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Low Rates of Insecticides and *Nosema locustae* (Microsporidia: Nosematidae) on Baits Applied to Roadsides for Grasshopper (Orthoptera: Acrididae) Control

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ABSTRACT Wheat-bran baits treated with either low dosages of chemical insecticides (carbaryl and dimethoate) or with *Nosema locustae* Canning were applied to reduce abundance of grasshoppers in roadside vegetation. Both carbaryl and dimethoate provided acceptable short-term reductions. Application of baits containing 4% carbaryl or 4% dimethoate (80 g/ha) resulted in 76 and 70% mortality after 4 d, respectively. After 31 d, 10% of the grasshoppers collected from the *N. locustae*-treated plots were infected. Infection rates were equal in roadside populations treated with 2 or 4 kg *N. locustae* bait per hectare.

KEY WORDS *Nosema locustae*, insecticide bait, carbaryl, dimethoate, bran carrier, grasshopper control

GRASSHOPPERS ARE periodic pests of grassland and cereal crops. In the Canadian prairie provinces, the most damaging species inhabit grassy roadsides along the edges of fields, causing extensive damage to crop margins. Efforts to reduce crop losses focus on the destruction of grasshoppers in these areas and often involve extensive use of insecticide in roadside spraying. Of the 1.5 million ha infested in Alberta in 1984 (Grace & Johnson 1985), >300,000 ha were sprayed with chemical insecticides to control grasshoppers. In 1985 and 1986, ca. 700,000 and 600,000 ha in Alberta were sprayed (D.L.J., unpublished data), often repeatedly because of a prolonged hatching period (typically 20 May–30 June) and movement of grasshoppers from roadsides and pastures into sprayed areas.

The advantages of spraying chemical insecticides are the availability of spray equipment and the relatively high rates of mortality usually achieved. The main disadvantages to spraying include destruction of nontarget arthropods (e.g., pollinators), environmental risk, and expense. An alternative to aqueous chemical sprays is the distribution of a solid carrier, such as wheat bran, impregnated with insecticide that kills grasshoppers that feed on it. Insecticide baits are potentially useful in grasshopper pest management because of the reduced rates, and consequently lower costs, of application. They also afford greater precision and efficiency of control in directing the insecticide to the pest: only insects that actually feed on the bait are killed, and drift is negligible. Bran bait can be spread under conditions that would be too windy for spraying.

Arsenic-treated baits were first used in fighting grasshoppers nearly a century ago and have attracted interest and experimentation many times since (Gibson 1915, Criddle 1931). Modern insecticide baits have also been tested. Charnetski & Hobbs (1974) concluded that carbofuran was 3-fold more effective than dimethoate on vermiculite. Foster et al. (1979) reported success with carbaryl bait for control of the Mormon cricket, *Anabrus simplex* Haldeman, a long-horned grasshopper. Onsager et al. (1980a,b) determined that carbaryl-treated bran bait had potential for controlling rangeland grasshoppers. Mukerji et al. (1981) and Mukerji & Ewen (1984) tested a total of six insecticides on bran carrier in pastures and found the greatest degree of control with dimethoate.

Most of the research on bran bait in the last decade has been directed toward its use as a carrier of spores of *Nosema locustae* Canning, a microsporidian that debilitates and kills grasshoppers within weeks of ingestion. Henry (1972) demonstrated that application of *N. locustae*-treated bait resulted in reductions in density of rangeland grasshopper populations of 50–60% and infection of 35–50% of the survivors. The disease also results in reduced feeding by grasshoppers (Oma & Hewitt 1984, Johnson & Pavlikova 1986) and reduced grasshopper reproduction (Henry & Oma 1981) at sublethal levels of infection.

Although interest has been generated in the bait method of application of insecticides and *N. locustae*, neither has been tested on roadside populations. We performed our experiments with roadside populations of grasshoppers in Alberta cropland. Our objectives were to assess the potential of carbaryl bait, to compare it with dimethoate bait, to determine whether lower rates of application of dimethoate than presently employed could be used, and to attempt to introduce the disease caused by *N. locustae* into roadside grasshopper

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populations. Carbaryl was included because it may be superior to dimethoate owing to its lower toxicity to mammals, lower volatility, and lack of a strong odor. Because bait must be transported and stored in bags after formulation, the objectionable odor of dimethoate is a disadvantage.

Materials and Methods

Experimental Design. Baits were applied to roadsides in northwestern Taber County, Alberta, at six locations (randomized complete blocks). In each block, seven plots were established; plots measured 800 m in length and consisted of two subplots (10 m wide strips), one on each side of the road. One control plot chosen at random in each block received no bait. Each block consisted of 5.6 km of road. The insecticides used were commercial formulations of carbaryl (Sevin XLR, Union Carbide Agricultural Products [Canada], Calgary, Alb.) or dimethoate (Cygon, Cyanamid Canada, Willowdale, Ont.). The seven treatments were 2 and 4% carbaryl bait, 2 and 4% dimethoate bait, *N. locustae* bait at 2 and 4 kg/ha, and an untreated control. The *N. locustae* bait consisted of 1.25×10^9 spores per kilogram. Chemical insecticide baits were applied at 2 kg/ha, resulting in application rates of 40 and 80 g (AI)/ha. No baits that combined insecticide with *N. locustae* were tested. Daily temperature maxima and minima, precipitation, and relative humidity were monitored at one location in the area.

Bait Formulation and Application. During application of the insecticide to the wheat bran, the bran was turned in an electric cement mixer (70 liter) at 65 rpm. Insecticide was applied using a hand-held sprayer. No water or wetting agents were added to the insecticide. Bran was treated in 5-kg batches, weighed, and bagged in packages sufficient to treat one plot. (Each plot covered 1.6 ha and received 3.2 kg of bait.)

Spores of *N. locustae* were produced at the USDA Rangeland Insect Laboratory, Bozeman, Mont., as described by Henry (1986). The spores were sprayed onto the bran in the cement mixer with a 103 kPa (15 psi) fine-mist sprayer, in 15 ml water/kg bran.

Application of baits to roadside plots was made within 2 d of mixing, with a truck-mounted blower (Buffalo Turbine) designed to blow bran out to the side in a 10-m swath. Untreated bran was used to calibrate the blower settings and truck velocity just before application of the treated bran. Treatments were applied between 0800 and 1200 hours in random order within blocks, so that the six blocks were treated over a 2-d period (three blocks on 21 June and three blocks on 22 June).

Roadside vegetation was chiefly crested wheat grass, *Agropyron cristatum* (L.) Gaertn., and smooth brome, *Bromus inermis* Leyss., ca. 20–50 cm high. Adjacent fields were wheat, stubble, or rangeland (in order of prevalence).

Ecological Sampling and Statistical Analysis. Species and age composition of the grasshoppers present in each block were determined by identifying grasshoppers collected in 100 sweeps with a net (38-cm diameter) from each replicate on the day of treatment.

Owing to the bias inherent in the use of sweep-net samples to assay grasshopper mortality in insecticide trials (Johnson et al. 1986), and because economic thresholds for grasshoppers are given in units of numbers per square meter and not numbers per sweep, we monitored changes in grasshopper abundance on a density basis. Each sampling area was 600 m in length centered in the treated plot (800 m long), with the two sides of the road as subplots. Eight sampling quadrats (0.25 m^2) were established in the two subplots per plot and marked with stakes. The locations of the 16 quadrats per plot were marked with stakes at the road margin. The numbers of grasshoppers observed in the quadrats were recorded just before bait application, and 2 and 4 d after application. Because of time limitations and occasional loss of stakes delimiting a quadrat, the grasshoppers in additional unmarked quadrats were counted after the 1st d of sampling. These new quadrats were always within a few meters of the original location. Counts were made either by a single observer (D.L.J.) or by two observers (D.L.J. and one of two trained surveyors), counting three blocks per day. Farmers owning adjacent land did not spray the roadsides during the 4 d following application.

Linear models appropriate to a randomized complete-block design with subplots and subsampling (SAS Institute 1982) were used to analyze the counts from the 0.25-m^2 quadrats. We tested for differences among replicates, treatments, and subplots. Treatment effects were further subdivided with orthogonal contrasts comparing concentrations and ingredients. Two basic analyses were performed: analysis of variance of the absolute reduction in number of grasshoppers (i.e., the reduction in abundance per 0.25 m^2), and of the relative reduction, $\log_e$(final abundance/initial abundance). To summarize and to compare the results with those of other research studies, the adjusted percentage of mortality for each bait treatment was calculated from the modified Abbott's formula (Connin & Kuitert 1952): $100\,[1 - \alpha]$, where $\alpha = (T_2C_1)/(T_1C_2)$, the cross-product ratio, and T_1, C_1, T_2, C_2 are the total counts in the treated and control groups before and after treatment. Density-dependence was assessed by comparing the percentage of mortality adjusted by the modified Abbott's formula among low-, medium-, and high-density plots. The data were divided into three groups of approximately equal size on the basis of initial density: 1–6, 7–11, or >11 grasshoppers per 0.25 m^2.

Infection by *N. locustae*. Two sweep-net samples of 50 sweeps each were collected from each of the *N. locustae*-treated plots and the control plots after 4 d and again after 31 d. Grasshoppers in these samples were frozen and taken to the lab-

Table 1. Age structure of the three dominant grasshopper species

Species[ab]	Instar					Adult	% of total[c]
	1st	2nd	3rd	4th	5th		
M. sanguinipes	3	5	9	29	52	2	78
C. pellucida	4	21	8	43	22	3	12
Melanoplus infantilis Scudder	2	4	11	67	15	0	5

[a] The percentage in each age class is shown for each of the dominant species.

[b] Other species that each accounted for ≤1% of the community were *Aerochoreutes carlinianus* (Thomas), *Aeropedellus clavatus* (Thomas), *Ageneotettix deorum* (Scudder), *Amphitornus coloradus* (Thomas), *Bruneria brunnea* (Thomas), *Dissosteira carolina* (L.), *Melanoplus bivittatus* (Say), *Melanoplus dawsoni* (Scudder), *Melanoplus femurrubrum* (De Geer), *Melanoplus packardii* Scudder, *Metator pardalinus* (Saussure), *Pseudopomala brachyptera* (Scudder), *Spharagemon collare* (Scudder), and *Trachyrhachys kiowa* (Thomas).

[c] The prevalence of each species, based on a total sample of 982 individuals.

Table 2. Adjusted percentage of mortality and change in abundance of grasshoppers

Bait formulation[a]	% killed[b]		Mean no. grasshoppers/0.25 m^2[c]		
	2 d	4 d	0 d	2 d	4 d
2% carbaryl	60	67	7.6	2.9	2.0
4% carbaryl	71	76	10.3	2.8	2.0
2% dimethoate	61	59	8.9	3.3	3.0
4% dimethoate	70	70	9.3	2.6	2.2
Untreated	—	—	8.9	8.4	7.1

[a] 2% (AI) = 40 g (AI)/ha.

[b] Adjusted for mortality in the untreated group with the modified Abbott's formula.

[c] There were 96 quadrats examined per treatment per date. SEM densities are 5–15% of the means.

oratory for assessment of infection. The grasshoppers were individually ground in 5 ml distilled water in 15-ml tissue grinders (Potter-Elvehjem), and the presence and relative abundance of *N. locustae* spores were determined by observing hanging-drop suspensions with a differential interference contrast microscope at 400×.

Results

At the time of application of bait, the most common species in all six blocks were the migratory grasshopper, *Melanoplus sanguinipes* (F.), and the clearwinged grasshopper, *Camnula pellucida* (Scudder) (Table 1). Grasshopper abundance in the plots ranged from 0 to 32 per 0.25 m^2. The blocks differed slightly in age structure (blocks × instars 1–4 versus 5–adult, $\chi^2 = 49.3$; df = 5; $P < 0.001$). Block 3, situated on an open, level roadside adjacent to stubble fields, had the oldest population (28% first–fourth instars). Block 4 was bordered by rangeland and may have warmed more slowly in the spring, causing this site to have the youngest population (68% first–fourth instars). The 1985 Alberta spring grasshopper survey indicated that rangeland populations were typically 1–3 wk behind nearby roadside populations in age (D.L.J., unpublished data).

Efficacy. The adjusted mortality percentages and the average reductions in the number of grasshoppers per 0.25 m^2 are shown in Table 2. The insecticide baits provided significant reductions after 2 and 4 d at both 2 and 4% (AI).

The efficacy of low and high rates of dimethoate did not differ on either sampling date. The 4% carbaryl bait resulted in significantly higher mortality than the 2% bait on both posttreatment sampling dates (Table 3). With carbaryl bait, grasshopper numbers declined significantly from 2 to 4 d (for 2% carbaryl bait, $t = 2.49$; df = 95; $P < 0.01$; for 4% carbaryl bait, $t = 2.08$; df = 95; $P = 0.02$); the dimethoate resulted in no decrease after 2 d (for 2% dimethoate, $t = 0.95$; df = 95; $P > 0.1$; for 4% dimethoate, $t = 1.22$; df = 95; $P > 0.1$). Overall, carbaryl and dimethoate did not differ significantly in efficacy ($P > 0.1$; Table 3).

Significant differences in relative mortality among replications of the experiment and between subplots (Table 3) indicated natural variability among populations and sites. In block 1, which did not differ from the others in initial population density, age, or species composition, the treatments inexplicably failed to reduce grasshopper abundance significantly. Age structure in block 1 did not differ significantly from that in the other five blocks. Without this aberrant block, both dimethoate bait and carbaryl bait resulted in adjusted reductions in grasshopper abundance of >80% after 4 d. However, all six blocks were included in the analysis of the data, and no observations have been omitted.

No precipitation occurred during application or monitoring. Maximum and minimum temperatures in the grass canopy at a height of 10 cm were 28 and 3°C, respectively.

Infection by *N. locustae*. A total of 1,005 grasshoppers of the two predominant species present at the sites was examined for the presence of spores. The background (natural) level of infection of *N. locustae* was very low. Only 2 of the 360 grasshoppers collected from the untreated plots had spores of *N. locustae*. The proportion of infected grasshoppers collected from plots treated with 2 and 4 kg/ha did not differ ($P > 0.05$). Population infection rates were 5.3% ($n = 323$) and 10.2% ($n = 322$) after 4 and 31 d, respectively. Nearly all of the infected grasshoppers had spore concentrations of less than three spores per microscopic field ("light" infection [Henry et al. 1973]). *Malameba locustae* (King & Taylor) and *Farinocystis* sp. were found in the grasshoppers assayed, but these are both easily distinguished from *N. locustae*.

The proportion infected did not differ significantly ($P > 0.05$) between *M. sanguinipes* and *C. pellucida*. No effect of grasshopper age on infection could be discerned, because of the advanced age structure of the grasshoppers at the time of sampling.

Density Dependence. In our study, the percentage control in the plots with the highest initial population densities was no lower than in those plots with low or moderate initial population densities. At both 2 and 4 d after application, the relative mortality in the plots with higher initial numbers of grasshoppers was at least as great as in plots with low initial numbers.

Table 3. Analysis of variance of the reduction in numbers of grasshoppers per 0.25 m^2 4 d after bait application

Source of variation	df	*P* value	
		Absolute reduction[a]	Relative reduction[b]
Blocks	5	0.12	0.001
Bait formulations	6	0.004	<0.001
Blocks × baits	30	0.023	0.052
Subplots	42	<0.001	<0.001
Quadrats	588		
Orthogonal contrasts			
2 vs 4% carbaryl	1	<0.001	0.005
2 vs 4% dimethoate	1	0.19	0.081
Carbaryl vs dimethoate	1	0.40	0.10
Insecticides vs control	1	<0.001	<0.001

[a] Day_0 count − day_4 count.

[b] $Log_e[(day_4 \text{ count} + 1)/(day_0 \text{ count} + 1)]$.

Discussion

Both chemical insecticide baits significantly reduced grasshopper numbers. The toxic baits reduced grasshopper numbers to densities below the economic threshold of 13–24 grasshoppers per square meter for roadsides bordering cereal crops (Western Committee on Crop Pests 1986). At the high rate of insecticide application, 80 g (AI)/ha, use of these baits represents only one-third and one-sixth of the lowest spray rates recommended for control of grasshoppers with dimethoate and carbaryl, respectively. Dimethoate on bran bait is presently recommended for grasshopper control at a rate of 110–165 g (AI)/ha on 2–3 kg bran/ha on seed alfalfa crops and pastures (Western Committee on Crop Pests 1986). Higher rates of carbaryl bait than those tested in our experiments may also be required for application in heavy canopies. However, the lower rates that we tested were effective in roadside grass and would probably perform as well or better on rangeland or pasture. The mortality caused by application of 2% carbaryl bait at 2 kg/ha is similar to the result achieved by Onsager et al. (1980a,b) with 2% carbaryl bait at 1.7 kg/ha applied to rangeland.

Mukerji & Ewen (1984) concluded from recent field trials that carbaryl-treated bran bait was not useful for protecting crops from grasshoppers. They treated a pasture with a range of rates and found that even 200 g carbaryl/ha applied on 3 kg wheat bran/ha resulted in <50% mortality. This result contrasts markedly with the average 76% mortality achieved in our experiments with 80 g (AI)/ha on 2 kg bait/ha. The formulation used by Mukerji & Ewen (1984) was Sevin 80S, a sprayable powder that may not adhere to bran. In our experiments, we observed that Sevin XLR formed a fine paint-like coating that adhered well to the particles. The difference in the formulations could account for the difference in observed mortality.

Low-level epizootics of *N. locustae* were initiated in plots at both the low and high rates of bait application. Infection rates of ca. 10%, 31 d after application, prove that this pathogen can be introduced into and maintained in roadside grasshopper populations for at least 31 d. We suspect that the infection rate 1 mo after application would have been higher had the application taken place earlier in the season. Application of *N. locustae* is most effective when most of the grasshoppers present are in the third instar (Henry 1972).

The similarity in proportion of the populations infected between the low- and high-dosage treatments corroborates the findings of Johnson & Henry (1984), who showed that the rate of infection does not increase with increasing rate of application. In replicated experiments in pastures, those authors found similar rates of infection 6 wk after application of 3.1 × 10^9, 6.2 × 10^9, 12.4 × 10^9, and 24.7 × 10^9 spores per hectare.

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