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Reduction of Consumption by Grasshoppers (Orthoptera: Acrididae) Infected with *Nosema locustae* Canning (Microsporida: Nosematidae)

D. L. JOHNSON[1] AND E. PAVLIKOVA*

*Agriculture Canada, Research Station, Lethbridge, Alberta T1J 4B1, and *Alberta Agriculture, Agriculture Center, Lethbridge, Alberta T1J 4C7, Canada*

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The effect of ingestion of *Nosema locustae* Canning spores on feeding by grasshoppers was measured in simultaneous laboratory and field experiments. After 21 days, fourth-instar *Melanoplus sanguinipes* (F.) nymphs, administered spores at the rates of 0, 2.0×10^4, 2.0×10^5, and 2.0×10^6 per grasshopper, showed dry matter consumption of 102, 87, 64, and 26 mg in 48 hr, respectively. Rate of inoculation was a significant factor in suppression of feeding after correction for the effects of developmental stage, sex, and body weight. The quantity of dry matter consumed decreased linearly with increasing rate of spore ingestion. Experiments on 50 caged 1-m^2 plots on pasture grass yielded similar trends in per capita consumption independent of the effects of mortality. Field consumption per integrated grasshopper-day was 108, 77, 31, and 27 mg dry wt at the four inoculation rates, over 20 days.

KEY WORDS: *Nosema locustae; Melanoplus sanguinipes;* feeding reduction; infection; inoculation; sublethal effects; consumption.

INTRODUCTION

The purpose of insect control is to reduce crop losses caused by insect damage to plant tissue. Most control measures are designed to kill sufficient numbers of insects so that the total amount of feeding is reduced to an acceptable level. Consequently, routine tests of the efficacy of insecticides, both chemical and microbial, tend to be based on assessment of mortality. This approach is so common that in the field of crop protection, efficacy is treated as a synonym of mortality. However, insecticides can be effective in protecting crops for a number of other reasons, including effects on consumption, development, reproduction, and activity. This is particularly true of microbial agents applied to debilitate pest insects while remaining endemic.

Nosema locustae Canning is a microsporidian pathogen of grasshoppers and crickets which occurs naturally at low levels. It is potentially useful in reducing damage to range and pasture grass by grasshoppers. It may also prove useful in long-term management of grasshopper outbreaks, allowing suppression of population growth. Initial research on the epizootiology of *N. locustae* in grasshoppers indicated that the disease could be established and would cause increased mortality (Henry, 1971, 1972). Application of spores at rates near 2.5×10^9/ha on 2 kg wheat bran/ha has been shown to be capable of reducing populations by about half in 4–9 weeks (Henry et al., 1973; Henry and Oma, 1974). The surviving population is typically 20–70% and up to 100% infected by the end of the growing season. Therefore, the effects of infection on feeding rates must be known if the value of *N. locustae* in plant protection is to be predicted. A recent experiment by Oma and Hewitt (1984) indicated a significant but unquantified reduction in feeding by infected female grasshoppers [*Melanoplus differentialis* (Thomas)]. In order to model the expected effects of wide-scale application of *N. locustae* on grasshoppers of rangeland, pas-

[1] To whom correspondence should be addressed.

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ture, and roadsides, it is necessary to quantify this reduction in feeding and verify the effect in the field. In this study, we quantified the effect of inoculation rate of *N. locustae* on subsequent consumption by grasshoppers in laboratory and field experiments.

MATERIALS AND METHODS

Food consumption by the migratory grasshopper, *Melanoplus sanguinipes,* inoculated with known quantities of *N. locustae* spores, was measured in simultaneous laboratory and field cage trials. Fourth-instar *M. sanguinipes* for both experiments were collected from a roadside (an area of ca. 100 m^2) near Turin, Alberta, Canada, on June 5, 1985. The spores used in both experiments were provided by J. E. Henry, and were produced at the USDA Rangeland Insect Laboratory, Bozeman, Montana, U.S.A. Prior to receipt for this experiment the spores were stored frozen in *M. differentialis* cadavers.

Experiment I: Measurement of feeding in the laboratory. The method of inoculation was the same for both experiments. The grasshoppers were individually caged in 19-ml vials with foam plugs for 24 hr. Spores were dispensed onto 7-mm diameter lettuce discs in 5-μl doses of distilled water and allowed to dry at room temperature. A total of 480 lettuce discs was prepared, 120 at each of four concentrations: 0, 10^4, 10^5, or 10^6 *N. locustae* spores per disc. Solution concentrations were determined via hemocytometer counts of the spores present in subsamples. A total of 120 grasshoppers was randomly assigned to each of the four concentrations and allowed to feed on 1 disc each for 24 hr at 25°C. Most of the discs were consumed within 1 hr. The treatment was repeated the following day, giving final known inoculation rates of 0, 2.0×10^4, 2.0×10^5, and 2.0×10^6 spores per grasshopper. Individuals that underwent ecdysis during the ingestion period were discarded, as were those which did not completely consume both lettuce discs.

To allow mortality to occur, the treated grasshoppers were placed in stainless steel screen cages (23 × 23 × 50 cm, width × length × height) by treatment, 30 per cage, in a controlled environment chamber (2.2 × 2.2 × 2.4 m; 8 hr dark at 15°C, 16 hr light at 25°C) for 21 days. They were fed crested wheat grass [*Agropryon cristatum* (L.)] from the field site, supplemented with fresh wheat (*Triticum aestivum* L. cv. Norstar) leaves.

The survivors remaining after 21 days were caged individually in white PVC pipe (diameter 8.5 cm, length 10 cm) screened on the ends. Consumption of fresh wheat leaves was measured over a 48-hr period at 25°C in a manner similar to the method of Holmberg and Hardman (1984). Wheat plants used in the trials were grown in a greenhouse at 25 ± 3°C. Plants in the three-leaf stage were cut at ground level, and the cut ends were immersed in water to allow maintenance of turgor during feeding. The wheat for each grasshopper was weighed fresh before feeding and weighed dry (24 hr at 70°C) after 48 hr of confinement with the grasshoppers. Undamaged wheat from each replicate was weighed, dried, and reweighed to provide an estimate of the proportion of dry matter. This value was used to calculate the initial dry weight of the wheat offered to the grasshoppers. Twenty grasshoppers from each of the four treatments were included in each replication of the experiment (three randomized blocks). Owing to the death of some grasshoppers after inoculation, the final number assayed was 166. At the end of the 48-hr feeding period, the consumption, weight, and sex of each grasshopper were recorded.

The quantity of dry matter eaten per survivor during 48 hr was subjected to analysis of variance appropriate to an unbalanced randomized block design with subsampling (GLM routine, SAS Institute Inc., 1982), and to analysis of covariance with body

weight as the concomitant variable. Orthogonal contrasts were constructed to compare the uninfected control groups with the groups which received spores, and to test for linear and nonlinear responses to rate of inoculation.

Experiment II: Estimation of feeding in field cages. The field experiment was designed to measure the effects of both mortality and feeding suppression caused by inoculation on consumption.

Estimates of grass consumption were made in cage trials on an ungrazed pasture at the Agriculture Canada Research Station, Lethbridge, Alberta. The pasture is a homogeneous stand of crested wheat grass which produced an average of 74.5 g dry matter/m^2 (SE = 4.30) in 1985. Fifty 1-m^2 plots with steel screen cages (0.8 × 1.25 × 1.0 m, width × length × height) were mowed on June 1 and October 2, 1984. The grass was oven-dried and weighed to give baseline estimates of relative differences in potential productivity of the plots. In 1985, the plots were raked in early spring and allowed to produce new growth before the feeding trial. The 50 bottomless cages were arranged in 10 randomized blocks on the pasture grass. One cage in each block contained no grasshoppers; this plot produced an estimate of the 1985 growth potential (CHECK85) relative to that of 1984 (CHECK84). Each of the other four cages in each block received 30 grasshoppers. Grasshoppers inoculated at the same rates as in the laboratory experiment were introduced into the experiment immediately (June 8) so that the result would reflect the combined effects of mortality and feeding suppression. At the end of 20 days, the surviving grasshoppers were recovered by vacuum and counted. The grass remaining in each of the 50 cages was cut and oven-dried to constant weight.

Estimates of the quantity of grass removed by feeding in each cage (REMOVED) were calculated by subtracting the grass remaining after 20 days of feeding (REMAIN85) from the grass in the respective cage without grasshoppers (CHECK85). Adjustment was made for the growth potential of each plot by reference to the 1984 dry matter production (GRASS84) as follows:

$$\text{REMOVED}_{ij} = (\text{CHECK85}_j \times \text{GRASS84}_{ij}/\text{CHECK84}_j) - \text{REMAIN85}_{ij}$$

for plot i (1,2,3,4) and block j (1,2,...,10). REMOVED is the estimate of consumption, equal to the difference between the potential and realized production of grass. The quantities of dry matter produced by the caged plots were compared by the analysis of variance. This variable includes the effects of both mortality and feeding suppression. Per capita consumption was calculated by dividing the quantity of dry matter removed from each caged plot by the corresponding number of grasshopper-days (GD) (Hewitt and Onsager, 1982; Onsager and Hewitt, 1982) for the plot. Feeding pressure in GD was estimated by the integral of the survival curve over the 20 days. Survival of grasshopper nymphs can be assumed to be exponential over short periods of time since r, the rate of change in numbers, does not vary, so that the number remaining at time t is defined by

$$N_t = 30e^{rt}.$$

The starting number (30 per cage) and the final number (counted at the end of the feeding trial) were known, so the GD for each cage was estimated by the integral

$$\int_0^{20} 30e^{rt}\,dt = 30(e^{20r} - 1)/r$$

where $r = \ln(N_{20}/30)/20$. Analysis of variance of the resulting estimates of per capita consumption ($= \text{REMOVED}_{ij}/\text{GD}_{ij}$) provided a test of the effects of feeding suppression independent of the effects of mortality.

RESULTS

Experiment I: Measurement of Feeding in the Laboratory

Mortality. Ingestion of *N. locustae* at inoculation rates of 2.0 × 10^4, 2.0 × 10^5 and

2.0×10^6 spores resulted in 32, 26, and 55% mortality, respectively, after 21 days (adjusted against the untreated group using the modified Abbott's formula, Henderson and Tilton, 1955). The results were similar to the rates of mortality reported from field trials (Henry et al., 1973).

Consumption 21 days after inoculation. Baseline consumption by the control group averaged 102 mg during the 48-hr period. Consumption by surviving grasshoppers was considerably reduced by administration of *N. locustae* (Table 1). On the average, the three rates of infection resulted in a reduction of feeding of approximately 40%, with an approximately linear relationship between inoculation rate and consumption.

Analysis of variance of the dry matter consumed by the individual grasshoppers indicated a highly significant effect of inoculation rate ($P = 0.005$) (Table 1). Partitioning of the significant effect of the treatment via orthogonal contrasts indicated that the wheat consumption by uninfected grasshoppers was significantly greater than that of the three infected groups ($P < 0.001$). A linear trend in consumption as a function of inoculation rate was indicated since the low and high rates differed significantly ($P < 0.001$). Their average did not differ from the central rate ($P = 0.40$), indicating no strong nonlinear effects in the range tested. Body weight attained by the infected survivors was not significantly less than that of the untreated group (Table 2), although the highest inoculation rate resulted in a reduction of 12%. It may be hypothesized that the difference in consumption is the result of the lower body weight attained by the most heavily infected grasshoppers, since consumption is closely related to body weight (Holmberg and Hardman, 1984; see also Table 5). However, it can be seen from the analysis of milligrams of dry matter consumed per milligram of body weight that this hypothesis can be rejected. Inoculation rate was a highly significant factor ($P = 0.002$) determining consumption even after body weight was adjusted for. In fact, the relationship between consumption and body weight was slightly but noticeably altered by infection with *N. locustae*, as evidenced by the lower slopes in regressions of consumption on body weight (Table 3). The relationship of body weight to consumption differed weakly between the uninfected group and the pooled infected groups ($P = 0.063$; multiple regression of orthogonally coded interaction variables).

Reduction in feeding was due entirely to slower development. The infected grasshoppers developed more slowly than the uninfected group (Table 4; $\chi^2_3 = 16.6$, $P < 0.001$). The individuals that attained adulthood within the time of the feeding trial were heavier than their fifth-instar cohorts (286 vs 230 mg) and consumed consider-

TABLE 1
MEAN DRY MATTER CONSUMPTION BY GRASSHOPPERS UNDER LABORATORY CONDITIONS, AS A FUNCTION OF RATE OF INOCULATION

N. locustae inoculation rate[a]	Consumption[b] in 48 hr (mg)	SE	*N*
0	102.3	5.8	48
2.0×10^4	86.6	7.5	43
2.0×10^5	64.0	5.0	44
2.0×10^6	26.3	5.5	31

[a] Spores administered per os.
[b] Dry weight, Norstar wheat.

TABLE 2
FRESH BODY WEIGHT AND CONSUMPTION PER BODY WEIGHT (SE) BY GRASSHOPPERS IN THE LABORATORY

N. locustae inoculation rate	Body weight, mg	Consumption/ body weight[a]
0	265 (6.7)	0.378 (0.017)
2.0×10^4	278 (8.8)	0.298 (0.026)
2.0×10^5	264 (6.7)	0.239 (0.017)
2.0×10^6	233 (8.7)	0.101 (0.023)

[a] Milligrams dry matter consumed divided by millligrams fresh body weight. The dry matter ratios were 1.28, 1.01, 0.81, and 0.34, respectively, since the grasshoppers consisted of 29.5% dry matter.

TABLE 3
PARAMETER ESTIMATES (SE) OF LINEAR REGRESSION ANALYSES OF CONSUMPTION (IN mg/48 h) AS A FUNCTION OF BODY WEIGHT (IN mg)

N. locustae inoculation rate	Intercept	Slope[a]	r^2
0	−65.9 (22.8)	0.635 (0.085)	0.539
2.0×10^4	−60.1 (29.9)	0.527 (0.105)	0.364
2.0×10^5	−33.1 (26.9)	0.367 (0.100)	0.224
2.0×10^6	−60.6 (22.7)	0.373 (0.095)	0.322

[a] All four models have slopes significantly greater than 0 ($P < 0.001$).

TABLE 5
ANALYSIS OF COVARIANCE OF CONSUMPTION BY *M. sanquinipes* AS A FUNCTION OF *N. locustae* INOCULATION RATE AND INSTAR, WITH BODY WEIGHT AS A COVARIATE

Source	df	SS	*F*	*P*
Blocks	2	9,851.7	6.29	0.043
Inoculation rate	3	49,843.6	21.23	0.003
Blocks × rate	5	3,913.3	0.96	0.444
Instar	1	25,816.5	31.75	<0.0001
Body weight	1	39,635.7	48.75	<0.0001
Residual	153	124,394.7		

ably more (97 vs 42 mg). However, the differences in stage of development were not great enough to account for the differences in dry matter consumption. Inclusion of instar and body weight in the model did not account for the treatment differences (Table 5), although both instar and body weight were significant factors affecting consumption.

Females consumed slightly more than males, e.g., 110 mg (SEM = 10.2) vs 85.7 mg (SEM = 8.90) in the untreated group. This difference was due partly to the greater weight of the females (272 vs 251 in the untreated group). When sex was included as a covariate in the model explaining differences in dry matter consumed, inoculation rate remained highly significant ($P = 0.006$) while sex alone (i.e., independent of body weight) was not important in determining consumption ($P = 0.29$).

TABLE 4
NUMBERS AND PERCENTAGES OF GRASSHOPPERS IN EACH DEVELOPMENTAL STAGE 21 DAYS AFTER INOCULATION OF FOURTH-INSTAR *M. sanguinipes* IN THE LABORATORY

	N. locustae inoculation rate			
Stage	0	2.0×10^4	2.0×10^5	2.0×10^6
IV	0	0	1 (2%)	1 (3%)
V	15 (31%)	17 (40%)	14 (32%)	22 (71%)
Adult	33 (69%)	26 (60%)	29 (66%)	8 (26%)

Experiment II: Estimation of Feeding in Field Cages

Over the 20 days of confinement in the field cages, the low, medium, and high rates of inoculation resulted in 29, 32, and 35% mortality, respectively (adjusted against the uninfected group via the modified Abbott's formula). The quantities of grass harvested from the caged plots at the end of the 20-day period, adjusted for growth potential in comparison with the 1984 harvest data, showed significant differences due to inoculation rate ($P = 0.012$, Table 6). The number of survivors remaining in the cages containing inoculated grasshoppers was significantly less than the number remaining in the control group ($P = 0.016$, Table 6). Owing to this mortality, the grass in the cages with infected grasshoppers was subjected to feeding by fewer grasshoppers.

The total GD, or "feeding pressure," on the cages differed between the inoculated and healthy groups (16% higher in the control group, $P = 0.060$, Table 6). However, this unequal feeding pressure did not explain all of the effect of the treatments. The per capita consumption, or forage loss per GD, differed significantly among treatments. These values showed a trend similar to that of the per capita consumption estimates derived from the laboratory study. The uninfected grasshoppers ate significantly more grass than the infected grasshoppers ($P = 0.034$), as was the case under laboratory conditions. However, the de-

TABLE 6
GRASS CONSUMPTION BY *M. sanguinipes* IN THE FIELD CAGES

N. locustae inoculation rate	Grass consumed[a] (g/m^2)	Survivors[b]	Grasshopper-days, GD	Consumption per GD (mg)
0	44.9 (12.9)	14.3 (2.2)	409 (37)	108 (29)
2.0×10^4	26.5 (16.7)	10.2 (1.8)	357 (28)	77 (45)
2.0×10^5	10.0 (10.2)	9.8 (0.9)	359 (13)	31 (32)
2.0×10^6	9.7 (9.0)	9.3 (1.6)	344 (27)	27 (24)

Note. Values are means (SE) of the results of 10 replications of the experiment.
[a] Adjusted to the 1984 potential.
[b] Number of the original 30 grasshoppers per cage which survived 20 days.

crease in the quantity consumed with increasing quantity of spores ingested was statistically discernible under laboratory conditions only, owing to the much greater variability of the field data. A linear trend was suggested by the relative values of the means (Table 6), but it was obscured by random error or other unmeasured factors operating in the field ($P = 0.162$).

The untreated grasshoppers consumed more dry matter in the field cages than their untreated cohorts did in the laboratory. This is not surprising, since crested wheat grass had a higher percentage dry matter (49.8%, SE = 7.2) than the greenhouse-grown wheat used in the laboratory experiment (12.1%, SE = 1.2). In addition, outdoor microclimatic conditions may have stimulated greater rates of feeding than were attained under artificial conditions. No measurable rain occurred during the outdoor trials, and air temperature minima and maxima fell between 4 and 13°C and 20 and 31°C, respectively. Perhaps the greatest difference between the techniques employed in the laboratory and field experiment concerns the measurement of wastage. While only consumption was measured in the laboratory, the per capita consumption estimates from the field cages included both consumption and wastage caused by clipping of vegetation.

DISCUSSION

Our results showed larger differences than were found by Oma and Hewitt (1984). They estimated forage consumption by *M. differentialis* based on average initial leaf weight and regressed these values on a posteriori ratings of infection. Males showed no effect of infection on food consumption, while females had significantly lower rates of feeding at higher rates of infection. It may be that the effects of infection on the smaller *M. sanguinipes* in our experiments were more dramatic, and that inoculation of the fourth instar in our experiments allowed more time for development of the disease. Oma and Hewitt (1984) inoculated fifth-instar grasshoppers.

Evaluation of the usefulness of *N. locustae* in management of grasshopper pests should be made in light of the effects of infection on consumption. The realized efficacy of application of spores on bran may be considerably higher than has appeared to be the case in some evaluations based on mortality assessment alone. Standard applications of around 2.5×10^9 spores/ha can reduce grasshopper density by 50–60% (Henry et al., 1973; Henry and Oma, 1981). Infection among the survivors varies between experiments. In trials conducted in Canada, infection rates from around 5% in Alberta (Johnson and Henry, 1984) to 50% in Saskatchewan (Ewen and Mukerji, 1980) have been observed 4 weeks after application, depending presumably on characteristics of the bait formulation, weather, and the method of assay of infection. The pathogen is transmitted through cannibalism, and, even in studies that provide low initial infection rates, the incidence of *N. locustae* spores can increase dramati-

cally. For example, Johnson and Henry (1984) found a fourfold increase in the proportion of the population infected between 4 and 6 weeks after application. In some cases, most of the survivors may carry the disease: Ewen and Mukerji (1980) recorded 95–100% infection between 9 and 12 weeks after application of spores on bran. Estimations of field infection rates are not directly comparable with known inoculation rates, since a qualitative scale of trace, light, moderate, and heavy is standard methodology. However, Henry and Oma (1981) refer to grasshoppers containing 10^5 spores each as being "lightly infected" and to *M. bivittatus* individuals containing 10^7 to 10^9 spores as "moderately infected." We believe that the suppression of feeding indicated in the results reported here occurs under field conditions, and that the degree of control of grasshopper damage afforded by application of *N. locustae* may previously have been underestimated. Future field experiments might be better assessed on the bases of both grasshopper abundance and reduction of damage to vegetation.

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