

Effect of Nitrogen Fertilization on Choice Among Grasses by the Grasshopper *Phoetaliotes nebrascensis* (Orthoptera: Acrididae)

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ABSTRACT The influence of nitrogen fertilization on host plant selection among grasses by the grasshopper *Phoetaliotes nebrascensis* Thomas was examined in the laboratory. Grasses used in the study are normally found in natural *P. nebrascensis* diets. Clear preferences among grass species were observed. Nitrogen fertilization resulted in decreased feeding on *Andropogon hallii* Hack. and possibly increased feeding on *A. scoparius* Michx. within grass-species trials. No significant differences in amounts eaten between high- and low-N fertilization treatments were observed in tests with the other grass species. Preference rankings among grass species by *P. nebrascensis* were somewhat altered because of the influence of fertilization on *Agropyron smithii* Rydb. However, nonfertilized *A. smithii* ranks higher than fertilized plants. In general, N fertilization did not clearly affect selection among grass species, although some unique responses were observed.

KEY WORDS Insecta, *Phoetaliotes nebrascensis*, foliar nitrogen, host selection

NITROGEN IS OFTEN a key nutrient which can influence survival (McGinnis & Kasting 1966, White 1984), reproduction (Smith & Northcott 1951, McCaffery 1975), and the degree of host plant use by insect herbivores (McGinnis & Kasting 1966, Mattson 1980, Bernays 1982). Not only is nitrogen usually limited in leaf material (Mattson 1980), but variation in nitrogen availability through time may greatly influence both spatial distributions (Bernays 1982, Miller & Strickler 1984) and population fluctuations of herbivorous insects (Rhoades 1983, White 1978, 1984).

Chemical, physical, and extrinsic (ecological) attributes of available plants direct host selection by grasshopper species (Mulkern 1967, Chapman 1974, Bernays & Chapman 1977, 1978, Otte & Joern 1977, Joern 1979). Clearcut choices among alternative host plants are often observed in preference studies, although results do not always predict actual diets observed under natural conditions (Heidorn & Joern 1984). Whereas differences in classes of defensive chemicals among species may be sufficient to explain host plant selection, especially among forbs (Chapman 1974, Bernays & Chapman 1978), attributes responsible for choices among grass species have been elusive (Bernays & Chapman 1978, Capinera et al. 1983, Heidorn & Joern 1984).

Although most studies of host plant selection by insect herbivores examine toxic defensive chemicals, primary nutrients (sugars, protein, free amino acids, etc.) may play an important role. Many sugars stimulate grasshopper feeding and act in an additive fashion to determine meal size (Cook 1977,

Bernays & Chapman 1978). The amino acids L-proline and L-serine are phagostimulants to grasshoppers as well, but they do not act in an additive fashion (Cook 1977). In studies to date, salts, phospholipids, and other possible nutrients have had little influence on feeding in grasshoppers (Bernays & Chapman 1978). Bernays (1982) suggests that host plants of high quality (for example, high N content) may decrease the amount of movement of individuals, an account which would be consistent with the above observations. All available evidence suggests that primary nutrients may play a central role in directing selection among host plants, especially for grasses.

We examined the role of nitrogen in the selection among grass species by examining grasshopper feeding responses to two levels of N-fertilization. Two predictions were tested: highly N-fertilized leaves should be chosen over those with lower N levels within a species, if nitrogen is an integral factor in the host plant selection process; and selection among grass species should be influenced by nitrogen fertilization, although nutritional nitrogen is only one of many chemical attributes which can simultaneously contribute to host selection. Factors other than nitrogen levels and grass species identity were not specifically examined in this study.

Materials and Methods

Phoetaliotes nebrascensis Thomas, a common North American range grasshopper which feeds on a variety of grasses (Mulkern et al. 1969, Pruess 1969, Joern 1979, 1985), was used. Grasshoppers

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were hatched from eggs laid by adults collected in the Nebraska sandhills (Arapaho Prairie, Arthur County, Neb.). Diets of developing nymphs consisted of wheat bran fortified with brewers yeast (5% by weight) and lettuce. Adults were used in the feeding experiments; adults were fed wheat bran supplemented with lettuce except during trials. Individuals were held without food overnight before testing began in the morning (approximately 0800–0900 hours CDST). All tests were performed in a temperature-controlled laboratory at room temperature (ca. 22–25°C). Cages were placed near large windows to provide natural light.

Four grass species were included in the tests (nomenclature from Great Plains Flora Association 1986)—*Agropyron smithii* Rydb. (western wheatgrass), *Andropogon hallii* Hack. (sand bluestem), *Andropogon scoparius* Michx. (little bluestem), and *Bouteloua gracilis* (H.B.K.) Lag. ex Griffiths (blue grama). Plants were grown from seed (Stock's Seed Farm, Murdoch, Nebr.) in a greenhouse and fertilized with modified Hoagland's solution with low (0.01 g N/liter) and high (0.32 g N/liter) nitrogen levels. Ammonium and nitrate were used as nitrogen sources. Grasses were watered daily with ca. 1 liter of nutrient solution for each greenhouse flat (35 by 50 cm). Fertilization treatments increased the foliar nitrogen levels in all grass species. Nitrogen levels of fertilizer treatments based on single bulk samples (Kjeldahl method, 1 g dry wt) for each grass species were (mg N/g dry wt): *A. smithii*, low N, 8.1, high N, 12.7; *A. hallii*, low N, 7.8, high N, 14.6; *A. scoparius*, low N, 8.6, high N, 11.2; and *B. gracilis*, low N, 15.5, high N, 22.0. Paired choice tests were begun just after grasses had begun flowering. Trials ran from 21 July until 8 August 1984. Order of the paired combinations was randomly assigned.

A balanced incomplete block experimental design was used to examine preferences among grass species in relation to fertilization and taxonomic identity of grass species. This design is appropriate when individuals make comparative ratings of different objects presented to them in situations when all objects cannot be simultaneously presented (Cochran & Cox 1957, 440). This problem is inherent in the present study. Because grasshoppers generally fill their gut while eating, it becomes increasingly difficult to distinguish among lower rated choices when all plants are presented simultaneously because of reduced intake. In our experiments, paired leaves of different treatments (combinations of fertilization and grass species) were presented to *P. nebrascensis* adults. Each paired combination was replicated 20 times. The amount of leaf material removed from grasshopper feeding was measured.

Choice tests with *P. nebrascensis* among grass leaves were conducted as follows: leaves of each experimental treatment to be compared were collected, immediately placed in water-filled vials, and kept cool. Outlines of leaves presented to grass-

hoppers were recorded using photograms (Heidorn & Joern 1984), where leaves are placed against a sheet of photographic paper and exposed to light to provide an image. A single leaf of each of two species was simultaneously presented to the grasshopper together in the same vial. Leaves were prepared just before the trials were begun. Although this does not eliminate the possibility of nutritional changes in the cut leaves, the elapsed time is minimized, and the physiological changes among species are as standard as can be expected. When a grasshopper finished eating (usually after 10–30 min), the vial was removed; grasshoppers were allowed up to two hours but most fed soon after food was introduced. Grasshoppers were continuously watched during the trials. Observed feeding time is consistent with available estimates of meal length in several grasshopper species (Parker 1984, Joern et al. 1986). In most trials, grasshoppers fed on both leaves. At the completion of a trial, the leaf area removed from feeding was recorded on the corresponding photographic image. Leaf area eaten was determined gravimetrically based on a standard relationship for weight of photographic paper of known area by knowing the weight of the eaten portions. Estimates of dry-weight of grasses eaten were calculated by multiplying leaf area eaten by species-specific constants.

Results were analyzed using the GLM Procedure of SAS (SAS Institute 1985). Because leaf nitrogen content was not determined for leaves in each trial, fertilization level was treated as a fixed effect in the analysis. Orthogonal contrasts and paired *t* tests were used to test specific hypotheses. In addition, Tukey's Student Range Test was performed to allow pairwise comparisons of means (treatment combinations were ranked for the entire experiment) (Table 1). This test was employed because it is conservative (Steel & Torrie 1980), and sufficient degrees of freedom were not available to allow calculations of orthogonal contrasts for all desired comparisons.

Results

Both fertilization and taxonomic identity of the grass species influenced the amount eaten by *P. nebrascensis* in paired-species tests (Table 1). Species-specific effects are evident, and fertilized plants were sometimes preferentially chosen. *Agropyron smithii* is a notable exception, in that the low fertilization level was more readily eaten than the high level, and indeed, was ranked second among the eight possibilities.

Amount eaten in these paired tests was greatly influenced by the grass species presented; orthogonal contrasts showed statistically significant differences ($P < 0.001$) in amount eaten by *P. nebrascensis* between all species pairs except *Andropogon scoparius* versus *Agropyron smithii* ($P < 0.08$).

Table 1. Mean amount eaten of each grass species according to N fertilization level^a

| Grass species | Fertilization level | \bar{x} amount eaten (mg) | SE | Tukey's groupings |
|---------------------|---------------------|-----------------------------|------|-------------------|
| <i>A. hallii</i> | High | 2.02 | 0.16 | a |
| <i>A. smithii</i> | Low | 1.53 | 0.14 | a, b |
| <i>A. hallii</i> | Low | 1.45 | 0.12 | b |
| <i>A. scoparius</i> | High | 1.28 | 0.10 | b, c |
| <i>A. smithii</i> | High | 1.25 | 0.11 | b, c |
| <i>A. scoparius</i> | Low | 1.12 | 0.11 | b, c |
| <i>B. gracilis</i> | High | 0.91 | 0.08 | c, d |
| <i>B. gracilis</i> | Low | 0.63 | 0.09 | d |

^a Treatments are ranked from most to least amount eaten. Means are based on all pairwise combinations of trials including the treatment. Tukey's pairwise groupings (Tukey's Student Range test) are presented to help identify treatment differences. Treatments with the same letter were not statistically distinguishable.

When the two fertilization treatments within the same grass species were compared, consumption does not support the a priori prediction that N fertilization greatly alters feeding patterns. Within grass species (Table 2), significant differences in amount eaten between fertilization levels was observed for *Andropogon hallii* ($P < 0.05$) and possibly *A. scoparius* ($P < 0.06$) but not in the other two species. On average, less of *A. hallii* in the high-nitrogen treatments is eaten than in low-nitrogen treatments; the same is true in three of the four grasses tested. High-nitrogen *A. scoparius* leaves, on the other hand, were preferred over low-nitrogen leaves.

Diet of *P. nebrascensis* from Arapaho Prairie (Table 3) has been determined by gut analysis (Joern 1983, 1985). All grass species used in this experiment are included in the natural diet. In terms of relative proportion of the diet, little correspondence exists between ranks from present experiments and actual contributions of these grass species to the observed diet. The low ranking of *A. hallii* in the naturally observed diet is problematical based on the present results.

Discussion

Although abundant evidence indicates that nitrogen is an important, often limiting nutrient, does

Table 2. Effect of nitrogen fertilization on choice within a grass species^a

| Grass species | Fertilization | | <i>t</i> value | <i>P</i> |
|---------------------|---------------|----------------|----------------|----------|
| | Low (mg) 2 SE | High (mg) 2 SE | | |
| <i>A. smithii</i> | 1.64 (0.72) | 1.55 (0.63) | 0.24 | >0.10 |
| <i>A. hallii</i> | 2.25 (0.67) | 0.98 (0.72) | 2.56 | 0.05 |
| <i>A. scoparius</i> | 1.82 (0.82) | 2.23 (0.66) | 2.04 | 0.06 |
| <i>B. gracilis</i> | 1.47 (1.0) | 0.81 (0.22) | 0.90 | >0.10 |

^a Statistical comparisons within species are shown using paired *t* tests.

Table 3. Actual contribution of grass species used in this study to the diet of *P. nebrascensis* at Arapaho Prairie

| Grass species ^a | Actual diet proportion ^b |
|----------------------------|-------------------------------------|
| <i>A. hallii</i> | 0.05 (4) |
| <i>A. smithii</i> | 0.11 (2) |
| <i>A. scoparius</i> | 0.14 (1) |
| <i>B. gracilis</i> | 0.10 (3) |

^a Listed in order of preference determined from this study.

^b Proportion of a host plant in the diet is indicated. Ranks of grass species in natural diets are shown in parentheses.

an insect herbivore such as *P. nebrascensis* actively select host plants based on its abundance? This question is important because it is commonly assumed that insect herbivores will select host plants which are highest in nutritional quality.

Phoetaliotes nebrascensis did not generally choose leaves grown with high nitrogen fertilizer in within-species choice trials. No statistically detectable difference was observed in amount eaten in high- versus low-N fertilization for two of the grass species. More of the low-N fertilization treatment was eaten in comparisons of *A. hallii*. Leaves of high-N *A. scoparius* were marginally preferred to low-N leaves. Such results argue against the overriding importance of foliar nitrogen levels alone in mediating selection by *P. nebrascensis* among leaves within a grass species.

Clear species-specific contributions to selection among grasses were also observed. Differences among grass species appeared much more important than did N fertilization treatments alone. Although the underlying mechanistic basis for selection among grass species was not uncovered in this or other studies (Capinera et al. 1983, Heidorn & Joern 1984), its overriding importance is obvious. Total foliar nitrogen was not an adequate explanation for patterns of choice among species. The foliar nitrogen concentration of *B. gracilis* is approximately double that of the other species (Heidorn & Joern 1984, unpublished), but it was the lowest ranked species in the preference tests. In addition, N fertilization had little influence in altering results of preference tests among species, especially in favor of high-N leaves.

Even though nitrogen has an important bearing on developmental rate, survivorship, and reproduction, it does not appear to be directly involved as a cue for selecting specific tissue by *P. nebrascensis*. In an evolutionary sense, this is surprising, because a grasshopper should select leaves which support increased survivorship or reproductive output. This contrasts with the additive effect of sugar concentration on meal size in locusts (Bernays & Chapman 1978) and the associated existence in a variety of grasshoppers of numerous sensillae on various mouthparts (chemoreceptors) which specifically respond to sugars and other chemicals (Chapman 1982). To our knowledge, no chemoreceptors have been identified in grasshoppers that respond directly to nutritional forms of nitrogen

(Chapman 1982), but such receptor responses have not been adequately studied to draw unequivocal conclusions concerning the sensory system.

Elevated foliar N levels may result in contradictory feeding responses that cannot be easily interpreted simply as a function of fertilizer treatment. Two examples illustrate the complexity of the problem. Insect herbivores may compensate for low N in their host plant by consuming more tissue while processing it faster (Slansky & Feeny 1977). Alternately, increased availability of N to the plant may be shunted into defensive chemicals such as alkaloids or cyanogenic compounds (Mooney et al. 1983). Such physiological responses by the grass may result in lowered feeding rates by the grasshopper. Grasses produce both alkaloids and cyanogens (Redak 1987). Present data are not sufficient to explore the likelihood that these or other mechanisms are operating.

Preference ranks from the present study do not adequately predict the diet of naturally occurring *P. nebrascensis*. Present results predict that *A. hallii* should constitute a substantial fraction of the diet in naturally occurring *Phoetaliotes*, but this was not observed. This difference cannot be explained by unavailability of *A. hallii*, because it was reasonably abundant at the site where natural diets were determined. Also, relative rankings of the other species in the diet are not well predicted.

Present results indicate that *P. nebrascensis* is not selecting food based on nutritional nitrogen content at the primary level of selection. However, additional evidence suggests that grass-feeding grasshoppers, including *P. nebrascensis*, do respond to nitrogen fertilization by aggregating in high-N patches (Heidorn & Joern in press). Behavioral responses directing host selection by grasshoppers at the point of actually eating is probably best explained as a compromise between inhibitory and excitatory chemical stimuli in leaf tissue (Bernays & Chapman 1978, Miller & Strickler 1984). Foliar nitrogen does not appear to be among the critical cues at this juncture in the feeding hierarchy. However, because nitrogen has important nutritional significance for important life history characteristics, grasshoppers appear to respond to the adequacy of the diet by finding patches of high quality if they exist (Heidorn & Joern in press). Both processes are important in understanding host selection among grass feeding species; additional detailed studies of each, including interactions between the two levels, will be profitable.

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