Feeding preference and spatial distribution of grasshoppers (Acrididae) in response to nitrogen fertilization of Calamovilfa longifolia

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Abstract. Feeding and distribution responses of grass-feeding grasshoppers to nitrogen fertilization of a native C₄ grass, Calamovilfa longifolia (Hook.), were examined in the field. When fertilized with ammonium nitrate, nitrogen concentration in the foliage increased. In paired choice tests, the grass-feeding Ageneotettix deorum (Scudder) typically consumed more leaf material fertilized at intermediate levels although preferences for leaves with altered levels of nitrogen were sometimes inconsistent. In field plots, total biomass of grasshoppers which fed on grasses increased with nitrogen application; grasshoppers with mixed diets exhibited no apparent distributional response to fertilizer treatments.

Introduction

Local distribution of grasshoppers (Acrididae) is patchy both within and among species (Joern, 1982a,b, 1986). Many factors may influence spatial distribution including: vegetation structure; plant species diversity; microclimate; availability and suitability of host plants; substrates which render individuals cryptic; and availability of oviposition sites (reviewed in Uvarov, 1977; Joern, 1982a, 1986). This study investigates the role of host plant quality on distributional patterns of grass-feeding grasshoppers.

Insect herbivores, such as grasshoppers, are often nitrogen limited (Mattson, 1980; Bernays, 1982; Prestige & McNeill, 1983; Scriber, 1984). Under these conditions, individuals should move to patches in which the foliar nitrogen content is highest, especially if food is limiting. It is presently unresolved whether foliar nitrogen concentration greatly influences distributional patterns and, if so, by what mechanism. Both overall satiation level of an individual or the strength of specific nutritional stimuli (or chemical defences) in leaf tissue may determine host plant acceptability and ultimately influence herbivore distribution by modifying searching behaviour (Bernays, 1982; Bernays & Simpson, 1982; Dethier, 1982; Miller & Strickler 1984).

Among possible explanations, two physiological mechanisms predict that grasshoppers should be attracted to and congregate in patches of high quality food. Grasshoppers may possess the sensory capabilities to distinguish among leaves of different nitrogen (or specific free amino acid) concentrations. If so, resulting distributional patterns will follow from a series of selective choices of leaves with higher nitrogen concentration and eventually, a large number of individuals will be located in the same, high-quality patches. Alternatively, grasshoppers may not be capable of actively discriminating among leaves with different nitrogen levels (Bernays, 1982; Chapman, personal communication) even though grasshopper species distributions are patchy and correspond with foliar nitrogen content of host plants. Though immediate discrimination is not possible in this view, nutrient feedback mechanisms could still lead to increased restlessness on nutritionally poor host plants where individuals leave poor patches but are effectively trapped by inactivity in the vicinity of higher quality foods (Bernays, 1982; Bernays & Simpson, 1982).

The hypothesis that grass-feeding grasshoppers are distributed according to foliar nitrogen concentration was tested in the field by application of N fertilizer. In addition, the hypothesis that grass-feeding grasshoppers preferentially eat leaves with higher foliar nitrogen content was tested using paired choice tests among leaves from the fertilization treatments.

Materials and methods

Study site

The study was performed at Arapaho Prairie in the sandhills region of Nebraska, USA (Arthur Co., T18N, R39W, sections 31 and 32). Sandhills prai-
rie, typical of north-central Nebraska, is a mixed-grass association which includes species of both tallgrass and shortgrass prairies. Keefer, Harrison & Vescio (1980) describe the plant community. Annual precipitation averages 40 cm, 80% of which falls between April and September. During 1984, 27-7 cm of rainfall was recorded 20 km north-east of Arapaho Prairie between April and September. A large and diverse grasshopper assemblage exists on the site; all species are univoltine (Joern, 1982b).

Patches (probably clones) of prairie sandreed, Calamovilfa longifolia (Hook.), were used because they provided large (c 10 m diameter), circular, nearly monospecific (c 98% relative cover) plots. Clones contained mature tillers. Generally, the system was relatively simple yet provided a natural setting for the distribution experiment. Clones were large enough to allow leaves to be sampled for the preference tests and chemical analyses with only a negligible reduction in leaf availability. In addition, grasshopper distribution patterns over the nitrogen concentration gradient would not be confounded by differences in plant species composition.

Methods

Two experiments were performed: one to determine the effect of leaf nitrogen concentration on food preference in Ageneotettix deorum (Scudder) in paired comparisons and another to test whether grasshoppers were distributed in response to nitrogen fertilization levels. The same fertilization plots were used in both experiments. Three large clones of C. longifolia, two of which measured c 9 m and one c 12 m in diameter, were employed. Each clone was equally divided into three triangular sections with unfertilized 1 m borders between the sections radiating out from an unfertilized 2 m diameter circle in the centre of the clone.

Three N fertilizer levels were used: 0, 3 and 6 g N m$^{-2}$. Nitrogen was applied as an aqueous solution of NH$_4$NO$_3$ (21 m$^{-2}$); the 0 g N m$^{-2}$ sections received equivalent water only. Clones were fertilized twice (30 June and 20 July 1984).

Total leaf nitrogen, per cent acid-pepsin digestibility of leaves, leaf water content and grass biomass for each of the fertilization levels on each clone were determined. Acid-pepsin digestibility has been suggested (Belovsky, 1986) as an alternative to foliar N as a measure of host plant quality. Leaf samples for determining N content and acid-pepsin digestibility were collected at the same time as experimental leaves used in the feeding trials. Leaves were weighed fresh, dried and reweighed to measure water content. After dried samples were ground, total N content (Kjeldahl analysis, Association of Official Agricultural Chemists, 1984) and acid-pepsin digestibility (Association of Official Agricultural Chemists, 1984) were measured with two subsamples per plot. Grass biomass was determined by clipping four randomly placed 0.1 m$^2$ subplots from each treatment plot, drying and weighing.

Preference and fertilization

Feeding trials began 6 days after the second fertilizer application and were conducted over a 9-day period (26 July–3 August, 1984). Paired leaves, each from a different N fertilizer treatment, were presented to the univoltine grasshopper, Ageneotettix deorum, in the field for a 2-h period (0900–1100 mountain daylight time [MDT]). Methods followed those of Heidorn & Joern (1985). Fresh C. longifolia leaves from the two nitrogen levels to be compared were collected, wrapped in moist paper towels in plastic bags and kept cool until used. Leaf pairs were presented in water-filled, capped vials with the leaves protruding through a slit in the cap. Vials were buried along a 50 m transect (one vial every 2 m) in the middle of circular, closely clipped areas measuring 60 cm in diameter; these leaves became the only available vegetation within a 30 cm radius. Grasshoppers were naturally recruited to the vials from surrounding vegetation. The area of each leaf blade eaten by one grasshopper in one feeding event was measured following specific procedures outlined by Heidorn & Joern (1985); leaf area was calculated from photograms.

Feeding trials were performed on each of the three possible pairs (0 vs 3, 0 vs 6 and 3 vs 6) within each clone. Each trial was replicated between 15 and 19 times depending on the number of stations used by A. deorum in a particular trial. Grasshoppers were allowed to freely choose grass leaves and only one grasshopper was allowed to feed at a vial. Grass pairs on which more than one grasshopper fed were eliminated from the analysis. After a grasshopper finished feeding (10–30 min), the vial was removed. The trial was ended after 2 h whether or not every station had been used.

Leaf area eaten was determined by comparing the damaged leaf against original leaf area before the test; leaf area eaten was gravimetrically determined. Leaf area was transformed into dry
Grasshopper distribution

Grasshopper distribution patterns were described 3 weeks after the last fertilizer application. Each section of each clone was carefully enclosed using a 1.5 m high fiberglass screen barrier. All grasshoppers in each section were then removed by systematically collecting with an insect net. Sampling ended when no new grasshoppers were caught in a 20 min period.

Statistical analyses

ANOVA was used to analyse the responses (grasshopper food preference or distribution) using fertilization classes as fixed levels. Addition of N fertilizer may have other physiological effects and consequently alter nutrient status of the plant in addition to increased foliar nitrogen, so the analysis evaluates the response by grasshoppers to any fertilizer effect. A balanced incomplete block design was used to compare the amount eaten among treatments in the preference comparisons. This specific problem and the statistical design is treated in Cochran & Cox (1957, p. 440). Mean values from each trial were used in the ANOVA since the replications within each experiment represent subsampling for this overall analysis and are not actual replications in such an experimental design. Use of means from each set of paired comparisons in the ANOVA is justified according to the Central Limit Theorem. Comparisons among fertilization levels were made using orthogonal contrasts based on a priori planned comparisons. Patterns of grasshopper distribution, total N content, acid-pepsin digestibility, water content and grass density were examined using a randomized complete block design. Calculations for statistical analyses were performed using SAS, PROC GLM (SAS Institute, 1983). For each feeding trial, mean amounts eaten per fertilizer treatment level and pairwise t-tests were also calculated for each of the nine pairs. Such t-tests provide an alternative method for examining the degree of variation in individual grasshopper response but must be interpreted cautiously as combined conclusions from these multiple t-tests are prone to type II errors and are not by themselves sufficient to draw conclusions.

Results

Total nitrogen content of C. longifolia leaves increased linearly ($P < 0.003$) and possibly curvilinearly (quadratic orthogonal contrast, $P < 0.07$) with increased fertilizer level (Fig. 1). Per cent acid-pepsin digestibility and per cent water content of C longifolia leaves showed no trend ($P > 0.1$) with increased fertilizer level. A general increase in C. longifolia biomass was observed ($P < 0.06$) even though the response was more variable at high N levels.

![Fig. 1. Mean total foliar nitrogen and grass dry weight in response to NH$_4$NO$_3$ fertilization of Calamovilfa longi\-folia. Vertical bars represent 1 SE; n = 3.](image)

Results of the food preference trials for A. deorum are presented in Fig. 2 and Table 1. ANOVAS for total amount eaten per treatment (Fig. 2) were not in total agreement with present hypotheses since the average amount eaten in response to fertilization level showed an initial increase followed by a clear decrease. The '0' vs '3' and 6' orthogonal contrast ($P < 0.08$) suggested that leaves from the fertilized plots may have been preferred to the control plot (0) leaves. However, the '3' vs '6' contrast ($P < 0.05$) indicates that the leaves from the 3 g N m$^{-2}$ sections were preferred to those from the 6 g N m$^{-2}$ sections. This contradicts the expected preference for leaves with highest total nitrogen levels although foliar N levels of 6 g treatments were not much greater than 3 g treatments (Fig. 1). Results of preference tests were also analysed within paired-comparison experiments (Table 1). Because of high levels of variation, only one of the individual paired comparisons produced a significant preference (clone 1, 0 vs 3, $P < 0.03$). However, inspection of the values of amounts eaten (based on means) clearly indicates that the four highest values given for consumption are for the 3 g N m$^{-2}$. 


Biomass of grasshoppers and different fertilizer levels are summarized in Fig. 3. Grasshoppers were split into two feeding guilds: primarily grass-feeders and mixed/forb-feeders (Table 2 based on Joern, 1983). Since different species, sexes and developmental stages often vary greatly in size and the amount they eat, distributional analyses were based on biomass. The total biomass m⁻² of grass-feeders showed a linear increase with fertilization \((P < 0.003)\) while the relationship showing a similar trend was not significant for mixed/forb-feeders \((P < 0.2)\). A comparison of distributions of grass-feeders in relation to fertilizer level (Fig. 3a) and the average level of total leaf nitrogen in relation to fertilizer level (Fig. 1) indicates that the two responses were very similar. We conclude that the grass-feeding guild of grasshoppers responded positively to increasing N fertilizer level, probably in response to increasing nitrogen concentration of foliage. However, responses of individual species are variable and complex (Fig. 3b).

Within the grass-feeding guild (Fig. 3b) none of the species-specific comparisons showed significant responses to N fertilizer \((P > 0.1)\). This is probably due to the small sample size. Of the grass-feeders, only *Mermiria bivittata* (Serville) and *Phoebaliotes nebrascensis* (Thomas) were found in all three clones at all fertilization levels. These two dominant grasshopper species showed similar and positive responses in distribution with increasing N fertilizer levels (Fig. 3b). The remaining species, combined, did not exhibit this result \((P > 0.9)\). While the experiment probably did not have significant power to adequately assess all individual species responses, others must have been important since grass-feeding species as a group did significantly respond to fertilizer rate.

**Discussion**

Abundant evidence supports the notion that nitrogen is extremely important and often limiting to insect herbivores, including grasshoppers (Smith & Northcott, 1951; McNeill & Southwood, 1978; Mattson, 1980; Bernays, 1982). Since time spent feeding is very short relative to the passage rate of food through the gut (Bernays & Simpson 1982, Joern, Mitchler & O’Leary, 1986) grasshoppers should take leaves with higher levels of nitrogen when possible. If host plants are of critical importance, the distributions of grasshoppers and foliagel quality should be congruent.

Distribution patterns of graminivorous grasshoppers were congruent with the applications of increased levels of nitrogen fertilization. For feeding guilds, total biomass of grass-feeders significantly increased with increasing nitrogen fertilization while biomass of mixed-feeders did

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**Table 1.** Pairwise t-tests for individual Ageneotettix deorum preference trials. Number of leaf pairs \((n)\), mean amount eaten for each of the two fertilization levels, difference between the mean amounts eaten, SE of the difference, the value of \(t\) and the probability that mean amounts eaten differ are given for each of the nine separate trials. An * indicates statistical significance at the 0.05 level.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Pair</th>
<th>n</th>
<th>Low level</th>
<th>High level</th>
<th>Difference</th>
<th>SE of difference</th>
<th>(t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 vs 3</td>
<td>16</td>
<td>0.156</td>
<td>0.563</td>
<td>-0.407</td>
<td>0.169</td>
<td>2.40*</td>
</tr>
<tr>
<td>1</td>
<td>0 vs 6</td>
<td>18</td>
<td>0.444</td>
<td>0.428</td>
<td>-0.016</td>
<td>0.254</td>
<td>-0.07</td>
</tr>
<tr>
<td>1</td>
<td>3 vs 6</td>
<td>19</td>
<td>0.658</td>
<td>0.484</td>
<td>-0.174</td>
<td>0.271</td>
<td>-0.64</td>
</tr>
<tr>
<td>2</td>
<td>0 vs 3</td>
<td>18</td>
<td>0.439</td>
<td>0.600</td>
<td>0.161</td>
<td>0.239</td>
<td>0.68</td>
</tr>
<tr>
<td>2</td>
<td>0 vs 6</td>
<td>18</td>
<td>0.572</td>
<td>0.356</td>
<td>-0.216</td>
<td>0.188</td>
<td>-1.15</td>
</tr>
<tr>
<td>2</td>
<td>3 vs 6</td>
<td>16</td>
<td>0.369</td>
<td>0.438</td>
<td>0.069</td>
<td>0.203</td>
<td>0.34</td>
</tr>
<tr>
<td>3</td>
<td>0 vs 3</td>
<td>18</td>
<td>0.394</td>
<td>0.922</td>
<td>0.528</td>
<td>0.390</td>
<td>1.35</td>
</tr>
<tr>
<td>3</td>
<td>0 vs 6</td>
<td>15</td>
<td>0.273</td>
<td>0.460</td>
<td>0.187</td>
<td>0.197</td>
<td>0.95</td>
</tr>
<tr>
<td>3</td>
<td>3 vs 6</td>
<td>16</td>
<td>0.850</td>
<td>0.581</td>
<td>-0.269</td>
<td>0.321</td>
<td>-0.84</td>
</tr>
</tbody>
</table>
not (Fig. 3). Not surprisingly, response of the mixed-feeders was highly variable since forbs, which were rare in the *C. longifolia* clones, often make up a large portion of their diets.

Do grasshoppers discriminate among leaves of different foliar nitrogen concentrations immediately upon feeding and prefer leaves with higher N levels? Paired choice tests with *A. deorum* indicated that preferences were found for leaves from the intermediate level, 3 g N m⁻². If *A. deorum* preferred eating leaves based solely on higher nitrogen levels, we expected that higher N concentrations would be preferentially eaten. While leaves from the fertilized plots (combination of both rates) were preferred to unfertilized leaves, results were not fully consistent with the hypothesis that leaves with highest N levels would be preferred in that leaves from the 3 g N m⁻² plots were preferred to those from the 6 g N m⁻² plots. Since average foliar N levels for fertilized leaves did not greatly differ (Fig. 1), a reasonable secondary hypothesis is that there should be no difference in the amount eaten between the 3 and 6 g N m⁻² treatments. This result was also not observed. Lack of linearity could result from a response to other nutrient changes caused by N fertilizer such as increased levels of chemical defenses (Coley, Bryant & Chapin, 1985) which may not vary in the linear manner observed for foliar nitrogen. High fertilization levels may also have resulted in higher foliar N quality as well as quantity in the form of different abundances of free amino acids (Prestige & McNeill, 1983). It is not yet presently known if defensive chemicals (e.g. nitrogen containing alkaloids or cyanogens) are present in *C. longifolia* and whether concentrations of either defensive compounds or free amino acids vary in response to N fertilizer. Alternately, any number of other physiological responses may have occurred in *Calamovilfa* with fertilizer application which could have resulted in lowered feeding on 6 g N treatments. However, increased accumulation on 6 g N treatments should not be expected if this mechanism is of primary importance for determining patterns of distribution.

Table 2. Average numbers of grasshoppers collected for each level of fertilizer treatment. Standard deviations are presented in parentheses when applicable. The number of clones (maximum of 3) and the number of sections within clones (maximum of 9) in which each species is found is indicated as another measure of the response.

<table>
<thead>
<tr>
<th>Grasshopper species</th>
<th>Number of clones</th>
<th>Number of sections</th>
<th>Fertilization level</th>
<th>0 g m⁻²</th>
<th>3 g m⁻²</th>
<th>6 g m⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass feeders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Agenetetettix deorum</em> (Scudder)</td>
<td>2</td>
<td>2</td>
<td>1 (0)</td>
<td>0</td>
<td>2 (0)</td>
<td></td>
</tr>
<tr>
<td><em>Amphitornus coloradus</em> (Thomas)</td>
<td>3</td>
<td>5</td>
<td>1.5 (0.71)</td>
<td>0</td>
<td>2.0 (1.0)</td>
<td></td>
</tr>
<tr>
<td><em>Arphia pseudonietana</em> (Thomas)</td>
<td>2</td>
<td>4</td>
<td>1.5 (0.71)</td>
<td>0</td>
<td>2.0 (1.0)</td>
<td></td>
</tr>
<tr>
<td><em>Cordillcris occipitalis</em> (Thomas)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Memiria bivittata</em> (Serville)</td>
<td>3</td>
<td>9</td>
<td>4.3 (1.2)</td>
<td>7.3 (4.0)</td>
<td>9.3 (3.5)</td>
<td></td>
</tr>
<tr>
<td><em>Opeia obscua</em> (Thomas)</td>
<td>2</td>
<td>6</td>
<td>3.0 (1.4)</td>
<td>5.5 (4.9)</td>
<td>5.0 (1.4)</td>
<td></td>
</tr>
<tr>
<td><em>Phoetaliotes nebrascensis</em> (Thomas)</td>
<td>3</td>
<td>9</td>
<td>9.0 (5.6)</td>
<td>14.0 (8.0)</td>
<td>15.0 (10.3)</td>
<td></td>
</tr>
<tr>
<td><em>Spharagemon collars</em> (Say)</td>
<td>1</td>
<td>2</td>
<td>1 (0)</td>
<td>0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Mixed/forb feeders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hesperetettix speciosus</em> (Scudder)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Hesperetettix viridis</em> (Thomas)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td><em>Melanopus bivittatus</em> (Dodge)</td>
<td>2</td>
<td>3</td>
<td>1.0 (0.0)</td>
<td>1.0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Melanopus femurrubrum</em> (DeGeer)</td>
<td>2</td>
<td>6</td>
<td>1.0 (1.4)</td>
<td>2.5 (2.1)</td>
<td>5.0 (5.7)</td>
<td></td>
</tr>
<tr>
<td><em>Melanopus foedus</em> (Scudder)</td>
<td>3</td>
<td>8</td>
<td>2.3 (1.2)</td>
<td>1.7 (1.2)</td>
<td>3.5 (0.7)</td>
<td></td>
</tr>
<tr>
<td><em>Melanopus sanguinipes</em> (F.)</td>
<td>2</td>
<td>2</td>
<td>2.0</td>
<td>0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><em>Schistocrates lineata</em> (Scudder)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Results concerning preference are probably applicable to other graminivorous grasshopper species besides *A. deorum*. In other paired-choice tests, the grass-feeding *P. nebrascensis* could not consistently discriminate between leaves of seedling grasses treated with low and high nitrogen fertilizer for each of four species under laboratory conditions (Joern & Alward, 1988).

Taken together, the two experiments indicate that, while there was no detectable preference for *C. longifolia* leaves with highest nitrogen levels, there was a general distributional response to increasing foliar nitrogen; more nutritious patches of grass support higher grasshopper biomass. Alternately, grass availability may explain grasshopper distributions since grass biomass increased in response to fertilization rate (*P < 0.08*); but it is unlikely that grasshoppers are food-limited (in a biomass sense) at this site. It must also be stressed that the preference experiments were performed with *A. deorum* which was not among the most frequently found grasshoppers in the *C. longifolia* clones although it is often the most abundant grasshopper species at Arapaho Prairie.

Does evidence support the view that grasshoppers were effectively trapped in patches of high-quality food resources because of feeding control mechanisms (satiety) and their resulting effects on restlessness (Bernays, 1982; Bernays & Simpson, 1982; Dethier, 1982; Miller & Strickler, 1984)? Since movements of individuals were not specifically tracked in response to fertilizer treatments or leaf nitrogen levels, the question cannot be strictly evaluated. However, evidence exists which is consistent with this interpretation. For example, hunger in the grasshopper *Melanoplus sanguinipes* (Fabr.) resulted in increased random search movement until an acceptable food source was encountered (Mulkern, 1969). Though not conclusive, the observed distribution of grasshoppers in the present experiment is also consistent with this interpretation. These results suggest that the impact of food quality on dispersal behaviour should be studied in more detail. We conclude that changes in host plant quality, as indicated by foliar nitrogen concentration, affect grasshopper distributions. That these results were observed under natural conditions, where many additional factors also vary, strengthens our conclusion.

Acknowledgments

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References


Grasshopper response to fertilization


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