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Multiple Logit Analyses of the Effects of Temperature and Humidity on the Toxicity of Propoxur to German Cockroaches (Orthoptera: Blattellidae) and Western Spruce Budworm Larvae (Lepidoptera: Tortricidae)

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ABSTRACT The effect of temperature and humidity on the toxicity of propoxur to adult female German cockroaches, *Blattella germanica* (L.), was examined. With high humidity, propoxur was found to be least toxic near 25°C. At either temperature extreme (10 or 35°C), increased mortality indicated a quadratic relationship between temperature and mortality. Mortality also increased at low humidities. In contrast, the effect of temperature on the toxicity of propoxur to western spruce budworm, *Choristoneura occidentalis* Freeman, was a simple linear relationship which increased with increasing temperature. Exposure time was also related to changes in the toxicity of propoxur to the spruce budworm in both a linear and quadratic fashion. Multiple logit models were used to evaluate these multifactor toxicity experiments.

CAN ENVIRONMENTAL factors, such as temperature and humidity, be exploited in order to reduce insecticide use in the management of insect pest populations? Multifactor toxicological studies are needed to evaluate this question. To date, most studies have shown that temperature affects the toxicity of insecticides, but have dealt with temperature using single parameter (dose-response) statistical models (Blum and Kearns 1956, Chalfant 1973, Dustan 1947, Guthrie 1950, Norment and Chambers 1970, Sparks et al. 1982, Vinson and Kearns 1952). Multiple logit and probit statistical models are now being used in the analysis of multifactor studies (Robertson et al. 1981, Savin et al. 1982); they present a powerful tool in the analysis of toxicological data in relation to any number of factors.

We used multiple factor statistical models to examine the toxicity of propoxur on German cockroaches, *Blattella germanica* (L.) (GCR), in relationship to temperature and humidity and the toxicity of propoxur on the western spruce budworm larvae, *Choristoneura occidentalis* Freeman (WSB), in relationship to temperature and exposure time.

Materials and Methods

Treatments. Adult female GCR averaging 77 mg wet weight (reared on Milkbone dog biscuits at 24°C, 60 to 80% RH, in a windowed room under natural photoperiod for May) were tested with a variety of doses of propoxur (0.0625–0.8 μg per animal, at least four doses per treatment), three different temperatures (10, 23, 35°C), and two levels of humidity (high and low). A total of 19 to 20 GCR were used per treatment. Preliminary experiments showed that no mortality occurred due solely to the temperatures and humidities used. Propoxur was dissolved in acetone. Control groups treated with acetone only were exposed to each temperature and humidity treatment combination. A 4-μl amount of the insecticide solution was applied ventrally between the coxae of each GCR, with a microapplicator. The high and low humidities were maintained using distilled water (ca. 100% RH) and Drierite (ca. 20% RH), respectively, in closed containers with potassium hydroxide as a carbon dioxide absorbant. Mortality was recorded after 48 h (Ishii and Sherman 1965). Abbott's formula was used to correct for control mortality, which was less than 10% (Ashton 1972).

Sixth-instar WSB averaging 50 mg wet weight (reared on artificial diet [McMorran 1965] at 20°C, 90–100% RH, 12L:12D photoperiod) were treated with a variety of doses of propoxur (20–180 μg per animal with at least five doses per temperature), and temperatures (10, 20, and 30°C). A total of 20 larvae were used per treatment. A 2-μl amount of the insecticide was applied dorsally (near midbody), with a microapplicator. Following treatment, the larvae were placed in petri dishes with cubes of WSB artificial diet. Because of the moisture in the artifical diet of the WSB, the humidity could not be varied as in the GCR experiments. Mortality was recorded at 24, 48, 72, and 96 h on the same group of larvae. No mortality was recorded in the control groups treated with acetone.

Table 1. Toxicity of topically applied propoxur to female GCR for various combinations of temperature and humidity

Temp (°C)	Humidity[a]	LD_{50} (95% CI) (μg/animal)
10	Low	0.034 (0.026–0.045)
10	High	0.056 (0.043–0.073)
23	Low	0.111 (0.086–0.142)
23	High	0.183 (0.142–0.234)
35	Low	0.050 (0.037–0.069)
35	High	0.083 (0.061–0.113)

[a] Low and high humidities were ca. 20 and 100%, respectively.

Statistical Methods. Maximum likelihood estimates for the parameters in the multiple logit model were calculated using an iteratively, reweighted Gauss–Newton algorithm (BMDP3R) (Jennrich and Ralston 1979, Brown 1971). The final model was checked using a goodness-of-fit test (C. C. Brown's test; Brown 1971). Models involving interactions between each of the variables were tested, but the coefficients for the interactions did not differ significantly from 0 based on 95% confidence intervals. Dummy variables were used in the logit model for humidity: high humidity was coded 0 and low humidity, 1 (Netter and Wasserman 1974).

A generalized model predicting mortality in the GCR was:

$$p = b_0 + b_1(d) + b_2(t) + b_3(t^2) + b_4(h) \quad (1)$$

where p = logit proportion dead, d = ln(dose), t = temperature (°C), h = humidity coded 0 or 1, and b_0, b_1, b_2, b_3, b_4, are regression coefficients to be estimated. A rearrangement of equation 1 allowed calculation of the LD_{50} of propoxur for any combination of temperature and humidity within the range of the experimental data (logit of 50% mortality = 0):

$$LD_{50} = -[b_0 + b_2(t) + b_3(t^2) + b_4(h)]/b_1 \quad (2)$$

For a specified temperature and humidity, the associated confidence interval of the LD_{50} can be calculated using standard formulae (Ashton 1972), after reduction of the original model, equation 1, to a two variable equation and calculation of the associated variance-covariance matrix (Savin et al. 1982):

$$\text{reduced equation } p = x + b(d) \quad (3)$$

where p = logit proportion dead, $x = b_0 + b_2(t) + b_3(t^2) + b_4(h)$, t = specified temperature (°C), h = specified humidity coded 0 or 1, d = ln(dose). The variance-covariance matrix for the reduced model is as follows:

$$\begin{bmatrix} 1 & 0 & t & t^2 & h \\ 0 & 1 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} \text{original} \\ \text{variance} \\ \text{covariance} \\ \text{matrix} \end{bmatrix} \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ t & 0 \\ t^2 & 0 \\ h & 0 \end{bmatrix}$$

$$= \begin{bmatrix} \text{var } x & \text{cov}(x,b) \\ \text{cov}(x,b) & \text{var}(b) \end{bmatrix}$$

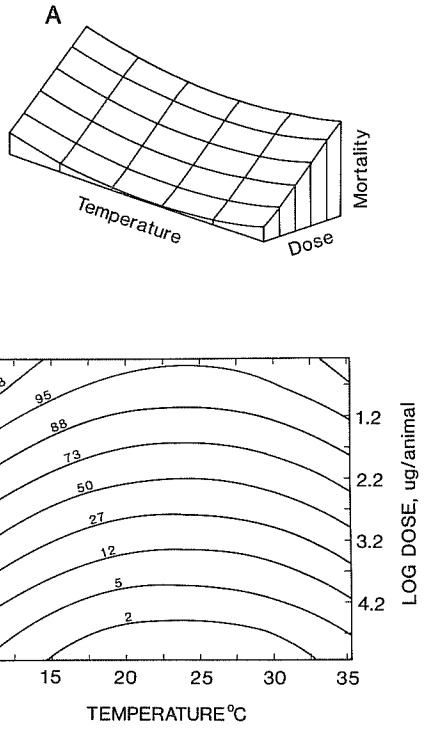

Fig. 1. (A) Response surface of equation 1, $p = 9.18 + 1.75$*(ln dose) $- 0.52$*(temp °C) $+ 0.01$*(temp^2) $+ 0.87$* (humidity), for the effect of the dose of propoxur and temperature on adult, female GCR (humidity is high; ca. 100% RH). (B) Contour plot of equation 1. Contours are the percent mortality for a given temperature and dose of propoxur (humidity is high; ca. 100% RH).

The generalized model for mortality in the WSB was:

$$p = b_0 + b_1(d) + b_2(t) \quad (4)$$

where p = logit proportion dead, t = temperature (°C), d = ln(dose). The procedures as detailed above for the GCR can then be applied to this model in the calculation of the LD_{50} values and their associated confidence intervals.

A repeated measures analysis of variance (ANOVA) model (BMDP2V) was used to analyze the effect of time on the toxicity of propoxur on WSB and determine when mortality was at its maximum. The Greenhouse–Geiser modification to the degrees of freedom in the model was used because the mortality recorded over time was on the same set of individuals (Winer 1962).

Results and Discussion

Examination of two-dimensional plots of the data by holding two of the remaining factors (temperature, humidity, or dose) constant and the fitting

Table 2. Regression coefficients for equation 4 and the asymptotic SDs for topical application of propoxur to 6th-instar WSB

Day[a]	Parameter		
	Intercept	ln(dose)	Temp (°C)
	b_0 (SD)	b_1 (SD)	b_2 (SD)
1	−8.00 (1.17)	1.27 (0.22)	0.09 (0.02)
2	−7.43 (1.04)	1.32 (0.20)	0.09 (0.02)
3	−8.54 (1.11)	1.58 (0.21)	0.12 (0.12)
4	−8.85 (1.12)	1.76 (0.22)	0.11 (0.02)

[a] Postexposure time at which mortality was recorded.

of higher-order models indicated that equation 1 provided an adequate representation of the GCR data. The results of the analysis of the GCR data indicated that low humidity increased the potency of propoxur, while temperature was correlated with mortality in a quadratic fashion (a parabolic shape). Values (SD) of the regression coefficients for each variable were b_0 (intercept, 9.18 (0.96); b_1 (ln[dose]), 1.75 (0.14); b_2 (temperature), −0.52 (0.08); b_3 (temperature)^2, 0.01 (0.002); and b_4 (humidity), 0.87 (0.24).

At the optimal temperature of the GCR, approximately 25°C (Cornwall 1968), mortality for any dose of propoxur was lowest, while at either temperature extreme, mortality increased (Table 1; Fig. 1). Thus the environmental factors, such as nonoptimal temperatures and humidities, enhanced the efficacy of the insecticide.

Similar graphical and statistical procedures as detailed above were performed on the data for the WSB and indicated that equation 4 adequately represented the data for each day separately. The results of the analysis of the WSB data indicated a simple linear relationship between temperature and toxicity of propoxur. Increasing the temperature increased the toxicity of propoxur (positive temperature coefficient, Table 3; Fig. 2). The slope value (b_1) for the relationship between the dose of propoxur and mortality increased from day 1 to day 4; hence, the LD_{50} decreased over time for a given temperature (Table 2). The trend analysis of the day effect in the repeated measures ANOVA indicated that both the linear and quadratic components were significant (linear component, $F = 50.4$, df = 1, 4, Greenhouse–Geiser modification df = 0.6, 2.4, $\alpha = 0.01$; quadratic component, $F = 15.4$, df = 1, 4, Greenhouse–Geiser modification df = 0.6, 2.4, $\alpha = 0.04$). This indicated that, by day 3, the recorded mortality had begun to stabilize and that the "true mortality" due to propoxur could be recorded on this or a later date.

Table 3. Toxicity of topically applied propoxur to WSB larvae for various combinations of temperature and time

Day	Temp (°C)	LD_{50} (95% CI) (μg/animal)
1	10	259.8 (156.5–363.1)
1	20	125.6 (95.0–55.4)
1	30	60.7 (36.0–85.8)
2	10	142.0 (123.4–161.8)
2	20	72.7 (60.9–84.5)
2	30	37.3 (26.1–48.5)
3	10	106.4 (84.0–129.4)
3	20	50.0 (41.2–58.8)
3	30	23.5 (11.2–35.8)
4	10	80.7 (63.3–98.1)
4	20	42.3 (35.1–49.5)
4	30	22.1 (15.2–29.0)

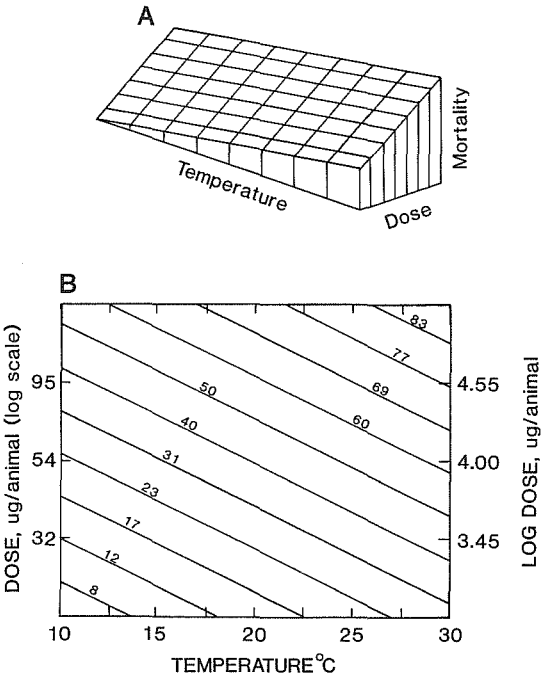

Fig. 2. (A) Response surface of equation 4, $p = -8.85 + 1.76*(\ln \text{dose}) + 0.11*(\text{temp °C})$, for the effect of the dose of propoxur and temperature on western spruce budworm larvae. (B) Contour plot of equation 4. Contours are the percent mortality for a given temperature and dose of propoxur.

The ratios of the LD_{50} values showed that, at 23°C and high humidity, as much as 5-fold more propoxur was needed to kill 50% of the GCR than at 10°C and low humidity (Table 4). Similar ratios using day 4 responses and temperature treatment combinations showed that as much as 3.6-fold more propoxur was needed to kill 50% of the WSB at 10°C than at 30°C (Table 5).

Increased toxicity at higher temperatures (positive temperature coefficient), as seen in the WSB, has been noted for organophosphates, carbamates, and some chlorinated hydrocarbon insecticides in both endo- and ectotherms, while a greater toxicity at low temperatures (negative temperature coefficient) has been noted for DDT and pyrethrins (Baetjer and Smith 1956, Blum and Kearns 1956, Chalfant 1973, Dustan 1947, Guthrie 1950, Hoffman et al. 1949, Hoffman and Linquist 1949, Meyer and Karel 1948, Norment and Chambers 1970, Sparks et al. 1982, Vinson and Kearns 1952). Increased toxicity of a carbamate insecticide at

Table 4. Relative toxicities for propoxur topically applied to female GCR for selected combinations of temperatures and humidities

		10°C		23°C		35°C	
		L	H	L	H	L	H
10°C	L	—	1.6	3.2	5.4	1.5	2.4
	H	0.6	—	2.0	3.2	0.9	1.5
23°C	L	0.3	0.5	—	1.6	0.4	0.7
	H	0.2	0.3	0.6	—	0.3	0.4
35°C	L	0.7	1.1	2.2	3.6	—	1.6
	H	0.4	0.7	1.3	2.2	0.6	—

Ratios were calculated using LD_{50} values from Table 1; LD_{50} (dose units) for environmental condition in the horizontal heading divided by LD_{50} for condition in the vertical heading. Low (L) and high (H) humidity were ca. 20 and 100% RH, respectively. The intersection of 23H and 10L is 5.4, indicating that approximately 5.4-fold more propoxur is needed at 23°C, high humidity than at 10°C, low humidity to cause 50% mortality.

both high and low temperatures, as reported herein for propoxur on the GCR, may also have occurred in crickets, *Gryllus pennsylvanicus* Burmeister, treated with carbaryl, though no formal test of this hypothesis was conducted by the authors (Harris and Kinoshita 1977). The interactions between temperature and dose of a pesticide are complex and involve penetration, distribution, excretion, metabolism, and action of the pesticide or other temperature dependent toxins in the insect's body (Busvine 1971, Hayes 1975). We found that the toxicity profile of propoxur differs in two insect species when temperature is varied. Consequently, broad generalizations concerning the effects of temperature on the toxicity of carbamate insecticides to insects should be deferred until additional data are acquired with other species to determine if the cricket (Harris and Kinoshita 1977) and the GCR are infrequent exceptions or if the positive temperature coefficient of carbamates should be questioned.

The results of multifactor toxicological tests in the laboratory may provide the basis for reducing the quantities of insecticide used in field applications. If an insecticide could be applied any time during a range of dates or time of day, then for the GCR, either high or low temperatures might be chosen to increase the killing efficiency of propoxur. However, the potency of propoxur applied as a residual at low temperatures may be adversely affected by the reduced physiological activity of GCR and their contact with the treated surface at cooler temperatures. The mode of exposure in our experiment (topical application) may not accurately reflect the tarsal contact toxicity of propoxur at reduced temperatures.

In contrast to the GCR, high temperatures might enhance the toxicity of chemical such as propoxur to the WSB. However, further studies will be necessary to determine the effects of temperature on the toxicity of chemicals actually used for WSB control.

The information from laboratory and field studies could then be incorporated into integrated pest management programs (Croft et al. 1976, Jones 1977, Valentine et al. 1976) to optimize the cost effectiveness of insecticide application, yet manage insect pests with decreased concentrations of insecticides in the environment.

Table 5. Relative toxicities for propoxur topically applied to WSB larvae

	10°C	20°C	30°C
10°C	—	0.5	0.3
20°C	1.9	—	0.5
30°C	3.6	1.9	—

Ratios of the LD_{50}s recorded 4 days postexposure for selected temperatures. For further details, see Table 4 legend.

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