

Sublethal Effects of Chlorpyrifos-Methyl on Reproduction in Female German Cockroaches (Dictyoptera: Blattellidae)

ROBERT L. HAMILTON AND COBY SCHAL

Department of Entomology,
Cook College, Rutgers University,
New Brunswick, New Jersey 08903

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ABSTRACT Longevity and lifetime fecundity were significantly reduced after topical application of LC₁₀, LC₂₀, and LC₆₀'s of chlorpyrifos-methyl to 2-d-old female German cockroaches, *Blattella germanica* (L.). Low concentrations (LC₁₀ and LC₂₀) often resulted in failure of the first ootheca to hatch. This effect is temporary, and ≥90% of subsequent oothecae hatched normally. Application of an LC₆₀ also resulted in a high percentage of abortions of first oothecae, but also caused >15% inviability in subsequent oothecae.

KEY WORDS Insecta, *Blattella germanica*, chlorpyrifos, reproduction

MOST STUDIES pertaining to effects of insecticides have concerned their acute toxicity to insects and related arthropods. Until recently, sublethal effects have received comparatively little attention. In most cases, female fecundity is reduced after exposure to sublethal doses of insecticides; in males, mate-finding and courtship appear to be readily affected (see reviews by Moriarty [1969] and Haynes [1988]).

The German cockroach, *Blattella germanica* (L.), is a major pest of economic importance. Current control strategies rely heavily upon various formulations of insecticides such as toxic baits and liquid applications to surfaces. Insects may receive sublethal doses of insecticide with either of these approaches. However, the effects of sublethal doses on reproduction in either males or females have not been well studied. Exposure to carbamate or organophosphate insecticides or their vapors causes some gravid female German cockroaches to drop their oothecae prematurely (Parker & Campbell 1940; Harmon & Ross 1987, 1988), which can result in failure to hatch. Cochran (1985) showed that feeding females low concentrations (<6 ppm) of an unrelated insecticide, avermectin B₁, resulted in a high percentage of the females failing to mate or produce oothecae.

Effects of administration of sublethal amounts of organophosphate or carbamate insecticides to female cockroaches before they produce oothecae have not been studied. Our study was done to investigate effects of a single sublethal concentration of an organophosphate insecticide, chlorpyrifos-methyl, applied soon after adult emergence, on longevity and lifetime fecundity of female German cockroaches.

Materials and Methods

Insects used in this study were from an established susceptible colony of German cockroaches

(VPI normal strain) maintained at 27°C with a photoperiod of 12:12 (L:D). Commercial dog food pellets and water were provided ad lib. Each newly emerged female was held in a filter paper-lined Petri dish with food and water for 48 h before treatment with insecticide. To minimize biological variation, females were individually weighed on the day of emergence and randomly assigned among treatments.

A preliminary concentration-response assay was done on 2-d-old females to estimate concentrations with sublethal effects. Technical chlorpyrifos-methyl (99%; The Dow Chemical Company, Midland, Mich.) was dissolved in acetone; 2 μl was topically applied with an Isco model M microapplicator (Instrumentation Specialties Company, Lincoln, Nebr.) between the mesothoracic legs of each cockroach. Thirty females were treated with each of four doses ranging from 0.8 to 1.6 μg per female. Control females (n = 42) were treated with 2 μl of acetone only. Mortality was evaluated 48 h after insecticide treatment (day 4).

Concentrations of insecticide, estimated from probit analysis (SAS Institute 1985) as the LC₁₀ (0.8 μg), LC₂₀ (1.0 μg), and LC₆₀ (1.4 μg), were topically applied to enough isolated 2-d-old females to yield 30-40 survivors in each treatment. Based upon actual survivorship 7 d after treatment, the amounts applied were the LC₁₂ (n = 30), LC₁₉ (n = 47), and LC₆₀ (n = 39). Two days after insecticide treatment, each surviving female was confined with two males (replaced as necessary) for her entire lifetime; however, mating was not specifically monitored. Females and oothecae were examined daily for reproductive events and survival. Timing of oothecal production and nymphal hatching, as well as numbers of hatched nymphs, eggs per ootheca, and longevity, were recorded. Data were analyzed with analysis of variance and Duncan's multiple range procedures (P = 0.05; SAS Institute 1985).

Table 1. Longevity and lifetime fecundity of *Blattella germanica* females after treatment with sublethal concentrations of chlorpyrifos-methyl

	Treatment			
	Acetone	Chlorpyrifos-methyl		
		LC ₁₀	LC ₂₀	LC ₆₀
Longevity (days)	176 (5.7) ^a	153 (8) ^b	158 (5.7) ^b	154 (4.6) ^b
Total oothecae per female	5.2 (0.2) ^a	4.6 (0.2) ^b	4.9 (0.2) ^{ab}	4.7 (0.1) ^b
Total nymphs per female	163 (5.4) ^a	128 (9.0) ^b	139 (6.7) ^b	134 (7.9) ^b
Age at hatch of first viable ootheca (days)	32 (0.3) ^a	32 (0.3) ^a	33 (0.4) ^a	33 (0.4) ^a
Age at drop of first nonviable ootheca (days)	10	31 (2.4) ^a	26 (3.6) ^a	31 (2.3) ^a

All values are $\bar{x} \pm \text{SEM}$; means within rows followed by the same letter are not significantly different ($P \geq 0.05$; Duncan's multiple range test [SAS Institute 1985]).

Results and Discussion

Lifespans of females that survived treatment with all concentrations of chlorpyrifos-methyl were significantly shorter than those of controls (Table 1). A significant reduction in the number of oothecae per treated female (Table 1), as well as a large percentage of nonviable first ootheca and in some cases, subsequent oothecae (Fig. 1), resulted in significantly lower lifetime fecundity in treated females (Table 1). Days to hatch of viable oothecae (defined as any ootheca that hatched, regardless of the number of nymphs emerging) was similar among all treatments (Table 1), as was percentage hatch (nymphs hatched per number of eggs) of viable oothecae (Table 2). Cochran (1985) also found that nymphal production per ootheca was similar in controls and in females fed avermectin. Progeny of insects fed avermectin produced more nymphs per ootheca and exhibited greater percentage hatch than control females (Cochran 1985). Hamilton & Schal (1988) showed that high protein diets affect reproductive parameters of female *B. germanica*, including the size of oothecae but not percentage hatch.

Nonviable oothecae of females treated with chlorpyrifos-methyl were carried full-term (to about day 30) before being dropped. The one control female that aborted her first ootheca did so on day 10 (Table 1). In control females, viability of oothecae was very high through the production of three oothecae and decreased to approximately 90% in

Table 2. Percentage hatch of viable oothecae of females treated with acetone and various concentrations of chlorpyrifos-methyl^a

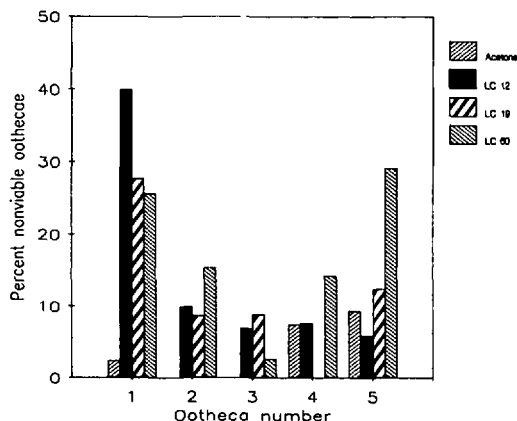
Ootheca no.	Treatment			
	Acetone	Chlorpyrifos-methyl		
		LC ₁₀	LC ₂₀	LC ₆₀
1	92.2 \pm 1.2	94.1 \pm 1.2	92.8 \pm 1.2	90.3 \pm 1.6
2	89.1 \pm 1.9	86.5 \pm 3.9	90.2 \pm 1.5	91.7 \pm 0.9
3	89.6 \pm 1.7	87.3 \pm 2.7	91.1 \pm 1.3	89.8 \pm 2.2
4	90.2 \pm 1.6	92.1 \pm 1.7	87.4 \pm 2.2	90.2 \pm 1.5
5	87.9 \pm 1.7	92.1 \pm 1.6	90.0 \pm 1.5	84.4 \pm 3.8

^a Percentage hatch \pm SEM was calculated by dividing the number of hatched nymphs by the number of eggs in the ootheca.

the fourth and fifth oothecae (Fig. 1). A similar pattern of increased inviability of later oothecae was previously documented in the German cockroach (Willis et al. 1958).

Low concentrations of chlorpyrifos-methyl had a transient effect on reproduction and viability of oothecae. Between 25 and 40% of all treated females failed to hatch their first ootheca, but $\leq 10\%$ of subsequent oothecae failed to hatch when females were treated with an LC₁₀ or LC₂₀ (Fig. 1). This effect may be mediated either through mating or events after mating (e.g., fertilization). If insecticide treatment suppressed mating for approximately 10 d, the virgin females would oviposit an infertile first ootheca. After dropping this infertile ootheca, females could mate, and subsequent oothecae would be fertile. However, in our colonies, most virgin females abort their infertile ootheca within 3 d of formation. Because retention times of nonviable first ootheca did not differ significantly (Table 1), these data suggest that most females treated with chlorpyrifos-methyl probably had mated and that the sublethal effects of the insecticide were expressed through events such as fertilization, ovulation, or egg case formation that occur after mating.

Application of the LC₆₀ resulted in fewer nonviable first oothecae than in the LC₁₀ (Fig. 1), per-

**Fig. 1.** Percentage nonviable oothecae of female German cockroaches after treatment with various dosages of chlorpyrifos-methyl.

haps as a result of selection by acute toxicity of more hardy females. Fifty percent of nonviable first oothecae were dropped before day 20 (i.e., approximately 8–10 d after formation), suggesting that some of these females had failed to mate and therefore aborted their first ootheca. However, unlike females treated with lower concentrations, females surviving the LC₆₀ continued to produce more nonviable oothecae (≥15%). Most of these females produced at least one viable ootheca. An occasional viable ootheca was preceded and followed by an infertile ootheca, indicating that lack of mating was only partially responsible for production of nonviable oothecae. This, combined with our observation that nonviable oothecae were not consistently produced by the same few females, further suggests that the insecticide interfered with events after mating.

Treatment with either LC₁₀ or LC₉₀ chlorpyrifos-ethyl did not affect viability of the first and subsequent oothecae when compared with controls (data not presented). Apparently, mating and oothecal production were not affected in females that survived treatment with chlorpyrifos-ethyl. Longevity was unaffected in females that survived treatment with the low concentration of chlorpyrifos-ethyl (174 d) but was significantly shortened (135 d) in females surviving the high concentration. Thus, these two closely related chemicals (chlorpyrifos-ethyl and chlorpyrifos-methyl) apparently have different sublethal effects on females: Chlorpyrifos-methyl reduces lifespans and viability of oothecae, whereas chlorpyrifos-ethyl does not affect viability of oothecae but reduces longevity at high sublethal doses.

Our observation that even low concentrations of chlorpyrifos-methyl can drastically affect reproduction of female German cockroaches merits further study. Previous reports (Parker & Campbell [1940], Harmon & Ross [1987, 1988]) indicate that insecticide treatment of gravid female German cockroaches causes abortion of the egg case. Our results indicate that topical application of sublethal concentrations of chlorpyrifos-methyl shortly after adult emergence may have long-lasting effects on

reproduction and may reduce lifetime fecundity significantly.

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