The Toxicity of Bran Baits, Formulated with Carbaryl, Chlorpyrifos and Dimethoate, on Yellow Mealworms (*Tenebrio molitor* L.)[1,2]

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ABSTRACT Laboratory feeding trials were conducted to assess the comparative impact of registered and new unregistered insecticide-treated bran baits on nontarget epigeal arthropods. Yellow mealworm beetles and larvae (*Tenebrio molitor,* L.) (a surrogate, surface-dwelling tenebrionid) were individually housed in containers and exposed to five different dosages (quantities) of bran bait treated with three different insecticides. Response to the baits (mortality in beetles and moribundity in larvae) was greatest in the chlorpyrifos bait treatment, and progressively lower in the dimethoate and carbaryl treatments. Mealworm beetles and larvae showed a probit response to increasing dosages of carbaryl and dimethoate bran bait as evidenced by the sigmoidal shape of their mortality curves (i.e., increasing dosages of bran bait corresponded to increased responses). The mortality curves for yellow mealworms exposed to these same dosages of chlorpyrifos bait lacked the characteristic sigmoidal shape and were low-sloped. Feeding trials with chlorpyrifos bait at low enough dosages to produce a sigmoid response were not conducted. It is postulated that field populations of tenebrionids could be negatively affected by the use of chlorpyrifos bait to control grasshopper populations.

KEY WORDS *Tenebrio molitor* L., bran bait, nontarget, carbaryl, dimethoate, chlorpyrifos, Coleoptera, Tenebrionidae.

The widespread use of insecticides by the agricultural industry makes the periodic exposure of nontarget organisms to these insecticides unavoidable. The increasing body of literature examining impact of insecticides on nontarget organisms (Ripper 1956, Newsom 1967, Pimentel 1971, McEwen et al. 1972, Ware 1980, Brandenburg 1985) attests to the perceived need for agents or formulations that provide suitable levels of pest control, and yet spare nontarget invertebrates.

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Bran bait formulations of insecticides have been suggested as such agents, and have proven to be efficient methods of pest control. Carbaryl (Foster et al. 1979, Onsager et al. 1980, Johnson 1986, Johnson and Henry 1987, Johnson et al. 1987, Quinn et al. 1989), chlorpyrifos (Johnson 1986, Johnson et al. 1987, Boetel et al. 1989a,b), and dimethoate (Mukerji et al. 1981, Johnson and Henry 1987) bran baits have all been shown to provide significant levels of grasshopper control. These insecticides are commonly used in spray formulations to reduce the damage caused by grasshopper infestations of cereal crops and grasslands. In 1986, an estimated 69,100 liters of Sevin XLR (carbaryl), 60,000 liters of Lorsban (chlorpyrifos), 42,000 liters of Cygon (dimethoate), and 15,500 kg of Hopper Stopper (dimethoate bran bait) were applied in Alberta for grasshopper control (D. Johnson and M. Dolinski [Alberta Agriculture], unpublished data). Presently in Canada, only dimethoate is registered in a bran bait formulation for grasshopper control. Studies of the potential impact on nontarget organisms are required prior to the registration of new bran baits.

Bran baits' lower concentrations of active ingredients and reduced rate of drift result in lower direct contact and less residue buildup on crops relative to spray formulations, factors which make these baits safer for most nontarget organisms. In addition, the impact of bran bait is greatest on those organisms which actively feed on it (i.e., grasshoppers). A consequence of this, however, is that organisms with feeding habits similar to those of grasshoppers are also affected. Such organisms include herbivorous species of beetles.

Quinn et al. (1990) noted that field populations of darkling beetles were reduced by 59% after exposure to carbaryl bait. McColloch (1918) determined that adult *Eleodes tricostata* Say (a tenebrionid) were susceptible to poisoned baits, but the larvae were not. George et al. (1992) noted that field populations of Coleoptera had not returned to pretreatment population densities 1 yr after exposure to carbaryl bran bait.

With over 1000 species in North America (Doyen and Tschinkel 1974), the Tenebrionidae family is prevalent in many different environments. The surface-dwelling members of this beetle family are detritivores (Arnett 1986), and thus are the group of nontarget insects most likely to be negatively affected by grasshopper control measures using bran bait.

The objective of the current research was to compare the effects of various levels of registered and unregistered bran baits on the survival of yellow mealworms *(Tenebrio molitor* L.), a nontarget tenebrionid species.

Materials and Methods

Mealworm beetles and larvae were individually housed in containers in the laboratory and exposed to bran bait treated with carbaryl, dimethoate, or chlorpyrifos insecticide, each at five dosages. The insects were reared at 20°C and 50% R.H. in 2-liter mason jars containing a mixture of four parts each of yellow cornmeal, wheat bran, wheat germ, white flour, and one part brewer's yeast (modified from Cotton 1956).

Separate trials were conducted for beetles and larvae. For the beetle trial, one newly-emerged adult beetle ($\leq$ 1 wk old) was placed in each of 200 50-ml plastic containers in a randomized complete block design. Each of the 10 blocks contained 20 treatments. The treatments consisted of five dosages of bran bait (0.007, 0.018, 0.050, 0.135, and 0.370 g), of each of three baits used, 4% carbaryl bait (A.I. by weight), 5.3% dimethoate bait, and 3% chlorpyrifos bait as well as five controls (no bait). These levels corresponded to a field rate of 5 kg/ha (0.007 g), and 2.5, 7, 19, and 53 times this rate. After 24 h, 10 g of a soil/peatmoss mixture (50:50), 2 g of the rearing medium (food), a No. 1 filter paper (refuge), and a small piece (approx. 1 g) of carrot were placed in each container to provide moisture and food. Additional carrot pieces were added every 3 d if necessary. Daily assessments were made of the number of beetles dead, moribund (limited response to probe stimulation), and unaffected by the bait (typical reaction to probe stimulation). The experiment was terminated after 22 d, at which time no further mortality due to the treatments was evident.

The larvae trial followed the same experimental design, procedure, and feeding regime as the adult trial, using larvae with a mean weight of 0.14 g (SD = 0.02). The experiment was terminated after 17 d.

Bran bait formulation. Commercial formulations of carbaryl (Sevin XLR, Rhone Poulenc Canada Inc., Montreal, Quebec), chlorpyrifos (Lorsban 4E, Dow Chemical Canada Inc., Sarnia, Ontario), and dimethoate (Cygon 480, Cyanamid Canada Inc., Markham, Ontario) were used to prepare the baits. Bran bait formulations of these insecticides were 4% carbaryl, 3% chlorpyrifos, and 5.3% dimethoate (A.I. by weight), which are concentrations typically used in grasshopper control studies (Johnson et al. 1987, Boetel et al. 1989a,b, Quinn et al. 1989). The carbaryl bait was formulated as described by Johnson and Henry (1987), and the chlorpyrifos bait was formulated as described by Johnson (1986). Commercially available dimethoate bran bait was used ("Hopper Stopper" - Peacock Industries, Saskatoon, Saskatchewan).

Statistical analyses. Data were analyzed using the S108-Multiline Quantal Bioassay computer program (Morse et al. 1987). The program fits dose-response regression equations to quantal data, using maximum likelihood estimation. The regression allows for estimation of the effective dose (ED), i.e., the dose necessary to precipitate a response (lethal or sublethal) in a specified proportion of the treated individuals (cf. Morse et al. 1987). The program also calculates a common regression line which is used to test the hypothesis that the individual probit lines are identical. If the probit lines for the treatments differ, tests are performed to determine if the difference is due to the intercepts, the slopes, or both (Finney 1971). Mealworm responses were analyzed cumulatively (referred to subsequently as 5 d, 10 d, and 15 d cumulative responses).

Plots of the observed percentage responses against $\log_{10}$(dose) of bait quantity, together with the curve of expected values (as calculated by the maximum likelihood regression of probit response on transformed bran bait doses) were generated using SAS (SAS Institute 1985).

Results

Bait had a lethal effect on beetles, with very few observed in moribund states and a significant number being affected within hours of exposure to the baits.

Larvae, on the other hand, remained moribund for many days with few dying within the time-frame of the experiment. Data analysis was, therefore, conducted on time-to-death for beetles, and time-to-moribundity for larvae.

Slopes of mortality curves for carbaryl and dimethoate in beetles were statistically indistinguishable on days 5 and 10. The mortality data for both compounds were therefore pooled on each day and common mortality curves generated. The slopes of the common mortality curves for beetles treated with carbaryl and dimethoate were calculated to be 2.60 and 3.46 for the 5 d and 10 d mortality responses, respectively (Table 1). The 5-d and 10-d common response curve slopes for larvae moribundity were 0.97 and 2.67, respectively (Table 1). The response curves for the beetles and larvae are presented in Figs. 1 and 2, respectively.

Table 1. Probit lines, significance tests and effective doses for yellow mealworm beetles and larvae.[a]

Day	Treatment	Probit Model (y=)	SE (slope)	X^2	(df)	ED_{50}	ED_{95}
			Adults				
1-5	Carbaryl	2.041 + 2.284 (x)	0.584	0.807	(3)	0.128	0.671
	Dimethoate	2.770 + 3.061 (x)	0.807	0.300	(3)	0.125	0.429
	Common[b]	2.345 + 2.605 (x)	–	0.012	(1)	0.126	0.539
1-10	Carbaryl	5.644 + 4.998 (x)	1.523	0.015	(3)	0.074	0.159
	Dimethoate	4.166 + 3.104 (x)	0.764	0.309	(3)	0.046	0.154
	Common[a]	4.288 + 3.457 (x)	–	3.183	(1)	0.058	0.172
1-15	Carbaryl	5.212 + 4.453 (x)	1.328	0.089	(3)	0.068	0.158
	Dimethoate	5.801 + 3.711 (x)	0.994	0.412	(3)	0.027	0.076
	Common[b]	6.217 + 3.977 (x)	–	0.203	(1)	0.043	0.141
			Larvae				
1-5	Carbaryl	0.428 + 1.651 (x)	0.700	2.402	(3)	0.550	5.456
	Dimethoate	0.539 + 0.880 (x)	0.341	5.094	(3)	0.244	18.090
	Common[c]	0.314 + 0.974 (x)	–	1.113	(1)	0.475	23.270
1-10	Carbaryl	1.145 + 2.128 (x)	0.726	2.335	(3)	0.290	1.718
	Dimethoate	3.751 + 3.864 (x)	1.080	7.465	(3)	0.107	0.285
	Common[b]	2.617 + 2.668 (x)	–	2.223	(1)	N/A	N/A

[a] N/A = not enough intermediate responses for analysis; ED = effective dose required to achieve 50% or 95% response; x = log dose.

[b] = identical lines (same slope $P > 0.05$, same intercept $P > 0.05$).

[c] = parallel lines (same slope $P > 0.05$, different intercepts $P < 0.05$).

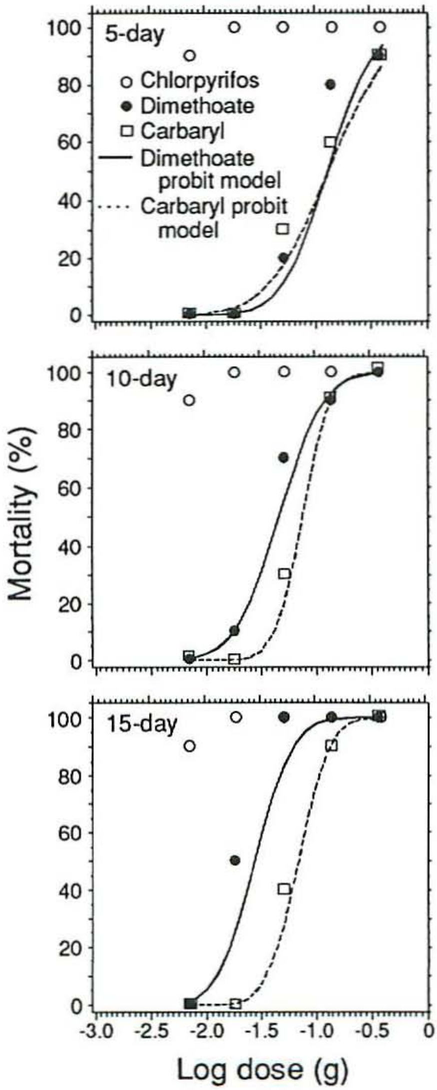

Fig. 1. Mortality response of adult beetles to chlorpyrifos, dimethoate, and carbaryl bran baits.

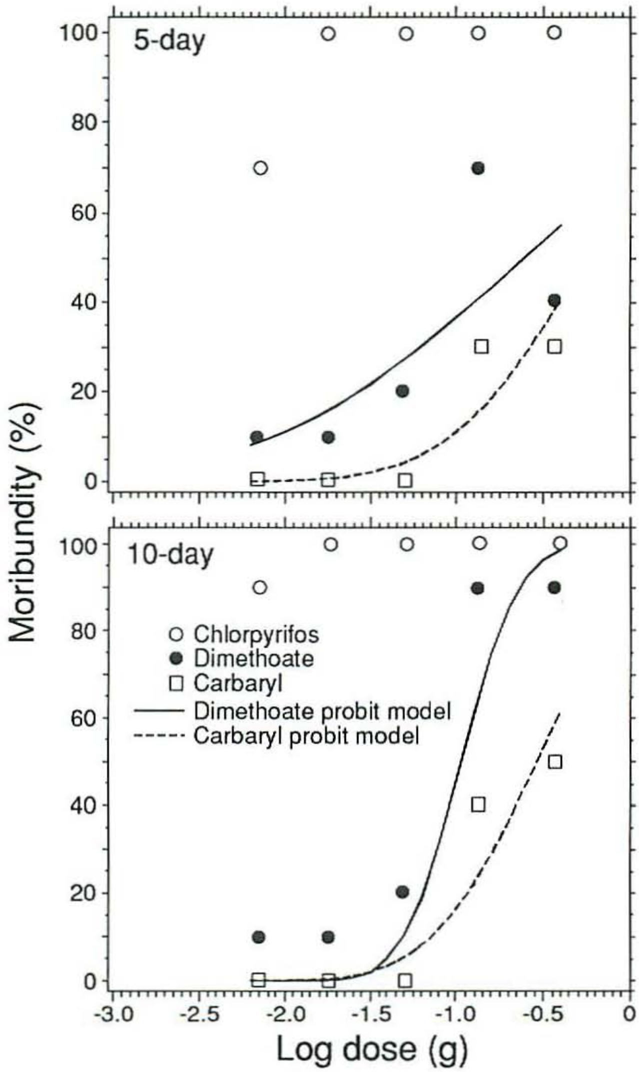

Fig. 2. Moribundity response of mealworm larvae to chlorpyrifos, dimethoate, and carbaryl bran baits.

The beetle 5-d effective dose for 50% response (ED_{50}), calculated from the common probit analysis (carbaryl and dimethoate), was 0.13 g as compared with 0.48 g for the larvae (Table 1).

The response of the larvae to bran bait was greatest in the chlorpyrifos bait treatments, less in the dimethoate and least in the carbaryl bait treatments (Fig. 2). Both beetles and larvae displayed an immediate and pronounced response to the chlorpyrifos treatments, succumbing within hours of exposure. The 5-d and 10-d responses for beetles in the carbaryl and dimethoate treatments were not significantly different from each other (Table 1). After 15 d, the mortality curves were parallel but with different intercepts, indicating a more pronounced mortality response for dimethoate-treated beetles than for carbaryl-treated beetles. The 5-d and 10-d dose responses for the larvae revealed that the carbaryl and dimethoate regression lines were parallel to each other, but had different intercepts (Table 1).

The ED_{50} values for carbaryl and dimethoate reveal similar relationships (Table 1). For the beetle 5-d and 10-d responses, the ED_{50} for carbaryl and dimethoate were equivalent, but for the 15-d response, the ED_{50} for dimethoate was less than that for carbaryl. For larvae, the ED_{50} for dimethoate was lower than that for carbaryl on days 5 and 10 (Table 1).

The sigmoidal shape of the dose-response mortality curves (Figs. 1, 2) indicates that increasing dosages of bran bait corresponded to increasing levels of response. The chlorpyrifos bait treatments reached nearly complete mortality at lower dosages. Mortality in beetles and moribundity in larvae reached 100% with only 0.018 g of chlorpyrifos bait (log dose - 1.74; Figs. 1, 2). When beetles and larvae were confined with similar amounts of carbaryl and dimethoate bait, 0 and 10% or less, respectively, they became moribund.

Total response of beetles and larvae to the bran bait was enhanced over time, as shown by the increasing slope of the functional response curves from the 5-d response through to the 15-d response (Figs. 1, 2, Table 1). The slopes of the common mortality curves for beetles were 2.61, 3.46, and 3.98 for the 5-d, 10-d, and 15-d responses, respectively. The same trend was evident for the larvae, the slope of the response curve increasing from 0.97 for the 5-d response to 2.67 for the 10-d response (Table 1).

Discussion

Chlorpyrifos-treated bran bait had a greater effect on beetles and larvae than either carbaryl- or dimethoate-treated bran bait. The slopes of the dose-response curves for carbaryl- and dimethoate-treated bait were high. Hudson et al. (1984) noted that the dose response curves for most of the chlorpyrifos-treated nontarget species he studied had low slopes. The implications of low slope response curves are that some individuals will be affected by the bait, even at treatment levels significantly lower than the LD_{50} (Tucker and Leitzke 1979). The steep slopes of the dose-response curves for carbaryl and dimethoate bran baits are characteristic of "safer" toxicants. They imply that dosages lower than the LD_{50} would not cause significant levels of mortality among yellow mealworms.

Mealworm beetles and larvae exhibited a probit response to the carbaryl and dimethoate baits (i.e., increasing rates of bran bait corresponded to increasing responses). A probit response was not observed for beetles or larvae in the chlorpyrifos treatments. High rates of mortality for beetles and high levels of moribundity for larvae, were observed even at the lowest levels of chlorpyrifos bait. This low level response further illustrates the toxic nature of chlorpyrifos bait. This study did not attempt to estimate the threshold below which no effects are produced by chlorpyrifos bait.

Mortality of beetles exposed to the carbaryl and dimethoate baits became more pronounced over time. Evidently, the bran bait was effective throughout the experimental period. The immediate and total response of the beetles and larvae to the chlorpyrifos bait precluded any time-response trend which otherwise might have been observed. It is doubtful whether the longevity of bran bait would be of significance in a field setting. Although rainfall and sunlight might begin to degrade the bait shortly after its deposition in the field, most would be ingested in a short period of time by both target and nontarget organisms (Mukerji et al. 1981).

Mealworm larvae were more tolerant of insecticide-treated bran baits than adults. Larvae have lower activity and respiration rates and presumably, absorb less insecticide. In addition, the larvae have endocrine systems which are particularly insensitive to organophosphorus and carbamate insecticides (McCaleb et al. 1980, Sparks and Hammock 1980).

Field studies would be necessary to determine if the use of bran bait in a grasshopper control program would have any impact on the field survival, age structure, and rate of growth of tenebrionid populations. Direct extrapolation of these results to a field setting is not possible. The mealworms were, in some cases, exposed to quantities of bran bait many times higher than what they could be expected to encounter in the field. However, these laboratory trials gave the mealworms a maximum and prolonged exposure under controlled conditions to the different bran baits, a necessary first step prior to field work. The results indicate that yellow mealworms would have to ingest or come into contact with relatively large amounts of carbaryl or dimethoate bait before appreciable losses would occur. Exposure to only small amounts of chlorpyrifos bait, however, could have a significant impact on tenebrionid populations.

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