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HERBIVORE FORAGING IN CHEMICALLY HETEROGENEOUS ENVIRONMENTS: NUTRIENTS AND SECONDARY METABOLITES

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Abstract. We provide an exemplar study for investigating the manner and extent to which plant secondary metabolites (PSMs) influence nutritional regulation in herbivores selecting among multiple foods. Two experiments were performed using the African migratory locust, *Locusta migratoria* (L.). In both cases, locusts were given access to multiple synthetic foods that varied in their concentration of the two most strongly regulated nutrients (protein and digestible carbohydrate) and a carbon-based PSM, tannic acid (TA). Insects in the first experiment were given two suboptimal but complementary foods: a high-protein, low-carbohydrate food and a low-protein, high-carbohydrate food. Tannic acid was then added to one food type but not the other, to both, or to neither food type. Here we could see how the addition of TA to a food with a specific protein : carbohydrate profile influenced nutritional regulatory responses. In a second experiment, locusts were given a high-protein, low-carbohydrate food and a low-protein, high-carbohydrate food, both of which contained TA. A third TA-free food containing one of five different protein : carbohydrate ratios was also provided. This experiment provided an opportunity to measure the extent to which a TA-free resource would be incorporated into the diet in relation to its nutrient content. Results indicated that the extent to which locusts regulated their protein : carbohydrate intake depended on the protein : carbohydrate composition of the TA-free foods in their environments. It was evident that TA is more effective as a feeding deterrent than as a post-ingestive toxin, but its effectiveness as a feeding deterrent is strongly linked to the protein : carbohydrate composition of the food in which it occurs. We discuss these findings within the context of plant defense theory and models of foraging behavior.

Key words: African migratory locust; chemical heterogeneity; feeding deterrent; foraging; herbivores; Locusta migratoria; *nutrition; plant secondary metabolites; protein : carbon ratio; state–space models; tannic acid.*

INTRODUCTION

Two hypotheses are regularly invoked in the literature to explain patterns of food selection by herbivores. The first states that herbivores are physiologically constrained in their capacity to detoxify plant secondary metabolites (PSMs) and, as a result, base their feeding decisions on a need to dilute the intake of any single PSM (Freeland and Janzen 1974). The second hypothesis states that because plants are nutritionally variable, both spatially and temporally, the overriding criterion driving food selection is the need to acquire an appropriate balance of nutrients (Pulliam 1975, Westoby 1978, Rapport 1980). It seems unlikely, however, that these two hypotheses would be mutually exclusive. Rather, herbivores probably select a mix of foods that represent a trade-off between minimizing the consumption of toxic PSMs and balancing nutrient intake, with the nature of the trade-off between toxic PSMs and nutrients reflecting the kind and range of host plants eaten. However, simply framing herbivore foraging as a trade-off between toxin intake and nutrient regulation can itself be problematic, because it is not always a straightforward issue to characterize specific plant metabolites as either toxins or nutrients (Bernays and Chapman 1987, Bernays 1991, Berenbaum 1996, Behmer and Elias 1999).

A few attempts have been made to disentangle the influence of PSMs and nutrients on the foraging behavior of herbivores (e.g., Pennings et al. 1993, Bernays et al. 1994, Hägele and Rowell-Rahier 1999, Dearing et al. 2000). However, the lack of quantitative information on the PSM and nutrient levels in the experimental foods has left interpretations somewhat incomplete. Quantifying a plant's chemical content and the amounts of tissue that a herbivore has eaten can be both difficult and time consuming, but the failure to do so prevents us from properly understanding how herbivores, particularly those that are mobile, confront the decisions of how much of a given food to eat and how to distribute feeding among a range of different food types. Despite its obvious ecological importance, from the viewpoint of both herbivore and plant evolution (Atsatt and O'Dowd 1976), there exist no studies that give a clear description of the interactive effects of PSMs and nutrients on herbivores that are foraging in

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environments with multiple foods that vary orthogonally in both their PSM and nutrient content.

Part of the challenge in establishing the relative importance of nutrient balancing and toxin dilution to the foraging patterns of herbivores is to develop an experimental approach that identifies key variables and quantifies their interactions. Given that the relationship between nutrients and PSMs comprises interactions among a suite of nutrients (proteins, carbohydrates, lipids, salts, vitamins, sterols, etc.) and classes of PSM (carbon-based, nitrogen-based, etc.), as well as between the behavioral and physiological control systems of herbivores, there is a need to break this complex system into manageable units. Our approach to dealing with the complexity of nutrient–nutrient and nutrient–PSM interactions and their impact on herbivore foraging behavior is founded on a set of state–space graphical representations of the animal within its nutritional environment, the ''geometric framework,'' (Simpson and Raubenheimer 1993, Raubenheimer and Simpson 1999). This approach has features in common with the resource allocation models of Tilman (1982) and with ecological stoichiometry (Reiners 1986), but places more emphasis on the physiology and behavior of individual animals. Our broad aim is to develop and test an experimental technology that can serve as an exemplar for ecologists and other scientists concerned with animal foraging and resource acquisition and allocation.

The first step in our program required choosing a model herbivore, and we selected the African migratory locust, *Locusta migratoria*. This mobile grass specialist is a particularly appropriate subject for a study on nutrient–nutrient and nutrient–PSM interactions for at least three reasons. First, it is the best known of all insect herbivores with regard to the physiological mechanisms controlling feeding behavior (Simpson and Raubenheimer 2000). Second, it is not specifically adapted to deal with most PSMs, yet is not extremely sensitive to their presence (Bernays and Barbehenn 1987). Third, there is a chemically defined artificial diet that supports excellent nymphal development (Dadd 1960). In our program to date, it has been necessary to work with synthetic diets, because only these offer the required level of control over the chemical composition of experimental foods. What follows is a summary of our research aims. The first three of these have been achieved and the fourth is the subject of the current paper.

1. *To identify the key nutrients that herbivores regulate, and the optimal intake of these over given periods in development*.—Protein and carbohydrate have been shown to be the most strongly regulated nutrient groups in insects, and much is now known of the physiological bases for such responses (Simpson and Raubenheimer 2000). Extensive studies have been undertaken on fifth-instar locust nymphs, which have been shown to precisely regulate the intake of both protein and carbohydrate when faced with different pairings of individually unbalanced but complementary foods (Chambers et al. 1995), and to compensate for both a fivefold dilution of their diet with cellulose (Raubenheimer and Simpson 1993) and for variation in the frequency with which two complementary foods occur in the environment (Behmer et al. 2001). Locusts also regulate their intake of salt and sterols (see Behmer et al. 1999, Simpson and Raubenheimer 2000), but these appear to be subservient to the mechanisms controlling protein and carbohydrate intake (e.g., see Trumper and Simpson 1993).

2. *To quantify the nature and fitness consequences of the trade-offs between ingesting too much of some nutrients and too little of others*.—There are fitness costs to an animal of not achieving its optimal intake of nutrients (Raubenheimer and Simpson 1997; S. J. Simpson, R. M. Sibly, K. P. Lee, S. T. Behmer, and D. Raubenheimer, *unpublished manuscript*). An animal may not be able to reach its intake target for various ecological reasons. One is that nutritionally suitable foods are not available, due either to absence of a nutritionally optimal food or of complementary food items that can be mixed to provide an optimal diet. In such cases, animals must balance eating an excess of some nutrients against a deficit of others. The GF (geometric framework) has provided a means of exploring and quantifying these trade-offs and relating them to the nutritional ecology of animals (Raubenheimer and Simpson 1997, Simpson et al. 2002).

3. *To quantify the influence of PSMs on nutrient trade-offs when herbivores are provided with single foods differing in nutritional composition*.—The presence of plant secondary metabolites provides another reason for failing to reach the intake target. In a recent study (Simpson and Raubenheimer 2001), we began an experimental analysis of the interaction between nutrients and PSMs by confining *L. migratoria* nymphs to a single food that varied in the content of dietary protein (P), digestible carbohydrate (C), and tannic acid (TA). We chose TA to begin the study of nutrient–PSM interactions because it is known to influence behavior and growth in *L. migratoria* both as a feeding deterrent and, post-ingestively, as a toxin (Bernays 1978, Bernays et al. 1980, Raubenheimer 1992), as it does in many other animals (Bernays et al. 1989, Schultz et al. 1992). Results showed that, for locusts, the consequences of ingesting TA varied depending on the ratio of protein to carbohydrate (P:C) in the food. When the P:C ratio was near optimal, addition of TA up to 10% by dry mass had no measurable deleterious impact, whereas addition of lower concentrations had marked negative effects when the P:C ratio was suboptimal. At lower than optimal P:C ratios, TA had a deleterious effect primarily as an antifeedant, whereas it served as a post-ingestive toxin when the P:C ratio was higher than optimal.

4. *To establish the extent to which herbivores con-*

tinue to regulate nutrient intake when they are able to select among foods of differing nutritional and PSM composition.—This is the aim of the experiments in the current study. We have undertaken two experiments. Locusts in the first experiment were given two suboptimal, but complementary, foods: a high-protein, low-carbohydrate food and a low-protein, high-carbohydrate food. Tannic acid was then added to one food type but not the other, to both, or to neither food type. If regulation of nutrient intake was paramount, then the presence of TA would not distort the pattern of intake between the two food types and locusts would achieve the same point of protein and carbohydrate intake irrespective of TA. If the effects of TA were asymmetrical, then its presence in one food type would have more impact on patterns of food intake than would its addition to the other food. In a second experiment, locusts were given a dish of a high-protein, low-carbohydrate food and a dish of low-protein, high-carbohydrate food, both of which contained TA. A third dish containing TA-free food (one of five different protein : carbohydrate ratios) was also present. This provided an opportunity to measure the extent to which a TA-free resource would be incorporated into the diet in relation to its nutritional quality.

We are also using the experimental designs and relationships established using TA to investigate the effects of nitrogen-based PSMs on locusts, and extending them to the study of other insect species. These data will be reported elsewhere. Having established and quantified the key variables and relationships under very controlled circumstances, we will be in a position to use our approach under more natural conditions. A useful bridge between chemically defined synthetic foods and the complexity of natural environments has been provided by single-gene mutants of *Arabidopsis* (Wright et al. 2000).

The ultimate goal of our research program is to use knowledge of the relationships established under aims (1) through (4) to explain in greater detail patterns of resource use by herbivores, allocation of resources in plants to defenses, and the consequences of these on herbivore populations and nutrient flow through ecosystems. Our contention is that the detailed understanding of the interactions among nutrients and PSMs arising from the GF provides unique insights into higher level processes (see Wright et al. 2001).

Methods

Insects and experimental chambers

Experimental locusts came from a culture kept under crowded conditions and maintained on a diet of seedling wheat and wheatgerm at the Department of Zoology, University of Oxford. Following ecdysis to the fifth stadium (day 0), locusts were removed from the culture, weighed, and placed singly into circular Plexiglass arenas (23 cm in diameter and 16 cm high). Each arena contained synthetic food in small plastic dishes (modified 3.5 cm and 5.5 cm diameter Petri dishes arranged to minimize spillage; Raubenheimer and Simpson 1990) and a small plastic container ($7 \times 4 \times 2$ cm with two 1.5 cm holes in the top) that provided drinking water. Within each arena, the water container was positioned centrally and the food dishes surrounded it in an equidistant and symmetrical arrangement. Expanded aluminium perches were positioned directly behind each food dish to permit the locusts to roost. Partitions were placed around the outside of each arena to screen the locusts from one another. All experiments were conducted in a constant-temperature room at 29–31°C under a L:D 12:12 h photo regime. Approximately equal numbers of male and female locusts were used in each experiment.

Synthetic foods

Dry, granular, chemically defined foods were made in a similar manner to those developed by Dadd (1961) and further modified by Simpson and Abisgold (1985). In total, we made five foods that varied in the ratio of protein (P) to digestible carbohydrate (C), expressed on a dry mass basis (P31:C11; P25:C17; P19:C23; P13:C29; P7:C35). The protein component of the diets was a 3:1:1 mix of casein, albumen, and peptone that closely matches the amino acid profile of seedling wheat (see Simpson and Abisgold 1985). Digestible carbohydrate comprised a 1:1 mix of sucrose and dextrin. The P19:C23 formulation is near nutritionally optimal for *L. migratoria* (Chambers et al. 1995), whereas the other foods represent suboptimal mixtures that are symmetrically imbalanced on either side of the P19:C23 food. All five foods contained the same total concentration of protein and carbohydrate (42%) and also contained identical percentages of the other ingredients (54% cellulose, with the remaining 4% consisting of salts, vitamins, and sterols). To some of the food dishes we added tannic acid powder (Sigma-Aldrich Company, Dorset, UK) at a concentration of 10% (dry mass), as described by Raubenheimer and Simpson (1990). We selected this concentration based on results from an earlier study, in which it was found that 10% TA had no measurable deleterious effects when in a near-optimally balanced food, but was detrimental when present in foods with a suboptimal P:C ratio (Simpson and Raubenheimer 2001). The tannic acid (TA) was added to the dry food, rather than being incorporated earlier in the mixing procedure, to avoid complexing with the dietary protein and cellulose (Mole and Waterman 1987, Raubenheimer and Simpson 1990).

Experimental protocol

Two separate experiments were performed. In the first, each arena contained two dishes of P31:C11 food (henceforth, ''P'') and two dishes of P7:C35 food (henceforth, ''C''); alone these foods are nutritionally

suboptimal, but together they are complementary. Tannic acid was then added to the different food types yielding four different treatments: (1) no TA added to either food type (treatment code NTA), (2) TA added to the P31:C11 food (PTA), (3) TA added to the P7:C35 food (CTA), and (4) both food types containing TA (BTA). After treatments had been assigned to the individual arenas using a randomized block design, each individual food dish within an arena was given a number (1–4). This allowed us to identify the nature of the contents of individual dishes and to return dishes after they had been removed for weighing to the same location in the arena. After each dish had been allocated its synthetic food and allowed to equilibrate under stable ambient room humidity levels for ~24 h, it was weighed to the nearest 0.1 mg. Control food dishes indicate that this procedure provides errors in estimation of consumption of ≤1%. Locusts were then allowed to feed for 48 h. At the end of this period, each dish within an arena was removed and replaced with a new pre-weighed dish of the same food. The dish that had been removed was then allowed to equilibrate to room humidity levels before it was reweighed. We repeated this procedure at the end of day 4. At the end of day 6 (the last day of the experiment), locusts were removed and their individual wet mass was recorded. We changed the food at 2-d intervals to determine whether food preference changed over time.

In the second experiment, the setup was similar except that each arena had one dish of TA-containing P31:C11 food, one dish of TA-containing P7:C35 food, and one dish of TA-free food (P31:C11, P25:C17, P19:C23, P13:C29, or P7:C35). As in the previous experiment, each food dish was labeled with a number; prior to being weighed, the foods were allowed to equilibrate to ambient humidity levels. This experiment ran for the duration of the fifth stadium and the amount of food eaten from each dish was recorded on the third, fifth, eighth, and last day of the fifth stadium. After locusts had successfully moulted to the adult stage, their wet mass was recorded. They were then dried in a desiccating oven at 40°C. Following desiccation, carcasses were weighed to the nearest 0.1 mg and were lipid-extracted in three 24-h changes of chloroform. At the end of the third chloroform wash, they were redried and reweighed to calculate lipid content. This chloroform extraction procedure had earlier been shown to be >98% efficient relative to Soxhlet distillation (Simpson 1983). The lipid-free carcasses were then analyzed for nitrogen content using the micro-Kjeldahl procedure.

To calculate lipid and nitrogen growth in our test insects, we first had to accurately estimate their starting body lipid and nitrogen content. We did this by performing lipid and nitrogen analyses, as previously described, on newly moulted fifth-stadium locusts. Regression equations, with locust mass on the *x*-axis and lipid or nitrogen mass on the *y*-axis, were then generated from these data (for body lipid, $y = 0.034x + 1.662$; for body nitrogen, $y = 0.023x + 1.066$) and were used to calculate our test insect's starting body lipid and nitrogen content.

Statistical analysis

Various aspects of food consumption and insect performance were analyzed using analysis of covariance (ANCOVA), multivariate analysis of covariance (MANCOVA), and failure time analysis (PROC LifeReg) techniques. For MANOVA analyses, we used the Pillai's test statistic, which is considered to be the most robust to violations of assumptions (Scheiner 1993). All analyses were performed using the statistical package SAS 6.12 (SAS Institute 1989). When it was necessary, the data were log-transformed to meet underlying assumptions. For most ANCOVA analyses, initial mass of locusts was used as a covariate to correct for size differences among the experimental animals (on average, females are larger than males). Where significant effects were observed, post hoc comparisons were performed using contrasts. For MANOVAs, contrasts followed the techniques employed by Scheiner (1993). Where multiple contrasts were made, α levels were adjusted using the Bonferroni method. For all ANCOVA and MANCOVA analyses, tests for heterogeneity of slopes were performed; in no case were significant effects observed.

RESULTS

Experiment 1: Paired complementary foods with or without tannic acid

Consumption patterns.—Our primary interest was to compare how the presence of tannic acid (TA) in the different food types influenced consumption patterns relative to the NTA treatment (with no TA in either food). Locusts on the CTA treatment ate relatively more P food and less C food across the 6-d experiment compared to locusts from the NTA treatment, whereas locusts on the PTA and BTA treatments did not differ from those on NTA (Table 1, Fig. 1A). This pattern of results was evident across days 0–2 and 2–4. During days 4–6, however, locusts from the BTA treatment ate more C food and less P food compared to those from the NTA treatment, whereas CTA and PTA treatments did not differ statistically from the NTA treatment (Table 1, Fig. 1B–D). Additionally, during days 0–2, a significant treatment-by-sex interaction was detected, revealing that females on the CTA treatment ate significantly less of the C food with 10% TA than did males. A significant covariate effect was also observed during this period, with larger insects eating more than smaller ones. However, the interaction term and covariate were not significant for days 2–4 and 4–6.

Nutrient intake.—Fig. 2 shows a bi-coordinate plot of the cumulative amounts of protein and carbohydrate eaten by fifth-stadium *Locusta migratoria* over the entire experiment (days 0–6), with locusts on the control

TABLE 1. Results of MANCOVA for food consumption by locusts from the first experiment.

Source	df (hypothesis, error)	*F* values: Days 0–6	Days 0–2	Days 2–4	Days 4–6
A) Amounts consumed from the different food dishes					
Treatment	6, 66	2.93**	3.59**	2.46*	3.05**
Sex	2, 32	0.29	0.05	1.03	0.50
Treatment × Sex	6, 66	2.10	3.27*	1.94	0.73
Initial mass	2, 33	2.78	4.12*	2.33	0.77
B) Multivariate contrasts					
NTA vs. PTA	2, 32	0.65	0.93	0.20	1.20
NTA vs. CTA	2, 32	4.74†	7.49†	6.12†	1.33
NTA vs. BTA	2, 32	0.95	0.22	0.73	6.55†

Notes: Part (A) reports overall *F* values (Pillai's Trace) for the entire time period (days 0–6) and for each of the different time intervals. Treatment refers to the four possible combinations of C and P food with or without tannic acid. Initial mass was used as a covariate to adjust for size differences among insects. Part (B) reports *F* values for multivariate contrasts between specific treatments: NTA, no tannic acid; PTA, tannic acid added to the protein 31 : carbohydrate 11 food; CTA, tannic acid added to the protein 7 : carbohydrate 11 food; CTA, tannic acid added to the protein 7 : carbohydrate 35 food; BTA, tannic acid added to both types of food.

* P; < 0.05; ** $P < 0.01$.

† Significant difference with $\alpha = 0.05/3$ (divided by three because there are three comparisons).

treatment (NTA) indicated by the solid circles and solid lines. MANOVA for the entire experiment revealed that a significant difference in protein and carbohydrate intake was observed among treatments. When specific comparisons were made, using contrasts, it was revealed that the protein–carbohydrate intake of locusts on the CTA and BTA treatments differed from that of locusts on the NTA treatment. For locusts on the CTA treatments, carbohydrate intake was low compared to that of NTA locusts. For BTA locusts, both protein and carbohydrate intake were reduced compared to those of NTA locusts. No statistical difference in protein–carbohydrate intake was observed between the PTA- and NTA-reared locusts.

Nutrient intake for each time period was also analyzed (see Fig. 2). As shown in Table 2A, significant differences among treatments in protein–carbohydrate intake were observed for each time period. Additionally, a significant treatment-by-sex interaction and covariate effect were detected during days 0–2; females on the CTA treatment ingested less carbohydrate than did males. A significant covariate effect was also found during the first time period, with larger insects having higher protein–carbohydrate intakes than smaller ones. Significant differences in protein–carbohydrate intake between the NTA and CTA treatments were observed during the first two time periods, whereas a difference between the NTA and BTA treatments was observed during the last time period. No differences in protein–carbohydrate intake were observed between the PTA and NTA treatments for any of the time intervals.

Total intake and growth.—Over the duration of the experiment, similar total amounts of food were eaten, on average, by insects on the different treatments (Table 3), but significant differences among the treatments were found in the total amount of tannic acid ingested (Table 3, Fig. 3). Above a threshold intake of tannic

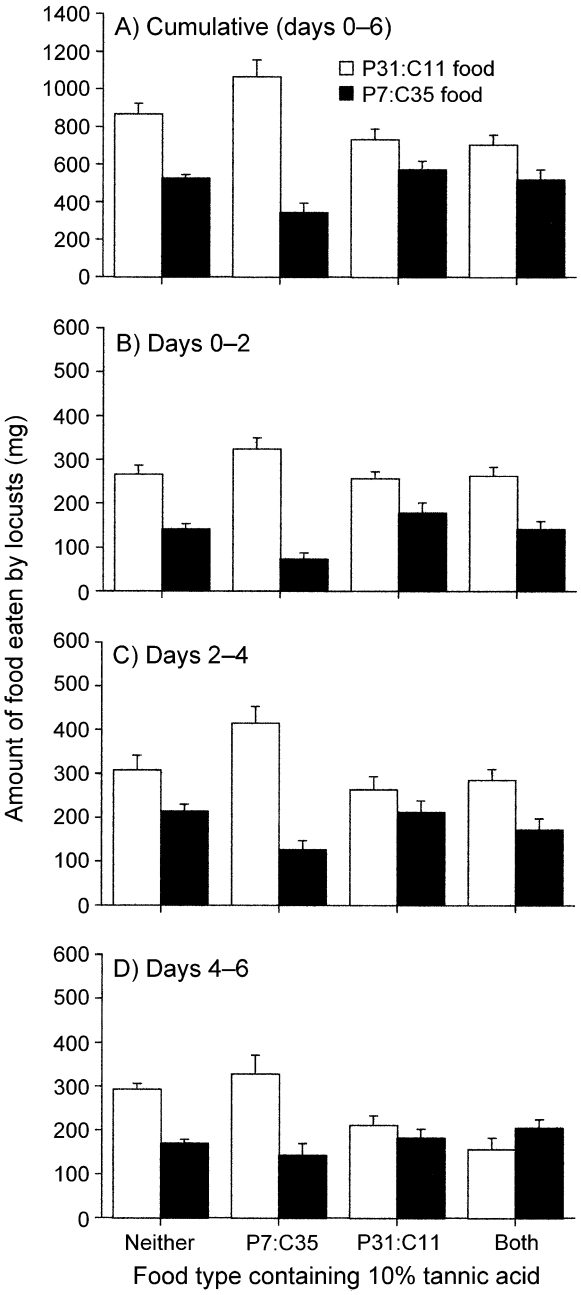

FIG. 1. The total amount of protein, P (P31:C11) and carbohydrate, C (P7:C35) food eaten (mean + 1 SE) from the first experiment, where locusts encountered two dishes each of P and C food, with or without 10% tannic acid (TA). Panel (A) represents consumption over the entire experiment (days 0–6); panels (B), (C), and (D) represent consumption over days 0–2, 2–4, and 4–6, respectively.

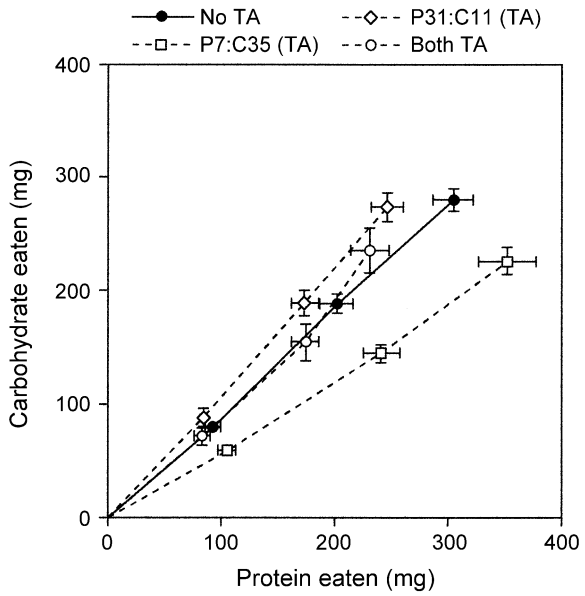

FIG. 2. Bivariate means (± 1 SE) for protein (P) and carbohydrate (C) intake for locusts from the first experiment. The solid line indicates the intake ''trajectory'' for locusts from the treatment containing no tannic acid (NTA) in either food type. The intake trajectories for treatments with tannic acid in one or both food types are indicated by dashed lines. The first, second, and third points along each trajectory indicate the cumulative intake points reached after two days, four days, and six days, respectively. For comparisons of intake points, see Table 2.

acid (~50 mg), mass gain was low relative to the NTA treatment (Fig. 3). To determine whether reduced growth was a direct effect of high tannic acid intake (toxicity) or, alternatively, an indirect result of reduced nutrient intake (deterrence), ANCOVAs were performed using total protein intake and total carbohydrate intake as covariates (see Raubenheimer and Simpson 1994). Our first analysis revealed that when both protein and carbohydrate intake were included as covariates, the difference among treatments became nonsignificant (Table 4A), suggesting that growth differences were due to differences in nutrient intake among treatments. To determine whether protein or carbohydrate intake was more of a determining factor explaining body mass gain, we conducted two separate ANCOVAs. Results from these analyses showed that protein intake (Table 4B), not carbohydrate intake (Table 4C), was more important in explaining mass gain.

Experiment 2: Multiple food types with and without tannic acid

Consumption patterns.—When consumption patterns of the three different food types (P + 10% TA (PTA), C + 10% TA (CTA), and a TA-free food) were analyzed over the entire fifth stadium (Fig. 4A), consumption of the TA-free food increased as the protein : carbohydrate ratio of the TA-free food increased (Table 5). Additionally, as the protein : carbohydrate ratio of the TA-free food increased, consumption of CTA food increased while consumption of PTA food decreased.

During days 0–3, a quadratic increase in consumption of the TA-free food was observed as the protein : carbohydrate ratio of the TA-free food increased. Consumption of the PTA food decreased in a quadratic fashion, however, as the protein : carbohydrate ratio of the TA-free food increased (Table 5B, Fig. 4B). Over days 3–5, consumption of the TA-free food again increased in a quadratic fashion, but now consumption of the PTA and CTA food decreased and increased, respectively, in a linear fashion (Table 5B, Fig. 4C). From day 5 until the ecdysis, no trend in consumption of the TA-free food was observed. There were, however, trends in consumption of the CTA and PTA foods. As the protein : carbohydrate ratio of the TA-free food increased, we observed a linear increase and decrease

TABLE 2. Results of MANCOVA for locust protein and carbohydrate intake from the first experiment.

Source	df (hypothesis, error)	*F* values Days 0–6	Days 0–2	Days 2–4	Days 4–6
A) Protein and carbohydrate intake					
Treatment	6, 66	6.10**	4.50**	3.52**	3.75**
Sex	2, 32	0.97	0.31	0.52	0.71
Treatment × Sex	6, 66	1.83	2.63*	1.43	0.68
Initial mass	2, 32	1.82	3.96*	0.85	0.13
B) Multivariate contrasts					
NTA vs. PTA	2, 32	1.93	1.66	0.60	2.91
NTA vs. CTA	2, 32	6.86†	8.60†	6.15†	0.81
NTA vs. BTA	2, 32	4.95†	0.34	2.20	9.92†

Notes: Part (A) reports overall *F* values (Pillai's Trace) for the entire time period (days 0–6) as well as for each of the different time intervals. Treatment refers to the four possible combinations of C and P food with or without tannic acid. Initial mass was used as a covariate to adjust for size differences among insects. Part (B) reports *F* values for multivariate contrasts between specific treatments, identified in Table 1.

* $P < 0.05$; ** $P < 0.01$.

† Significant difference with $\alpha = 0.05/3$ (divided by three because there are three comparisons).

TABLE 3. ANCOVAs for the first experiment comparing the total amount of food consumed, the amount of tannic acid consumed, and body mass gained by locusts.

Source	df	*F* values Total consumption	Tannic acid consumption	Mass gain
Treatment	3, 2†	1.40	27.17**	3.30*
Sex	1	2.87	2.95	0.09
Treatment × Sex	3, 2†	1.43	1.28	1.89
Initial mass‡	1	2.72	4.84*	1.99
Error	33, 24†			

* $P < 0.05$; ** $P < 0.01$.

† Degrees of freedom for tannic acid consumption.

‡ Initial mass was used in each of these analyses as a covariate to adjust for size differences among individuals.

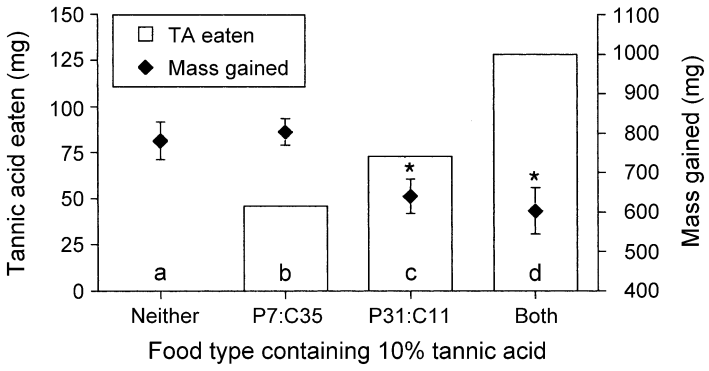

FIG. 3. Total tannic acid (TA) intake (bars) and mass gained (diamonds) by fifth-stadium locusts during the first experiment. Different letters at the base of the bars indicate significant differences in tannic acid intake (Tukey test, $\alpha = 0.05$). An asterisk above a diamond indicates a significant difference in mass gain compared to the no tannic acid treatment (Dunnett's test, $\alpha = 0.05$).

in consumption of CTA and PTA food, respectively (Table 5B, Fig. 4D).

Nutrient intake.—There was a significant difference among treatments in protein : carbohydrate intake (Table 6A, Fig. 5). Locusts from the treatment with TA-free P19:C23 food regulated protein : carbohydrate intake to the same point as locusts reared on TA-free P and C foods (data taken from experiment 1). For this reason, we used the TA-free P19:C23 treatment as the reference point to which protein-carbohydrate intake from the others treatments is compared. Three comparisons were made. First, the difference in protein : carbohydrate intake between the reference treatment (TA-free P19:C23) and the treatment with the lowest P:C intake (TA-free P13:C29) was not significant. Next, protein : carbohydrate intake on the TA-free P25:C17 treatment differed significantly from the reference treatment. Additionally, intakes on the TA-free P19:C23 and P31:C11 treatments were significantly different.

Protein : carbohydrate intake across the stadium is also plotted in Fig. 5. As shown in Table 6A, significant differences in protein : carbohydrate intake were observed for each time period. When we compared protein : carbohydrate intake on the different treatments to the TA-free P19:C23 treatment, though, the only difference detected was with the TA-free P31:C11 treatment, and only up until day 5. Among the remaining treatments, protein : carbohydrate intake for each time period did not differ statistically from that achieved by locusts on the TA-free P19:C23 treatment.

Total intake and growth.—Similar total amounts of food were eaten among the different treatments, but significant differences in the amount of tannic acid consumed were observed (Table 7). Intake of TA was highest for locusts from the TA-free P7:C35 treatment and was significantly greater than from any of the other treatments, but no differences were observed among these other treatments (Fig. 6A). A significant treatment effect was noted in mass gain (Table 7), with the TA-free P25:C17 treatment yielding greater mass than all but the TA-free P31:C11 (Fig. 6B); no other differences among the treatments were observed. The effect of sex was also significant, with females gaining more mass during the fifth stadium than males. An ANCOVA was performed to determine whether nutrient intake or tannic acid consumption might explain differences in mass gain. This analysis revealed that protein intake as a covariate was significant ($F = 8.72$, df = 1,34, $P = 0.006$); when protein intake was included in the model, the treatment effect, but not the sex effect, became nonsignificant. With regard to developmental time, no significant difference among the treatments was observed (failure time analysis, $\chi^2 = 7.39$, df = 4, $P = 0.117$).

Total body nitrogen (N) and lipid gain during the fifth stadium were also compared across treatments. MANOVA revealed a significant treatment effect ($F = 2.97$, df = 8,66, $P = 0.007$), but sex, the treatment-by-sex interaction, and initial mass as the covariate were not significant. Post hoc comparisons showed that N and lipid gain on the TA-free P7:C35 and P13:C29 treatments differed significantly from the TA-free P31:

TABLE 4. ANCOVAs for the first experiment, using intake of specific nutrients as covariates to explore the physiological basis of differences in growth (mass gain) in locusts: (A) the effect on growth of both protein and carbohydrate, and the separate effects of (B) protein and (C) carbohydrate. Values in boldface indicate significant effects.

Source	df	MS	*F*	*P*
A) Growth corrected for both protein and carbohydrate intake				
Treatment	3	1151.1	0.20	0.896
Sex	1	6654.0	0.63	0.431
Treatment × Sex	3	5680.3	0.99	0.411
Protein intake	1	300 303.5	52.18	**<0.001**
Carbohydrate intake	1	64 161.1	11.15	0.002
Error	33	5755.1		
B) Growth corrected for protein intake				
Treatment	3	3792.6	0.51	0.680
Sex	1	1975.1	0.26	0.611
Treatment × Sex	3	8834.4	1.18	0.331
Protein intake	1	356 166.8	47.66	**<0.001**
Error	42	254 079.8		
C) Growth corrected for carbohydrate intake				
Treatment	3	92 680.6	6.43	**0.001**
Sex	1	37 201.2	2.58	0.118
Treatment × Sex	3	35 366.9	2.45	0.080
Carbohydrate intake	1	120 024.4	8.32	**0.007**
Error	42	1 195 051.9		

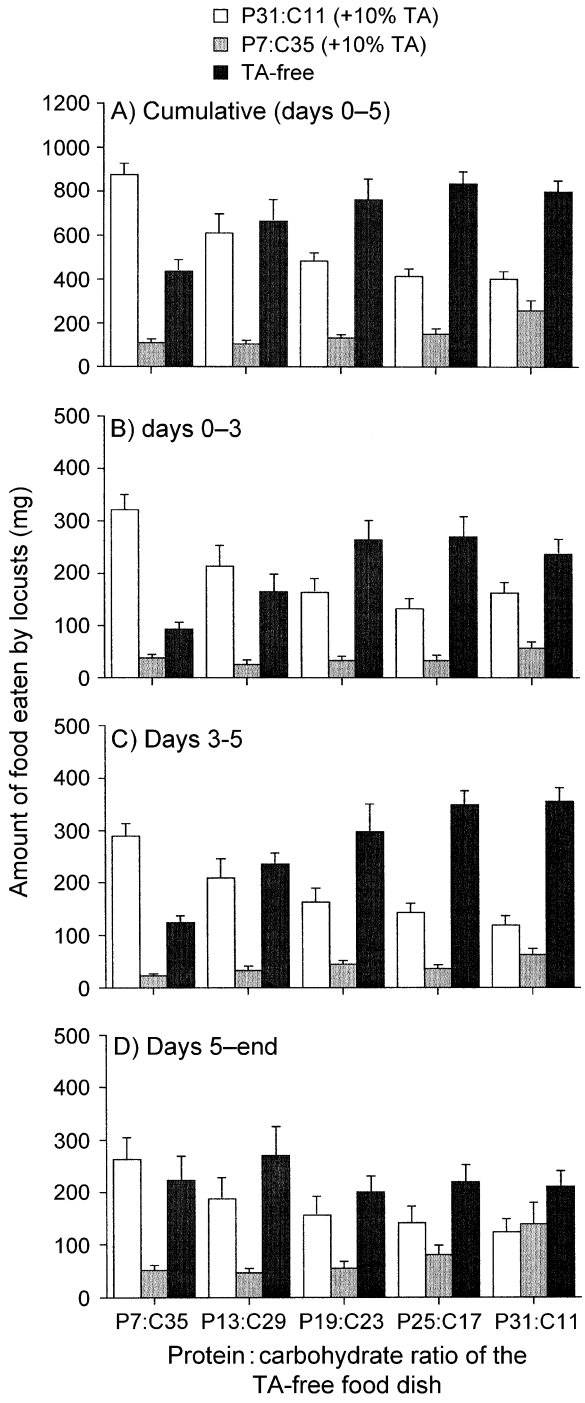

FIG. 4. The total amount of P (P31:C11) + TA, C (P7:C35) + TA, and TA-free (P7:C35, P13:C29, P19:C23, P25:C17, or P31:C11) food eaten by locusts (mean + 1 SE). Panel (A) represents consumption over the entire fifth stadium; panels (B), (C), and (D) represent consumption over days 0–3, 3–5, and 5–end, respectively.

C11 treatment. The TA-free P19:C23 and P25:C17 treatments did not differ from the three previously mentioned treatments. One-way ANOVAs and subsequent contrasts showed, however, that differences in lipid gain existed between the TA-free P31:C11 treatment and the TA-free P25:C17 and P19:C23 treatments (P31:C11 vs. P25:C17, $F = 5.45$, df = 1,35, $P = 0.020$; P31:C11 vs. P19:C23, $F = 3.806$, df = 1, $P = 0.030$). Finally, we analyzed the efficiency of conversion of ingested protein and carbohydrates into nitrogen (N) and lipid gain, respectively, using two ANCOVA designs (see Raubenheimer and Simpson 1994): (1) lipid gain as the dependent variable and carbohydrate as the covariate, and (2) nitrogen gain as the dependent variable and protein intake as the covariate. The rationale for using carbohydrate intake as a covariate for an analysis of lipid gain is that sugars and dextrose are the only nutrients in the experimental foods that are used for lipogenesis: lipids are present in trace quantities, and *L. migratoria* do not use ingested proteins to any significant extent for lipogenesis (D. Raubenheimer and S. J. Simpson, *unpublished manuscript*). Similarly, protein intake was used as a covariate in the analysis of nitrogen gain because casein, albumin, and peptone were the sole source of nitrogen in the foods. Results demonstrate that when N gain was reanalyzed in this manner, protein intake as a covariate was significant ($F = 13.187$, df = 1,35, $P = 0.001$), but the main treatment effect (protein–carbohydrate ratio of the TA-free food) now became nonsignificant. Similarly, when lipid gain was reanalyzed, carbohydrate as a covariate was found to be significant ($F = 4.246$, df = 1,35, $P = 0.048$), but the main treatment effect became nonsignificant. However, sex as a main effect then became significant ($F = 6.438$, df = 1,35, $P = 0.016$), with males showing greater lipid gain over the final stadium.

DISCUSSION

When the plants available to an herbivore vary in both their nutritional and PSM composition, foraging decisions should represent a trade-off between the benefits gained from obtaining an optimal balance of nutrients and the costs of ingesting PSMs that may be deleterious. In the current study, we have addressed this question by presenting locusts with multiple foods that vary in their protein, carbohydrate, and tannic acid content, and by examining locust foraging decisions within the context of the geometric approach.

Before investigating the ways in which the chemical constituents of different foods in an herbivore's environment might interact to influence foraging decisions, it is important first to identify which nutrient intake point (''intake target'') is regulated in the absence of PSMs. For example, locusts on the NTA (no tannic acid) treatment in the first experiment regulated to a protein–carbohydrate intake target of 21.3% protein and 19.4% digestible carbohydrate, which is similar to what has been demonstrated in previous studies (Rau-

TABLE 5. Results of MANCOVAs for food consumption by locusts for the entire fifth stadium (days 0 to end) and for three time intervals (days 0–3, 3–5, and 5–end) within the stadium.

Source	df (hypothesis, error)	*F* values			
		Days 0–end	Days 0–3	Days 3–5	Days 5–end
A) Amounts consumed from the different food dishes					
Treatment	12, 99	3.87**	2.63**	3.50**	3.22**
Sex	3, 31	0.33	0.31	0.38	1.20
Treatment × Sex	12, 99	0.77	0.90	0.76	1.23
Initial mass	3, 31	0.59	0.19	0.69	1.10
B) Linear and quadratic contrasts (* significant one-tailed test, $\alpha = 0.05$)					
P31:C11 + 10% TA					
Linear	1	8.27*	⋯	3.55*	4.86*
Quadratic	1	2.05	3.52*	0.60	0.99
P7:C35 + 10% TA					
Linear	1	10.91*	0.21	7.81*	8.76*
Quadratic	1	2.15	2.37	0.01	2.33
TA-free					
Linear	1	⋯	⋯	⋯	0.05
Quadratic	1	11.07*	10.07*	11.26*	0.61

Notes: Part (A) reports overall *F* values (Pillai's Trace) for the entire fifth stadium and for each of the three different time intervals. Treatment refers to the P:C (protein : carbohydrate) ratio of the TA (tannic acid)-free food, and initial mass was used as a covariate to adjust for size differences. Part (B) reports *F* values for linear and quadratic trends in consumption among the three different food types. When higher order trends are significant, lower order trends are not reported and are replaced by ellipses.

* $P < 0.05$; ** $P < 0.01$.

benheimer and Simpson 1993, 1997, Chambers et al. 1995, Simpson and Raubenheimer 2000, Behmer et al. 2001). Having identified this reference intake target, it was next possible to explore how the addition of TA to the different food types influenced foraging patterns. A key finding of the first experiment was that the nature of the influence of TA on foraging decisions depended on which food type contained it. For instance, when TA was present only in the C (high-carbohydrate) food (CTA treatment), much more of the P food was eaten relative to the C food and the intake target was not reached. In contrast, when TA was present only in the P (high-protein) food (PTA treatment), the ratio of P and C foods eaten remained similar to that on the NTA treatment and the intake target was reached. However, the PTA-reared locusts ingested a greater quantity of TA than did the CTA-reared locusts. When locusts could not limit TA intake because it occurred in both foods (BTA treatment), the amounts of P and C foods eaten were similar to amounts eaten in the NTA treatment. However, because a portion of the total mass of the food was TA, the total amounts of protein and carbohydrate ingested between these treatments were unequal. Nonetheless, BTA-reared locusts did regulate to

TABLE 6. Results of MANCOVAs for protein and carbohydrate intake by locusts from the second experiment.

Source	df (hypothesis, error)	*F* values			
		Days 0–end	Days 0–3	Days 3–5	Days 5–end
A) Protein and carbohydrate intake					
Treatment	8, 66	4.95**	4.16**	6.93**	4.17**
Sex	2, 32	1.59	0.22	0.32	3.12
Treatment × Sex	8, 66	0.76	0.92	0.79	1.08
Initial mass	2, 32	1.06	0.19	0.97	2.50
B) Multivariate contrasts					
P19:C23 vs. P13:C29	2, 32	1.98	0.66	1.57	3.25
P19:C23 vs. P25:C17	2, 32	4.94†	0.45	3.27	3.48
P19:C23 vs. P31:C11	2, 32	15.46†	16.70†	16.43†	4.20

Notes: Part (A) reports overall *F* values (Pillai's Trace) for the entire time fifth stadium as well as for each of the three different time intervals. Treatment refers to the P:C ratio of the third food dish, which contained no tannic acid; initial mass was used as a covariate to adjust for size differences among insects. Part (B) reports *F* values for multivariate contrasts between specific treatments.

* $P < 0.05$; ** $P < 0.01$.

† Significant difference with $\alpha = 0.05/3$.

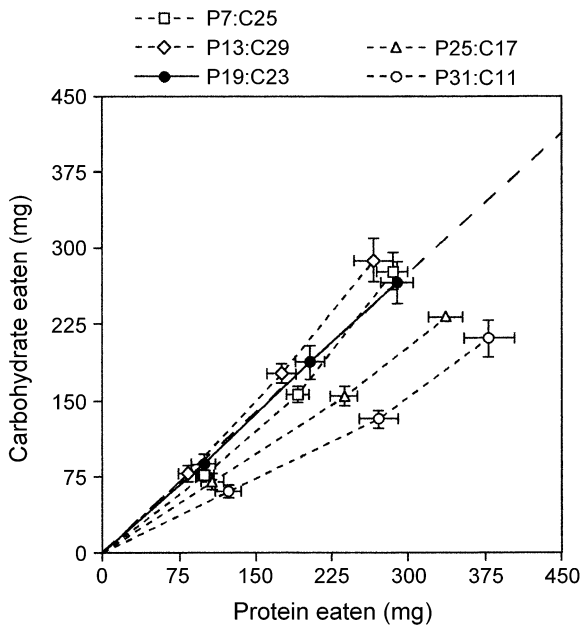

FIG. 5. Bivariate means (± 1 SE) for protein (P) and carbohydrate (C) intake for locusts from the second experiment. Treatment groups are represented by the type of TA-free food available. The solid line indicates the intake trajectory for locusts from the treatment containing the TA-free P19:C23 food (the near-optimal food). Intake trajectories for the other treatments are indicated by dashed lines. The first, second, and third points along each trajectory indicate the cumulative intake points reached after day 3, day 5, and the end of the experiment, respectively. Finally, for reference, the thin dotted line represents the intake trajectory selected by locusts on the TA-free treatment from the first experiment. For comparisons of intake points, see Table 6.

a protein : carbohydrate intake ratio similar to that of the NTA-reared locusts.

Why should locusts regulate their nutrient intake on the PTA and BTA treatments, but not on the CTA treatment? There are two likely explanations, one mechanistic and the other functional, as discussed in detail in Simpson and Raubenheimer (2001). First, because protein and TA can complex once they enter the preoral cavity, TA is likely to have a greater deterrent effect on feeding when combined with low-protein foods, because more unbound TA will be available to interact with mouthpart taste receptors. Second, perhaps for locusts the costs of undereating protein and overeating carbohydrate (as would happen if intake of the P food were to be reduced when it contained TA) are greater than the costs of ingesting the amount of TA required to reach the target level of protein intake. In contrast, the costs of overeating protein and undereating carbohydrate (by avoiding the C food when it contains TA) are less than the deleterious effects of ingesting the amount of TA associated with achieving the intake target for carbohydrate.

With regard to the first of these explanations, it has been shown that TA is most deterrent to locusts and

TABLE 7. Results of ANCOVAs for the second experiment comparing the total amount of food eaten, tannic acid consumed, and mass gained by locusts.

Source	df	*F* values: Total consumption	Tannic acid consumption	Mass gain
Treatment	4	0.29	2.97*	4.15**
Sex	1	3.04	0.02	10.30**
Treatment × Sex	4	0.36	1.11	1.28
Initial mass	1	1.98	0.01	0.38
Error	83			

Notes: Treatment refers to the P:C (protein : carbohydrate) ratio of the third food dish, which contained no tannic acid. All values were recorded over the duration of the fifth stadium. Initial mass was used as a covariate to adjust for size differences among insects.

* $P < 0.05$; ** $P < 0.01$.

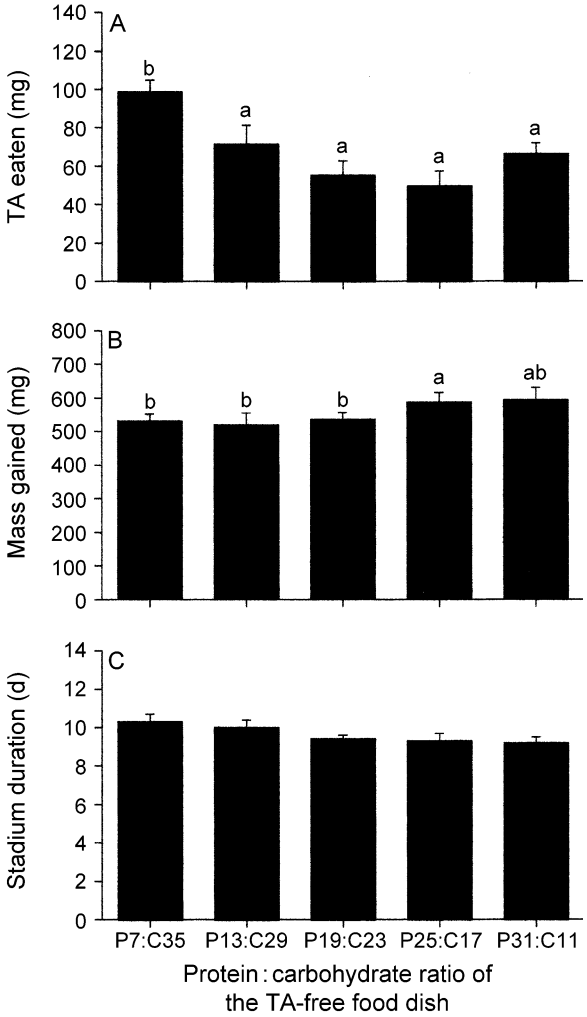

FIG. 6. Mean (+ 1 SE) total tannic acid (TA) consumption (A), mass gain (B), and stadium duration (C) of fifth-stadium locusts from the second experiment. Different letters above a bar indicate a significant differences.

other animals when combined in a food with a low protein : carbohydrate ratio (Raubenheimer 1992, see discussion in Simpson and Raubenheimer 2001). However, this cannot be the entire explanation, because such an effect would predict that the locusts with TA in both the P and C foods (BTA treatment) should also have failed to achieve their intake target.

With regard to the second explanation, it may be relevant that protein may be deaminated and used to supplement limited carbohydrate intake, whereas ingested carbohydrate cannot substitute for protein. There is some evidence that *Locusta migratoria* has a limited ability to subject protein to gluconeogenesis (Zannotto et al. 1993, Simpson and Raubenheimer 2001), although this seems to be better developed in the polyphagous desert locust, *Schistocerca gregaria* (D. Raubenheimer and S. J. Simpson, *unpublished manuscript*). Specifically, *L. migratoria* does not to any significant extent convert ingested amino acids to lipid stores (see *Methods*), whereas *S. gregaria* does. Detailed biochemical studies in caterpillars (Thompson 1997, 1998, 1999) have shown that gluconeogenesis is most pronounced on foods with an excess of protein.

The effect on forging of interactions between foods with different nutrient and TA content was further explored in the second experiment by increasing the number of food types available in the arenas. Here, locusts had access to one dish each of TA-containing C and P foods and a dish of TA-free food of varying protein : carbohydrate ratio. Results from this experiment showed that whether locusts regulated to their intake target depended on the protein : carbohydrate ratio of the TA-free food available to them. When the protein : carbohydrate ratio of the TA-free food was below the intake target ratio (21.3% protein:19.4% digestible carbohydrate), locusts reached the intake target, largely by substituting the TA-containing C food with the TA-free food. By contrast, when the protein : carbohydrate ratio of the TA-free food was above the intake target ratio, animals persisted in avoiding the TA-containing C food and, hence, overingested protein. These responses are very similar to those observed in the first experiment and confirm the importance of the relationship between TA and protein : carbohydrate ratio in determining the deterrent properties of the food, with TA exerting a more powerful deterrent effect when foods contain a low protein : carbohydrate ratio. Consumption of C food with TA was relatively low in all treatments. By contrast, TA-containing P food was eaten in relatively large quantities and tended to increase as the protein : carbohydrate ratio of the TA-free food decreased (Fig. 4A). This was probably due to the need to maintain protein intake, forcing insects to ingest more of the TA-containing P food, rather than allowing them to attain protein from the TA-free food without concurrently over-ingesting carbohydrate.

Another factor that might affect the deterrence of a PSM is an herbivore's current nutritional needs, which are known to change both quantitatively and qualitatively during growth, development, and reproduction. For example, cuticle growth, fueled by protein, dominates the first half of the fifth stadium in *L. migratoria* (Hill and Goldsworthy 1968), whereas fat body carbohydrate growth, particularly glycogen, dominates the latter half of the stadium (Hill and Goldsworthy 1968, Simpson 1982). In the second experiment, nutritional requirements associated with these shifts in growth may explain the trend of locusts during the second and third time periods (days 3–5 and 5–end, respectively) to increase their consumption of TA-containing C food as the protein : carbohydrate ratio of the TA-free food increased. This was particularly the case during the last time period.

It is interesting to note that, in experiment 1, CTA-reared locusts, which did not regulate to the intake target, had gained more mass by the end of the experiment (day 6) than had the PTA- and BTA-reared locusts. At first glance, this difference might be attributed solely to direct toxic effects of TA because PTA- and BTA-reared locusts ingested more TA than did the CTA-reared locusts. However, an ANCOVA indicated that differences in mass gain were best explained by differences in protein intake, with the CTA-reared insects ingesting more protein as a result of avoiding the TA-containing C food (Table 4). In experiment 2, locusts that regulated to their intake target (the TA-free P7:C35, P13:C29, and P19:C23 treatments) also had lower body mass than those on the TA-free P25:C17 treatment that did ingest more protein. Again, however, ANCOVA shows that this outcome was best explained by the indirect influence of TA on protein intake, and not by the amount of TA ingested. There may, however, be a potential cost of failing to regulate carbohydrate intake, because lipid storage reserves tended to decrease as locusts ingested excess protein (experiment 2; see Fig. 7). One manner in which this cost can express itself is in decreased resistance to starvation (Raubenheimer and Simpson 1997). Failing to regulate carbohydrate intake during nymphal development may also have serious repercussions for adults because lipids provide a source of fuel for flight (important for either migration or mate seeking).

Finally, our data may have important implications for plant defense theory, particularly when viewed in the context of the availability of resources to plants (Coley et al. 1985, Bazzaz et al. 1987). For example, it is predicted that plants in resource-poor habitats will be slow growing, have low leaf protein content, and will be defended by carbon-based compounds such as tannins, whereas resource-rich habitats will result in the use of nitrogen-based defensive compounds in conjunction with fast growth rates and high leaf nitrogen contents. A major premise underlying this prediction is that low nitrogen availability in resource-poor environments will place constraints on the types of defense that will be favored through evolutionary time. Our results suggest that a fundamentally important ad-

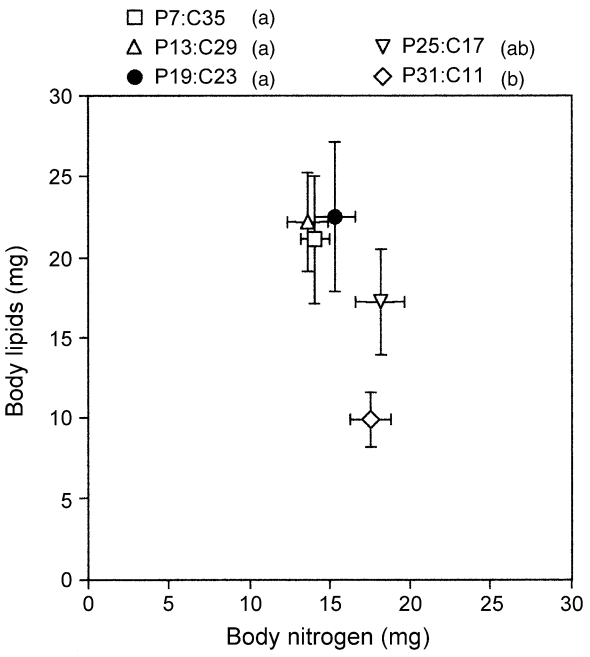

FIG. 7. Bivariate means (± 1 SE) for body nitrogen (N) and lipid accumulation during the fifth stadium for locusts from the second experiment. Different letters for the five food types in the figure key indicate significant differences.

ditional factor favoring carbon-based defensive compounds (or at least those that act in the manner demonstrated here by tannic acid) in resource-poor environments is that their anti-herbivore effects are strongest when food protein content is low. The results also support the suggestion that, by providing a heterogeneous spatial distribution of PSMs and nutrients, plants can redistribute feeding within the canopy and offer a form of protection even though the total amount of feeding is unchanged (Edwards et al. 1992, Hoy et al. 1998). We are currently analyzing the interactions between nutrients and nitrogen-based PSMs and comparing them with the present data on a carbon-based compound.

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LITERATURE CITED

Atsatt, P. R., and D. J. O'Dowd. 1976. Plant defense guilds. Science **193**:24–29.

Bazzaz, F. A., N. R. Chiariell, P. D. Coley, and L. F. Pitelka. 1987. Allocating resources to reproduction and defense. Bioscience **37**:58–67.

Behmer, S. T., and D. O. Elias. 1999. The nutritional significance of sterol metabolic constraints in the generalist grasshopper *Schistocerca americana*. Journal of Insect Physiology **45**:339–348.

Behmer, S. T., D. O. Elias, and E. A. Bernays. 1999. Post-ingestive feedbacks and associative learning regulate the intake of unsuitable sterols in a generalist grasshopper. Journal of Experimental Biology **202**:739–748.

Behmer, S. T., D. Raubenheimer, and S. J. Simpson. 2001. Frequency-dependent food selection in locusts: a geometric analysis of the role of nutrient balancing. Animal Behaviour **61**:995–1005.

Berenbaum, M. R. 1996. Turnabout is fair play: secondary roles for primary compounds. Journal of Chemical Ecology **21**:925–940.

Bernays, E. A. 1978. Tannins: an alternative viewpoint. Entomologia Experimentalis et Applicata **24**:244–253.

Bernays, E. A. 1991. Relationship between deterrence and toxicity of plant secondary compounds to the polyphagous grasshopper *Schistocerca americana*. Journal of Chemical Ecology **17**:2519–2526.

Bernays, E. A., and R. Barbehenn. 1987. Nutritional ecology of grass foliage-chewing insects. Pages 147–175 *in* F. Slansky and J. G. Rodriguez, editors. Nutritional ecology of insects, mites, spiders and related invertebrates. John Wiley, New York, New York, USA.

Bernays, E. A., K. L. Bright, N. Gonzalez, and J. Angel. 1994. Dietary mixing in a generalist herbivore: tests of two hypotheses. Ecology **75**:1997–2006.

Bernays, E. A., D. J. Chamberlain, and P. McCarthy. 1980. The differential effects of ingested tannic acid on different species of Acridoidea. Entomologia Experimentalis et Applicata **28**:158–166.

Bernays, E. A., and R. F. Chapman. 1987. Evolution of deterrent responses in plant-feeding insects. Pages 159–173 *in* R. F. Chapman, E. A. Bernays, and J. G. Stoffolano, editors. Perspectives in chemoreception and behavior. Springer-Verlag, New York, New York, USA.

Bernays, E. A., G. Cooper-Driver, and M. Bilgener. 1989. Herbivores and plant tannins. Advances in Ecological Research **19**:263–302.

Chambers, P. G., S. J. Simpson, and D. Raubenheimer. 1995. Behavioural mechanisms of nutrient balancing in *Locusta migratoria* nymphs. Animal Behaviour **50**:1513–1523.

Coley, P. D., J. P. Bryant, and F. S. Chapin, III. 1985. Resource availability and plant antiherbivore defense. Science **230**:895–899.

Dadd, R. H. 1960. The nutritional requirements of locusts. II. Utilization of sterols. Journal of Insect Physiology **5**: 161–168.

Dadd, R. H. 1961. The nutritional requirements of locusts. IV. Requirements for vitamins of the B complex. Journal of Insect Physiology **6**:1–12.

Dearing, M. D., A. M. Mangione, and W. H. Karasov. 2000. Diet breadth of mammalian herbivores: nutrient versus detoxification constraints. Oecologia **123**:397–405.

Edwards, P. J., S. D. Wrateen, and E. A. Parker. 1992. The ecological significance of rapid wound-induced changes in plants—insect grazing and plant competition. Oecologia **91**:266–272.

Freeland, W. J., and D. H. Janzen. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. American Naturalist **108**:269–289.

Hägele, B. F., and M. Rowell-Rahier. 1999. Dietary mixing in three generalist herbivores: nutrient complementation or toxin dilution? Oecologia **119**:521–533.

Hill, L., and G. J. Goldsworthy. 1968. Growth, feeding activity, and the utilization of reserves in larvae of *Locusta*. Journal of Insect Physiology **14**:1085–1098.

Hoy, C. W., G. P. Head, and F. R. Hall. 1998. Spatial heterogeneity and insect adaptation to toxins. Annual Review of Entomology **43**:571–594.

Mole, S., and P. G. Waterman. 1987. Tannins as antifeedants to mammalian herbivores—still an open question? Pages 572–587 in G. R. Waller, editor. Allelochemicals: role in

agriculture and forestry. ACS Symposium Series 330. American Chemical Society, Washington, D.C., USA.
Pennings, S. C., M. T. Nadeau, and V. J. Paul. 1993. Selectivity and growth of the generalist herbivore *Dolabella auricularia* feeding upon complementary resources. Ecology **74**:879–890.
Pulliam, R. H. 1975. Diet optimization with nutrient constraints. American Naturalist **109**:765–768.
Rapport, D. J. 1980. Optimal foraging for complementary resources. American Naturalist **116**:324–346.
Raubenheimer, D. 1992. Tannic acid, protein, and digestible carbohydrate: dietary imbalance and nutritional compensation in the African migratory locust. Ecology **73**:1012–1027.
Raubenheimer, D., and S. J. Simpson. 1990. The effects of simultaneous variation in protein, digestible carbohydrate and tannic acid on the feeding behavior of larval *Locusta migratoria* and *Schistocerca gregaria*—I: short-term studies. Physiological Entomology **15**:219–233.
Raubenheimer, D., and S. J. Simpson. 1993. The geometry of compensatory feeding in the locust. Animal Behaviour **45**:953–964.
Raubenheimer, D., and S. J. Simpson. 1994. The analysis of nutrient budgets. Functional Ecology **8**:783–791.
Raubenheimer, D., and S. J. Simpson. 1997. Integrative models of nutrient balancing: application to insects and vertebrates. Nutrition Research Reviews **10**:151–179.
Raubenheimer, D., and S. J. Simpson. 1999. Integrating nutrition: a geometrical approach. Entomologia Experimentalis et Applicata **91**:67–82.
Reiners, W. A. 1986. Complementary models for ecosystems. American Naturalist **127**:59–73.
SAS Institute. 1989. SAS Software Release 6.12. SAS Institute, Cary, North Carolina, USA.
Scheiner, S. M. 1993. MANOVA: multiple response variables and multispecies interactions. Pages 94–112 *in* M Scheiner and J. Gurevitch, editors. Design and analysis of ecological experiments. Chapman and Hall, New York, New York, USA.
Schultz, J. C., M. D. Hunter, and H. M. Appel. 1992. Antimicrobial activity of polyphenols mediates plant–herbivore interactions. Pages 621–637 *in* R. W. Hemingway, editor. Plant polyphenols: biogenesis, chemical properties, and significance. Plenum Press, New York, New York, USA.
Simpson, S. J. 1982. Changes in the efficiency of utilisation of food throughout the fifth instar nymphs of *Locusta migratoria*. Entomologia Experimentalis et Applicata **31**:265–275.
Simpson, S. J. 1983. Changes during the fifth instar of *Locusta migratoria* in the rate of crop emptying and their relationship to feeding and food utilisation. Entomologia Experimentalis et Applicata **33**:235–243.
Simpson, S. J., and J. D. Abisgold. 1985. Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. Physiological Entomology **10**:443–452.
Simpson, S. J., and D. Raubenheimer. 1993. A multi-level analysis of feeding behaviour: the geometry of nutritional decisions. Philosophical Transactions of the Royal Society of London **342B**:381–402.
Simpson, S. J., and D. Raubenheimer. 2000. The hungry locust. Advances in the Study of Behavior **29**:1–44.
Simpson, S. J., and D. Raubenheimer. 2001. The geometric analysis of nutrient–allelochemical interactions: a case study using locusts. Ecology **81**:422–439.
Simpson, S. J., D. Raubenheimer, S. T. Behmer, A. Whitworth, and G. A. Wright. 2002. A comparison of nutritional regulation in solitarious and gregarious phase nymphs of the desert locust, *Schistocerca gregaria*. Journal of Experimental Biology **205**:121–129.
Thompson, S. N. 1997. Glucogenesis in an insect, *Manduca sexta* L., estimated from the ^{13}C isotopomer distribution in trehalose synthesized from [1,3-$^{13}C_2$]glycerol. Biochimica et Biophysica Acta **1336**:110–116.
Thompson, S. N. 1998. Long-term regulation of gluconeogenesis by dietary carbohydrate and relevance to blood sugar level the insect *Manduca sexta* L. International Journal of Biochemistry and Cell Biology **30**:987–999.
Thompson, S. N. 1999. Blood sugar formation from dietary carbohydrate is facilitated by the pentose phosphate pathway in an insect *Manduca sexta* Linnaeus. Biochimica et Biophysica Acta **1472**:565–575.
Tilman, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, New Jersey, USA.
Trumper, S., and S. J. Simpson. 1993. Regulation of salt intake by nymphs of *Locusta migratoria*. Journal of Insect Physiology **39**:857–864.
Westoby, M. 1978. What are the biological bases of varied diets? American Naturalist **112**:627–631.
Wright, G. A., D. Raubenheimer, S. Hill, and S. J. Simpson. 2000. The feeding behaviour of *Schistocerca gregaria*, the desert locust, on two starch mutants of *Arabidopsis thaliana*. Chemoecology **10**:59–76.
Wright, G. A., S. J. Simpson, D. Raubenheimer, and P. C. Stevenson. 2002. An analysis of herbivore feeding decisions in the field: the relationship between feeding behaviour and host-plant chemistry in the weevil, *Exopthalmus jekelianus* (White), feeding *ad libitum* on Central American mahogany, *Cedrela odorata* L. Oikos, *in press*.
Zannotto, F. P., S. J. Simpson, and D. Raubenheimer. 1993. The regulation of growth by locusts through post-ingestive compensation for variation in the levels of dietary protein and carbohydrate. Physiological Entomology **18**:425–434.