We evaluated the levels of resistance to these new insecticides to gain a better understanding of the levels of variation that exist among populations prior to commercial use of the materials for fly control. While there was some variation among populations, house flies were susceptible to fipronil at all poultry facilities as there was

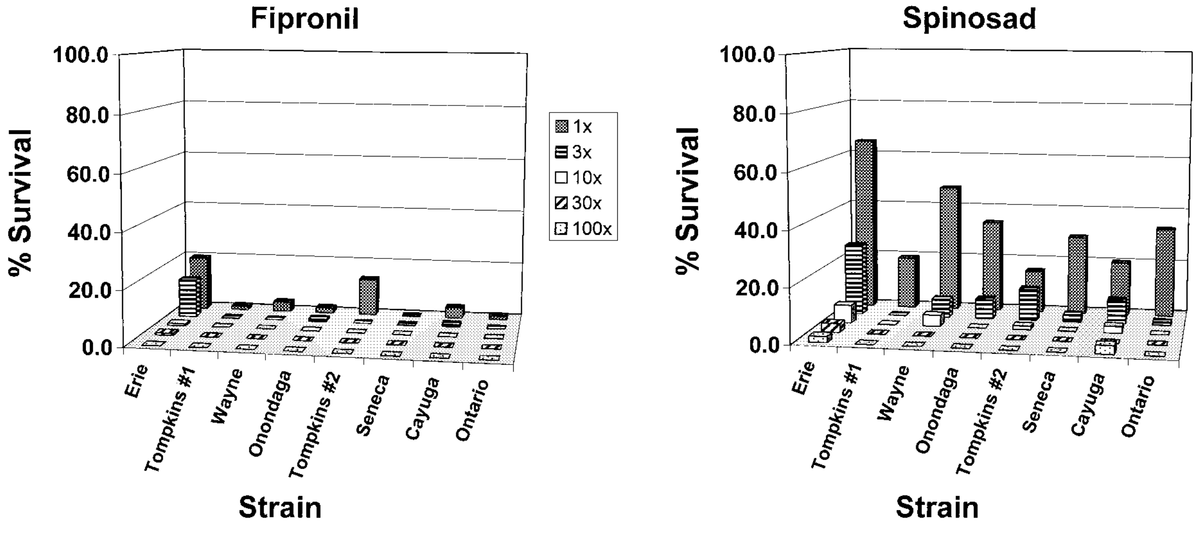

Figure 4. Percentage survival of house flies from eight poultry facilities across New York state exposed to fipronil and spinosad as described in Fig 1.

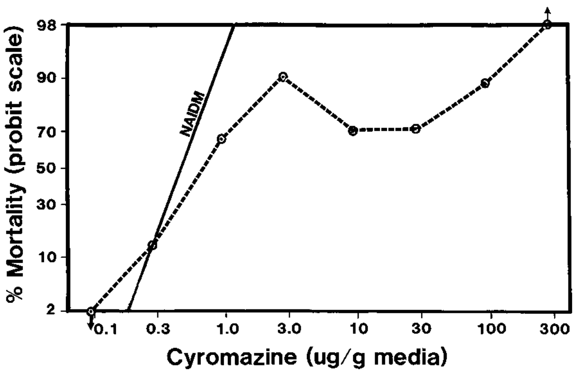

Figure 5. Concentration-response line for cyromazine against CS house fly larvae. The NAIDM line was taken from Iseki and Georghiou.[17] Survivorship indicates adult flies that successfully emerged from larvae that were reared on the treated media. The data points for each concentration represent 11 replications carried out over six different days using three independent solutions of cyromazine. Each data point represents 220 house flies.

$<20\%$ survival, even at the LC_{99} (Fig 4). The pattern for spinosad was similar, although there was generally slightly higher survival (Fig 4). At the Erie facility there was $>60\%$ survival at the spinosad LC_{99} and $>20\%$ survival at $3 \times LC_{99}$. For both of these insecticides, the highest percentage survival was at the Erie county facility. This facility also had among the highest levels of resistance to the other insecticides as well.

Evaluation of the toxicity of cyromazine against the CS strain did not produce a linear concentration–response curve (Fig 5). Although mortalities at the lower concentrations were similar to those reported by Iseki and Georghiou[17] fly mortality decreased when cyromazine concentration increased from 2.7 to $9.2\,\mu g\ g^{-1}$ and 100% kill was not obtained until a concentration of $270\,\mu g\ g^{-1}$ was used. While one possible explanation for these results might be that the CS strain is heterogeneous in its response to cyromazine, this would not explain the decrease in mortality between 2.7 and $9.2\,\mu g\ g^{-1}$. In addition, the CS strain has been evaluated against dozens of insecticides and appears to be a reliable, homogeneous susceptible strain. Further, the CS strain has no known history of exposure to cyromazine. Another possible explanation for the results is that at concentrations $>3.0\,\mu g\ g^{-1}$ exposure does not increase with increasing concentration. This could occur, for example, if there was an antifeedant effect at these concentrations. Given the results with the CS strain we decided to add two additional strains to our survey: OCR and Cornell-R.[16] These strains offer two benchmarks for the effectiveness of cyromazine (see below) as neither of these strains has been exposed to this larvicide.

Similar to the results obtained for the other insecticides, the levels of apparent cyromazine resistance varied considerably among facilities (Fig 6). The greatest use of cyromazine occurred on the Erie, Tompkins #1 and Ontario facilities which had among the highest levels of resistance. Cyromazine was the only insecticide we tested that did not show a clear trend of decreasing survival with increasing concentration. Thus, the results obtained with the CS strain do not appear to be unique for that strain. It seems that monitoring for resistance to cyromazine is not as straight forward as for the other insecticides tested. For more reliable estimates of cyromazine resistance, it appears that an annual assessment should be carried out to compare results at a given facility over time.

The results of our study provide an interesting comparison to the 1987 survey of insecticide resistance at dairy farms in New York. On dairy farms, the similar pattern of resistance, independent of the geographical

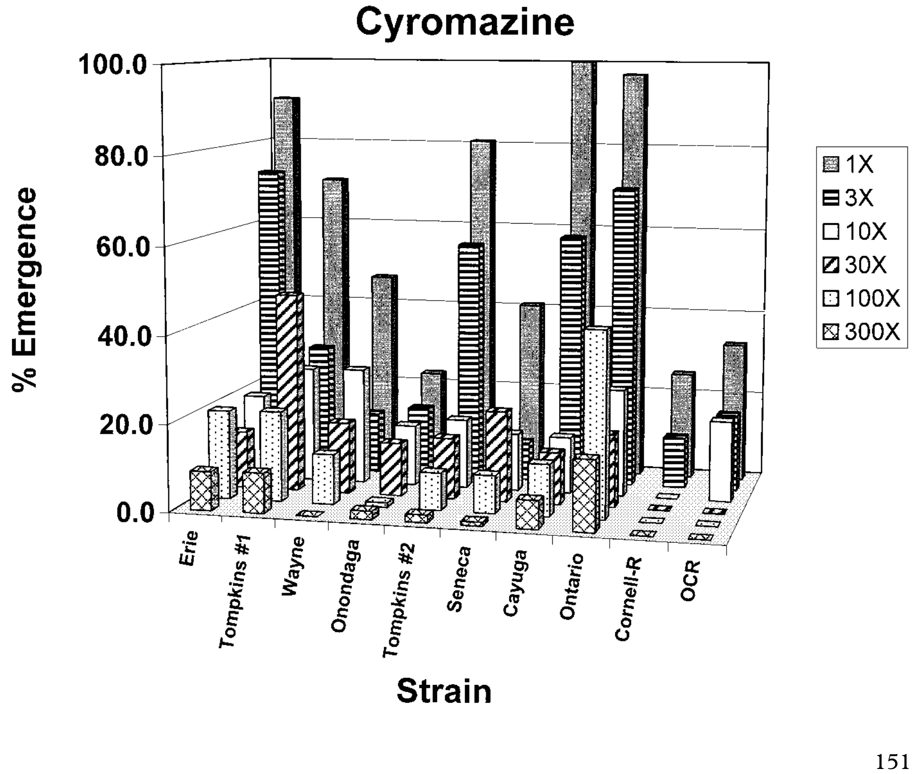

Figure 6. Percentage of cyromazine-treated larvae emerging as adults. House flies from eight poultry facilities across New York state, and two laboratory colonies, were tested using the cyromazine-susceptible strain LC_{99}, $3 \times LC_{99}$, $10 \times LC_{99}$, $30 \times LC_{99}$, $100 \times LC_{99}$ and $300 \times LC_{99}$. SE (of the mean) was $<5\%$ for nearly all points.

location or insecticide use, suggested that house flies were quite mobile, perhaps facilitated by the open architecture at dairy farms. Our results for caged-layer poultry facilities are in marked contrast to this because the levels of resistance varied considerably from one facility to another (even if from the same county), and levels of resistance were generally correlated to use of that insecticide at that facility. Another remarkable contrast between the current study and the previous results from dairy farms is the level of permethrin resistance. On New York dairy farms in 1987 there was $<60\%$ survival at the LC_{99} and there were no survivors at any farm at $30 \times LC_{99}$. While there are probably some differences in the levels of resistance between dairy farms and poultry facilities, these results suggest there was a large increase in permethrin resistance in house flies over the last decade across New York. The levels of resistance to tetrachlorvinphos and dimethoate found at dairy farms in 1987 were higher than we observed at poultry facilities in 1998. Given the low levels of resistance against dimethoate, this insecticide may still be useful for house fly control in New York caged-layer poultry facilities.

Our results suggest that there is limited dispersal of house flies into and out of caged-layer poultry facilities in New York. This suggests that resistance management at these facilities is thus feasible. While resistance management on a regional scale is certainly desirable, the required coordination and cooperation are not always obtainable. Given the very localized patterns of resistance in this study, it appears that caged-layer poultry facilities, at least in northern states, could devise workable resistance management schemes, perhaps even on a facility-by-facility basis.

Overall, very little is known about the resistance mechanisms present in these strains. A previous study on flies collected from New York (Learn dairy) determined the mechanisms of pyrethroid resistance to be increased monooxygenase-mediated metabolism, decreased cuticular penetration and decreased sensitivity of the nervous system.[22] In this strain, the monooxygenase-mediated metabolism was less effective against pyrethrins, resulting in lower levels of resistance to this insecticide. This is one possible explanation for the lower levels of pyrethrins resistance observed in this study.

Our results suggest limited efficacy of tetrachlorvinphos, permethrin and cyfluthrin because the resistance levels are very high. Fipronil and spinosad appear to be promising new materials for house fly control, yet uncertainty about new insecticide regulations appears to be discouraging the development of these materials for house fly control.

One concern about resistance monitoring has been that field-collected populations might be generally more 'robust', and able to survive insecticide exposure better than laboratory-reared, susceptible strains. Our results indicate this is not the case. The field-collected flies were highly susceptible to fipronil, an insecticide to which they have never been exposed and to which cross-resistance appears to be low.[13] Thus, the survival observed in our resistance monitoring assays (at least in the case of the adulticides) is due to resistance.

ACKNOWLEDGEMENTS

We thank F Harrison for technical assistance and the New York poultry producers who cooperated in this study. This research was supported by Hatch Projects 139414 and 139418 and Regional Project S-274.

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