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14. THE EFFECT OF FOLIAR NUTRIENTS UPON THE GROWTH AND FEEDING OF A LEPIDOPTERAN LARVA

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SUMMARY

It is suggested that levels of available leaf nitrogen, rather than total leaf nitrogen, are relevant to the growth and development of insect herbivores. A simple assay based on enzyme inhibition is described which estimates the levels of leaf protein complexing agents with host plant foliage. These estimates are then combined with total leaf protein values to give the levels of protein actually available to a feeding insect.

It is shown that available, rather than total, leaf protein levels are related to the performance of larval winter moths (*Operophtera brumata* L.), when they are fed on leaves from several host plant species.

Nitrogen availability is discussed with reference to the feeding biology of both sucking and chewing insect herbivores, and briefly with respect to the population levels of the winter moth.

INTRODUCTION

The relevance of the nutritional status of a food plant to the feeding and growth of phytophagous insects has been the subject of many studies during the past 30 years (Fraenkel 1953; House 1961; Feeny 1970; Southwood & McNeill 1978). Research has been concentrated largely on two categories of chemicals: nitrogen and its component amino acids (Slansky & Feeny 1977; Southwood & McNeill 1978), and those compounds, notably the tannins, which combine with plant proteins to reduce the availability of the latter to feeding herbivores (Feeny 1970; Swain 1976a; Bernays 1978).

The defensive function of plant phenolics, particularly against chewing insects, is well documented (Swain 1976a; Ibrahim, Axtell & Oswalt 1973; Reese & Beck 1976; Feeny 1970; Levin 1971; Oates, Swain & Zantovska 1977). However, there is evidence that the effects of phenolics upon herbivores in general, and insects in particular, are by no means simple. For example,

tannins seem to have little effect upon the insects of *Eucalyptus* spp. (Fox & Macauley 1977), or upon the scale insect *Mycetapsis personatus* when feeding on varieties of *Mangifera indica* (Salama & Saleh 1972). Furthermore, the relative ineffectiveness of the hydrolysable tannins as a defence against the winter moth, reported by Feeny (1966), has been brought into question by evidence suggesting that they may be more effective than are the condensed tannins (Swain 1976a; Van Summere *et al.* 1975; Bernays 1978).

Nitrogen is well established in ecological lore as an important determinant of phytophagous insects' success (Southwood & McNeill 1978). Most of the clearest evidence comes from work on leaf-sucking insects. Thus high dietary nitrogen levels promote rapid development and increased growth in aphids (Dixon 1969) and can affect the distribution of several hemipteran species in the field (McNeill 1973; Gibson 1980). With chewing insects the evidence is less clear. Artificial diet experiments have shown that high nitrogen levels lead to larger lepidopteran larvae (House 1965), and some studies show that total plant nitrogen is positively correlated to an insect's feeding and growth (Fox & Macauley 1977). Much of the literature shows, however, that total nitrogen levels are unrelated to a chewing insect's growth (Slansky & Feeny 1977; Baker 1975), though they have distinct effects upon its digestion and feeding processes. Theoretical objections have also been raised to question the assumption that low nitrogen levels act as a defence against herbivores. Hamilton & Moran (1980) point out that low nutrient levels may well enhance insect consumption, so that, 'genes conferring lowered nutritive quality (in host plants) could even increase herbivore damage in some circumstances'.

Thus, total nitrogen as an indicator of diet quality or host plant defence strategy appears to be ambiguous: in some cases it is predictive while in others it is not. As a result, many studies have focussed on the components of dietary nitrogen—the amino acids (e.g. Prestidge & McNeill, p. 257 this volume). An alternative is to consider nitrogen availability. Many 'quantitative' plant defences (Feeny 1976) are assumed to act by complexing with leaf proteins to reduce their availability and yet most studies in the literature have looked at total nitrogen and the defence compounds as separate entities. As far as the author is aware, only Feeny (1970) makes any allowance for this interaction to provide estimates of the leaf proteins that are actually available to a feeding herbivore.

This paper reports on investigations of the associations between host plant quality and the performance of winter moth (*Operophtera brumata* L.) larvae when fed on the leaves of various ages from six common food plant species. Host plant quality was measured throughout the growing season by qualitative screening of foliar phenols, and by quantitative estimates of leaf toughness, leaf water content, total leaf nitrogen content and levels of leaf

'nutrient-reducing agents'. This last estimate was obtained from an enzyme assay and then combined with the foliar protein values to provide a measure of the amounts of protein available to the feeding larvae. Larval performance was assessed by monitoring 'overall' performance (pupal fresh weight, larval growth rate and weight increase) and mortality in 1976 and 1977, and feeding processes (consumption levels and feeding efficiencies) in 1977. Associations between the plant and animal parameters were then examined using standard correlation techniques.

METHODS

Analyses of leaf material

Six host plant species were selected: *Crataegus monogyna, Malus sylvestris* (cultivated apple), *Prunus spinosa, Corylus avellana, Fagus sylvatica* and *Quercus robur* (as a control). These species are all common winter moth host plants in the field which reduced the possibility of larval performance being affected by toxins and deterrents.

Leaves for chemical analyses and feeding trials were collected from the field into polythene bags which were pre-filled with nitrogen and generous quantities of ice to minimize chemical decay before analysis. All the material consisted of lower sun leaves and was collected in mid-morning.

Percentage leaf water was assessed gravimetrically using preweighed freeze-dried materal; leaf toughness was estimated using a modified penetrometer (Moreau 1965); percentage total nitrogen was measured from freeze-dried material using the micro-Kjeldahl method (Allen *et al.* 1974). Preliminary investigations of the leaf phenolics were performed by two-dimensional chromatography (Haslam 1966; Feeny 1966; Harborne 1973), using 2% acetic acid and butanol/acetic acid/water (12:3:5) as the first and second solvents respectively. These were developed with vanillin and $FeCl_3/K_3Fe(CN)_6$ sprays, and were examined under u.v. light with and without ammonia vapour. The analyses were carried out on leaves extracted in 70% aqueous acetone, stabilized by the addition of a little KCN, and stored in the dark at 4°C.

The available methods for quantitative estimates of nutrient-reducing agents like the phenolics (Brown, Love & Handley 1962; Feeny 1966; Haslam, pers. comm.) are all subject to several drawbacks. They often require extraction of the relevant compounds from the leaf tissues and there is no guarantee that these compounds remain unaltered by the extraction process (Brown, pers. comm.). Also, the identity of the substances extracted is frequently difficult to establish due, at least partly, to their heterogeneity.

Furthermore, the value of information concerning a single class of phenols such as the tannins, or even the phenols as a whole, is not only difficult to assess in terms of their effect upon the potential food plant quality, but may also represent one of a number of types of compound which can act as nutrient-reducing agents. Finally, the time needed to perform many of the standard methods prevents frequent screening of several plant species.

Professor G. C. Varley suggested that it might be possible to use the inhibition of bacterial amylase by tannins, observed by Feeny (1966), as the basis of a quantitative assay which avoids these problems. Such inhibition is well known and has been reported for a wide variety of enzymes including proteinases, invertases, lipases, phosphatases, decarboxylases, ureases and β-glucosidases, as well as amylases (Benoit & Starkey 1968).

There is also evidence that the level of inhibition of β-glucosidases and amylase is related to the amount of tannin present (Goldstein & Swain 1965; Boudet & Gadal 1965b). This suggests that any inhibition of amylase caused by leaf extract is paralleled by the effect of the leaf tissues upon the digestive enzymes of a feeding herbivore. Furthermore, the chemical similarity of nutrient proteins and enzymes suggests that an assay using enzyme inhibition mimics another major effect of nutrient-reducing agents—that is, the complexing between the nutrient proteins and compounds like tannins. Therefore, any inhibition detected by an assay of this type should reflect similar processes occurring in the gut of an herbivorous insect.

Examinations of the basic starch–amylase reaction revealed three relationships. First, when varying amounts of enzyme were added to constant amounts of starch, the end-point time (i.e. the isocolorimetric point as detected by an iodine indicator) was proportional to the inverse of the enzyme concentration. Second, aqueous extracts of the leaves of several host plant species (*Corylus, Crataegus* and a *Viburnum* sp.) were able to inhibit the starch digestion such that adding a fixed quantity of leaf extract to the enzyme solutions, prior to their admixture with starch, caused a delay in the end-point time which was linearly related to the inverse of the enzyme concentration. An example of this relationship using *Corylus* leaves is presented in Fig. 14.1a. The gradient of this line could provide a measure of the degree of inhibition of the starch digestion that was caused by the contents of the leaf extracts.

A third series of experiments showed that aqueous solutions of oak tannin extract (provided by B.R. Brown) inhibited the amylase in a manner identical to that shown by the leaf extracts. Also, the 'inhibition gradient' was linearly related to tannin concentration (Fig. 14.1b). Using this relationship, it was possible to describe the degree of enzyme inhibition caused by the leaf extracts in terms of the amount of oak tannin that produced an equivalent effect.

In addition, gallic acid, quercetin and (+)-catechin inhibited the starch–

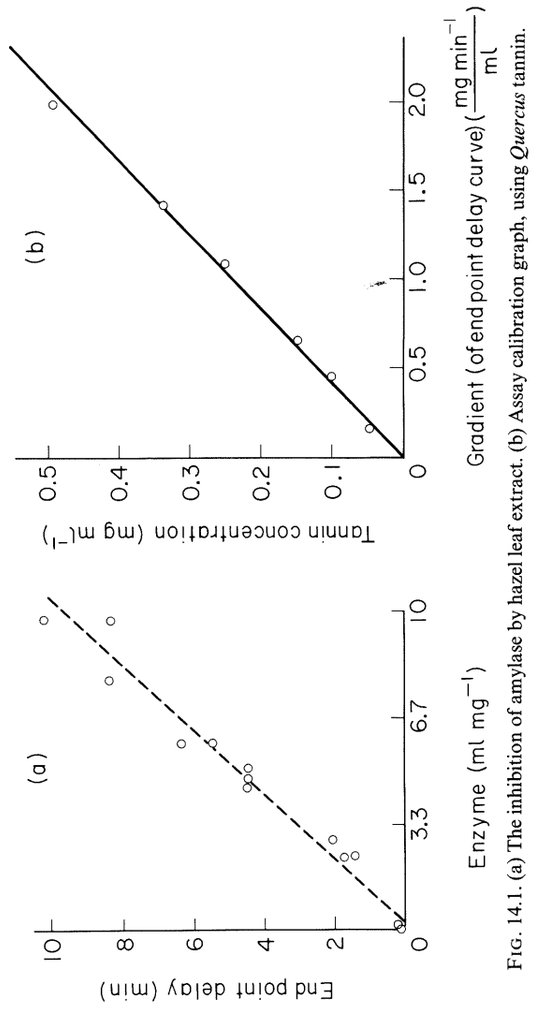

FIG. 14.1. (a) The inhibition of amylase by hazel leaf extract. (b) Assay calibration graph, using *Quercus* tannin.

amylase reaction, though the degree of inhibition was less than that produced by tannins. Thus gallic acid is about half (58%) as effective an inhibitor as the oak tannin extract, and quercetin and (+)-catechin are, respectively, 35% and 7% as effective. These inhibitory capacities parallel the reduction in pupal weight that is caused by the addition of these compounds to artificial diets of larval winter moth (Feeny 1966). This indicates that the effects of these compounds upon amylase activity closely mimics their effect upon larval growth.

As a result of this information, and after taking into consideration several additional factors—notably the complexing time between enzyme and extract, extract concentrations, the effect of leaf extracts on the starch iodine reaction and solution stabilities—a standard assay procedure was developed (see Appendix 1). This produced estimates of the levels of 'Inhibitors' within the host plant foliage and was calibrated in terms of 'Oak tannin equivalents'.

It has been shown that the degree of complexing between oak tannins and casein is related to the tannin/protein ratio (Feeny 1969). By calculating the ratio between the levels of leaf protein and the tannin equivalent (i.e. inhibitor) estimated by the enzyme assay, it is possible to obtain a measure which should reflect the amount leaf protein rendered unavailable by the inhibiting compounds. This allows calculation of the available protein levels.

The degree of tannin protein complexing also varies according to the type of tannin involved (Feeny 1969), and the calibration tannin extract contained both hydrolysable and condensed tannins. As the latter are much more effective at complexing with protein, it is likely that they produced most of enzyme inhibition caused by the calibration extract. On this assumption, the graph shown in Fig. 14.2, which represents the complexing characteristics of condensed tannins with casein, was used to calculate estimates of available protein. Only approximate levels of complexing were assessed due to the many uncertainties involved, so the reduction of protein availability was estimated to the nearest 5%. Total protein concentrations were obtained by multiplying the total nitrogen levels by 6.25 according to Allen *et al.* (1974).

Feeding trials

On eclosion, experimental animals were taken from laboratory stock in batches of at least 20, and fed on the experimental food plants. Feeding trials were conducted at 20°C, in 75% r.h. and 16:8 L/D, using leaves fresh from the field which were renewed every two days. Each larva was reared in numbered air-tight pots to reduce leaf water loss. Larvae were weighed daily from the fourth (penultimate) instar until they stopped feeding. Consumption and defaecation levels were estimated gravimetrically, and assimilation levels then

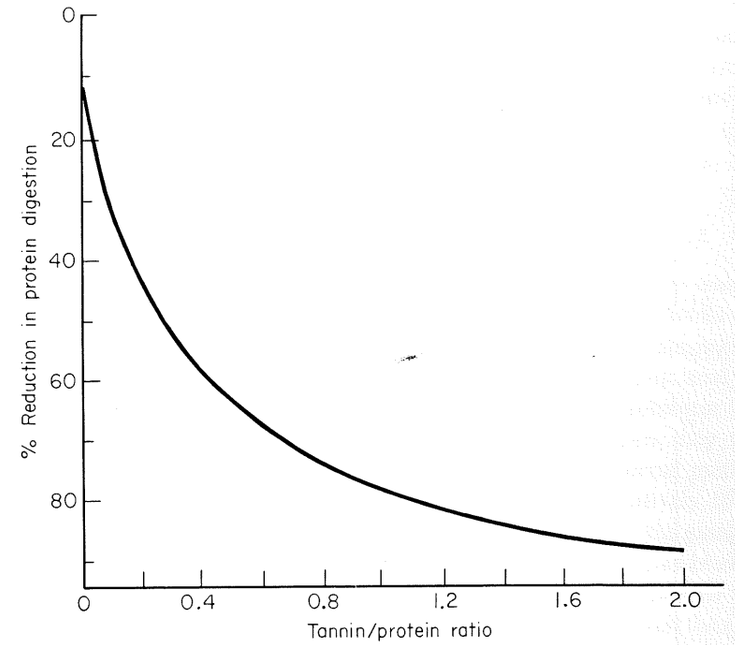

FIG. 14.2. Available protein estimation: the effect of the tannin/protein ratio upon the digestion of casein by trypsin (after Feeny 1966).

determined by difference (assimilation = consumption − faeces). Relative growth rates, consumption indices and feeding efficiencies (Assimilation Efficiency, AD; Growth Efficiency, ECD; Overall Conversion Efficiency, ECI) of the fifth instar larvae were defined and calculated according to Waldbauer (1964).

Data preparation and correlation

The mean values of each measure of larval performance were calculated for each feeding trial, using only those larvae which survived to pupation. The estimates of food plant quality relevant to each feeding trial were calculated from graphs of the type shown in Fig. 14.3, employing standard geometric methods. The values obtained were then corrected to allow for changes that were found to occur in the experimental food while it was being eaten. As no relationship was detected between leaf water content and any of the animal variables measured, both the larval performance data and the plant quality

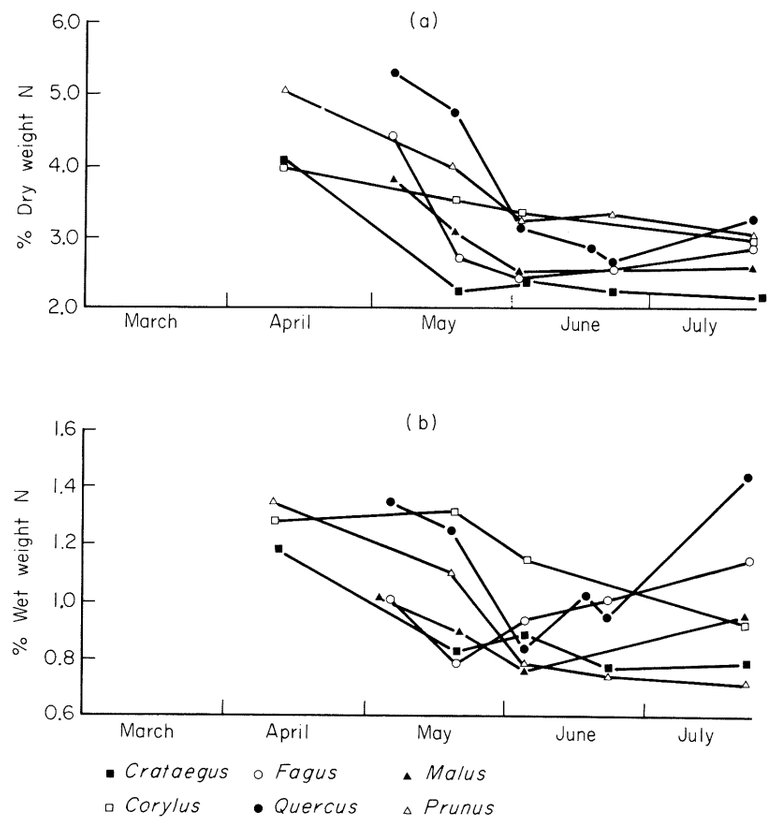

FIG. 14.3. Seasonal variations in % total leaf nitrogen for 1977. (a) % Dry weight. (b) % Wet weight.

values were calculated in terms of fresh weight throughout. Though less convenient, such values are likely to be of greater biological relevance than are the dry weight values. This is emphasized by a comparison of the dry and wet weight total nitrogen figures shown in Figs 14a & b. Though the range of values obtained for both variables shows a similar proportional variation, the pattern of general decline with time shown by the dry weight levels is not evident in the wet weight values. Indeed, in *Q. robur* leaves the July levels are higher than the April ones. Furthermore, the relative levels of wet and dry weight nitrogen levels in the different host plant species differ considerably.

Associations between the animal and plant variables were examined using both simple and partial correlation techniques (Snedecor & Cochran 1962).

The correlations were performed on the data from all the feeding trials together (transformed to give the best fit), on the assumption that a deductive approach is of more value than an inductive one, as well as to take advantage of the wider range of plant quality presented by the use of several plant species.

RESULTS

The enzyme bioassay

An example of the leaf inhibitor levels obtained from the enzyme assay is presented in Fig. 14.4a. It is conspicuous that the highest inhibitor levels were found in *Crataegus*, and the lowest in *Malus*. This agrees well with the number and intensity of the phenolic spots detected by the chromatographic analyses. In addition, there is good agreement between the maximum inhibitor level shown by the assay and the levels of tannins against which they were calibrated, as reported by Feeny (1970) using a specific chemical method. These points strongly suggest that the assay reacts quantitatively to the presence of phenols within leaf tissues. However, the data from the assays of *Corylus* foliage indicate that the assay detects compounds other than phenols. Thus the phenol content of 1977 *Corylus* leaves was considerably less than that of 1976, while the inhibitor content was higher. This implies that the assay

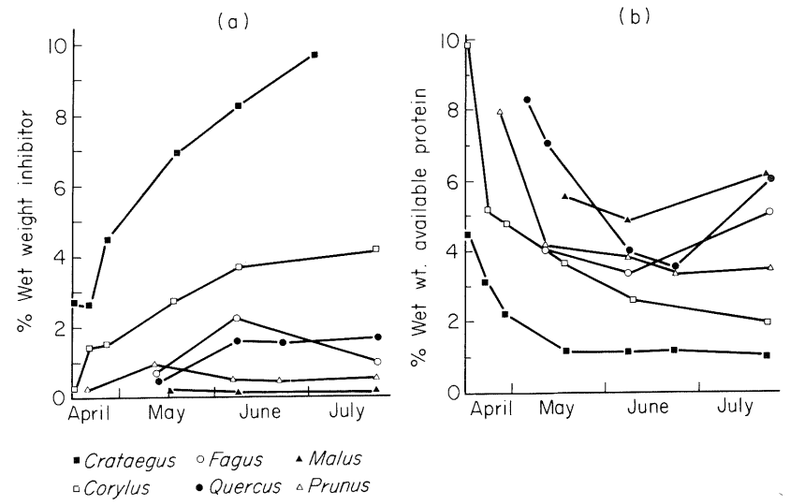

FIG. 14.4. Seasonal variations in leaf contents for 1977. (a) % Wet weight inhibitor. (b) % Wet weight available protein.

fulfills its desired role as an indicator of compounds, in addition to phenols, which may act as nutrient-reducing agents either by directly inhibiting herbivore enzymes or by complexing with foliar proteins.

This method has several advantages over the more chemical techniques. Firstly, as it uses similar principles to those involved in insect feeding (i.e. maceration, mixing then digestion), any inhibition detected is likely to be due to substances that also reduce the potential food quality of the leaves. Secondly, the assay can be performed on aqueous leaf extracts, so that the compounds detected are more likely to be identical to those present in the living leaf tissues than are those found in the acetone or methanol extracts which are necessary for the more chemical methods. Thirdly, this assay is sensitive not only to the presence of phenols, but is probably affected by other compounds with similar effects such as protein inhibitors (Buonocore, Petrucci & Silano 1977), and so it provides an estimate of all the nutrient-reducing agents within leaf tissues, regardless of chemical identity. Such a method is likely to have wider biological relevance than those which encompass a specific type of chemical. Lastly, this technique is easily and rapidly performed, which permits frequent analyses of several host plant species.

Available protein estimations

The value of these estimations can be seen by comparing the total leaf nitrogen and available protein levels for 1977 (Figs 14.3b & 4b). The available protein levels, in contrast to the total nitrogen values, fall below the 6.25% (equivalent to 1% nitrogen) levels suggested by some authors to be limiting to lepidopteran growth (Fox & Macauley 1977). This implies that larval performance may well be limited by nitrogen availability. In addition, the relative nutrient status of the six host plant species differs markedly according to the measure examined, particularly in the cases of *Crataegus* and *Corylus*. There is also a contrast in the phenologies of the two variables. The available protein levels tend to fall more rapidly in April and May, as well as rise less markedly in July, than do the corresponding total nitrogen contents.

The arguments used to develop these estimates are open to a number of criticisms. Firstly, there is no guarantee that the inhibitors detected act upon the feeding insect by reducing the available protein rather than directly inhibiting its digestive enzymes, and hence the assumption that all inhibitors detected act to reduce the available protein is speculative. Secondly, the use of a single class of phenolics and a simple protein to provide the conversion graph is open to censure—not only are many types of compound likely to be detected by the assay, but also there are many types of protein within the leaf

tissues, most of which are more complex than casein. Thirdly, the conditions used by Feeny to produce the complexing curve bear little resemblance to conditions found either within a leaf, within an insect, or within the enzyme assay employed.

Associations between host plant quality and measure of larval performance

Overall larval performance

Of the independent plant variables, only the leaf inhibitor content is significantly correlated with pupal fresh weight, fifth instar weight increase and relative growth rate (Table 14.1a). The correlations are significant at the 1% level for the pupal fresh weights and weight increases, and at 2% for the relative growth rates: all are negative. These results suggest that while a rise in foliar inhibitor levels is related to a decrease in larval performance, changes in either leaf toughness or total leaf nitrogen contents are not reflected by changes in larval growth.

If, however, the interaction between leaf inhibitors and foliar nitrogen is considered, it becomes evident that nitrogen levels within the host plant leaves are of importance to the larval performance. The correlations between the animal variables and the available leaf protein levels are all significant at the 5% level or less, and are all positive, implying that an increase in larval growth accompanies a rise in available protein levels.

Using standard multiple regression (Steele & Torrie 1960), the proportion of the variation in pupal fresh weight explained by leaf toughness and available protein together (R^2) is 0.46 ($P<0.01$, $n=23$). Though significant, this suggests that many other factors are important in determining larval growth. While fifth instar weight increase and relative growth rate are measurements relevant to the fifth instar alone, the pupal fresh weight includes the effects of all five instars. 77% ($P<0.01$, $n=23$) of the variation in the last parameter is explained by the variation in the larval weight at the beginning of the fifth instar. Events leading to pupation may be triggered well in advance of the cessation of feeding (Varley, pers. comm.) and so may only be partially dependent upon variation in food quality after the 'trigger' has operated.

Larval consumption

As larval consumption rate (mg day^{-1}) is significantly related to the Averaged Mean Weight (Waldbauer 1964) of the larvae ($r=0.80$, $P<0.01$, $n=13$), correlations with the larval consumption index (that is the consumption rate

TABLE 14.1. Partial correlation coefficients between larval performance and host plant quality. (Significance levels in brackets. *⇒log transformation.)

Animal variables	Plant variables: Leaf inhibitor content	Total leaf nitrogen	Leaf toughness	Available leaf protein
(a) Overall measures of larval performance, 1976 & 1977 data, $n=23$.				
Pupal fresh weight	−0.6217*(0.01)	0.2744 (NS)	−0.3054 (NS)	0.5851*(0.01)
Fifth instar weight increase	−0.5587*(0.01)	0.1379 (NS)	−0.3775 (NS)	0.4905 (0.05)
Fifth instar growth rate	−0.5018 (0.02)	0.0423 (NS)	−0.2193*(NS)	0.4727*(0.05)
(b) Feeding processes, 1977 data, $n=13$.				
$\frac{C}{T.W.}$ (CI)	0.3867*(NS)	−0.2450*(NS)	−0.1766*(NS)	−0.3777*(NS)
G/A (ECD)	0.3167 (NS)	−0.5030 (NS)	−0.4349 (NS)	−0.2968*(NS)
A/C (AD)	−0.7397 (0.01)	0.7665 (0.01)	0.7087 (0.02)	0.6724*(0.05)
G/C (ECI)	−0.3966 (NS)	−0.0611 (NS)	−0.1273*(NS)	0.4563*(NS)

C=consumption (mg wet wt.) T=feeding times (days) W=averaged mean weight (mg wet wt.) G=weight increase (mg wet wt.) A=assimilation (mg wet wt.)

TABLE 14.2. Correlation coefficients between larval nutrient intake levels and measures of larval performance. (Significance levels in brackets; 1977 data, $n=13$, *⇒log transformation used.)

Animal variables	Plant variables: Total nitrogen (Partial correlation technique)	Inhibitor	Available protein (Simple correlation technique)
Fifth instar weight increase	0.4975* (NS)	−0.6261* (0.05)	0.6465* (0.05)
Pupal fresh weight	0.4805 (NS)	−0.6804* (0.02)	0.6933* (0.01)

corrected for larval size) were examined: none were significant (Table 14.1b). If the larval intake levels of nitrogen, inhibitor and available protein are calculated using the mean consumption levels for each feeding trial and the corresponding values of each plant parameter (Table 14.2), a different answer

emerges. While the total nitrogen intake is not correlated to either fifth instar weight increase or to pupal fresh weight, the inhibitor and available protein intake levels are. This suggests that while diet quality does not influence larval consumption levels, consumption levels influence larval performance by virtue of their effect upon nutrient intake levels. Again the importance of leaf nitrogen only becomes apparent when its availability is considered.

This evidence is at variance with the published data (Tanton 1962; Iversen 1974; Reese & Beck 1976) which indicates that insect consumption levels respond to many plant variables. In the case of the winter moth, larval consumption appears to be primarily related to size which may mask any effects of plant quality.

Larval feeding efficiencies

The variation in leaf contents and in leaf toughness are not closely related to either larval ECI or ECD (see Table 14.1b). In contrast, the larval assimilation efficiency (AD) is significantly related to all four measures of host plant quality. This suggests that the major effect of host plant nutrient status upon larval digestion is the determination of the proportion of leaf tissues that can be digested. The highest correlation is with total leaf nitrogen level while the lowest is with available leaf protein content, though this may be due to the differences in correlation technique employed. The signs of the correlation coefficients show that larval AD increases in association with a rise in leaf nutrient availability, as represented by high nitrogen and available protein levels and a low inhibitor content. However, the larval assimilation efficiency also increases with rising leaf toughness. Tougher leaves are likely to contain more cellulose and lignin and therefore less nutrients. The non-significant relationship between larval consumption index and leaf toughness (see Table 14.1b) invalidates the possibility that rising toughness leads to reduced feeding rates and hence high assimilation efficiencies, as was reported by Soo Hoo & Fraenkel (1966) and Mehta & Saxena (1973). In the absence of further evidence, it seems possible that leaf toughness is related to a quantitative change within the leaves that itself affects the larval assimilation.

As the associations between the plant variables and the larval AD are not reflected by the Overall Conversion Efficiencies (ECI), it is probable that the larval ECD responds to entirely different parameters of food quality (e.g. the proportion of different amino acids), and that this contrast in response is evident as a lack of correlation between any of the measured plant variables and the larval ECI.

Larval mortality

The total larval mortality is the cumulative mortality of all five instars, and so examining it in relation to the diet quality during any one instar is meaningless. Such cumulative errors are reduced if the early instar mortalities are considered. First instar mortality may well be influenced by the egg quality and so the second instar cumulative mortality is the first which it is profitable to investigate. No chemical measure of food plant quality was correlated to this mortality (arcsine transformation), but leaf toughness was highly so ($r=0.74$, $P<0.001$, $n=23$). Hence, while leaf toughness exerts a direct effect upon early instar survival, the influence of the chemical aspects of diet quality is manifest by its effects on pupal fresh weight rather than larval mortality. Pupal fresh weight is closely correlated to subsequent adult survival and fecundity (Andrzejewska, in Gradwell 1973; Wint 1979), which suggests that any influence of nutrient availability upon the survival of the winter moth acts via reproductive success.

DISCUSSION

The evidence I have presented suggests that in terms of overall larval performance, it is the availability of nutrients in the host plant leaves, rather than other aspects of plant quality, that is most closely related to the growth of winter moth larvae. An increase in inhibitor levels, or a fall in available nitrogen content is associated with a decline in larval performance. Total nitrogen is, however, independent of larval growth. This last piece of evidence supports many of the reports in the literature concerning chewing insects, but it contrasts with the majority of evidence derived from leaf-sucking herbivores (Slansky & Feeny 1977; Southwood & McNeill 1978) as well as Fox & Macauley's (1977) assertion that the growth of *Eucalyptus* phytophages is closely allied to the foliar total nitrogen levels.

Such contradictions may be reconciled by considering the effects of nitrogen availability. In the case of Fox & Macauley's work, there is no correlation between the leaf tannin levels and the insects' growth. This suggests little or no interaction between the secondary compounds and either the insect digestion or the leaf proteins, and so nitrogen availability is unaffected by the defences. Insect growth is therefore closely related to total nitrogen concentrations. Many hemipterans extract their nitrogen directly from the host plant's vascular system and so avoid the majority of the quantitative defences which are located largely in the cells of the non-vascular tissues, and are present in the sap as inactive soluble glucosides (Siegler 1977). The plant proteins in the sap do not interact with the protein-complexing

agents and so the insect's growth is closely related to the total (and available) nitrogen levels. The difference between sucking and chewing insects is further underlined if their digestion is considered. Proteolytic enzymes are used by chewing insects to hydrolyse the leaf-bound proteins to their constituent amino acids. These enzymes are likely to be affected by protein-complexing agents and are essential for the chewing insects' successful extraction of much of their dietary nitrogen. In the case of leaf-sucking insects, much of their nitrogen intake is already in small units and so their digestion is less vulnerable to inhibition.

If this is the case, both sucking and chewing insects are liable to be affected by the quantity (and by inference, the quality) of available nutrients, but only the latter are subject to many quantitative defences. The distribution of chemical defences is also likely to be an important factor, be they clumped, as in resin canals or essential oil glands, or evenly distributed throughout the non-vascular tissues. Such a dichotomy in the vulnerability of sucking and chewing insects to chemical plant defences may well be a contributing factor to the composition of phytophage communities on any specific host plant species.

The present evidence also suggests that plant nutrient levels affect the populations of chewing insects at the level of their reproductive capacity. In univoltine species such as the winter moth, this may mean that a host plant's nutrient status in any particular year may influence the insect population densities in the next. Conversely, the population levels in any particular year may be unrelated to the nutrient quality of the host plant tissues in that year. This concept is supported by preliminary evidence from a study now in progress (Southwood, Kennedy & Wint, unpublished data) in which the insect fauna of four oak species is being investigated alongside, among other factors, their nutrient status. Population levels of the major chewing herbivores (*Operophtera brumata* and *Tortrix viridana*) were estimated but were not related to the available protein content of the leaves on which they fed. Furthermore, no convincing evidence was found to relate the insect population densities to the initial nutrient content of the flushing leaves. Considerably more information is needed before any firm conclusions can be drawn.

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APPENDIX 1

THE QUANTITATIVE ESTIMATION OF THE NUTRITIVE INHIBITOR CONTENT OF EXPERIMENTAL HOST PLANT LEAVES: THE ENZYME ASSAY—REAGENTS AND PROCEDURES

THE BASIC STARCH–AMYLASE REACTION

The methodology of the basic starch–amylase reaction was modified from that used in undergraduate biochemistry practical experiments (P.C.J. Brunet, pers. comm.).

Reagents used

Stock starch reagent mixture

50 ml soluble starch solution (1 g in 500 ml water)
20 ml salt (NaCl) solution (1% w/v)
20 ml phosphate buffer, for pH 6.6
The buffer solution was made up from two solutions, in the ratio of 3:2, i.e. 150 ml solution 1 and 100 ml solution 2:
1. 2.269 g KH_2PO_4 in 250 ml water
2. 4.75 g Na_2HPO_4 $12H_20$ in 250 ml water

Enzyme solutions

An aqueous solution of α-amylase (Type III-A, from *Bacillus subtilis*, obtainable from Sigma London Chemical Co. Ltd) was prepared at 0.4 mg ml^{-1}. This was subsequently diluted to give four enzyme solutions—0.4 mg ml^{-1}, 0.3 mg ml^{-1}, 0.2 mg ml^{-1} and 0.1 mg ml^{-1}.

Iodine indicator solution

5 ml 0.2% I_2 in 5% KI
245 ml water

Standard reaction procedure

4.5 ml of the starch reagent mixture was added to 1 ml of each of the four enzyme solutions, held in a water bath at 20°C. Every 15 seconds, 0.25 ml of each reacting mixture was withdrawn and added to 4 ml of the iodine indicator solution until the end-point was reached, as indicated when no change in the colour of the iodine solution was produced by the addition of the enzyme–starch reactant mixture (i.e. the isocolorimetric point). This was originally determined by colorimetric comparison at 550 nm of the experimental solutions with 4 ml of indicator solution to which 0.25 ml of reagent blank solution had been added. It was subsequently discovered that reproducible end-point determinations could be obtained using simple visual comparisons, against a white background, in daylight and without fluorescent lighting. As the visual comparisons were considerably less cumbersome to perform, these were used in all the experimental enzyme assays.

THE ASSAY

Fresh leaves, collected as described under 'Methods', were macerated in distilled water, to give a standard extract concentration of 60 mg ml^{-1}. 0.25 ml of this was then added to 1 ml of each of the four enzyme solutions, which were left at 20°C to 'complex' for ten minutes precisely. 4.5 ml of the starch reagent mixture was then added to each extract enzyme mixture, and the resultant end-points determined as described above. However, as well as 0.25 ml of reagent blank, 0.25 ml of leaf extract was added to the control iodine indicator sample, to allow for any direct effects of the leaf extract upon the iodine–starch colour reaction.

A control assay was performed alongside the experimental assays in which water was substituted for the leaf extract. The end-points of this test were then subtracted from those of the leaf extract assays to give the delays in the end-point time of each enzyme–starch reaction that were caused by the addition of the leaf extracts. These figures were then plotted against the inverse of the relevant enzyme concentrations, and the slope of the resulting line calculated using linear regression. This gradient was used for subsequent calculations (see below) only if the regression was significant at $P < 0.001$. If this condition was not fulfilled, the assay was repeated.

Leaf extracts were diluted when necessary to ensure that the levels of enzyme inhibition were equivalent to those of the calibration graph (Fig. 14.1b), and to ensure that the leaf extracts themselves did not affect the iodine–starch reaction.

The foliar levels of inhibitor were calculated from the gradients described above as follows:

$$\%\text{ wet weight inhibitor} = \frac{GC}{E} \times 100$$

G = gradient of the plot of end-point delay/enzyme.

C = gradient of the calibration curve. This was 0.25 in the 1976 and 1977 assays, but can vary according to the batch of enzyme used.

E = weight of leaf added to each enzyme solution (mg fresh wt.).

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15. NITROGEN IN DEFENCE AGAINST INSECTS

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INTRODUCTION

The possible protective roles of secondary metabolites, including nitrogenous ones, is a topic which has recently attracted much attention, and been the subject of various generalizations and theories. Proof of the supposed protective roles is not always easy to obtain, and attempts to gather it have often failed. In this paper work is reviewed on the relationship between insect herbivores and the potentially deterrent or noxious compounds containing nitrogen which they may encounter in plants. What is the evidence for the protective role of such nitrogen-containing compounds in the biology and ecology of plants?

THE PLANTS

Several major groups of nitrogenous compounds from plants have toxic effects on man or domestic animals. Most important are the cyanogenic compounds, alkaloids, unusual amino acids and amines, and the glucosinolates, which are all loosely grouped as secondary metabolites. A number of unusual proteins and a few rarer chemicals which have been little studied to date may also affect animals. For the plant, there are two separate aspects of the occurrence of such chemicals. Firstly, they must be able to synthesize them and secondly they must have the capacity to store them. For example, most plants have the capacity to synthesize cyanide at least in minute amounts (Gewitz *et al.* 1974; Hegnauer 1976), but only certain species store cyanogenic compounds in measurable quantities. Storage of secondary metabolites generally involves spatial separation from active cytoplasm into vacuole or cell wall, or in cuticle, laticifers, glands and hairs (Schnepf 1976). They are also commonly retained in non-toxic chemical complexes (Müller 1976). Special biochemical tolerance mechanisms operate in some plants (Fowden & Lea 1979) and a further discussion of the relative importance of these mechanisms is given by McKey (1979).

Different types of compound tend to occur in different quantities—both in