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λ-Cyhalothrin Resistance Detection in the German Cockroach (Blattodea: Blattellidae)

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ABSTRACT λ-Cyhalothrin resistance levels of 13 recently collected field strains of German cockroach, *Blattella germanica* (L.), were determined by topical insecticide bioassay. The resistance levels ranged from 21- to 67-fold. Residual bioassays were conducted simultaneously against the Orlando insecticide-susceptible strain, the HRDC strain (carbamate resistant), the Village Green strain (pyrethroid resistant), and the Marietta strain (pyrethroid, carbamate, and organophosphorus resistant) to determine a diagnostic concentration for use in resistance detection bioassays. The LC_{99} of the Orlando susceptible strain (0.9 μg per jar) and 5 times the Orlando LC_{99} (4.5 μg per jar) were chosen for use in λ-cyhalothrin resistance detection bioassays. A highly significant relationship was observed between mortality at 5 times the LC_{99} of Orlando (4.5 μg per jar) and resistance ratio (LD_{50} in topical bioassays). The relevance of this relationship to λ-cyhalothrin resistance detection is discussed.

KEY WORDS *Blattella germanica*, diagnostic concentration, resistance detection, insecticide resistance, λ-cyhalothrin

THE GERMAN COCKROACH, *Blattella germanica* (L.), is a domiciliary pest found in populations defined by the structure they inhabit (Valles 1998). Isolation by structure provides the unique opportunity to evaluate a sample of individuals from the population for the presence of insecticide resistance before treatments are chosen or dispensed. Insecticide resistance in German cockroaches typically has been assessed by collecting cockroaches from the field and rearing them in the laboratory to acquire large numbers needed to conduct topical or residual bioassays (Valles and Yu 1996). These methods are time consuming, labor intensive, and may require special equipment or knowledge. Hence, these techniques are not conducive for use in the field by pest control operators.

For those concerned with insect pest control, the main objectives of insecticide resistance detection methods are to determine quickly whether insecticide resistance is present and if the level is sufficiently high to result in control failure. From a pest control perspective, the most important information that can be provided about a resistant population is which insecticide or alternative control tactic would be most effective against the population (Ball 1981, ffrench-Constant and Roush 1990).

One of the most difficult problems with development of insecticide resistance detection bioassays is choosing a diagnostic dose (ffrench-Constant and Roush 1990). For example, when employing a diagnostic dose bioassay, false negatives and positives may result when a dose is chosen that is too high or low, respectively. The task of assigning a diagnostic dose becomes especially difficult when laboratory insecticide-susceptible reference strains are no longer representative of susceptible insects in the field (Brown and Brogden 1987). The objective of this study was to identify a diagnostic concentration or relationship that could be used to evaluate resistance in field populations by determining mortality at several concentrations of λ-cyhalothrin of 13 German cockroach strains exhibiting a range of resistance levels.

Materials and Methods

All comparisons were made to the Orlando insecticide-susceptible German cockroach strain (Koehler and Patterson 1986). Adult males were used in all bioassays to eliminate developmental effects on insecticide tolerance (Valles et al. 1996). Topical bioassays were first conducted (Valles et al. 1996) to ascertain the relative resistance levels to λ-cyhalothrin among the 13 field strains. Collection information is summarized in Table 1.

Residual bioassays were conducted with the Orlando susceptible strain to provide a starting point for choosing a diagnostic concentration of λ-cyhalothrin. Residue bioassays also were conducted with a carbamate resistant strain, HRDC (Wadleigh et al. 1989); a pyrethroid resistant strain, Village Green (Atkinson et al. 1991); and the Marietta strain which is cross resistant to pyrethroid, organophosphorus, and carbamate insecticides (Valles and Yu 1996). Technical grade λ-cyhalothrin was pipetted into a 118 ml (4-ounce) mason jar (Ball, Muncie, IN), with an interior surface area of 90 cm^2, in a small volume (<100 μl) of acetone followed by 400 μl of additional acetone. The

Table 1. German cockroach field strain collection information

Strain	Date collected	City/State	Collection site	Control history[a]
Woodland	14 Feb. 1996	Gainesville/FL	House	Unk
Levy 324	24 June 1996	Chiefland/FL	House	Unk
Levy 616	25 June 1996	Chiefland/FL	House	Unk
Levy 405	11 July 1996	Chiefland/FL	Duplex	Unk
Union 507	25 July 1996	Lake Butler/FL	Duplex	Unk
Union 511	25 July 1996	Lake Butler/FL	Duplex	Unk
Malo	31 July 1996	Gainesville/FL	Trailer home	Pyr
Pinellas 214	16 Aug. 1996	Dunedin/FL	Apartment	Hyd
Pinellas 417	16 Aug. 1996	Dunedin/FL	Apartment	Hyd
Fuerte	16 Oct. 1996	Sacramento/CA	Restaurant	OP, CB, Pyr, BA, Hyd
Sacramento	16 Oct. 1996	Sacramento/CA	Bakery	OP, CB, Pyr
Swine	14 Nov. 1996	Gainesville/FL	Swine Rearing Unit	CB, Pyr
NASJAX	10 Apr. 1997	Jacksonville/FL	Restaurant	Hyd, IGR

[a] Insecticide type known to have been used against the population: organophosphorous (OP), carbamate (CB), pyrethroid (Pyr), hydramethylnon (Hyd), insect growth regulator (IGR), boric acid (BA), unknown history (Unk).

jars were rotated by hand until the acetone evaporated. At least 5 concentrations causing between 0 and 100% mortality were chosen for each bioassay. Ten adult male German cockroaches (≈1 wk old) were placed into each jar after the treatment dried. At least 3 replications per concentration were conducted. Jars were placed at 26°C on a photoperiod of 12:12 (L:D) h and mortality was assessed after 24 h. Mortality was corrected for control mortality using Abbott's formula (Abbott 1925). Control jars were treated with 500 μl acetone.

Residue bioassays were conducted with the Levy 405, Levy 616, Union 507, Pinellas 214, and Woodland strains at 0.9, 1.8, 4.5, and 9 μg λ-cyhalothrin per jar representing 1, 2, 5, and 10 times the Orlando LC_{99}, respectively, and at 110 μg per jar (the labeled rate of 0.03% applied at 3.8 liters/9.3 m^2). These bioassays were replicated 5 times for each strain with 10 cockroaches per jar. Mortality was assessed 24 h after placement on the residue. The remaining field strains then were bioassayed at 4.5 μg per jar (5 times the LC_{99} of Orlando). A minimum of 5 replications was conducted for each of the field strains in the residue bioassays with 10 insects per jar.

Bioassay data were analyzed with probit analysis using the probit procedure (SAS Institute 1988). The relationship between resistance ratio (determined from topical bioassay) and percentage of mortality (at 4.5 μg per jar) were analyzed using regression analysis (with the anticipation of a linear relationship) or Pearson product-moment correlation. Mortality at 4.5 μg λ-cyhalothrin per jar was compared by the Dunnett multiple comparison procedure using the response of the Orlando strain as control (SAS Institute 1988).

Results

λ-Cyhalothrin resistance level, determined at the LD_{50} in the topical bioassays, ranged from 21- to 67-fold (Table 2). Residue (jar) bioassay results for the established laboratory strains, Orlando, HRDC, Village Green, and Marietta are summarized in Table 3 and Fig. 1. The LC_{99} value for the Orlando strain was 0.9 μg λ-cyhalothrin per jar. This value corresponded to the LC_{80} in HRDC, the LC_{15} in Village Green, and less than the LC_{01} in Marietta. Resistance levels of Village Green and Marietta in the residue (jar) bioassays measured at the LC_{50} were 12.9- and 15.6-fold

Table 2. Toxicity of topically applied λ-cyhalothrin among field-collected German cockroach strains

Strain	*n*	Slope ± SE	LD_{50} (95% CI)[a]	LD_{90} (95% CI)[a]	df	χ^{2b}	RR[c]
Orlando	200	5.68 ± 1.10	0.008 (0.007–0.011)	0.014 (0.011–0.036)	3	6.9	1
Levy 405	150	2.41 ± 0.34	0.024 (0.019–0.031)	0.083 (0.059–0.15)	3	0.5	2.9 (−12.2–17.9)
Swine	120	3.68 ± 0.55	0.039 (0.027–0.039)	0.073 (0.058–0.11)	3	3.4	3.9 (−15.9–23.6)
Sacramento	150	2.46 ± 0.34	0.044 (0.034–0.055)	0.15 (0.11–0.24)	3	3.3	5.1 (−12.9–23.1)
Levy 616	120	1.49 ± 0.37	0.082 (0.056–0.13)	0.59 (0.28–4.5)	2	1.3	9.6 (−14.7–34)
Union 507	150	2.11 ± 0.34	0.18 (0.12–0.23)	0.73 (0.53–1.2)	3	0.5	21.1 (5.5–36.7)
Levy 324	150	2.21 ± 0.31	0.20 (0.15–0.25)	0.75 (0.52–1.3)	3	2.5	23.1 (8.2–38)
Malo	150	2.54 ± 0.36	0.25 (0.19–0.31)	0.80 (0.60–1.2)	3	1.6	29.4 (14.9–43.8)
Union 511	150	2.37 ± 0.45	0.28 (0.22–0.42)	0.98 (0.59–2.8)	3	1.8	33.1 (17.5–48.7)
Pinellas 417	120	2.70 ± 0.61	0.31 (0.25–0.42)	0.92 (0.59–2.7)	2	2.5	36.2 (20.6–51.8)
Pinellas 214	150	3.21 ± 0.60	0.32 (0.27–0.38)	0.80 (0.59–1.5)	3	0.8	37.3 (22.6–52)
NASJAX	200	3.42 ± 0.38	0.37 (0.32–0.43)	0.88 (0.72–1.2)	3	3.5	43.7 (29.8–57.6)
Woodland	150	3.48 ± 0.49	0.45 (0.38–0.53)	1.1 (0.83–1.5)	3	0.4	52.8 (38.8–66.7)
Fuerte	120	2.99 ± 0.54	0.57 (0.44–0.70)	1.5 (1.1–2.7)	2	2.6	66.6 (52.7–80.5)

[a] μg λ-cyhalothrin per insect.
[b] No chi-square values are significant ($P = 0.05$).
[c] Resistance ratio at the LD_{50} and 95% CI calculated by the method of Robertson and Preisler (1992).

Table 3. Toxicity of λ-cyhalothrin in residue bioassays to the German cockroach

Strain	*n*	Slope ± SE	LC_{50} (95% CI)[a]	LC_{99} (95% CI)[a]	df	χ^{2b}	RR[c]
Orlando	140	5.7 ± 1.2	0.34 (0.31–0.38)	0.86 (0.63–1.8)	3	2.6	1
HRDC	100	5.2 ± 1.0	0.61 (0.53–0.71)	1.71 (1.28–3.14)	3	0.7	1.8 (−0.3–4.0)
Village Green	140	1.7 ± 0.4	4.34 (3.20–6.55)	107.65 (37.52–1364)	3	2.1	12.9 (9.8–15.9)
Marietta	140	3.4 ± 0.6	5.23 (4.39–6.46)	25.54 (16.34–58.92)	3	5.6	15.6 (12.8–18.4)

[a] μg λ-Cyhalothrin per jar.
[b] No chi-square values significant ($P = 0.05$).
[c] Resistance ratio at the LD_{50} and 95% CI calculated by the method of Robertson and Preisler (1992).

compared with the Orlando strain, respectively. The HRDC strain was not resistant.

Evaluating 0.9 (Orlando LC_{99}), 1.8 (2 times the Orlando LC_{99}), 4.5 (5 times the Orlando LC_{99}), 9 (10 times the Orlando LC_{99}), and 110 μg λ-cyhalothrin per jar (labeled rate) against the Levy 405, Levy 616, Union 507, Pinellas 214, and Woodland field strains allowed a series of increasing diagnostic concentrations to be compared against field strains exhibiting a range of resistance levels to λ-cyhalothrin. As indicated in Fig. 2, mortality was independent of resistance level (based on topical bioassays) at the 2 lowest (0.9 and 1.8 μg per jar) and 2 highest (9 and 110 μg per jar) residue concentrations. However, at the middle concentration (4.5 μg per jar or 5 times the LC_{99} of Orlando) mortality among the strains ranged from 10 to 98%. When this concentration was evaluated against all of the field strains, a highly significant relationship between resistance level (topical bioassay equivalent) and percentage mortality was observed (Fig. 3).

Discussion

The insecticide-susceptible Orlando strain of the German cockroach has been in culture for nearly 4 decades bringing into question how well it represents insecticide-susceptible cockroaches in the field. However, 0.9 μg λ-cyhalothrin per jar (LC_{99} for the Orlando strain) caused 47% mortality in the Levy 405 field strain, which was taken from the field recently (Table 1). Similarly, 80 and 15% of the HRDC and Village Green strains were killed at this concentration, respectively. Mortality in these recently collected strains at the LC_{99} of the Orlando strain suggests that the Orlando strain is still representative of susceptible field cockroaches.

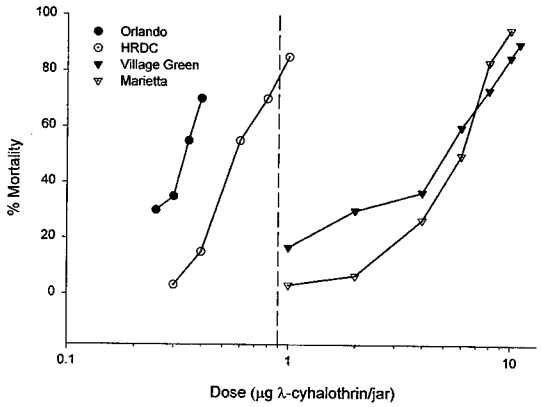

Fig. 1. Concentration-mortality lines for the Orlando, HRDC, Village Green, and Marietta German cockroach strains exposed to λ-cyhalothrin. Dashed vertical line represents the LC_{99} (0.9 μg λ-cyhalothrin per jar) of the Orlando strain.

ffrench-Constant and Roush (1990) noted that resistance detection may be conducted at many different levels of sensitivity depending on the desired purpose. For example, a stringent assay is required for detecting changes in the frequency of resistant genotypes, whereas a comparatively less rigorous assay is necessary to identify an insecticide that is least affected by the resistance (Brent 1986). Use of the Orlando LC_{99} in jar bioassays appears to be an acceptable means of providing a nominal response with respect to the presence of λ-cyhalothrin resistance in a field population of German cockroaches. ffrench-Constant and Roush (1990) reported that bioassays based on a diagnostic concentration are more efficient than lethal concentration values for detecting low frequencies of resistance. However, a diagnostic concentration bioassay based on a susceptible strain LC_{99} provides no information about the magnitude of the resistance. Moreover, if this method predicts the presence of resistance in a population (i.e., cockroaches survive at the diagnostic concentation), does it necessarily mean that control failure will occur? This is the most relevant question from a pest control operators perspective. Cochran (1996) reported that control difficulty will occur in German cockroaches exhibiting a resistance level of 3- to 5-fold in residual bioassays. However, such resistance ratios are highly dependent on the concentration of insecticide used in the bioassay (Cochran 1997). For example, the resistance ratio can be compressed by overwhelming the resistance mechanism with an increased insecticide concentration. Therefore, in an effort to relate the magnitude of the resistance level, a range of λ-cyhalothrin concentrations (multiples of the Orlando strain LC_{99}) were evaluated against the field strains exhibiting a range of resistance levels to λ-cyhalothrin (Table 2).

Interestingly, mortality was independent of resistance level at the 2 lowest (0.9 and 1.8 μg per jar) and 2 highest (9 and 110 μg per jar) residue concentrations. Conversely, mortality and resistance level were significantly correlated at the middle concentration (4.5 μg per jar or 5 times the LC_{99} of Orlando); mortality among the strains

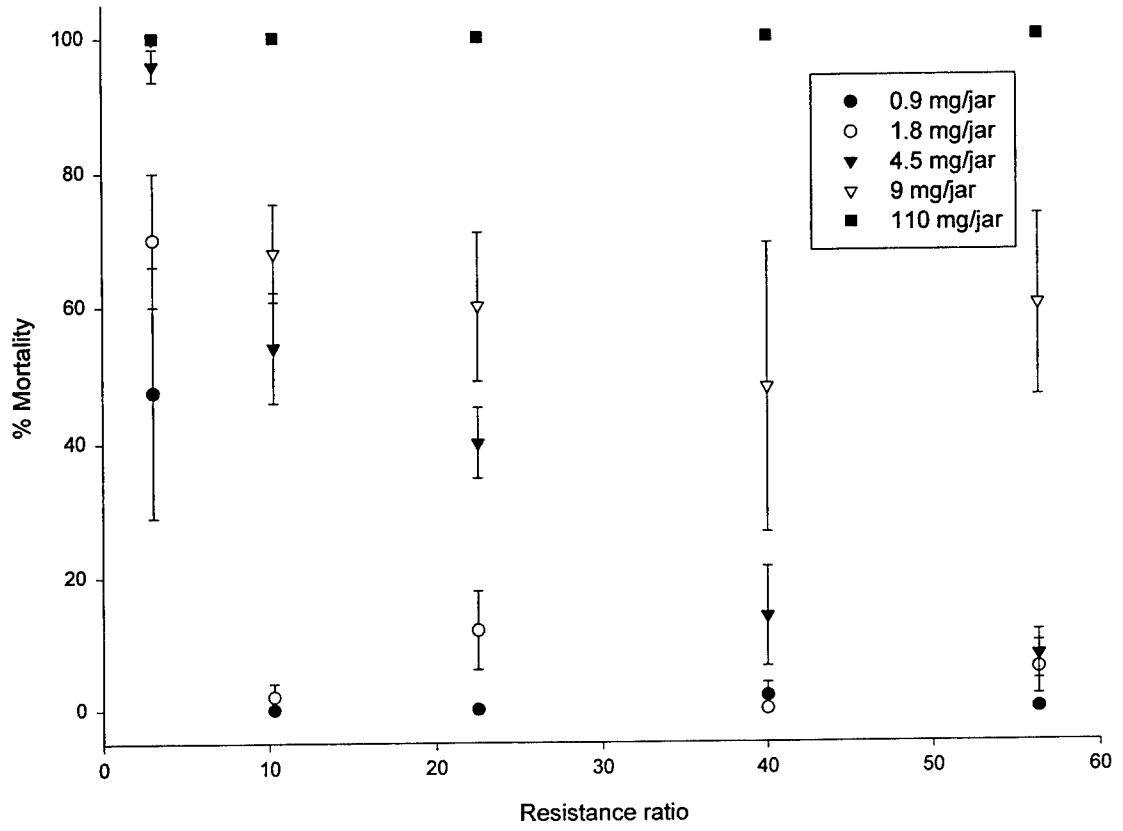

Fig. 2. Relationship between resistance ratio (determined at the LD_{50} in topical insecticide bioassays) and percentage of mortality at 0.9 (LC_{99} of the Orlando strain), 1.8, 4.5, 9, and 110 μg λ-cyhalothrin per jar in the Levy 405, Levy 616, Union 507, Pinellas 214, and Woodland German cockroach strains. Data points indicate the mean ± SE for 5 replications of 10 insects per replicate.

ranged from 10 to 98%. As mortality data from additional strains were added to the initial regression curve generated with Levy 405, Levy 616, Union 507, Pinellas 214, and Woodland field strains, each maintained the relationship between mortality at 4.5 μg per jar and resistance level (determined in topical bioassays at the LD_{50}). This relationship was strengthened further by converting the abscissa to a log scale (Fig. 3). However, it is not clear whether this relationship is capable of providing an estimate of λ-cyhalothrin resistance or is merely fortuitous. Such a simple model may not predict λ-cyhalothrin resistance level among heterogeneous populations or whether multiple mechanisms are responsible for the resistance. However, the field strains employed in these experiments have been reported to exhibit multiple mechanisms of resistance, including *kdr*-type resistance (Dong et al. 1998) as well as enhanced metabolism (Valles 1998). Multiple mechanisms of resistance appear to be common among insecticide resistant German cockroaches (e.g., Siegfried and Scott 1991). To my knowledge, this method of diagnostic concentration estimation has not been demonstrated previously.

The method of detection proposed involves capturing cockroaches from the field by vacuum (Valles 1997) and placing adult male cockroaches in a jar (90 cm^2) coated with 0.9 μg λ-cyhalothrin. Mortality is assessed 24 h after treatment. Resistance is indicated in the sample population if <90% mortality is achieved. Additionally, survival on the 4.5 μg λ-cyhalothrin per jar concentration (5 times the LC_{99} of Orlando) indicates severe resistance within the population. The practice of employing multiple diagnostic doses to evaluate the presence of resistance has been reported previously for purposes of gaining information about the magnitude of the resistance (Scott et al. 1989). Further testing is necessary to determine whether mortality at 4.5 μg per jar and subsequent use of the regression equation in Fig. 3 can provide a reliable estimate of the resistance level. Possibly, it could be used to generate a better understanding of the relationship between resistance level and control failure in the field.

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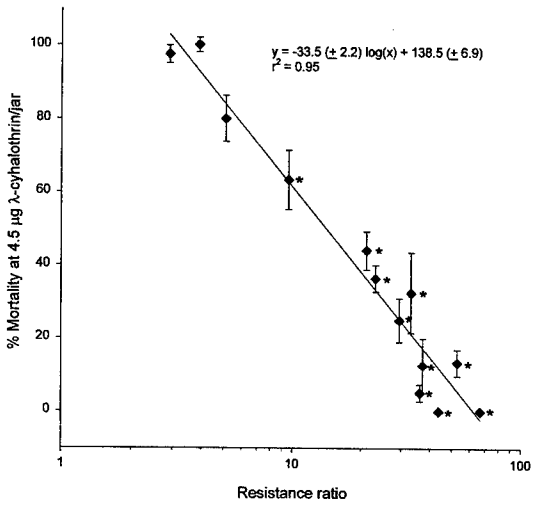

Fig. 3. Relationship between mortality at 5 times the LC_{99} of the Orlando strain (4.5 µg λ-cyhalothrin per jar) and resistance ratio (determined at the LD_{50} in topical insecticide bioassays). Each data point represents the mean ± SE for a single strain ($n = 5$). An asterisk indicates significant ($P = 0.05$) difference in percentage mortality between the indicated field strain and the insecticide-susceptible Orlando strain. Mortality for Orlando was 100%.

the Sacramento and Fuerte strains. This research was supported in part by the Strategic Environmental Research and Development Program, Project PP-1053.

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