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The Effects of Timing and Frequency of Application of *Nosema locustae* (Microspora: Microsporida) on the Infection Rate and Activity of Grasshoppers (Orthoptera: Acrididae)

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The influence of the timing of application of spores of *Nosema locustae* on the prevalence and degree of infection of grasshoppers was assessed in a field experiment. The application treatments were early season, late-season, double (both early and late), and no bait (untreated). To assess effects on population density and activity, two methods of sampling were used: density estimates based on permanent quadrats, and sequential sampling in which the number of sweeps required to collect 100 grasshoppers was recorded. Late-season application resulted in a higher prevalence of infection than did early application. The degree of infection was also a function of timing: late-season application resulted in more moderate to heavy infections than did early application. The infection rates that resulted from double application did not differ significantly from that of late-season application. Application of *N. locustae* provided moderate reductions, ca. 30%, in grasshopper density. Differences in the required sweep sampling effort were greater, suggesting that reduction in grasshopper activity was caused by the disease.

KEY WORDS: Insecta; biological control; grasshoppers; *Nosema locustae*; insect control—timing and frequency; infection rate; sequential sampling.

INTRODUCTION

Extensive areas of rangeland and cropland of the Canadian Prairies and the U.S. Great Plains have been threatened by high grasshopper densities in recent years, resulting in the application of insecticide to millions of hectares of government and private lands. Concern over economic and environmental costs has stimulated research on alternatives to chemical insecticides. The focus of the alternative grasshopper control research conducted by the Research Branch of Agriculture Canada and by the U.S. Department of Agriculture/Agricultural Research Service has been biological control using the microsporidium *Nosema locustae*.

Nosema locustae is able to debilitate grasshoppers and locusts by attacking the fat body and other internal organs (Canning, 1953, 1962; Henry, 1971, 1972), suggesting potential use as an environmentally safe, long-term management tool.

Although the detrimental effects of *N. locustae* on grasshopper growth and survival can be demonstrated easily in laboratory experiments, the results of field experiments designed to measure its effectiveness vary widely. N. Foster [Animal and Plant Health Inspection Service (U.S. Department of Agriculture); pers. commun. based on unpublished data] reports no discernible effects of *N. locustae* on grasshopper populations, while Ewen and Mukerji (1980) reported 60% mortality and infection of 95–100% of the surviving grasshoppers collected 12 weeks (700 degree-days >10°C) after field application of *N. locustae* bait. More typical results indicate 30–50% grasshopper mortality caused by *N. locustae*, and infection of 20–50% of the survivors (Henry et al., 1973, 1978, 1985; Henry and Oma, 1974). In atypical situations, such as application against roadside grasshopper populations, only about 10% are infected (Johnson and Henry, 1987).

Because the primary goal of experimental and practical field applications of *N. locustae* has been to reduce the number of grasshoppers in the treated area, the more susceptible third and fourth nymphal in-

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stars are targeted (Henry et al., 1973; Henry and Onsager, 1982). Late-season application is considered inadequate, or at best inferior to earlier application (J. E. Henry, pers. commun.), because the larger and more robust adults are less likely to succumb to the disease. However, the nonlethal symptoms of infection include significant suppression of feeding (Johnson and Pavlikova, 1986) and of reproduction (Henry and Oma, 1981). Also, it is reasonable to assume that higher rates of infection of the adults would increase the rate of transmission of the pathogen to the next generation (Henry and Oma, 1981). In the interest of increasing the percentage of the grasshopper population successfully infected, I examined the effects of the timing and frequency of *N. locustae* application without the constraint of maximizing grasshopper mortality. Because of the controversy surrounding efficacy, the comparisons were made as part of a rigorous field experiment.

MATERIALS AND METHODS

Bait Preparation

The *N. locustae* bait was formulated in a stainless-steel mixer (Marion Mixer Model 6041, Rapids Machinery Co., Marion, Iowa) designed for mixing dry feed. The mixer produced 200 kg of bait per run, requiring about 1 hr.

The spores were produced in vivo using the general methods recommended by Henry (1986). They were stored in water at −10°C until preparation of the spore suspension used in the bait mixer. For each run, a suspension was prepared from measured quantities of distilled water, previously frozen *N. locustae* spores, and molasses (1% final concentration), added as a sticking agent. The suspension was sprayed onto rapidly mixing wheat bran (weighted mean flake diam 0.63 mm), at 250 kPa through three nozzles (Delavan LF1 65°) spaced 38 cm apart on a spray boom mounted in the mixing tank. The mixer shaft and four 45-cm blades attached to it turned at 40 rpm during spraying. After the suspension had been sprayed out, 5 ml of distilled water per final kilogram of bait was sprayed to wash the pressure lines and nozzles. The final moisture content of the mixed bait was 14–18%. The spore concentration of the formulated bait was 1.3×10^9 spores/kg of bait (wet weight). The bait was packaged in 16-kg quantities in burlap bags and stored indoors at room temperature overnight before field application.

Field Plot Design

Four blocks of native and improved grass pasture near Taber, Alberta, Canada, were each divided into four 16-ha plots. Each plot measured 200 × 800 m running N–S. Vegetation in blocks 1 and 2 was almost entirely crested wheatgrass, *Agropyron cristatum*. Blocks 3 and 4, 11 km away, consisted of ca. 80% *Poa* spp., 10% June grass, *Koeleria gracilis*, and 10% needle-and-thread, *Stipa comata*. Standing crop (mean dry weight of sixteen 0.25-m^2 aboveground forage samples per block) was 93.6, 98.0, 122.4, and 98.8 g/m^2 in blocks 1–4, respectively.

A complete set of the following four treatments was randomly assigned to the plots in each block: early season bait application (June, when younger instars normally predominate), late-season application (July, when a majority of grasshoppers are normally in the adult stage), double application (both early and late application to the same plot), and no bait application. The treated plots received wheat bran bait containing 3.3×10^9 spores/ha at each application.

Field Application Methods

The bait was first applied 25 June 1986 at a rate of 2.5 kg/ha using a truck-mounted bran blower (Peacock Industries, Saskatoon, Saskatchewan) modified to deliver bait directly into the blower duct. The late-season application (16 July 1986) was made using a dry fertilizer spreader attached to a

Pawnee Brave aircraft (Kinniburgh Spray Service, Purple Springs, Alberta). The aerial spreader was tested and calibrated at the airstrip to give the same distribution of bait particles and application rate as the truck-mounted applicator.

Estimation of Infection Rate

The infection rate, defined as the percentage of the grasshoppers found to contain *N. locustae* spores, was estimated on three posttreatment sampling dates, 5, 7, and 9 weeks after the early bait application. Sixty grasshoppers randomly selected from frozen samples that had been collected live from each of the 16 plots were ground individually in 5 ml of water, using a Potter–Elvehjem tissue grinder. Hanging drop suspensions of the tissue were examined under Nomarski differential interference contrast at 400×. A 0–4 rating scale [nil, trace, light, moderate, and heavy (Henry, 1972)] was used to quantify the degree of infection of each grasshopper assayed. The assays were performed blind: only sample date, plot number, subplot number, and species codes were recorded on the samples. Infection rate was compared among the treatments and species, and between the sexes, in contingency tables with χ^2 tests.

Population Estimation

Two methods of estimating grasshopper population density were used: counts of grasshoppers observed in quadrats and collection of grasshoppers with sweepnets. The two methods were employed because under typical field conditions the first provides an unbiased estimate of population density of active and lethargic insects alike, while the latter method depends on grasshopper condition and activity (Johnson et al., 1986). Fewer sweeps are required to collect more active grasshoppers, since they jump when startled and spend more time resting on vegetation than on the ground.

For the purpose of sampling, each plot was subdivided into two subplots (the north and south ends). Grasshopper population density was monitored by weekly counts of the number of living grasshoppers in permanent 0.25-m^2 steel quadrats. The quadrats were simple open squares constructed of 0.6-cm-diam. steel rod squares and did not confine grasshopper movement. They were placed in the plots at the beginning of the experiment (20 per plot, in one 10-quadrat transect per subplot). The positions of the 320 quadrats did not change during the experiment, but 5 quadrats were lost. The grasshoppers were counted before treatment and 2, 3, 4, 5, 7, 8, and 9 weeks after the initial treatment. During sampling, observers were generally unaware of which of the 16 plots had received which treatments.

During collection of sweepnet samples, observers recorded the number of sweeps required to collect 100 grasshoppers (there were two such samples per plot, per sampling date). Sampling was always carried out in clear weather, between the hours of 0900 and 1700. The number of sweeps per 100 grasshoppers was adjusted in cases in which the actual number collected exceeded 100 (e.g., a sample found on examination to contain 123 grasshoppers that were captured in 225 sweeps would be recorded as 100 grasshoppers in 182.9 sweeps). Sweepnet samples were frozen and returned to the laboratory for identification of grasshopper species, age class (five nymphal stadia and the adult stage), and infection status. Species identifications were based on taxonomic keys and descriptions by Brooks (1958), Otte (1981, 1984), and Vickery and Kevan (1983).

The $\log_e$-transformed count data and the number of sweeps per 100 grasshoppers were analyzed separately for each posttreatment sampling date with analysis of covariance, using the general linear model routine of SAS (1982). Since the quadrat positions did not change during the experiment, the pretreatment observation (week 0) was the concomitant variable. Treatment effects were tested with experimental error

(block by treatment interaction), experimental error was tested with subplot error, and subplot error was tested with sampling error among quadrats. Orthogonal contrasts were used to compare the following treatment combinations: early vs late treatment, single vs double treatment, and treated vs untreated. The correlation coefficient (r) between sweeps per 100 grasshoppers and grasshopper density was calculated for each treatment.

RESULTS AND DISCUSSION

Grasshopper Species and Age Composition

Three species (Table 1) accounted for 88 and 85% of the grasshoppers collected at the times of the early and late applications, respectively. The age structure of the target was the major difference in conditions between the early and late treatments. The early treatment was applied when only 2.9% of the grasshoppers were in the adult stage (Table 2), while the late application was directed primarily at adults (84%, Table 2).

Infection Rate

Some infected individuals were collected from the untreated plots. These are interpreted as being immigrants from treated plots, because *N. locustae* spores were not detected in grasshoppers collected before

TABLE 1
GRASSHOPPER SPECIES PRESENT AT THE STUDY SITE[a]

Species	Percentage of total collection	
	Early treatment (N = 5497)	Late treatment (N = 6239)
Melanoplus infantilis	46.9	39.5
Camnula pellucida	24.4	30.6
M. sanguinipes	17.0	14.4
M. packardii packardii	2.8	2.8
Aeropedellus clavatus[b]	2.0	2.0
Ageneotettix deorum	1.9	2.8
Trachyrhachys kiowa kiowa	1.5	3.1
Trimerotropis campestris	1.5	1.6
Phlibostroma quadrimaculatum	<1.0	1.8
Aerochoreutes carlinianus carlinianus	<1.0	<1.0
Amphitornus coloradus coloradus	<1.0	<1.0
Anabrus simplex	<1.0	<1.0
Arphia conspersa	<1.0	<1.0
Bruneria brunnea	<1.0	<1.0
Dissosteira carolina	<1.0	<1.0
Ecoptolophus costalis	<1.0	<1.0
Hadrotettix trifasciatus	<1.0	<1.0
Hesperotettix viridis pratensis	<1.0	<1.0
M. bivittatus	<1.0	<1.0
M. confusus	<1.0	<1.0
M. dawsoni	<1.0	<1.0
M. femurrubrum femurrubrum	<1.0	<1.0
Metator pardalinus	<1.0	<1.0
Phoetaliotes nebrascensis	<1.0	<1.0
Pseudopomala brachyptera	<1.0	<1.0
Spharagemon collare	<1.0	<1.0

[a] The grasshoppers were collected from 16 field plots by sweepnet just before application of the bait.
[b] *A. clavatus* was excluded from the study. It constitutes a small proportion of the total community and attains adulthood very early in the season. It was rarely seen in the plots during this experiment.

TABLE 2
GRASSHOPPER SPECIES AND AGE STRUCTURE AT THE TIME OF APPLICATION OF *Nosema locustae* BAIT[a]

Species	Instar 1	2	3	4	5	Adult	Total
				Early application[b]			
Melanoplus infantilis	2.6	8.4	19.7	40.9	27.3	1.1	2584
Camnula pellucida	0.0	0.5	6.7	34.2	52.0	6.6	1341
M. sanguinipes	3.5	10.5	22.3	29.5	30.7	3.5	935
M. packardii	3.2	11.6	21.3	30.3	30.3	3.2	155
Ageneotettix deorum	0.0	4.7	9.4	44.9	41.1	0.0	107
Trimerotropis campestris	11.8	29.4	35.3	22.4	1.2	0.0	85
Trachyrachys kiowa	3.8	3.8	17.5	30.0	45.0	0.0	80
Rare species (combined)	11.8	13.7	31.4	33.3	8.8	1.0	102
All species	2.4	7.2	17.2	36.4	33.9	2.9	5389
				Late application[c]			
M. infantilis	0.0	0.0	0.7	3.7	12.4	83.1	2468
C. pellucida	0.0	0.0	0.0	0.3	2.7	97.0	1914
M. sanguinipes	0.0	0.0	2.2	6.5	21.7	69.5	902
T. kiowa	0.0	0.0	1.0	0.5	6.3	92.2	192
A. deorum	0.0	0.0	0.0	2.3	8.5	89.3	177
M. packardii	0.0	1.1	2.9	9.7	28.0	58.3	175
T. campestris	0.0	1.0	8.2	16.3	25.5	49.0	98
Rare species (combined)	0.0	0.0	0.0	11.7	41.0	47.3	188
All species	0.0	0.0	0.9	3.5	12.0	83.6	6114

[a] The total number collected and the percentage of each species in each age class are shown.
[b] 25 June 1986.
[c] 16 July 1986.

the experiment, or from untreated fields more than 2 km away from the treated fields. Johnson and Henry (1987) noted a natural infection rate of about 0.5% in the same geographic area in 1985.

Although the infection rate that resulted from the early application of *N. locustae* bait was initially higher than that caused by the late application (Table 3), the late application of spores infected more individuals by the end of the experiment ($\chi^2 = 7.97$, $P < 0.01$). The effect occurred consistently: later treatment resulted in a higher rate of infection at all four block locations (Table 4).

The degree of infection also differed between the early and the late treatments. Although the percentage infected at the trace and light levels did not depend on timing of application, the late application resulted in approximately twice as much moderate to heavy infection by the ninth week ($\chi^2 = 21.5$, $P < 0.001$). The double treatment did not result in greater intensity of infection (i.e., trace and light vs moderate and heavy) than the late treatment, but by 9 weeks after the early application the late and double treatments had resulted in more moderate to heavy infections than did the early treatment (19.8 vs 8.3%, $\chi^2 = 18.1$, $P \leq 0.001$).

The three major species in the plots treated with *N. locustae* bait differed significantly in their rates of infection; the separation was not as great on the last sampling date (week 5: $\chi^2 = 40.8$, $P < 0.001$; week 7: $\chi^2 = 25.5$, $P < 0.001$; week 9: $\chi^2 = 7.6$, $P = 0.02$). The species differences were consistent over blocks and sampling dates: *Camnula pellucida* had the lowest and *Melanoplus infantilis* had the highest percentage infected. The prevalence of infection also differed between the sexes. At 9 weeks, more females than males were infected (38% vs 27%; $\chi^2 = 13$, $P < 0.001$), and nearly four times as many females as

TABLE 3
PERCENTAGE OF GRASSHOPPERS INFECTED WITH *Nosema locustae* SPORES

	Camnula pellucida	*Melanoplus infantilis*	*M. sanguinipes*	All species
	5 weeks after the early[a] and 2 weeks after the late[b] bait application			
Untreated	2.1	0.8	3.1	1.7
Early	19.7	41.0	12.9	27.0
Late	10.3	10.5	5.3	9.2
Both	22.2	45.9	10.0	32.5
Sample size	241	474	233	961
	7 weeks after the early and 4 weeks after the late bait application			
Untreated	3.1	6.6	5.2	5.8
Early	12.8	39.3	30.8	32.9
Late	13.5	26.0	15.4	22.1
Both	14.3	42.8	22.7	34.6
Sample size	143	569	206	960
	9 weeks after the early and 6 weeks after the late bait application			
Untreated	7.7	5.7	6.2	5.8
Early	23.1	24.2	20.6	22.9
Late	20.8	38.4	32.3	34.6
Both	14.3	42.6	35.0	38.8
Sample size	90	579	260	960

[a] 25 June 1986.
[b] 16 July 1986.

males contained heavy (class 4) concentrations of *N. locustae* spores (15% of 366 females vs <4% of 354 males collected from treated plots). Females and males did not differ in combined trace and light infections (16% of each sex), but the combined moderate and heavy categories accounted for 22.1% of females and 9.6% of males ($\chi^2 = 21.8$, $p < 0.001$). The wet body weight of *M. sanguinipes* females was only 19% greater than that of males ($n = 40$ males, 40 females), and *C. pellucida* females were 51% heavier than males. However, the differences in the prevalence and degree of infection were much greater and were probably due in part to differences between the sexes in fat content or other physiological variables, and not to body size alone.

TABLE 4
REPEATABILITY OF THE GREATER INFECTION RATE RESULTING FROM LATE TREATMENT[a]

	Block 1	Block 2	Block 3	Block 4
Untreated	6.7	8.3	3.3	5.0
Early	16.7	33.3	23.3	18.3
Late	46.7	35.0	30.0	26.7
Both	36.7	43.3	41.7	33.3

[a] The values are percentages of grasshoppers infected 9 weeks after the early and 6 weeks after the late bait application ($n = 240$ grasshoppers assayed per block).

Grasshopper Abundance

Mean grasshopper population densities before and after treatment are shown in Table 5. The pretreatment estimates (quadrat counts and sweepnet samples) were independent of the treatments ($P > 0.2$).

Application of *N. locustae* bait provided moderate early season reductions at 2 and 4 weeks after the initial application (Table 5, orthogonal contrasts, $P = 0.06$) and hastened the decline in numbers of grasshoppers in the pastures at the end of the season ($P < 0.01$). On no date did early application result in a greater or lesser decline in grasshoppers than resulted from later application (up to 8 weeks, $P > 0.2$). At 4 weeks, the overall adjusted mortality (Abbott, 1925) in the treated plots was 39% or 8, 44, 35, and 14% in blocks 1–4, respectively. (Calculation of adjusted mortality for separate vs combined blocks is discussed by Johnson et

TABLE 5
MEAN[a] GRASSHOPPER DENSITY (NO. PER 0.25 m^2) BEFORE AND AFTER APPLICATION OF *N. locustae* BAIT

	Weeks after application							
Early[b]:	0[c]	2	3	4	5	7	8	9
Late[d]:	−3	−1	0	1	2	4	5	6
Untreated	6.34	4.49	5.05	5.14	7.06	3.74	2.12	0.71
Early	5.00	3.14	3.81	2.87	4.90	4.13	0.89	0.59
Late	7.49	4.40	5.45	4.03	4.96	3.37	1.54	0.81
Both	6.20	2.65	3.38	3.13	3.67	3.25	0.80	0.53
	Orthogonal comparison of treated (3) vs untreated P (>F)							
	0.91	0.06	0.62	0.06	0.16	0.90	0.01	0.08

[a] Each mean is based on $n = 80$ quadrat counts.
[b] 25 June 1986.
[c] The "week 0" counts were made just before treatment application, so these data were used to estimate the pretreatment densities used in the analysis of covariance and in calculating the adjusted mortalities discussed in the text.
[d] 16 July 1986.

al., 1986.) Nine weeks after the initial treatment, the mean grasshopper densities declined to <3 grasshoppers/m^2 in all blocks and treatments. At this low level, sampling precision is reduced since most of the observations are 0 or 1, so sampling was terminated.

The blocks differed significantly in the reduction in grasshopper density (analysis of covariance, $P < 0.05$ on dates), although the differences were not clearly explained by block attributes such as age structure, species composition, initial population density, or vegetation.

The number of sweeps required to collect a fixed number of grasshoppers increased significantly in the plots treated with *N. locustae* (Table 6). The estimates of the reduction in density from the quadrat samples were less than the estimates based on sweepnet samples. The greater sweeping effort required in the plots treated with *N. locustae* reflects symptoms of the disease: a reduction in activity or a change in posi-

TABLE 6
MEAN[a] NUMBER OF SWEEPS REQUIRED TO CAPTURE 100 GRASSHOPPERS, BEFORE AND AFTER APPLICATION OF *N. locustae* BAIT

	Weeks after application							
Early[b]:	0[c]	2	3	4	5	7	8	9
Late[d]:	−3	−1	0	1	2	4	5	6
Untreated	165	121	172	117	91	138	213	360
Early	173	180	206	213	145	160	291	493
Late	111	109	139	181	94	141	232	232
Both	128	183	252	208	140	159	321	548
	Orthogonal comparison of treated vs. untreated P (>F)							
	0.52	0.11	0.31	0.05	0.05	0.09	0.02	0.10

[a] Each mean is based on eight separate samples (one per subplot).
[b] 25 June 1986.
[c] The "week 0" counts were made just before treatment application, so these data were used to estimate the pretreatment densities used in the analysis of covariance, and in calculating the adjusted mortalities discussed in the text.
[d] 16 July 1986.

tion of the grasshoppers. I have observed in the field that infected individuals spend more time in a lethargic condition on the ground and are not as likely as healthy grasshoppers to climb vegetation, jump, or take flight. This means that the infection rates recorded in experiments of this type may underestimate the true prevalence of infection.

Both methods of sampling were probably adversely affected by the windy conditions during week 3 when a mean wind speed of 35.7 km/hr was recorded (Table 7). Sampling on all other dates was carried out when mean wind speed was <25 km/hr, and between temperatures of 20° and 32°C. During the 65-day experiment, a total of 82 mm of rainfall was recorded, none of which occurred during sampling or bait application. The decline in late August was not caused by rainy or otherwise adverse weather.

The correlation of mean quadrat counts to required sweeping effort varied among treatments. Over 3–7 weeks after treatment, the correlation coefficient (r, paired subplot observations) between these variables was -0.625 ($P < 0.001$), -0.584 ($P < 0.001$), -0.505 ($P = 0.003$), and -0.282 ($P = 0.12$), for untreated, early, late and double treatments, respectively.

The scatterplot in Figure 1 illustrates the relationship between the two sampling methods. It shows a typical hyperbolic relationship between sampling effort and availability. Each point represents a pair of observations on one subplot: the mean number of grasshoppers/0.25 m^2 and the number of sweeps required to collect 100 grasshoppers. Although the relationship is clearly variable, *N. locustae* application did reduce density somewhat, and this reduction would increase the effort required to

TABLE 7
WEATHER DURING TREATMENT APPLICATION AND SAMPLING[a]

Month	Day	Sample week[c]	Air temperature[b]			Rain (mm)	Sun (hr)	Mean wind (km/hr)
			Maximum	Minimum	DD_{10}			
June	25	0 (treatment)	31.0	9.5	380	0.0	12.1	14.2
	26	0 (treatment)	21.5	6.0	384	0.0	9.6	9.9
	27		25.5	12.0	393	0.2	13.0	13.7
July	3	1	23.0	12.0	443	0.0	9.5	25.2
	4	1	17.0	8.0	446	0.6	4.8	9.2
	10	2	25.5	9.5	490	0.0	9.3	14.3
	11	2	23.0	13.0	498	1.2	9.5	14.9
	16	(treatment)	17.0	10.0	528	0.6	0.0	7.5
	17	3	19.5	8.5	533	0.0	5.6	35.7
	18	3	22.5	6.5	538	0.0	11.1	18.2
	22	4	30.5	9.0	572	0.0	9.0	14.0
	23	4	24.0	12.5	581	17.8	9.3	12.5
	29	5	19.5	7.0	615	0.0	10.2	13.9
	30	5	22.0	4.0	620	0.0	12.0	12.8
August	12	7	29.0	12.5	739	5.2	13.1	13.7
	13	7	24.0	15.0	749	0.0	4.8	11.0
	21	8	23.0	5.5	810	0.0	0.2	15.2
	22	8	28.0	6.0	818	0.0	11.2	9.9
	26	9	25.0	8.0	847	0.0	11.4	10.3
	27	9	30.0	7.0	856	0.0	11.8	10.4

[a] Based on daily weather data collected at the Agriculture Canada Research substation, Vauxhall, Alberta.
[b] Degree-days above 10°C were calculated from maximum and minimum daily temperatures by integrating a cosine function that simulated temperature over each 1-day period (FORTRAN program available on request).
[c] Sampling and treatment were carried out in block order, blocks 1 and 2 first, followed by blocks 3 and 4 the following day.

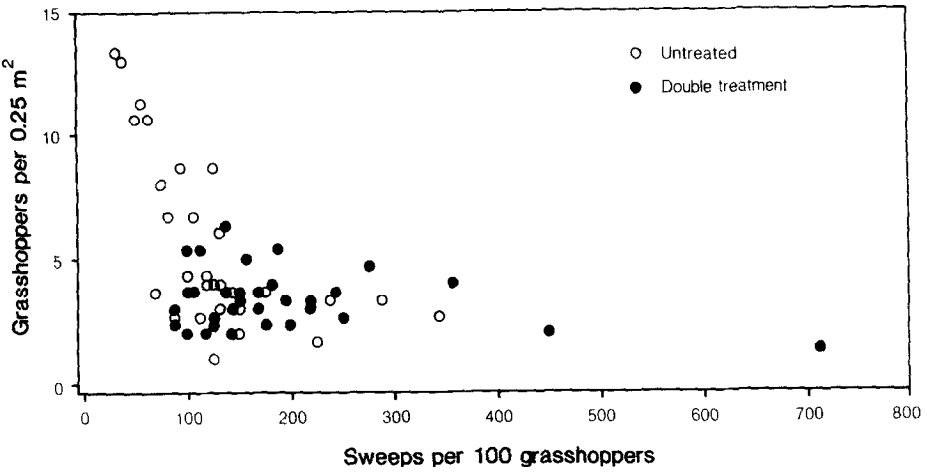

FIG. 1. The effect of *Nosema locustae* application on the relationship of the two sampling methods.

collect 100 grasshoppers. As discussed earlier, since the treatments affected sweepnet sampling to a greater extent, the increase in required sweeping effort evident in Figure 1 may be caused by changes in grasshopper behavior (due to lethargy) as well as by scarcity (due to mortality). This effect and the lack of a strong relationship between density and sweepnet records at low density illustrate the inadequacy of sweepnet sampling alone as the basis for assessment of the effectiveness of grasshopper control methods. It may explain the high rate of mortality recorded in some small plot experiments, e.g., as reported by Ewen and Mukerji (1980). The sample unit used by those authors for estimating reductions in grasshopper numbers was 10 sweeps per subplot in unreplicated plots.

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REFERENCES

ABBOTT, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18, 265–267.

BROOKS, A. R. 1958. Acridoidea of southern Alberta, Saskatchewan, and Manitoba (Orthoptera). *Canad. Entomol. Suppl.*

CANNING, E. U. 1953. A new microsporidian *Nosema locustae* n. sp., from the fat body of the African migratory locust, *Locusta migratoria migratorioides* R. and F. *Parasitology*, 43, 287–290.

CANNING, E. U. 1962. The pathogenicity of *Nosema locustae* Canning. *J. Insect Pathol.*, 4, 248–256.

EWEN, A. B., AND MUKERJI, M. K. 1980. Evaluation of *Nosema locustae* (Microsporida) as a control agent of grasshopper populations in Saskatchewan. *J. Invertebr. Pathol.*, 35, 295–303.

HENRY, J. E. 1971. Experimental application of *Nosema locustae* for control of grasshoppers. *J. Invertebr. Pathol.*, 18, 389–394.

HENRY, J. E. 1972. Epizootiology of infection by *Nosema locustae* Canning (Microsporida: Nosematidae) in grasshoppers. *Acrida*, 1, 111–120.

HENRY, J. E. 1986. Effect of grasshopper species, cage density, light intensity, and method of inoculation on mass production of *Nosema locustae* (Microsporida: Nosematidae). *J. Econ. Entomol.*, 78, 1245–1250.

HENRY, J. E., FOWLER, J. L., WILSON, M. C., AND ONSAGER, J. A. 1985. Infection of West African grasshoppers with *Nosema locustae* Canning (Protozoa: Microsporida: Nosematidae). *Trop. Pest. Manage.*, 31, 144–147.

HENRY, J. E., AND OMA, E. A. 1974. Effect of prolonged storage of spores on field applications of *Nosema locustae* (Microsporidia: Nosematidae) against grasshoppers. *J. Invertebr. Pathol.*, 23, 371–377.

HENRY, J. E., AND OMA, E. A. 1981. Pest control by *Nosema locustae*, a pathogen of grasshoppers and crickets. *In* "Microbial Control of Pests and Plant

Diseases 1970–1980" (H. D. Burgess, Ed.), pp. 573–584. Academic Press, New York.

HENRY, J. E., OMA, E. A., AND ONSAGER, J. A. 1978. Relative effectiveness of ULV spray applications of spores of *Nosema locustae* against grasshoppers. *J. Econ. Entomol.*, **71**, 629–632.

HENRY, J. E., AND ONSAGER, J. A. 1982. Large-scale test of control of grasshoppers on rangeland with *Nosema locustae*. *J. Econ. Entomol.*, **75**, 31–35.

HENRY, J. E., TIAHRT, K., AND OMA, E. A. 1973. Importance of timing, spore concentrations, and levels of spore carrier in applications of *Nosema locustae* (Microsporida: Nosematidae) for control of grasshoppers. *J. Invertebr. Pathol.*, **21**, 263–272.

JOHNSON, D. L., AND HENRY, J. E. 1987. Low rates of insecticides and *Nosema locustae* (Microsporidia: Nosematidae) on baits applied to roadsides for grasshopper (Orthoptera: Acrididae) control. *J. Econ. Entomol.*, **80**, 685–689.

JOHNSON, D. L., HILL, B. D., HINKS, C. F., AND SCHAALJE, G. B. 1986. Aerial application of the pyrethroid deltamethrin for grasshopper (Orthoptera: Acrididae) control. *J. Econ. Entomol.*, **79**, 181–188.

JOHNSON, D. L., AND PAVLIKOVA, E. 1986. Reduction of consumption by grasshoppers (Orthoptera: Acrididae) infected with *Nosema locustae* Canning (Microsporidia: Nosematidae). *J. Invertebr. Pathol.*, **48**, 232–238.

OTTE, D. 1981. "The North American Grasshoppers: Vol. I. Acrididae. Gomphocerinae and Acridinae." Harvard Univ. Press, Cambridge.

OTTE, D. 1984. "The North American Grasshoppers: Vol. II. Acrididae, Oedipodinae." Harvard Univ. Press, Cambridge.

SAS Institute. 1982. "SAS User's Guide: Statistics." SAS Institute, Cary, North Carolina.

VICKERY, V. R., AND KEVAN, D. K. McE. 1983. A monograph of the orthopteroid insects of Canada and adjacent regions. *Lyman Entomol. Museum and Research Laboratory Memoir*, No. 13, Vol. II., Ste-Anne-de-Bellevue, Québec.