Laboratory Evaluation of the Effects of Carbaryl and Chlorpyrifos Bran Baits and Sprays Used in Grasshopper Control, on Alfalfa Leafcutting Bees (*Megachile rotundata* [F.])[1]

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J. Agric. Entomol. 9(2):109-115 (April 1992)

ABSTRACT Caged populations of alfalfa leafcutting bees, *Megachile rotundata* (F.), were exposed to alfalfa plants treated with spray or wheat-bran formulations of carbaryl or chlorpyrifos. Carbaryl bran bait had no significant impact on male or female bees. Bee mortality from chlorpyrifos was greater than from carbaryl, and occurred sooner. Mortality from spray formulations of insecticide was greater than mortality from insecticide-treated bran baits. Male bees were more susceptible to these toxicants than were female bees. The results indicate that a grasshopper control program using carbaryl bran bait would have no significant impact on leafcutting bee populations.

KEY WORDS *Megachile rotundata* (F.), bran bait, nontarget, carbaryl, chlorpyrifos, pollinator, Acrididae, Hymenoptera, Megachilidae.

The lack of suitable native pollinators in western Canada necessitates a heavy reliance on the introduced alfalfa leafcutting bee, *Megachile rotundata* (F.), to pollinate alfalfa seed crops (Richards 1984). The use of contaminated leafcuttings to construct nests makes this insect particularly susceptible to the toxic effects of insecticide sprays (N.R.C.C. 1981).

Recommendations to reduce the effects of insecticides on bees include delaying bee emergence until after insecticide application (and until after residues dissipate to a safe level), moving the bees or covering their shelter during application, or spraying the insecticide when the bees are inactive (Anderson and Atkins 1968, Johansen 1982). In spite of these precautions, however, the heavy reliance of the alfalfa seed industry on insecticides[3] for pest control makes the periodic exposure of bees to insecticide unavoidable. Thus, an insecticide or formulation that provides acceptable levels of pest control, yet is safe for pollinators, is required.

Bran bait formulations of insecticide have been proposed to control target acridid populations, while being relatively harmless to nontarget fauna. Carbaryl (Foster et al. 1979, Onsager et al. 1980a,b, Johnson and Henry 1987, Johnson et al. 1987a), Quinn et al. 1989), chlorpyrifos (Johnson 1986, Johnson et al. 1987a), and dimethoate (Mukerji et al. 1981, Johnson and Henry 1987) bran baits all have been shown to provide significant levels of grasshopper

[1] Received for publication 18 March 1991; accepted 14 October 1991. L. R. S. Contribution No. 3879057.

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[3] In 1986, an estimated 69,100 L of Sevin XLR (carbaryl), 60,000 L of Lorsban (chlorpyrifos), 42,000 L of Cygon (dimethoate), and 15,000 L of Hopper Stopper (dimethoate bran bait) were sprayed in Alberta for acridid control on alfalfa seed and other crops (D. Johnson and M. Dolinski, Alberta Agriculture, unpublished data).

control. The lower concentrations of active ingredient and reduced degree of drift makes baits safer than sprays for pollinators. The impact of bran bait on pollinators should be further reduced because pollinators would be expected to contact a properly formulated bait less frequently than would targeted acridids. In spite of this, few bran bait-pollinator studies have been performed. Using various combinations of dimethoate and carbofuran-treated vermiculite and styrofoam baits, Charnetski and Hobbs (1974) found no apparent effect on leafcutting bees. Mukerji et al. (1981) noted that leafcutting bees were unaffected by a field application of a variety of bran baits, even though the bees were foraging at the time of application. As these studies were anecdotal only, a more rigorous testing procedure was designed to examine the effect of unregistered formulations of bran bait (carbaryl and chlorpyrifos) on leafcutting bees. The objective of this study was to determine the relative toxicity of the insecticides carbaryl and chlorpyrifos in bait and spray formulations to male and female leafcutting bees, when applied at dosage rates recommended for grasshopper control.

Materials and Methods

Alfalfa leafcutting bee prepupae were acquired from the Agriculture Canada Research Station, Lethbridge, Alberta, and reared according to the methods of Richards (1984). Prepupae were stored over the winter at 5°C, and then incubated at 30°C and 70% R.H. in compartmentalized incubation trays. Newly emerged male and female bees were added randomly to ten 1.3-m^3 fiberglass cages and housed within greenhouse facilities maintained at 25 ± 4°C and 50 ± 10% R.H. The greenhouse was supplemented with fluorescent (high output, 105 W) and incandescent (300 W) lighting to allow the bees to continue activity in the absence of sunlight. Artificial lighting was used a total of 57 h, 40% of the duration of the experiment. Each cage contained three 2-liter pots of flowering alfalfa (*Medicago sativa* L. cv. Beaver) with seven plants per pot. Six 28-ml vials with cotton wicks, containing a 50:50 mixture of liquid honey and distilled water, were placed on the top of each cage as a food supplement for the bees. No nest tubes or additional sources of water were provided.

Initially, 40 male and 40 female newly emerged adult bees were added to each of 10 cages. The populations were allowed to stabilize for 3 d, during which time any bees that died were removed and replaced with live bees. Cages were arranged in a randomized complete block design, with two complete replications of the experiment. Each experimental block contained five treatments: a control (no bait or spray), 0.33 g of 5% carbaryl bait, 0.33 g of 3% chlorpyrifos bait, 4 ml of carbaryl spray (A.I. = 0.22 g), and 4 ml of chlorpyrifos spray (A.I. = 0.06 g). Alfalfa plants were sprayed with a hand-held aspirator at a pressure of 70 kPa.

A 5% (A.I. by weight) carbaryl bait was formulated as described by Johnson and Henry (1987), and a 3% (A.I. by weight) chlorpyrifos bait was formulated as described by Johnson (1986). The baits were manually sprinkled on the alfalfa foliage in a single dose. The bran bait rates were equivalent to 2.5 kg/ha, rates that provide effective acridid control (Johnson 1986, Johnson et al. 1987a,b). Spray rates corresponded to the recommended rate for adult grasshoppers on barley or wheat crops of 877 ml/ha for chlorpyrifos (Lorsban 4E, Dow Chemical Canada Inc., Sarnia, Ontario; Jones 1991), and 3459 mL/ha for carbaryl (Sevin XLR, May and

Baker Canada Inc., Mississauga, Ontario; Jones 1991). The bees remained in the cages until death or 13 d after insecticide application.

Statistical Analyses. Survival rates for the two different trials were transformed using an arcsin procedure (Southwood 1978), and analyzed using the General Linear Model (GLM) ANOVA procedure of SAS (SAS Institute 1985). Data from the two replicates for each trial were pooled as there were no significant replicate by trial or treatment by trial interactions. The analysis was run as a weighted measures analysis to correct for the unequal numbers of male and female bees and variable levels of attrition. Orthogonal contrasts were performed for males, females, and gender differences on a daily basis to determine differential insecticide, formulation (bran bait versus spray), and insecticide by formulation relationships. A least-squares means analysis was conducted to determine significant relationships among treatments.

Results

Chlorpyrifos, in bait and spray formulation, had a greater impact on leafcutting bee survival than did carbaryl. Male bee mortality from chlorpyrifos (bait or spray) was significantly higher than from carbaryl (bait or spray). This trend was significant for Days 1-13 (Day 1: $F = 52.50$, df = 1, $P < 0.001$). Female bee survival was higher in the carbaryl bait treatments than in the chlorpyrifos spray treatments. This trend was significant for Days 3-5 (Day 3: $F = 4.42$, df = 1, $P < 0.05$). Carbaryl bait had no significant effect on male or female bee survival during the entire experiment.

Male bees incurred significantly greater mortality than did female bees in the chlorpyrifos spray treatment from Days 1-13 (Day 1: $F = 27.59$, df = 1, $P < 0.001$) and in the chlorpyrifos bait treatment from Days 2-3 (Day 2: $F = 20.51$, df = 1, $P < 0.001$; Table 1, Days 1-4 presented, Fig. 1).

Male bee mortality in the chlorpyrifos bait treatment was significantly greater than in the chlorpyrifos bait treatment from Days 1-3 (Day 1: $F = 18.69$, df = 1, $P < 0.001$; Table 1, Fig. 1). In contrast to chlorpyrifos, carbaryl spray did not reduce male survival significantly more than did carbaryl bait for days 1-9, but did for days 10-13 (Day 10: $F = 40.03$, df = 1, $P < 0.001$; Table 2, Fig. 1).

Table 1. Percent survival of male *Megachile rotundata* exposed to chlorpyrifos bait versus chlorpyrifos spray (Days 1-4).

	Day[a,b]			
Treatment	1	2	3	4
Bait	80.8	55.0	36.0	30.6
Spray	22.7***	12.8**	11.4*	10.1 NS
Control	100.00	96.1	92.1	92.1

[a] ***$P < 0.001$, ** $P < 0.01$, * $P < 0.05$, NS = no significant effect.
[b] No significant effects were observed after day 4.

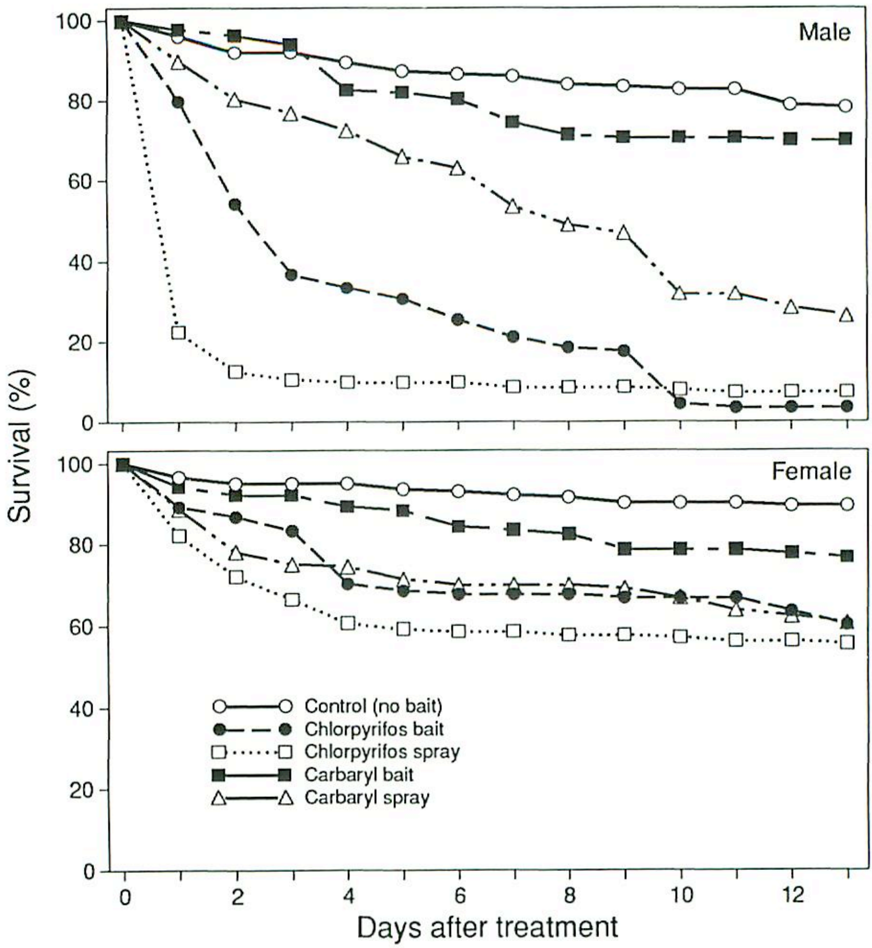

Fig. 1. Percent survival of male and female *Megachile rotundata* after exposure to chlorpyrifos and carbaryl bran baits or sprays.

Discussion

Bee mortality was greater and earlier in the chlorpyrifos treatments than in the carbaryl treatments. While most of the mortality in the chlorpyrifos treatments (bait and spray) occurred within the first 3 d of exposure, mortality from carbaryl spray occurred very gradually until 10 d after the initial exposure, and then increased. These patterns are typical of bee poisoning found after application of these two insecticides. Chlorpyrifos has been classified as a hazard to bees in the field 2-3.5 d after application (N.R.C.C. 1981). Carbaryl does not

Table 2. Percent survival of male *Megachile rotundata* exposed to carbaryl bait versus carbaryl spray (Days 9-13).

	Day[a,b]				
Treatment	9	10	11	12	13
Bait	74.1	73.6	73.6	72.7	72.6
Spray	48.7 NS	31.4**	31.3**	27.4**	24.6**
Control	90.2	90.2	90.2	89.5	89.5

[a] ** $P < 0.01$, NS = no significant effect.
[b] No significant effects were observed prior to day 9.

degrade as quickly as chlorpyrifos, and is classified as hazardous to bees in the field 3-7 d after application (N.R.C.C. 1981). A greater proportion of the bees was able to withstand the effects of the less toxic carbaryl over the short term. However, as carbaryl does not break down as rapidly as chlorpyrifos, carbaryl-treated bees were effectively exposed to toxic residues for a longer period.

Bee mortality was lower in the bran bait treatments than in the spray treatments, regardless of which insecticide was used. We conclude that although the bees were initially exposed to the bran bait toxicants, they did not receive a long-term exposure. Bran bait coverage of the foliage was intermittent and short-lived because any bait that was intercepted by the alfalfa soon fell to the cage bottom, resulting in lower effective exposure to the bees. The chlorpyrifos bait was toxic enough to cause high mortality within the first 3-4 d of exposure, whereas the bees were able to tolerate the lower toxicity of the carbaryl bait for the short time they were exposed to it.

The spray treatments provided a more thorough and longer lasting coverage of the alfalfa foliage, with a higher concentration of active ingredient. Leafcutting activity was evident, and thus, contact of the bees with this foliage probably gave them a higher dermal and oral exposure to the insecticides. Contaminated nectar and pollen may also have contributed to insecticide exposure and increased mortality.

In the chlorpyrifos treatment, male bees experienced significantly higher mortality rates than did females. This may result in part from the lesser efficiency with which male leafcutting bees deactivate insecticides (Guirguis and Brindley 1975). The greater susceptibility of male leafcutting bees to insecticides also may be related to their smaller body mass (Klostermeyer et al. 1973, Rothschild 1979) and consequent greater surface to volume ratio.

Bee mortality in the field from carbaryl bait might be expected to be lower than that observed in these cage trials. The toxicity of the bait could be reduced within a short period of time as a result of weather conditions. In addition, as leafcutting bees generally do not forage within a crop canopy (N.R.C.C. 1981), bait dislodged by wind from the canopy would be lost to potential contact with the bees. Although the present findings were obtained in the laboratory, they suggest that an acridid-control program utilizing bran bait formulation of carbaryl may have no significant impact on resident populations of leafcutting bees. A similar program using chlorpyrifos bran bait or spray, however, could be expected to seriously affect leafcutting bee populations.

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