Stage-Dependent Bendiocarb Tolerance in the German Cockroach (Dictyoptera: Blattellidae)[1]

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Abstract Topical insecticide bioassays revealed that last-instar German cockroach, *Blattella germanica* (L.), nymphs were up to 19.8-fold more tolerant of bendiocarb than adult males. Pretreatment with the cytochrome P450 monooxygenase inhibitor, piperonyl butoxide, eliminated this difference. Acetylcholinesterase activity was equally inhibited by bendiocarb in both the last-instar nymphal and adult male stages. The bimolecular rate constant (*ki*) for adult males was $3.58 \times 10^5 \pm 1.0$ $M^{-1}min^{-1}$, which was not statistically different from nymphs at $3.33 \times 10^5 \pm 1.1$ $M^{-1}min^{-1}$. Similar *ki* values indicate that altered acetylcholinesterase does not contribute to the increased nymphal tolerance to bendiocarb. These results indicate that increased detoxification catalyzed by microsomal oxidases is responsible for the enhanced nymphal tolerance to bendiocarb in the Village Green strain.

Key Words German cockroach, *Blattella germanica,* bendiocarb, insecticide tolerance, stage-dependent susceptibility

Stage-dependent tolerance has been reported to occur in the German cockroach, *Blattella germanica* (L.), toward insecticides in several chemical classes. Dewey (1942) reported that fourth- and fifth-instar nymphs were more tolerant to sodium fluoride dusts than male and female adults. Similar results also were reported by Fales and Bodenstein (1963) using chlordane and by Woodbury (1938) using pyrethrum sprays. Koehler et al. (1993) provided the most comprehensive comparison among developmental stages of an insecticide-susceptible and resistant strain of German cockroach. They demonstrated that mixed-sex late-instar German cockroach nymphs (fourth to sixth instars) were significantly more tolerant to bendiocarb, chlorpyrifos, and cypermethrin than adult males. Although many reports document the phenomenon of stage-dependent insecticide susceptibility in German cockroaches, very little documentation exists concerning the mechanisms responsible. The purpose of this investigation was to examine factors responsible for stage-dependent differential susceptibility to the carbamate insecticide, bendiocarb, in the Village Green German cockroach strain.

Materials and Methods

Adult male (1 to 2 wks old) and nymph (sixth instar mixed-sex) German cockroaches of the insecticide-resistant Village Green strain were used in all tests. The

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cockroaches were reared as described by Koehler and Patterson (1986). Nymphs were staged by the method described by Koehler et al. (1993). Cockroaches were anesthetized with CO_2 and treated topically with bendiocarb dissolved in 1 µl acetone (Valles and Koehler 1994). The insecticide solution was applied to the first abdominal sternite in five concentrations producing >0% and <100% mortality. Each concentration was replicated three times. Piperonyl butoxide (100 µg/insect) was applied to the first abdominal sternite 2 h prior to insecticide application. Mortality was recorded 24 h after insecticide treatment, and data were analyzed by probit analysis (Finney 1971).

Acetylcholinesterase activity was measured with acetylthiocholine as substrate as described by Ellman et al. (1961). Fifty adult males or last-instar nymphs were decapitated and the heads homogenized in 10 ml of 0.1 M sodium phosphate buffer, pH 8.0. The homogenate was filtered through two layers of cheesecloth, then centrifuged at 1,000 g_{max} for 15 min. The supernatant was used as the enzyme source. Bimolecular rate constant (*ki*) for inhibition of acetylcholinesterase by bendiocarb was determined by the method of Aldridge (1950).

Results and Discussion

Bimolecular rate constants (*ki*) for the inhibition of acetylcholinesterase by bendiocarb were the same for both nymphs and adults. The *ki* for adult males was $3.58 \times 10^5 \pm 1.0\ M^{-1}min^{-1}$, which was not statistically different from nymphs at $3.33 \times 10^5 \pm 1.1\ M^{-1}min^{-1}$. Similar *ki* values indicate that altered acetylcholinesterase does not contribute to the increased nymphal tolerance to bendiocarb.

Nymphs were 19.8 times more tolerant to bendiocarb than adult males at the LD50 value (Table 1). The increased tolerance to bendiocarb observed in nymphs was effectively eliminated by piperonyl butoxide, an established cytochrome P450 monooxygenase inhibitor. Adults and nymphs pretreated with piperonyl butoxide were equally susceptible to bendiocarb as determined by overlapping 95% fiducial limits. These results suggest that increased microsomal oxidase activities play a major role in the observed stage-dependent differential susceptibility to bendiocarb. Similar results were reported by Valles et al. (1996) who found that last-instar male and female nymphs of the Village Green German cockroach strain were significantly more tolerant of topically-applied propoxur than adult males. They determined that nymphs oxidatively metabolized propoxur nearly three times faster than adult males. This

Table 1. Toxicity of bendiocarb and bendiocarb + piperonyl butoxide applied topically to adults and final instar nymphs of the Village Green German cockroach.

Insecticide	LD50 (µg/insect)		
	Adult	Nymph	N/A*
Bendiocarb	5.5 (4.0-9.8)**	109.5 (58.6-484.6)	19.8
Bendiocarb + PBO	2.5 (1.7-3.2)	3.4 (1.5-5.5)	1.3

* Nymph LD50/Adult LD50.
** 95% Fiducial Limits.

result was supported by synergist bioassay data (which nearly eliminated the stage dependent difference) and a two-fold increase in cytochrome P450 content in nymphs as compared with adult males.

Adult male German cockroaches are routinely used to assess insecticide resistance levels and to evaluate insecticide efficacy. Because nymphs are the most insecticide-tolerant stage, the level of resistance may be underestimated and insecticide efficacy exaggerated unless nymphs are included in insecticide evaluations. Furthermore, nymphs have been estimated to comprise >80% of field populations (Ross and Mullins 1995). Consequently, an increased understanding of the way in which nymphs cope with insecticide exposure may lead to more effective control measures for German cockroach populations.

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