Origin and Extent of Resistance to Fipronil in the German Cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae)

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ABSTRACT Fipronil, a phenylpyrazole insecticide, was made available in 1999 in bait formulations for use against the German cockroach, *Blattella germanica* (L.). We have investigated resistance to fipronil in the descendants of cockroaches collected just before, or contemporaneously with, the introduction of fipronil baits. Cockroaches were obtained in two types of settings: homes that either had or had not been serviced by a pest management professional while occupied by their current residents. Thorough inspections by us turned up no evidence that fipronil had been used in any of the homes, and in addition, no residents claimed to have used baits containing fipronil. Resistance to fipronil was detected by topically dosing adult males with the LC_{99} of fipronil, the value of which was determined in a dose–response assay with males of an insecticide-susceptible strain. Fewer than 99 of 100 males of all field-collected strains died within 72 h of being treated. Moreover, substantial numbers of males survived doses three and 10-fold greater than the LC_{99}. Regression analysis showed that 67% of the variation in the percentage of males that died after being treated with fipronil was explained by a linear relationship with the percentage that died after being treated with dieldrin. Therefore, it appears that resistance to fipronil in German cockroaches—whose ancestors had never been exposed to it—is attributable to enduring resistance to the cyclodienes, which were formerly used for cockroach control and have a similar mode of action as fipronil. Lastly, we found that insects resistant to topically administered fipronil were likewise resistant, and to a similar degree, to ingested fipronil.

KEY WORDS Fipronil, insecticide resistance, cross-resistance, cyclodienes, *Blattella germanica*

FIPRONIL (COLLIOT ET AL. 1992) is a relatively new insecticide that is beginning to see widespread use against an array of arthropod pests of agricultural, medical, and veterinary importance. Baits containing fipronil have already, in the 5 yr since their introduction, become popular among consumers and professionals alike for control of domestic cockroaches and ants. The great appeal of fipronil can be attributed, in large part, to its considerable lethality (Kaakeh et al. 1997), but equally attractive is its distinctly greater toxicity to insects than mammals (Gant et al. 1998, Hainzl et al. 1998).

Fipronil kills insects by interacting agonistically with gamma-aminobutyric acid (GABA)-gated chloride channels (Gant et al. 1998), a mode of action that Colliot et al. (1992) called unique. This assertion is, however, tenable if one considers solely contemporary insecticides, for cyclodienes, which were formerly used extensively in insect control, also act on GABA channels (Ghiasuddin and Matsumura 1982, Wafford et al. 1989). This similar mode of action of cyclodienes and fipronil has prompted some to speculate that cockroaches, as well as other insects, may show resistance (cross-resistance) to fipronil without their ancestors ever having been exposed to it (Kaakeh et al. 1997, Scott and Wen 1997, Valles et al. 1997). Such conjecture seems appropriate given that cockroach mitigation was, just after the Second World War, carried out almost exclusively with cyclodienes, mainly chlordane (Cochran 1995). As a result, cockroaches developed remarkably high levels of resistance to these insecticides; the German cockroach, *Blattella germanica* (L.), was first suspected resistant to chlordane as early as 1951 (Grayson 1960a), and investigators, soon thereafter, reported levels of chlordane, aldrin, and dieldrin resistance exceeding two, and sometimes three, orders of magnitude (Fisk and Isert 1953, Clarke and Cochran 1959, Green et al. 1961, Ishii and Sherman 1965, McDonald et al. 1969).

Recent investigations have indeed shown that insects resistant to cyclodienes are more tolerant of fipronil. Scott and Wen (1997), for example, found that a strain of German cockroach selected in the laboratory for high resistance to dieldrin was almost eightfold more tolerant of fipronil than was an insecticide-susceptible strain. Interestingly, this same strain was >553-fold resistant to topically applied JKU-0422,

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another phenylpyrazole (Bloomquist 1994). At any rate, Valles et al. (1997) found that fipronil killed within 24 h a lower percentage of dieldrin-resistant German cockroaches than dieldrin-susceptible ones. And lastly, it was demonstrated that the GABA channels of cyclodiene-resistant insects were less affected by fipronil, and other phenylpyrazoles, than the GABA channels of insecticide-susceptible ones (Bloomquist 1994, Hosie et al. 1995). The mechanism of cross-resistance has, in fact, been well characterized: a single amino acid substitution in the second transmembrane domain of the GABA receptor (ffrench-Constant et al. 1993b, Thompson et al. 1993, Kaku and Matsumura 1994) imparts decreased sensitivity to both fipronil and cyclodienes in resistant insects (ffrench-Constant et al. 1993a, Hosie et al. 1995).

Therefore, it seems incontrovertible that resistance to cyclodienes imparts at least slight cross-resistance to fipronil, but as yet, no practical significance can be attributed to this finding. The prevalence of cyclodiene resistance in feral populations of the German cockroach is unknown and, quite reasonably, might be expected rather low. The universal abandonment of organochlorines in cockroach control has unquestionably resulted in relaxed selection for cyclodiene resistance. What's more, the large expanse of time—nearly 40 yr—since cyclodienes were last uniformly used against cockroaches may very well have been sufficient for resistance to diminish substantially in field populations. Indeed, when German cockroaches resistant to chlordane were reared in the laboratory, unexposed to insecticide for 25 generations, their resistance decreased 30-fold (Grayson 1960b). Nevertheless, despite its attenuation, chlordane resistance was still 12-fold higher in males of the deselected strain than in those of a strain never exposed to the insecticide (Grayson 1960a, b). Therefore, it is conceivable that some feral populations remain resistant to cyclodienes and, because of this, might tolerate fipronil. However, although collections from six Virginia apartments yielded cockroaches some three to 33-fold resistant to chlordane, a strain, 28-fold resistant to the insecticide, showed just 1.7-fold heightened tolerance for fipronil (Bloomquist and Robinson 1999).

Assessing the pervasiveness of resistance to cyclodienes in cockroaches would have heuristic value, but it is far more important to investigate resistance to fipronil, especially since cyclodienes will, in all likelihood, never again be used in cockroach control. Fipronil, by contrast, is relatively new to urban pest control and very likely to remain on the market for many years. Our primary objective in the current study was to examine resistance to fipronil in German cockroach populations before widespread use of the insecticide. The results reported herein provide a baseline against which future levels of resistance can be compared.

Materials and Methods

Insect Rearing and Collecting. German cockroaches of an insecticide-susceptible strain, obtained from American Cyanamid, were reared at 27 ± 0.5°C under a photoperiod of 12:12 (L:D) h. The insects were provided rat chow (no. 5012, Purina Mills, St. Louis, MO) and water ad libitum. Males were separated from the colony just after becoming adults and transferred to small plastic boxes (13.5 cm × 18.5 cm × 9.5 cm high), where they were given rat chow, water, and a cardboard shelter. They were maintained under the same temperature and photoperiod as the colony.

Feral cockroaches were collected in homes, mainly single-family dwellings, in central, southeastern, and northeastern North Carolina. County Agents of the North Carolina Cooperative Extension Service were asked to locate infested homes, which they did with the help of social workers, public health officers, and housing inspectors. Upon visiting a home, we asked its residents how long they had lived there and whether the home had been serviced by a pest management professional while they were living there. Feral cockroaches were ultimately collected in 29 homes in nine counties of North Carolina (Table 1). The majority of the homes were single-family dwellings—stick-built structures, trailers, and mobile homes—but some were apartments situated in buildings comprising no more than four residential units. Most of the homes, while occupied by their current residents, had not been professionally treated, and more importantly, according to the responses of residents to our queries, baits containing fipronil had never been used in any of the homes. This we verified by inspecting homes for bait products.

Cockroaches were collected with a vacuum apparatus similar to one described by Wright (1966). If >50 viable cockroaches were obtained in a single dwelling, they were returned to the lab and reared as a distinct strain. The rearing conditions of field-collected cockroaches, and of their descendants, were similar to those of the laboratory strain. Adult males of all strains were at least 12-d-old, but no >51-d-old, at the time they were used in experiments. In all cases, experimental insects were kept at 27 ± 0.5°C under a photoperiod of 12:12 (L:D) h.

Chemicals. Fipronil [(±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-ρ-tolyl)-4-trifluoromethylsulphinylpyrazole-3-carbonitrile], at 97.1% purity, was obtained from Rhône-Poulenc Ag Company (Research Triangle Park, NC; now Bayer Environmental Science, Montvale, NJ); cypermethrin [cyano(3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate], at 96.0% purity, was provided by S. C. Johnson Wax (Racine, WI); and dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4α,5,6,7,8,8α-octahydro-1,4-*endo*-*exo*-5,8-dimethanonaphthalene), at 98.1% purity, was purchased from Chem Service (West Chester, PA). All stock solutions containing insecticide and all dilutions of them were made with pesticide grade acetone (Fisher, Pittsburgh, PA).

Dose–Response Assays and Detection of Resistance. For each of the three insecticides (fipronil, cypermethrin, and dieldrin), the relationship between insecticide dose and insect mortality was established with adult males of the insecticide-susceptible strain.

Table 1. Strains of cockroaches and where they were collected

Strain	Collection location	Collection date (mo/da/yr)	Dwelling	Treatment history
AE-AI	Burlington, Alamance Co.	11/12/97	H	N
HC-AI	Burlington, Alamance Co.	11/17/97	M	P
Cr-Al	Graham, Alamance Co.	11/12/97	A	P
Gi-Al	Graham, Alamance Co.	11/12/97	A	P
Wa-Al	Graham, Alamance Co.	11/12/97	A	P
Ru-Al	Graham, Alamance Co.	11/17/97	M	N
Hi-Al	Mebane, Alamance Co.	01/29/98	H	N
SG-Ca	Newport, Carteret Co.	02/18/98	M	N
BD-Du	Greenevers, Duplin Co.	08/20/97	M	P
B1-Du	Greenevers, Duplin Co.	08/20/97	M	P
B2-Du	Greenevers, Duplin Co.	08/20/97	M	P
SN-Ha	Scotland Neck, Halifax Co.	07/17/98	H	P
Pl-No	Rich Square, Northampton Co.	07/17/98	M	P
Wa-No	Garysburg, Northampton Co.	07/17/98	M	N
Ch-On	Hubert, Onslow Co.	04/02/98	H	N
SL-On	Hubert, Onslow Co.	04/02/98	M	N
GC-On	Jacksonville, Onslow Co.	02/18/98	M	N
TH-On	Jacksonville, Onslow Co.	02/10/98	H	N
Cr-Or	Chapel Hill, Orange Co.	11/18/97	A	N
Go-Or	Chapel Hill, Orange Co.	01/26/98	A	N
Jo-Or	Chapel Hill, Orange Co.	01/26/98	A	N
Sy-Or	Chapel Hill, Orange Co.	11/18/97	A	P
La-Or	Hillsborough, Orange Co.	01/26/98	A	N
Lw-Or	Hillsborough, Orange Co.	11/18/97	H	N
MC-Or	Mebane, Orange Co.	11/18/97	M	N
BL-Ra	Asheboro, Randolph Co.	11/25/97	M	N
HC-Ra	Asheboro, Randolph Co.	11/25/97	M	N
HI-Ra	Randleman, Randoph Co.	11/25/97	M	N
Ea-Wa	Raleigh, Wake Co.	11/22/97	H	N

Cockroaches, of all ages, were collected in mobile homes or trailers (M), apartments in buildings comprising no more than four residential units (A), or single family, stick-built structures (H). During the time in which the current residents had lived in a home, it either had been treated by a pest management professional (P) or had not (N).

Males were briefly anesthetized with CO_2 and treated on the ventral thorax, between the coxae, with 1-μl acetone containing either no insecticide or one of 10 concentrations of it. Each dose was administered to three sets of 10 males, which after being treated were placed in 150 $\times$ 25 mm plastic petri dishes and given food and water. The males were inspected for mortality 72 h later. Moribund insects, as defined by Clarke and Cochran (1959), were considered dead in these and all other assays.

The concentrations of insecticides used in dose–response assays were selected in such a way that most caused >60% of insects to die. The objective of this was to increase the precision of the LC_{99} estimates that were obtained in logit models (Robertson et al. 1984). Furthermore, the LC_{99} of fipronil was determined empirically. One thousand males of the insecticide-susceptible strain were treated with the logit model's estimated LC_{99}, an additional thousand were treated with a dose equivalent to the upper confidence limit of the estimated LC_{99}, and a final thousand were treated with a dose halfway between the estimated LC_{99} and its upper confidence limit. After being dosed with insecticide, all males were placed by the hundred in small plastic boxes, each containing a single cardboard shelter. The insects were given food and water and inspected for mortality 72 h later. The dose that killed most closely 99% of insects was considered the LC_{99}.

Resistance to topically administered fipronil was detected using a discriminating-dose technique (Roush and Miller 1986, Cochran 1995). One hundred adult males from each of 20 field strains were treated on the ventral thorax with the empirically determined LC_{99}. After 72 h, dead insects were counted, and if significantly fewer than 99 of a strain had died, separate sets of 100 males from the same strain were dosed with three- and ten-fold greater fipronil than the LC_{99}. Insects were, once again, inspected for mortality 72 h later.

Diets and Assays Used to Detect Resistance to Ingested Fipronil. Six diets were prepared containing rat chow and either no fipronil or five different concentrations of it. Rat chow biscuits were ground in a blender to a powder, which was sifted through a 710 μm sieve to remove large particles. A gram of ground rat chow was then dispensed into each of six scintillation vials, which also received 750 μl acetone containing 0, 1, 2.5, 5, 10, or 25 μg fipronil. The acetone was eliminated from the rat chow by placing the vials under a vacuum. During this procedure, the acetone-rat chow slurry was intermittently stirred with a spatula, and when the solvent had completely evaporated, the mixture was vortexed. The resulting diets were composed of $\approx$0, 0.0001, 0.00025, 0.0005, 0.001, and 0.0025% fipronil by weight.

The relative toxicity of the six diets was ascertained by feeding each to three sets of 12 adult males of the

Table 2. Results of logit analyses on the relationship between insecticide dose and insect mortality

Insecticide	*n*	Model parameters[a]		Lethal concentrations[b]		Model fit		
		Intercept ± SE	Slope ± SE	LC_{50} (95% CI)	LC_{99} (95% CI)	χ^2	df	*P*
Fipronil	300	58.39 ± 6.75	9.40 ± 1.10	2.0 (1.9–2.1)	3.3 (3.0–3.6)	6.21	8	0.48
Dieldrin	300	20.00 ± 2.21	7.81 ± 0.90	77.3 (73.4–81.4)	139.3 (124.2–156.1)	4.25	8	0.83
Cypermethrin	300	13.52 ± 1.56	4.26 ± 0.53	41.8 (38.4–45.6)	123.0 (98.0–154.3)	11.12	8	0.20

Adult males of an insecticide-susceptible strain were treated with fipronil, dieldrin, or cypermethrin and examined for mortality 72 h later.
[a] The intercept and slope parameters are for models in which the independent variable is natural logarithm of dose.
[b] Lethal concentrations are expressed in ng/insect.

insecticide-susceptible strain. Males were placed by the dozen in 150 × 25 mm plastic petri dishes without food but with water for 24 h. Then, at the onset of the next scotophase, they were given one of the experimental diets and examined for mortality 2.5 h later and subsequently at 0.5–5 h intervals through 72 h. The experimental diets were removed 6 h after they were initially provided and replaced with biscuits of rat chow.

Resistance to ingested fipronil was examined using a similar protocol, but only a single insecticide concentration, 0.0005%, was used. This concentration was selected because it was the lowest to kill almost all insecticide-susceptible males within 72 h. Four sets of 12 males of a strain were fed this diet for 6 h, and the amount they consumed in this time was measured. The males were inspected for mortality at 0.5–6 h intervals for 72 h.

Assay for Cross Resistance. Cross-resistance was assessed by treating 50 adult males of a strain with the LC_{99} of fipronil, 50 more with twice the LC_{99} of dieldrin, and an additional 50 with 25-fold the LC_{99} of cypermethrin. These concentrations were selected because they caused, in preliminary experiments, wide-ranging mortality in males of various field-collected strains. After being treated, males were collectively weighed, placed in groups of 25 in 150 × 25 mm petri dishes, given food and water, and inspected for mortality 72 h later.

Statistical Analysis of Data. Dose-mortality relationships were analyzed with logistic regression (logit analysis). Natural logarithm of dose was the independent variable in all analyses, and the parameters of all logit models were estimated with PROC LOGISTIC in SAS 6.12 for the personal computer (SAS Institute 1990, 1996). Regression coefficients and their variances and covariances were used to calculate the doses expected to kill 50 and 99% of cockroaches as well as the 95% CL of these doses (Collett 1991). None of the cockroaches that were treated solely with acetone died, so Abbott's formula was never invoked to adjust for control mortality.

The relationship between the percentage of insects that died after being treated with fipronil and weight of the insects was analyzed using Spearman rank correlation (Zar 1996). Males of different strains were weighed in groups of 100 after they had been treated with the LC_{99}, 3X LC_{99}, or 10X LC_{99} of fipronil. At each of the three doses, the weights were ranked, as were the percentages of males that died. A Z-test was then used to determine whether the ranks were correlated.

Death rates of insects fed fipronil were quantified and compared using a proportional hazards model (Cox regression model). Each of the coefficients in the model represented a different field strain and explained the effect of an insect's strain on its survival. Hazard ratios were obtained by exponentiating the coefficients, a hazard ratio essentially being the instantaneous death rate of a field-strain male divided by that of an insecticide-susceptible male. All model parameters were estimated with PROC PHREG in SAS (SAS Institute 1996).

Factors influencing the percentage of a strain's males that died after being treated with fipronil were identified using multiple regression, which was carried out with PROC REG in SAS (SAS Institute 1990). The model included four predictors: the total weight of the males that had been treated with fipronil, the percentage of a different set of males of the same strain that died after being treated with dieldrin, the percentage of another set of males that died after being treated with cypermethrin, and the history of insecticide use in the home in which the strain was collected. This last variable was categorical and coded '0' if a home had not been serviced by a professional and '1' if it had.

A Z-test with a correction for continuity, as described by Roush and Miller (1986), was used to determine whether the percentage of insects killed with fipronil differed from, or was less than, 50 or 99%. The decision criterion for rejecting null hypotheses was $P = 0.05$. Standard error of the mean is given with all means.

Results

Determining the LC_{99} of Fipronil. The protocol we selected for detecting resistance called for treating adult males with the LC_{99} of fipronil. An estimate of this value was therefore needed and was obtained by analyzing the relationship between insect mortality and fipronil dose with adult males of the insecticide-susceptible strain. Ten concentrations of fipronil, ranging from 1.5 to 3.2 ng per insect, were administered topically to males, and killed between 3 and 97% of them in 72 h. A logit model (Table 2) was fit to the data and predicted the LC_{99} to be 3.3 ng per insect. The 95% confidence interval of this estimate, 3.0–3.6

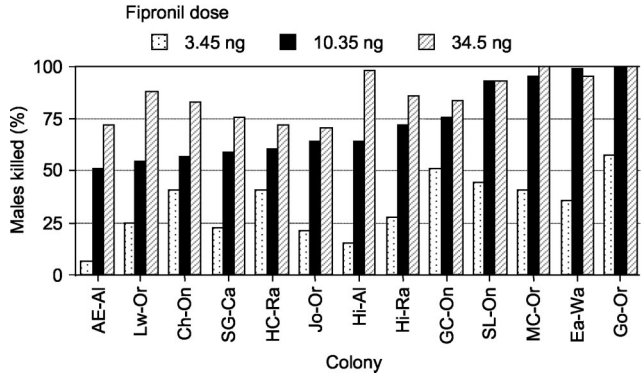

Fig. 1. Mortality of adult males treated topically with fipronil. The 13 shown strains were collected in homes that had not been serviced by a pest management professional while occupied by the current residents. Each bar shows the number of males out of 100, or percentage, killed in 72 h by one of three doses of fipronil.

ng, was narrow probably because eight of the ten insecticide concentrations killed >63% of males.

We tested empirically whether the estimated LC_{99} actually killed 99%. Ten sets of 100 males were topically dosed with 3.3 ng of fipronil and examined for mortality 72 h later. Just 98.3 ± 0.56% of them died, significantly <99% ($Z = 2.07$, $P = 0.019$). This indicated that the logit model slightly underestimated the LC_{99}, so 1,000 more males, in ten sets of 100, were treated with 3.45 ng of fipronil, and an additional 1,000 with 3.6 ng. The higher of these doses killed 99.7 ± 0.15% of males, significantly >99% ($Z = -2.38$, $P = 0.009$), whereas the lower dose killed 99.2 ± 0.33%, a value that did not differ from 99% ($Z = -0.79$, $P = 0.21$). Consequently, 3.45 ng of fipronil per insect was deemed a reasonable estimate of the LC_{99} and used in screening field strains for resistance.

Screening for Resistance. The primary objective of our investigation was to determine whether feral cockroaches were resistant to fipronil. We initially examined cockroaches whose ancestors had been collected in homes that had not been serviced by a professional while occupied by the current residents. Adult males of 13 of the 19 strains collected in this type of setting were topically dosed with 3.45 ng of fipronil, and this amount killed significantly fewer than 99 of 100 males in all 13 strains ($Z \leq 41.71$, $P < 0.001$). The highest percentage killed was 57, whereas the lowest was six (Fig. 1). All the strains, therefore, exhibited a degree of resistance in relation to the insecticide-susceptible strain. To assess the amplitude of resistance, we treated 100 males of each strain with 10.35 ng fipronil, threefold the LC_{99}, and another 100 with 34.5 ng fipronil, 10-fold the LC_{99}. These higher doses caused greater mortality, with the 3X LC_{99} killing 50–99% of males and the 10X LC_{99} killing 70–100% (Fig. 1). Nevertheless, a substantial number of males of many strains, at least 13% from eight and 25% from four, survived the highest dose.

Resistance to fipronil was also screened for in seven of the 11 strains collected in homes that had been professionally treated (Table 1), though not with fipronil. The results (Fig. 2) were similar to those in Fig. 1. The LC_{99} of fipronil killed 4–66% of males, but always <99% ($Z \leq 32.66$, $P < 0.001$). The higher doses of fipronil, 10.35 ng and 34.5 ng, killed up to 98% of males, but at least 18% of males of three strains survived the 10X LC_{99}. How tolerant a strain's males were of fipronil did not appear to be influenced by whether the strain's domicile had been professionally treated. Whereas the LC_{99}, 3X LC_{99}, and 10X LC_{99} killed on average 32.4 ± 4.2, 71.8 ± 5.0, and 85.1 ± 3.0% of the males in the 13 strains in Fig. 1, the same doses killed 38.4 ± 7.7, 80.7 ± 8.4, and 83.0 ± 8.3% of the males in the seven strains in Fig. 2. There was no difference between these percentages at any dose (LC_{99}: $t = -0.61$, df = 18, $P = 0.55$; 3X LC_{99}: $t = -1.06$, df = 18, $P = 0.30$; 10X LC_{99}: $t = 0.12$, df = 18, $P = 0.90$).

Of the 20 screened strains, the most resistant was Cr-Al, which had been collected in public housing. Significantly <50% of males of this strain died after being treated with either 10.35 ng ($Z = 2.10$, $P = 0.002$) or 34.5 ng ($Z = 2.50$, $P = 0.006$) of fipronil. The higher of these doses, by contrast, killed >50% of males in all other tested strains ($Z \geq -4.10$, $P < 0.001$), whereas the lower dose killed no different than 50% in three of the strains ($-0.10 \geq Z \geq -1.30$, $0.46 \leq P \leq 0.097$).

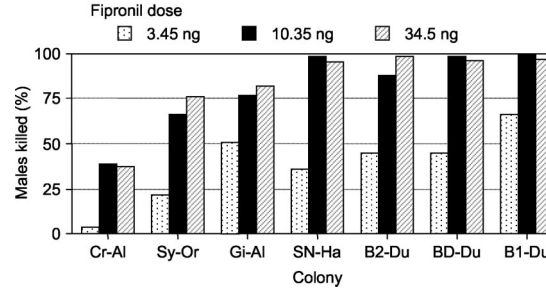

Fig. 2. Mortality of adult males 72 h after being treated topically with fipronil. The seven shown strains were from homes that had been professionally serviced while occupied by the current residents. Each bar shows the number of males out of 100, or percentage, killed by one of three doses of fipronil.

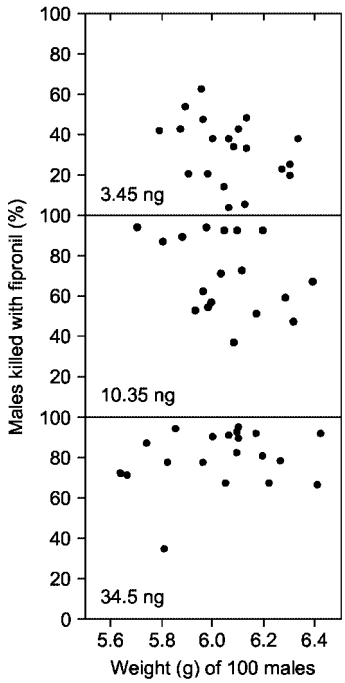

Fig. 3. Relationship between mortality after treatment with fipronil and insect weight. All groups of 100 males in Figs. 1 and 2 were weighed after they were treated with fipronil. The percentage of a group's males that died is plotted against their collective weight. A data point within a graph represents one of the 20 strains in Figs. 1 and 2, and all strains are represented in each graph.

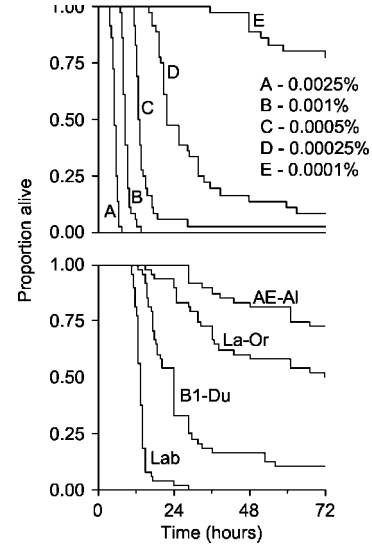

Fig. 4. Mortality of adult males that had ingested fipronil. Each of five concentrations of fipronil (0.0001%, 0.00025%, 0.0005%, 0.001%, and 0.0025%) in ground rat chow was fed for 6 h to 36 adult males of the insecticide-susceptible strain. The decline over time in the proportion of males remaining alive is shown in the 72 h after males received one of the fipronil-laced diets (top graph). The curves are step functions that remain at a plateau between deaths and decrease at the time of a death or deaths (Parmar and Machin 1995). The lower graph shows the survival over time of males of four different strains, 48 males per strain, given a diet containing 0.0005% fipronil.

We addressed the effect of insect weight on fipronil tolerance by determining whether weight and mortality were correlated at the three fipronil concentrations across all 20 strains in Figs. 1 and 2 (Fig. 3). Weight was not related to mortality at the LC_{99} (Spearman rank correlation: $Z = -1.53$, $P = 0.13$), 3X LC_{99} ($Z = -1.36$, $P = 0.17$), and 10X LC_{99} ($Z = 0.809$, $P = 0.42$). Moreover, the relationships were still not significant when the 13 strains in Fig. 1 and seven in Fig. 2 were analyzed separately (results not shown).

Resistance to Ingested Fipronil. Because fipronil is currently available only in bait formulations for use against cockroaches, we screened strains for resistance to ingested fipronil. We first identified the lowest concentration of fipronil in the diet that would kill almost all insecticide-susceptible males after a single bout of feeding. Adult males were starved for 24 h, and then given for 6-h ground rat chow containing varying amounts of fipronil. No males fed a control diet lacking fipronil died, but all did so within 14 h when fed high concentrations (0.0025% and 0.001%) of fipronil (Fig. 4, upper graph, A and B). Ten-fold lower concentrations (0.00025% and 0.0001%), by contrast, killed no >93% of males after 72 h (Fig. 4, upper graph, D and E). Rat chow with 0.0005% fipronil (Fig. 4, upper graph, C), however, killed over 90% of insects in 18 h and 35 of 36 in 28.5 h. This dose was consequently judged ideal for screening field-collected strains for resistance.

Three strains (B1-Du, Lw-Or, and AE-Al), with distinctly different tolerances for topically administered fipronil (Figs. 1 and 2), were examined for resistance to ingested fipronil. Males of the three strains, and of the insecticide-susceptible strain, were fed rat chow containing 0.0005% fipronil and observed periodically for death over the next 72 h (Fig. 4, lower graph). More than half the insecticide-susceptible males died within 13.5 h, while the rest did so in the next 15 h, in contrast to AE-Al and Lw-Or males, at least half of which survived for 72 h. Males of the B1-Du strain died more quickly; only 50% survived for 24 h, and just five of the initial 48 were still alive after 72 h.

Death rates of fipronil-fed cockroaches were quantified by fitting the time-mortality data (Fig. 4, lower graph) to a Cox regression model, which with three coefficients, one for each of the field strains, described better the relationship between mortality and time than did a null model with no coefficients (likelihood ratio statistic, $\chi^2 = 202.04$, df = 1, $P < 0.001$). The hazard ratios (exponentiated regression coefficients) corresponding to the three strains, and their 95% confidence intervals, were as follows: B1-Du, 0.107 (0.065–0.178); Lw-Or, 0.026 (0.014–0.48); and AE-Al, 0.012 (0.006–0.024). These values showed that males of the B1-Du, Lw-Or, and AE-Al strains died at ≈10.7, 2.6,

Table 3. The effects of various factors on the percentage of a strain's males that died after being treated with fipronil

Variable	Estimate	SE	*t*	*P*
Percentages not transformed				
Intercept	66.279	68.320	0.970	0.34
Male weight	−18.006	22.363	−0.805	0.43
Cypermethrin mortality	−0.003	0.095	−0.037	0.97
Dieldrin mortality	0.682	0.111	6.159	<0.001
Treatment history of home	−1.392	4.591	−0.303	0.76
Percentages transformed[a]				
Intercept	46.347	43.612	1.063	0.30
Male weight	−10.520	14.151	−0.743	0.47
Cypermethrin mortality	−0.071	0.084	−0.845	0.41
Dieldrin mortality	0.708	0.114	6.197	<0.001
Treatment history of home	−1.691	2.842	−0.595	0.56

[a] Percentages of males that died after being treated with cypermethrin, dieldrin, and fipronil were arcsine square root transformed.

and 1.2% the rate of males of the insecticide-susceptible strain. The confidence intervals of none of the hazard ratios encompassed the value 1, so the males of the three strains died more slowly than did insecticide-susceptible males.

In the foregoing experiment, males of different strains ingested unequal amounts of fipronil-laden rat chow in the 6 h it was provided, and this might have accounted, at least in part, for the measured differences in their rates of death. Indeed, insecticide-susceptible males, which died fastest, consumed 47.3 ± 3.09 mg rat chow (n = 4 sets of 12 males), whereas B1-Du males, which died more gradually, consumed just 41.5 ± 2.72 mg. Nevertheless, males of the AE-Al strain consumed more rat chow (48.8 ± 1.65 mg) than those of any other strain yet died the slowest of all.

Cross Resistance. Fipronil could, in no way, have been directly responsible for the resistance we detected because the ancestors of all males we examined had never been exposed to it. Therefore, we determined, using multiple regression, whether any of four other factors had an effect on fipronil resistance. The regression model consisted of a single dependent variable—the percentage of a strain's males that died after being treated with 3.45 ng of fipronil—and four independent variables: the weight of the males that had been treated with fipronil, the percentage of different males of the same strain that died after being treated with either 278 ng of dieldrin or 3.07 μg of cypermethrin (two separate variables), and the treatment history of the home in which the strain was collected (professionally treated or not). The doses of dieldrin and cypermethrin were twofold and 25-fold their respective LC_{99} values estimated in logit models with insecticide-susceptible males (Table 2).

All variables were measured in each of 27 strains, and a main-effects regression model was significant (F = 12.15; df = 4, 22; $P < 0.001$) with a large coefficient of multiple determination, R^2 = 0.69. Nevertheless, the only variable having a significant effect on the mortality of males treated with fipronil was the mortality of males of the same strain treated with dieldrin (Table 3). This variable was therefore included as the sole independent variable in a new model (Fig. 5, top graph), which remained significant (F = 50.86; df = 1, 25; $P < 0.001$) with a large coefficient of determination, R^2 = 0.67. In comparison, whether or not males of a strain were tolerant of cypermethrin had little effect on their ability to withstand a dose of fipronil (Fig. 5, bottom graph).

Because it is common to normalize percentages before performing regression analysis, an additional main-effects model was constructed in which all percentages were arcsine square root transformed. There was, however, no change in results. The model was, again, significant (F = 11.10; df = 4, 22; $P < 0.001$) with

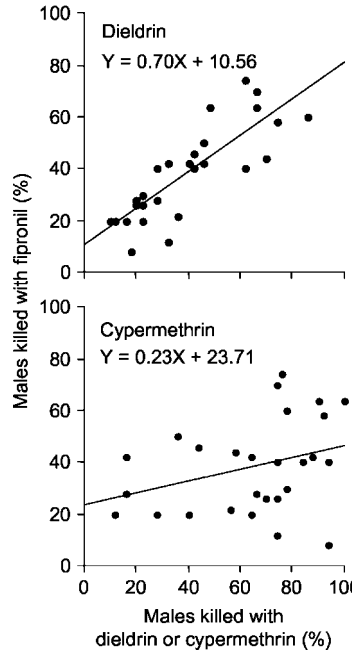

Fig. 5. Relationship between mortality after treatment with fipronil and mortality after treatment with dieldrin or cypermethrin. The percentage of 50 males of a strain that died after being treated with 3.45 ng of fipronil is plotted against the percentage of 50 different males of the same strain that died after being treated with 278 ng of dieldrin (top graph) or 3.07 μg of cypermethrin (bottom graph). All males were examined for mortality 72 h after they were treated. The data are from 27 different strains, all collected in homes in which fipronil had never been used.

an R^2 of 0.67, and the mortality of males treated with dieldrin was, yet again, the only variable of any value in predicting the percentage of a strain's males that would die after being dosed with fipronil (Table 3).

Discussion

Cause of Resistance to Fipronil. Resistance to the cyclodienes first appeared in scattered populations of the German cockroach in the early 1950s and soon thereafter became ubiquitous, reaching its zenith late in the same decade (Grayson 1966, Cochran 1995). Subsequently, these compounds, because of their escalating inefficacy, were almost wholly abandoned in cockroach control and largely superseded by other organic insecticides, chiefly organophosphates (Grayson 1966, Cochran 1995). Thenceforth, little effort was made to survey feral cockroach populations for cyclodiene resistance.

We can now say that resistance to the cyclodienes has endured to the present, although it has certainly lapsed. A dose of dieldrin, twice the LC_{99} and roughly 4X the LC_{50} of the susceptible strain, killed just 10% of adult males from one field-collected strain and 12, 16, and 18% from three others (Fig. 5). It ought be realized, though, that this dose—and for that matter, much higher ones—probably would have killed no males of most strains when resistance was rampant. Nevertheless, the fact that some resistance has persisted is quite remarkable given that the use of cyclodienes against cockroaches fell off dramatically nearly 40 yr ago. We suspect that resistance has diminished only gradually owing to small differences in the fitness of resistant and susceptible individuals. However, resistance may also have been sustained, in part, by the once standard practice of using chlordane around homes to control termites. Indeed, because of chlordane's long residual life, it is quite plausible that cockroaches are still being exposed to it. In this regard, detectable residues of chlordane can still be recovered from people (Whyatt et al. 2002), though the insecticide's use around homes was terminated more than a decade ago.

The strong, positive linear relationship we detected between the percentage of a strain's males that died after being treated with fipronil and the percentage that died after being treated with dieldrin is convincing evidence that the past use of cyclodienes has given rise to present fipronil resistance. But it can be argued that the relationship between the two variables is spurious and that the susceptibility of a strain's males to fipronil is determined by a variable other than, yet still correlated with, the susceptibility of the strain's males to dieldrin. Indeed, the prior use of chlordane, the major cyclodiene used in cockroach control (Cochran 1995), probably accounts for most current resistance to both dieldrin and fipronil. Nevertheless, the central issue is not whether resistance to fipronil is owing to a particular cyclodiene, but to cyclodienes in general. And in our study, we selected dieldrin to represent all compounds of this type, though chlordane or aldrin would have equally sufficed. In any case, it seems reasonable that resistance to fipronil is in reality cross-resistance to the cyclodienes because fipronil and the cyclodienes have a similar mode of action (Wafford et al. 1989, Cole et al. 1993) and because the GABA receptors of cyclodiene-resistant insects show decreased sensitivity to fipronil and related phenylpyrazoles (Bloomquist 1994, Hosie et al. 1995).

We attempted, without success, to identify other factors affecting the susceptibility of males to fipronil. Cockroaches from professionally treated homes were no more tolerant of fipronil than were those from homes that had been treated only by their occupants—that is, if they had been treated at all (compare Fig. 1 and 2; Table 3). And unexpectedly, some of the most resistant cockroaches we discovered were from strains that originated in rural dwellings that had seen scant insecticide use (SG-Ca and HI-Ra in Fig. 1). So it is apparent that neither a home's treatment history nor its location, are of any value in predicting the fipronil tolerance of its indwelling cockroaches. Cypermethrin mortality (tolerance) was another factor with a negligible effect on fipronil mortality. This finding, though negative, was informative because it showed that resistance to fipronil was not the result of a general resistance phenomenon, for example, decreased permeability of the cuticle to insecticide. Lastly, we found, through both rank correlation (Fig. 4) and linear regression analyses (Table 3), that the size of males and their susceptibility to fipronil were unrelated. On the whole, it should perhaps come as no surprise that none of these factors were useful predictors, because how tolerant males were of dieldrin accounted for so much of the variation (67%) in their susceptibility to fipronil.

Fipronil in Baits. Our results show that feral cockroaches are resistant to ingested fipronil (Fig. 4), but it cannot be said whether this resistance is currently affecting fipronil's efficacy or, for that matter, whether resistance is expanding in cockroach populations. These issues should certainly be examined in a future investigation, but nevertheless, a persuasive case can be made that fipronil is now entirely effective and likely to remain so, as long as its use continues to be restricted to baits. It is widely held that adult German cockroaches consume at least 1 mg of food in a single meal (Reierson 1995), a view supported by our own preliminary investigations. Because bait formulations contain, at minimum, 0.01% fipronil (Maxforce FC gel, Bayer Environmental Science, Montvale, NJ), a cockroach that has consumed 1 mg of bait will be exposed to 100 ng of fipronil. This amount is roughly threefold the quantity that—when topically administered—killed almost all males of most field-collected strains (Figs. 1 and 2). It seems, therefore, that the concentration of fipronil in bait is more than adequate to kill even the most tolerant of individuals, and for this reason, resistance to fipronil may not increase in cockroach populations.

However compelling the preceding reasoning, there is ample reason to suggest it specious. Cockroaches, in fact, are given considerable opportunity to

ingest or be exposed to concentrations of fipronil lower than those found in baits. First, fipronil is often excreted in a dilute form by moribund cockroaches. These exudates are, in turn, readily ingested by other cockroaches, primarily by small nymphs (Buczkowski and Schal 2001), which are expected to suffer extensive secondary mortality (Buczkowski et al. 2001). In any event, translocated baits containing partially metabolized fipronil may serve as a powerful selective pressure for resistance development. Second, fipronil's recent popularity in ant and termite control products may contribute to development of resistance in cockroaches. Baits formulated for ants contain substantially less fipronil, as little as 0.001%, than baits for cockroaches. Cockroaches with a degree of tolerance for fipronil may disproportionately survive following consumption of these baits and in this manner frequency of resistance will increase in a population. Third, cockroaches may also become exposed to fipronil that has been used to treat for termites. Insecticide residues are expected on surfaces over which cockroaches walk, and the heterogeneous nature of the residues may, once again, select for survival of tolerant individuals. If the ambient concentration of fipronil is at a level that allows resistant individuals, but not susceptible ones, to survive, the frequency of resistance will increase in a population (Georghiou and Taylor 1986, Denholm and Rowland 1992).

Resistance to fipronil may be slow to increase, or may not increase at all, under current conditions, but overzealous optimism is nonetheless unwise because active ingredients in baits have, in the past, failed on account of resistance. Schal (1992), for instance, found that a significant number of cockroaches from several field-collected strains survived for many weeks when given, as their sole source of food, a sulfluramid-laden bait. The resistance of these insects was, however, probably not attributable to sulfluramid itself because many of the tested strains were collected before the insecticide had been first used in cockroach control. Schal (1992) hypothesized that the resistance sprang from the prior, prolonged exposure of cockroaches to household cleaning agents, which often contain compounds chemically similar to sulfluramid. Regardless, it is evident that cross-resistance can rob an insecticide of its efficacy in baits, which we speculate might have happened with fipronil had it been introduced many years ago when resistance to cyclodienes abounded.

The Future of Fipronil in Cockroach Control. Fipronil is currently available only in bait formulations for use against cockroaches, but it—or some other phenylpyrazole—could someday be deployed in spray formulations as well. As such, fipronil has shown itself to be highly effective against a variety of arthropods (Postal et al. 1995, Sulaiman et al. 1997, Davey et al. 1998, Lecoq and Balança 1998) and would probably be equally successful, at least initially, in controlling cockroaches. It is important to realize, though, that most residual insecticides currently or formerly used to control cockroaches have, in the end, impeded their own efficacy by bringing about resistance (Cochran 1995). We suspect the same would happen, in due time, with fipronil, so the issue of interest is not if, but how soon, fipronil would lose its efficacy after its first use as a residual insecticide.

Another issue of considerable import is whether the mechanism underlying cyclodiene resistance—GABA receptor insensitivity (ffrench-Constant et al. 1993a, Bloomquist 1993)—can on its own undermine the ability of fipronil to control cockroaches. If it can, resistance to fipronil may soon become a practical problem. Nevertheless, an earlier investigation showed that a strain of German cockroach with an astonishingly high resistance ratio to dieldrin of ≈17,000 was just 7.7-fold resistant to fipronil (Scott and Wen 1997). The implication of this finding is that resistance to cyclodienes does not yield high-level resistance to fipronil and thus probably does not diminish fipronil's utility as a cockroach control agent. However, we have now identified a strain (Cr-Al), whose resistance ratio to fipronil surpasses 17X: a dose of fipronil ≈17 times the LC_{50} of the susceptible strain killed significantly fewer than 50% of Cr-Al males. Two reasons, one or both possible, may account for this strain's high level of resistance: (1) the mechanism of cyclodiene resistance can, in fact, increase by >7.7-fold an insect's tolerance of fipronil or (2) one or more other mechanisms impart resistance exceeding 7.7-fold. Regardless, further investigation on the Cr-Al strain is certainly merited, especially since resistance ratios >10 have been associated with the failure of the residual insecticide chlorpyrifos (Rust et al. 1993).

In our opinion, the current moderate levels of resistance to fipronil in the German cockroach militate against its use as a residual insecticide, for the historical weight of evidence clearly indicates that cockroaches can become highly resistant to most insecticides used in this manner (Cochran 1995). It is indeed imaginable that German cockroaches could ultimately become as resistant to residual fipronil as they once were to the cyclodienes. If this were to occur, fipronil would almost certainly become ineffectual in either spray or bait formulations. It would be particularly troubling for fipronil to lose its efficacy in baits, for consumers and pest management professionals alike are increasingly turning to them in their efforts to control cockroaches. This trend is unlikely to let up, largely for regulatory reasons.

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