THE PERFORMANCE OF

URABA LUGENS WALKER (LEPIDOPTERA: NOLIDAE)

IN RELATION TO NITROGEN AND PHENOLICS IN ITS FOOD.

BY

JANET DAWN FARR

B.Ag.Sc.(Hons.) University of Adelaide, South Australia.

A thesis submitted for the degree of Doctor of Philosophy

in the Faculty of Agricultural Science at

The University of Adelaide.

Department of Entomology

Waite Agricultural Research Institute.

September 1985

TO

MY MOTHER

DOREEN WINIFRED FARR

Advice from a caterpillar -

"One side will make you grow taller, and

the other side will make you grow shorter."

Lewis Carroll: Alice's Adventures in Wonderland.

A late instar larvae of Uraba lugens Walk. and Oxyopes sp. (Oxyopidae: Araneae) on Eucalyptus camaldulensis.

TABLE OF CONTENTS

SUMMARY

The performance of Uraba lugens Walker was investigated in relation to the influence of nitrogen and phenolic components present in its food plants, using fresh and synthetic diets. The seasonal and annual variation of these components in Eucalyptus camaldulensis, a good food plant for U. lugens, was also examined. This was done in order to test the hypothesis that nitrogen is normally a limiting nutrient for phytophagous insects, but may become more available when its food plant is subjected to "water stress", leading to increased survival of early instars and therefore a population increase or "outbreak".

Larvae fed water stressed E. camaldulensis were potentially more fecund, although survival did not increase. For artificial diets incorporating leaf powder of either good (E. camaldulensis) or poor (E. platypus) food plants, the amino acids proline and valine increased larval performance compared with the respective base diets. However, although proline concentrations varied markedly in E. camaldulensis foliage, valine concentrations remained relatively constant throughout the study period.

The major influencing factor on the performance of U. lugens was the phenol quercetin, which decreased nitrogen assimilation to a much greater degree than other phenols studied (caffeic acid, chlorogenic acid and gallic acid).

In the food plant E. camaldulensis total nitrogen and phenols were negatively correlated. Total nitrogen reached its maximum level in early spring and declined to its minimum in winter but total phenols

reached maximum levels in winter and minimum levels in early spring.The variation of individual amino acids in E. camaldulensis foliage was also examined.

The results of this study are discussed in relation to existing hypotheses on insect host / plant interrelations and population dynamics and an alternative hypothesis is proposed.

DECLARATION

The work presented in this thesis is my own unless otherwise acknowledged, and has not previously been submitted to any university for the award of any degree or diploma. This thesis may be made available for loan or photocopying provided that an acknowledgement is made in the instance of any reference to this work.

Signed:

(J.D. Farr)

September 1985

ACKNOWLEDGEMENTS

I am extremely grateful to my supervisor, Dr. F.D. Morgan, for his untiring advice, criticism and encouragement given throughout the course of this study. I am also indebted to Dr. A.C. Jennings who gave advice on the biochemical aspects of this project including the procedure for extraction of amino acids, and Mr. G.S. Taylor who assisted in all aspects of this study.

Mr. D. Boehm operated the amino acid analyser and Mr. C. Rivers helped in the determination of total nitrogen. The experimental figures were photographed by Mr. B. Palk, and Mr. R. Coles photographed the front plate. Dr. R. Laughlin introduced me to the "Apple" computer on which this thesis was typed and printed, and Mrs. C. Schonfeldt photocopied the thesis. To all these people I extend my appreciation of their patience and excellent help.

I gratefully acknowledge the Univeristy of Adelaide for the award of an University Research Grant Scholarship during this study.

I also wish to thank my fellow students and members of the entomology department for their assistance, encouragement and friendship.

Chapter 1

Insect / Host Plant Relations-

Literature Review.

CHAPTER 1

Insect / host plant relations - Literature Review.

1.1 INTRODUCTION.

Although insects are well known to be amongst the most abundant and successful of terrestrial animals, the evolutionary path of the phytophagous insect species has not been an easy one. Less than a third of insectan evolutionary pathways have achieved the ability to feed on the Spermatophyta (Southwood, 1973). Dethier (1982) said:

"As primary producers, green plants are the synthesizers and bankers of biochemical wealth. Herbivores are the have nots, the pillagers of this biochemical wealth."

This characterises well the relationship between the phytophagous insect and its food plant.

The insect/plant relationship may essentially be considered in terms of shelter, food and transport. Plants provide food and/or shelter for insects, while the mobility of insects has led to the plant's dependence on insects for the transport of pollen and propagules. In utilizing green plants for food and shelter insects have had to overcome a number of problems: (a) surviving the extremes of temperature and dessication; (b) attachment to, and penetration of, what may be a tough, waxy, hairy or spiny surface; (c) locating a particular plant species amongst a great variety on which the insect cannot survive and (d) ingestion, consumption and digestion of a food source which may be

suboptimal for nutritional or other reasons (Southwood, 1973).

In this review on insect/plant relations the latter two "evolutionary hurdles", host selection and the provision of adequate nutrition, especially the role of nitrogen, will be considered with some emphasis on the physiology of the plant and its relationship to the growth and survival of insect leaf feeders.

Most adult insects are able to select their own diets, but many larval forms are influenced by the choice of food plant made by the ovipositing female. Such larvae however, in common with those that experience a greater or lesser degree of foraging are none-the-less endowed with the ability to identify a preferred diet. Whereas this ability may serve no apparent purpose in the more sedentary forms, it is as critical to free foraging larvae as to resultant dispersing adults. This is especially true in cases where the female may not oviposit on the food plant (Erlich and Raven, 1964) directly but in its vicinity. Some species may oviposit in the vicinity of the preferred host (eg. Ectropis excursaria Guenée) and others, such as some Hepialidae and Hisperiinae, eject eggs into space. Even in the situation where an ovipositing adult selects the food plant of its offspring, the larvae may still select actual feeding sites upon it or, in instances where the plant has been damaged or consumed by the time the eggs hatch, another plant must be found. If young are dislodged from their food they again must have the ability to find another food plant or perish. Thus recognition and selection of food plants and feeding sites is a function of the parent and the offspring (Dethier, 1959).

While vision, phototaxis, geotaxis and hygrotaxis all may play parts in directing insects to their oviposition and feeding sites, the ultimate forces working at close range and operating in the final recognition of preferred plants are largely chemical.

1.2 HOST PLANT SELECTION: an historical perspective.

It is usually assumed that the first insects, whose fossil record starts in the Carboniferous era (Smart and Hughes, 1973), were not plant feeders but were saprophagous (living on dead organic matter), the ability to deal with the greater problems of feeding on green plants evolved later. The chemicals which enabled these insects to detect their decomposing plant food were probably metabolites such as carbon dioxide, ammonia based compounds, alcohols and acids. These chemicals are generally related to respiration, fermentation and chemical breakdown associated with the decomposition of organic matter, and the insects responding to them probably lived in soil or leaf litter. The move from saprophagy to plant feeding is a fundamental step, one which probably occurred several times and is still actively debated in the literature (Smart and Hughes, 1973; Swain, 1978).

Harbourne (1977) stated that most plants are toxic. It had long been recognised that insects discriminate as to their host plants. One species of plant is not utilized by all phytophagous insects just as one phytophagous insect does not utilize all species of plants. Approaching the explanation to this phenomena Stahl (1888) suggested that a myriad of diverse botanical compounds, the metabolic functions of which were

obscure, probably afforded the plant's protection against attack by enemies. The earliest experimental evidence that compounds of this nature (often called secondary plant compounds) played this role was by Verschaffelt (1910) who investigated the effect of mustard oil glycosides on the acceptance and consumption of food plants by Pieris rapae (L.) and P. brassicae (L.). It is interesting to note that these compounds were found to stimulate insect feeding rather than preventing herbivory as originally postulated.

Verschaffelt's work directed the line of research for the next fifty or so years in the investigation of biochemical cues or token stimuli (secondary plant compounds of no nutritive value) for host selection (Dethier, 1941,1953; Thorsteinson, 1953, 1955, 1958 b; Fraenkel, 1959 a and b; Fraenkel et al, 1960; Nayar and Fraenkel, 1963; Nayar and Thorsteinson, 1963; Chambis and Jones, 1966; David and Gardiner, 1966). The presence of token stimuli were considered the major means by which insects chose their food plants despite a review by Dethier (1954) which discussed the question of whether host plant selection was guided by token stimuli or perception of plants that contained a satisfactory compliment of nutrients. Secondary plant compounds are often characteristic of botanical groupings and include particular glycosides, alkaloids, flavonoids and terpenes. Fraenkel (1959 b) argued that many secondary plant substances which now encourage feeding by particular insects are in fact evolved by plants to resist insect attack; insect races adapted to such compounds now accept them as recognition stimuli.

The emphasis thus far in host selection research had been on the role of feeding stimulants. Jermy (1958,1966) and Thorsteinson (1960) placed the attractive and stimulatory features of plant chemicals in balanced perspective by also directing attention to the importance of repellents and deterrents in host selection. Therefore since this early period the direction of research has changed toward an emphasis on deterrents with a consequent neglect of volatile attractive stimuli, and from individual token stimuli to the idea that a complex of compounds is involved in this recognition process (Thorsteinson, 1958 a and b; Schoonhoven, 1968,1982; Dethier, 1970, 1974; Alfaro, et al 1980).

Parallel to the study of token stimuli as phytochemical cues for insect host selection, existed the hypothesis that the perception of nutritionally adequate food is more important in the selection of host plants. Discussion and evidence for this hypothesis is scant, however it is considered to some degree by Painter (1953) and Kennedy (1953), and disputed by Lipke and Fraenkel (1956). Kennedy and Painter emphasised the fact that the favoured host plant is not merely something fed on, it is something lived on and in this respect looked at the possible role in the variation of the host plant's physiology and the influence of this on the performance and the fecundity of the insect. Examples were drawn from work with Aphis fabae Scop. where it was observed that female aphids settled and fed on leaves which produced a greater number of young (Kennedy 1953) and pea aphids (Macrosiphum pisi (Kltb.)) which produced more progeny on flowering branches than vegetative branches of alfalfa plants (Painter, 1953).

In the same light Fennah (1953) considered the influence of environmental variations including soil fertility and water status on the plant's physiology and thus the carbohydrate and amino-nitrogen content of the leaves. This was brought into perspective with the incidence of homopterans on their host plants and fluctuations in insect population. Unlike Fraenkel (1953, 1959 a) who expounded that all plants provided an adequate supply of nutrients to the insect, Fennah implied that plants may vary in their nutritional compliment and may not be nutritionaly adequate at all times. This idea could be supported by Wittwer and Haseman (1946) who found that thrips preferred spinach plants grown on a nutrient high in nitrogen.

Apart from such general approaches to the question of the role of nutrients in host selection, very little work had been done on the influence of individual nutrients on insect feeding behaviour. Thorsteinson (1953) found that certain nutrients enhanced the phagostimulatory effect of token stimuli and later reported that ascorbic acid and sucrose induced feeding in several diverse species of insects (Thorsteinson, 1958 a).

The use of artificial diets and electrophysiological studies played a major role in the investigation of nutrients as feeding stimulants (Davis, 1968; Schoonhoven, 1968, 1973; Vanderzant, 1974), for example sucrose has been demonstrated to be a phagostimulant for many phytophagous insects (Beck, 1956; Ito, 1960; Mittler and Dadd, 1963,1964,1965; Heron, 1965; Hsiao and Fraenkel, 1968; Cook, 1977; Hatfield et al, 1982). Variations in the concentration of sucrose in the diet has been found to influence the insect's response

(Thorsteinson, 1960; Hsiao and Fraenkel, 1968; Cobbinah et al, 1982; Hatfield et al, 1982). Insect feeding is proportional to the concentration of sucrose in the diet up to a point where the the insect's feeding response peaks with any further increase in the concentration of sucrose having little effect until at some higher level the feeding response declines.

Electrophysiological studies have revealed the presence of a sucrose receptor in all lepidopteran larvae investigated (Schoonhoven, 1973). The sugars glucose and fructose however are not consistent as phagostimulants but may often act synergistically with sucrose (House, 1977; Cobbinah et al, 1982).

Other nutrients have also been found to act as phagostimulants for insects: for example phospholipids (Thorsteinson and Nayar, 1963; Hsiao and Fraenkel, 1968); sterols, ascorbic acid and B vitamins (Thorsteinson, 1960: Beck, 1965); and amino acids (Auclair et al, 1957; Auclair, 1963; Beck and Hanec, 1958; Davis, 1965; Hsiao and Fraenkel, 1968; Schoonhoven, 1969; Cook, 1977; Hatfield et al, 1982; Sivastava et al, 1983). Alanine and aminobutyric acid have been found to stimulate feeding in pea aphids (Sivastava et al, 1983), the European corn borer (Beck and Hanec, 1958) and Pieris brassicae (Schoonhoven, 1969). Proline has been shown to stimulate feeding in P. brassicae (Schoonhoven, 1969), Locusta migratoria (L.) (Cook, 1977), and the spruce budworm Choristoneura fumiferana (Clem.) (Heron, 1965), however it inhibits feeding in the pea aphid Acyrthosiphon pisum (Harris) (Srivastava et al, 1983). Often an insect's feeding response is intensified by the combination of several amino acids (Davis, 1965) or by combining these

with some other substances such as sucrose (Mittler and Dadd, 1964; Heron, 1965), or phosphate buffer (Robins et al, 1965).

Phytophagous insects thus obtain detailed information about their food (Schoonhoven, 1968, 1973; van Emden, 1971). The complete compliment of a plant's chemistry may be involved in an insect's selection of a host plant. Kennedy and Booth's (1951) "dual discrimination theory" proposed that the sensory stimuli for insect feeding reponses were not solely plant secondary substances but included universal substances of fundamental nutritional importance to plants and insects alike, or at least token stimuli physiologically associated with universal substances. This theory was based on the observation that insects may discriminate not only between plant species but also within a species (see also Williams et al, 1983). Therefore it is evident that in a chemical context host selection depends on both the stimulatory or deterrent effect of secondary plant substances and the quantitative balance of a plant's nutritive constituents.

1.3 NUTRITION.

House (1966 a, 1969) and Gordon (1959) pointed out that the nutritional superiority of a food depends on its nutrient balance, that is, the proportion of nutritionally important substances in the food with respect to the proportions required by the insect. This is also supported by the work of Vanderzant (1963). Many phytophagous insects have adapted to their host plant and their composition over many millions of generations. The optimum amino acid mixture for the pink bollworm,

Pectinophora gossypiella corresponds closely to that of cotton protein (Vanderzant, 1958) and the content of essential amino acids in the bud anther of cotton is similar to the amino acid content of the boll weevil Anthonomus grandis grandis (Boheman) (Lindig et al, 1980). Nevertheless many problems of balance remain. Methionine, an amino acid relatively scarce in plants (Lord, 1968; Boyd, 1970), is an essential dietary requirement for most insects. The water soluble B-vitamins, especially thiamine, riboflavin, nicotinic acid, folic acid, pyridoxine, pantothenic acid, inositol, choline and biotin are also essential for insects (Dadd, 1963; Gothilf and Waites, 1968; Auclair, 1969; House, 1969), however plant tissues differ greatly in the quantitative and qualitative representation of these substances (Lord, 1968). Other studies have shown that differences in the relative concentrations of amino acids and glucose found between varieties of peas correlate with resistance to the pea aphid Acyrthosiphon pisum (Harr.) (Auclair et al, 1957; Maltais and Auclair, 1957).

It is also evident from the literature that different species of insects have different nutrient requirements. This is demonstrated by the investigations into amino acid nutrition reviewed by Chen (1966). Investigations have revealed that the ten "essential" amino acids which are known to be necessary for growth of mammals are also essential for insects (Lipke and Fraenkel, 1956; Singh and Brown, 1957; Kasting and McGinnis, 1958, 1962; Kasting et al, 1962; House, 1962). These amino acids are: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. However some exceptions have been noted. For example, tyrosine is essential for

Myzus persicae (Sulzer) (Strong and Sakamoto, 1963); Drosophila needs glycine (Hinton et al, 1951); while methionine, phenylalanine and threonine are not required by Blattela germanica (L.) (House, 1949; and see Gordon, 1959).

Thus it is seen from the extensive work of House (1965,1966,1969,1977) and others that nutrient requirements differ subtly from one species to the next, and that many insects, but not all (see Chew, 1980), eat the food most suited to their requirements.

As discussed earlier, Fraenkel (1953, 1959) suggested that green leaves are excellent sources of all food materials required by insects. Mittler's (1953) studies on aphid excretion, suggesting that the free amino acids and amides ingested by the aphids from phloem sap were in excess of the aphid's requirements, supported this view. However Gordon (1961) and Schoonhoven (1969) suggested that host plants are often nutritionally suboptimal. This opinion is supported by the work of Smith and Northcott (1951) who found that the grasshopper Melanoplus mexicanus (Sauss.) failed to develop normally on wheat when low soil-nitrogen levels led to low levels of leaf protein; and by Rodriguez (1951) who demonstrated that populations of the mite Tetranychus bimaculatus Harvey increased more rapidly on host plants supplied with abundant nutrients. The fecundity and reproductive rate of M. persicae and Brevicoryne brassicae (L.) were found to be directly correlated with the total soluble nitrogen present in brussels sprout plants (van Emden, 1966; van Emden and Bashford, 1971). Later studies have also found that larval survival, growth and adult fecundity increase on host plants high in nitrogen (McClure, 1979, 1980; Iheagwam, 1981; Myers and Post, 1981;

Rausher, 1981; Tabashnik, 1982). Using artificial diets containing a higher lysine-arginine ratio and higher total amino acid content than present in the natural food of the boll weevil Anthonomus grandis grandis, Lindig et al (1980) found the diets resulted in an increase in larval survival , growth, oviposition and hatch. Studying a mangrove ecosystem Onuf et al (1977), found a greater proportion of herbivory and greater abundance of lepidopterans on leaves and buds of trees growing in high nutrient sites richer in nitrogen; and Pradham (1971) has related that the application of nitrogen fertilizers to crops increases their susceptibility to insect pests.

It therefore appears from such evidence that nitrogen may be a limiting nutrient for insect growth.

1.4 NITROGEN AS A LIMITING NUTRIENT.

In the past decade many studies have examined the relationship between larval growth and plant nitrogen content (see reviews by Mattson, 1980; Scriber and Slansky, 1981). Most of these studies support the view that nitrogen is a limiting nutrient for larval growth (Slansky and Feeny, 1977; Fox and Macauley, 1977; Lincoln et al, 1982; Tabashnik, 1982), but there are exceptions (Schroeder and Malmer, 1980).

Plants generally contain lower concentrations of essential nutrients, particularly proteins and their constituent amino acids, than insects (Southwood, 1973; McNeill and Southwood, 1978). The mean protein content of a sample of nearly 400 different species of trees and shrubs

is only 13.4% of dry weight (Russell, 1947) however to be successful, synthetic diets for insects must contain 25% dry weight of protein or more and insects consist approximately of 50% dry weight protein (Southwood, 1973). Therefore obtaining an adequate and balanced intake of amino acids and proteins from the host plants on which they feed may be a finely balanced problem for herbivorous insects.

In considering this problem a number of authors have used a quantitative nutritional approach (Shroeder, 1977; Slansky and Feeny, 1977; Scriber, 1978; Schroeder and Malmer,1980; Auerbach and Strong, 1981; Scriber and Slansky, 1981; Tabashnik, 1982). This involves measuring the amount of food consumed, digested and assimilated, excreted, metabolized and converted into biomass (Waldbauer, 1968). Analysis of these measurements reveals how insects respond to different foods and which food components exert the greatest effects on growth.

The nutritional parameters and indicies of larval growth are summarized here (after Waldbauer, 1968; and Slansky and Feeny, 1977).

GR: Growth rate (mg/day) = biomass gained per day.

RGR: Relative growth rate = biomass gained per unit mean larval mass per day.

CR: Consumption rate (mg/day) = food ingested per day.

RCR: Relative consumption rate = food ingested per unit mean larval mass per day.

AD: Approximate digestibility or assimilation efficiency (%) = (food ingested - faeces / food ingested) X 100.

ECD: Efficiency of conversion of digested food (%) = (biomass gained / food ingested - faeces) X 100

ECI: Efficiency of conversion of ingested food (%) = (biomass gained / food ingested) X 100

NCR: Nitrogen consumption rate (mg/day) = nitrogen ingested per day.

NAR: Nitrogen accumulation rate (mg/day) = biomass nitrogen gained per day.

NUE: Nitrogen utilization efficiency (%) = (biomass nitrogen gained / nitrogen ingested) X 100.

RNCR: Relative nitrogen consumption rate = nitrogen ingested per gram mean larval dry mass per day.

RNAR: Relative nitrogen accumulation rate = biomass nitrogen gained per gram mean larval dry mass per day.

Using quantitative means in studying insect nutrition it becomes clear that together with water (Scriber, 1977,1979; Reese and Beck, 1978; Scriber and Feeny, 1979) nitrogen is one of the most limiting nutrient resources for larval growth (Slansky and Feeny, 1977; Scriber, 1978; Scriber and Slansky, 1981).

Working with fifth instar Pieris rapae on a variety of wild and cultivated food plants, Slansky and Feeny (1977) found that in general there was no correlation between plant nitrogen content and larval growth rate (GR). However as plant nitrogen content increased, consumption rate (CR) declined, and efficiency of conversion of ingested food or gross growth efficiency (ECI) increased together with an increase in assimilation efficiency (AD). Thus the relatively constant larval growth rate on plants with increasing nitrogen contents resulted from compensation between declining CR and increasing ECI.

Similar findings can be drawn from the results of Taylor and Bardiner (1968) working with the chrysomelid Phaedon cochleariae Fab. and the lepidopteran Putella maculipennis Curtis on turnip and radish. Both species were seen to have a greater CR and AD on older plants with a low protein content than on younger plants with a higher protein content.

It would be expected that compensatory changes of larval CR and ECI in relation to plant nitrogen content are likely to reflect compensatory changes at the level of nitrogen utilization. Slansky and Feeny (Ibid.) found that although CR decreased with increasing plant nitrogen the rate of consumption of nitrogen (NCR) increased with increasing plant nitogen content and by contrast nitrogen utilization

efficiency (NUE) declined. Therefore P. rapae maintained a relatively stable rate of nitrogen accumulation into larval biomass (NAR) due to the compensation between NCR and NUE.

The experiments using variation in nitrogen fertilization of food plants conducted by Slansky and Feeny (1977) showed a similar compensation between CR and ECI. Larval growth on the plants with the highest dose rates of fertilizer however, was exceptionaly high together with high values for ECI and NUE. This suggests that the higher GR and NAR of larvae on these plants resulted from an increase in feeding efficiency, the higher proportion of nitrogen present in the highly fertilized plants being in a more digestible form than the proportion typical of the other food plants.

Auerbach and Strong (1981) working with several insect species ranging from polyphagous to monophagous on a number of different host plants with variable nitrogen contents, found that the oligophagous species studied did not vary their relative consumption rates (RCR) with increasing nitrogen content in the host plant . Although AD and ECI were found to increase on hosts with a higher nitrogen content the nitrogen utilization efficiency (NUE) also increased. Therefore the nitrogen accumulation rate (NAR) was lower on the low nitrogen hosts which is reflected in the lower GR observed. Slansky and Feeny (1977) concluded that the stabilization of growth rates among larvae on different hosts is a consequence, not a cause, of stabilization of nitrogen accumulation rates. Therefore if P. rapae larvae were not able to maintain their homeostatic ability to stabilize NAR, they probably would have exhibited lower growth rates on low-nitrogen hosts, as observed by Auerbach and

Strong (1981) for the oligophagous species studied.

Slansky and Feeny's concept that stabilization of the nitrogen accumulation rate is achieved by larvae adjusting their feeding rates, implies an active process and also concerns the inverse relationship between gross growth efficiency (ECI) and consumption rate as observed by other authors (Soo Hoo and Fraenkel, 1966; Waldbauer, 1968). However Auerbach and Strong (1981) have demonstrated that similar NAR on hosts with different nitrogen levels can be fulfilled by other means.

Studying larvae of two monophagous beetle species with extremely long life cycles, Auerbach and Strong (1981) found that for one species (Cephaloleia consanguinea) there was no difference in consumption rates or ECI between the low and high nitrogen hosts. NAR remained constant due to lower nitrogen utilization efficiencies (NUE) on the high nitrogen plants. Chelobasis perplexa did exhibit an inverse relationship between CR and ECI, however the relative consumption rate (RCR) increased as the nitrogen of the host plant increased and ECI was seen to decrease. Therefore the constant NAR observed results from low NUEs on the high nitrogen plants. Auerbach and Strong proposed two possible explanations for this phenomenon. Firstly they suggested that nitrogenous compounds such as amino acids were functioning as feeding stimulants, high nitrogen compounds therefore inducing an increase in consumption rate which in turn resulted in a reduction in ECI. The second explanation mentions the possibility that other features of the high-nitrogen plants resulted in their being less efficiently converted to insect biomass by the monophagous species studied; therefore NAR stabilization could be achieved only through increased consumption rates. In this case the

higher consumption rates would be a consequence of low growth efficiencies (ECI).

Insects may therefore compensate for low nitrogen content in their diet by varying feeding rates and efficiencies of digestion. Other strategies however may also be employed to compensate for low and changing host plant nitrogen levels. These are discussed by Mc Neill and Southwood (1978) and include; (a) varying feeding sites on the same plant; (b) movements to different plants; (c) modification in the physiology of the plant; (d) nitrogen from other sources; and (e) phenological adaptions.

In examining the question of whether nitrogen is limiting in the natural diet of phytophagous insects, two forms of variation in nitrogen content have been considered: (1) variation among different host plant species; and (2) variation within a host plant species. The study of larval growth in relation to inter specific variation in nitrogen content has yielded much insight into plant-herbivore relationships, but interpretation of results can be complicated by differences among plant species other than nitrogen content. For example qualitative differences among plant species in secondary chemistry may effect larval growth (Soo Hoo and Fraenkel, 1966; Slansky and Feeny, 1977). This issue is moderated by studying larval growth in relaton to variation in nitrogen content within a host species. Fertilization experiments have been used to achieve wide ranges in intraspecific variation of leaf nitrogen content (Smith and Northcott, 1951; Slansky and Feeny, 1977; McClure, 1980; Auerbach and Strong; 1981). However as Tabashnik (1982) pointed out, the variations in nitrogen attained through these fertilization experiments

may be exaggerated in comparison with the range experienced in the field; or as mentioned by Auerbach and Strong (1981) and SLansky and Feeny (1977), the nitrogen fertilizer itself may alter the balance of free amino acids in the plant which in turn would influence the insect's feeding behaviour. Another aspect of using nitrogen fertilizer in such studies is its possible influence on the water content of the plant (SLansky and Feeny, 1977). Several studies have found that leaf water and nitrogen content are positively correlated, and that low leaf water content may limit larval growth (Reese, 1978; Scriber and Slansky, 1981). Addition of high levels of nitrogen fertilizer however, may lead to salt stress in the treated plants, influencing the nitrogen water balance. This problem was apparent in Auerbach and Strong's (1981) work which mentions the occurrence of salt stress symptoms on the leaves of the highly fertilized treatments.

Considering these problems Tabashnik (1982) studied the intraspecific field variation in leaf nitrogen content and its influence on larval growth using two species of Colias larvae on two species of legume. The response of Colias larvae to intraspecific variation in leaf nitrogen content were consistent with the broad interspecific trend that larval growth is positively correlated with leaf nitrogen content (Slansky and Feeny, 1977; Scriber and Feeny, 1979; Mattson, 1980; Scriber and Slansky, 1981). Some variation in larval response to different leaf nitrogen levels did exist however between the different species on vetch and alfalfa. For example: larvae fed vetch showed a strong negative correlation between RCR and leaf nitrogen content and a positive correlation between ECI and leaf nitrogen content, corresponding to

Slansky and Feeny's (1977) findings; whereas larvae fed on alfalfa did not alter their consumption rates as leaf nitrogen varied, which is comparible to studies by Schroeder and Malmer (1980) and Auerbach and Strong (1981). Tabashnik concluded, as did Slansky and Feeny, and Auerbach and Strong, that nitrogen is a limiting nutrient for the growth of Colias larvae, even though the two species were fed on legumes which have a high nitrogen content compared to the host plants of most other herbivoes (see Scriber and Slansky 1981). Schroeder and Malmer (1981), working with 34 species of Hymenoptera and Lepidoptera where their larvae were fed leaves of black cherry, concluded otherwise, stating that if nitrogen is limiting it most likely would be manifested as an imbalance of required amino acids.

Auerbach and Strong (1981) have pointed out the possibility that low plant nitrogen content may be a form of plant defence. If an herbivore has equivalent growth rates on hosts with different foliar levels of nitrogen as a result of increasing its consumption rate on low nitrogen plants, then low nitrogen plants suffer more feeding damage. However in some cases the nitrogen content may be too low for any stabilization of nitrogen accumulation, which would result in lower growth rates, survival and fecundity. The feeding time must also be considered in relation to a low nitrogen diet since increased feeding time often represents a period of increased susceptibility to parasites and predators for phytophagous insects (Hassel and Southwood, 1978).

The nitrogen and water content of tree foliage is much lower than in herbaceous species (Scriber and Feeny, 1979; Scriber and Slansky, 1981). The maximum RGR of larvae confined to mature tree leaves

(with water generally less than 70% and nitrogen usually less than 3%) is retricted to generally half that observed for herbaceous plant feeders (Scriber and Feeny, 1979). Thus it is considered that tree leaves are a poor substrate for rapid and or efficient growth in comparison to herbs, due to low AD. Unlike some herb feeders, tree feeders seem limited in their ability to compensate for low AD by increasing RCR or ECD (Scriber, 1978). Larvae feeding on herbaceous or ephemeral plants have been found to feed faster and grow more rapidly than larvae feeding on perennial shrub or tree leaves, taking less time to complete each stadium (Scriber and Feeny, 1979) having higher NAE and NAR (Rhoades and Cates, 1976). Herbaceous plant feeders appear more efficent at converting food and nitrogen into biomass (Scriber and Feeny, 1979).

1.5 FEEDING EFFICIENCIES OF SPECIALISTS AND GENERALISTS.

A number of authors have suggested that herbaceous plant feeders tend to be monophagous or oligophagous whereas insect species feeding on shrub or tree leaves tend toward polyphagy (Brues, 1924; Futuyama, 1976; Slansky, 1976; Scriber and Feeny, 1979). It has been postulated that the evolutionary gain for dietary specialization is an increase in food use efficiency (Dethier, 1954; Gordon, 1959; Waldbauer, 1968; Krieger et al, 1971; Whittaker and Feeny, 1971;) which may be due to an appreciable reduction in metabolic costs, for example in tolerating defensive chemicals present in the food plant (Feeny, 1975). Tests of this hypothesis however have produced conflicting results. Rhoades and

Cates (1976) and Auerbach and Strong (1981) concluded that the degree of specialization of a phytophagous insect did influence food use efficency. Futuyama and Wasserman (1981) and Schroeder (1976, 1977) however did not find that feeding specialization resulted in greater efficiencies of resource utilization (see also Futuyama et al, 1984).

To determine whether efficiency of utilization of a host plant depends on the degree of feeding specialization, plant growth form must also be considered, since as previously discussed, evidence suggests that shrub and tree species are utilized less efficiently than herbaceous or ephemeral plants. The question is further complicated by different races of an insect species which are regionally specialized on different hosts. Individuals from one geographic range may be better adapted than individuals from another part to a particular food species which is locally abundant or one on which larval survivorship is relatively high (Brues, 1924; Gilbert and Singer, 1975; Morrow, 1977; Hsiao, 1978). Cates (1981) found that some populations of widely distributed polyphagous species are more specialized in their diet than host plant lists would suggest.

Considering these influences Scriber and Feeny (1979) concluded that although specialist feeders did appear in some cases to utilize their host plants more efficiently, other examples existed where generalist species seemed capable of growth rates comparable to those of specialist feeders. Therefore larvae of generalist feeding habits are not necessarily less efficient than species with more restricted feeding habits in exploiting many of their food plants. They suggest that the "metabolic load" of detoxifying capacity carried by some generalists

(Krieger et al, 1971; Brattsen et al, 1977; Schoonhoven and Meerman, 1978) has by itself only a small effect on larval growth rates. This view is supported by both Bernays (1978), who indicated that digestion in grasshoppers is not reduced by the addition of tannin to the diet; and Morrow and Fox (1980) who found that levels of leaf nitrogen rather than oil content better explained the variation in herbivore growth on Eucalyptus. However since generalists are by definition likely to attack a greater and chemically more diverse range of food plants, they are less likely than specialists to be equally well adapted to growth on all their food plants (Waldbauer, 1968). It therefore follows that individuals of generalist species are more likely than specialists to occur on plants on which they are relatively inefficient. The specialized physiological adaptions to the host in host-specific insects may be the consequence rather than the cause of host specificty (Futuyama et al, 1984).

1.6 PLANT APPARENCY OR ESCAPE IN SPACE AND TIME

Rhoades and Cates (1976) and Feeny (1975, 1976) have suggested that a general relationship may exist in nature between the diversity of mechanical and chemical defences in plants (or particular plant tissues) and the "predictability and availabilty" of a plant resource in space and time (Rhoades, 1979). For example the dominant trees of a temperate zone climax forest are according to Feeny (1975), "bound to be found" by insects in ecological time, owing to their large size, long life relative to that of insects, and possibly also as a result of lower vegetation diversity. These plants are therefore classified as "apparent".

Herbaceous or ephemeral plants however, characteristic of early stages of community succession, are faster growing and have a shorter life span. These plants are therefore likely to be less predictable as a food source and relatively "hard to find" by herbivores. Relying primarily on escape in time and space as a defence strategy such plants are classified as "unapparent".

Unapparent plants and plant tissues are normally characterised by qualitative defences such as those which disrupt the metabolic processes of herbivores, and include compounds such as alkaloids, pyrethrins, cardiac glycosides, cyanogenic glycosides, saponins, non-protein amino acids and insect hormone analogues (Rhoades and Cates, 1976). These plant toxins are generally present and active in small quantities, and are cheap to produce in terms of metobolic cost to the plant. However the qualitative plant toxins are vulnerable to counter adaption by insects and may be used as attractants or sequestered as defence systems by more specialized herbivores (Fraenkel, 1959, 1969; Rothschild, 1972; Freeland and Janzen, 1974; Dowd et al, 1983). Scriber and Feeny (1979) found that larvae of the southern army worm grow equally well on cyanogenic and acyanogenic varieties of their host plant. Insects that can detoxify such defence compounds, are then influenced by the nitrogen levels in the food plant (van Emden, 1972, 1973). Larvae of monophagous and oligophagous insects tend to prefer young leaf tissues (Cates, 1980) even though they are highest in qualitative defences, because they are easier to digest and normally contain higher relative nutrient and water concentrations (Feeny, 1976; Rhoades and Cates, 1976; Cates and Rhoades, 1977; Scriber and Feeny, 1979). This view is disputed

with good examples of slected feeding induced by antifeedants or unacceptable levels of certain compounds by researchers on pine sawflies (Ikeda et al, 1977). Unapparent plants are r-selected, short lived and continually producing new growth or seeds, consequently there is usually some region of the plant at any one time that contains an adequate supply of nitrogen for the herbivores. Despite the availability of food during the growing season of an ephemeral plant (or plant tissue) escape in space and time would probably be successful against specialist herbivores since they lack an alternative food source. Specialist herbivores must allocate time and energy into the search for their food plant. Therefore the more ephemeral the resource the greater will be the specialist herbivores mortality during the search (Chaplin, 1980). This defence strategy however would not be effective against generalist herbivores. Since generalist herbivores are by definition "less highly co-evolved" with any given host plant, selection pressure would favour a wide diversity of toxic defence chemicals in the ephemeral plants (Cates and Rhoades, 1977). Therefore the distribution of plant defence compounds may depend on herbivore feeding pressures (Rhoades and Cates, 1976; Cates and Rhoades, 1977; Rodriguez, 1977).

In contrast, apparent plants or plant tissues are exposed both in space and time; they are long-lived and grow in association with large numbers of the same species. Besides general physical defence methods such as hairs, silica and tough fibrous leaves (Denno and Donnelly, 1981) an important defence for these plants is the lowering of the levels of available nitrogen, or making the essential flushes of nitrogen as short and unpredictable in time and space as possible (McKey, 1974; Rhoades and

Cates, 1976; Mooney et al, 1980). Apparent resources often contain high concentrations of dosage dependant quantitative defences such as phenols, tannins, resins and refractory carbohydrates which act within the gut of the herbivore to reduce the availability of plant nutrients. Quantitative defences are thought to be the most difficult for all herbivores to overcome but may be particularly directed against monophagous and oligophagous herbivores (Feeny, 1976; Rhoades and Cates, 1976; Cates and Rhoades, 1977; but see Fox and Macauley, 1977; and Bernays, 1978). Cates (1980) found that the tough, mature, less nutritious woody perennials were preferred by polyphagous insects. Denno and Donnelly (1981) also found that specialist herbivores avoided the tough mature leaves of apparent plants. Working under a range of laboratory and field conditions a number of investigators have observed greater levels of herbivory on young leaves in both apparent and unapparent plants (Kennedy and Booth, 1951; Feeny, 1970; Reichle et al, 1973; Hamilton et al, 1978; Ives, 1978; Milton, 1979; Oates et al, 1980; Colely, 1980, 1983).

Such observations would seem to support the hypothesis that young leaves of both apparent and unapparent plants are less well defended chemically and physically than mature leaves (McKey, 1974; Feeny, 1976; Rhoades and Cates, 1976). Investigations of temperate species have revealed that young leaves contain lower concentrations of quantitative defences such as tannins, although they may have higher concentrations of qualitative toxins (Feeny, 1970; Dement and Mooney, 1974; Lawton, 1976; Rhoades and Cates, 1976; Mckey, 1979). Although Macauley and Fox (1980) found no variation in the tannin concentration of Eucalyptus leaves with age, young eucalypt leaves are more available

(i.e. apparent) to their herbivores than are for example young leaves of oak, since they are initiated several times during the growing season. However research on tropical species has revealed higher phenol and tannin concentrations in young leaves compared to mature leaves (Milton, 1979; Oates et al, 1980; Coley, 1983).

Plant secondary compounds, and particularly phenols, have been considered as major defences against herbivores (Whittaker and Feeny, 1971; Levin, 1971, 1976; Rhoades, 1979). However a number of studies have found no correlation between herbivory and phenol or tannin content (Fox and Macauley, 1977; Balick et al, 1978; Bernays, 1976; Morrow and Fox, 1980; Oates et al, 1980; Coley, 1983). The recent literature suggests that the importance of plant secondary compounds in defence may have been overemphasised (Bernays, 1981). Leaf toughness and quality may play a more important role in herbivore grazing patterns than previously realised (Scriber, 1978; Scriber and Feeny, 1979; Scriber and Slansky, 1981; Coley, 1983). Coley (1983) has therefore proposed an alternative hypothesis to the plant apparency model, suggesting that habitat quality determines the type and extent of a plant's commitment to anti-herbivore defences.

The variation in insect / plant interactions indicate that generalisations, as with the plant apparency model, have many exceptions. For example the spondyliaspids Cardiaspina spp. and Eucalyptolyma maideni Frogg. and the lepidopteran Uraba lugens (Walk) are specialist feeders on Eucalyptus, feeding on mature leaves (Cobbinah, 1983; Morgan, 1984). The plant apparency projection appears to be an extrapolation from specific cases to a general conceptual model. Extending the postulates made in

relation to specific and general defence for plant apparency, it follows that early successional or unapparent communities should be primarily composed of chemical specialists, whereas climax or apparent communities should be primarily composed of chemical generalists (Feeny, 1976; Rhoades and Cates, 1976). These predictions however are not consistent with observations made by Levin (1976 b) who found from a world wide floral sample that 33% of annuals contained alkaloids compared with 20% of the perennials.

Although the plant apparency model has been useful in considering the relationships between plant defence allocation and insect herbivory it should be viewed with caution in attempting to apply a general rule to insect / plant interrelations. Plant apparency may of course be due more to the fact that commonness, density and variability of a plant family or genus such as the Myrtaceae or Eucalyptus have provided a range of usable substrates stimulating the evolution of predators to take advantage of them. Non-apparency by contrast may be due to the reverse relationship, for example Ficaceae and Ficus in particular. The number of herbivores on Eucalyptus greatly outnumber those on Ficus in Australia, possibly due to the differences in the areas colonised by the indigenous species together with the possible differences in the chemical defences of both groups.

The more profitable approach is perhaps, one that considers the basis for viable interactions between food plants and monophagous, oligophagous and polyphagous herbivores with the prime objective being to understand the relationships and from this basis, to speculate upon the development of it. There is no doubt that many insect herbivores have

essentially similar requirements of their food plants and some of these requirements tend to be so similar as to question the niche concept (Elton, 1927). Good examples appear to be the scolytid bark beetles, many species of which, compete for the same tissues of the same food plant. Yet it is known that even closely related species use different components of the general complex of volatile signals that issue from the food plant in finding their host (Perttunen, 1957; Chapman, 1963; Atkins, 1966; Rudinsky, 1966) and moreover convert certain of these compounds present to communicate with their own species (Anderson, 1948; Wood and Vite, 1961; McMullen and Atkins, 1962; Chapman, 1966; Pitman, 1966).

Different insect species on a similar range of host plants may develop a successional relationship possibly stimulated by changes in the food plant's physiology. This can be demonstrated by considering an insect host plant complex such as that of Eucalyptus camaldulensis Dehnh.. Miles et al (1982 b) found that consumption of E. camaldulensis leaves by Paropsis atomaria Oliver resulted in an increase in the phenol content of the eucalypt's leaves and a decrease in the nitrogren content. Wounding has been found to increase the activity of phenylalanine ammonia-lyase (PAL), the first and limiting enzyme in the pathway of phenolic biosynthesis (Wong et al, 1974; Bidwell, 1979). Other authors have also found similar relationships between defoliation and the nitrogen / phenol ratios (Benz, 1974; Wratten et al, 1981; Haukioja, 1982; Schultz and Baldwin, 1982; Edwards and Wratten, 1983; Tuomi et al, 1984). It is therefore possible that as the physiology of the plant changes with the impact of herbivory, the fauna associated with the plant will also change. For example, in the case of E. camaldulensis a

predominance of flush feeders such as Glycaspis brimblecombei Moore and P. atomaria may give way to insects often found on moderate phenol / nitrogen herbage such as Cardiaspina albitextura Taylor (Morgan, 1984), Uraba lugens (Cobbinah, 1978) and Perga spp. These latter insects being able to tolerate or even utilize the proportions of secondary plant compounds in relation to nitrogen present in their food. It appears that the proportion of nitrogen to plant secondary compounds (see: Lunderstadt and Reymers, 1980; Williams et al, 1983), or the balance of physiological compounds within a plant may determine a successful faunal association. If insect herbivores co-evolve with their host plants and become adapted or "conditioned" to the nutrient levels present in their food then the selection of feeding sites apparent in nature may be adequately explained where no physical determinants are present. Thus in many instances the balance between nutrients, phagostimulants and feeding inhibitors present in the different foliage classes on a tree may provide a range in their proportions that permit a choice for each grazer. This allows for changes in food preference with age of herbivore (Isman and Duffey, 1982) and may constitute a basis for lack of competition for food resource between predators of the same food plant.

1.7 ENVIRONMENT AND PLANT PHYSIOLOGY.

Much work has been done on environmental influences on the physiology of the plant especially in the realm of water stress on nitrogen metabolism. Kemble and Macpherson (1954) working with perennial rye grass, were the first researchers to find a dramatic increase in

proline during wilting. Other researchers followed, finding that "water stress", namely drought or wilting, induced significant increases in the free amino acid content of the plant especially proline, and a decrease in the plant's protein content (Chen et al, 1964; Barnett and Naylor, 1966; Durzan and Ramaiah, 1971; Stewart, 1972 a and b; Hsiao, 1973; Singh et al, 1973 a and b; Blum and Ebercon, 1976; Boggess et al, 1976; Ferreira et al, 1979; Hanson et al , 1979; Huang and Cavalieri, 1979; Dubetz and Gardiner, 1980; Jones et al, 1980; Stewart and Hanson, 1980; Fukutoku and Yamada, 1981; Labanauskas et al, 1981; Tanabe et al, 1982). Further work showed that the response of the plant to environmental stress such as heat and salinity closely paralleled that of water deficit (Chu et al, 1974, 1976; Huber, 1974; Treichel, 1975; Liu and Hellebust, 1976; Bar-Nun and Poljakoff-Mayler, 1977; Wyn Jones and Storey, 1978; Cavalieri and Huang, 1979, 1981; Jefferies et al, 1979; Lawlor, 1979; Spyropoulos and Lambris, 1979; Tatt, 1980; Labanauskas et al, 1981; Cavalieri, 1983; Dreier, 1983). A number of recent reviews on this subject outline the physiological impact of water stress on the plant (Hanson, 1980; Stewart and Larher, 1980; Turner and Kramer, 1980; Paleg and Aspinall, 1981; Hanson and Hitz, 1982). Water stress has been shown to decrease photosynthesis (Lawlor, 1976; Liu and Hellebust, 1976; Lawlor and Fock, 1977, 1978; Naidenova, 1979; Benecky, 1980; Ludlow et al, 1980; Osonubi and Davis, 1980; Bhardwaj and Singhal, 1981; Dougherty and Hinckley, 1981) and respiration (Hsiao, 1973; Brown and Thomas, 1980). In response to water stress the accumulation of abscisic acid (ABA) a potent growth inhibitor (Wright, 1969; Wright and Hiron, 1969; Mizrahi et al, 1970), is thought to induce the dramatic increase in proline observed in

the plant (Aspinall et al, 1973; Aspinall, 1980). Proline is also thought to accumulate in plants due to four main changes in the plant's metabolism: (1) stimulation of proline synthesis from glutamic acid; (2) inhibition of proline oxidation; (3) the incorporation of free proline into protein is impaired, and (4) the export of proline via the phloem is reduced (Hanson, 1982). A number of authors have hypothesised that the possible value of proline accumulation is as a nitrogen-store, energy store, or a NH_3-detoxification product (Stewart and Hanson, 1981). However combining physiological and genetic approaches, some researchers have suggested that accumulation of proline may be an incidental response to water stress (Wyn Jones and Storey, 1978; Hanson, 1982).

In contrast to the influence of environmental stress or water stress on the nitrogen metabolism of the plant, studies on the influence of environment on secondary plant metabolism is sparse. Vaadia et al (1961) stated that, in studying plant water deficits and physiological processes, greater emphasis should be given to the relation between water deficits and metabolism. This is certainly still true today. Although much more is known on the implications of water stress on the "primary" nitrogen and carbohydrate pathways, very little is known of the influence on production of secondary plant compounds (with the exception of plant wounding). This is perhaps due to the early concept that secondary plant compounds were indeed just that - anomalies of plant metabolism which just happened to repel herbivores. However recent work has shown that the term "secondary plant compound" should be revoked as a misnomer (Seigler, 1977) and that these compounds should, more appropriately be designated as allelochemics. With the advent of improved biochemical techniques such

as gas liquid chromatography, gas chromatography - mass spectrometry and high pressure liquid chromatography, this gap in our knowledge of plant metabolism may be resolved in the near future.

One aspect of plant "secondary metabolism" is its metabolic cost to the plant. If the metabolic cost to the plant for the production of secondary plant substances is high as suggested by Levin (1971) and McKey (1979), it would be logical to assume that environmental stress, such as water stress or high temperature, would result in a decrease of these compounds due to the observed decrease in photosynthesis and respiration as suggested by Rhoades (1979) and Temple (1981). The evidence for such metabolic costs are however circumstantial and are discussed by McKey (1979).

In considering nutrient stress it has been found that a well nourished plant is more tolerant of disease (Graham, 1983). However in the case of soil nitrogen, limiting nitrogen conditions induces synthesis and accumulation of phenolics and alkaloids, while supraoptimal soil nitrogen results in the reduction of phenol and alkaloid synthesis (Hoque, 1982; Graham, 1983). It therefore appears that phenol and nitrogen metabolism are negatively correlated. However in considering this information one cannot extrapolate to the implications that since in a water stressed plant the free amino acids increase, the phenols will therefore decrease, because water stress decreases protein synthesis (see Parker and Patton, 1975). The answer therefore lies in the understanding of secondary metabolism and its cost to the plant.

Mukherjee and Choudhuri (1981) found that water stress increaseed the activity of indoleacetic acid (IAA) which is known to inhibit phenylalanine ammonia-lyase (PAL) an important enzyme involved in the synthesis of a number of plant defence compounds (Bidwell, 1979). Inhibitors of protein synthesis are also known to prevent the increase of PAL (Bidwell, 1979). It would therefore be expected from such evidence that water stress may result in a decrease in plant secondary compounds. This has been observed by Miles et al (1982 b) and implied by Temple's (1981) research on bracken fern. Hence the concept, that a plant under physiological stress may by virtue of a decrease in its ability to produce sufficient of its protective chemicals, becomes vulnerable to its enemies. Graham (1983) has indicated that relatively small changes of this kind in a host plant may result in a considerable effect on the severity of a disease affecting it.

1.8 ENVIRONMENTAL STRESS AND INSECT OUTBREAKS.

From the preceeding literature discussed it appears that an insect's "performance" on a host plant, is, to some extent, dependent on the quality of food available and this, in turn, may be influenced by environmental factors. A number of authors have suggested that the success or capacity for increase of an insect is dependent on the quality of its food resource (Davidson and Henson, 1929; Evans, 1938; Rawlings, 1953; Clark, 1962, 1963; House, 1962, 1965, 1966 a and b; van Emden, 1969; Kimmins, 1971; van Emden and Way, 1973; Southwood, 1973; Williams et al, 1983) and this has been discussed at some length in previous

sections of this review.

White (1966, 1974, 1976, 1978) proposed that plant stress or water deficit increases the "available nitrogen" in the plant which in turn increases the survival of the first instar of insect herbivores resulting in an outbreak of the insect. Occasions of increased insect performance such as lower mortality, increased development rates and higher fecundity have recently been recorded on plants supplied with nitrogen fertilizers (Rahier, 1978; Sementer et al, 1980; McClure, 1980; Myers and Post, 1981; Prestige, 1982). However the influence of a supraoptimal supply of nitrogen on plant phenolics, as described by Graham (1983), must be considered when assessing such data. Insect performance has also been found to increase when considering favourable field variations in nitrogen content (Kimmins, 1971; Fox and Macauley, 1977; Onuf et al, 1977; Webb and Moran, 1978; Myers and Post, 1981; Tabashnik, 1982). Nevertheless there is a paucity of evidence for the induction of insect outbreaks or increased survival as a consequence of plant water stress. The evidence available in many records is more coincidental or circumstantial than factual. See: Wellington et al 1950; Taylor, 1955; Van der Laan, 1959; Grimalskii, 1977; Wenz, 1977; West, 1979; Mason, 1981; Moriondo and Covassi, 1981; von Seitschek, 1981; who recorded incidences of disease outbreaks associated with the occurrence of drought or abnormally low rainfall.

Working with aphids it has been found that water stress may produce negative effects on their performance such as reduced feeding, increased number of alatae, decreased survival and fecundity (Kennedy, 1958; Kennedy et al, 1958; Kennedy and Booth, 1959; Sumner et al, 1983).

However using aphids or phloem feeding insects to test the influence of plant water deficiency on insect populations leads to difficulties in interpreting the results, since these insects rely on plant turgor pressure for feeding, and lower plant turgor pressure will reduce the uptake of food (Kennedy et al, 1958; Kennedy and Booth, 1959). Consequently a number of conflicting results have been reported concerning the performance of aphids on water stressed plants. De Vries and Manglitz (1982) found no variation in the survival and reproduction of the spotted alfalfa aphid (Therioaphis maculata Buckton) on droughted lucerne, and Wearing (1972) found that intermittent water stress increases the survival and fecundity of Myzus persicae and Brevicoryne brassicae, as opposed to the general detrimental effects of continuous water stress.

Very little work has been done using chewing phytophagous insects. Haglund (1982) reports that proline and valine, amino acids which commonly increase in water stressed plants, increases feeding in grasshoppers. Contrary to these findings Bright et al (1982) found no increase in the feeding or performance of a number of insects, snails and powdery mildew on high proline barley varieties, and Miles et al (1982 a and b) found no increase in survival, rate of development and fecundity of Pieris rapae and Paropsis atomaria on water stressed plants.

Research has shown that insects accept a range of nutrient concentrations in their food (Auclair, 1965; Dadd and Mittler, 1965; van Emden, 1966; Dadd and Krieger, 1968; Mittler and Klienjan, 1970; van Emden and Bashford, 1971; Beck, 1974; Cobbinah et al, 1982). Variations beyond the acceptable range of host nitrogen content may be

disadvantageous (Carrow and Graham, 1968) unless insects have been preconditoned to accept them (Cobbinah et al, 1982), or are able to vary their ingestion rates to accommodate the change in their food (Slansky and Feeny, 1977; Miles et al, 1982 a and b). Low nitrogen levels may be critical to insects adapted or conditioned to a high nitrogen diet and high nitrogen critical to insects normally feeding on low nitrogen foods. This may explain the variation in response to water stress observed between the two aphid species studied by Wearing (1972).

In view of the contradictions apparent in the considerable body of research attempted on these matters and the problems with such a general theory by White (1966), I decided to select a test animal that was oligophagous within the Myrtaceae. Additionally I wanted one which had been shown to respond mainly to secondary plant compounds such as antifeedants in selecting its food and moreover, one which might demonstrate a number of survival curves indicated by Slobodkin (1962). Uraba lugens (Lepidoptera: Nolidae) demonstated all of these charactistics and, as well fed reasonably well on artificial diets (Cobbinah, 1978). This enabled me to examine stress in the food plants on the survival, fecundity and growth rate of U. lugens and to test the influence of nitrogen components in the diet both upon Slobodkin I and Slobodkin IV survival curves. In the former, increased available nitrogen could be tested for an increase in survival of the late instars where in nature, most mortality is recorded for U. lugens. In the latter I could determine whether increased nitrogen could compensate for observed mortalities where a diet was in some way inadequate. By these researches

I hoped to be able to indicate clearly where and at what level, if any, increased nitrogen affected survival and the other biological parameters I might wish to examine. My reason being that if nitrogen did influence U. lugens in a way similar to that suggested by White (1966) for other insects, then it may well be, at some critical level, a rise in total nitrogen that might be involved whereas at another, perhaps higher level, the influence could be found in sensitive increases of particular amino acids necessary to insect nutrition (Dadd and Mittler, 1965; House, 1965, 1969; Dadd and Krieger, 1968).

Chapter 2

Introduction

Figure 2.1.1.

Eucalyptus microcarpa in South Australia subjected to continual damage by *Uraba lugens*.

CHAPTER 2

Introduction.

2.1 THE BIOLOGY OF URABA LUGENS WALK.

The gum leaf skeletonizer Uraba lugens Walk. is an important defoliator of many Eucalyptus species and certain closely related species in the genera Angophora and Tristania. Since the insect was first described by Walker (1863) its taxonomy has been subject to considerable tergiversation as discussed by Cobbinah (1978). Thirty years prior to Common's (1975) revision of this species it was known as Roeselia lugens (Wlk.) and before that, had been assigned to a variety of different names (Seitz, 1933; Cobbinah, 1978).

Uraba lugens has been recorded in all Australian states, outbreaks of severe defoliation of host plants being recorded in New South Wales, Queensland, South Australia (fig. 2.1.1), Victoria and Tasmania (Brimblecombe, 1962; Campbell, 1962; Harris, 1974) and Western Australia (Morgan pers. comm.,1984). In South Australia U. lugens is widespread occurring from sea level to about 1000m elevation and from coastal to inland and riverine habitats (Morgan and Cobbinah, 1977). Campbell (1962, 1966, 1969) recognised two biological forms of the species in New South Wales. The highland form which has thirteen larval instars and eggs are laid in a flat raft of up to 200 with adjacent eggs touching; and the coastal or lowland form which has eleven larval instars, eggs are laid in masses of up to 100 and arranged in parallel rows one egg diameter apart. Morgan and Cobbinah (1977) suggested that the two

Figure 2.1.2.

Biology and feeding patterns of *Uraba lugens* during initial instars.

(a) Egg batch of *U. lugens* on *Eucalyptus camaldulensis*.

(b) Feeding pattern of first instar larvae after eclosion.

(c) Feeding damage by first and second instar larvae on *E. camaldulensis*. Also demonstrates the gregarious nature of early larval instars.

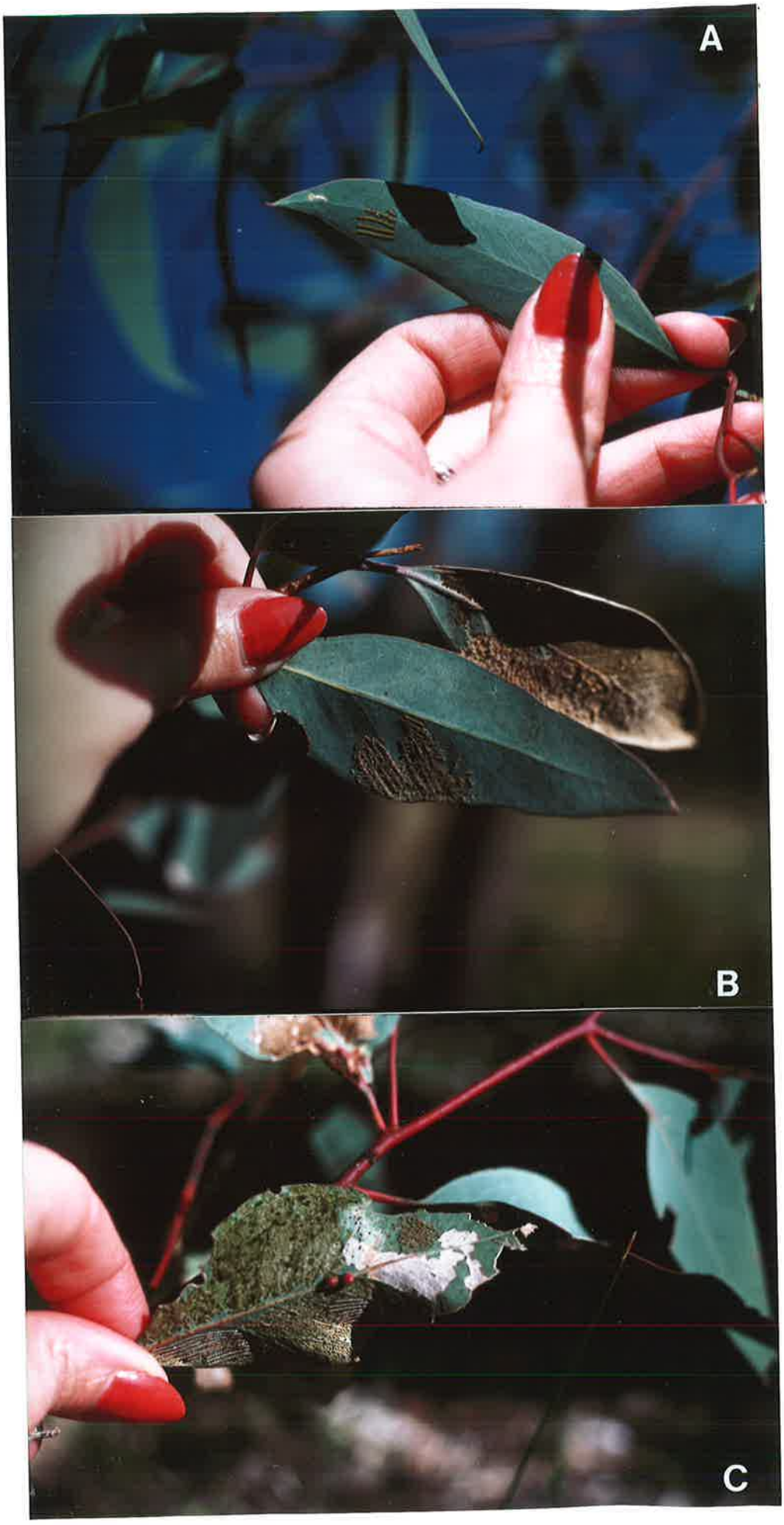

A
B
C

Figure 2.1.3.

Biology and feeding patterns of *Uraba lugens*.

(a) Second and third instar larvae feeding on *Eucalyptus camaldulensis*.

(b) Skeletonization of an *E. microcarpa* leaf following eclosion. Also note the presence of larval moulting casts.

(c) Fifth and sixth instar larvae plus moulting casts.


A
B
C

Figure 2.1.4.

Feeding damage by *Uraba lugens* on *Eucalyptus camaldulensis*.

(a) Skeletonization by initial instars (1st - 5th), known as flagging.

(b) Defoliation of a sapling by late instar larvae.


A


B

biomorphs described by Campbell may be temperature induced. The biological form of U. lugens in South Australia has characteristics ascribed to both biomorphs. The larvae have an average of eleven instars ranging from eight to thirteen, while egg batches range in size from 20 to over 500 (Cobbinah, 1978) and are arranged in essentially parallel rows where they may be almost touching to more than one egg diameter apart (fig. 2.1.2 a).

The insect is bivoltine in all regions of Australia except Western Australia where it is univoltine (Curry pers. comm. 1984). The winter generation is completed in late October to early December and the summer generation is completed within the period of March to May. Univoltine insects are adults in February / March.

The egg incubation period is dependant on temperature and varies from three to four weeks in summer and four to six weeks in winter (Morgan and Cobbinah, 1977; Cobbinah, 1978). The larvae of U. lugens are overtly gregarious during the first to fourth stadia (fig. 2.1.2 c), skeletonizing the leaves around the egg mass immediatly after eclosion (fig. 2.1.2 b) and later extending their feeding over the remainder of the leaf (figs. 2.1.2 b,c and 2.1.3 a, b, c) The skeletonizing habit of the larvae persists to the fifth stadium, when individuals move to feed on leaf edges (fig. 2.1.4 b) accompanied by a breakdown in the gregarious behaviour pattern apparent in the earlier larval stages. Larval development is completed in 60 to 85 days during summer and 130 to 180 days in winter (Morgan and Cobbinah, 1977). Pupation occurs mainly in the soil however pupae have also been observed under bark or on leaves of the host tree. Pupal duration also varies with temperature, laboratory

observations have revealed that at 15°C the pupal period is approximately 26 days whereas at 28°C the pupal period is decreased to 10 days (Morgan and Cobbinah, 1977).

The adults of U. lugens do not feed. The females oviposit on a wide range of Eucalyptus and related species (Brimblecombe, 1962; Campbell, 1966; Cobbinah, 1978) however survival of the larvae on the hosts selected is not assured (Morgan and Cobbinah, 1977). The majority of "egg hosts" are either unsuitable for larval establishment or the larvae that begin feeding do not survive to adults. Acceptance of any "egg host" by larvae normally occurs soon after eclosion and may vary from ready acceptance and mass feeding to feeding that is not sustained after initial tasting followed by larval dispersion. Thus the egg hosts of U. lugens include highly suitable plants on which larval survival is high and less suitable plants on which larval survival is low or non-existant.

2.2 FOOD PLANTS OF U. LUGENS

The host plants of U. lugens can therefore be divided into three categories:

(1) Good host plants, high survival (greater than or equal to 80%) of 1st - 4th instars (figs. 2.2.1 and 2.4.1 e,f).

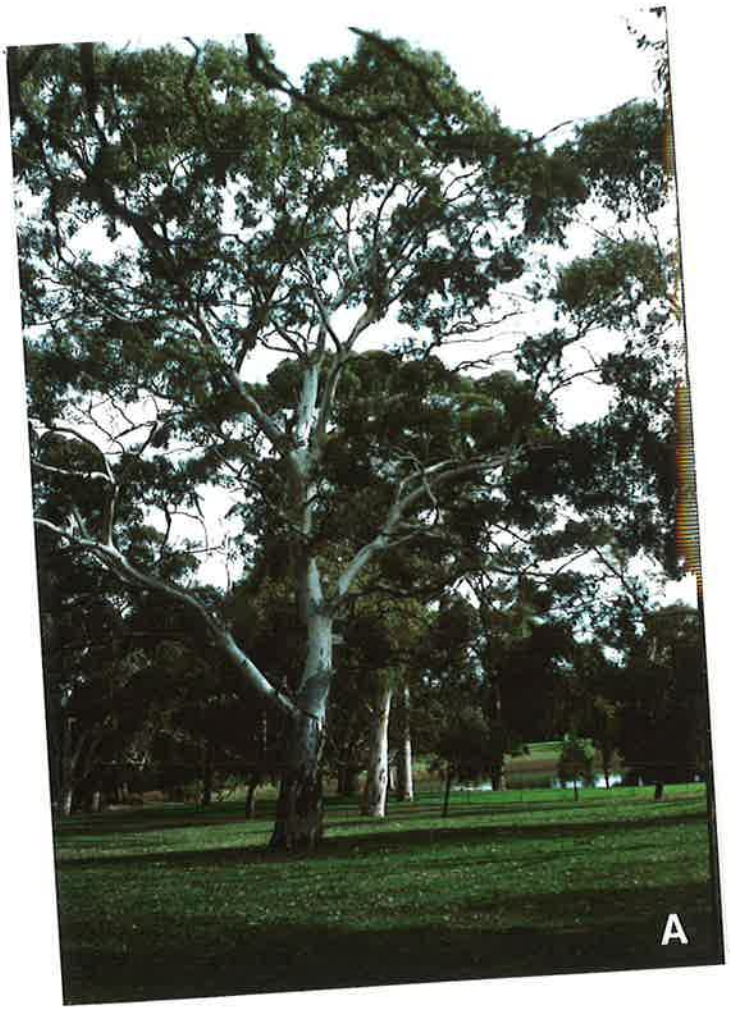
(2) Intermediate host plants, where 20 -50% of larvae survive to 4th instar (fig. 2.4.1 c,d).

Figure 2.2.1.

Eucalyptus camaldulensis a good food plant for Uraba lugens.

(a) In foreground, an example of a mature E. camaldulensis tree.

(b) Newly mature leaves of E. camaldulensis.


A


B

Figure 2.2.2.

Eucalyptus platypus a poor food plant for Uraba lugens.

(a) A mature E. platypus in centre foreground.

(b) Leaves and flower buds of E. platypus.


A


B

(3) Poor host plants, where fewer than 15% of the larvae survive to 4th instar (figs. 2.2.2 and 2.4.1 a,b).

Cobbinah et al (1982) explained the variable acceptance by larvae of food plants by the presence of antifeedants in a threshold effect. However Cobbinah pointed out that the presence of antifeedants does not necessarily explain the variable suitability of "good" food plants when assessed on growth rate, survival and fecundity, and credited nutritional variation to be an influencing factor.

2.3 SLOBODKIN'S SURVIVAL CURVES.

Slobodkin (1962) postulated four basic forms of survivorship curves for animal populations (fig. 2.3.1). In the Type I survival curve the mean, median and maximum life expectancy at birth are almost equal, so that the mortality is concentrated on the old animals.

Type II represents a system in which a constant number of animals die per unit time, regardless of the number of animals remaining (i.e. a constant number of deaths per unit time).

Type III represents a system in which the risk of death is constant with age (i.e. a constant proportion of the animal population die at each age).

Type IV represents a population in which mortality mainly affects the young animals (i.e. the mean life expectancy of any individual increases with age).

The Type IV curve is probably the most common in nature, although natural populations in general do not conform to any one of

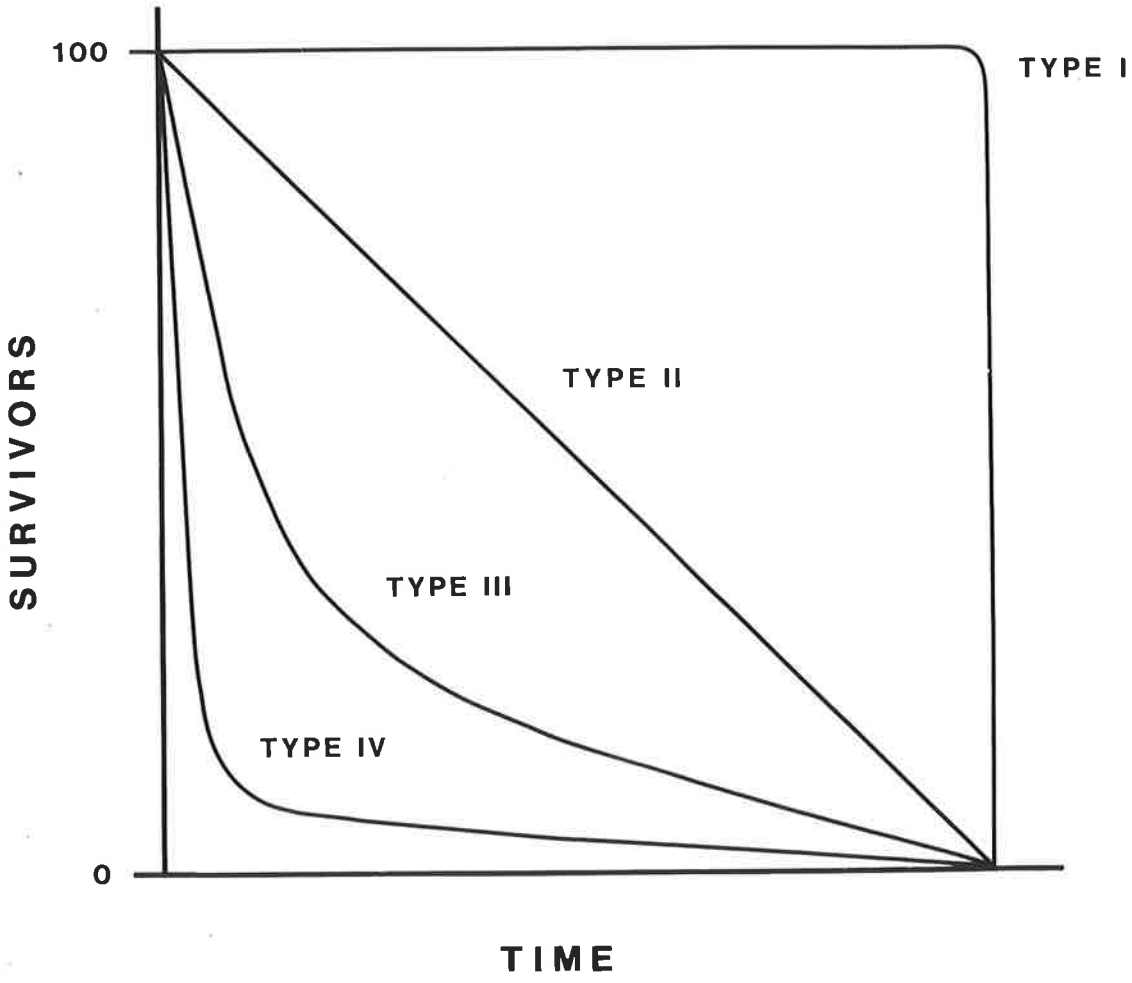
Figure 2.3.1 Slobodkin's Survival Curves.

After Slobodkin (1962) p 35

"Growth and Regulation of Animal Populations."

SLOBODKIN'S

SURVIVAL CURVES



these curves. Typically, dfferent ages are susceptable to different sources of mortality, producing a survivorship curve that is a composit of the various curves possible. Most animals that have a distinct larval stage followed by metamorphosis into an adult condition have extremely high mortality in the young stages or in the transition from one mode of life to another. This group includes most fish, coelenterates, crustacea and insects. Slobodkin points out however that the survivorship curve is not a constant character of a population or even a species. Although the general shape of the curve does not alter greatly, the actual duration of life and the details of curve shape are extremely sensitive to the sex and genotype of the individuals and more importantly to environmental conditions.

2.4 SURVIVAL OF U. LUGENS ON DIFFERENT HOST PLANTS IN THE CONTEXT OF SLOBODKIN'S SURVIVAL CURVES.

Morgan and Cobbinah (1977) and Morgan (unpublished data) examined the acceptability of different "egg hosts" by U. lugens and the insect's subsequent survival on these plants. Considering these observations, the following pattern emerges (fig 2.4.1). On a poor host, for example Eucalyptus astringens or E. platypus the survival curve is comparable with that of a Slobodkin Type IV (fig. 2.4.1 a and b). A small number of larvae may survive to adults in some years, however this is rare and larvae generally do not survive past the fourth stadium. Other food plants on which U. lugens follows a survival curve similar to that of E. platypus and E. gardineri (fig. 2.4.1 b) are: E. dives;

Figure 2.4.1 Survival of U. lugens larvae on different egg hosts.

(a) Survival curve on a Poor Food Plant, viz. E. astringens, E. globulus, and E. obliqua.

(b) Survival curve on a Poor Food Plant, viz.

E. gardneri ——————

E. platypus – – – – – – –

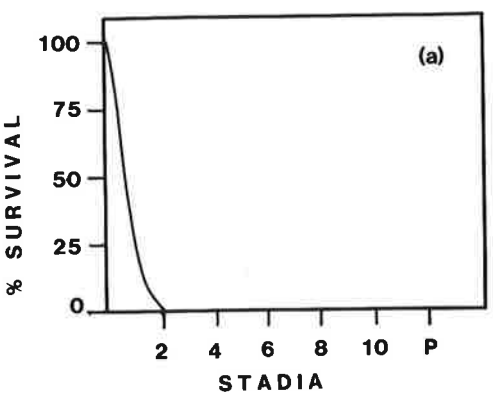
(c) Survival curve on an Intermediate Food Plant, viz. E. sideroxylon, and E. punctata.

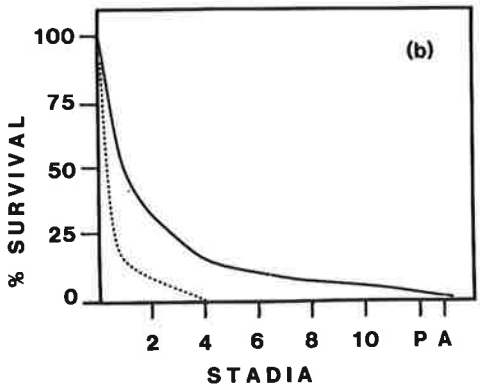
(d) Survival curve on an Intermediate Food Plant, viz. E. fasiculosa.

(e) Survival curve on a Good Food Plant, viz. E. camaldulensis.

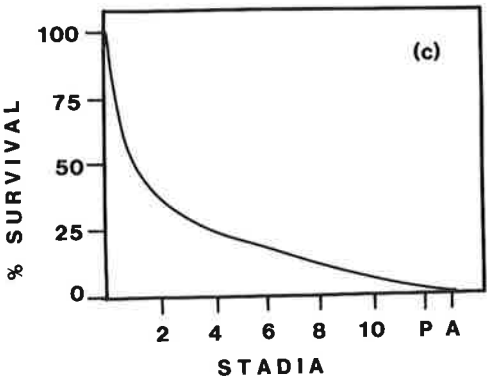
(f) Survival curve on a Good Food Plant, viz. E. lehmannii.

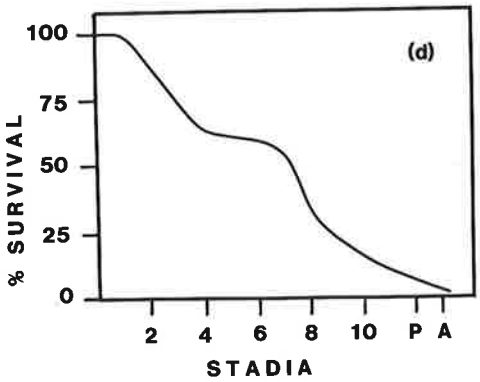
POOR HOST PLANTS



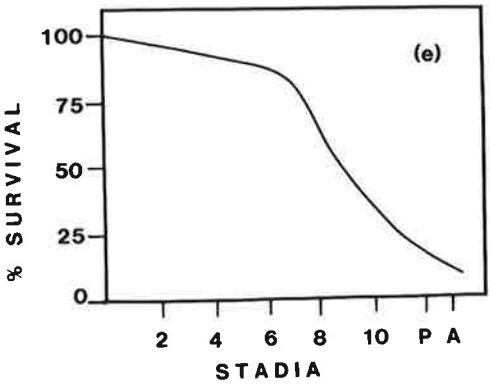


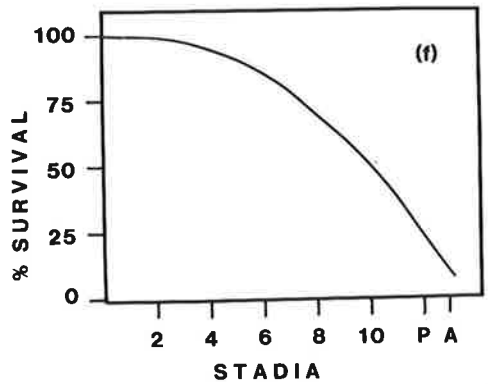
INTERMEDIATE HOST PLANTS





GOOD HOST PLANTS





E. drepanophylla; E. eremophila; E. fergusoni; E. gillii; E. kybeanensis; E. longifolia; E. largeana; E. mulleriana; E. ovata and E. paniculata.

For an intermediate host such as E. sideroxylon and E. punctata (fig. 2.4.1 c) the survival curve is similar to a Slobodkin Type III. In this case survival past the fourth stadium is greater than 20%. Other examples of intermediate hosts for U. lugens are: E. angophoroides; E. aromophloia; E. botryoides; E. dawsoni; E. guilfoylei; E. largiflorens; E. nortoni; E. orgadophylla; E. propinqua; E. robusta; E. tereticornis and E. viminalis.

The survival curve for U. lugens on E. fasiculosa (fig. 2.4.1. d) is similar to that of a Slobodkin Type II curve and approaches the survival curve representative of a good host. For larvae on a good food plant such as E. camaldulensis and E. lehmannii, the survival curve approaches that of a Slobodkin Type I (fig. 2.4.1 e and f). Survival is high in the initial stadia but decreases rapidly after the eighth stadium. Other examples of good food plants for U. lugens are: E. citriodora; E. maculata; E. moorei; E. occidentalis; E. odorata and E. stellulata.

White (1966, 1978) proposed that most herbivorous insects usually remained at a low level of abundance relative to the apparent abundance of their food because most of them die in the early instars from a relative shortage of nitrogen in their food. This idea has been further substantiated by the work of Mc Clure (1979, 1980) on elongate hemlock scale, Forina externa Ferris; Myers and Post (1981) on cinnabar moth; and Tabashnik (1982) on Colias spp.. All these authors found that larval survival was related to the nitrogen content of the plant. So that

Figure 2.4.2.

White's Hypothesis in the context of Slobodkin's Survival curves.

(a) An insect with survival curve Type IV.

Host plant at normal conditions. ——————

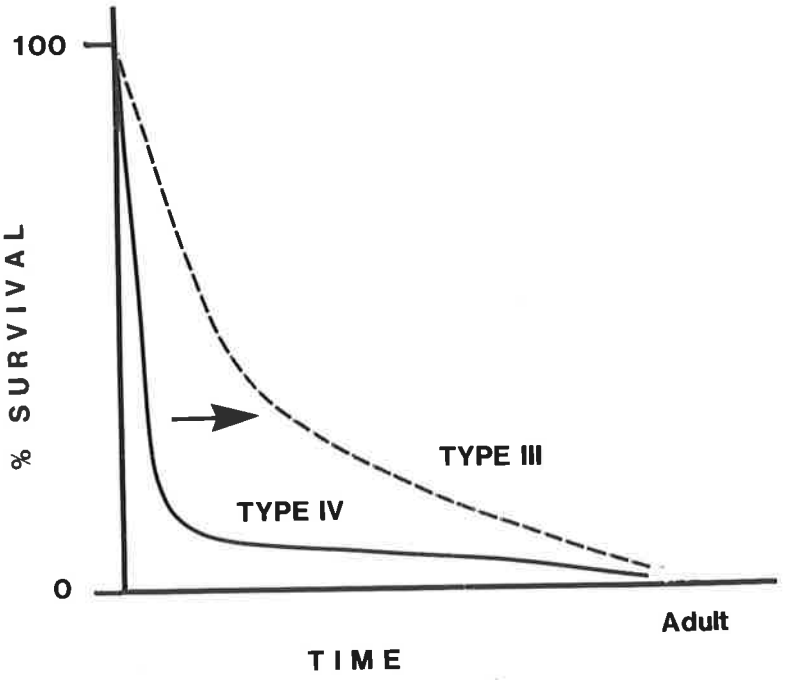
Host plant with a higher nitrcgen content. — — — — — —

(b) An insect with survival curve Type I.

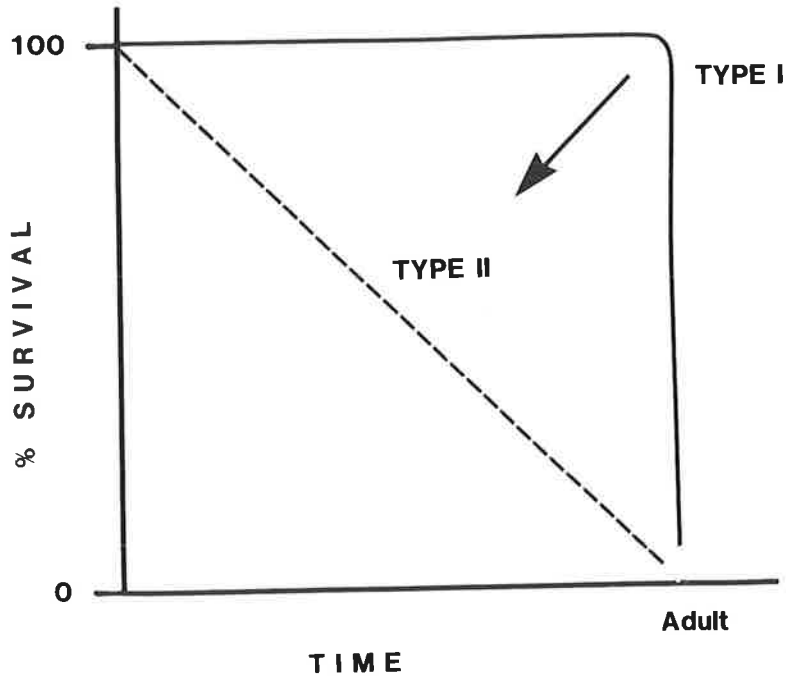
Adequate host plant ——————

Inadequate or low nitrogen host — — — — — —

(a)


100
% SURVIVAL
0
TYPE III
TYPE IV
Adult
TIME

(b)


100
% SURVIVAL
0
TYPE I
TYPE II
Adult
TIME

nitrogen was purported to be the limiting nutrient for larval growth, survival and adult fecundity.

It is known that plants subjected to drought respond with an increase in free amino acids (Hsiao, 1973; Singh et al, 1973 a and b). White (1966, 1974, 1976) therefore hypothesised that plants become a richer source of nitrogen to herbivores when stressed by random fluctuations in the summer and winter rainfall, this higher availability of nitrogen in the host plant leading to a higher proportion of young insects surviving. Relating White's hypothesis to Slobodkin's Survival Curves it is considered that most phytophagous insects generally follow a Type IV curve (fig. 2.4.2 a). An increase in the leaf nitrogen content due to an environmental stress such as drought may therefore shift this curve toward that of Type III. The converse may also occur (fig. 2.4.2 b). An insect with a survival curve of Type I while feeding on a good food plant may show a shift toward a Type II curve if fed on an inadequate food plant or one having a lower nitrogen concentration .

Chapter 3

The Influence of Individual Amino Acids Incorporated Into Artificial Diets, on the Biological Performance of *Uraba lugens* *Walk.*

CHAPTER 3

The influence of individual amino acids incorporated into artificial diets, on the biological performance of *Uraba lugens* *Walk.*

3.1 INTRODUCTION.

Many studies on the nutritional attributes of amino acids in the insect diet have been made. Taylor and Bardner (1968) concluded that the larvae of both Phaedon cochlaeriae Fab. and Eutella maculipennis Curt. developed at a greater rate on younger plants with a higher protein content. While Davis (1975) determined that Tenebrio molitor L. required the same ten essential amino acids as most other insects (i.e. L-valine, L-leucine, L-threonine, L-lysine, histidine, arginine, methionine, phenylalanine, tryptophan and iso-leucine). Pseudosarcophaga affinis (Fall.),reared aseptically on chemically defined media, showed that food lacking these amino acids failed to support larval growth (House, 1954). Omission of glycine from its diet also lowered the rate of growth and few individuals developed beyond the first instar. Using the European corn borer, Pyrausta nubilalis Hubner, Beck (1950) also found that growth rate and development of the larvae was suppressed on nitrogen deficient diets. Incorporation of a number of amino acids in an agar-based purified diet for P. nubilalis increased the average feeding time of the larvae (Beck and Hanec, 1958), this being most noticeable for the diets containing L-alanine, DL-α-amino-n-butyric acid, L-serine and L-threonine.
A negative effect on feeding time occurred on diets containing

L-tryptophan, L-arginine, β-alanine. The effects of L-amino acids on feeding was found to be correlated with their molecular weights and their water solubilities. Hsiao and Fraenkel (1968) demonstrated that the molecular configuration of an amino acid had a profound influence on its effectivness as a feeding stimulant. All amino acids that stimulate insect feeding have molecular weights less than 126 (Hsiao and Fraenkel, 1968), while those that can be categorised as attractants are water soluble. Feeding experiments on spruce budworm Choristoneura fumiferana (Clem) larvae revealed that it was stimulated by the presence of L-proline (Heron, 1965) an amino acid highly soluble in water and of low molecular weight. Proline is also a phagostimulant for Locusta migratoria (L.), the amino acids L-threonine, L-cysteine and L-valine showing similar but somewhat less activity (Cook, 1977). However, Bright et al (1982) found no increase in the feeding or performance of a number of insects and molluscs fed barley varieties containing high levels of proline, over those fed barley with lower levels of proline.

Proteins and /or the presence of free amino acids, both appear to influence phagostimulation and growth rate of insects. Female bushflies fed high protein diets immediately develop eggs (Jones and Walker, 1974). Dabrowski and Bielk (1978) found mite fecundity to be positvely correlated with the nitrogen content of their diet and the presence of specific amino acids. Labeyrie (1969,1978) has commented on the influence of food quality and quantity on the reproductive potential of female insects and van Emden (1966) showed that fecundities of Brevicoryne brassicae (L.) and Myzus persicae (Sulzer) were positively correlated with the amount of soluble nitrogen in their diets. Since the

development of an insect from pupa to adult is achieved through the process of cell differentiation and this involves the formation and utilization of proteins, dietary proteins and free amino acids must play a major role in growth, development and reproduction of insects.

The influence of a number of free amino acids on growth, survival and fecundity of Uraba lugens Walk. was therefore investigated. Since adult females of this species select plants for oviposition and there is a range of survival and growth rates of their progeny on the different egg hosts, it seemed possible that additional dietary nitrogen may increase acceptance, growth, survival and fecundity of U. lugens larvae, that by adult selection, were located on poor food plants (see Morgan and Cobbinah, 1977). This view is supported by Beck and Hanec (1958) and Hsiao and Fraenkel (1968) who indicated that different amino acids may vary in their influence on insect performance. It seemed possible that proline and valine may significantly increase larval phagostimulation and / or acceptance of test diets, and that larval growth rate and adult fecundities could also be enhanced because of the low molecular weights (less than 126) and high water solubilities of these amino acids. Proline is also of considerable interest because of its marked increase in stressed plants (Singh et al, 1972, 1973). On the other hand an excess of these amino acids in an artificial diet may reduce the insect's feeding response by "masking" the nutritional value of the diet and hence result in a decrease in insect performance (White, 1974).

It seemed appropriate therefore, to test U. lugens on diets that embraced a range of concentrations of free amino acids previously shown to effect insect performance. One might be able to demonstrate whether its performance could be influenced not only by the amino acid supplied but also by its concentration in the diet. Cobbinah et al (1982) had demonstrated that U. lugens was influenced by the proportion of leaf powder from a good food plant. Thus they showed that thresholds of compounds in diets may be the basis upon which a food plant may be rated on a scale from non-food plant to good-food plant. That is, components of the food governed its acceptability and suitability to an insect (see also Feeny, 1968, 1970).

3.2 MATERIALS AND METHODS.

3.2.1 Diet.

A meridic base diet was prepared using, Davis' Bacteriological Agar, cellulose, sucrose and leaf powder. The proportions of these main ingredients were varied from those used by Cobbinah (1978) whose original agar diet formulated for *U. lugens* was found to be of unsuitable consistency.

Artificial diets representing good and poor host plants were prepared from the two egg hosts (Cobbinah et al, 1982) *Eucalyptus camaldulensis* a preferred host for *U. lugens* in nature and *Eucalyptus platypus* upon which oviposition is frequently recorded but no larvae survive to the final instar.

The leaf powder component was prepared as follows: Fresh leaves were collected from the field and immediately placed and retained in liquid nitrogen to arrest metabolic processes and to ensure minimal deterioration of their chemical components. They were then ground in a mortar and pestle under liquid nitrogen, the resulting fragments being freeze-dried for 48 hours. After freeze-drying the leaf material was further ground in a Wiley Mill until it passed through a 0.5 millimeter screen, the resultant powder being stored in a dry environment at -20°C until use.

The base diet was made up in the proportions 1.0g Davis' Bacteriological Agar, 1.5g cellulose, sucrose at 0.01 molar and 6% (by weight) leaf powder, to 30ml water. An antifungal agent, Tegosept M® ($HOC_6H_4COOCH_3$ supplied by Merck) at 0.05% (by weight) was also added,

this delayed fungal growth on the diet for approximately ten days once the diet had been exposed to aerial spores.

Sucrose was the phagostimulant for the insect (Cobbinah, 1978). Cobbinah showed that larvae of U. lugens were stimulated to feed on sugar diets at concentrations ranging from 0.01 molar to 0.1 molar sucrose, the feeding response increasing with increasing concentration from poor or a low level of larval feeding to a point where all larvae fed on the diet presented. The concentration chosen for this experiment was the point at which larval feeding responses were intermediate so that consumption of the diets would not be due only to larval response to sugar.

Cellulose was added to maintain a "hard" consistency, thereby increasing the attractiveness and mechanical quality of the diet since U. lugens prefers to feed on firm to tough newly mature and mature leaves of its food plant (Cobbinah, 1978). In the E. platypus diet it was necessary to incorporate a greater amount of the binding agent, agar (1.5g), since the E.platypus diet was found to be inferior in consistency and texture when containing the same proportions of ingredients as the E. camaldulensis diet. The added agar, though a protein, is nutritionally inert to insects (Vanderzant, 1969).

The amino acid additives were selected by means of scoring individual amino acids according to their influence on feeding, growth, and development of insects previously studied. A spectrum of amino acids was therefore obtained ranging from amino acids with considerable influence on growth and development to those with little such influence. The amino acids selected (table 3.2.1.1) include four of the essential amino acids, i.e. L-arginine, L- methionine, L-valine and L-tryptophan,

as well as L-proline, often present in large quantities in stressed plants and aspartic acid which attained a low score in the selection procedure. The selection provided a spectrum that was likely to encompass negative to strongly positive responses.

Table 3.2.1.1 Amino acids incorporated into test diets.

Amino Acid	Abreviated	Molecular weight
Proline	Pro	115
Valine	Val	117
Aspartic acid	Asp	133
Methionine	Met	149
Arginine	Arg	174
Tryptophan	Try	204

A further treatment incorporated all test amino acids in the the base diet.

The amino acids were incorporated at two concentrations, 0.01 molar and 0.05 molar. The diets were prepared in the following manner: an 0.05% Tegosept solution was made up with distilled water. Thirty milliliters of this was then transferred to a beaker containing weighed amounts of agar, cellulose, sucrose and the respective amino acid. This mixture was brought to the boil whilst being rapidly stirred. After the mixture had boiled, it was removed from the heat source and vigorously stirred while cooling. Just before the mixture solidified, the pre-weighed amount of lyophilized leaf powder was added, the diet being poured into a heat sterilized petri dish. The diets were prepared in advance and were stored at +1° C where they could be kept for up to three

weeks without obvious deterioration.

Two control diets were used: (1) fresh diet; (2) base diet (artificial diet containing leaf powder of the species under study but no added amino acids). The test diets consisted of base diet plus the test amino acid. The experiment consisted of six individual amino acid treatments each at two concentrations - high (0.05 M) and low (0.01 M) and a combination of the test amino acids. There was therefore a total of 2+ 2+ (7)2+ (7)2 = 32 treatments with 10 replicates per treatment.

The diet was presented as a 16 mm diameter disk cut with a sterilized cork borer with a triangular cavity cut into the top since the larvae feed on edges. This was placed in a sterilized 9 cm diameter petri dish and five caterpillars that had just moulted to the fourth instar were placed in each control and test arena (see section 3.2.2).

3.2.2 Insects.

Early instar larvae were collected from the field from Eucalyptus microcarpa, a good food plant not included in the test diets.This was done to avoid the effect of possible "parent imprint" (Jermy, 1965; Hovanitz, 1969). Larvae were fed on fresh leaves of E. microcarpa in laboratory cages until they reached the fourth stadium. The fourth instar larvae were fasted for twenty four hours before being placed on the test diets. Larvae younger than this stadium were found to have a high mortality rate due mainly to their gregarious behaviour and a dfficulty in their utilization of the diet as presented. Young larvae tended to fall off the test diets and were unable to re-establish themselves on the diet due possibly to poor locomotor ability, unless in

a high concentration of individuals. Five fasted larvae were weighed and then placed on each of the test diets in a heat sterilized petri dish and reared at a constant temperature of 25°C and a 15 hour light period.

Treatments were each scored for the number of insects on or off the diets at 15 minute intervals over two hours. This was to determine diet acceptability. The insects were then retained on the diets for five days after which the diets were renewed. From then on the diets were renewed every five days with the exception of the fresh diet which was renewed daily. When the test animals moulted faecal pellets in each arena were counted and the newly ecdysed insects were re-weighed before they began feeding again. This data was used to estimate weight gained on the individual test diets. Since production of faeces is positively correlated with the amount of diet consumed (Cobbinah, 1978) an estimate of diet consumption could also be made. Survival was also recorded at this time. Following pupation, the pupae were sexed and the females individually weighed to estimate their potential fecundity using the regression established by Cobbinah (1978): $y = 5.94x - 193.26$ where x = female pupal weight (mg), and y = potential number of eggs.

3.3 RESULTS AND DISCUSSION.

3.3.1 Diet acceptance

Since there was no significant difference in the larval acceptance of any presented diet with time, time 4 (60 miutes on diet), which was considered adequate for test insects to settle and respond to the diet, was analysed to determine any trends present in the data (fig. 3.3.1.1 and table 3.3.1.1; see appendix 3.3.1.1 for raw data).

By comparison with the control diets, base and fresh, the test diets including leaf powder of *E. camaldulensis* may be related as follows:

Base diet: Equal to: *E. camaldulensis* + proline
+ methionine
+ arginine
and *E. platypus* + combined amino acids
+ proline
+ methionine (0.01 M)

Inferior to: *E. camaldulensis* + valine
+ tryptophan
+ combined amino acids
Fresh
and *E. platypus* + valine
+ tryptophan

Superior to: E. camaldulensis + aspartic acid
and E. platypus + methionine (0.05 M)
+ arginine
+ aspartic acid
Fresh

Fresh E. camaldulensis:

Equal to: E. camaldulensis + valine
+ tryptophan
+ combined amino acids
and E. platypus + valine
+ tryptophan (0.05 M)
Superior to: all other diets including
base and fresh E. platypus

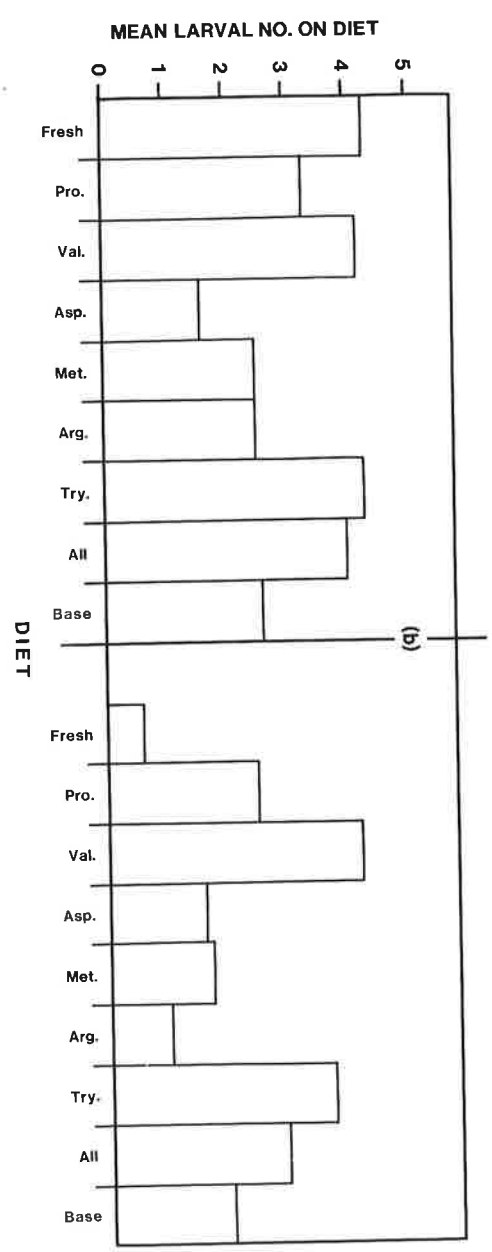
The acceptance by larvae of fresh E. platypus compared with the other test diets is as follows:

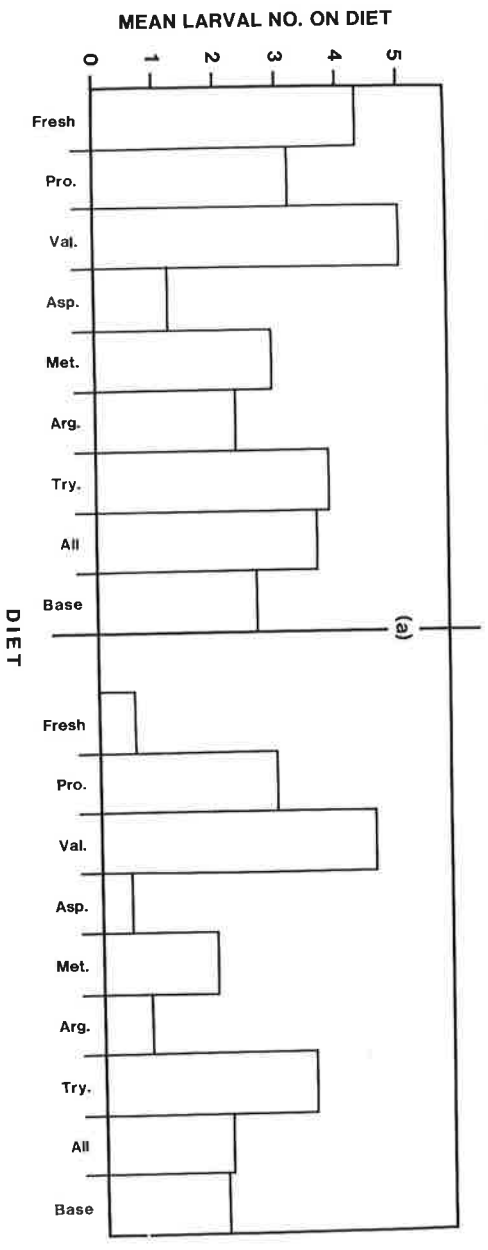
Equal to: E. camaldulensis + aspartic acid (0.01 M)
and E. platypus + aspartic acid (0.01)
Inferior to: all other diets.

Figure 3.3.1.1 Acceptance by larvae of diet at Time 4 (measured as mean larval number on diet).

(a) Amino acids at 0.01M.

(b) Amino acids at 0.05M.





E. camaldulensis

E. platypus

Table 3.3.1.1 Mean number of larvae on diets at time interval IV (one hour after placement on diet) indicating larval acceptance of diet.

Diets		*E. camaldulensis* + amino acid		*E. platypus* + amino acid	
Amino acid	Conc.				
Val	0.01	5.0	a	4.5	a
	0.05	4.2	b	4.2	a
Try	0.01	3.8	b	3.5	ab
	0.05	4.3	ab	3.7	ab
Combined	0.01	3.6	b	2.1	c
	0.05	4.0	b	2.9	bc
Pro	0.01	3.2	bc	2.9	bc
	0.05	3.3	bc	2.5	bc
Met	0.01	2.9	bc	1.9	cd
	0.05	2.5	bc	1.7	cd
Arg	0.01	2.3	cd	0.8	e
	0.05	2.5	bc	1.0	de
Asp	0.01	1.2	e	0.5	e
	0.05	1.6	de	1.6	cd
Base	-	2.6	bc	2.0	c
Fresh	-	4.3	ab	0.6	e

L.S.D. = 1.58, P = 0.001.
L.S.D. = 0.79, P = 0.05.
Different letters in columns 3 and 4 indicate those values that are significantly different at P = 0.05.

The most acceptable test diets were not significantly different from fresh leaves of the common food plant E. camaldulensis. Perhaps the most interesting results were those where test diets were inferior to base diet and where the poor food plant leaf powder diets were superior to both base diet and fresh diet of E. platypus. Such results demonstrate that addition of certain nutrient compounds can induce U. lugens to respond poorly to diets where they are incorporated (examples here are arginine and aspartic acid at the lower concentration) while incorporation of other individual components such as valine and tryptophan induce much greater acceptance of the diets presented. It is also apparent that specific amino acids and their concentrations in the diets affected the larval response such that a gradient from high acceptability (e.g. fresh diet) to low acceptability (i.e. equivalent to base diet) may be obtained.

As valine and tryptophan both induced high acceptability of diets based upon E. platypus leaf powder one may conclude one of two reasons for this. Either these amino acids are acting as arrestants or they are improving the palatability of the diets into which they are incorporated. Which of these is responsible may be discovered by examination of relative diet consumption rates, survival rates and growth rates presented in the next sections.

3.3.2 Diet consumed

This was determined by counting the accumulated number of faecal pellets over 5 days in each arena and calculating mean values for each diet since consumption is positively correlated with faecal pellet production (Cobbinah, 1978). The data is represented in table 3.3.2.1 and figure 3.3.2.1.

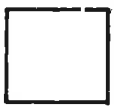
Table 3.3.2.1 Mean faecal count ± SE per test arena for 5 days

Diet	E. camaldulensis		E. platypus	
	Conc. 0.01M	Conc. 0.05M	Conc. 0.01M	Conc. 0.05M
Pro	621 ± 36.4 ad	624 ± 18.0 ad	243 ± 26.7 ae	326 ± 42.3 ad
Val	435 ± 47.1 bd	588 ± 34.0 ad	414 ± 48.8 ad	353 ± 36.0 ad
Asp	630 ± 47.5 ad	603 ± 30.5 ad	287 ± 26.4 ad	317 ± 24.7 ad
Met	1142 ± 50.6 ad	248 ± 20.6 cf	432 ± 45.9 ad	217 ± 12.8 ae
Arg	914 ± 60.4 ad	407 ± 21.5 cd	320 ± 38.7 ad	307 ± 24.9 ad
Try	572 ± 76.2 ad	468 ± 38.2 bd	115 ± 16.8 af	87 ± 15.3 af
*All	233 ± 40.1 cf	89 ± 8.9 cf	112 ± 28.3 af	77 ± 10.5 af
Base	364 ± 24.3 c	-	215 ± 21.7 a	-
Fresh	495 ± 67.8 d	-	45 ± 12.6 f	-

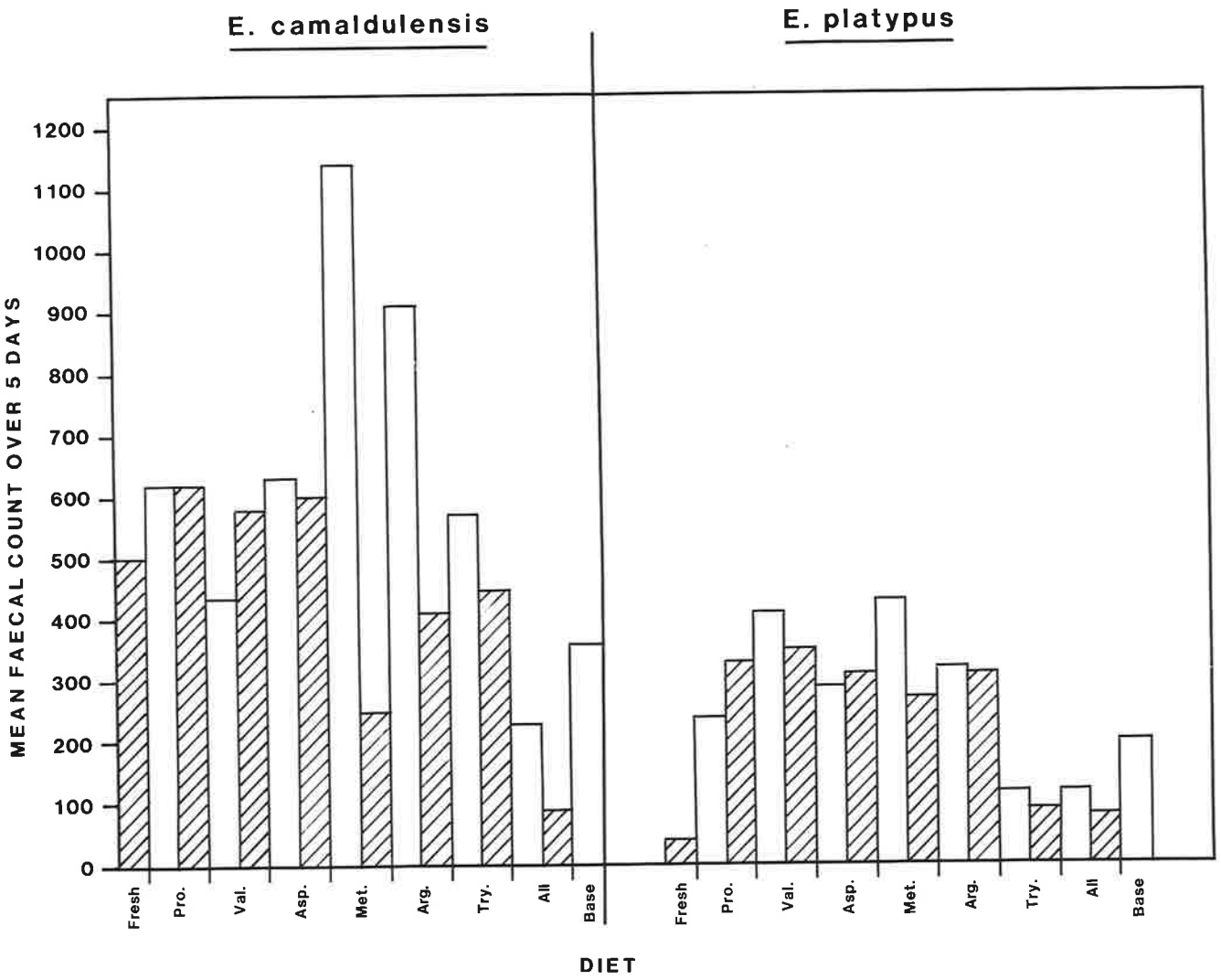
* refers to diet incorporating all the test amino acids.
Analysed as a split plot design, significance at $P < 0.001$.
Letters in columns refer to respective fresh and base diets as follows:

a. significantly higher than fresh diet.
b. comparable with fresh diet.
c. significantly lower than fresh diet.
d. significantly higher than base diet.
e. comparable to base diet.
f. significantly lower than base diet.

Figure 3.3.2.1 Mean faecal count on test diets over 5 days

amino acids at 0.01M 

amino acids at 0.05M 


E. camaldulensis
E. platypus
MEAN FAECAL COUNT OVER 5 DAYS
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
Fresh
Pro.
Val.
Asp.
Met.
Arg.
Try.
All
Base
DIET

In all test diets with the exception of valine at 0.01M and the combined amino acid diet at 0.05M, the production of faecal pellets by larvae on diets containing E. camaldulensis leaf powder was significantly greater than on diets containing leaf powder of E. platypus (see fig. 3.3.2.1 and table 3.3.2.1). For E. camaldulensis the production of faecal pellets by larvae on different test diets in descending order is (see fig 3.3.2.1): methionine (0.01M); arginine (0.01M); proline, aspartic acid, valine (0.05M) and tryptophan (0.01M); fresh diet, tryptophan (0.05M), valine (0.01M) and arginine (0.05M); base diet; methionine (0.05M) and combined amino acids (0.01M); combined amino acids (0.05M).

For E. platypus the production of faecal pellets by larvae on the different test diets in decending order is: methionine (0.01M) and valine; Proline (0.05M), arginine and aspartic acid; proline (0.01M); methionine (0.05M) and base diet; combined amino acids (0.01M) and tryptophan (0.01M); tryptophan (0.05M) and combined amino acids (0.05M); fresh diet.

In other words, for E. camaldulensis addition to the base diet of methionine, arginine and tryptophan at low concentrations, proline and aspartic acid at both concentrations and valine at the higher concentration significantly increases consumption of the diet above that of fresh leaf material. For E. platypus the addition to the base diet of methionine, valine, proline, aspartic acid and arginine all at both concentrations, significantly increases diet consumption above that of the fresh and base diet although consumption does not significantly approach that on fresh E. camaldulensis.

If consumption of diet is influenced by its nutrient value, its acceptability and suitability, then the results achieved in this study indicate that:

(i) food of low acceptability may be made more acceptable by the addition of certain amounts of specific amino acids (nutrient components) e.g. valine and tryptophan.

(ii) food acceptability may be decreased by addition of certain amounts of specific amino acids (nutrient components) e.g. arginine and aspartic acid.

(iii) incorporation of all test amino acids did not increase consumption over base diet or fresh diet for either eucalypt.

Reasons for this result may include:

(a) that diets rich in nutrients may decrease consumption compared with those of lesser nutrient value (Beckwith, 1976; Slansky and Feeny, 1977).

(b) that incorporation of one component that decreases consumption in a combination of components may mean that it still dominates the insects' response to that diet.

N.B. The fact that certain diets induced the production of many more faecal pellets than similar larvae on fresh leaves of a known food plant could mean that the test diets were increasing ingestion rate because of a serious deficiency in the nutrient value of those diets. That is lower nutrient value induces greater ingestion rates as higher nutrient value

dereases ingestion rate - "incorporation" of nutrient may govern ingestion rate.

3.3.3 Growth

Table 3.3.3.1 Mean fresh weight gain (mg) per test arena during the fifth stadium ± SE.

Diet	E. camaldulensis		E. platypus	
	Conc 0.01M	Conc 0.05M	Conc 0.01M	Conc 0.05M
Pro	21.6 ± 1.5 bd	17.8 ± 1.7 cd	- 1.1 ± 2.7 e	9.1 ± 2.3 ad
Val	15.3 ± 2.1 ce	6.9 ±10.0 cf	- 3.0 ± 1.4 cf	-3.7 ± 1.6 cf
Asp*	-	-	-	-
Met	-0.1 ± 1.6 cf	-11.8 ± 1.1 cf	- 4.0 ± 1.9 cf	-12.8 ± 1.2 cf
Arg	24.3 ± 1.9 bd	11.0 ± 1.6 ce	- 7.5 ± 7.3 cf	- 1.0 ± 2.2 be
Try	-4.7 ± 1.8 cf	- 6.2 ± 1.8 cf	-10.2 ± 1.3 cf	- 9.5 ± 0.9 cf
All	6.6 ± 1.8 cf	- 1.8 ± 1.6 cf	1.3 ± 1.8 be	- 2.9 ± 1.7 ce
Fresh	26.8 ± 2.9 d	-	- 0.4 ± 0.3 e	-
Base	13.4 ± 1.6 c	-	- 0.04 ± 1.6 b	-

* missing data.
Analysed as a split plot design significance, $P < 0.05$.
Letters in columns refer to respective fresh and base diets as follows:

a. significantly higher than fresh diet.
b. comparable with fresh diet.
c. significantly lower than fresh diet.
d. significantly higher than base diet.
e. comparable to base diet.
f. significantly lower than base diet.

These results show that insect growth on the test diets was generally poor, indicating that such artificial diets do not usually compare favourably with natural diet. Nevertheless they are of interest because they show that growth rate may well be influenced by specific components in the diet. For instance, valine while increasing the acceptility of diet from the poor food plant, had little effect on consumption and growth rate when compared with controls. Arginine however, had little or no effect on acceptability but generally increased consumption and increased weight gain (growth rate) compared with the base diet in E. camaldulensis diets only. This amino acid is known to be an indispensible dietary component for insects (House, 1958), however it did not improve the performance of U. lugens on the E. platypus diets. This was possibly due to the lower consumption rates on the E. platypus diet compared to the E. camaldulensis arginine diet. Proline on the other hand, had a slight affect on acceptability, generally increased consumption and weight gain at 0.05M compared with the base and fresh diets for E. platypus.

Methionine and tryptophan significantly decreased weight gain compared to base diet in both the test species studied, despite an increase in acceptability in the case of tryptophan and consumption in the case of both amino acids. It would seem therefore that acceptability and consumption are not positively correlated as found by Beckwith (1976) and Slansky and Feeny (1977). Methionine in particular appears to render the diet inferior to that of the base diet, the insect compensating for this by consuming greater quantities.

The concentration of amino acids also affects the growth rate of U. lugens, weight gain significantly decreasing for larvae on the higher amino acid concentration. This is particularly evident in the diets containing E. camaldulensis and as would be expected appears to be related to diet consumption (tables 3.3.3.1 and 3.3.2.1) which also decreases on the higher concentration amino acid diets. Therefore the excessively high concentration of amino acids may "mask" the real nutritional quality of the diet, the insect responding to this apparent high quality by consuming less and consequently having a lower growth rate. Consumption rate and nutrient value therefore appear to have a combined effect on growth rate and therefore may influence fecundities of adults from larvae fed those types of diet.

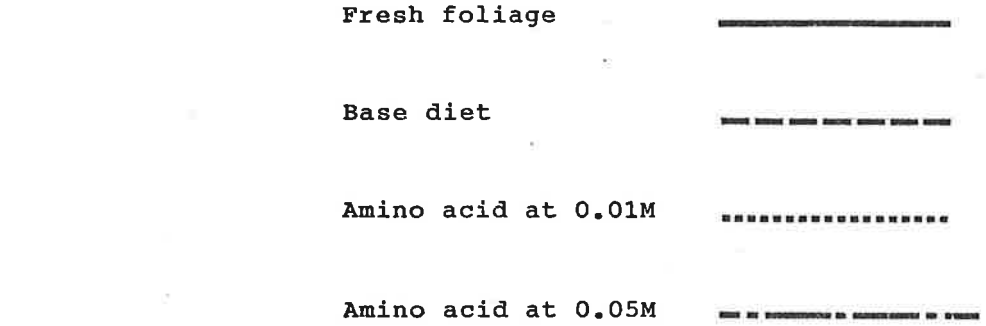
3.3.4 Survival

For E. camaldulensis although the final survival of larvae on artificial diets is significantly lower than the survival of larvae on fresh diet a number of patterns have emerged (see fig. 3.3.4.1, and tables 3.3.4.1 and 3.3.4.2).

(1) Base diet + proline (fig. 3.3.4.1 a):

For the early experimental instars survival of larvae on the proline diet follows closely the survival of larvae on the fresh diet material. The final survival of larvae on the proline diet at 0.01M is significantly greater than that on the base diet but lower than that on fresh diet.

Figure 3.3.4.1 Survival of U. lugens larvae on different test diets (percentage based on numbers entering each stadium).



(a) Proline
(b) Valine
(c) Aspartic acid

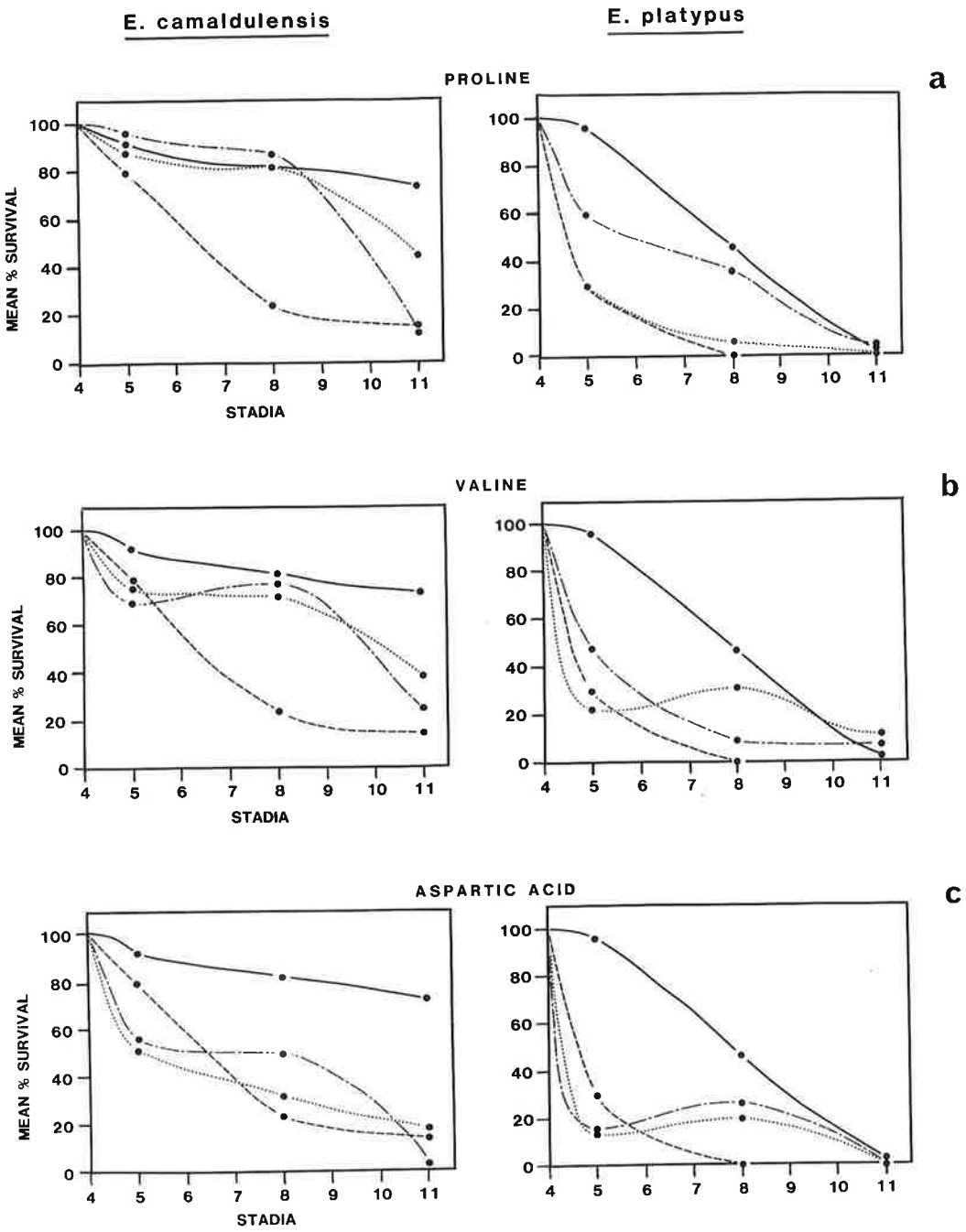

E. camaldulensis
E. platypus
PROLINE
a
VALINE
b
ASPARTIC ACID
c
MEAN % SURVIVAL
STADIA
0
20
40
60
80
100
4
5
6
7
8
9
10
11

Figure 3.3.4.1 cont.

Fresh foliage

Base diet

Amino acid at 0.01M

Amino acid at 0.05M

(d) Methionine
(e) Arginine
(f) Tryptophan

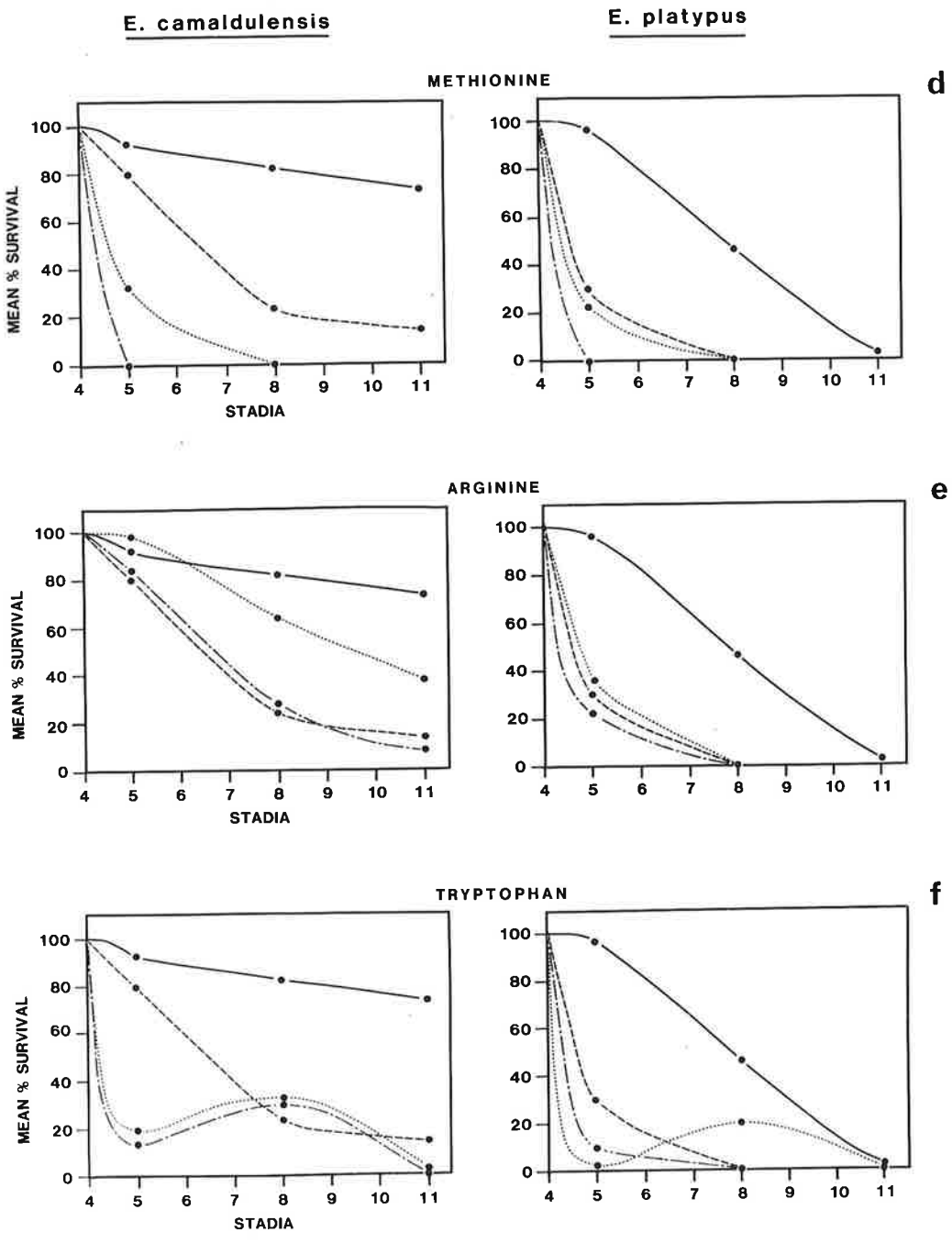

E. camaldulensis
E. platypus
METHIONINE
d
ARGININE
e
TRYPTOPHAN
f
MEAN % SURVIVAL
STADIA
0
20
40
60
80
100
4
5
6
7
8
9
10
11

Figure 3.3.4.1 cont.

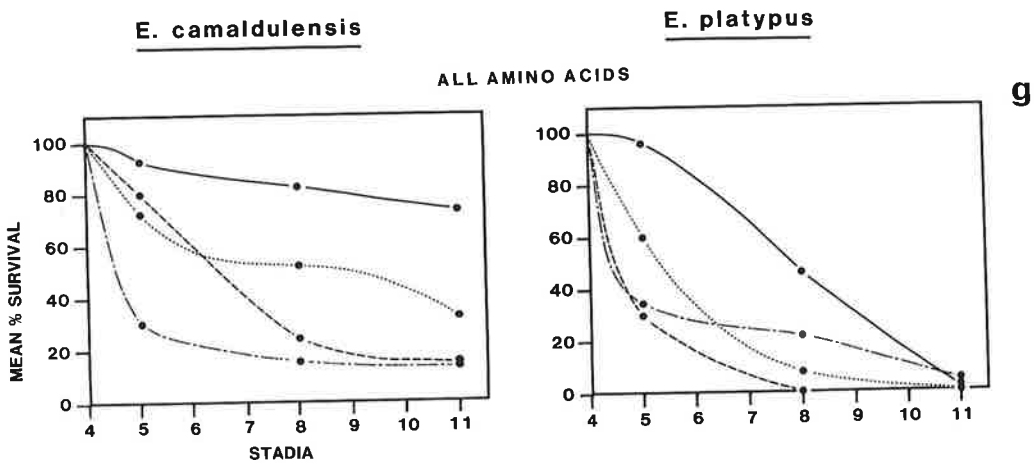
Fresh foliage

Base diet

Amino acids at 0.01M

Amino acids at 0.05M

(g) All test amino acids combined.


E. camaldulensis
E. platypus
ALL AMINO ACIDS
MEAN % SURVIVAL
100
80
60
40
20
0
4
5
6
7
8
9
10
11
STADIA

g

(2) Base diet + valine (fig. 3.3.4.1 b):

Is similar in pattern to that of the proline diet with the exception of the final survival for the high concentration valine diet. This is significantly higher than survival on the base diet although lower than survival on the low concentration valine diet.

(3) Base diet + aspartic acid (fig. 3.3.4.1 c):

Although survival to the 8th stadium on the high concentration aspartic acid diet is significantly greater than survival on the base diet, the survival curve follows more closely that of the base diet curve.

(4) Base diet + methionine (fig. 3.3.4.1 d):

The curve for the methionine diet is significantly inferior to that of the base diet. It appears that methionine in concentrations above that normally occurring in natural diet results in a "toxic" effect on U.lugens.

(5) Base diet + arginine (fig. 3.3.4.1 e):

For this amino acid at the higher concentration the survival curve follows closely the curve for the base diet. Although survival on the lower concentration diet is comparable with that on the fresh diet for the 5th stadium, survival at the 11th stadium is significantly lower than survival on the fresh diet.

(6) Base diet + tryptophan (fig. (3.3.4.1 f):

Again this diet is significantly inferior to that of the base diet, the curve very closely approaching a Slobodkin Type IV.

(7) Base diet + all amino acids (fig. 3.3.4.1 g):

For the low concentration diet the curve appears intermediate between that of fresh diet and base diet. Survival is significantly less than on fresh diet but significantly greater than on base diet. For the high concentration diet the curve is somewhat inferior to that for the base diet at first but final survival is not significantly different to that on base diet.

Table 3.3.4.1 Mean % Survival (± SE) of *U. lugens* in continuous culture on test diets with 0.01M amino acid added; survival recorded at the end of the 5th., 8th. and 11th. stadia.

Diet	*E. camaldulensis*			*E. platypus*		
	Stadia			Stadia		
	5	8	11	5	8	11
Prol	88 ± 3.3 bc	82 ± 4.7 bc	44 ± 8.9 c	30 ± 7.3 e	6 ± 5.7 c	0 ± 0 b
Val	76 ± 5.0 e	72 ± 6.1 bc	38 ± 6.9 c	22 ± 6.3 e	31 ± 5.9 b	11 ± 5.9 a
Asp	52 ± 6.8 d	32 ± 7.4 e	18 ± 5.9 e	14 ± 6.0 d	20 ± 0 c	0 ± 0 b
Met	32 ± 6.8 d	0 ± 0 d	-	22 ± 6.9 e	0 ± 0 e	-
Arg	98 ± 2.0 bc	64 ± 2.7 c	38 ± 6.3 c	36 ± 9.8 e	0 ± 0 e	-
Try	20 ± 6.0 d	33 ± 4.2 e	3 ± 3 d	2 ± 2 d	20 ± 0 c	0 ± 0 b
All	72 ± 5.3 e	52 ± 9.0 c	31 ± 7.3 c	60 ±12.2 c	8 ± 3.6 c	0 ± 0 b
Fresh	92 ± 4.4 c	82 ±10.1 c	73 ± 8.4 c	96 ± 4.0 c	46 ± 7.9 c	2 ± 2 c
Base	80 ± 6.6 b	24 ± 6.5	14 ± 3.7	30 ± 6.8	0 ± 0	-

Analysed as split plot design, significance $P < 0.05$.
Letters in columns refer to respective fresh and base diets as follows:

- a significantly higher than respective fresh diet.
- b comparable to respective fresh diet.
- c significantly higher than respective base diet.
- d significantly lower than respective base diet.
- e comparable with respective base diet.

All values significantly lower than fresh diet unless otherwise indicated.

Table 3.3.4.2 Mean % Survival (± SE) of *U. lugens* in continuous culture on test diet with 0.05M amino acids added; survival recorded at the end of the 5th., 8th. and 11th. stadia.

Diet	*E. camaldulensis*			*E. platypus*		
	Stadia			Stadia		
	5	8	11	5	8	11
Prol	96 ± 2.7 ab	88 ± 4.4 ab	12 ± 5.3 d	60 ± 8.9 b	36 ± 9.3 ab	3 ± 2.9 ab
Val	70 ±10.0 d	77 ± 7.0 ab	24 ± 4.4 b	48 ± 8.5 b	8 ± 4.4 b	7 ± 6.6 ab
Asp	56 ± 7.1 c	50 ± 8.0 b	2 ± 2.2 c	16 ± 5.8 c	26 ± 6.7 b	0 ± 0
Met	0 ± 0 c	-	-	0 ± 0	-	-
Arg	84 ± 4.7 ad	28 ± 6.1 d	8 ± 3.7 d	22 ± 6.2 d	0 ± 0 d	-
Try	14 ± 6.7 c	30 ± 5.8 d	0 ± 0 c	10 ± 10 c	0 ± 0 d	-
All	30 ± 6.8 c	15 ± 7.3 d	13 ± 13 d	34 ± 9.0 d	22 ± 8.1 b	4 ± 4 ab
Fresh	92 ± 4.4 b	82 ±10.1 b	73 ± 4.7 b	96 ± 4.0 b	46 ± 7.9 ab	2 ± 2 b
Base	80 ± 6.6 a	24 ± 6.5	14 ± 3.7	30 ± 6.8	0 ± 0	-

Analysed as split plot design, significance at $P < 0.05$.
Letters in columns refer to respective fresh and base diets as follows:

- a comparable to respective fresh diet.
- b significantly higher than respective base diet.
- c significantly lower than respective base diet.
- d comparable with respective base diet.

All values significantly lower than fresh diet unless otherwise indicated.

On the E. platypus diets U. lugens survival did not approach that on E. camaldulensis diets and only in the case of valine at the lower concentration is its survival significantly greater than that on fresh diet of E. platypus (table 3.3.4.1 and 3.3.4.2, fig.3.3.4.1 b).The following patterns in survival may be seen for the E. platypus diets (fig. 3.3.4.1).

(1) Base diet + proline (fig. 3.3.4.1 a):

At the higher concentration survival on the proline diet approached that of the fresh diet curve. At the lower concentration the survival curve follows that on the base diet although survival is significantly higher at the 8th. stadium than on base diet.

(2) Base diet + valine (fig. 3.3.4.1 b):

Survival on the diets of both concentrations follows closely the survival curve for the base diet at first then is significantly greater than that on base diet at the 8th. stadium and significantly greater than survival on fresh diet at the final stadium.

(3) Base diet + aspartic acid (fig. 3.3.4.1 c):

Survival at first is significantly lower than on base diet. Although survival is higher than on base diet at stadium 8, but no larvae survive to pupation (see section 3.3.5).

(4) Base diet + methionine (fig. 3.3.4.1 d):

shows a similar pattern to that observed for larvae on E. camaldulensis diets incorporating this amino acid (i.e. Slobodkin type IV curve).

(5) Base diet + arginine (fig. 3.3.4.1 e):

The survival curve for larvae on diets at both concentrations is not significantly different from larval survival on base diet.

(6) Base diet + tryptophan (fig 3.3.4.1 f):

The survival of larvae to the end of the 5th. stadium is lower on the tryptophan diet than on the base diet. On the lower concentration diet survival is significantly greater than on the base diet at stadium 8 but no larvae survive to pupation.

(7) Base diet + all test amino acids (fig. 3.3.4.1 g):

Survival on the low concentration diet is significantly greater than survival on the base diet although no larvae survive to pupation. Survival on the high concentration diet is not significantly different from survival on base diet at stadium 5, however at stadium 8 it is significantly higher than on base diet and some larvae survive to pupation.

In consisdering the implications of these data two points need to be emphasised:

(1) larvae were fed on a good host (E. microcarpa) to the begining of stadium 4 before being presented with the test diets

and (2) the base diet was not designed as an adequate diet for U. lugens larval development.

Therefore larvae at the fifth stadium feeding on the artificial diets may still have been influenced by nutrients and fat body stored during the period on their previous diet. This may explain the considerably high survival observed on the E. camaldulensis base diet (table 3.3.4.1). Since consumption and growth was much lower on the E. platypus diets, survival would naturally be lower. This is particularly evident in the E. platypus base diet for which there is very low survival in the fifth stadium and none thereafter. However the high survival on fresh E. platypus may be due to the nutrient value of these leaves despite the presence of an antifeedant (Cobbinah et al, 1982). U. lugens in nature usually do not survive past the fourth stadium on this egg host, however larvae fed on an acceptable plant up to this stage and subequently transferred to a non prefered host may survive to pupation (Cobbinah, 1978).

Therefore in considering the survival data for the fifth stadium the interesting feature is survival rates below that of the base diet. Methionine and tryptophan to a lesser degree appear to be toxic to U. lugens. This is supported by the growth rates found on these amino acids (table 3.3.3.1). Similar findings have also been reported by Davis (1956) concerning the deleterious effects of specific amino acids on

insect performance when incorporated into artificial diets. Although methionine and tryptophan are "essential" amino acids for insect development, their concentration in fresh foliage is exceptionally low compared to other amino acids such as valine (see chapter 6). Therefore the considerable excess of these two amino acids even in the low concentration diet was probably significant enough to interfere with the insects natural feeding patterns and performance.

For survival to the later stadia it is interesting to note those diets on which the larval performance was greater than that on the respective base diets. Considering both the E. camaldulensis and E. platypus diets, the incorporation of valine significantly increased survival above that of the base diets (fig. 3.3.4.1 b). However on the combined amino acid diet survival was higher than for larvae on the respective base diet at the low concentration for both eucalypts. At the higher concentration survival was comparable with the base diet for the diet incorporating the good host and higher than the respective base for poor host diets (fig. 3.3.4.1 g). This may be due to consumption by U. lugens of this diet (fig. 3.3.2.1) which was significantly lower than that for other E. platypus diets with the exception of tryptophan. Although consumption was much lower on the combined amino acid diet the amount of amino acids ingested in the high concentration diet may have been sufficient to maintain survival without the toxic effect of methionine contributing to larval performance.

3.3.5 Potential fecundity.

Table 3.3.5.1 Mean potential fecundity ± SE, estimated from female pupal weight (mg) from the regression $y = 5.94x - 193.26$ (Cobbinah, 1978), where y = potential fecundity and x = pupal weight (mg).

Diet	E. camaldulensis		E. platypus	
	Conc. 0.01M	Conc. 0.05M	Conc. 0.01M	Conc. 0.05M
Pro	72.6 ± 18.1 bd	73.7 ± 22.8 bd	*	*
Val	91.2 ± 16.9 bd	83.7 ± 21.1 bd	** 7.9 ad	** 127.5 ad
Asp	48.3 ± 26.0 e	*	*	*
Met	*	*	*	*
Arg	73.0 ± 13.1 bd	65.3 ± 43.2 bd	*	*
Try	*	*	*	*
All	86.6 ± 20.0 bd	*	*	** 42.6 ad
Fresh	84.5 ± 12.2 d	-	*	-
Base	38.6 ± 15.8 c	-	*	-

* no survival to pupation.
** only one individual.
Analysed as a split plot design.
Due to low pupal numbers replicates from each treatment pooled, significance at $0.001 < P < 0.05$.
Letters in columns refer to respective fresh and base diets as follows:

a significantly higher than fresh diet.
b comparable to fresh diet.
c significantly inferior to fresh diet.
d significantly superior to base diet.
e comparable to base diet.

For larvae fed the E. camaldulensis test diets potential fecundities of surviving individuals was similar to those fed:- base diet plus valine (at both concentrations), base diet plus all test amino acids (at 0.01M), base diet plus arginine (at 0.01M) and fresh leaf material.

For larvae fed the E. platypus test diets the data is limited due to low survival, however potential fecundities of the surviving individuals were improved for larvae fed diets containing valine and the combined test amino acids (at 0.05M) over those on both fresh and base diets (table 3.3.5.1).

It must be remembered however that fecundity is also a function of survival. Therefore taking an overall view, the fecundity of larvae fed the E. camaldulensis test diets was significantly lower than fecundities on the fresh diet although significantly greater than for larvae fed the base E. camaldulensis diet. Whereas larvae fed the E. platypus diets did have higher fecundities than the fresh diet together with the base diet.

Although the overall performance of U. lugens on artificial diets does not compare favourably with that on the fresh diet of a good host (E. camaldulensis) a number of conclusions on the influence of excess amounts of amino acids on the performance of U. lugens may be drawn from this experiment.

(i) Individual amino acids influence the acceptance, consumption and performance on a diet by varying degrees compared to the base diet. Valine and proline to a lesser extent significantly improve larval performance on E. platypus diets compared to fresh and base diets.

(ii) The incorporation of specific amino acids (methionine and tryptophan) at concentrations in excess of those experienced in nature are deleterious to insect performance.

(iii) The presence of excessive amounts of nitrogen "mask" the real nutritional quality of the diet since consumption is significantly reduced on the high concentration diets.

The amino acids valine and proline increase dramatically in water deficient plants (Singh et al, 1973; Hsiao, 1973). Since the incorporation of valine in E. platypus diets leads to an increase in the insects performance, the performance of U. lugens on a poor host plant may be enhanced when this plant is subjected to adverse environmental conditions such as drought. Therefore the oviposition habit of U. lugens adult females may leave open the possibility of coevolution of this species with egg hosts on which the larvae do not often survive. Investigation of the effects of water stressed host plants on the survival and fecundity of U. lugens and variation in the chemical composition of the host plant in the following chapters may further explain the results of this experiment.

Chapter 4

The Influence of Variable Nitrogen on the Performance of *Uraba lugens* Walk. Feeding on Fresh Diet.

CHAPTER 4

The influence of variable nitrogen on the performance of Uraba lugens Walk feeding on fresh diet.

4.1 INTRODUCTION.

The outbreak hypothesis proposed by White (1966, 1969, 1974, 1976, 1978) suggests that the abundance of an insect is dependent on the proportions of its population surviving in the very early stages of its development, for example survival in the first stadium. This survival is thought to be influenced by the proportion of soluble nitrogenous compounds present in the food plant which in turn is influenced by the water status of the plant. White's hypothesis therefore consists of two parts: (1) "water stress" results in an increase in the free amino acids in the plant; (2) this increase in free amino acids results in a higher survival rate of the early instars of a phytophagous insect.

It is well known that water stress dramatically increases the amount of certain free amino acids in the plant's foliage (see review section 1.7). Implicit in the second part of this hypothesis is the assumption that nitrogen is the limiting nutrient for an insect feeding on a plant; moreover the amount of nutrient provided depends on the growing conditions of the food plant which therefore indirectly regulate the insect population feeding on it.

A number of researchers have found that the rate of growth of phloem feeding insects is directly correlated with the amount of soluble nitrogen in their food plants (van Emden and Bashford, 1971;

McClure, 1980; Miles et al, 1982a). However working with such insects on water stressed plants leads to conflicting results, possibly due to the insect's reliance on the plant's turgor (Kennedy et al, 1958; Kennedy and Booth, 1959).

Chewing insects therefore offer greater opportunities for determining the influence of a plant's physiological status on the performance of its insect population. However very little work of this nature has been done using such a phytophage.

Fox and Macauley (1977) found that the performance of Paropsis atomaria Oliver on various species of Eucalyptus was closely related to the leaf nitrogen content. However Slansky and Feeny (1977) found that Pieris rapae (L) had a similar growth rate on different species of crucifers with variable nitrogen content. A high level of proline, an amino acid which increases dramatically in water stressed plants has also resulted in a variable response from phytophagous insects. Haglund (1982) found that high proline levels increases the feeding rate of grasshoppers. However Bright et al (1982) working with high proline barley varieties found no increase in the performance of a number of insect species, including a grasshopper, fed on these plants. Similarly Miles et al (1982b) found no increase in the performance of P. atomaria when fed water stressed leaves of E. camaldulensis.

Because of such variations in the response of insects to nitrogen fluctuations in their diet, it was decided to test the influence of variations in nitrogen content in fresh diet on the performance of U. lugens by two means. Experiment I: subjecting potted plants of E. camaldulensis to water stress and recording the survival, development

and fecundity of larvae fed on these plants. Experiment II: varying the free amino acid content in the leaves of a range of egg hosts for U. lugens by standing branches in a number of different aqueous solutions of amino acids and recording the survival of the first instar.

Eucalyptus camaldulensis is a good food plant for U. lugens (Cobbinah, 1983), survival of early instars is high, it is only in the eighth stadium that the survival curve follows the decline typical of Slobodkin's type I curve (see Introduction chapter 2 fig. 2.3.1 and 2.4.1 e). It would therefore be expected that survival of the early stadia would not vary significantly from that of the control plants. If White's hypothesis holds true however survival may be greater in the latter stadia on plants with a high nitrogen content.

Eucalyptus platypus and E. astringens are poor hosts for U. lugens (see Chapter 2 fig.2.4.1 a and b). For larvae on E. platypus there is usually no survival past the fourth stadium and on E. astringens, no survival past the second stadium. The low survival of U. lugens on these species has been attributed to the presence of antifeedants (Morgan and Cobbinah, 1977; Cobbinah et al, 1982; Cobbinah, 1983). However in some seasons on such poor hosts the survival of U. lugens has been observed to increase, some insects surviving to adults (Morgan and Cobbinah, 1977; Cobbinah, 1978). Applying White's (1969, 1978) hypothesis, an increase of free amino acids in the plant's foliage may influence this change in survival. Therefore in Experiment II an increase in the survival of first instars feeding on poor food plants artificially provided with a higher complement of amino acids, could support the second part of White's hypothesis. This would arise, not

because nitrogen was in critically low concentrations in the plants, but because additional amounts of amino nitrogen may counter the affects of the antifeedants present (see Cobbinah et al, 1982).

4.2 MATERIALS AND METHODS.

4.2.1 Experiment I: The influence of water stress on the performance of U. lugens

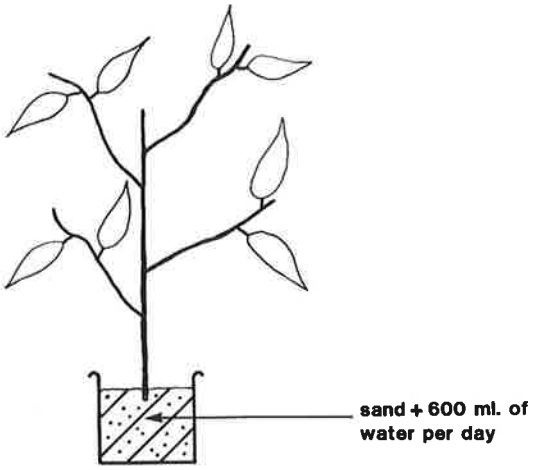
4.2.1.1 Food plants

Saplings of river red gum (E. camaldulensis) were grown in five litre pots containing river sand and maintained in an insect proof field cage. The saplings were watered at two daily intervals and supplied monthly with the water soluble fertilizer "Thrive" prior to the experiment. For the experiment, saplings of a uniform height (approx. 1m) and vigor were selected and randomly allocated to three watering regimes (fig. 4.2.1.1.1):

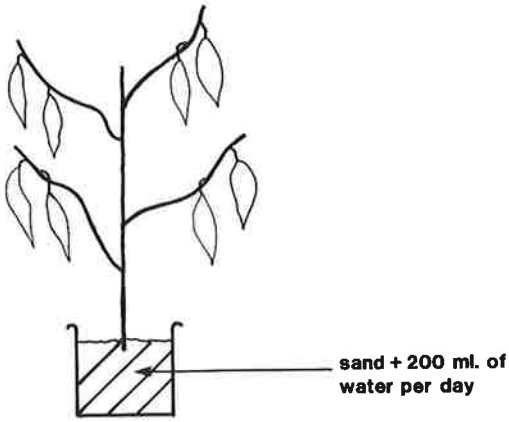
(1) Control: Soil kept in a moist condition by applying 600ml of distilled water per day, so that the leaves remained turgid. This was assesed by measuring the angle of the leaf to the stem (Elias, 1981; Miles et al, 1982 b).

Figure 4.2.1.1.1 Watering regimes for Experiment I.

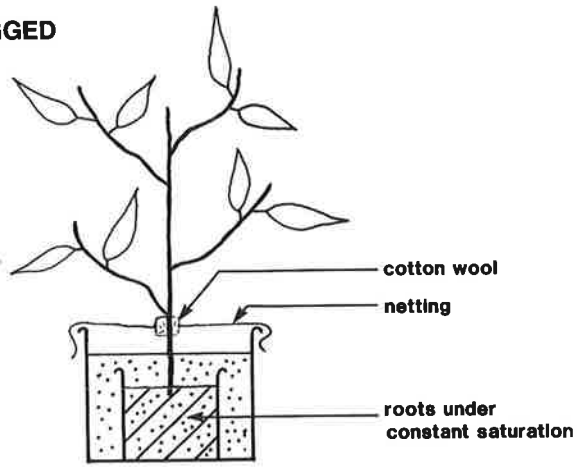
CONTROL



DROUGHTED



WATERLOGGED



(2) Droughted: Watering was ceased until the water potential reached - 25 bars (measured on a pressure bomb psychrometer). The saplings were then watered with 200ml of water per day in order to maintain their stressed condition, such that the angle of the leaves to the stem was between 30 - 40 degrees, a predicted water potential of - 24 bars (Miles et al 1982 b).

(3) Waterlogged: The potted saplings were placed in 10 litre water proof containers so that the plants could be continually flooded with water.

The experiment was conducted in a controlled environment of 30°C and with a 12 /12 hour light / dark period with four replicates per treatment. Saplings were equilibrated for three days in the above conditions and thereafter subjected to the three watering regimes and equilibrated for a further three days before placing the insects in the treatments.

4.2.1.2 Insects

Egg batches of U. lugens were collected from E. camaldulensis in the field. One hundred newly ecloded larvae from each egg batch were placed on the test plants. This was done by cutting around the egg batch when the eggs were at the black head stage and attaching the remaining leaf portion and eggs to a leaf on the test plant by means of a paper clip. Care was taken to ensure a minimal amount of the original oviposition site remained without harming the attached eggs so that the

newly eclosed larvae would have to move quickly onto the test plant to feed. At the sixth stadium larvae were transferred to new test plants which had been subjected to the same test watering regimes for the same time. Their numbers were reduced to 20-30 on each plant in order to decrease the feeding load on the plant. This also reduced disruption of the larvae by frequently tansferring them to new food plants.

Larvae were reared through to pupation and the following parameters were recorded: survival of the first, second, sixth and eleventh instars; duration of the first seven stadia measured as time taken in days for 60% of the population to moult to the next instar; potential fecundity using the regression established by Cobbinah (1978, 1983; see also chapter 3, section 3.2.2).

4.2.2 Experiment II: The influence of variations in nitrogen concentration in three different food plants on the survival of first instar larvae for *U. lugens*.

Branches with a number of mature leaves were collected from, *E. camaldulensis*, *E. platypus* and *E. astringens* from the Waite Agricultural Research Institute aboretum and placed immediately in water. These branches were transported back to the laboratory where they were placed in a number of preprepared aqueous solutions of amino acids for twenty four hours at 25°C.

Egg batches of *U. lugens* were collected from the field on *E. microcarpa* in order to prevent the influence of "parent imprint"

(Jermy, 1965; Hovanitz, 1969) and transferred to the treatments at the black head stage as described above (section 4.2.1.2). The number of newly ecloded larvae were recorded so that an estimate of first stadium survival could be made. The treatments were maintained at 25°C and in a twelve hour light period per day for the duration of the experiment.

The treatments were as follows: distilled water; proline at 0.02% (W/W); proline at 0.02% plus glutamic acid, histidine, cysteine, threonine, leucine, valine, all at 0.01%. The treatments were replicated four times.

4.3 RESULTS AND DISCUSSION.

4.3.1 Experiment I

4.3.1.1 Length of stadia

Table 4.3.1.1.1 Mean stadial length in days ± SD.

Stadium	Watering Regime		
	Control	Droughted	Waterlogged
1	4.0 ± 0	4.0 ± 0	4.0 ± 0
2	4.5 ± 1.0	3.8 ± 0.5	3.8 ± 0.5
3	4.5 ± 0.6	4.5 ± 0.6	4.5 ± 0.6
4	4.3 ± 0.5	4.5 ± 0.6	4.0 ± 0
5	3.5 ± 0.6	3.3 ± 0.5	4.0 ± 0
6	4.5 ± 0.6	4.5 ± 1.0	4.0 ± 0
7	4.3 ± 0.5	4.0 ± 0	4.0 ± 0

Analysed using 2 way analysis of variance.
No significant difference in length of stadia between treatments ($P > 0.05$).

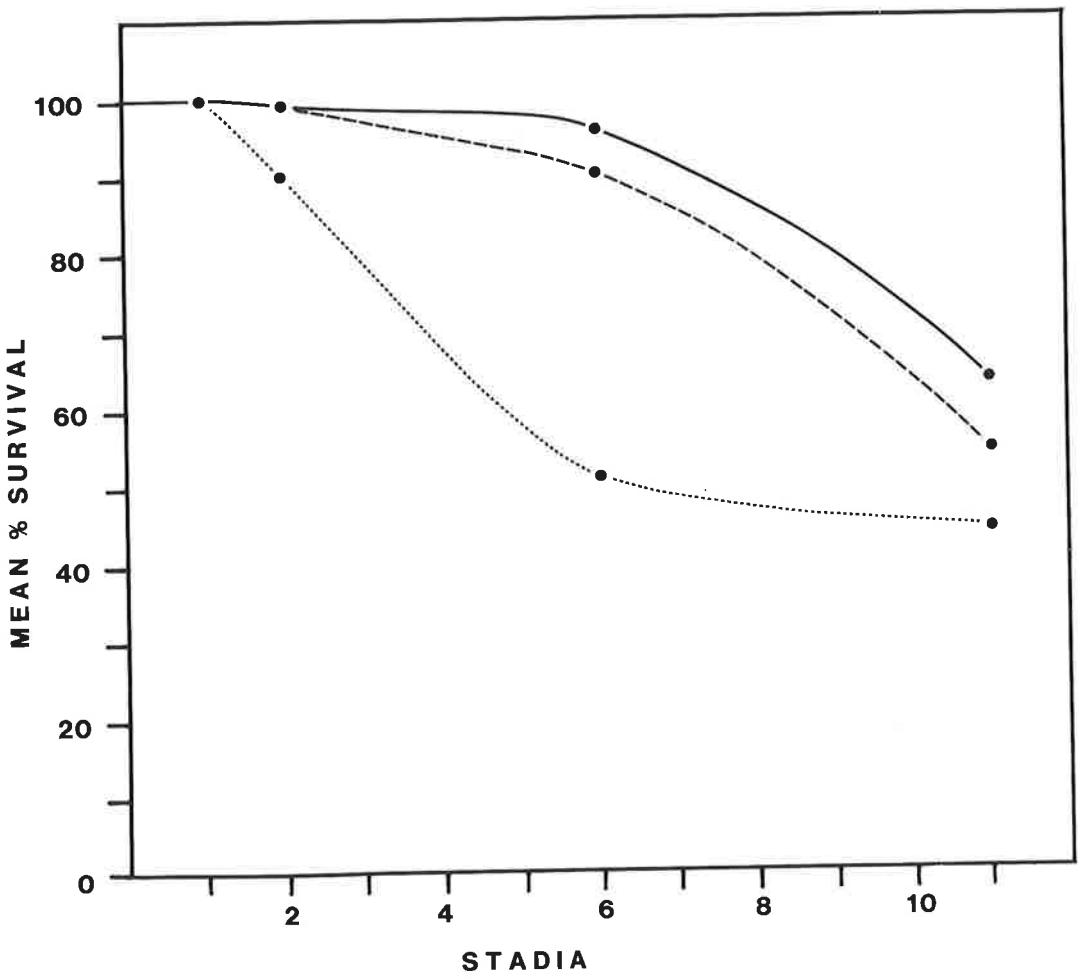
The water status of the plant did not influence the development rate of U. lugens as measured by stadial length. According to Beck (1950) dietary deficiencies suppress or retard moulting, therefore it may be assumed that the diets used were nutritionaly adequate for U. lugens.

Figure 4.3.1.2.1 Mean percentage survival of *U. lugens* larvae on *E. camaldulensis* subjected to three different watering regimes.

Control ——————

Droughted – – – – – –

Waterlogged .....


100
80
60
40
20
0
MEAN % SURVIVAL
2
4
6
8
10
STADIA

4.3.1.2 Survival. (fig. 4.3.1.2.1)

Table 4.3.1.2.1 Mean percentage survival ± SE, of U. lugens on potted E. camaldulensis in different watering regimes.

Stadium	Watering Regimes		
	Control	Droughted	Waterlogged
I	100 ± 0	100 ± 0	100 ± 0
II	98.2 ± 0.5	97.9 ± 0.7	90.0 ± 1.6
VI	95.7 ± 1.0	90.1 ± 1.8	*51.4 ±16.3
XI	63.3 ± 7.7	54.1 ± 14.2	43.8 ± 9.4

Analysed using 2 way analysis of variance.
* significantly different at $P < 0.05$ from control.

In general terms the overall survival of U. lugens on E. camaldulensis was not influenced by the treatment watering regimes. The significantly lower survival of larvae on the waterlogged plants at stadium VI was due in part to the experimental design. The larvae at stadium VI were significantly more active than larvae of the previous stadia, this behaviour pattern being considerably more obvious in the stressed treatments probably due to stress - induced changes in the quality of the food. Larvae at this stage of their development no longer exhibit the gregarious behaviour necessary to the survival of early instars (Cobbinah, 1978) and become more active in searching for fresh feeding sites but this did not explain the noticeable increase in searching by larvae on the stressed plants. A number of larvae from the waterlogged treatment were therefore drowned in the water reservoir

despite the netting secured around the base of the tree which was designed to prevent this occurring. At stadium VI a fresh netting trap of stronger material was secured around the base of the sapling which was surrounded by a non-absorbant cotton wool plug. This measure prevented such losses in the succeeding stadia.

4.3.1.3 Potential fecundity

Table 4.3.1.3.1 Mean potential fecundity (MPF) ± SE, of U. lugens fed E. camaldulensis subjected to different watering regimes.

	Watering Regimes		
	Control	Droughted	Waterlogged
MFP	164 ± 26 a	242 ± 18 b	229 ± 21 b
*CNG	5191	6546	5015

Analysed using one way analysis of variance due to no significant difference between the replicates. Different letters indicate significant difference at, $P < 0.001$.

* Potential contribution to next generation by test population based on a sex ratio of 1.

The water status of the plant significantly influenced the potential fecundity of U. lugens. In other words larvae feeding on E. camaldulensis subjected to drought or waterlogging were 40% potentially more fecund than larvae feeding on plants under an adequate watering regime. Since survival was not influenced by the different watering regimes the potential population for the succeeding generation of U. lugens fed droughted or waterlogged plants should be greater than

for larvae fed plants not subjected to water stress. The inference from these results is that stress leading to higher amounts of certain amino acids in leaves of a good food plant may slightly decrease survival of certain instars but significantly increases the size of individuals and therefore their fecundities.

4.3.2 Experiment II: The influence of variations in nitrogen concentration in three different food plants on the survival of first instar larvae of U. lugens.

Table 4.3.2.1 Mean % survival of first instar larvae ± SE.

Treatment	Food Plant		
	E. camaldulensis	E. platypus	E. astringens
Dist. water	63 ± 22	14 ± 2	0.5 ± 0.5
Pro	99 ± 1	14 ± 3	1.3 ± 0.8
Pro + Val	100 ± 0	20 ± 3	4.8 ± 1.8
+ Glu	94 ± 1	16 ± 2	1.8 ± 0.6
+ His	91 ± 6	14 ± 4	1.3 ± 0.8
+ Leu	85 ± 9	14 ± 3	2.0 ± 1.7
+ Thr	86 ± 2	14 ± 3	1.8 ± 1.8
+ Cys	98 ± 2	14 ± 2	1.5 ± 0.7
* LP	100 ± 0	15 ± 3	0 ± 0

* Living Plant.
Analysed by one way Anova, no significant difference between amino acid treatments or from living plant control ($P > 0.05$).

Survival of the first stadium was significantly lower on the poor egg hosts E. platypus and E. astringens than on E. camaldulensis. However increasing free amino acids in the leaves of these plants did not significantly increase larval survival (table 4.3.2.1) as might be expected from White's (1969, 1978) hypothesis. Although the survival of larvae on E. camaldulensis in distilled water was observed to be lower than the live plant control or the proline + valine treatment (table 4.3.2.1), this value was not significantly different. This result can be attributed to the variation between replicates in the distilled water treatment where, in one replicate no larvae survived past the first instar. Since egg batches for this experiment were obtained from the field and not from laboratory culture it is probable that an unkown biological factor outside the control of this experiment, may have influenced the survival of the insects in this treatment.

The effect of the proposed antifeedants (Cobbinah et al, 1982) present in the poor egg hosts was therefore not significantly overcome by increasing the soluble nitrogen components of the leaves, although there appears to be a trend of higher survival on the proline + valine treated plants. This is particularly evident for larvae fed E. astringens, where survival is nine times greater on the proline + valine treatments than for the distilled water or living plant controls and two to three times greater than for the other E. astringens treatments. This trend supports the findings discussed in chapter 3, where the addition of valine to the E. platypus base diet significantly increased survival of U. lugens larvae compared to survival on the base and fresh diets of this eucalypt

(tables 3.3.4.1 and 3.3.4.2). If there is any support for White's (1966) thesis that increased nitrogen in the food plant influences the outbreak dynamics of defoliators in these data, it may not be a general increase in the total nitrogen in the food but rather the increased availability of specific amino acids that may initiate improved survival of early instars of a phytophage.

These results suggest that the survival of U. lugens does not increase on a good host plant subjected to water stress. Although the survival of U. lugens is high in the initial stadia on such a plant it might be expected that survival of the latter stadia may be increased if White's (1966, 1969, 1978) hypothesis is valid, but this did not occur. This is supported by the work of Miles et al (1982 a and b). However the capacity for increase in an insect population is also dependant on the insect's fecundity. The potential fecundity of U. lugens dramatically increased on water stessed plants (table 4.3.1.3.1). Therefore as the survival of U. lugens was not influenced by the different watering regimes its capacity for population increase was dramatically enhanced, unlike the findings of Miles et al (1982 b), where no increase in fecundity was observed for P. atomaria fed water stressed E. camaldulensis.

Increasing the soluble nitrogen component in poor egg hosts for U. lugens also did not significantly increase survival of the first instar. This suggests that poor survival of U. lugens on these plants is mainly influenced by the secondary compounds of these plants as Cobbinah et al (1982) has indicated. Increasing the soluble nitrogen in plant

foliage by means other than water stress may not involve the same physiological processes in the plant's metabolism. Miles et al (1982b) reported an increase in free amino acids and a decrease in plant phenols in water stressed E. camaldulensis saplings. It has been proposed by a number of authors (Feeny, 1968, 1969; Todd et al, 1971; Chan et al, 1978; Mansour, 1981, 1982; Isman and Duffey, 1982; Mansour et al, 1982; Sutherland et al, 1982) that phenols render plant nitrogen less available to phytophagous insects. If plant nitrogen and phenols are negatively correlated (Miles et al, 1982 b; Morgan, 1984) then it is possible that nitrogen will be more available to insects feeding on water stressed plants which may also contain lower levels of antifeedants giving a two-way benefit to the phytophage attacking them.

The influence of phenols on nitrogen utilization by U. lugens is therefore investigated in the next chapter.

Chapter 5

The Influence of Phenols on Assimilation of diet by *Uraba lugens* Walk.

CHAPTER 5

The influence of phenols on assimilation of diet by Uraba lugens.

5.1 INTRODUCTION.

Plants are known to contain a wide range of secondary compounds or allelochemics (long considered as non-nutritional compounds produced by one organism which affect another, Whittaker 1970). These terpenoids, steroids, alkaloids and phenols (Whittaker and Feeny, 1971; Harbourne, 1972, 1976, 1977) appear to be involved in the protection of plants against herbivores, such as primates (Oates et al, 1980), snails (Geilsman and McConell, 1981), phytophagous insects (Feeny, 1968, 1975, 1976; Levin, 1976 a; Rhoades and Cates, 1976) and fungi (Chattopadhyay and Bera, 1980; Kritzman and Chet, 1980; Haars et al, 1981; Alfensas et al, 1982). Bernays (1978, 1981) considers that generalisations concerning the role of these compounds can be misleading since it has been found that the presence of some tannins in insect food are necessary for their continued growth, development and reproduction (see also Kato, 1978; Bernays and Woodhead, 1982 a and b; McFarlane and Distler, 1982). In another example, high levels of phenols and tannins in the natural diet of Paropsis atomaria Oliver does not retard this insect's performance (Fox and Macauley, 1977).

However, considering those plant secondary compounds having plant protective capacities, their specific mechanisms (see Klocke and Chan, 1982) include:

(1) reducing the availability of dietary proteins and amino acids (Feeny, 1970; Davis et al, 1978; Reese, 1978; Lunderstadt and Reymers, 1980) and / or inhibition of enzymatic digestive activity in the gut (Appelbaum et al, 1964; Goldstein and Swain, 1965; Appelbaum and Konijin, 1966; Feeny, 1969;Deloach and Spates, 1980).

(2) direct toxicity (Feeny, 1970).

and (3) repellence or antifeedant effects (Lipke and Fraenkel, 1976; Reese, 1978).

The inhibition of growth in Heliothis zea (Boddie) larvae fed variable amounts of condensed tannin in artificial diets was found to be due to reduced diet consumption rather than to a decrease in assimilation of the diet (Klocke and Chan, 1982). A number of other studies have also reported the influence of plant secondary compounds as feeding deterrents for insects (Asakawa et al, 1980; Fisk, 1980; Gillenwater et al, 1980; Montenegro et al, 1980; Mansour, 1981; Hatfield et al, 1982; Mansour et al, 1982; Russell et al, 1982; Miller and Feeny, 1983; Hutchins et al, 1984). However the incorporation of low molecular weight phenolics such as chlorogenic acid and rutin into artificial diets did not inhibit feeding of H. zea despite their toxic effects (Isman and Duffey, 1982).

There is a paucity of evidence for a "direct toxic" action of plant secondary compounds (i.e. decreased insect performance due to other than decreased diet consumption or assimilation of dietary nitrogen.

Although a number of studies have indicated that certain allelochemics are toxic (Applebaum et al, 1969; Todd et al, 1971; Thorpe and Briggs, 1972; Sutherland et al, 1982) diet consumption and / or assimilation of the test diet is often not reported. Bernays (1978) studied the effect of condensed and hydrolysable tannins on the survival, growth, consumption, digestion and efficiency of conversion of ingested food for a number of grasshopper species. Digestion was not reduced by the addition of tannin to their diets. The deleterious effect of hydrolysable tannins on Locusta migratoria L. was found to result from tannin passing through the peritrophic membrane damaging the epithelium of the midgut and caecae thereby decreasing assimilation.

The phenols chlorogenic acid and rutin (Isman and Duffey, 1982) did not significantly decrease diet consumption, digestability and utilization by late instar larvae of H. zea despite a chronic inhibition of growth of the early larval instars. Since larval growth in this experiment was rapidly improved when larvae were transferred to the control diet, the effect of dietary phenols on this insect was most likely not the same as that observed by Bernays (1978) for L. migratoria. Inhibition of feeding behaviour and / or limitation of the availability of some essential nutrients during the early stadia of H. zea seems a probable explanation with the later instars overcoming these effects as they became "conditioned" to the diets.

The influence of plant allelochemics on the availability of dietary nutrients to phytophagous insects has been investigated by Feeny (1968, 1969), Mansour (1981) and Miller and Feeny (1983). In each case the presence of increasing amounts of plant secondary compounds reduced

Table 5.1.1 Phenols present in Egg Hosts of U. lugens.

Rating as a Food Plant for U.lugens	Species	Phenols																					
		a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v
Good	E. camaldulensis*	-	-	-	-	4	T	5	5	2	T	T	1	T	1	-	-	1	-	-	-	2	1
	E. lehmannii	-	-	-	1	4	-	5	5	3	1	-	-	-	3	-	-	2	-	-	-	T	-
	E. odorata	-	-	-	-	4	-	5	5	2	2	-	-	-	-	-	-	-	-	-	-	3	1
	E. moorei	-	T	-	2	5	-	2	5	3	-	-	-	-	-	-	-	-	-	-	-	T	-
Intermediate	E. fasiculosa	2	-	-	4	1	0	2	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	E. punctata	-	-	-	-	2	-	5	5	3	1	2	2	-	2	-	-	1	-	-	-	3	1
	E. sideroxylon	-	3	T	-	3	-	5	3	2	1	-	-	-	2	-	-	-	-	-	-	2	2
	E. viminalis	-	T	-	-	3	-	5	5	2	-	-	1	-	1	-	-	-	-	-	-	1	1
Poor	E. astringens	-	T	-	1	5	T	4	5	3	-	-	-	-	1	-	2	2	-	-	-	1	-
	E. gardneri	-	T	-	-	5	-	1	2	2	-	-	-	-	2	-	-	-	-	-	-	1	-
	E. obliqua	2	1	-	2	4	-	3	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-
	E. platypus	-	-	-	1	3	-	5	5	3	-	-	-	-	2	-	-	-	-	-	-	1	-

Adapted from Hillis (1966 a and b, 1967 a b c and d).
* Mean of results from Hillis (1966 a) table 1 p 552.
Numbers (1 - 5) represent an increasing scale of the relative amounts;
T = trace; - = not present (concentrations ranked as to strength of chromatogram spot).
Letters represent the following phenols:

a Leucodelphinidins
b Leucocyanidins
c "Leuopelargonidins"
d Myricetin
e Quercetin
f Kaempferol
g Ellagic acid
h Gallic acid
i Genistic acid
j Caffeic acid
k p-Coumaric acid
l Sinapic acid
m Ferulic acid
n Macrantherin
o Renatherin
p Taxifolin
q Aromadendrin
r Astringin
s Rhapontin
t Piceid
u Chlorogenic acid
v p-coumarylquinic acid

both growth efficiencies and efficiencies of conversion of the test diets. However, Slansky and Feeny (1977) have pointed out the capacity of some insects to compensate for low availability of nutrients by adjusting their feeding rates, but in the studies by Mansour (1981) and Miller and Feeny (1983) stabilization of nutrient accumulation did not occur since feeding rates also decreased.

Uraba lugens Walk increased its biting response when fed artificial diets containing the phenols gallic acid and chlorogenic acid (Cobbinah, 1978). It is therefore possible that, unless these compounds are acting exclusively as phagostimulants, they may act as "cues" for U. lugens thus compensating for decreased nutrient availability in diets (see also Slansky and Feeny, 1977).

A series of chemotaxonomic surveys by Hillis (1966 a and b, 1967 a b c and d) outline the phenolic compounds present in Eucalyptus. Table 5.1.1 summarises this work in the context of egg hosts assigned to three categories of food plants of U. lugens. The phenols listed present no obvious differentiation between the egg host plants tabulated and their rating as food plants for larvae of U. lugens. However, the concentrations of phenols such as quercetin varies within an individual species (see Hillis, 1966a), compared with gallic and ellagic acids which remain relatively constant within a species (see table 5.1.1 and Hillis 1966a).

Therefore in investigating the influence of phenolics on the assimilation of diet and, in particular, dietary nitrogen by U. lugens, a spectrum of phenols was selected representing the range of concentrations present in Eucalyptus. These phenols were: gallic acid which is present

in high concentrations and remains relatively constant within and between species; quercetin which although present in high quantities, varies in concentration within and between species; and caffeic and chlorogenic acids, present at relatively low concentrations in the leaves of Eucalyptus spp.

It might be expected that, since chlorogenic and caffeic acids are present at low concentrations in the food plants of U. lugens (table 5.1.1), an increase in their concentrations may significantly affect feeding and / or assimilation of diets. However, the performance of U. lugens fed gallic acid may not be influenced by increasing concentrations of this phenol since it is present at consistently high concentrations in its natural food. That is, U. lugens may have developed a "detoxifying" mechanism for this phenol whereas quercetin may influence the assimilation of diet and dietary nitrogen since its concentration is variable within and between Eucalyptus species. It could therefore represent a component of diet which could perhaps influence feeding and nutrition of defoliators as its concentration varies under the influence of currently unknown factors in the environment of a species such as U. lugens.

5.2 MATERIALS AND METHODS.

5.2.1 Diet

A modified version of Wardojo's (1969) diet for Pieris brassicae was used into which was incorporated a number of phenols at different concentrations. The specifications and preparation of the diet are outlined below:

5.2.1.1 Stock solutions and mixtures.

(a) An aqueous solution of potassium hydroxide made such that 50ml KOH solution contains 0.56g of KOH.

(b) Vitamin B stock solution containing:-

Niacinamide	0.1 g
Ca pantothenate	0.05 g
Pyridoxine HCl	0.025 g
Riboflavin	0.025 g
Thiamine HCl	0.025 g
Folic acid	0.025 g
Biotin	0.001 g
ρ-amino benzoic acid	0.025 g

made up to 100ml with distilled water.

(c) Fatty acid - sterol mixture:-

β-sitosterol	0.036 g
stigmasterol	0.02 g
cholesterol	0.009 g

dissolved in -

Oleic acid	0.45 ml
Linoleic acid	0.075 ml
Linolenic acid	0.225 ml

in a water bath at 100 degrees Celsius.

5.2.1.2 Preparation

2.0g of vitamin free casein was dissolved in 50ml of KOH stock solution. To this was added the following:-

	Amount in grams
Albumin	4.0
Sucrose	2.0
Glucose	0.5
Fructose	0.5
Potato starch	4.0
Cellulose	4.0
Inositol	0.04
Ascorbic acid	0.4
Menadione	0.005
Wessons Salt Mix.	0.5
Vitamin A palmitate	0.01
Sorbic acid	0.15
Methyl ρ-hydrobenzoate	0.1
Streptomycin sulphate	0.02
Lecithin	0.15
Fatty acid sterol mixture	0.75 ml
B vitamin stock solution	0.5 ml

The resultant mixture was then gently homogenised, after which 6g of lyophilised leaf material of E. camaldulensis (prepared as in chapter 3, section 3.2.1) was added. Davis Bacteriological Agar (3.5g) was dissolved in 40ml of hot distilled water and added to the above mixture which was stirred throughout preparation, then poured into a heat sterilised petri dish as the mixture cooled. The diet was stored at 1°C until use.

From this base diet the following phenolic treatments were prepared: base diet alone (diet + no phenol); diet + quercetin; diet + gallic acid; diet + chlorogenic acid; diet + caffeic acid. Each of these phenols was incorporated into the diet at a range of concentrations for each phenol of 0.05%, 1.0%, 5.0%, 10.0% (W/W), with the exception of chlorogenic acid, which, due to its high cost, was incorporated at the following concentrations, 0.1%, 0.5%, 1.0%, 1.8%. These concentrations included the range of concentrations of total phenolics found in mature leaves of E. camaldulensis; viz. 4 - 10% per gram dry wieght of leaf or 2 - 5% per gram fresh weight of leaf (G.S.Taylor, pers. comm.; also see chapter 6).

Determination of the formulation of the base diet (diet with no added phenol) involved a number of feeding trials. Larval feeding on the original diet (Wardojo 1969) was poor compared with feeding on the E. camaldulensis base diet used in chapter three. Therefore a number of modifications were made incorporating knowledge of the dietary components that influence the feeding of U. lugens (see Cobbinah, 1978). The modifications were as follows.

Casein hydrolysate was deleted from all the diets since larval feeding improved with its omission. For the same reason the amount of potato starch was reduced and choline chloride was omitted. The sugars glucose and fructose were added since, in combination with sucrose, they act synergistically to dramatically improve larval phagostimulation (Cobbinah, 1978). Since U. lugens prefers to feed on diets of a very hard consistency the amounts of agar and cellulose were increased. Although these modifications alone increased larval feeding significantly above that of the original Wardojo (1969) diet, it did not compare favourably with feeding on fresh diet and was insufficient to maintain a comparable survival with that on fresh diet through several successive stadia. A number of volatiles present in Eucalyptus spp such as cineol, limonene, caryophylene, α- and β- pinene, cymene, α- and γ- terpine, sabine and carene were therefore tested for their potential as phagostimulants, however these were unsuccesful. It was therefore necessary to include E. camaldulensis leaf powder in the experimental diet. The minimum amount of leaf powder necessary for improved feeding by the larvae was therefore added. It is apparent from these preliminary feeding trials and Cobbinah's (1978) work that there is a phagostimulant or a combination of phagostimulants present in leaves of good food plants of U. lugens that have not as yet been recognised.

5.2.2 Insects

Larvae of U.lugens were collected from E. camaldulensis in the field and reared on fresh leaves of this eucalypt in the laboratory until they moulted to the seventh instar. These were fasted for twenty four hours before being assigned randomly to the test diets which were presented as decribed in chapter three. Four larvae were placed on each test diet and the treatments replicated four times. The larvae were fed the test diets for five days at 25 degrees Celsius in a 12 hour light regime. The diet was changed every two days and all diets were presented to test insects in sterilised 9 centimeter glass petri dishes.

5.2.3 Experimental procedures

The mean dry weight of each diet was calculated by weighing ten samples from each test diet prior to a test. Each diet was the oven dried at 110 degrees Celsius and reweighed to give the data from which both dry weight and moisture content were obtained. Before the larvae were placed on a test diet its fresh weight was recorded. When the diet was changed, the food remaining was removed from the test arena, dried and weighed so that the dry weight of diet consumed could be calculated. Frass was also collected from each test arena at this time, dried and weighed to calculate the diet utilized and discarded by the insects.

The total nitrogen in both test diets and frass was estimated using the Micro Kjeldahl technique as follows:

A pre-weighed amount of oven dried, ground diet or frass was placed in a 100ml pyrex test tube to which one Keltab and 4ml of concentrated sulphuric acid was added using an automatic dispenser. The samples were then heated on a digestion block using a pre-programed Tecator Controller. the temperature program was as described in table 5.2.3.1.

Table 5.2.3.1 Temperature program for Micro Kjeldahl procedure.

Step	Time hr. min.	Temperature °C
1	6. 00	120
2	0. 15	155
3	1. 00	317
4	2. 15	359
5	5. 00	cool to room temp

After the solutions had cleared the digests were cooled before adding 50ml of double distilled water and shaking. The total nitrogen present in the sample was then estimated on an auto analyser.

Utilization (assimilation) of nitrogen was then calculated using Taylor and Bardner's (1968) equation:

% utilization of diet =

[(Dry weight of food consumed - Dry weight of faeces)

/ Dry weight of food consumed] X 100

which was modified to read:

% utilization of nitrogen =

[(Amount of nitrogen consumed from diet

- Amount of nitrogen in faeces)

/ Amount of nitrogen consumed from diet] X 100

The percentage utilization of diet is equivalent to Waldbauer's (1968) approximate digestability or assimilation efficiency.

Since some assimilated nitrogen is excreted in the form of uric acid, allantoin, allantoic acid and other compounds (Bursell, 1967; Kahn et al, 1976) and the Kjeldahl technique used was not modified to include nitrate and other organic compounds (see Bradstreet, 1965), the data probably underestimated the true level of nitrogen assimilation or nitrogen assimilation efficency recorded.

Table 5.3.1.1 Mean amount of diet consumed (mg) per insect over 5 days ± SE.

Diet	% Concentration of Phenol in Diet						
	0	0.1	0.5	1.0	1.8	5.0	10.0
Control	31.9 ± 3.3 a	-	-	-	-	-	-
Caffeic acid	-	-	35.1 ± 0.7 a	40.4 ± 2.2 ab	-	24.8 ± 1.8 a	41.9 ± 4.5 ab
Chlorogenic acid	-	30.3 ± 2.7 a	25.2 ± 4.3 a	54.5 ± 1.6 c	74.4 ± 3.3 d	-	-
Gallic acid	-	-	45.2 ± 1.0 bc	39.7 ± 7.8 b	-	45.2 ± 0.5 bc	34.0 ± 1.4 a
Quercetin	-	-	37.5 ± 2.6 ab	26.8 ± 4.0 a	-	38.2 ± 3.7 ab	38.8 ± 5.0 ab

Analysed by Duncan's Multiple Range Test.
Different letters in rows and columns indicate significance at $P < 0.05$.

5.3 RESULTS AND DISCUSSION.

See appendix 5.3.1.

5.3.1 Diet consumption

It might be expected that if the presence of phenols in a plant are an evolutionary adaption for means of "direct" or immediate plant protection, then consumption of a diet would be inversely proportional to the amount of secondary plant compounds present in that diet. This has been recorded in a number of studies on the effects of phenolics on insect consumption (Asakawa et al, 1980; Fisk, 1980; Gillenwater et al, 1980; Montenegro et al, 1980; Mansour, 1981; Hatfield et al, 1982; Isman and Duffey, 1982; Klocke and Chan, 1982; Mansour et al, 1982; Miller and Feeny, 1983; Hutchins et al, 1984), the reduced rate of consumption being closely paralled by the reduced growth efficiencies recorded.

However, for the artificial diets presented to U. lugens, consumption did not decrease significantly from that of the control diet in any instance (table 5.3.1.1). Consumption of the diets containing caffeic acid and quercetin were comparable to that of the control diet (table 5.3.1.1; figs. 5.3.1.1 a and 5.3.1.2 b). Incorporation of chlorogenic and gallic acids into the base diet significantly increased consumption rates (table 5.3.1.1; figs. 5.3.1.1 b and 5.3.1.2 a).

Figure 5.3.1.1 Amount of test diet consumed (mg) per insect over 5 days.

(a) Caffeic acid
Regression: $y = 30.92 + 0.72x$
Not significant ($T = 1.15$, 19 df; $P > 0.05$)

(b) Chlorogenic acid
Regression: $y = 23.20 + 27.20x$
Significant ($T = 7.59$, 18 df; $P < 0.001$)

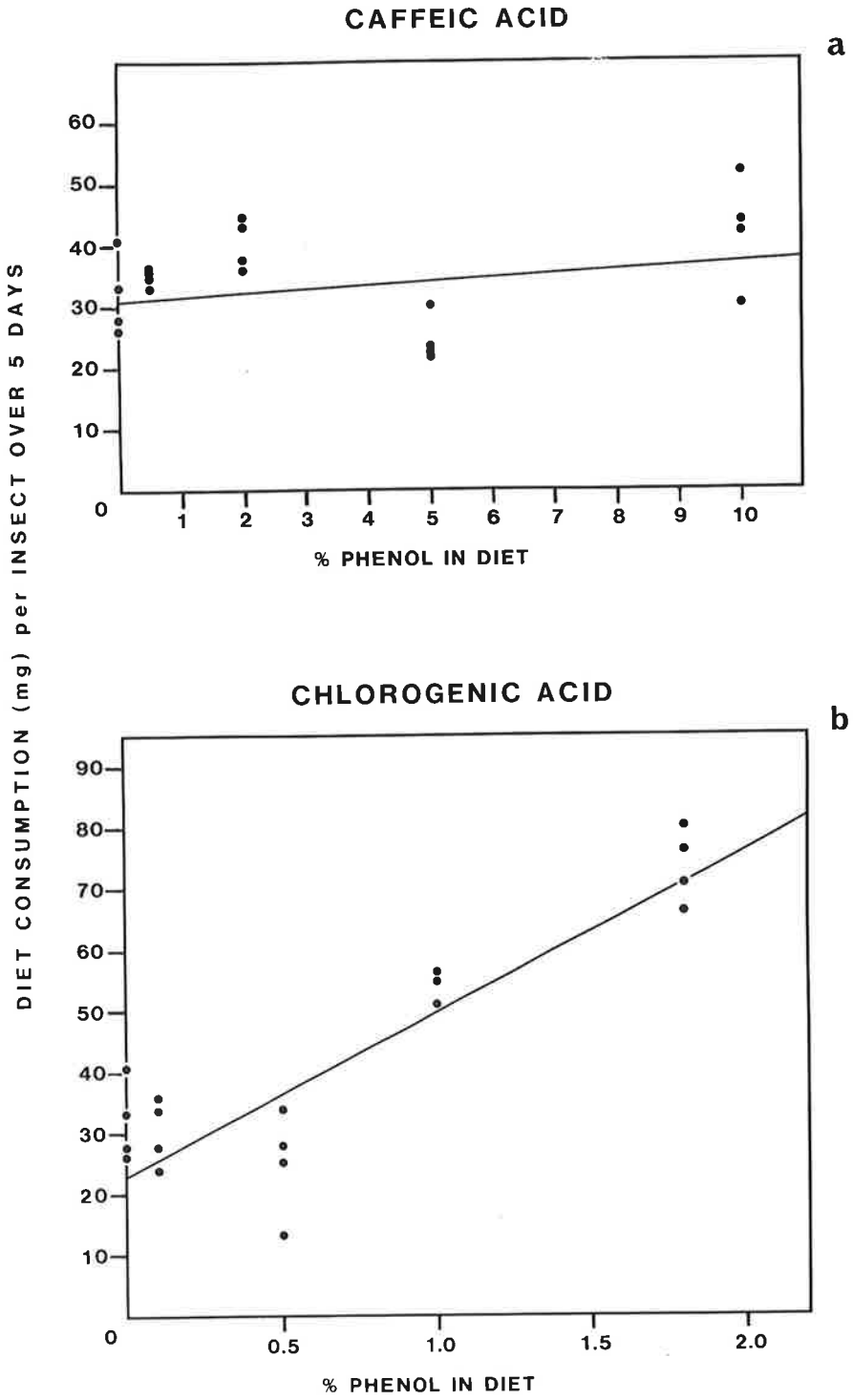
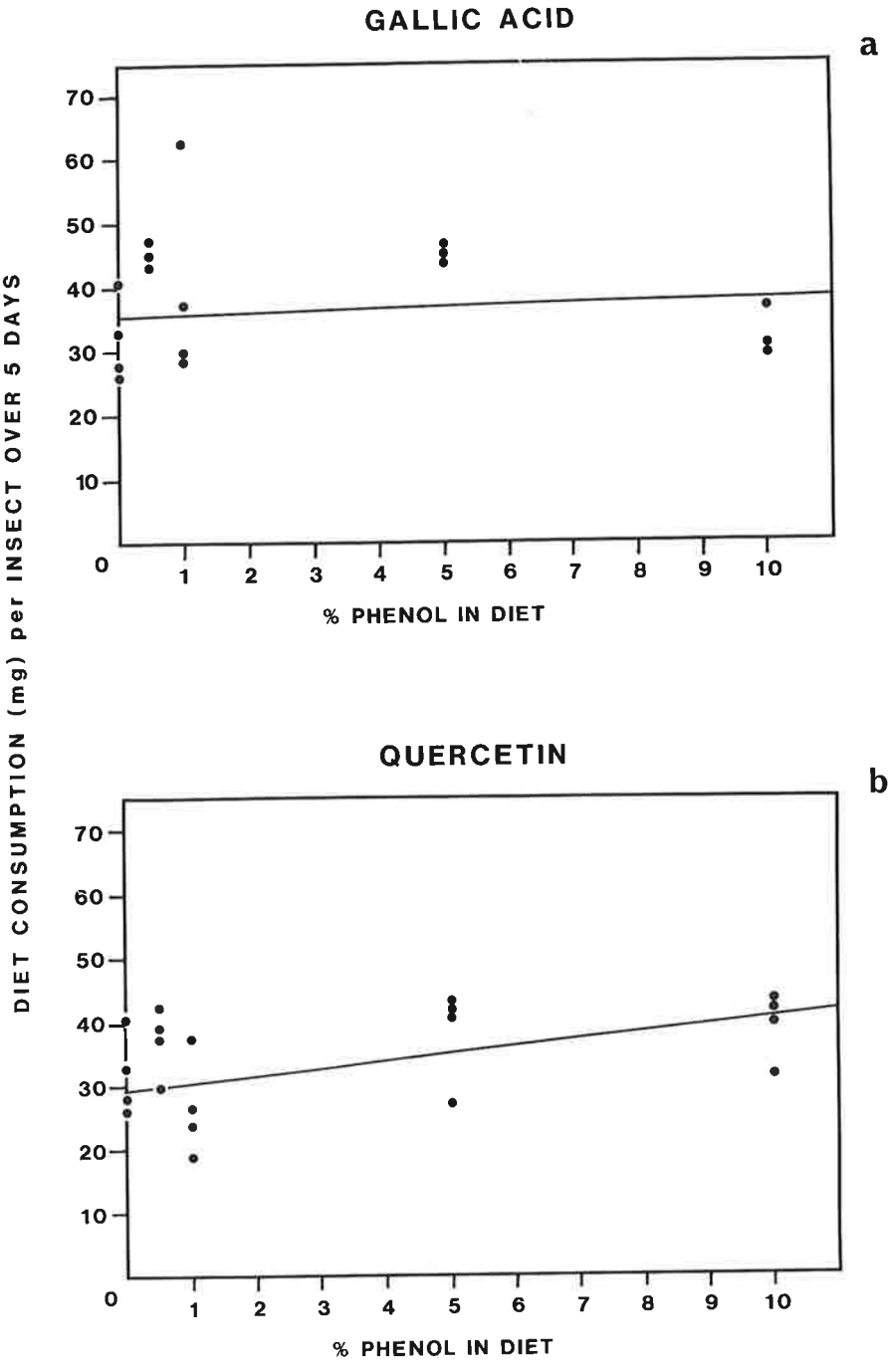

CAFFEIC ACID
a
DIET CONSUMPTION (mg) per INSECT OVER 5 DAYS
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
% PHENOL IN DIET
CHLOROGENIC ACID
b
90
80
70
60
50
40
30
20
10
0
0.5
1.0
1.5
2.0
% PHENOL IN DIET

Figure 5.3.1.2 Amount of test diet consumed (mg) per insect over 5 days.

(a) Gallic acid
Regression: $y = 35.78 + 0.31x$
Not significant ($T = 0.36$, 16 df; $P > 0.05$)

(b) Quercetin
Regression: $y = 29.62 + 1.08x$
Not significant ($T = 1.87$, 19 df; $P > 0.05$)


GALLIC ACID
a
DIET CONSUMPTION (mg) per INSECT OVER 5 DAYS
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
% PHENOL IN DIET
QUERCETIN
b
DIET CONSUMPTION (mg) per INSECT OVER 5 DAYS
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
% PHENOL IN DIET

If the presence of phenols decrease an insect's utilization of dietary components, then, according to Slansky and Feeny (1977), consumption may increase to compensate for the lower nutritional value of the diet. Consumption of the chlorogenic acid diet significantly increased with progressively higher concentrations of this phenol (fig. 5.3.1.1 b). However for the other phenols tested this trend did not occur (figs. 5.3.1.1 a and 5.3.1.2 a, b).

Some phenolic compounds, for example chlorogenic acid are known to stimulate feeding (Hsiao and Fraenkel, 1968). Cobbinah (1978) found that the incorporation of chlorogenic and gallic acids into a plain agar diet increased the biting response of U. lugens while caffeic acid reduced larval feeding response and quercetin did not influence feeding compared with the artificial diet controls. In this experiment caffeic acid did not deter feeding which may have been due to the additional nutrients and other components present in the artificial diet used as opposed to the simple diet used by Cobbinah (1978). Thus the additional components may have overridden the deterrent effect of caffeic acid reported by Cobbinah. The response of U. lugens to gallic acid and chlorogenic acid in particular, may be due either to larval compensation for a decrease in diet quality or that these phenols act directly to stimulate feeding.

Table 5.3.2.1 Utilization of diet (means as %of total diet provided) ± SE.

Diet	% Concentration of Phenol in Diet						
	0	0.1	0.5	1.0	1.8	5.0	10.0
Control	42.7 ± 4.7 ab	-	-	-	-	-	-
Caffeic acid	-	-	11.0 ± 0.2 d	32.7 ± 2.6 bc	-	16.2 ± 4.3 d	36.2 ± 3.8 b
Chlorogenic acid	-	36.1 ± 2.5 b	23.3 ± 1.3 c	30.2 ± 3.1 bc	35.2 ± 3.1 b	-	-
Gallic acid	-	-	30.5 ± 0.7 bc	38.4 ± 3.7 b	-	35.9 ± 1.0 b	32.3 ± 8.1 bc
Quercetin	-	-	32.1 ± 3.6 bc	51.1 ± 5.4 a	-	23.8 ± 3.7 c	11.9 ± 4.3 d

Analysed by Duncan's Multiple Range Test.
Different letters in rows and columns indicate significant difference at $P < 0.05$.

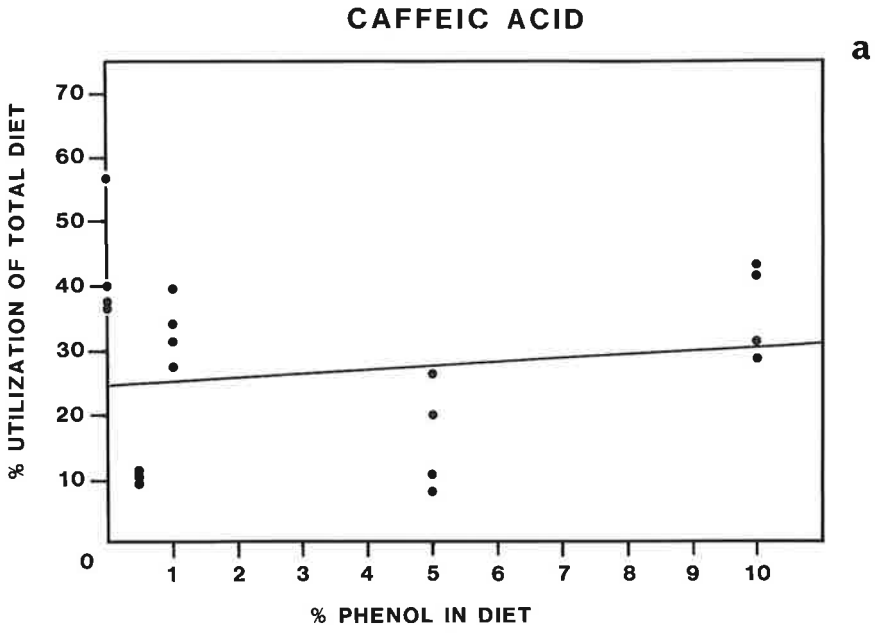
5.3.2 Utilization of total diet.

Although consumption of diet was not decreased by the phenols caffeic acid and quercetin, utilization or assimilation efficiency was significantly reduced (table 5.3.2.1). With increasing proportions of quercetin in the diet assimilation by U. lugens is progressively reduced such that it can be expressed as a negative linear regression (fig. 5.3.2.2 b). However, for the phenols chlorogenic and gallic acids assimilation efficiencies were not significantly different from that of the control (table 5.3.2.1; figs. 5.3.2.1 b and 5.3.2.2 a).

Figure 5.3.2.1 Utilization of Total Test Diet containing phenols at different concentrations by *U. lugens* larvae.

(a) Caffeic acid
Regression: $y = 24.54 + 0.60x$
Not significant ($T = 0.69$, 19 df; $P > 0.05$)

(b) Chlorogenic acid
Regression: $y = 31.88 + 0.19x$
Not significant ($T = 0.05$, 18 df; $P > 0.05$)


CAFFEIC ACID
a
% UTILIZATION OF TOTAL DIET
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
% PHENOL IN DIET

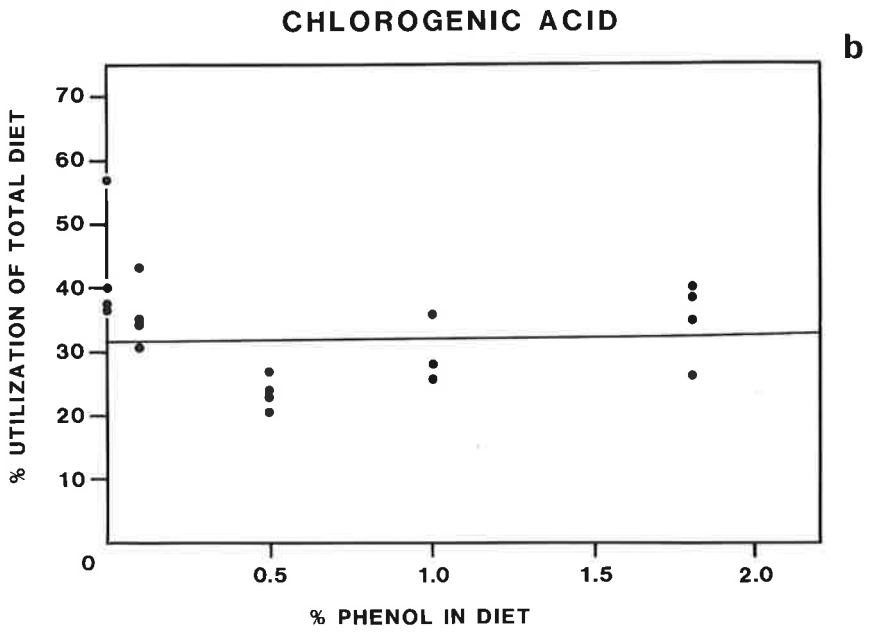
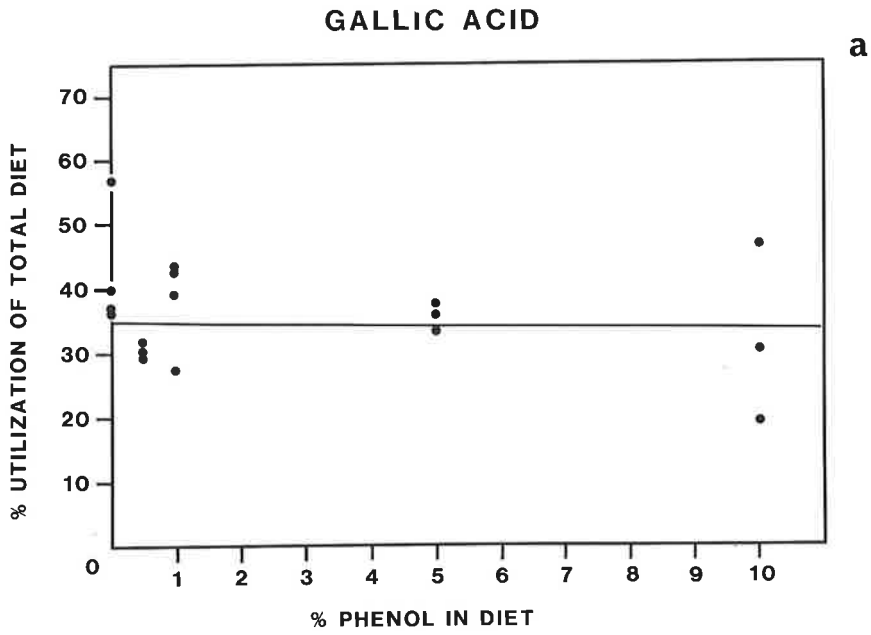

CHLOROGENIC ACID
b
% UTILIZATION OF TOTAL DIET
70
60
50
40
30
20
10
0
0.5
1.0
1.5
2.0
% PHENOL IN DIET

Figure 5.3.2.2 Utilization of Total Test diet containing phenols at different concentrations by *U. lugens* larvae.

(a) Gallic acid
Regression: $y = 34.86 - 0.14x$
Not significant ($T = -0.18$, 16 df; $P > 0.05$)

(b) Quercetin
Regression: $y = 39.87 - 2.78x$
Significant ($T = -3.37$, 19 df; $P = 0.002$)


GALLIC ACID
a
% UTILIZATION OF TOTAL DIET
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
% PHENOL IN DIET

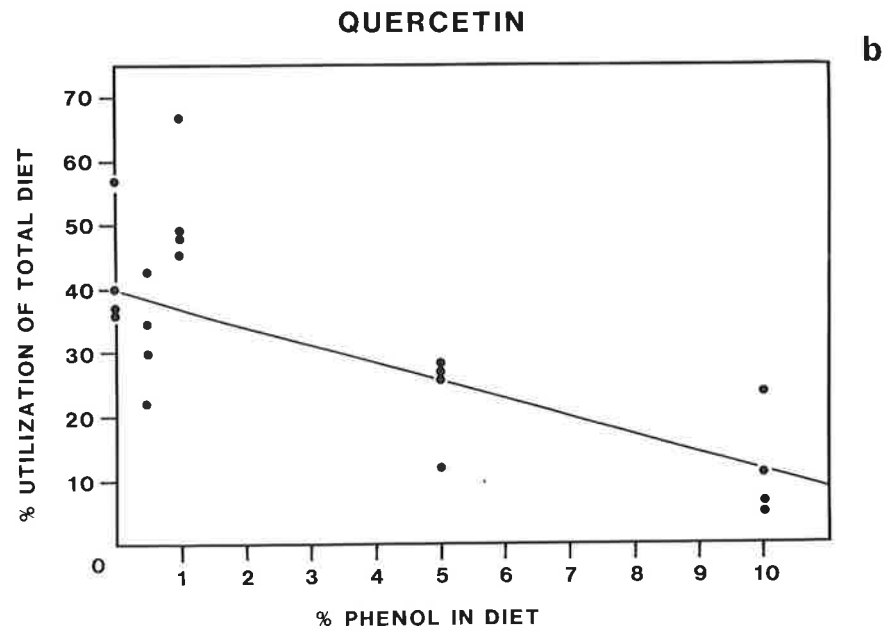

QUERCETIN
b
% UTILIZATION OF TOTAL DIET
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
% PHENOL IN DIET

Table 5.3.3.1 Utilization of dietary nitrogen (means as % of total nitrogen provided) ± SE.

Diet	% Phenol Content in Diet						
	0	0.1	0.5	1.0	1.8	5.0	10.0
Control	66.0 ± 5.4 a	-	-	-	-	-	-
Caffeic acid	-	-	9.8 ± 3.7 d	32.2 ± 1.1 b	-	23.4 ± 3.0 c	27.1 ± 2.2 bc
Chlorogenic acid	-	31.7 ± 3.1 b	36.5 ± 6.7 b	47.5 ± 21.0 ab	38.7 ± 5.5 b	-	-
Gallic acid	-	-	26.3 ± 1.1 bc	38.4 ± 7.0 b	-	45.6 ± 2.9 b	42.5 ± 11.4 b
Quercetin	-	-	32.9 ± 3.1 b	34.0 ± 3.9 b	-	6.3 ± 2.0 d	-7.6 ± 3.7 e

Analysed by Duncan's Multiple Range Test.
Different letters in rows and columns indicate significant difference at $P < 0.05$

5.3.3 Utilization of dietary Nitrogen.

The assimilation of dietary nitrogen was significantly reduced in all the test diets containing additional phenols with the exception of chlorogenic acid at 1.0% (table 5.3.3.1). The greatest reduction in nitrogen assimilation was observed in the quercetin test diets, where nitrogen utilization decreased with increasing concentrations of this phenol (fig. 5.3.3.2 b). This trend however was not apparent in the other phenol test diets (figs. 5.3.3.1 a b and 5.3.3.2 a).

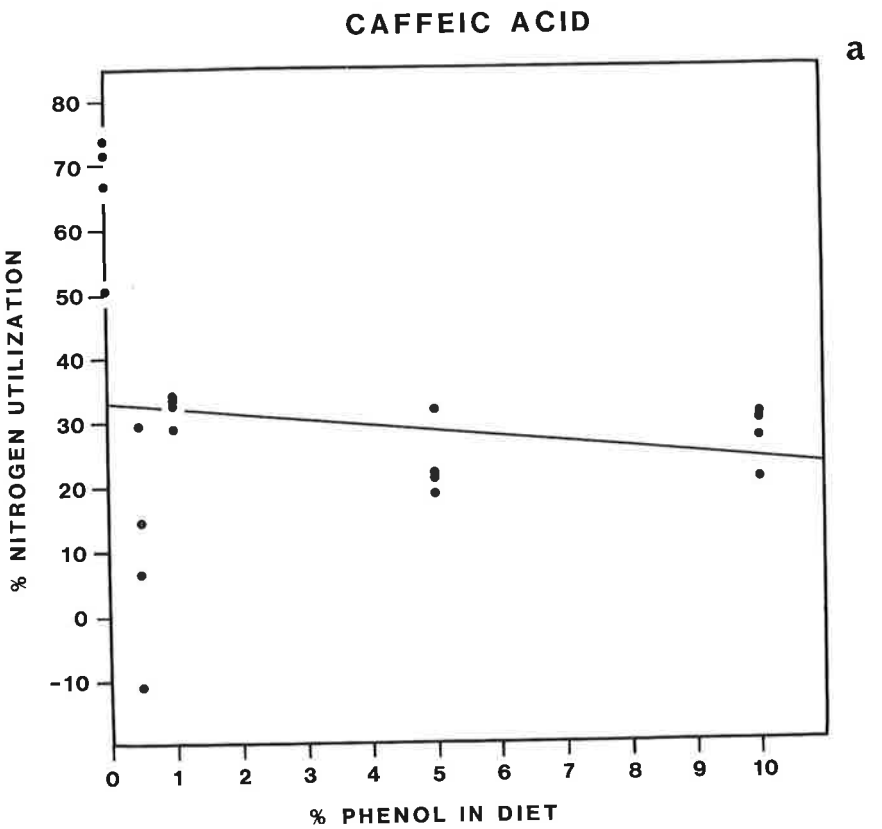
These results show that the phenols caffeic acid and quercetin reduce larval assimilation of diet and in the case of quercetin, the reduction in diet and nitrogen assimilation can be expressed linearly with increasing amounts of this phenol (figs. 5.3.2.2 b, and 5.3.3.2 b). The phenols chlorogenic and gallic acids however did not influence diet and nitrogen assimilation markedly (tables 5.3.2.1 and 5.3.3.1; figs. 5.3.2.1 b, 5.3.2.2 a, 5.3.3.1 b, 5.3.3.1 b and 5.3.3.2 a), so that the increase in consumption on these diets (and thus an increase in nitrogen consumption, Appendix 5.3.1), particularly chlorogenic acid, may compensate for the decrease in nitrogen assimilation observed (table 5.3.3.1).

The survival data (Appendix 5.3.1) is not a good estimate of larval performance on the test diets since larvae were assigned to the diets at the seventh stadium for only five days and thus had stored nutrients from their previous diet. However survival of larvae on the quercetin diet was much lower than survival on the other test diets, indicating that the presence of this phenol dramatically reduces larval performance. A better measure of larval performance would have been

Figure 5.3.3.1 Utilization of dietary nitrogen by *U. lugens* larvae in the presence of phenols.

(a) Caffeic acid
Regression: y = 33.27 - 0.99x
Not significant (T = -0.78, 19 df; P > 0.05)

(b) Chlorogenic acid
Regression: y = 45.88 - 1.40x
Not significant (T = -1.15, 16 df; P > 0.05)


CAFFEIC ACID
a
% NITROGEN UTILIZATION
80
70
60
50
40
30
20
10
0
-10
0
1
2
3
4
5
6
7
8
9
10
% PHENOL IN DIET

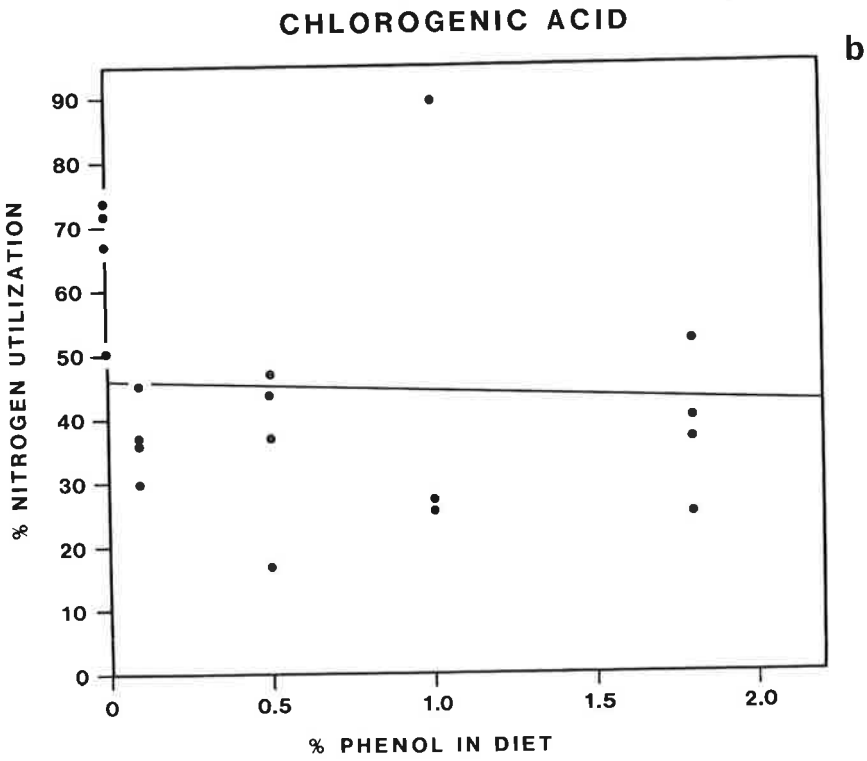
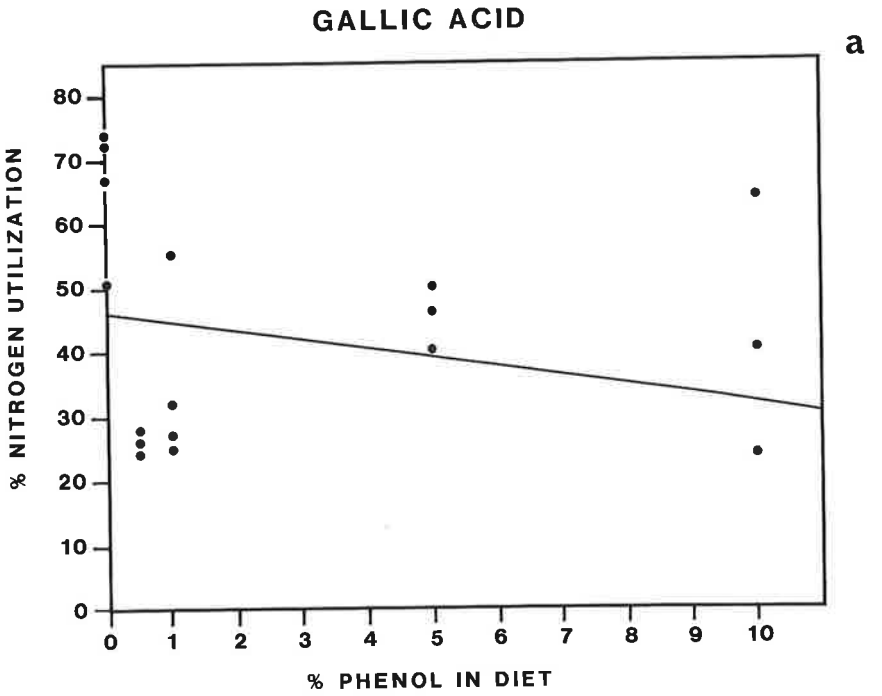
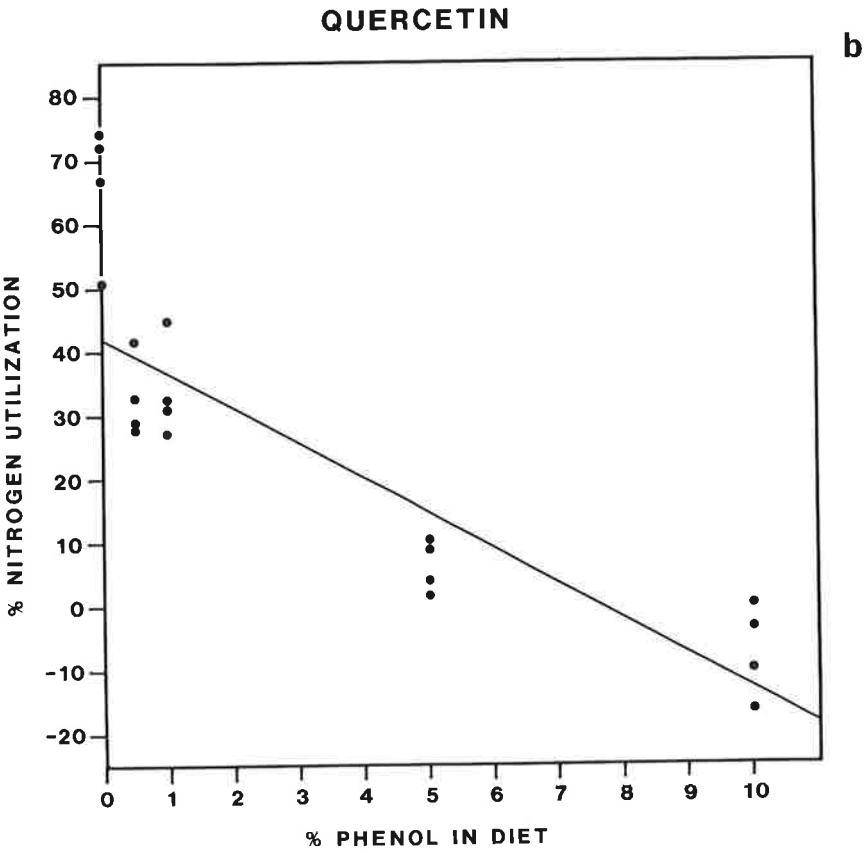

CHLOROGENIC ACID
b
% NITROGEN UTILIZATION
90
80
70
60
50
40
30
20
10
0
0
0.5
1.0
1.5
2.0
% PHENOL IN DIET

Figure 5.3.3.2 Utilization of dietary nitrogen by *U. lugens* larvae in the presence of phenols.

(a) Gallic acid
Regression: $y = 44.89 - 3.33x$
Not significant ($T = -0.47$, 18 df; $P > 0.05$)

(b) Quercetin
Regression: $y = 42.30 - 5.48x$
Significant ($T = -5.57$, 19 df; $P < 0.001$)


GALLIC ACID
a
% NITROGEN UTILIZATION
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10
% PHENOL IN DIET


QUERCETIN
b
% NITROGEN UTILIZATION
80
70
60
50
40
30
20
10
0
-10
-20
0 1 2 3 4 5 6 7 8 9 10
% PHENOL IN DIET

weight gained by larvae in the test diets. Due to the shortage of larvae available at the time of this experement and the time involved in measuring this additional parameter, this was not done. The performance of early instar larvae on phenol diets would also be interesting to investigate since Isman and Duffey (1982) found that plant phenolics influence the performance of early instars of H. zea more profoundly than the performance of late instar larvae. However the feeding habit of U. lugens (see Introduction chap. 2) does not facilitate such an experiment with early larval stadia on artificial diets, although Cobbinah's (1978) work on the host preferences of U. lugens does suggest that plant secondary compounds may influence performance of early stadia to a greater degree than later stadia.

The response of U. lugens larvae to the presence of gallic acid in their diet suggests that this insect may have developed a "detoxifying" or compensation mechanism. The presence of a relatively constant yet high concentration of this phenol in the food plants of U. lugens (section 5.1 and table 5.1.1) lends credence to this hypothesis.

It might have been expected however that the response of U. lugens to chlorogenic acid may have paralled that on quercetin diets since this phenol although at low concentrations in the insect's food plants, fluctuates considerably (table 5.1.1). Also it is thought that the presence of ortho-hydroxyl groups may influence the toxicity of phenols since Todd et al (1971) found that phenols having ortho-hydroxyl groups (viz. catechol, tannic acid, procatechuic acid, quercetin and chlorogenic acid) were the most detrimental of the compounds tested, to

the growth and survival of Schizaphis graminum (Rondani). However Alfensas et al (1982), working on fungi, found the converse to be true, the lower the number of hydroxyl groups the more toxic the phenol to fungal growth.

Although caffeic acid reduces diet assimilation for U. lugens it probably has little influence on the insects when feeding on fresh foliage since its concentration is very low (see table 5.1.1). Quercetin therefore appears as the major phenol tested in this experiment that may influence the performance of U. lugens on natural food plants. Although often at high concentrations, this phenol fluctuates greatly within and between the eucalypt food plant species of U. lugens (see section 5.1 and table 5.1.1) and may therefore be a major regulatory factor in the population variation of U. lugens on its host plants.

Chapter 6

Seasonal and Annual Variation of Nitrogen and Phenols Present in Mature Eucalyptus camaldulensis Foliage.

CHAPTER 6

Seasonal and annual variation of nitrogen and phenols present in mature Eucalyptus camaldulensis foliage.

6.1 INTRODUCTION.

In the previous chapters it has been demonstrated that the performance of Uraba lugens is influenced by the inclusion of specific amino acids (chap. 3) and phenols (chap. 5) in artificial diets and by the water status of its food plant (chap. 4). Although Miles et al (1982 b) found no increase in the survival and fecundity of Paropsis atomaria Oliver fed water stressed E. camaldulensis, this insect feeds on young eucalypt leaves as opposed to the mature leaves preferred by U. lugens. Ohmart et al (1985) however, found that P. atomaria fed Eucalyptus blakelyi Maiden of variable nitrogen content, significantly increased its fecundity in response to higher nitrogen food, although the viability of its eggs was lower than that of adults fed leaves with lower nitrogen levels.

It has been demonstrated by Journet and Cochrane (1978) that the young leaves of E. blakelyi contain a higher concentration of free amino acids than mature leaves. Since P. atomaria feeds on young, nitrogen rich foliage it seems possible that variation solely of nitrogen in the food plant by fertilizer application would not necessarily influence the insects' overall performance unless the variation in other leaf components such as allelochemics (eg. phenols) played an important role in the insects' ability to utilize the extra nitrogen present.

Uraba lugens, however, normally feeding on low nitrogen foliage may well be influenced by variation in the nitrogen concentration of its food, particularly soluble nitrogen. Since it has been demonstated that phenols (eg. quercetin) decrease the capacity of U. lugens to utilize nitrogen (chap. 5), variation in both phenols and nitrogen in the food plant must be considered.

Since Cooper-Driver et al (1977) showed that seasonal variation in the plant secondary compounds of bracken influenced feeding of the locust Schistocerca gregaria Forskal and Miles et al (1982 b) demonstrated that water stress both increased the soluble nitrogen present in E. camaldulensis foliage and decreased the concentration of total phenols, it seemed appropriate to investigate the seasonal and annual variation of nitrogen and phenols in field grown E. camaldulensis. Two aspects of these relationships were of interest to me:

(i) If nitrogen and phenols are negatively correlated as they fluctuate in concentrations in the leaves then, if nitrogen is high and phenols are low during periods of water stress, U. lugens may be assisted in droughts because (a) antifeedants may be below their threshold of effect (Cobbinah et al, 1982) and (b) nutrients may be more efficiently utilized because phenols such as quercetin may be in too low a concentration to adversely affect nitrogen assimilation from the gut (see chap. 5).

(ii) A similar relationship to that apparent during drought may result seasonally due to the usual fluctuations in seasonal precipitation or temperature. Stress in the trees may be caused either by waterlogging of soils for lengthy periods in winter - spring or by periods of high temperatures, which results in high evapotranspiration rates, during summer.

6.2 MATERIALS AND METHODS.

Mature leaves of Eucalyptus camaldulensis were collected near midday at two monthly intervals during 1980 to 1982 from 2 - 3m high trees on the southern slopes of Mount Osmond in South Australia. The sampling sites were arranged such that the North Eastern samples (NE) were taken from an area on a higher level than the North Western (NW) and South Western (SW) samples.

The leaves were plunged into liquid nitrogen immediately after removal from the plant, then ground under liquid nitrogen and freeze dried for 24hrs.. It was recognised that freeze drying of biological material may result in the loss of some compounds of low and intermediate molecular weight (viz. amino acids, flavonoids and glycosides etc.; see van Sumere et al, 1983).Since each sample was treated similarly any such loss of materials through the freeze drying procedure was assumed to be constant. The freeze dried material was stored at -18°C to ensure minimum enzymatic degradation (Dr. A.C. Jennings, pers. comm.) prior to extraction and analysis of the leaf components.

6.2.1 Determination of total nitrogen.

Dried, ground leaf material (0.25g) was weighed into a 50ml pyrex test tube and digested and analysed by the Micro Kjeldahl technique as described in chapter 5 (sec. 5.2.3).

6.2.2 Determination of total phenols.

The phenols present in the freeze dried leaf material were extracted in the following manner.

To 0.1g. of lyophilized leaf powder was added 30ml. of 80% (v/v) methanol, the mixture being boiled under reflux in a water bath for 10min.. The mixture was filtered into a separating funnel using Whatman no. 4 filter paper and the procedure repeated with the remaining residue; 50ml. hexane were added to these bulked filtrates, and this was shaken for 1min., and the phases left to separate. The upper layer was then discarded. This was repeated with a further 50ml. hexane. The resultant extract was then evaporated down to less than 5ml. in a rotary evaporator then made up to 25ml. with distilled water. In order to maintain consistency in the extraction procedure for each sample, the extraction time was standardized at 1hr..

Determination of total phenols present in the extracted samples was achieved using the techniques of Swain and Hillis (1959). 0.01ml. of the extract was diluted to 7ml. with distilled water in a graduated 10ml. tube and mixed thoroughly; 0.5ml. of Folin - Dennis reagent was then added, the solution again being shaken vigorously. Precisely 3min. after addition of the Folin - Dennis reagent, 1.0ml. of saturated sodium carbonate was added and the solution was made up to 10ml. with distilled water and thoroughly mixed. This was then filtered through a 0.45μm Gelman Acrodisc® and the absorbance immediately read on a Shimadzu spectrophotometer at 725nm. The amount of phenol present in each sample was calculated as mg. per ml. catechin equivalents, by comparison of the sample's absorbance with that of a standard curve for catechin.

The catechin standard curve was constructed as follows. From a catechin stock solution (1mg. per ml. of catechin in ethanol) 0.1, 0.2, 0.5 and 1.0 ml. was added to four graduated tubes and made up to 1.0ml. with ethanol. Using 0.1ml. of the standard solutions the procedure for the Folin - Dennis reaction was then followed as described above. For the blank, 0.1ml. ethanol replaced the test sample in the procedure described. A standard curve was established for each set of samples analysed so that any variation in the analytical procedure or conditions during analysis would be accounted for.

6.2.3 Determination and extraction of free amino acids.

Extraction of free amino acids from eucalypt leaves presented a number of problems due to the presence of a high proportion of tannins, resins and phenols etc. common in plants of this genus. These compounds interfered with both extraction and analysis. The procedures for reducing this interference was achieved through a number of trial extractions. The results of these trials will be discussed in the context of the final extraction procedure used.

The extraction of free amino acids from lyophilized *E. camaldulensis* leaves was as follows:-

The test sample of freeze dried leaf material (2.0g.) was extracted in methanol chloroform and water (MCW, 20:10:8). Extractions were performed under oxygen free nitrogen (OFN) and all solvents used were kept at 1°C and flushed with OFN in order to minimize oxidation of the leaf components. The sample was therefore homogenized with 40ml. of MCW for bursts of 1min. in an iced packed homogenizer, then centrifuged

FIGURE 6.2.3.1. Chromatograms of E. camaldulensis leaf extracts.

(a) Chromatography of chloroform (Chl.fr.) and aqueous fractions from 2g MCW extractions to which had been added 0.01g alanine with and without the addition of glacial acetic acid (Ac) to the extraction procedure.
Spotted as follows (amino acid standards (st.) listed in order of appearence from solvent front).

4 ul							15 ul			4 ul	3 ul
ALA	TYR	MET	VAL	PRO			TEST	TEST	TEST	ALA	All
SER	GLU	TRP	ASP	GLY	:	:	ALA	ALA	+ Ac	GLY	st.
ORN	THR	LYS	GLN	HIS	Chl.fr.		+Ac			HIS	
	ARG	CYE	CYS								

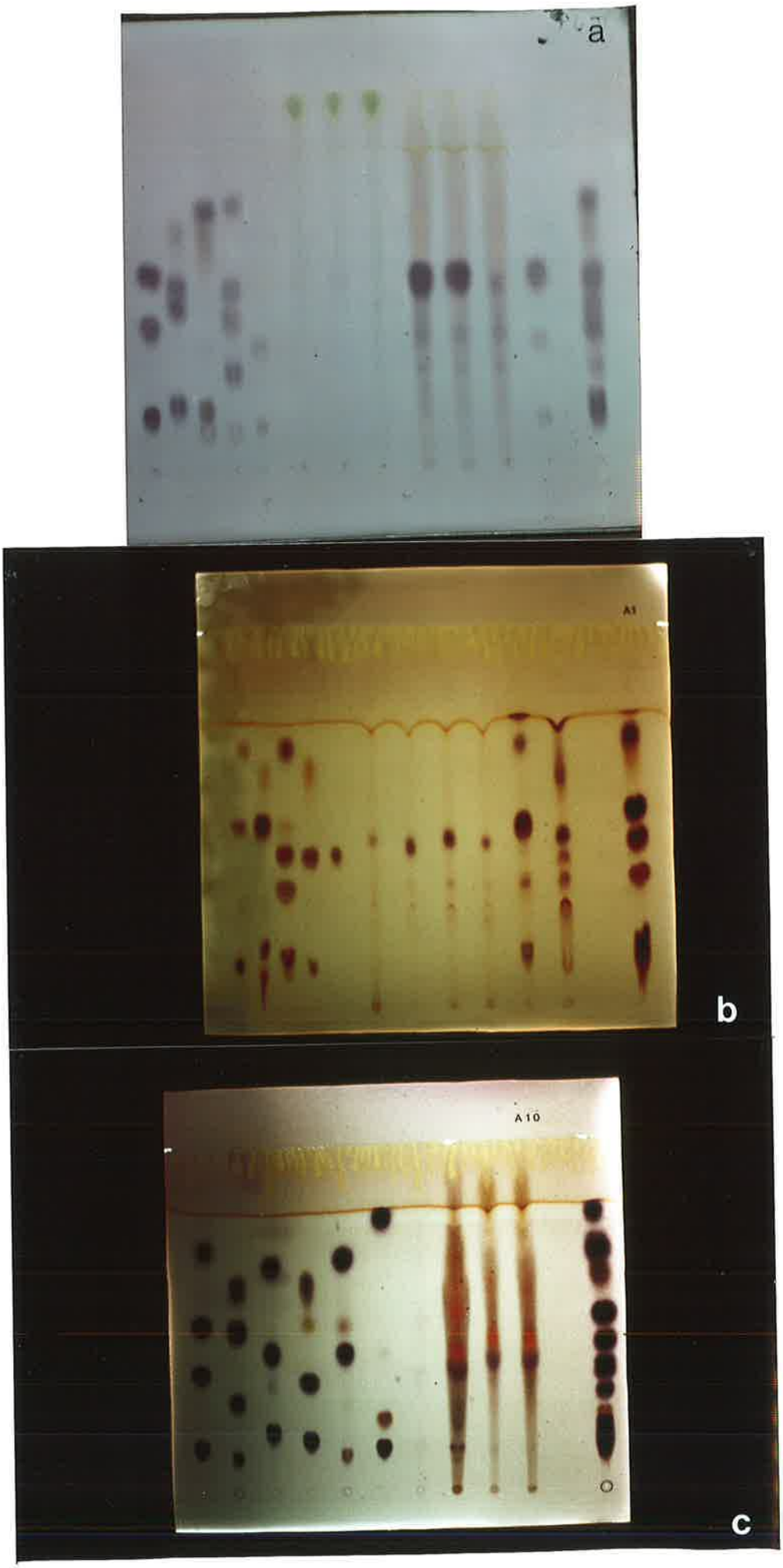
(b) Chromatography of leaf samples extacted in boiling ethanol (ETOH) and MCW with and without the incorporation of PVP- Amberlite (Amb) and glacial acetic acid in the extraction procedure. Plus a set of standards (1mg/ml) extracted in MCW.
Spotted as follows:

1 ul					20 ul						1 ul
LEU	TYR	VAL	TRP		TEST	TEST	TEST	TEST	TEST	st	All
ALA	GLU	PRO	ASP		ETOH	ETOH	MCW	MCW	MCW	MCW	st
ORN	GLN	THR	HIS			+PVP	+Ac		+PVP		
	ARG	GLY				+Amb			+Amb		
	CYS	LYS									
		CYE									

(c) Chromatography of leaf samples extracted in MCW with glacial acetic acid, with and without the addition of PVP and Amberlite.
Spotted as follows:

1 ul							30 ul			1 ul
PHE	TYR	MET	TRP	VAL	LEU		TEST	TEST	TEST	All
ALA	GLU	THR	PRO	PRO	ASN			+PVP	+PVP	st
SER	GLN	ARG	GLY	ASP	HIS				+Amb	
ORN	CYS		LYS	CYE						

Amino acid abbreviations: CYE, cysteine; CYS, cystine; other amino acids abbreviated as for appendix 6.3.3.1 and tables 6.3.3.1 and 6.3.3.2.


a
A1
b
A10
c

at 1150 rpm for 15min at 1°C. The supernatant was collected in a chilled separating funnel and the residue extracted three more times with 30ml. of MCW flushed with OFN. To each MCW extraction was added 3 drops of glacial acetic acid which maintained the extract at a low pH, reducing the complexing of amino acids and phenols (Dr. A.Jennings, pers. comm.) which results in precipitation and thus loss of amino acid material, and difficulty in phase separation. The increased efficiency of this extraction method using acetic acid was verified using thin layer chromatography (see figure 6.2.3.1 a and b; the procedure used for chromatography is outlined in appendix 6.2.3.1).

Poly vinyl-polypyrrolidone (PVP) and Amberlite XAD-4 were also added to each extraction of the sample with MCW (in the quantities: 1st extraction 2.0g each of PVP and Amberlite; succeeding extractions 1.0g each of PVP and Amberlite). These components were added to the extract in order to remove phenolic and tannin compounds during the extraction procedure, further reducing the interference of these compounds in the extraction and analysis of the free amino acids present in the sample. A similar method was used by Jung and Fahey (1981) to remove phenolics present in alphalpha and crown vetch for the purpose of studying forage digestability in the absence of these compounds. The improved efficiency of extraction using PVP and Amberlite is demonstrated in figures 6.2.3.1 b and c and 6.2.3.3 a. In figure 6.2.3.1 b, portions (2g) of the same lyophilyzed leaf material were extracted in (a) boiling ethanol and (b) MCW, with and without the incorporation of PVP and Amberlite. This plate demonstrates that (1) extraction in MCW is superior to extraction in boiling ethanol and (2) extraction with PVP and Amberlite is superior to

all other extraction procedures exhibited on this chromatogram. Determination of the total free amino acids (see appendix 6.2.3.2 for procedure) present in a leaf sample extracted with and without PVP and Amberlite further demonstrates the improved efficiency resulting from the incorporation of these materials (see table 6.2.3.1).

Table 6.2.3.1 Total free amino acids (measured as µmoles per ml. equivalent leucine per g. dry wt. of leaf) present in lyophilized E. camaldulensis leaves extracted in MCW with and without the incorporation of PVP and Amberlite.

	Sample Extraction Procedure		
	-ve PVP ,& Amb	+ PVP	+ PVP & Amb
Conc.	8.60	19.75	48.00

Although incorporation of PVP and Amberlite improved extraction results, a certain amount of amino acid impurities were present in the amino acid extract (see fig 6.2.3.3 a,b and c). The plates pictured in figure 6.2.3.3 demonstrate that samples extracted with unwashed PVP and Amberlite exhibit stronger spots particularly in the glutamic acid, alanine and glycine regions. It was therefore necessary to wash these materials before adding them to the extraction (see fig 6.2.3.3 c). This was done by washing the PVP and Amberlite five times in 80% ethanol and drying the residue prior to extraction. The number of washings was determined by extraction and chromatography of these materials

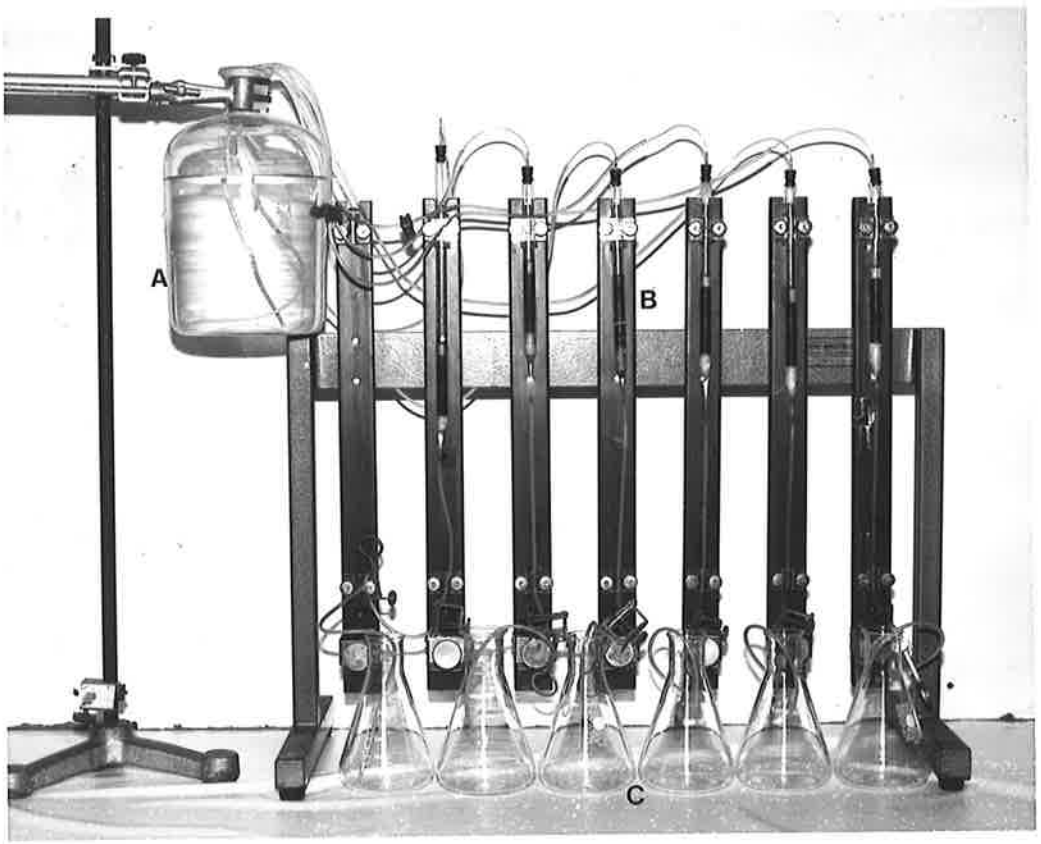
FIGURE 6.2.3.2.

Ion exchange columns used in extract purification.

A. Solvent resevoir.

B. Ion exchange column.

C. Collection flask for eluate


A
B
C

(not pictured). It was found that after five washings no impurities were evident on a chromatogram.

After extraction in MCW, sufficient chloroform and water was added to the bulked supernatants to alter the ratio of MCW to 20:20:18 thus separating the aqueous and chloroform phases. The extract was shaken for 1min. then allowed to stand overnight or until separation was complete at 1°C. The aqueous layer was then collected and evaporated down to less than 5ml. in a rotary evaporator. The resultant concentrate was then made up to 5ml with distilled water prior to loading the extract onto a cation exchange column.

It was necessary to purify the extract further by the use of an ion exchange column since although the PVP and Amberlite removed a proportion of the components in the extract responsible for interference, a considerable amount of "tailing" and interference was still present in some samples (see figs. 6.2.3.1 c, 6.2.3.3 a and b). The ion exchange system used (see fig 6.2.3.2) was a 1cm. diameter glass tube packed with a glass wool plug and 5cm of Dowex 50X-8. Prior to sample loading the ion exchange resin was set up in the acid phase so that distilled water run through the column was at pH 5. A sample was loaded onto the column, washed with 250ml. of distilled water then eluted with 100ml. of 1.0M ammonium hydroxide. A similar purification technique was used by Kaiser et al (1974) prior to amino acid analysis by gas-liqud chromatography. The benefit of this step is demonstrated in figure 6.2.3.3 a,b and c. From these chromatograms it can be seen that samples eluted from the ion exchange column (fig 6.2.3.3 b and c) yield more discrete spots than samples not subjected to the column purification (figs. 6.2.3.1 c,

FIGURE 6.2.3.3. Chromatograms of E. camaldulensis leaf extracts.

(a) Chromatography of MCW extracts with and without washed (wa) PVP and Amberlite.
Spotted as follows:

1 ul							30 ul		1 ul
PHE	TYR	MET	TRP	VAL	LEU	TEST	TEST +PVP +Amb wa	TEST +PVP +Amb	All st
ALA	GLU	THR	PRO	PRO	ASN				
SER	GLN	ARG	GLY	ASP	HIS				
ORN	CYS		LYS	CYE					

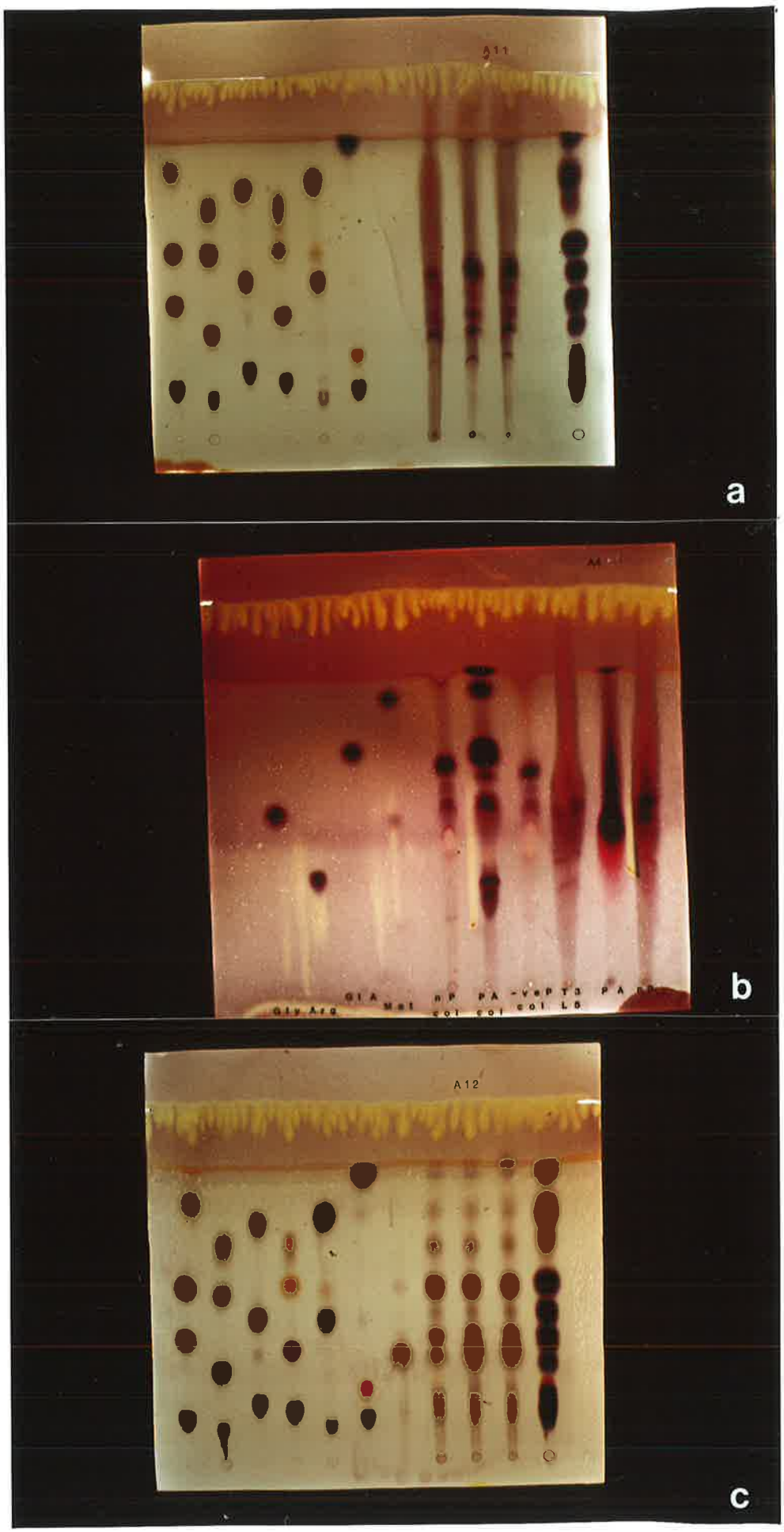
(b) Chromatography of MCW extracts with and without washed PVP and Amberlite and column (col) purification step.
Spotted as follows:

1 ul				30 ul					
GLY	ARG	GLU	MET	TEST +PVP +Amb wa col	TEST +PVP +Amb col	TEST col	TEST	TEST +PVP +Amb	TEST

(c) Chromatography of MCW extracts subjected to column purification with and without washed PVP and Amberlite. Plus the third washing extract of PVP and Amberlite.
Spotted as follows:

St. as in a					30 ul				1 ul
					PVP +Amb 3 wa	TEST +PVP +Amb wa col	TEST +PVP +Amb col	TEST +PVP col	All st

See fig 6.2.3.1 for amino acid abbreviations.


A11
a
A4
b
A12
c

6.2.3.3 a and b). The eluate was collected and evaporated down to dryness in a rotary evaporator, redissolved in 50% methanol (in 0.1M HCl) and again evaporated down to dryness in a vacuum dessicator. The samples were then analysed on a Beckman amino acid analyser.

6.3 RESULTS AND DISCUSSION.

6.3.1 The variation of total nitrogen.

(see appendix 6.3.1.1 for raw data)

Table 6.3.1.1 Variation in Total Nitrogen concentrations (mean ± SE, for n=3; in mg/g dry wt of leaf) present in mature Eucalyptus camaldulensis foliage from January 1980 to January 1982.

YEAR	MONTH					
	Jan	Mar	May	July	Sep	Nov
1980	15.03 ± 1.13 ab	13.19 ± 1.06 bcde	12.80 ± 0.13 bcde	14.77 ± 0.53 abc	15.69 ± 0.61 a	13.72 ± 0.99 abcd
1981	12.66 ± 0.35 cde	11.08 ± 0.13 e	11.87 ± 1.34 de	13.59 ± 1.45 abcd	13.85 ± 0.13 abcd	11.61 ± 0.61 de
1982	12.93 ± 0.61 bcde	-	-	-	-	-

* Analysed by 2 way analysis of variance.

** Different letters in rows and columns signify significant difference at $P < 0.05$.

Total nitrogen reached a maximum in spring during September - October (table 6.3.1.1 and fig 6.3.1.1) as would be expected, and reaches its lowest point around March - April. This trend is supported by Morgan's (1984) work on the variation of total soluble nitrogen on the same group of trees throughout the time period surveyed in this study. Together with seasonal variation there appears to be a trend throughout Jan. 1980 - Jan. 1982 of progressively lower total nitrogen in mature E. camaldulensis foliage (see fig. 6.3.1.1). Although this trend is not significant within the total nitrogen data, it becomes more apparent when considering the variation of free amino acids discussed later (see section 6.3.3).

There is also a difference in total nitrogen between the sampling sites (see table 6.3.1.2).

Table 6.3.1.2 Total Nitrogen (as mg/g dry wt. of leaf; mean ± SE) at different sampling sites.

	SAMPLE SITE		
CONC.	NE	NW	SW
Mean	14.03	13.03	12.88
SE	±0.47 a	±0.47 ab	±0.37 b

* Analysed by 2 way analysis of variance.
** Different letters signify significant difference at $P < 0.05$.

FIGURE 6.3.1.1.

Variation of total nitrogen (mg/g dry weight of leaf) and phenols (mg equivalent catechin per g dry weight of leaf) from January 1980 to April 1982 in mature E. camaldulensis foliage (as means for n=3).

Legend

Total nitrogen ——————

Total phenols - - - - - -

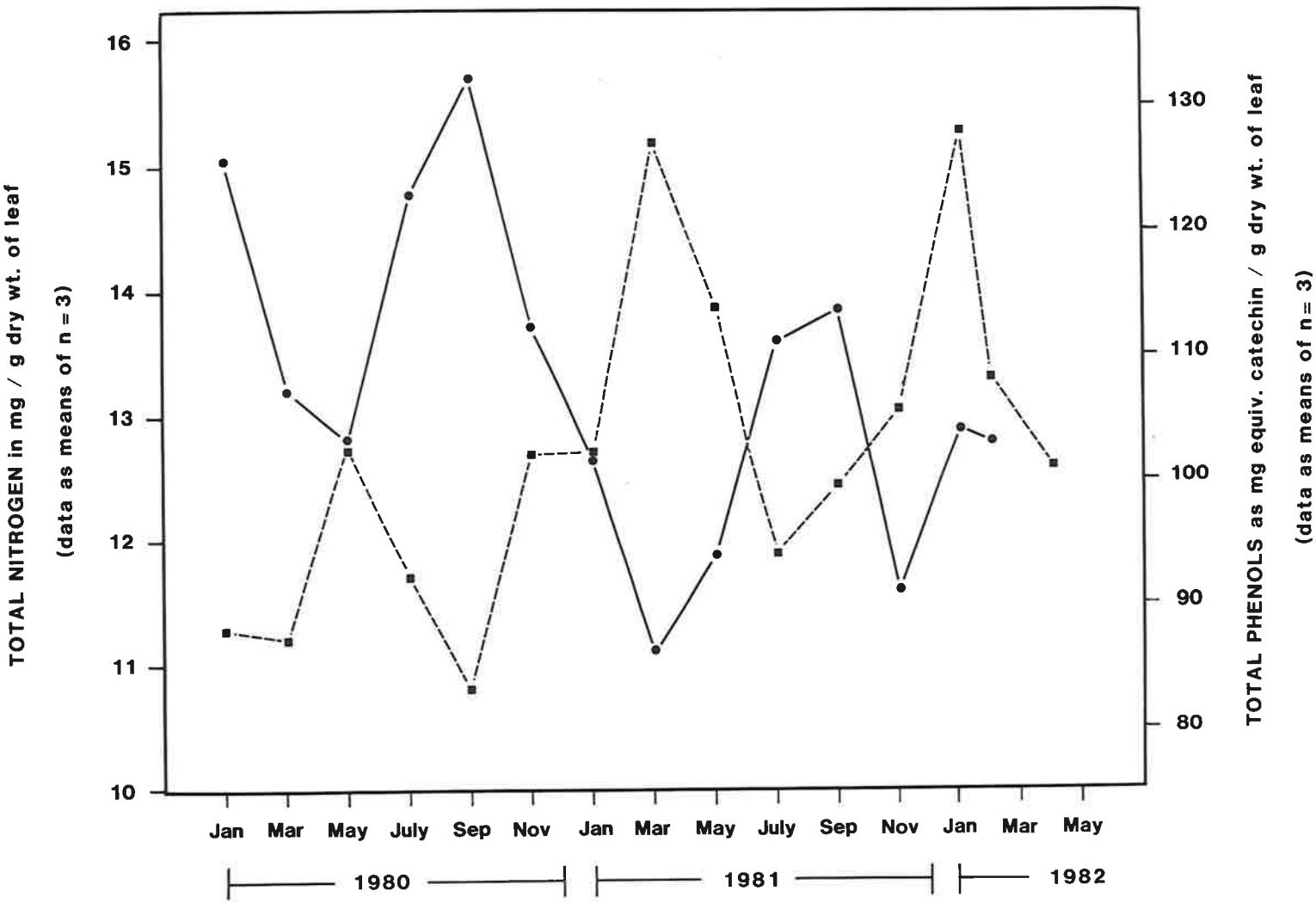

TOTAL NITROGEN in mg / g dry wt. of leaf
(data as means of n = 3)
TOTAL PHENOLS as mg equiv. catechin / g dry wt. of leaf
(data as means of n = 3)
16
15
14
13
12
11
10
130
120
110
100
90
80
Jan
Mar
May
July
Sep
Nov
Jan
Mar
May
July
Sep
Nov
Jan
Mar
May
1980
1981
1982

Table 6.3.2.1 Variation of total phenols (mean ± SE, for n=3; as mg/g equivalent catechin per g dry wt. of leaf) present in mature E. camaldulensis foliage from January 1980 to April 1982.

YEAR	MONTH							
	Jan	Feb	Mar	Apr	May	July	Sep	Nov
1980	87.70 ± 8.53 d	-	86.91 ± 10.26 d	-	102.67 ± 3.44 bcd	92.43 ± 6.27 cd	82.97 ± 3.61 d	101.88 ± 16.62 cd
1981	102.12 ± 14.26 cd	-	126.69 ± 6.51 ab	-	113.78 ± 5.76 abc	94.15 ± 10.41 cd	99.15 ± 9.46 cd	105.50 ± 12.09 abcd
1982	127.88 ± 11.18 a	108.18 ± 3.44 abc	-	101.09 ± 3.16 cd	-	-	-	-

* Analysed by 2 way analysis of variance.

** Different letters in colomns and rows indicate significant difference at $P < 0.05$.

Samples from the North East site were significantly higher in nitrogen than samples taken from the South Western site. The North Eastern sample site was at a higher level on the hillside than the other two sites so that the water table at this elevation may have been much lower in respect to the trees' root systems than for the sampling sites on the lower slopes, particularly that of the South Western site. It is possible therefore that trees on the North Eastern site were subjected to a greater degree of water stress.

6.3.2 Total phenols (see appendix 6.3.2.1 for raw data)

The total phenols rise to their highest level around May - June 1980 and March 1981 (when total nitrogen is at its lowest) and falls to its lowest point around September when total nitrogen is at its highest level (see fig. 6.3.1.1). Over the period surveyed phenols progressively increase in mature E. camaldulensis foliage (see table 6.3.2.1) as opposed to the total nitrogen which tends to decrease from January 1980 to January 1982. Considering a scatter diagram for total nitrogen and phenols (fig. 6.3.2.1) it can be seen that these two leaf components are negatively correlated with a correlation coefficient for the means of $r = -0.732$ ($P < 0.05$). A negative correlation between nitrogen and phenols in E. camaldulensis plants subjected to water stress was also observed by Miles et al (1982 b). However this negative correlation is not reflected in the difference in phenol content between the sample sites (see table 6.3.2.2). Samples from the North Eastern and Western sites have a higher phenol content than samples from the South Western site.

FIGURE 6.3.2.1.
Scatter diagram for means of total nitrogen and phenols.

Regression: $y = 203.37 - 7.62x$
y = total phenols, x = total nitrogen.
Significant ($T = -3.72$, 12 d.f.; $P < 0.01$)

Correlation coefficient: $r = -0.732$
Significant ($P < 0.05$).

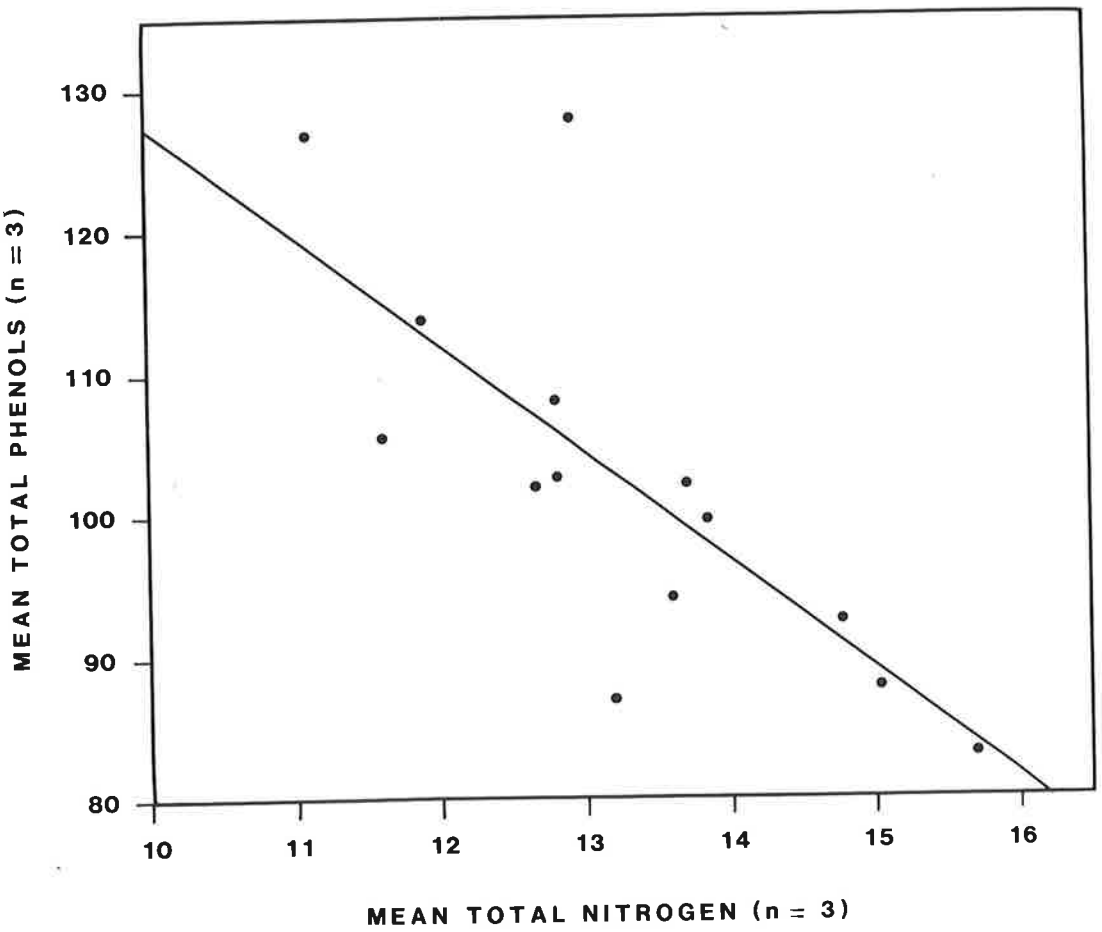

MEAN TOTAL PHENOLS (n = 3)
130
120
110
100
90
80
10
11
12
13
14
15
16
MEAN TOTAL NITROGEN (n = 3)

Table 6.3.2.2 Variation of total phenols (mean ± SE, as mg/g equivalent catechin per g dry wt. of leaf) between sample sites.

	SAMPLE SITE		
CONC.	NE	NW	SW
Mean	105.55	107.76	93.38
SE	± 4.50 a	± 4.75 a	± 4.47 b

* Analysed by 2 way analysis of variance.
** Different letters indicate significant difference at $P < 0.05$.

The data presented accord with our current knowledge of the metabolism of amino acids and phenols and their relationship via the shikimic acid pathway (see Bidwell, 1979 and Graham, 1983). Phenylalanine ammonia-lyase (PAL) catalyses the deamination of phenylalanine to cinnamic acid, an important precursor of flavonoid compounds. Thus PAL catalyses the branching reaction that leads from the shikimic acid pathway to formation of a wide range of secondary plant compounds including the phenolics. The activity of this enzyme (PAL) is affected by a number of external and internal factors such as the development rate of the plant. It, for example, is stimulated in plants by wounding, infection by disease and by the growth regulating substance ethylene. Other hormones, such as indolacetic acid (IAA), inhibit PAL. The activity of IAA is increased when a plant is subjected to drought (Mukherjee and Chouduri, 1981). Therefore a decrease in PAL activity as a consequence of

increased IAA due to water stress would result in a decrease in the production of plant secondary compounds. This is of importance in that attack by U. lugens at such a time would, initially at least, be advantageous to success of that attack for the reasons mentioned at the end of 6.1 above.

PAL activity is also stimulated by light (see Bidwell, 1979), and since the northern slopes of the sample site would receive more light daily, both in intensity and longevity, than the Southern slope because of shading of the high hills to the East and West of the study area, this may explain the higher concentration of phenols in trees on the Northern sample sites (table 6.3.2.2).

6.3.3 Free Amino Acids. (see appendix 6.3.3.1 for raw data)

Total free amino acid concentrations vary throughout the study period, July 1980 to January 1982, with a similar trend to that for total nitrogen discussed earlier (sec. 6.3.1). A maximum of 1183.5 µg/g dry weight of leaf was reached in September 1980, from there the total free amino acids decrease to a low of 271.1 µg/g dry weight of leaf in March 1981 (see fig 6.3.3.1 a and table 6.3.3.2). Although the total soluble nitrogen slowly increased after March 1981 the highest level reached in September - November of 1981 is much lower than the 1980 September maximum and did not differ significantly from the low reached in March 1981 (see table 6.3.3.2).

The individual amino acids which significantly varied throughout the period studied are: phosphoserine, threonine, glutamine, proline, glutamic acid, glycine, alanine, iso-leucine, leucine, tyrosine,

Table 6.3.3.1 Variation of amino acid concentration (means for n=3, as µg/g dry weight of leaf) in mature E. camaldulensis foliage from July 1980 to January 1982.

SAMPLING TIME		AMINO ACIDS													
Year	Month	PSE	ASP	THR	SER	ASN	GLN	PRO	GLU	GLY	ALA	VAL	CYS	CYN	MET
1980	July	119.56 ab	1.18	7.61 de	33.06	13.46	59.98 a	37.57 b	282.77 bc	4.10 d	17.93 cd	7.34	6.52	13.69	0.03
	Sep	56.31 cd	4.85	18.46 ab	75.66	9.91	8.46 bc	185.69 a	504.25 a	25.49 a	26.85 a	9.12	23.49	27.79	2.69
	Nov	42.06 d	2.75	25.74 a	63.58	25.60	3.96 bc	235.73 a	303.87 b	20.05 ab	22.80 ab	12.47	7.25	28.53	1.65
1981	Jan	35.58 d	2.16	17.48 bc	60.59	6.48	0.34 c	20.59 b	134.64 cd	15.49 bc	19.31 bcd	9.59	11.87	26.19	2.24
	Mar	33.87 d	1.37	9.50 de	33.84	5.15	24.72 b	18.12 b	65.52 d	8.32 cd	19.86 abc	4.21	5.42	5.25	3.80
	May	54.01 cd	11.78	7.33 de	132.72	2.63	10.26 bc	9.06 b	67.84 d	8.88 cd	9.78 e	3.48	9.58	7.92	0.08
	July	72.37 bcd	24.93	3.44 e	19.56	-	4.19 bc	13.20 b	143.92 cd	2.70 d	9.45 e	4.69	6.98	13.09	0.48
	Sep	127.62 a	15.81	3.28 e	26.66	1.28	10.59 bc	9.01 b	185.13 bcd	2.65 d	7.78 e	7.87	3.65	12.92	-
	Nov	97.85 abc	1.38	7.13 de	38.60	8.09	5.84 bc	21.59 b	201.30 bcd	8.50 cd	12.52 de	6.62	5.70	12.32	-
1982	Jan	71.73 bcd	2.27	11.74 cde	53.69	19.46	0.55 c	20.06 b	156.03 bcd	9.40 cd	21.81 abc	5.81	16.92	27.07	0.03
			n.s.		n.s.	n.s.						n.s.	n.s.	n.s.	n.s.

PSE, phosphoserine; ASP, aspartic acid; THR, threonine; SER, serine; ASN, asparagine; GLN, glutamine; PRO, proline; GLU, glutamic acid; GLY, glycine; ALA, alanine; VAL, valine; CYS, half cystine; CYN, cystathionine; MET, methionine.

* Each amino acid analysed by 2 way analysis of variance.

** Different letters in columns indicate significant difference at $P < 0.05$.

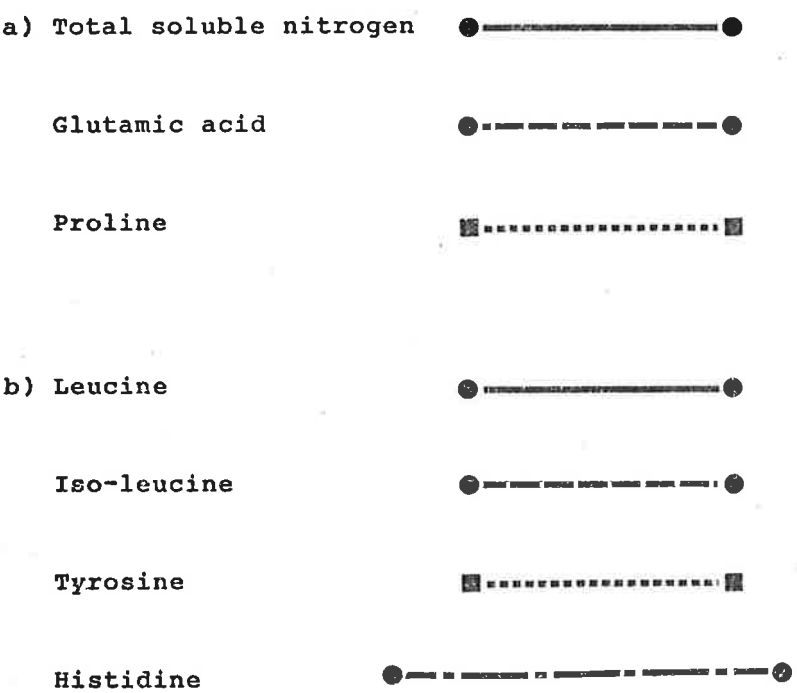
Table 6.3.3.2 Variation of amino acid concentration (means for n=3, as µg/g dry weight of leaf) in mature E. camaldulensis foliage from July 1980 to January 1982.

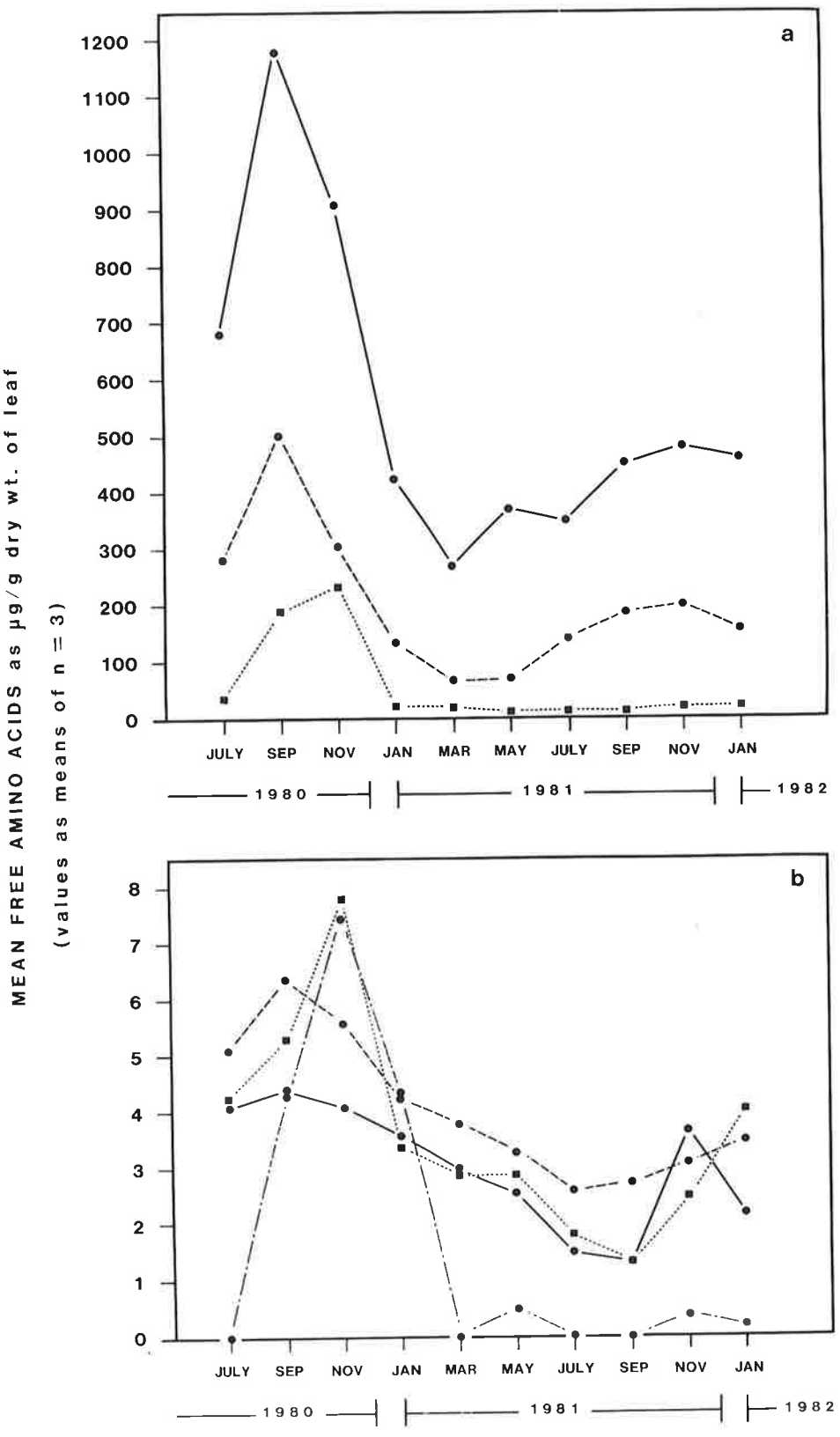
SAMPLING TIME		AMINO ACIDS												
Year	Month	ILE	LEU	TYR	PHE	BALA	GABA	ORN	ETH	LYS	HIS	ARG	TOTAL	GLU +GLN
1980	July	5.08 ab	4.09 ab	4.28 bc	21.21 a	3.27	23.99 b	1.40 b	-	-	- b	13.80 bc	681.93 bc	342.76 b
	Sep	6.39 a	4.43 a	5.31 ab	20.63 a	3.52	55.90 a	5.91 a	23.94	5.39	4.39 ab	68.46 a	1183.47 a	512.72 a
	Nov	5.61 ab	4.09 ab	7.80 a	12.97 bc	0.48	22.42 b	2.29 b	3.87	4.61	7.52 a	40.54 b	908.23 ab	307.81 bc
1981	Jan	4.26 bcd	3.58 abc	3.04 bcd	15.31 ab	-	11.57 b	6.06 a	4.81	5.33	4.31 ab	5.20 c	426.01 cd	134.97 d
	Mar	3.80 bcd	2.97 abcd	2.92 bcd	7.89 c	-	14.78 b	1.58 b	-	-	- b	2.10 c	271.11 d	90.24 d
	May	3.28 cd	2.56 bcd	2.90 bcd	8.29 c	-	12.62 b	2.90 b	0.56	2.17	0.51 b	1.18 c	372.31 d	78.10 d
	July	2.58 d	1.54 d	1.85 cd	8.11 c	1.58	15.47 b	1.02 b	-	-	- b	0.10 c	351.27 d	148.12 d
	Sep	2.77 d	1.36 d	1.34 d	11.40 bc	0.85	17.93 b	0.63 b	-	-	- b	0.51 c	450.47 cd	195.72 bcd
	Nov	3.12 d	3.73 abc	2.55 cd	15.50 ab	6.04	18.94 b	1.56 b	-	1.90	0.44 b	1.89 c	482.35 cd	207.15 bcd
1982	Jan	3.48 cd	2.22 cd	4.05 bcd	7.79 c	0.25	19.92 b	2.15 b	-	3.87	0.21 b	2.85 c	463.35 cd	156.58 cd
						n.s.			n.s.	n.s.				

ILE, iso-leucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; BALA, β alanine; GABA, γ amino-butyric acid; ORN, ornithine; ETH, ethanolamine; LYS, lysine; HIS, histidine; ARG, arginine; TOTAL, total free amino acids.

* Each amino acid analysed by 2 way analysis of variance.
** Different letters in columns indicate significant difference at $P < 0.05$.

FIGURE 6.3.3.1.
Variation of total soluble nitrogen and individual amino acids (as µg/g dry weight of leaf, mean for n=3) in mature E. camaldulensis foliage from July 1980 to January 1982.




MEAN FREE AMINO ACIDS as µg/g dry wt. of leaf
(values as means of n = 3)
a
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
JULY SEP NOV JAN MAR MAY JULY SEP NOV JAN
1980
1981
1982
b
8
7
6
5
4
3
2
1
0
JULY SEP NOV JAN MAR MAY JULY SEP NOV JAN
1980
1981
1982

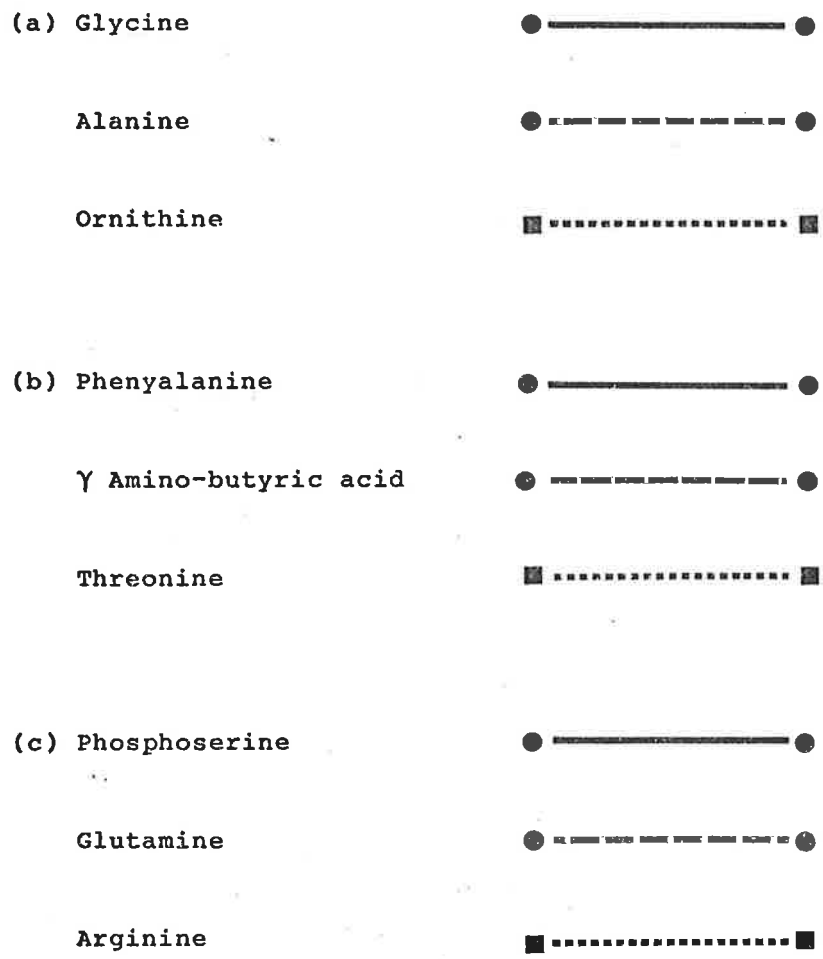
phenylalanine, γ amino-butyric acid, ornithine, histidine and arginine (see table 6.3.3.1 and 6.3.3.2). The variations in these amino acids are plotted in figures 6.3.3.1 and 6.3.3.2. With the exception of phosphoserine and glutamine (fig. 6.3.3.2 c) all the amino acids reach their highest level in September - November 1980 (figs. 6.3.3.1 a,b and 6.3.3.2 a,b,c), the most dramatic variation being observed for glutamic acid and proline (see also tables 6.3.3.1 and 6.3.3.2). Studying Ericaceae leaves Eder and Kinzel (1983) found similar trends in the variation of free amino acids.

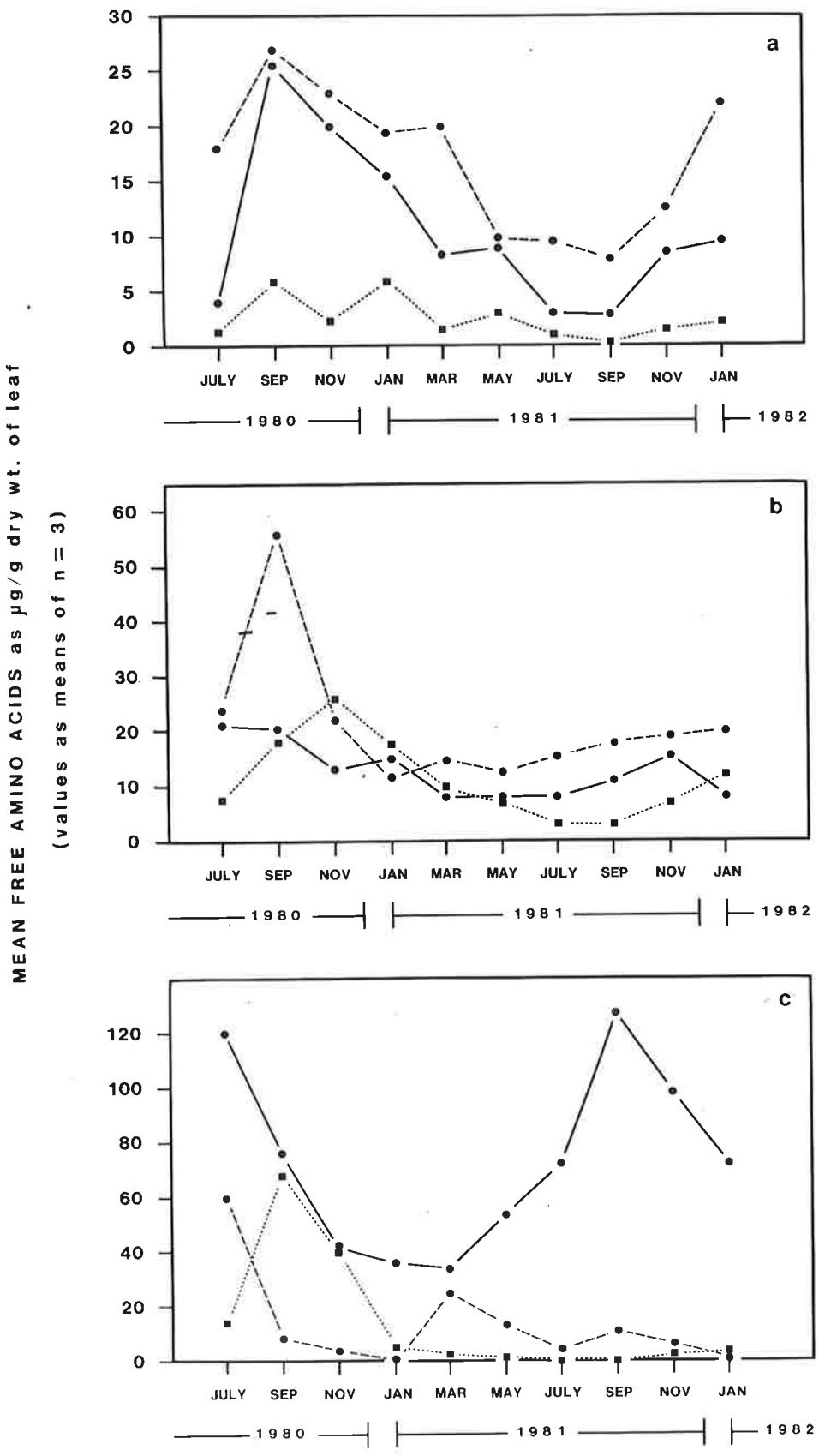
The occurrence of phosphoserine in the leaf material analysed is unusual (Dr. A.C.Jennings, pers. comm.). Journet and Cochrane (1978) investigating the amino acid content of E. blakelyi did not record the presence of this amino acid. The presence of phosphoserine in these samples could be attributed to biochemical breakdown during storage of the leaf samples prior to extraction and analysis (Dr. A.C.Jennings, pers. comm.). If this had occurred then it might be expected that the highest concentration of phosphoserine would be in the 1980 samples and it would progressively decline in the samples taken at a later date but this trend was not observed (see fig. 6.3.3.2 c and table 6.3.3.1). This compound is the first to be eluted off the column, emerging with the solvent front. Its ocurrence is therefore an artifact of the column elution and can be explained as a combination of compounds such as cysteic and laevulinic acids which react positively with ninhydrin and are thus assigned as phosphoserine (D. Boehm, pers. comm.).

The variation in glutamine also indicates that biochemical degradation of the sample by enzymic reactions was small. Since glutamine

FIGURE 6.3.3.2

Variation of individual amino acids (µg/g dry weight of leaf, mean for n=3) in mature E. camaldulensis foliage from July 1980 to January 1982.




MEAN FREE AMINO ACIDS as µg/g dry wt. of leaf
(values as means of n = 3)
a
b
c
JULY SEP NOV JAN MAR MAY JULY SEP NOV JAN
1980
1981
1982

is one of the less stable amino acids (Dr. A.C.Jennings, pers. comm.) it might be expected that glutamine would be at a lower concentration in the earlier samples, however, as with phosphoserine it reaches a maximum in July 1980. It is likely however, that some glutamine may have been hydrolysed to glutamic acid by the methanol-HCl solution used to redissolve the test extracts. It is therefore more accurate to combine the amounts of these two amino acids (table 6.3.3.2) and consider the trends of the resultant combination which follows very closely the curve observed for glutamic acid (see tables 6.3.3.1, 6.3.3.2 and fig. 6.3.3.1 a).

Despite the variation across time, two way analysis of variance for total soluble nitrogen and each individual amino acid did not reveal a significant difference between the sampling sites although the trend observed for total nitrogen is still apparent (see tables 6.3.1.2 and 6.3.3.3).

Table 6.3.3.3 Variation of total soluble nitrogen (mean ± SE, as µg/g dry wt. of leaf) between sampling sites.

	SAMPLE SITE		
CONC.	NE	NW	SW
Mean	624.12	537.89	515.14
SE	± 91.29	±116.21	± 94.80

Analysed by 2 way analysis of variance, $P > 0.05$.

The high values for soluble nitrogen observed in the September - November samples for 1980 (figs. 6.3.3.1 and 6.3.3.2) indicates the possibility that the trees sampled were subjected to a water deficit since free amino acids, particularly proline, are known to increase when a plant is droughted (Barnett and Naylor, 1966; Hsiao, 1973; Singh et al, 1973 a and b; Huang et al, 1979; Miles et al, 1982 b; see also Review sec. 1.7).

Meteorological data from the Waite Agricultual Research Institute (table 6.3.3.4) show that the monthly rainfall for January, February and March 1980 and again for August and September 1980, were much lower than that of the running mean taken over a 56 year period. Since the pan evaporation at these times is high (see table 6.3.3.4) it is possible that the level of the water table dropped sufficiently to subject the test trees to water stress; the succeeding rainfall replenishing the water table so that the water depleted months of February and April 1981 did not reduce its level sufficiently to result in another significant water deficit for the trees in this period.

No soil moisture data are available from the study area to support this viewpoint. However, two phenological observations were made which provide support for the weather data which were recorded less than 3 km West of the study area. The first was that in spring - summer 1980 ground vegetation died or wilted about 2 months earlier than it did in the same period of 1981. The second was that, in 1980, the typical bent flaccid petioles of the leaves of the test trees, indicating severe stress (see Miles et al, 1982 b) were a noticeable feature during February, March and September. In 1981, this was noticeable in late

Table 6.3.3.4 Monthly rainfall (mm) and pan evaporation (mm) from the Waite Agricultural Research Institute meteorological station.

YEAR	STATISTIC		MONTH											
			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1980	Rainfall	MT	9.8	0.8	1.0	75.0	43.4	92.2	104.2	22.8	27.8	107.2	46.8	27.2
		RM 1	23.7	27.7	21.0	55.9	79.3	72.9	85.3	72.5	62.7	55.1	38.8	30.0
	Pan Evap.	MT	202.2	208.8	170.3	117.2	74.2	46.6	48.2	78.4	106.2	142.6	176.6	225.2
		RM 2	232.2	200.6	172.5	107.0	64.6	46.5	48.5	65.6	94.5	140.7	175.2	212.6
1981	Rainfall	MT	26.8	2.8	66.4	3.0	61.2	179.0	137.0	126.2	51.0	32.2	35.6	26.8
		RM 3	23.8	26.6	21.8	55.0	79.0	74.0	86.7	73.4	62.5	54.7	38.7	29.9
	Pan Evap.	MT	262.6	221.0	124.0	119.0	70.6	42.2	53.4	65.2	104.4	145.0	169.2	197.6
		RM 4	236.4	201.5	170.4	107.5	64.9	46.3	48.7	65.6	95.0	140.9	175.0	212.0

MT, monthly total; RM 1, running monthly mean over 56 yrs.;
RM 2, running monthly mean over 22 yrs;
RM 3, running monthly mean over 57 yrs.;
RM 4; running monthly mean over 23 yrs.

February only (Dr. F.D.Morgan, pers. comm.).

Stress related to high temperatures and low air moisture is indicated by the pan evaporation figures which indicate that low rainfall was probably the major contributor to stress in the trees in summer of 1980 whereas evapotranspiration may have been more important in the same period of 1981. In the latter, the good summer precipitation probably offset the higher temperatures so that water stress in the trees was not as noticeable as in 1980.

Chapter 7

General Discussion.

CHAPTER 7

General Discussion.

"We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time."

T.S. Elliot: The Gidding

Nitrogen has often been considered as a limiting nutrient for the growth and survival of herbivores. As early as 1953, Fennah suggested that plants vary in their nutritional complement and may not be nutritionally adequate at all times. Many latter studies have emphasized the role of nitrogen in the performance of phytophages (van Emden, 1966; Pradham, 1971; Onuf et al, 1977; McClure, 1979, 1980; Lindig et al, 1980; Iheagwam, 1981; Myers and Post, 1981; Rausher, 1981; Tabashnik, 1982). This evidence generally indicates that the proportion of nitrogen present in plant foliage is, at times, inadequate for herbivore growth and survival (Southwood, 1973; Fox and Macauley, 1977; Slansky and Feeny, 1977; McNeill and Southwood, 1978; Mattson, 1980; Scriber and Slansky, 1981; Degabriele, 1981; Lincoln et al, 1982; see also chap. 1, sec. 1.4). In line with this body of evidence White (1966, 1969, 1978) proposes that water stress triggers the production of increased soluble nitrogen in plant foliage rendering it more nutritious and thereby resulting in a population increase or outbreak of herbivores feeding on such improved diets. This stimulus for outbreak is higher survival in the early stages

of their life cycles, due solely to the increase in available nitrogen.

Uraba lugens Walker offered alternative ways of examining this and opposing hypotheses because of its differential performances on plants across its food plant range (see chap. 2) and its acceptance of synthetic diets. Firstly, White's theory was no longer as general as he had inferred in 1966, so it was appropriate to look both at insect performance on natural or simulated diets and diets selected for the influence of specific components. Secondly it was already well known that water stress influenced the nitrogen content of foliage on affected plants (see chap. 1, sec. 1.7). That part of his theory was therefore not in question. What had not been adequately demonstrated was the link between increase in stress-induced components of available nitrogen and significant increase in population density of the early instars of the phytophage (see chap. 1, sec. 1.8), especially in the stimulation of pest outbreaks.

The studies completed therefore followed a careful sequence. As U. lugens is an eruptive rather than a cyclic outbreaking pest (see Berryman, 1980; Morgan and Cobbinah, 1977) it could be stimulated to increase population density if the critical components of its environment that synchronize to cause such increases were known and were manipulated properly. This insect, in nature, does not appear to be regulated by parasitoids and predators (Campbell, 1962, 1966; Harris, 1972; Morgan and Cobbinah, ibid.). Thus examination of its performance relative to changes in the quality of its food plant was justified in attempting to understand its population dynamics. Also adults do not feed, which permitted studies to be concentrated on the caterpillars and

"performance" therefore could be restricted to developmental time, survival and potential fecundity, the latter derived from female pupal weight using Cobbinah's (1978) positive correlation of these factors.

Possible manipulation of nutrient levels in food were derived from chemical analysis of natural food as well as monitoring variation in nitrogen seasonally for consecutive generations of the insect in the field (figs. 6.3.1.1, 6.3.3.1, 6.3.3.2 and tables 6.3.1.1, 6.3.3.1, 6.3.3.2). Because of the physiological interaction between certain phenolic or total phenolics and the nitrogen in foliage (Graham, 1983) and especially the possibilities that:

(i) Phenolics themselves may reach toxic levels (Morgan, 1984).

and (ii) Phenolics may influence the assimilation of nitrogen from the gut (Reese, 1978).

These likely interactions were included in attempts to determine those factors that might provide the critical components of the relationship between U. lugens and its food.

Because of time limits, selected components only could be studied, those included being either, necessary to insect nutrition or known to vary significantly with effects of stress on food plants.

I attempted to determine "adequate controls" for a number of treatments by including at least two, fresh diet and base diet. Often another, base diet+combined components of the test diets, was available.

Let us now examine in sequence the following components of theories relating food quality and quantity to insect performance and pest population dynamics and then compare my results with them.

I (a) Plants are usually an adequate source of nutrients for herbivores (Fraenkel, 1953, 1959).

(b) Plants are often inadequate sources of nutrients for herbivores (White, 1978; McNeill and Southwood, 1978).

Uraba lugens successfully colonises a number of eucalypts and near relatives, the foliage of which ranges from about 0.8 - 3.0% total nitrogen (see Cobbinah,1978; Cobbinah et al, 1982; Lambert and Turner, 1983). Satisfactory food plants, those on which the insect completes development in low numbers, and good food plants with better larval survival, occur throughout the range. Its most common food plant in South Australia is river red gum (Eucalyptus camaldulensis) which in nature seasonally varies in total nitrogen in mature foliage from about 1.1 - 3.0% with highest levels in spring / summer and lowest levels in winter (fig. 6.3.1.1; Morgan 1984). Peak or outbreak populations have been recorded in both winter and summer generations (Campbell, 1962, 1966; Morgan and Cobbinah, 1977).

The evidence tends therefore to support Fraenkel rather than White. Yet I have demonstrated that available food may not always be adequate for U. lugens (see fig. 2.4.1). Host selection, or selection of egg hosts and food plants, is therefore not apparently due to nutrient value (see also Cobbinah et al, 1982). Good food plants such as river red gum do apparently appear to regulate caterpillar nutrition by altering the Nitrogen / Phenolic ratio in the food (figs. 5.3.2.1b, 5.3.3.1b, 6.3.1.1 and tables 5.3.2.1, 5.3.3.1; see also Miles et al 1982 b; Morgan 1984).

Total nitrogen may never be at critically low levels but because phenolics such as quercetin decrease the assimilation of nitrogen from the gut when above a concentration threshold in the food, caterpillar performance may decline. This evidence supports the findings of Reese (1978). It indicates that a pest / tree interaction provides adequate food for a herbivore until the tree is damaged to a level where it must protect itself. It appears to do this by altering the Nitrogen / Phenolic ratio with nitrogen decreasing and phenol increasing. Increasing phenol is the critical factor (Morgan, 1984) and artificially increasing nitrogen with fertilizer may offset the disadvantage to the herbivore.

It seems therefore that nitrogen may always be adequate for U. lugens but the level of assimilation may not be at certain times. If this is what White means by "available nitrogen" and apparent shortage of nitrogen, my data provide support. The problem is not that the nitrogen is not available but that it is prevented from being assimilated via the gut membrane (Reese, 1978). This evidence indicates that "available" needs specifying as to its meaning or should be replaced by "assimilable" to indicate its transfer to the "blood" for utilization in metabolism.

II (a) Water stress increases available nitrogen in foliage which results in survival of more first instars feeding on it and thus leads to pest outbreaks (White, 1978).

(b) Water stress leads to a manifold increase in proline and often an increase in total nitrogen (Miles et al, 1982b, Singh et al, 1973) but plants high in proline do not improve the performance of a number of arthropods and molluscs (Bright et al, 1982).

As it has already been shown (Miles et al, 1982b) that water stressed E. camaldulensis plants did have increased proline and total nitrogen in their leaves, I concentrated on plants having no water stress, low water availability (drought) and waterlogging (flooding). The latter was included because of the reported effects of flooding of forests of river red gum on U. lugens populations (Campbell, 1962; Harris, 1972).

My data (fig. 4.3.1.2.1; tables 4.3.1.1.1, 4.3.1.2.1, 4.3.1.3.1) do not clearly support White's or Bright et als' hypothesis. What happened, was that pupae from the two stress treatments were larger and adults potentially more fecund. There was no improved survival in the treatments over the control but "performance" of those larvae reared on the stressed plants was improved in the form of significantly larger size. There was a tendency toward lower survival in the flooded treatment, though significantly lower than the control only at instar VI ($P < 0.05$; see table 4.3.1.2.1). If significant also at instar XI, this would have supported the results of Campbell (1962) and Harris (1972) and be in accord with the present knowledge of the physiology of waterlogged plants. For although waterlogging is known to increase proline in the foliage (Singh and Singh, 1981), ethylene which stimulates the activity of phenylalanine ammonia-lyase also increases (Blake and Reid, 1981;

Bidwell, 1979; Bradford and Yang 1980; Hunt et al, 1981) and may therefore lead to an increase in the plant's phenols. This is contrary to the physiology of the plant during drought, where phenolics present in the foliage have been observed to decrease (Miles et al, 1982b; see also chap. 6, sec. 6.3.2 and figs. 6.3.1.1 and 6.3.2.1), although ethylene has also been observed to increase in plants subject to water deficit (Apelbaum and Yang, 1981). Thus the influence of waterlogging on plant allelochemics requires further investigation.

Water stress may therefore lead to better growth rates of the herbivore and higher fecundities which may stimulate a pest outbreak. This may however be due to the fact that stressed plants (especially plants subject to drought) are unable to alter the Nitrogen / Phenolic ratio as unstressed plants appear to do as foliage damage levels increase (Benz, 1974; Wratten et al, 1981; Haukioja, 1982; Schultz and Baldwin, 1982; Edwards and Wratten, 1983; Morgan, 1984; Tuomi et al, 1984).

Supporting studies:

I felt that at least two factors might be involved in outbreaks of U. lugens. Firstly, increases in nitrogen in leaves or increases of particular amino acids in leaves over others, might result in improved overall performance of this moth. Secondly, as Cobbinah et al (1982) had found, that poor or non food plants occasionally became satisfactory for development and survival of this insect, perhaps this was due to stress, plus improved levels of nitrogen or a more satisfactory Nitrogen / Phenol ratio. Therefore I looked at changing the survival curve at two points. By adding nitrogen to diets (chap. 3) or to foliage (chap. 4) I could

(a) increase survival of early instars

and (b) increase survival of late instars.

That is, I could change a Slobodkin type II, III or IV survival curve into, respectively a type I, II or III. Alternatively, I could change the Slobodkin I curve into another where survival improved in the late instars to what I will term a Slobodkin Ia survival curve (see figs. 2.4.1 and 2.4.2 for comparison).

Four possibilities were available. I could modify the nitrogen level in good or poor food plant leaves, or I could manipulate diets from both good food plant leaf powder or poor food plant leaf powder.

Encouragement for the latter combination was the remarkable studies of Cobbinah et al (1982) where they demonstrated that the threshold of effect of poor food plant could be derived by diets where increased proportions of one were added to the other.

My results of these experiments indicated:

(a) On diets containing good food plant leaf powder, that increased proline and valine in diets gave survival similar to those on fresh diets. Base diet + arginine was also superior to other diets (fig. 3.3.4.1 and tables 3.3.4.1, 3.3.4.2).

(b) On diets incorporating leaf powder of the poor food plant, diets with added valine, proline and combined amino acids all produced some pupae. Though the valine and combined treatments were 3 and 2-fold higher in survival than that on fresh diet, variability in the replicates was high.

The interest here is that valine and proline alone, as additives were at least as successful as combined amino acids and fresh leaves. These two amino acids are known to increase in leaves following water stress of a plant (Hsaio, 1973; Singh et al, 1973). In my study proline increased markedly but valine did not (table 6.3.3.1 and Fig. 6.3.3.1a). There is a case for increased valine in the diet as a possible stimulator of better larval survival and fecundity with proline also having some effect. There may even be an "additive effect" when both are increased in the diet of this moth (table 4.3.2.1). This also should be further investigated.

Thus for U. lugens, an oligophagous mature leaf feeder, an increase in the proline / valine complement of a food plant's foliage together with a decrease in its phenolic content (viz. quercetin) at a critical stage in its life cycle (e.g. after eclosion) may result in an increase in its development rate, growth and survival leading to a greater number of potentially more fecund individuals.

The results of this study therefore suggest that it is a balance between the deleterious and advantageous components of a plant that determine the performance of its associated phytophages. So that if environmental conditions alter this balance such that components deleterious to its associated phytophage decrease and this change is synchronized with a critical stage in the insect's life cycle then a population "outbreak" may occur. In other words water stress may render the nitrogen component of a plant's foliage more "assimilable" to its phytophagous insect population. If however a phytophage can adjust its feeding rate or digestive physiology to compensate for levels of "toxins"

in its diet (Slansky and Feeny 1977; Auerbach and Strong, 1981) or has evolved a detoxifying mechanism for these compounds (Brattesten et al, 1977) then this change in the insect's performance may not occur as observed by Miles et al (1982b) (see also Bernays, 1978).

Therefore if a chewing phytophagous insect is susceptable to its food plant allelochemics such that they act as (a) antifeedants, inhibiting ingestion, and / or (b) inhibit the assimilation of avialable nutrients in the ingested food, and these compounds decrease with water stress together with a corresponding increase in specific beneficial nutrient components at a critical point in the insect's feeding life, then an increase in the insect's population may occur in nature.

References

REFERENCES CITED

ALFARO, R.I., PIERCE, H.D., BORDEN, J.H. and OEHLSCHLAGER, A.C. (1980). Role of volatile and nonvolatile components of Sitka spruce bark as feeding stimulants for Pissodes strobi Peck (Coleoptera: Curculionidae). Can. J. Zool. 58: 626-632.

ALFENSAS, A.C., HUBBES, M. and COUTO, L. (1982). Effect of phenolic compounds from Eucalyptus on mycelial growth and conidial germination of Cryphonectria cubensis. Can. J. Bot. 60: 2535-2541.

ANDERSON, R.F. (1948). Host selection by the pine engraver. J. Econ. Entomol. 41: 596-602.

APELBAUM, A. and YANG, S.F. (1981). Biosynthesis of stress ethylene induced by water deficit. Plant Physiol. 68: 594-596.

APPLEBAUM, S.W., BIRK, Y., HARPAZ, I. and BONDI, A. (1964). Comparitive studies on proteolytic enzymes of Tenebrio molitor L.. Comp. Biochem. Physiol. 11: 85-103.

APPLEBAUM, S.W. and KONIJN, A.M. (1966). The presence of a Tribolium - protease inhibitor in wheat. J. Insect Physiol. 12: 665-669.

APPLEBAUM, S.W., MARCO, S. and BIRK, Y. (1969). Sapponins as possible factors of resistance of legume seeds to the attack of insects. Agric. Food Chem. 17:618-622.

ASAKAWA, Y., TOYOTA, M., TAKEMOTO, T., KUBO, I. and NAKANISHI, K. (1980). Insect antifeedant secoaromaden-drane-type sesquiterpenes from Plagiochila species. Phytochemistry 19: 2147-2154.

ASPINALL, D. (1980). The role of abscisic acid and other hormones in adaption to water stress. In: Adaption of plants to water and high temperature stress.(ed. Turner, N.C. and Kramer, P.J.), John Wiley and Sons.,1980 pp. 155-171.

ASPINALL, D., SINGH, T.N. and PALEG, L.G. (1973). Stress metabolism. V. Abscisic acid and nitrogen metabolism in barley and Lolium temulentum L. Aust. J. Biol. Sci. 26: 319-327.

ATKINS, M.D. (1966). Behavioural variation among scolytids in relation to their habitat. Can. Entomol. 98: 285-288.

AUCLAIR, J.L. (1963). Aphid feeding and nutrition. Ann. Rev. Entomol. 8: 439-490.

AUCLAIR, J.L. (1965). Feeding and nutrition of the pea aphid Acrythosiphon pisum (Homoptera; Aphidae) on chemically defined diets of various pH and nutrient levels. Ann. Entomol. Soc. Am. 58: 855-875.

AUCLAIR, J.L. (1969). Nutrition of plant sucking insects on chemically defined diets. Entomol. Exp. Appl. 12: 623-641.

AUCLAIR, J.L., MALTAIS, J.B. and CARTIER, J.J. (1957). Factors in resistance of peas to the pea aphid, Acrythosiphon pisum (Harr.) (Homoptera: Aphididae). II. Amino acids. Can. Entomol. 89: 457-464.

AUERBACH, M.J. and STRONG, D.R. (1981). Nutritional ecology of Heliconia herbivores: experiments with plant fertilization and alternative hosts. Ecol. Monogr. 51: 63-83.

BALIK, M.J., FURTH, D.G. and COOPER-DRIVER, G. (1978). Biochemical and evolutionary aspects of arthropod predation on ferns. Oecologia (Berlin) 35: 55-89.

BARNETT, N.M. and NAYLOR, A.W. (1966). Amino acid and protein metabolism in Bermuda grass during water stress. Plant Physiol. 41: 1222-1230.

BAR-NUN, N. and POLJAKOFF-MAYBER, A. (1977). Salinity stress and the content of proline in roots of Pisum sativum and Tamarix tetragyna. Ann. Bot. 41: 173-179.

BECK, S.D. (1950). Nutrition of the European corn borer, Pyrausta nubialis (Hubner). II. Some effects of diet on larval growth characteristics. Physiol. Zool. 23: 353-361.

BECK, S.D. (1956). A bimodal response to dietary sugars by an insect. Biol. Bull. 110: 219-228.

BECK, S.D. (1965). Resistance of plants to insects. Ann. Rev. Entomol. 10: 207-232.

BECK, S.D. (1974). Theoretical aspects of host plant specificty in insects. In: Proc. Summ. Inst. Biol. contr. plant insect and diseases. (ed. Maxwell, F.G. and Harris, F.A.). Univ. Mississippi Press. pp. 290-311.

BECK, S.D. and HANEC, W. (1958). Effect of amino acids on feeding behaviour of the European corn borer, Pyrausta nubialis (Hubn). J. Insect Physiol. 2: 85-96.

BECKWITH, R.C. (1976). Influence of host foliage on the Douglas-fir tussock moth. Environ. Entomol. 5: 73-77.

BENECKE, U. (1980). Photosynthesis and transpiration of Pinus radiata D. Don under natural conditions in a forest stand. Oecologia (Berlin) 44: 192-198.

BENZ, G. (1974). Negative Rückkoppelung durch Raum-und Nahrungkonkurrenz sowie zyklische Veränderungder Nahrungsgrundlage als Regelprinzip in der Populationsdynamik des Graun Lärchenwicklers, Zeiraphera diniana (Guenée) (Lep. Tortricidae). Z. Angew. Entomol. 76: 196-229.

BERNAYS, E.A. (1978). Tannins an alternative veiwpoint. Entomol. Exp. Appl. 24: 44-53.

BERNAYS, E.A. (1981) Plant tannins and insect herbivores: an appraisal. Ecol. Entomol. 6: 353-360.

BERNAYS, E.A. and WOODHEAD, S. (1982)a. Incorporation of dietary phenols into the cuticle in the tree locust Anacridium melanorhodon. J. Insect Physiol. 28: 601-606.

BERNAYS, E.A. and WOODHEAD, S. (1982)b. Plant phenols utilised as nutrients by a phytophagous insect. Science 216: 201-202.

BERRYMAN, A.A (1980). General constructs for risk decision models. Proc. 1980 Conv. Soc. Am. Foresters, Spokane, Washington, pp. 123-128.

BHARDWAJ, R. and SINGHAL, G.S. (1981). Effect of water stress on photochemical activity of chloroplasts during graining of etiolated barley seedlings. Plant Cell Physiol. 22: 155-162.

BIDWELL, R.G.S. (1979). Plant Physiology. 2nd. ed., Macmillan Pub. Co., Inc. New York. pp 238-242.

BLAKE, T.J. and REID, D.M. (1981). Ethylene, water relations and tolerance to waterlogging of three Eucalyptus species. Aust. J. Plant Physiol. 8: 497-505.

BLUM, A. and EBERCON, A. (1976). Genotypic responses in sorghum to drought stress. III Free proline accumulation and drought resistance. Crop Sci. 16: 428-431.

BOGGESS, S.F., STEWART, C.R., ASPINALL, D. and PALEG, L.G. (1976). Effect of water stress on proline synthesis from radioactive precursors. Plant Physiol. 58: 398-401.

BOYD, C.E. (1970). Amino acid, protein and calorie content of vascular aquatic macrophytes. Ecology 51: 902-906.

BRADDSTREET, R.B. (1965). The Kjeldahl method for organic nitrogen. Academic Press, New York.

BRADFORD, K.J. and YANG, S.F. (1980). Xylem transport of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor, in waterlogged tomato plants. Plant Physiol. 65: 322-326.

BRATTESTEN, L.B., WILKINSON, C.F. and EISNER, T. (1977). Herbivore-plant interactions: Mixed function oxidases and secondary plant substances. Science 196: 1349-1352.

BRIGHT, S.W.J., LEA, P.J., KUEH, J.S.H., WOODCOCK, C., HOLLOMAN, D.W. and SCOTT, G.C. (1982). Proline content does not influence pest and disease susceptability of barley. Nature 295: 592-593.

BRIMBLECOMBE, A.R. (1962). Outbreaks of the eucalypt leaf skeletonizer. Qld. J. Agric. Sci. 19: 209-217.

BROWN, K.W., and THOMAS, J.C. (1980). The influence of water stress preconditioning on dark respiration. Physiol. Plant 49: 205-209.

BRUES, C.T. (1924). The specificity of food plants in the evolution of phytophagous insects. Am. Nat. 54 127-144.

BURSELL, E. (1967). The excretion of nitrogen in insects. Adv. Insect Physiol. 4: 33-61.

CAMPBELL, K.G. (1962). The biology of Roeselia lugens (Walk.) the gum leaf skeletonizer moth with particular reference to the Eucalyptus camaldulensis Dehn. (River red gum) forests of the Murray Valley region. Proc. Linn. Soc. N.S.W. 87: 316-338.

CAMPBELL, K.G. (1966). Aspects of insect-tree relationships in forests of Eastern Australia. In: Breeding pest resistant trees. (ed. Gerhold, H.D., Schneiner, E.J., McDermott, R.E. and Winieski, F.A.) Pergamon Press, New York. pp 239-250.

CAMPBELL, K.G. (1969). Note on the biology of Uraba (Roeselia) lugens Walker, the gum leaf skeletonizer. J. Entomol. Soc. Aust. (N.S.W.) 6: 30.

CARROW, J.R. and GRAHAM, K. (1968). Nitrogen fertilisation of the host tree and population growth of the balsam woolly aphid, Adelges piceae (Homoptera: Adelidae). Can. Entomol. 100: 478-485.

CATES, J.F., WATERMAN, P.G. and CHOO, G.N. (1980). Food selection by the Southern Indian leaf monkey, Probetis johnii in relation to leaf chemistry. Oecologia (Berlin) 45: 45-56.

CATES, R.G. (1980). Feeding patterns of monophagous, oligophagous and polyphagous insect herbivores: The effect of resource abundance and plant chemistry. Oecologia (Berlin). 46: 22-31.

CATES, R.G. (1981). Host plant predictability and the feeding patterns of monophagous, oligophagous and polyphagous insect herbivores. Oecologia (Berlin) 48: 319-326.

CATES, R.G and RHOADES, D.F. (1977). Patterns in the production of antiherbivore chemical defenses in plant communities. Biochem. Syst. Ecol. 5: 185-193.

CAVALIERI, A.J. (1983). Proline and glycine-betaine accumulation by Spartina alterniflora Loisel. in response to NaCl and nitrogen in a controlled environment. Oecologia (Berlin) 57: 20-24).

CAVALIERI, A.J. and HUANG, A.H.C. (1979). Evaluation of proline accumulation in the adaption of diverse species of marsh Halophytes to the saline environment. Am. J. Bot. 66: 307-312.

CAVALIERI, A.J. and HUANG, A.H.C. (1981). Accumulation of proline and glycine-betaine in Spartina alterniflora Loisel. in response to NaCl and nitrogen in the marsh. Oecologia (Berlin) 49: 224-228.

CHAMBISS, O.L. and JONES, C.M. (1966). Cucurbitacins: Specific insect attractants in Cuccurbitaceae. Science 153: 1392-1393.

CHAN, B.G., WAISS, A.C. Jr., BINDER, R.G. and ELLIGER, C.A. (1978). Inhibition of lepidopterous larval growth by cotton constituents. Entomol. Exp. Appl. 24: 94-100.

CHAPLIN, S.J. (1980). An energetic analysis of host plant selection by the large milkweed bug, Oncopeltus fasciatus. Oecologia (Berlin) 46: 254-261.

CHAPMAN, J.A. (1963). Field selection of different log odours by scolyted beetles. Can. Entomol. 95: 673-676.

CHAPMAN, J.A. (1966). The effect of attack by the ambrosia beetle Trypodendron Lineatum (Oliver) on log attractivness. Can. Entomol. 98: 50-59.

CHATTOPADHYAY, S.B. and BERA, A.K. (1980). Phenols and polyphenol oxidase activity in rice leaves infected with Helminthosporium oryzae. Phytopathol. Z. 98: 59-63.

CHEN, P.S. (1966). Amino acid and protein metabolism in insect development. Adv. Insect Physiol. 3: 53-114.

CHEW, F.S. (1980). Food plant preferences of Pieris caterpillars (Lepidoptera). Oecologia (Berlin) 46: 347-353.

CHU, T.M., ASPINALL, D. and PALEG, L.G. (1974). Stress metabolism. VI Temperature stress and the accumulation of proline in barley and radish. Aust. J. Plant Physiol. 1: 87-97.

CHU, T.M., ASPINALL, D. and PALEG, L.G. (1976)a. Stress metabolism. VII. Salinity and proline accumulation in barley. Aust. J. Plant Physiol. 3: 219-228.

CHU, T.M., ASPINALL, D. and PALEG, L.G. (1976)b. Stress metabolism. VIII. Specific ion effects on proline accumulation in barley. Aust. J. Plant Physiol. 3: 503-511.

CLARK, L.R. (1962). The general biology of Cardiaspina albitextura (Psyllidae) and its abundance in relation to weather and parasitism. Aust. J. Zool. 10: 537-586.

CLARK, L.R. (1963). Factors affecting the attractiveness of foliage for oviposition by Cardiaspina albitextura (Psyllidae). Aust. J. Zool. 11: 20-34.

COBBINAH, J.R. (1978). The biology and food preference of the gum leaf skeletonizer Uraba lugens (Walk.). PhD Thesis. University of Adelaide.

COBBINAH, J.R. (1983). Suitability of various eucalypts for growth of the larvae of Uraba lugens (Walk.). For. Ecol. Man. 6: 361-366.

COBBINAH, J,R., MORGAN, F.D. and DOUGLAS, T.J. (1982). Feeding responses of the gum leaf skeletonizer Uraba lugens (Walk.) to sugars, amino acids, lipids, sterols, salts, vitamins and certain extracts of eucalypt leaves. J. Aust. Entomol. Soc. 21: 225-236.

COLEY, P.D. (1980). Effects of leaf age and plant life history patterns on herbivory. Nature 284: 545-546.

COLEY, P.D. (1983). Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecol. Monogr. 53: 209-233.

COMMON, I.F.B. (1975). Insects of Australia - A text book for students and research workers. Melbourne Univ. Press. pp 765-865.

COOK, A.G. (1977). Nutrient chemicals as phagostimulants for Locusta migratoria.Ecol. Entomol. 2: 113-121.

COOPER-DRIVER, G., FINCH, S. and SWAIN, T. (1977). Seasonal variation in secondary plant compounds in relation to the palatability of Pteridium aquilinum. Biochem. Sys. Ecol. 5: 177-183.

DABROWSKI, Z.T. and BIELAK, B. (1978). Effect of some plant chemical compounds on the behaviour and reproduction of spider mites (Acarina: Tetranychidae). Entomol. Exp. Appl. 24: 317-326.

DADD, R.H. (1963). Feeding behaviour and nutrition in grasshoppers and locusts. Adv. Insect Physiol. 1: 47-109.

DADD, R.H. and KRIEGER, D.L. (1968). Dietary amino acid requirements of the aphid Myzus persicae. J. Insect Physiol. 14: 741-764.

DADD, R.H. and MITTLER, T.E. (1965). Studies on the artificial feeding of the aphid Myzus persicae. III Some major nutritional requirements. J. Insect Physiol. 11: 717-743.

DAVID, W.A.L. and GARDINER, B.O.C. (1966). The effect of sinigrin on the feeding of Pieris brassicae L. larvae transferred from various diets. Entomol. Exp. Appl. 9: 95-98.

DAVID, W.A.L. and GARDINER, B.O.C. (1966). Mustard oil glucosides as feeding stimulants for Pieris Brassicae larvae in a semi-synthetic diet. Entomol. Exp. Appl. 9: 247-255.

DAVIDSON, J. and HENSON, H. (1929). The internal condition of the host plant in relation to insect attack, with special reference to the influence of pyridine. Ann. Appl. Biol. 16: 458-471.

DAVIES, A.M.C., NEWBY, V.K. and SYNGE, R.L.M. (1978). Bound quinic acid as a measure of coupling of leaf and sunflower-seed proteins with chlorogenic acid and congeners: loss of availability of lysine. J. Sci. Food Agric. 29: 33-41.

DAVIS, G.R.F. (1956). Amino acid requirements of Oryzaephilus surinamensis (L.) (Coleoptera: Silvanidae) for pupation. Can. J. Zool. 34: 82-85.

DAVIS, G.R.F. (1965). Phagostimulatory effects of amino acids on Ctenicura destructor (Coleoptera; Elateridae). Arch. Int. Physiol. Biochem. 73: 610-626.

DAVIS, G.R.F. (1968). Phagostimulation and consideration of its role in artificial diets. Bull. Entomol. Soc. Am. 14: 27-30.

DAVIS, G.R.F. (1975). Essential amino acids for the growth of larva of the yellow meal worm Tenebrio molitor. J. Nutr. 105: 1071-1075.

DEGABRIELE, R. (1981). A relative shortage of nitrogenous food in the ecology of the koala (Phasolarctos cinereus). Aust. J. Ecol. 6: 139-141.

DELOACH, J.R. and SPATES, G.E. (1980). Effect of soybean trypsin inhibitor-loaded erthrocytes on fecundity and midgut protease and hemolysis activity of stable flies. J. Econ. Entomol. 73: 590-594.

DEMENT, W.A. and MOONEY, H.A. (1974). Seasonal variation in the production of tannins and cyanogenic glucosides in the chaparral shrub, Heteromeles arbutifolia. Oecologia (Berlin) 15: 65-76.

DENNO, R.F. and DONNELLY, M.A. (1981). Patterns of herbivory on Pasiflora leaf tissues and species by generalized and specialized feeding insects. Ecol. Entomol. 6: 11-16.

DETHIER, V.G. (1941). Chemical factors determining the choice of food plants by Papilio larvae. Am. Nat. 75: 61-73.

DETHIER, V.G. (1953). Host plant perception in phytophagous insects. 9th Int. Congr. Entomol. Amsterdam 2: 81-87.

DETHIER, V.G. (1954). Evolution of feeding preferences in phytophagous insects. Evolution 8: 33-54.

DETHIER, V.G. (1959). Egg laying habits of Lepidoptera in relation to available food. Can. Entomol. 91: 554-561.

DETHIER, V.G. (1970). Chemical interactions between plants and insects. In: Chemical ecology (eds. Sondhiemer, E. and Sermone, J.B.). Academic Press, New York pp. 83-102.

DETHIER, V.G. (1974). The sensitivity of the labellor chemoreceptors of the blowfly and the response to natural foods. J. Insect Physiol. 20: 1859-1869.

DETHIER, V.G. (1982). Mechanism of host plant recognition. Entomol. Exp. Appl. 31: 49-56.

DE VRIES, N.E. L., MANGLITZ, G.R. (1982). Spotted alfalfa aphid (Therioaphis maculata (Buckton)) (Homoptera: Aphididae): Water stress, amino acid content, and plant resistance. J. Kansas Entomol. Soc. 55: 57-64.

DOUGHERTY, P.M. and HINCKLEY, T.M. (1981). The influence of a severe on net photosynthesis of white oak. (Quercus alba). Can. J. Bot. 59: 335-341.

DOWD, P.F., SMITH, C.M. and SPARKS, T.C. (1983). Detoxification of plant toxins by insects. Insect Biochem. 13: 453-468

DREIER, W. (1983). The content of proline and the salt resistance of plants. Biol. Plant. 25: 81-87.

DUBETZ, S. and GARDINER, E.E. (1980). Protein content and amino acid composition of seven wheat cultivars subjected to water stress: effects of nitrogen fertiliser treatments. J. Plant Nutr. 2: 517-523.

DURZAN, D.J. and RAMAIAH, P.K. (1971). The metabolism of L-proline by jack pine seedlings. Can. J. Bot. 49: 2163-2173.

EDER, M. and KINZEL, H. (1983). Seasonal variations in free amino acid contents of deciduous Ericaeae leaves. Z. Pflanzenphysiol. 112: 269-273.

EDWARDS, P.J. and WRATTEN, S.D. (1983). Wound induced defences in plants and their consequences for patterns of insect grazing. Oecologia (Berlin) 59: 88-93.

EHRLICH, P.R. and RAVEN, P.H. (1964). Butterflies and plants: a study in coevolution. Evolution 18: 586-608.

ELIAS, P. (1981). Visual estimation of water stress in Mercurialis perennis L. Biol. Plant. 23: 456-461.

ELTON, C. (1927). Animal ecology. London, Sidgwick and Jackson.

EMDEN, H.F. van (1966). Studies on the relations of insect and host plant. III Acomparison of the reproduction of Brevicoryne brassicae and Myzus persicae: (Hemiptera: Aphididae) on brussels sprout plants supplied with different rates of nitrogen and potassium. Entomol. Exp. Appl. 9: 444-460.

EMDEN, H.F. van (1969). The differing reactions of Brevicoryne and Myzus to leaf age, turgidity and soluble nitrogen in brassicas. Ann. Appl. Biol. 149: 546-547.

EMDEN, H.F. van (1972). Aphids as phytochemists. In: Phytochemical Ecology (ed. Harbourne, J.B.). Proc. Phytochemical Soc. Symp. No. 8., Academic Press, New York and London. pp. 25-43.

EMDEN, H.F. van (1973). In: Perspectives in aphid biology. (ed. Lowe, A.D.). Entomol. Soc. N.Z., Bull. No. 2, pp. 54-64.

EMDEN, H.F. van and BASHFORD, M.A. (1971). The performance of Brevicoryne brassicae and Myzus persicae in relation to plant age and leaf amino acids. Entomol. Exp. Appl. 14: 349-360.

EMDEN, H.F. van and WAY, M.J. (1973). Host plant population dynamics of insects. Insect / Plant Rel. Roy. Entomol. Soc. London Symp. 6: 181-199.

EVANS, A.C. (1938). Physiological relationships between insects and their host plants. I The effect of chemical composition of the plant on reproduction and production of winged forms in Brevicoryne brassicae L. (Aphididae). Ann. Appl. Biol. 25: 558-572.

FEENY, P.P. (1968). Effect of oak leaf tannins on larval growth of the winter moth Operophtera brumata. J. Insect Physiol. 14: 805-817.

FEENY, P.P. (1969). Inhibitory effect of oak leaf tannins on the hydrolysis of proteins by trypsin. Phytochemistry 8: 2119-2126.

FEENY, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51: 565-581.

FEENY, P. (1975). Biochemical coevolution between plants and their insect herbivores. In: Coevolution of animals and plants. (eds. Gilbert, L. and Raven, R.). Univ. Texas Press, Austin Texas, U.S.A., pp. 2-19.

FEENY, P. (1976). Plant apparency and chemical defense. In: Biochemical Interaction between plants and insects. (eds. Wallace, J.W., Mansell, R.L.). Recent Adv. Phytochem. 10: 1-36.

FENNAH, R.G. (1953). Some aspects of the food-problem of homoptera in the humid areas of the neotropical region. 9th. Int. Congr. Entomol. Amsterdam 2: 260-265.

FERREIRA, L.G.R., DE SOUZA, J.G. and PRISCO, J.T. (1979). Effects of water deficit on proline accumulation and growth of 2 cotton genotypes of different drought resistance. Z. Pflanzenphysiol. 93: 189-200.

FISK, J. (1980). Effects of HCN, phenolic acids and related compounds in Sorghum bicolor on the feeding behaviour of the plant hopper Peregrinus maidis. Entomol. Exp. Appl. 27: 211-222.

FOX, L.R. and MACAULEY, B.J. (1977). Insect grazing on Eucalyptus in response to variation in leaf tannins and nitrogen. Oecologia (Berlin) 29: 145-162.

FRAENKEL, G. (1951). The nutritional requirements of insects for known and unknown vitamins. Trans. 9th. Congr. Entomol. (1952) 1: 277-280.

FRAENKEL, G. (1952). The nutritional value of green plants for insects. 9th. Int. Congr. Entomol. Amsterdam 2: 90-105.

FRAENKEL, G. (1953). Insects and plant biochemistry. The specificity of food plant for insects. XIV Int. Congr. Zool. pp. 383-387.

FRAENKEL, G. (1956). Insects and plant biochemistry: the specificity of food plants for insects. Proc. 14th. Int. Congr. Zool., Copenhagen pp. 383-387.

FRAENKEL, G. (1959)a. The chemistry of host specificity of phytophagous insects. 4th. Int. Congr. Biochem. 12: 1-14.

FRAENKEL, G. (1959)b. The Raison d'Etre of secondary plant substances. Science 129: 1466-1470.

FRAENKEL, G. (1969). Evaluation of our thoughts on secondary plant substances. Entomol. Exp. Appl. 12: 473-486.

FRAENKEL, G., NAYAR, J., NALBANDOV, O. and YAMAMOTO, R.T. (1960). Futher investigations into the chemical basis of the insect host plant relationships. XI Int. Kongr. F. Entomol. Wein. Verh.β. 3: 122-126.

FREELAND, W.J. and JANZEN, D.H. (1974). Strategies in herbivory by mammals: The role of plant secondary compounds. Am. Nat. 108: 269-289.

FUKUTOKU, Y. and YAMADA, Y. (1981). Diurnal changes in water potential and free amino acid contents of water-stressed and non-stressed soybean plants. Soil Sci. Plant Nutr. 27: 195-204.

FUTUYAMA, D.J. (1976). Food plant specialisation and environmental predictability in Lepidoptera. Am. Nat. 110: 285-292.)

FUTUYAMA, D.J., ROBIN, P.C. and van NOORDWIJK, I. (1984). Adaption to host plants in the fall cankerworm (Alsophila pometaria) and its bearing on the evolution of host affiliation in phytophagous insects. Am. Nat. 123: 287-296.

FUTUYAMA, D.J. and WASSERMAN, S.S. (1981). Food plant specialization and feeding efficiency in the tent caterpillars Malacosoma disstria and M. americanum. Entomol. Exp. Appl. 30: 106-110.

GEISELMAN, J.A. and McCONNEL, O.J. (1981). Polyphenols in brown algae Fucus vesiculosus and Ascophyllum nodosum: Chemical defenses against the marine herbivorous snail, Littorina littorea. J. Chem. Ecol. 7: 1115-1133.

GILBERT, L.E. and SINGER, M.C. (1975). Butterfly ecology. Ann. Rev. Ecol. Syst. 6: 27-54.

GILLENWATER, H.B., JURD, L. and McDONALD, L.L. (1980). Repellency of several phenolic compounds to adult Tribolium confusum. J. Georgia Entomol. Soc. 15: 168-175.

GOLDSTEIN, J.L. and SWAIN, T. (1965). The inhibition of enzymes by tannins. Phytochemistry 4: 185-192.

GORDON, H.T. (1959). Minimal nutritional requirements of the German roach, Blattella germanica L. Ann. New York Acad. Sci. 77: 290-351.

GORDON, H.T. (1961). Nutritional factors in insect resistance to chemicals. Ann. Rev. Entomol. 6: 27-54.

GOTHILF, S. and WAITES, R.E. (1968). Inhibition of growth and increased mortality of Mexican bean beetle larvae fed with thiamine and pyridoxine antagonists and reversal of effect with vitamin supplementation. Entomol. Exp. Appl. 11: 261-268.

GRAHAM, R.D. (1983). Effects of nutritional stress on susceptibility of plants to disease with particular reference to the trace elements. Adv. Biochem. Res. 10: 222-276.

GRIMALSKI, V.I. (1977) [Influence of droughts on the massive reproduction of needle-eating pests of pine.] Ekologiya 2: 94-96. Transl. in Soviet J. Ecol. 8: 175-177.

HAARS, A., CHET, I. and HUTTERMANN, A. (1981). Effect of phenolic compounds and tannin on growth and laccase activity of Fomes annosus. Eur. J. For. Path. 11: 67-76.

HAGLUND, B.M. (1980). Proline and valine - cues which stimulate grasshopper herbivory during drought. Nature 288: 697-698.

HAMILTON, W.J. III, BUSKIRK, R.E. and BUSKIRK, W.H. (1978). Omnivory and utilization of food resources by chachma baboons, Papio ursinus. Am. Nat. 112: 911-924.

HANSON, A.D. (1980). Interpreting the metabolic responses to water stress. Hort. Sci. 15: 623-629.

HANSON, A.D., and HITZ, W.D. (1982). Metabolic responses of mesophytes to plant water deficits. Ann. Plant Physiol. 33: 163-203.

HANSON, A.D., NEILSEN, C.E., PEDERSEN, A.R. and EVERSON, E.H. (1979). Capacity for proline accumulation during water stress in barley and its implications for breeding drought resistance. Crop Sci. 19: 489-493.

HARBOURNE, J.B. (1972). Phytochemical Ecology. Proc. Phytochem. Soc. Symp. Royal Holloway College Englefield Green, Surrey. 1971. Academic Press.

HARBOURNE, J.B. (1976). Phytochemical methods. Chapman and Hall, London.

HARBOURNE, J.B. (1977). Flavonoids and the evolution of angiosperms. Biochem. Syst. Ecol. 5: 7-22

HARRIS, J.A. (1972). The effect of flooding on population density of the gum leaf skeletonizer moth, Uraba lugens (Walk) in Barmah State forest. For. Comm. Vic. Res. Rep. 25 (unpublished).

HARRIS, J.A. (1974). The gum leaf skeletonizer, Uraba lugens. For. Tech. Papers 21. Forest Commission, Victoria.

HASSEL, M.P. and SOUTHWOOD, T.R.E. (1978). Foraging strategies of insects. Ann. Rev. Ecol. Syst. 9: 75-98.

HATFIELD, L.D., FRAZIER, J.L. and FERREIRA, J. (1982). Gustatory discrimination of sugars, amino acids, and selected allelochemics by the tarnished plant bug, Lygus lineolaris. Physiol. Entomol. 7: 15-23.

HAUKIOJA, E. (1982). Individual defences of white birch to a geometrid defoliator, Epirrita autumnata. Proc. 5th. Int. Symp. Insect - Plant Relationships, Wageningen 1982; pp 199-203 Pudoc Wageningen.

HERON, R.J. (1965). The role of chemotactic stimuli in the feeding behaviour of the spruce budworm larvae on white spruce. Can. J. Zool. 43: 247-269.

HILLIS, W.E. (1966)a. Variation in polyphenol composition within species of Eucalyptus L'Herit. Phytochemistry 5: 541-556.

HILLIS, W.E. (1966)b. Polyphenols in the leaves of Eucalyptus L'Herit: A chemotaxonomic survey - I. An introduction to the series Globulares. Phytochemistry 5: 1075-1090.

HILLIS, W.E. (1967)a. A chemotaxonomic survey. II. The sections Renantheroideae and Renantherae. Phytochemistry 6: 259-274.

HILLIS, W.E. (1967)b. A chemotaxonomic survey - III. The series Transversae, Exsertae, Subexsertae, Microcarpae, Semiunicolores, Viminales, Argyrophyllay and Paniculatae of the section Macrantherae. Phytochemistry 6: 275-286.

HILLIS, W.E. (1967)c. A chemotaxonomic survey - IV. The sections Porantheroideae and Terminales. Phytochemistry 6: 373-382.

HILLIS, W.E. (1967)d. A chemotaxonomic survey - V. The series cornutae and subcornutae of the section Macrantherae and the section Platyantherae. Phytochemistry 6: 845-856.

HINTON, T., NOYES, D.T. and ELLIS, J.F. (1951). Amino acids and growth factors in a chemically defined medium for Drosophila. Physiol. Zool. 24: 335-353.

HOQUE, E. (1982). Biochemical aspects of stress physiology of plants and some considerations of defensive mechanisms in conifers. Eur. J. For. Path. 12: 280-296.

HOUSE, H.L. (1949). Nutritional studies with Blattela germanica (L.) reared under aseptic conditions. III. Five essential amino acids. Can. Entomol. 81: 133-139.

HOUSE, H.L. (1958). Nutritional requirements of insects associated with animal parasitism. Exp. Parasit. 7: 555-609.

HOUSE, H.L. (1962). Insect nutrition. Annu. Rev. Biochem. 31: 653-672.

HOUSE, H.L. (1965). Effects of low levels of the nutrient content of a food and nutrient imbalences on the feeding and nutrition of a phytophagous larvae, Clerio euphorbiae (Lineaus) (Lepidoptera: Sphingidae). Can. Entomol. 97: 62-68.

HOUSE, H.L. (1966)a. Effects of varying the ratio between the amino acids and the other nutrients in conjunction with a salt mixture on the fly Agria affinis (Fall.). J. Insect Physiol. 12: 299-310.

HOUSE, H.L. (1966)b. Effects and interactions of varied levels of temperature, amino acids and vitamins on the rate of larval development in the fly, Pseudosarcophaga affinis. J. Insect Physiol. 12: 1493-1501.

HOUSE, H.L. (1969). Effects of different proportions of nutrients on insects. Entomol. Exp. Appl. 12: 651-669.

HOUSE, H.L. (1977). Interaction between amino acids and glucose in larval nutrition of the fly Agria housei (Diptera: Sarcophagidae). Can. Entomol. 109: 87-91.

HOVANITZ, W. (1969). Inherited and / or conditioned changes in host plant preference in Pieris. Entomol. Exp. Appl. 12: 729-735.

HSAIO, T.C. (1973). Plant responses to water stress. Ann. Rev. Plant Physiol. 24: 519-570.

HSIAO, T.H. (1978). Host plant adaptions among geographic populations of the Colorado potato beetle. Entomol. Exp. Appl. 24: 437-447.

HSIAO, T.H. and FRAENKEL, G. (1968). The influence of nutrient chemicals on the feeding behaviour of the Colorado potato beetle, Leptinotarsa decemlineata (Coleoptera: Chrysomelidae). Ann. Entomol. Soc. Am. 61: 44-54.

HUANG, A.H.C. and CAVALIERI, A.J. (1979). Proline oxidase and water stressed-induced proline accumulation in spinach leaves. Plant Physiol. 63: 531-535.

HUBER, W. (1974). Über den einfluß von NaCl - oder abscisinsaurebehandlung auf den proteinmetabolismus und einige weitere enzymedes amino säurestoffwechsels in keimlingen von Pennisetum typhoides. Planta 121: 225-235.

HUNT, P.G., CAMPBELL, R.B., SOJKA, R.E. and PARSONS, J.E. (1981). Flooding-induced soil and plant ethylene accumulation and water status response of field grown tobacco. Plant Soil 59: 427-439.

HUTCHINS, R.F.N., SUTHERLAND, O.R.W., GNANASUDERAM, C., GREENFIELD, W.J., WILLIAMS, E.M. and WRIGHT, H.J. (1984). Toxicity of nitrocompounds from Lotus pedunculatus to grass grub (Costelytra zealandica) (Coleoptera: Scarabaeidae). J. Chem. Ecol. 10: 81-93.

IHEAGWAM, E.U. (1981). The relationship between weight of insect, age, hardness and nitrogen content of cabbage leaves and fecundity of the cabbage white fly, Aleyrodes brassicae Wlk. (Homoptera, Aleyrodidae). Z. Angew. Entomol. 91: 349-354.

IKEDA, T., MATSUMURA, F. and BENJAMIN, D.M. (1977). Mechanism of feeding discrimination between matured and juvenile foliage by two species of pine sawflies. J. Chem. Ecol. 3: 677-694.

ISMAN, M.B. and DUFFEY, S.S. (1982). Toxicity of tomato phenolic compounds to the fruit worm, Heliothis zea. Entomol. Exp. Appl. 31: 370-376.

ITO, T. (1960). Effects of sugars on feeding larvae of the silk worm Bombyx mori. J. Insect Physiol. 5: 95-107.

ITO, T. and INOKUKCHI, T. (1981). Nutritive effects of D-amino acids on the silk worm, Bombyx mori. J. Insect Physiol. 27: 447-453.

IVES, P.M. (1978). How discriminating are cabbage butterflies. Aust. J. Ecol. 3: 261-276.

JEFFERIES, R.L., RUDMIK, T. and DILLON, E.M. (1979). Responses of Halophytes to high salinities and low water potentials. Plant Physiol. 64: 989-994.

JERMY, T. (1958). Untersuchungen über auffinden und wahl der nahrungbum kartoffelkäfer (Leptinotarsa decimlineata Say). Entomol. Exp. Appl. 1: 179-208.

JERMY, T. (1965). The role of rejective stimuli in the host selection of phytophagous insects. Proc. XII Int. Congr. Entomol. London, 1964 p. 547.

JERMY, T. (1966). Feeding inhibitors and food preference in chewing phytophagous insects. Entomol. Exp. Appl. 9: 1-12.

JONES, R.E. and WALKER, J.M. (1974). Some factors affecting feeding and egg developement in the Australian bush fly Musca vetustissma. Entomol. Exp. Appl. 17: 117-125.

JOURNET, A.R.P. and COCHRANE, P.M. (1978). Free amino acids in the leaves of Eucalyptus blakelyi. Phytochemistry 17: 1789-1790.

JUNG, H-J.G., and FAHEY, G.C. (1981). Effect of phenolic compound removal on in vitro forage digestibility. J. Agric. Food Chem. 29: 817-820.

KAHN, M.A.A., VINSON, S.B. and Mitlin, N. (1976). Effect of the parisitoid, Cardiochiles nigriceps on the nitrogen excretion of its host, Heliothis virescens. J. Insect Physiol. 22: 51-56.

KAISER, F.E., GEHRKE, C.W., ZUMWALT, R.W., KUO, K.C. (1974). Amino acid analysis. Hydrolysis, ion exchange clean up, derivitization, and quantitation by Gas-liquid chromatography. J. Chromatography 94: 113-133.

KASTING, R., DAVIS, G.R.F. and McGINNIS, A.J. (1962). Nutritionally essential and non essential amino acids of the prairie grain wire worm, Ctenicera destructor Brown determined with glucose-U-C^{14}. J. Insect Physiol. 8: 584-596.

KASTING, R. and McGINNIS, A.J. (1958). Use of glucose labelled with carbon - 14 to determine the amino acids essential for an insect. Nature 182: 1380-1381.

KASTING, R. and McGINNIS, A.J. (1962). Nutrition of the pale western cut worm Agrostis orthogonia Morr. (Lepidoptera, noctuidae) IV. Amino acid requirements determined with glucose-U-C^{14}. J. Insect Physiol. 8: 97-103.

KATO, M. (1978). Phenols as indespensible components of the synthetic diet of the silkworm, Bombyx mori. Entomol. Exp. Appl. 485-490.

KEMBLE, A.R. and MACPHERSON, H.T. (1954). Liberation of amino acids in perennial rye grass during wilting. Biochem. J. 58: 46-50.

KENNEDY, J.S. (1953). Host plant selection in Aphididae. 9th. Int. Congr. Entomol. Amsterdam 2 : 106- 113.

KENNEDY, J.S. (1958). Physiological condition of the host-plant and susceptability to aphid attack. Entomol. Exp. Appl. 1: 50-65.

KENNEDY, J.S. and BOOTH, C.O. (1951). Mechanisms of host plant selection. Ann. Appl. Biol. 56: 317-322.

KENNEDY, J.S. and BOOTH, C.O. (1959). Responses of Aphis fabae Scop. to water shortage in host plants in the field. Entomol. Exp. Appl. 2: 1-11.

KENNEDY, J.S., LAMB, K.P. and BOOTH, C.O. (1958). Responses of Aphis fabae Scop. to water shortage in host plants in pots. Entomol. Exp. Appl. 1: 274-291.

KIMMINS, J.P. (1971). Variations in the foliar amino acid composition of flowering balsam fir (Abies balsamea (L.) Mill.) and white spruce (Picea glauca (Moench) Voss) in relation to outbreaks of the spruce budworm (Choristoneura fumiferana (Clem)). Can. J. Zool. 49: 1005-1011.

KLOCKE, J.A. and CHAN, B.G. (1982). Effects of cotton condensed tannin on feeding and digestion in the cotton pest, Heliothis zea. J. Insect Physiol. 28: 911-915.

KRITZMAN, G. and CHET,I. (1980). The role of phenols in the pathogenicity of Botrytis alli. Phytoparasitica 8: 27-37.

KRIEGER, R.T., FEENY, P.P. and WILSON, C.F. (1971). Detoxification enzymes in the gut of caterpillars: An evolutionary answer to plant defenses. Science 172: 579-581.

LABANAUSKAS, C.K., SHOUSE, P., STOLZY, L.H. and HANDY, M.F. (1981). Protein and free amino acids in field-grown cowpea seeds as affected by water stress at various growth stages. Plant and Soil 63: 355-368.

LABEYRIE, V. (1969). The variability of the physiological and ethological activities and the population growth of insects. In: Statistical Ecology. (eds. Patil, C.P., Pielou, E.C. and Waters, W.E.), Univ Park, Penn. Univ. Press. 2: 314-336.

LABEYRIE, V. (1978). Reproduction of insects and co-evolution of insects and plants. Entomol. Exp. Appl. 24: 496-504.

LAMBERT, M.J. and TURNER, J. (1983). Soil nutrient relationships with particular reference to Eucalyptus sub genera. Aus. For. 46: 200-209.

LANSBERG, J. and WYLIE, F.R. (1983). Water stress, leaf nutrients and defoliation: a model of dieback of rural eucalypts. Aust. J. Ecol. 8: 27-41.

LAWLOR, D.W. (1976). Water stress induced changes in photosynthesis, photorespiration, respiration and CO_2 compensation concentration of wheat. Photosynthetica 10: 378-387.

LAWLOR, D.W. (1979). Effects of water and heat stress on carbon metabolism of plants with C_3 and C_4 photosynthesis. In: Stress physiology in crop plants. (eds. Musell, H. and Staples, R.C.), Wiley and Sons, pp. 304-326.

LAWLOR, D.W. and FOCK, H. (1977). Water stress induced changes in the amount of some photosynthetic assimilation products and respiratory metabolites of sunflower leaves. J. Exp. Bot. 28: 329-337.

LAWLOR, D.W. and FOCK, H. (1978). Photosynthesis, respiration and carbon assimilation in water-stressed maize at two oxygen concentrations. J. Exp. Bot. 29: 579-593.

LAWTON, J.H. (1976). The structure of the arthropod community on bracken. Bot. J. Linn. Soc. 73: 187-216.

LEVIN, D.A. (1971). Plant phenolics: An ecological perspective. Am. Nat. 105: 157-181.

LEVIN, D.A. (1976)a. The chemical defenses of plants to pathogens and herbivores. Ann. Rev. Ecol. Syst. 7: 121-159.

LEVIN, D.A. (1976)b. Alkaloid-bearing plants: An ecogeographic perspective. Am. Nat. 110: 261-284.

LINCOLN, D.E., NEWTON, T.S., EHRLICH, P.R. and WILLIAMS, S. (1982). Coevolution of the checkerspot butterfly Euphydras chaledona and its larval food plant Diplacus aurantiacus: larval response to protien and leaf resin. Oecologia (Berlin) 52: 216-223.

LINDIG, O.H, POE, W.E.and HENDIN, P.A. (1980). Essential amino acids in dietary prtein sources and the nutrional status and oviposition of boll weevils. J. Econ. Entomol. 73: 172-175.

LIPKE, H. and FRAENKEL, G. (1956). Insect nutrition. Ann. Rev. Entomol. 1: 17-44.

LIU, M.S. and HELLEBUST, J.A. (1976). Effects of salinity changes on growth and metabolism of the marine centric diatom Cyclotella cryptica. Can. J. Bot. 54: 930-937.

LORD, C. (ed.) (1968). Biochemists hand book. Spon., London, pp. 1192.

LUNDERSTADT, J. von and REYMERS, A. (1980). Zur nahrungsqualität von fichtennadeln für fortliche schadinsekten. 14. Wechselwirkung zwischen proteinen und phenolen nach verfütterung von nadeln der fichte (Picea abies Karst.) an larven von Gilpinia hereyniae (Hym., Diprionidae).Z. Angew. Entomol. 90: 113-126.

LUDLOW, M.M., NG, T.T. and FORD, C.W. (1980). Recovery after water stress of leaf gas exchange in Panicum maximum var. trichoglume. Aust. J. Pant Physiol. 7: 299-313.

MACAULEY, B.J. and FOX, L.R. (1980). Variations in total phenols and condensed tannins in Eucalyptus: leaf phenology and insect grazing. Aust. J. Ecol. 5: 31-35.

McCLURE, M.S. (1979). Self regulation in populations of the elongate hemlock scale, Forina externa (Homptera: Diaspididae): Role of food quantity and quality. Misc. Publ. Entomol. Soc. Am. 11: 33-40.

McCLURE, M.S. (1980). Foliar nitrogen: a basis for host suitability for elongate hemlock scale, Forina externa (Homoptera: Diaspididae). Ecology 61: 72-79.

McFARLANE, J.E. and DISTLER, M.H.W. (1982). The effect of rutin on growth, fecundity and food utilization in Acheta domesticus (L.). J. Insect Physiol. 28: 85-88.

McKEY, D. (1974). Adaptive patterns in alkaloid physiology. Am. Nat. 108: 305-320.

McKEY, D. (1979). The distribution of secondary compounds within plants. In: Herbivores: their interactions with secondary plant metabolites. (eds. Rosenthal, G.A. and Janzen, D.H.). Academic Press , New York. pp. 55-133.

McMULLEN, L.H. and ATKINS, M.D. (1962). On the flight and host selection of the Douglas fir beetle Dendroctonus pseudsugae Hopk. (Coleoptera: Scolytidae). Can. Entomol. 94: 1309-1325.

McNEILL, S. and SOUTHWOOD, T.R.E. (1978). The role of nitrogen in the developement of insect / plant relationships. In: Biochemical aspects of plant and animal co-evolution. (eds. Harbourne, J.B. and van Emden, H.F.). Academic Press London. pp. 77-98.

MALTAIS, J.B. and AUCLAIR, J.L. (1957). Factors in resistance of peas to the pea aphid, Acyrthosiphon pisum (Harr.) (Homoptera: Aphididae). I. The sugar - nitrogen ratio. Can. Entomol. 89: 365-370.

MANSOUR, M.H. (1981). Efficiency of two allelochemics on the conversion of ingested and digested food into the body tissues of Spodoptera littoralis (Boisd) (Lepidoptera, Noctuidae). Z. Angew. Entomol. 92: 493-499.

MANSOUR, M.H. (1982). The chronic effects of some allelochemics on the larval development and adult reproductivity of the cotton leaf worm Spodoptera littoralis Boisd. Z. Pflanzenkr. (Pflanzenpathol.) Pflanzenschutz, Sonderh 89: 224-229.

MANSOUR, M.H., DIMITRY, N.Z. and ROFAEEL, I.S. (1982). The role of coumarin as secondary plant substance in the food specificty of the cow pea aphid Aphis craccirora Kock. Z. Angew. Entomol. 93: 151-157.

MASON, R.R. (1981). Host foliage in the susceptability of forest sites in central California to outbreaks of the Douglas-fir tussock moth, Orgyia pseudotsugata (Lepidoptera: Lymantriidae). Can. Entomol. 113: 325-332.

MATTSON, W.J. Jr. (1980). Herbivory in relation to plant nitrogen content. Ann. Rev. Ecol. Syst. 52: 216-223.

MILES, P.W., ASPINALL, D. and ROSENBERG, L. (1982)a. Performance of the cabbage aphid Brevicoryne brassicae (L.) on water-stressed rape plants, in relation to changes in their chemical composition. Aust. J. Zool. 30: 337-345.

MILES, P.W., ASPINALL, D. and CORREL, A.T. (1982)b. The perfomance of two chewing insects on water-stressed food plants in relation to changes in their chemical composition. Aust. J. Zool. 30: 347-355.

MILLER, J. and FEENY, P. (1983). Effects of benzylisoquinoline alkaloids on the larvae of polyphagous Lepidoptera. Oecologia (Berlin) 58: 332-339.

MILTON, K. (1979). Factors influencing leaf choice by howler monkeys (Alouatta): A test of some hypotheses of food selection by generalist herbivors. Am. Nat. 114: 362-378.

MITTLER, T.E. (1953). Amino acids in phloem sap and their excretion by aphids. Nature 172: 207.

MITTLER, T.E. and DADD, R.H. (1963)a. Studies on the artificial feeding of the aphid Myzus persicae (Sulzer) - I. Relative uptake of water and sucrose solutions. J. insect Physiol. 9: 623-645.

MITTLER, T.E. and DADD, R.H. (1963)b. Studies on the artificial feeding of the aphid Myzus persicae (Sulzer) - II. Relative survival, development, and larviposition on different diets. J. Insect Physiol. 9: 741-757.

MITTLER, T.E. and DADD, R.H. (1964). Gustatory discrimination between liquids by the aphid Myzus persicae (Sulzer). Entomol. Exp. Appl. 7: 315-328.

MITTLER, T.E. and DADD, R.H. (1965). Differences in the probing responses of Myzus persicae (Sulzer) elicited by different feeding solutions behind a parafilm membrane. Entomol. Exp. Appl. 8: 107-122.

MITTLER, T.E. and KLEIJAN, J.E. (1970). Effects of artificial diet components on wing production by the aphid, Myzus persicae. J. Insect Physiol. 16: 833-850.

MIZRAHI, Y. BLUMENFELD, A. and RICHMOND, A.E. (1970). Abscisic acid and transpiration in leaves in relation to osmotic stress. Plant Physiol. 46: 169-171.

MONTENEGRO, G., JORDAN, M. and ALJARO, M.E. (1980). Interactions between Chilean mattorral shrubs and phytophagous insects. Oecologia (Berlin) 45: 346-349.

MOONEY, H.A., EHRLICH, P.R., LINCOLN, D.E. and WILLIAMS, K.S. (1980). Environmental controls on the seasonality of a drought deciduous shrub, Diplacus aurantiacus and its predator, the checkerspot butterfly, Euphydryas chalcedona. Oecologia (Berlin) 45: 143-146.

MORGAN, F.D. (1984). Psylloidea of South Australia. Hand book of flora and fauna of South Australia, D.J. Woodman, Gov. Printer.

MORGAN, F.D. and COBBINAH, J.R. (1977). Oviposition and establishment of Uraba lugens (Walker), the gum leaf skeletonizer. Aust. J. For. 40: 44-55.

MORIONDO, F. Von and COVASSI, F. (1981). Tannesterben in Italien. Forstwissenschafliches Centralblatt 100: 168-170.

MORROW, P.A. (1977). Host specificity of insects in a community of three co-dominant Eucalyptus species. Aust. J. Ecol. 2: 89-106.

MORROW, P.A. and FOX, L.R. (1980). Effects of variation in Eucalyptus essential oil yeild on insect growth and grazing damage. Oecologia (Berlin) 45: 209-219.

MUKHERJEE, S.P. and CHOUDHURI, M.A. (1981). Effects of water stress on some oxidative enzymes and senesence in Vigna seedlings. Physiol. Plant. 52: 37-42.

MYERS, J.H. and POST, B.J. (1981). Plant nitrogen and fluctuations of insect populations: A test with cinnabar moth - tansy ragwort system. Oecologia (Berlin). 48: 151-156.

NAIDENOVA, T.S. (1979). [Effect of a short drought on the rate of photosynthesis of Quercus robur.] Gorkostopanska Nauka 16: 3-12.

NAYAR, J.K. and FRAENKEL, G. (1963). The chemical basis of the host selection in the catalpa sphinx, Ceratoma catalpae (Lepidoptera, Sphingida). Ann. Entomol. Soc. Am. 56: 119-122.

NAYAR, J.K. and THORSTEINSON, A.J. (1963). Further investigations into the chemical basis of insect-host plant relationships in an oligophagous insect, Putella maculipennis (Curtis) (Lepidoptera: Plutellidae). Can. J. Zool. 41: 923-929.

OATES, J.F., WATERMAN, P.G. and CHOO, G.M. (1980). Food selection by the South Indian leaf-monkey, Presbytis johnii, in relation to leaf chemistry. Oecologia (Berlin) 45: 45-56.

OHMART, C.P., STEWART, L.G. and THOMAS, J.R. (1985). Effects of nitrogen concentrations of Eucalyptus blakelyi foliage on the fecundity of Paropsis atomaria (Coleoptera: Crysomelidae). M.S.

ONUF, C.P., TEAL, J.M. and VALIELA, I. (1977). Interactions of nutrients, plant growth and herbivory in a mangrove ecosystem. Ecology 58: 514-526.

OSONUBI, O. and DAVIES, W.J. (1980). The influence of water stress on the photosynthetic performance and stomatal behaviour of tree seedlings subjected to variation in temperature and irradiance. Oecologia (Berlin) 45: 3-10.

PAINTER, R.H. (1953). The role of nutritional factors in host plant selection. 9th. Int. Congr. Entomol. Amsterdam 2: 101-105.

PALEG, L.G. and ASPINALL, D. eds. (1981). The physiology and biochemistry of drought resistance in plants. Academic Press.

PARKER, J. and PATTON, R.L. (1975). Effects of drought and defoliation on some metabolites in roots of black oak seedlings. Can. J. For. Res. 5: 457-463.

PERTTUNEN, V. (1957). Reaction of two bark beetle species Hylurgops palliatus Gyll and Hyiastes ater Payk (Coleoptera: Scolytidae) to the terpene α pinene. Ann. Entomol. Fenn. 23: 101-110.

PITMAN, G.B. (1966). Studies on the pheromone of Ips confusus (LeConte). III. The influence of host material on pheromone production. Contrib. Boyce Thompson Inst. 23: 147-158.

PORT, G.R. and THOMPSON, J.R. (1980). Outbreaks of insect herbivores on plants along motor ways in the United Kingdom. J. Appl. Ecol. 17: 649-656.

PRADHAN, S. (1971). In the tropics, protection research more needed than production research. Indian J. Entomol. 33: 233-259.

PRESTIDGE, R.A. (1982). The influence of nitrogenous fertilizer on the grassland Auchenorrhyncha (Homoptera). J. Appl. Ecol. 19: 735-749.

RAHIER, M. (1978). Performance of Myzus persicae and production of its host plant, Brassica rapa related to plant mineral nutrition. Entomol. Exp. Appl. 24: 421-424.

RAUSHER, M.D. (1981). Host plant selection by Battus philenor butterflies: The roles of predation, nitrition, and plant chemistry. Ecol. Monogr. 51: 1-20.

RAWLINGS, G.B. (1953). Insect epidemics on forest trees in New Zealand. N.Z. For. 6: 405-412.

REESE, J.C. (1978). Chronic effects of plant allelochemics on insect nutritional physiology. Entomol. Exp. Appl. 24: 625-631.

REESE, J.C. and BECK, S.D. (1978). Interrelationships of nutritional indicies and dietary moisture in the black cutworm (Agrotis ipsilon) digestive efficiency. J. Insect Physiol. 24: 473-479.

REICHLE, D.E., GOLDSTIEN, R.A., van HOOK, R.I. Jr. and DODSON, G.J. (1973). Analysis of insect consumption in a forest canopy. Ecology 54: 1076-1084.

RHOADES, D.F. (1979). Evolution of plant chemical defense against herbivores. In: Herbivores: Their interaction with secondary plant metabolites. (eds. Rosenthal, G.A. and Janzen, D.H.). Academic Press, New York, London. pp. 3-54.

RHOADES, D.F. and CATES, R.G. (1975). Toward a general theory of plant antiherbivore chemistry. In: Biochemical interaction between plants and insects. (eds. Wallace, J.W. and Mansell, R.L.). Rec. Adv. Phytochem. 10: 168-205.

ROBINS, W.E., THOMPSON, M.J., YAMAMOTO, R.T. and SHORTER, T.J. (1965). Feeding stimulation for the female house fly, Musca domestica Linneaus. Science 147: 628-630.

RODRIGUEZ, E. (1977). Ecogeographic distribution of secondary constituents in Parthenium (Compositae). Biochem. Syst. Ecol. 5: 207-218.

RODRIGUEZ, J.G. (1951). Mineral nutrition of the two-spotted spider mite, Tetranychus bimaculatus Harvey. Ann. Entomol. Soc. Am. 44: 511-526.

ROTHSCHILD, M. (1972). Some observations on the relationship between plants, toxic insects and birds. In: Phytochemical Ecology. (ed. Harbourne, J.B.). Proc. Phytochemical Soc. Symp., Academic Press. pp. 1-12.

RUDINSKY, J.A. (1966). Scolytid beetles associated with Douglas fir: Response to terpene. Science 152: 218-219.

RUSSELL, F.C. (1947). The chemical composition and digestibility of fodder shrubs and trees. Joint Publs. Commonw. agric. Bur. 10: 185-231.

RUSSELL, G.B., SUTHERLAND, O.R.W., CHRISTMAS, P.E. and WRIGHT, H. (1982). Feeding deterrents for black beetle larvae, Heteroychus arator (Scarabeidae), in Trifolium repens. N.Z. J. Zool. 9: 145-150.

SCHOONHOVEN, L.M. (1968). Chemosensory bases of host plant selection. Ann. Rev. Entomol. 13: 115-136.

SCHOONHOVEN, L.M. (1969). Gustation and food plant selection in some lepidopterous larvae. Entomol. Exp. Appl. 12: 555-564.

SCHOONHOVEN, L.M. (1973). Plant recognition by lepidopterous larvae. Insect / Plant Rel. Roy. Entomol. Soc. London Symp. 6: 87-99.

SCHOONHOVEN, L.M. (1982). Biological aspects of antifeedants. Entomol. Exp. Appl. 31: 57-69.

SCHOONHOVEN, L.M. and MEERMAN, J. (1978). Metabolic cost of changes in diet and neutralization of allelochemics. Entomol. Exp. Appl. 24: 489-493.

SCHROEDER, L.A. (1976)a. Effect of food deprivation on the efficiency of utilization of dry matter , energy, and nitrogen by larvae of the cherry scallop moth Calope undulata. Ann. Entomol. Soc. Am. 69: 55-58.

SCHROEDER, L.A. (1976)b. Energy, matter and nitrogen utilization by larvae of the monarch butterfly Danaus plexippus. Oikos 27: 259-264.

SCHROEDER, L.A. (1977). Energy, matter and nitrogen utilization by larvae of the milkweed tiger moth Euchaetias egle. Oikios 28: 27-31.

SCHROEDER, L.A. and MALMER, M. (1980). Dry matter, energy and nitrogen conversion by Lepidoptera and Hymenoptera larvae fed leaves of black cherry. Oecologia (Berlin) 45: 63-71.

SCHULTZ, J.C. and BALDWIN, I.T. (1982). Oak leaf quality declines in response to defoliation by gypsy moth larvae. Science 217: 149-150.

SCRIBER, J.M. (1977). Limiting effects of low leaf-water content on the utilization, energy budget, and larval growth of Hyalphora cercropia. Oecologia (Berlin). 28: 269-287.

SCRIBER, J.M. (1978). The effects of larval feeding specialization and plant growth form on the consumption and utilization of plant biomass and nitrogen: An ecological consideration. Entomol. Exp. Appl. 24: 694-710.

SCRIBER, J.M. (1979). Effects of leaf-water supplimentation upon post-ingestive nutritioal indices of forb-, vine-, and tree feeding Lepidoptera. Entomol. Exp. Appl. 25: 240-252.

SCRIBER, J.M. and FEENY, P. (1979). Growth of herbivorous caterpillars in relation to feeding specialization and to the growth form of their food plants. Ecology 60: 829-850.

SCRIBER, J.M. and SLANSKY, F. Jr. (1981). The nutritional ecology of immature insects. Ann. Rev. Entomol. 26: 183-211.

SEIGLER, D.S. (1977). Primary roles for secondary compounds. Biochem. Syst. Ecol. 5: 195-199.

SEITSCHEK, O. Von (1981). Verbreitung und bedeutung der tannenerkrankung in Bayern. Fortwissenschaftliches Centralblatt 100: 138-148.

SEITZ, A. (1933). Macro Lepidoptera of the world. Alfred Kernen, Publisher, Stuttgart. vol. 10, p 112.

SEMTNER, P.J., RASNAKE, M. and TERRIL, T.R. (1980). Effect of host-plant nutrition on the occurence of tobbacco hornworms and tobacco flea beatles on different types of tobacco. J. Econ. Entomol. 73: 221-224.

SINGH, K.R.P. and BROWN, A.W.A. (1957). Nutritional requirements of Ades aegypti L. J. Insect Physiol. 1: 199-220.

SINGH, M. and SINGH, T.A. (1981). Free proline accumulation in maize (Zea mays L.) subject to prolonged waterlogging. Plant Soil 59: 349-351.

SINGH, T.N., PALEG, L.G. and ASPINALL, D. (1973)a. Stress metabolism. I. Nitrogen metabolism and growth in the barley plant during water stress. Aust. J. Biol. Sci. 26: 45-56.

SINGH, T.N., ASPINALL, D., PALEG, L.G. and BOGGESS, S.F. (1973)b. Stress metabolism. II. Changes in proline concentration in excised plant tissues. Aust. J. Biol. Sci. 26: 57-63.

SLANSKY, F. Jr. (1976). Phagism relationships among butterflies. J. New York Entomol. Soc. 84: 233-242

SLANSKY, F. and FEENY, P. (1977). Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecol. Monogr. 47: 209-228.

SLOBODKIN, L.B. (1962). Growth and regulation of animal poulations. Holt, Rinehart and Winston, New York. 184 pp.

SMART, J. and HUGHES, N.F. (1973). The insect and the plant: progressive paleoecological integration. Symp. Roy. Entomol. Soc. London 6: 143-155.

SMITH, D.S. and NORTHCOTT, F.E. (1951). The effects on the grasshopper, Melanoplus mexicanus mexicanus (Sauss.) (Orthoptera: Acrididae), of varying the nitrogen content in its food plant. Can. J. Zool. 29: 297-304.

SOO HOO, C.F. and FRAENKEL, G. (1966)a. The selection of food plants in a polyphagous insect, Prodenia eridania (Cramer). J. Insect Physiol. 12: 693-709.

SOO HOO, C.F. and FRAENKEL, G. (1966)b. The consumption, digestion and utilization of food plants by a phytophagous insect, Prodenia eridania (Cramer). J. Insect Physiol. 12: 711-730.

SOUTHWOOD, T.R.E. (1973). The insect / plant relationship: an evolutionary perspective. Symp. Roy. Entomol. Soc. London 6: 3-30.

SPYROPOULOS, C.G. and LAMBRIS, M.P. (1979). Influence of temperature on the effects of water stress on Quercus species. Ann. Bot. 44: 215-220.

SRIVASTAVA, P.N., AUCLAIR, J.L. and SRIVASTAVA, U. (1983). Effect of nonessential amino acids on phagostimulation and maintenance of the pea aphid, Acyrthosiphon pisum. Can. J. Zool. 61: 2224-2229.

STAHL, E. (1888). Pflanzen und Schnecken. Jena Z. Med. Naturw. 22. NF. XV: 557-584

STEWART, C.R. (1972)a. The effect of wilting on proline metabolism in excised bean leaves in the dark. Plant Physiol. 51: 508-511.

STEWART, C.R. (1972)b. Proline content and metabolism during the rehydration of wilted excised leaves in the dark. Plant Physiol. 50: 679-681.

STEWART, C.R. and HANSON, A.D. (1980). Proline accumulation as a metabolic response to water stress . In: Adaption of plants to water and high temperature stress. (eds. Turner, N.C. and Kramer, P.J.). Wiley and Sons; New York, Chichester, Brisbane, Toronto. pp. 173-190.

STEWART, G.R. and LARHER, F. (1980). Accumulation of amino acids and related compounds in relation to environmental stress. In: The biochemistry of plants. Academic Press. 5: 609-635.

STRONG, F.E. and SAKAMOTO, S.S. (1963). Some amino acid requirements of the green peach aphid, Myzus persicae (Sulzer) determined with glucose-U-C^{14}. J. Insect Physiol. 9: 875-879.

SUMERE, van C., GEIGER, H., BRAL, D., FOCKENIER, G., VANDE CASTEELE, K., MARTENS, M., HANSELAER, R. and GEVAERT, L. (1983). Freeze drying and analysis of plant and other biological material. Anal. Biochem. 131: 530-532.

SUMNER, L.C., NEED, J.J., McNEW, R.W., DORSCHNER, K.W., EIKENBARY, R.D. and JOHNSON, R.C. (1983). Response of Schizaphis graminum (Homoptera: Aphididae) to drought stressed wheat, using polyethylene glycol as a matricum. Environ. Entomol. 12: 919-922.

SUTHERLAND, O.R.W., HUTCHINS, R.F.N. and GREENFIELD, W.J. (1982). Effect of lucerne saponins and lotus condensed tannins on survival of grass grub, Costelytra zealandica. N.Z. J. Zool. 9: 511-514.

SWAIN, T. (1978). Plant-animal coevolution: a synoptic veiw of the Paleozoic and Mesozoic. In: Biochemical aspects of plant and animal coevolution. (ed. Harbourne, J.B.). Academic Press, London, San Fran Sisco. pp. 3-19.

SWAIN, T. and HILLIS, W.E. (1959). The phenolic constituents of Prunus domestica I. The quatitative analysis of phenolic constituents. J. Sci. Food Agric. 10: 63-68.

TABASHNIK, B.E. (1982). Responses of pest and non-pest Colias butterfly larvae to intraspecific variation in leaf nitrogen and water content. Oecologia (Berlin) 55: 389-394.

TANABE, Y., SANO, M. and KAWASHIMA, N. (1982). Changes in free amino acids in white and green tissues of variegated tobbacco leaves during water stress. Plant Cell Physiol. 23: 1229-1235.

TATT, O.H. (1980). Effects of manitol induced water stress on the ribosomes of intact leaves of Azuki bean seedlings. Biol. Plant. 22: 249-254.

TAYLOR, C.E. (1955). Growth of the potato plant and aphid colonization. Ann. Appl. Biol. 43: 151-155.

TAYLOR, W.E. and BARDNER, R. (1968). Leaf injury and food consumption by larvae of Phaedon cochleariae (Coleoptera: Crysomelidae) and Plutella maculipennis (Lepidoptera: Plutellidae) feeding on turnip and radish. Entomol. Exp. Appl. 11: 177-184.

TEMPLE, A.S. (1981). Field studies of the relationship between herbivore damage and tannin concentration in bracken (Pteridium aquilinum Kuhn). Oecologia (Berlin) 51: 97-106.

THORP, R.W. and BRIGGS, D.L. (1972). Mortality in immature leaf cutter bees in relation to alfalfa saponins. Environ. Entomol. 1: 399-401.

THORSTEINSON, A.J. (1953). The chemotactic responses that determine host specificity in an oligophagous insect (Plutella maculipennis (Curt) Lepidoptera). Can. J. Zool. 31: 52-72.

THORSTEINSON, A.J. (1955) The experimental study of the chemotactic basis of host specificity in phytophagous insects. Can. Entomol. 87: 1-19.

THORSTEINSON, A.J. (1958)a. Acceptability of plants for phytophagous inscts. Proc. 10th. Int. Congr. Entomol. 2: 599-602.

THORSTEINSON, A.J. (1958)b. The chemotactic influence of plant constituents on feeding by phytophagous insects. Entomol. Exp. Appl. 1: 23-27.

THORSTEINSON, A.J. (1960). Host selection in phytophagous insects. Ann. Rev. Entomol. 5: 193-218.

THORSTEINSON, A.J. and NAYAR, J.K. (1963). Plant phospholipids as feeding stimulants for grasshoppers. Can. J. Zool. 41: 931-935.

TODD, G.W., GETAHUN, A. and CRESS, D.C. (1971). Resistance in barley to the green bug, Schizaphis graminum. I. Toxicity of phenolic and flavonoid compounds and related substances. Ann. Entomol. Soc. Am. 64: 718-722.

TREICHEL, S. (1975). Der einfluβ von NaCl auf die prolinkonzentration verschiedener halophyten. Z. Pflanzenphysiol 76: 56-68.

TUOMI, J., NIEMELA, P., HAUKIOJA, E., SIRON, S. and NEUVONEN, S. (1984). Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. Oecologia (Berlin) 61: 208-210.

TURNER, N.C. and KRAMER, P.J. eds. (1980). Adaption of plants to water and high temperature stress. Wiley and Sons., New York, Chichester, Brisbane, Toronto.

VAADIA, Y., RANEY, F.C. and HAGAN, R.M. (1961). Plant water deficits and physiological processes. Ann. Rev. Plant Physiol. 12: 265-292.

VAN DER LAAN, P.A. (1959). Correlation between rainfall in the dry season and the occurence of the white rice borer (Scirpophaga innotata Wlk.) in Java. Entomol. Exp. Appl. 2: 12-20.

VANDERZANT, E.S. (1958). The amino acid requirement of the pink boll worm. J. Econ. Entomol. 51: 309-311.

VANDERZANT, E.S. (1963). Nutrition of the adult boll weevil: oviposition on defined diets and amino acid requirements. J. Insect Physiol. 9: 683-691.

VANDERZANT, E.S. (1969). Physiological aspects of artificial diets. Entomol. Exp. Appl. 12: 642-650.

VANDERZANT, E.S. (1974). Development, significance, and application of artificial diets for insects. Ann. Rev. Entomol. 19: 139-166.

VERSCHAFFELT, E. (1910), The cause determining the selection of food in some herbivorous insects. Proc. Roy. Acad. Amsterdam. 13: 536-542.

WALDBAUER, G.P. (1968). The consumption and utilization of food by insects. Adv. Insect Physiol. 5: 229-288.

WALKER, F. (1863). Tortricidae - Nolinae. List species. Lepid. Coll. Brit. Mus. 35: 17-28.

WARDOJO, S. (1969). Artificial diet without crude plant material for two oligophagous leaf feeders. Entomol. Exp. Appl. 12: 698-702.

WEARING, C.H. (1972). Responses of Myzus persicae and Brevicoryne brassicae to leaf age and water stress in brussels sprouts grown in pots. Entomol. Exp. Appl. 15: 61-80.

WEBB, J.W. and MORAN, V.C. (1978). The influence of the host plant on the population dynamics of Acizzia russellae (Homoptera: Psyllidae). Ecol. Entomol. 3: 313-321.

WELLINGTON, W.G., FETTES, K.B., TURNER, K.B. and BELYEA, R.M. (1950). Physical and biological indicators of the development of outbreaks of the spruce budworm, Choristonura fumiferana (Clem.) (Lepidoptera: Tortricidae). Can. J. Res.,D 28: 308-331.

WENZ, J.M. (1977). 1976 and 1977 drought / pest-caused tree mortality in national forests of Northern California. Rep. For. Insect and Disease Management staff. Reg. 5., For. Ser. U.S.D.A.

WEST, P.W. (1979). Date of onset of regrowth dieback and its relation to summer drought in eucalypt forests of southern Tasmainia. Ann. Appl.Biol. 93: 337-350.

WHITE, T.C.R. (1966). Food and outbreaks of phytophagous insects with special reference to Cardiaspina densitexta Taylor (Psyllidae, Homoptera) on Eucalyptus fasiculosa F.U.M. (Myrtaceae) in South Australia. PhD Thesis, University of Adelaide.

WHITE, T.C.R. (1969). An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. Ecology 50: 905-909.

WHITE, T.C.R. (1974). An hypothesis to explain outbreaks of looper caterpillars with special reference to populations of Selidosema suavis in a plantation of Pinus radiata in New Zealand. Oecologia (Berlin) 16: 279-301.

WHITE, T.C.R. (1976). Weather, food and plagues of locusts. Oecologia (Berlin) 22: 119-134.

WHITE, T.C.R. (1978). The importance of a relative shortage of food in animal ecology. Oecologia (Berlin) 33: 71-86.

WHITTAKER, R.A. (1970). The biochemical ecology of higher plants. In: Chemical Ecology. (eds. Sondheimer, E. and Simeone, J.D.). Academic Press, New York. pp. 43-70.

WHITTAKER, R.H. and FEENY, P.P. (1971). Allelochemics: Chemical interactions between species. Science 171: 757-770.

WILLIAMS, K.S., LINCOLN, D.E. and EHRLICH, P.R. (1983). The coevolution of Euphydryas chalcedona butterflies and their larval host plants. I. Larval feeding behaviour and host plant chemistry. Oecologia (Berlin) 56: 323-329.

WITTWER, S.H. and HASSERMAN, L. (1946). Soil nitrogen and thrips injury on spinach. J. Econ. Entomol. 38: 615-616.

WONG, P.P, ZUCKER, M. and GREASY, L.L. (1974). Induction of phenylalanine amonia-lyase in strawberry leaf disks. Plant Physiol. 54: 659-665.

WOOD, D.L. and VITE, J.P. (1981). Studies on the host selection behaviour of Ips confusus (Le Conte) (Coleoptera: Scolytidae) attacking ponderosa pine. Contr. Boyce Thompson Inst. 21: 79-96.

WRATTEN, S.D., GODDARD, P. and EDWARDS, P.J. (1981). British trees and insects: the role of palatability. Am. Nat. 118: 916-919.

WRIGHT, S.T.C. (1969). An increase in the "inhibitor β" content of detached wheat leaves following a period of wilting. Planta 86: 10-20.

WRIGHT, S.T.C. and HIRON, R.W.P. (1969). Increase in abscisic acid, the growth inhibitor, in detached leaves by a period of wilting. Nature (Lond.) 224: 719-720.

WYN JONES, R.G. and STOREY, R. (1978). Salt stress and comparitive physiology in the Gramineae. II. Glycinebetaine and proline accumulation in two salt- and water-stressed barley cultivars. Aust. J. Plant Physiol. 5: 817-829.

Appendix 3.3.1.1 Acceptance of *E. camaldulensis* diets by *U. lugens* larvae.
(L = amino acid at 0.01M; H = amino acid at 0.05M)
Mean no. of larvae (mean for n = 10) on Diet ± SD
(Time interval = 15 min)

Diet	Time Interval 1	2	3	4	5	6	7	8
Base	2.0 ± 1.3	2.7 ± 1.3	2.7 ± 1.3	2.6 ± 1.1	2.4 ± 1.2	2.8 ± 1.8	2.7 ± 1.7	2.7 ± 1.6
+ Pro. L	2.7 ± 1.1	2.5 ± 1.5	3.3 ± 1.6	3.2 ± 1.5	2.5 ± 1.6	2.5 ± 2.0	2.1 ± 1.8	2.0 ± 1.9
+ Pro. H	3.9 ± 0.9	4.0 ± 0.8	3.6 ± 0.7	3.3 ± 1.3	3.3 ± 1.3	2.7 ± 1.3	2.6 ± 1.5	3.0 ± 1.3
+ Val. L	5.0 ± 0	5.0 ± 0	5.0 ± 0	5.0 ± 0	5.0 ± 0	5.0 ± 0	5.0 ± 0	5.0 ± 0
+ Val. H	4.5 ± 0.5	4.8 ± 0.4	4.5 ± 0.5	4.2 ± 0.6	4.2 ± 0.5	4.5 ± 0.6	4.2 ± 0.6	4.3 ± 0.7
+ Asp. L	0.6 ± 0.5	0.8 ± 0.9	1.0 ± 0.8	1.2 ± 0.6	0.8 ± 0.8	1.2 ± 0.9	1.0 ± 1.1	0.6 ± 0.8
+ Asp. H	2.6 ± 1.3	2.6 ± 1.3	2.4 ± 1.1	1.6 ± 0.8	1.2 ± 1.0	0.8 ± 0.6	0.7 ± 1.1	1.1 ± 1.0
+ Met. L	1.6 ± 1.3	1.8 ± 1.6	3.2 ± 1.4	2.9 ± 1.4	2.9 ± 1.7	2.7 ± 1.4	2.7 ± 1.6	2.1 ± 1.3
+ Met. H	3.5 ± 1.8	3.3 ± 0.8	2.1 ± 1.4	2.5 ± 1.0	2.5 ± 1.1	2.2 ± 0.6	1.6 ± 1.2	1.1 ± 1.2
+ Arg. L	3.1 ± 1.1	2.4 ± 1.0	2.5 ± 1.1	2.3 ± 1.3	2.2 ± 1.4	2.4 ± 1.2	2.6 ± 1.3	3.0 ± 0.9
+ Arg. H	2.7 ± 1.2	2.4 ± 1.1	2.8 ± 1.0	2.5 ± 1.0	2.5 ± 1.0	2.9 ± 1.3	2.6 ± 1.2	2.6 ± 1.4
+ Try. L	4.4 ± 0.7	4.0 ± 0.7	3.9 ± 1.1	3.8 ± 1.1	3.9 ± 1.1	3.8 ± 1.2	3.8 ± 1.2	3.6 ± 1.3
+ Try. H	4.5 ± 0.5	4.3 ± 0.8	4.4 ± 0.7	4.3 ± 0.7	4.4 ± 0.5	4.1 ± 0.7	4.2 ± 0.6	3.9 ± 0.7
+ All L	3.6 ± 1.2	3.4 ± 1.1	3.5 ± 0.9	3.6 ± 1.0	3.2 ± 1.0	3.5 ± 1.0	3.8 ± 0.8	3.4 ± 0.8
+ All H	4.5 ± 0.7	4.0 ± 0.7	4.1 ± 0.7	4.0 ± 0.7	3.9 ± 0.7	3.9 ± 0.7	4.0 ± 0.8	4.1 ± 0.7
Fresh	4.2 ± 0.6	4.2 ± 0.6	4.3 ± 0.7	4.3 ± 0.7	4.4 ± 0.5	4.4 ± 0.7	4.5 ± 0.7	4.6 ± 0.7

Appendices

Appendix 3.3.1.2 Acceptance of *E. platypus* diets by *U. lugens* larvae
(L = amino acids at 0.01M; H = amino acids at 0.05M)
Mean no. of larvae (mean for n = 10) on Diet ± SD
(Time interval = 15 min.)

Diet	Time Interval 1	2	3	4	5	6	7	8
Base	1.4 ± 1.1	2.0 ± 1.6	2.2 ± 1.7	2.0 ± 1.4	2.0 ± 1.4	1.8 ± 1.8	1.5 ± 1.7	1.7 ± 1.5
+ Pro. L	4.0 ± 1.1	3.3 ± 1.1	3.2 ± 1.2	2.9 ± 1.2	2.6 ± 1.0	2.7 ± 1.3	2.0 ± 1.4	2.2 ± 1.1
+ Pro. H	3.2 ± 1.5	3.4 ± 1.5	2.9 ± 1.6	2.5 ± 1.7	2.8 ± 1.6	2.6 ± 1.3	2.8 ± 1.0	2.5 ± 1.3
+ Val. L	5.0 ± 0	4.5 ± 1.0	4.5 ± 0.5	4.5 ± 0.5	4.5 ± 0.5	4.5 ± 1.0	4.0 ± 1.1	4.1 ± 1.1
+ Val. H	4.3 ± 1.1	4.3 ± 1.1	4.1 ± 1.4	4.2 ± 1.1	3.7 ± 1.3	3.7 ± 1.3	3.6 ± 1.4	3.5 ± 1.5
+ Asp. L	0.6 ± 0.7	0.8 ± 0.8	1.1 ± 0.9	0.5 ± 0.5	0.5 ± 0.7	0.3 ± 0.7	0.4 ± 0.5	0.4 ± 0.7
+ Asp. H	3.1 ± 1.5	2.6 ± 1.3	2.4 ± 1.1	1.6 ± 0.8	1.2 ± 1.0	0.8 ± 0.6	0.7 ± 1.1	1.1 ± 1.0
+ Met. L	2.0 ± 1.2	1.6 ± 1.3	1.5 ± 1.3	1.9 ± 1.2	1.6 ± 1.0	1.5 ± 1.2	1.8 ± 1.3	1.2 ± 1.2
+ Met. H	2.2 ± 1.6	2.4 ± 1.4	2.0 ± 1.2	1.7 ± 1.1	1.6 ± 1.1	1.0 ± 1.1	1.2 ± 1.2	1.1 ± 1.1
+ Arg. L	0.7 ± 0.8	0.6 ± 0.7	0.7 ± 0.7	0.8 ± 0.6	0.5 ± 0.5	0.7 ± 0.8	0.9 ± 0.7	0.7 ± 0.8
+ Arg. H	1.1 ± 1.1	1.2 ± 1.4	1.1 ± 1.1	1.0 ± 1.1	1.3 ± 1.2	1.0 ± 0.9	1.2 ± 1.0	1.0 ± 1.1
+ Try. L	3.7 ± 1.2	3.9 ± 0.9	3.8 ± 1.0	3.5 ± 1.0	3.1 ± 1.1	3.1 ± 1.2	3.0 ± 1.1	3.0 ± 1.1
+ Try. H	4.4 ± 0.7	4.2 ± 0.6	4.0 ± 0.7	3.7 ± 0.9	3.2 ± 0.8	3.4 ± 1.0	3.3 ± 0.9	3.1 ± 1.0
+ All L	2.1 ± 1.2	2.0 ± 1.2	1.8 ± 0.9	2.1 ± 1.3	2.1 ± 1.2	1.8 ± 1.4	2.0 ± 1.2	1.9 ± 1.3
+ All H	4.2 ± 1.0	2.9 ± 1.7	3.1 ± 1.7	2.9 ± 1.6	2.9 ± 1.5	2.9 ± 1.4	2.7 ± 1.6	2.7 ± 1.6
Fresh	0.6 ± 0.7	0.7 ± 0.7	0.7 ± 0.5	0.6 ± 0.7	1.0 ± 1.1	0.9 ± 1.0	1.2 ± 1.0	0.8 ± 0.8

Appendix 5.3.1 Utilization of diet by *U. lugens* larvae on diets of differing phenol content and specifications of the diets used.

Test Diet	%Conc.	%MC	TND	Mean TND	DC	NC	FW	TFN	%UD	%UND	S1	S2	Mean S2
Control		29.66	19.91	21.19	25.78	0.55	11.15	12.59	56.75	74.30	3	3	3.50
			21.07		40.57	0.86	25.57	16.64	36.97	50.50	3	3	
		± 1.32	21.97	± 0.87	33.15	0.73	20.90	11.13	36.93	66.87	4	4	± 0.58
			22.02		28.23	0.60	16.88	9.81	40.21	72.32	4	4	
			20.98										
Caffeic acid	0.5	25.23	28.55	29.15	32.99	0.96	29.30	27.97	11.18	14.78	4	2	1.25
			27.00		36.54	1.07	32.66	30.49	10.60	6.50	3	1	
		± 0.43	31.64	± 1.75	35.75	1.04	31.75	36.40	11.19	-10.89	3	1	± 0.50
			29.98		35.23	1.03	31.36	23.27	10.99	28.94	4	1	
			28.59										
	1.0	25.29	29.71	29.58	44.38	1.31	29.50	29.42	33.52	33.89	4	3	3.25
			26.61		35.76	1.06	21.72	32.77	39.26	32.71	4	4	
		± 0.23	32.03	± 1.96	43.71	1.29	31.96	27.11	26.88	32.99	4	3	± 0.50
			29.31		37.77	1.12	26.00	30.50	31.16	29.02	4	3	
			30.25										
	5.0	28.40	26.61	29.21	22.00	0.64	19.57	26.69	11.00	18.72	4	0	1.25
			25.84		23.47	0.68	17.27	26.88	26.45	32.30	4	0	
		± 0.46	36.28	± 4.16	30.22	0.88	24.28	28.64	19.67	21.24	3	2	± 1.50
			29.18		23.79	0.70	22.00	24.88	7.50	21.22	4	3	
			28.14										
	10.0	33.09	21.20	21.89	30.02	0.66	20.75	22.02	30.88	30.47	4	3	2.25
			20.43		43.71	0.96	31.25	22.36	28.51	26.97	4	2	
		± 1.0C	24.68	± 1.73	51.71	1.13	29.60	30.28	42.75	20.82	4	3	± 0.96
			22.40		42.18	0.92	24.30	29.65	42.50	30.01	3	1	
			20.76										

%MC = % moisture content ± SD, n = 10; TND = total nitrogen in diet (mg / g dry weight); Mean TND = mean total nitrogen in diet ± SD (n = 5); DC = dry weight of diet consumed per insect (mg); NC = nitrogen consumed per insect (mg); FW = dry weight of frass produced per insect (mg); TFN = total nitrogen in frass (mg/g dry wt. of frass); %UD = % Utilization of total diet; %UND = % Utilization of nitrogen in diet; S1 = number of larvae after 2 days on diet (original number of larvae = 4); S2 = number of larvae surviving after 5 days on diet; Mean S2 = mean of S2 ± SD.
Data on larval diet consumption and utilization taken over 5 days.

Appendix 5.3.1 Cont.

Test Diet	%Conc.	%MC	TND	Mean TND	DC	NC	FW	TFN	%UD	%UDN	S1	S2	Mean S2
Chlorogenic acid	0.1	25.37 ± 0.65	28.16 36.67 31.64 34.89 30.22	32.32 ± 3.45	23.79 33.74 35.47 28.00	0.77 1.09 1.15 0.91	16.38 21.90 20.17 18.16	25.69 31.35 39.80 31.77	31.17 35.09 43.13 35.14	45.29 37.04 29.97 36.24	4 4 3 3	4 4 3 3	3.50 ± 0.58
	0.5	26.33 ± 0.85	28.94 27.00 30.10 27.46 28.69	28.44 ± 1.24	25.07 13.41 28.30 34.02	0.71 0.38 0.80 0.97	18.33 10.65 21.80 26.20	32.19 19.99 19.53 23.14	26.90 20.55 22.97 22.99	17.26 44.18 47.10 37.34	3 2 4 3	2 2 3 3	2.50 ± 0.58
	1.0	28.00 ± 0.87	28.94 28.55 34.74 30.25 29.31	30.36 ± 2.53	51.44 55.23 56.68	1.56 1.67 1.72	32.70 39.90 41.70	4.99 31.27 29.92	36.43 27.76 26.43	89.55 25.59 27.50	4 3 3	4 3 3	3.30 ± 0.58
	1.8	27.93 ± 0.91	27.00 26.33 32.80 31.56 28.14	29.15 ± 2.89	80.42 65.78 70.60 76.98	2.34 1.92 2.06 2.24	49.35 47.16 52.10 49.11	23.71 25.76 29.52 27.29	38.63 40.24 26.20 35.62	52.32 36.64 25.52 40.27	4 4 4 3	4 3 4 3	3.50 ± 0.58

Appendix 5.3.1 Cont.

Test Diet	%Conc.	%MC	TND	Mean TND	DC	NC	FW	TFN	%UD	%UND	S1	S2	Mean S2
Gallic acid	0.5	24.75 ± 0.22	27.78 26.64 32.88 30.56 28.24	29.22 ± 2.49	44.99 47.02 43.58	1.32 1.37 1.27	31.68 32.05 30.49	29.80 32.39 30.83	29.50 31.84 30.04	28.19 24.45 26.18	4 4 4	3 3 3	3.00 ± 0
	1.0	26.62 ± 0.53	27.78 27.00 31.92 28.45 29.32	28.89 ± 2.87	63.39 29.20 29.60 37.67	1.80 0.84 0.86 1.09	45.20 17.65 17.40 21.45	29.85 32.61 21.96 36.97	27.50 39.55 43.38 43.06	25.15 31.77 55.32 27.13	3 2 3 3	3 2 3 3	2.75 ± 0.50
	5.0	26.02 ± 0.57	23.52 23.91 26.76 25.03 24.87	24.82 ± 1.26	44.69 44.65 46.23	1.11 1.11 1.15	29.50 27.90 29.44	18.69 23.74 20.99	33.97 37.50 36.32	50.29 40.23 46.14	2 4 4	2 4 3	3.00 ± 1.00
	10.0	31.85 ± 1.32	21.97 20.51 22.41 21.31 22.49	21.74 ± 0.83	36.62 33.31 32.10	0.78 0.72 0.70	19.35 23.14 25.95	14.75 18.68 20.32	47.16 30.53 19.16	63.71 40.30 24.43	3 3 3	2 3 3	2.66 ± 0.57

Appendix 5.3.1 Cont.

Test Diet	%Conc.	%MC	TND	Mean TND	DC	NC	FW	TFN	%UD	%UND	S1	S2	Mean S2
Quercetin	0.5	25.65	31.52	32.25	30.15	0.97	19.75	35.26	34.49	28.38	4	3	3.50
			31.26		39.29	1.27	22.38	40.41	43.04	28.63	4	4	
		±,1.23	33.96	± 1.08	42.61	1.37	29.88	30.82	29.87	32.98	3	3	± 0.58
			32.57		38.10	1.23	29.20	24.52	23.36	41.73	4	4	
			31.95										
	1.0	27.33	27.78	29.51	18.84	0.53	10.30	39.45	45.32	26.91	3	1	1.00
			27.00		23.66	0.69	7.86	48.82	66.78	45.05	4	0	
		± 0.68	32.80	± 2.27	37.71	1.11	21.60	35.38	42.72	31.33	4	2	± 0.80
			30.02		26.87	0.79	13.59	39.28	49.42	32.66	3	1	
			29.97										
	5.0	28.95	24.68	25.69	42.90	1.10	31.40	32.04	26.81	8.71	4	1	1.00
			22.75		40.58	1.04	29.10	32.02	28.29	2.08	3	2	
		± 0.65	29.32	± 2.48	42.33	1.09	30.65	31.76	27.59	10.48	3	1	± 0.80
			26.78		27.06	0.70	23.70	28.17	12.42	3.96	3	0	
			24.92										
	10.0	29.21	20.43	21.50	31.52	0.68	27.91	24.27	11.45	0.05	3	2	1.25
			20.04		43.22	0.93	32.78	29.43	24.16	-3.80	4	3	
		± 0.65	23.91	± 1.51	39.65	0.85	37.65	24.91	5.00	-10.02	3	0	± 1.50
			21.68		40.82	0.88	38.06	26.93	6.90	-16.79	3	0	
			21.42										

APPENDIX 6.2.3.1 Amino acid chromatography.

Chromatograms were run on 20 cm square glass plates spread with Sigma-cell Type 100® (20% slurry) on an automatic plate spreader at 0.4mm slit width, and dried for 24 hrs. Samples and standards were spotted onto plates and run upwards in a chromatography tank in the following solvent solution: iso-propanol 60ml.; methyl-ethyl-ketone 15ml.; 1M hydrochloric acid 25ml. The plate was then removed from the chromatography tank and allowed to dry in a fume hood.

Plates were sprayed with 0.1% ninhydrin in ethanol (50 ml.), to which was added 15ml. glacial acetic acid and 2ml. 2:4:6 collidine, and dried in a 100°C oven for 10min. or until the plate had developed sufficient colour.

Amino acid standards were prepared as 1mg. standard amino acid per ml. of 50% methanol in 0.1M hydrochloric acid.

APPENDIX 6.2.3.2 Determination of total free amino acids by spectrophotometry.

Ref: Rosen, H.A. (1957). Arch. Biochem. Biophys. 67: 10-15.

REAGENTS:

Stock NaCN:	49mg NaCN in 100ml distilled water.
Acetate Buffer:	1080g sodium acetate trihydrate in 800ml distilled water to which was added 20ml. glacial acetic acid. Made up to 3 litres with distilled water (pH 5.3 - 5.4).
Acetate-Cyanide:	20ml. of stock NaCN made up to 1 litre with acetate buffer (0.2 mM NaCN in acetate buffer).
Ninhydrin:	3g of recrystalized ninhydrin in 100ml. ethylene glycol monomethyl-ether (3% ninhyrin in methyl cellusolve).
Diluent:	Isopropyl alcohol and water 1:1.
Leucine standard:	52mg D-leucine in 1 litre water (0.4mM). Solutions for standard curve were prepared as follows: 0.1, 0.2, 0.5 and 1.0 ml of leucine standard made up to 1.0ml. with distilled water (0.04 to 0.4 µmoles per ml.).

APPENDIX 6.2.3.2 cont.

METHOD:

To the test solution or standard (1.0ml.) was added 0.5ml. acetate-cyanide buffer and 0.5ml. ninhydrin solution. The blank was prepared using 1.0ml of distilled water to replace the test and standard solutions. This was heated in a water bath for 15min. at 100°C. After heating, 5.0ml diluent was added to the solution and mixed thoroughly. The solution was then cooled to room temperature and the absorbance read against a blank on a Shimadzu spectrophotometer at 570nm. Concentration for the test samples were calculated from the standard curve as µmole equivalents of leucine per ml.

APPENDIX 6.3.1.1 Variation in Total Nitrogen (mg/g dry weight of leaf) present in mature E. camaldulensis foliage from January 1980 to February 1982.

SAMPLE DATE	SAMPLE SITE					
	NE	NW	SW	Mean	SD	SE
7. 1.80	14.11	17.27	13.72	15.03	1.95	1.13
11 .3.80	15.30	12.14	12.14	13.19	1.83	1.06
10. 5.80	12.53	12.93	12.93	12.80	0.23	0.13
10. 6.80	14.11	12.14	12.53	12.93	1.05	0.68
1. 7.80	15.03	15.30	13.72	14.77	0.91	0.53
1. 9.80	16.48	14.51	16.09	15.69	1.05	0.61
3.11.80	11.74	14.90	14.51	13.72	1.72	0.99
6. 1.81	12.14	12.53	13.32	12.66	0.60	0.35
4. 3.81	10.35	10.95	10.95	11.08	0.23	0.13
4. 5.81	14.51	10.16	10.95	11.87	2.32	1.34
3. 6.81	16.48	13.72	14.11	14.90	1.43	0.83
1. 7.81	16.48	12.14	12.14	13.59	2.51	1.45
1. 9.81	13.72	13.72	14.11	13.85	0.25	0.13
6.11.81	12.14	10.56	12.14	11.61	0.91	0.53
6. 1.82	13.32	13.72	11.74	12.93	1.05	0.61
8. 2.82	15.69	11.74	10.95	12.79	2.54	1.47
Mean	14.03	13.03	12.88	-	-	-
± SD	1.88	1.88	1.46	-	-	-

APPENDIX 6.3.2.1 Variation of Total Phenols (mg equiv. catechin per g dry weight of leaf) present in mature E. camaldulensis foliage from January 1980 to April 1982.

SAMPLE DATE	SAMPLE SITE					
	NE	NW	SW	Mean	SD	SE
7. 1.80	82.98	104.25	75.88	87.70	14.77	8.53
11. 3.80	87.70	104.25	68.78	86.91	17.75	10.26
10. 5.80	108.98	101.88	97.15	102.67	5.95	3.44
1. 7.80	104.25	90.05	82.98	92.43	10.84	6.27
1. 9.80	90.05	80.60	78.25	82.97	6.25	3.61
3.11.80	82.98	134.98	87.70	101.88	28.76	16.62
6. 1.81	84.15	91.95	130.25	102.12	24.67	14.26
4. 3.81	127.88	137.33	114.88	126.69	11.27	6.51
4. 5.81	123.15	114.88	103.30	113.78	9.97	5.76
1. 7.81	102.35	106.60	73.50	94.15	18.01	10.41
1. 9.81	108.98	108.98	80.60	99.52	16.38	9.46
6.11.81	129.30	90.05	97.15	105.50	20.92	12.09
6. 1.82	132.60	144.43	106.60	127.88	19.35	11.18
8. 2.82	113.70	101.88	108.98	108.18	5.95	3.44
6. 4.82	104.25	104.25	94.78	101.09	5.47	3.16
Mean	105.55	107.77	93.39	-	-	-
± SD	18.04	18.39	17.34	-	-	-

APPENDIX 6.3.3.1 Individual free Amino Acid concentrations (µg/g dry weight of leaf) in mature *E. camaldulensis* foliage from 3 sampling sites.

Amino acids listed in this appendix are abreviated as follows:

ALA	alanine	ILE	iso-leucine
ARG	arginine	LEU	leucine
ASN	asparagine	LYS	lysine
ASP	aspartic acid	MET	methionine
BALA	β alanine	ORN	ornithine
CYN	cystathionine	PHE	phenylalanine
CYS	half cystine	PRO	proline
ETH	ethanolamine	PSE	phosphoserine
GABA	γ amino-butyric acid	SER	serine
GLN	glutamine	THR	threonine
GLU	glutamic acid	TRP	tryptophan
GLY	glycine	TYR	tyrosine
HIS	histidine	VAL	valine

APPENDIX 6.3.3.1 (a) Individual free Amino Acid concentrations (µg/g dry weight of leaf) in mature *E. camaldulensis* foliage from 3 sampling sites at sampling date July 1, 1980.

AMINO ACID	SAMPLE SITE				
	SW	NW	NE	Mean	SD
PSE	137.14	130.11	91.43	119.56	24.60
ASP	1.52	1.04	0.96	1.18	0.31
THR	5.24	9.63	7.98	7.61	2.22
SER	28.13	43.01	28.03	33.06	8.62
ASN	10.78	-	29.60	13.46	14.98
GLN	35.51	42.30	102.14	59.98	36.70
PRO	32.84	41.43	38.45	37.57	4.36
GLU	309.30	331.75	207.28	282.77	66.33
GLY	2.11	4.43	5.76	4.10	1.85
ALA	17.22	21.69	14.88	17.93	3.46
VAL	6.20	10.28	5.55	7.34	2.56
CYS	3.79	14.38	1.38	6.52	6.92
CYN	15.39	14.56	11.12	13.69	2.26
MET	0.09	-	-	0.03	0.05
ILE	5.73	5.73	3.78	5.08	1.13
LEU	3.84	5.27	3.18	4.09	1.07
TYR	2.65	6.37	3.81	4.28	1.90
PHE	23.71	26.58	13.34	21.21	6.96
BALA	9.80	-	-	3.27	5.66
GABA	17.06	23.29	31.63	23.99	7.31
ORN	-	1.44	2.80	1.40	1.40
ETH	-	-	-	-	-
LYS	-	-	-	-	-
HIS	-	-	-	-	-
TRP	-	-	-	-	-
ARG	2.87	38.03	0.49	13.80	21.02
TOTAL	670.93	771.29	603.58	681.93	84.39
GLU+GLN	344.81	374.05	309.42	342.76	32.36

APPENDIX 6.3.3.1 (b) Individual Amino Acid concentrations (µg/g dry weight of leaf) in mature *E. camaldulensis* foliage from 3 sampling sites at sampling date September 1, 1980.

AMINO ACID	SAMPLE SITE				
	SW	NW	NE	Mean	SD
PSE	13.44	70.35	85.15	56.31	56.31
ASP	6.64	4.04	3.88	4.85	1.55
THR	20.30	15.67	19.42	18.46	2.46
SER	78.77	67.99	80.23	75.66	6.69
ASN	8.51	18.78	2.44	9.91	8.26
GLN	-	-	25.39	8.46	14.65
PRO	217.02	151.10	188.96	185.69	33.08
GLU	553.30	436.15	523.31	504.25	60.86
GLY	24.68	20.54	31.24	25.49	5.40
ALA	27.32	26.94	26.30	26.85	0.52
VAL	12.51	6.19	8.52	9.12	3.19
CYS	23.14	40.29	7.04	23.49	16.63
CYN	11.60	63.74	8.02	27.79	31.19
MET	7.55	-	0.51	2.69	4.22
ILE	7.37	6.60	5.72	6.39	1.11
LEU	4.81	3.17	5.31	4.43	1.12
TYR	4.57	3.44	7.92	5.31	2.33
PHE	20.72	20.43	20.73	20.63	0.17
BALA	-	-	10.57	3.52	6.10
GABA	75.17	30.16	62.35	55.90	23.19
ORN	6.23	5.33	6.19	5.91	0.51
ETH	53.12	-	18.69	23.94	26.94
LYS	8.85	2.92	4.40	5.39	3.09
HIS	7.87	2.29	3.00	4.39	3.04
TRP	-	-	-	-	-
ARG	95.45	34.81	75.12	68.46	30.96
TOTAL	1288.94	1030.93	1230.54	1183.47	135.29
GLU+GLN	553.30	436.15	548.70	512.72	66.35

APPENDIX 6.3.3.1 (c) Individual free Amino Acid concentrations (µg/g dry weight of leaf) in mature E. camaldulensis foliage from 3 sampling sites at sampling date November 3, 1980.

AMINO ACID	SAMPLING SITE				
	SW	NW	NE	Mean	SD
PSE	45.89	33.31	46.98	42.06	7.60
ASP	0.87	3.93	3.44	2.75	1.64
THR	16.62	38.42	22.17	25.74	11.33
SER	62.37	59.51	68.87	63.58	4.80
ASN	5.38	46.97	24.44	25.60	20.82
GLN	11.81	-	-	3.94	6.82
PRO	52.98	392.81	261.40	235.73	171.36
GLU	83.68	482.23	345.70	303.87	202.54
GLY	11.30	26.80	22.05	20.05	7.94
ALA	22.40	26.29	19.72	22.80	3.30
VAL	5.71	14.30	17.39	12.47	6.05
CYS	9.25	10.60	1.89	7.25	4.69
CYN	6.81	59.12	19.65	28.53	27.26
MET	3.33	-	1.60	1.65	1.67
ILE	4.68	6.62	5.54	5.61	0.98
LEU	3.99	3.74	4.55	4.09	0.41
TYR	3.50	10.07	9.84	7.80	3.73
PHE	1.77	18.57	18.58	12.97	9.70
BALA	1.44	-	-	0.48	0.83
GABA	16.31	10.12	40.84	22.42	16.25
ORN	4.14	-	2.73	2.29	2.10
ETH	1.62	-	-	3.87	6.71
LYS	78	-	4.06	4.61	4.91
HIS	12.34	-	9.74	7.52	6.70
TRP	-	-	-	-	-
ARG	8.91	23.33	89.40	40.54	42.92
TOTAL	417.39	1266.73	1040.56	908.23	439.86
GLU+GLN	95.49	482.23	345.70	307.81	196.13

APPENDIX 6.3.3.1 (d) Individual free Amino acid concentrations (µg/g dry weight of leaf) in mature *E. camaldulensis* foliage from 3 sampling sites at sampling time January 6, 1981.

AMINO ACID	SAMPLE SITE				
	SW	NW	NE	Mean	SD
PSE	19.16	58.80	33.77	35.58	17.39
ASP	0.98	0.77	4.71	2.16	2.22
THR	13.56	17.64	21.24	17.48	3.84
SER	42.42	59.28	80.08	60.59	18.87
ASN	3.17	9.25	7.02	6.48	3.07
GLN	-	-	1.02	0.34	0.59
PRO	9.20	21.94	30.63	20.59	10.78
GLU	83.34	30.27	290.29	134.64	137.38
GLY	7.31	18.59	20.58	15.49	7.15
ALA	8.75	23.27	25.91	19.31	9.24
VAL	5.54	14.19	9.06	9.59	4.35
CYS	8.92	5.50	21.18	11.87	8.25
CYN	0.77	11.07	66.73	26.19	35.49
MET	1.15	0.67	4.91	2.24	23.2
ILE	4.48	4.12	4.18	4.26	0.19
LEU	3.53	4.16	3.06	3.58	0.55
TYR	1.85	4.73	2.55	3.04	1.50
PHE	11.07	19.26	15.60	15.31	4.10
BALA	-	-	-	-	-
GABA	9.48	16.57	8.66	11.57	4.35
ORN	4.26	9.99	3.92	6.06	3.41
ETH	4.54	5.17	4.72	4.81	0.32
LYS	8.55	-	7.44	5.33	4.65
HIS	6.70	-	6.22	4.31	3.74
TRP	-	-	-	-	-
ARG	8.13	0.35	7.13	5.20	4.23
TOTAL	266.85	330.61	680.59	426.01	222.76
GLU+GLN	83.34	30.27	291.30	134.97	137.96

APPENDIX 6.3.3.1 (e) Individual free Amino acid concentrations (µg/g dry weight of leaf) in mature *E. camaldulensis* foliage from 3 sampling sites at sampling time March 4, 1981.

AMINO ACID	SAMPLE SITE				
	SW	NW	NE	Mean	SD
PSE	8.39	55.39	37.84	33.87	23.74
ASP	1.82	0.97	1.32	1.37	0.43
THR	6.64	11.29	10.58	9.50	2.51
SER	36.85	22.37	42.30	33.84	10.30
ASN	-	6.62	8.83	5.15	4.60
GLN	22.43	23.61	28.11	24.72	2.99
PRO	13.56	14.79	26.00	18.12	6.86
GLU	59.98	7.38	129.20	65.52	61.10
GLY	4.65	11.75	8.57	8.32	3.56
ALA	18.83	23.21	17.54	19.86	2.97
VAL	3.24	4.85	4.53	4.21	0.85
CYS	3.89	9.92	2.48	5.42	3.96
CYN	2.72	8.27	4.76	5.25	2.81
MET	-	-	-	-	-
ILE	3.17	3.81	4.43	3.80	0.63
LEU	2.18	3.10	3.64	2.97	0.74
TYR	1.87	2.94	3.96	2.92	1.05
PHE	7.71	7.46	8.49	7.89	0.54
BALA	-	-	-	-	-
GABA	5.58	18.52	20.23	14.78	8.01
ORN	-	3.60	1.15	1.58	1.84
ETH	-	-	-	-	-
LYS	-	-	-	-	-
HIS	-	-	-	-	-
TRP	-	-	-	-	-
ARG	1.14	0.87	4.29	2.10	1.90
TOTAL	204.39	240.70	368.24	271.11	86.06
GLU+GLN	82.41	30.99	157.31	90.24	63.52

APPENDIX 6.3.3.1 (f) Individual free Amino acid concentrations (µg/g dry weight of leaf) in mature *E. camaldulensis* foliage from 3 sampling sites at sampling time May 4, 1981.

AMINO ACID	SAMPLE SITE				
	SW	NW	NE	Mean	SD
PSE	50.56	25.62	85.85	54.01	30.26
ASP	0.62	1.99	32.73	11.78	18.16
THR	5.61	9.82	6.55	7.33	2.21
SER	348.74	45.32	4.09	132.72	188.22
ASN	4.04	3.85	-	2.63	2.28
GLN	16.55	-	14.22	10.26	8.96
PRO	10.44	7.92	8.82	9.06	1.28
GLU	10.71	16.87	175.95	67.84	93.67
GLY	7.13	14.21	5.31	8.88	4.70
ALA	8.72	11.81	8.81	9.78	1.76
VAL	3.11	3.06	4.26	3.48	0.68
CYS	8.48	18.12	2.14	9.58	8.04
CYN	3.63	8.78	11.36	7.92	3.93
MET	-	-	0.25	0.08	0.14
ILE	2.71	3.69	3.44	3.28	0.51
LEU	2.30	2.60	2.77	2.56	0.24
TYR	2.15	4.89	1.65	2.90	1.75
PHE	6.86	10.23	7.79	8.29	1.74
BALA	-	-	-	-	-
GABA	11.96	13.55	12.33	12.62	0.83
ORN	1.94	4.96	1.80	2.90	1.78
ETH	-	1.68	-	0.56	0.97
LYS	-	6.51	-	2.17	3.76
HIS	-	1.54	-	0.51	0.89
TRP	-	-	-	-	-
ARG	-	1.36	2.19	1.18	1.10
TOTAL	506.27	218.36	392.29	372.31	144.99
GLU+GLN	27.26	16.87	190.17	78.10	97.19

APPENDIX 6.3.3.1 (g) Individual free Amino acid concentrations (µg/g dry weight of leaf) in mature E. camaldulensis foliage from 3 sampling sites at sampling time July 1, 1981.

AMINO ACID	SAMPLE SITE				
	SW	NW	NE	Mean	SD
PSE	132.27	6.30	78.56	72.37	63.21
ASP	4.87	0.96	68.95	24.93	38.18
THR	1.44	6.97	1.92	3.44	3.07
SER	15.08	25.77	17.84	19.56	5.56
ASN	-	-	-	-	-
GLN	5.04	7.53	-	4.19	3.84
PRO	3.99	31.64	3.98	13.20	15.96
GLU	201.13	36.08	194.57	143.92	93.46
GLY	0.64	6.64	0.83	2.70	3.41
ALA	10.94	8.91	8.48	9.45	1.32
VAL	3.56	3.66	6.86	4.69	1.88
CYS	12.80	7.14	1.02	6.98	5.89
CYN	22.93	3.86	12.48	13.09	9.55
MET	-	1.43	-	0.48	0.84
ILE	2.89	2.26	2.60	2.58	0.32
LEU	1.71	1.48	1.42	1.54	0.15
TYR	1.96	1.71	1.89	1.85	0.13
PHE	7.34	8.57	8.43	8.11	0.67
BALA	4.74	-	-	1.58	2.74
GABA	9.55	19.54	17.32	15.47	5.24
ORN	1.58	1.49	-	1.02	0.89
ETH	-	-	-	-	-
LYS	-	-	-	-	-
HIS	-	-	-	-	-
TRP	-	-	-	-	-
ARG	0.30	-	-	0.10	0.17
TOTAL	444.74	181.91	427.17	351.27	146.94
GLU+GLN	206.17	43.61	194.57	148.12	90.69

APPENDIX 6.3.3.1 (h) Individual free Amino acid concentrations (µg/g dry weight of leaf) in mature *E. camaldulensis* foliage from 3 sampling sites at sampling time September 1, 1981.

AMINO ACID	SAMPLE SITE				
	SW	NW	NE	Mean	SD
PSE	134.09	116.30	132.45	127.62	9.83
ASP	24.81	0.71	21.93	15.81	13.17
THR	4.75	2.52	2.57	3.28	1.27
SER	25.51	26.45	28.03	26.66	1.28
ASN	3.84	-	-	1.28	2.22
GLN	8.43	10.25	13.08	10.59	2.34
PRO	6.40	11.07	9.55	9.01	2.38
GLU	156.55	189.28	209.58	185.13	26.76
GLY	3.73	2.01	2.21	2.65	0.94
ALA	6.24	7.49	9.60	7.78	1.70
VAL	3.53	7.40	12.68	7.87	4.59
CYS	5.42	1.75	3.77	3.65	1.84
CYN	12.18	16.55	10.04	12.92	3.32
MET	-	-	-	-	-
ILE	2.54	2.41	3.37	2.77	0.52
LEU	1.22	1.23	1.65	1.36	0.24
TYR	1.07	1.25	1.70	1.34	0.33
PHE	11.32	11.37	11.51	11.40	0.10
BALA	2.55	-	-	0.85	1.47
GABA	19.73	20.67	13.39	17.93	3.96
ORN	-	1.89	-	0.63	1.09
ETH	-	-	-	-	-
LYS	-	-	-	-	-
HIS	-	-	-	-	-
TRP	-	-	-	-	-
ARG	0.38	0.95	0.19	0.51	0.39
TOTAL	434.28	431.55	485.59	450.47	30.44
GLU+GLN	164.98	199.53	222.66	195.72	29.03

APPENDIX 6.3.3.1 (i) Individual free Amino acid concentrations (µg/g dry weight of leaf) in mature *E. camaldulensis* foliage from 3 sampling sites at sampling time November 6, 1981.

AMINO ACID	SAMPLE SITE SW	NW	NE	Mean	SD
PSE	106.96	75.90	110.68	97.85	19.10
ASP	1.59	0.77	1.79	1.38	0.54
THR	9.08	3.75	8.56	7.13	2.94
SER	39.41	32.26	44.13	38.60	5.97
ASN	8.46	6.67	7.15	8.09	0.83
GLN	-	17.54	-	5.84	10.12
PRO	19.33	21.18	24.26	21.59	2.49
GLU	195.74	192.77	215.39	201.30	12.29
GLY	12.09	4.39	9.02	8.50	3.88
ALA	9.78	13.78	14.00	12.52	2.38
VAL	9.67	5.39	4.80	6.62	2.66
CYS	3.40	12.66	1.05	5.70	6.14
CYN	12.64	15.61	8.70	12.32	3.47
MET	-	-	-	-	-
ILE	3.01	4.13	2.24	3.12	0.95
LEU	2.78	6.30	2.10	3.73	2.26
TYR	2.56	2.25	2.77	2.53	0.26
PHE	17.06	14.70	14.73	15.50	1.36
BALA	6.07	12.05	-	6.04	6.03
GABA	7.90	30.31	18.61	18.94	11.21
ORN	2.81	1.88	-	1.56	1.43
ETH	-	-	-	-	-
LYS	5.69	-	-	1.90	3.29
HIS	1.32	-	-	0.44	0.76
TRP	-	-	-	-	-
ARG	-	1.81	3.85	1.89	1.93
TOTAL	477.38	475.86	493.80	482.35	9.95
GLU+GLN	195.74	210.31	215.39	207.15	10.20

APPENDIX 6.3.3.1 (j) Individual free Amino acid concentrations (µg/g dry weight of leaf) in mature *E. camaldulensis* foliage from 3 sampling sites at sampling time January 6, 1982.

AMINO ACID	SAMPLE SITE				
	SW	NW	NE	Mean	SD
PSE	60.07	52.02	103.09	71.73	27.46
ASP	1.75	2.79	2.28	2.27	0.52
THR	13.25	10.51	11.45	11.74	1.39
SER	61.91	41.13	58.03	53.69	11.05
ASN	41.33	4.92	12.14	19.46	19.28
GLN	-	-	1.64	0.55	0.95
PRO	20.22	22.86	17.10	20.06	2.88
GLU	124.94	143.64	199.52	156.03	38.80
GLY	8.62	11.84	7.74	9.40	2.16
ALA	29.60	18.91	16.93	21.81	6.82
VAL	6.54	6.38	4.51	5.81	1.13
CYS	16.24	19.28	15.24	16.92	2.11
CYN	20.96	33.72	26.53	27.07	6.40
MET	-	-	0.08	0.03	0.04
ILE	3.14	3.94	3.36	3.48	0.41
LEU	2.28	1.50	2.89	2.22	0.70
TYR	3.44	3.05	5.67	4.05	1.41
PHE	9.05	6.52	7.81	7.79	1.27
BALA	-	0.74	-	0.25	0.42
GABA	11.67	29.50	18.60	19.92	8.99
ORN	2.08	4.37	-	2.15	2.19
ETH	-	-	-	-	-
LYS	-	11.60	-	3.89	6.70
HIS	-	0.62	-	0.21	0.36
TRP	-	-	-	-	-
ARG	3.08	1.15	4.31	2.85	1.59
TOTAL	440.18	430.97	518.89	463.35	48.32
GLU+GLN	124.94	143.64	201.16	156.58	39.72