

Impact of diet quality on demographic attributes in adult grasshoppers and the nitrogen limitation hypothesis

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Abstract. 1. Various formulations of the nutrient stress hypothesis predict that insect herbivore populations will respond positively to increased nutrient quality of host plants, especially dietary nitrogen. Survival and reproduction by adult females of two grasshopper species [Acrididae; *Melanoplus sanguinipes* (Fabricius) and *Phoetaliotes nebrascensis* (Thomas)] were evaluated in response to defined diets that varied factorially in both total nitrogen (1–7%) and total soluble carbohydrate (4.3–26.7%). These grasshopper species coexist naturally but are typically shifted phenologically so that specific developmental stages normally encounter host plants of different nutritional quality under natural conditions.

2. Demographic responses by adult females of both species varied according to diet quality, but not in the same fashion. Diet quality affected survival significantly in *P. nebrascensis* but not in *M. sanguinipes*. Survival in *P. nebrascensis* was greatest on diets containing the lowest nitrogen concentrations; carbohydrate level had no effect.

3. Diet quality influenced reproduction significantly in both species. Egg production rate (eggs/day) in *M. sanguinipes* exhibited a negative linear response to increased carbohydrate, coupled with a significant quadratic response to nitrogen that reached a maximum at an intermediate level of about 4% total N. A significant quadratic response to total N for pod production rate (indicating the timing of reproduction) was also observed. Clutch size in *M. sanguinipes* exhibited a negative relationship with total carbohydrate in the diet, but no response to nitrogen. No interaction was observed between nitrogen and carbohydrate levels. For *P. nebrascensis*, response to diet quality was much weaker, with only a suggestive maximum at 4% total-N for both egg production rate (eggs/day) and clutch size (eggs/pod) and a suggestive linear response for pod production rate as carbohydrate level increased. Female body weight did not contribute to any reproductive response as a covariate variable.

4. Combined with a similar, previous analysis of demographic responses by the grass-feeding grasshopper, *Ageneotettix deorum*, these results challenge the ability to draw generalizations about host plant nutritional quality and grasshopper demographic responses. These three grasshopper species respond quite differently to defined diets that vary in total nitrogen and carbohydrate levels. Thus, although host plant quality can contribute significantly to grasshopper population responses, a uniform explanation is not likely.

Key words. *Ageneotettix deorum*, carbohydrate, dietary nitrogen, fecundity, food quality, grasshopper, *Melanoplus sanguinipes*, nutrient stress–insect performance hypothesis, *Phoetaliotes nebrascensis*, survival.

Introduction

Insect ecologists (McNeill & Southwood, 1978; White, 1978, 1984, 1993; Rhoades, 1979, 1983; Mattson, 1980; Slansky, 1982; Scriber, 1984; Price, 1991; Tallamy & Raup, 1991) and physiologists (Dadd, 1960, 1963, 1985; House, 1961, 1969; Simpson & Simpson, 1992; Hinks *et al.*, 1993) have repeatedly emphasized the influence of plant nutritional quality, especially dietary nitrogen, on key insect herbivore demographic responses such as survival, developmental rate, growth rate and reproduction. As concentrations of key nutrients increase in the available food, the insect herbivore can shunt more of these nutrients into key physiological processes that underlie demographic attributes such as survival and reproduction.

Insect herbivores such as grasshoppers typically encounter abundant food as bulk under most natural conditions. Yet the major nutritional problem that must be solved by most chewing insects concerns ingesting sufficient critical nutrients, not just total bulk (Behmer & Joern, 1993, 1994; Yang & Joern, 1994a,b). However, the nutritional quality of food plants that insect herbivores encounter varies considerably within and among years, among sites and among tissues within a single plant (Denno & McClure, 1983; Mattson & Haack, 1987; Jones & Coleman, 1991; A. Joern & S. Mole, unpublished observation). To a grasshopper that exhibits a long digestion time relative to feeding time (Yang & Joern, 1994a,b), the total nutrients that can be obtained over the time and climatic conditions available for feeding each day may be highly constrained by plant nutritional quality. A grasshopper can exert some control over diet quality and has the ability to extract available nutrients by manipulating the length of time that food remains in the gut (Yang & Joern, 1994a,b), or balance nutritional needs by selecting alternate plants (Bernays & Bright, 1991; Bernays *et al.*, 1991; Simpson & Simpson, 1992). But even under the best conditions encountered, grasshoppers typically eat food of poor quality relative to nutritional needs (Bernays, 1982; Hinks *et al.*, 1993). As such, food may often be limiting to insect herbivores such as grasshoppers.

Multiple lines of evidence suggest that nutritional quality, especially nitrogen, can limit insect herbivores (McNeill, 1973; McNeill & Southwood, 1978; White, 1978, 1984, 1993; Denno & McClure, 1983). The basis of this claim revolves around the generally low nitrogen levels in food plant tissue relative to nutritional requirements, and to the generally responsive nature of insect herbivores to increased nitrogen in their diets. Other nutrients, minerals and energy can also be limiting, of course, making the search for key limiting factors a potential empirical nightmare (Clancy, 1992; Clancy & King, 1993). By focusing on the nitrogen limitation hypothesis, however, it is assumed that the energy needs and needs for trace nutrients and minerals of insect herbivores are met by consuming sufficient plant material to satisfy nitrogen needs.

Ecologically, multiple intrinsic and extrinsic factors influence nitrogen availability in host plants encountered by insect herbivores (Denno & McClure, 1983; Mattson & Haack, 1987; Jones & Coleman, 1991).

1. Different tissues and developmental states routinely vary in nitrogen levels (Scriber, 1984; Slansky, 1993). However, selective feeding on specific tissues or growth stages within plants by insect herbivores can do much to ameliorate an otherwise poor resource, provided that enough exists (Simpson & Simpson, 1992).

2. Host plants are regularly stressed, resulting in routine and rapid physiological change (Jones & Coleman, 1991). Extensive observational and experimental evidence documents that various forms of stress on plants often make available plant tissues more palatable with increased levels of soluble nitrogen that is readily available to herbivores (Rhoades, 1983; Mattson & Haack, 1987; Jones & Coleman, 1991; White, 1993).

3. Under field conditions, phenological matches between host plant senescence and the insect herbivore determine both the level and quality of limiting nutrients that will typically be encountered by the herbivore. As plants develop and senesce, critical concentrations of key nutrients often drop as the season progresses (Scriber, 1984; Slansky, 1993). For example, total nitrogen levels in typical dominant grasses from the primary site range from about 4–5% d.w. in late-May to \approx 1% d.w. in mid-August in most years.

Improved nutritional quality in plants could lead to increased success by insect herbivores, possibly even resulting in population outbreaks of the herbivore (McNeill & Southwood, 1978; White, 1978, 1984, 1993). Although White (1984) stressed the nitrogen needs of developing juveniles, he did not address the equally important issue of how adults should respond to variable diets. From a population perspective, reproductive output will also be important unless survivorship is so low that few adults actually reproduce.

Three issues are addressed. First, does varied nutritional quality of the diet in the form of nitrogen potentially contribute to the population dynamics of representative generalist feeding grasshoppers through effects on the demographic attributes of survival and reproduction? Secondly, what is the relative importance of energy (carbohydrates) vs. nitrogen (protein) as a limiting agent? Finally, do generalist feeding grasshoppers that normally encounter very different nutritional landscapes during normal life cycles because of displaced timing of phenologies respond to variable host plant quality in basically the same way? Demographic responses using adult females of two grasshopper species reared on defined diets that vary in total protein and carbohydrates within naturally occurring levels are compared. Protein and carbohydrate levels in plants typically eaten by these grasshopper species in the field vary significantly both seasonally and among years. In this context, the consequences of nutritional differences to demographic responses of adult grasshoppers given a single diet throughout the study are explored.

Materials and methods

Grasshoppers

Adults of two melanopline grasshoppers species, *Melanoplus sanguinipes* (Fabricius) and *Phoetaliotes nebrascensis*

(Thomas), were studied. Both species are widespread throughout western North American grasslands and are common in Nebraska sandhills grassland, the source of individuals used in these experiments. *Melanoplus sanguinipes* is a polyphagous species that feeds on a combined diet of grasses and forbs, whereas *P. nebrascensis* is primarily graminivorous, although it eats many grass species (Joern, 1979a, 1985). The exact seasonal appearance by these species is weather-dependent, although there is a general phenological difference between the species. Typically, *M. sanguinipes* hatches in late May with adults appearing in early to mid-July whereas *P. nebrascensis* is a late season species that emerges in late June with adults appearing in late July to mid-August.

Individuals used in these experiments hatched from eggs laid by field-caught adults collected in Arthur and Keith Counties, Nebraska, in 1992, shortly after adults emerged. These adults were transferred to an insectary and fed a combination of dry wheat bran and 7–10-day-old rye seedlings while obtaining eggs. Egg diapause was accommodated by storing eggs at 6 °C for four and a half months in sand moistened with the antifungal agent methylparaben. Following diapause and hatching, developing nymphs were kept in an insectary at 30 °C (photophase LD 14:10 h) with 40 W incandescent light bulbs placed adjacent to rearing cages to allow grasshoppers to thermoregulate above ambient insectary temperatures (*thermophase*, LD 10:14 h). Newly hatched nymphs were fed a mixed diet of wheat bran and 7–10-day-old rye seedlings *ad libitum* throughout the nymphal developmental period. Males were separated from females at the fifth instar. By feeding nymphal grasshoppers in this way, all grasshoppers had the opportunity to acquire the necessary macro- and micro-nutrients associated with normal nymphal development. Additionally, these first generation eggs approximated to the genetic background of field populations. The treatment of field-collected females during egg collection minimized but may not have eliminated possible maternal effects.

Experimental design and analysis

Survival and reproductive performance of the two grasshopper species reared on a range of diets was examined using a randomized complete block experimental design. Within this framework, fifteen unique treatment combinations of dietary protein and carbohydrates were employed using a 5 × 3 factorial arrangement. Five total nitrogen (1, 2.5, 4, 5.5 and 7% dry weight) and three carbohydrate (4.3, 16.7 and 26.7% dry weight) levels spanned a range reported in naturally occurring grasses and forbs on which *M. sanguinipes* and *P. nebrascensis* typically feed (Lyttleton, 1973; Joern, 1979a, 1985; A. Joern & S. Mole, unpublished observations). Each treatment was replicated six times.

ANCOVA (SuperAnova, General Linear Model, Abacus Concepts Inc., 1989) was used to evaluate reproductive performance and survival in response to diet treatments, with individual female weight treated as a covariate. Because female weight might affect targeted responses independent of adult

diet quality, this variable was used as a covariate. Data were distributed normally and otherwise met the underlying assumptions of ANCOVA. By assessing the presence of linear and quadratic contrasts for each main effect, anticipated responses to increased protein or carbohydrate in the diet were assessed, if any existed.

Defined diets

Agar-based defined diets (Table 1) containing commercially prepared Purina Horse Charge® (Ralston Purina Co., St Louis, Missouri, U.S.A.), casein, sucrose, starch and cellulose allowed quantitative manipulation of food quality. Horse Charge is sold as a horse feed supplement containing high quality plant protein fortified with vitamins and minerals. Ingredients found in Horse Charge include: plant protein products, grain products, forage products, processed grain by-products, molasses products, animal fat preserved with ethoxyquin, vitamins A, E, B₁₂ and D₃ supplements, riboflavin supplement, calcium pantothenate, calcium carbonate, sodium selenite, dicalcium phosphate, calcium iodate, salt, cobalt carbonate, ferrous carbonate, copper sulphate, manganous oxide and zinc oxide. Agar-based diets containing only Horse Charge successfully support both development and reproduction in grasshoppers (Yang & Joern, 1994a,b).

The fifteen unique diet combinations all contained equal amounts of Horse Charge and sorbic acid so that the concentration of micronutrients was constant across treatment diets. Both agar and methylparaben were also held constant across all treatments. As such, only the levels of carbohydrates, nitrogen and cellulose varied across the fifteen diet combinations.

Analysis of Horse Charge indicated that total nitrogen and total non-structural carbohydrates (TNC) each constituted 6% of the total dry weight (Y. Yang & A. Joern, unpublished observations). However, the basal amount of Horse Charge in each of the present diets contributed 2% and 1% (dry weight) to the total carbohydrate and nitrogen levels in each diet, respectively. Casein was added to further elevate protein levels and a combination of sucrose and starch was added to elevate carbohydrate levels (Table 1). Casein is a standard supplement to artificial diets when manipulating dietary nitrogen (Clancy, 1992), as it contains all common amino acids and is rich in the essential ones. In addition it is vitamin- and fat-free, providing more control over supplementation. Although casein does vary somewhat from common plant proteins in amino acid composition, grasshoppers have been shown to compensate effectively for diet imbalances much greater than this (Simpson *et al.*, 1995). The use of casein, an animal protein, to supplement artificial diets in this case should provide only minimal problems for interpretation in the context of questions addressed here. Cellulose was also added so that the dry weight portion (20%) and water content (80%) of all diets were held constant.

Experimental protocol

As female grasshoppers moulted to adults, they were caged individually and assigned randomly to one of the fifteen diet

Table 1. Agar-based artificial diet components and concentrations, with ingredients expressed both as mass (g) and per cent dry mass of the total. Cellulose was added to each diet so that the total dry mass of each diet was 200 g. Diets were prepared using standard procedures. Agar was dissolved in 800 ml of boiling water and allowed to cool before the addition of the remaining components. Addition of remaining components to the diet followed after a sufficient cooling period prevented denaturation of the protein.

Base ingredients	Mass (g)	% Dry weight		
Horse Charge®	16.6	8.3		
Ascorbic acid	1.6	0.8		
Methyl-paraben	1.4	0.7		
Agar (corn based)	21.4	10.7		
Supplemental ingredients				
% Nitrogen	Casein (g)	% Dry weight		
1.0	0.0	0.0		
2.5	18.8	9.4		
4.0	37.5	18.8		
5.5	56.2	28.1		
7.0	74.5	37.3		
% Carbohydrate	Sucrose (g)	% Dry weight	Starch (g)	% Dry weight
4.3	2.0	1.0	4.7	2.3
16.7	8.0	4.0	23.3	11.7
26.7	14.0	7.0	37.3	18.7

treatment combinations. Cages (8 cm diameter × 11 cm high) were constructed of fine-meshed wire screen and each contained a vial with drinking water. Each cage was placed over a cup with moistened sand to accommodate oviposition. Each female received food twice daily for the remainder of its life. All experiments were conducted in an environmental chamber set at 30 °C (photophase, LD 14:10 h). In addition, individual cages were placed next to 40 W incandescent light bulbs so that individual grasshoppers could self-regulate body temperatures (thermophase, LD 6:18 h).

Females were paired with males at the beginning of each trial. Males were rotated regularly among females representing different diet treatments to minimize any effect of diet quality on the male's contribution to reproduction. Eggs were collected from *M. sanguinipes* approximately every 3 days, which allowed estimation of the number of days until the first egg pod was produced. Eggs were collected from *P. nebrascensis* only at the end of the experiment, so the number of days until the first egg pod could not be estimated. Egg pods were dissected to count the number of eggs produced. All experiments ended after 50 days, even if females were still alive. This period corresponds roughly to the number of days available to females to lay eggs under field conditions before first frost.

Results

Survivorship

Diet quality had a significant effect on survival for adult *P. nebrascensis*, but not adult *M. sanguinipes* (Table 2). In *P. nebrascensis*, survival was greatest at low nitrogen levels and decreased in a significant linear fashion as nitrogen levels increased (Fig. 1), over a range of values commonly encountered in natural vegetation. Carbohydrate levels had no

effect. There was no significant interaction between nitrogen and carbohydrate levels for either species. Likewise, female body size as a covariate did not contribute to survival responses in a significant way for either species.

Reproduction

Three attributes of reproduction in response to diet quality were assessed: rate of egg production (eggs/day), clutch size (eggs/pod) and pod production (pods/day). Different responses to diet nutritional quality were observed in the two grasshopper species.

Egg production rate. Diet quality exerted a significant effect on reproduction in several ways. In *M. sanguinipes*, egg production rate exhibited a significant negative, linear response to carbohydrate levels coupled with a significant quadratic response to nitrogen levels (Table 2, Fig. 2). Average rate of egg production at 4.3% carbohydrate was 1.4-fold and 3.4-fold greater than that observed at 16.7% and 26.7% carbohydrate, respectively. The greatest rate of egg production was observed at intermediate nitrogen levels (4%). There was no interaction between nitrogen and carbohydrate levels on reproduction.

In general, the impact of variation in diet quality on reproduction in *P. nebrascensis* was much less than for *M. sanguinipes*. No main effect of nitrogen or carbohydrate on reproduction in *P. nebrascensis* was observed, although there was a suggestive quadratic trend for the effect of nitrogen on egg production rate (Table 2, Fig. 2). The number of eggs/day reached the maximum level at intermediate nitrogen levels. There was no significant carbohydrate × nitrogen interaction and no effect of initial female weight as a covariate.

Clutch size. Different nutritional components of diet quality affected clutch size (eggs/pod) for both species (Table 2). For

Table 2. Results of ANCOVA (with initial grasshopper weight as covariate) measuring survival and reproductive responses to five nitrogen (1, 2.5, 4, 5.5, and 7% dry weight) and three carbohydrate (4.3, 16 and 26.7% dry weight) levels for *Melanoplus sanguinipes* and *Phoetaliotes nebrascensis*.

Source	d.f.	Days alive		Eggs/day		Eggs/pod		Pods/day		Days to first pod [†]	
		F	P	F	P	F	P	F	P	F	P
<i>Melanoplus sanguinipes</i>											
Nitrogen	4	1.07	NS	1.13	NS	0.64	NS	1.69	NS	1.02	NS
Linear	1	2.89	NS	0	NS	0	NS	0	NS	3.38	NS
Quadratic	1	0.03	NS	4.37	*	2.12	NS	6.64	*	0.16	NS
Carbohydrate	2	0.20	NS	3.27	*	3.94	*	0.75	NS	0.62	NS
Linear	1	0.17	NS	6.53	*	7.41	*	1.40	NS	0.04	NS
Nitrogen × carbohydrate	8	1.73	NS	1.58	NS	0.98	NS	0.65	NS	0.43	NS
Weight	1	0.57	NS	0.19	NS	1.59	NS	0.53	NS	1.73	NS
Error	56										
<i>Phoetaliotes nebrascensis</i>											
Nitrogen	4	5.44	**	1.01	NS	1.42	NS	0.74	NS		
Linear	1	19.04	**	0.37	NS	0.00	NS	1.89	NS		
Quadratic	1	0.98	NS	3.52	0.06	5.07	*	1.41	NS		
Carbohydrate	2	0.23	NS	0.84	NS	0.08	NS	0.69	NS		
Linear	1	0.18	NS	0.70	NS	0.63	NS	3.39	0.07		
Nitrogen × carbohydrate	8	1.63	NS	1.00	NS	1.95	NS	1.09	NS		
Weight	1	0.27	NS	1.99	NS	0.38	NS	1.72	NS		
Error	51										

[†]This analysis only has 18 degrees of freedom in the error term. * = $P < 0.05$; ** = $P < 0.01$; NS = $P > 0.05$.

M. sanguinipes, clutch size was affected significantly by carbohydrate level; clutch sizes decreased significantly as carbohydrate levels in the diet increased (Fig. 3). Average clutch size was 1.4-fold and 2.3-fold greater at low carbohydrate levels (4.3%) than at intermediate (16.7%) or high (26.7%) levels, respectively. For *P. nebrascensis*, neither the main effect of nitrogen or carbohydrate was significant, although there was a suggestive quadratic effect of nitrogen level (Table 2, Fig. 3). The nitrogen × carbohydrate interaction was not significant for either species. Likewise, the covariate (initial female weight) was not correlated with average clutch size.

Pod production. Pod production rates in these two grasshopper species were affected by different dietary features. There was a significant quadratic trend in pod production in response to nitrogen for *M. sanguinipes* (Fig. 4) with a maximum response at intermediate levels. A suggestive ($P = 0.07$) linear response to carbohydrate level was observed for *P. nebrascensis*, where pod production rate increased as carbohydrate levels increased. There was no significant carbohydrate × nitrogen interaction and no effect from female body size as a covariate on pod production.

The response of the time to production of the first pod from eclosion to the adult stage by *M. sanguinipes* was also examined in response to varied nitrogen and carbohydrate in the diet. On average, the first pod was laid 22.2 ± 1.6 days after eclosion, but diet treatments in the adult stage exhibited no significant impact on this response. Comparable data are not available for *P. nebrascensis*.

Discussion

Food quality can limit overall nutrient intake by an insect herbivore, as evidenced by demographic consequences in both grasshopper species. While feeding on one of a range of diets of variable quality, grasshoppers were provided food and water *ad libitum* and allowed to self-regulate body temperatures. Thus, individuals had as much control of food intake and processing rate as was physiologically possible, so that nutrient intake was limited by food quality as defined by protein (nitrogen) and carbohydrate levels. The nitrogen limitation hypothesis (White, 1978, 1984, 1993) is evaluated for adults based on the diversity of responses by adult females of three grasshopper species to variable quality diets.

Effect of dietary protein and carbohydrates on survival and reproduction

Adult grasshoppers studied here respond to variation in dietary protein and carbohydrate levels. Variation in protein levels typically exhibited much more impact on survival and reproduction than did carbohydrate levels, with few detectable interactions between carbohydrate and protein components. Finally, if an effect existed, survivorship responded linearly to diet quality, whereas reproductive responses were often quadratic, reaching a maximum near 4% total nitrogen.

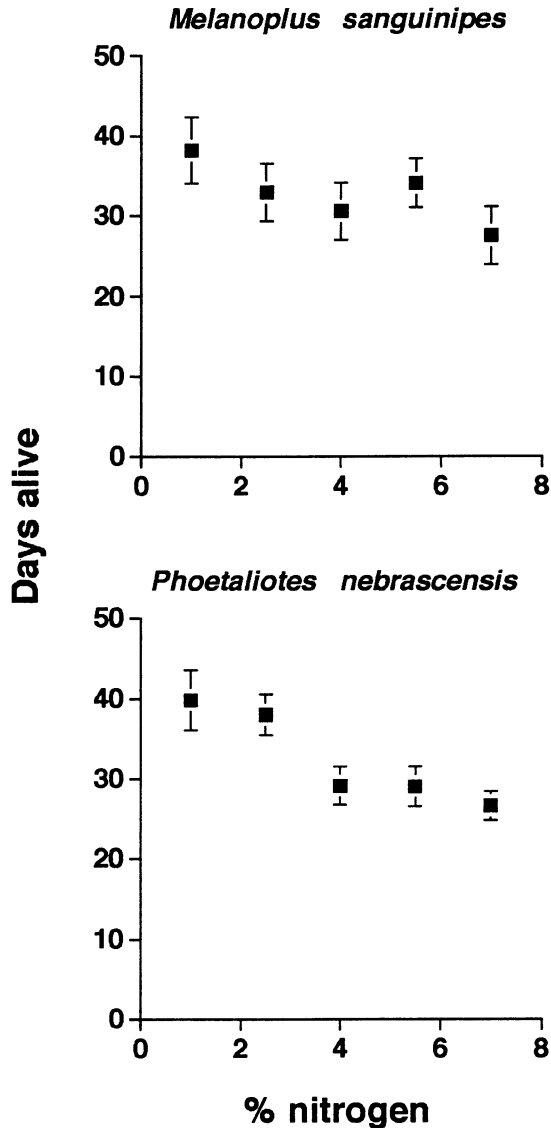


Fig. 1. Survival of adult females of *M. sanguinipes* and *P. nebrascensis* on diets constituted with different levels of total nitrogen. Mean number of days alive (\pm SE) is indicated for each diet. Because no response to carbohydrate was observed, responses to nitrogen levels include all carbohydrate levels.

By comparison, a related study with the strictly grass-feeding grasshopper *A. deorum* from the same site yielded a third pattern of response to the same diets studied here (Joern & Behmer, 1997). In adult *A. deorum*, survival exhibited a positive linear response with increased nitrogen levels and a significant carbohydrate \times nitrogen interaction resulted. Reproduction in *A. deorum* in response to diet quality was in general agreement with the other two species, and was strongly affected by dietary nitrogen, whereas carbohydrate contributed no significant effect. A quadratic effect of nitrogen with a maximum near 4% was observed for egg production rate in *A. deorum* and a linear increase in eggs/pod was observed as N-levels in the diet increased.

Three main patterns emerge. Reproduction in all three grasshopper species responded strongly to dietary nitrogen levels in a quadratic fashion with a peak near 4% N. However, adult female survivorship among three species exhibited widely divergent responses to dietary nitrogen and carbohydrate. With some exceptions, carbohydrates seldom proved limiting, whereas nitrogen levels exerted great impact.

Clearly, reproductive responses by these three species converge on the same general pattern, a quadratic response with a peak near 4%. Grasshoppers at this site seldom, if ever, encounter significant amounts of high quality leaf material during reproductive periods under natural conditions (usually $< 2\text{--}4\%$ nitrogen dry weight in good years, A. Joern & S. Mole, unpublished observations). Consequently, only one side of the quadratic peak actually contributes much towards understanding the interaction from an ecological standpoint, and a linear response in reproduction to increased nitrogen levels in the diet results. Further support comes from the fact that egg production in grasshoppers is nitrogen intensive and limiting (McCaffery, 1975). This response seems reasonable, given that nitrogen is perhaps the primary limiting nutrient to terrestrial plant production, with significant related consequences for plant species composition, foliar nutrient constituents for herbivores and resulting food web structure (Tilman, 1988; DeAngelis, 1992; Ritchie & Tilman, 1992; Schmitz, 1994; Whitehead, 1995; Elser *et al.*, 1996). The observed decrease in performance at higher nitrogen levels (beyond the hump) suggests that constraints in nutrient balance exist and are no longer within this suitable nitrogen range, and that increased nitrogen in the diet must be balanced by suitable complements of carbohydrates or some other mineral nutrient (Clancy & King, 1993; Raubenheimer & Simpson, 1993; Simpson & Raubenheimer, 1993).

General patterns of survivorship in response to diet variability among grasshopper species are not detectable from the results of this study. Each of these three grass-feeding species exhibited a completely different response to food quality, including no detectable effect for *M. sanguinipes*. The most likely ecological explanation for survival responses to dietary nitrogen concerns differences in the normal range and variation of nutritional environments that each of these species encounters naturally (described below).

Grasshoppers used in these experiments were confined to single diets and responses represent forced outcomes to specific combinations. Ideally, grasshoppers choose nutrient combinations that permit maximum efficiency in post-ingestive processes, which, in turn, optimizes metabolism and growth responses (Simpson & Raubenheimer, 1993). Late nymphal *Locusta migratoria*, for example, exhibited an experimentally determined intake target that approximated a 1:1 ratio of digestible carbohydrate to protein (w/w) (Simpson & Raubenheimer, 1993). Carbohydrate and protein components of diet are regulated separately in nymphal *Locusta*, and each contributes to growth. When allowed to compensate for dietary imbalances in carbohydrate:protein ratios, either by adjusting food intake by selecting combinations of alternate diets or through unknown post-ingestive physiological mechanisms, locusts achieve similar growth patterns.

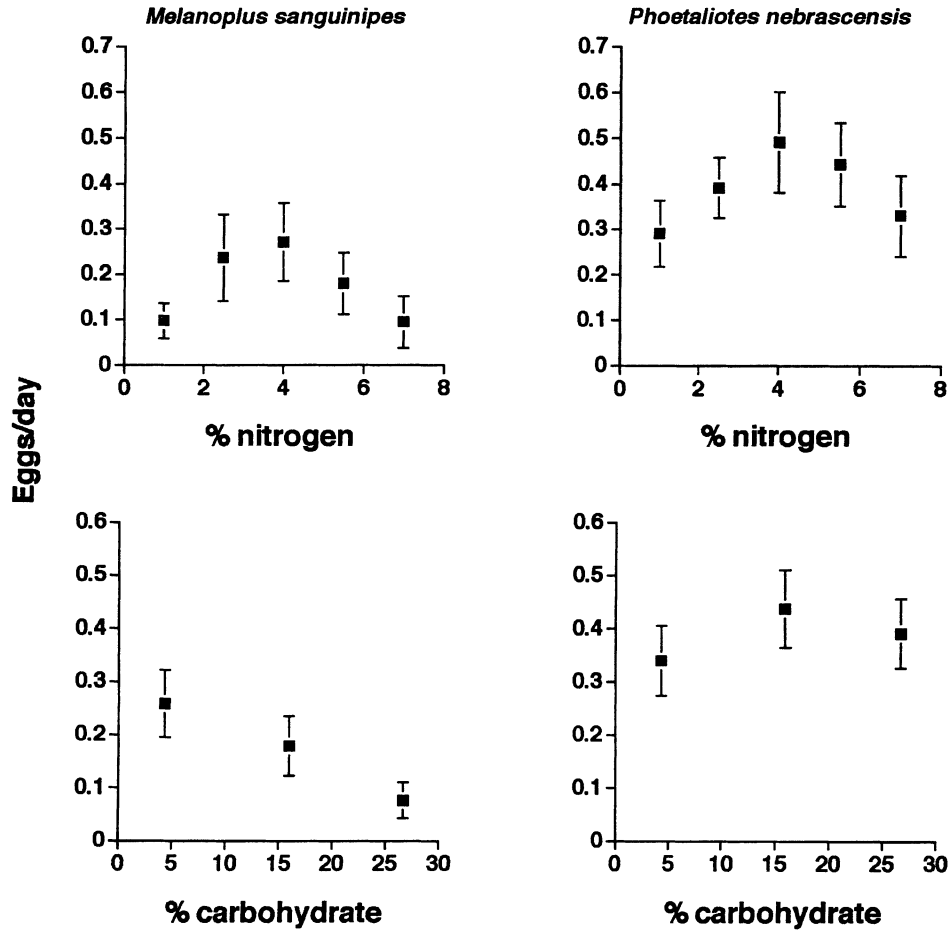


Fig. 2. Egg production rate (mean eggs/day \pm SE) in response to different levels of nitrogen and carbohydrate in the diet for two grasshopper species, *M. sanguinipes* and *P. nebrascensis*.

In terms of ecologically relevant performance, nitrogen clearly dominated egg production and contributed greatly to survivorship in the present study. Interactions between dietary nitrogen and carbohydrate were scarce, and carbohydrates contributed very little to variation in reproduction or survival in three grass feeding species. These results correspond to those of Raubenheimer & Simpson (1993), in that grasshoppers feeding on widely variable combinations of carbohydrate and protein reach similar performance objectives. However, results of the present study highlight the importance of diet quality on demographic responses, especially concerning effects of nitrogen on egg production. Clearly, full post-ingestive compensation is not possible and significant effects of different diets remain for adult survivorship and reproduction.

Egg production did not vary with female weight. Responses similar to this study have also been reported for other insect groups (Leather, 1988; Klingenberg & Spence, 1997). In the current study, however, the range of female weight was probably not as great as that often observed when females encounter a broader range of food qualities throughout development, so this conclusion must be tempered.

Possible consequences of phenological shifts and environmental expectations

Seasonal appearances by coexisting grasshopper species routinely exhibit phenological shifts (Joern, 1979b; Joern & Gaines, 1990). Although underlying mechanisms are seldom disentangled, species-specific phenologies are probably timed to provide a close match to the optimal needs of the organisms involved (Tauber *et al.*, 1985), either to best match resource availability and physiological needs, or to mediate among biotic interactions with other species. Independent of the causes driving actual shifts in seasonal appearance among species, long association with a restricted range of nutritional conditions should mould demographic responses to anticipated conditions.

Coupled with species-specific differences in host plant selection, differences in the responses to diet quality may reflect the nature of the environment typically encountered by adults of each species. For example, foliar nitrogen levels in grasses are typically highest early in the growing season and decline steadily until they reach the lowest levels in late July or early August (A. Joern & S. Mole, unpublished observations).

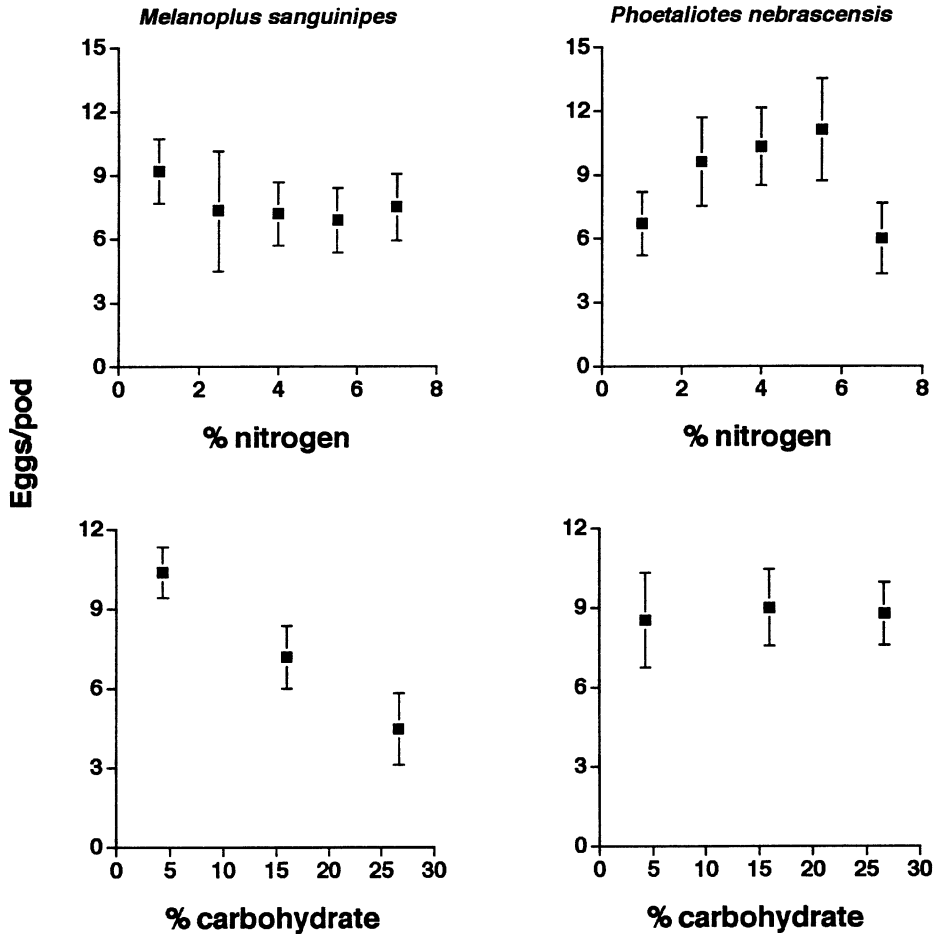


Fig. 3. Clutch size as a function of total nitrogen and dietary carbohydrates in the diet (mean eggs/pod \pm SE) for *M. sanguinipes* and *P. nebrascensis*.

This drop can be significant in terms of total nitrogen (from 5–6% to 0.5–1.0%, dry weight). Following late summer rains (mid- to late August), the foliar nitrogen levels often increase somewhat, but do not approach early season levels. In addition, significant among-year variation also exists, such that different years may be classified generally as good or bad, with the foliar nitrogen levels at any given time in the phenological cycle of a grasshopper shifting from year to year. In analyses of natural vegetation (A. Joern & S. Mole, unpublished observations), total non-structural carbohydrates do not generally exhibit repeatable seasonal patterns among years and overall levels vary unpredictably, both seasonally and among years.

As such, grasshopper species that exhibit different phenological patterns may normally encounter very different nutritional environments, both in terms of the absolute nutrient levels and the variation of such levels among years. These differences in the nutritional environment can be expected to exert very different selective pressures on the types of anticipated responses to variable nutritional resources. In this study, the three grasshopper species normally experience different situations. *Ageneotettix deorum* (Joern & Behmer, 1997) is the earliest species to reach the adult stage, a period

that corresponds to a rapid drop in grass nutritional quality. *Melanoplus sanguinipes* typically appears next, but typically exhibits an extended hatch so that adults from this species may encounter a wide range of nutritional conditions, both within and among years. In addition, *M. sanguinipes* routinely eats both grasses and forbs so that the dietary variability among seasons may be greatly reduced. Finally, *Phoetaliotes nebrascensis* is one of the latest emerging species and adults appear in late July and early August, during the period when plant quality remains at its lowest quality for about 4–6 weeks, the most productive portion of the adult reproductive period.

Phenological shifts and different nutritional environments commonly encountered among these species did not alter responses to food quality significantly. Each species exhibited roughly the same quadratic response to nitrogen levels, indicating that reproduction was nitrogen limited. Patterns of survivorship may correspond to combined phenological and dietary differences. *Ageneotettix deorum* adults commonly experience the greatest change in foliar nitrogen concentrations. As such, these individuals routinely inhabit a variable, but somewhat predictable nutritional environment. Rapid responses to increased nitrogen concentration in diets in the form of increased survival might be expected. Alternately,

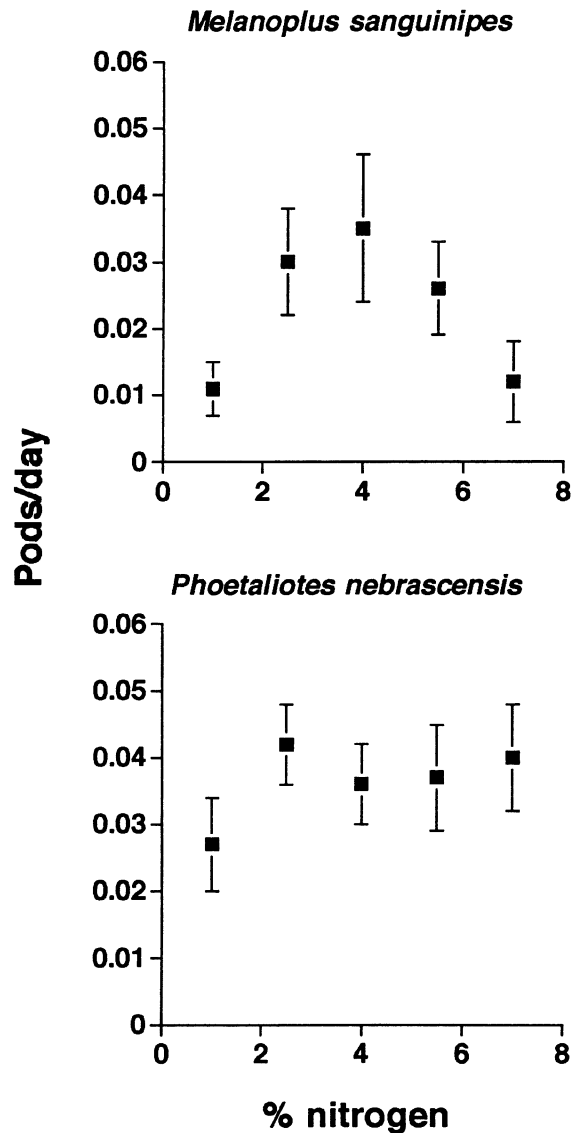


Fig. 4. Rate of egg pod production in response to total nitrogen content in the diet (mean pods/day \pm SE). Because no response to carbohydrate was observed, responses to nitrogen levels include all carbohydrate levels.

P. nebrascensis typically encounters a low nutritional quality environment as an adult. Here, one expects survival to be less affected by low nitrogen levels than for species from higher quality nutritional environments. However, it is not clear why the species should exhibit a negative response to increased nitrogen levels in food. Some unrecognized physiological response may be involved. For *M. sanguinipes*, combined effects of extended hatch, phenological timing and mixed diet generally buffer this species against radical shifts in the nutritional environment, especially nitrogen. This may explain the relative lack of survivorship response to changes in either carbohydrate or protein levels of the food for this species.

Consequences for the nitrogen limitation hypothesis

Dietary nitrogen levels figure prominently in many explanations of insect herbivore population dynamics (Mattson & Haack, 1987; White, 1993). Assuming that plant nutritional quality typically occurs at low levels, especially for nitrogen, insect herbivores often face what White refers to as an *inadequate environment*. In particular, young nymphal stages may be most susceptible to low nitrogen concentrations in food (White, 1984, 1993). As such, any variation in nitrogen levels, such as those accompanying plant stress, may provide opportunities for flushes of high quality nutrients that can be tracked by the insect herbivore populations. When limiting nutrients increase in availability, nymphal survival improves and populations may increase dramatically in size. If all stages of an insect herbivore's life cycle respond to variable nutritional quality of host plants in a similar manner, positive reinforcement of trends early in life should ensue.

For the adults of grasshopper species examined here, pronounced species-specific differences exist for demographic responses to variable quality food, especially with respect to nitrogen levels. Adult survival of three common grasshopper species exhibits three different responses to increased levels of dietary nitrogen. Alternatively, adults of these species may be energy limited rather than nitrogen limited. However, no species exhibited a significant response in survivorship over a naturally occurring range of carbohydrate levels. In addition, egg production was more responsive to variation in diet quality than was survival where nitrogen was clearly most important. Carbohydrate levels had some impact on *M. sanguinipes*, but a negative relationship was observed rather than the more intuitive positive one. In sum, adult demographic responses to different quality diets are not in qualitative agreement with anticipated nymphal responses based on White's inadequate environment scenario. As such, the nutrient stress hypothesis, developed to explain population responses by insect herbivores to the altered quality of host plants in response to stress, either only requires nymphal responses to explain patterns, or is insufficient as an explanation if egg production is important for explaining patterns of population dynamics in any significant way. Given the large number of factors that contribute to the population dynamics of insect herbivores (Price, 1991; Cappuccino & Price, 1995), including grasshoppers (Belovsky & Joern, 1995), it is not yet possible to distinguish among alternatives.

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