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Monitoring insecticide resistance in house flies (Diptera: Muscidae) from New York dairies

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

Abstract: House flies were collected from dairies across New York state and the levels of resistance to seven insecticides were measured using standard laboratory assays with three to five diagnostic concentrations. The highest levels of resistance were found for tetrachlorvinphos, permethrin and cyfluthrin. Although levels of resistance to methomyl were somewhat lower, they were among the highest ever reported for field-collected house flies. Resistance to pyrethrins was limited primarily to the lowest diagnostic concentration. House flies were susceptible to fipronil at all dairies, suggesting that this material would be highly effective for fly control. The levels of resistance were similar at all the dairies, irrespective of their insecticide use, suggesting substantial movement of flies between facilities. Relative to resistance levels found at New York dairies in 1987, resistance levels had increased for permethrin, were unchanged for tetrachlorvinphos and had decreased for dimethoate. To identify a single diagnostic concentration that could be used in the laboratory assays to assess accurately resistance levels in future studies, we carried out a 'simulated' field bioassay using formulated materials. A diagnostic concentration for each insecticide is proposed on the basis of a comparison of our bioassays.

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

1 INTRODUCTION

House flies, *Musca domestica* L, are major pests in and around dairy housing systems. Recently, entero-haemorrhagic *Escherichia coli* O157:H7 was isolated from house flies collected at a cattle farm in Japan, documenting for the first time that house flies carry this serotype.[1] Many dairy farmers practice integrated pest management, but chemical control still plays a major role in fly management on New York farms. House fly insecticide resistance is a global problem[2] and has specifically been documented in New York state.[3–5] The importance of resistance management has increased lately, due in large part to the implementation of the Food Quality Protection Act (FQPA) of 1996. With few effective insecticides currently available, the removal of any insecticide will reduce the chances for a successful resistance management program.

In a 1987 survey of flies from NY dairies,[4] the frequency of resistance was high for crotoxyphos, dimethoate and tetrachlorvinphos, moderate for permethrin and low for dichlorvos. Patterns of resistance in these open architecture dairies were similar across the state, with resistance present on farms with little or no pesticide use, suggesting widespread movement of resistant flies. Conversely, a recent resistance survey found substantially different resistance patterns (between facilities) at enclosed poultry facilities, suggesting limited movement of flies into these sites.[5]

Although resistance levels can be determined accurately with laboratory bioassays, in the case of house flies it is frequently unknown what level of resistance in these assays equates with control problems in the field. The level of resistance that correlates with control problems can be small or large and varies between insects, insecticides and type of bioassay. Thus, it can be difficult to determine an accurate diagnostic dose for monitoring resistance (ie one that does not overestimate or underestimate the problem).

This study was conducted with field-collected populations of house flies to measure the level of resistance to commonly used insecticides. We then compared these results with the level of control observed under a 'simulated' field exposure (wood panels treated with formulated materials at label rate). Our goals were to survey the levels of resistance at New York dairies and to determine a single diagnostic concentration for future monitoring efforts.

2 MATERIALS AND METHODS

2.1 Insects, farms and chemicals

House flies were collected by sweep net from within

* Correspondence to: Phillip E Kaufman, Department of Entomology, Comstock Hall, Cornell University, Ithaca, New York 14853, USA
E-mail: pek4@CORNELL.edu
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dairy barns and around calf hutches across New York state during June 1999. Laboratory colonies of each strain were established from house flies free of pathogens and ectoparasites. House flies were reared as previously described.[5] Flies were bioassayed beginning with the first generation of adults produced by the field-collected flies. The Cornell susceptible (CS) strain served as the standard laboratory strain to which all field strains were compared.[6]

Farms in Wyoming, Schuyler and Saratoga counties served as collecting sites for insecticide-treated fly populations, while a certified organic farm in Tioga county provided flies from a site where no insecticides had been used (~10 years). The Schuyler county farm was included in a previous house fly resistance survey.[4]

Eight technical grade insecticides were tested: cyfluthrin (91%, *cis:trans* 40:60, Bayer, Kansas City, MO), dimethoate (99.7%, American Cyanamid, Princeton, NJ), fipronil (96.3%, Rhone-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), piperonyl butoxide, (PBO, Aldrich Chem, Milwaukee WI) and tetrachlorvinphos (99.5%, Chem Service, West Chester, PA).

Five formulated insecticides were examined: pyrethrins + PBO (PY-80 Premium (0.5% pyrethrins, 4.0% PBO), Universal Cooperatives, Minneapolis, MN), dimethoate (Bonide Cygon 234 g litre^{-1} EC, Bonide Products, Yorkville, NY), tetrachlorvinphos (Rabon 500 g kg^{-1} WP, Fermenta, Kansas City, MO), permethrin (Ectiban 557 g litre^{-1} EC, Universal Cooperatives, Minneapolis, MN), and cyfluthrin (Tempo 200 g kg^{-1} WP, Bayer, Kansas City, MO).

2.2 Bioassays

2.2.1 Laboratory bioassays

Technical grade insecticides were examined using either a residual contact or feeding assay. For the residual contact method 10–25 (2- to 5-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 technical grade insecticide as described previously.[4,5] Methomyl is formulated as a bait, 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.[5] For all insecticides at all concentrations, a minimum of 100 house flies were tested. Bioassays were done at 25 °C with a 12:12 h light:dark photoperiod. Mortality was assessed after 48 h and flies were considered dead if they were ataxic.

The insecticide-susceptible CS strain was used to generate a complete concentration-response line for pyrethrins + PBO. Bioassay data from five replications were pooled and analyzed by standard probit analysis,[7] as adapted to personal computer use by Raymond[8] using Abbott's correction[9] for control mortality. The LC_{99} for the CS strain was previously determined for the other insecticides used.[5]

Bioassays of field-collected colonies were carried out using three to five diagnostic concentrations (susceptible strain LC_{99}, $3\times LC_{99}$, $10\times LC_{99}$, $30\times LC_{99}$ and $100\times LC_{99}$), as this method is considered optimal for the detection of resistant individuals. To ensure that these concentrations remained accurate, the CS strain was periodically tested at the LC_{99}, and complete mortality was always observed (data not shown). Ultimately, such diagnostic tests are most useful in detecting resistance where mortality at a diagnostic concentration can be correlated with control failure. At the time these studies were completed, however, such correlations had not been made for house flies and, as such, diagnostic concentrations had not been established. Therefore, we covered a 100-fold range of concentrations to avoid either over- or underestimating the extent of the problem.

2.2.2 Simulated field bioassays

Flies were assayed using commercially available, formulated materials either as space sprays or as residual contact applications. The pyrethrins + PBO formulation was examined as a space spray in a 27.38 m^3 (3.7 m × 3.7 m × 2 m) enclosed shed. To maintain air movement, two 61-cm fans, delivering a velocity of 3.25 m s^{-1} (Schalen-Anemometer Model 3007, Germany), were positioned to circulate air throughout the room. Flies were anaesthetized with carbon dioxide and 25 (2- to 5-day-old) female house flies were placed into 15 cm × 15-cm nylon mesh bags (14 squares cm^{-2}) that were folded to create an open-air pocket and suspended 30 cm below the ceiling. All four field-collected fly strains and the CS strain were exposed simultaneously in identical treated and untreated buildings. The insecticide application was made according to label directions (aerosol sprayed for 2–3 s per 28.3 m^3) and flies were removed from the buildings following a 2-h exposure. The chemical exposure portion of this assay was conducted at *c* 22.2 °C with natural, window-derived sunlight and was replicated three times.

Dilutions of the remaining formulated insecticides (dimethoate, tetrachlorvinphos, permethrin and cyfluthrin) were prepared according to label directions and applied to unpainted plywood panels at a uniform rate of 5 ml per 929 cm^2 (= 1 gal per 750 ft^2). The plywood panels were exposed to natural summer weather conditions for 10 days before the insecticides were applied. Calibrated trigger-pump hand sprayers were used to apply insecticides and a water treated control to individual 929-cm^2 plywood panels. All panels were allowed to dry for 1 h before test insects were placed on them.

Flies were exposed to treated panels by anaesthetizing them with carbon dioxide and transferring 25 flies to each panel. Flies were confined to panels by placing wooden embroidery hoops (14.5 cm inner diameter, 1 cm thick) that had been covered with coarse mesh screen cloth (14 squares cm^{-2}) (Fig 1). Prior to fly transfer, a strip of duct tape (3 cm × 9 cm) was affixed near the bottom of the hoop for the duration of the exposure and prevented fly contact with the treated board while the insects were anaesthetized or immobilized. Hoops were secured to the plywood with two rubber bands stretched across the hoop and fastened to push pins. After the hoop was secured, boards were hung vertically for a 2-h holding period at 25 °C under constant fluorescent lighting. This design was an attempt to replicate what flies in dairies would experience, including the choice of resting on a treated surface or moving to untreated areas. Throughout the holding period, flies were observed walking on the surface of the plywood.

Following exposure to either a treated board or space spray, flies were again anaesthetized and transferred to 118-ml plastic cups with screened lids. Flies were provided with a dental wick soaked in 10% sugar-water and held at 25 °C under constant fluorescent lighting. Mortality was assessed after 48 h and flies were considered dead if they were ataxic. The assays were replicated three times (100 insects per replication), with four boards (or screened bags) per farm (including the CS strain) and insecticide at each replication. The labour intensity of these simulated field bioassays precludes them from being an effective means of monitoring resistance from large numbers of collection sites.

3 RESULTS AND DISCUSSION

3.1 Laboratory bioassays

The two pyrethroid insecticides most commonly used for house fly control in New York are permethrin and cyfluthrin. Very high levels of resistance to permethrin were observed, with >65% survival at the 3× LC_{99} (Fig 2). These levels are much higher than those reported in 1987 (one-half of the farms reported >40% survival at the 1× LC_{99}),[4] and probably result from the extensive use of permethrin in the late 1980s and early 1990s. Cyfluthrin resistance also was very high, with >50% survival at the 10× LC_{99} and >20% survival on three of the four farms at 100× LC_{99}. Cyfluthrin was originally registered in New York state in August 1991, indicating that resistance developed rapidly to this compound.

For pyrethrins there was 60–80% survival at the 1× LC_{99} of flies from the four dairies (Fig 3). Given that pyrethrins are usually applied in combination with PBO (at various ratios) at New York dairies, we examined each field-collected colony against pyrethrins + PBO. PBO synergized pyrethrins by 22-fold, resulting in an LC_{99} of 39 ng cm^{-2} to the CS strain. Compared with pyrethrins alone, there was a slight decrease in percentage survival in the LC_{99} for pyrethrins + PBO (percentage survival was greatly reduced at the Schuyler dairy), although there was a slight increase in percentage survival at 3× LC_{99}. The differences observed between pyrethrins and pyrethrins + PBO suggests that it is probably more

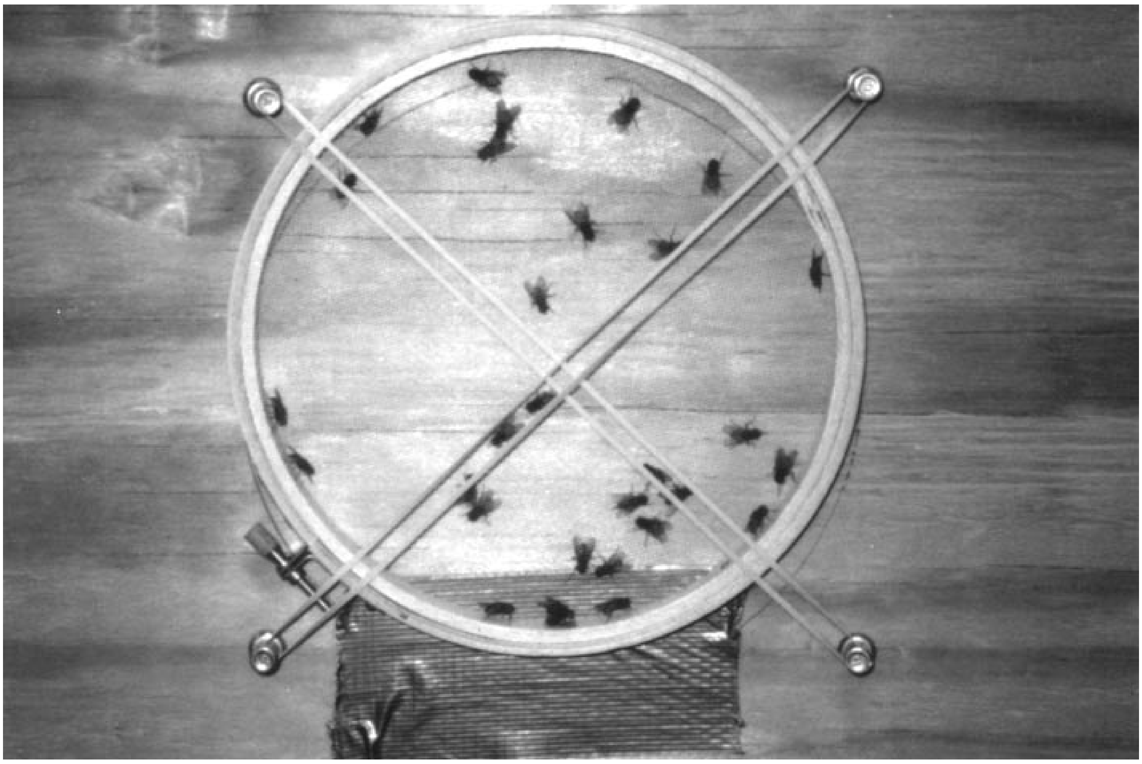

Figure 1. House flies confined to treated plywood panel (ie simulated field bioassay).

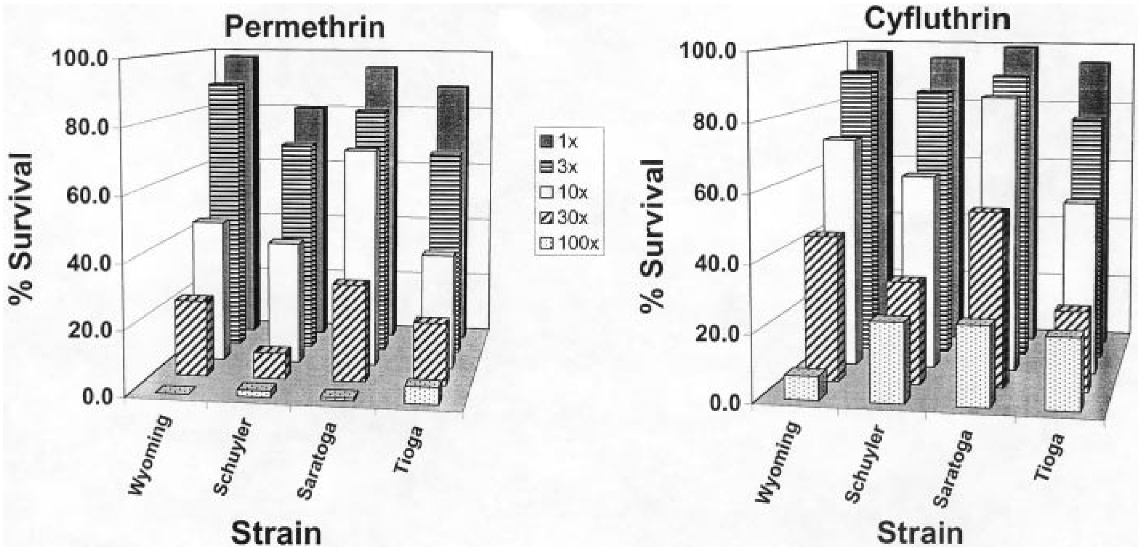

Figure 2. Percentage survival of house flies from four dairy farms across New York state exposed to two pyrethroid insecticides at the susceptible strain LC_{99}, $3\times\ LC_{99}$, $10\times\ LC_{99}$, $30\times\ LC_{99}$, $100\times\ LC_{99}$.

realistic to use the latter in resistance monitoring programs.

The two most widely used organophosphate insecticides for house fly control on New York dairies are tetrachlorvinphos and dimethoate. Resistance to tetrachlorvinphos was very high, with >50% survival at the $30\times\ LC_{99}$ concentration on all farms (Fig 4). This pattern of resistance closely follows that reported in 1987.[4] Only low levels of resistance to dimethoate were detected in house flies from the four dairies using the laboratory bioassay. Resistance levels were much higher in a 1987 dairy survey (eg survival at $2\times\ LC_{99}$ was 60–95%).[4] This suggests a substantial loss of resistance to dimethoate over the last decade. Due to the stability of tetrachlorvinphos resistance during a period when dimethoate resistance was substantially reduced, cross-resistance between these two compounds appears limited.

Methomyl is formulated only as a bait for fly control. Using our feeding assay, there was ≥50% survival at $3\times\ LC_{99}$ for flies from all the dairies and >40% at $10\times\ LC_{99}$ on two farms (Saratoga and Tioga) (Fig 5). Methomyl resistance in house flies from dairies was generally much greater than on poultry farms,[5] consistent with the greater usage of these fly baits on dairy farms.[10,11]

Fipronil is a relatively new and highly promising insecticide that is effective against house flies and for which only low levels of cross-resistance have been detected.[12] We evaluated the level of resistance to this new insecticide to gain a better understanding of the levels of variation that exist among populations prior to commercial use of this material for fly control. House flies were susceptible to fipronil at all dairy facilities as there was <10% survival, even at the LC_{99} (Fig 5). Consistent with our 1998 poultry study, the highest percentage survival (8% at the LC_{99}) was observed at the farm with the highest levels of resistance to other insecticides (Saratoga).[5]

The results of our bioassays allow us to speculate on

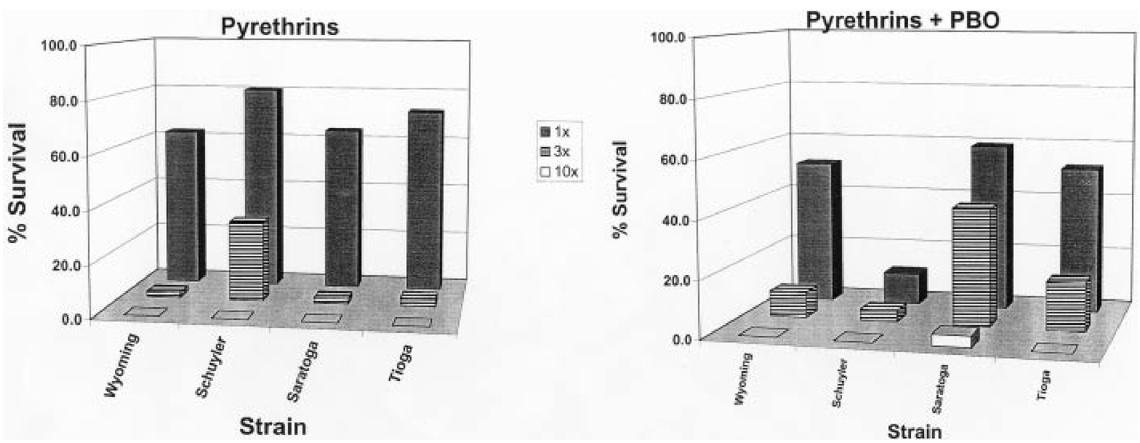

Figure 3. Percentage survival of house flies from four dairy farms across New York state exposed to pyrethrins and pyrethrins + piperonyl butoxide (PBO) as described in Fig 2.

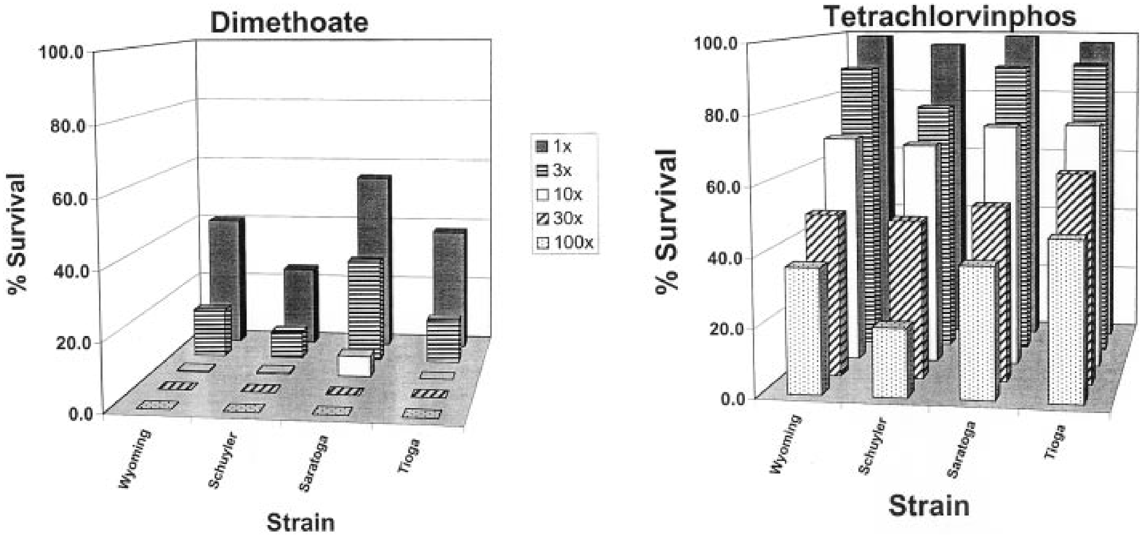

Figure 4. Percentage survival of house flies from four dairy farms across New York state exposed to two organophosphate insecticides as described in Fig 2.

the resistance mechanisms involved. Due to the lack of reduction in pyrethrins resistance by addition of PBO, it is likely that *kdr* is involved (ie the only other mechanism known to confer resistance to pyrethrins).[13,14] Given that *kdr* confers resistance to pyrethroids, this is undoubtedly one of the factors involved in resistance to permethrin and cyfluthrin. However, given that the levels of permethrin and cyfluthrin resistance are very high in these strains, it seems likely that CYP6D1 mediated (ie monooxygenase) resistance is conferring resistance as well. This resistance mechanism has been well studied in a strain of house flies previously collected from the dairy in Schuyler country.[15,16] Previous studies on methomyl in house flies have implicated altered acetylcholinesterase and increased detoxification as the mechanisms of resistance.[17] Given that the levels of resistance are similar in our study and that of Price and Chapman,[17] it seems probable that these are the mechanisms involved. The mechanisms involved in resistance to the two organophosphates that we studied are much less easy to discern. For these insecticides, there are a variety of detoxification enzymes that have been implicated in resistance[18,19] as well as a change in the target site (acetylcholinesterase). Unlike the *kdr* mechanism discussed above, however, this altered target site resistance mechanism does not confer cross-resistance to all organophosphates.[20] Thus, although the mechanisms of resistance to dimethoate and tetrachlorvinphos seem to be different, it is unclear exactly what mechanisms are involved.

3.2 Simulated field bioassays

Permethrin was ineffective against all field-collected strains, but was very effective against the CS strain (Fig 6), suggesting this insecticide retains little or no efficacy at these dairies. Survival of flies exposed to cyfluthrin was less than 30% on three of the four farms, but cyfluthrin was highly effective against the

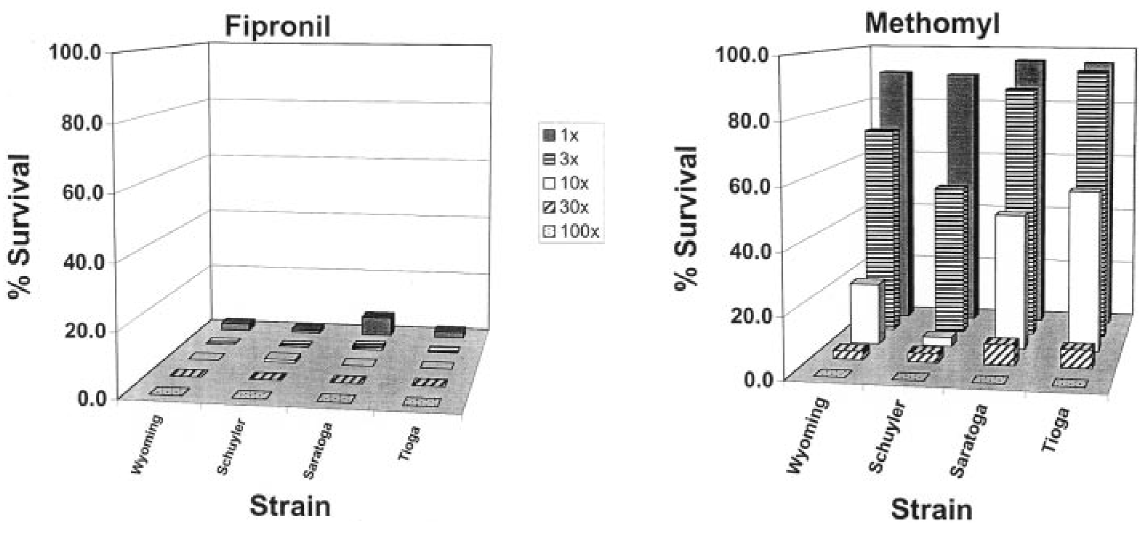

Figure 5. Percentage survival of house flies from four dairy farms across New York state exposed to methomyl and fipronil as described in Fig 2.

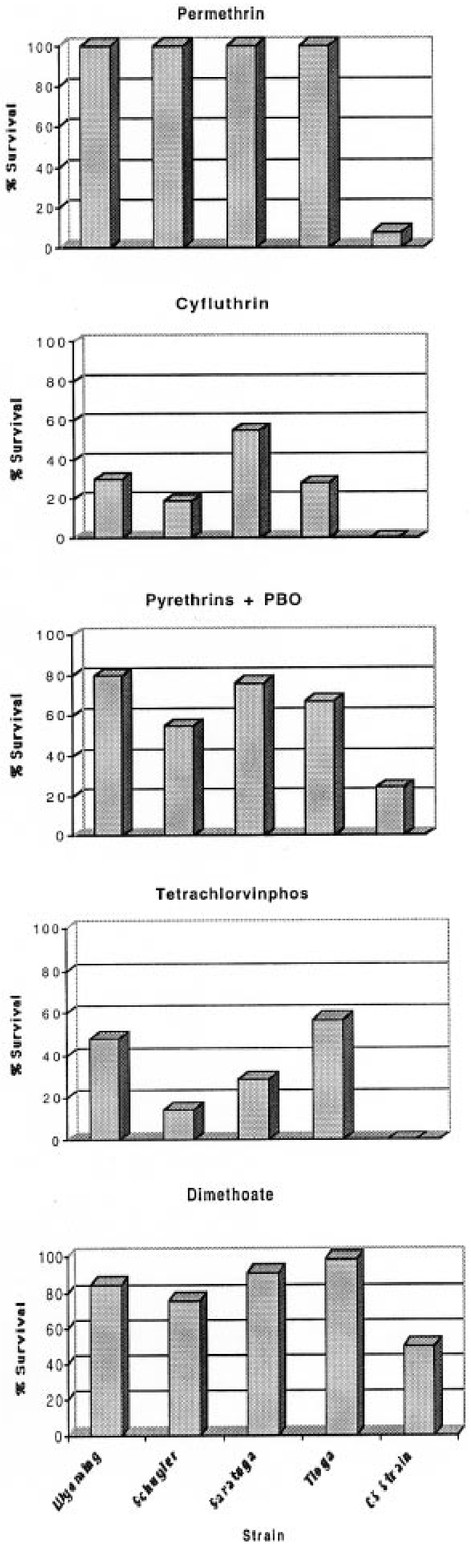

Figure 6. Percentage survival of house flies from four dairy farms across New York state and a laboratory-susceptible strain (CS) exposed to the labeled rate of Ectiban™, Tempo™, Cygon™ and Rabon™ on treated plywood boards and a space spray of the labeled rate of PY-80 Farm and Dairy Spray™.

CS strain (100% kill). For the space spray containing pyrethrins + PBO, was more than 50% survival observed with all field-collected strains and 20% survival among laboratory-susceptible flies.

Tetrachlorvinphos was highly effective against the CS strain (100% kill), and moderately effective against the field-collected strains, with the percentage survival ranging from 14% (Schuyler) to >50% (Tioga) (Fig 6). Dimethoate was largely ineffective in controlling house flies in the simulated field bioassay. We observed >75% survival on all field-collected strains and 50% survival of the insecticide-susceptible (CS) strain. This does not appear to be due to a problem with the Cygon™ *per se*, because the same results were observed with two batches of Cygon™. Furthermore, when formulated dimethoate (diluted in acetone) was used to treat glass jars (to the label rate) CS flies died (100%) within 2 h (Scott JG, unpublished). However, dimethoate is still an efficacious insecticide at many regional dairies (Kaufman PE, unpublished). Although it appears that the simulated field bioassays are resulting in unrealistically low mortality for this insecticide (relative to fly control on dairies), the results clearly show that resistance to dimethoate, *via* contact with treated glass, is low at these four dairies.

A goal of this study was to identify which concentration in the laboratory bioassay was most appropriate for monitoring resistance for each insecticide. This concentration was determined by comparing results of the 'simulated field test' with those of the laboratory bioassay. The best diagnostic concentrations for permethrin, cyfluthrin and pyrethrins + PBO appear to be 3× LC_{99}, 30× LC_{99} and 1× LC_{99}, respectively. Although cyfluthrin is apparently still effective, reports of field failures continue to increase (Kaufman PE, unpublished). For tetrachlorvinphos, 100× LC_{99} appears to be the best concentration for monitoring resistance, as this concentration most closely matches the 'simulated field test' results. By a similar rationale, the best diagnostic concentration for dimethoate appears to be 3× LC_{99}. This concentration documented well the decrease in resistance from 1987 to 1999. This concentration also mirrored the relatively low levels of resistance seen in the simulated field assays. Deciding the most appropriate diagnostic concentration for methomyl is more difficult because we have neither simulated field assays nor reliable field efficacy information from these dairies. By comparison of our current results with those from poultry facilities,[5] it would appear that 3× LC_{99} would be a reliable indicator of resistance (although perhaps not of overall field efficacy).

The surprising survival of the CS flies (and associated variability among mesh bags holding flies) following exposure to pyrethrins + PBO suggests that spray coverage throughout the treated building may not have been uniform. It is unlikely that space sprays applied in dairy barns would be distributed any more evenly than in our controlled environment. Therefore, the relatively low resistance to pyrethrin + PBO observed with house flies in the technical assay may be the result of incomplete spray coverage and relatively short half-life of the pyrethrins, which allows for survival of susceptible flies at dairies.

Surveys of insecticide use on New York dairy farms

Table 1. Summary of and changes in patterns of insecticides used for fly control in and around barn areas on New York state dairy farms in 1986, 1991 and 1997[a]

Chemical	*Survey*	*Estimated percentage of farms treated*	*Percentage change from* 1986	1991
Dimethoate	1986	4.1		
	1991	9.0	+119.5	
	1997	4.2	+2.4	−53.3
Tetrachlorvinphos	1986	6.0		
	1991	13.8	+130.0	
	1997	8.4	+40.0	−39.1
Permethrin	1986	5.8		
	1991	48.1	+729.3	
	1997	23.4	+303.4	−51.3
Cyfluthrin	1986	na		
	1991	0.0	—	
	1997	4.2	—	—
Pyrethrins + PBO	1986	21.6		
	1991	67.2	+211.1	
	1997	39.0	+80.6	−42.0
Methomyl	1986	20.7		
	1991	44.4	+114.5	
	1997	44.9	+116.9	+1.1

[a] Data adapted and compiled from three pest and pesticide use surveys of New York state. References 10, 21, 22.

were completed in 1986,[21] 1991[22] and 1997[10] (Table 1). These surveys indicate to which insecticides house fly populations on New York dairies have probably been exposed. It should be noted that since 1986 a number of insecticides included in these surveys have not been reregistered. Furthermore, few new compounds have been registered. This has resulted in increased usage of the few remaining compounds as well as increased selection pressure on house fly populations. For example, in the 1986 survey there were seven active ingredients registered for use in fly baits, but in 1997 methomyl was the only bait formulation. It is expected that this trend will continue as the implementation of the FQPA progresses. In general, farmers are now using less insecticide than in 1991. We attribute this decrease to both the adoption of integrated pest management practices and the loss of efficacy. Currently producers rely primarily on permethrin, pyrethrins, methomyl and, since its introduction, cyfluthrin. The high levels of resistance to permethrin, methomyl and cyfluthrin, combined with the lack of suitable alternative insecticides, suggests that control of house flies on dairies could face grave limitations in the near future.

Dimethoate resistance, as measured in the technical assay, was lower than in the 1987 study; however, tetrachlorvinphos resistance levels were similar. This differing response, given that the reported application of both materials dropped from 1991 levels, may have resulted from several factors. First, available data did not allow for a determination of the number of applications made per year on farms, but only provided use/no use responses. Thus, total use of dimethoate and tetrachlorvinphos could have been substantially different. Second, it is possible that the mechanisms house flies used in developing resistance were different for these two organophosphates. A better understanding of the factors involved is important in planning resistance management strategies utilizing rotations.

The similar levels of resistance at all four dairies, independent of their prior insecticide use, is consistent with the patterns observed in 1987.[4] Although insecticide use was prevalent at three of the current farms (Wyoming, Schuyler and Saratoga), no insecticides had been used on the Tioga county farm for more than 10 years. The nearest dairy farm to this facility is *c* 1.6 km, well within the dispersal range of the house fly.[23] It has been hypothesized that movement of flies between farms is limited[24,25] or may occur over an extended period of time.[4] However, house fly resistance to cyfluthrin, which was registered for use in New York in 1991, was already at a high level on the Tioga county farm. This suggests that movement of resistant individuals happened within eight years; however, cyfluthrin resistance may be due to a combination of both movement and cross-resistance with pyrethrins and permethrin.

With the exception of dimethoate, resistance to insecticides on New York dairy farms has increased since 1987.[4] Furthermore, resistance levels were higher on New York dairies than on New York poultry farms.[5] Our comparison between laboratory assays and standard field application methods allowed us to determine single diagnostic concentrations for future resistance monitoring efforts. The development of this efficient monitoring technology is a critical first step in the development of a successful resistance management strategy.

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