THE BIOLOGY AND FOOD PREFERENCES OF THE GUM LEAF SKELETONIZER, *URABA LUGENS* (WALK.)

by

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TABLE OF CONTENTS

Summary

The biology of Uraba lugens, the gum leaf skeletonizer, has been examined with emphasis upon the selection and relative suitability of hosts. The adult in selecting the foodplant, governs the establishment and survival of its progeny. Though about 60% of species within the closely related genera Eucalyptus and Angophora available during this study, attract oviposition, comparatively few are superior foodplants based upon their effects upon survival, growth and fecundity of the insect. The egg hosts (those attracting oviposition) may therefore be classified according to their suitability as foodplants.

Evidence is provided that indicates a selection behaviour of adults that is determined by volatile emanates from the trees. The presence of either oviposition stimulants or substances strongly inhibitory to oviposition seem to govern the selection of some species and the avoidance of others respectively. There are data which suggest that a sign-stimulant may be present in Eucalyptus which identifies to adults this group of plants from others. Caterpillars appear to accept foodplants that have lower concentrations of certain hydrocarbon terpenoid components in their leaves, are low or lacking in toxins and adequate, though not necessarily high, in common nutrient levels when compared with poor or non-hosts. The interaction between levels of antifeedants and toxins on the one hand and phagostimulants and basic nutrients on the other provide the substrate upon which caterpillars discriminate between the plants selected for them by their parents.

Declaration

The work presented in this thesis is my own unless otherwise acknowledged, and has not previously been published or submitted for the award of any degree.

J.R. COBBINAH

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CHAPTER 1. GENERAL INTRODUCTION

The range of food for the different species of phytophagous or plant feeding insects varies from monophagy (feeding upon a single species of host plant e.g. Coccus fagi) through oligophagy (having a host range restricted to closely related species of plants e.g. Uraba lugens) to polyphagy (where host plants may include many species not closely related e.g. Ectropus excursaria). Even polyphagous insects are for some reason, unable to feed upon certain plants or parts of plants. The polyphagous desert locust, Schistocerca gregaria will not feed on the seeds of neem, Azadirachta indica (Butterworth and Morgan, 1971) just as the oligophagous Uraba lugens cannot feed upon certain species of Eucalyptus (Morgan and Cobbinah, 1978 in press). Such diversity in food plant selection and utilization reflects variability in both degree and mode of adaptation that have evolved between the plants and insects concerned.

Evolution tends toward simplicity and early workers (Brues, 1920; Dethier 1954) considered polyphagy to be primitive and monophagy advanced. Much current evidence indicates that co-evolution between plants and insects has proceeded which involves a wide spectrum of host specificity (Ehrlich and Raven 1964, 1969, Dethier, 1970, Singer 1971, Beck, 1974) the direction of which may be modified by natural processes of selection operating upon both host and insect. Thus a general trend toward monophagy may reverse. The reversal may be in a sense temporary but because of the time it takes to demonstrate such relationships, evidence obtained over a few decades may be insufficient to determine the actual trend be it temporary or otherwise.

The primary factors determining host plant specificity are physical

and chemical. Shape of objects, colour and texture may be important (Wallace, 1958) but because of the general nature of these visual factors, they do not explain fully the basis of foodplant specificity. Since the pioneering work of Verschaeffelt (1910) which demonstrated that larvae of Pieris brassicae L. and P. rapae L., are stimulated to feed on plant hosts containing mustard oil glycosides, the importance of plant chemicals in regulating foodplant specificity has been demonstrated widely (Hamamura, 1965; Thorsteinson, 1953; Nayar and Thorsteinson, 1963; Nayar and Fraenkel, 1963; Keller et al., 1962; Maxwell et al., 1963; Butterworth and Morgan 1971). To stress the importance of plant chemicals in insect foodplant interrelationships, Dethier (1947) defined the 3 kinds of feeding habits of phytophagous insect:- monophagy, oligophagy and polyphagy on the basis of the number and kinds of plant chemicals to which they respond.

Uraba lugens (Walk) is commonly known as the gum leaf skeletonizer, due to the characteristic skeletonizing habits during the early stages of larval life. It has been recorded in all states of Australia and more than 10 outbreaks have been recorded in the red gum forests of the Murray Valley region since 1900 (Campbell, 1962; Harris 1974). The insect is essentially a pest of eucalypts but successfully develops on a few species of the closely related genera - Angophora and Tristania (Campbell, 1962, 1966; Brimblecombe, 1962; Morgan and Cobbinah, 1977). The adults have a wider range of 'egg-hosts' than the larvae have foodplants (Morgan and Cobbinah, 1977) and, until the present studies were initiated, nothing was known about the factors governing host selection and acceptability by the moths and the larvae respectively. Morgan and Cobbinah (1978) suggested that the unacceptability of certain egg-hosts to the larvae may be due to the absence of specific feeding stimulants or

to the presence of inhibitors of feeding behaviour.

Of course, if we are to understand clearly the interactions between Uraba lugens and its foodplants, then a knowledge of the precise chemical and, perhaps, physical factors implicated in the interrelationship is essential. The present study examines the bases of foodplant specificity and preference in Uraba lugens in some detail. It includes: (1) basic understanding of the insect's biology (2) evaluation of its preference, acceptability and the developmental suitability of representatives of the different chemical and botanical groups of the main host genus, Eucalyptus (3) feeding, growth rate and fecundity of Uraba lugens under varied conditions (4) the influence of physical characteristics of foodplants on larval feeding responses and foodplant suitability (5) isolation of chemical substances that initiate and maintain larval feeding (6) determination of the role of nutrient substances and the suitability of foodplants which contain them, for growth of the insect and (7) the role of feeding inhibitors on the foodplant specificity of larvae. The results of these researches are discussed in the context of mechanisms of foodplant specificity in phytophagous insects.

CHAPTER 2. LITERATURE REVIEW

2.1 Early concepts

The pioneer study of host plant selection is attributed to Verschaeffelt (1910). Since that time several workers have postulated theories to explain the intricate relationships between phytophagous insects and their host plants. Some of these early theories have been found to be grossly generalized, however, we owe our present knowledge of the subject to the testing of some of these generalizations. An early principle on this subject was that "a healthy plant is an immune plant". Another postulates that "an insect's normal host plant meets that insect's specific nutritional and ecological requirements in a completeness not offered by other plant species". Such generalized statements have been supported by some research and rejected by other studies. The Hopkins (1910) host selection principle, for instance, which postulates that "the larval foodplant strongly influences adult oviposition preferences", has been variously confirmed (Jermy et al., 1968, Hovanitz and Chang, 1964; Hovanitz, 1969) and rejected (Thorpe, 1930; Dethier, 1954 and Wiklund, 1974). The present review is intended to cover only the general mechanisms involved in the insect host plant interrelationship. References to specific research papers published on the subject will be made in connection with the content of each subsequent section of the thesis. The modern era of our thinking on the relationship between insects and their foodplants probably started with the symposium on "the physiological relations between insects and their foodplants" in Amsterdam in 1951. In that symposium Fraenkel (1953) proposed the 'odd substance' theory that host specificity and resistance are based on the presence of 'odd' or 'secondary' substances such as essential oils, alkaloids and glycosides produced within the plant tissues

This became known as the Theory of token stimuli. A similar view was advanced less categorically by Dethier (1947, 1953). However, Kennedy and Booth (1951), Kennedy (1953) and Fennah (1953) had advanced the 'Dual discrimination theory' which postulates that both nutritive and secondary substances in plants are important in foodplant selection.

Fraenkel (1959, 1960), Lipke and Fraenkel (1956) gave several reasons to support their odd substance theory. They asserted that basic food requirements of all insects seem to be very similar to those of higher vertebrates; and that all plants are equally nutritious and could equally well serve as food provided the insects ate enough of them. Unfortunately, the 'odd substance theory', valid as it is to a point, does not explain all the insect foodplant relationships observed in nature. Fraenkel's contention that all plants are equally nutritious cannot be sustained in view of the evidence of House (1961, 1962, 1967) who showed that nutrient requirements of insects differ subtly from one species to another and that many insects are found feeding on food most suited to their requirements. House's (1967) experiment with Agria affinis (Fallen) showed that a significantly greater number of larvae chose the most effective diet. One may therefore ask "Do nutrient substances provide the chemotactic stimuli that elicit feeding by insects not repelled by any of the other constituents of the food?" Thorsteinson (1960) argued that many nutrients in plants act as gustatory indicators of a suitable food and wondered why such nutrients could not be included in a class of token simuli with the so-called secondary plant substances. Waldbauer (1964) demonstrated that the success of a given plant as a host not only depends on the quantity of it that is eaten, but also on its digestibility and the degree of its utilization by the insect. There are several examples in the literature demonstrating the relationship between

certain nutrients in plants and their susceptibility to insects or the preference for them exhibited by insect pests. Nevertheless, it is not clear whether this relationship is causal or coincidental.

Kennedy (1953) showed that the physiological conditions of plants can determine selection by insects. For instance Aphis fabae has as its summer host, sugarbeet, and as its winter host, Euonymus. When the 2 hosts are offered in the same stage of growth, the winter host, Euonymus is preferred. But under natural conditions the aphids show preference for the summer host in summer because the foliage of the winter host, Euonymus is mostly in an unacceptable condition, neither growing nor senescing. If growth and senescence are induced in summer in Euonymus the aphids colonize it readily.

Fraenkel (1969) modified his statement that "All plants are equally nutritious ....." but, though recognizing the probable influence of adverse nutrient chemicals and physical characteristics, his basic argument remained unchanged.

Whilst the theory of Kennedy and his co-workers has an advantage of flexibility it suffers the disadvantage of having been formulated in rather subjective terms, such as the postulated necessity for the insect to be capable of making a nutritional assessment of the food value of its host (see Beck, 1965). Moreover, the nutrient argument presupposes that changes in nutrient substances associated with different cultural and climatic conditions would fundamentally alter host selection at least within varieties, races and/or clones. Insufficient analytical data exist to support this contention. It is clear that, in spite of changes in chemical constitution of plants as a result of seasonal, growth and edaphic factors, there exists some measure of constancy which sets one species apart from another and delimits the basic feeding habits of its predators. The literature lacks examples in which a non-host plant

has become attractive to a predator as a result of induced changes in its physiological state. The customary approach has been a relationship between one nutrient or a few nutrient substances and preference or susceptibility to a pest. As Fraenkel (1969) rightly asserted; changes in composition are always complex and never affect only one nutrient. Therefore one would have to consider the effect of a particular change in the context of a whole spectrum of factors.

A recapitulation of our present understanding of the physiological mechanism of foodplant selection shows that the phenomenon depends largely upon olfactory and contact chemical stimulation by compounds in the foodplants. Food selection is in relation to (a) stimulation by nutrient substances (sugars, amino acids) (b) stimulation by 'token stimuli' (e.g. Sinigrin in Pieris rapae) and (c) the insect's ability to deal with toxic substances in foodplants(Miles, 1969, 1972; Brattsten, 1977).

2.2 Behavioural mechanisms

There is a voluminous body of literature covering the behavioural mechanisms leading to selection of host plants (Dethier, 1954, 1970; Wigglesworth, 1953; de Wilde, 1958; Cathy, 1958; Thorsteinson, 1953, 1960; Chin, 1950; Beck, 1965, 1974; Feir and Beck, 1963; Yamamoto and Fraenkel, 1960; Fraenkel and Gunn, 1940; Hamamura, Hayashiya, Naito, Matsumura and Nishida, 1962; Dethier and Schoonhoven, 1969; Saxena, 1969). The traditional concept was that insects find their host plants by 'chemotropism' (McIndoo, 1926). Thorsteinson (1960) however, asserted that until the insect encounters the immediate vicinity of the foodplant, foraging is random. Mulkern (1969) believes that the feeding behaviour of grasshoppers cause them to remain in areas of favourable host plants

through a general type of orthokinesis. While vision, phototaxis, geotaxis and hygrotaxis undoubtedly play their specific roles in directing insects to the proper environment for oviposition and feeding, the ultimate forces working at close range and operating in final recognition of preferred plants are largely chemical.

On the basis of the present knowledge of the subject, insect establishment on a foodplant involves 5 main stereotyped behavioural components: (i) orientation towards the plant (ii) recognition of the host plant (iii) initiation of feeding (exploratory biting or piercing) (iv) maintenance of feeding (v) cessation of feeding followed by dispersal of more mobile forms. The classification adopted here differs slightly from that of Dethier (1954) de Wilde (1958) and Hsiao (1969) and is based on the observed behaviour of Uraba lugens Walk. Steps I and II constitute only one step in Dethier's classification and steps III and IV are one step in Hsiao's classification. Step III in de Wilde's and step IV in de Wilde's and Hsiao's classifications were omitted in the present classification.

Each of these steps is manifested in response to various physical (e.g. visual, tactile) or chemical (e.g. humidity, smell, taste) stimuli. In each step the insect's response to different plants may be positive or negative. Its positive response may differ in intensity from one plant or part of a plant to another. Only when the insect's response to one plant is positive, at one step can the next response follow. For example only when the insect orients towards and thus finds its host plant can recognition occur and, subsequently, feeding be initiated. In many associations between insects and host plants, the first 2 steps of the behavioural mechanism leading to host selection are the functions of the gravid female.

2.3 Electrophysiological studies

Electrophysiological studies have provided further insight into basic aspects of foodplant selection and specificity by phytophagous insects. There is evidence that phytophagous insects are able to make subtle choices between 2 apparently acceptable foodplants (Hanson and Dethier, 1973). This indicates that insects get fairly detailed information about the chemical composition of those foodplants. Waldbauer and Fraenkel (1961) and Waldbauer (1962, 1964) demonstrated that removal of the maxillae (organs each bearing 2 sensillae styliconica) of Manduca sexta, results in larvae feeding on non-host plants. This simple experiment indicates that without maxillary sense organs the insect's ability to distinguish a host and non-host is lost. Thus the maxillae contain sensillae which dominate host selection by caterpillars of M. sexta.

The ultrastructure and functions of insect sense organs have been studied intensively by Schneider (1957, 1963, 1964, 1969) and Dethier (1941, 1955, 1958). As in the vertebrates, taste and olfactory receptors are anatomically distinct. The taste organs consist of innervated cuticular bristles, hairs and pegs with open tips. There are 4 or more morphological types of olfactory organs, (i) sensillae trichodeae - long, thick walled hairs or pegs found in moths; (ii) sensillae basiconicae - shorter, thin walled hairs or pegs found in many insects; (iii) sensillae placodeae - plate-like organs found in bees and other Hymenoptera and (iv) sensillae coeloconicae - pit pegs found in lepidoptera, hymenoptera and many other orders of insects.

The taste (gustatory) receptors respond mainly to water-soluble substances such as salt and sugar. The olfactory receptors are responsive to air- or water-borne substances which are either water insoluble or water

and lipoid soluble.

Perhaps the most intriguing aspect of chemoreceptor activity is the range of chemicals that initiate response by a given receptor. Schneider (1969) recognised 2 groups of chemoreceptors which he called generalist and specialist. The generalist responds to a wide range of chemically unrelated compounds by increasing (excitation), decreasing (inhibition) or maintaining their basal rate of "spike activity". Different compounds are, however, associated with different patterning of the neural spike activity. For example Dethier and Schoonhoven (1969) showed that 2 receptors of Manduca sexta larvae respond to odours of tomato, geranium and linalool. However, the spike activity pattern to the 3 substances were different, reproducible and consistent with each compound. The 'specialist' chemoreceptors respond to one or a few closely related chemicals. 'Specialist' receptors sensitive to feeding stimulants, feeding deterrents and oviposition stimulants have been demonstrated (Schoonhoven, 1967; Ishikawa et al., 1969). The carrion receptor of Calliphora and Nechrophorus (Dethier, 1947), the quinine chloride (Glycoalkaloid) receptor of Pieris brassicae (Ma, 1969) and the sinigrin sensitive receptors of P. brassicae (Schoonhoven, 1967) are well known examples.

With electrophysiological techniques, it has been possible to gain further insight into synergistic and antagonistic interactions. Morita et al. (1966) reported that salt enhanced the spike activity of the labellar sugar receptor of the fleshfly. An opposite type of interaction has been reported by many workers (Hodgson, 1957; Takeda, 1961).

The behavioural responses of food-seeking phytophagous insects are few, in view of the multitude of sensory information provided by the chemical and physical attributes of the foodplant. The options open to the insect are: (i) acceptance followed by feeding or (ii) rejection followed

by starvation or migration. It is believed that the decision is a function of the central nervous system which monitors and integrates the incoming information. The decision whether or not a plant is accepted depends on the degree to which the information received approximates the overall information sought (Schoonhoven, 1969), the receptor activity being influenced by both the qualitative and quantitative components of the stimulus. Dethier (1968) for instance, showed that with Phormia regina, a chemical stimulus acting on a single receptor may influence the central nervous system to order acceptance at one concentration and rejection at another concentration of the chemical concerned. Schoonhoven (1969) observed that, for insects which possess 2 or more phagostimulant receptors e.g. Pieris brassicae, feeding response may correspond to the summation in the CNS of inputs from the phagostimulant receptors. For the plant that contains both phagostimulants and deterrents, the outcome of any feeding situation may depend on the relative intensities of the spike activities of the receptors sensitive to the stimulants and deterrents. Thus if the feeding stimulant dominates, the insect will feed and vice versa. At the same time, it is known that other factors such as degree of distension of foregut and the haemolymph osmotic pressure may influence the decision (Dethier and Bodenstein, 1958; Chapman, 1968).

2.4 Definitions

Various operational terms have been used in the literature to describe insect behavioural responses towards foodplants. The terms "attractant" and "repellent" have commonly been employed to describe stimuli eliciting respectively, positive or negative behavioural responses to hosts. The 2 terms as defined in their strictest sense do not relate to all possible types of reactions of insects to chemicals, however. Consequently, new

terms such as acceptants, rejectants, phagostimulants, token stimulants, sapid nutrients and saccharotropism have been introduced (Thorsteinson, 1953, 1955, 1958; Lipke and Fraenkel, 1956; Beck, 1956). The proliferation of the new terms also led to their misuse in many instances. In attempting to resolve the confusion surrounding their terminology in insect foodplant relationship, Dethier et al. (1960) proposed a terminology with which to deal with insect feeding behaviour and other chemosensory responses. The terms 'arrestant' and 'locomotor stimulant' describe the effect of the chemicals which cause insects to aggregate or disperse, whereas the terms feeding, mating or ovipositional stimulant and deterrent designate chemicals which elicit or inhibit feeding, mating or oviposition. The terms 'attractant' and 'repellent' are limited to the effects of chemicals which cause insects to orient towards or away from the source. These authors also recognised that: (a) the same compound may have multiple effects on behaviour even of the same insect; (b) any given effect may be elicited by chemicals which in other respects act differently.

Hamamura et al. (1962), in a study of food selection by silkworm larvae recognised and proposed terminology different from other workers, (a) attractants (b) biting factors (c) swallowing factors and (d) co-factors. The terms were, however, self-explanatory.

Beck (1965) found a real need for introduction of additional terms and broadening existing ones. He accepted the definition of classes of stimuli involved in orientation responses as stipulated by Dethier and associates (1960), except that the terms should be applicable to physical as well as chemical stimuli. Because initiation and maintenance of feeding are known to be separable phenomena he coined the words 'feeding incitant' and 'feeding suppressant' to describe stimuli that evoke and inhibit initiation of feeding respectively.

2.4.1 Classification of insects

Insects have been classified in a variety of ways according to their food habits. These include groupings according to agronomic criteria, recognising such groups as vegetable, fruit, forage and cereal crop insects and groups according to the part of a plant injured e.g. foliage feeders, bark or stem borers, root feeders. Other groupings have been erected on phytogeographical basis. Hering (1951) coined the terms 'xenophobe' and 'xenophile' which refer respectively to rejection and acceptance of plants indigenous to a region other than the aboriginal home of an insect. However, a classification which is based on the number of plant species accepted as food (monophagy, oligophagy and polyphagy) has had a much wider application.

Monophagy is defined as feeding on a single plant genus or species. Hering (1951) recognised 3 subgroups of monophagy:

(i) Specific Monophagy (first degree) - restriction to only one species of plant.

(ii) Sectional Monophagy (second degree) - the insect lives only on one section of a specific plant genus.

(iii) Generic Monophagy (third degree) - the phytophage eats some or all species of a plant genus.

Oligophagy is defined as feeding on several plant genera. Hering (1951) defined 4 subgroups but these subgroups have not had any appreciable application since then. Polyphagy is defined as feeding on members belonging to different families or orders. Hering (ibid.) distinguished between first degree polyphagy and second degree polyphagy, referring to species that attack different orders of plant belonging to the same or to several classes. Dethier (1947) and Thorsteinson (1953) offered an entirely new interpretation to the concept of monophagy, oligophagy and polyphagy. They classified phytophagous insects on their response to

chemical plant constituents. Dethier (ibid.) classified those insects as monophagous which respond positively to only one chemical or group of related chemical plant constituents, whereas, in his scheme, oligophagous insects are those that respond to distinct and unrelated chemicals. Under his scheme a homochemotactic oligophagy refers to a situation in which response is to only one trophic stimulant or a group of related substances. Heterochemotactic oligophagy refers to a situation in which response is to several chemically unrelated trophic stimuli.

2.4.2 Classification of plants

A classification of plants according to plant constituents and other host relationships of significance in relation to attack by a given phytophagous insect was given by Thorsteinson (1953): (i) plants which are neither attractive to ovipositing females nor acceptable to larvae; (ii) attractive plants - plants that contain chemicals which probably by olfaction attract gravid females of the insect species, and stimulate them to oviposit on them; (iii) acceptable plants - plants that contain chemical constituents which by olfaction and gustation stimulate an insect to chew and ingest them. The last group may be further classified as:

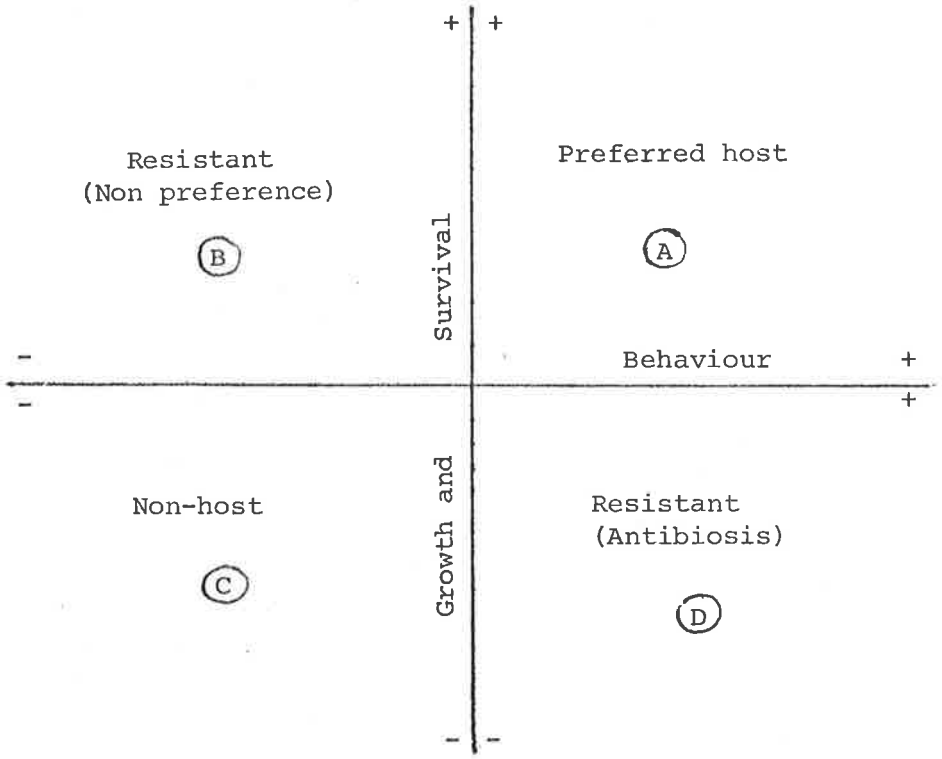
(a) Plants on which the insect will feed but which contain toxic chemical constituents which hinder vigorous development resulting in low fecundity of the pest.

(b) Plants that possess morphological characteristics such as hairyness or toughness of cuticle which deter feeding by insects.

(c) Plants that contain trophic stimulants and are free of chemical constituents and physical characteristics which inhibit feeding but that do not contain a complete complement of nutrients, vitamins and minerals required by the insect.

(d) Plants that contain stimulants and are free of chemical constituents and physical characteristics which inhibit feeding.

Beck's (1974) classification was simple but informative. It considered both the behavioural and dietary responses of the insect. The classification is given below incorporating the present writer's view.



The relative effect of the plant on the insect's growth and well being is represented on the ordinate, in which the arbitrary range is from very good (+) to quite deleterious (-). The relative scale on the abscissa expresses the insect's behavioural responses to the plant, ranging from avid acceptance (+) to complete avoidance (-). Plants that would fall in quadrant A (++) for a particular insect would be those that are very attractive, offer good feeding and orientation stimuli, and are both nutritious and non-toxic, these would be suitable and preferred hosts. Plants that would fall in quadrant B would be non preferred e.g. for oviposition, but were nevertheless as suitable for development and

growth as those in quadrant A. Plants in quadrant C are unattractive, toxic and/or nutritionally inferior and would be non-host plants. Plants in quadrant D would be attractive to insects but unable to sustain larval development due to the presence of toxic substances and/or nutrient deficiencies.

2.5 Role of nutrient and secondary plant substances

The role of chemical factors in insect foodplant interrelationships has been clearly demonstrated. Both nutrient and secondary plant substances have been implicated. Through such effects as attraction, repellence, stimulation and inhibition, nutrient and secondary plant substances are known to modify the behaviour of insects.

2.5.1 Attractants and repellents

The definitions of attractant and repellent given by Dethier et al. (1960) are adopted here. An attractant or repellent is defined as a chemical which causes insects to orient towards or away from the source respectively. The property of volatility is thus inferred for the candidate compound. It is therefore not surprising that most of the plant attractants and repellents are secondary plant substances. Hedin et al. (1974) classified the known attractants by their chemical structure and by insect group odour. They found insect attractants included 21 terpenes, 7 alcohols, 4 esters, 4 acids and 2 phenolics.

There is very little information on naturally occurring repellents. Some of the compounds reported are capsaicin (Schreiber, 1958) myrcene and limonene (Smith, 1966).

2.5.2 Oviposition stimulants

Many of the compounds reported as stimulating oviposition were also identified as feeding stimulants or attractants, but not necessarily for the same insect. The secondary plant substances also predominate in this class of chemo-stimulation. The cabbage root fly is stimulated by sinigrin and β--phenylethylamine (Traynier, 1965). Matsumoto and Thorsteinson (1968) reported that the onion maggot was stimulated by n-propyl disulphide, methyl disulphide, n-propyl mercaptan and n-propyl alcohol. Lucillia cuprina is stimulated to lay eggs by gasses given off by solutions of ammonium carbonate and the vapour of Indole (Barton-Browne, 1965). Stadler (1974) found that D-∝-pinene and L-β-pinene stimulate egg-laying of the Eastern spruce budworm and allyl-isothiocyanate was reported for the diamond-back moth (Gupta and Thorsteinson, 1960). In the orthoptera, 4 terpenes were reported for the desert locust (Carlisle et al., 1965). A nutrient substance, Lecithin, was reported for the Colorado potato beetle (Grison, 1958).

2.5.3 Feeding incitants

Factors that induce initiation of feeding include sinigrin and related mustard oil glycosides for diamond-back moth, Plutella maculipennis Curtis and a number of other insects that feed on Crucifers. Alkaloids, phaseolunatin and lotaustrin act as feeding incitants for the Mexican bean beetle, Epilachna varivestis Mulsant (Nayar and Fraenkel, 1963).

2.5.4 Feeding stimulants

There is no clear distinction between feeding incitant and feeding stimulant, and many substances may have both effects. Some feeding stimulant have been shown to be host-specific substances, but others have proved to

be common chemicals of universal botanical distribution, such as glucose, sucrose, fructose, ascorbic acid, inositol; L-alanine, DL- -aminobutyric acid, L-serine, L-threonine, proline; β-sitosterol and phospholipids (Beck and Hanec, 1958; Thorsteinson, 1960; Gothilf and Beck, 1967; Heron, 1965; Hsiao and Fraenkel, 1966; Hamamura, 1965). Examples of feeding stimulants of restricted botanical distribution include catalposides for the larvae of Catalpa moth, Ceratomiae catalpae (Boisduval) (Nayar and Fraenkel, 1963); Cucurbitacins from Cucurbitaceae for Diabrotica undecimpunctata howardi Barb (Chambliss et al., 1966) and a series of related essential oils from Umbelliferae that attracted larvae of Papilio ajax (Dethier 1941).

2.5.5 Feeding deterrents (inhibitors)

The plant chemicals that militate against feeding of various insects are of primary importance in determining which plants are eaten (Hsiao, 1969; Thorsteinson (1960), and the potential value of such chemicals has been pointed out already (Jermy, 1965; Munakata 1970). An exhaustive review of the plant feeding inhibitors is given by Chapman (1974). Many different chemicals are involved, some of them amongst the commonest constituents of plants. A majority of amino acids and sugars are phago-stimulatory at the concentrations normally found in plant leaves, but a number of cases are known in which the amount of feeding is reduced by high concentrations (Hsiao and Fraenkel, 1968; Beck, 1956, 1960; Beck and Hanec, 1958; Brett et al., 1965). Akeson et al. (1970) found that arabinose reduced the amounts of sucrose treated discs eaten by Sitona cyllindricollis.

The secondary plant substances, however, provide us with the best known examples of feeding inhibitors. Even the most voracious phytophages

e.g. the desert locust, Schistocerca gregaria Forsk, will not feed on seeds of neem, Azadirachta indica. This behaviour was shown to be caused by the presence of the terpenoid, azadirachtin which acted as a potent feeding deterrent (Butterworth and Morgan, 1971). The alkaloid, isoboldine from Loculus trilobus affects feeding by Spodoptera littoralis (Wada and Munakata, 1968). Other secondary plant substances (inhibitors) that have been isolated and characterized include Phlorizon from leaves of apples (Montgomery and Arn, 1974), Gossypol from glanded cotton (Maxwell et al., 1965); Melantriol from Azadirachta indica (Lavie et al., 1967) Demissine and tomantine from wild potato and tomato (Kuhn and Low, 1955) Nornicotine and Lupinine from tobacco (Harley and Thorsteinson, 1967). In addition, plant substances such as inorganic salts (Ma, 1972; Khalifa et al., 1974; Salamy and El Sharaby, 1973) acids and bases (Dethier, 1937, 1939; Touhey and Bray, 1961) alcohols (Khalifa et al., 1974), aldehydes and ketones (Coaker and Finch, 1973; Ishikawa and Hirao, 1965) juvenile hormone analogs,(Bowers, 1966, 1968; Mansingh et al., 1970) have been recorded as feeding inhibitors.

2.5.6 Miscellaneous categories

Coumarin has been found to be a flight termination agent for sweet clover weevil (Heidwig and Thorsteinson, 1961). Cellulose, silica and potassium phosphate were reported as swallowing factors for the silkworm larvae (Hamamura et al., 1962). Trans-2-hexanal was reported as a mating factor for the polyphemus moth (Riddiford and Williams, 1967). Kato and Yamata (1966) reported the 3,4-dihydroxybenzene structure of chlorogenic acid as a growth factor for the silkworm larvae.

It is apparent from the many examples given above that the plant chemicals play very important roles in the feeding behaviour of insects and

may alone or in conjunction with physical factors of the plant determine foodplant selection. It is also evident that the secondary plant substances have assumed an unparalleled significance in this behaviour.

2.6 Induced feeding

Induction of specific food preference is an area which is beginning to make an impact in the insect-foodplant relationship. Some investigators have successfully changed the host specificity of an insect by a process of accommodation or conditioning to a new host (Craighead, 1921; Thompson and Parker, 1927; David and Gardiner, 1966; Stride and Straatman, 1962; Jermy et al., 1968; Hovanitz and Change, 1962 a, b; Hovanitz, 1969). The term "conditioning" in host selection is reserved for the effect that a particular association between host and insect has on the choice of host by subsequent stages of the insect (Montieth, 1955). The Hopkins host selection principle that contends that larval food conditioning determines adult oviposition preference is of historical interest.

The degree of conditioning obtained by different authors varied considerably. Craighead (1921), Hovanitz and Change (1962 a, b) reported high degrees of conditioning. Jermy et al. (1968) showed that preference once induced was not lost after 2 larval moults and subsequent feeding on artificial diet. Thompson and Parker (1927) found a small degree of conditioning in Pyrausta nubilalis (Hbn.) without any change of preferred host. Although larval conditioning could be demonstrated in Colorado potato beetle the conditioning did not influence the choice of plant for either feeding or oviposition by the adult beetle (Bongers 1965).

2.7 Quantitative studies

Studies on the rate of food intake, efficiencies of digestion and

utilization have disclosed differences in the suitability of different plants as hosts. Some of the information available in this area ~~are~~ is difficult to interpret because measurements have not been standardized, methods have varied in accuracy, vertebrate nutritional indices have been employed in some cases unrealistically and errors involved in dry weight estimates have been too high in certain cases. Waldbauer (1964) reviewed the subject and pointed out some of the shortcomings inherent in some of the earlier techniques.

The most thoroughly studied phytophagous insect with respect to food utilization is the commercial silkworm Bombyx mori (Legay, 1958; Hassenein and El Shaarawy, 1961 a, b; Mukaiyama and Ito, 1962 a, b; Takeuchi and Kosaka, 1961). Differences in food efficiencies can be demonstrated only by measuring intake, digestibility and growth. The efficiency with which digested food is used for growth will vary not only with the maintenance requirement for energy but also with the balance of nutrients (Gordon, 1959).

The importance of measures of intake and utilization cannot be overemphasized. For example, instances of poor growth may not be due to the nutritional inadequacy of the diet but to a low rate of intake due to the absence of a feeding stimulant or the presence of a deterrent. The addition of nutrient with a phagostimulatory activity might lead to increased growth, although the nutrient is neither required nor utilized (Dadd, 1960). Sand (1959) suggested that there may be more than one optimal diet for a given insect species. Measures of intake and utilization can give an indication of this, since patterns of utilization may be different although diets are similar in their ability to support growth. For instance, low intake might be offset by high digestibility or a high utilization of digested food for growth or vice versa. Poor digestibility

might be offset by efficient utilization of digested food for growth or vice versa.

2.8 Evolutionary trends

The evolution of host plant specificities has been little studied, however, and more investigation of the subject is badly needed. Various workers have attempted to explain the origin of insects feeding habits and the evolution of host plant specificity. Brues (1920), Dethier (1954), Fraenkel (1959) and Waldbauer (1963) were of the opinion that the evolution of host specificity was from a primitive polyphagy to the more restricted food habits of oligophagy and monophagy. Ehrlich and Raven (1964, 1969) and Singer (1971) gave evidence to support the contention that there has been a co-evolution of plants and insects, involving a wide spectrum of host plant specificities.

Dethier (1970) proposed a hypothesis of congruency to explain the origin of feeding diversity. The hypothesis assumes that changes in neural systems of insects and the chemical systems of plants occur randomly by mutation. If there is a change in receptor sensitivity and/or central interpretation such that somewhere in the plant kingdom there is a chemical that will stimulate feeding, the plant possessing that chemical will be eaten. Similarly, if there are neural changes such that chemicals formerly acting as repellents or deterrents are no longer detected, the plants involved may be eaten If there are neural changes such that formerly non-stimulating chemicals can now be detected and/or sensory input is now interpreted as unacceptable, the plant involved will not be eaten. I think this hypothesis is too simplistic. In the first place the presence of a feeding stimulant does not necessarily guarantee acceptance of that plant by an insect. It also assumes that any substance which is

not detected by the insect's sensory mechanism could not be of any effect physiologically.

Wiklund (1974) advanced a hypothesis that hostplants range and habitat predictability are intimately correlated. Thus (1) a 'monophagic strategy' being favoured in a predictable habitat, makes it possible to maximize reproductive success on one hostplant per habitat; or (2) a 'polyphagic strategy' is compulsively adopted in unpredictable habitats where 2 or more hostplants per habitat have to be utilized for the maintenance of the species. By extension of Van Valens (1965) niche variation hypothesis, which postulates a positive relationship between niche width and morphologic (or genetic) variability, one may deduce that polyphagous species are endowed with greater genetic variability concerning a number of ecological parameters than are monophagous species and are thereby able to utilize a greater number of foodplants and habitats than the latter.

It is clear that plants are evolving in an atmosphere of multiple pressures, of which insect predation is but one. The avenues of escape from these pressures include emigration to new habitats and evolving physical and chemical defensive mechanisms (Dethier 1970). The secondary plant substances are believed to have been elaborated to protect plants from predation by herbivores and insects (Fraenkel, 1959). It has been suggested that insects responded to these chemicals. Thus a host preference arose when a given insect species, by mutation, genetic recombination or selection, overcame the repellent or toxic effect of such a material thereby gaining a new source of food (Fraenkel, 1959). This hypothesis is essentially similar to Dethier's (1970).

We do not have sufficient information to explain evolution of different host plant specificities on the basis of genetic mutation or recombination.

Geographical, ecological and genetic isolation mechanisms may be important but again direct evidence is lacking here. The plant and insect species we see around us are those that have been able to survive the course of evolution. There is reason to believe that certain plant and insect species have perished because they could not make the necessary adjustment to their changing or new environment of which the insect host plant interaction is only a part. This belief is reinforced by the fact that the number of living species of organisms is less than one tenth the total number of fossil species (Wallace, 1961). Since survival depends on the capacity of the organisms to cope with their total environments, it follows that all the surviving plant and insect species exhibit adaptations in one way or another. The fact that insects are so often attracted to plants that are nutritionally satisfactory may probably be due to the fact that the 2 organisms have evolved together; and that insects that have been attracted to unsuitable hosts have tended to become extinct.

CHAPTER 3. GENERAL BIOLOGY

3.1 Introduction

The gum leaf skeletonizer, Uraba lugens (Walk), an important pest of eucalypt forests was first recorded in high numbers by Froggatt (1900) on the Western Australian crimson flowered gum, Eucalyptus ficifolia at Botany in New South Wales. He referred to it as Nola metallopa Meyr. It has been recorded in all Australian states with periodic outbreaks of severe defoliation reported for Queensland, New South Wales, Victoria, South Australia and Tasmania. This chapter is mainly descriptive, with a few ad-hoc experiments to clear some of the uncertain aspects of the biology.

3.2 Taxonomy

The insect which was first described by Walker (1863) as Uraba lugens (Arctiidae:Nolinae) has gone through many taxonomic vicissitudes. Walker (1866) redescribed the insect as Caesa viduella and Felder and Rogenhofer (1874), unaware of Walker's description of the holotype in the British museum collection, referred it to a new genus and species Toxoloma australe Felder. In a revision of Australian lepidoptera, Meyrick (1886) placed the species in the genus Nola (Leach, 1815) and listed Uraba lugens (Walk), Caesa viduella (Walk) and Toxoloma australe Felder as synonyms. Lucas (1890) placed it in Sorocostia (Rosentock, 1885) and erected a new species interspersia with Nola lugens (Walk), Nola metallopa Meyr and Mosoda jucunda Walk as synonyms. However, Nola lugens and Nola metallopa had each been accorded specific rank by Meyrick (1886). Froggatt (1900) in an apparent description of the habits of the insect refers to it as Nola metallopa Walk, the seedling gum caterpillar. Strand (1920) and Spencer (1928) both used Meyrick's nomenclature, Nola lugens (Walk). At about this time both Turner (1926) and Meyrick (1927) raised

the Nolinae to family rank and later, Turner (1943) referred N. lugens to the genus Roeselia (Huebner 1827) a treatment which was to last for about 30 years until Common (1975) reverted to Walker's original description and name Uraba lugens on the following bases: "According to Forbe's Lepidoptera of New York and neighbouring states - Roeselia (Huebner, 1825) is a synonym of Nola (Leach, 1815). Grote (1874) fixed the type species of Roeselia as cucullatella L. Roeselia includes European species, the genus being congeneric with Nola. The Australian species lugens is however, not congeneric with Nola in my view and was erroneously considered so by Meyrick in 1866. It must therefore be referred to another more appropriate genus. Because Uraba has priority over Caesa Walker, Toxolama Felder and Sorocostia Rosentock and is, in other respects, available, lugens is reassigned to it. Uraba lugens automatically becomes the type species as it is the only species in the genus. This treatment satisfies the Law of Priority (ICSN) which requires that: the oldest valid name is maintained".

3.3 Biology

3.3.1 Introduction

Campbell (1962, 1966, 1969) recognized 2 biological forms of U. lugens in New South Wales which are distinguished as follows:

(a) The alpine and southwestern form: 13 larval instars, eggs laid in a flat raft of up to 200 with adjacent eggs touching.

(b) The coastal and lowland form: 11 larval instars, eggs laid in masses of up to 100 but arranged in parallel rows one egg diameter apart. He found that the larvae and adults of the 2 biomorphs were indistinguishable.

Uraba lugens is widespread in South Australia, occurring from sea level to about 1000 m elevation and from coastal to inland and riverine habitats.

There appears to be only one biological form which possesses some characters similar to the coastal and lowland form of Campbell. There are a number of differences in its biology which might indicate that the 2 biomorphs described by Campbell from New South Wales are temperature induced (Morgan and Cobbinah, 1977).

There are 2 discrete generations a year, one which completes in November-December and the other in March-May. The former is referred to as the winter generation and the latter, the summer generation.

3.3.2 The egg

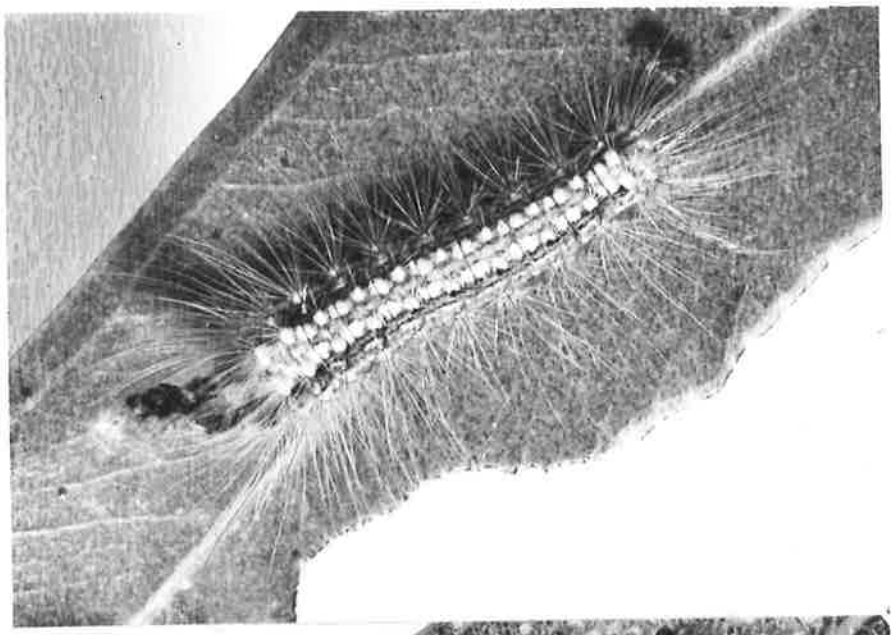
Eggs are circular, dorso-ventrally flattened measuring about 0.25 mm high and 0.50 mm wide. They each have a central disc-like operculum bordered by a series of pits. The transparent chorion renders embryonic development visible. The eggs are arranged in more or less parallel rows and may be almost touching to more than one egg diameter apart. Egg batches range in size from 20 to over 500 and occasionally contain eggs of two ovipositions. The incubation period is about 3-4 weeks in summer and 4-6 weeks in winter. Moisture influences both oviposition and eclosion of eggs (Morgan and Cobbinah, 1977), large emergences occurring over a short time following showers of rain or wetting of fully developed eggs in the laboratory. Under drier conditions, the larvae of a single egg batch eclode sporadically over a relatively longer period. Newly laid eggs are green but after about 5 days, depending on temperature, the colour changes to pale yellow and subsequently to yellowish brown and finally to brown. All these changes indicate different stages of embryonic development. Just before hatching the developed embryo can be seen through the transparent chorion, particularly its prominent head capsule and pro-thoracic shield both pigmented black. In natural conditions the proportion

Figure 3.3.3.1a Life history studies

top - empty egg cases

middle - gregarious feeding by young larvae

bottom - mature larvae showing previous head capsules stacked on prothorax.



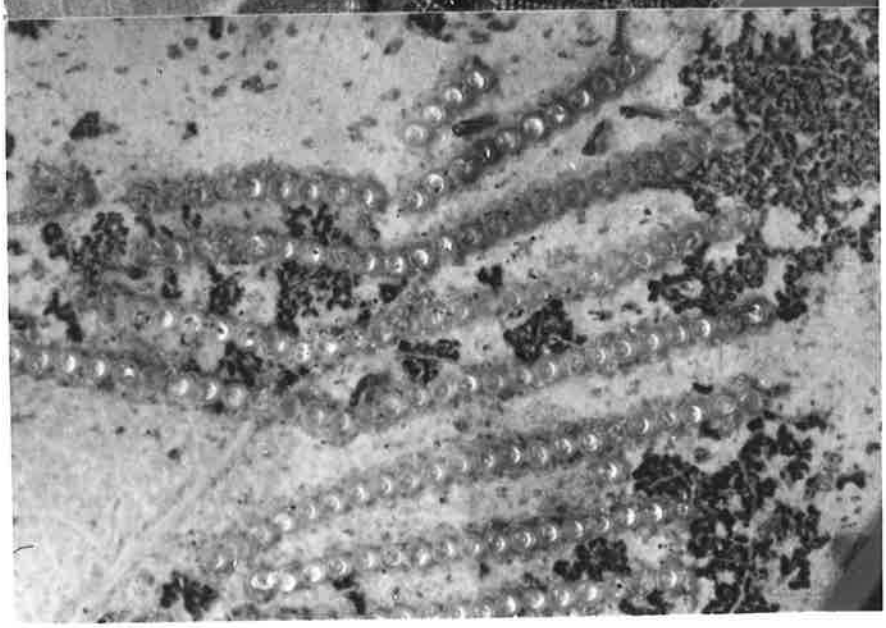
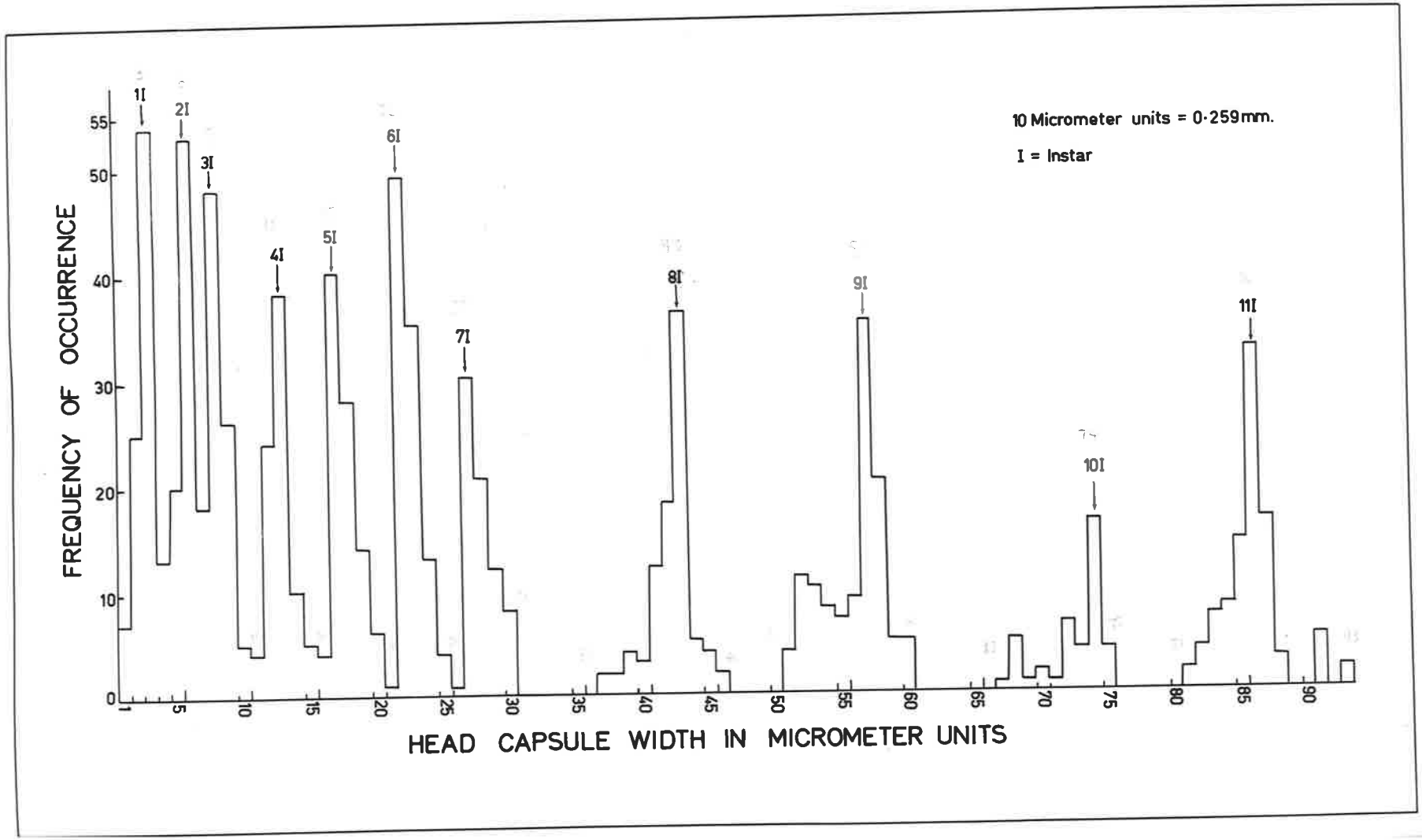


Figure 3.3.3.1b Number of instars determined by head-capsule measurements.


10 Micrometer units = 0·259mm.
I = Instar
FREQUENCY OF OCCURRENCE
HEAD CAPSULE WIDTH IN MICROMETER UNITS
1I
2I
3I
4I
5I
6I
7I
8I
9I
10I
11I
0
10
20
30
40
50
55
1
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90

of infertile eggs is very small, rarely exceeding 0.1%. Infertile eggs can easily be distinguished by their failure to develop colour and to pass through the characteristic stages of the fertile eggs. They remain pale yellow green, their opercula collapse after a few days and they shrink noticeably.

3.3.3 The larva

(i) Morphological features

Larvae when first ecloded average about 2 mm in length. A fully grown larva, however, measures up to 25 mm in length and its head capsule is light to dark brown in colour (see Fig. 3.3.3.1a). Thoracic and abdominal segments bear three pairs of protuberances - one pair dorsally and the other two pairs laterally. Each protuberance has a tuft of hairs, those on the lateral ones being longer. Also arising from the lateral tufts of thoracic segments are a series of very long black hairs projecting anteriorly whilst those on the penultimate abdominal segment are posteriorly directed.

The protuberances are yellowish in colour but the dorsal ones are often encircled with pink tufts. The lower lateral ones have four pink tufts surrounding them. Between the dorsal and lateral protuberances is a broken black line running the whole length of the body.

Brimblecombe (1962) recorded eight larval instars, but Campbell (1962, 1966, 1969) recorded 11-13 instars. The South Australian form has a variable number of larval instars ranging from 8 to 13 with majority of larvae completing 11 stages (see Fig. 3.3.3.1b). The number of larval stages appears to be determined by a variety of factors including food and climate, particularly temperature.

(ii) Larval Behaviour

The larvae of Uraba lugens are overtly gregarious during the first

four larval stages. Egg clusters tend to hatch over a short period in optimum conditions. After eclosion the first caterpillars wander over the egg mass and eventually onto the leaf surface spinning chains of silk which they lay over the surfaces of the eggs and leaf by rhythmic side to side movements of the head.

The silk spinning by U. lugens appears to be a factor in the maintenance of early aggregation. The larvae that are the last to emerge, follow the existing silken trails produced by the early ecloders to the area where they have settled and are feeding. Gregarious feeding during the first four stages is restricted to the area covered with silken mat. The progeny from one egg batch may however separate into two discrete groups when the time between first and last hatchings exceeds about 5-7 days; and it is then found that one group has moulted before the other and the groups are in different stadia. The unified feeding rhythm evident in the group is probably due to physiological uniformity and the close proximity of one larva to another. Thus, movement of an individual larva together with its sense of direction is conveyed to adjacent larvae either by direct contact between the larval skins or perhaps more commonly by movement of the lateral sensory hairs of individuals that appear to be invariably touching or interlocked.

The skeletonizing habits of the larvae, to which the insect owes its common name, persist up to the fifth stage, when individuals move to feed on the edges of the leaves instead of upon the surface. The small larvae bore down through the lamina leaving the reticulum of veins and the opposite epidermis - thus the leaf skeleton is left.

The most noticeable behaviour pattern from the fifth stage onwards is the breakdown of the gregariousness. As the feeding area is enlarged main veins in the leaf are encountered, which are large enough to break the contact between adjacent individuals and this results in periods of

isolation for members of a group. As these periods increase the aggregation of the whole group ultimately breaks down. By the eighth stage, larvae are mainly found as individuals or pairs.

It is also a remarkable fact that from the fifth stage, when skeletonizing no longer occurs, the head capsules are retained, following ecdysis, upon the prothoracic setae. As moults succeed one another the head-capsules are stacked one upon the other above the prothorax. Both head-capsule widths (Fig. 3.3.3.1b) and number stacked on top of the prothoracic segment are useful indices for estimating larval age. In the last stage, the larvae exhibit a strong positive geotaxis and move towards the base of the tree in search of pupation sites. During later stadia, any disturbance of the host tree causes larvae to drop or suspend on silk. In such situations they may pupate where they locate without completing additional instars or they may cross fallen debris to ascend the same or a new tree.

3.3.4 The pupa

This is generally medium to dark brown in colour and in the natural state is located in an ovoid firm silken cocoon in which is incorporated particles from the surface upon which the cocoon is built, the string of larval head-capsules, and many of the setae which are removed from the body surface of the prepupa during cocoon manufacture. In laboratory rearings, insects may be stimulated to pupate without producing cocoons, by holding prepupae in clean glass containers. With no soft surface to chew to produce a cocoon site the prepupa does not spin one or else it produces an imperfect or partial cocoon (see Fig. 3.3.4.1a). In the field, pupation occurs mainly in soil but pupae have also been observed under loose bark and on leaves. Wherever pupation

Figure 3.3.4.1a Pupae

top - imperfect cocoons produced on cotton material

bottom - structure and coloration of cocoons produced by larvae reared in different places.

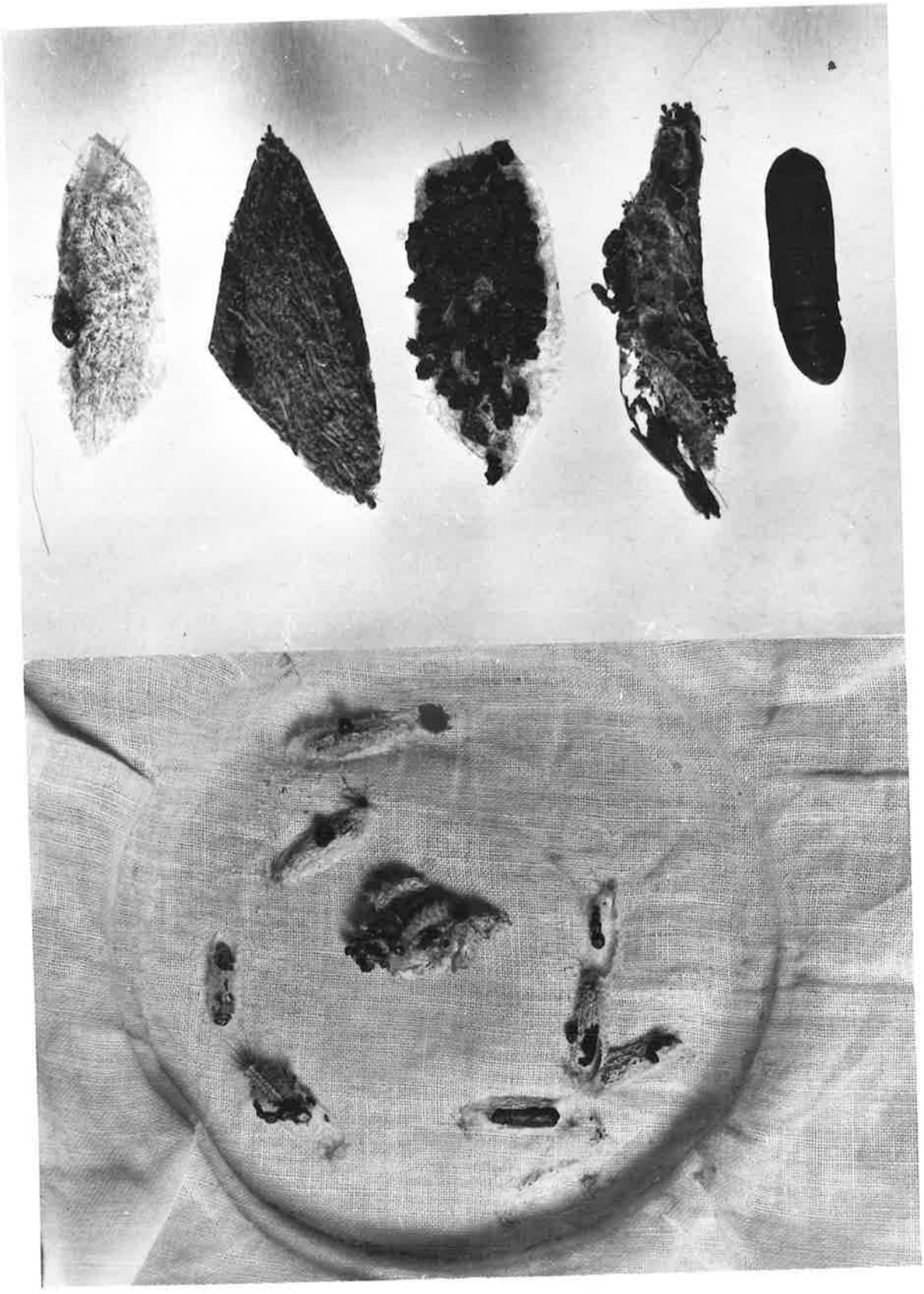
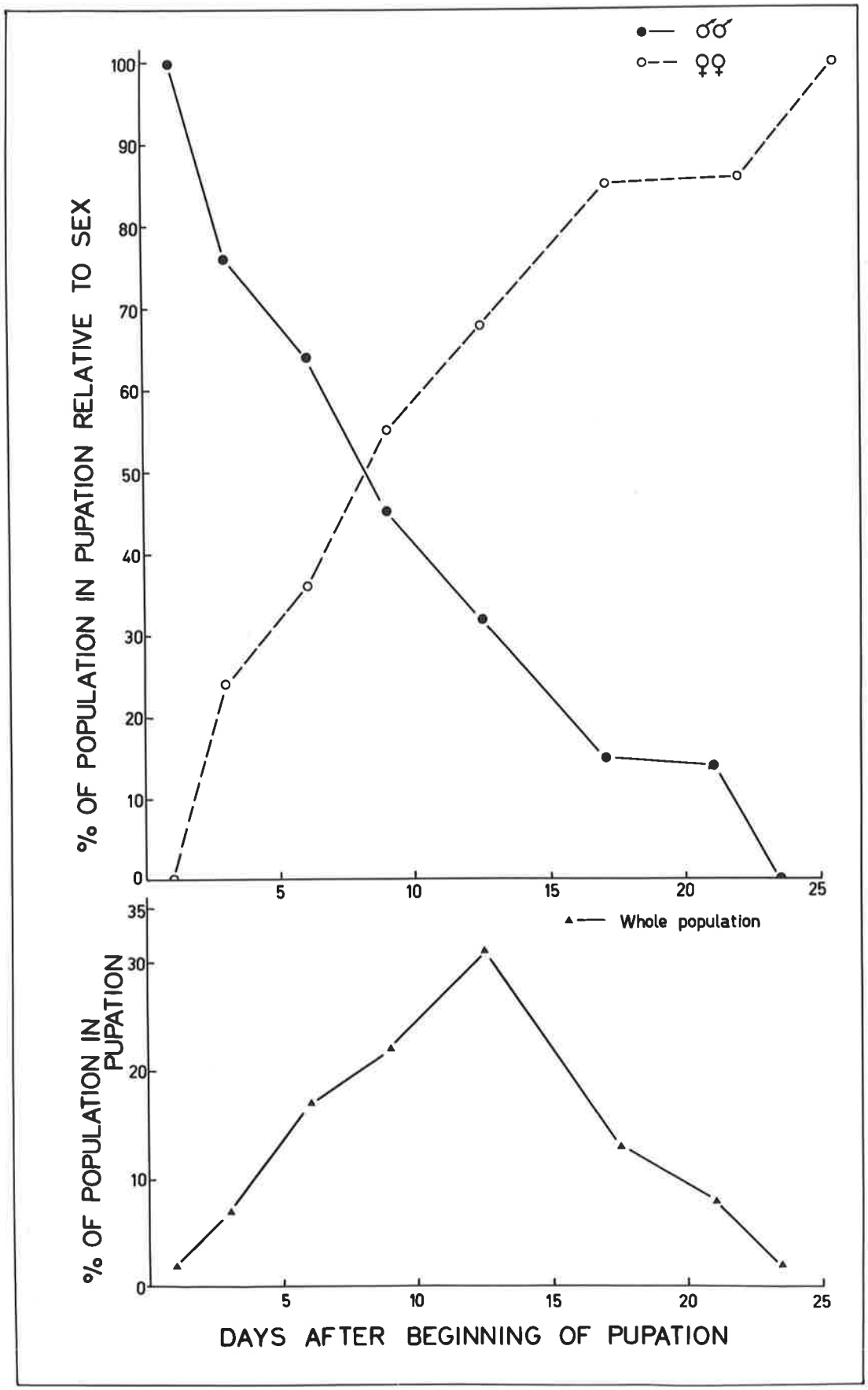


Figure 3.3.4.1b Pupation pattern of U. lugens

top - ● males o females

bottom - whole population.


♂♂
♀♀
% OF POPULATION IN PUPATION RELATIVE TO SEX
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
Whole population
% OF POPULATION IN PUPATION
35
30
20
10
0
5
10
15
20
25
DAYS AFTER BEGINNING OF PUPATION

occurs the cocoons blend with the surroundings (see Fig. 3.3.4.1a). The males usually are the first to pupate (Fig. 3.3.4.1b) and are about half the weight of the females.

The pupal period is about 2-4 weeks and depends mainly on ambient temperature. A test of 5 temperature regimes (5°C, 10°C, 13°C, 16°C and 25°C) showed that the fastest developmental period of 16 days took place at 25°C. At 16°C the pupal duration was 27 days. None emerged at lower temperatures, although some did when then transferred to 25°C. Prior to emergence, the obtect pupa (strictly, more probably the pharate adult) wriggles its way out of the cocoon and the adult bursts out of the pupal case to dry and spread its wings on nearby surfaces.

3.3.5 The adult

The adults are present in the field from late March to early May and from early November to late December each year. They are small sized greyish-brown moths. The hindwings are lighter in colour, each with a dark spot in the middle. The forewing has raised tufts and darker broken bands of scales forming a more or less typical pattern. The small eyes are ventrally located and the mouthparts are represented by only 2 palpi and a vestigial proboscis. The moth therefore does not feed and has a very short life lasting two to eight days.

The males can be distinguished from the females by their bipectinate antennae and narrow cylindrical abdomens ending in a truncated tuft of scales. The females having ciliated antennae and more or less conical abdomens. Considerable variations in size exist. Climatic factors are involved but food has been shown to play a leading role (see Chapter 5). The body length of males varies from 8.5 mm to 11.5 mm and forewing length from 10.5 mm to 12.0 mm. The corresponding measurements for females are from 10 mm to 13 mm, and 12 mm to 15.5 mm, respectively.

Adults of both sexes are positively phototactic, although U.V. light trap catches favour males by about 9:1 (see Appendix table 3.3.5.1). Two hypotheses were put forward to explain this: a) the sex ratio may greatly favour males over females and/or the females have a shorter flight range. The 2 alternatives were tested.

3.3.5.1 Test I. Sex ratio determination

Method:

Larvae were randomly collected from trees in the campus of the Waite Agricultural Research Institute in winter, 1975, summer and winter 1976 and from an Inman Valley redgum plantation in summer, 1977. The larvae were reared to the pupal stage in an insectary, on cut foliage at 25°C ± 5°C and 60% R.H. The pupae were sexed and the proportion of males to females recorded for each collection.

Results:

The ratio of the sexes during the four generations are shown in Table 3.3.5.1.1. The ratio recorded approached 1:1 in all generations,

Table 3.3.5.1.1 Sex ratio of pupae reared from larvae collected from arboretum of W.A.R.I. and Inman Valley redgum*plantation.

Generation	Total number	Males	Females	Ratio ♂♂ : ♀♀
Winter 1975	680	335	345	1 : 1.0298
Summer 1976	750	317	433	1 : 1.3659
Winter 1976	393	212	181	1.1712 : 1
*Summer 1977	312	158	154	1.0259 : 1
Totals	2135	1022	1113	1 : 1.09

* Collected from Inman Valley

though it slightly favoured the females in summer, 1976 and the males in winter, 1976. The slight difference may be due to timing of the collection. As shown in Fig. 3.3.4.1b a greater proportion of the males pupate early. Thus the proportion of females among those pupating increases with time and may account for nearly 100% of all pupae towards the end of the pupation period. Thus if larval collection starts late a proportion of the males would have pupated already. Similarly if larval collection is abandoned early a greater proportion of the females is left to pupate in the field. Even allowing for the effects of timing, however, the results clearly show that the paucity of females in light trap cannot be ascribed to differences in sex ratio.

3.3.5.2 Test II. Flight activity of the male and female

The second hypothesis that the smaller number of females in U.V. light traps may be due to shorter flight range of the females was then tested.

Method:

The test was carried out in a flight chamber. The working principles, design and operation of the flight chamber is as described by Kennedy and Booth (1963). The inside of the flight chamber is painted black and a 15-W globe projecting from a hole in the roof provides light in the chamber. A cylindrical stick fitted with a camel hair brush was used as a probe. A distortion free microphone connected to a cassette recorder was used to record events in the flight chamber. Thirty moths made up of 13 males and 17 females of different physiological age were used. The first group (four pairs) were 24 hr old moths, the second group (four pairs) were 48 hr old, the third group (five pairs) were mated pairs, and the last group (four females) had commenced egg-laying in the laboratory. Three

vine moth (two females and one male), were included in the study as a control group. The moths were introduced into the flight chamber singly and flight activity recorded separately over a period of 10 minutes. To stimulate take-off the upward airflow was increased or the moth was touched with a probe.

Results:

Vine-moth - All the vine moths flew. The male made four flights in 10 minutes and the females made three and five flights each lasting 20 to 180 secs.

Gum leaf skeletonizer

24 hour old moth: None of the eight moths in this group flew though they spread their wings when the upward airflow was increased or when touched with the probe. They all, however, walked on the floor of the flight chamber.

48 hour old moth: Only one male was airborne for about 5 secs. However, all the others spread, flicked and vibrated their wings when touched, they produced buzzing sounds and made a series of wriggling movements on the floor.

Mated pairs: Two males and two females flew in this group. The flights were all brief and erratic. There was very little vertical flight. One female made five take-offs totalling 28 secs. Two males and one female made two take-offs each lasting seconds only. Vibration of wings and wriggling movements were more pronounced in this group. Also a series of jumps were observed among members in this group.

Ovipositing females: None of the four females in this group took-off, though they spread, flicked and vibrated their wings when touched. Their movements were relatively sluggish. They continued their egg-laying in the flight chamber. Perhaps the act of laying eggs dominated all other

physiological and behavioural activities.

The failure of many of the moths to take-off may be due to the conditions prevailing in the flight chamber. The air current used to control the flight activity was cooler and lowered the chambers temperature to ca. 20°C. In natural conditions the moths are in flight in late November/December and March to late April, periods during which the mean temperature is above 20°C. Perhaps the low temperature might have inhibited take-offs, but other factors such as visual stimulation and smell which in natural conditions may stimulate flight were absent and these might have contributed to the results obtained.

These studies were inconclusive as far as the variation in flight capabilities of the male and female are concerned. However, it did indicate that Uraba lugens is probably not as good a flier as the vine moth or probably requires some specific stimuli to fly. Harris (1974) ascribed the regularity of infestation of the same trees to the poor flight capabilities of the female though other reasons such as proximity and/or induced preference may also account for selection of the same trees.

The high wingloading of the females (mean forewing expanse females - 84 mm, mean weight of females - 84.1 mg; mean forewing expanse males - 56.3, mean weight of males - 47.9) coupled with the fact that the insects do not feed as adults tends to indicate that long distance flight may not be physiologically advantageous, though some must presumably do so in certain generations to account for the variability in the disposition of eggs noted from generation to generation and from tree to tree.

3.4 Factors affecting biology

3.4.1 Natural factors of control

Campbell (1962) recorded a number of parasites and predators but

indicated that a fungus, Aspergillus flavus accounted for the major part of the mortalities. Brimblecombe (1962) recorded the crab spider, Philodromus sp. as the most noticeable natural enemy in the outbreaks covering about 1000 square miles and extending through much of the Brisbane, Lockyer and Fassifern Valleys in 1960 and 1961.

Little is known of the native parasites of Uraba lugens in South Australia. The objective of the work reported here was to determine the identities and impact of native natural control agents on the gum leaf skeletonizer in South Australia.

Methods

Immature stages of Uraba lugens were collected from several locations in Adelaide and Inman Valley from time to time in 1975 through 1976. The eggs and larvae were brought to the insectary and placed in rearing jars containing fresh eucalypt foliage which was replaced when needed and insects were reared to determine the extent of parasitism. Emerging parasites were removed daily, identified, and the emergence data recorded by species.

Observation on acts of predation were usually made first in the field, and then confirmed in the laboratory. Nymphs and adults suspected as predators were caged with various stages of U. lugens larvae and observed. During the growth season the eggs of predators and preferred oviposition sites were identified by observing oviposition in the Waite Institute arboretum. Also eggs suspected of being those of predators were reared in the insectary to stages that permitted identification.

Diseased larvae and pupae were brought to the laboratory and pathogens cultured for identification.

Results

The following records of parasites, predators and pathogens were obtained during the course of this study.

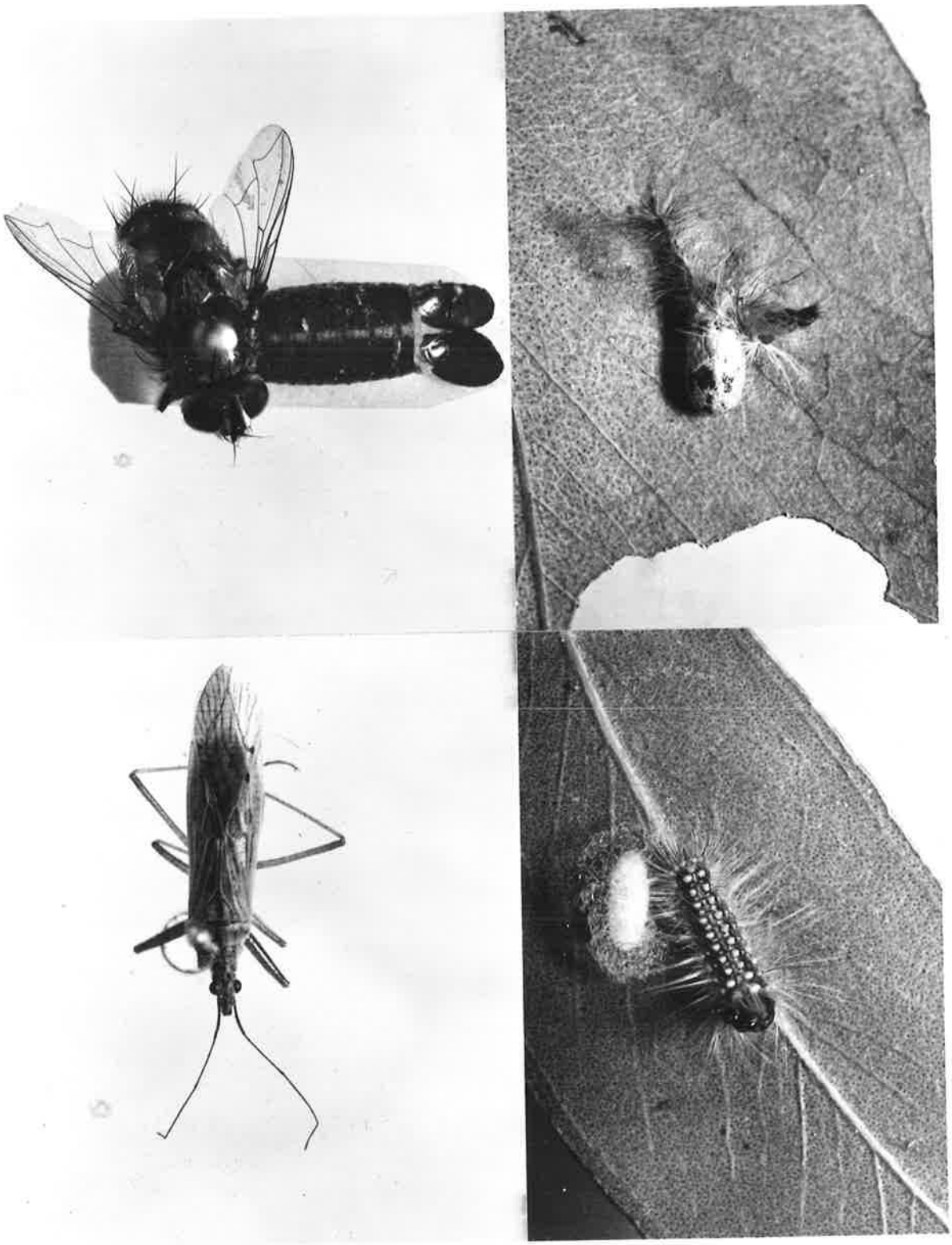
Figure 3.4.1.1a Natural enemies

top left - *Apanteles* sp.

top right - Nabid bug

bottom left - *Casinaria* sp.

bottom right - *Exorista* sp.



Species	Family	Parasitized stage
Endoparasites		
Casinaria sp.	Ichneumonidae	Larva 5-11
Dioctes sp.	"	Larva 5-8
* *Apanteles* sp.	Braconidae	Larva 5-8
* *Trichogramma* sp.	Trichogrammatidae	Egg
Winthemia lateralis (Macq.)	Tachinidae	Larva/Pupa
Exorista sp.	"	Larva/Pupa
** *Brachymeria froggati*	Chalcididae	Larva
Ectoparasites		
† *Trichoplectrus* sp.	Eulophidae	Larva
Predators		
Chrysopa edwardsi	Chrysopidae	Egg/Larva
C. ramburi	"	Egg/Larva
Oechalia schellenbergii	Pentatomidae	Larva
Cermatulus nasalis nasalis	"	Larva
Nabis tasmanicus	Nabidae	Larva
Pathogens		
Aspergillus parasiticus	Eurotiaceae	
* *Aspergillus flavus*	Eurotiaceae	Larva/Pupa
Chaetomium sp.	Melanosporaceae	Pupa
Beauveria bassiana	Moniliaceae	Larva/Pupa

* Recorded by Campbell (1962)

** Recorded by Brimblecombe (1962)

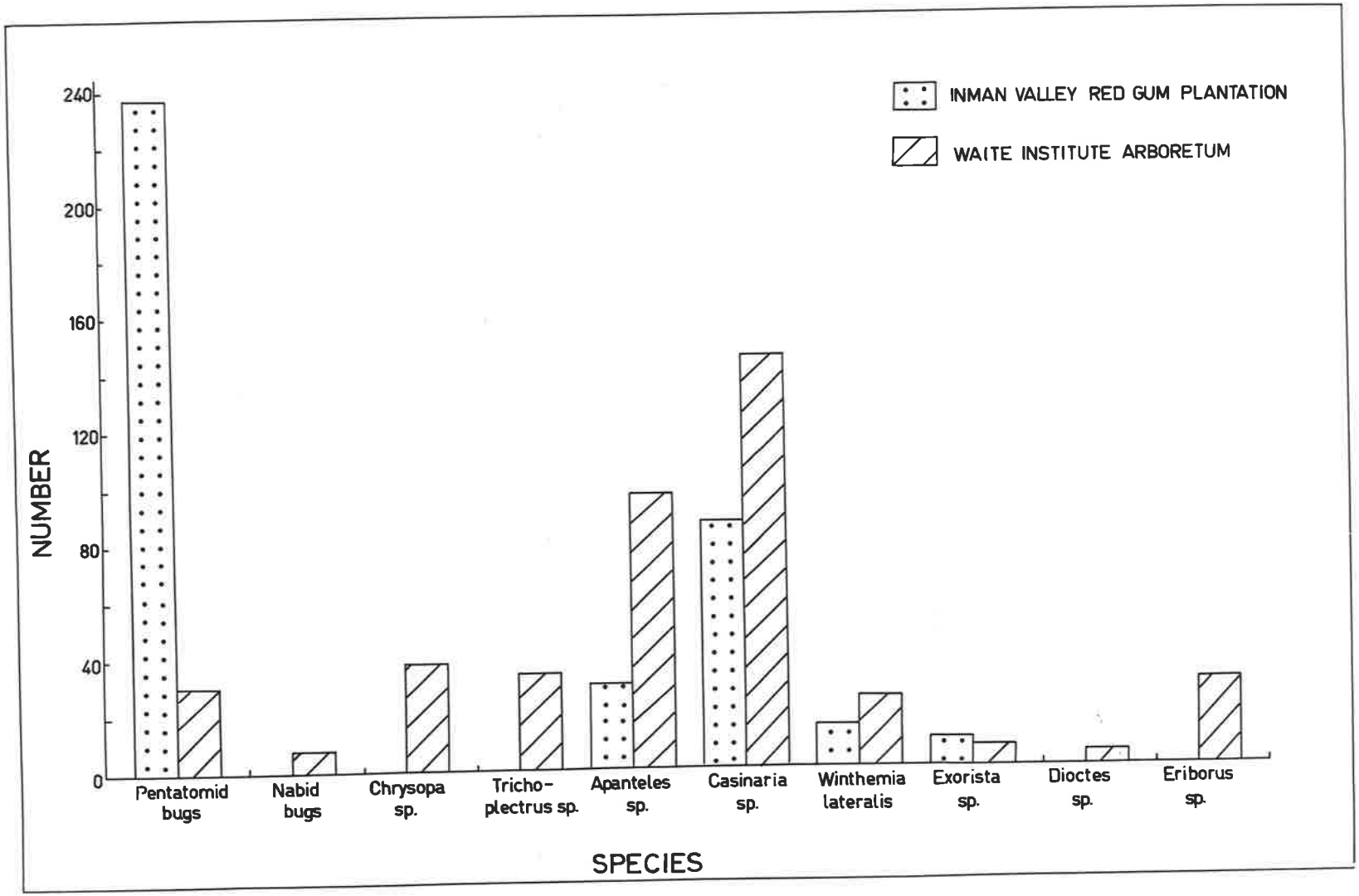
† Name under review.

In terms of numbers, voracity, and searching abilities *Casinaria* sp., *Apanteles* sp., *Trichoplectrus* sp., *C. edwardsi*, *O. schellenbergii* and *C. nasalis nasalis* were clearly the most important natural enemies of *Uraba lugens* in the course of this study. *Casinaria* sp. and *Apanteles* sp. were the most important endoparasites (see Fig. 3.4.1.1a) and the 2 accounted for well over 90% of all parasitism. Incidence of *Apanteles* was lower

Figure 3.4.1.1b Natural enemies - continued

Beauveria bassiana on dead pupa.

Figure 3.4.1.2 Occurrence of natural enemies of *U. lugens* at Inman Valley and the arboretum of W.A.R.I.


INMAN VALLEY RED GUM PLANTATION
WAITE INSTITUTE ARBORETUM
NUMBER
240
200
160
120
80
40
0
Pentatomid bugs
Nabid bugs
Chrysopa sp.
Tricho-plectrus sp.
Apanteles sp.
Casinaria sp.
Winthemia lateralis
Exorista sp.
Dioctes sp.
Eriborus sp.
SPECIES

in Winter, 1976 at Inman Valley but relatively high in the arboretum of W.A.R.I. It attacks larvae from 5-8th larval stage. The parasite grub emerges from the host and spins a yellow or whitish cocoon which adheres to the host which is destined to die. Only one individual develops in each host.

Casinaria sp. attack 5-8th instar larvae. The grub emerges before the prepupal stage of the host and soon spins a greyish cocoon with black markings near the edges. Death of the host occurs at the time of parasite emergence and both parasite and host may adhere to the surface of leaf for a long period.

The ectoparasite, Trichoplectrus sp. attacks early larval stages (1-4 instars). The attacked larvae usually do not moult again, and they become sluggish and turn brown in colour before death.

Oechalia schellenbergii and Cermatulus nasalis nasalis are two of the more abundant predators of Uraba lugens. They attack late larval stages. There was a high incidence of the two species during a minor outbreak on redgum at Inman Valley in the winter generation, 1976. Prior to this they had been encountered only sporadically in the field.

Other species of parasites, predators and all the pathogens were of relatively minor importance during these studies, however, the dominating influence of Aspergillus flavus during the outbreaks of the gum leaf skeletonizer in the redgum forests of the Murray Valley region (Campbell, 1962) and that of the crab spider, Philodromus sp. during the outbreaks in Queensland (Brimblecombe, 1962) indicate that natural control agents may assume different roles under different phenological and environmental conditions.

3.4.2 Food

Apart from climatic factors and the natural enemies, a third factor

that may exert a major influence on biology is food. Food exerts its influence through both chemical and physical characteristics of the plant, the former including nutritive substances as well as compounds commonly referred to as 'secondary plant substances'. This complex of substances alone or in conjunction with the physical features of the plant may affect the biology of the insect to a smaller or larger extent. Comprehensive studies of the ways in which the physical and chemical components of food affect the biology of Uraba lugens are discussed in subsequent chapters.

3.4.3 The effect of density of larvae on the biology of Uraba lugens

1. Introduction

The effect of population density of lepidopterous larvae on morphology, physiology and behaviour has been extensively reported (Long and Zaher, 1958, 1960; Zaher and Moussa 1961; Hanneberry and Kishaba 1966; Rivney and Meisner, 1966; Hodjat, 1970). The effects of rearing density on survival, rate of development, growth and adult morphometrics were examined for Uraba.

2. Materials and Methods

One egg batch of approximately 400 eggs was reared until eclosion was completed. The newly hatched larvae were randomly allocated to five density groups - 2, 4, 8, 16 and 32 larvae per 250 ml plastic vial each fitted with a snap-on perforated lid. A damp filter paper 9 cm in diameter was placed at the bottom of each dish. All larvae were reared on excised leaves of Eucalyptus maculata, the egg host. New foliage was introduced as required to prevent dispersal of larvae. The vials were kept clean by removing faeces and changing filter papers daily. All rearings were done at $25^{o}C \pm 1^{o}C$, a relative humidity of 50% $\pm$ 10% and in a 16 hr photoperiod. There were 6 replicates of each treatment. Larval mortalities, rate of

development, pupal weights and adult morphometrics were recorded.

3. Results and Discussion

(i) Effect of density on mortality

Campbell (1962) had previously recorded low survivals of about 7% in what he termed a crowding experiment. A number of records over about 20 generations (Morgan and Cobbinah, 1978) have shown that most larval mortality occurs in later stadia. The results obtained in the present investigation tended to confirm these trends (Table 3.4.3.1) and supported the view that development of early gregariousness was advantageous to survival in this insect. For example 50% mortality occurred by the fifth week in the lowest density replicates compared with <16% in the highest density groups. In later stadia the mortality trends of the instars was reversed, such that about 60% of the total mortality occurred in the two highest densities after the 8th week of development. This was about twice that recorded in the two lowest densities for the same period. All larvae dying in the course of this study were examined to determine the causes of death. The causes of death of a large number of early instars particularly in the low density groups were not due to pathogens or other clear factors. The caterpillars appeared to die of "starvation" brought about possibly by changes in innate physiological and behavioural processes resulting from the absence of natural gregariousness. Deaths due to such causes were few in those groups where aggregation occurred from the outset; in these deaths, particularly from the eighth stadium onward, were invariably associated with the pathogenic fungi Aspergillus flavus, Chaetomium sp. and Beauveria bassiana. The comparatively higher mortalities due to pathogens in the high density groups would be expected as frequent body contacts may enhance transmission of diseases. Infection by pathogenic diseases is enhanced by particular ranges of temperature and

TABLE 3.4.3.1 Effect of density on larval mortality where food and climate were optimum.

Relative Density*	Cumulative percent mortality (average of six replicates) by week after beginning of experiment:-											
	1	2	3	4	5	6	7	8	9	10	11	12
2-Larvae Group	0	25	25	25	50	50	50	50	50	50	75	75
4-Larvae Group	0	0	12.5	25	25	37.5	37.5	37.5	37.5	62.5	62.5	62.5
8-Larvae Group	0	12.5	18.75	18.75	25	25	43.75	56.25	68.75	81.25	81.25	81.25
16-Larvae Group	0	3.12	6.25	12.5	15.62	18.75	21.87	28.12	37.5	71.87	81.25	87.5
32-Larvae Group	3.22	9.37	9.37	9.37	10.93	15.62	21.87	34.37	54.68	71.87	84.37	95.31

* number of larvae in a 250 ml vial.

high relative humidities. High humidities prevailed in all treatments due to the comparatively small containers containing damp filter papers, fresh leaves, faeces and active larvae. However, greatest mortalities in field populations occur over the same stadia, namely those after the caterpillars have segregated. The majority of rearings completed over more than 20 generations under laboratory conditions have also demonstrated this trend (Morgan, pers. comm.). Under the conditions of rearing in these studies, crowding could have contributed to the vulnerability of larvae in the highest density groups to the quantities of spores ingested or contacted. In conditions of constant space, crowding effects will increase as the insects grow.

(ii) Effect of density on developmental rate of Uraba

The effect of larval density on rate of development is shown in Table 3.4.3.2. The mean number of days taken for development decreases as

TABLE 3.4.3.2 Effect of density on rate of development.
(Host: E. maculata*)

	Relative Density**				
	2	4	8	16	32
Mean larval period (days)	88.5	83.9	81.3	77.0	76.9
Range	61-119	67-102	52-103	61-109	57-92
Standard error	± 11.9	± 6.5	± 4.5	± 3.5	± 2.0
Mean pupal period (days)	10	16	17.8	18.5	20.8
Range	-	9-20	10-22	16-23	14-24
Standard error	-	± 2.5	± 2.2	± 0.9	± 2.3
Mean larval plus pupal period	98.5	99.9	99.1	95.5	97.7

* Eggs laid upon this species

** Number of larvae in 250 ml vial : c/f Table 3.4.3.1

the number of larvae per 250 ml plastic vial increases, but the pupal period was significantly longer in high density groups ($P < 0.02$). It appears a longer larval period is compensated for by a shorter pupal period. The larval plus pupal periods, therefore were not significantly different and thus were not affected within the range of densities examined. Hodjat (1970) also found no significant difference in the larval and pupal period in different densities of Spodoptera littoralis though significant differences were evident in the larval and pupal developmental rates among the treatments. The greater variation in larval developmental time (see Table 3.4.3.2) in the lower density groups may be due to absence or lower 'communal stimulation', a behavioural mechanism normally initiated by few members within gregarious groups which directs all members within the group to feed, rest or move to a new foliage. Through such unique forms of communication the groups tend to be physiologically and developmentally uniform.

There was no significant difference in the pupal weight but this result may have been affected by the smaller number of larvae that pupated. The important feature of pupal weight in Uraba lugens is its relationship to fecundity (see Chapter 5). Female pupal weight is positively correlated with the number of eggs in the ovaries. When females emerging in various treatments were dissected and their eggs counted, the means were: 262 for the 2-larval density group, 318 for the 8-larval density group, 453 for the 16-larval density group and 294 for the 32-larval density group. No female emerged from the 4-larval treatment. Because of the small sample size, the degree of significance of these differences in number of eggs were not determined but the trend appears to support the survival data in relation to the advantages of moderate larval densities and gregarious behaviour for the gum leaf skeletonizer.

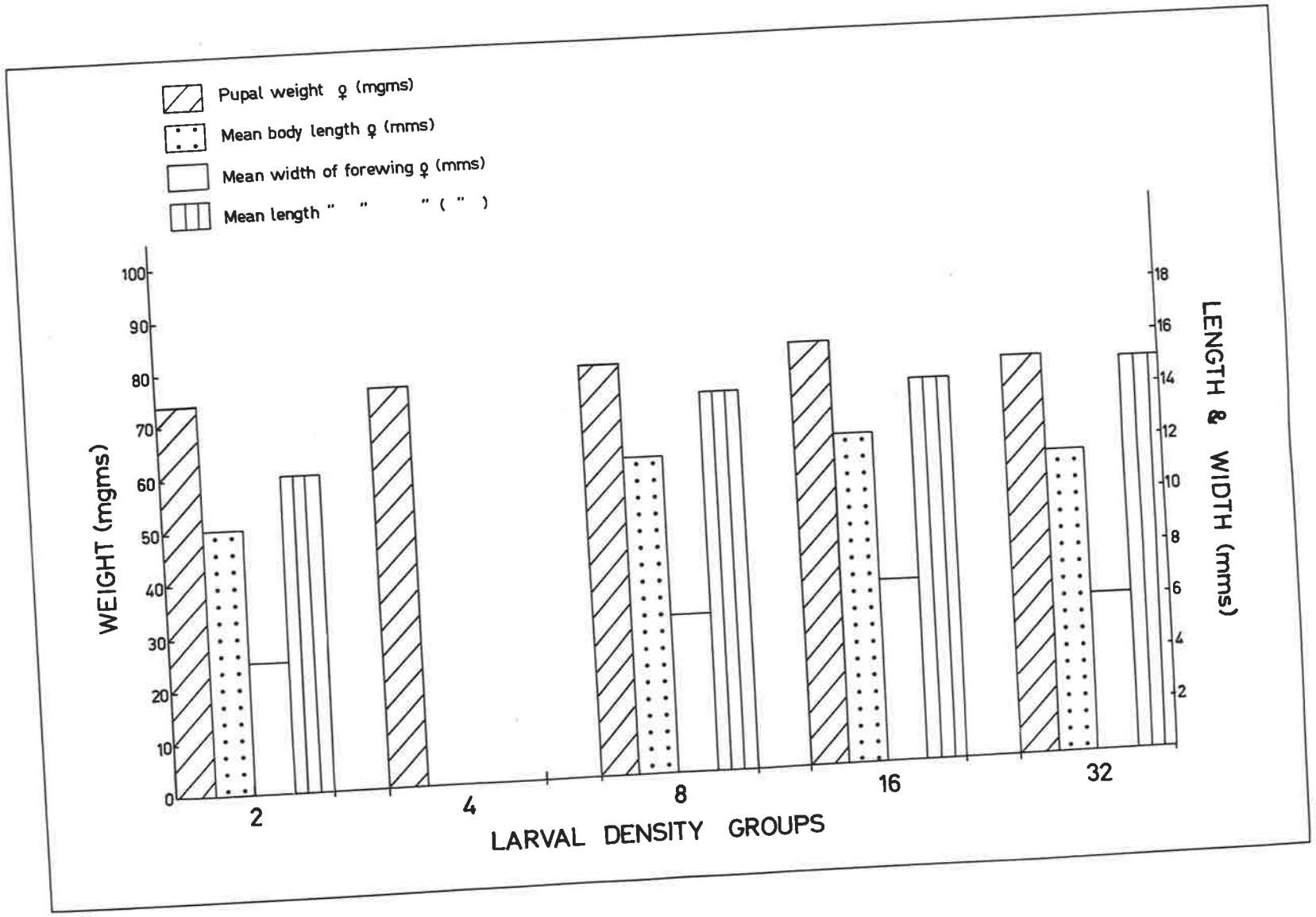
(iii) Effect of density on adult morphological characters (Morphometrics)

The morphometrics of pupae and moths obtained from the various treatments are shown in Fig. 3.4.3.1. As in the other subsections, the 16-larval cultures seem to show slightly higher values for the characters measured. The relatively higher number of eggs recorded for this group, and slightly shorter larval plus pupal development time together indicate that under the conditions of this experiment this group represents a near optimum larval density. There was no distinct colour pattern associated with the degree of crowding as recorded for some insects (Cobbinah, 1971; Hodjat, 1970) though subtle differences were evident in all treatments.

Density affects animal and plant populations in a variety of ways. Density effects resulting either directly or indirectly from co-actions between the group components, are influential in affecting population growth (Allee et al., 1949). Responses such as the post embryonic development of insects, the growth of individual organisms, rate of oxygen consumption, protection from environmental poisons, resistance of marine forms to hypotonic sea water, determination of sex in certain organisms and morphological expressions such as development of wings by aphids and initiation of phases in locusts are well-known examples of the influence of high population density on species.

Although the small number of larvae completing development precluded statistical analysis of some of the present results, nevertheless, the overall consistency of the trends clearly demonstrated advantages and disadvantages of gregarious and crowded conditions respectively. Whilst 8-16 per container could be considered advantageous, 32 represented over-crowding and 2-4 appeared to be somewhat below the threshold for aggregation effects natural to Uraba. It was evident that the gregariousness of the

Figure 3.4.3.1 Effect of crowding on pupal weight and certain adult morphological characters.


Pupal weight ♀ (mgms)
Mean body length ♀ (mms)
Mean width of forewing ♀ (mms)
Mean length " " " (")
WEIGHT (mgms)
100
90
80
70
60
50
40
30
20
10
0
18
16
14
12
10
8
6
4
2
LENGTH & WIDTH (mms)
2
4
8
16
32
LARVAL DENSITY GROUPS

first to fifth larval stages had a survival value and also enhanced rate of development.

Some of the disadvantages thought to be associated with gregariousness are (i) attraction of parasites and predators and (ii) easy transmission of diseases. For instance, some parasites such as Tachinomyia sp. which attacks spruce budworm, are more attracted to clusters than to isolated larvae (Morris, 1955). However, because the aggregations of U. lugens begin to disperse before most of the natural enemies appear in the field, it is expected that this situation may be avoided to some extent. It is not known whether the dissolution of the aggregations in the fifth larval stadium is advantageous to the parasites and predators or to the host. It may be advantageous to the parasites and predators if preference is shown for larger larvae or the reverse if the dissolution of the aggregation results in more diffuse signals from host to predator and perhaps fewer contacts between them.

Over-crowding may occur in the field during outbreaks as was observed in the winter generation, 1976 at Inman Valley. This condition may manifest itself only during later stages of larval development and rates of development and mortality ~~are~~ similar to those recorded in the 32-larvae treatments (Table 3.4.3.1 and 3.4.3.2) were observed. Over-crowding is normally harmful to species both under natural and experimental conditions. The effects usually increased mortality, decreased growth rate and reduced fecundity. Accumulation of excreta and their decomposition products may assist natural control factors as could induction of more frequent movements from host to host as severe defoliation occurs. Decreased larval growth rates and the subsequent reduced fecundity of adults may be due to scarcity of food either through the higher larval/food ratio or the periods without food when migrating to new resources. Another

factor may be the change in quality or age of foliage brought about by defoliation and the subsequent effect lower quality may have on development and survival.

In summary it would appear that the mortality trends in populations of Uraba lugens may vary according to whether early instars are able to develop particular aggregations, while later instars may be affected adversely by segregation, mobility or dispersive behaviour and voraciousness making them contact and ingest more pathogenic organisms. Though such early deaths are not recorded in natural aggregations the trends in mortality for late instars are a feature of natural populations.

CHAPTER 4. OVIPOSITION STUDIES

4.1 General Introduction

Uraba lugens (Walk) lays eggs on eucalypts and related genera, such as Angophora and Tristania (Campbell 1962, 1966; Brimblecombe, 1962) but the larvae do not necessarily survive on all species on which the females deposit their eggs (Morgan and Cobbinah, 1977). The young larvae rarely move more than a few millimetres from the egg site and this indicates that host selection is mainly a function of the females in selecting oviposition sites.

Accordingly field and laboratory studies were carried out to examine this aspect of the biology of the insect.

4.2 Host selection (arboretum studies)

4.2.1 Study area

This study was conducted in the arboretum of the Waite Agricultural Research Institute. The area is virtually frost free with its exact location 34° 58'S : 138° 38'E and altitude about 105-122 metres above sea level. Mean annual rainfall and temperature are 24.48" (609.6 mm) and 61.4°F (18.4°C) respectively. The trees are sited on 3 blocks referred to in this study as Urrbrae, Dam and Fullarton. These 3 blocks have one of the most extensive collections of Eucalyptus species in existence. The original vegetation was an open savannah of E. odorata, E. leucoxylon and E. camaldulensis; a few of which still remain. The soil type is grey sandy loam. Because of differences in date of planting and inherent variation in growth among species, there exists a wide range of sizes and shapes; but over 98% of the species surveyed during this study had branches within 3 metres of the ground.

4.2.2 Methods

Five hundred and eighty trees representing species of Eucalyptus, Angophora, Acacia and Tristania were inspected regularly during six consecutive generations of Uraba lugens between 1974 and 1977. Another 440 trees of plants representing about 150 genera were examined from time to time. The strong preference shown by ovipositing insects for the basal crown region (Morgan and Cobbinah, 1977) enables most inspections of trees without climbing aids. The crown of each tree was divided into 3 equal sections from base to top and the twigs in the basal sections inspected 3 times at fortnightly intervals after each oviposition period. During the second and third inspections, larval survival was recorded.

4.2.3 Results and discussion

The results of this study are presented in Appendix Table 4.2.3.1 and Appendix Fig. 4.2.3.1a-c. Oviposition was recorded in one or more generations on 174 species of eucalypts and 2 of Angophora representing about 60% of the species regularly surveyed. With the exception of section Micrantherae which was represented by only one species with linear foliage, oviposition was recorded in all eleven botanical sections of the genus Eucalyptus (Blakely, 1965). The nearest neighbour technique (Walloff and Blackith, 1962) was used to determine dispersion of egg hosts in the arboretum. Chi-square analysis gave non-significant results indicating that egg hosts were not clumped. It seems therefore, that selection of a host may not necessarily influence the selection of its nearest neighbour in the same or in subsequent generations.

The differing shapes, growing habits and leaf sizes of egg hosts were variables which were not controlled in this study, and these may have had some influence on the egg-laying behaviour of the female.

Most eucalypts with narrow lanceolate leaves such as E. xanthonema, E. linearis, E. viridis, E. cneorifolia, E. augustissima and E. foecunda were free of eggs and larvae during the survey period. This may be due to relatively small surface areas of the leaves, for where leaves were broad or intermediate in cross-sectional area eggs were always deposited on the broadest section of the leaves. However, the narrow surface available on leaves of E. spathulata did not preclude oviposition on it, thus size and shape alone may not be major factors in the selection of egg hosts by Uraba.

It is clear that the females of U. lugens prefer to oviposit on leaves that are less than 3 metres above ground (Campbell, 1962; Harris, 1974; Morgan and Cobbinah, 1977). As nearly 99% of species compared in this survey had leaves less than 3 metres above ground few species would have been excluded from selection on this count.

The consistent failures of females to oviposit on some species with apparently adequate growth characteristics would indicate that selection is governed either by the presence of repellents, the absence of attractants or the dominance of repellence over attraction in these trees. Such a conclusion is consistent with similar situations examined for the other insect-host relationships (Beck, 1974; Dethier, 1970). The arboretum also contains 440 trees belonging to plant genera other than Eucalyptus and Angophora, some belonging to the same family Myrtaceae and others with no close relationship to these genera. Periodic examinations during the survey failed to locate eggs or larvae of Uraba on them which further suggests that oviposition sites are positively selected by the female and are not the result of chance encounters with certain species.

The choice of Eucalyptus and Angophora by Uraba would be expected if the insect had evolved with its hosts, because these genera are closest

relatives from within the Myrtaceae. Indeed, it has been suggested from comparisons of various characters that the eucalypt subgenera Corymbia and Blakella are more closely related to Angophora than to other subgenera of Eucalyptus (Baker and Smith, 1920; Johnson, 1972).

Appendix Table 4.2.3.1 shows that in some 40% of the egg hosts there is virtually no survival by the end of the fourth stadium. This is discussed in detail in Section 5.1. In general, trees on which there was poor larval survival were not common egg hosts; indeed they attracted eggs in comparatively few generations studied. Notable exceptions were E. gardneri and E. annulata. Examples of lepidopterous insects that lay eggs on species on which their young larvae cannot survive have been commonly recorded (Wiklund, 1973; Dethier, 1940, 1941; Sevastopulo, 1964; Straatman, 1962).

It would appear that the discriminatory ability of females of the gum leaf skeletonizer is not highly developed for selecting larval foodplants since unsuitable larval focdplants are invariably found within the egg hosts of each generation. The artificial nature of the arboretum, where most of these studies were completed, may have influenced the results obtained but this would have been somewhat offset by the interaction of the moth with the arboretum over more than fifty years of its development. Nevertheless, in natural forest other mechanisms of orientation may have made it easier for the females to select suitable host plants. These studies may, therefore, demonstrate evolving newer interactions than those in natural forest areas. The insect is a major pest of young trees in home gardens indicating its successful entry into more organised situations than occurs in wilderness. Whatever the merits of these studies in terms of natural interactions, the results show that larval survival depends upon a high degree of host selectivity by the

female moths and that this selective behaviour is vital for continued survival of U. lugens. They also show that the requirements for oviposition are apparently met by many eucalypts and by at least one related genus, while food requirements are more restricted.

4.3 Host selection (Field cage studies)

4.3.1 Introduction

Free choice has frequently been used to determine behavioural responses of insects to host stimuli. Insects may orient to certain intensities of light, to shapes, humidity, gravity, compass direction and to wind (Roth and Willis, 1951; Henson, 1964; Hovanitz and Chang, 1962; Greene and Morrill, 1970; Weseloh, 1971). It is therefore desirable to design experiments so that all host plants are presented to insects in a way that will randomize the effects of these criteria. No control over the arrangement, sizes and shapes of trees and leaf surface area of trees surveyed in the preceding studies was possible, so a cage study was designed to minimize the possible effects of some of those variables found to influence behaviour of insects selecting oviposition sites.

4.3.2 Methods

Seven Eucalyptus species growing in pots were randomly placed in a 3 x 6 x 3 m nylon mesh cage so that each was represented once in each of the 5 rows. The species were selected as providing a range of suitabilities for oviposition by Uraba. E. camaldulensis (control) E. citriodora, E. gardneri and E. scoparia are often selected for oviposition. E. torquata is selected in some generations while E. lehmannii and E. globulus had not been recorded as egg host previously, although larvae can be reared on them experimentally. Each plant was one metre from

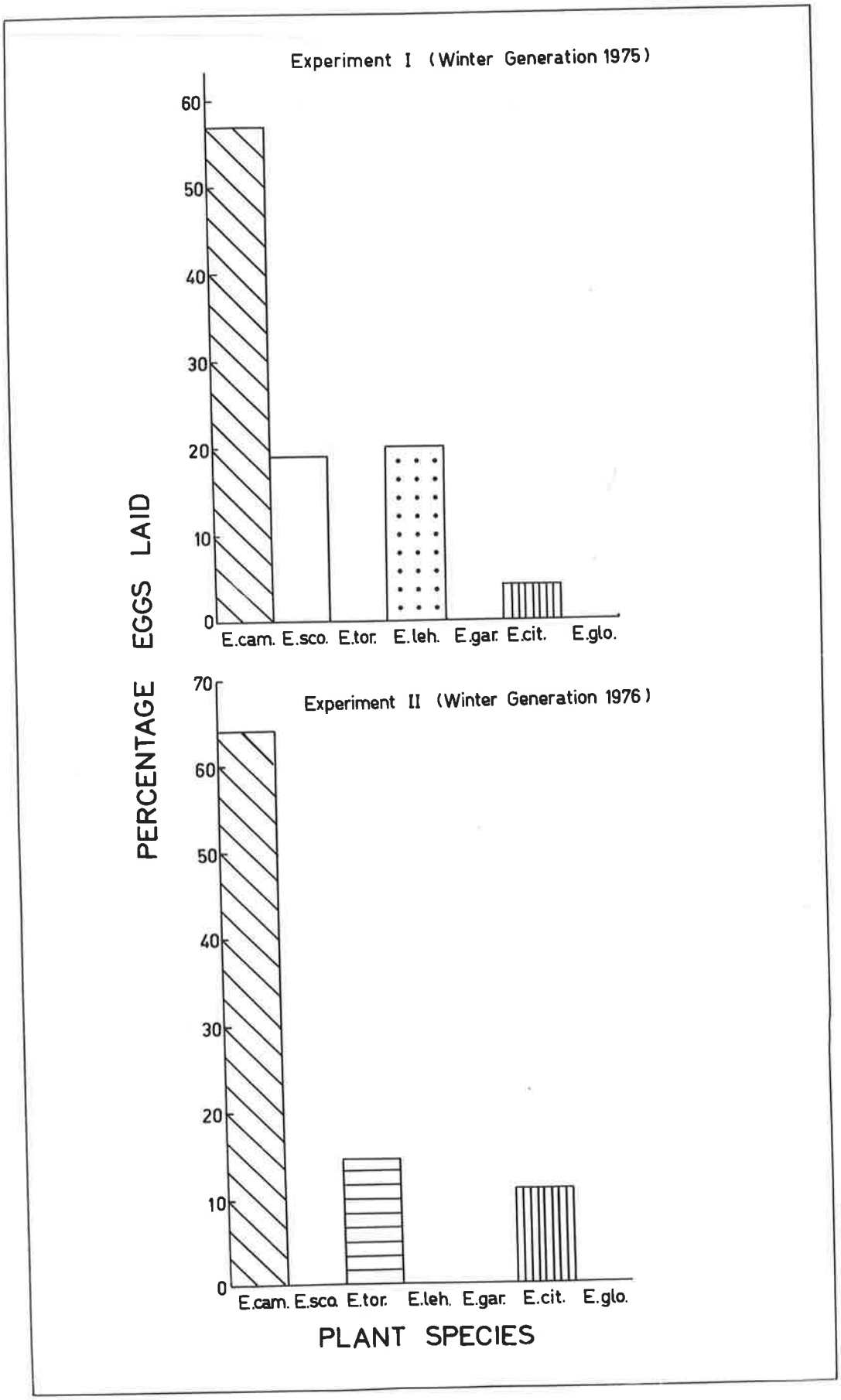
its neighbour and all were trimmed so that their heights and surface areas were approximately the same. Thirty pairs of moths that were obtained from laboratory cultures were released into the cage in October, 1975. The experiment was repeated in October, 1976. In order to test for possible 'host imprint', groups of 5 pairs of pupae, one group reared on each of the 7 eucalypts under test, comprised the 35 pairs used in October, 1976. In both experiments the egg masses were located and counted following deaths of the moths. The number of eggs per Eucalyptus species was used to indicate preference by the female for the oviposition sites.

4.3.3 Results and discussion

In both experiments E. camaldulensis was favoured as egg host on the assessment used (Fig. 4.3.3.1a, b). Some interesting results were among the other selections. E. gardneri a favoured egg host in the arboretum was not selected in either experiment. There seems to be no explanation for this except that E. gardneri is represented in the arboretum by an adult tree whilst all trees used in field cage experiments had juvenile foliage. The term 'juvenile foliage' as used in this thesis refers to a particular form of foliage and tree in its early stages of growth. It should be noted that 'juvenile foliage' nevertheless passes through the same cycle from bud to senescence "young to overmature stages" as the adult type of foliage of mature trees. Leaves of many juvenile eucalypts vary from corresponding adult leaves in both physical and chemical characteristics. Although transition from juvenile to adult foliage may take place very early in the life of the tree e.g. E. camaldulensis, some species of Eucalyptus may have no adult leaves for several years e.g. E. globulus (Penfold and Willis, 1961). Variation in leaf maturity may therefore have influenced the overall results obtained.

Figure 4.3.3.1a Choice of hosts by ovipositing females of U. lugens top - Winter 1975; bottom - Winter 1976.

E. cam. = E. camaldulensis; E. sco. = E. scoparia; E. tor. = E. torquata; E. gar. = E. gardneri; E. cit. = E. citriodora; E. glo = E. globulus.


Experiment I (Winter Generation 1975)
60
50
40
30
20
10
0
E.cam. E.sco. E.tor. E.leh. E.gar. E.cit. E.glo.
PERCENTAGE EGGS LAID
Experiment II (Winter Generation 1976)
70
60
50
40
30
20
10
0
E.cam. E.sco. E.tor. E.leh. E.gar. E.cit. E.glo.
PLANT SPECIES

The number of eggs laid in the two tests represents only a tenth of the potential for the females used which suggests that (i) only 10% of the females laid eggs or (ii) most laid fewer eggs than they could have in more natural conditions. It is possible that the egg laying stimulus is not strong enough in fresh juvenile foliage.

The females oviposit on foliage of both juvenile and mature trees of E. camaldulensis. Larval establishment on this species is very good (see Chapter 5). It is also the most widespread of all eucalypts. Consequently, notwithstanding the problem of interpreting the data obtained solely with juvenile trees, it does at least underscore the preference of Uraba lugens for this species indicated by other kinds of experiments.

4.4 Spatial distribution of eggs

4.4.1 Introduction

Animal populations may be distributed according to 3 broad patterns, namely - uniform, random and clumped (Odum, 1964). Determination of the type of distribution of any insect population and its movement within its habitat are necessary if a real understanding of the nature of this population is to be obtained and especially if overall density is to be correctly measured. The distribution pattern not only affects the sampling programme (Rojas, 1964) and the method of analysis of the data (Southwood, 1966), but it may also provide important information on the innate behaviour of the insect. This study was undertaken to obtain some ecological and behavioural information on spatial distribution of egg masses that could assist subsequent attempts to determine other population parameters.

4.4.1.1 Distribution of eggs in the crowns of trees

Female Uraba lugens prefer to lay their eggs on leaves that are less than 3 metres above ground (Morgan and Cobbinah, 1977). But young eucalypts and many mallee forms of the genus rarely exceed 3 metres in total height.

In the studies reported here, distribution of eggs with respect to crown levels and compass quadrants were determined.

4.4.1.2 Methods

Three small river redgums (E. camaldulensis) and two Illyarie gums (E. erythrocorys) each measuring about 3 metres high were selected for this study. Two methods were used to assess distribution of egg masses on the selected trees. The first was called 'The Handglass Method'; in which all twigs on selected trees were examined with the aid of a hand-glass for egg masses. Egg counts were made using the technique of Harris (1974). The total number of eggs observed on each tree was recorded. The second method was called "damage index". This method relied on signs of larval feeding to assess distribution of the eggs. The crown of each tree was divided into 3 equal vertical parts : terminal, mid, and basal and into 4 compass quadrants. The boundary of each vertical region or quadrant was marked with soft texta pen and edge branches were tied with different coloured tapes. There were varying numbers of twigs in the different regions.

4.4.1.3 Results and discussion

Crown Levels

Both methods showed that the insect exhibited strong preference for the basal third of the crown (see Tables 4.4.1.3.1 and 4.4.1.3.2).

TABLE 4.4.1.3.1 Egg masses distribution obtained by examining foliage of Red Gum for eggs (Handglass Method).

Crown Level	Number of egg batches	Number of eggs	Number of twigs	Number of eggs/twig
Terminal	0	0	353	0
Mid	14	1710	181	10
Basal	66	6103	195	31

TABLE 4.4.1.3.2 Egg distribution obtained by assessment of twig damage.

Crown Levels	E. camaldulensis			E. erythrocorys		
	damaged twigs	undamaged twigs	Totals	damaged twigs	undamaged twigs	Totals
Terminal	12	25	37	2	56	58
Mid	31	3	34	9	36	45
Basal	44	2	46	41	4	45

X^2 analysis of vertical distribution of damaged twigs was highly significant ($P < 0.001$) for the two species of eucalypts. Because the two redgum trees showed similar trends, the results of the two trees have been combined (Table 4.4.1.3.1).

The results of the two methods indicate a high oviposition by females and high survival of larvae on the basal third of the crown. About 78% of all eggs examined were laid at basal crown levels. However, a shift towards utilizing leaves in the mid and to a lesser extent terminal regions of the trees examined was evident in the following generation. This was due to the fact that most of the twigs in the lower crown had lost their leaves through larval feeding. General observations indicated that females prefer the basal twigs and that in

tall trees if these twigs are dead no eggs are laid on the tree. The strong preference for the leaves located in the basal crown region may be due to poor flight capability of female (Harris, 1974) or negative response to light and/or positive response to gravity or air moisture.

The distribution of eggs of many forest insects is influenced by height above ground. Both Neodiprion swainei Middleton (Lyons, 1964) and Choristoneura fumiferana (Clemens) (Morris, 1955) tend to oviposit at upper tree levels, while 60-80 percent of egg masses of Hemerocampa pseudotsugata McD. were found in the bottom half of the crown (Luck and Dahlsten, 1967). With Archips semiferanus, 67-80% of the egg masses were found on limbs and bole sections in the middle half of trees (Ellenberger and Cameron, 1977).

TABLE 4.4.1.3.3 Twig damage in 4 compass quadrants.

Quadrant	Damaged	Undamaged	Total
North	16	13	29
South	14	17	31
West	12	18	30
East	12	15	27
	54	63	117

X^2 analysis of distribution according to compass direction was not significant. It appears the eggs were randomly placed in the 4 quadrants.

Damage index whilst it would be useful in determining larval distribution on large trees does not provide precise information as to the number or distribution of egg masses laid, while larval movement and subsequent feeding could result in damage on twigs on which no eggs were laid. Whilst the 'handglass method' gives a better measure of number and distribution of egg-masses, it is too slow and too difficult

to be of any practical value in quantitative survey work involving big trees.

This study was not undertaken to test sampling techniques. But results indicate that samples taken from basal third of crowns based on damage signs may be adequate for estimating populations and determining population distribution in field surveys of this pest.

4.4.1.4 Egg distribution on leaves

Egg distribution on leaves of different ages, upper (adaxial) and lower (abaxial) surfaces of leaves and on different sections of leaves were recorded to determine whether the age, surface or position on the leaf play any part in the processes of selection of oviposition sites.

4.4.1.5 Methods

In 3 consecutive generations between 1974 and 1975, the age of leaves of mature trees of E. camaldulensis on which eggs were laid were recorded. Leaves were placed in one of the three physiological age classes a) young b) mature c) overmature (senescent). These correspond to 3 classes described by White (1966) as young, mature and old.

a) 'Young' - Soft, flaccid, yellowish to pale green, or incompletely expanded new leaves. Located in terminal region of twigs.

b) 'Mature' - Bright green, flexible, and fully expanded leaves free of blemishes other than occasional early gall formation. Situated in mid-section of twigs.

c) 'Overmature' - These are thick, yellow green to dark green, rigid and frequently with necrotic and discoloured patches. They are generally situated at the base of twigs.

To obtain information on relative abundance of the three age groups

of leaves, 30 twig samples were collected mainly from the basal crown levels of a redgum tree. The twigs were taken to the laboratory where the proportion of each of the 3 groups (young, mature and overmature) was recorded.

The distribution of eggs relative to lower and upper surfaces of leaves were also recorded. Due to twisting of petioles during development in some eucalypts, it is not uncommon to find the original lower surface directed upwards, however, in most species the lower surface with its slightly prominent midrib could be recognised even where petioles were twisted.

Finally, the distribution of eggs according to positions on the leaf were determined by dividing each leaf arbitrarily into 3 equal (lengthwise) sections and recording eggs on each third separately.

4.4.1.6 Results and discussion

TABLE 4.4.1.6.1 Distribution of eggs according to the age of leaf.

Age class	Total number of eggs	% total no. of eggs	% relative abundance of leaves (a)	% number of egg batches (b)	Proportional distribution (b)/(a)
Young	4,695	5.8	8.7	6.4	0.74
Mature	57,713	71.8	68.3	71.1	1.04
Overmature	18,012	22.4	23.0	22.5	0.98

Table 4.4.1.6.1 shows that relative to abundance of the various age groups of foliage, the females seem to show preference for mature foliage. A proportional distribution (b)/(a) values above or lower than one reflected greater or lesser preference for each group.

TABLE 4.4.1.6.2 Egg distribution on Lower and Upper surfaces of leaves.

Leaf Surface	Number of egg batches	Total Number of eggs	Percent total of eggs	Mean number of eggs/batch
Upper	124	12,303	32.5	98.9
Lower	237	25,556	67.5	107.8

About twice as many egg batches and eggs are laid on lower surface of leaves. The female may be reacting to some feature of the lower surface of the leaf. The only conspicuous physical difference of the 2 sides of leaf is the slightly more prominent midrib on the basal side of the lower surface. White (1966) asserted that the midrib acts as a tactile oviposition stimulant for the lerp, Cardiaspina densitexta Taylor. He went further to show a direct relationship between oviposition stimulus and the size of the midrib relative to the thickness of the leaf blade. Tactile stimulus may play a relatively minor role in the oviposition responses of the gum leaf skeletonizer, because the female freely lays eggs on smooth surfaces such as glass petri dishes, plastic cannister jars, wooden frames of cages, paper towels and even concrete walls in laboratory studies.

It seems possible that the preference which the females showed for laying eggs on the lower surface of horizontal leaves may be due to a combination of factors. Perhaps, the females prefer to hang upside down during oviposition. The choice of lower surface of leaf and lower crown level (see above) may be caused by the adult preference for higher air moisture as well as positive geotaxis. The response to gravity starts late in the larval period, when all larvae move down trees to find pupation sites on the ground, loose bark, forest litter and lower surfaces of

lowest branches. A negative response to light could also explain why the female deposits her eggs on the shadier lower surface and branches in the lower crown level. These views are further supported by the fact that in nearly 80% of the cases in which eggs were recorded on upper surfaces, the leaves were found either hanging vertically or due to the characteristic petiole twisting of some eucalypts, the morphologically upper surface was directed towards the ground. Mansour (1976) showed that Aphidoletes aphidomyza prefers to lay eggs on leaves exposed to dim light, and at higher light intensities females only laid on lower surfaces of the plant leaves.

TABLE 4.4.1.6.3 Egg distribution on different sections of leaves.

Site of oviposition	Number of egg batches	Percent of total	Total number of eggs	Percent of total
Basal	181	50.14	19,847	52.40
Mid	139	38.50	13,466	35.46
Tip	41	11.36	4,588	12.04

The data in Table 4.4.1.6.3 show that a majority of egg batches and eggs were laid on the basal section of each leaf and the number of egg batches and eggs decreased with increasing distance from the petiole. It is possible that the firmness and the big surface area of the basal section may be responsible for the choice of this area for deposition of eggs. On broad leaved species such as E. polyanthemos and E. baxteri egg batches are commonly laid on the mid and terminal sections of leaves. It appears from the nature of the egg mass that a broad flat area would be a more appropriate site upon which to produce the consecutive parallel lines of eggs.

4.5 Effect of host plant chemicals on egg-laying

4.5.1 Introduction

Appendix Table 4.2.3.1 shows that members of both Eucalyptus and Angophora which show apparent physical differences such as rugosity, pubescence and texture are suitable for Uraba lugens to lay its eggs. A most likely explanation for this is the existence of similar chemical stimuli presented by the leaves of these different trees. The following studies extend the analysis of oviposition behaviour of Uraba lugens by examining the role of some of the chemical constituents of species of Eucalyptus and Angophora.

4.5.2 Materials and methods (General)

4.5 litre plastic cannister jars were used as oviposition chambers and held at $25^{o}C \pm 2^{o}C$. Each cannister jar was partitioned into 2 chambers with a wire gauze. Paper towel was hung on the wall of upper chamber of cannister jars to serve as the oviposition substrate. Test substances and water in 150 ml plastic vials were placed in the lower chamber. Mated or paired moths were released into the upper chamber and allowed to mate and lay eggs.

4.5.2.1 Test I - Effect of crude leaf homogenate

Crude leaf homogenate was prepared by blending 10 gm of fresh leaves of E. camaldulensis in 100 ml distilled water. This was placed in the lower chamber of test unit together with a vial containing water. The standard unit had a vial containing only water whilst the control had neither homogenate nor water. There were 3 replicates of each treatment and homogenates were changed after every two days. Two pairs of adults were introduced into the upper chamber of each test unit and egg counts were made after all female moths were dead.

4.5.2.2 Test II - Effect of homogenates of 'egg host' and 'non egg host'

The preparation of homogenates and experimental set up was as described above. Mature foliage of Eucalyptus camaldulensis (egg host) and Harpulla pendulla (non egg host) were used for preparation of homogenates.

4.5.2.3 Test III - Effect of leaf homogenates of various species

Leaf homogenates of seven eucalypts: E. camaldulensis, E. torquata, E. lehmannii, E. scoparia, E. gardneri, E. citriodora and E. globulus were tested. Preparation of homogenates and setting up of the test units were as described in Test I except that vials containing water were omitted in this study. Humidity in the room was maintained at 75 ± 5% with humidifiers and there were 3 replicates of each test.

4.5.2.4 Test IV - Effect of major essential oils of Eucalyptus and Angophora

The following substances were tested as oviposition stimulants as there is evidence that they occur in host plants (Baker and Smith, 1920; Penfold, 1965; Pryor and Bryant, 1958; Willis, McKern and Hellyer, 1971). The substances were: crude leaf distillate of E. camaldulensis, Cineol (Eucalyptol), Pinene mixture (α and β), Limonene, Camphene, and Caryophyllene. They were presented in aqueous emulsions at 0.25% concentrations -- Experimental conditions and set up as described for Test III.

4.5.3 Results and discussion

Test I. The results of Test I are presented in Table 4.5.3.1.

TABLE 4.5.3.1 Effect of leaf homogenate of E. camaldulensis on egg-laying.

Treatment	Number of egg batches	Number of eggs	Mean number of eggs/treatment ± s.e.
Dry chamber (control)	1	43	[a] 14.3 ± 14.3
Water (standard)	4	321	[b] 107.0 ± 6.0
*Water+Leaf homogenate	6	803	[c] 267.7 ± 29.2

* Leaves obtained from 3 yr old E. camaldulensis tree

Analysis of variance of transformed data ($\sqrt{x}$) showed a significant difference among treatments ($P < 0.001$). Comparisons of treatment means by L.S.D. showed that all the three treatments differ from each other. The results therefore suggests that water (moisture) plays a role in releasing the oviposition behaviour of U. lugens. This confirms the findings of Morgan and Cobbinah (1977). Water has also been found to promote oviposition in the cucumber looper, Anadevidia peponis (Fabricius) (Ichinose and Sasaki, 1971) and Pieris rapae (Hovanitz and Chang, 1964). However, the significant increase in number of eggs laid in test units containing leaf homogenate suggests that chemical factors from the plant provide the key stimuli for egg-laying. As the test units bear no resemblance to plants and the gravid females were not in physical contact with substances tested, it seemed logical to infer that the oviposition behaviour was induced by odoriferous substances present in the plant.

Test II. The results of test of homogenates of an egg host, Eucalyptus camaldulensis and a non egg host, Harpulla pendulla are given in Table 4.5.3.2.

TABLE 4.5.3.2 Effect of leaf homogenates of E. camaldulensis and H. pendulla on egg-laying.

Treatment	Number of egg batches	Total number of eggs	Mean number of eggs/treatment
E. camaldulensis	8	613	204.3 ± 16.2
H. pendulla	0	0	0

The gravid females failed to lay eggs in treatments containing leaf homogenates of H. pendulla. The results suggest that H. pendulla may contain oviposition repellents of some sort since oviposition chambers containing water alone elicit oviposition (see Table 4.5.3.1).

Test III. The results of a test of effect of leaf homogenates of various species of eucalypt in inducing egg-laying are presented in Table 4.5.3.3.

TABLE 4.5.3.3 Effect of leaf homogenate of seven eucalypt species on egg-laying.

Treatment†	Number of egg batches	Number of eggs	Mean number of eggs/treatment ± s.e.	Treatment means of transformed data ($\sqrt{x}$)*
E. globulus	0	0	0	0^{d}
E. camaldulensis	10	587	195.7 ± 50.6	13.7^{b}
E. scoparia	1	15	5.0 ± 5.0	1.3^{d}
E. lehmannii	11	729	243.0 ± 74.4	15.1^{ab}
E. torquata	6	366	122.0 ± 91.0	8.4^{c}
E. gardneri	18	1118	372.6 ± 69.1	19.1^{a}
E. citriodora	2	410	136.7 ± 120.5	8.5^{c}

† All leaves were collected from 3 year old trees

* Groups with the same letter are not significantly different.

Analysis of variance was done on transformed data ($\sqrt{x}$) due to high variances within treatments. The results showed a significant difference ($P < 0.01$) among treatments. The L.S.D. for the transformed data was 5.066 showing significant differences between E. gardneri and E. camaldulensis, E. citriodora, E. torquata, E. globulus and E. scoparia. E. lehmannii and E. gardneri were not significantly different from each other as were E. lehmannii and E. camaldulensis. The relative number of eggs laid in the different treatments were not comparable with numbers laid on intact plants in the field cage (see Fig. 4.3.3.1 a,b). A laboratory test of plant homogenates seemed, therefore, not to be a direct test of the chemostimulation afforded by the constituents of the plant. Perhaps some volatile constituents are lost during the process of preparation of homogenates or that homogenizing results in changes in concentration of substances which affects the responses of females. In general only about 50% of the females used in this study laid eggs, although, this figure was an improvement on the proportion that laid eggs in the field cage. Perhaps homogenizing increased the perception of oviposition stimulants which are probably present in low concentrations in juvenile leaves. Eggs were laid in all treatments except those containing E. globulus. In both the arboretum studies (Table 4.2.3.1) and the field cage test (Fig. 4.3.3.1), E. globulus failed to elicit egg-laying. It appears, therefore, as if E. globulus lacks the constituent that elicits oviposition, or possesses an oviposition inhibitor of some sort.

Although E. gardneri was not selected for egg-laying in the field cage test, it was one of the favoured species in the arboretum (Table 4.2.3.1), it is therefore not entirely surprising that more eggs were laid in test units containing homogenate of this species.

Test IV. Table 4.5.3.4 shows that egg-laying stimulus was strongest

in test units containing leaf homogenates ($P < 0.001$).

TABLE 4.5.3.4 Effect of major volatile constituents of eucalypts on egg-laying.

Treatment	No. of egg batches	Number of eggs laid	Mean number of egg/treatment ± s.e.	Treatment* means of transformed data ($\sqrt{x}$)
†Leaf homogenate	26	2068	517 ± 36.78	22.7^{a}
Steam distillate	11	1078	269 ± 56.73	16.1^{b}
**Pinene (mixture)	15	1261	315.3 ± 57.06	17.5^{b}
Cineol	7	275	68.75 ± 15.58	8.1^{c}
Camphene	3	92	23.0 ± 14.98	3.3^{de}
Caryophyllene	0	0	0	0^{e}
Limonene	3	173	43.0 ± 15.55	5.9^{ed}
Water standard	19	1334	333.5 ± 79.84	17.8^{b}

* Groups with the same letters are not significantly different

** Pinene mixture - is a mixture of α and β Pinene.

† Leaves were obtained from mature (> 30 yr old) E. camaldulensis tree.

The mean number of eggs laid per female in test units containing leaf homogenates was 258.5. This was close to the mean number of eggs in females of the winter generation, (see Appendix Fig. 5.3.3.2). The mean number of eggs per female in test units containing juvenile foliage of E. camaldulensis (see Table 4.5.3.3) was 97.8, about a third of the number of eggs in females in the winter generation 1977, suggesting that the foliage of juvenile trees are less effective in eliciting oviposition response.

Steam distillate of foliage and Pinene mixture stimulated about similar amounts of egg-laying which was not significantly different from that of water. Although water or high moisture has been shown to promote egg-laying in U. lugens, the evidence indicates that chemical factors provide the key stimuli. On the basis of the present results, it appears that the Pinene mixture and the steam distillate had no effect since they

did not differ significantly from the water treatment, however, the concentration of substances used might have affected the results. All the pure chemicals were tested at 0.25% concentration. The results also indicate that Limonene, Cineol, Camphene and Caryophyllene significantly inhibit oviposition at concentrations tested under the conditions of the experiment. Response to terpene solutions in bioassay is difficult to correlate to natural levels encountered in vivo (All et al., 1975), however, some basic understanding can be derived by comparing larval response to terpenes tested at the same concentration.

4.6 General discussion

The overall studies suggest that the females do show some degree of discrimination in selection of host and oviposition sites. Basal crown level and underside of leaves are preferred to upper crown levels and inner surfaces of leaves. Among the age classes of trees and leaves, juvenile trees and young foliage are infrequently selected. Basal third of leaves are preferred in narrow-leaf species, but, compass directions of tree crowns do not seem to influence selection within a tree crown.

The two prominent factors, therefore, appear to be physical and chemical factors arising in part from the age and position of a leaf or branch within crown and in part from the nature of the leaf. There are numerous examples in the literature showing that chemical rather than physical properties of host plants are the main stimuli for host selection (Schoonhoven, 1968; Fraenkel, 1969; Grison, 1958; Traynier, 1965; Gupta and Thorsteinson, 1960; Applebaum et al., 1965). This was confirmed in this study. It has also been demonstrated that several chemicals together are responsible for selection and oviposition preference (Gupta and Thorsteinson, 1960; Matsumoto and Thorsteinson, 1968; Carlisle et al.,

1965; Perry and Fay, 1967). In the study reported above the leaf homogenate stimulated significantly more egg-laying than did pure volatile constituents. This seems to indicate that perhaps a complex of 'signature' of odoriferous substances are involved in inducing egg-laying.

It appears that the relationships between U. lugens and its host plants are based on 3 major responses of the insect:- (i) a positive response to a common stimulus present in a majority of Eucalyptus and some Angophora (see Table 3.2.3.1) (ii) a positive response to a more specific stimulus which directs the adult females to certain preferred species e.g. E. camaldulensis (iii) a negative response to inhibitory substances or perhaps inhibitory concentrations of some substances which enables females to avoid plant species outside its 'egg host range'.

CHAPTER 5. FOOD PLANT ACCEPTANCE AND SUITABILITY FOR LARVAL DEVELOPMENT

5.1 Larval acceptance of foodplants

5.1.1 Introduction

Food selection in U. lugens (Walk) is the primary role of the adult. The egg hosts, however, include highly suitable foodplants as well as Eucalyptus species that militate against larval establishment and survival (Morgan and Cobbinah, 1977). Acceptance of any egg host by larvae normally takes place soon after eclosion and involves behaviour patterns from ready acceptance and mass feeding at one extreme to feeding that is not sustained after initial 'tasting' followed by dispersion of the larval group and no further attempts to feed at the other. The degree of acceptability of any potential host is presumably determined by the physical and chemical characteristics of the host either in combination or alone.

The following study was designed to measure the degree of acceptability of various potential and actual foodplants of Uraba lugens.

5.1.2 Materials and methods

The acceptability of various plants to Uraba lugens larvae was determined by observing larval establishment and survival to the end of the fourth stadium of larvae ecloding on egg hosts and some selected plants in the arboretum of W.A.R.I. For the non egg-hosts selected for this study, egg masses were attached to mature leaves. A total of 192 plant species belonging to 10 genera and 7 families were examined.

5.1.3 Results and discussion

The data in Appendix Table 4.2.3.1 columns (a) and (b) and

TABLE 5.1.3.1 Larval establishment and survival to fourth instar for *Uraba lugens* (Walker) on some non-egg hosts related to *Eucalyptus camadulensis*.

Plant species	Plant Family	Insect Performance	
		Establishment	Survival to 4th instar
Eucalyptus socialis	Myrtaceae	+	●
Eucalyptus rariflora	"	●	-
Eucalyptus decipiens	"	+	●
Eucalyptus pressiana	"	-	-
Eucalyptus leptophleba	"	●	-
E. cinerea var. *multiflora*	"	●	-
Eucalyptus obliqua	"	+	●
Eucalyptus carnei	"	●	-
Eucalyptus staigeriana	"	●	●
Eucalyptus camaldulensis	"	++++	+++
Acacia pycnantha	Mimosaceae	-	-
Dysoxylon fraseranum	Meliaceae	-	-
Arbutus unedo	Ericaceae	-	-
Prunus lusitanica	Rosaceae	++	●
Syzycium paniculatum	Myrtaceae	-	-
Angophora floribunda	"	+	+
Ceratonia siliqua	Caesalpinaceae	-	-
Harpulla pendulla	Sapindaceae	-	-

\+ = 25% survival

● = few survivors usually less than 15% of total eggs that hatched

\- = 100% mortality

Table 5.1.3.1 summarizes the degree of acceptability of different plants to the larvae of *Uraba lugens*. Appendix Table 4.2.3.1 has been presented according to the classification proposed by Blakely (1965). In general, there was no discernible relationship between acceptability and botanical grouping. The first instar larvae accept the plants to varying degrees,

ranging from immediate acceptance to rejection even after 24 hr. On about a third of the egg hosts there is little or no survival through the first stadium.

The fact that a plant was accepted by a caterpillar was a poor indicator of its ability to support that larva. While acceptance must precede survival and growth it did not necessarily assure them. Though larval survival and growth were good on highly acceptable plants such as E. camaldulensis, E. moorei and E. scoparia, several other accepted plants proved inadequate in each respect. Survival also did not necessarily indicate a good growth rate (see Section 5.3). Apart from Eucalyptus and Angophora species the Portugal Rose, Prunus lusitanica was the only plant species that was found to be acceptable to some extent. The survival trend for caterpillars on various plant species could be determined by the end of the fourth stadium and before the caterpillars segregated. The trends shown (Appendix Table 4.2.3.1 and Table 5.1.3.1) could be explained by any or all of a complex of factors such as the presence of toxins and feeding deterrents, lack of feeding stimulants, nutritional inadequacy, insufficient consumption, low utilization and physical barriers. Immediate rejection as observed on species such as Acacia pynantha, Dysoxylon fraserum, Ceratonia siliqua, Syzycium paniculatum and Harpulla pendulla tend to indicate that these species contain some toxic substances as the larvae tend to die much faster than do larvae that are simply starving because of lack of any kind of food. All these species were not egg hosts. Although the evidence presented here showed that about 60% of the egg hosts were unacceptable as larval foodplants, it was perhaps, worth noting that in some generations a few larvae have managed to complete development on certain apparently poor foodplants.

Since these observations were made in the field, it is conceivable

that other and non-nutritional factors, such as control by natural enemies, could have influenced the results obtained. If this influence had dominated it probably would have been more noticeable on superior hosts than records show. Indeed, 75% survival at the end of the fourth stadium is common on good host plants and natural control factors are usually recorded as significantly affecting populations older than eighth instars (see Chapter 3).

5.2 Larval selection and food preferences

5.2.1 Introduction

The first instar larvae of Uraba lugens show limited locomotory activity and are therefore committed to accepting foodplants selected by their parents or in default, they starve to death. In contrast the late instar larvae show high locomotory activity in the field and are capable of movement from one young tree to another under conditions of heavy infestations. These conditions, which appear periodically in eucalypt forests (Campbell, 1962; Brimblecombe, 1962; Harris, 1974), thus result in host plant selection by the larvae themselves.

The study reported herein was undertaken to determine the relationship between larval selection and preference and the suitability of selected food to support growth.

5.2.2 Materials and methods

In April, 1975 mature healthy foliage of ten Eucalyptus species was collected from the arboretum of the Waite Agricultural Research Institute. They were selected as representatives of the sections of the genus Eucalyptus based on their essential oils (Baker and Smith, 1920). The species chosen also represented 10 'botanical series' recognized by Blakely (1965). Preliminary survey of the host/insect relationship

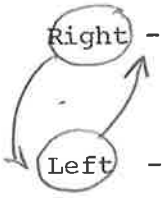
indicated that, of the 10 selected, E. camaldulensis, E. citriodora and E. melanophloia were common foodplants, periodically heavily infested by U. lugens. Moderate infestations of E. cosmophylla and E. punctata had been recorded, whilst E. moorei, E. platypus, E. alpina and E. oreades had not been found infested.

Leaf discs 21 mm in diameter were punched out from leaves of each plant species to be examined. The species were compared in 2-choice tests. Three leaf discs from each of the 2 species to be compared were arranged alternately and equidistant from each other (see Fig. 5.2.2.1). This arrangement provided each test caterpillar with equal chances of encountering either plant even where a first discovery resulted in rejection of one. Each arrangement was replicated six times. To prevent desiccation of leaf discs, the petri dishes were lined with moist filter papers. Six undamaged, active, fifth instar larvae were used for each replicate because it is in this stage of the life history that the larvae begin to segregate and are therefore more likely to make intra- or inter-tree selections. Up to the fourth stadium, caterpillars either accept or reject the foodplant selected by their parent but remain within the aggregation. Two larval release methods were employed.

Test A: One larva was placed on each of the six discs in a petri dish.

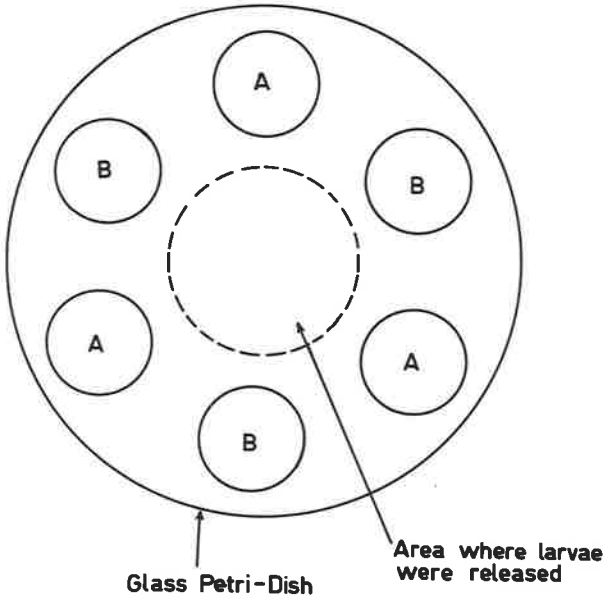
Test B: All six larvae were released in the centre of the petri dish, equidistant from the leaf discs.

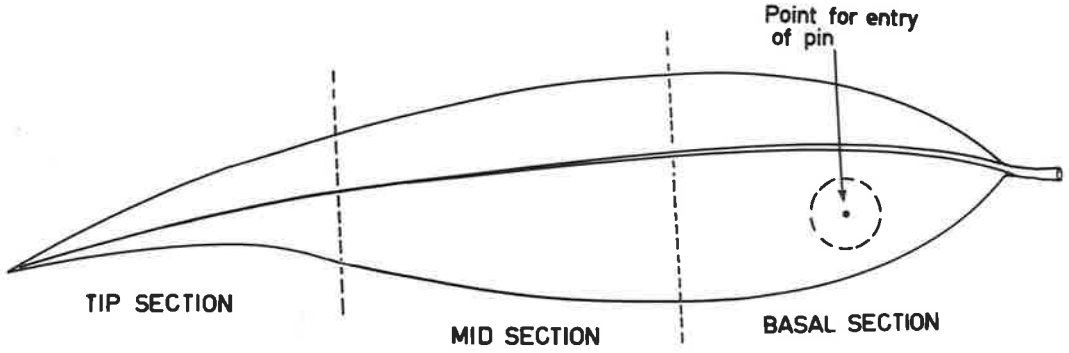
Tests were conducted at 25^{o}C, 60% relative humidity and with a 16 hr photophase. Larval distribution on both leaf discs and filter paper were scored at 15 minute intervals over a 3 hr period. The data were plotted as percentages responding to each plant species. At the end of a 3 hr period, the leftover food was placed on gridded perspex and the area consumed in mm^2 was recorded. Different species of Eucalyptus may

Figure 5.2.2.1 

- leaf discs arrangement in test arena.

- leaf showing the basal, middle and tip sections.


A
B
B
A
A
B
Glass Petri-Dish
Area where larvae were released


Point for entry of pin
TIP SECTION
MID SECTION
BASAL SECTION

vary in the sizes and contents of their leaves so food consumed by caterpillars was also calculated on a dry weight basis using the following formula:

Dry wt. of food consumed (DWC)	=	Estimated dry wt. of food introduced (EDWI)	-	Dry wt. of food leftover (DWL)

The estimated dry weight of each leaf disc was based upon the dry weight of an uninfested check disc. Check and test discs were cut as sister pairs from test leaves and were then assigned to their respective treatments. The magnitude of the difference between the characteristics of the check and test of any disc-pair may be reduced greatly by choosing least variable representatives. In preliminary studies, it was found that for broad leafed species such as E. alpina, discs from left and right halves of the same leaf were less variable than discs from different leaves. For narrow leafed species such as E. citriodora, E. melanophloia and E. intertexta, in which the desired size of discs could not be obtained from either halves of the same leaf, the least variable representatives were discs obtained from nearest neighbour leaves of the same twig (see also Waldbauer, 1966). Each matched pair of discs were placed together and chosen on a random selection of left or right so that each had an equal opportunity of assignment to check or to test roles.

5.2.3 Results and discussion

The results in Table 5.2.3.1 section A indicate that E. platypus, E. alpina, E. oreades are less preferred for feeding by larvae than E. punctata, E. moorei and E. melanophloia. However, E. cosmophylla and E. camaldulensis were not significantly different. E. intertexta was preferred to E. citriodora in Test A but not in Test B. This may be due

TABLE 5.2.3.1 Mean areas consumed and estimated dry weights of food consumed in 2-choice tests.

Host Plant	Mean area consumed in mm^2 ± s.e.		Mean dry weight of food consumed (mg) ± s.e.	
	Test A	Test B	Test A	Test B
Section A				
1. *Eucalyptus citriodora*	6.53 ± 3.00	8.16 ± 1.89	8.66 ± 2.17	9.2 ± 2.82
E. intertexta	23.56 ± 3.64**	5.75 ± 2.63	33.33 ± 8.52	17.98 ± 4.19
2. *E. camaldulensis*	16.3 ± 4.01	11.31 ± 3.70	19.5 ± 4.84	14.7 ± 3.70
E. cosmophylla	22.21 ± 3.24	10.23 ± 2.40	25.33 ± 1.30	21.2 ± 3.64
3. *E. platypus*	0.23 ± 0.23	3.85 ± 1.11	4.0 ± 2.72	12.5 ± 2.20
E. punctata	23.65 ± 5.13**	37.85 ± 3.91***	54.2 ± 9.06***	47.0 ± 3.71**
4. *E. alpina*	14.00 ± 3.46	1.98 ± 0.89	18.08 ± 11.32	0.33 ± 4.89
E. mocrei	23.23 ± 2.58*	21.00 ± 2.58***	23.95 ± 4.65	31.48 ± 5.65***
5. *E. oreades*	16.47 ± 3.02	4.78 ± 2.09	28.0 ± 4.99	16.48 ± 2.66
E. melanophloia	17.75 ± 1.89	15.86 ± 2.44**	30.2 ± 5.25	29.5 ± 4.1*
Section B				
6. *E. alpina*	3.81 ± 1.56	3.31 ± 0.87	6.80 ± 4.88	11.0 ± 2.86
E. oreades	8.86 ± 1.75*	8.98 ± 1.46**	16.31 ± 4.78*	15.3 ± 3.56
7. *E. citriodora*	6.06 ± 1.33	2.56 ± 1.34	12.8 ± 1.13	8.83 ± 2.94
E. camaldulensis	21.00 ± 4.52**	25.62 ± 5.32**	24.0 ± 12.48*	40.33 ± 7.37**

..cont'd.

TABLE 5.2.3.1 continued

Host Plant	Mean area consumed in mm^2 ± s.e.		Mean dry weight of food consumed (mg) ± s.e.	
	Test A	Test B	Test A	Test B
8. E. intertexta	5.38 ± 1.92	0.58 ± 0.45	12.38 ± 2.50	3.90 ± 2.32
E. moorei	17.50 ± 2.27**	14.35 ± 2.51***	30.71 ± 3.27**	26.33 ± 3.11***
9. E. punctata	18.20 ± 3.51	24.5 ± 4.79	22.58 ± 4.61	22.41 ± 4.26
E. melanophloia	8.10 ± 3.94	9.4 ± 2.52	17.38 ± 6.28	16.30 ± 3.45
10. E. platypus	5.13 ± 1.09	1.33 ± 0.88	14.50 ± 9.76	10.83 ± 2.72
E. cosmophylla	22.26 ± 4.90**	22.98 ± 2.57***	31.56 ± 6.52	41.2 ± 4.23***

t - test significance between paired means

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Figure 5.2.3.1 Percent larval response to hosts in two host choice tests (A) Each larva placed on leaf disc (B) All larvae placed equidistant from leaf discs in the middle of each arena.

PERCENTAGE RESPONSE
HOST PLANTS
70 60 50 40 30 20 10 0 10 20 30 40 50 60 70
E. oreades
E. alpina
Outside discs
E. camaldulensis
E. citriodora
Outside discs
E. intertexta
E. Moorei
Outside discs
E. melanophloia
E. punctata
Outside discs
E. platypus
E. cosmophylla
Outside discs
TEST B
TEST A
E. alpina
E. Moorei
Outside discs
E. oreades
E. melanophloia
Outside discs
E. punctata
E. platypus
Outside discs
E. camaldulensis
E. cosmophylla
Outside discs
E. intertexta
E. citriodora
Outside discs
TEST B
TEST A

to low larval response to E. intertexta in Test B (see Fig. 5.2.3.1). Whilst larvae placed on E. intertexta would stay on it and feed, the results indicate that when given the choice between the 2 species, preference is shown for E. citriodora. In general, inter-specific differences were greater when larvae are given a direct choice than when set to feed upon a single host.

The results in section B of Table 5.2.3.1 and Fig. 5.2.3.1b show that E. oreades, E. camaldulensis, E. moorei and E. cosmophylla are preferred to E. alpina, E. citriodora, E. intertexta and E. platypus respectively. Because of the free choice type of tests it was not possible to establish whether the feeding differences among species were due to a feeding deterrent, toxins, nutritional inadequacy, physical barriers or whether the differences were due to expressions of gustatory preferences. All the preferred species in Table 5.2.3.1 support complete larval development though E. oreades is inferior to the others. Of the 3 species that were significantly less preferred in sections A and B of Table 5.2.3.1 only E. citriodora supports full larval development. E. citriodora was less preferred because it was tested against species that are relatively superior. In spite of the short period in which the experiment was carried out, the larvae showed a distinct preference for E. moorei over E. intertexta for each of the three criteria used. Both species support full larval development (see Section 5.3). Among the ten species tested, mortality was lowest on E. moorei, also the developmental time was the shortest and pupal weight attained was highest (see below).

Although all possible combinations were not tested, the evidence indicates that larval food selection, unlike that of the adult, was related to the suitability of the selected species to support growth. These results support the view of Schoonhoven (1969) that 'larvae get fairly detailed information about the chemical composition of plants'.

This is of very real interest, in view of the fact that host selection in Uraba lugens is the responsibility of the adult female. The larvae upon hatching are in consequence limited to the narrow choice of either accepting or rejecting the immediate substrate. Only under unusual circumstances such as when the entire plant is consumed before the larvae reach maturation, as in outbreaks on small trees, is larval migration and subsequent foodplant selection likely to occur.

5.3 Food plant suitability

5.3.1 Introduction

The gum leaf skeletonizer, U. lugens (Walk) is an important defoliator of many species of Eucalyptus and certain near relatives such as Angophora and Tristania (Brimblecombe, 1962; Campbell, 1962, 1966; Morgan and Cobbinah, 1977). Brimblecombe (ibid.) considered that E. racemosa, E. umbellata and E. melanophloia were more satisfactory hosts for U. lugens than E. tessalaris, E. drepanophylla, Tristania sp. and Angophora sp. Campbell (1966) listed 6 favoured and 26 suitable hosts for U. lugens including those referred to by Brimblecombe. However, both Brimblecombe and Campbell failed to indicate the criteria used to determine suitability and/or favourability. They seem to have based their classification on frequency of infestation and size of populations associated with foodplants at any one time. It appears that the criteria used by them will be appropriate in determining the relative preference of the different foodplants; but a preferred species is not necessarily a favourable or suitable species.

Since the gum leaf skeletonizer infests many species of eucalypts and it is not known how the different species affect growth and egg production, this study was undertaken to compare, on the basis of 3 criteria, the relative suitability of some foodplants.

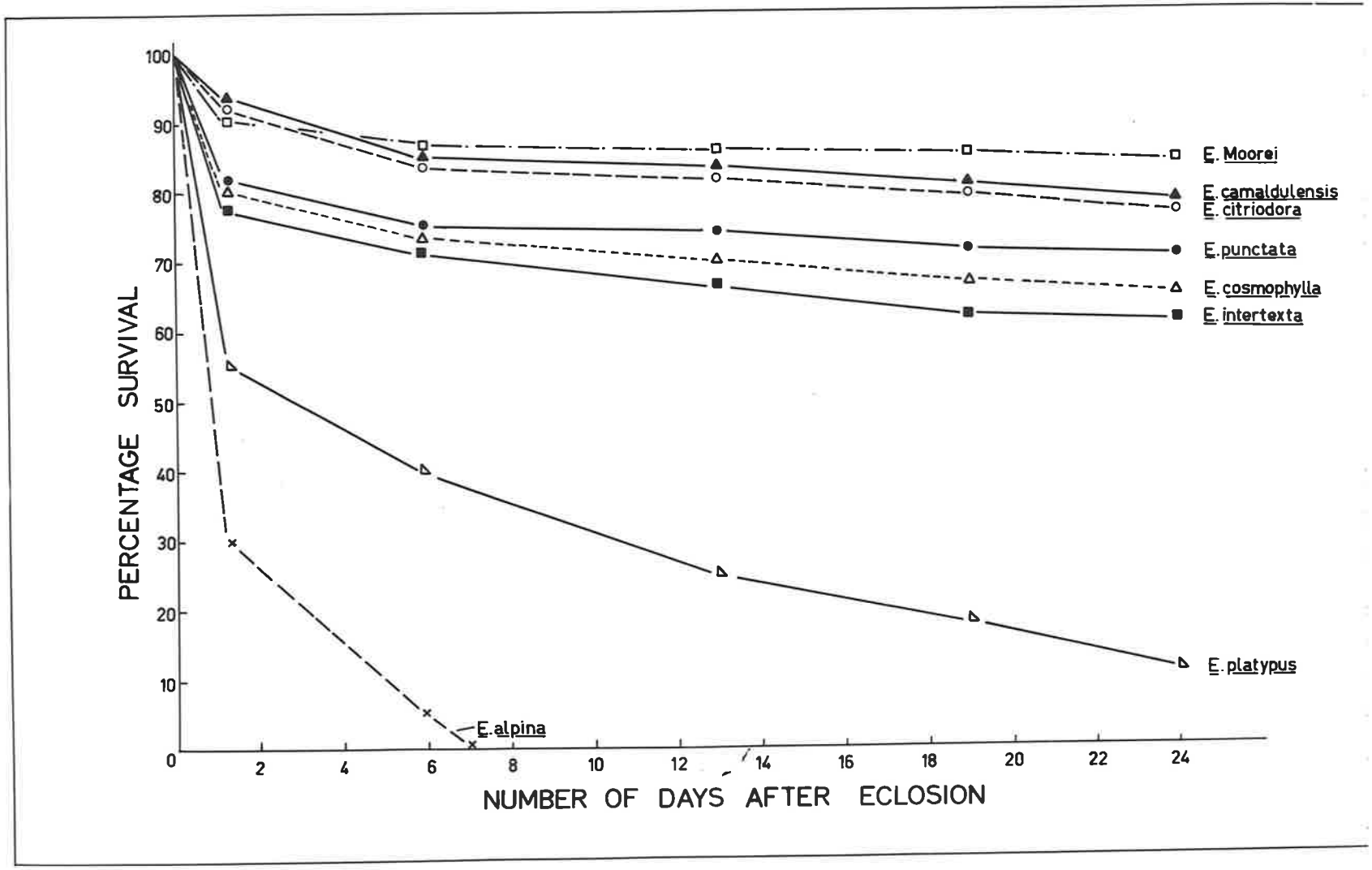
5.3.2 Materials and methods

The suitability of various species of Eucalyptus to the gum leaf skeletonizer, was determined by the following tests. The tests were conducted in both summer (Test 1) and winter (Test 2) generations with mature and juvenile plants respectively. Egg masses (on pieces of leaf) were attached to leaves of test trees with stainless steel clips. In each case leaves of about the same age were chosen by counting back from the terminal bud on selected twigs. Egg masses were obtained from a single species of Eucalyptus not included in the treatments. This was done to avoid the effect of any possible 'parent imprint' (Hovanitz, 1969; Jermy, 1965).

When egg masses were divided to provide sufficient batches for the treatments, the division was at right angles to the linear sequence in which they were laid. This was done to ensure that each batch contained about equal numbers of the first and last eggs laid in the egg mass. Though no evidence was available for U. lugens, it was shown that egg masses of Malacosoma disstria contained eggs that give rise to superior and inferior individuals (Wellington, 1957, 1959). Hence an attempt was made to provide egg batches for each treatment as uniform as possible with respect to any innate qualities that might be associated with order of oviposition. All egg masses were dampened at the black head stage to promote even eclosion. Tests were carried out on trees in the field until the larvae entered the last stadium when they were collected and rearing was completed on cut sections of test plants in the laboratory at $25^{o}C \pm 5^{o}C$, 70% R.H. and a 16 hour photophase.

Three criteria were used to determine the suitability of the various foodplants to U. lugens. The first of these was larval establishment, measured as percent survival, or conversely mortality at the end of fourth stage. The second criterion was larval developmental time, measured as

Figure 5.3.3.1 Establishment and early survivals of *U. lugens* upon 8 species of *Eucalyptus* (Test 1).


PERCENTAGE SURVIVAL
NUMBER OF DAYS AFTER ECLOSION
E. Moorei
E. camaldulensis
E. citriodora
E. punctata
E. cosmophylla
E. intertexta
E. platypus
E. alpina

days spent from hatching to pupation. In the case of the winter generation, the growth rates of larvae on the various plants were determined also by randomly measuring ten larvae of each group every fourth day and obtaining the mean length. The measurements of the larvae were made between the third and sixth stadia because prior to this time the larvae were too small and after the sixth stadium the populations on some of the young trees were so small as to make meaningless the procedure of 'random selection' for measurements.

The third criterion for the two tests, was pupal weight, which serves as an index of potential fecundity of the adult female (see Fig. 5.3.3.2). Life table studies using these same methods but on caged juvenile trees were also made in the winter generation (see Appendix Table 5.3.3.2).

5.3.3 Results

Test 1 (Summer generation - Trees > 20 years old)

Fig. 5.3.3.1 shows the percentage survivals of Uraba lugens on 8 species of Eucalyptus. At the end of the fourth stadium, 88% and 100% of the caterpillars had died on E. platypus and E. alpina respectively. On the other six hosts, relatively low mortalities ranged from 15% on E. moorei to 37% on E. intertexta.

The mean developmental periods of larvae on these six species ranged from 70 days on E. moorei to 73 days on E. citriodora (see Table 5.3.3.1).

Pupal weight was used as a measure of potential fecundity of the adult female (Fig. 5.3.3.2) after testing it against such morphological characters as forewing width, forewing length and forewing expanse. A backward elimination multiple regression procedure (Draper and Smith, 1966) which permits dropping of the least important variable in a stepwise computer analysis (see Appendix Table 5.3.3) ranked the four variables in

Figure 5.3.3.2 Relationship between pupal weight and potential fecundity of U. lugens. (Data from females reared upon 10 species of eucalypts).

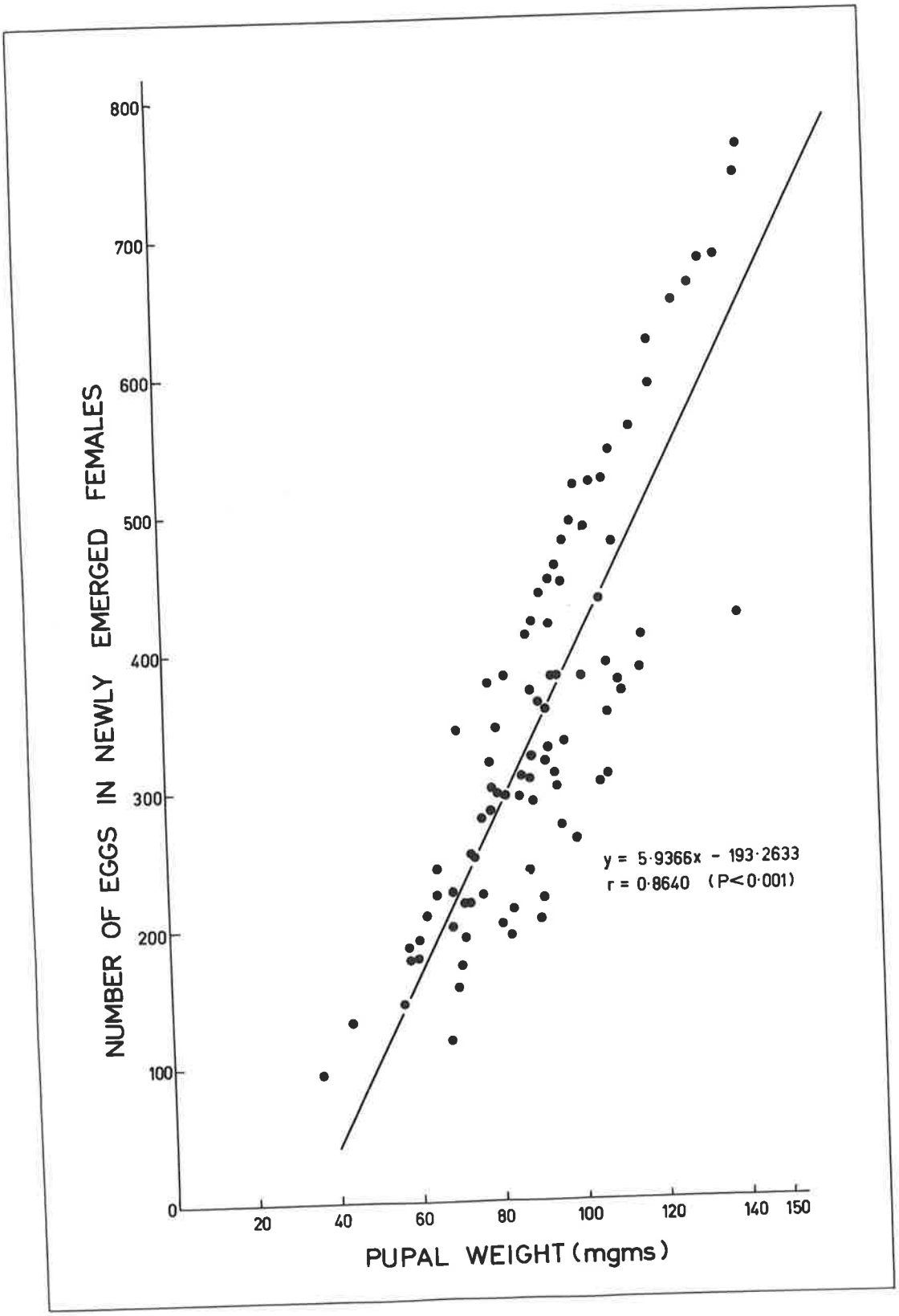

NUMBER OF EGGS IN NEWLY EMERGED FEMALES
800
700
600
500
400
300
200
100
0
20
40
60
80
100
120
140
150
PUPAL WEIGHT (mgms)
y = 5·9366x - 193·2633
r = 0·8640 (P<0·001)

TABLE 5.3.3.1 Developmental days for individuals of Uraba lugens (Wlk.) reared on six satisfactory foodplants in the genus Eucalyptus. (Summer Generation - 1975 Mature plants).

Days after eclosion	% of total pupation per foodplant					
	E. camaldulensis	E. citriodora	E. punctata	E. intertexta	E. cosmophylla	E. moorei
61	-	4	3	-	-	5
64	7	4	23	9.5	-	33
67	25	8	13	9.5	-	17*
70	18*	13	25*	9.5	33	12
73	22	25*	22	47.5*	67*	12
76	11	38	11	19	-	6
79	17	8	3	5	-	9
82	-	-	-	-	-	6
84	-	-	-	-	-	-
Mean developmental days ± s.e.	72.5 ± 0.7	72.6 ± 0.7	70.3 ± 0.9	72.1 ± 0.9	72 ± 0.6	69.7 ± 0.7

* Ca 50% pupated by this day.

this order of increasing importance - forewing width, forewing expanse, forewing length and pupal weight in predicting the potential fecundity of the adult female. The scatter for the four variables recorded in the winter generation, 1977 are shown in Appendix Fig. 5.3.3.1-5.3.3.4. Only the pupal weight was significant ($P < 0.001$) in step one of the multivariate analysis. However, when the least important variable, forewing width, was dropped, wing length became a significant factor ($P < 0.05$). This implies that wing width as a factor in the multivariate analysis affected the sensitivity of using wing length to predict potential fecundity. Although wing length became a significant factor in step 2 it was not as sensitive

as pupal weight for predicting potential fecundity. Baker (1968), Klomp (1968) Wiklund (1973) also found that potential fecundity was proportionate to pupal weight.

The heaviest pupae were produced on E. moorei and the smallest on E. citriodora (Table 5.3.3.2) emphasizing the suitability range of hosts recorded for relative developmental period (above).

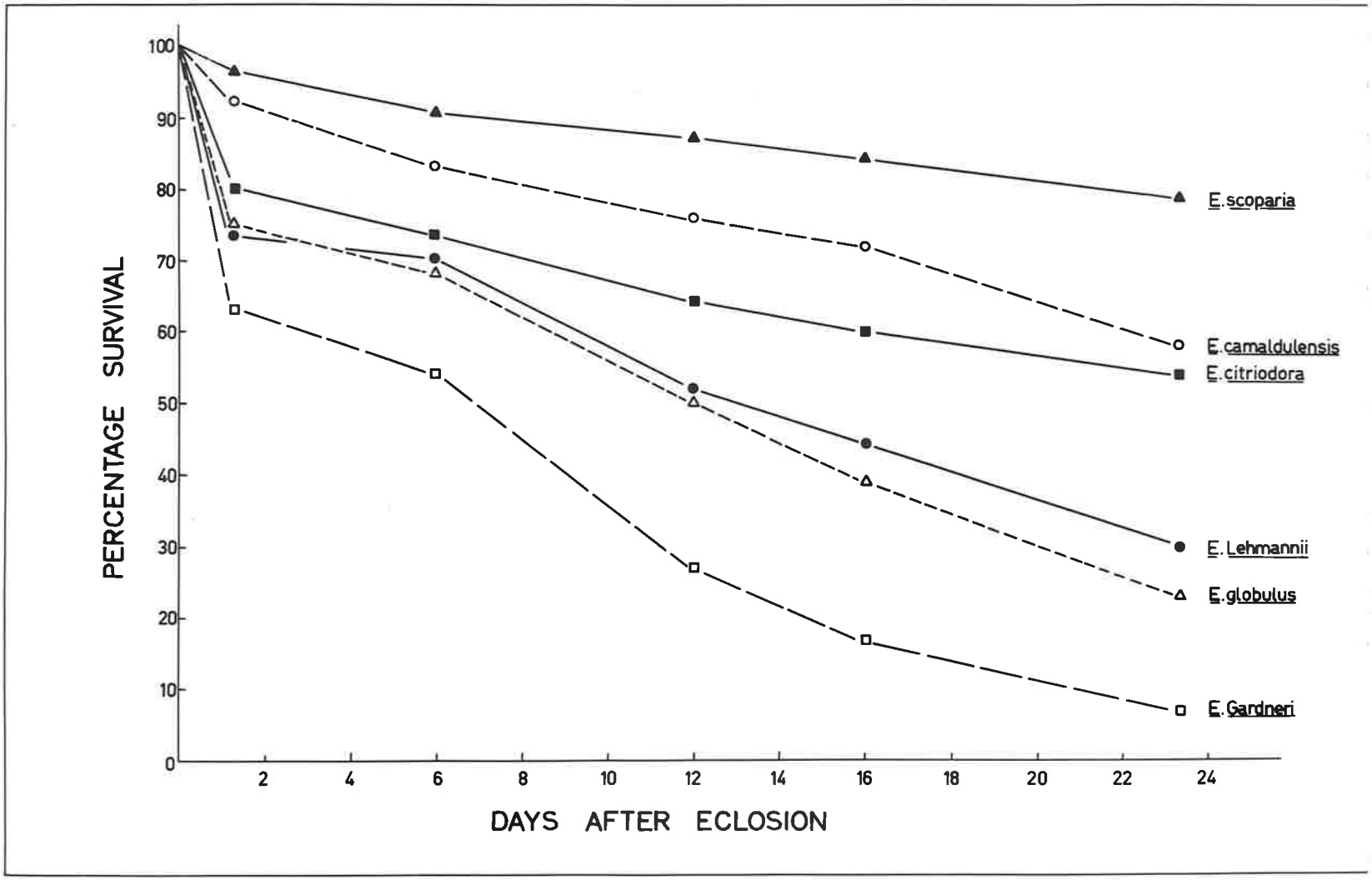
TABLE 5.3.3.2 Estimates of average and total potential fecundity for the female pupae reared on adult and juvenile species of Eucalyptus - Total pupae reared per food-plant are shown in brackets.

Host	Number of female pupae	Mean weight of female pupae	Potential fecundity	
			Mean	Total
A Adult plants				
E. camaldulensis	10 (18)	96.8	381	3,810
E. citriodora	21 (50)	87.3	325	6,824
E. cosmophylla	3 (3)	90.7	345	1,035
E. intertexta	13 (21)	103.8	423	5,499
E. moorei	31 (61)	109.0	454	14,074
E. punctata	11 (29)	90.6	345	3,795
All species	89 (182)	96	379	35,038
B Juvenile plants				
E. camaldulensis	70 (162)	88.7	333	23,310
E. citriodora	59 (120)	74.7	250	14,750
E. scoparia	91 (186)	89.3	335	30,485
E. globulus	6 (18)	68.4	210	1,260
E. lehmannii	60 (111)	86.3	317	19,020
E. gardneri	11 (14)	96.8	381	4,191
All species	309 (633)	85.1	304	93,016

Test II (Winter generation - 3 year old trees)

The highest survival in the winter generation was obtained on

Figure 5.3.3.3 Establishment and early survivals of *U. lugens* upon 6 species of *Eucalyptus* (Test II).


PERCENTAGE SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
2
4
6
8
10
12
14
16
18
20
22
24
DAYS AFTER ECLOSION
E. scoparia
E. camaldulensis
E. citriodora
E. Lehmannii
E. globulus
E. Gardneri

E. scoparia and the lowest on E. gardneri (Fig. 5.3.3.3). The developmental period in the winter generation ranged from 136 days on E. scoparia to 172 days on E. globulus (Table 5.3.3.3). The growth rates

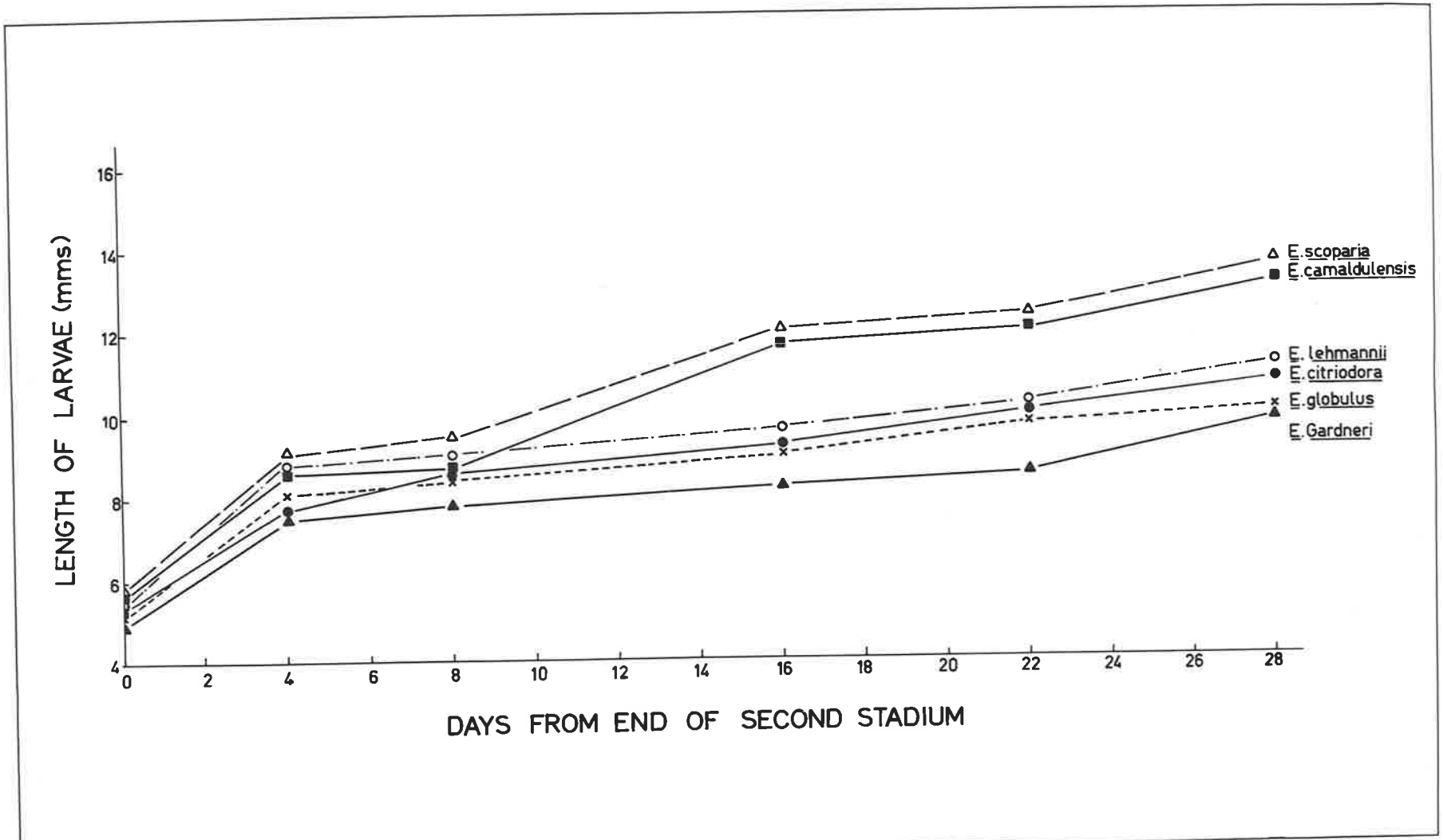
TABLE 5.3.3.3 Developmental times for individuals of Uraba lugens (Wlk) reared on 6 foodplants in the genus Eucalyptus [Winter generation - 1976 : Young 3 yr old plants]

Days after eclosion	Percent of total pupation					
	E. scoparia	E. camaldulensis	E. citriodora	E. lehmannii	E. globulus	E. gardneri
122	1.0	0	0	0	0	0
124	4.3	2.3	1.6	0	0	0
127	8.2	2.3	1.6	0	0	0
129	1.0	2.3	0	0	0	0
131	13.0	3.5	8.0	0	0	0
134	21.2*	6.4	8.0	4.5	0	0
137	23.7	7.6	9.6	0	0	0
142	15.0	5.8	19.2	12.6	0	0
145	5.8	11.6	4.0*	20.7	0	0
148	4.3	11.6*	11.2	12.6*	0	13.3
152	1.0	11.6	11.2	17.1	25	13.3
155	0.5	10.5	11.2	18.0	15	6.7
163	1.0	11.6	6.4	8.1	5	26.7*
165	0	8.1	7.2	0	15*	26.7
168	0	4.7	0.8	6.3	25	13.3
171	0	0	0	0	10	0
175	0	0	0	5	0	0
Mean no. of days ± s.e.	136 ± 0.5	148 ± 0.9	146 ± 0.1	150 ± 0.8	162 ± 1.8	160 ± 1.8

Kruskal-Wallis H ($P < 0.001$)

* Ca 50% pupated by this day

Figure 5.3.3.4 Growth rates of larvae of U. lugens reared on various plants (Data as means of 10 larval measurements per host per time).


LENGTH OF LARVAE (mms)
16
14
12
10
8
6
4
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
DAYS FROM END OF SECOND STADIUM
E. scoparia
E. camaldulensis
E. lehmannii
E. citriodora
E. globulus
E. Gardneri

on the 6 species as measured by larval length can be arranged in this order of decreasing growth E. scoparia > E. camaldulensis > E. lehmanni > E. citriodora > E. globulus > E. gardneri (Fig. 5.3.3.4). Perhaps the most surprising results were in connection with the pupal weights in which E. gardneri which was associated with very high early larval mortalities, low growth rates and relatively long developmental periods produced some of the heaviest pupae (see Table 5.3.3.2). In all other cases, survival, rate of development and pupal weight were positively related e.g. data for E. moorei.

The life tables shown in Appendix Table 5.3.3.2 a-f were prepared mainly to determine the influence of host plants on age-specific mortality. Apart from food, weather was perhaps the only other factor that had a major influence in this study. However because the experiment was carried out in one locality, weather was not expected to affect larvae on one host any more than the other. It is apparent from the tables that the mortalities in the early larval stages was a good reflection of numbers pupating, indicating that food quality was a 'key factor' here. In general, a greater part of the mortalities was associated with establishment of ecloding larvae on hosts. This ranged from 14% in E. scoparia to 72% on E. gardneri. Most of the mortalities in the later stages were caused by pathogenic organisms.

5.3.4 Discussion

The differential survival, growth rates, and pupal weights recorded in these studies could be due to a variety of factors within host plant species. A particular host plant may be deficient in certain nutrients necessary for proper growth and development or may contain various secondary chemicals which deter or inhibit larval feeding, interfere with

digestion, assimilation and/or the utilization of food.

The high mortality of larvae within 24 hours of eclosion on E. alpina may be due to toxins. Antifeedants affecting the adequacy of food intake or inferior nutritional value may have been the cause of high mortalitites recorded on E. platypus at the end of the fourth stadium. Among the species on which complete larval development occurred in Test I, mortality was lowest on E. moorei, the developmental period was the shortest and pupal weight attained was the highest. On E. citriodora, the developmental period was the slowest and pupal weight was the lowest. However, the relatively high numbers completing development resulted in a higher potential contribution to the next generation than all the other species with exception of E. moorei (Table 5.3.3.2). The relatively high survival of U. lugens on E. citriodora, slow growth and small size attained, all contribute to the conclusion that leaves of this species are probably nutritionally inferior or may be deficient in some essential nutrient(s) necessary for growth. One would expect a toxin to cause high mortality which would tend to rule out this possibility. The ready acceptance of E. citriodora by caterpillars and their continued feeding decreases the likelihood of feeding inhibitors, but a good rate of feeding combined with slow growth rate strongly infers low nutrient value or nutritional deficiency which in itself need not be lethal.

The results obtained on E. gardneri are worthy of comment. This species was associated with low ingestion rates and high mortalities of early instars (see also Appendix 5.3.3.2 a-f). Growth rates were slow for most of the early stadia (see Fig. 5.3.3.4), yet development was not apparently affected, moultings occurring at about the expected time for each stadium. Though relatively small amounts of food were eaten by early

instars, the sixth and subsequent ones began to grow at a much faster rate, resulting in relatively large pupae. The initial low ingestion rate and high mortality suggests the presence of toxins or antifeedants which selected the caterpillars much in the same way as a particular dose of an insecticide does. That is, the most susceptible or low quality larvae died while superior individuals survived. It would appear that the survivors had to possess some system, probably enzymatic to overcome the "toxic components" of the diet. Given this, the growth rate equalled or surpassed larvae on that of the control plants (E. camaldulensis). Because of the unusual nature of the results obtained on E. gardneri, the experiment was repeated in winter generation 1977, but 100% mortality was recorded by the end of the fourth stadium, again suggesting seasonal variation.

E. gardneri is represented in the arboretum of Waite Agricultural Research Institute by only one tree. Though it is a favoured egg host (see Appendix Table 4.2.3.1), complete development had never been observed on it prior to the test above. The results in Appendix Table 4.2.3.1 and the present study suggest that different preference systems exist for adults and larvae. The evidence tends to indicate that the adults select from a wider host plant range than the larvae, possibly due to wider distribution of the oviposition stimulant or due to the ability of different substances to stimulate oviposition. Eucalyptus alpina and E. platypus both of which are egg hosts did not support full larval development.

The suggestion of a selection process on larvae by E. gardneri is probably the same as that operating for other non-hosts and poor to moderate host plants, the range of effect upon caterpillars possibly indicative of different levels of the toxin/antifeedants in the leaves. Logical extension of this thesis would be toward development of resistance

to the substance(s) concerned followed by extension of the foodplant range. The rate that such a phenomenon might develop would then depend upon the chances of resistant or superior individuals mating with each other. This might be greater in U. lugens than in other species because of their adult tendency to remain close to pupation sites (Campbell, 1962; Harris, 1974; Morgan and Cobbinah, 1977).

Another factor suggesting the selection of 'superior quality' individuals by E. gardneri is the large size of the pupae. As Wellington (1957, 1959) and others have observed, superior individuals are more resistant to hazards, more active and more voracious. This usually results in a larger more robust adult and moreover one that is more fecund.

At both assessments natural control agents were recorded. It was observed that parasitism of larvae was highest on E. camaldulensis, otherwise parasitism was correlated with the numbers of surviving larvae on each host plant. The reason for a higher level of parasitism of larvae reared on E. camaldulensis is not easily discernible. However, since it is most common and widely distributed foodplant, it may be that the major parasites such as Casinaria sp. and Apanteles sp. have evolved a sensitivity to signals from this tree and not from others. There are several reports of hosts that are readily attacked when occurring on one foodplant but not another (Arthur, 1962; Clausen, 1941; Smith, 1957; Stary, 1964; Walker, 1940). For example, Apechthis rufata a parasite of oak and fir tortricid, Choristoneura murinana, is attracted primarily by Oak, and only secondarily by the host itself (Zwolfer and Kraus, 1957).

The overall study has shown that eucalypts differ in the degree of suitability to U. lugens. In most extreme cases, all larvae may die, however, most other foodplants may be ranked in order of their desirability with regard to the ability of larvae to survive and grow. Estimates of average and total potential fecundity (Table 5.3.3.2) of females reared

on various foodplants indicate that the reproductive capacity is greater on some foodplants than on others.

CHAPTER 6 BIOASSAY TECHNIQUES AND INFLUENCE OF PHYSICAL FACTORS ON LARVAL FEEDING RESPONSES

6.1 Bioassay techniques

6.1.1 Introduction

Bioassay techniques are essential in detecting substances that affect feeding and growth of insects. The techniques in use are many but can be classified into 3 groups:

(1) Inert Media bioassay - The test substance or extract is incorporated into more or less inert material such as plant pith (Harris, 1963; Heron, 1965), plain filter paper (Yamamoto and Fraenkel, 1960; Thorsteinson and Nayar, 1963) charred filter paper (Niimura and Ito, 1964), Styropore lamellae (Meisner et al., 1971), and agar (Sutherland, 1971).

(2) Host Tissue bioassay - The test substance is applied directly to the host tissue e.g. leaf vacuum infiltration (Harris and Mohyuddin, 1965) and twig application (All and Benjamin, 1975).

(e) Artificial Diet bioassay - The test substance or extract is incorporated into a basic diet (Soo Hoo and Fraenkel, 1966; Hsiao and Fraenkel, 1968; Feeny, 1970).

In a study of larval feeding behaviour of Uraba lugens I examined a number of these techniques. The host tissue and the artificial diet methods were abandoned after preliminary tests because of the possible influence of some of the nutrients present in these media on the feeding of the larvae, whether through interaction with substance under test or by an additive effect increasing the concentration of the substance to a point at which it may exert an abnormal positive or negative influence on the experimental results. For these reasons, the inert substrate bioassay was investigated further. As Harris and Mohyuddin (1965) point out, no single technique is suitable for all insects though they found the agar base carriers and filter paper impregnations readily acceptable to caterpillars.

6.1.2 Methods

(i) Agar base carrier

The agar base carrier was prepared by dissolving equal weights (1 gm) of agar and cellulose powder in boiling water (30 ml). This was the 'plain diet' and materials to be tested were added to the cooling fluid and stirred rapidly until gelling commenced. The viscuos fluid was poured into 19 cm glass petri dishes and, once cooled, 5.5 cm discs were cut out with a cork borer and placed into 48 x 12 mm plastic petri dishes for feeding trials. When heat stable substances were tested, appropriate amounts of each were dissolved in a suitable solvent before the cellulose powder and agar were added. The plain diet was always prepared with distilled water, however. Artificial 'feeding holes' were made in the surfaces of the diets before presentation to caterpillars as this appeared to stimulate quicker acceptance of a diet by young larvae. The plain diet was always used in these studies as a control and hence they differed somewhat from similar studies of Feeny (1970), Soo Hoo and Fraenkel (1966) Hsiao and Fraenkel (1968) in which the control contained other nutrient substances apart from each test substance.

(ii) Filter paper bioassay

This was used to test components of natural and synthetic origin and involved 5.5 cm diameter No. 1 Whatman filter papers dipped into standard quantities of the test materials and dried at 30°C. The control paper discs were dipped in solvent only, but were otherwise treated in the same way as experimental discs. The discs were arranged in alternate fashion around the edges of 19 cm plastic petri dishes. Larvae were placed in the centre of the arena or equidistant from each disc.

(iii) Experimental Animals

All larvae used in each experiment were of similar physiological

age and vigour and were usually offspring of the same parent. When shortages of sibling cultures occurred, some of the larvae had to be obtained from different sources. These caterpillars were of the same age group and were retained as sibling groups rather than mixing them with test animals from other origins. The groups were then assigned randomly to treatments and controls for each test. All larvae used in experiments were starved for 24 hours before a test unless otherwise stated. This ensured that larvae had evacuated their gut and that their physiological condition was more or less standardized (Hsiao, 1968).

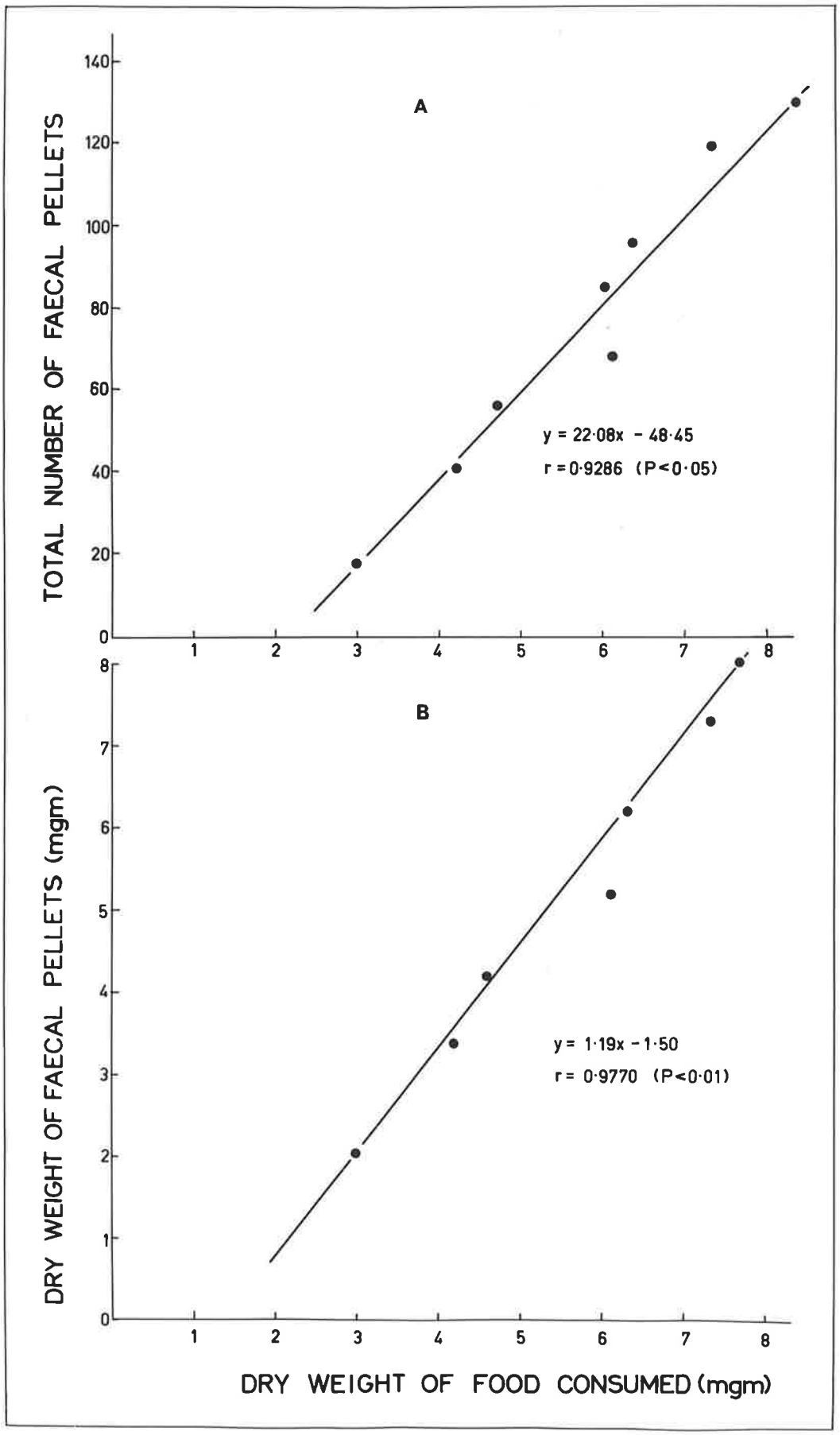
6.1.3 Assessment of larval responses

(i) Agar base carrier

The biting response was rated subjectively on the observed variation in attack behaviour. Consistent attack on the feeding site was rated high (++++), intermittent attack as moderate (++), initial attack only as low (+) and no attack as (0). The feeding response was based on either faecal count or dry faecal weight, whichever was most appropriate under particular experimental conditions. The use of faecal counts as an index of amount of food consumed has been criticised by both Kasting and McGinnis (1962) and Waldbauer (1964). In the studies reported here, faecal counts were used as an index of amount of food consumed on diets containing single nutrient substances usually of similar complexity. Sutherland (1971) has pointed out that where faecal pellets are relatively constant in size and do not fragment, and are used to determine differences of single nutrient diets, the count represents a reliable index of food ingested. Preliminary trials with Uraba lugens on leaf powder diets and single nutrient diets corroborates Sutherland's findings (see Fig. 6.1.3a,b). However, because of the greater reliability of faecal weights

Figure 6.1.3 Relationship between dry weight of food consumed (mg) and (top) total number of faecal pellets produced and (bottom) dry weight of faecal pellets produced.

A - top; B - bottom.


TOTAL NUMBER OF FAECAL PELLETS
A
y = 22·08x - 48·45
r = 0·9286 (P<0·05)
DRY WEIGHT OF FAECAL PELLETS (mgm)
B
y = 1·19x - 1·50
r = 0·9770 (P<0·01)
DRY WEIGHT OF FOOD CONSUMED (mgm)

($P < 0.01$) as an index of food consumed, it was preferred to faecal counts ($P < 0.05$) whenever larvae used in tests were obtained from different parents and sources.

(ii) Filter paper bioassay

The feeding behaviour of larvae to test substances applied to filter paper discs was determined by observing biting responses and positions of larvae at 15 minute intervals over 3 hours. The filter paper had serious drawbacks: (a) the larvae often moved the filter discs from their original positions and so disrupted the original arrangement of the arena, (b) when the larvae did not feed on the papers it was not possible to tell whether there had nevertheless been a biting response, which may be distinguishable from a continuous feeding response, and may be regulated by different chemicals - e.g. in Bombyx mori (Hamamura et al., 1962).

Due to these shortcomings, the agar base technique was used more extensively than the filter paper technique as it enabled the biting response and the continuous feeding response to be distinguished and recorded.

6.2 Effect of physical factors of plants on larval feeding

6.2.1 Introduction

Physical and chemical attributes of plants are recognized as the two major factors that determine selection and preference by phytophagous insects - (Painter, 1951, 1958; Beck, 1965, 1974; Dethier, 1947; Friend, 1958; Soo Hoo and Fraenkel, 1966; Augustine et al., 1964; Hsiao, 1966 and Erickson, 1975).

Several plant physical characteristics such as colour (Ilse, 1937) thickened cuticle (Tanton, 1962; Williams, 1954; Feeny, 1970) hairs, spines, thorns (Johnson, 1953; Bernays and Chapman, 1970) high silica content (Patanakamjorn and Pathak, 1967) have shown positive correlation

with the resistance of plant species to insect attack. In spite of these studies, there is a considerable degree of uncertainty as to the real significance of physical factors of foodplants in insect - host plant interactions (Augustine et al., 1964; Painter, 1951). Dethier (1953) thought that the role of leaf texture may have been over-emphasized in such relationships.

The results in Chapter 5 indicate clearly that different species of Eucalyptus vary in their degree of acceptability, suitability and preference to the larvae of Uraba lugens. Because these may be influenced by hardness of leaves an attempt was made to determine the role of physical factors (specifically hardness) in the relationship between Uraba and its food-plants.

6.2.2 Materials and methods

Two techniques were used to determine directly or indirectly the effect of leaf toughness or hardness on larval feeding.

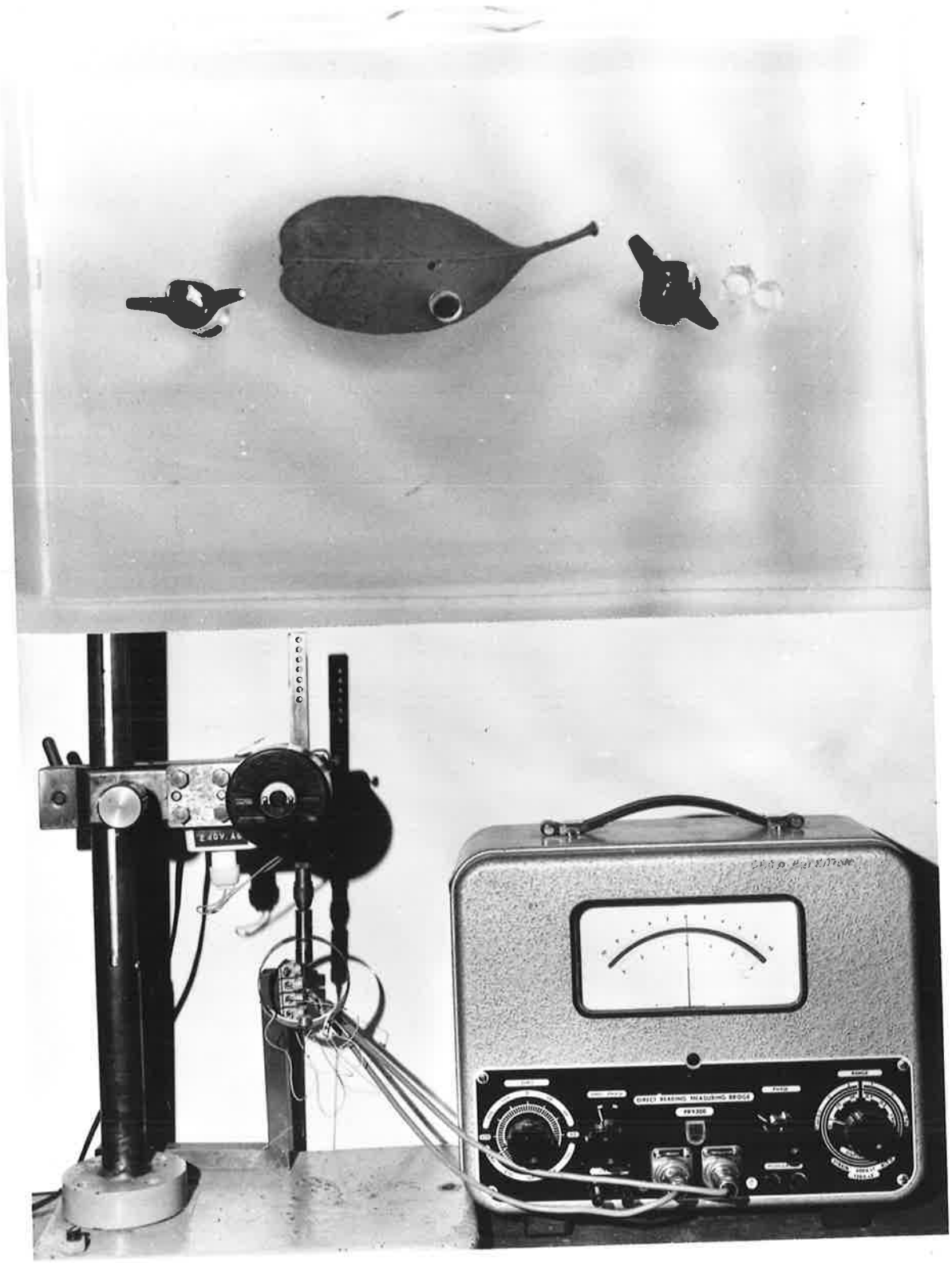
(i) Leaf hardness determination with penetrometer

The penetrometer was used to determine the hardness of leaves whose acceptability to young larvae had been determined in earlier experiments (Chapter 5). A detailed description of the penetrometer used is given by Cockroft, Barley and Greacen (1969). The probe used in the study reported here was made of stainless steel and measured 1.25 mm with a tip angle of 45^{o}. The forces were measured with small proving rings equipped with electrical resistance strain gauges; the transducers were connected to a millivolt recorder. The stainless steel probe is driven into the leaf by an electric motor operating a rack and pinion drive. The probe's downward movement is at a rate of 2.9 mm/minute. The toughness of a leaf is expressed as $T = F/c$ where F is the point resistance and c

Figure 6.2.2 Leaf hardness measurements.

top - perpex boards in which leaves are clamped for measurement.

bottom - penetrometer set up.



is the cross-sectional area of probe tip in mm^2. Calibration of the probe was made prior to this study. A pointer recording of 2.2 represented a pressure of 100 gm. However, because the gram weight measurement is subject to changes depending on diameter and angle of probe, an arbitrary toughness scale from one to ten was established or (equivalent to a pointer recording range of 1 to 6, with 0.5 pointer recording increments equal to 1 leaf toughness unit).

(ii) Leaf samples

Five 12-inch twig samples were collected from each of the species tested from the part of the crown normally chosen as an oviposition/feeding site by U. lugens. The twigs were placed in plastic bags and taken into the laboratory in an ice-packed box. Two mature leaves were selected randomly from each twig and each leaf was in turn clamped between 2 perspex boards (see Fig. 6.2.2) in such a way that the probe pressure was applied to the midpoint of the basal third of leaf. The reason for selecting this site for test is related to the selection of oviposition sites by U. lugens and the fact that ecloding larvae will invariably start feeding near where eggs are laid. In all, ten leaves from each tree were measured for toughness.

(iii) Removal of physical characters of leaves as a comparative technique in studies upon feeding of caterpillars

The effect on larval feeding of removing physical characters of leaves was studied. Mature leaves of the 10 species of Eucalyptus used in earlier studies were collected from the field in June, 1975. They were taken into the laboratory packed in ice and freeze-dried for 48 hours. The dried leaves were ground in a Wiley mill until the powder passed through a 0.5 mm screen. Then the powder was incorporated into the 'plain diet' agar medium until it comprised 6% by weight of the total mixture.

Three 6th instar larvae which had been starved for 24 hours were placed on each diet and each treatment was replicated 6 times. After 24 hours the number of faecal pellets were counted and used as the comparative index of food consumed (see above).

6.2.3 Results and discussion

6.2.3.1 Penetrometer determination

The toughness and acceptability of 20 Eucalyptus are shown in Table 6.2.3.1. In general, there was no positive relationship between the acceptability of species and toughness of leaves. Eucalyptus cinerea and E. crenulata which were associated with low acceptability and high first instar mortalities had lower toughness ratings than 13 species which were rated more acceptable and suitable to caterpillars whereas E. erythrocorys and E. melanophloia were accepted yet had very different toughness values. Also E. stricklandii, E. woodwardi and E. alpina were of similar toughness but differed greatly in their acceptability to the young larvae (Table 6.2.3.1).

From these results it may be inferred that factors other than leaf toughness make soft species such as E. cinerea and E. crenulata less acceptable to young larvae than other species. Based on reasons advanced earlier (Chapter 5) it is logical to infer that such factors may be of a chemical nature. Moreover leaf toughness, even if it has some effect may not be a very important factor in nature, in view of the fact that young larvae are capable of moving from older, tougher leaves to less tough young tissues without sacrificing food quality (Morgan and Cobbinah, 1978). Again, the characteristic skeletonizing habit of the early instar larvae, which involves feeding on softer tissues between veins, tends to reduce to a minimum the possible inhibiting influence of hard fibrous leaf material on young larvae.

TABLE 6.2.3.1 Relationship between acceptability of food and toughness of foliage.

Plant species	Acceptability & suitability for larval growth*	T-Value
E. camaldulensis	Good	4
E. moorei		4
E. erythrocorys		7
E. stricklandii		6
E. pumila		4
E. melanophloia		3
E. citriodora	Satisfactory	3
E. robusta		3
E. intertexta		3
E. sidroxylon		3
E. woodwardi		7
E. cosmophylla		9
E. punctata		4
E. cladocalyx nana	Poor	4
E. astringens		4
E. crenulata		1
E. platypus		7
E. alpina		7
E. gardneri		3
E. cinerea		1

* Good Acceptability - supports > 70% of initial pop. to 4th instar
Satisfactory " - supports Ca 30-70% of initial pop. to 4th instar
Poor " - supports < 30% of initial pop. to 4th instar.

The use of a pin probe penetrometer (Williams, 1954; Tanton, 1962) was not considered as appropriate for chewing insects (Feeny, 1970; Bernays and Chapman, 1970). Feeny (ibid.) replaced the pin with a cutting rod which punch out leaf discs by a shearing and tearing action.

Though, it was recognized that the pin probe penetrometer would give a more meaningful value for sucking insects, I did not consider the punching rod or the cutting edge type instruments as superior to the pin penetrometer for an insect which, during early life, avoids all fibres and veins except perhaps the most delicate.

A major shortcoming of the methods used by the earlier workers in which sand or water was used to exert pressure on the penetrometer was the control of the flow of the fluid material. Williams (1954) and Tanton (1962) did not describe how this was achieved. Feeny (1970) regulated the flow using a chromatography column but it is evident that the 'end point' score may not actually represent the exact pressure that punctured the leaf. The magnitude of the error will depend upon the movement of the flow regulator and the sizes of the sand particles. Bernays and Chapman (ibid.) stated that 'with soft leaves, the weight of the funnel alone was often sufficient to cut the leaf. This implies that when two or more of the test leaves were soft enough to be cut by the weight of the funnel, the method could not be used to quantify and compare their hardness. The penetrometer used in this study is based on a strain gauge transducer. The sensitivity and direct transmission of force from the strain gauge to the bridge recorder via transducers overcomes the inaccuracies associated with the other methods. I therefore believe my tests were more sensitive and accurate than those reported in the literature quoted herein.

6.2.3.2 Leaf powder

The degree of food consumption was estimated by the number of faecal pellets produced in 24 hours. The trends shown by results in Table 6.2.3.2 are similar to those described in Chapter 5. Significantly more faecal pellets were produced on good and satisfactory host plants than on

the unacceptable species, yet E. alpina and E. platypus the unsuitable foodplants were superior to the plain diet alone. The plain diet was

TABLE 6.2.3.2 Biting and feeding responses of fifth instars U. lugens on diet containing 6% leaf powder of various species of eucalypts.

Diet	Biting response	Frass pellets per larva/day	Arbitrary scale* based on faecal pellets
1. Plain diet	+	3	1
2. Diet + E. camaldulensis	++++	38	8
3. Diet + E. moorei	++++	44	10
4. Diet + E. citriodora	++++	37	8
5. Diet + E. intertexta	++++	35	7
6. Diet + E. cosmophylla	++++	38	8
7. Diet + E. punctata	++	26	5
8. Diet + E. melanophloia	++++	33	7
9. Diet + E. oreades	++	25	5
10. Diet + E. platypus	++	22	4
11. Diet + E. alpina	++	14	2

* Arbitrary scale <12 = 1; 12 - 15 = 2; 16 - 19 = 3; 20 - 23 = 4 24 - 27 = 5; 28 - 31 = 6; 32 - 35 = 7; 36 - 39 = 8; 40 - 43 = 9 >44 = 10.

made up of agar and cellulose powder and lacked nutrient substances, feeding stimulants and/or sign stimulant which even an unacceptable plant may contain. The low feeding rate on E. alpina and E. playtpus cannot be explained by the toughness of leaves of these species since powdering removed any such differences that may exist among the ten species, though I expect that the proportion of fibrous tissue to non-fibrous tissue in the powders would be characteristic for each species tested.

Two objections to this explanation which were recognized by Soo Hoo and Fraenkel (ibid.) are that: (1) resistance principles of a chemical

nature may have been destroyed in the drying and powdering process; (2) chemical feeding deterrents may have been diluted in the basic diet. These may not be important here because all the leaves were handled in a similar manner and the freeze-drying technique used in this study as against oven drying of other methods would minimize chemical distintergration.

The similarity in trends of the results in Chapter 5 and Section 6.2 of Chapter 6 suggest that physical characteristics of leaves play a relatively minor role in the relationship between U. lugens and its foodplant.

CHAPTER 7. STIMULANTS IN EUCALYPTUS LEAVES AFFECTING FEEDING BEHAVIOUR OF LARVAE OF URABA LUGENS

7.1 Introduction

The chemical factors that influence feeding behaviour of larvae of phytophagous insects are mainly (1) feeding stimulants and (2) feeding deterrents (inhibitors). These chemicals have not been investigated before for the gum leaf skeletonizer, U. lugens. The study reported in this chapter was designed to identify the chemical constituents of the plants that elicit chemotactic and/or phagostimulatory responses in this insect. Larval feeding responses to some of the constituents of the chemical groups covered in this chapter are presented in Chapter 8, however, references have been made to some of the results in this chapter.

7.2 Materials and methods

All foliage for extraction was obtained from eucalypt trees in the arboretum of the Waite Agricultural Research Institute. The preparation of the foliage and procedure used in bioassay were essentially as described in Chapter 6.1.

The experiment was designed to provide 6 larvae of about the same age, in each of five or six replicates, with a choice between a test and a control of filter paper discs arranged in alternate fashion in a 9 x 1 cm plastic petri dish. Discs were dipped in either a test solution or the control (solvent) and the solvent evaporated before discs were presented to the insects. The second technique involved counting the number of faecal pellets produced by 15 larvae held on agar diets containing either a test solution or a solvent (control). All experiments were carried out at $25^{\circ} \pm 2^{\circ}C$, 50%-70% relative humidity and a 16 hr photophase. Host plant extracts for feeding tests were prepared from a carefully weighed

lyophilized host plant powder or from fresh plant material. Extraction was carried out in a soxhlet or Erlenmeyer flask for the appropriate period, usually 24 or 48 hours duration. Excess solvent was removed using a rotary evaporator. Extracts being prepared for comparison tests were made from equal weights of material and were concentrated to the same final volume. To ascertain the effectiveness of the lyophilized leaf material as a source for isolation of feeding stimulants, a preliminary test was carried out to determine the responses of larvae to leaf powder.

Results of preliminary tests of leaf powder

The results of the preliminary test are shown in Fig. 7.2.1. The data showsthat the amount of feeding on agar medium increased to a maximum as the amount of leaf material is increased to 50% but decreased at higher concentrations. This result is consistent with the hypothesis that at least one component of the leaf powder was not acceptable at too high an absolute- or relative-concentration.

7.3 Isolation procedure and results

7.3.1 Step 1 - Solubility in 3 solvent systems (Water, 80% Alcohol, Diethyl ether)

The lyophilized leaf powder was divided into three 10 gm portions each of which was extracted with 100 ml of one of the following solvents - distilled water, 80% alcohol and diethyl ether. The extracts and the residues were incorporated into agar medium in 6% portions. The effect of extracts and residues on feeding of larvae was measured by counting faecal pellets produced by 15 larvae in 24 hours. The results are given in Tables 7.3.1.1 and 7.3.1.2.

These data indicate that the feeding stimulants are largely soluble in water and 80% alcohol extracts. However,

Figure 7.2.1 Feeding responses of 6th instars of U. lugens to various amounts (%) of leaf powder in diet.

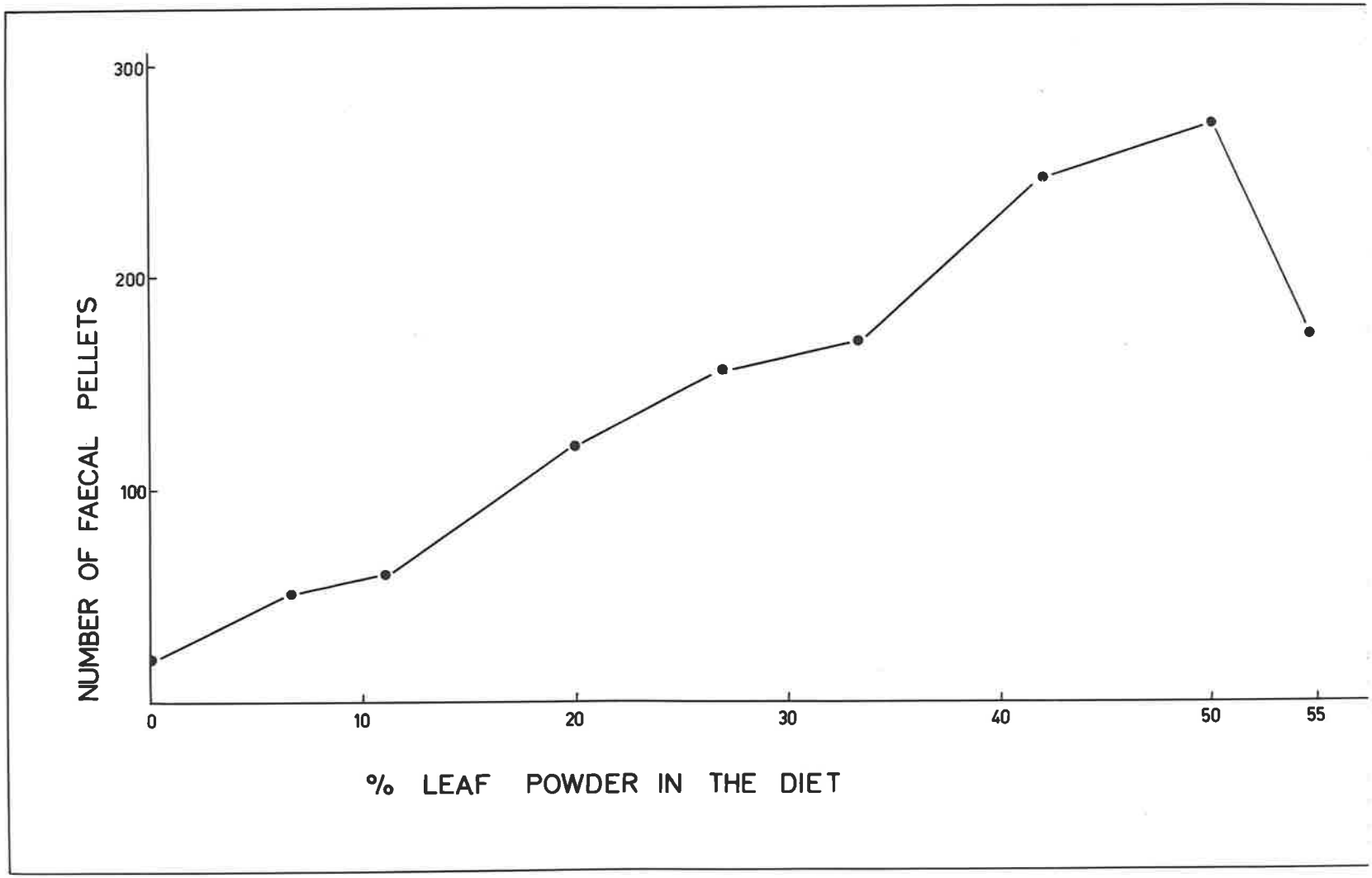

300
200
100
NUMBER OF FAECAL PELLETS
0
10
20
30
40
50
55
% LEAF POWDER IN THE DIET

TABLE 7.3.1.1 The influence of various residues of E. maculata on feeding by 5th instar larvae of U. lugens.

Residue	Biting Intensity	Accumulated faecal pellets in 24 hrs
Plain diet	0	2
Diet + residue of water extract	++	49
Diet + residue of 80% alcohol extracts	++	52
Diet + residue of ether extract	+++	120
Diet + Leaf powder	++++	192

Kruskal-Wallis H test ($P < 0.01$)

TABLE 7.3.1.2 Biting and feeding responses of 6th instar U. lugens to various extracts.

Extract	Biting Intensity	Accumulated faecal pellets in 24 hrs
Plain diet	++	46
Diet + water extract	++	81
Diet + 80% alcohol extract	+++	122
Diet + ether extract	+	28

Kruskal Wallis H ($P < 0.01$)

the fact that the residues of leaf powder extracted with either water or alcohol showed some activity suggests that some water and alcohol insoluble active substance(s) could be involved. On the otherhand, it is possible that the activity in water and alcohol residues may be due to incomplete removal of feeding stimulants from original leaf powder. The results in Table 7.3.1.2 suggest that the ether extract probably contains a feeding deterrent of some kind since the larval response to this diet

was lower than the response to the plain diet. The 80% alcohol extract was the most active, therefore, it was used as a starting material for further investigation of the chemical nature of the feeding stimulants.

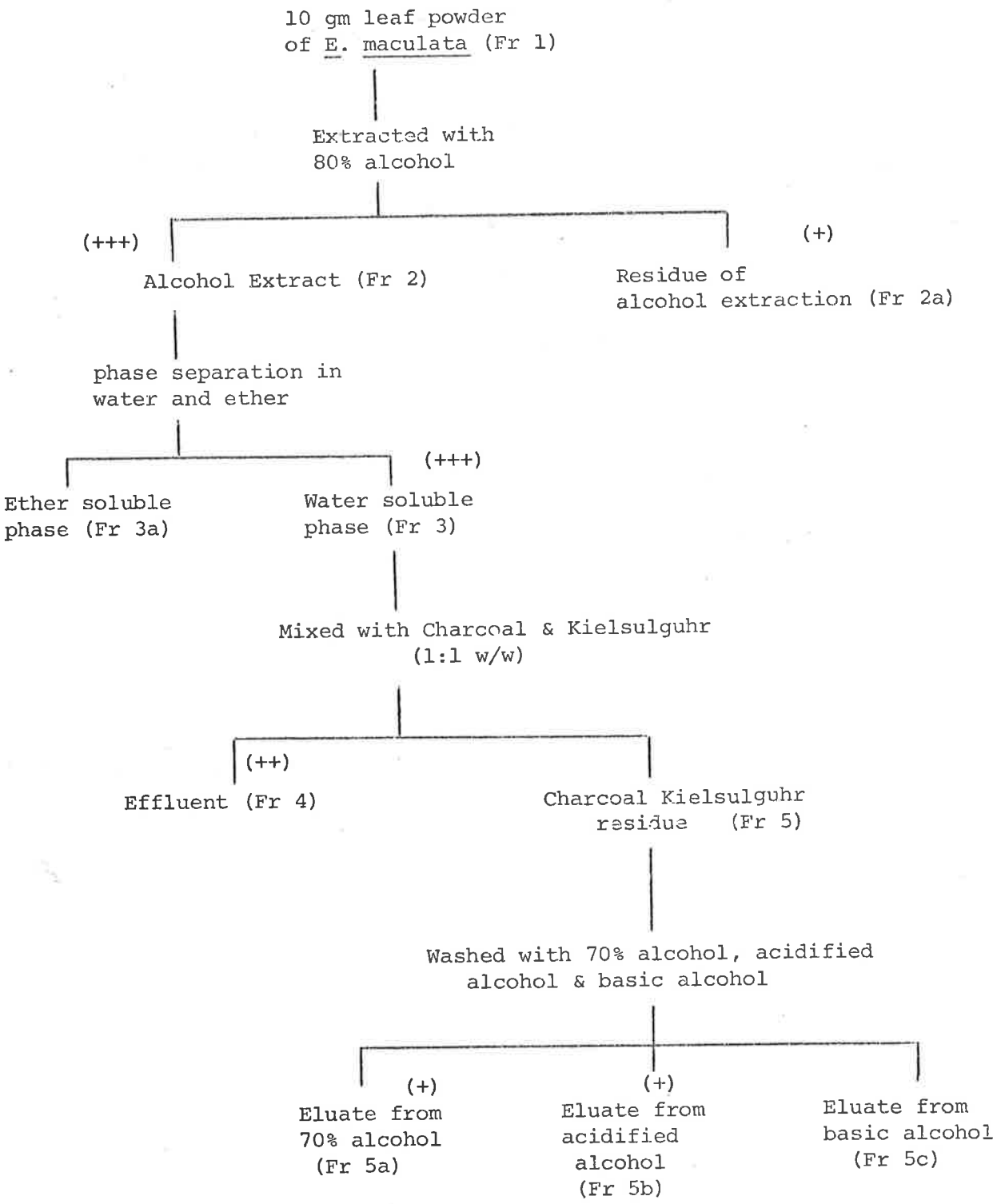
7.3.2 Step 2 - Isolation of feeding stimulants from 80% alcohol extract

The alcohol extract was concentrated to near dryness in a rotary evaporator at room temperature. The extract was taken up in water and ether (1:1 v/v) and separated into 2 phases by shaking. The 2 phases were collected in separate flasks and concentrated to the same final volume in the rotary evaporator. Sixth instar larvae were exposed to Whatman No. 1 filter paper discs dipped into these 2 phases. Larval aggregation was recorded.

The water soluble phase (Fr 3) elicited strong aggregation response but the ether soluble phase (Fr 3a) was ineffective indicating that the active substance in the 80% alcohol extract had passed on to the water phase. Water and alcohol soluble constituents of plants may consist mainly of amino acids, amines, sugars, sugar alcohols, water and alcohol soluble organic acids and phenolic glycosides.

The water soluble phase was next concentrated in a rotary evaporator at 40^{o}C and the concentrate was mixed with activated charcoal and Kielsulghur (1:1 wt/wt). Charcoal permits selective recovery of adsorbed compounds and has often been used for rapid and simple separation of aromatic substances such as phenolic compounds from aliphatic and inorganic compounds in aqueous solutions of crude mixtures (Asatoor and Dalgeish, 1956). The effluent (Fr 4) was filtered off. This fraction consists largely of aliphatic water soluble substances such as amino acids and sugars The filter cake was eluted in succession with 70% alcohol, acidified alcohol (alcohol containing 5% HCl) and basic alcohol (alcohol containing

Fig. 7.3.2.1 Fractionation of the 80% alcohol soluble extract.



10% KOH) to obtain subfractions 5a, 5b and 5c (Loschiavo, 1965). Fraction 4 and subfractions 5a, 5b and 5c were tested by the paper disc impregnation technique. A schematic representation of the procedure is shown in Fig. 7.3.2.1. The results of tests are shown in Table 7.3.2.1.

TABLE 7.3.2.1 Aggregation responses of larvae of U. lugens to extracts of leaf powder adsorbed on filter paper discs.

Fraction	Mean number of larvae aggregating on		Mann-Whitney U test
	Test	Control	
Alcohol extract (Fr. 2)	31.4	3.4	***
Ether soluble phase of Fr. 2 (Fr. 3a)	3.2	5.8	N.S.
Water soluble phase of Fr. 2 (Fr. 3)	29	9.2	***
Effluent from charcoal treatment of Fr. 3 (Fr. 4)	30.4	8.8	***
Alcohol eluate of charcoal (Fr. 5a)	26.4	9.8	***
Acid eluate of charcoal (Fr. 5b)	27.5	9.9	***
Alkaline eluate of charcoal (Fr. 5c)	11.2	10	N.S.

*** Mann Whitney U ($P < 0.001$)

The effluent fraction 4 elicited strong response so did subfractions 5a and 5b. Subfraction 5c, however, did not induce aggregation. The positive responses to subfraction 5a and 5b indicate that a water soluble aromatic substance such as phenolic glycoside may be involved in the stimulatory activity of the water soluble phase. Phenolic glycosides are stable in weak acids but unstable in weak alkaline solutions. Alkaline solutions usually open the heterocyclic rings of phenols and this may account for the ineffectiveness of the basic alcohol extract. However since disaccharides are also appreciably adsorbed by charcoal and easily eluted with aqueous

alcohol, samples of subfraction 5a and 5b were tested for presence of sugars. Samples of subfraction 5a and 5b were applied to a baseline about 2.5 cm from end of 20 x 35 cm Whatman No. 1 filter paper. The samples were chromatographed in n-Butanol-acetic acid-water (4:1:5 v/v) for 16 hours. The dried chromatograms were developed with alkaline silver nitrate solution. The results showed that both fractions contained sugars. Chemical test with Ninhydrin also showed that traces of amino acids were present. The results suggested that the activity in subfraction 5a and 5b could not be ascribed to phenolic substances without further investigation. Consequently, another fractionation procedure was adopted to separate the phenolic glycosides. The details of the procedure are shown in Fig. 7.3.3.1.

7.3.3 Isolation of phenolic substances

To evaluate the response to the phenolic fraction of alcohol extract, I concentrated the alcohol to dryness in rotary evaporator. The dried extract was dissolved in water and neutralized with barium hydroxide. Lead acetate was used to precipitate phenolic compounds (Seshadri, 1962; Nayar and Fraenkel, 1963). The solution was centrifuged and supernatant filtered through a fluted filter paper. The precipitate was suspended in water and regenerated with H_2S to precipitate lead as lead sulphide. The supernatant (Fr. 3a) was freed of excess H_2S by passing through nitrogen gas. The supernatant (Fr. 2) was treated in a similar manner. The two fractions (2a and 3a) were tested by the filter disc impregnation method. The results are shown in Table 7.3.3.1.

The results suggest that the larvae do not respond to the phenolic compounds. However, a comparison of the response to fraction 2a and the parent extract, fraction 1, indicate that some of

Fig. 7.3.3.1 Scheme for isolation of phenolic glycosides

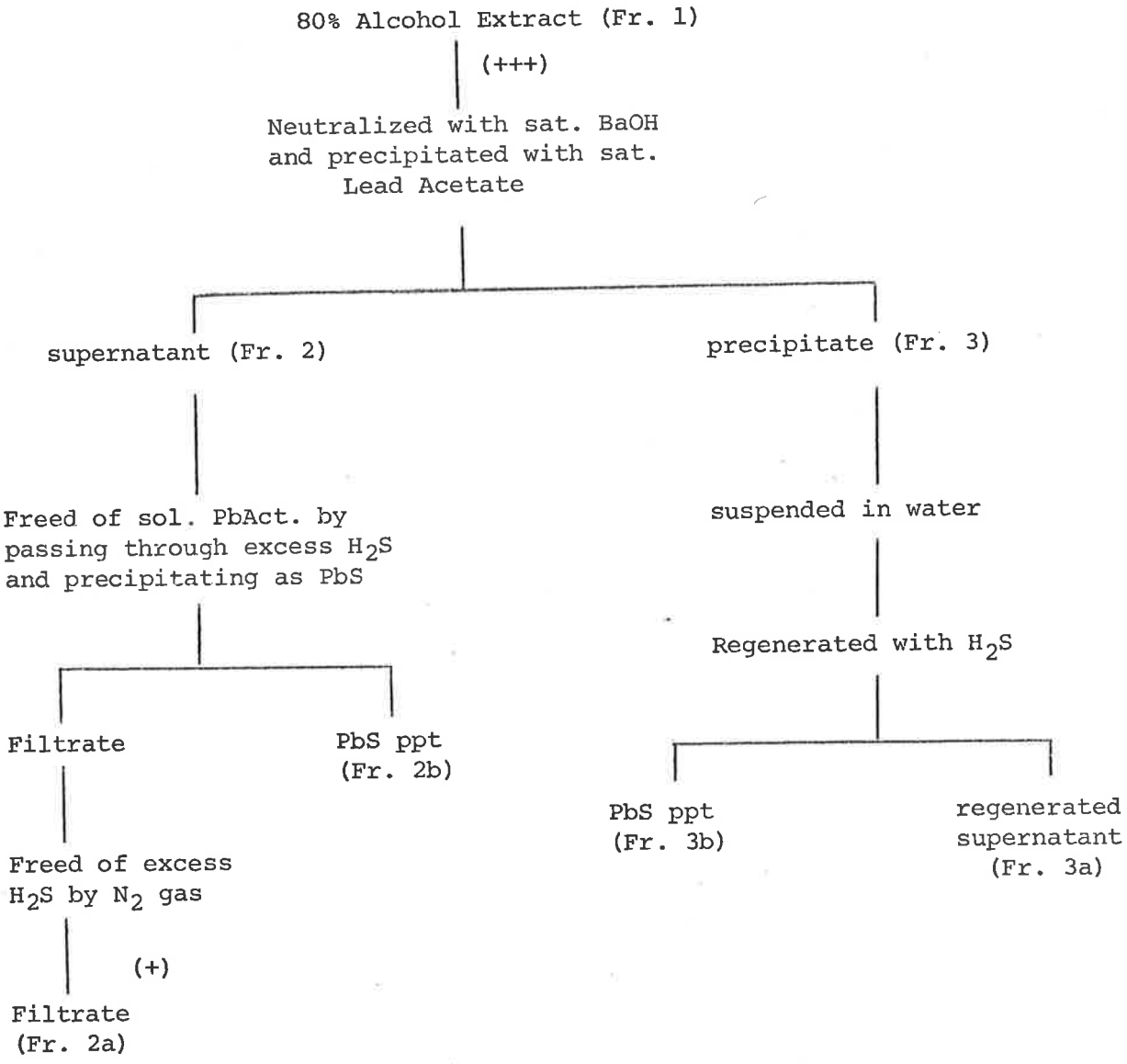


TABLE 7.3.3.1 Influence of phenolic substances on larval aggregation.

Fraction	Mean number of larvae per		Mann Whitney U-significance
	Test	Control	
Alcohol extract (Fr. 1)	25.2	3.2	***
Fraction 2a	19.6	10.8	*
Fraction 3a	7.6	5.6	N.S.

Mann Whitney U *** ($P < 0.001$)

* ($P < 0.05$)

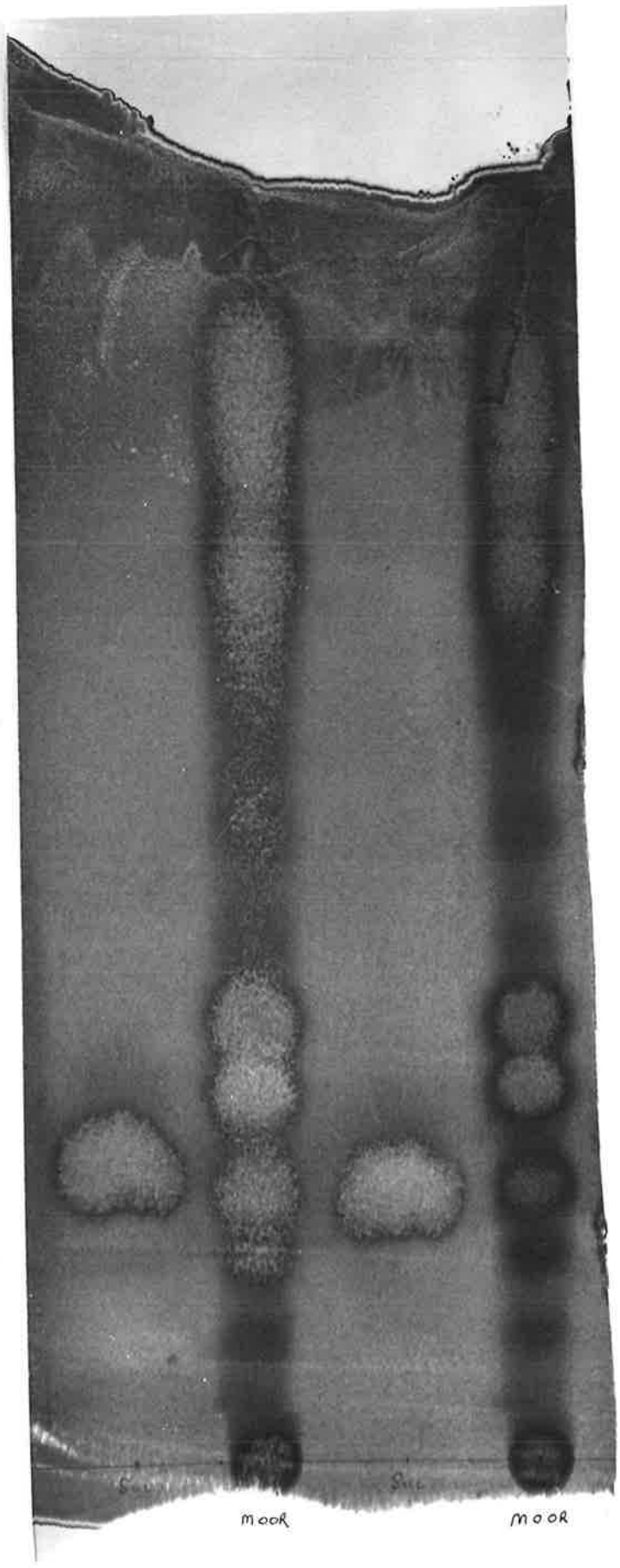
the activity in fraction 1 was lost through fractionation. Hsiao and Fraenkel (1968) observed that lead deters feeding when used to precipitate glycosides. In order to ascertain the influence of phenolic substances on larval feeding, some of the most common phenolic substances identified in Eucalyptus (Hillis, 1966) were tested for phagostimulation by incorporation in agar medium. None of the 7 phenolic compounds including chlorogenic acid was active when tested singly (see Chapter 8). Thus the possibility of phenolic substances acting as gustatory stimulants in their own right was ruled out.

7.3.4 Isolation of amino acids and sugars

Further investigation of the chemical nature of the feeding stimulants was made by adsorption chromatography on cation exchange Dowex-50 (H+) and Darco-celite columns. The fractionation scheme is shown in Fig. 7.3.4.2.

Due to possibility of a loss of labile active substances during drying, fresh leaves were used. Fresh leaves of E. maculata were collected in the arboretum and taken to the laboratory in boxes containing

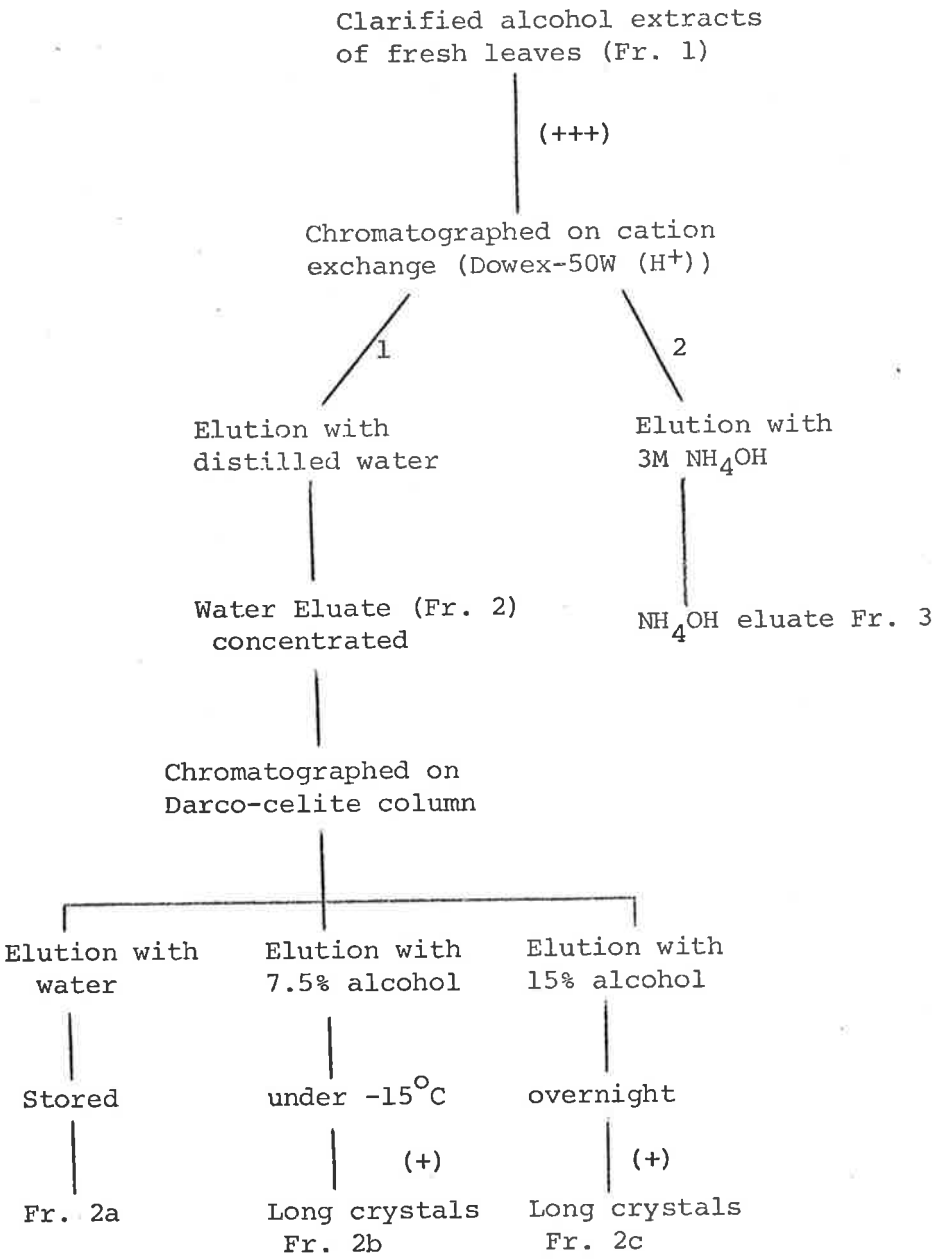
Figure 7.3.4.1 Paper chromatography of sugars in alcohol extract of E. moorei. (Sucrose standard).


MOOR
MOOR

dry ice. Weighed samples of the foliage were quickly dropped into boiling alcohol. The extract was cooled, and centrifuged. The supernatant was concentrated in a rotary evaporator at 30°C to near dryness and placed on Dowex-50W (H^+) column. The column was first eluted with distilled water, to obtain eluate fraction 2 and then with 3M NH_4OH to obtain eluate fraction 3. Fraction 2 consisted mainly of anionic and neutral substances such as sugars whereas fraction 3 consisted mainly of amino compounds. To obtain some preliminary information about the nature of sugars and amino compounds in the 2 fractions, paper chromatography, 2 dimensional thin layer chromatography and the amino acid analyzer were employed. Chemical tests with Anthrone and Ninhydrin had shown that fractions 2 and 3 consisted largely of sugars and amino compounds respectively. A sample of the water eluate was streaked on Whatman No. 1 paper and developed with upper phase of Butanol-acetic acid-water (4:1:5 v/v). The dried chromatogram was dipped in $AgNO_3$, dried and sprayed with ethanolic NaOH. Eight sugar spots were identified including glucose, fructose, sucrose and raffinose (see Fig. 7.3.4.1). Similar chromatographic analysis of clarified alcohol extracts of ten eucalypt species listed in Table 5.2.3.1 showed that all the 10 species have similar sugars with sucrose as the major component. A 2-dimensional T.L.C. of the amino group in Butanol-acetic acid-water (4:1:5 v/v) and 2, 6 Lutidine-collidine -water (1:1:2 v/v) and analysis using an amino acid analyzer demonstrated the presence of at least 12 amino acids including serine, glycine, valine, Isoleucine, Tyrosine, Phenylalanine, Aspartic acid and alanine. Fraction 3 was concentrated to dryness and taken up in water and incorporated to agar diet. Fraction 2 was also concentrated to dryness and placed on a Darco-celite column for separation of sugars (Whistler et al., 1950). The column was first eluted with water, followed by 7.5% alcohol and 15% alcohol to obtain subfractions

2a, 2b and 2c (see Fig. 7.3.4.2).

Fig. 7.3.4.2 Scheme for separation of sugars and amino acids.



The eluates subfractions 2a, 2b and 2c were stored below -15°C overnight and long crystals were found in 2b and 2c after 48 hr. All subfractions were tested but only 2b and 2c showed high activity. Chemical tests with Anthrone and Fehlings reagents, showed the presence of carbohydrates, sugars and reducing sugars (Cheronis and Enterikin 1957).

TABLE 7.3.4.1 Influence of amino compounds and sugars on larval feeding responses.

Fraction	Biting Intensity	Accumulated faecal pellets in 24 hours
Plain Diet	+	6
Diet + Fr. 1	++++	218
Diet + Fr. 2	+++	172
Diet + Fr. 3	+	11
Diet + Fr. 2a	+	23
Diet + Fr. 2b	++	56
Diet + Fr. 2c	++	66

Kruskal-Wallis H $P < (0.001)$

Preparatory paper chromatography substantiated this finding. The principal sugars found in all the 3 subfractions were glucose, fructose and sucrose. It appears the 3 subfractions differ only in amounts, though water, 7.5% alcohol and 15% alcohol elutions separate monosaccharides, disaccharides and higher oligosaccharides respectively. The results of the chromatographic analysis indicated that both the 7.5% alcohol and 15% alcohol elutions contained monosacchardies and disaccharides. This suggests that there was incomplete separation of groups. Paper chromatography of the acid hydrolyzate of subfractions 2b and 2c yielded glucose and fructose. Bioassay of glucose, fructose, sucrose and 19 other sugars showed that only fructose and sucrose were active alone. From this evidence it is concluded that the principal chemicals responsible for the activity in the water soluble phase are fructose and sucrose. Nevertheless, it was evident from the results that the activity of the original alcohol extract declined

with purification. This stresses the importance of other substances in attaining optimal response.

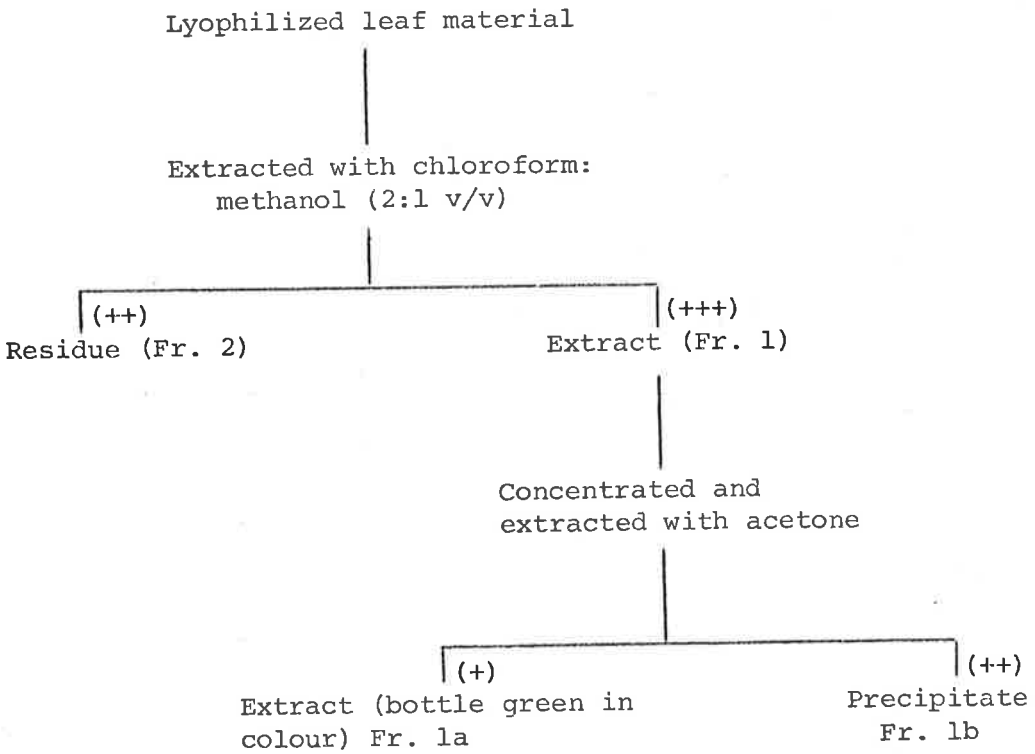
Glucose, fructose and sucrose are the principal sugars in virtually all higher plants. Thus the 3 sugars do not explain the specificity shown by U. lugens for the eucalypts. Nevertheless, the bioassay of the many plant substances (see Chapter 8) including secondary plant substances such as phenolic glycosides and terpenoids showed that fructose and sucrose are important for initiation and maintenance of feeding. Similar findings have been made for many phytophagous insects (Mithra et al., 1964; Heron, 1965; Loschiavo, 1965; Hsiao and Fraenkel, 1968; Akeson et al., 1968; Sutherland, 1971).

7.3.5 Isolation of water and alcohol insoluble stimulants

In comparing the feeding response to various extracts and residues as shown in Tables 7.3.1.1 and 7.3.1.2; it appears that feeding stimulants other than water and alcohol soluble substances may be present in eucalypt leaves. For instance, both the water and alcohol extracted residues were fairly active. Moreover, the ether extracted leaf residue was not as active as the original leaf powder. It was, therefore, postulated that in addition to alcohol and water soluble active substances there may also be 'fat soluble' feeding stimulants. This possibility was investigated by using the chloroform: methanol solvent method (Hanahan, 1960). The methanol has the property of rupturing the lipid-protein linkage whilst the chloroform extracts mainly the water insoluble substances. The extract was filtered through a Buchner funnel. The residue was re-extracted with two separate volumes of the same solvent system and the extracts pooled. The total chloroform: methanol extract was concentrated and aliquots of the concentrated extract were tested for biological activity. Part of the concentrated extract was re-extracted with acetone to separate the

phospholipids from the neutral fats, sterols and steroids (Hanahan, 1960; Robinson, 1964). The acetone insoluble phospholipids were washed with water, redissolved in a small quantity of chloroform and tested. The acetone soluble extract was concentrated and tested in a similar manner. The fractionation procedure and the results are shown in Fig. 7.3.5.1 and Tables 7.3.5.1 respectively.

Fig. 7.3.5.1 Scheme for separation of phospholipids from neutral fats.



The results in Table 7.3.5.1 indicate that all the 4 fractions elicit feeding responses. The chloroform:methanol extract elicited comparable biting response to the original leaf powder suggesting the presence of a 'fat soluble' feeding stimulant. Although chloroform:methanol are used largely for extraction of 'fat soluble' substances, analysis of fraction 1 and subfractions 1a and 1b with Anthrone and Ninhydrin indicated that the

TABLE 7.3.5.1 Biting and feeding responses of 7th instar U. lugens to chloroform:methanol extract and its subfractions.

Fraction	Biting Intensity	Accumulated faecal pellets in 24 hours
Plain diet	+	36
Diet + chloroform:MeOH extract (Fr. 1)	++++	237
Diet + residue from Fr. 1	+++	176
Diet + acetone eluate of Fr. 1 (Fr. 1a)	+++	107
Diet + ppt. of acetone treatment of Fr. 1 (Fr. 1b)	+++	129
Diet + original leaf powder	++++	288

Kruskal-Wallis H ($P < 0.01$)

major fraction and its subfractions contained carbohydrates and amino acids. Paper and thin layer chromatography substantiated these findings. Thus, the biological activity in fraction 1 and subfraction 1a and 1b may not necessarily be due to the lipid substances alone. The least appreciated facet of lipid purification is the marked tendency for lipid preparations to trap non-lipid components (Hanahan, 1960). To ascertain whether some of the activity of fractions 1 and subfractions 1a and 1b were due to free fatty acids, steroids and phospholipids, some commercial grade 'fat soluble' nutrient substances were bioassayed. None was effective in eliciting feeding singly (see Chapter 8). It is possible that the strong response to fraction 1 and the subfractions may be due to combination of lipid substances and sugars. The methanol fraction of the solvent system may have been responsible for extraction of the sugars.

7.4 Discussion

Investigation into leaf constituents that evoke feeding showed that

the principal component is soluble in water, ethanol and insoluble in ether. The major constituents of the alcohol extracts are amino acids, sugars, sugar alcohols, organic acids and phenolic compounds. Further investigation ruled out phenolic compounds as having any positive influence on feeding. Paper chromatography of clarified alcohol extract in upper phase of equilibrated mixture of 1-butanol-90% formic acid-water (10:3:10 v/v) with bromothymol blue (Brown, 1950) as the detection reagent showed only traces of organic acids. It is possible that eucalypt leaves do not accumulate organic acids from the citric acid cycle to any appreciable level as do citrus, blackberries or apples (Robinson, 1960).

Elution chromatography using Cation exchange and Darco-celite columns separated out crystals which were found to be biologically active. Taste and chemical tests (with Anthrone) and paper chromatography before and after HCl hydrolysis suggested that the active substance was sucrose with glucose and fructose contaminants. Apart from spots for glucose, fructose and sucrose, 5 other spots were observed on chromatograms. A test of 19 sugars including glucose, fructose, sucrose (see Chapter 8) showed that fructose and sucrose are strong feeding stimulants.

It was evident that the activity of the crude extract declined with purification. This suggests that some important substances are lost during the process of extraction and purification. It is possible that the decline in activity may be due to loss of synergistic or additive effects brought about by purification.

The residual activity in the alcohol and water extracted residues and the strong response to the chloroform:methanol extract suggest that the leaf may contain stimulants additional to sugars. Investigation into the chemical nature of the water and alcohol insoluble feeding stimulants was however, inconclusive. It appears that much more work is needed

to elucidate and identify the 'fat soluble' feeding stimulant(s). The fat soluble substances include fatty acids, phospholipids, steroids and terpenoids, flavonoids, porphyrins, long chain hydrocarbons, alcohols and ketones etc. (Robinson, 1960). Some of the common fatty acids, sterols and phospholipids were bioassayed to determine their influence on larval feeding responses. These substances were ineffective when tested alone, though the phospholipids slightly enhanced feeding when combined with sucrose. Further investigations of the nature of the 'fat soluble' feeding stimulant(s) are reported in Chapter 10.

CHAPTER 8 FEEDING BEHAVIOUR RELATED TO GROUPS OF PLANT COMPOUNDS

8.1 Introduction

The effects of chemical components of plants in the feeding behaviour of phytophagous insects has been stressed in many reviews on insect host plant relationships (Thorsteinson, 1960; Beck, 1965, 1974; Schoonhoven, 1968). The chemicals themselves have been categorized into attractants, repellents, arrestants, suppressants, feeding stimulants and feeding deterrents based upon the sensory responses they induce. Some of these substances are secondary plant substances specific to certain species or groups of species of plants, but others are nutrient substances of common botanical distribution.

Provided that the physical characteristics of the food are acceptable to an insect, the feeding stimulants or phagostimulants, alone or in combination with feeding deterrents, determine the amounts consumed. In Chapter 7 it was shown that the major feeding stimulants for larvae of U. lugens were soluble in water and 80% ethanol. Aqueous and ethanol extracts consist largely of plant carbohydrates, amino acids, water soluble vitamins, mineral salts and phenols. Feeding responses to these groups of substances have been demonstrated for many species of insects (Beck, 1957; Davies, 1965, 1975; Schoonhoven 1968; Harvey, 1974; Cook, 1977).

Nothing is known of the role these substances play in the feeding behaviour of U. lugens and it was therefore considered appropriate to determine the effects of individual substances on the feeding behaviour of this insect. The experiments embraced the water soluble components as well as the lipids and sterols to obtain a general picture of the roles of nutrient substances in determining feeding patterns.

8.2 Materials and methods

The bioassay method used for evaluating the effect of test substances was the same as that described in Chapter 6. Lipids and sterols, were first dissolved in ether or chloroform and then mixed with cellulose powder used in the plain diet. After evaporation of the solvent, the treated cellulose powder was combined with agar solution. All substances used were of "technical grade" and were generally tested at two concentrations for biting and feeding responses. Subsequently certain combinations of nutrients were presented to determine the presence or absence of interaction between them. A total of 15 larvae were used in each treatment. Each test was conducted at $25^{o}C \pm 2^{o}C$, 60% ± 5% RH, under 16 hr photophase.

8.3 Results and discussion

Due to differences in physiological age and pre-test feeding experiences of caterpillars, intertest comparisons of faecal counts could not be made. The differences recorded did not affect the validity of the results of any one experiment, however, as caterpillars used in a particular test all came from the same age group and had similar pre-test feeding experiences. Nevertheless, it was apparent that caterpillars that had fed upon meridic diet prior to test produced significantly more faecal pellets than those that had not, suggesting that pre-test experience had resulted in some adaptation to diet.

8.3.1 Carbohydrates

The effects of carbohydrates on larval feeding behaviour are summarized in Tables 8.3.1.1 and 8.3.1.2. Of the 24 sugars and related substances tested only fructose, sucrose and sucrose/glucose/fructose

mixture elicited strong biting and continued feeding responses. Moreover, the feeding response was positively related to increase in their concentration in the diet. Sucrose has been found to stimulate feeding of many phytophagous insects while pentoses are generally poor phagostimulants and such trends were confirmed here for U. lugens. The disaccharides and oligosaccharides, Lactose, Trehalose, Melizitose and Dextrin evoked moderate biting responses but did not stimulate feeding. No obvious relationship between activity of sugar and its chemical configuration could be discerned.

None of the sugar alcohols were feeding stimulants for the gum leaf skeletonizer. Sucrose, glucose and fructose, the most commonly occurring plant sugars (Wykes, 1952) were found in all 10 species of Eucalyptus listed in Table 5.2.3.1. The molar concentration of these 3 common sugars tested to determine the optimum feeding response showed that glucose alone had no effect while sucrose and fructose both elicited response at .001 molar concentration and produced peak feeding at about 0.1 molar concentration. The data suggest that the increases in concentration above this level resulted in decreased feeding of larvae (Fig. 8.3.1.1). The effect of very high concentrations of phagostimulants on food intake by phytophagous insects still remains an unsolved phenomenon. Schoonhoven (1969) posed 3 questions: "Does a low impulse frequency in the chemoreceptory cell concerned induce a stimulation of feeding, whereas high frequencies inhibit? Is a high frequency too much out of balance with activity levels of other chemoreceptors and therefore becoming inhibitory? Or does high concentrations of feeding stimulants also 'excite' deterrent cells?" More work in sensory physiology is required to answer these questions, but Bernays and Chapman (1974) suggest that the internal physiological conditions of individuals may also be important.

TABLE 8.3.1.1 Biting and feeding responses of larvae of Uraba lugens to carbohydrates and related compounds.

Test compound	Concentration molar %	Biting response	Faecal[1] counts	Molar concentration	Biting response	Faecal[2] counts
1 Plain diet	-	+	25	-		
2 Pentoses						
L-Arabinose	0.01M	+	14	.05	+	19
L-Rhamnose	0.01	+	23	.05	+	32
D-Ribose	0.01	+	18	.05	+	17
D-Xylose	0.01	+	20	.05	+	22
3 Hexoses						
D-Fructose	0.01	+++	155	.05	++++	300
D-Galactose	0.01	+	27	.05	+	16
D-Glucose	0.01	+	22	.05	+	30
D-Mannose	0.01	+	19	.05	+	27
L-Sorbose	0.01	+	17	.05	+	19
4 Disaccharides						
D-Cellobiose	0.01	+	10	.05	+	15
D-Lactose	.01	++	63	-		
D-Maltose	.01	++	34	-		
D-Melibiose	.01	++	45	-		
D-Sucrose	.01	+++	168	.05	++++	346
D-Trehalose	.01	+	42	.05	++	46

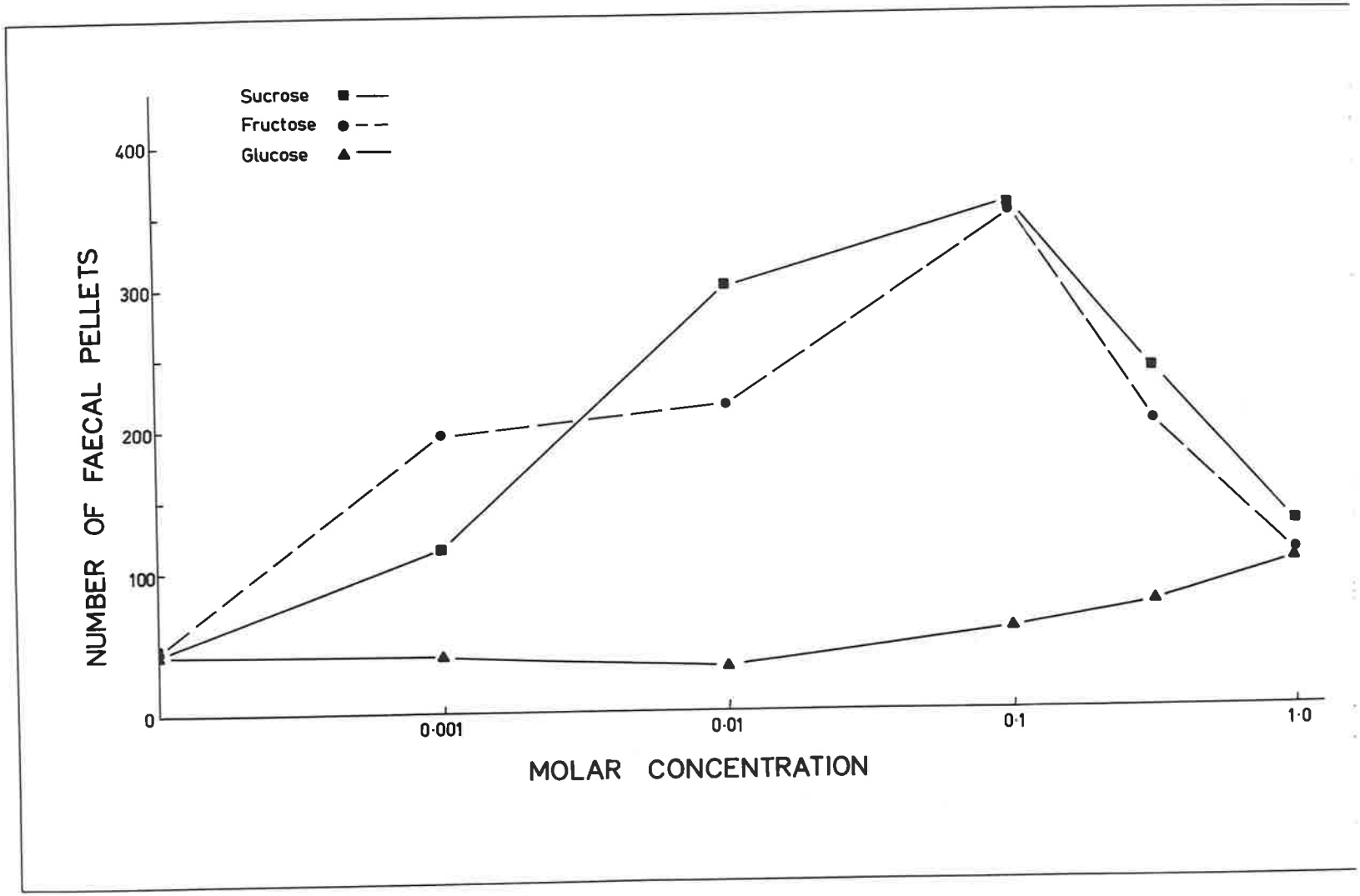
continued ....

TABLE 8.3.1.1 continued

Test compound	Concentration molar %	Biting response	Faecal[1] counts	Molar concentration	Biting response	Faecal[2] counts
5 Tri and Polysaccharides						
D-Melizitose	.01	++	53	.05	++	60
D-Raffinose	.01	+	21	.05	+	28
Dextrin	2.5%	++	54	-		
Starch	2.5%	+	15	-		
Fructose/Glucose/Sucrose	0.5%	++++	272	-		
6 Polyhydric alcohols						
D-Dulcitol	.01	+	30	.05	+	31
D-Inositol	.01	+	14	.05	+	21
D-Mannitol	.01	+	35	-		
D-Sorbitol	.01	+	35	-		
7 Glucoside						
Methyl- -D glycopyranoside	.01	+	29	.05	+	38

Kruskal Wallis $H^{(1,2)}$ ($P < 0.001$)

Figure 8.3.1.1 Feeding responses of 7th instar Uraba lugens to the major water and alcohol soluble sugars.


Sucrose
Fructose
Glucose
400
300
200
100
0
NUMBER OF FAECAL PELLETS
0·001
0·01
0·1
1·0
MOLAR CONCENTRATION

They have shown that higher concentrations of substances such as sugars, amino acids, salts and mixtures of these, produced high haemolymph osmotic pressure in Locusta migratoria L. The immediate effect of such a high haemolymph concentration or osmotic pressure is reduction in amount of food ingested. This condition does not necessarily mean that the feeding stimulant has become a deterrent at high concentration merely that less food may be necessary under such conditions.

As the larvae encounter a mixture of sugars in their natural food, the response of larvae to mixtures of some of the sugars were investigated. The results are shown in Table 8.3.1.2. In experiments with the house-fly (Gallun and Fraenkel, 1957), the blowfly (Dethier, 1955) and the sweetclover weevil (Akeson et al., 1970) it was found that many slightly active or inactive sugars, when mixed with an active stimulant strongly inhibited feeding. On the otherhand (Meisner et al., 1970, 1971; Heron 1965; Yamamoto and Fraenkel 1962; Hsiao and Fraenkel, 1968; Cook, 1977) have reported that mixtures of some sugars enhance feeding. In the present study, the outcome of interactions permitted classification of the sugars into 3 groups. Maltose, sorbitol and α-methyl glucopyranoside, were considered inert because their mixtures with sucrose evoked about the same level of feeding as the corresponding sucrose diet. With galactose, raffinose, trehalose, inositol and dextrin the amount of feeding was increased to a level expected for the sum of the effects of the 2 constitutents. Perhaps the most interesting observation as far as feeding stimulation is concerned is the result obtained with mixtures of sucrose and glucose. The combination led to a much greater feeding response than the effect of either chemical (Table 8.3.1.2). Although glucose was not stimulative alone, it is always accompanied by fructose and sucrose in Eucalyptus

TABLE 8.3.1.2 The biting and feeding responses of 7th instar Uraba lugens larvae to combinations of sugars and related compounds.

	Test compound	Molar concentration	Biting response	Faecal weights in mg*
1	Plain diet	-	+	1.8
2	Diet+sucrose	.01	++++	8.0
3	Diet+sucrose/glucose	.01 + .01	++++	16.8
4	Diet+sucrose/galactose	.01 + .01	++++	10.9
5	Diet+sucrose/lactose	.01 + .01	++	4.9
6	Diet+sucrose/maltose	.01 + .01	++++	7.9
7	Diet+sucrose/raffinose	.01 + .01	++++	9.2
8	Diet+sucrose/trehalose	.01 + .01	++++	10.1
9	Diet+sucrose/inositol	.01 + .01	++++	10.9
10	Diet+sucrose/sorbitol	.01 + .01	++++	8.9
11	Diet+sucrose/dextrin	.01 + 5%	++++	9.7
12	Diet+sucrose/starch	.01 + 5%	+	2.9
13	Diet+sucrose/methyl glucopyranoside	.01 + .01	++++	7.8

* Larvae used were obtained from different sources.

Kruskal-Wallis Anova ($P < .01$)

foliage and therefore must, on these results, play a significant role in the overall palatability of the food to U. lugens. Interaction of this nature confer a high degree of efficiency on mixtures. Hsiao (1966) showed that dilution of mixtures to a level in which the individual components of mixtures failed to evoke feeding does not affect the phagostimulation of the mixtures. Lactose and starch, however, showed antagonistic effects when combined with sucrose. Lactose was slightly active when tested alone but in combination with sucrose the amounts of food eaten were much less than for sucrose alone. Starch not only failed

to stimulate feeding when tested alone, but also reduced significantly the amount of food ingested when presented in mixture with sucrose. It is not clear why starch has an inhibitory effect on feeding. All leaves contain starch and there is little doubt that starch is consumed by phytophagous insects. Fraenkel (1940) showed that the blowfly did not feed on a starch diet though its gut contains amylase. But this is understandable in an insect which feeds largely on meat as a larva and nectar as an adult.

The results shown above stressed the importance of sugars in insect/foodplant relationships. Sugars are known to provide easily available energy for insect activity and development. In some adult insects sugars serve as the exclusive food. Glaser (1923) in his experiments with adult houseflies showed that sugar or some form of starch that can be assimilated are important to the longevity of the flies. Beck (1957) has shown that apart from the apparent nutritional role of sugars, larval 'saccharotropism' results in orienting larvae to those host plant tissues which by virtue of their higher sugar content, offer the greatest protection against the action of resistant factors.

Phagostimulation extends the list of the important roles played by sugars in insect-host plant interactions. The 3 common sugars - glucose, fructose and sucrose were all found to be important in feeding stimulation of larvae of U. lugens though the last two are considered the most important in view of the fact that they each induced strong feeding responses. The strong positive response to fructose and sucrose is not unique since the substances have been shown to evoke strong feeding responses by the European corn borer (Beck, 1957), the Mexican bean beetle (Augustine et al., 1964), the spruce budworm (Heron, 1965), and the clear-winged grasshopper (Thorsteinson, 1960). Perhaps their common occurrence

in higher plants may explain why they are frequently associated with feeding stimulation of phytophagous insects.

8.3.2 Amino acids

The effects of 22 amino acids on the biting and feeding responses of Uraba lugens are summarized in Table 8.3.2.1. None tested singly elicited a strong feeding response from the larvae, though the basic amino acids as a group evoked slight to moderate biting and feeding responses. Other amino acids that elicited biting response include 2 mono-aminocarboxylic acids; Glycine and Leucine and one monodicarboxylic acid - Asparagine. In general, .05M concentration was associated with higher biting response. For combinations of sucrose and amino acids, selection was restricted to eight of the most effective single amino acids. The results are shown in Table 8.3.2.2. All the eight amino acids with exception of DL-α-aminobutyric acid enhanced feeding in the presence of sucrose. A very simplistic classification of the eight amino acids was undertaken. DL-α-aminobutyric acid was considered as inert; Histidine monohydrochloric acid, L-Leucine, L-Asparagine, DL-alanine, L-Methionine and L-Valine showed additive effect, however, L-Arginine monohydrochloric acid was considered as synergistic because the net effect was greater than what would be expected by summation of individual effects. It is probably of even more interest to note that the mixture was more effective than a 2-fold concentration of sucrose.

8.3.3 Lipids and sterols

Fatty acids, triglycerides and phospholopids as well as free sterols have all been investigated as feeding stimulants for insects. Loschiavo (1965) found palmitic acid to be a strong phagostimulant for the confused

TABLE 8.3.2.1 Biting and feeding responses of Uraba lugens larvae to amino acids.

Test compound	Concentration molar %	Biting response	Faecal[1] counts	Molar concentration	Biting response	Faecal[2] counts
1 Plain diet	-	+	25			19
2 Aliphatic Amino Acids						
2.1 Monoaminocarboxylic acids						
Glycine	.01	+	37	.05	++	43
L-Alanine	.01	+	15	.05	+	13
DL-Alanine	.01	+	22	.05	+	39
L-Serine	.01	+	23	.05	+	29
L-Valine	.01	+	25	.05	+	39
L-Leucine	.01	+	30	.05	++	44
L-Threonine	.01	+	19	.05	+	34
DL-α-Aminobutyric acid	.01	+	32	.05	++	49
2.2 Monodicarboxylic acids and their amides						
L-Aspartic acid	.01	+	11	.05	+	21
L-Asparagine	.01	++	39	.05	++	51
L-Glutamic acid	.01	+	21	.05	+	23
L-Glutamine	.01	+	20	-		
2.3 Basic Amino acids						
L-Lysine-HCl	.01	++	40	.05	++	49
Arginine-HCl	.01	++	47	.05	++	62
Histidine-HCl	.01	++	53	.05	++	78

continued .....

TABLE 8.3.2.1 continued

Test compound	Concentration molar %	Biting response	Faecal[1] counts	Molar concentration	Biting response	Faecal[2] counts
2.4 Sulphur-containing Amino acids						
L-Cysteine	.01	+	17	.05	+	27
L-Cystine	.01	+	23	.05	+	34
L-Methionine	.01	+	25	.05	+	39
3. Aromatic Amino acids						
DL-Phenylalanine	.01	+	11	.05	+	10
L-Tyrosine	.01	+	19	.05	+	22
4. Heterocyclic Amino acids						
L-Tryptophan	.01	+	41	.05	+	30
L-Proline	.01	+	25	.05	+	36
5. Miscellaneous						
Adenine	.01	++	71	.10	++	84

Kruskal Wallis $H^{(1,2)}$ ($P < 0.001$)

TABLE 8.3.2.2 The biting and feeding responses of 8th instar Uraba lugens larvae to combinations of sucrose and various amino acids.

	Test compound	Molar concentration	Biting response	Faecal weights in mg*
1	Plain diet		+	4.4
2	Diet+sucrose	.05	++++	14.1
3	Diet+sucrose	.1	++++	21.9
4	Diet+sucrose/Histidine HCl	.05 + .05	++++	18.7
5	Diet+sucrose/L-Leucine	.05 + .05	++++	19.6
6	Diet+sucrose/L-Asparagine	.05 + .05	++++	19.0
7	Diet+sucrose/L-Arg-HCl	.05 + .05	++++	24.0
8	Diet+sucrose/DL-Alanine	.05 + .05	++++	15.6
9	Diet+sucrose/L-Methionine	.05 + .05	++++	15.6
10	Diet+sucrose/L-Valine	.05 + .05	++++	16.8
11	Diet+sucrose/DL- -Amino butyric acid	.05 + .05	++++	13.8

* Larvae used were collected from population on different trees.

Kruskal-Wallis H ($P < 0.05$)

flour beetle, Tribolium confusum. Thorsteinson and Nayar (1963) showed that phospholipids - Lecithin, phosphatidyl inositol, phosphatidyl serine are stimulatory to Melanoplus bivittatus and Camnula pellucida. Dadd (1960) showed that wheat germ oil stimulated feeding in Schistocerca gregaria. Hamamura et al. (1962) found β-sitosterol a powerful feeding stimulant for the silkworm Bombyx mori and Hsiao and Fraenkel (1968) found the sterols-cholesterol and β-sitosterol stimulated biting but not feeding in Leptinotarsa decemlineata. The phospholipids-phosphatidyl inositol, phosphatidyl serine and phosphatidyl ethanolamine were, however, feeding stimulants.

In the present study, none of the fatty acids, sterols and phospho-

lipids promoted feeding on agar medium alone (Table 8.3.3.1). However,

TABLE 8.3.3.1 Biting and feeding responses of 5th instar Uraba lugens larvae to lipids and sterols.

	Test compound	Concentration	Biting response	Faecal weights in mg
A_1	Plain diet	-	++	4.0*
2	Diet+Palmitic acid	0.01	+	1.4
3	Diet+Oleic acid	0.01	+	1.9
4	Diet+Linoleic acid	0.01	+	2.7
5	Diet+Stearic acid	0.01	+	3.4
6	Diet+Cholesterol	0.01	+	3.1
				Faecal counts
B_1	Plain diet	-	+	72***
2	Diet+Phosphatidyl ethanolamine	0.15%	+	49
3	Diet+Phosphatidyl inositol	0.15%	+	56
4	Diet+Phosphatidyl choline	0.15%	+	49
5	Diet+Sucrose	0.1M	++++	438

* Kruskal Wallis ($P < .05$)

*** Kruskal Wallis ($P < .001$)

when some of the best known phospholipids were tested in combination with sucrose, all enhanced feeding with phosphatidyl inositol and phosphatidyl choline being slightly more effective (Table 8.3.3.2). In view of the results in this section, it is just possible that the phagostimulatory activity of the phospholipid leaf fraction demonstrated in Chapter 7 was due in part to contamination with e.g. sucrose, since pure reagent grade phospholipids failed to stimulate biting and feeding responses alone.

TABLE 8.3.3.2 Biting and feeding responses of 5th instar larvae to combination sugars and phospholipids.

	Test compound	Concentration	Biting response	Accumulated faecal pellets by 15L
1	Plain diet		++	104
2	Diet+Sucrose	.01	++++	270
3	Diet+Sucrose/ Phosphatidyl ethanolamine	.01 + .15%	++++	285
4	Diet+Sucrose/ Phosphatidyl inositol (Cephalin)	.01 + .15%	++++	316
5	Diet+Sucrose/ Phosphatidyl choline (Lecithin)	.01 + .15%	++++	337

Kruskal Wallis ($P < 0.01$)

8.3.4 Vitamins

A test of phagostimulatory activity of the vitamins was restricted to the water-soluble vitamins of the B-complex and Ascorbic acid because these are the major ones which have been shown to be required by phytophagous insects (House, 1954, 1958; Dadd 1961; Fraenkel and Blewett, 1943; Davis, 1975). The vitamins tested included thiamine hydrochloride, Pyrodoxine hydrochloride, Choline chloride, Panthothenic acid, Niacin, Riboflavin, brewers yeast, Vitamin B_{12} and Ascorbic acid. Panthothenic acid and Ascorbic acid showed moderate and strong feeding responses respectively (Table 8.3.4.1). Due to the strong response to Ascorbic acid, it was tested at different concentrations to determine threshold of response and optimal response. The threshold of response and optimal response were .001M and 0.1M concentrations respectively (Fig. 8.3.4.1). Though Ascorbic acid was as effective as sucrose and

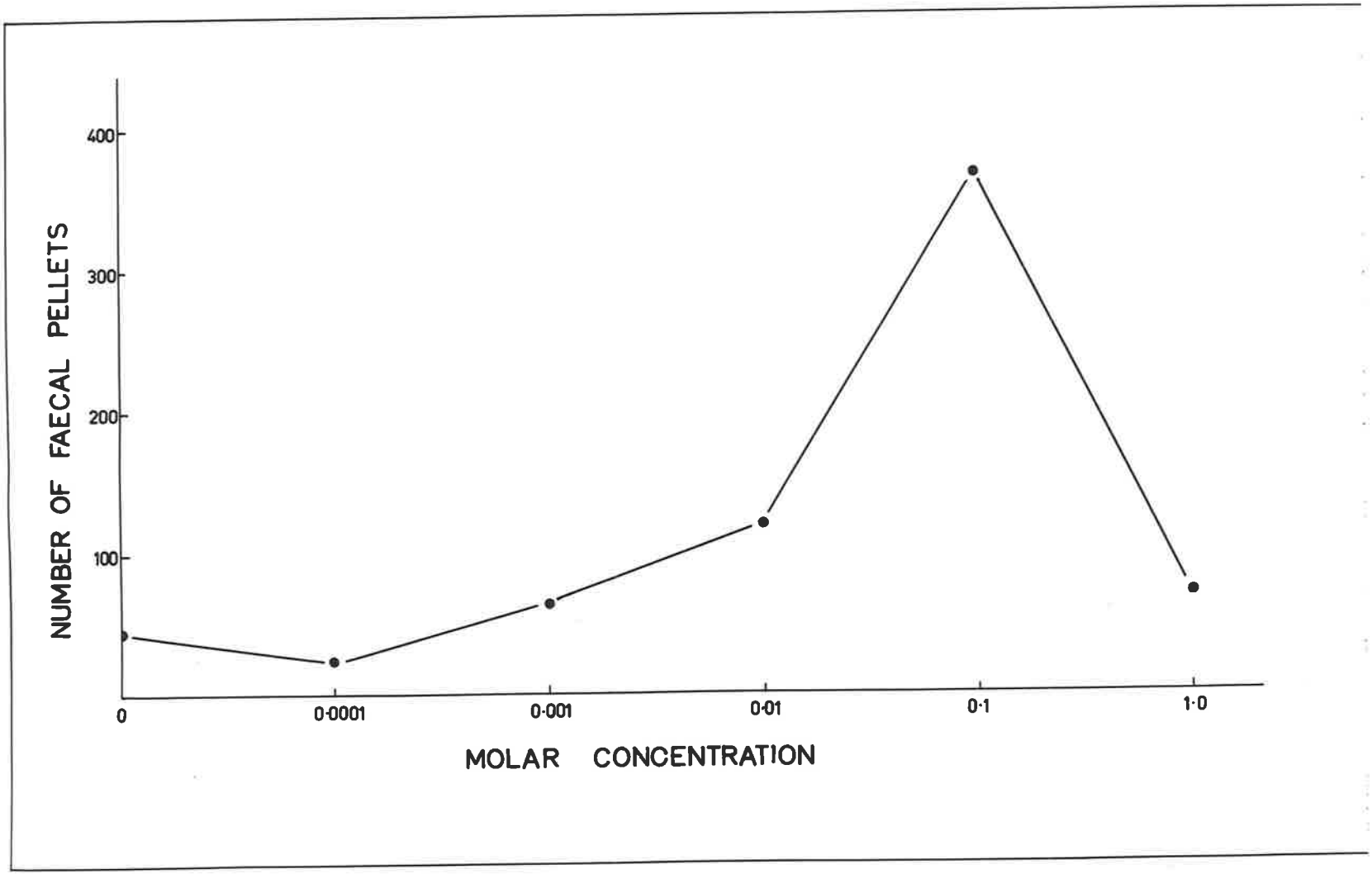
TABLE 8.3.4.1 Biting and feeding responses of 6th instar *Uraba lugens* larvae to vitamins.

	Test compound	Molar concentration	Biting response	Faecal counts
1	Plain diet	-	+	7
2	Diet+Thiamine hydrochloride	.01	+	9
3	Diet+Pyrodoxine hydrochloride	.1	+	6
4	Diet+choline chloride	.01	0	1
5	Diet+Panthothenic acid	.1	++	76
6	Diet+Niacin	.1	+	20
7	Diet+Riboflavin	.01	+	28
8	Diet+Ascorbic acid	.01	++	119
9	Diet+Vitamin B_{12}	.01	+	23
10	Diet+Brewers Yeast	1.5%	+	24

Kruskal-Wallis H ($P < 0.001$)

fructose at 0.1M concentration, it showed a relatively low activity at concentrations below optimum. Perhaps the effectiveness of Ascorbic acid in inducing intense biting and feeding responses may be due to structural similarity between this vitamin and some of the 6-carbon sugars tested. Vitamins as a group have been demonstrated as phagostimulants for a few phytophagous insects. Thorsteinson (1958) found Ascorbic acid and thiamine to be stimulatory for the grasshopper, *Chorthippus longicornis* (Latrielle). Thorsteinson (*ibid.*) reported that thiamine and Ascorbic acid elicit feeding responses from the potato beetle, though this result was not confirmed by Hsiao and Fraenkel (1968). Sutherland and Hillier (1974) found .01M Ascorbic acid to evoke intense

Figure 8.3.4.1 Feeding responses of 7th instar Uraba lugens to various concentrations of Ascorbic acid in diet.


NUMBER OF FAECAL PELLETS
400
300
200
100
0
0·0001
0·001
0·01
0·1
1·0
MOLAR CONCENTRATION

feeding activity though 0.1M Ascorbic was ineffective. Cook (1977), however, did not find Ascorbic acid an active stimulant for Locusta migratoria L. though it enhanced feeding in the presence of sucrose. Gothilf and Beck (1967) found Ascorbic acid to be a feeding deterrent for Trichoplusia ni.

8.3.5 Inorganic salts

The mineral requirements of insects constitute, perhaps, the least known factor in insect nutrition. Loeb (1915) demonstrated the qualitative requirement for some of the major elements - potassium, phosphorus and magnesium, however, very little has been added to this in the years following. The mineral constituents of certain Eucalyptus spp. have been investigated (Lamb, 1976; Ashton, 1975; Bevage, 1977; Fox and MaCauley, 1977). N, P, K, Ca and Mg exist in fairly high levels but Na, B, Fe, Zn and Cu were detected in trace amounts.

Inorganic salts have been shown by many workers to influence feeding behaviour of phytophagous insects (Gothilf and Beck, 1967; Akeson et al., 1969; Salama and El-Sharaby, 1973). Mallis et al. (1962) found that inorganic salts K_2HPO_4, KCl, NaCl and Na_2HPO_4 stimulate feeding of larvae of the webbing clothes moth, Tineola bisselliela (Hummel) and the furniture carpet beetle, Anthrenus flavipes Le Conte. Hamamura et al. (1962) found K_3PO_4 stimulatory to Bombyx mori. However, the influence of inorganic salts on feeding behaviour of phytophagous insects has been demonstrated adequately only in the presence of a strong phagostimulant. Thorsteinson (1960) found KH_2PO_4 to be ineffective alone, but when presented with sucrose the amount of food eaten by Camnula pellucida was significantly increased. Hsiao and Fraenkel (1968) found a mixture of NaCl, KCl, KH_2PO_4, $Ca_3(PO_4)_2$, $CaCO_3$ and $MgSO_4$, when given in addition to sucrose, enhanced feeding for Leptinotarsa decemlineata.

Chloride, dihydrogen phosphate and orthohydrogen phosphate were the most effective anions. Cook (1977) showed that potassium dihydrogen phosphate enhanced feeding of Locusta migratoria L.

On the otherhand, NaCl inhibits feeding on sucrose impregnated paper and leaves for L. migratoria and Danaus plexippus (Haskell and Schoonhoven, 1969; Dethier, 1937). Sodium nitrate reduces feeding by P. brassicae and Sitona cylliadricollis (Ma, 1972). Potassium chloride also inhibits the feeding response of Camnula pellucida (Scud.) to sugar (Thorsteinson, 1960). It appeared the extent of inhibition by the inorganic salts depends on relative concentrations of phagostimulant and salt. For example, the addition of low concentration of NaCl to diets containing sucrose increases consumption by Pieris brassicae (L.) and L. decemlineata, but at higher concentrations of NaCl, consumption is reduced.

In the study reported here 'salt mixture W' was tried initially. The results are summarized in Tables 8.3.5.1 and 8.3.5.2. The salt mixture failed to evoke feeding response alone at the 2 concentrations tested. However, when the mixture was tested in the presence of sucrose it enhanced feeding at both 2% and 5% concentrations (Fig. 8.3.5.1).

Some of the major constituents of the 'salt mixture W' are KCl, KH_2PO_4, $Ca_3(PO_4)_2$, $CaCO_3$ and NaCl. These together with Na_2PO_4 and $MgCl_2$ were tested with 0.1M sucrose to determine their influence on feeding behaviour of larvae of U. lugens. The results in Table 8.3.5.2 showed that NaCl at 0.05% and $MgCl_2$ at 0.1% concentrations significantly reduced feeding on agar medium. However, Na_2PO_4 significantly enhanced feeding on agar medium but its effectiveness could not be ascribed to either the cation or the anion.

Figure 8.3.5.1 Feeding responses of 8th instar Uraba lugens to a mixture of sucrose and salt mixture 'W'.

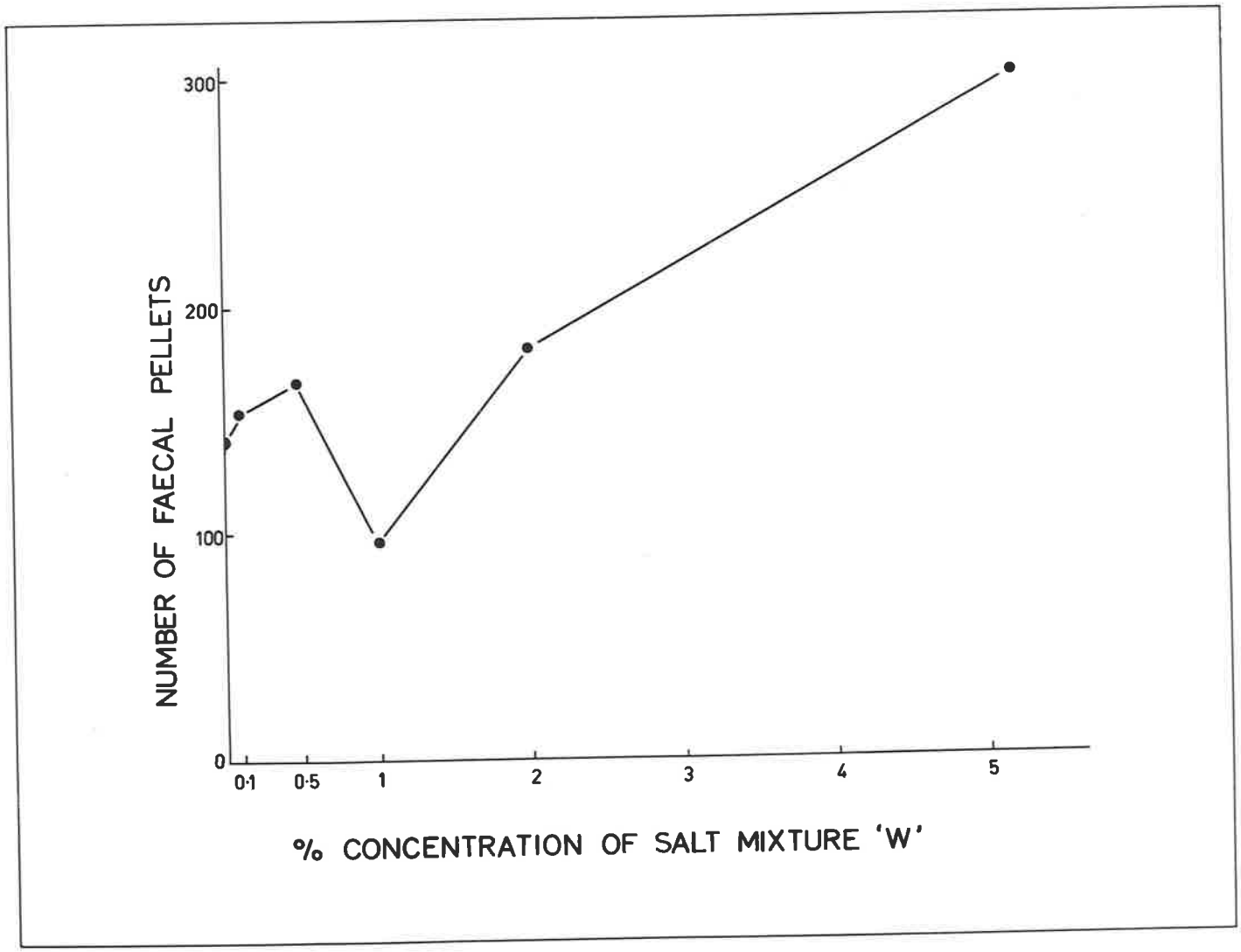

300
200
100
0
NUMBER OF FAECAL PELLETS
0·1
0·5
1
2
3
4
5
% CONCENTRATION OF SALT MIXTURE 'W'

TABLE 8.3.5.1 Biting and feeding responses of 8th instar Uraba lugens larvae to salt mixture W.

	Test compound	Concentration	Biting intensity	Accumulated faecal pellets by 15 larvae
1	Plain diet	-	+	6
2	Diet+salt mixture	0.1%	+	15
3	Diet+salt mixture	0.5%	+	20
4	Diet+sucrose	0.1M	++++	149

Kruskal-Wallis H ($P < 0.001$)

TABLE 8.3.5.2 Biting and feeding responses of 8th instar Uraba lugens larvae to a combination of .1M sucrose and various components of salt mixture W.

	Test compound	Concentration	Biting intensity	Accumulated faecal pellets by 15 larvae
1	Plain diet	-	+	88
2	Diet+sucrose	0.1M	++++	318
3	Diet+sucrose/KCl	0.1M + 0.3%	++++	309
4	Diet+sucrose/KH_2PO_4	0.1M + 0.3%	++++	311
5	Diet+sucrose/$Ca_3(PO_4)_2$	0.1M + 0.2%	++++	299
	Diet+sucrose/$CaCO_3$	0.1M + 0.2%	++++	299
6	Diet+sucrose/NaCl	0.1M + 0.5%	++	167
7	Diet+sucrose/Na_2PO_4	0.1M + 0.05%	++++	496
8	Diet+sucrose/$MgCl_2$	0.1M + 0.1%	++++	231

Kruskal-Wallis ($P < .05$)

8.3.6 Phenols

Quantitatively, phenols may be the most important non-nutrient water and alcohol soluble substances in Eucalyptus. Hillis (1966) found

large quantities in all Eucalyptus species he investigated. The role of phenols in plant defence has been a subject of many comprehensive reviews (Levin, 1971; Rodriguez et al., 1976; Whittaker and Feeney, 1971; Kosuge, 1969). With such large quantities of substances commonly considered as providing immunity from insect attack, Eucalyptus should be free from insect attack. However, Eucalyptus trees in Australia are known to be subjected to heavier defoliation than many forest tree species in the northern hemisphere (Burdon and Chilvers, 1974; Carne, 1966; Elton, 1966; Gosz et al., 1972; Mattson and Addy, 1975).

Some phenolic substances e.g. chlorogenic acid, are, however, known to stimulate feeding (Hsiao and Fraenkel, 1968). In Chapter 7 it was shown that the water and alcohol extracts of Eucalyptus contain substances that evoke biting and feeding response. Since phenols are largely soluble in water and alcohol extracts of leaves, It was of interest to determine the influence of these phenols on larval feeding. The phenols known to exist in eucalypts include gallic acid, caffeic acid, p-coumaric acid, ferulic acid, chlorogenic acid, trans-stilbene and quercetin (Hillis, 1966). These substances were tested for their effect on larval feeding by incorporation in agar medium alone or in combination with sucrose. The results are summarized in Tables 8.3.6.1 and 8.3.6.2. None of the phenols alone significantly induced feeding response though chlorogenic acid and gallic acid elicited moderate biting responses. However when the phenols were tested in equimolar concentrations with sucrose, very interesting results were obtained. Coumaric acid, cafeic acid and ferulic acid depressed larval feeding by at least 40%, however, ethyl gallate slightly enhanced larval feeding. It appears that larval response to phenols would depend not only upon the relationship between amounts of sugars and phenols but also on the quantitative relationship among the phenols that enhance larval feeding and those that depress feeding.

TABLE 8.3.6.1 Biting and feeding responses of 6th instar larvae of Uraba lugens to phenolic substances.

	Test compound	Molar concentration	Biting response	Accumulated faecal counts by 15 larvae
1	Plain diet	-	+	14
2	Diet+Chlorogenic acid	.01M	++	32
3	Diet+Gallic acid	.01	++	44
4	Diet+Ferulic acid	.01	+	20
5	Diet+Caffeic acid	.01	+	10
6	Diet+p-Coumaric acid	.01	+	8
7	Diet+Trans stilbene	.01	+	9
8	Diet+Quercetin	.01	+	9
9	Diet+Sucrose	.01	++++	181

Kruskal-Wallis ($P < 0.011$)

TABLE 8.3.6.2 Biting and feeding responses of 7th instar Uraba lugens to combination of phenolic substances and sucrose.

Test compound	Concentration	Biting response	Accumulated faecal pellets
Plain diet	-	+	15
Diet+Sucrose	.01M	++++	180
Diet+Sucrose+Chlorogenic acid		++++	141
Diet+Sucrose+Ethyl gallate		++++	209
Diet+Sucrose+Cafeic acid		+++	99
Diet+Sucrose+p-Coumaric acid	.01M	+++	108
Diet+Sucrose+Quercetin		++++	159
Diet+Sucrose+Ferulic acid		+++	94

Kruskal-Wallis ($P < 0.01$)

8.3.7 Leaf factor or sign stimulant?

To determine whether in addition to sucrose, fructose, ascorbic acid, foodplants contain a 'leaf factor' that enable larvae to recognize them, agar diets were prepared containing sucrose, leaf powder or a combination of the two. Seventh instar larvae which had been starved for 24 hours were placed on these diets, and their biting responses relative to the control were recorded subjectively at 60 minute intervals over the first eight hours and at the end of 24 hours. Amounts of food eaten estimated by the number of faecal pellets produced was also recorded at the end of 24 hours.

The results of the test are shown in Table 8.3.7.1. Although the results in the table indicate that 0.01M sucrose is about twice as effective as 0.5% leaf powder in evoking feeding, no biting mark was evident on the diet lacking leaf powder until the end of fifth hour. On the otherhand diets containing leaf powder with or without sucrose were fed upon during the first hour of exposure to larvae and feeding was obvious during the second hour.

TABLE 8.3.7.1 Biting and feeding responses of 7th instar U. lugens to diet containing leaf powder and/or sucrose. (records at hourly intervals for 8 hours for biting response)

Time	Biting response								Accumulated faecal pellets at end of 24 hours
	1	2	3	4	5	6	7	8	
Plain diet	0	0	0	0	0	0	0	+	24
Diet+0.01M sucrose	0	0	0	0	+	++	+++	++++	150
Diet+0.01M sucrose +0.5% leaf powder	+	++	+++	++++	++++	++++	++++	++++	251
Diet+0.5% leaf powder	+	++	+++	+++	+++	+++	+++	+++	69

Kruskal-Wallis H ($P < 0.01$)

The results suggest that on diet lacking leaf powder, the larvae, perhaps, spend some time to get adapted to the diet before feeding is sustained. The period of adaptation may depend on the physiological state and past experience of individuals to such diets. In the present study, the effect of the interaction of the leaf powder and sucrose was greater than would be expected for the sum of individual effects (see Table 8.3.7.1) indicating that perhaps, the 'leaf factor' and the common feeding stimulants together may determine the palatability of the foodplant. If the leaf factor is peculiar to eucalypts and related genera, it may serve as a sign stimulant for caterpillars of Uraba lugens and may also be important in selection of egg-hosts by adults.

8.4 General discussion

It is, perhaps, appropriate to point out at this stage that the results presented emphasized palatability. Some of the substances which were ineffective in inducing feeding responses may be essential nutrient substances required for survival, growth and reproduction of Uraba lugens. Of the many substances tested individually for phagostimulation few evoked significant biting and feeding responses. These include sucrose, fructose and ascorbic acid. However, a considerable number of the substances from virtually all the groups tested influenced feeding in the presence of sucrose and have been considered as having additive, synergistic or antagonistic effects.

In nature the palatability of a foodplant will depend on the interaction of a complex of substances in the foodplant. This complex includes the feeding stimulants, the substances which interact to produce additive and synergistic effects, the feeding inhibitors and feeding co-factors (such as optimum pH and water level). It is evident from the data presented that optimal phagostimulation may depend not only on the presence

of feeding stimulants but also upon the proper combination and concentrations of these chemicals and of course the physiological state of the insects.

With possible exception of some polyphagous insects such as the desert locust, Schistocerca and the southern armyworm, Prodenia eridania (Dodd 1960; Soo Hoo, 1963) all phytophagous insects require gustatory stimuli to initiate and maintain feeding. Substances that are in themselves nutrients provide these stimuli in most phytophagous insects. In Uraba lugens little feeding occurs in the absence of sucrose, fructose and ascorbic acid. This underscores the importance of these nutrient substances for this insect though the common occurrence of these substances suggests that the selectivity shown by U. lugens toward Eucalyptus species seems more logically explained by a combination of olfactory responses from the adult and the presence of larval feeding inhibitors in some 'egg-hosts'.

CHAPTER 9 RELATIONSHIP BETWEEN NUTRIENT COMPOSITION AND THE ACCEPTABILITY OF THE FOODPLANT FOR URABA LUGENS (WALK)

9.1 Introduction

The role of nutrient substances in foodplant selection and preference has been a subject of controversy in recent times. Some workers (Kennedy and Booth, 1951; Kennedy, 1953; Fennah, 1953, 1956) stressed their importance, but others (Dethier, 1947; Fraenkel, 1953a, 1953b; Lipke and Fraenkel, 1956) did not. The core of Fraenkel's argument was that nutritional requirements of all insects are much the same and all green leaves are excellent sources of all the food materials which insects seem to require. However, a lot of evidence has subsequently accumulated which shows that whilst qualitative requirements may be similar, striking differences exist in the quantitative requirements of nutrients by phytophagous insects. It is also known that nutrient composition of green plants differs both in kind and amounts and these may influence reactions of phytophagous insects (see below). For example whilst total nitrogen in two samples of leaves from different plants or parts of the same plant may be similar, the amounts of component amino acids may vary greatly from one to the other (Kasting et al., 1958).

Carbohydrates, amino acids, lipids, vitamins and inorganic salts have all been shown to influence feeding of phytophagous insects through either acting as feeding stimulants or feeding co-factors. But perhaps the role of nutrient substances in insect-foodplant relationship is of even greater significance in cases where they determine the susceptibility or resistance of plant species or varieties.

Beck (1950, 1956, 1957) demonstrated the relationship between nutrient composition of corn plants and their resistance to insects. He (1957b) found a significant correlation between the feeding responses of the larvae,

resistance factors in the plants, and the sugar contents of the plant tissues. Dobie (1974) showed that the resistance of maize varieties was related to hardness which was closely correlated with amylase content. Chalfant and Gaines (1973) showed that positive correlations exist between concentrations of reducing and non-reducing sugars, total starches, nitrogen and susceptibility of the southern pea, Vigna sinensis to cowpea curculio, Chalodermis aeneus (Boheman).

Auclair, Maltais and Cartier (1957) found, for example, that the susceptibility of different varieties of peas to aphid attack is associated with their amino acid contents and their relative amounts of nitrogen and sugars. Infestations and distributions of both the cocoa thrips, Selenothrips rubrocinctus (Giard.) (Fenna, 1962) and the psyllid, Cardiaspina densitexta (White, 1966) are associated with leaf nitrogen content.

Nutrient and trace element levels may also be associated with the suitability of plants for particular pests. The Pinus sylvestris resistant var. uralensis has significantly lower N, P, Na, Mg and B than the susceptible vars. iberica and haguenensis (Wright et al., 1967) and boron deficient olive trees are associated with low populations of the olive leaf Eriophyid (Oxypleurites maxwelli K.), which was common in healthy trees not boron-deficient (Rodriguez, 1960). Creighton (1938) also found that copper and zinc deficiencies in cotton increased the mortality rate of the cotton leafworm Alabama argillacea (Hb.).

Even water has been shown to influence insect-foodplant relationships and may be responsible for preference or susceptibility of a plant. Kennedy (1958) showed that the selection of oviposition sites by apterous heteroecious females of Aphis fabae is related to the developmental physiology of the plant and that, it may be possible to increase aphid resistance in plants physiologically by cultural methods such as controlling the amount of water available to them. For example different

severities of droughting results in diverse effects in plants; in some species, the nitrate uptake exceeds the capacity of the drought-stricken foliage to reduce it (Flynn et al., 1957; Meyer and Anderson, 1952). A moderate loss of water in the leaf causes sugars to be converted to starch, but a more pronounced loss causes the reverse reaction (Meyer and Anderson, 1952). Water deficiency either by causing fall in cell turgor (Kennedy and Booth, 1959) or changes in chemical composition may affect reactions of phytophagous insects due possibly to induced proportionate variations in key components of their diets (Kasting et al., 1958).

The objectives of the present studies were to determine (a) whether quantitative differences exist in the levels of total soluble sugars, total N, P, K, Ca and water of ten species of Eucalyptus which differed in their susceptibility to attack by the gum leaf skeletonizer, Uraba lugens (Walk.) and (b) the relationships of these differences to the ranges of host suitability demonstrated by these trees.

9.2 Materials and methods

9.2.1 Foliage collection and handling

All leaves used were mature, sub-opposite members of leaves on which eggs were attached for studies of foodplant suitability reported in Chapter 5.3. They were picked from the outer sections of tree crowns about 1.5 to 1.8 metres from the ground and placed in polythene bags for transfer to the laboratory in a portable box containing dry ice. Subsamples for analysis of total soluble sugars were freeze-dried (48 h at 0.1 mm Hg; -15°C). Subsamples for analyses of N, P, K, Ca were first cleaned with wet cotton wool, rinsed in deionized water and left in a dish to drain for 15 minutes prior to drying in an oven (48 h at 60°C). Dried plant material was ground in a Wiley mill to pass through a 0.5 mm screen and stored in tightly capped vials at -15°C.

9.2.2 Analyses

9.2.2.1 Soluble sugars

1. Extraction

The plant samples were extracted in 150 ml Erlenmeyer flasks with boiling ethanol (0.1 g/m^{-1} ethanol). The extracts were cooled and centrifuged for 10-15 minutes and the supernatant decanted. Extracts may be deproteinized with Lead acetate. However, because of the difficulties of removing Lead and its subsequent interference with results, this step was omitted. Deproteinizing steps are frequently omitted with Anthrone procedures (Ebell, 1969). The crude extracts were, however, clarified with powdered charcoal. Ebell (ibid.) obtained identical values for charcoal clearing alone and from Lead acetate plus charcoal treatment indicating that interfering substances were adequately removed by adsorption on charcoal. The clarified extracts were filtered off into 100 ml volumetric flasks.

2. Reagents

Anthrone: For quantitative reagent, 2 gm of Anthrone was dissolved in 1 litre of 95% H_2SO_4.

Standard solutions: Comparisons with the unknowns from the leaves were made using glucose in the (wt/vol.) range 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5% and 4%.

3. Determination

An Anthrone procedure (adapted from Morris, 1948) was selected for its simplicity, reproducibility and its direct applicability to estimation of total soluble sugars. 4 ml each of diluted sample, standard and blank, in 20 x 150 mm tubes were reacted with 8 ml of Anthrone. The test tubes were capped with aluminium foil, shaken and allowed to stand at room temperature for about 10 minutes for development of colour. The optical densities of the solutions were determined at

620 nm. All readings were made against a blank of Anthrone reagent.

9.2.2.2 Total nitrogen analysis (Micro-Kjeldahl Technique)

1. Digestion

0.250 gms ground plant samples were weighed into 100 ml Kjeldahl flasks containing 1 Keltab and 2 glass beads. 4 ml conc H_2SO_4 was added to each sample which was then heated on a Maloney electric heater till the solution cleared. The heating was then continued at higher temperature for at least another 15 minutes following which the solution was diluted to 50 ml with deionized water.

2. Reagents

NaOH Soln.: 400 gm of NaOH was dissolved in 1000 ml distilled water.

Methyl red - Methylene blue (mixed indicator):

0.2% Methyl red in 95% ethanol was mixed with 0.2% Methylene blue in 95% ethanol in 2:1 proportions.

Boric acid - Indicator soln.:

10 gm A.R. Boric acid and 5 ml mixed indicator solution were dissolved in 500 ml distilled water. The mixture was left overnight to dissolve and the final solution was stored out of bright sunlight.

$KH(IO_3)_2$ Soln. 0.01N

3.899 gm of $KH(IO_3)_2$ was dissolved in 1 litre distilled water.

3. Determination procedure

A 5ml aliquot of digested plant material was placed in a distillation flask, then 10 ml NaOH were added. The flask was connected to a distillation head and 10 ml distilled off into 5 ml of Boric acid -

Indicator solution. The distillate was titrated with $KH(IO_3)_2$ to a lilac end point. The amount of acid used to reach the end point was recorded. Three replicates were run for each sample.

4. Calculations

The percent N was calculated using this formula:

$$\%N = \frac{0.1401 \times \text{ml acid to reach EP}}{\text{mgm plant material}} \times \frac{\text{dilution}}{\text{ml aliquot}} \times 100$$

N determination of the "digests" was repeated using the automatic N-analyzer as a check.

9.2.2.3 Phosphorus determination

1. Digestion

500 mg of oven dried ground leaf material was placed into a 100 ml Kjeldahl flask and 7 ml nitric perchloric acid mixture (prepared by mixing 500 ml conc. nitric acid with 83 ml of 70-72% perchloric acid) was added. The flask was heated on a Maloney electric heater until fumes of brown nitrous oxide were no longer evolved. The digestion time was approximately 40 minutes. After cooling, the "digest" was diluted to 20 mls with deionized water and filtered through a No. 2 filter paper into a 100 ml volumetric flask.

2. Reagents

i. Mixed Reagents:

500 ml conc HNO_3

500 ml 0.25% NH_4VO_3 (2.5 g Ammonium Vanadate/litre)

500 ml 5.0% $NH_4MO_7O_{24}$ (50 g Ammonium molybdate/litre)

The Nitric acid and Ammonium molybdate were added to Ammonium vanadate in succession, and the mixture was thoroughly mixed and allowed to cool.

ii. Standard P solution

Stock solution: 2.195 g oven dried KH_2PO_4 was dissolved in 500 ml deionized water. (This solution contained 1000 p.p.m. phosphorus).

Working solution: The working solution was made by diluting 20 ml of stock solution with deionized water to make 1 litre.

3. Determination Procedure

To prepare a standard calibration curve, 1, 2, 3, 4 and 5 ml of the working solution were pipetted into five 100 ml volumetric flasks respectively, and each brought to 70 ml with deionized water. Then 10 ml of mixed reagent was added and each solution made to the required volume with deionized water. The optical densities of these solutions were determined on a Unicam S.P. 600 at 390 mμ after 30 minutes.

Aliquots of diluted digest were prepared and determined in the same manner as the working standards and the values compared directly with the Standard calibration curve.

9.2.2.4 Potassium and calcium determination

1. Digestion

Samples were digested in Nitric-perchloric acid as described for phosphorus.

2. Determination Procedure

K and Ca concentrations were determined directly on a Varian Techtron Atomic Absorption spectrophotometer as follows:

Potassium

Standard solution: 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm, 6 ppm and 7 ppm KH_2PO_4 solutions (wt/v) were prepared.

Technical details (determination): Wavelength 766.5 nm. Slit width - 300 (0.1 nm), Lamp Current - 5 mA, fuel - Acetylene (10 p.s.i.), support air (15 p.s.i.).

Calcium

Standard solutions: 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm and 10 ppm of $CaCO_3$ were prepared.

Technical details (determination): Wavelength 422.7 nm.

Slit width - 23 (0.08 nm), Lamp Current - 3 mA, support - Nitrous oxide (15 p.s.i.); fuel - Acetylene (10 p.s.i.).

9.2.2.5 Moisture determination

Moisture content of leaves was determined by taking the fresh weight of mature leaves and oven drying them at 100°C. After 24 hr the samples were reweighed and the loss in weight was regarded as a measure of moisture content of the leaves.

9.3 Results and discussion

Little can be deduced from the nutrient status of the foodplants without a good knowledge of the insects qualitative or quantitative requirements. However, as long as there is a correlation between gross response such as survival or growth rates and plant tissue nutrient content, plant analysis can be useful in pointing to components that could be influencing insect behaviour. Where existing differences in quantities of foodplant nutrients seem to have little or no relation to insect survival or growth rates, the roles of other plant components in insect foodplant relationships should be examined.

As little is known about the food requirements of Uraba lugens discussion in this study has centred on the few nutrients whose roles in the nutrition of other species have been adequately demonstrated. The

Backward Elimination Multiple regression procedure (Draper and Smith, 1966) which determines the contribution of each variable to the regression sum of squares was used to determine the influence of soluble sugars, total nitrogen, phosphorus, potassium, calcium on feeding behaviour of larvae. Using the overall and partial F-tests it was found that the nutrient substances acting singly or in combination do not explain fully the variation in survival, growth rates and pupal weights of Uraba.

The same results were analysed in a different way, by plotting scatters of soluble sugar, nitrogen, and phosphorus levels in the various plants to determine whether any biological meaningful relationship was missed by the statistical analysis. Scatters relating calcium, potassium and the major nutrient substances are shown in Appendix Fig. 9.3.1(a-f).

Nitrogen/sugar relationship of the 10 Eucalyptus spp.

When a scatter diagram relating concentrations of leaf total nitrogen and leaf soluble sugars was plotted no distinct groups were apparent (see Fig. 9.3.1a) though there was a wide range in both variables with leaf total soluble sugars ranging from 0.9% in E. melanophloia to 1.9% in E. citriodora and leaf nitrogen concentrations ranging from 0.94% in E. cosmophylla to 1.5% in E. melanophloia (Table 9.3.1).

These dry weight values convert to green (wet) leaf molar concentrations of 0.025 (E. moorei), 0.03 (E. camaldulensis), 0.014 (E. melanophloia), 0.024 (E. intertexta), 0.042 (E. citriodora), 0.019 (E. punctata and E. cosmophylla), 0.036 (E. oreades), 0.026 (E. alpina), 0.02 (E. platypus). The species lowest in sugar is E. melanophloia, a a good food plant for Uraba lugens, and although its sucrose concentration may not be much above the threshold for feeding response (0.001M; Chapter 8) it is probable that all ten eucalypts have sufficient sucrose and fructose to stimulate feeding (see Fig. 8.3.1.1.). Of interest are the similar values for E. moorei, E. intertexta

TABLE 9.3.1 Nutrient content of leaves of 10 species of Eucalyptus (values as percentages of dry weight of leaves).

Plant species	Soluble sugars	Nitrogen	Phosphorus	Potassium	Calcium	H_2O	Sugar/H_2O ratio (a)	N/H_2O ratio (b)	P/H_2O ratio (c)
E. moorei	1.50	1.27	0.224	0.43	1.01	49.2	.0305	.0258	.0045
E. camaldulensis	1.63	1.06	0.108	0.60	1.10	50.0	.0326	.0212	.0022
E. melanophloia	0.90	1.5	0.210	0.78	1.03	45.1	.0199	.0332	.0046
E. intertexta	1.20	1.28	0.105	1.48	2.16	51.7	.0232	.0247	.0020
E. citriodora	1.90	1.36	0.077	0.76	2.21	61.2	.0310	.0222	.0013
E. punctata	1.03	1.16	0.135	0.76	1.01	49.9	.0206	.0232	.0027
E. cosmophylla	1.04	0.94	0.079	0.52	1.09	45.2	.0230	.0207	.0017
E. oreades	1.80	1.26	0.070	0.64	0.96	55.5	.0324	.0227	.0013
E. alpina	1.25	1.06	0.133	0.42	1.35	60.8	.0204	.0174	.0021
E. platypus	1.25	0.81	0.118	0.54	1.01	46.3	.0269	.0174	.0025

Figure 9.3.1 Scatter showing the relationship between the suitability of foodplant for growth and its nutrient composition.

a (top) - (Nitrogen/sugar)

b (bottom) - (Phosphorus/sugar)

o *E. melanophloia*

□ *E. punctata*

▲ *E. intertexta*

Δ *E. camaldulensis*

∇ *E. alpina*

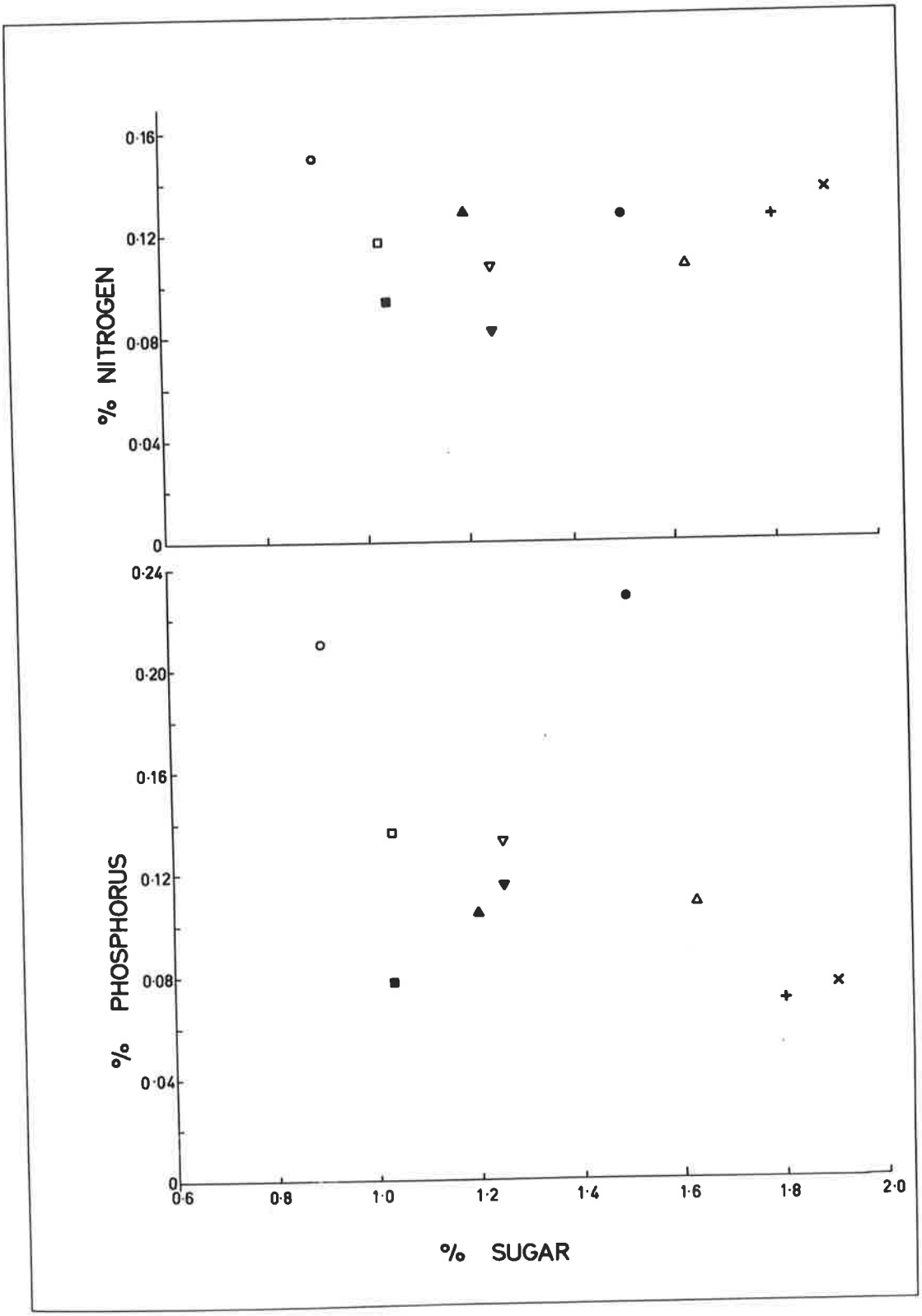
\+ *E. oreades*

x *E. citriodora*

● *E. moorei*

■ *E. cosmophylla*

▼ *E. platypus*


% NITROGEN
0·16
0·12
0·08
0·04
0
% PHOSPHORUS
0·24
0·20
0·16
0·12
0·08
0·04
0
0·6
0·8
1·0
1·2
1·4
1·6
1·8
2·0
% SUGAR

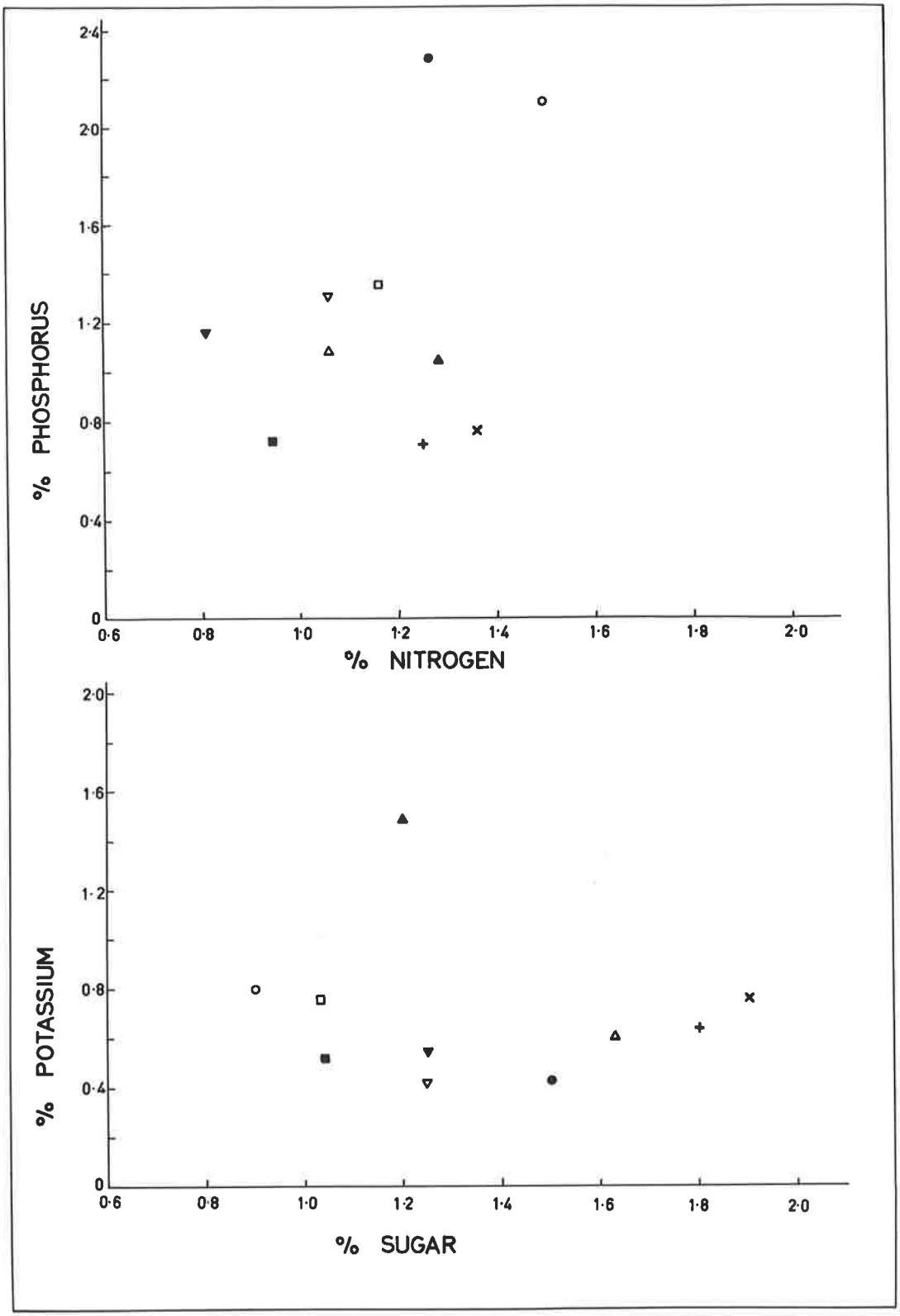
and E. alpina which for feeding rate, growth and survival of U. lugens represent a host suitability gradient from superior through intermediate to a non-food plant. Again, if sugar concentration in leaves alone determined feeding response, the gradient of suitability should be from E. citriodora (high) to E. melanophloia (low), which is not the case. (see chapters 5 and 6).

Of the scatter diagrams mentioned above, the most convincing trend in host suitability was obtained when water/nitrogen and water/sugar concentrations were related. (Appendix Fig. 9.3.1. a). The good food plants E. moorei and E. melanophloia located near the top of the scatter, the intermediate hosts E. intertexta and E. punctata were in an intermediate position with E. alpina and E. platypus, the non-food plants, at the bottom. Good hosts E. camaldulensis and E. citriodora, however located along with the poor hosts, E. oreades and E. cosmophylla, in the intermediate category. There was no discernible relationship between the degree of host suitability and location of the respective species within the intermediate group, which indicates that other factors than sugar, nitrogen and water may be involved in explaining their degrees of suitability to Uraba lugens.

Phosphorus/sugar relationship

When a scatter diagram relating phosphorus and sugar was plotted, 2 distinct groups were observed (Fig. 9.3.1b and Appendix Fig. 9.3.1b). E. moorei and E. melanophloia were located on the top section of the scatter (high P) separated from the others. Insects require substantial amounts of phosphorus (Dadd, 1973). Among the species on which complete larval development occurred, E. cosmophylla and E. citriodora were lower in ratings in regard to rate of development and pupal weight, this might have been influenced by their lower phosphorus level. However, the relatively higher amounts found in E. alpina and E. platypus, two species

Figure 9.3.1c Scatter showing relationship between the suitability of foodplant for growth and its nutrient composition (N/P).
(See Figure 9.3.1 for key to symbols).


% PHOSPHORUS
2·4
2·0
1·6
1·2
0·8
0·4
0
0·6
0·8
1·0
1·2
1·4
1·6
1·8
2·0
% NITROGEN
% POTASSIUM
2·0
1·6
1·2
0·8
0·4
0
0·6
0·8
1·0
1·2
1·4
1·6
1·8
2·0
% SUGAR

which do not support full larval development suggests that factors other than P levels may be important in explaining the degree of suitability of the different species (Chapter 5.3).

Nitrogen/phosphorus relationship

When nitrogen was plotted against phosphorus, the relationship between them was found to be fairly similar to the relationship between phosphorus and sugar (see Fig. 9.3.1c, Appendix Fig. 9.3.1c). One group was made up of E. moorei and E. melanophloia (high P and N) and another was made up of the other 8 species. In view of the importance of N and P for growth, it is expected that E. moorei and E. melanophoia may be more suitable for growth than the others. This seems to have been borne out by the suitability test (Section 5.3) in which larvae maintained on E. moorei, had the highest survival rate, the shortest rate of development and attained the heaviest pupal weight. E. melanophloia was not included in the test referred to. Again the fact that E. alpina and E. platypus occupied intermediate positions in this respect suggests that other factors, possibly, feeding inhibitors, might have accounted for the 100% mortalities recorded on these species.

The relationship between potassium, calcium and sugars, nitrogen and phosphorus are shown in Appendix Figures 9.3.1d, e, f, g. No discernible relationship could be found between these factors and suitability of foodplants.

The overall results suggest that factors, probably of no nutritional value, may be important in explaining more fully, the variation in survival and growth of Uraba on the various host plants. The multiple regression analysis has shown categorically that the variation in suitability of foodplants could not be explained by the differences in the contents of nutrients measured. This was largely confirmed by the scatter diagrams

though the results of some of the scatters (see Appendix Fig. 9.3.1a) suggest certain trends in suitability may be influenced to a certain extent by nutrients or combinations of nutrients within leaves of the foodplant. This is not entirely surprising since the growth performance of an insect would necessarily be affected by nutrients in its foodplant, though it is apparent that a nutritious food would be of no avail in presence of inhibitory substances.

Studies of the nature reported here have one major weakness. They tend to emphasise the relationship between absolute nutrient levels and insect feeding behaviour. As House (1965) pointed out the nutrient balance may be more important for phytophagous insects. However, in absence of a thorough knowledge of both qualitative and quantitative requirements of Uraba lugens, discussion of the importance of nutrient balance could be at best speculative.

Recently other studies (Nielsen and Palzer, 1977) have shown that eucalypts, whether under stress or not, have similar levels of total nutrients and trace elements. Indeed the levels recorded for the species studied compared favourably with those reported herein. Such data indicate that, if differences in nitrogen or other nutrients and trace elements are indeed important in foodplant suitability for phytophagous species such as U. lugens, it will probably be due to differences or inbalance in the proportions of key components. A fruitful area in terms of components of total N in leaves could be significant changes in the proportions of key amino acids for the specific phytophage considered. If such stimuli vary for the species of insect, the evidence would offer an explanation for the intriguing question of why some species outbreak while a closely related one on the same host plant does not.

CHAPTER 10. FEEDING INHIBITORS

10.1 Introduction

The gum leaf skeletonizer, U. lugens is essentially a pest of eucalypts. Host selection by the adult and larval food preferences, however, do not coincide and many egg hosts do not support full larval development (Morgan and Cobbinah, 1977). Unacceptability of some of the egg hosts and certain species of eucalypts by the larvae may be due to one or more of the following characteristics: (1) absence of feeding stimulant(s) (2) physical characteristics, such as spines or toughness which suppress feeding (3) presence of feeding inhibitors including toxins. The evidence in an earlier study (Chapter 6) suggests that physical characters such as toughness play a relatively minor role if any in the selection of food-plants by U. lugens. Investigation into the chemical factors that initiate and maintain continuous feeding responses of larvae showed that the ubiquitous plant constituents, fructose, sucrose and ascorbic acid are the major feeding stimulants. Moreover their general presence in similar quantities in many eucalypts would indicate that feeding stimulants are not the reason for the lack in acceptability of certain species to Uraba.

The study reported here evaluates the hypothesis that the inability of the larvae to survive on some of the egg hosts and other species of eucalypts may be due to the presence of inhibitory substances which dominate feeding stimulants in these hosts.

10.2 Materials and methods

Fresh leaves of test plants were collected, freeze-dried for 48 hr and ground in a Wiley mill to pass through a 0.5 mm sieve. All the species used in these studies were egg hosts differing in their suitability for larval growth and development. E. moorei, and E. camaldulensis are suitable

foodplants for larval development but E. platypus does not sustain full larval development whilst E. punctata is intermediate in suitability for larval development (see Chapter 5).

In earlier studies it was found that the water and alcohol extracts of leaf powder of various species of eucalypts contained substances that enhanced larval feeding. The ether extract, on the other hand, showed some weak negative activity, consequently ether was used to isolate possible feeding inhibitors from the lyophilized leaf powder. Aliquots of 10 gm of leaf powder of E. moorei and E. platypus, species which represent two extremes in regard to suitability for larval development were extracted with 100 ml of Diethyl ether for 48 hr. The ether extracts of the two species were concentrated to the same final volume and each incorporated into agar medium containing 0.1M sucrose as a feeding stimulant. The procedure used for preparing the diet was essentially that described in Chapter six. The diet was prepared by mixing the ether extract with the cellulose powder of the diet to form a paste. After evaporation of the ether, 30 ml of 0.1M sucrose was added and the mixture heated on a magnetic stirrer hotplate. The agar was added as the mixture warmed up. The hot diet was poured into glass petri dishes and allowed to gel. The plain diet was prepared in similar manner except that it was devoid of sucrose and leaf extract : the 'standard diet' contained 0.1M sucrose in addition to ingredients in the plain diet.

The effect of leaf powder concentrations on larval feeding was determined by incorporating 0.5%, 4.5%, 19% and 32% leaf powder of each of the two species in two 'standard diets'. Three active fifth instars were placed on each diet in 47 x 10 mm plastic petri dishes. There were five replicates of each treatment and the number of faecal pellets or the dry weight of faeces produced by a total of 15 larvae in 24 hr was taken as a measure of food ingested. All experiments were carried out at

25 ± 2°C, 40-60% RH and with a 16 hr photophase.

Data was subjected to non-parametric Kruskal-Wallis analysis of variance to detect whether significant differences between treatments and controls existed.

10.3 Results and discussion

The results of the feeding trials using ether extracts of E. moorei and E. platypus are shown in Table 10.3.1

TABLE 10.3.1 Larval responses to ether extracts of E. moorei and E. platypus compared to plain and standard diets.

Diet	Biting Intensity	Accumulated faecal pellets in 24 hr
Plain diet (PD)	+	79
Diet + 0.1M sucrose (standard)	++++	353
Standard + ether extract of E. moorei	++++	502
Standard + ether extract of E. platypus	++	184

Kruskal-Wallis Anova ($P < 0.01$)

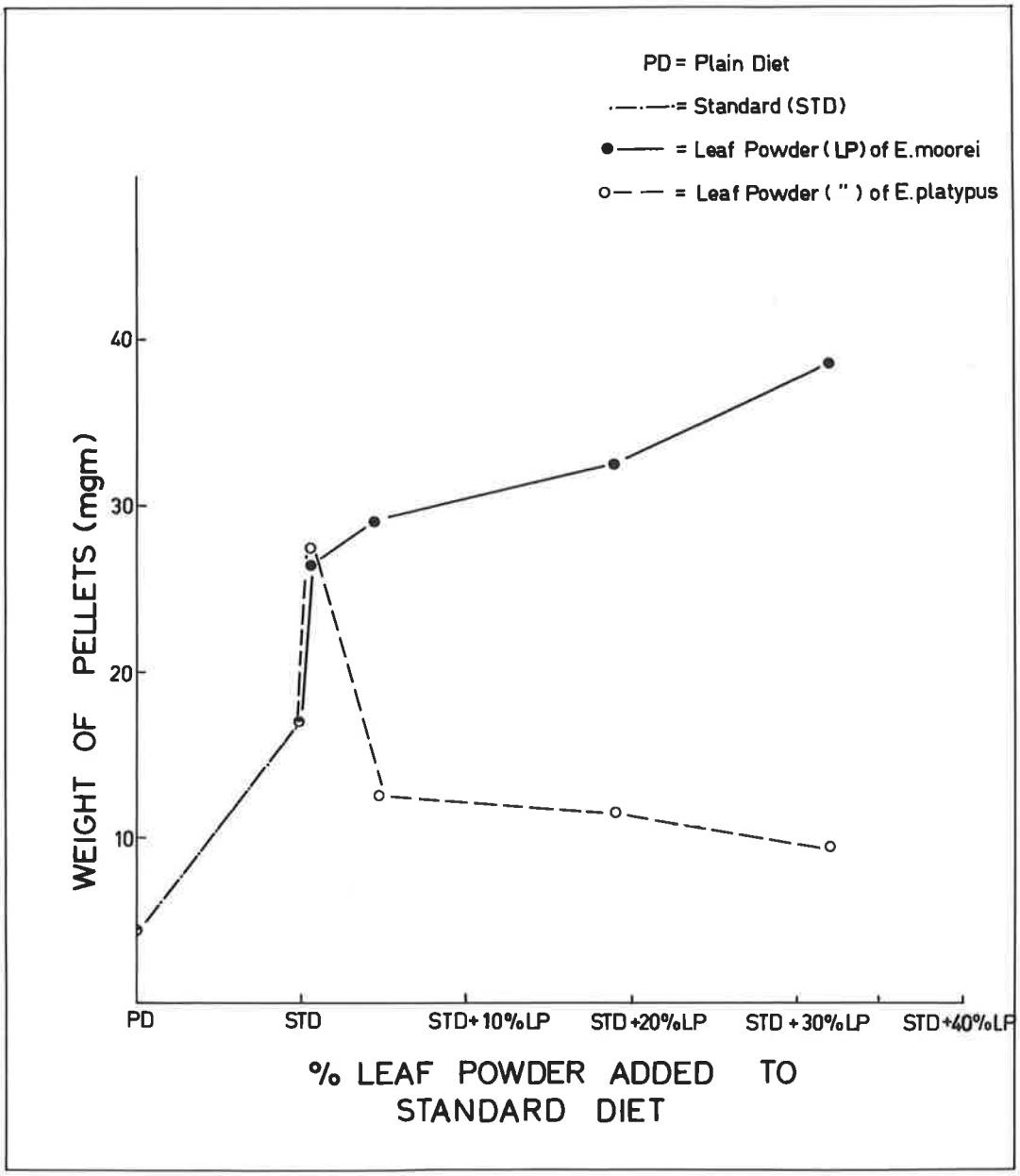
The crude ether extract of E. platypus significantly reduced biting and feeding responses on an otherwise highly acceptable diet (standard). Whereas larvae that were offered a standard diet showed a high biting response and produced 353 faecal pellets in 24 hr, those exposed to diet containing ether extract of E. platypus showed reduced biting response and produced only 184 faecal pellets. On the other hand, larvae exposed to identical diets containing ether extract of E. moorei increased both their biting and feeding responses to a level significantly higher than those on the standard diet and nearly 3-times that on the

diet containing E. platypus extract. The results of this test not only provide clear evidence of the existence of inhibitory substance(s) in the ether extract of the poor larval host, E. platypus but also the presence of a potent feeding stimulant or synergist in E. moorei, the effect of which is probably masked in unacceptable foodplants by the inhibitory substance(s). Ether soluble inhibitory substances have been demonstrated by a number of workers (McGinnis and Kasting, 1962; All and Benjamin, 1975; Hosozawa et al., 1974). In the present studies, 0.1M sucrose was added to diets containing leaf extracts and compared to the standard diet. This is a very important step because failure to eat a plant or diet does not necessarily provide evidence of the presence of inhibitors, since the same result could arise from an absence of suitable phagostimulant(s). Thus even though the plain diet did not contain any phagostimulant or inhibitor it elicited some biting and feeding response.

It is of interest to note that the levels of feeding on plain diet in the absence of phagostimulation and inhibition was lower than that recorded on the diet containing ether extract of E. platypus.

The existence of feeding inhibitory substances in the lyophilized leaf powder of E. platypus was substantiated by a quantitative study that involved incorporation of different amounts of leaf powder in agar medium. In these tests leaf powder was used in preference to ether extracts in an attempt to overcome any variability in the extraction of substance from the different samples of plant material. The results of the test are shown in Fig. 10.3.1. With 0.5% leaf powder of the two test species added to the standard diet, feeding was enhanced on both diets. However, as the leaf powder is increased, larval feeding decreases on diet containing leaf powder of E. platypus but continues to increase on the E. moorei diet. These data suggest that the inhibitory substance in E. platypus

Figure 10.3.1 Production of frass by U. lugens relative to the proportion of leaf powder in the diet.


PD = Plain Diet
·—·—· = Standard (STD)
●—— = Leaf Powder (LP) of E.moorei
o— — = Leaf Powder (") of E.platypus
WEIGHT OF PELLETS (mgm)
40
30
20
10
PD
STD
STD+10%LP
STD+20%LP
STD+30%LP
STD+40%LP
% LEAF POWDER ADDED TO STANDARD DIET

was masked or dominated by phagostimulants in the diet until the proportion of leaf powder was increased to a level where the inhibitory substance(s) was in sufficient quantity to reverse this effect. The higher feeding response recorded for 0.5% leaf powder concentration relative to the standard diet may have been due to the combination (additive or synergistic) of the leaf powder stimulant(s) and the sucrose. Since there is no evidence to suppose that the inhibitory substance(s) is absent in suitable or acceptable species, it may be speculated that the degree of acceptability of a host to larvae may be dependent upon the ratio between the feeding stimulants and the inhibitory substances.

As a first attempt to determine the nature of the antifeedant(s) in the ether extract of E. platypus, extracts of three host plants each selected from the three suitable categories - poor, satisfactory and good hosts were chromatographed on thin layer silica gels. The solvent system was n-Hexane-Diethyl ether-Acetic acid (83:16:1 v/v). The chromatograms were viewed under u/v light after lightly spraying with 0.05% Berberidine hydrochloride in methanol. Nine identical bands were apparent from extracts of each of the three leaf extracts, six of which had similar Rf values to standards of authentic phospholipids, sterols, free fatty acids triglycerides, sterol esters, and hydrocarbons (terpenoids) respectively (see Fig. 10.3.2a).

In order to isolate the active substance in amounts large enough for bioassay, thick layer silica gels (3 mm thickness) were prepared. Ether extracts of E. platypus were chromatographed as described above and each of the nine bands were scraped separately into non-absorbent paper and transferred into a funnel lined with filter paper. Each band was eluted three times with 5 ml aliquots of Diethyl ether. The eluates were

Figure 10.3.2 Bands obtained by chromatographing ether extracts on thick layer silica gels.

(a) top - chromatograms of 3 species of eucalypts and standards

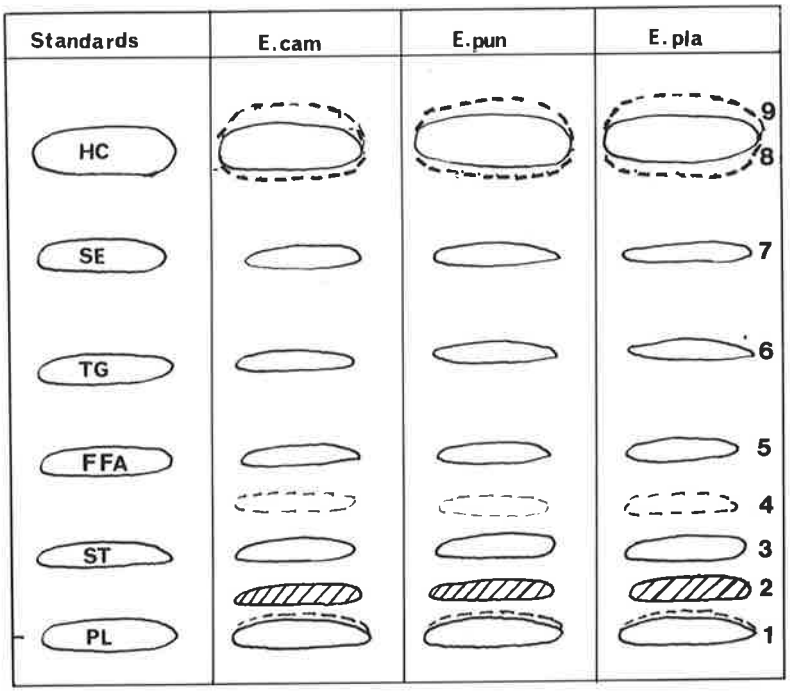
E. cam. = E. camaldulensis;

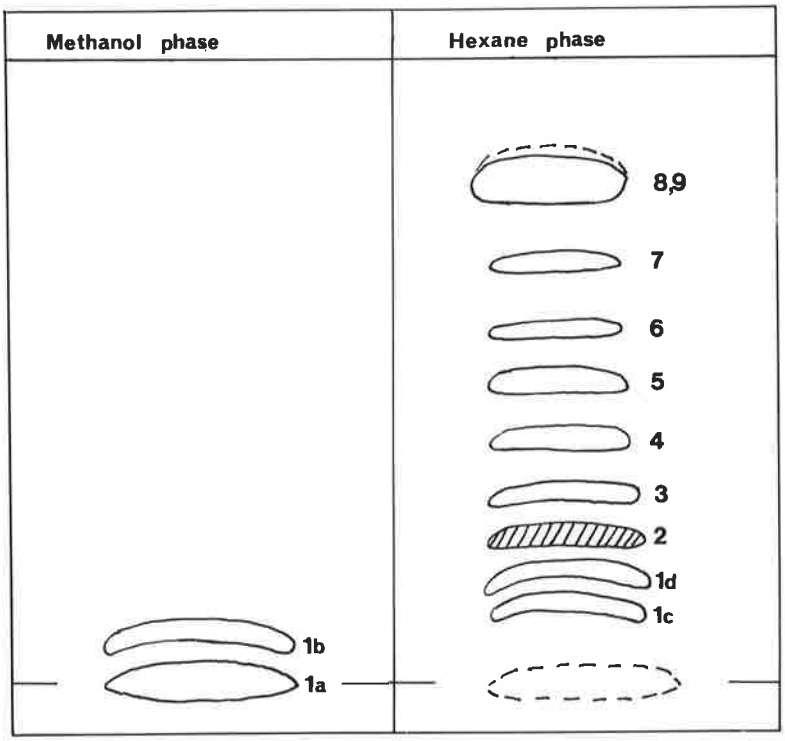
E. pun. = E. punctata

E. pla. = E. platypus.

8, 9 is one band (see text).

(b) bottom - chromatograms after phase separation of ether extracts in hexane and aqueous methanol.


Standards
E.cam
E.pun
E.pla
HC
SE
TG
FFA
ST
PL
9
8
7
6
5
4
3
2
1


Methanol phase
Hexane phase
8,9
7
6
5
4
3
2
1d
1c
1b
1a

concentrated to dryness under a stream of nitrogen, taken up in 1 ml ether and then incorporated into agar medium containing 0.01M sucrose as a feeding stimulant. Larval biting and feeding responses to the components of the bands were compared with a plain diet and a standard diet (plain diet + 0.01M sucrose) which had 1 ml ether incorporated into them and were treated exactly as the diets which included plant extracts. The results of the test are shown in Table 10.3.2.

TABLE 10.3.2 Larval biting and feeding responses to the constituents of ether extracts of E. platypus.

Diet/band	Biting response	Relation to authentic substance	Accumulated faecal pellets by 15 larvae
Plain diet	+	-	25
Diet + 0.01M sucrose (std.)	++++	-	174
Std. + band 1	+++	(PL, pigments polar substances?)	120
Std. + band 2	++++	(unknown - blue-green band)	200
Std. + band 3	++++	steroid	168
Std. + band 4	++++	(unknown, DG?)	163
Std. + band 5	++++	FFA	166
Std. + band 6	++++	TG	165
Std. + band 7	+++	ST.E	141
Std. + band 8	+++	HD	99
Std. + band 9	+++	HD	103

Kruskal-Wallis H ($P < 0.05$)

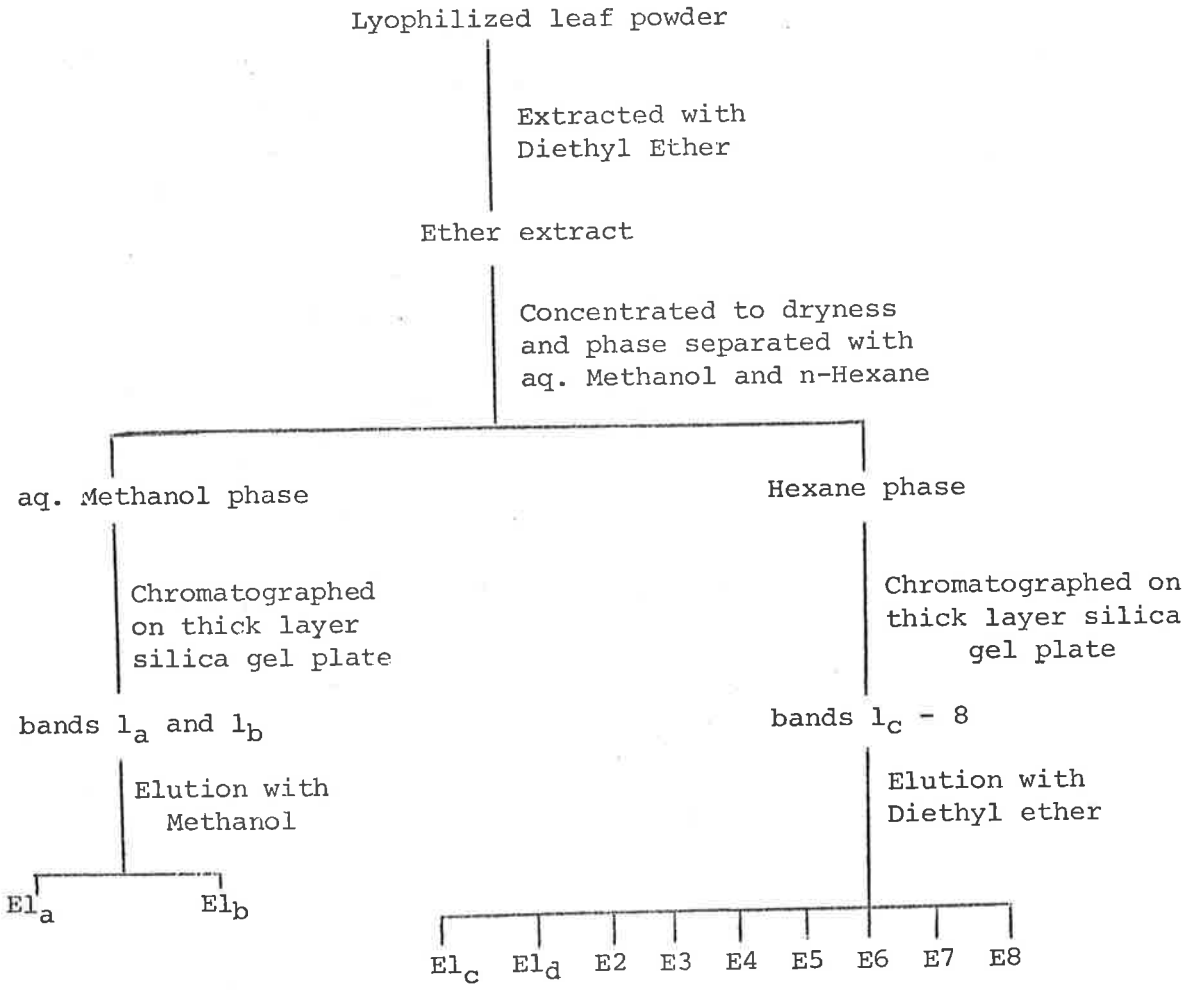
PL - phospholipids; ST - sterol; DG - diglyceride; FFA - free fatty acid; TG - Triglyceride; ST.E - sterol esters; HD- hydrocarbon.

The results in Table 10.3.2 indicate that bands 1, 8 and 9 depress feeding. On the basis of the reference substances used, bands 8 and 9 co-chromato-

graphed with triterpene hydrocarbon and was probably of terpenoid origin. The two bands were one band but were split for the bioassay largely because of the greater area it occupied on the gels. Band 1 which had the same Rf as standard phospholipid, also had a green pigmented substance and probably other, polar substances associated with it. Bands 3-7 showed hardly any inhibitory activity, but band 2 appeared to promote feeding activity of larvae.

Due to the complexity of the constituents of band 1 another fractionation step was adopted to separate the more polar constituents of the ether extract from the non-polar groups. The fractionation procedure is shown in Fig. 10.3.3. The ether extract of E. platypus was concentrated to dryness and the dried extract was shaken with 1:1 mixture of n-Hexane and aqueous methanol (20% H_2O and 80% Methanol). The upper Hexane phase contained mainly non-polar substances whilst the lower aqueous Methanol phase contained the more polar ether extractable substances. The Hexane and the Methanol fractions were chromatographed as described above. The band 1 of the ether extract fractionated into four bands, two of which were soluble in either of the two solvent systems (see Fig. 10.3.2b). In addition, bands 2 to 9 of the Ether extract appeared in the Hexane fraction. The bands were eluted and the eluates concentrated to dryness. The concentrated materials were prepared and presented to larvae as previously described. Due to shortage of larvae only the new bands and the bands which showed biological activity in the previous test (see Table 10.3.2) were considered. For the same reason all the bands could not be tested at the same time. The results of the tests are shown in Tables 10.3.3a and 10.3.3b. Although the results in Table 10.3.3a and 10.3.3b cannot be compared directly in view of the fact that the larvae used differed in age, rate of food consumption and possibly physiological state, certain inferences

Fig. 10.3.3 Fractionation procedure of Ether extract of E. platypus. E = eluates. El_a, l_b, l_c, l_d were previously band 1 (see Table 10.3.2)



could be made based on comparison with the larval response to the standard diet. Bands l_a and 8 both showed strong inhibitory activity. On the other hand, bands l_b, l_c, l_d and 2 all enhanced larval feeding activity, with band l_d increasing feeding 3-fold over the standard diet. This result corroborates earlier findings (see Chapter 7) that in addition to the alcohol and water soluble feeding stimulants, there exists a "fat soluble"

TABLE 10.3.3a Biting and feeding responses of 10th instar *U. lugens* to components of Ether extract of *E. platypus*.

Diet/band	Biting response	Faecal weights (mg)
Standard diet	+++	25.6
Standard diet + band 1a	+	8.6

TABLE 10.3.3b Biting and feeding responses of 8th instar *U. lugens* to components of ether extract of *E. platypus*.

Diet/band	Biting response	Faecal wt. (mg)
Standard diet	++	8.4
Standard diet + band 1b	+++	12.9
Standard diet + band 1c	+++	15.0
Standard diet + band 1d	++++	29.0
Standard diet + band 2	+++	14.5
Standard diet + band 8	+	3.0

Kruskal-Wallis H ($P < 0.01$)

feeding stimulant. Since the 'fat soluble' feeding stimulant is possibly not a nutrient substance (see Chapter 8), there is at least a theoretical possibility for it acting as a 'sign' stimulant for *U. lugens*.

Due to the inhibitory activity shown by bands 1_a and 8, these 2 bands were investigated further by TLC and GLC and monitored by feeding trials. Chromatographic analysis of band 1_a on silica gel plates in chloroform: Ethyl acetate and Formic acid (50:40:10 v/v) and sprayed with

Fast Blue B salt and basic alcohol (0.2 NaOH in 80% alcohol) showed the presence of at least 5 phenolic substances. Since all but one of the common phenolic substances in eucalypts (see Hillis, 1966) inhibits feeding to certain degrees (see Chapter 8), it is possible that these substances are responsible for the inhibitory activity of band 1_a, though further investigations would be required to substantiate this point. Phenols as a group have been shown to inhibit feeding and growth of many insects and pathogens (Byrne et al., 1966; Levin, 1971; Chapman, 1974).

Investigations of the active principle of the hydrocarbon band 8, was restricted to determining differences of the composition of the hydrocarbon bands of 3 host plants, E. camaldulensis*, E. punctata and E. platypus which represent a range of foodplant suitability for U. lugens. The hydrocarbon bands of these three species were eluted with ether and concentrated to dryness. The dried extracts were redissolved in acetone and the constituents of the eluates analysed with a Hewlett-Packard HP-5840A Gas Chromatograph.

A 5 µl sample of each of the 3 species was injected into a gas chromatograph by means of a gas tight syringe. The GLC used a metal column 1.D. 2mm, length 20 in of 10% UC. W982, 80-100 WAW DMCS F16 with N_2 as carrier gas (15 ml/min). The hydrogen and air flow pressures were 20 psi and 30 psi respectively. The column oven temperature was programmed as follows: 50-140-230°C. Injection and detector temperatures were 225°C and 250°C respectively. Figures 10.3.4a, b show the GLC profiles of some standard hydrocarbons and that of the three species of eucalypts tested. The resolution times of the standards (monoterpenes,

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* E. camaldulensis was used here in place of E. moorei because the only tree of E. moorei in the arboretum died. E. moorei is not a native of South Australia.

Figure 10.3.4a GLC profiles of standards

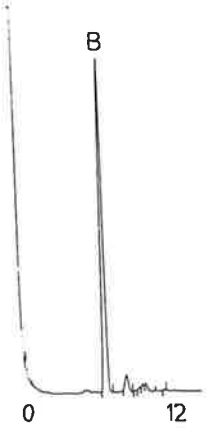
A - Pinene; B - Limonene

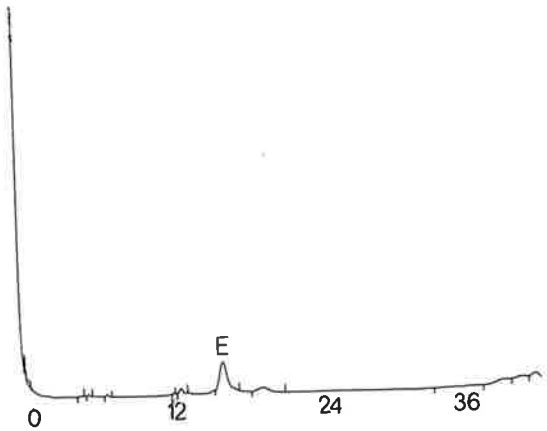
C - Camphene; D - Cineol

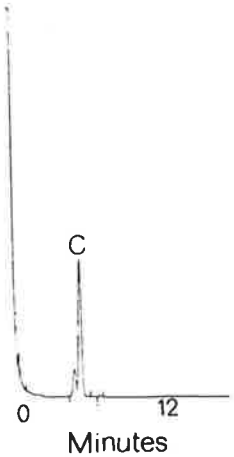
E - Caryophyllene; F - squalene.


A
0
12


D
0
12


B
0
12


E
0
12
24
36


C
0
12
Minutes

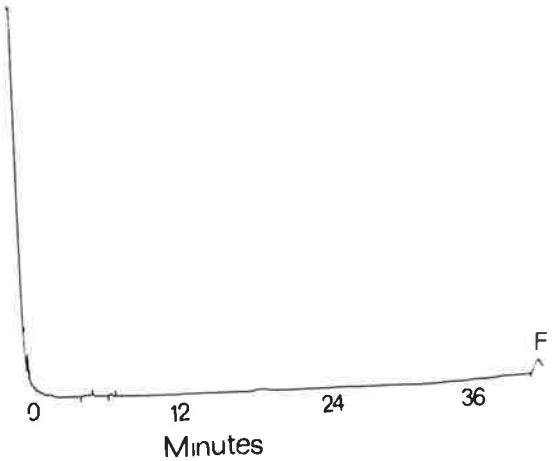
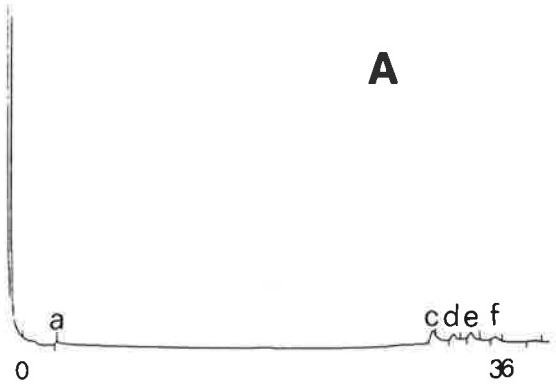

F
0
12
24
36
Minutes

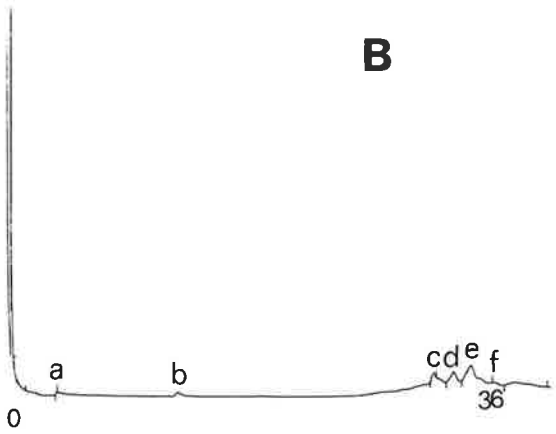
Figure 10.3.4b GLC profiles of host plant extracts

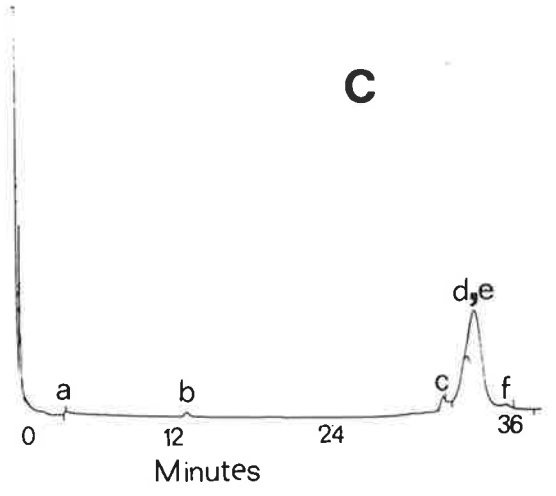
A = *E. punctata*

B = *E. camaldulensis*

C = *E. platypus*


A
a
c d e f
0
36


B
a
b
c d e f
0
36


C
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Minutes

sesquiterpene and a triterpene) and the peaks in the hydrocarbon bands of the extract suggest that the constituents of the bands are possibly sesquiterpenes or diterpenes. The stability of the constituents of the band to the freeze drying procedure and heat point to the possibility that they are diterpenes. Diterpenes have been recorded as antifeedants to some insects (Hosozawa et al., 1973, 1974; Kato et al., 1972; Wada et al., 1968).

Analysis of the profiles show that E. platypus, the species that appears to inhibit the feeding activity of the larvae has about six times as much of substances 'd' and 'e' as the other species tested.
A possible interpretation of these results is that when these substances exist in a high concentration in a foodplant they may be responsible for making it a poor host. It is true that E. punctata, which is less favourable as a foodplant than E. camaldulensis, contains a lower level of substances 'd' and 'e' and of total heat stable hydrocarbons and hence some caution should be exercised in the interpretation of these profiles. Nevertheless the relatively low level of total soluble sugars in E. punctata (see Chapter 9) could well be the decisive factor in making this species a relatively poor host.

Evidently, there is the need for further investigations. For instance, the substances shown in the profiles need to be isolated and assayed to determine their biological activity on larvae.

The feeding responses of larvae to some of the most common volatile hydrocarbon constituents of eucalypts were tested to see whether some of these substances play a role in the feeding inhibition of U. lugens. The results shown in Table 10.3.4 indicate that Pinene and Limonene at 0.5% concentration have a pronounced inhibitory effect on feeding; nevertheless, because of the volatility of these substances, the inhibitory effect of

the hydrocarbon band could not be ascribed to these substances. Pinene and Limonene have both been recorded as antifeedants for some phytophagous insects (All and Benjamin, 1973; Smith, 1966). Cineol and the crude leaf distillate show only weak activity even at high concentrations tested.

TABLE 10.3.4 Biting and feeding responses of 7th instar U. lugens to major volatile constituents of eucalypts.

Diet	Concentration	Biting response	Faecal weights (mg)
Plain diet	-	+	1.9
Diet + sucrose (2)	0.1M	++++	16.4
(2)+Crude leaf distillate	0.75%	+++	10.1
(2)+Crude leaf distillate	0.5%	+++	10.4
(2) + Pinene	0.5%	++	7.5
(2) + Pinene	0.15%	+++	9.4
(2) + Cineol	0.5%	+++	11.9
(2) + Cineol	0.15%	++++	15.8
(2) + Limonene	0.5%	++	8.5
(2) + Limonene	0.15%	+++	11.7

Kruskal-Wallis H ($P < 0.01$)

On the basis of the above results, it seemed reasonable to assume that many other plant constituents may also exhibit feeding inhibitory activity and that acceptability of a foodplant may be due to interaction of a complex of substances.

The present investigation has shown that food preference and suitability for development by U. lugens is associated with the occurrence of effective concentrations of feeding inhibitors in the unacceptable hosts. Inhibition may arise through interruption of normal feeding at different points (Beck, 1965). The insect may be actively repelled by the properties of the plant making directed movements away from it (Dethier et al., 1960);

or having made contact, further feeding activity such as biting may be suppressed either by physical or chemical characteristics of the plant. Again, having bitten the leaf, the insect may be deterred from feeding. The chemicals involved in inhibiting feeding behaviour at these various points are known as repellents, suppresants and deterrents respectively. Field observation and results of the present test indicate that the inhibitor acts as a deterrent or antifeedant upon the larvae of U. lugens.

The presence of inhibitory effects in the lyophilized leaf powder and fractions therefrom suggests that the inhibitory substance(s) are possibly not volatile in nature. Bailey et al. (1961) have shown that the freeze-drying technique completely eliminates many volatile materials in plants and this is further substantiated by the fact that the activity of the crude extracts of eucalypt leaves were not destroyed by heat in the course of the preparation of these diets. In summary it has been shown that at least two components of E. platypus deter feeding of larvae of U. lugens and probably represents the basis for their recognition of host and non-host plants. It seems clear that this determinant of caterpillar behaviour has little or no effect upon the selection of egg-hosts by adults.

CHAPTER 11 GENERAL DISCUSSION

The concept of host plant preference by phytophagous insects is fundamental to many aspects of agricultural and forest entomology. Preference patterns may be influenced by geographical, ecological and seasonal factors, as well as by factors innate to both the host and the pest. Innate factors may be 'general' in that they affect the feeding of most insects, or cause a given insect to feed on most plants, or 'specific' in that they stimulate a particular relationship between an insect species and its host plant. In this study both general and specific factors innate to either host or insect, and especially those relating to the selection by the insect of certain species of plants within the same geographical and ecological range, have been examined for Uraba lugens.

The stepped activities leading to the finding and acceptance or rejection of plants by phytophagous insects often begin with the selection of oviposition sites by gravid females. Oviposition involves a series of behavioural events which may be controlled by different stimuli. Although all of those involved in selection of oviposition sites by Uraba are not defined, field and laboratory observations and experimental results provide clear evidence for a generalised behavioural sequence resulting in host acceptance. The initial step is orientation to and recognition of the foodplant (De Wilde, 1958; Beck, 1965; Hsiao, 1969). In Uraba, this step may be influenced by humidity, visual cues and chemical stimuli. Humidity has been demonstrated not only to enhance egg-laying (see Chapter 4) but also to affect significantly the eclosion of eggs (Morgan and Cobbinah, 1977). The role of visual cues in host selection is not easily discernible, although the fact that gravid females do not select yellowing leaves, seedlings and very small

trees of acceptable hosts irrespective of their age, suggests that perhaps colour, shape and/or size of trees may be of some significance in host selection either through restricted negative geotropic behaviour or related adult dispersal phenomena. The bulk of the data suggest that chemical stimuli originating from the host is of major significance in the selection of plants by Uraba (see Chapter 4). The plant species selected by adults throughout these studies belong to two closely related genera - Eucalyptus and Angophora (see Baker and Smith, 1920; Penfold and Willis, 1961; Johnston, 1972). The choice of species within these two genera is a result of positive discrimination as indicated by the relative distribution of eggs and non-egg hosts in the study area. No egg mass or larvae were recorded on trees of other plants intermixed with the egg hosts during seven successive generations of the insect. Moreover, the choice of egg host did not influence the selection of its neighbour in the same or subsequent generations of the insect. Thus adult selection was not the result of chance encounter. Indeed, selection or rejection of plant is apparently regulated by plant-produced stimuli or attractants and inhibitors (see Chapter 4). This concept implies that those plant species that the insect actively avoids either lack the oviposition attractant or contain an oviposition inhibitor, and allows the possibility that selection or rejection will depend upon the relative influence of variable admixtures of both attractant and inhibitor, or of the dominance of one over the other.

The next stage in the insect-foodplant interrelationship, after recognition and selection of plant species by the adult, is larval feeding. To facilitate discussion, this behavioural process has been subdivided into 3 successive steps. These are: (1) initiation of biting (exploratory biting) (2) feeding action and continuous feeding (3) cessation of feeding (resting and locomotion). In insects in which the adults

select suitable foodplants for larvae, feeding follows this sequence and is interrupted only by larval ecdysis. In Uraba lugens, however, adult selection of a plant does not necessarily guarantee larval acceptance, feeding or survival upon it (see Chapters 4 and 5). On some egg hosts larvae may cease feeding after the exploratory biting and ultimately die without attempting to find other more suitable food. Hunger does not seem to affect the threshold of acceptance to an entirely unacceptable food plant implying the presence of repellent factors, not merely the absence of attractive ones.

The feeding responses of U. lugens to its host during its first stadium always result in high mortality upon unsuitable foodplants, because the caterpillars rarely move more than a few millimetres from the site of eclosion during the stadium and always remain upon the leaf where the eggs were laid. However, in situations in which a whole plant is eaten before pupation, the later instars may be forced to search for other foodplants, as has been observed during outbreaks. Therefore, in the course of this project, the ability of larvae to discriminate between foodplants was investigated and it is suggested that the suitability of the food (in relation to growth and survival) is strongly correlated with larval host selection (see Chapter 5.2). Larval acceptance or rejection of a leaf is preceded by test biting indicating that gustatory stimuli provide critical information about the chemical composition of the plants (see also Schoonhoven, 1969). Adults on the otherhand apparently select some egg hosts upon criteria other than their suitability for larval growth and survival.

This discrepancy between the adult and the larval selection of hosts suggests that at least two responses recognized by Wiklund (1975) are possible from an evolutionary point of view. A strong selective advantage will be conferred upon those females that predominantly oviposit on the

egg hosts on which the larvae survive. Conversely, there will be a strong selective pressure against females that predominantly oviposit on egg hosts on which larval mortality is, or approximates, 100%. Consequently, granting that the larvae are restricted in the range of plants on which they can feed successfully, the adaptive response of the adults should be towards a restriction of oviposition preferences leading to the acquisition of an adult host plant range at least as narrow as that of the larvae.

On the otherhand, selection pressure affecting survivals upon poor hosts could result in the development of 'resistant strains'. That is, given that the adults lay eggs on species of plant acceptable to few larvae, strains may be selected that are able to feed on a wider host range than most, assuming the occurrence of matings between individuals that survive and genetic basis for survival on such difficult foodplants. In either of these two alternatives, the ultimate result should be that all larvae feed and survive on all plant species oviposited on by all adults. It is reasonable, therefore, to seek reasons why such a correspondence has not been achieved in U. lugens.

Fecundity of Uraba is greater when larvae feed upon some foodplants than upon others (see Chapter 5). On the basis of selected species and other criteria used in these studies, the following plants may be arranged in the order of suitability for Uraba, E. moorei > E. camaldulensis > E. intertexta > E. citriodora > E. punctata > E. cosmophylla > E. platypus > E. alpina. Eucalyptus punctata and E. cosmophylla occupy an intermediate position in this sequence and provide a threshold for discussion of the biological significance of variable suitabilities within host range. The most suitable or superior hosts (E. moorei, E. camaldulensi E. intertexta etc.) in providing larger numbers of caterpillars in

succeeding generations attract higher degrees of parasitism and predation than the lower more dispersed numbers of U. lugens typical of intermediate and inferior hosts. Such a trend leads to low densities of the insect on superior hosts perhaps significantly lower than those on hosts of intermediate to poor suitability. Also the higher larval densities severely defoliate superior hosts with the distinct possibility of a resultant decrease in their number of their foliage complement. The combined effects of such decreased food and higher predation upon populations of U. lugens result in local eradication of the insect. While this has been found to occur on such hosts as E. camaldulensis, it has not been recorded upon hosts of intermediate suitability where trends in Uraba populations and defoliation seem to be similar from generation to generation where insect density is always low and defoliation insignificant. Foodplants such as E. punctata and E. cosmophylla are therefore valuable components of the biosystem, assuring a residual population of the insect to recolonize superior hosts that recover from damage during outbreaks.

If the suitability of a foodplant is determined either by its physical or its chemical properties, it should be possible to differentiate between these categories by comparing rates of consumption by larvae when fed fresh leaves or leaf powder diets. Such tests suggest that physical characteristics of leaves are not important in food selection by U. lugens (see Chapter 6).

On the otherhand, overwhelming evidence supports the findings of Verschaeffelt, 1910; Dethier, 1953; Fraenkel, 1953, 1959, 1969; Beck, 1965, 1974 that chemical factors are largely responsible for host specificity of larval U. lugens (see Chapters 6, 7, 8, 9 and 10). The chemicals that regulate the feeding responses of these larvae include both feeding stimulants and feeding inhibitors. In the absence of feeding

stimulants little or no feeding occurs (see Chapter 8). In the presence of feeding deterrents feeding is depressed despite the presence of feeding stimulants (see Chapter 10). The major feeding stimulants for Uraba are fructose, sucrose and ascorbic acid. Other substances enhance the activity of sucrose and all are common plant constituents and common feeding stimulants of insects. Although there were variable amounts of these substances in different plants, no direct relationship could be found between these and the specific suitability of any foodplant for U. lugens (see Chapter 9).

Feeding inhibitors have been shown in many cases as the primary determinants of foodplant specificity in phytophagous insects (Thorsteinson, 1960; Hsiao, 1969; Harley and Thorsteinson, 1967; Wada and Munakata, 1968). The search for the factors that inhibited feeding of U. lugens should ideally include not only isolation and identification of effective substances but also the respective concentrations of each in superior, intermediate, inferior and non hosts. As well one should endeavour to determine the effects of other components of the natural food upon each effective substance. Such an ideal is beyond the limits of a study of this kind but certainly the basis built herein provides the future opportunity to explore these areas of science more specifically.

The weight of data indicates a dominance of feeding inhibitors over feeding stimulants in inferior or non-food plants and a prima facie case for inhibition to be in the hydrocarbon fraction of leaf extracts is made. In feeding trials, some results strongly indicate volatile components govern feeding but such results are not clear cut for certain plant species. That a complexity in the determination of feeding exists in an insect which is naturally associated with two genera which include a total of > 500 species is not surprising.

It is however, pertinent to point out that under the limited

tenure of the SCAAP* award, it has not been possible to explore interesting aspects of the relationship between Uraba lugens and its foodplants that have emerged as the study progressed. For instance, the small survivals often of larger than normal individuals that occur from time to time on species which for most generations do not support larval development. Was it due to differences in the quality of certain larvae that enabled them to overcome substances apparently toxic to their brothers and sisters or was it due to changes in physiology of the plant or the leaf that supported such survival? Perhaps, a basic approach to this would be the use of electrophoretic techniques to characterize differences in enzyme systems of the larval population. This is a logical approach because host specificity is not due only to the presence or absence of feeding stimulants and feeding deterrents but also to the ability of the insect to deal with toxic substances (Miles, 1969).

It has been reported that the physiological condition of a plant may influence food selection of phytophagous insects (Kennedy, 1953; Fenna, 1953). Although such a possibility was strongly contested by Fraenkel (1953, 1959, 1969), this aspect of host plant interrelationship could well be worth investigating for U. lugens the host preferences of which appear to differ for different agroclimatic areas (Campbell, 1966). Thus while the alpine populations show preference for E. dalrympleana, E. stellulata and E. pauciflora, the favoured inland hosts are E. robusta, E. botryoides and E. camaldulensis respectively. The question is: What is the reason for differences in preferences in different habitats? Is it due to a process of co-evolution? Are local preferences genetically determined or a result of conditioned responses? Would the potential host range of a local population become narrower as the population becomes increasingly adapted to its preferred host?

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* SCAAP - Special Commonwealth African Assistance Plan.

Providing answers for these and other questions may take many years, and may require some elegant techniques to answer some of the fundamental questions involved.

In final consensus, this wide ranging study has pointed to clear differences between host selection by adults and by larvae, the bases for these enabling a distinct hierarchy of host suitability to be established. Ecologically such a sequence of host suitability is seen as an advantageous component of the biosystem of Uraba lugens and some evidence that the insect may be increasing its host range is provided by occasional survivals of larvae upon otherwise unsuitable foodplants consistently found among selected egg-hosts.

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APPENDIX TABLE 3.3.5.1 Light trap catches of Uraba lugens moth in Summer and Winter generations 1975.

Month	Number of Uraba lugens ♂	♀
Summer Generation		
March 1975	18	0
April 1975	16	4
May 1975	3	1
	37	5
Winter Generation		
October 1975	17	0
November 1975	17	5
December 1975	4	8
	38	13

During the flight periods of Summer and Winter generations in 1975, a light trap was operated to obtain information on the abundance of and activity of male and female moth. The light trap used is as described by Cullen (1969).

As well as the number of U. lugens caught each night, the total number of moths was also recorded. Records from individual nights were pooled to give monthly values which are presented in Table 3.3.5.1.

APPENDIX TABLE 4.2.3.1

Field records of oviposition, larval establishment and survival to fourth instar for Uraba lugens (Walk.). (Only species on which eggs were recorded are shown here).

Host Plant	No. of trees	Oviposition	Establishment (a)	Survival to 4th instar (b)
Section - MACRANTHERAE Subsection - Cordiformes Series - EUDEMESDES				
·Eucalyptus erythrocorys	3	++++	++	++
E. tetragona	1	+	·	
E. ebbanoensis	1	+++	++	
E. curtisii	2	+++	++	
Subsection - Longiores Series - TETRAPTERAE				
E. steedmanii	2	++	++	+
E. papuana	2	+	+	·
E. dicromphloia	1	+	+	·
Series - CORYMBOSAE-PELTATE				
E. trachyphloia	1	++	++	+
E. bloxsomei	1	++	++	+
E. watsoniana	1	++		
E. maculata	3	++++	++++	+++
E. citriodora	3	++++	++++	+++
Series - TRANSVERSAE				
E. diversicolor	1	++	+	+
E. grandis	1	++	+	
E. deanei	1	+		
E. botryoides	1	++	++	+
·E. robusta	2	++++	++	+
E. resinifera	2	++	+	
·E. pumila	3	+++++	++	+
E. propingua	2	++++	++	+
·E. punctata	5	+++	++	+
E. longifolia	1	++	·	
·E. cosmophylla	1	+++	+	·
Series - OBLIQAE				
E. caesia	5	++	·	
·E. woodwardi	3	+++	·	
·E. stricklandii	1	++++	++	++
Series - CORNUTAE				
E. gomphocephala	2	++	+	
·E. platypus	3	++	·	
E. annulata	3	+++	·	
E. megacornuta	2	++	·	
E spathulata	4	++	++	·

Host Plant	No. of trees	Oviposition	Establishment	Survival to 4th instar
E. eremophila	3	++	·	
E. stowardi	5	+++	+	·
E. nutans	3	+	+	
E. burdettiana	2	++	+	
E. macrandra	3	+++	·	
E occidentalis	4	+++	+++	+++
Series - SUBCORNUTAE				
E. redunca	7	+++	++	
E. gardneri	1	++++	++	·
Subsection - Truncatae Series - MICROCORYTHAE				
E. caldocalyx nana	5	++++	+	+
Series - DUMOSAE				
E. accedens	2	++++	++	+
E. desmondensis	1	++	·	
E. dundasi	1	++++	++	++
E. merrickiae	5	++	·	
E. loxophleba	2	++++	++	+
E. incrassata	5	++	·	
E. conglobata	4	++	+	
E. anceps	2	++		
E. dumosa	4	+++	+	·
E. platycorys	2	++		
E. Le souefii	5	++	+	·
E. goniantha	5			
E. torquata	3	++	·	
E. concinna	4	+		
E. stoatei	1			
Subsection - Orbiculares Series - ANISOMELEAE				
E. pimpiniana	2	+	++	·
Series - DECURVAE				
E. falcata	1	++		
Subsection - Tereticornes Series - ELONGATAE				
E. urrbrae		++	+	+
E. erythronema	4	+++	++	+
E. dielsii	2	+		
E. cylindriflora	2			
Series - EXSERTA				
E. exserta	3	+++++	++++	++
E. morrisii	9	++		
E. tereticornis	3	++		
E. dealbata	1	+++	++	
E. parramentensis	2	++	++	+
E. camaldulensis	4	+++++	++++	+++
E. McIntyrensis	1	+++	+	+

Host Plant	No. of trees	Oviposition	Establishment	Survival to 4th instar
Series - SUBEXSERTAE				
E. alba	1	+	+	·
E. platyphylla	2	+	·	
E. ovata	1	++	+	·
Section - MACRANTHERAE Series - MICROCARPAE				
E. nicholi	1	+++	+	+
E. scoparia	2	++	+++	+++
E. praecox	1	+	·	
E. aromaphloia	1	++++	++	+
Series - GLOBULARES				
E. angophoroides	1	+++++	++	+
E. dunii	1	++	++	+
E. nortonii	1	+++	++	+
E bridgesiana	1	++	·	
E. mannifera	1	++	++	
E. rubida	2	++	++	
E. darylympleana	1	+	+	
E. glaucescens	2	+	+	
E. kruseana	5	++	+	+
E. crenulata	2	++	++	
E. brachyphylla	5	++		
E. megacarpa	1	++	++	+
E. maideni	2	++	+	+
E. goniocalyx	1	++	+	
E. nitens	2	++++	++	+
E. cyperllocarpa	2	++	++	
Series - SEMIUNICOLORES				
E. neglecta	1	++	+	
Series - VIMINALES				
E. baeuerleni	2	++	·	
E. quadrangulata	1	++		
E. macarthuri	1	+++	+++	++
E. smithii	1	+		
E. viminalis	2	++	++	+
E. pryoriana	1	+		
Series - ARGYROPHYLLAE				
E. cinerea	3	+++	++	
Series - PANICULATAE				
E. intertexta	3	++++	++	++
E. cloeziana	1	++		
Section - RENANTHEROIDEAE Series - DIVERSFORMAE				
E. diversifolia	3	+++++	++	+
Section - RENANTHERAE Subsection - Cordatae Series - OCCIDENTALES				
E. staerii	1	+		

Host Plant	No. of trees	Oviposition	Establishment	Survival to 4th instar
Subsection- Papilionantherae Series - OCHROXYLON				
E. guilfoylei	1	++	++	+
Section - RENANTHERAE Series- PSEUDO-STRINGYBARKS				
E. pilularis	3	+		
E. muelleriana	1	++	·	·
Series - STEATOXYLON				
E. microcorys	2	++	·	
Series - PACHYPHLOIAE				
E. blaxlandi	1	++		
E. baxteri	2	++	++	+
E. alpina	2	+		
E. eugenioides	1	++	++	+
E. oblonga	1	+	·	
E. laseroni	1	++	++	++
E. fastigata	1	++	·	
E. regnans	1	+	++	·
Series - FRAXINALES				
E. oreades	1	++	·	
E. triflora	1	+		
E. obtusiflora	1	++	·	
E. kybaenansis	1	++	+	·
Series - LONGITUDINALES				
E. stellulata	1	+++	++++	+++
E. mitchelliana	2	++	+	+
E. moorei	1	+++	++++	+++
Series - PEPERITALES				
E. dives	1	++	·	·
E. andrewsi	2	++		
Section - PORANTHEROIDEAE Series - FRUCTICOSAE				
E. uncinata	5	+	·	
Section- PORANTHEROIDEAE (Normales) Series - SUBBUXEALES				
E. lansdowneana	6	+++	++	·
E. odorata	10	++++++	++++	++
E. polybractea	1	+	·	
E. currabubula	1	++	++	
E. porosa	1	+	·	
Series - BUXEALES				
E. largiflorens	3	++++	++	+
E. largeana	1	+++	·	

Host Plant	No. of trees	Oviposition	Establishment	Survival to 4th instar
E. moluccana	2	++	+	
E. bosistoana	2	+++	+++	+++
E. behriana	1	++	++	+
E. woolsiana	5	++	++	+
E. albens	1	++	++	+
E. populifolia	2	++	++	+
E. rariflora	2	+		
E. microtheca	1	++	++	·
E. racemosa	3	++	+++	++
E. staigeriana	1	++	++	
E. melanophloia	2	+++++	+++	+++
E drepanophylla	1	++++	++	·
E. orgadophylla	1	+++	++	++
E. argophloia	1	++	+	·
Section - TERMINALES				
E. paniculata	2	++	++	·
E. fergusoni	1	++	·	
E. caleyi	5	+++	+	
E. affinis	1			
E. sideroxylon	4	+++	++	+
E. leucoxylon	8	+++++	++++	++
Series - MELLIODORAE				
E. melliodora	1	++	++	++
Series - HETEROPHLOIAE				
E. conica	1	+	·	
E. rudderi	2	+++	+	·
E. dawsonii	2	+++	++	·
E. polyanthemos	1	++	++	+
Section - GRACILES Series - ARIDAE				
E. celastroides	1	++	·	
Section - MICRANTHERAE Series - EREMOPHILAE				
E. cneorifolia	3			
Section - PLATYANTHERAE Series - SUBULATAE				
E. squamosa	1	+		
E. longicornis	2	++	+	·
E. oleosa	3	+	·	
E. transcontinentalis	5	+	·	
E. gilii	1	++	+	·
E. flocktoniae	1	++	++	
E. cooperiana	1	++	·	
E. brockwayi	1	+	·	
Series - LEPTOPODAE				
E. orbifolia	3	+	·	
E. lane-poolei	1	++	+	+
E. drummondi	1	++	++	+
E. salmonophloia	2	+		

Host Plant	No. of trees	Oviposition	Establishment	Survival to 4th instar
Series - CONTORTAE				
E. salubris v. glauca	3	++		
Series - XYLOCARPAE				
E. oldfieldii	4	+++	+	+
E. macrocarpae	1			
E. pyriformis	2	++	·	
E. pachyphylla	2	++	+	+
E. burracoppinensis	4	++	++	·
E. youngiana	3	+	+	·
NON-EUCALYPT SPECIES				
Angophora subvelutina	2	++	+	+
Angophora costata	1	+	+	+

N.B. For oviposition each cross represents a generation; in other columns, each cross represents about 25% of each generation so that two crosses represent about 50% survival as an average of the generations recorded. A dot (·) represents a few survivors and usually less than 15% of the total egg population.

Appendix Figure 4.2.3.1 Spatial arrangements of egg host and non-egg hosts in the arboretum of W.A.R.I.

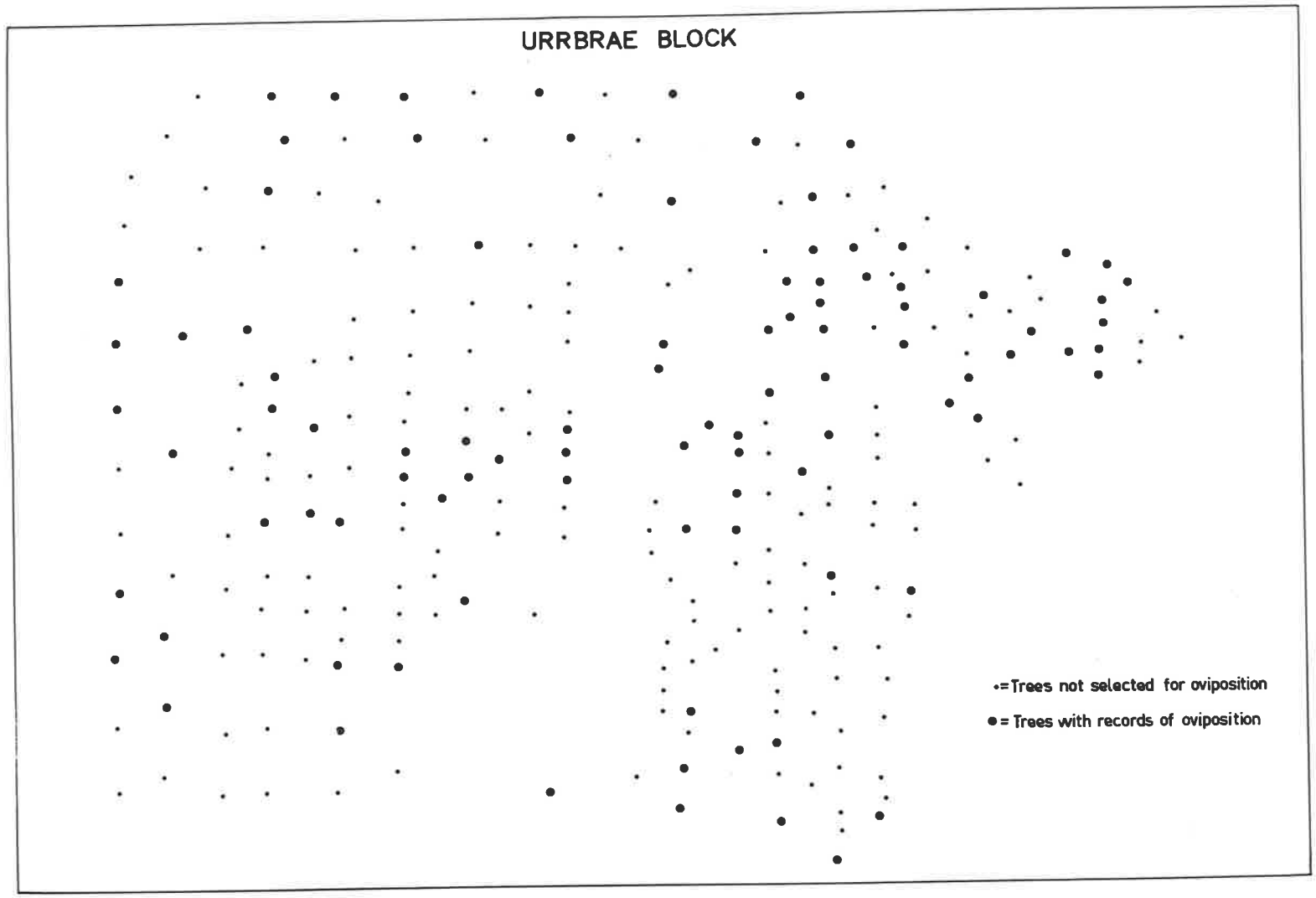
● Egg host

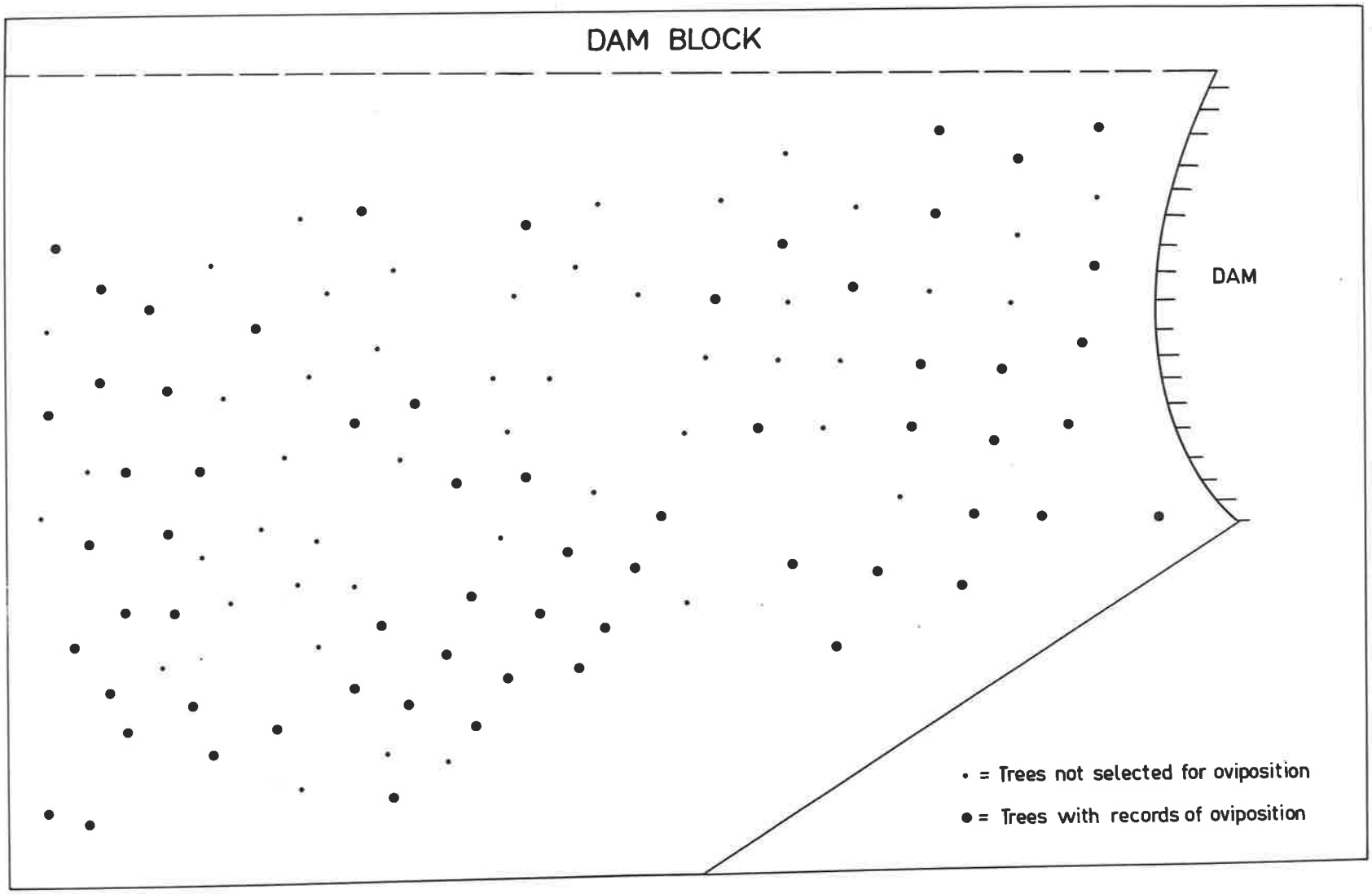
• non- egg host.

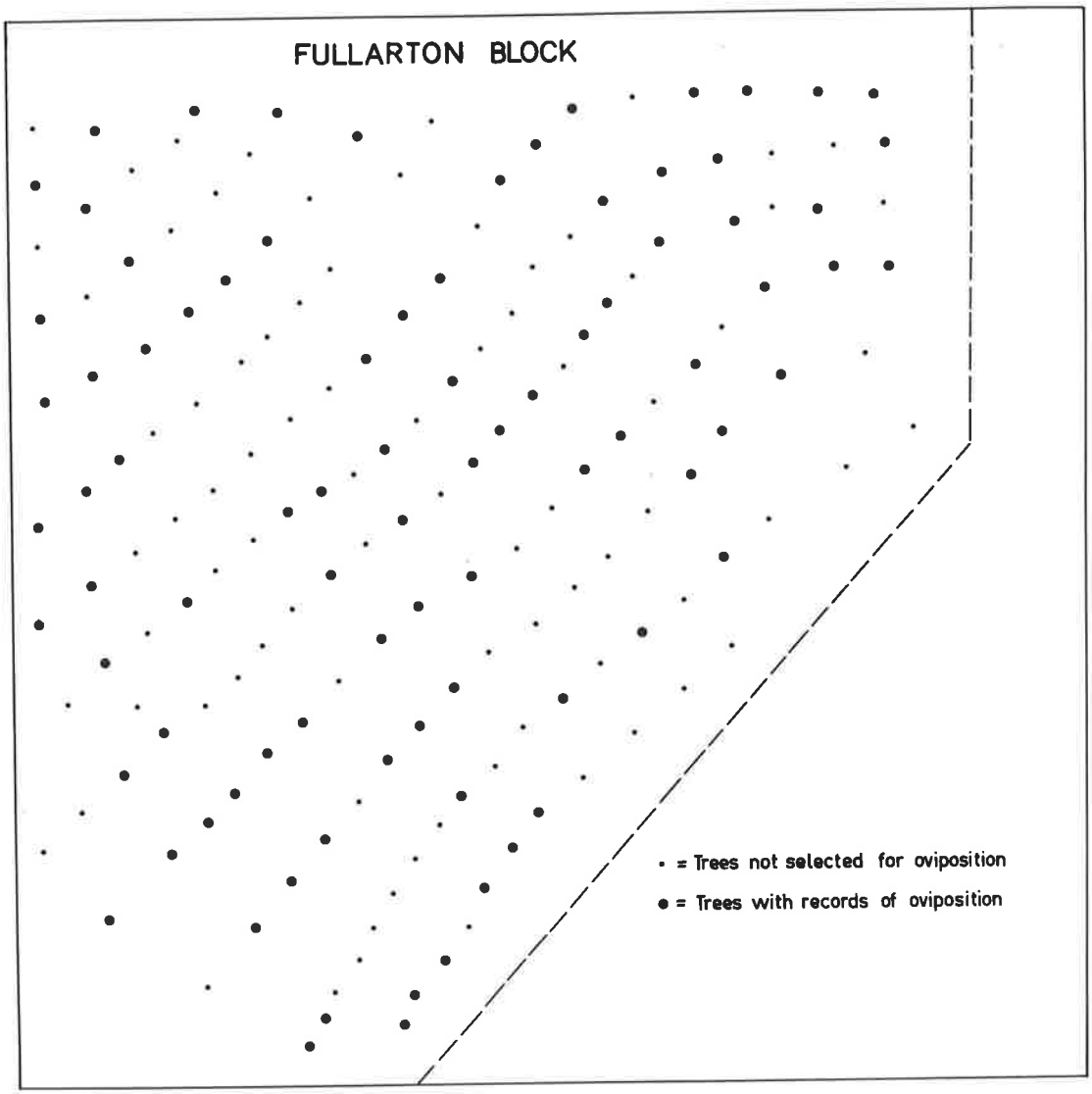
a) Urrbrae block

b) Dam block

c) Fullarton block.


URRBRAE BLOCK
•=Trees not selected for oviposition
●= Trees with records of oviposition


DAM BLOCK
DAM
• = Trees not selected for oviposition
● = Trees with records of oviposition


FULLARTON BLOCK
· = Trees not selected for oviposition
● = Trees with records of oviposition

Appendix Figures 5.3.3.1 - 5.3.3.4

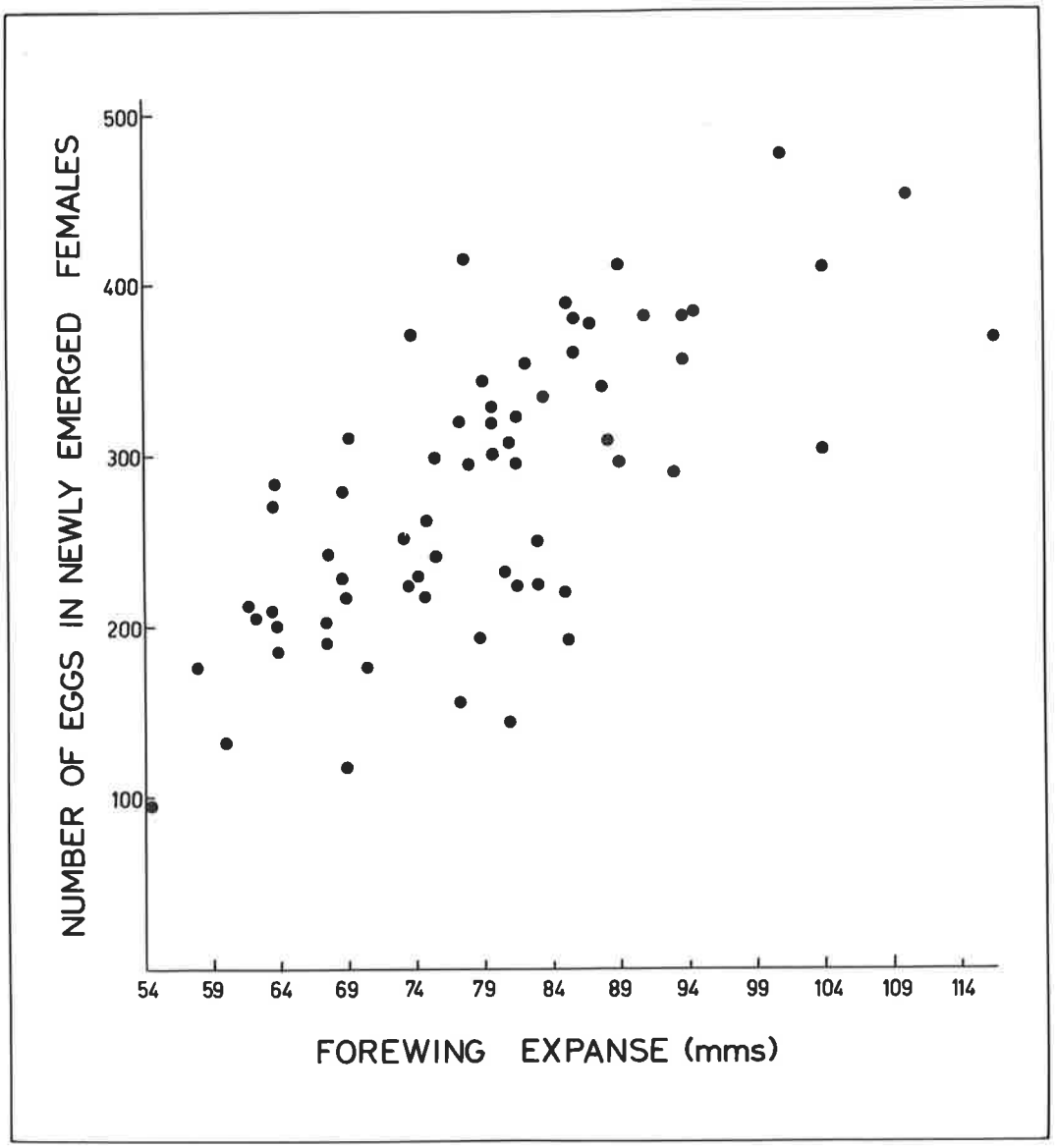
Relationship between pupal and adult morphological characters and potential fecundity of *U. lugens*. (All larvae reared on *E. camaldulensis* in Winter 1976).

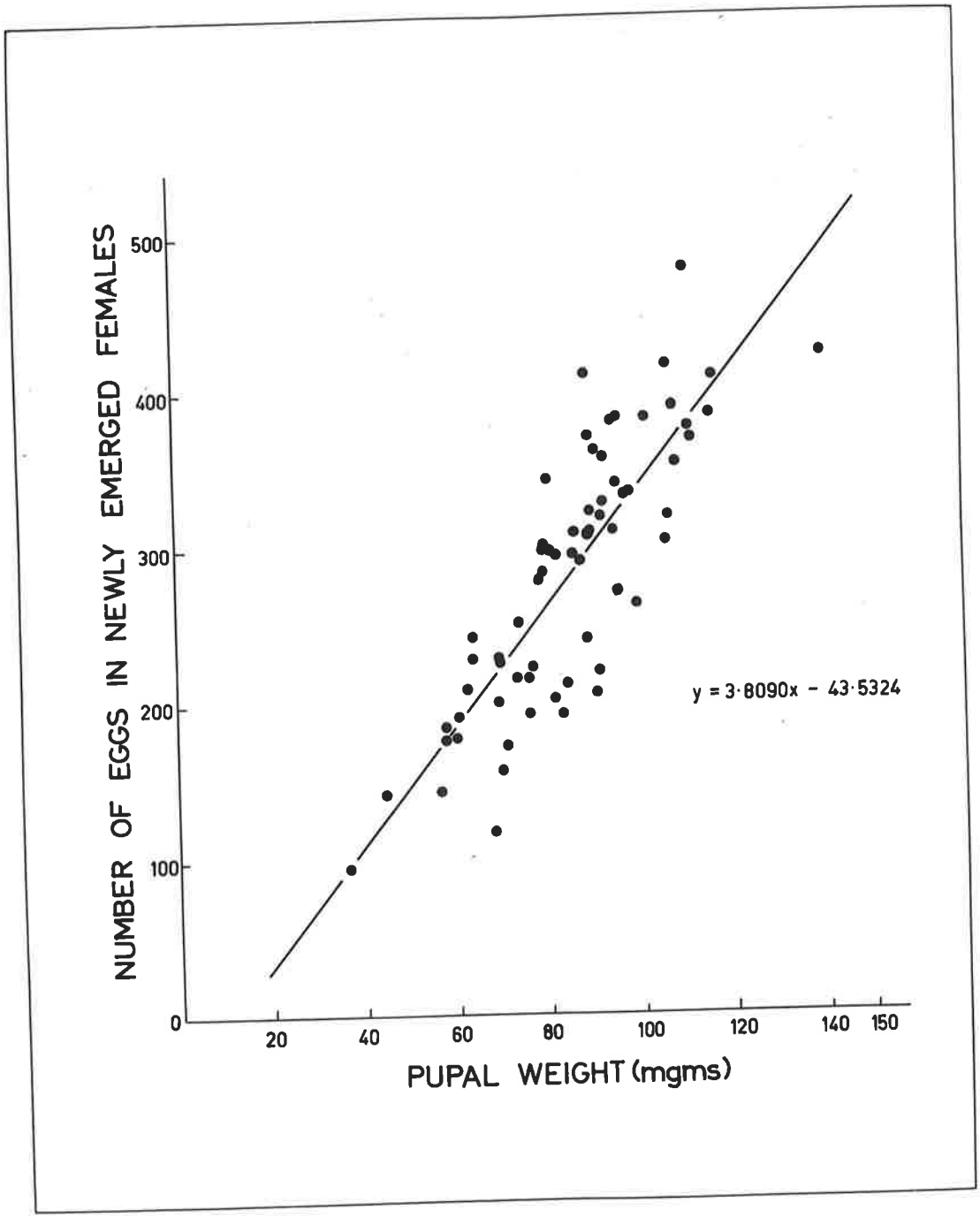
5.3.3.1 Forewing expanse and potential fecundity

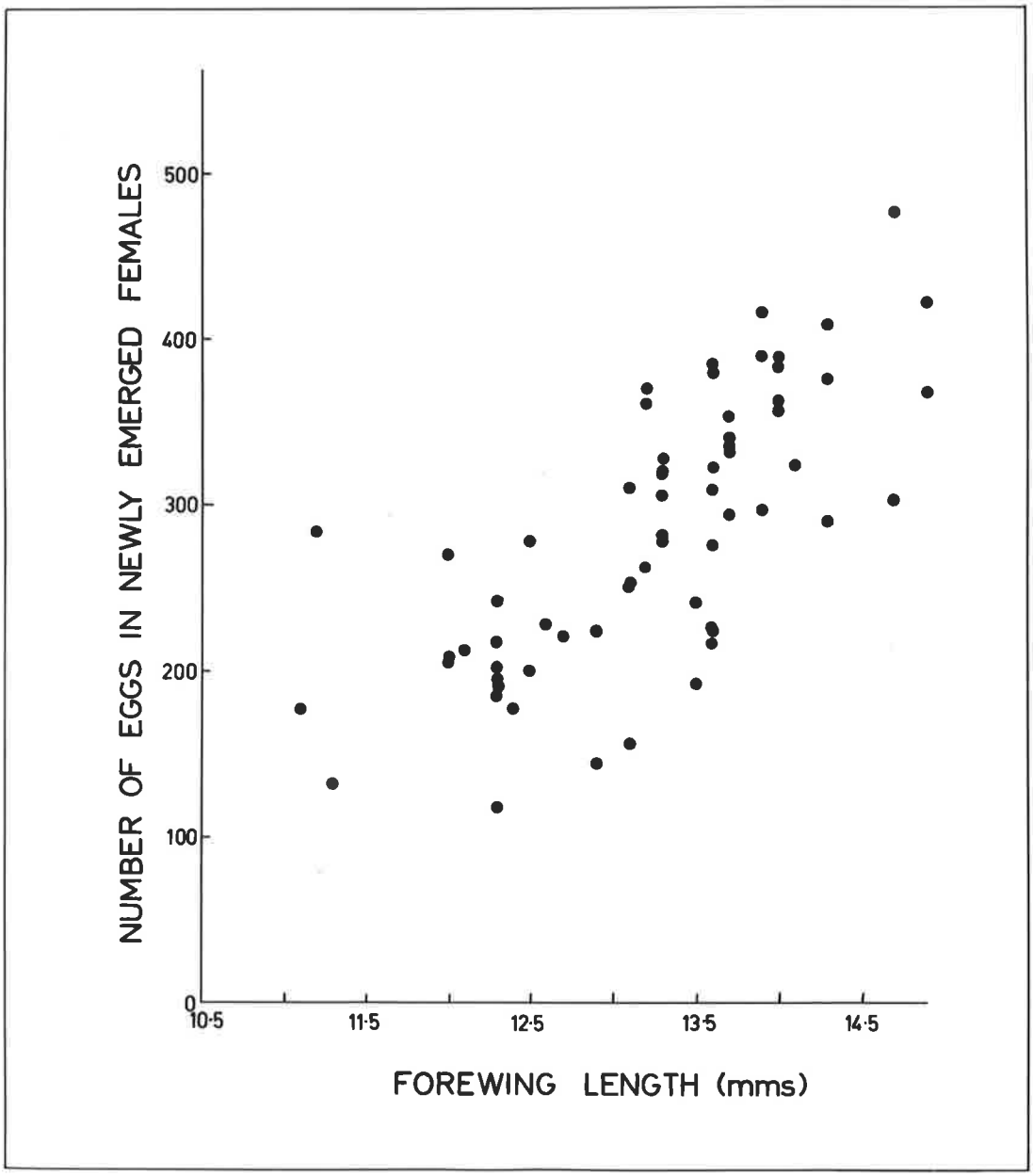
5.3.3.2 Pupal weight and potential fecundity

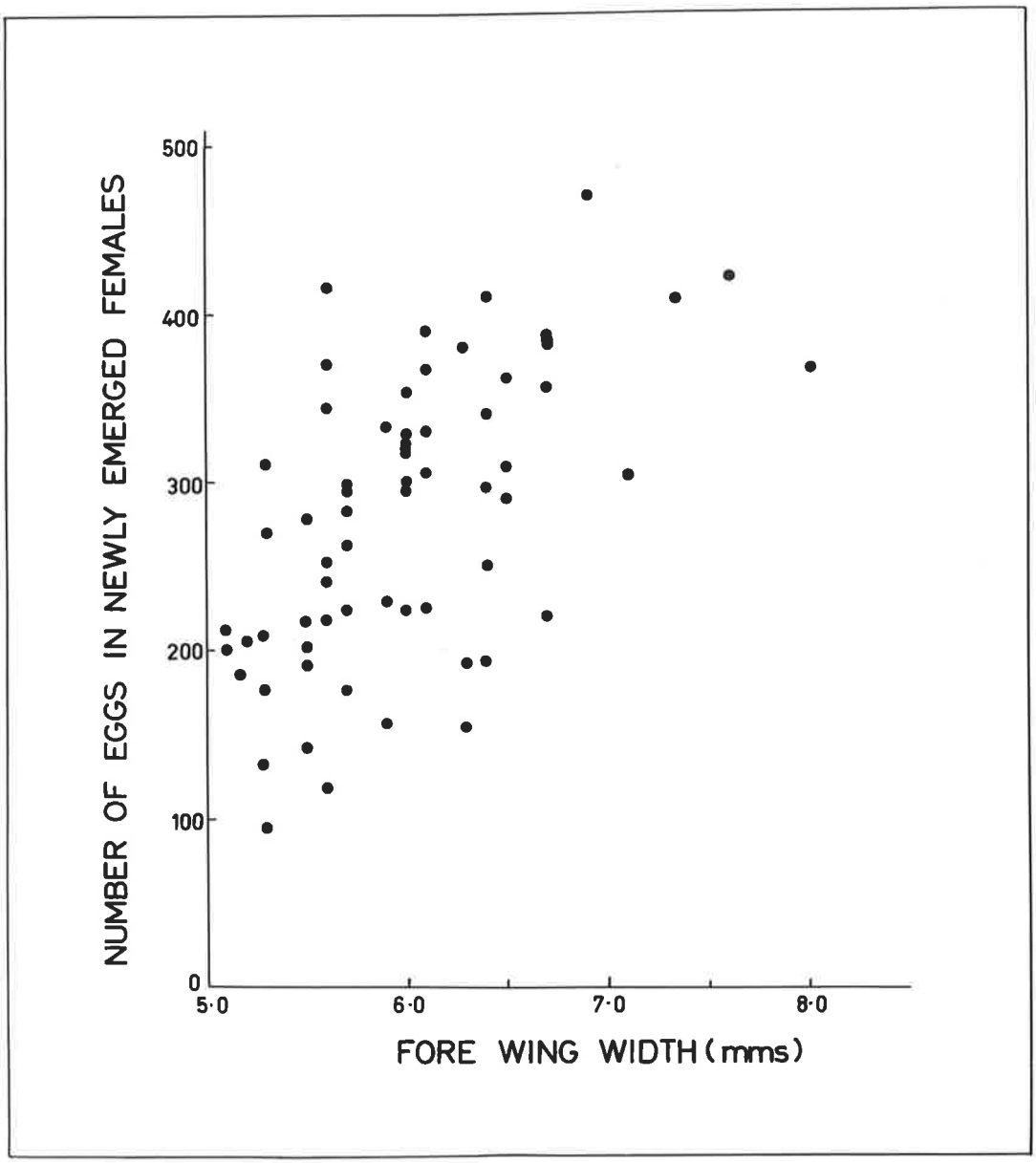
5.3.3.3 Forewing length and potential fecundity

5.3.3.4 Forewing width and potential fecundity.


NUMBER OF EGGS IN NEWLY EMERGED FEMALES
500
400
300
200
100
54
59
64
69
74
79
84
89
94
99
104
109
114
FOREWING EXPANSE (mms)


NUMBER OF EGGS IN NEWLY EMERGED FEMALES
0
100
200
300
400
500
20
40
60
80
100
120
140
150
PUPAL WEIGHT (mgms)
y = 3·8090x - 43·5324


NUMBER OF EGGS IN NEWLY EMERGED FEMALES
500
400
300
200
100
0
10·5
11·5
12·5
13·5
14·5
FOREWING LENGTH (mms)


NUMBER OF EGGS IN NEWLY EMERGED FEMALES
500
400
300
200
100
0
5·0
6·0
7·0
8·0
FORE WING WIDTH (mms)

APPENDIX TABLE 5.3.3.2a Life-table for *Uraba lugens* with caged *E. camaldulensis* as host plant.

Stage of development	Number entering each x (lx)	Predominant behaviour and mortality factors dxF	Number dying in each x (dx)	dx as percent of lx	Percent real mortality 100 x dx / n	Survival rate within x
EGG	n = 245	Failed to hatch	6	2.45		
		Washed by rain	10	4.08		
		Damaged by clips	7	2.85		
		Unfertilized	8	3.26		
		Subtotal	31	12.64	12.65	.87
LARVAE [Instars 1 + 2]	214	Establishment of aggregation and establishment on host-1				
		Host factors interacting with weather and unknown causes	72	33.64		
		Subtotal	72	33.64	29.39	.66
LARVAE [Instars 3 - 4]	142	Establishment of aggregation and establishment on host-2				
		Host factors	32	22.53		
		Disease	13	9.15		
		Subtotal	45	31.68	18.36	.68
LARVAE 5th instar- 8th instar	97	Dispersal stage disease				
		Aspergillus spp.	8	8.24		
		Beauveria bassiana	2	2.06		
		Subtotal	10	10.30	4.08	.90

APPENDIX TABLE 5.3.3.2a continued

Stage of development	Number entering each x (lx)	Predominant behaviour and mortality factors dxF	Number dying in each x (dx)	dx as percent of lx	Percent real mortality $\frac{100 \times dx}{n}$	Survival rate within x
9-11th instar	87	Prepupal stage	2	2.29		
		Subtotal	2	2.29	0.82	.98
PUPAL	85	Unknown	2	2.35		
		Beauveria bassiana	3	3.52		
ADULT			5		2.04	.94

APPENDIX TABLE 5.3.3.2b Life-table for Uraba lugens with caged E. scoparia as host plant.

Stage of development	Number entering each x	Mortality factors (dxF)	Number dying in each x (dx)	dx as percent of 1x	Percent real mortality 100 x dx / n	Survival rate within x
EGG	n = 251	Failed to hatch	10	3.98		
		Unfertilized	4	1.59		
		Washed by rain	4	1.59		
		Subtotal	18	7.16	7.17	.93
LARVAE [Instars 1 + 2]	233	Establishment of aggregation and establishment on host-1				
		Host factors, Weather, Unknown causes	32	13.73	12.75	.86
LARVAE [Instars 3 + 4]	201	Maintenance of aggregation and establishment on host				
		Host factors, Weather, Unknown	20	9.95	7.96	.90
		Subtotal	20			
LARVAE [Instars 5th-8th]	181	Dispersal stage	7	3.86		
		Disease - A. flavus	21	11.60	11.15	.85
		Unknown	28	25.46		
		Subtotal				
LARVAE [Instars 9-11th]	153	Prepupal				
		Disease - A. flavus	15	9.80		

APPENDIX TABLE 5.3.3.2b continued

Stage of development	Number entering each x	Mortality factors (dxF)	Number dying in each x (dx)	dx as percent of 1x	Percent real mortality $\frac{100 \times dx}{n}$	Survival rate within x
		Disease - *A. flavus*	15	9.80	9.96	.84
		Unknown	10	6.54		
		Subtotal	25	16.34		
PUPAL	128	Pupal				
		2-*Chaetomium* sp.	2	1.56	1.19	.98
		Beauveria bassiana	1	0.78		
		Subtotal	3	2.34		
ADULT	125					

APPENDIX TABLE 5.3.3.2c. Life-table for *Uraba lugens* with caged *E. lehmanni* as host plant

Stage of development	Number entering each x	Predominant behaviour and mortality factors (dxF)	Number dying in each x (dx)	dx as percent of 1x	Percent real mortality $\frac{100 \times dx}{n}$	Survival rate within x
EGG	n = 280	Failed to hatch	24	8.57		
		Damaged by clips	10	3.57		
		Subtotal	34	12.14	12.14	.88
LARVAL INSTARS (1 - 2)	246	Establishment of aggregation and establishment on host-2				
		Host factors / Unknown	159	64.63	56.78	.35
LARVAL INSTARS (3 - 4)	87	Maintenance of aggregation and establishment on host-2				
		Host factors / Unknown	17	19.54	6.07	.80
LARVAL INSTARS (5th - 8th)	70	Dispersal stage				
		Aspergillus spp.	9	11.25		
		Virus disease	3	3.75		
		Subtotal	12	15.00	4.29	.85
LARVAL INSTARS (9th - 11th)	58	Prepupal stage				
		No mortality	0	0	0	1.0
PUPAL	58	Pupal				
		Chaetomium sp.	2	3.45	0.71	0.97
ADULT	56					

APPENDIX TABLE 5.3.3.2d. Life-table for *Uraba lugens* with caged *E. globulus* as host plant.

Stage of development	Number entering each x	Predominant behaviour and mortality factors (dxF)	Number dying in each x (dx)	dx as percent of 1x	Percent real mortality $\frac{100 \times dx}{n}$	Survival rate within x
EGG	n = 214	Unfertilized	3	1.40		
		Washed away	20	9.35		
		Subtotal	23	10.75	10.75	.89
LARVAL INSTARS (1 - 2)	191	Establishment of aggregation and establishment on host				
		Host factors Unknown	121	63.35	56.54	.37
LARVAL INSTARS (3 - 4)	70	Maintenance of aggregation and establishment on host				
		Host factors Unknown	19	27.14	8.88	.73
LARVAL INSTARS 5th - 8th	51	Dispersal stage				
		Diseases				
		Aspergillus spp.	3	5.88		
		Virus	3	5.88		
		Beauveria bassiana	4	7.84		
		Subtotal	10	19.60	4.67	.80
LARVAL INSTARS 9 - 11	41	Prepupal No mortality	0	0	0	1.0
PUPAL	41	Pupal No mortality	0	0	0	1.0
ADULT	41					

APPENDIX TABLE 5.3.3.2e. Life-table for Uraba lugens with caged E. citriodora as host plant.

Stage of development	Number entering each x	Predominant behaviour and mortality factors (dxF)	Number dying in each x (dx)	dx as percent of 1x	Percent real mortality 100 x dx / n	Survival rate within x
EGG	n = 288	Failed to hatch	29	10.07		
		Unfertilized	5	1.74		
		Damaged by clips	7	2.43		
		Subtotal	41	14.24	14.24	.86
LARVAL INSTARS 1 - 2	247	Establishment of aggregation and establishment on host				
		Host factors } Unknown }	86	34.81	29.86	.65
LARVAL INSTARS 3 - 4	161	Maintenance of aggregation and establishment on host-2				
		Host factors } Unknown }	68	42.23	23.61	.58
LARVAL INSTARS 5th - 8th	93	Dispersal stage				
		Host factors } Unknown }	28	30.11		
		Diseases				
		Aspergillus spp.	14	15.05		
		Virus	8	8.60	17.36	.46
		Subtotal	50	53.76		
LARVAL INSTARS 9th - 11th	43	Prepupal				
		Aspergillus spp.	2	4.65	0.69	.95
PUPAL	41	Pupal	0	0	0	1.0
ADULT	41					

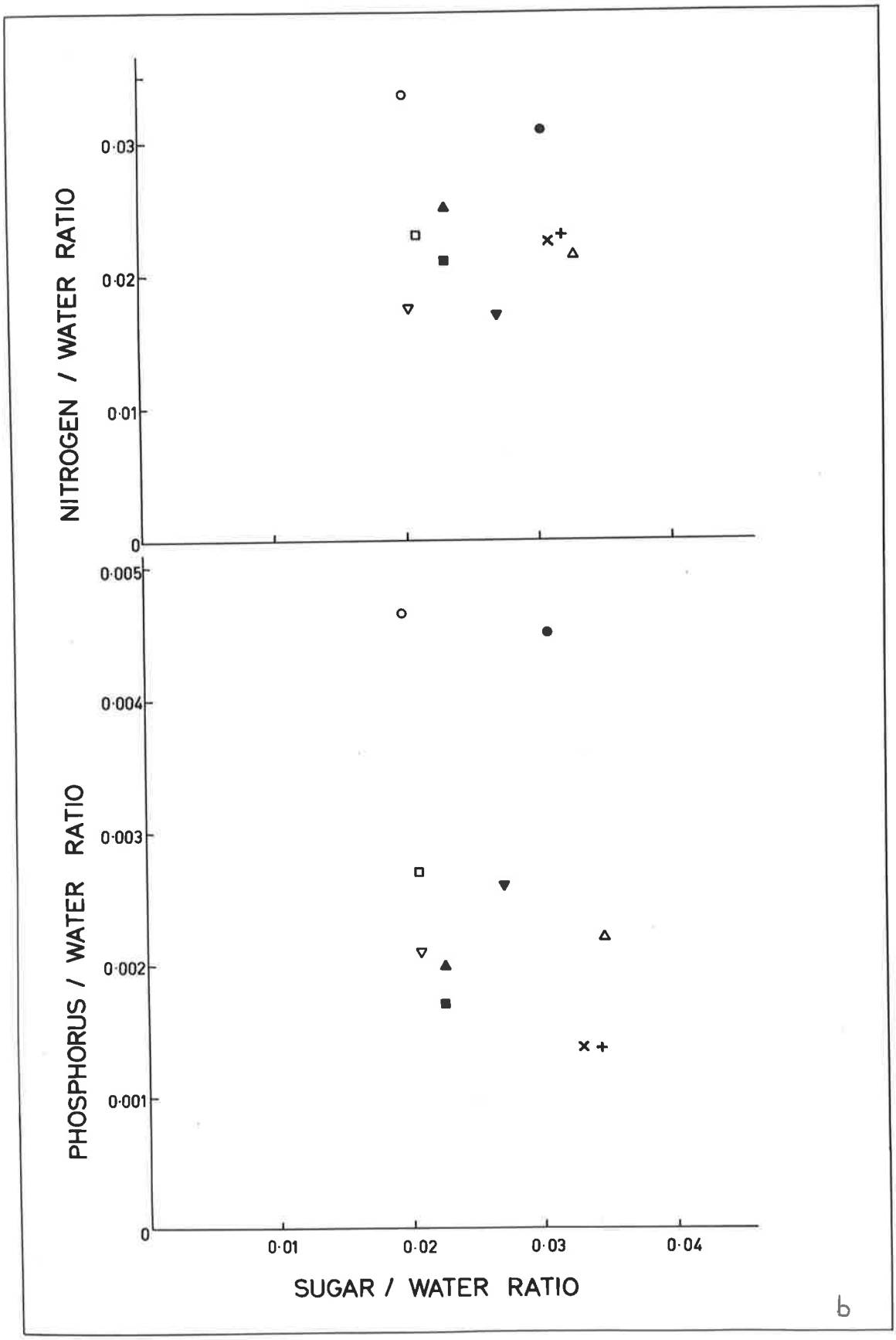
APPENDIX TABLE 5.3.3.2f Life-table for *Uraba lugens* with caged *E. gardneri* as host plant.

Stage of development	Number entering each x	Predominant behaviour and mortality factors (dxF)	Number dying in each x (dx)	dx as percent of 1x	Percent real mortality 100 x dx / n	Survival rate within x
EGG	n = 245	Failed to hatch	10	4.08		
		Damaged by clips	5	2.04		
		Subtotal	15	6.12	6.12	.94
LARVAL INSTARS (1 and 2)	230	Establishment of aggregation and establishment on host				
		Host factors } Unknown }	165	71.74	67.35	.28
LARVAL INSTARS (3 - 4)	65	Maintenance of aggregation and establishment on host				
		Host factors } Unknown }	58	89.23	23.67	.11
LARVAL INSTARS (5 - 8)	7	Dispersal stage				
		Host factors } Unknown }	3	42.86		
		Aspergillus spp.	4	57.14		
		Subtotal	7	100.00	2.85	0.0

Appendix Figures 9.3.1

Scatter showing the relationship between the suitability of foodplant for growth and nutrients composition - (Ca, K and others). (See Figure 9.3.1 for key to symbols).

a


NITROGEN / WATER RATIO
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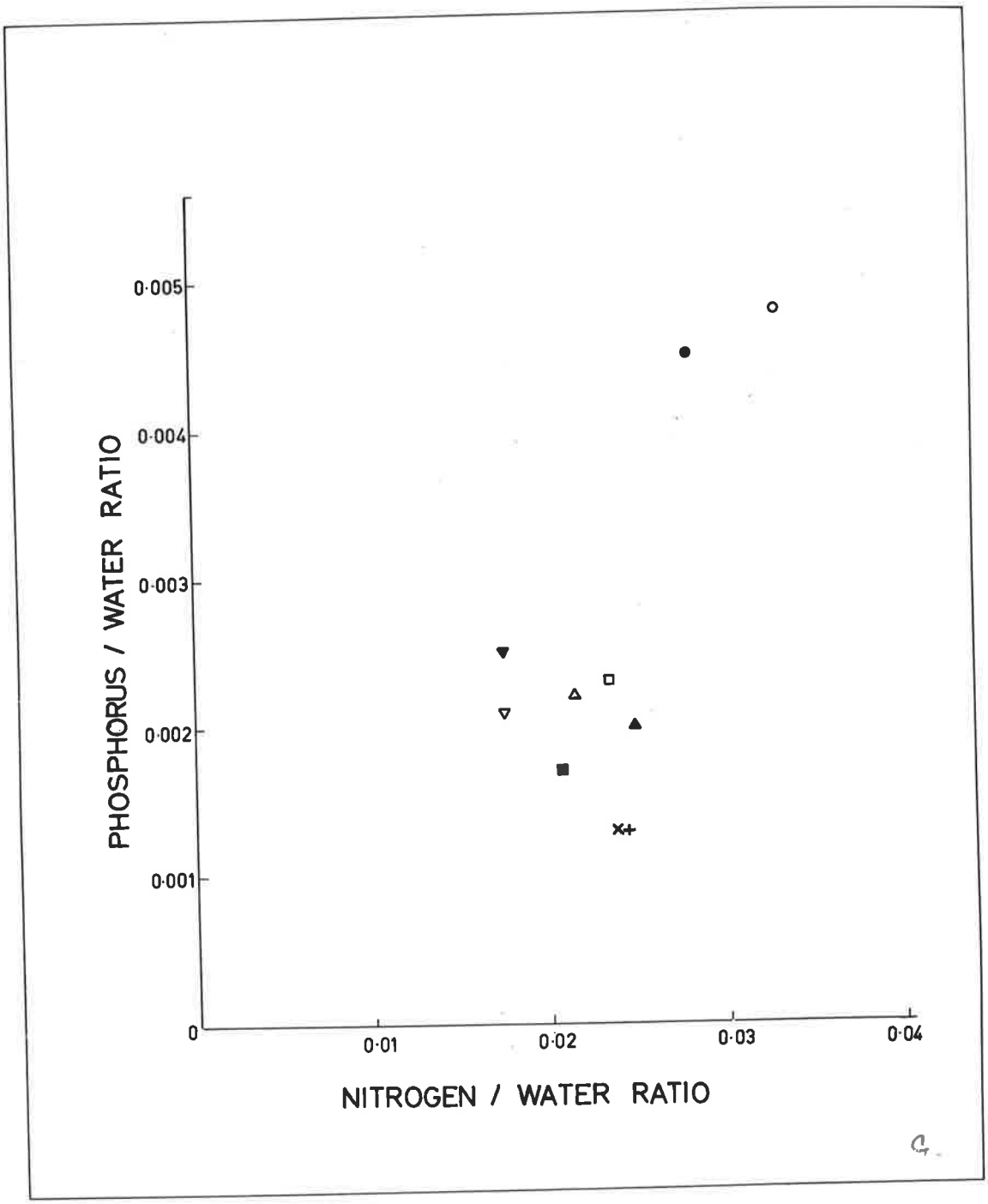
0·02
0·01
0
PHOSPHORUS / WATER RATIO
0·005
0·004
0·003
0·002
0·001
0
0·01
0·02
0·03
0·04
SUGAR / WATER RATIO

b


PHOSPHORUS / WATER RATIO
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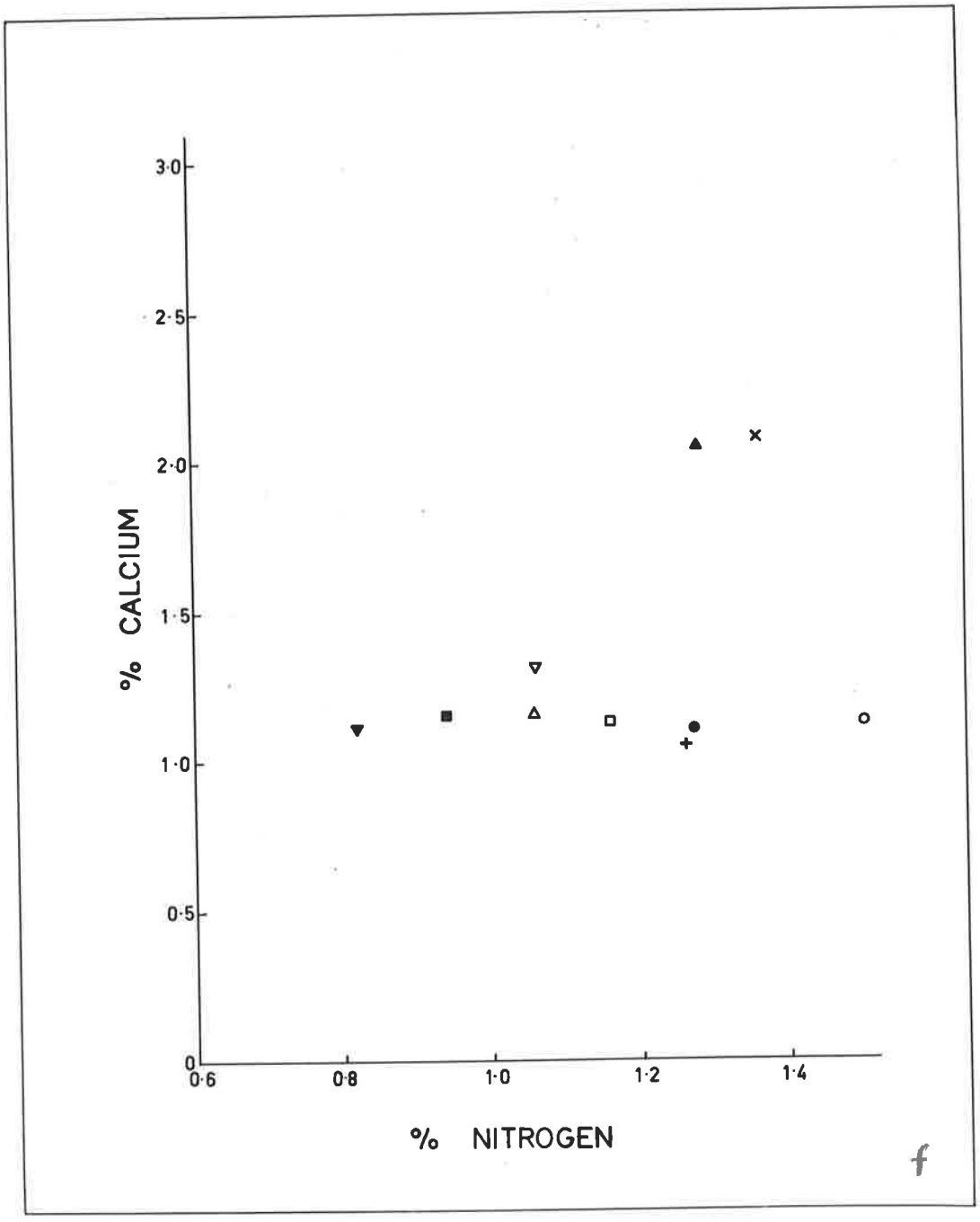
0·004
0·003
0·002
0·001
0
0·01
0·02
0·03
0·04
NITROGEN / WATER RATIO



f