

TEMPERATURE EFFECTS ON λ -CYHALOTHRIN TOXICITY IN
INSECTICIDE-SUSCEPTIBLE AND RESISTANT GERMAN
COCKROACHES (DICTYOPTERA: BLATTELLIDAE)

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ABSTRACT

Pre- and post-treatment temperature effects on λ -cyhalothrin toxicity were determined in insecticide-susceptible and resistant German cockroach, *Blattella germanica* (L.), strains. Acclimation at 19, 26, or 31°C for 10 days before insecticide treatment had no effect on λ -cyhalothrin toxicity in either strain. No differences were observed in aldrin epoxidase and glutathione S-transferase activities when "Orlando" (susceptible) cockroaches were incubated for 10 days at 19, 26, and 31°C. When temperature treatment followed insecticide application, a negative temperature coefficient of toxicity (greater toxicity at lower temperature) toward λ -cyhalothrin was observed for the Orlando but not the *kdr*-type resistant "Village Green" cockroaches. Piperonyl butoxide synergized λ -cyhalothrin in "Orlando" cockroaches 3 and 5-fold at 26 and 31°C, respectively. Conversely, piperonyl butoxide pre-treatment did not cause a significant reduction in the LD₅₀ value in the "Village Green" strain regardless of temperature.

Key Words: *Blattella germanica*, insecticide resistance, synergism

RESUMEN

Se determinó el efecto de la temperatura, antes y después de la aplicación del insecticida, sobre la toxicidad de λ -cicalotrina en una raza susceptible y otra resistente de la cucaracha alemana, *Blattella germanica* (L.). La aclimatación a 19, 26, ó 31°C durante 10 días, antes del tratamiento con λ -cicalotrina, no tuvo efecto sobre la toxicidad en ninguna raza. No se observaron diferencias en las actividades de aldrín epoxidasa y glutatión-S-transferasa cuando las cucarachas de la raza "Orlando" (raza susceptible) se incubaron durante 10 días a 19, 26, y 31°C. Cuando las cucarachas se mantuvieron a diferentes temperaturas después de la aplicación de λ -cicalotrina, se observó un coeficiente de toxicidad negativamente relacionado con la temperatura (mayor toxicidad a menor temperatura) en la raza "Orlando", pero no en la raza "Vi-

llage Green", que es resistente por *kdr*. El butóxido de piperonilo sinergizó 3 y 5 veces la toxicidad de λ -cialotrina en la raza "Orlando" a 26 y 31°C, respectivamente. Sin embargo, la aplicación de butóxido de piperonilo no disminuyó significativamente la DL₅₀ en la raza "Village Green" en las diferentes temperaturas evaluadas.

The relationship between temperature and insecticide toxicity in insects has been studied widely (Scott 1995). Although this phenomenon has been examined extensively in many insect species, few studies have compared the responses of insecticide-susceptible with insecticide-resistant strains at different temperatures. Currently, only one study compares the temperature-toxicity relationship between insecticide-susceptible and resistant German cockroaches, *Blattella germanica* (L.). Scott (1987) reported a positive temperature coefficient of toxicity for the pyrethroid, cypermethrin, in an insecticide-susceptible (CSMA) German cockroach strain. However, an insecticide-resistant strain (VPIDLS) with a *kdr*-type mechanism exhibited a negative temperature coefficient of toxicity for cypermethrin (Scott 1987). Conversely, Wadleigh et al. (1991) reported a negative temperature coefficient of toxicity toward cypermethrin in an unrelated insecticide-susceptible German cockroach strain, "Orlando". These inconsistencies and lack of information warrant further investigation into the temperature-toxicity relationship, especially among insecticide-resistant German cockroaches.

The purpose of this research was to compare the effect of different temperature treatments made before and after insecticide application on λ -cyhalothrin toxicity in insecticide-susceptible and resistant German cockroaches. We also examined the effect of the synergist piperonyl butoxide on λ -cyhalothrin toxicity at different temperatures in these strains.

MATERIALS AND METHODS

The insecticide-susceptible "Orlando" (Koehler & Patterson 1986) and resistant "Village Green" strains (Atkinson et al. 1991) were used in all experiments. The "Village Green" strain has been reported to exhibit nerve insensitivity (*kdr*-type) as the major mechanism of resistance to pyrethroid insecticides (Bull & Patterson 1993). The cockroaches were reared at 26°C, 55% relative humidity, on a photoperiod of 12:12 (L:D) as described by Koehler & Patterson (1986).

To assess pre-treatment temperature effects on toxicity, four groups of 100 adult male cockroaches (1-3 weeks old) were removed from a rearing tub and placed into four-liter glass jars with cardboard harborage, #5001 laboratory rodent diet (Purina, St. Louis, MO), and two cotton-stoppered 20 ml scintillation vials of water. Each jar of 100 cockroaches was held for 10 days at 19, 26, or 31°C in environmental chambers at 80% relative humidity on a 12:12 (L:D) photoperiod (Walker et al. 1993). At the end of the 10 day incubation period, the cockroaches were removed from the environmental chamber and placed into 15 by 100 mm Petri dishes (10 cockroaches per Petri dish). Cockroaches were bioassayed immediately after removal from the environmental chambers by topical insecticide application in 1 μ l of acetone to the first abdominal sternite as described by Valles & Yu (1996a). Cockroaches were held at 26°C, and mortality was assessed 24 h after insecticide application.

To assess post-treatment temperature effects on insecticide toxicity, adult male German cockroaches (1-3 weeks old, mean weight of 47 and 51 mg for "Orlando" and Village Green, respectively) were placed into Petri dishes and treated topically with

insecticide as described above. At least 5 insecticide concentrations causing >0% and <100% mortality were chosen for each bioassay. Immediately after treatment, the cockroaches were placed into the environmental chambers at 80% relative humidity on a 12:12 (L:D) photoperiod at 19, 26, or 31°C. Mortality was assessed 24 h after insecticide application. Three to five replications containing 10 cockroaches per concentration were conducted. When the synergist bioassay was performed, piperonyl butoxide (100 µg per cockroach) was applied to the first abdominal segment 1 h before insecticide treatment.

Pre-treatment temperature effects on detoxification enzymes were assessed by quantifying cytochrome P450 monooxygenase and glutathione *S*-transferase activities using surrogate substrates. All enzyme reactions were conducted within linear ranges of incubation time and protein concentration determined previously (Valles & Yu 1996a). Three experiments, each with duplicate determinations, were conducted for all enzymes. "Orlando" cockroaches were maintained with food, water, and harborage as described above for 10 days at 19, 26, or 31°C. Detoxication enzymes were assayed immediately after the incubation period.

Microsomal epoxidase activity was measured by the epoxidation of aldrin to dieldrin (Valles & Yu 1996b). Microsomes were prepared by homogenizing 10 decapitated adult male "Orlando" cockroaches for 30 s in 20 ml of ice-cold 0.1 M sodium phosphate buffer, pH 7.5, containing 10% glycerol, 0.1 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, and 1 mM 1-phenyl-2-thiourea using a Potter-Elvehjem teflon pestle and glass mortar. The homogenate was filtered through 2 layers of cheesecloth, then centrifuged at 10,000 g_{max} for 15 min. The supernatant was filtered through glass wool and further centrifuged at 105,000 g_{max} for 1 h. The resulting pellet (microsomes) was suspended in sodium phosphate buffer, pH 7.5 and used as the enzyme source.

Glutathione *S*-transferase activity was measured with *p*-nitrophenyl acetate (PNPA) as substrate as described by Yu & Nguyen (1992). Enzyme preparation was performed as described above for the microsome preparation. The 105,000 g_{max} supernatant was used as the enzyme source. Homogenization and centrifugation took place in 0.1 M sodium phosphate buffer, pH 7.0. Protein determinations were made by the method of Bradford (1976) using bovine serum albumin as the standard.

Insecticide bioassay data were subjected to probit analysis (Finney 1971). Enzyme activity means were analyzed by analysis of variance followed by Scheffé's multiple comparison procedure when appropriate.

RESULTS AND DISCUSSION

Table 1 summarizes the bioassay results for pre-treatment temperature effects on insecticide toxicity. No significant differences in toxicity (based on non-overlapping 95% confidence limits) were observed among the temperatures tested for either strain of German cockroach. Resistance ratios ranged from 14 to 15 in the "Village Green" strain.

Most studies have revealed that acclimation has no effect on toxicity in insects (Rai 1967, Fisher & Hansell 1964, Scott 1995). However, acclimation temperature has been reported to significantly influence insecticide toxicity in cockroaches. For example, Munson (1953) and Baldus & Mutchmor (1988) reported a negative temperature coefficient of toxicity for DDT in American cockroaches, *Periplaneta americana* (L.), acclimated at different temperatures for 10 to 14 days. This effect was attributed to changes in cuticle lipid saturation in response to temperature treatment which affected DDT solubility and mobility toward the target site.

TABLE 1. PRE-TREATMENT TEMPERATURE EFFECTS ON INSECTICIDE TOXICITY IN THE GERMAN COCKROACH.

Insecticide	Strain	Temperature (°C)	LD ₅₀ (95% CL) (µg/insect)	Slope ± SE	χ ²	df	RR ^a
λ-Cyhalothrin	Orlando	19	0.0053 (0.0049-0.0057)	6.7 ± 0.4	8.2	4	—
		26	0.0047 (0.0045-0.005)	8.4 ± 0.5	1.6	4	—
		31	0.0044 (0.0043-0.0046)	10.5 ± 0.6	8.9	4	—
	Village Green	19	0.073 (0.058-0.092)	2.1 ± 0.2	9.4	4	14
		26	0.067 (0.052-0.086)	2.1 ± 0.06	0.9	3	14
		31	0.066 (0.050-0.085)	1.9 ± 0.1	4.6	4	15

^aLD₅₀ Village Green/LD₅₀ Orlando

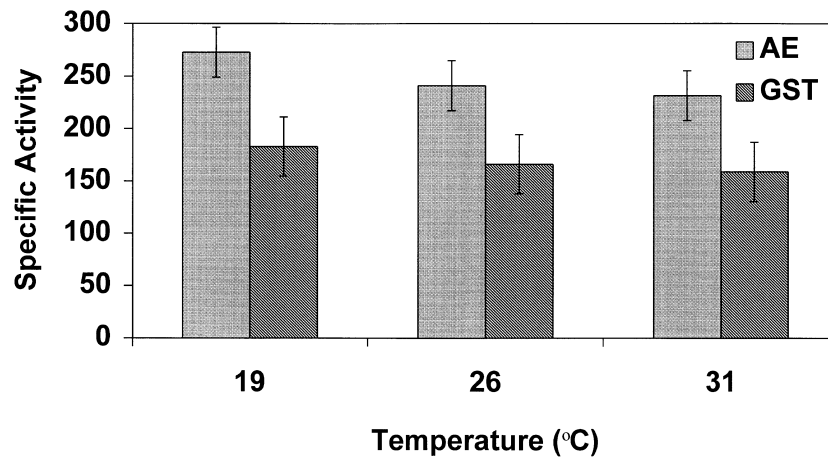


FIG. 1. Pretreatment temperature effects on aldrin epoxidase (AE) (pmol/min/mg protein) and glutathione *S*-transferase (GST) (nmol/min/mg protein) activities in the "Orlando" German cockroach strain.

Stressors such as heat often induce the synthesis of proteins that prevent stress-induced damage and limit further cellular damage from occurring (Hightower 1991). Cytochromes P450 are the most widely studied group of these proteins (Ryan & Hightower 1996). However, no significant differences in cytochrome P450-catalyzed aldrin epoxidation ($F = 0.71$; $df = 12, 3$; $P = 0.56$) or PNPA conjugation ($F = 0.08$; $df = 12, 3$; $P = 0.97$) at different acclimation temperatures were observed in "Orlando" cockroaches (Fig. 1). Apparently, these temperature pretreatments did not influence the production of these detoxication enzymes.

"Orlando" cockroaches exhibited a negative temperature coefficient of toxicity for λ -cyhalothrin (Table 2). λ -Cyhalothrin was nearly 3-fold more toxic at 19°C than at 31°C. Interestingly, "Village Green" cockroach susceptibility to λ -cyhalothrin was not significantly affected by the temperature treatments. As a result, the resistance ratio increased with decreasing temperature. The resistance ratio was 10-fold at 31°C, 14-fold at 26°C, and 20-fold at 19°C. This is an unusual observation. Typically, pyrethroid toxicity is affected by temperature changes. For example, Scott (1987) reported a negative temperature coefficient of toxicity toward *S*-bioallethrin in susceptible (CSMA) and *kdr*-type resistant (VPIDLS) cockroaches.

Pretreatment with piperonyl butoxide eliminated temperature-dependent λ -cyhalothrin toxicity differences in "Orlando" cockroaches (Table 3). Piperonyl butoxide synergized the toxicity of λ -cyhalothrin in 26 and 31°C treated "Orlando" cockroaches 3 and 5-fold, respectively. However, no synergism was observed at 19°C (Tables 2 and 3). McKinlay (1965) also reported that piperonyl butoxide only synergized insecticide toxicity at higher temperatures (21 and 32°C) in the migratory grasshopper, *Melanoplus bilituratus* (F.). In the "Village Green" strain, no synergism was observed at any temperature tested, nor were there any differences in λ -cyhalothrin toxicity at the different temperatures.

The mechanism responsible for pyrethroid temperature-toxicity relationships remains a mystery. However, Narahashi et al. (1995) have demonstrated recently that sodium ion flow through tetramethrin-modified sodium channels at low temperatures

TABLE 2. POST-TREATMENT TEMPERATURE EFFECTS ON INSECTICIDE TOXICITY IN THE GERMAN COCKROACH.

Insecticide	Strain	Temperature (°C)	LD ₅₀ (95% CL) (µg/insect)	Slope ± SE	χ ²	df	RR ^a
λ-Cyhalothrin	Orlando	19	0.0022 (0.0019-0.0026)	3.6 ± 0.3	9.8	4	—
		26	0.0046 (0.0043-0.0050)	7.5 ± 0.5	2.3	3	—
		31	0.0057 (0.0053-0.0060)	6.1 ± 0.5	6.1	4	—
	Village Green	19	0.044 (0.036-0.054)	2.4 ± 0.2	3.1	3	20
		26	0.065 (0.054-0.080)	1.9 ± 0.1	3.5	4	14
		31	0.058 (0.043-0.075)	1.7 ± 0.1	3.5	4	10

^aLD₅₀ Village Green/LD₅₀ Orlando.

TABLE 3. POST-TREATMENT TEMPERATURE EFFECTS ON INSECTICIDE TOXICITY IN THE GERMAN COCKROACH.

Insecticide	Strain	Temperature (°C)	LD ₅₀ (95% CL) (µg/insect)	Slope (± SE)	χ ²	df	RR ^a
λ-Cyhalothrin + PBO	Orlando	19	0.0015 (0.0012-0.0020)	2.4 ± 0.2	0.6	3	—
		26	0.0016 (0.0011-0.0028)	1.3 ± 0.1	4.2	3	—
		31	0.0012 (0.0009-0.0017)	1.6 ± 0.1	2.2	5	—
	Village Green	19	0.031 (0.023-0.042)	1.5 ± 0.1	6.9	5	21
		26	0.039 (0.030-0.050)	1.7 ± 0.3	14.4	4	24
		31	0.032 (0.023-0.052)	1.5 ± 0.1	0.5	3	27

^aLD₅₀ Village Green/LD₅₀ Orlando.

augmented the depolarizing after potential in rat cerebellar Purkinje neurons. Although Narahashi et al. (1995) correlated changes in action potential and sodium ion currents in the presence of pyrethroid insecticides at different temperatures, the mechanism(s) causing this phenomenon remains unknown. The toxicity of λ -cyhalothrin in the "Village Green" strain, which exhibits *kdr*-type pyrethroid resistance, was unaffected by temperature treatments in this study. Perhaps the altered target site (voltage-gated sodium channels) in this insecticide-resistant strain was responsible for the independence of insecticide treatments with respect to temperature.

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