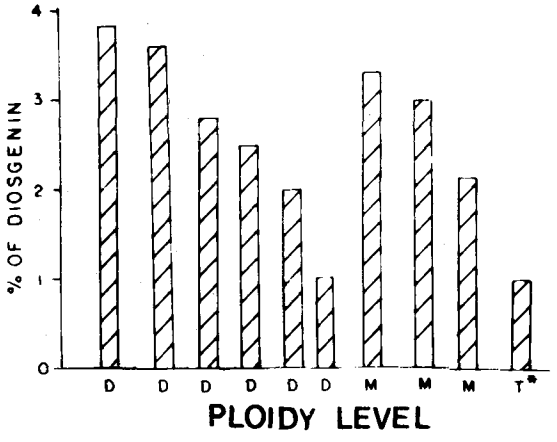

Figure 9. Percentages of diosgenin contents in plants with different ploidy at the third vegetative growth cycle in the field (D = diploid, M = mosaic, T = tetraploid; * represents diosgenin content in the first year of vegetative growth in the field).

in characters, such as the absence of white spotting in leaves or increased stomatal frequency appearing in two diploid individuals, may require further genetic tests to trace their origin.

There are several records of variants amongst regenerants in asexual species, but the genetic nature of such variability has not been fully clarified[4, 18–20]. In the present case, plants of *D. floribunda* were regenerated from stem tissue culture and variability could be documented in regenerants. The observed range of variations offers scope for an exploration of their economic potential.

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Selection of high-level abamectin resistance from field-collected house flies, *Musca domestica*

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Summary. Abamectin is a novel, highly promising insecticide with activity against many pests. To determine if resistance to abamectin could occur, we collected house flies from several New York dairies and selected them in the laboratory. Resistance developed rapidly and to a high level (36 or > 60,000-fold, depending upon test technique and/or adjuvant) that could not be overcome by the synergists piperonyl butoxide or *S,S,S*-tributylphosphorotrithioate. There was no increase in (cross)resistance to crotoxyphos, dichlorvos, dimethoate, tetrachlorvinphos, permethrin, dieldrin or lindane following abamectin selection. Our results suggest the potential for abamectin resistance is high, at least in house flies, and that the judicious use of abamectin will be needed to prolong its usefulness as an insecticide.
Key words. Insecticide resistance; abamectin; ivermectin; house fly; selection.

Pesticide resistance is a severe problem that limits our ability to control pests of agricultural and medical importance. Historically, resistance problems have necessitated the use of new compounds, particularly those belonging to new pesticide classes. However, the time taken for resistance to develop to a new insecticide is extremely variable, ranging from only a few applications to decades of use. One of the most important factors that could

accelerate the evolution of resistance is cross-resistance to the new insecticide resulting from previous insecticide use [1].

Abamectin is a new and highly promising insecticide belonging to the class of pesticides known as avermectins [2, 3]. As the uses of abamectin continue to expand, and new avermectins are discovered [4], there is an increasing need to evaluate how rapidly abamectin resistance might develop, and to investigate the biochemistry and genetics of the resistance in order to attempt to design and implement strategies to delay the onset of this problem.

In a previous study [5], pyrethroid-resistant house flies of the LPR strain were found to be 25-fold resistant to abamectin, demonstrating the potential for cross-resistance. In this study we selected house flies recently collected from the field to determine if higher resistance to abamectin could occur, how rapidly it would evolve, what effect synergists would have on the resistance, and if there would be cross-resistance to seven other insecticides.

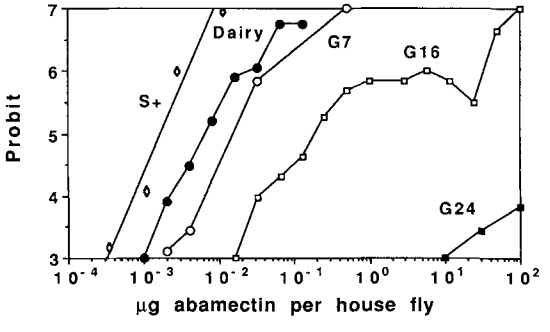
Materials and methods

House flies were selected with formulated abamectin (Merck Sharp and Dohme, Three Bridges, New Jersey, USA) via a residual exposure. Formulated abamectin (18 g/l emulsifiable concentrate) was dissolved in acetone immediately before use. A 1-ml volume of the desired concentration was then rolled on the inner surface of a 450-ml (250 cm^2 internal surface area) jar until dry. Flies were provided with a solution of powdered milk and sugar in a 25-ml flask inside the jar for the first selection and were given a 2.5-cm sucrose-saturated dental wick in all other selections. Mortality was scored at 72 h. To mimic the intensive selection often experienced during field applications and to minimize selection for minor resistance genes [6], abamectin concentrations used for the selection were initially sufficient to kill > 99% of the treated flies and were always greater than those needed to kill 95% of the individuals in several susceptible strains (18 µg/jar [7]). To provide a large sample size for selection, flies were collected from each of 10 dairies across a more than 460 km geographical range in New York State [8] and reared in the laboratory for approximately 3–6 generations prior to selection. Approximately 1000 flies from each strain were used in the initial selection and their survivors pooled in succeeding generations to create the strain hereafter called 'AVER'. Flies were sexed prior to selection during the last three generations of selection, but the females were not necessarily virgins prior to treatment.

After selection was completed, insecticide bioassays were conducted by topical application to the thoracic notum of female flies [5], or by exposure to a residue [8]. To investigate the possible role of metabolism as a mechanism of resistance, piperonyl butoxide (PBO, purified by eluting from a silica gel column with 10% ether in hexanes), an inhibitor of the cytochrome P450 microsomal monooxygenases, *S,S,S*-tributylphosphorotrithioate (DEF, Chem Service, West Chester, Pennsylvania, USA), an inhibitor of hydrolases, or diethyl maleate (DEM, Sigma, St. Louis, Missouri), an inhibitor of glutathione (GSH) conjugation, was applied topically in 0.5 µl acetone 1 h prior to abamectin at doses of 2, 10, and 10 µg/fly, respectively. For comparative purposes, an insecticide-susceptible strain, S+, originally obtained from F. W. Plapp Jr, Texas A & M University (College Station, Texas, USA), was similarly bioassayed. To investigate possible cross-resistance to other insecticides, 48 h LD_{50} values were determined for dieldrin and lindane (recrystallized from technical, sources unknown) by topical assay and 24 h LC_{50} values for crotoxyphos, dichlorvos, dimethoate, permethrin and tetrachlorvinphos (whose sources and purity have been previously reported [9]) by the residual technique to compare to previous data [8]. Probit analysis was carried out by the method of Finney [10] as adapted for personal computer use by Raymond [11].

Results

Over 60,000-fold resistance developed to technical abamectin applied topically (fig.) after selection (summarized in table 1) of just seven generations. 34 males sur-



The toxicity of topically applied technical abamectin to laboratory susceptible (S+), field-collected (dairy), and AVER flies at generations 7 (G7), 16 (G16), and 24 (G24). The data for the dairy strain have been reported previously [5] and are shown as an approximation of the parental strain used for the selections.

Table 1. History of abamectin selection of the AVER house fly strain

Generation[a]	Selecting dose	No. flies selected	% Mortality
Parental	29	10,000	> 99%
3	20	9,000	> 95%
5	58	12,000	> 95%
10	60	10,000	> 80%
12	300 (♂)	6,000	> 80%
	500 (♀)	8,000	> 80%
16	300 (♂)	4,000	> 70%
	500 (♀)	6,000	> 70%
23	300 (♂)	3,000	> 70%
	500 (♀)	5,000	> 70%

[a] Generation 1, 2, 4, 6–9, 11, 13, 14, 15, and 17–22 were not selected.

vived the initial selection and were crossed to more than 100 virgin field-collected females plus four females that had survived the selection. Although only 7 generations were selected, the process actually took 24 generations. Due to the low number of progeny produced by flies surviving the abamectin selection, additional generations were needed between selections to build up the colony. During the second, third, and fourth selections it appeared that fewer males than females were surviving. For this reason the fifth, sixth, and seventh selections were done using a slightly higher dose of abamectin on females than males. The mortality reported in table 1 (72 h) is in effect a minimum because flies continued to die after being placed in the colony cage.

The level of resistance observed by topical application of technical abamectin (> 60,000-fold) was much larger than that observed by residual exposure to formulated abamectin. The 48-h LC_{50} values for the latter were 0.05 and 1.8 μg/cm^2 for S+ and AVER, respectively, a resistance ratio increase of 36-fold. To determine if the difference in resistance levels between the two experiments was due to the test technique (i.e., topical vs residual) and/or the material (i.e., technical vs formulated), we attempted two other experiments. In the first we topically applied formulated material to S+ and AVER flies. However, 0.5 μl of the formulation blank killed > 50% of the S+ flies within 48 h and thus we were unable to determine an LD_{50} for S+ using formulated abamectin by topical application. Next we tested the toxicity of technical abamectin by the residual method. The 72-h LC_{50} values were 0.033 and > 6.3 μg/cm^2 for S+ and AVER, respectively, a resistance ratio of > 190. This resistance ratio is higher than we observed for the residual bioassay of formulated abamectin, suggesting that some component of the formulation reduces the level of resistance observed. The exact nature of the resistance mechanisms and how the formulation acts to mitigate the expression of part of the resistance will require further study.

The synergists PBO, DEF, and DEM were ineffective in substantially altering the toxicity of abamectin in either fly strain (table 2), suggesting abamectin is not rapidly metabolized by monooxygenases, hydrolases, or GSH conjugation in these strains. This is not the case for all house fly strains, however, as a previous report found abamectin toxicity to be slightly synergized by PBO (3.6-fold) in a susceptible strain. The lack in measurable reduction of the resistance ratio in the presence of the synergists suggests that metabolism is not the major mechanism of resistance in the AVER strain. However, synergist data must be interpreted with care[12], and further studies will be needed to clarify the role of metabolism in this resistance.

Table 2. Toxicity of abamectin alone or with synergist to susceptible (S+) and resistant (AVER) house flies

	S+				AVER		
Treatment	n	LD_{50}[a] (95% CL)	Slope (SEM)	SR[b]	n	LD_{50}[a]	RR[c]
Abamectin	160	0.0017 (0.0014–0.0022)	2.9 (0.5)	--	50	> 100 (14)[d]	> 60,000
Abamectin + PBO	160	0.0015 (0.0012–0.0019)	2.8 (0.4)	1.1	50	> 100 (8)[d]	> 60,000
Abamectin + DEF	200	0.0015 (0.0012–0.0018)	3.6 (0.6)	1.1	50	> 100 (22)[d]	> 60,000
Abamectin + DEM	160	0.0017 (0.0013–0.0024)	3.1 (0.5)	1.0	50	> 100 (30)[d]	> 60,000

[a] In units of μg per fly. Toxicity was evaluated at 48 h. [b] Synergistic ratio = LD_{50} abamectin/LD_{50} abamectin + synergist. [c] Resistance ratio = AVER LD_{50}/S+ LD_{50}. [d] Percent mortality at 100 μg per fly.

Table 3. Percent mortality of five insecticides to the S+ (susceptible), AVER (abamectin selected) and Parental (unselected strain used to produce AVER) house fly strains

		Strain			
		S+	AVER	Parental[a]	Change in resistance level
Insecticide	Concentration[b]	% Mortality	% Mortality	% Mortality	
Crotoxyphos	38	99	93	0–10	Loss of resistance
	114		99	0–25	
	380		100	0–41	
Dichlorvos	450	40	11	0–53	No observable change
	1350	100	92	58–100	
	4500		100	98–100	
Dimethoate	2.5	100	97	0–13	Loss of resistance
	7.5		99	6–42	
	25		100	42–90	
Permethrin	35	99	36	33–93	No observable change
	105		71	60–100	
	350		100	84–100	
Tetrachlorvinphos	29	100	37	3–18	Loss of resistance
	87		73	2–22	
	290		97	10–53	

90–390 flies were tested at each dose for each strain. [a] Expressed as the range of percent mortality of the field strains before being combined into the AVER (unselected) strain[8]. [b] Concentrations are given as μg/jar and represent the susceptible strain's LC_{99}, $3 \times LC_{99}$, and $10 \times LC_{99}$, respectively[8].

To determine if resistance to abamectin conferred cross-resistance to other insecticides, we evaluated the toxicity of five insecticides to the AVER strain before and after selection. The results (table 3) show a decrease in resistance toward crotoxyphos, dimethoate, and tetrachlorvinphos, while resistance to dichlorvos and permethrin was relatively unchanged. Considering the large increase in abamectin resistance following selection, it would appear that there is no strong cross-resistance to any of these five insecticides from the mechanism(s) producing the abamectin resistance. The decrease in resistance to crotoxyphos, dimethoate and tetrachlorvinphos may have been due to selection against organophosphate resistance genes by abamectin or more simply due to reproductive disadvantages inherent to the genes. The stability of the dichlorvos and permethrin resistance suggests that either there is no strong reproductive disadvantage in having these resistance genes, that they are tightly linked to the genes conferring resistance to abamectin, or that they are involved in conferring part of the abamectin resistance. The LD_{50} values for lindane and dieldrin were 30 ng and 6 ng per fly for S+, and 52 ng and 16 ng per fly for AVER, respectively. Therefore, the low resistance ratios of 1.7- and 2.7-fold for lindane and dieldrin, respectively, suggest there is no cross-resistance to these chlorinated hydrocarbons following abamectin selection.

Discussion

Only low to moderate levels of resistance have been previously selected by avermectins in either arthropods [13, 14] or nematodes [15–17]. In contrast, we obtained extremely high levels of resistance to technical abamectin. Further, it is clear that the potential for development of abamectin resistance in house fly populations may be very high. It is remarkable that we could select high-level abamectin resistance with only seven selections. By comparison, a similar study of field-collected house flies required about 22 consecutive generations of equal or greater selection intensity (measured as percent mortality) to develop high-level permethrin resistance [18]. Our results suggest that the alleles for resistance to abamectin may already exist at a relatively high frequency compared to other classes of insecticides prior to their introduction. One possibility for this may be that there was cross-resistance to abamectin due to previous insecticide use. Although insects having carbamate, organophosphate, DDT, cyclodiene, or *kdr*-mediated pyrethroid resistance do not appear to be cross-resistant to abamectin [7], other mechanisms of pyrethroid resistance present in the LPR strain of house flies can cause cross-resistance to abamectin [5]. It will be necessary to identify the biochemical mechanisms of resistance in the AVER strain before we can understand if there is a link between the mechanisms causing high levels of resistance in the AVER strain and the mechanisms of pyrethroid-induced cross-resistance to abamectin in the LPR strain.

Based on our current results we have little basis to judge what resistance mechanisms are involved in conferring protection to abamectin. PBO, DEF, and DEM were ineffective at measurably suppressing the abamectin resistance, suggesting that monooxygenase, hydrolase, or GSH conjugation-mediated metabolism may not be involved in the resistance. Decreased cuticular penetration may be involved in the resistance although this mechanism usually confers only low levels of resistance [12]. These arguments suggest that one of the mechanisms of abamectin resistance could be a type of target site insensitivity. If the AVER strain does in fact have an altered target site mechanism of resistance, it is interesting that there was no cross-resistance to dieldrin or lindane, which are thought to act at the same general target site as abamectin [19–22]. This is in agreement with the finding that cyclodiene resistant house flies were not cross-resistant to abamectin [7]. The rapid rate and high levels of resistance achieved in this study, combined with the potential for cross-resistance to abamectin [5] associated with use of pyrethroids, suggest that avermectins should be used carefully to avoid the development of resistance.

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