

The effects of simultaneous variation in protein, digestible carbohydrate and tannic acid on the feeding behaviour of larval *Locusta migratoria* (L.) and *Schistocerca gregaria* (Forsk.). I. Short-term studies

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ABSTRACT. The influence of simultaneously varying the levels in artificial diets of protein, digestible carbohydrate (14% or 28%) and tannic acid (absent or 10%) on the feeding behaviour of the oligophagous *Locusta migratoria* (L.) and the polyphagous *Schistocerca gregaria* (Forsk.) (Acrididae) was investigated. Total consumption and detailed feeding behaviour were recorded over a 12 h period in choice and no-choice experiments. In addition, amounts eaten by *Schistocerca* of the 14% protein, 14% carbohydrate diet with and without tannic acid were measured at regular intervals throughout the fifth stadium, and insect growth over this period was recorded. There were no interactive effects of nutrient levels and tannic acid, despite the fact that both species compensated for dilution of dietary protein by increasing consumption. Only male *Locusta* compensated for dilution of dietary carbohydrates, and this compensation was much less marked than for protein. Tannic acid did influence feeding as a main effect, however. It caused an increase in amounts eaten by *Schistocerca* in both choice and no-choice experiments. This increased consumption was due to an increase in the number of meals taken. A shorter latency period before and a longer duration of the first meal by naive insects suggested a phagostimulatory rather than a post-ingestive effect of tannic acid. The stimulatory effect was only apparent for the first 24 h of continuous exposure, but this temporary enhancement none the less resulted in the insects being heavier at adult ecdysis. Stadium duration was also somewhat reduced. In a no-choice situation, no effect of tannic acid on the feeding behaviour of *Locusta* was observed. When given a choice, however, this species took significantly more meals on the tannic acid-free diet, these being of similar average size to meals taken on the tannic acid diet. Significantly more insects took their first meals on the tannic acid-free diet in the choice test, indicating a deterrent effect of tannic acid in *Locusta*.

Key words. *Locusta migratoria*, *Schistocerca gregaria*, nutrient-allelochemical interactions, tannic acid, protein, digestible carbohydrate, dietary selection behaviour, feeding patterns.

Introduction

There have been numerous studies into the presumed defensive role of plant-produced tannins against insect herbivores, yet any general conclusions have so far eluded us (Bernays, 1981). This is partially due to the structural diversity and complexity of these compounds (Zucker, 1983), and to the inherent difficulties of studying the effects of plant-produced chemicals on herbivorous insects.

One such difficulty arises from the simultaneous needs of the experimenter to minimize uncontrolled variability due to the chemical complexity of plant tissue (e.g. see Risch, 1985), while avoiding the dangers of over-simplification, by taking into account possible interactive effects between chemicals. Such interactions include the intake of a particular nutrient being limited by an insect's ability to cope with other (nutrient or non-nutrient) chemicals occurring in the food. For instance, many phytophagous insects are known to compensate for dilution of dietary nutrients by increased consumption (Simpson & Abisgold, 1985; Simpson & Simpson, 1989). A consequence of eating more of a food to ingest sufficient of a limiting nutrient is that more of any potentially deleterious compounds present are also ingested. This should manifest itself as an interaction term in any analysis of an experiment in which levels of dietary nutrients are reduced in the presence of a deleterious allelochemical.

A second difficulty is the proper interpretation of the insect's response to a given diet. Thus an insect might eat more of one diet than another either because the former is more phagostimulatory (or less deterrent), or because it is nutritionally *inferior* and to provide the required level of nutrition more of it needs to be consumed (Simpson & Simpson, 1989). Similarly, diminished performance resulting from the ingestion of an allelochemical could be due to deterrence, toxicity, or both (Blau *et al.*, 1978; Bernays & Chapman, 1986; Cottee *et al.*, 1988). Deterrence could be due to an innate response of an insect to the sensory properties of food containing allelochemicals or due to post-ingestive effects of eating the diet (Lee & Bernays, 1988).

The study of insect nutrition has come some way in recent years towards approaching these problems of the chemical complexity of plants, possible interactive effects between nutrients

and the interpretation of the insects' response to diets varying in nutrient composition. This progress has been in part due to the introduction of techniques combining precise manipulation of chemicals in artificial diets with detailed observations of the patterns of feeding (Simpson & Abisgold, 1985; Simpson *et al.*, 1988, 1989; Simpson, 1989). While a few studies have recently investigated possible interactive effects on insect herbivores between nutrients and allelochemicals (Lincoln *et al.*, 1982; Hare, 1987; Broadway & Duffey, 1988; Johnson & Bentley, 1988; Slansky & Wheeler, 1989), none have combined this with detailed behavioural studies to elucidate the mechanisms underlying the insects' response. One purpose of this paper is therefore to introduce to the study of plant/insect interactions the technique of detailed behavioural analysis combined with the simultaneous variation of nutrients and allelochemicals in chemically defined diets.

This is the first in a series of papers in which we report on the detailed behaviour of two acridids, *Locusta migratoria* (L.) and *Schistocerca gregaria* (Forsk.) in response to simultaneous variation in the levels of protein (14% or 28%), digestible carbohydrate (14% or 28%) and the hydrolysable tannin, tannic acid (10% or absent). *Locusta migratoria* is oligophagous, feeding only on grasses, in which hydrolysable tannins do not occur, while the polyphagous *S. gregaria* feeds on a wide range of dicotyledonous plants known to contain these compounds. This leads to the expectation that the two species should respond differently to tannins, and previous studies have shown that *S. gregaria* is indeed tolerant of these compounds while *L. migratoria* suffers reduced growth and increased mortality due to their presence in food. (Bernays & Chamberlain, 1980; Bernays *et al.*, 1980). This background, together with the considerable amount known about acridid feeding biology (Bernays & Chapman, 1978; Simpson & Bernays, 1983; Bernays, 1985), made the comparison of *L. migratoria* and *S. gregaria* a convenient model system for the kind of study reported here.

Materials and Methods

Experimental insects. *Locusta migratoria* and *Schistocerca gregaria* were reared under similar conditions (Hunter-Jones, 1961) at the Depart-

ment of Zoology, Oxford, using seedling wheat and wheat bran as a food source. Insects were collected from the stock cages at the beginning of the fifth stadium (termed Day 0) so that their age fell within a 6 h range and their weights fell within 1 standard deviation of the mean of a large sample previously weighed. For *Locusta* this weight range was 440–550 mg (males) and 520–650 mg (females), and for *Schistocerca* 529–634 mg and 602–737 mg for males and females respectively. The insects were then placed individually in clear plastic containers and provided with ample seedling wheat and wheat bran, which were replaced daily until the start of Day 3. The plastic containers used in two-choice experiments (28×16×9 cm) were larger than those used in no-choice experiments (17×12×6 cm), to accommodate a second Petri dish containing diet. The containers had in them a strip of expanded aluminium to provide a perch, and were kept in a constant environment room at 30°C under a LD 12:12 h photoregime. Even illumination was provided during light phases by overhead fluorescent strip lights.

Artificial diets. The nutrient composition of the four dry, granular, cellulose-based artificial diets used was as in Simpson & Abisgold (1985): PC, 28% protein, 28% digestible carbohydrate; Pc, 28% protein, 14% digestible carbohydrate; pC, 14% protein, 28% digestible carbohydrate, and pc which had 14% of both nutrients.

The commercial brand of tannic acid used (Sigma Ltd) may contain up to 8% impurities, including free glucose and gallic acid. However, feeding experiments using pure tannic acid (BDH Ltd) later confirmed that it is the tannic acid and not the impurities which accounts for results reported here. The tannic acid powder (10% by dry weight) was added to the test diets only after they had dried to avoid complexing with protein and cellulose (Mole & Waterman, 1987) before being ingested by the insects. This meant that the tannic acid would form a surface coating over the lumps of diet, resulting in a variable ratio of tannic acid to diet depending on the size of individual lumps. The dry diet was therefore passed through wire sieves to produce particles of a standard size (425–850 µm) before adding the tannic acid. At the 10% level, however, not all the tannic acid adhered to the lumps of diet, with some settling at the bottom of the feeding dishes during the course of the experiments. Weighing of the settled tannic acid

suggested that the actual concentration in the diet was no less than 7.5%, however.

Locusts compensate for low protein levels in their food by eating more of diets containing 14% than 28% protein (Simpson & Abisgold, 1985; Abisgold & Simpson, 1987, 1988). It was therefore necessary to ensure that any observed effects resulting from the addition of tannic acid to the test diets were due to the chemical properties of tannic acid and not simply to the dilution of protein. Dilution of the control diet with additional cellulose was considered inappropriate because volumetric feedback from the gut plays an important role in determining meal size in locusts (Bernays & Chapman, 1973; Simpson, 1983), and the bulky cellulose fibres would contribute a disproportionate effect compared to that due to the finely powdered tannic acid. The slight uncertainty regarding the exact concentration of tannic acid in the food ingested by the insects further justified this decision. To control for the dilution of protein due to the addition of tannic acid to the test diets, the data for amounts eaten over 12 h, and individual 24 h periods in the longer-term experiment on *Schistocerca* (Fig. 3), were therefore corrected for the maximum possible dilution effect (assuming 10% tannic acid and 100% compensation by the insects for the dilution of dietary protein). Whether or not this correction was made had no effect on statistical significance in any of the tests reported. Because of this, the same correction was not performed on the data for cumulative amounts eaten in the longer term experiment on *Schistocerca* (Fig. 3). In such a case, the error introduced by the conservative nature of this step would compound with each successive addition of amounts eaten on subsequent days.

Amounts eaten. Shortly before the start of the light phase of Day 3, the insects were weighed and placed in clean plastic containers along with a 5.5 cm Petri dish (no choice experiments) containing artificial diet (approximately 1.5 g). For two-choice experiments, each Petri dish was glued to the centre of a larger Petri dish (9 cm), thus forming a central dish surrounded by a moat to prevent the spillage and mixing of the test and control diets. Before each experiment the dishes of diet had been dried in an oven at 45°C until they reached a constant weight (for 24 h) and weighed to within 1 mg, then placed open in the constant environment chamber for 12 h to equilibrate to ambient relative humidity;

the equilibrated diets contained a maximum of 4% water. After the experiment the dishes were again dried and re-weighed to obtain a measure of the amounts eaten.

When the insects were weighed at the start of the experiments, they had an unknown amount of residual wheat and wheat bran in the digestive tract. This had cleared the gut by the end of the experiment and wheat and wheat bran faeces were easily distinguishable from artificial diet ones. To obtain an accurate measure of insect weight, the wheat and wheat bran faeces were therefore dried and weighed after the experiment, and the equivalent fresh weight (as obtained from a previously prepared regression equation of frass dry weight versus fresh weight) was subtracted from the weight of each insect.

Statistical analyses were performed using ANCOVA, with sex and tannic acid (absent or 10%) and, where appropriate, protein and carbohydrate (14% or 28%) as main effects and insect weight as a covariate. For two-choice experiments, the term [amount eaten (control diet - tannic acid diet)] was calculated for each insect and the mean for all insects was tested using a *t*-test for significant difference from 0. For the longer-term experiment (see Fig. 3), ANCOVA with diet as a main effect and insect weight at the start of the experiment as a covariate was used to test for differences in cumulative amounts eaten of test and control diets. To test amounts eaten over each individual period, the same design was used but with insect weight at the preceding measurement as a covariate.

Behavioural observations. Insects were observed for 12 h and the behaviour of individuals recorded once a minute. The cages were screened from each other using white cardboard strips. Three categories of behaviour were recognized: feeding (and in a two-choice situation, it was noted on which diet), locomotion and quiescence. In subsequent analysis of these data, intermeal intervals were distinguished from intrameal pauses by the use of bout criteria obtained using log-survivorship functions (Simpson, 1982); the pooled data for each of the four experimental groups (males and females feeding on control and test diets) were analysed separately. The bout criteria ranged between 2 and 3 min, with a majority being 2 min. In this way it was possible to obtain the following measures of individual insect feeding behaviour:

latency period before the first meal; duration of the first meal (from the start of feeding until the first pause of longer than 2 min, i.e. including intrameal pauses (see Simpson, 1989)); number of meals; average meal duration; average intermeal interval; average meal size and mean ingestion rate (amount eaten/total time spent feeding).

The behavioural parameters, together with total amounts eaten, were analysed statistically using a 2×2 factorial ANOVA with sex and diet (0% or 10% tannic acid) as main effects. In addition, trends over time may be represented for use in the same ANOVA design as coefficients from the best fitting quadratic curve for the plot of parameter value versus time since the beginning of the first meal (Simpson & Abisgold, 1985). Best fit equations were obtained individually for each insect using the least squares method. For two-choice experiments, the same statistical test described above for total amounts eaten was used to test for significant differences in behavioural parameters.

Experiments. Interactions between tannic acid and nutrients. To investigate possible interactive effects between protein, digestible carbohydrate and tannic acid on total amounts eaten during a 12 h period, eighty male and eighty female *Locusta* and sixty male and sixty female *Schistocerca* were given one of eight diet mixtures: PC, Pc, pC, or pc each with or without 10% tannic acid. For logistical reasons, the experiment on *Locusta* was conducted in four stages of forty insects each, and *Schistocerca* in two stages, one of forty and one of eighty insects. Equal numbers of each sex were used in each stage, and treatments were as far as possible distributed equally between the stages. Using ANCOVA it was shown that 'experimental replicate' (with four levels for *Locusta*, and two for *Schistocerca*) made no significant contribution, either as a main effect or in two-way interaction terms, towards explaining the variance in the dependent variable, amount eaten. Males and females were positioned alternately, and equal numbers of each diet were allocated randomly within each sex.

Behavioural observations, no-choice. In order to investigate in detail the effect of tannic acid on the feeding behaviour of insects, thirty-two insects (sixteen males and sixteen females) of both species were offered pc diet with or without 10% tannic acid. The species were tested separately in groups of thirty-two insects, and their detailed

behaviour was recorded and total amounts eaten measured over a 12 h period. Males and females were positioned alternately, and the control and test diets were allocated alternately within each sex.

Two-choice experiments. In this series of experiments, twenty-two insects (eleven males and eleven females) of each species were offered a choice of pc diet with and without 10% tannic acid, and the total amounts of each diet eaten over a 12 h period were measured. This experiment was then repeated for *Locusta*, while recording patterns of feeding behaviour.

Longer-term experiment. To investigate whether the effects of tannic acid on feeding persisted beyond 12 h, twenty *Schistocerca* females were given pc diet with or without 10% tannic acid at the start of Day 3 and their progress was monitored through the remainder of the fifth stadium. Amounts eaten were measured and fresh diet was provided at the end of that light phase (12 h), then again at the end of the following dark phase, and at 24 h intervals thereafter until all the insects had moulted. The insects were weighed at 24 h intervals, but no account could be taken of residual food in the digestive tract. However, an accurate measure of total growth through the experimental period was obtained by weighing the insects while their guts were still empty after they had moulted to the adult stage. The duration of the stadium for each insect was recorded to the nearest 12 h.

In short-term (12 h) experiments, insects were not provided with free water (see Simpson & Abisgold, 1985). However, for periods of longer than this water is required in addition to the dry diets, and this was provided in rectangular plastic containers (7.8×4.7×2.0 cm) which had two 1.5 cm holes drilled into their lids. As a control to verify that the absence of water really was not important in short-term experiments, insects were kept without as well as with water for 48 h. The absence of water caused no difference in amounts of pc diet (with and without tannic acid) eaten over this period.

Results

Interactions between tannic acid and nutrients

Table 1 shows the results of the experiment in which protein, carbohydrate and tannic acid

TABLE 1. Summary of *F*-ratios from the ANCOVA for amounts eaten by fifth stadium locust nymphs of diets with two levels of protein, digestible carbohydrate (14% or 28%) and tannic acid (absent or 10% by dry weight). **P*<0.05; ****P*<0.001.

Source	<i>F</i> -values	
	<i>Locusta</i>	<i>Schistocerca</i>
Covariate		
Insect weight	4.1*	4.6*
Main effects		
Sex (S)	0.2	0.1
Tannic acid (T)	1.6	42.8***
Protein (P)	31.2***	20.3***
Carbohydrate (C)	5.4*	0.1
Two-way interactions		
S×T	1.1	<0.1
S×P	0.2	0.8
S×C	4.0*	0.2
T×P	1.3	<0.1
T×C	1.3	<0.1
P×C	1.3	1.8

levels were varied simultaneously. Tannic acid as a main effect had no measurable influence on total amount eaten by *Locusta*, but caused a significant increase in total consumption by *Schistocerca* (Fig. 1). Significantly more of the low protein (p) than the high protein (P) diets were eaten by both species, thus confirming the results of Simpson & Abisgold (1985) for *L. migratoria*, and demonstrating that *S. gregaria*, too, regulates food intake to compensate for dietary protein levels. Total amounts eaten (mean ±SE) by *Locusta* were 78.6±2.6 mg of p-diets and 62.2±2.4 mg of the P-diets, while *Schistocerca* ate 110.5±5.3 mg and 84.5±3.9 mg of the p and P diets respectively. There were no main effects of dietary carbohydrate for *Schistocerca* or female *Locusta* but, contrary to the findings of Simpson & Abisgold (1985), there were for *Locusta* males. The males ate more of the low (69±2.9 mg) than the high (55±3.2 mg) carbohydrate diets; the equivalent figures for females were 78.9±5.3 mg and 78.4±3.3 mg respectively. The fact that this effect due to carbohydrate is only seen in one case (in male *Locusta* in the present study) suggests that it is of marginal importance relative to compensatory feeding for protein. There were no significant interaction terms for either species between tannic

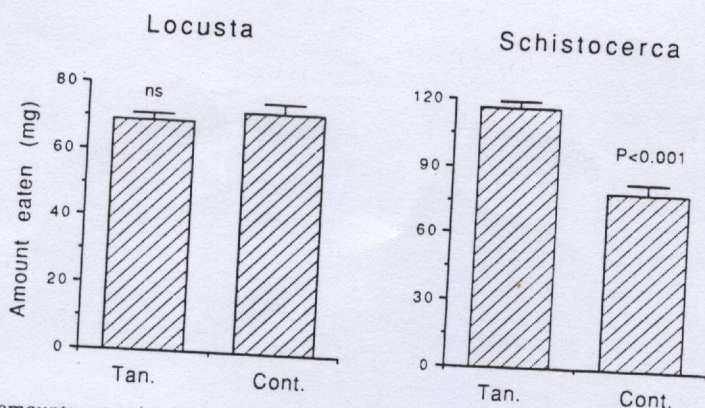


FIG. 1. Total amounts eaten (means \pm SE) over 12 h by fifth stadium locust nymphs of PC, Pc, pC and pc diets (see text) with (Tan.) or without (Cont.) 10% tannic acid.

acid and protein or carbohydrate levels or between tannic acid and sex. Clearly, then, increased consumption of tannic acid due to compensation for low dietary nutrients did not result in a difference in the effect of the allelochemical.

Behavioural observations, no choice

The presence of tannic acid in pc diet caused a significant increase in total amounts eaten over

12 h by *Schistocerca* (Table 2, Fig. 2a), confirming the results obtained in the previous experiment. This increase was primarily due to the insects taking significantly more meals (Fig. 2b), by shortening the average intermeal interval (Fig. 2c). There was no significant difference in the size of meals taken on the two diets (Fig. 2d). The presence of tannic acid also caused a significant reduction in the latency period before the first meal (Fig. 2e) and an increase in the duration of the first meal (Fig. 2f). Since the

TABLE 2. Summary of *F*-ratios from the ANCOVA for various behavioural parameters for fifth stadium locusts given pc diet (see text) with or without 10% tannic acid. **P*<0.05; ***P*<0.01; ****P*<0.001.

Source	<i>F</i> -values							
	Amount eaten	No. of meals	Average intermeal interval	Average meal size	Average meal duration	Ingestion rate (amount eaten/time feeding)	Time to first meal	Duration of first meal
<i>Locusta</i>								
Covariate								
Insect weight	0.3	0.6	1.7	1.8	6.1*	11.0**	2.2	0.2
Main effects								
Sex	0.3	0.4	1.6	3.9	7.8**	8.1**	1.0	1.5
Tannic acid	0.7	1.2	0.6	1.2	3.0	2.1	0.1	0.8
Two-way interaction								
Sex \times Tan	0.6	0.1	<0.1	<0.1	<0.1	0.1	0.1	0.3
<i>Schistocerca</i>								
Covariate								
Insect weight	0.4	0.1	<0.1	<0.1	<0.1	<0.1	0.3	<0.1
Main effects								
Sex	0.1	0.8	0.1	1.3	1.1	2.2	0.3	0.1
Tannic acid	12.6**	22.8***	7.1*	2.5	2.8	<0.1	5.9*	4.6*
Two-way interaction								
Sex \times Tan	0.8	1.1	0.6	2.0	1.5	2.4	1.4	3.9

insects at this stage had never before contacted either artificial diet, this suggests that tannic acid causes increased consumption through a phago-stimulatory effect, rather than through some post-ingestive feed-back mechanism.

Locusta showed no significant change due to tannic acid in any of the behavioural parameters measured (Table 2). There was, however, a significant sex effect on average meal duration, with females taking on average longer meals than males (5.1 and 2.4 min respectively).

Longer meals did not result in increased meal size, however, because the average consumption rate of males (2.0 mg/min) was significantly higher than that of females (1.1 mg/min; these figures, as well as those for average meal duration above, represent means adjusted for the significant effect of the covariate insect weight).

There was no significant effect due to tannic acid on the quadratic or linear coefficients for meal duration or intermeal interval versus

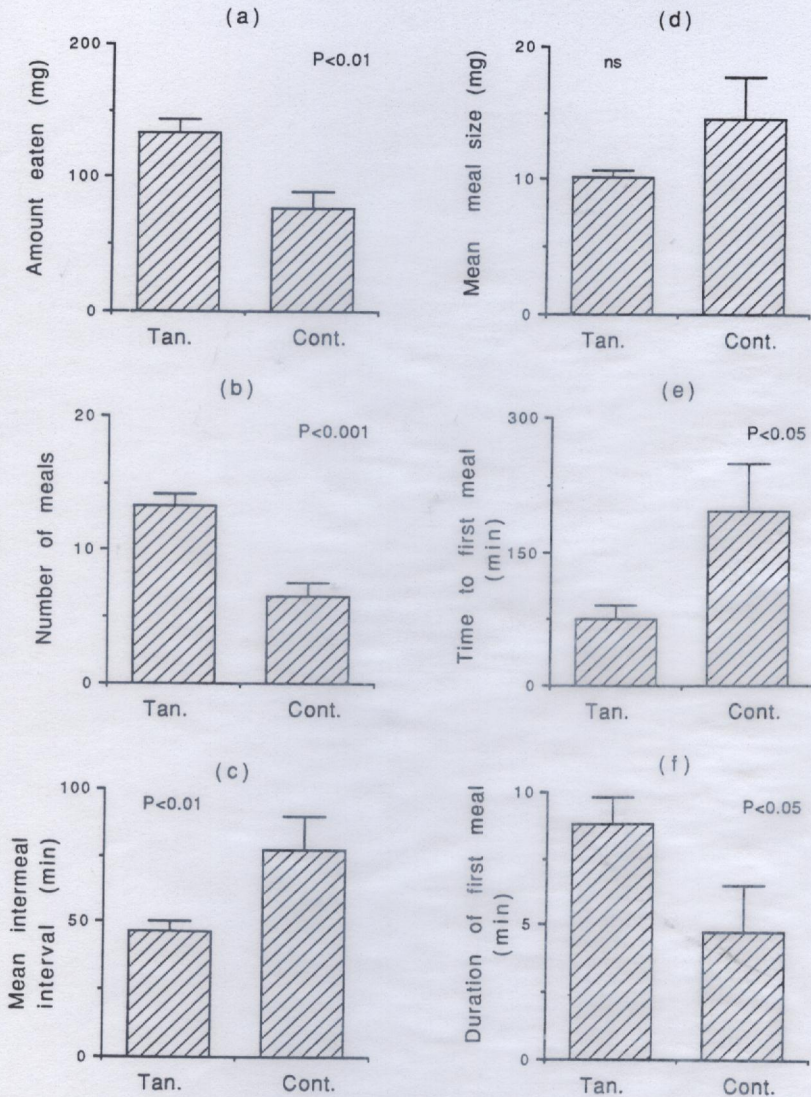


FIG. 2. Values of various behavioural parameters (mean \pm SE) for fifth stadium *Schistocerca* nymphs fed pc diet (see text) with (Tan.) or without (Cont.) 10% tannic acid.

TABLE 3. Means (\pm SE) of the choice term (control diet - tannic acid diet) of various behavioural parameters of fifth stadium locusts given pc diet (see text) with and without 10% tannic acid. Results from two experiments with *Locusta* and one with *Schistocerca* are presented. *P*-values are for a two-tailed *t*-test of significance between means and 0, the expected value if there was no discrimination between the diets.

Behavioural parameter	[(Control diet) - (tannic acid diet)]	<i>t</i>	Sign.
<i>Schistocerca</i>			
Amount eaten	-51.6 \pm 11.1	4.6	<i>P</i> <0.001
<i>Locusta</i>			
Amount eaten (expt 1)	50.4 \pm 6.4	7.9	<i>P</i> <0.001
Amount eaten (expt 2)	34.5 \pm 8.4	4.1	<i>P</i> <0.001
No. of meals (expt 2)	7.8 \pm 1.6	5.0	<i>P</i> <0.001
Average meal size (expt 2)	-0.6 \pm 1.3	0.4	ns

time since the first meal, indicating that the trends over time with respect to these parameters were the same on both diets (Simpson & Abisgold, 1985).

Two-choice experiments

When given a choice between control and tannic acid diets, *Schistocerca* ate more of the tannic acid diet (Table 3), confirming, as suggested above, that this compound causes increased consumption through direct peripheral sensory stimulation rather than through post-ingestive

feedback. *Locusta*, on the other hand, ate significantly more of the non-tannic acid diet in both the initial two-choice experiment and the repeat in which behavioural data were recorded (Table 3, experiments 1 and 2 respectively). The behavioural data in Table 3 show that this increased consumption was due to the insects taking meals more frequently on the control diet, which were of similar average size to those taken on the tannic acid diet. Significantly more of the insects took their first meal on the control diet (*P*<0.001; probability calculated using the binomial distribution), indicating a pre-ingestive deterrent effect due to tannic acid.

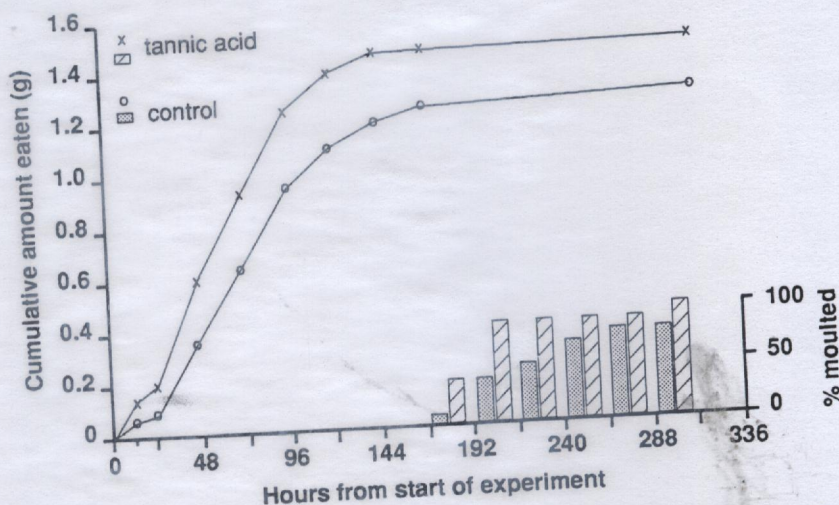


FIG. 3. Cumulative amounts eaten and the percentage moulted of fifth stadium *Schistocerca* nymphs fed pc diet (see text) with or without 10% tannic acid. Differences in cumulative amounts eaten of the test and control diets are significant at all points (using ANCOVA with two levels for diet and insect weight as a covariate). Amounts eaten of the two diets over individual periods differed significantly for periods 0-12 h and 12-24 h (see text for levels of significance).

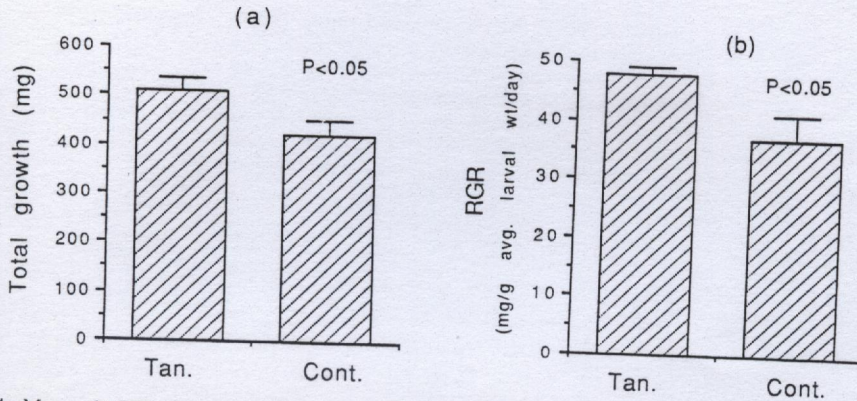


FIG. 4. Means (\pm SE) of total growth (a) and relative growth rate (total growth per day/average larval weight) (b) from the beginning of the third day of the fifth stadium to the final moult of female *Schistocerca* nymphs fed pc diet (see text) with (Tan.) or without (Cont.) 10% tannic acid.

Longer-term experiment

Fig. 3 shows the cumulative amounts eaten of control and tannic acid diets by *Schistocerca* females from the third day of the fifth stadium; the stadium duration of both groups is also represented. Over the first 12 h period, significantly more ($P < 0.01$) of the tannic acid diet was eaten, as could be expected from results of the previous experiments. This effect persisted over the second 12 h period (the first dark phase in the experiment) ($P < 0.01$), but thereafter there were no significant differences in amounts eaten over individual 24 h periods. However, the cumulative amounts eaten by the tannic acid-insects remained significantly greater throughout the rest of the experiment, indicating that the difference in amounts eaten due to enhanced consumption over the first 24 h of exposure to tannic acid diet was not made up by the control insects over the remainder of the fifth stadium. The probability levels for the successive measurements were as follows: 48 h, 72 h, 96 h and 120 h ($P < 0.01$); 144 h, 168 h, and the total amounts eaten after all the insects had moulted (hour 312) ($P < 0.05$).

Total growth over the course of the experiment (from the beginning of Day 3) and relative growth rate were both significantly greater on tannic acid diet ($P < 0.05$; Fig. 4a and b). When the data for total growth were analysed in an ANCOVA using total amount eaten as a covariate, the covariate was significant ($P < 0.001$) and the effect of diet on total growth fell away ($P = 0.3$). This suggests that the effect on growth of tannic acid was due to increased amounts eaten rather than to increased

nutritional value of the food. There were also indications of shortened stadium duration due to tannic acid diet (Fig. 3), but this was not statistically significant. A control insect which had not moulted by 14 days after the rest was considered abnormal and excluded from the analysis.

Discussion

The concentrations of protein and tannic acid in the synthetic diets used in this study were chosen as realistic levels likely to be encountered in plants, and the stage during the stadium at which the insects were tested is a period of high feeding activity (Simpson, 1982). Digestible carbohydrate levels in the diets were rather higher than in real plants. While insects of both species increased consumption to compensate for low levels of protein, and male *Locusta* for carbohydrate, there were no significant interaction terms between tannic acid and nutrients. This indicates that, over a 12 h period, the presence of tannic acid in the diet does not affect the ability of *Locusta* or *Schistocerca* nymphs to compensate for reduced levels of nutrients by increasing consumption. From a functional viewpoint, these results are not altogether surprising. The nutrient requirements of herbivorous insects and the quality and quantity of available food are continuously changing and compensatory feeding is an important mechanism whereby the insects maintain a nutritionally balanced diet (Simpson & Simpson, 1989). The ability to adjust food intake according to nutrient levels

despite the presence in the food of plant allelochemicals would therefore be advantageous. However, depending on the toxicity of the allelochemical, a point may be reached at which the nutritional advantage of increased intake of the food is offset by the deleterious effects of the allelochemical. In an insect adapted to cope with this relationship between nutrients and toxic chemicals in plants, it is at this point that compensatory feeding should be affected. It is known that in the long-term tannic acid has toxic effects on *Locusta* (Bernays, 1978), and in future research we will therefore be examining the effects on compensatory feeding by this species of prolonged exposure to tannic acid.

Previous studies into the effects on herbivorous insects of combined changes in dietary nutrients and allelochemicals have produced varied results. Hare (1987) raised larvae of the potato beetle (*Leptinotarsa decemlineata*) on artificial diets containing measured quantities of protein and one of five glycoalkaloids. For four of the alkaloids there was no significant interaction term with protein in larval weight gain, but for the most toxic, tomatine, there was. Johnson & Bentley (1988) examined the effects of protein and the alkaloid sparteine on growth and survivorship of larval *Spodoptera eridania*. The effects of protein and sparteine were independent when the artificial diet contained high levels of wheat germ, but when the major protein component was casein there was a significant interaction term. There was also an apparent interaction between protein and sparteine in influencing developmental rate, since the effects of protein concentration on larval stages beyond the first stadium was primarily evident when sparteine was present at high concentrations. The importance of protein quality in determining the effect of allelochemicals has also been shown by Broadway & Duffey (1988) for *Spodoptera exigua*. Unfortunately, neither Hare (1987) nor Johnson & Bentley (1988) measured consumption rates or other behavioural parameters of the larval stages for which a significant interaction term existed. It is therefore not possible to determine whether reduced performance was due to the toxic effects of the allelochemicals, or to reduced consumption as an adaptive behavioural response to maximize nutrient intake while minimizing the toxic consequences of ingesting the allelochemicals.

Lincoln *et al.* (1982) studied the response of larval checkerspot butterflies (*Euphydryas chalcedona*) to varying concentrations of the leaf resin from their foodplant and of protein. While larval survivorship, growth rates and the size of larvae at diapause were enhanced with increasing protein and depressed with increasing resin concentrations, there were no significant multiplicative interactions between these factors. The larvae ate more of the low protein diets, indicating an attempt at dietary compensation. There were no direct effects of the resin on consumption rates, but total consumption was reduced through its effects on larval size, growth rate and survivorship. It is, however, difficult to determine the extent to which these data represent the situation in the field, since in the preparation of the artificial diets the dry ingredients were mixed with water thus providing a medium in which the phenolic resins and proteins could form chemical complexes before the insects encountered the diet. The resins occur as a surface layer on the intact leaves of the host plant, so they would not normally contact soluble leaf proteins until after the insect bites the leaf. The experimental situation may therefore have had an important effect on the relationship between sensory input from resin and protein, and hence on the insects' behavioural response to these chemicals.

Slansky and Wheeler (unpublished data) found a significant interaction between caffeine and the degree of dietary dilution in affecting growth and survivorship of the velvetbean caterpillar (*Anticarsia gemmatilis*). Increased consumption to compensate for the lowered nutrient levels in the diluted diets resulted in the ingestion by the insects of deleterious quantities of caffeine. There was no significant effect on relative consumption rate, indicating that reduced total consumption was a secondary effect of reduced growth rather than due to a deterrent effect of caffeine on feeding.

In the present study, in addition to a lack of interactions between tannic acid and nutrients, there were no interactive effects of tannic acid with sex. However, male and female *Locusta* responded differently to dietary carbohydrate levels, with only the males showing a weak compensatory response to reduced dietary carbohydrates. Also, the patterns of feeding differed between the sexes, with the males of this species taking on average shorter meals at a higher

ingestion rate, but this was independent of the presence of tannic acid. Despite the lack of interactions of tannic acid with dietary nutrients or sex, it is none the less clear that tannic acid did in itself have a profound but different effect on the feeding behaviour of both *L. migratoria* and *S. gregaria*.

Locusta

In a no-choice situation there were no measurable effects of tannic acid on the feeding behaviour of *Locusta*, but when given a choice the insects took fewer meals, thereby decreasing consumption on the tannic acid diet relative to the control. These results do not accord with those of Bernays & Chapman (1977), who found that in a no-choice situation 1.2% tannic acid applied to cornflour wafers caused a 50% reduction in amount eaten over a 1–2 h period by fifth stadium *Locusta* nymphs. However, cornflour wafers are considerably less phagostimulatory than the diets used in the present study, and this probably explains why the insects were deterred by tannic acid in that study and not in the no-choice experiments in the present study. In the present two-choice experiments, significantly more of the naive insects took their first meal on the control diet, indicating a pre-ingestive deterrent effect of tannic acid. Such deterrence could be due to responses from specific deterrent receptors, or to interference with receptors responsible for phagostimulation. For example, tannic acid is known to deter feeding in some lepidopterous larvae by inhibiting the sugar receptors responsible for phagostimulation (Dethier, 1982; see also Mitchell & Sutcliffe, 1984). Whatever the sensory mechanism, it is known that there are physiological costs associated with the ingestion of tannic acid for *L. migratoria* (Bernays, 1978; Bernays *et al.*, 1980), and it would therefore be adaptive if, when a choice is available, these insects avoid consuming food containing this compound.

In accordance with the results of the present study, Bernays & Chamberlain (1982) found that in a choice assay *L. migratoria* nymphs showed a preference for controls over wheat leaves which had a surface coating of tannic acid (10% and 20%), and this effect was not apparent in a no-choice test. It is not surprising that such an effect may be found only in a choice test, since this form of assay is known to be more sensitive

than are no-choice tests in which only total consumption is measured (Cook, 1976). However, that the effect was not seen in the patterns of feeding in the no-choice assay in the present study is more surprising. For instance, Barton Browne, Simpson and van Gerwen (unpublished) found that analysis of feeding patterns in a no-choice test was a more sensitive indicator of deterrence for the Australian sheep blowfly than choice and no-choice assay in which only total amounts eaten were measured. A partial explanation for the lack of such an effect in the present study is suggested by considering the mechanisms whereby a feeding deterrent can possibly influence the patterns of feeding. Initiation and termination of feeding in locusts are determined, all else being equal, by the balance between the stimulatory properties of the food and the inhibition from gut stretch receptors and blood composition due to the previous meal (Simpson, 1989). A feeding deterrent can raise the threshold for initiating feeding, so that feeding begins only when the inhibitory feedback from the gut and blood is lower, or it can lower the threshold for meal termination, so that the insect stops feeding at a lower gut volume. Both possibilities may on their own result in reduced consumption; in the first case this would be due to the insect taking meals less frequently than it would in the absence of the deterrent, and in the second case, to a reduction in the size of the meals taken. However, if the thresholds for initiation and termination of feeding are altered equally, such that the mean difference between the gut volume at which feeding begins and ends is left unchanged, then the patterns of feeding would be expected to be unaffected by the feeding deterrent (L. Barton Browne, S. J. Simpson and A. C. M. van Gerwen, in preparation). This possibly explains why, in the no-choice assay, there were no differences between tannic acid and control insects in the amounts eaten, number of meals, meal size and intermeal intervals, but it does not explain why there was no difference in the latency period before the first meal. A raised threshold for the initiation of feeding due to tannic acid should result in the test insects starting to feed later than the controls; or, as the results for the choice experiments illustrated, on the first meal being taken on the less inhibitory of two diets.

Our data are insufficient to explain this interesting phenomenon, but it seems that any

differences in latency to feed in no-choice tests were hidden by the variability between insects and the fact that latencies on the diets were long relative to insects fed wheat (Simpson, 1982). Both tannic acid and control diets were initially novel to the insects and, apparently, to some extent deterrent. Experiments are currently underway to investigate this further.

Irrespective of the mechanism, it is interesting that a chemical which was shown in the choice experiments to be a feeding deterrent did not result in a reduction in the overall amount eaten when no choice was available. Such behaviour might be adaptive since it would enable the insects to exercise strong discretion where a choice of foodplants is available, yet make the most of a sub-optimal food source otherwise. These results illustrate how subtle the effects of feeding deterrents can be, and emphasize the value of detailed behavioural observations as well as the need to perform both choice and no-choice tests in studies of this kind (see also Schoonhoven, 1982).

Schistocerca

Schistocerca ate more of the tannic acid than the control diets in both the choice and no-choice experiments, this increased consumption being due to a shortening of the intermeal interval. That the naive tannic acid-fed insects started feeding sooner and the first meal was of a longer duration than in the controls, indicates a pre-ingestive stimulatory effect due to tannic acid. This does not accord with the results of Bernays & Chamberlain (1980). They found that for *S. gregaria* nymphs, consumption of artificial diet was reduced by the presence of 18% tannic acid over the first day of the fifth stadium. However, in the preparation of the diet the mixture was wet after combining the tannic acid with 18% protein, thus providing a medium in which the tannic acid and protein could react forming insoluble complexes at the favourable ratio of 1:1 (Goldstein & Swain, 1965). 60% of the protein was casein, which is known to react with tannins (Gstirner & Korf, 1966; Feeny, 1968). Therefore, the tannic acid would not have been available to the chemoreceptors to cause increased consumption, but it is uncertain how complexed tannic acid and protein could actually reduce consumption. Although proteins are not known to be themselves stimulatory, some amino acids

are (Cook, 1977) and precipitation of these with the tannin (Mole & Waterman, 1987) might have reduced the stimulatory properties of the test diet. Furthermore, it is known that diets lacking protein altogether are eaten in small amounts (Simpson *et al.*, 1988), and it could therefore be that consumption was reduced because complexing effectively removed the protein from the diet. It is perhaps significant in this regard that no reduction in consumption occurred when Bernays & Chamberlain (1980) applied the tannic acid (20%) to the surface of wheat where it could not contact the soluble leaf proteins. Also, since in that study there was no reduction in consumption of wheat containing 20% tannic acid, it seems unlikely that the difference between the results of the artificial diet studies of Bernays & Chamberlain (1980) and those of the present study is due to the different concentrations of tannic acid used in the two studies (18% and 10% respectively). On the other hand, Bernays & Chamberlain (1980) found no reduction in growth due to the presence of tannic acid in the artificial diet, and presumably therefore in available protein, and this might at first sight appear not to support the possibility that reduced consumption was due to the formation of tannin-protein complexes. However, tannin-protein complexes are known to be reversible by surfactants (Goldstein & Swain, 1965) such as those found in the gut of *S. gregaria* (Martin *et al.*, 1987), so that any complexed protein in the diet would none the less be made available to the digestive enzymes. Indeed, there is evidence that tannins can, under certain conditions, induce conformational changes in the structure of proteins increasing their digestibility by tryptic enzymes (Mole & Waterman, 1985).

Bernays & Chamberlain (1980) found that the reduced consumption due to tannic acid in artificial diet persisted for only 24 h, after which consumption was similar to that on the control diet. Similarly, in the present work, the stimulatory effects of tannic acid on *Schistocerca* nymphs also persisted for only 24 h. This indicates that behavioural habituation may occur not only to feeding deterrents (Szentesi & Bernays, 1984), but also to non-nutrient feeding stimulants.

Despite the waning after 24 h of the stimulatory effects of tannic acid in the present study, the insects fed diet containing this compound achieved a significantly greater adult weight and

a slightly shorter stadium duration than the controls. Similarly, Bernays (1978) and Bernays & Chamberlain (1980) found that *S.gregaria* nymphs fed wheat containing a surface coating of 20% tannic acid grew significantly more than controls over the fifth stadium with indications of reduced stadium duration. Both consumption and the efficiency of conversion of digested food were slightly higher for the tannic acid-fed insects in that study, but neither was significantly so. Bernays (1978) found that approximately two-thirds of the tannic acid was hydrolysed on its passage through the gut and suggested that *S.gregaria* might benefit from the sugars released during this hydrolysis. The present work has demonstrated that increased consumption which occurs during the first 24 h of exposure to tannic acid can account for the increased growth over the fifth stadium.

On the other hand, Bernays (1978) found no significant increase due to tannic acid in total growth through all the larval stadia of *S.gregaria*. From this it appears that the transitory effect on consumption may be beneficial to growth over a limited period only, at least when the insects are exposed to tannic acid continuously. It will be interesting to see whether the stimulatory effect can be reset by alternately feeding the insects tannic acid and control diets. This would have important implications for optimal foraging theory, since it might provide a selective basis other than dietary self-selection (Waldbauer & Friedman, 1988) for the frequent switching of plants while feeding by polyphagous insects. We are currently investigating this question.

While it is known that tannic acid stimulates feeding in the larvae of some tree feeding Lepidoptera (Bernays, 1981), the same has not hitherto been demonstrated for orthopterans. At least one species of acridid, *Anacridium melanorhodon*, is known to benefit from ingesting this compound (Bernays *et al.*, 1980; Bernays & Woodhead, 1982), but in the present study there were no indications of a nutritional effect on *S.gregaria* over and above the increased consumption. This is surprising since compounds which stimulate feeding by insects are often themselves nutritious or otherwise beneficial to the insect, or signal the presence in plants of such compounds. Perhaps insects stimulated to increase consumption of diets containing tannins have evolved to do so to compensate for some

costs associated with the detoxification of these compounds. For example, acridids that regularly ingest tannins have more substantial peritrophic membranes which can, in extreme cases, entail an investment of up to 10% of ingested protein (Bernays & Simpson, 1989). We have found that, when deprived of water, *S.gregaria* nymphs fed pc diet containing 10% tannic acid lost significantly more weight over a 48 h period than controls, despite the fact that their cumulative consumption remained higher than that of the controls (Raubenheimer & Simpson, unpublished). This suggests that there may well be physiological costs due to ingesting tannic acid, at least when water is absent. Additionally or alternatively, there may be ecological advantages to the ingestion of tannins, and these would not be apparent in laboratory studies of the kind reported here.

In conclusion, detailed observations of the insects' behavioural response to manipulation of chemicals in artificial diets has provided new insights into the effects of tannic acid on feeding by *S.gregaria* and *L.migratoria*, and has identified several unanswered questions regarding the mechanisms whereby insects respond to allelochemicals. Some of these will be the subject of future papers in this series.

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