Comparisons Between the Response of German Cockroach Field-Collected Strains (Dictyoptera: Blattellidae) to Vapors and Contact with a Cyfluthrin Formulation[1]

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ABSTRACT Research on differences in insecticide-induced behavior of German cockroach field-collected strains was continued. Late instar nymphs (5th-6th stadia) were drawn from the Fairbanks, an insecticide susceptible strain, and two pyrethroid resistant strains, the Jacksonville and Forest Green. Dispersal induced by vapors of a cyfluthrin flowable concentrate (FC) and the FC formulation base (blank) was compared with response to the FC and the FC blank when dry. Jacksonville nymphs avoided the dried FC, but not as strongly as Fairbanks strain nymphs. The AI played a major role in eliciting avoidance by Fairbanks strain nymphs but, in the Jacksonville strain, avoidance was due more to an ingredient(s) of the formulation base. Forest Green nymphs did not avoid the dried FC or the FC blank. Vapors of the FC and the FC blank caused rapid dispersal of all strains, but dispersal of resistant strain nymphs was slower than that of susceptible strain nymphs. Although Jacksonville nymphs responded more strongly to the dried formulation than Forest Green nymphs, the response to vapors was weaker than that of Forest Green nymphs. It is suggested that localized populations of the German cockroach have developed many different combinations of behavioral modifications and physiological/biochemical resistance.

KEY WORDS German cockroach, behavior, pyrethroid, cyfluthrin, *Blattella germanica.*

Relatively little attention has been paid to behavioral modifications of insect pests, although it has been emphasized that behavior, as well as physiological-biochemical resistance, can affect the ability of an insect to survive in a toxic environment (Lockwood et al. 1984, Pluthero and Singh 1984, Sparks et al., 1989). The discovery of a strain difference in the response of the German cockroach, *Blattella germanica,* to vapors of a propoxur formulation suggested that *B. germanica* is one of the insects in which behavior has been modified in ways that affect the efficacy of insecticide treatments (Bret and Ross 1985). Comparisons between additional field-collected strains that were exposed to vapors of several different propoxur formulations confirmed that German cockroach populations have undergone behavioral modifications (Wooster and Ross 1989).

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Pyrethroids are used widely for cockroach control. Therefore, continued investigation of strain differences in insecticide-induced behavior included experiments on dispersal induced by vapors of several commercial pyrethroids (Ross 1992). The strongest dispersal response occurred when late instar nymphs of several field-collected strains were exposed to vapors of a cyfluthrin flowable concentrate (FC). Another study revealed that strain differences also occur in response to dried formulations (Ross and Cochran 1992).

Continued research on strain differences in insecticide-induced behavior include comparisons of the response of several field-collected strains to vapors and residual deposits of a cyfluthrin FC and the FC blank (base without the AI). The results are reported here.

Materials and Methods

The experiments were conducted in a laboratory maintained at 24 - 27°C, ambient humidity, and photoperiod of 10:14 (L:D). They were begun at 9:00 a.m. and concluded at times that varied with the particular experiment.

Late instar nymphs were drawn from the Fairbanks strain, an insecticide susceptible strain collected in Alaska in 1985, and two pyrethroid resistant strains, the Jacksonville and Forest Green strains, collected in Florida in 1988 and 1989, respectively. Resistance profiles are estimates of LT_{50}s based on probit analysis (Cochran, pers. comm.; Ross and Cochran, 1992). Both strains are highly resistant (>100X) to pyrethrins, allethrin, permethrin, and phenothrin. The Forest Green strain is also resistant to fenvalerate (42X), cypermethrin (>60X) and cyfluthrin (14.5X). The Jacksonville is less resistant than the Forest Green strain to cyfluthrin, fenvalerate, and cypermethrin (2.3X, 7.2X, and 3.0X, respectively).

Groups of 10 late instar nymphs from each strain under study were exposed to vapors and dried deposits (residues) of a cyfluthrin FC obtained from Mobay, Kansas City, Mo. (AI 1%). The response to vapors was tested in an apparatus described by Wooster et al. (1990). Cockroaches were released in one part (aggregation dish) of the apparatus and allowed to settle on sections of screen wire that served as harborage. Vapors were introduced by a filter paper collar placed around the top of the aggregation dish after impregnation with 0.3 ml of the test material. Controls used water treated papers. Cockroaches used for controls were from the strain used in the particular experiment. After placement of the collar, the aggregation dish was covered with a glass plate and a plug preventing access to a second dish, the dispersal dish, was removed. The number of cockroaches in the dispersal dish was recorded photographically at 5 min intervals for 40 min.

Contact repellency tests used squares of filter papers (4 × 7 cm) that were treated with 0.35 ml of either the FC or the FC blank. After drying thoroughly under a hood, the papers were folded in four and placed in the center of a glass battery jar (diam 15 cm). Ten late instar nymphs were released in the jar and their distribution (on or off papers) recorded at 30 min intervals for the first hour, and subsequently at hourly intervals for the next 5 hours. Controls used Fairbanks strain nymphs.

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Each experiment was replicated 6X. After each experiment, cockroaches were placed in clean containers for 48 h and checked for mortality.

Analysis of covariance with Tukey's studentized range test ($P = 0.05$) (SAS Institute 1985) was used to test for significant differences between numbers of cockroaches on treated papers in the contact repellency experiments. In the dispersal experiment, Abbott's (1925) formula was used to adjust each replicate for dispersal in the controls. This assured that strain differences in behavior were those induced by the insecticide exposure. However, cockroaches in the controls, regardless of strain, rarely left the screen wire harborage on which they settled prior to placing a water-treated collar around the aggregation dish (mean dispersal of controls <2%). The time required for 50% dispersal (DT_{50}) of each replicate was estimated from probit analysis (SAS Institute 1985). The data were analyzed by analysis of variance (ANOVA) and means compared using Duncan's multiple range test (SAS Institute 1985).

Results

Nymphs of the Fairbanks strain (insecticide susceptible) were strongly repelled by the dried cyfluthrin FC (Table 1). Jacksonville cockroaches also avoided papers treated with the FC, but the percentage on treated papers was significantly higher than in the experiment with Fairbanks strain nymphs. Forest Green strain nymphs were not repelled. The percentage of Forest Green nymphs on FC treated papers did not differ significantly from the control data. None of the cockroaches died in either the experiments on contact repellency or vapor-induced dispersal.

In experiments on contact with the dried FC blank, Fairbanks strain nymphs avoided the treated paper, but the response was weaker than in the experiment with the complete formulation (Table 1). The strongest response to the blank was in the experiment with Jacksonville nymphs rather than with the susceptible strain. Nevertheless, the percentage of Jacksonville nymphs on the FC treated paper, like that of Fairbanks nymphs, was significantly less than on the paper treated with the FC blank. The data also suggest that the FC blank was slightly repellent to Forest Green nymphs, but the mean percentage on the treated papers did not differ significantly from the control.

A small experiment was undertaken to test the possibility that locomotory behavior of Jacksonville nymphs in the absence of an insecticide did not influence the results of the contact repellency tests (Table 1). Initially the nymphs moved on and off papers more frequently than Fairbanks strain nymphs, but within 2 - 2½ h > 90% settled on the untreated papers, similarly to Fairbanks strain nymphs (4 replicates, $n = 40$).

In the experiments on vapor-induced dispersal, Fairbanks strain nymphs dispersed more quickly than nymphs of the Jacksonville and Forest Green strains (Table 2). Dispersal induced by vapors of the FC blank was similar to that induced by the complete formulation. In the experiment with the complete FC, both resistant strains dispersed more slowly than the Fairbanks strain, but it took longer for 50% of the Jacksonville nymphs to disperse than either Fairbanks or Forest Green strain nymphs in the experiment with the FC blank. In experiments with both the FC and the FC blank, percentage dispersal of

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Table 1. Response of German cockroaches to a dried cyfluthrin FC and the FC formulation blank.

	Mean % on treated paper[†] (30 - 300 min)	
Strain*	FC[‡]	FC blank[‡]
Control	80.3 a (a)	82.6 a (a)
Fairbanks (S)	3.9 c (a)	67.3 b (b)
Forest Green (R)	80.8 a (a)	71.7 ab (a)
Jacksonville (R)	28.1 b (a)	57.5 c (b)

* S, insecticide susceptible; R, resistant to pyrethroids (cyfluthrin resistance of Forest Green > Jacksonville).

[†] Control, paper water-treated and dried; FC and FC blank, papers were treated with a cyfluthrin flowable concentrate (FC) and the formulation base, respectively.

[‡] Means followed by the same letter are not significantly different. First letter in each column, comparison between strains; in parentheses, comparison between FC and FC blank. [$P = 0.05$; analysis of covariance, Tukey's Studentized range test (HSD) on the means].

Table 2. Time required for 50% dispersal (DT_{50}) of German cockroach field-collected strains during exposure to vapors of a cyfluthrin FC formulation and the formulation blank.

	DT_{50} ($x \pm$ se), min*	
Strain	Cyfluthrin FC	Blank
Fairbanks (S)	9.9 ± 1.4 a (a)	9.3 ± 2.0 a (a)
Forest Green (R)	14.8 ± 0.8 b (a)	12.8 ± 1.8 a (a)
Jacksonville (R)	16.8 ± 2.7 b (a)	23.6 ± 1.7 b (a)

* Mean DT_{50}s from six replicates adjusted by Abbott's formula (1925) for dispersal in the control (mean dispersal in controls < 2%); numbers in columns followed by the same letter are not significantly different ($P > 0.05$, Duncan's multiple range test (SAS Institute 1985); numbers in rows followed by the same letter in parentheses are not significantly different (Students *t*-test).

Jacksonville nymphs at the end of the experiments was less than in the other strains (68% in both experiments compared with ≥ 80% in the Fairbanks and Forest Green strains). Dispersal of all strains was induced by exposure to vapors. As noted earlier, the data were adjusted for dispersal in the controls although it was <2% (see Materials and Methods).

Discussion

Insecticide usage has selected for altered behavior, as well as physiological/biochemical resistance, in insect populations (Lockwood et al. 1984, Pluthero and Singh 1984). For several decades, efforts to control the German cockroach have relied mainly on insecticides, but treatments of

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localized populations vary in respect to formulation type, AIs, application method, and application frequency. Also, the effects of treatment vary with the particular environment and the genetic composition of the target population. Variations in insecticide-induced behavior found here and in earlier studies on field-collected strains (Bret and Ross 1985, Wooster and Ross 1989, Ross 1992, Ross and Cochran 1992) are likely the result of the ways in which populations adapted to localized conditions. Many combinations of physiological/biochemical resistance with behavioral modifications have been reported in the literature (Lockwood et al. 1984, Sparks et al. 1989). It appears that, in *B. germanica*, the complexity of these combinations exists in a single species.

Pyrethroid resistance in *B. germanica* typically involves reduced nerve sensitivity (*kdr* factor) (Rashatwar and Masumura 1985, Umeda et al. 1988). In the housefly, reduced sensitivity to DDT, pyrethrum, and two pyrethroids was associated with *kdr* and *pen* resistance mechanisms (Virgona et al. 1983). Likewise, the generally reduced responses of the pyrethroid resistant strains to the cyfluthrin FC suggest that the observed behavioral modifications were, in part, an ancillary effect of the resistance mechanism, presumably *kdr*. The near complete avoidance of the dried FC by Fairbanks strain nymphs is largely attributable to susceptibility because the presence of the AI was the major factor in the repellent response. The nymphs were only slightly repelled by contact with the FC blank.

Dependence of altered behavior on the resistance mechanism could also account for greater avoidance of the dried FC and also a dried cyfluthrin EC by Jacksonville than Forest Green strain nymphs (Ross and Cochran 1992). The Jacksonville strain was less resistant to cyfluthrin than the Forest Green strain and presumably contained a higher frequency of cyfluthrin susceptible nymphs.

Behavioral differences resulting from selection by ingredients of solvent systems are likely to have arisen independently of resistance mechanisms. One example is a strain difference in response to an ingredient of the formulation base (D-glucose) of a commercial bait that led to the development of behavioral resistance (Silverman and Bieman, unpubl.). The greater response of Jacksonville than susceptible strain nymphs to the FC blank suggests that the Jacksonville strain was either selected for or contained a gene(s) conferring an enhanced avoidance of an ingredient(s) of the formulation blank. In contrast, Forest Green cockroaches were not repelled by contact with the complete FC formulation or, to any significant extent, by the FC blank. Although the former might be associated with a resistance mechanism, it is unlikely that this also explains the low response to the FC blank. Perhaps it is advantageous for cockroaches to remain in or near a favorable harborage if they are sufficiently resistant to overcome lethal effects of a toxicant.

In general, the FC blank (dried material) was not highly repellent, yet vapors of the FC blank caused rapid dispersal. Clearly, repellent substances were lost through vaporization. It is not surprising that the relative response of the resistant strains to vapors differed from that to the dried materials. Also, the cockroaches presumably sensed dried materials by contact chemoreception and vapors by olfaction. Jacksonville nymphs were more strongly repelled than Forest Green nymphs by contact with FC, yet they not only tended to disperse more slowly than Forest Green nymphs when exposed to vapors of the FC but,

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at the end of the experiment, percentage dispersal was less than that of the Forest Green nymphs. On the other hand, Forest Green strain nymphs did not avoid the dried formulation or its blank, yet vapor-induced dispersal was nearly as rapid as that of susceptible strain nymphs. To my knowledge, these were the first experiments to show that strain differences in response to residues (dried formulation) were unlike those induced by vapors of a commercial formulation.

Concealment of a response to the AI, if vaporized, by a strong response to repellents from the formulation base is the likely explanation of the similarity between dispersal induced by vapors of the blank and the complete formulation in the present and earlier (Ross 1992) experiments with the FC. A greater difference between the response of Forest Green and Jacksonville nymphs and between them and susceptible strain nymphs would probably have occurred if vapors had not caused such rapid dispersal. In flushing experiments with pyrethroid ECs, susceptible strain cockroaches dispersed but Forest Green and Jacksonville nymphs either did not disperse or dispersal was much reduced compared with the susceptible strain (Ross and Cochran 1992). Only one strain, K-851, has been found that is so insensitive that it would be difficult to flush with vapors of the cyfluthrin FC (Ross 1992).

None of the strains studied thus far avoid the insecticide when dried but fail to respond to the vapors. Nevertheless, the possibility of a residue response without a vapor response cannot be ruled out. In some formulations, possibly those that are water-based, vaporization might include very few or no repellents.

Insecticide-induced behavior of field-collected strains clearly varies in ways that affect the efficacy of flushing, especially with pyrethroids, and also the response to residual deposits. The type of formulation also influences flushing activity (Elbert and Behrenz 1986) and presumably is a determining factor in the development of strain differences. One would not expect selection for an altered response to vapors if vapors have little or no flushing activity. That repellency has a major effect on the efficacy of residual deposits has long been recognized (Ebeling et al. 1966). A multiplicity of other factors also affect residual activity, such as surface type and environment (Braness and Bennett 1990, Roper and Wright 1983). A model was constructed to predict the residual effectiveness of chlorpyrifos and cyfluthrin formulations, although the writers recognized that repellency of the deposits was not taken into account (Braness et al. 1991). Control strategies could be improved significantly if we knew more about insecticide-induced behavior in target populations.

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