THE HOST-PLANT RELATIONSHIPS OF PAROPSIS CHARYBDIS STAL (COLEOPTERA:CHRYSOMELIDAE)

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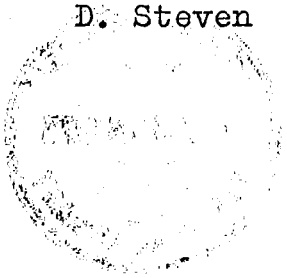

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CHAPTER I

INTRODUCTION

Paropsis charybdis Stal, the eucalypt tortoise beetle, is an Australian chrysomelid accidentally introduced into New Zealand early this century. Since then it has caused considerable damage to various species of *Eucalyptus* growing in this country. Both the larvae and adults of this insect species are defoliators, feeding on the young leaves and shoots of the host plant. *Paropsis* was at first of less concern than the scale *Eriococcus coriaceus* Maskell and the weevil *Gonipterus scutellatus* Bois-Duval but, with the introduction of parasites to suppress these two and the gradual spread of *Paropsis* throughout New Zealand, it became the chief pest of eucalypts in the country. The eucalypts are not at present a major component of forests in New Zealand although with the necessity to diversify away from a virtual *Pinus* monoculture and to provide for specialist products derived from hardwood and hardwood pulp, they are a group of trees with considerable potential value. Henry (1972) gave particular mention to this genus for use as a short-rotation timber crop to allow a more rapid and immediate expansion of the wood-product industry in New Zealand. He stated that Brazil was programming to grow eucalypts on a seven-year rotation on a large scale.

In the Canterbury region where the present work was undertaken there are no large forest plantings of any *Eucalyptus* species but there are many of these trees in amenity plantings which range from fairly large farm forestry areas (especially shelterbelts) down to single trees used

for shade and shelter or as ornamentals. Many of those trees in agricultural areas have resulted from early plantings, being survivors, seedlings or regrowths. Indeed, Olsen (1958) reported that in the South Island, eucalypts and E. globulus in particular, had been much sought after in the 1850s and 1860s and that the decade 1880-1890 saw the boom era of their use in the southern regions of New Zealand. The same author wrote that with the accidental importation of the blue-gum scale, Eriococcus coriaceus, at the turn of the century, serious damage to established trees occurred with a decline in the popularity of eucalypts in general and a decrease in subsequent plantings. The severity of the damage caused by the scale and by insect pests introduced later, such as Paropsis, was in part due to insufficient attention being paid to the climatic and soil conditions under which particular species of Eucalyptus had evolved. E. globulus was widely planted throughout New Zealand because it is quick growing and provides useful timber, but this gum is a Tasmanian species adapted to growing in cool, wet areas on freely draining soil and so it is not surprising that its tolerance of insect defoliation has been greatly reduced in unsuitable areas such as the Canterbury plains. In spite of these local adverse environmental conditions there are occasional vigorous and majestic trees even of E. globulus. These serve to demonstrate that selection for resistance or tolerance to insect pests and for suitability for environmental conditions could be a fruitful avenue of practical research. Several of the men active in the New Zealand Farm Forestry Association, especially Mr N.A. Barr of Northland, have done much along these lines

to help and encourage the establishment of farm woodlots of eucalypts as valuable economic assets.

However, in order to determine the effectiveness of the various silvicultural methods proposed to alleviate the damage caused by Paropsis, more information was required about the host-plant relationships of this insect. The data on host range present in the literature tended to be vague and confusing because they were derived solely from observations of damage, but they did indicate that this was a line of inquiry worth pursuing. The present research was undertaken in response to this need to know more about the interactions between Paropsis charybdis and its host plants. A twofold approach has been adopted; the presence of antibiotic or antifeeding factors in various Eucalyptus species has been investigated in two series of laboratory rearings, while in several series of feeding trials the ability of the larvae to discriminate between different eucalypt foliages has been checked. An attempt was also made to determine whether any of the chemicals known to be present in eucalypts could be used as an attractant for adult Paropsis.

CHAPTER II

REVIEW OF THE LITERATURE

1. THE PRINCIPLES OF INSECT-PLANT INTERACTIONS

The study of the relationship between phytophagous insects and their host plants has evolved slowly from very early and simple observations until today it is recognised as a topic worthy of study in its own right. From the beginning man has noticed that certain plants and insects have always appeared in association. This was shown in the common names given to insects such as the cabbage white butterfly, Colorado potato beetle, grass grub and even the eucalypt tortoise beetle. Along with other data, the host range of a phytophagous insect has often been used to help distinguish it as a distinct species. While some insects appeared restricted to one plant, others seemed to eat most species occurring in their geographic range. However the majority of cases lay in a continuous spectrum between these two extremes. Conversely although no plant was known to be absolutely free of insect attack, none was eaten by all the plant feeders occurring in the same region as it. These observations gave rise to the first rough classification of host-plant interactions depending on the breadth of the host range - monophagy, oligophagy and polyphagy, according to the number of plant types eaten. However as various authors have used species, genera or even families of plants as the measure of host range, the usefulness of this definition has been limited.

An example of the confusion arising from the vagueness

of these terms, and of the variability of observations on the host range of an insect, is provided by the literature on the silkworm Bombyx mori L.. Hamamura (1970) states that,

".....(it) has been regarded as one of the most typical examples of a monophagous insect. Although it can be experimentally fed on some other Moraceae and other plants, it feeds almost exclusively on mulberry leaves."

Ishikawa, Hirao and Arai (1969), however, make the contradictory statement that,

"The silkworm Bombyx mori, is an oligophagous insect which feeds under natural conditions on several species of Moraceae, Ulmaceae, Compositae, etc. especially Mulberry leaves."

Dethier (1954) suggested that the definitions of host range should be made a step closer to the insects' selection behaviour and instead of plant taxa, defined these terms on the basis of chemical interaction between insect and plant. Thus monophagy was considered due to the attractance to the insect of a single chemical, or a group of chemicals confused by the insect as one, oligophagy the attractance by several different chemicals, and polyphagy the acceptance of any plant free of feeding inhibitors. Although useful this view was criticised when the importance of inhibitors was shown in the selection of its host plant by an oligophagous beetle Leptinotarsa decemlineata (Jermy, 1958; Thorsteinson, 1960), and by a supposedly monophagous insect, the silkworm Bombyx mori (Ishikawa, 1966; Ishikawa and Hirao, 1966; reviewed by Ishikawa et al, 1969; Hamamura, 1970).

Thorsteinson (1960) in an early review of host selection by phytophagous insects, regarded host range as being determined by the probability with which plant types providing a signal pattern capable of inducing feeding in an insect occurred in the space-time range of that insect. Thus

monophagous insects require a "signal pattern" that only one or very few plant species can provide, whereas the "signal pattern" inducing feeding in polyphagous insects can be provided by most species. This view enlarged Dethier's earlier definitions of host range type to embrace all the varieties of stimuli offered by a plant and not just the chemical ones. However Thorsteinson re-emphasised the importance of chemicals by giving a new chemotactic classification of food-plant preference into two basic types. In the first of these feeding and oviposition were induced by chemical stimuli present in virtually all plants, but feeding was restricted by inhibitors occurring in some plant species. This class of insect-plant relationship was subdivided depending on whether the inhibitors were found more or less at random among plant groups or in most plants except some belonging to certain taxonomic groups. In the second type feeding and oviposition were induced by chemical stimuli, one or more of which was unusual and of restricted distribution among plants. This category was subdivided depending on whether the unusual chemicals were restricted to natural plant taxa (such as sinigrin and other mustard oil glycosides of the Cruciferae) or found more randomly in the plant kingdom.

More recent and detailed behavioural studies have elaborated the steps in the sequence of events resulting in the location and consumption of an acceptable food-plant by an insect, and have made apparent the error in both these earlier works of contrasting attraction with inhibition when these are separate and distinct events in this sequence.

The relationship between insects and secondary plant

substances was noticed as early as 1910 by Verschaffelt working with Pieris rapae and the mustard oil glycosides. Secondary plant substances are by definition compounds of unknown function not involved in the primary metabolic pathways. Many of these are toxic to some animals, and to date their existance has been staisfactorily explained only if they are considered a defense against herbivores, both insects and vertebrates. The picture of co-evolution of insect and plant that has arisen gives a fascinating story. Ehrlich and Raven (1965) and Raven (1969) found that among the larvae in the lepidopterous group Papilionoidea some divisions were restricted and fed on a narrow range of plant families (such as the Pierinae on Capparidaceae and Brassicaceae, and the Troidini on Aristolochiaceae). Others such as the large and varied groups Lycaeninae and Nymphalinae feed on a wide variety of plants. The most interesting observation made by those authors was that the plants fed on by the species having a restricted host-plant range were never represented among the food plants of those species having a wide range of hosts, and that the food plants of the former contained toxic secondary plant chemicals. The plants appear to have evolved a biochemical arsenal against indiscriminately browsing phytophages and some insects have in turn evolved means to counter these defenses. Not only have the plant defensive barriers been broached but often the deterrent chemical has come to be used by the insect in the behavioural sequence by which the host plant is recognised because the chemical may be a distinctive characteristic of the plant (Raven, 1969; Dethier, 1970b).

The separate stages in the behaviour pattern of an

insect in finding and recognising an acceptable host plant were recognised by Beck (1965) and are, directed movement towards a plant, cessation of movement on reaching the plant, the initiation of biting or piercing, and finally, continued feeding.

Beck also gave a classification of the stimuli involved in these stages (Table 1).

Table 1. The sequence of events in host selection by an insect (after Beck, 1965).

Response	Stimulus	
	Positive	Negative
1	attractant	repellent
2	arrestant	repellent
3	incitant	suppressant
4	stimulant	deterrent

Not all insects will show all stages; for example some grasshoppers appear to move at random rather than in a directed way (Mulkern, 1967, 1969). The stimulus at any stage may be chemical or it may depend on the physical properties of the plant. There is more reference to chemical than physical stimuli probably because the latter, although obvious, are often subjective in nature (for example leaf toughness or pilosity) and less suitable for quantitative investigation. However some aphids show a clear response to physical stimuli at stage two of the above sequence, preferring to land on yellow reflecting surfaces rather than on other colours (Moericke, 1969).

Similarly a dispute that seemed to ignore the fact

that host selection does not necessarily depend on a single choice situation was the argument as to whether selection depended on secondary plant substances or substances of nutritive value for the insect. Fraenkel (1959, 1969), on the grounds that all insects have essentially similar nutritional requirements, postulated that these nutritional substances could not be a factor in determining host-plant specificity. Thus the so called secondary chemicals (such as essential oils, alkaloids and glycosides) of no known function in plant metabolism were seen as the sole factors regulating host selection.

In his 1969 paper Fraenkel modified slightly the stand taken in earlier work but still claimed that,

"..... host selection is guided by the presence or absence of secondary plant substances, and that qualitatively or quantitatively nutrients can play only a very minor role, if one at all, in this context."

The opposing view was put by Schoonhoven (1968) in his review of the chemosensory basis of host-plant selection (p130),

"Monophagy and oligophagy could then well be based on a fairly subtle combination of a number of common plant components, combined with absence of several plant substances."

However in summing up Schoonhoven (1968) gave a more balanced view of the interaction of nutrients and secondary plant substances in determining the host selection of a phytophagous insect. He wrote that,

"When combining the present knowledge about the type of sensory information which the insect central nervous system receives from its gustatory receptors, it appears that sapid nutrients as well as secondary plant substances together characterise a particular plant for its commensal.... In some insect-plant relations the accent may lay on the stimulating or deterrent effect of odd substances, in other cases the amounts or ratios of the nutritive constituents may form the decisive factors. Several volatile factors, acting as sign stimuli to release feeding behaviour or

oviposition belong to the category of the secondary plant substances."

Distinctive chemicals which are restricted to one or a few plant species in one habitat would provide a quick means of identification for any insect capable of perceiving them. If the preferred host contained such a chemical any plant without this could be rapidly rejected as a nonhost, while if the host lacked a distinctive chemical any plant containing one could also be rejected. Such index chemicals could be expected to be used by an insect in the initial steps of the behavioural sequence leading to the successful establishment of the insect on a favourable plant host, because the more rapidly that the assessment of host/nonhost is accomplished the greater the efficiency of the host-finding process and the higher the chances of finding a suitable host. If volatile, an index chemical could act at a distance either to attract the insect to a favourable plant or to repell it from an unsuitable one; if nonvolatile, it could function as a contact stimulus either to halt the insect in the presence of the host plant or to make it move away from a nonhost.

The plant materials serving as insect nutrients vary in quality and quantity not only between different plant species but also between different parts of a plant, between strains and varieties of the one species and in the same plant part during different stages of growth. (This was also one of the arguments put forward by Fraenkel (1959, 1969) in order to show that the absolute and relative amounts of nutrients present although theoretically providing a sufficiently large number of combinations to account for interspecific host-plant discrimination, were in fact too variable within each species for this to apply in practice.) Even if

an insect was attracted to or arrested on a plant by an index chemical, it would probably be advantageous to the insect to be able to respond to the quality and quantity of nutrients present in the food eaten. For the same distinctive index chemical to function also as a marker for the most beneficial feeding site it would have to be localised in the plant part that provided the most nutritious food and the concentration of chemical would have to correlate closely with nutrient quality as the latter varied with the age and physiological state of the plant. This is unlikely to be the general case in view of the fact that the basic metabolic processes are common to all plants and so could not provide the substances sufficiently distinctive to function as index chemicals. Only the so called secondary plant substances which by definition are not closely connected to basic plant metabolism have enough individuality. The secondary plant substances could function as nutrients for a plant-feeding insect but the similarity between main metabolic pathways in both plants and animals and the low level of secondary substances present would make the major use of these chemicals exceptional. Research into the nutrition of phytophagous insects has not yet demonstrated such a case (House, 1969). Thus nutrients could be expected to operate as stimuli once the insect has located as acceptable host in order to localise feeding on the most nutritional areas of a host.

However an exception to this proposal has been demonstrated by the work of Rees (1969) on the imago chrysomelid beetle Chrysolina brunsvicensis Gr. on its food-plant Hypericum hirsutum. He found that the greatest densities of feeding insects per unit of apparent surface

area of the plant occurred on the upper third of the plants and especially on the flowerheads. Beetle density was directly correlated with the concentration of hypericin (hexahydroxy-dimethyl-naphthodianthrone), a quinoid substance naturally occurring only in a few species of the genus Hypericum. Electrophysiological investigations of trichoid chemoreceptor sensilla on the fifth tarsomeres of all legs of C. brunsvicensis showed that cells that responded specifically to hypericin were present. The highest concentrations of hypericin occurred in black stalked organs on the sepals and petals which underwent lysis as the flowers matured releasing hypericin into an extracellular aqueous solution. Thus this secondary plant substance would be available for direct contact with the tarsal chemoreceptors sensitive to it. In this way hypericin could mediate the observed aggregation of beetles on the flowerheads and upper plant parts. Whether this factor alone did control the distribution of C. brunsvicensis on the host was not shown. Other cells on the tarsi were found to be responsive to 1 : 1 electrolytes such as sodium or potassium chloride, to water, and to mechanical flexing. No response to sucrose, glucose or fructose could be revealed. The electrolyte receptor was inhibited by calcium salts and the concentration of calcium ions in the plant varied inversely with the height of the plant part above ground. The significance of this information with respect to the distribution of the beetle is unknown.

Rees summarises the situation investigated by writing;

"We may conclude that although no information has been collected about the manner in which C. brunsvicensis finds its food-plant, there is nevertheless substantial evidence that hypericin is responsible for mediating its choice of feeding site when once in contact with Hypericum hirsutum. Hypericin is almost certainly a nutritionally

insignificant stimulus, and it would be interesting to determine whether the regions of the plant that contained the greatest concentrations of it were also those where there were highest concentrations of nutrients."

(In view of the work of House (1969) the last section of this should be rephrased as the regions 'where the levels and ratios of nutrients provided optimal conditions for C. brunsvicensis.')

It would also be interesting to investigate other steps in this particular host selection sequence to see whether hypericin is involved in long-range host discrimination, and whether any nutrients can be discerned by gustatory receptors and have a role to play in the initiation or continuation of ingestion.

An amalgamation of the two methods proposed for chemosensory host discrimination - that is by the signal stimuli of secondary plant substances or by nutrients - would result if the secondary plant substances primarily controlled the location of its specific host by an insect and the nutrients controlled the continuation of feeding. The initiation of feeding and the site selection on the host could probably result from factors of either type.

At present only a few insects have been studied in sufficient detail for the factors controlling all the various facets of host selection and ingestion to be known. The data available for the silkworm Bombyx mori L. (Hamamura, 1970) serves as an example of the inter-relationships of the various chemical factors. The isoprenoids present in mulberry leaves were all found to be attractive (citral, linalyl acetate, linalool, terpinyl acetate) while some that were not present were found to be unattractive. Hexenol was also found to be attractive. These are all secondary

plant substances. Factors initiating biting were β-sitosterol, isoquercitin, and morin. Hamamura labelled the factors stimulating the next step in the feeding sequence as "swallowing factors". Inositol, sucrose, silica and potassium phosphate were active at this stage. All these substances when combined with several nutrients in an agar base evoked feeding in Bombyx larvae. However growth and development were poor unless twenty per cent of the diet was mulberry leaf powder. Chlorogenic acid, linolenic acid and oleic acid were identified as growth promoting factors present in mulberry leaves. Moreover acetylcholine was found to stimulate the moulting of larvae and phenolic acids (such as gallic acid or protocatechuic acid) promoted the growth of the younger larvae. When these additional factors were added to the artificial diet without leaf powder growth and survival were improved, but the average weight of the cocoon produced was still below that of larvae fed a leaf-based diet.

This example serves to show both the complexity of the insect-plant interaction and that the volatile secondary plant substances may be involved in the initial phases of host location while nutrients act during later stages.

It must be pointed out too that in most insects the larvae have only a restricted ability to move and it is the ovipositing female which plays the dominant part in the initial choice of hostplant (Fraenkel, 1969). In the cases where the adults and larvae do not feed on the same plant host or plant part (for example in all Lepidoptera and Diptera) most nutrients which have a low volatility will be unable to be sensed by the ovipositing female and so are unavailable as a source of data for host-plant discrimination. Volatile

compounds including many secondary plant substances will be the important factors in these cases.

The concepts of host-plant selection discussed so far have been derived largely from behavioural studies. This approach to the field has gained impetus from, and has in turn stimulated, the research necessary to develop artificial diets. Because many scientific investigations require a large or continuous year-round supply of a particular insect at the same stage of growth and physiological state, artificial rearing has developed into a study of its own (Smith, 1966). To develop a successful programme of artificial rearing which is freed from the laborious and often uneconomic use of plants or plant extracts while still providing the conditions for optimum growth and development, an understanding of all the factors affecting feeding is a necessity. It is not sufficient to simply provide a diet containing a high level of individual nutrients, these must be in the correct ratio for that insect (House, 1969) and the diet must include any cofactors and any non-nutritious substances which initiate or maintain ingestion in the insect under consideration. Such a detailed investigation has been attempted for only a few phytophagous insects among them the study of the silkworm Bombyx mori L. by Hamamura (1970) and co-workers mentioned earlier. This showed the multiplicity of the factors involved.

In other cases physical factors have been demonstrated to affect the feeding process. For example Vanderzant (1969) in discussing physical aspects of artificial diets for chewing phytophagous insects mentions the hardness, texture, homogeneity and water content as important characteristics of any

diet affecting the success with which an insect can feed on it. The work of Auclair and Cartier (Auclair, 1967; Cartier, 1965, 1966; Cartier and Auclair, 1964, 1965; reviewed by Auclair, 1969) showed that both the quality and quantity of light affected the settling and feeding responses of aphids on chemically defined diets. Acyrthosiphon pisum (Harris) and Macrosiphum euphorbiae (Thomas) preferred diets illuminated by orange and/or yellow light and larvae confined on diets and subjected to light of these frequencies had higher survival and greater growth rates than on diets illuminated by white, red or blue lights or in darkness. Similarly Aphis gossypii Glover grew, reproduced and survived best on diets illuminated by light of wavelengths from 525 to 595 mμ, and poorest on those at 485 mμ. If these aphids were reared under a broad spectral light (white light) they preferred and performed better under low intensities than high.

Thus it appears that the physical factors of the environment can markedly influence both the selection of host plants and the success with which these hosts can be utilised as food by herbivorous insects. However many, if not most, of such interactions remain to be investigated. The study of the chemical interaction between plants and insects remains the aspect on which most work has been done.

The most recent advances in the development of the present concept of the chemosensory basis of host selection have occurred using neurophysiological techniques. This work has extended and complimented behavioural studies. It has resulted in the identification of the chemicals that the insect can perceive and the threshold levels at which they become active. That is the chemical stimuli that are

capable of causing a modification of behaviour in an insect can now be recognised. However, whether or not such a behavioural change occurs depends on the state of the central nervous system which has to integrate and interpret the neural input from all sensory modalities. Attempts to understand the processes involved in the central nervous system during this interpretation have been begun but have not yet progressed very far (Yamada, 1970; Dethier, 1970a, 1970b).

Schneider (1965, 1969) and Yamada (1970) recognised two basic types of cells responding to chemical stimuli. These receptors were classified as generalists and specialists; the generalists were cells which responded to a wide variety of chemicals while the specialists only responded to a restricted group of substances. However, the generalist receptors did not respond equally to all the chemicals to which they were sensitive but in such a way so as to give neural action spectra that overlapped but were still individually distinct. Dethier (1970a) wrote that although this appeared to be characteristic of the olfactory system, the gustatory system appeared to consist solely of receptors individually specific to restricted groups of compounds. However not enough was known about either sensory system to be certain that these generalisations would prove correct. More recently Dethier (1972) has studied the effect of concentrated vapours on the contact (gustatory) chemoreceptors of the blowfly.

Chemosensory reception in the tobacco hornworm Manduca sexta (Johan.) which feeds on the Solanaceae has been investigated from the aspects both of larval feeding (Schoonhoven and Dethier, 1966; Schoonhoven, 1969; Dethier

and Schoonhoven, 1969) and adult oviposition selection (Yamamoto and Fraenkel, 1960; Yamamoto, Jenkins and McClusky, 1969). Dethier and Schoonhoven (1969) reported on extracellular electrophysiological recordings from olfactory receptors of the antennae of these hornworm larvae. Four different kinds of receptors (all generalists) could be recognised from their responses to various chemicals and plant odours, but as 16 of the bipolar neurons serving each antenna are believed to serve olfactory receptors, it is not known whether these recognised types are duplicated or whether all 16 receptors are unique. Early electrophysiological work (Schneider, 1962) showed that as in vertebrates receptor cells in general do not have an on-off reaction to stimulation. Instead the cells have a basic rate of firing which can be increased or decreased by an incoming stimulus. This increases the discriminatory power of a group of receptors in a factorial way. However the research by Dethier and Schoonhoven (1969) showed that the response of a receptor cell is not limited simply to alterations of the frequency of firing but can involve other aspects of impulse transmission. The cells reacted to different odours with changes in the latency of response, rate of adaptation and in alternations of frequency increase and decrease. Thus the complete temporal patterns of neural activity could function as a code conveying olfactory information to the brain.

Schoonhoven (1969) investigated the gustatory chemoreceptor cells localised in two sensilla styloconica on each maxilla of Manduca sexta. These sensilla each had a mechanoreceptor and four chemoreceptor cells. The medial sensillum styloconicum of this insect had one cell apparently

specifically activated by the feeding stimulant inositol, one cell specific for some alkaloids which typically occur in their solanaceous food plants, one labelled (Schoonhoven, 1969, figure I) as responding to 'salt and acid' (although only sodium and potassium chlorides are mentioned as stimuli in the text) and the fourth cell was designated a feeding 'stimulant' receptor in order to explain the total activity caused by host-plant sap. This same paper gave as present in the lateral sensillum an inositol receptor, one responding to sucrose and glucose, one to salt and acid and the last activated by feeding deterrents. The deterrent receptor was thought to have a wide activity spectrum with salicin giving the strongest stimulation. The inositol receptors on each sensillum are apparently not identical.(Schoonhoven and Dethier (1966, Table IV) list the receptors of the medial sensillum as being of three types - responding to water and salt, sucrose and glucose, and acid - and those of the lateral sensillum as being of four types - responding to water, salt, sucrose and glucose, and inositol. The changed interpretation given in the later paper is not fully explained, and Dethier (1970b) quotes the earlier account.)

The generality of some receptors, such as the deterrent receptor mentioned above in Manduca sexta, will probably only be fully explained when the nature of the reaction between the receptor sites and olfactory molecules is understood. At present there are many speculative theories about this phenomenon but little experimental evidence. Most of these theories have been derived from a consideration of a single property of the excitant molecules (Ottoson and Shepherd, 1967, p114), but den Otter (1972) has approached the problem

through the physiology of cell membranes.

Some evidence has been accumulated concerning ways in which the multitude of sensory input from the receptors is integrated in order to bring about the observed changes in behaviour. At the peripheral level both synergism and inhibition have been demonstrated. For example sodium chloride decreases the reaction to sucrose in Pieris brassicae L. (Schoonhoven, 1969), whereas the same salt enhances the deterrent reaction to strychnine in Bombyx mori L. (Ishikawa, 1966). The question as to how some chemicals stimulate in dilute concentration and inhibit in greater concentrations is possibly due to integration away from the peripheral receptors although in some cases it is no doubt similar to the situation in M. sexta where stronger concentrations of salts apparently stimulated the deterrent receptor as well as the specific salt receptor.

Within the central nervous system integration probably occurs at various levels. Dethier (1953) showed that stimuli applied simultaneously to chemoreceptors on different tarsi of the blowfly, Phormia regina Meig., were integrated within the central nervous system. He also found that antagonistic stimuli were more likely to result in rejection when applied to the same leg than to separate legs. The review by Gelperin (1971) dealt with the neural mechanism underlying the regulation of feeding in the blowfly, Phormia. In this case stretch receptors sensitive to the distension of both the crop and the foregut played a major role in terminating and initiating feeding. The crop acted as a reservoir releasing food slowly over several hours after a meal. The rate at which the food was returned to the midgut depended

on the osmotic pressure of the blood which in turn depended largely on the amount of the sugar trehalose (the main energy metabolite) present in the blood. In this way allowance could be made if the ingested food was of low nutritive value and contributed only little to the blood trehalose levels. Thus in the blowfly feeding is regulated by the sum of input from external sensory receptors and feedback from the internal stretch receptors. A similar increase in feeding to compensate for food of decreasing nutritive value was also shown by House (1965) for the phytophagous caterpillar Celerio euphorbiae (L.).

Other factors that have been shown to influence the interpretation of the sensory data are the general state of excitation of the insect, and the past history of feeding. The first, although obvious and causing many bioassays to incorporate an allowance such as time for the insect to become accustomed to the test situation, has been clearly demonstrated by Haskell, Paskin and Moorhouse (1962) (see also Kennedy and Moorhouse, 1969). In this work on the desert locust, Schistocerca gregaria (Forsk), starved but quiescent hoppers responded to wind-borne grass odours by moving upwind. Freshly fed hoppers ignored the odours and responded similarly to starved hoppers in 'clean' odourless wind. However, further research showed that stimulatory odours were but one type of sensation triggering wind-orientated movement. For example agitating the locusts by roughly handling them immediately prior to testing also caused this anemotaxis (Kennedy and Moorhouse, 1969).

The second factor capable of modifying the behaviour resulting from the stimulation of an insect is the previous

experience of the insect under consideration. Early work showed that insects were capable of conditioned learning. (Alloway (1972) regarded the olfactory conditioning as a dubious type of 'learning'.) For example there are the classic experiments of von Frisch who trained honey bees to visit coloured discs (von Frisch, 1950, 1967), and Thorpe (1939) who showed that Drosophila by selective breeding could be conditioned to prefer a diet flavoured by the normally repulsive peppermint oil. In spite of this and the fact that such 'learned' behaviour had been postulated as affecting insect-plant interactions even earlier (Walsh, 1864, 1865, in Jermy, Hanson and Dethier, 1968; Hopkins, 1917) little work has been done on this aspect. One detailed investigation was that done by Jermy et al (1968) who worked on the caterpillars of two phytophagous moths - Manduca sexta (an oligophage) and Heliothis zea (Boddie) (a polyphage). When last-instar larvae of these moths which had been raised on different host plants were tested individually for feeding preferences they showed a clear choice for the plant on which they had been reared. Such conditioned or learned behaviour could only be demonstrated for plant species within the innate host range of the insect and not for nonhost plants. The induced preference was specific for the inducing plant species and not simply an alteration in the general threshold of food acceptability. 'Naive' larvae reared on an artificial diet lacking plant extracts showed no or only slight preference; however as little as 24 hours feeding on plants or plant extracts could result in a strong induction. In one experiment designed to test the permanence of induced changes, diet-reared larvae were exposed consecutively to two different

plant species in either order. The subsequent preference was always to one particular plant although the other species was more eaten by 'naive' diet-reared larvae. As conditioned responses survived both feeding on artificial diet and moulting it was postulated that the basic change that had occurred was within the central nervous system. This deduction was further argued by Dethier (1970b). However Schoonhoven (1967) has shown changes in the electrophysiological responses of gustatory receptors in larvae reared on artificial diet, so the changes might have been at peripheral level. Wherever the actual induction occurred the results of this research are a striking demonstration of the marked effect that previous experience can have in modifying the behavioural response of an insect to the same set of stimuli.

From this review it can be seen that the interaction between phytophagous insects and their host plants may be complex. The selection process by which an insect locates and feeds on a suitable host involves a variable sequence of events and is affected by numerous factors, many of them chemical. Insects have well developed chemosensory systems - both olfactory and gustatory - and are capable of finely discriminating between plants on the basis of the chemicals present. This can be by both qualitative and quantitative differences in the chemical composition. Secondary plant substances and plant metabolites can both be distinguished and both appear to play a role in host-plant selection. It appears that volatile secondary plant substances are more important in the initial phases of host selection, and that the non-volatile nutrients and their absolute and relative concentrations operate principally in later phases. However

with the present paucity of detailed knowledge any generalisations made may be premature. Above all it must be remembered that the insect host-plant relationship has not evolved in and does not operate in a void, but must be considered within the framework of all environmental influences affecting the life and development of both plant and insect.

2. PAROPSIS CHARYBDIS STAL

The literature concerning Paropsis charybdis Stal is not extensive and includes as many unpublished reports as published references. Access to unpublished material was generously given by the Forest Research Institute, in particular by Dr Colin Basset, then head of the Pathology and Entomology Branch, and Mr John Styles. Dr Carne of the Commonwealth Scientific and Industrial Research Organisation, Australia, kindly provided a copy of his unpublished report produced after a visit to New Zealand from January to April 1967 to study the problem caused by this insect.

The unpublished forestry reports are; firstly by White who in 1962 presented an analysis of the problem of eucalypt defoliation in New Zealand; secondly by Dugdale who reviewed the situation in 1963 and 1965 and gave some observations on field oviposition in 1966; and finally by Styles who in 1966 reported on some brief laboratory rearing experiments and in 1969 summarised the known biology of Paropsis charybdis. Much of Styles' work was done in conjunction with the visit of Carne during the 1966-67 season. In 1970 Styles published a paper which included almost all the information given in his 1969 unpublished report. Future reference in this thesis to these unpublished forestry reports

and also that of Dr Carne (1967) will be simply by the author and year in which they were written; full details however will be given in the bibliography.

(1) Taxonomy

The true identity of the Australian chrysomelid which had established as a pest on eucalypts in New Zealand was determined by Selman (1963a, 1963b). In the course of his work attempting to identify the species of Paropsis reared by Carne of the Commonwealth Scientific and Industrial Research Organisation, Australia, Selman clarified the confused situation regarding the taxonomy of this group. This involved verification of the identity and validity of the various specific names within the genus. The result was that the Paropsini species present in New Zealand was recognised as Paropsis charybdis Stal (1860) and not as had been previously recorded, Paropsis dilatata Erickson (1842). Confirmation of this was obtained by directly comparing specimens from New Zealand with the types.

The genus Paropsis belongs in the Tribe Paropsini (family Chrysomelidae; sub-family Chrysomelinae) which is a large group of beetles indigenous to the Australian continent and associated islands. Paropsis charybdis is an uncommon species and Carne (1967) stated that it was not known where in Australia this beetle occurred most abundantly. Adults had been taken occasionally in the Australian Capital Territory but larval populations had not been found there. He gave the areas where P. charybdis would be most likely to occur as the south coast of New South Wales, the eastern coastal portion of Gippsland, and northern Tasmania. In New Zealand the species has been associated since its introduction with

Eucalyptus globulus - a Tasmanian species of eucalypt.

(2) Distribution

Paropsis was first recorded in New Zealand in 1916 from two sites in the Lyttleton area (Thomson, 1922, p294; Clark, 1930). (Dugdale (1965) mentioned a 'doubtful' record from this area and gave the date as 1912 but as the collector mentioned is that first referred to by Clark (1930) this is quite probably simply an error in the dates.) Clark (1938) thought that the initial introduction was probably as eggs or larvae on young plants, but also possibly the overwintering adults on poles. Miller (1925) followed the latter view.

By the time Paropsis was mentioned by Miller (1925) the distribution was recorded as being confined to the Canterbury region but in a footnote (p42) he recorded a single adult taken that year near Bulls in the North Island. Clark (1930) wrote that the beetle was infesting the eastern portion of the South Island from Dunedin and probably further south into Southland, northwards almost to Kaikoura. He doubted the 1925 North Island finding because it had not been since substantiated. In fact it was not until 1956 that Paropsis was again recorded in the North Island, once again in the Bulls region (Gurr, 1957) and also at Titahi Bay, Wellington (Morrison, 1957). In view of the conspicuous nature of the insect and the widespread use of eucalypts on farms in the region, Gurr considered that it was more probable that the early record was an isolated immigrant which did not establish, rather than an infestation which remained undetected and confined for thirty years and then began an explosive spread throughout the North Island. Clark (1938) repeated his doubt concerning the 1925 record and this view has been followed by

most authors, for example Miller (1944), Cottier (1956) and White (1962).

The spread of Paropsis charybdis in the South Island was hindered by the Southern Alps to the west, the Kaikoura Ranges to the north and the cold arid areas of Central Otago to the south. These areas would have provided not only physical barriers to dispersal but would have also lacked the eucalypts needed as hostplants (Clark, 1930). In the same paper, Clark, in discussing the mode of distribution of this beetle thought that the prevailing winds (norwesterly and southerly) could account for the initial dispersal from Lyttleton. This spread was more rapid in a general north-westerly direction and slower to the south. Accidental transportation by road and rail vehicles was considered probable on the basis of his personal experience.

By 1932 Paropsis had extended its range north to Kaikoura (figure 1) and probably into Southland (Clark, 1938). White (1962) reported that the insect collection of Cawthron Institute contained specimens collected in Nelson in 1921 and 1939, but that Paropsis penetrated only slowly into the Nelson and Marlborough regions. Dugdale (1965) commented that Paropsis records are more common for Nelson after 1940 and also that "it had been recorded from four widely separated areas in Southland prior to 1938".

Records from 1938 to 1952 are scarce but in the early 1950s specimens from north-eastern areas of the South Island were sent either to the Entomology Division of the Department of Scientific and Industrial Research or to the Forest Research Institute for identification (Dugdale, 1965). Paropsis was slow to colonise the remainder of the South Island, being

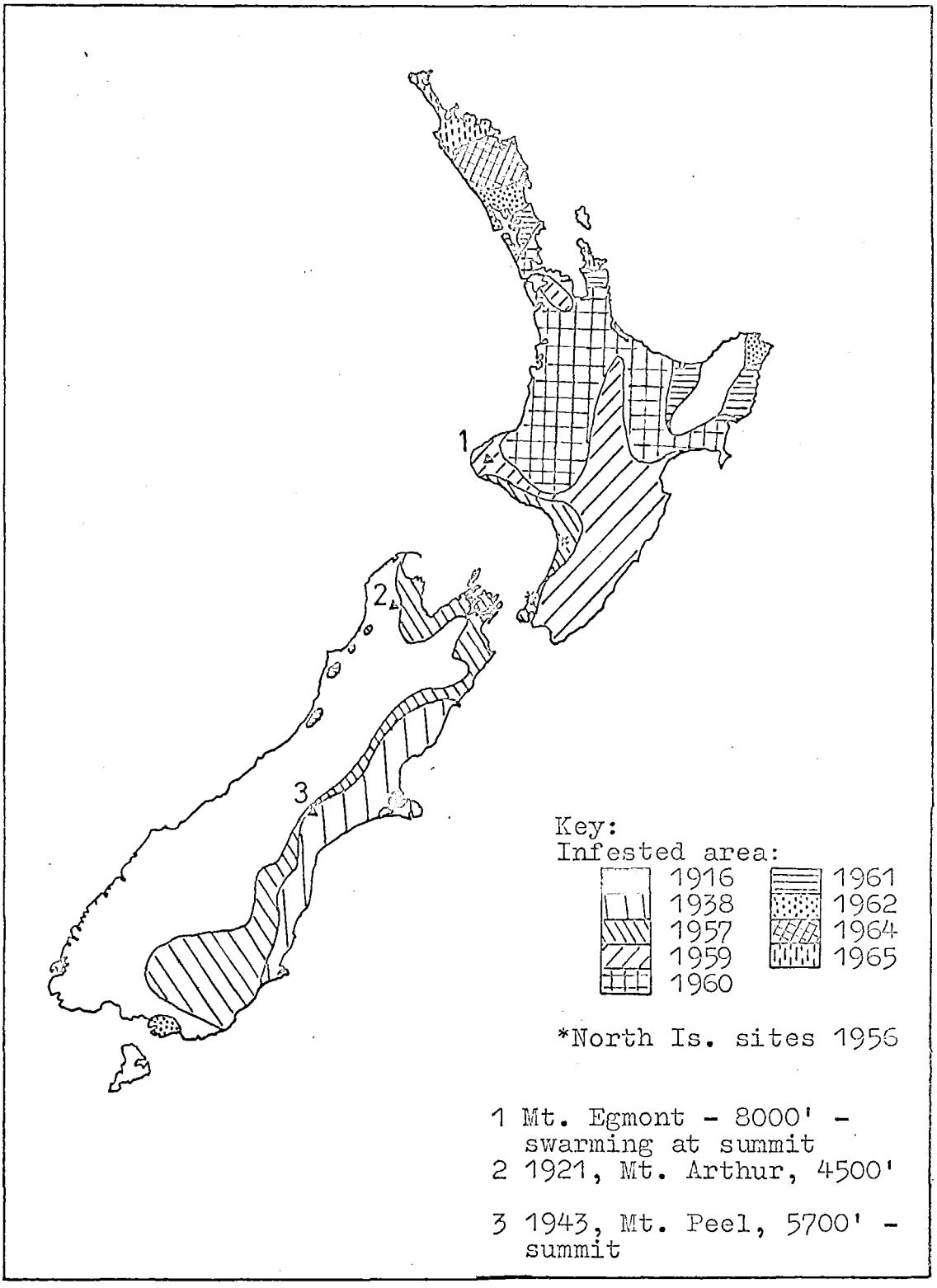


Figure 1. Map showing the colonisation of New Zealand by Paropsis charybdis (after White, 1962; Styles, 1970, pers. comm.).

limited by obvious physical barriers and a lack of host material but Forest Biological Survey records show a spread into Westland in 1961 and 1962 and to the far south near Bluff in the 1962-63 season.

The colonisation of the North Island is more fully documented. Gurr (1957) reported that four specimens of P. charybdis were taken from differing localities in the Palmerston North area in February/March 1956, with damage also to eucalypts in a nursery at Bulls. In 1956 specimens were also found at Titahi Bay, Wellington (Morrison, 1957). By Autumn 1957 the infestation had spread throughout the Manawatu and north to Hawera (Gurr, 1957). Forest Biological Survey reports recorded subsequent spread and the following account is taken from Dugdale (1965) who summarised these. By the autumn of 1958 Taranaki, Manawatu and Hawkes Bay had been colonised and there was a narrow strip of infestation extending up the centre of the island, with a minor spread up the east coast to near Gisborne and possibly one up the west coast north of New Plymouth. During the spring of 1958 and the summer of 1958-1959 the new areas colonised by the beetle were limited and largely resulted in an increase in the amount of land infested in the centre of the island. An isolated infestation was also recorded based on Puketutu Island near Auckland. In the following summer the spread was rapid with the limit of infestation by winter 1960 being just north of Auckland but with areas of the Coromandel Peninsula and East Cape not yet colonised. From 1960 to 1965 Paropsis spread slowly north up the North Auckland Peninsula and also reached the East Cape.

In 1970 Styles was able to report,

"The only areas still free of Paropsis charybdis are the Ureweras in the North Island, the upper Waiau Valley and parts of Westland to the Fiords in the South Island, and Stewart Island".

All these are rugged areas predominately covered in native vegetation.

White (1962) considered that the colonisation of the North Island by Paropsis could be attributed to windborne adults crossing Cook Strait from Nelson, whereas the pocket of infestation centred on Puketutu Island was probably due to coastal shipping movements. The rapidity with which the infestation spread throughout the North Island has been remarked at by various authors (White, 1962; Dugdale, 1965; Carne, 1967), but the mode of spread has not drawn comment. Clark (1930) remarked that, "Flight is strong and takes place during the heat of the day". That the adult beetles do have good powers of flight is also shown by Gibbs and Ramsay (1962) who reported sighting Paropsis at 4,800 ft on Pukematawa in the Tararua Range in November 1956, and who also recalled that Paropsis was observed "quite commonly" in January 1959 in the Southern Tararua Range on the peaks Alpha (4,400 ft) and Hector (5,000 ft) flying in hot sunshine. Similarly the isolated records from mountain peaks shown on the map (figure 1) and taken from specimens at the Department of Scientific and Industrial Research, Nelson, also point to a good flight ability. It should be noted however that, in accord with Clark's (1930) experience that the beetle could be readily transported by man, the initial spread in the North Island into the central region and along the coasts was along the main road-rail transportation routes.

(3) General Biology

The life cycle of P. charybdis is depicted in figure 2 which shows all stages from egg to adult. There are four larval instars and a motionless prepupal phase followed by the pupal stadium. The adult male is generally smaller and darker in colour than the female but size varies greatly.

Miller (1925, p42) used Paropsis as an example of a chrysomelid attacking trees. He gave a brief description of the larvae, and included small photographs of the adult, eggs, "late" larvae and a pupa. The larvae were reported as moving by a "looping" motion and mention was also made of the defense glands that the larvae can protrude from the dorsal surface of the abdomen. Clark (1930) made the major contribution to the early study of this insect, describing in some detail the adult, egg, all four larval instars and the prepupal and pupal stages. All except the second instar are included in figures as well as in the text. (Unfortunately the figure of the egg appears unfinished, lacking detail in the head region; for example the conspicuous larval eye-spots are not drawn in whereas the hatching spines which only appear later are shown.) Clark gave the life history with the adult overwintering beneath the bark of eucalypts until September when it emerged and began feeding. He found that egg laying began in October with the eggs deposited in two irregular rows on a shady portion of a leaf. Batch size was reported as "variable... seldom less than twelve and twice that number may be reached".

Without referring to the conditions under which the data was obtained Clark (1930) gave the developmental periods for the eggs, first, second, third, and fourth instar larvae and the pupae as 7-10 days, 5-7 days, 5-7 days, 4-6 days, 12-15 days and 12-14 days respectively. Of the 12-15 days

Figure 2. The complete life cycle of Paropsis charybdis, showing eggs, the four larval instars, a prepupa, a pupa and a pair of adults (the female is on the left).

taken by the fourth instar 4-5 were spent in active feeding and 8-10 in a motionless prepupal state. Adults began feeding 2-3 days after emergence. The larval instars were all found to be distinct and to differ not only in size but also in colouration and marking; for example the first was the only instar to have a black, sclerotised, prothoracic shield, while only the fourth instar had a light-coloured head capsule (figure 2). Pupation occurred in the ground among the litter at a depth of one to two inches. No pupal cell was found (Clark, 1930). Styles (1966) recommended for laboratory rearing the use of cellulose wadding as a medium "into which the prepupae could tunnel to construct pupal chambers". However, in the present work no structure definite enough to be called a pupal chamber has been observed and the prepupae have pupated readily among scraps of tissue paper, in dry frass, or even on exposed open areas of the container floor without appearing to have formed as much as a distinct hollow.

From field observations Clark observed that there were at least two generations per year with a considerable period of overlap. Hibernation was thought to depend on the local weather conditions but generally to begin in May or June.

Dugdale (1965) recorded that the account of the life history given by Clark (1930) had been corroborated by trial rearings carried out at Rotorua in order to establish laboratory and insectary populations of Paropsis charybdis prior to the attempted introduction of parasites. Further details that have been added largely concern oviposition and related phenomena. Thus Dugdale (1965) recorded that a single female "left unmolested and given a supply of males" could produce up to 800 eggs in 45 days. From the fact also

that a female allowed to continue laying for a period of three months produced over 1700 eggs this author also deduced that egg-laying was uniform throughout adult life and not markedly cyclic in pattern. Styles (1970) gave the potential fecundity of each female as from 1500 to 2000 eggs. This was based on the findings from six females kept by Dugdale during the 1963-64 season which produced an average of 1763 eggs (range 1318 - 2102) (Carne, 1967; Styles, 1970) and also from a single female kept by Styles in 1966-67 which laid 1791 eggs in 74 batches over 123 days (Styles, 1969, 1970). In both his 1969 and 1970 papers Styles gave the results of his observation of oviposition in the form of a graph showing the number of eggs in each batch as the season progressed. He commented, "The long egglaying period of the female with three to four high peaks is very similar to that of the Australian species P. atomaria (P.B. Carne, pers. comm.) ..... ". However the peaks graphed represented increases in batch size, and as the frequency of laying varied, these do not appear to be necessarily peaks in the overall rate of oviposition.

Dugdale in 1966 presented an unpublished report on various aspects of oviposition in the field at Puhipuhi State Forest. He found that P. charybdis normally laid a batch of between 10 and 45 eggs in one to three rows, flat on the apical half of a mature eucalypt leaf. On several trees that had been severely defoliated he found many batches laid either on terminal twigs or on bark strips above epicormic growth. Oviposition occurred from near ground level to the tops of the dominant trees, and was confined largely to old hardened leaves. This latter was shown by branches which had both young and old leaves; the young foliage had very few egg

Table 2. Number of egg batches from September to March on 8 ft coppice growth of E. resinifera at Puhipuhi State Forest (after Dugdale, 1966).

Height	Date									
	22/9	23/9	6/10	29/10	17/11	10/12	4/2	16/2	March	Total
0'-2'	1	3	0	0	0	3	0	0	0	7
2'-4'	21	15	10	0	0	1	2	2	0	51
4'-6'	3	1	4	0	0	1	0	0	0	9
6'-8'	1	0	0	0	0	0	0	0	0	1
Total	26	19	14	0	0	5	2	2	0	68

Table 3. Eggs and rafts on Eucalyptus species in Puhipuhi State Forest, 23 September 1965; and on coppice E. resinifera, 10 December 1965 (Dugdale, 1966).

Host species	Eggs	Rafts	Average No. Eggs per Raft
E. pellita 70'	386	23	16.7
E. resinifera 45'	595	32	18.2
E. resinifera 3'-6'	837	57	14.6
E. ovata 3'-6'	328	18	18.2
E. resinifera 3'-6' (coppice)	63	4	15.7

batches while these were recorded as very numerous on the older foliage. On an 8 ft high regeneration tree which was sampled for eggs within four strata of equal height, most batches were laid between two and four feet above ground level in the region where the greatest number of old leaves occurred (Table 2).

The crowns of three species of eucalypt were sampled and no significant differences in the number of eggs per batch were found between species (Table 3). This led Dugdale to the opinion that the adults mingled freely and were highly mobile within an area of eucalypts. The average number of eggs per batch ranged from 14 to 18 with occasional batches containing up to 45 eggs. The differences in the number of egg-batches per 1000 leaves at various heights were deemed not significant as it was felt that more sampling would show an overlapping variation at each height.

It had been intended to determine the number of generations in the season by regular egg samples. Unfortunately regular sampling was not achieved and the number of egg batches was very low on some sampling dates. The sequence shown in Table 4 ~~was inferred to indicate~~ suggests a generation every two months from September to February.

Table 4. Number of eggs, number of rafts per 1000 mature leaves, E. resinifera coppice growth, Puhipuhi Cpt 6. (Figures in brackets = hatched eggs per raft)(Dugdale, 1966).

	Date						
	23/9	29/10	17/11	10/12	4/2	March (a)	March (b)
No. of rafts	57	3	13 (13)	4	7 (6)	0	0
No. of eggs	837	42	(183)	63	115+ (100+)	0	0

Styles (1966) found that, in an open insectary at Rotorua, eggs laid in September gave rise to adults by November.

One feature noticed during sampling and commented on by Dugdale (1966) was the small number of mature leaves in a eucalypt crown (only 1,300 in the 45 foot tall E. resinifera and less than 3,000 in others). The oviposition in this particular tree on the 24 September was enough to give a potential of one larva per 1.8 mature leaves. It was felt that at the rate egglaying was continuing the total spring oviposition would provide potentially one larva per old leaf.

During the sampling in September 1965 Dugdale kept a record of the approximate state of development of the egg batches, and whether or not they had been damaged. Of a total of 130 batches, 64 were recorded as 'green', 48 as 'yellow', 18 as 'hatching'and 14 of these batches were damaged. The 'green' and 'yellow' were taken to represent early and later stages in development, the 'hatching' represented either a few larvae hatched or eggs containing fully developed larvae. From observations during the present research such a colour change, while it does frequently occur, is not an accurate indication of egg development. Some female Paropsis charybdis lay eggs which are yellow from the moment of oviposition, while the eggs of others are a dark brown shade throughout development and might be mistaken for developed eggs or those attacked by fungus. No details were given about how extensive the recorded damage was, that is what proportion of each egg batch was affected. There is also no indication of what had caused the damage.

Dugdale (1965) made the comment that,

"(the) viability of eggs in the laboratory is scarcely a fair guide and is better examined on egg batches collected from the field. A variable number in each batch were infertile; few batches had all fertile eggs".

Provided that mating and oviposition can be successfully accomplished under laboratory conditions there does not appear to be any reason why the fecundity and fertility of any insect cannot be measured in the laboratory. Such results will refer to the reproductive potential of that insect while the actual performance will depend on many factors, for example the probability that females will mate successfully, the longevity of the mated females, and the mortality factors affecting the egg stage. It appears that in the above quotation Dugdale has confused the terms fertility and viability. 'Fertility' is taken here to mean the number or proportion of eggs produced that are fertilised and so contain an embryo, whereas 'viability' is taken to mean the number or proportion of eggs that give rise to first instar larvae. To rephrase, it is considered that fertility concerns the production of embryos, while viability concerns the survival of these embryos during the egg stage. The latter factor is very vulnerable to changes in the external environment during incubation of the eggs.

It was found by Dugdale (1965) that there was a variation in the length of time taken by the eggs to develop between the batches laid first and those laid later in the season. The first and second egg batches took 9 to 16 days to hatch while the sixth and seventh took only 7 to 9 days. No particulars are given about either the number of females on which this observation was based, or the temperature conditions under which the eggs were kept.

Other details noted by Dugdale (1965) were that there

was no consistent weight loss in females during oviposition and that in a few cases females had been fertilised before they had begun their winter diapause. From the reports of the Forest Biological Survey observers it appeared that this diapause was not obligatory in the very warmest areas of New Zealand and that feeding could occur in most areas during unseasonable warm spells. No eggs and larvae have ever been found before early spring so that Paropsis charybdis may undergo a strict reproductive diapause in spite of its ability to break the overwinter cessation of feeding. Hibernation sites that have been recorded are in leaf litter (Styles, 1970), among thick, evergreen invariably-dry foliage, among coils of bark or beneath flaking bark on Eucalyptus trunks (Dugdale, 1965). Dugdale also noted that drifts of adult beetles have been found on beaches, and that one observer in Nelson saw a group of Paropsis flying along a beach and then inland to a five-acre area of E. viminalis. The flight appeared purposeful in that no beetles were flying in other directions (Dugdale, 1963).

A final point about the biology of P. charybdis arising from the 1965 paper of Dugdale was his comment that from the experience of the Commonwealth Scientific and Industrial Research Organisation, Australia, ultraviolet light is necessary for successful laboratory rearing, or rather that normal closed laboratory conditions were not suitable. The present work has shown no such need and Carne (pers. comm.) knew of no importance to Paropsis of ultraviolet light as such. It is possible that Dugdale was referring to the 1966 paper of Carne in which the effect of rearing P. atomaria under constant artificial light was given

as preventing adult diapause and the associated accumulation of fat-body. However this was almost certainly a response to daylength (Carne, 1966, p662).

In the field the beetle was described by Clark (1930) as conspicuous when feeding along the leaf edge, but well camouflaged when it dropped among the plant debris under the tree as it did when disturbed. If not dislodged in the initial moments of a disturbance the beetle clung tenaciously to the leaf or stem.

The larvae when disturbed made "short sharp movements, raising and lowering the posterior abdominal segments in short jerky motions and protruding repulsive glands" (Clark, 1930). These glands which appeared as horn-like processes from the dorsal integument of the eighth abdominal segment (figure 3) secreted a liquid which Clark described as having "a pungent and unpleasant odour of eucalyptus". The larvae also had the ability to eject some yellowish fluid from the mouth when antagonised (Clark, 1930).

Moore (1967) analysed the defense secretion of the closely related species Paropsis atomaria Olivier, Chrysophtharta variicollis Chapius and C. amoena Blackburn. Using standard chemical tests and thin layer chromatography, he identified as present in all three species hydrogen cyanide, benzaldehyde and glucose. He was unable to detect hydrogen cyanide in the sap of the eucalypt hosts of P. atomaria and from this inferred that this chemical had been synthesised by the larvae. The level of a cyanide glycoside that Moore estimated would be necessary in a plant in order to provide larvae with cyanide if the larvae were not capable of synthesising it for themselves was "in the order of 0.13

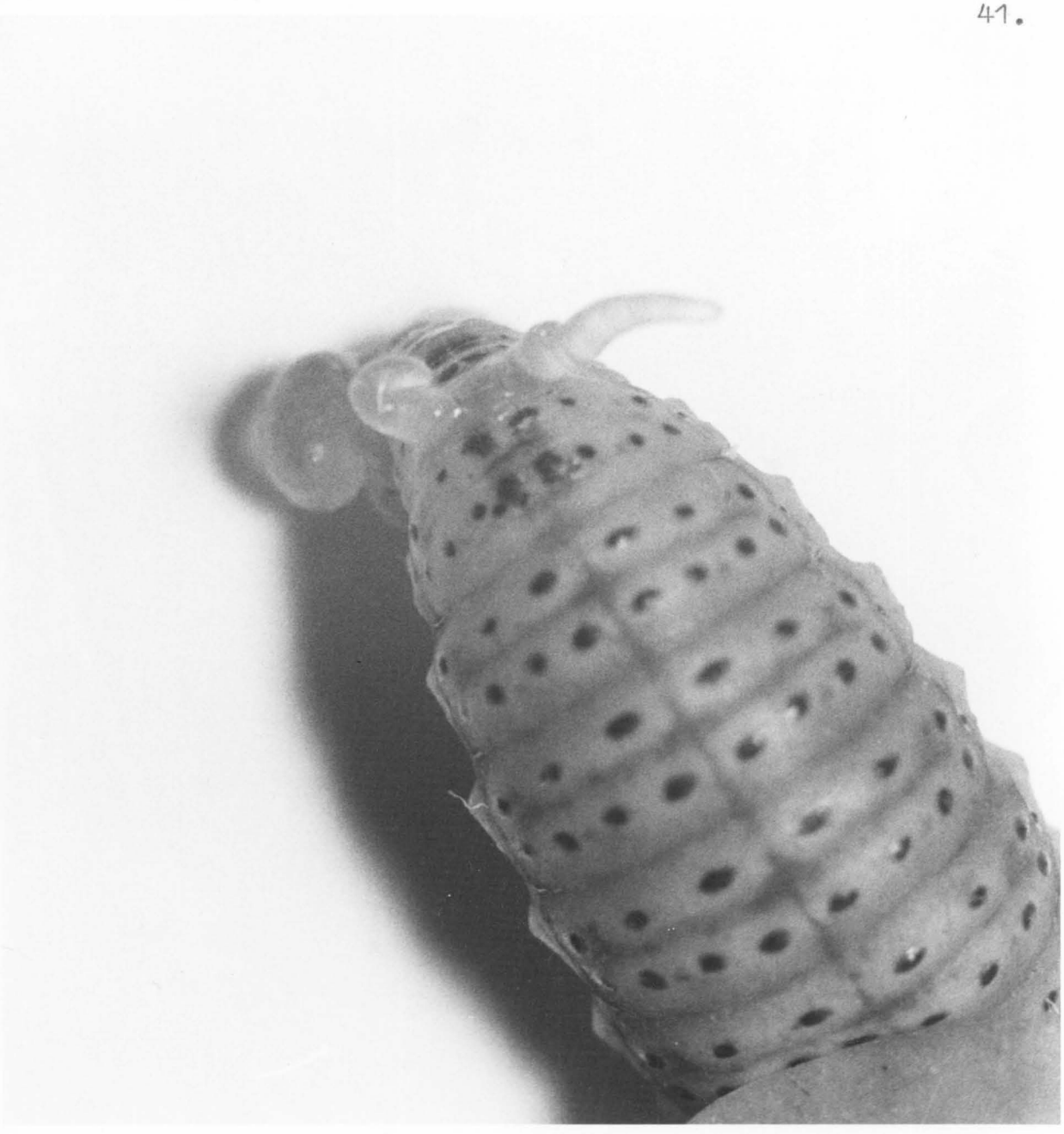


Figure 3. Everted defense glands shown on a fourth instar larva of P. charybdis.

per cent". Moore claimed that this was "impossibly high", thus verifying the conclusion that the larval Paropsini do themselves synthesize the main components of their defense secretion. Results of experiments performed during the present research tend to confirm that hydrogen cyanide, benzaldehyde and glucose are generally present in the Paropsini, because these substances were also detected in the defense secretion of Paropsis charybdis. The secretion of P. charybdis appeared as a mobile milky liquid which under the microscope was seen to be a suspension of oily droplets in an aqueous phase in accord with Moore's observations. However in this present case the secretion smelt strongly of neither almonds (Moore, 1967) nor Eucalyptus oil (Clark, 1930) but had an unidentified but distinctive odour. Hydrogen cyanide is of uncommon occurrence in insects and related invertebrates, being recorded from certain zygaenid moths and their larvae (Jones, Parsons & Rothschild, 1962) and several millipede species (Roth and Eisner, 1962; Jacobson, 1966). The secretion of P. atomaria was active against meat ants (Iridomyrex detectus Smith) giving knockdown about twenty seconds after contact and death generally within two minutes (Moore, 1967). The efficiency or otherwise of the secretion of P. charybdis against predators recorded in New Zealand is unknown. Personal observations from handling the larvae showed that the glands were everted with sufficient force to spray droplets of secretion to a maximum distance of at least 5 cm. The way in which an alarmed larva with glands everted raised the tip of the abdomen and moved it towards the sector from which the attack had originated meant that residual droplets of secretion left on the glands were wiped onto any attacker.

The glands can be fully replenished within 24 hours (Moore, 1967). In P. charybdis the glands of late fourth-instar larvae and prepupae were observed to regress and in many prepupae the reduced glands (less than half the maximum size attained) were permanently everted and apparently functionless. Fully developed but unhatched first-instar larvae have been seen to have functional glands in cases when the eggshell has been removed from over their abdominal tips. In such cases everted glands have been seen and the characteristic smell of the secretion has been detected.

(4) Economic Significance and Natural Mortality Factors

Clark (1930) inspected the area infested by Paropsis charybdis in New Zealand using a sample plot method and of the 3,800 acres of eucalypts surveyed found that 120 acres were between 0 and 25 % infested, 980 acres were from 26 to 50 % infested, 650 acres were between 51 and 75 % infested and the remaining 2,050 acres suffered an infestation of from 76 to 100 %. Unfortunately he did not define what constituted an "infested" tree for the purpose of this survey, nor did he detail how the sample plots were chosen and inspected. The survey was performed in order to determine the exact status of the various insects attacking eucalypts in New Zealand and the severity and extent of Paropsis damage in Canterbury (the area surveyed) was one of the most striking results of this work. Clark in the same paper discussed control measures, and due to the scattered nature of infested areas and their small size, and the apparent inability of the New Zealand environment (both climatic and biotic) to limit the beetle to a level below that causing economic damage, suggested that biological control appeared

the most hopeful method.

Introductions of parasites from Australia have been attempted since that time but without success; in fact to date no parasite introduction has reached the stage of releasing individuals in New Zealand following a quarantine period. This work has not been helped by the early identification of P. charybdis as P. dilatata, or by the scarcity of P. charybdis (and also of P. dilatata) in Australia. All parasites selected as possibilities have been members of the complex parasitising P. atomaria which is the most common Paropsini dealt with in Australia. No information is available as to how host specific any of these insects are.

The initial shipment was made to Clark by A.L. Tonnoir of the Commonwealth Scientific and Industrial Research Organisation in 1934 (Clark, 1938). It consisted of a trial shipment of parasitised eggs, but no parasites were obtained from these. In 1934-1935 further shipments of field-collected parasitised eggs and larvae took place. From the larvae several species of tachinids and one species of ichneumonid were reared. Again no egg parasites survived. Clark (1938) mentioned the difficulties during quarantine in determining primary and secondary parasites and states that it would be "some time before the introduction of a suitable parasite is decided upon". The tachinids being the commonest parasite encountered (the ichneumonids were "not plentiful" and the egg parasite "rare") it was thought that these would probably be used.

Miller, Clark and Dumbleton (1936) in a survey of biological control examples in New Zealand mentioned briefly

that the work to introduce a parasite of P. charybdis into New Zealand had been "begun only". White (1962), Dugdale (1965) and Carne (1967) mentioned this unsuccessful attempt at biological control and gave two reasons for the failure. Firstly, Forest Service records showed that no releases were made from early shipments because of both severe losses in transhipment and uncertainty as to which insects were parasites and which hyper-parasites. Secondly, Clark, who at this time was the sole entomologist working for the Forest Service, left the Service before this point had been clarified and with his departure all such entomological work was terminated. The parasites brought into New Zealand were Neopolycystus insectifurax Gir., Froggattimyia tillyardi Malloch and Meteorus sp. (Dugdale, 1965; Carne, 1967). The first is a chalcid parasitic on the eggs of P. atomaria, the second a tachinid larval parasite and the last a braconid parasite of the larvae (Carne, 1967). The braconid has been variously referred to as belonging to the genera Westwoodiella, Aridelus and Meteorus. Dr. Riek of the Commonwealth Scientific and Industrial Research Organisation has identified it as an undescribed species of Meteorus (Carne, 1972 - pers. comm.). Although Clark (1938) wrote that no egg parasites (Neopolycystus) were obtained from the shipments, Carne (1967) states that this insect proved capable of attacking P. charybdis eggs under insectary conditions. In nature P. atomaria normally lays its eggs in batches around the stems of terminal shoots with each egg projecting radially from the stem axis (Carne, 1966, p653 and plate I, figure I; Cumpston, 1939, p359 and plate X, figures 5 and 6). P. charybdis lays its eggs flat against the lower side of a leaf with adhesion

given by the full length of the egg and by blobs of cuticular adhesive at each end. Thus these two species vary considerably in their ovipositional patterns. A further factor which might affect an egg parasite is that the eggs of P. atomaria have a pattern of external cuticular ornamentation comprising four horns projecting from the free end of each egg and four longitudinal ridges along each egg, whereas the eggs of P. charybdis appear smooth but are actually very finely reticulated. Cumpston (1939) was able to get Neopolycystus to oviposit freely in P. atomaria eggs, and yet unable to get oviposition into egg batches of Chrysophtharta varicollis which are laid flat and have a tuberculate chorion. It is interesting to note that Cumpston found that the seven closely related Paropsini species she studied had similar eggs but that the arrangement of the eggs by the ovipositing female varied markedly. Indeed these differences were distinctive enough to form the basis of a key enabling species identification of the egg stage. In view of this variation and the selective pressure it could exert on the behaviour of the parasite it would not be surprising to find that Neopolycystus insectifurax is a parasite specifically of P. atomaria. Another difference between P. charybdis and P. atomaria that could be of vital importance to the successful transference of any parasite from one to the other is that these two species appear to attack contrasting ranges of eucalypts.

Because of the high cost of alternative chemical or cultural methods postulated for the control of P. charybdis in New Zealand, biological control has been kept under consideration in spite of this early failure. The fact that

eucalypts can tolerate a certain level of defoliation before economically significant damage is incurred to the eventual product - the timber or shelter provided by the tree - also makes the pest situation suitable for the successful use of biological controls. White (1962) discussed the possibility of introducing one or more parasites from the point of view of the work necessary to establish whether any particular parasite should be introduced or not. For example, he considered that it would be necessary to evaluate how well the parasite life cycle was synchronised to that of P. charybdis under New Zealand conditions, and whether the parasite needed supplementary feeding which might or might not be available in New Zealand. He also urged that the introduction be preceded and followed by adequate surveys in order to measure whether or not the introduction was operating as a regulatory factor on the populations of P. charybdis. Although White advocated that the first stage was obviously to locate P. charybdis in its indigenous habitat and there study its ecology especially the associated complex of parasites, predators and diseases, the next stage actually undertaken was to establish contact with the Commonwealth Scientific and Industrial Research Organisation in Australia and see what Paropsini parasites were available. This was the result of urgings of the Farm Forestry Association at the time when Paropsis was rapidly spreading throughout the North Island and causing extensive damage to farm plantations. Both the Forest Research Institute and the Department of Scientific and Industrial Research were involved. Dugdale (1965) recorded that the parasites most discussed were Neopolycystus, Meteorus and Froggattimyia species and that in

1963 a consignment of Meteorus species was received at the Forest Research Institute. However this was not successful as either hyperparasites, Perilampus sp., emerged or the parasite pupae were dead. The project was then handed over to the Department of Scientific and Industrial Research as the Forest Research Institute wished to concentrate on the introduction of parasites against the wood wasp Sirex noctilio. The Department of Scientific and Industrial Research established a supply of host plants and an insectary population of Paropsis and were ready to accept introductions for quarantine when the question of work priorities arose again. The work ceased and was later handed back to the Forest Research Institute (Dugdale, 1965). In this report Dugdale recorded that little was known about any of the parasites, the normal host of which is P. atomaria. In view of the differences in egglaying habits between P. atomaria and P. charybdis discussed earlier Dugdale doubted whether Neopolycystus would be able to transfer to the new host and decided that the parasites of the larval stage would be more likely to succeed. Also the Commonwealth Scientific and Industrial Research Organisation, who were to supply the parasites, had access to the braconid Meteorus rather than to the tachinid. However the most prolific generation of Meteorus (the autumn brood) was heavily attacked by the chalcidoid hyperparasite, Perilampus. In fact the high level of hyperparasitism (92% was reported by Styles (1970)) prevented any subsequent importations being made in 1966.

Carne (1967) referred again to the necessity to locate P. charybdis in Australia and study the parasite-predator complex in that area before deciding what import-

ations to make. Thus attempts to implement biological control, first by Clark in 1934 and then later by various officers in both the Forest Research Institute and the Department of Scientific and Industrial Research have failed to develop to the stage where field releases have been possible. The question of whether or not biological agents could effect a satisfactorily low level of infestation in New Zealand remains unanswered.

Although Carne (1967) reported that no disease had been noted in field populations of Paropsini larvae in Australia, there is an unidentified pathogen attacking P. charybdis in New Zealand. This was first apparent in 1964 when it killed large numbers of larvae in Canterbury. At the Forest Research Institute an unstrained suspension made from infected larvae collected in Canterbury was smeared onto an acceptable foliage (E. viminalis). Larvae at all stages of development when fed these leaves died within 24 to 48 hours (Styles, 1970). The epidemiology of the disease has been described in Carne (1967) and in the Forest Research Institute Annual Report for 1963 (p45-46). The outward diagnosis was given as a general moribund condition with darkening of the body, dark brown fluid excreta and a bloated appearance.

Histologically the only noted changes were a hypertrophy of the nuclei of the cells of the midgut epithelium, and that once a state of torpidity had been reached there was general tissue decomposition except for the nervous system (The Forest Research Institute Annual Report, 1963). Carne in his report described the larvae as becoming sluggish and dull in colour, ceasing to feed and rapidly becoming darker and dying. He found that "within 48 hrs of

the onset of recognizable symptoms, the larvae were reduced to structureless, semi-liquid black masses". The dead and dying larvae were usually seen hanging from the foliage by the anal segments. Diseased larvae were also seen in the field by Carne during his visit and arrangements were later made to send samples to Dr Goodwin, an insect pathologist with the Commonwealth Scientific and Industrial Research Organisation, for identification. In the 1966/1967 season only one sample of suspected diseased larvae was dispatched and these larvae proved not to be diseased. Styles (1970) considered that this scarcity was possibly due to dry weather reducing the incidence of the pathogen because Dugdale had found that the disease could be induced by holding the larvae under excessively moist conditions (Carne, 1967).

During the present work the author has kept a watch for the disease without avail. Occasional larval deaths during laboratory rearing have been suspected as being caused by a pathogen but in no case has it proved possible to reinfect healthy larvae by smearing foliage with a suspension from the corpses. In one case reinfection was thought to have occurred but the deaths proved to be due to an unknown contamination of the plastic of the rearing cages which was not transferable in the way described above to healthy larvae reared in other containers. A small trial which attempted to induce the disease by rearing the larvae in extremely humid conditions failed to increase the larval mortality significantly.

The extent of naturally occurring pre-adult mortality has been investigated by Styles in conjunction with Carne (Styles, 1970; Carne, 1967). The overall larval mortality

was assessed by placing batches of fifty first-instar larvae on selected regeneration trees four to six feet high. The numbers present and the state of development of these were checked daily. The results given in Styles (1970) are reproduced in Table 5. These results are of accumulated data from a varying number of trials per host so that the variation between hosts is readily apparent, but no notion is given in these papers of the variation between replicates on the one host. Styles (1969) includes the data from daily counts.

Table 5. Larval counts, survival and mortality of P. charybdis on selected eucalypt trees (Styles, 1970).

	Average number of days/instar	Number of larvae placed on selected trees		
		E. obliqua (10 trees)	E. viminalis (4 trees)	E. macarthuri (1 tree)
Instar 1	6	500	300	100
Instar 2	8	204	250	82
Instar 3	5	92	203	76
Instar 4	5	45	183	66
Number of larvae reaching maturity	-	24	174	60
Percentage reaching maturity	-	4.8	58	60
Percentage mortality	-	95.2	42	40

Carne (1967) gives these same results in a summarised form but includes some parallel experiments carried out at Nelson and also gives the range in total survival for each host species (Table 6).

Once again it is the obvious difference between the

foliages that attracts attention. However it should be remembered that these results show only total survival and do not distinguish between the mortality due directly to the host plant and that due to predation and other factors such as climate. External differences would be minimised at least for the Rotorua results, because the four eucalypt species grew together at the study site. Indirect effects of the host on survival such as the possibility that differences in tree growth habit could either affect the searching behaviour of predators or the exposure to climatic conditions cannot be eliminated. The results do suggest, though, that the host plant could be having a marked direct effect on the survival of larvae of Paropsis charybdis.

Table 6. Survival of P. charybdis larvae in field trials (Carne, 1967).

Locality	Host	Trials	Overall % survival	Range (%)
Rotorua	E. fastigata	4	0.0	-
	E. obliqua	10	6.8	0 - 15
	E. viminalis	6	58.0	40 - 72
	E. macarthuri	2	60.0	50 - 70
Nelson	E. globulus (j)*	2	0.0	-
	E. globulus (m)*	1	12.0	-
	E. obliqua	3	3.9	2 - 5
	E. macarthuri	3	8.6	4 - 14

* (j) = juvenile foliage

* (m) = young mature foliage

The low percentage of survival of larvae feeding on E. macarthuri at Nelson was unexpected because this foliage had been used successfully in laboratory cultures at that

place. This suggested to Carne that the mortality recorded for this eucalypt was due largely, if not wholly, to predation. He recorded a similar situation in Australia with P. atomaria and its host eucalypt, E. blakelyi, where laboratory survival frequently approached 100% but where field trials gave overall survivals ranging from 1.7% for 10 colonies in 1961 to 5.1% for 30 colonies in 1966 (Carne, 1967). The differences between survival on E. macarthuri at the two sites (low at Nelson and high at Rotorua) he thought possibly due to the fact that at Rotorua, the more recently invaded area, the local predator populations had not at that time fully adjusted to the rapid increase of abundance of this potential prey.

Styles (1970) quoting from earlier unpublished Forest Research Institute records listed as predators two species of pentatomid bugs, Cermatulus nasalis (Westwood) and Oechalia schellembergi (Guérin-Menéville), the European wasp, Vespula germanica Fab. and birds such as starlings and sparrows. He also recorded the possibility that birds and hedgehogs preyed on the soil-dwelling stages. Field predation was stated by White (1962) to be "insignificant". However Cox (in Dugdale, 1966) when sampling in coppice growth at Puhipuhi State Forest noted that, "of the pentatomid bugs Cermatulus was not seen; Oechalia schellembergi adults averaged about one per tree and were in many cases attacking the Paropsis larvae".

Concurrently with these experiments on larval survival, trials were carried out both at Rotorua and Nelson to investigate the mortality affecting the soil-living stages. Mature, fully-fed larvae were placed in cylindrical cages sited in an area of felled eucalypts. The cylinders contained three inches of sieved soil covered with sphagnum moss in which the

20 larvae could pupate. Two series of trials were performed, one in which the lower end of each cylinder was covered with fine mesh netting in order to exclude the large soil predators; the other with these ends open permitting the access of these predators. A summary of the results obtained are given in Table 7. These were for a total of 500 larvae in each trial carried out at Rotorua while details of the Nelson experiments are unknown.

Table 7. Mortality of the soil-inhabiting stages of P. charybdis; Trials A and C excluding predation, B not excluding predation (after Styles, 1970; Carne, 1967).

Trial	Adults Emerged			Dead or Diseased Adults, Pupae or Larvae	Not Accounted For	Percent Mortality
	Male	Female	Total			
Rotorua						
A 1	160	258	418	23	59	16.4
2	138	179	317	53	130	36.6
B 1	162	165	327	-	-	34.6
2	188	180	368	-	-	26.4
Nelson						
C	-	-	-	-	-	62 - 64

Once again the mortality incurred in the Nelson region was much greater than that at Rotorua. In view of the range of mortalities between trials done at different times (again the only indication of variability between replicates is given in the tabulated raw data of Styles (1969) for experiments at Rotorua), it appeared that the climate markedly affected survival of the soil-dwelling stages of P. charybdis. Styles (1970) recorded that during trial B 2 the weather was very dry.

The little difference obtained between predator-

including and predator-excluding cages at Rotorua was interpreted as showing that predation had only limited effect on the survival of the soil dwelling stages, whereas disease, fungal attack and other agents including desiccation did contribute greatly to the mortality at this stage. However, as Styles (1970) remarked, it would "have been preferable to have sited the experiment in a mature eucalypt stand with a large population of P. charybdis and where there may have been a higher number of predators in the soil".

No details of the limited trials carried out at Nelson are given but the mortalities for predator excluding cages (62-64%) were of the same order as those recorded for P. atomaria in the Australian Capital Territory (58-70%) while considerably higher than those at Rotorua (16-37%) (Carne, 1967). This again was presumably because the predator complex at Rotorua which had only recently been colonised by P. charybdis had not at that time adjusted to the level of prey populations available.

(5) Chemical Control

In 1962, Baker and de Lautour reported on an attempt to effect chemical control of P. charybdis by aerial spraying with DDT. Eucalypts planted on Puketutu Island for both ornamental and agricultural purposes were being heavily attacked by Paropsis in the spring of 1960. In response to a request by the owner of this island, laboratory tests were performed to determine the insecticide to be used. The results of these tests (Table 8) were interpreted as indicating that P. charybdis larvae and adults were very susceptible to insecticides. It was noted, however, that "the high mortality figures obtained in the controls showed

that the available laboratory conditions were not very good for conducting trials of this nature". DDT was selected because it gave a more rapid kill than lindane or dieldrin (Forest Research Institute Annual Report, 1960, p30).

Table 8. Laboratory trials to determine the effects of insecticides on P. charybdis (Baker and de Lautour, 1962).

A. Larvae placed on deposits from acetone solution in petri dishes.

Material	Rate (lb/acre)	% Mortality after :- 3 hr.	6 hr.	24 hr.	48 hr.
DDT	½	50	57	78	92
DDT	1	38	80	93	100
DDT	5	70	88	100	100
Lindane	½	24	52	82	96
Dieldrin	½	13	46	81	96
Control	-	0	3	44	82

B. Insect on foliage sprayed with an aqueous solution of DDT

Test	% Mortality after :- 6 hr.	12 hr.	24 hr.	48 hr.	72 hr.
Larvae	14	24	56	93	100
Mature larvae and adults	7	22	58	79	93
Control	0	2	16	56	66

Twenty-five acres of mixed species of eucalypts (E. viminalis Labill, hybrid E. viminalis - E. macarthuri, E. maideni F.v. M., E. ficifolia F.v. M., E. delegatensis R.T. Baker and E. punctata DC were recorded as badly infested; E. radiata was also present and attacked) were sprayed from the air at the rate of 1 lb para-DDT per acre.

Nine badly defoliated trees in an experimental block were examined before and after spraying and the infestation visually assessed. The numbers of dead and dying insects found on sacks placed beneath these nine trees were also recorded 2 hours, 24 hours and 48 hours after spraying. These results showed that most Paropsis (200 larvae and 16 adults of the final accumulated totals of 217 and 22 respectively) died within 24 hours of the spraying. Neither adults nor larvae were detected on the trees 48 hours after spraying. Follow up surveys showed very low numbers of beetles and good tree recovery until early March when the new growth was reported as suffering heavy defoliation. The reinfestation was thought to have originated from unsprayed trees elsewhere on the island.

A second spray of DDT at the same concentration as the first was applied in mid-March. The effectiveness of this was similarly assessed by observation and by determining the numbers of dead and alive beetles found on sacks placed under five trees and on plots in four other areas. This was carried out for several weeks. Within 12 hours of spraying "the ground was covered with dead and apparently dying beetles and very few were still active on the foliage". However the next day numerous adults were seen flying from the ground into the trees and although dead insects were recorded falling from the trees until late March the mortality was estimated as approximately 80%. This did not compare favourably with the initial spraying, which was primarily against the larvae, for which a 100% kill was estimated. One possible reason given for this was that some adults had, at the time of the second spray, ceased feeding and were entering diapause.

Styles (1970) recorded that a further trial was made in this same locality in 1967 using Gusathion 50 (azinphos-methyl). This chemical was effective but also only for a short time because once again reinfestation by immigration from unsprayed areas was not hindered.

If chemical control requires two applications per year, or the complete coverage of many small areas of eucalypts within a large total area in order to give adequate protection the cost factor becomes prohibitive. Other alternatives were discussed by White (1962) and Carne (1967). These include silvicultural methods such as the selection of non-susceptible species for future plantings, the elimination of susceptible species to prevent high populations causing damage by "spill-over" from susceptible to resistant eucalypts in mixed species stands, the use of mixed species stands of resistant and semi-resistant eucalypts, and the selection of eucalypts more suited to the environment. The last factor is important because as Carne noted in his 1967 report, Paropsis attack is only one of the many stresses to which eucalypts may be subjected in New Zealand. If a tree was already thrifty through being planted in, for example, an area too cold or too dry, the added stress of insect attack might be sufficient to overcome the lowered level of tolerance of the tree to such defoliation resulting in extensive dieback or death. This appeared to be the case when E. globulus, originally a component of wet sclerophyll forest in Tasmania, had been planted in dry areas such as Canterbury.

(6) Host Range

In order to decide whether or not the first three

silvicultural methods advanced above would be effective as control measures a detailed understanding of the insect host-plant interaction is needed. The first definite records of which eucalypts serve as hosts to P. charybdis is the following statement by Clark (1930, p121),

"The insect attacks the following trees, having a decided preference for the first three: Eucalyptus globulus, E. radiata, E. viminalis, E. regnans, E. gunnii, E. macarthuri, E. obliqua, E. eugenoides".

In the same paper the earliest record of Paropsis is given as the finding of overwintering Paropsis adults beneath the bark of fallen E. globulus trees. No new discussion concerning the host plants of this beetle occurred until the forestry report of White (1962) who recorded that the Forest Biological Survey was being used to gather more accurate information about the host spectrum. He also remarked that "It feeds on a wide range of species of Eucalyptus, and is causing severe damage to farm shelterbelts and woodlots". White later in this paper expanded this view in the statement that,

"So far field records would indicate that P. (charybdis) has a reasonably catholic taste within the genus Eucalyptus, but it has not been recorded attacking anything other than eucalypts. There are indications that some eucalypts may have partial immunity to attack, but an accurate measure of any such immunity can only be gained from carefully controlled rearings in the laboratory. The effect on the life history of P. (charybdis) of feeding exclusively on a range of different species must be measured."

White advocated such steps to gain a more detailed knowledge of the insect itself and its interaction with its host plants as the best approach to solving the problem caused by this beetle. The initial stages of any such research were to ~~be to find out~~ determine whether or not Paropsis could successfully complete its lifecycle when fed exclusively on any one species of eucalypt, and how such restricted feeding would affect the rate of increase or decrease of a population of beetles over a

number of generations; the rate being relative to that attained on one arbitrarily chosen species of Eucalyptus. To detect any effect of the host on the insect it would obviously be necessary to measure those parameters which could indicate the relative success with which Paropsis used each species as a food source; such as the duration, mortality and size of each stage in the lifecycle from egg to adult and the behaviour of adults and larvae.

In White's own words,

"... in any attempt to assess the food preferences of an insect the basic question which must be asked is, 'Can this insect maintain and increase itself while feeding exclusively on this food source'? In many instances the insect will be able to maintain itself, but its ability to multiply may vary considerably from species to species. Furthermore, it may take several generations of controlled rearing to enable such variation to be measured (e.g. there may be a gradual lowering of vitality which is expressed in decreasing fecundity, on the inability to mate successfully, or increased susceptibility to disease, high humidity and temperature, or the successful oclusion from the egg, ...)."

This quotation serves to show that at the time this report was written in 1962, nothing was known in detail about the insect-plant interactions; there was only the indication from field observations that certain Eucalyptus species might have some partial immunity to attack.

Dugdale (1965) reviewed the problem caused by Paropsis in New Zealand, drawing his information from published literature and from what was on file at the Forest Research Insitute. The report was divided into four sections; colonisation, defoliation, biology, and biological and chemical control. The largest section is that dealing with defoliation. In this, Dugdale gave an analysis of the great quantity of information concerning defoliation damage that had accumulated from observers of the Forest Biological Survey. In these field reports defoliation was assessed in four grades of severity within each

of two classes depending on whether the foliage attacked was normal adult foliage or epicormic growth. This distinction was made because Paropsis could induce, by severe defoliation, epicormic growth over the defoliated section of the tree. Dugdale's analysis was made in order to assist selection of eucalypts resistant to Paropsis damage. He wrote:-

"I assume that Paropsis will undergo no radical behavioural change. I also assume that a tree will endure a one-season in five years defoliation of new growth with little evidence of setback, and that such set-back is not economically detectable or deleterious. On these assumptions I suggest the Eucalyptus spp in New Zealand be grouped into three classes, based on the observed number of "defoliation-seasons". Thus species in Class I are, for aesthetic charm, shelter, timber and maybe pulp, a poor risk. Species in Class II are not such a poor risk, and probably can be grown with assurance in most suitable areas, but have a propensity for being defoliated because of (at present) inexplicable reasons. Species in Class III are, in the main, those that show but the slightest nibbling by adults, either in newly colonised areas or areas for the moment carrying a high Paropsis population on other species. These Class III species would be no risk at all to plant, from the viewpoint of Paropsis defoliation. It will be noticed that the majority of species fall in Class III.

Class I. Species liable to severe defoliation by Paropsis over 1-2 seasons in any three year period.

Eucalyptus deanei
" globulus *
" leucoxylon
" macarthuri *
(? " resinifera)
" viminalis *

* commonest species in New Zealand.

Class II. Species liable to severe defoliation by Paropsis in at least one season in any five year period, under conditions that may relate to site, seed-source, silvicultural treatment.

Eucalyptus amplifolia
" botryoides
" fastigata
" gunni
" maideni
" obliqua
" ovata
" pauciflora
" punctata

Eucalyptus radiata (see Clark, 1930; but not on Rotorua-Tokoroa experience)
" saligna (doubtfully in this class)

Class III. Species liable only to light or negligible defoliation by Paropsis, with perhaps one season in 10 years of obvious (i.e. light to moderate) defoliation, under conditions that may relate to site, seed source, silvicultural treatment.

Eucalyptus	aggregata	E.	maculata
"	baueriana	"	microcorys
"	blaxlandi	"	muelleriana
"	bosisyoana	"	niphophila
"	bridgesiana	"	nitens
"	camaldulensis	"	oreades
"	capitellata	"	paniculata
"	cinerea	"	pellita
"	cornuta	"	pilularis
"	dalrympleana	"	piperita
"	delegatensis	"	propinqua
"	eugenioides	"	regnans
"	ficifolia	"	scabra
"	fraxinoides	"	sieberiana
"	gummifera	"	sideroxylon
"	haemostoma	"	tasmanica
"	linearis	"	tereticornis
"	longifolia	"	umbellata
		"	urnigera

There are, of course, exceptions. In isolated localities in Canterbury, E. sideroxylon, E. tasmanica, E. urnigera suffer Class II defoliation, as do E. delegatensis, E. nitens, E. longifolia at Te Wera (Taranaki). In North Auckland, south of Whangarei, E. umbellata, E. tereticornis also suffer Class II defoliation. As incidence of this defoliation Class is not widespread for these species, and as there may be many factors concerned with these exceptions, the species are left in Class III. Conversely, E. pauciflora is possibly better placed in Class II, as is E. radiata."

The situation is complicated not only by site differences but also by differences in susceptibility to Paropsis attack within Eucalyptus species. This was reported later in this same paper of Dugdale's :-

"Observers have noted that, in E. viminalis there is the occasional specimen that is not as severely attacked as its neighbours. Obviously not all specimens of one species will be uniformly palatable. There is evidence, in E. botryoides and E. saligna, that seed source is of importance in the degree of defoliation; South African seed of both species yielded more palatable plants. There are clonal variations also. Below is a table of defoliation by clone and species observed by T. Faulds, on 14.12.62. He notes that the grafts were 7-16 feet high with intermingled branches,

and that the scions came from mature Tolaga Bay and Northland trees.

Table II. Clonal variation of Paropsis attack.

Species	Clone no.	Defoliation
E. botryoides	209	- Only this clone (out of 3) attacked; negligible.
E. gigantea	210	- "Severe attack" to 1 branch touching that of E. pilularis Clone 199.
E. muelleriana	203	- No attack.
E. pilularis	198	- Light attack on current season's growth.
	199	- Severe attack on current season's growth.
	* 200	- No attack on current season's growth.
	201	- Light attack on current season's growth.
E. saligna	(3 clones)	- No attack on any clones.

*Clone 200 has now been determined as E. pilularis x obliqua. "

As can be seen from Dugdale's footnote to these remarks some part of the variability noticed within Eucalyptus species can be traced to problems of identification of Eucalyptus species; problems which are confounded by the ready occurrence of hybridisation within the sections of this very large genus.

Dugdale continued his report by discussing the likely effects of eliminating the most favoured hosts of P. charybdis, because he felt that much damage to species he placed in his defoliation Class II resulted from emigration from Class I trees as these became eaten out during a season. Although if this elimination occurred there is a possibility that Paropsis would more extensively damage one or more species at present not so heavily attacked, Dugdale considered this unlikely.

This was because of his experience that whereas such phyto-omnivorous defoliators as Selidosema suavis and Declana floccosa had changed hosts in New Zealand, the related species with restricted feeding habits such as Selidosema fenerata, S. leucelaea and Declana hermione had retained a confined host range.

The only hosts recorded for Paropsis charybdis in Australia are E. maculosa and E. viminalis (Carne, 1963, personal comment in Dugdale, 1965).

The preceeding sections are intended to summarise present knowledge about the biology of P. charybdis and the status of this insect in New Zealand. As can be seen many aspects need to be more fully understood in order to determine the best approach to prevent damage by this beetle. One of the most important facets requiring investigation is the relationship between Paropsis charybdis and its host plants.

3. THE GENUS EUCALYPTUS

During the present research into the interactions between P. charybdis and some of the various Eucalyptus trees which are its host plants, problems were encountered in identifying the particular eucalypts used. This short section covers the literature used in tackling these difficulties and some references pertaining to the chemical composition of these trees.

The difficulty in eucalypt identification stems partly from the size of the genus, partly from the variety of characters needed to separate various species, partly from the variability of features within the species and also from the inadequacy of some of the descriptions. That the problem

is a general one and not restricted to the author is shown by the following quotations. Chippendale and Johnston who wrote the text for Kelly (1969) commented that,

"Identification of Eucalyptus specimens still presents a major problem. Various keys to assist in identification have been devised, but none is completely satisfactory." (p ix).

Similarly Penfold and Willis (1961, pv) wrote that

" ... the problem that exercised our minds continually was the vexed and difficult question of nomenclature. The genus presents special difficulties which are emphasised by the number of classification systems that have been proposed."

Most of the references (e.g. Penfold and Willis, 1961; Kelly, 1969) refer to eucalypts in their natural environment and for identification some dependence is made on the locality in which they occur. This feature is worthless when the trees under consideration are growing in areas beyond the boundaries of the natural distributions of any member of the genus, such as New Zealand. Penfold and Willis (1961) was the most comprehensive work encountered in this brief survey, with good sections on many aspects of the history, biology, culture and use of eucalypts including taxonomy and identification. The sections describing the variability of the key factors used in identification - the adult and juvenile leaf characteristics, the shape and number of flower buds, the shape of the fruit capsules and the types of bark - were especially valuable. Only two works refer specifically to eucalypts grown in New Zealand; Simmonds (1927) and McWhannell (1960). The former although limited to 70 species has full descriptions and a series of large, clearly drawn plates, while the latter depicts only the flower buds and seed capsules and is less useful for identification purposes.

In addition to all the above references and Grimwade (1920), a punched card key by Hall and Johnston (1964) was used in attempting to define the trees used. The tentative identifications arrived at were verified at the Forest Research Institute, Rotorua, to where preserved specimen sheets were sent. One plant could not be identified using this procedure and a specimen was sent to Mr. L.A.S. Johnson, Director of the Royal Botanic Gardens, Sydney. He was able to reply that the tree appeared "to be a hybrid of E. delegatensis R.T. Baker with another species ... it is possible one of the Tasmanian peppermints may be involved. The characteristics of the tree, fruit, etc suggest a backcross to E. delegatensis from the F1 ... ".

This ability of eucalypts to form hybrids within certain groupings of species (Penfold and Willis, p56 - 59) and the inherent variability of some species is both important and a complication. It is important because it shows the potential available for breeding programmes by the selection of strains and by the rearrangement of genetic material to give completely new combinations of desirable characteristics. The nuisance value comes from the problems of identification and the inability to predict accurately the characteristics of a tree once it has been identified. For example Pryor (1952) noticed that only one of a group of 30 E. rubida trees withstood scarab defoliation. The progeny of this tree showed it to be of hybrid origin, with the resistant species E. maculosa as the other parent.

The system of classification followed has been that used by Penfold and Willis. This is the antherial classification of Blakely, first published in 1934 and is, "the most elaborate and complex of all Eucalyptus classif-

ication systems, and is the one in general use today." (Penfold and Willis, p78). In this the genus is divided into eight 'sections' and 16 'subsections', by antherial characteristics and then other features are used to group species within these divisions into Series. "The various subsections are very difficult and sometimes impossible to separate" and "the relationship of the anther sections to the various Series and Subseries is not always consistent, and is sometimes confusing, while the scheme is often difficult to apply" (Penfold and Willis, p333 and p83).

The genus Eucalyptus belongs in the family Myrtaceae and the infra-familial classification is :-

family: Myrtaceae

subfamily: Leptospermoidene

tribe: Leptospereae

subtribe: Eucalyptinae

genus: Eucalyptus

There are approximately 525 species and 150 varieties of Eucalyptus recognised but the relationship and status of many of these are still undergoing change. The relationship of the 16 species used in the different stages of the research are shown in Table 9.

Table 9. The infra-generic classification of eucalypts, showing the taxonomic positions of the species used.

Sections and subsections		Series
A: Macrantherae		
a: Cordiformes	-	1 Eudesmieae
b: Ovoideae	-	2 Miniatae
c: Longiores	-	3 Tetrapterae, 4 Corymbosae (Nonpeltate)
		5 Corymbosae - Peltate *[1]
		6 Transversae, 7 Obliquae,
		8 Cornutae, 9 Subcornutae

d: Truncatae	-	10 Microcorythae
e: Subtruncatae	-	11 Dumosae
f: Orbiculares	-	12 Anisomeleae
g: Ovulares	-	13 Decurvae
h: Tereticornes	-	14 Elongatae, 15 Exsertae *[2], 16 Subexsertae
A: Macrantherae(Normales)	-	17 Microcarpae, 18 Globulares*[3,4,5], 19 Semiunicolores, 20 Viminales*[6,7], 21 Argyrophyllae, 22 Paniculatae
B: Renantheroideae	-	23 Diversiformae
C: Renantherae		
i: Cordatae	-	24 Occidentales
j: Papilionantherae	-	25 Ochroxylon
C: Renantherae(Normales)	-	26 Pseudo-stringybarks
		27 White mahoganies,
		28 Steatoxylon
		29 Pachyphloiae (Stringybarks)*[8,9,10]
		30 Fraxinales (Ashes) *[11]
		31 Longitudinales (Snow gums)
		32 Piperitales (Peppermints)*[12,13,14]
		33 Psathyroxyla (Snappy gums)
k: Brachyandrae	-	34 Myrtiformes
D: Porantheroideae		
l: Obliquiantherae	-	35 Fruticosae (Mallee gums)
D: Porantheroideae(Normales)		36 Subbuxeales (Mallee boxes)
		37 Buxeales (Boxes)
m: Attenuatae (species no series)		
n: Oblongae (Species +)		38 Siderophloiae (Ironbarks)
o: Elongatae (species no series)		

E: Terminales (Species +)* 15, 16

39 Melliodorae (Yellowboxes)

40 Heterophloiae

F: Graciles - 41 Aridae

G: Micrantherae - 42 Eremophilae

H: Platyantherae - 43 Subulatae, 44 Leptopodae

p: Emarginatae - 45 Contortae

q: Adenophorae - 46 Quadricostatae

r: Pyriformes - 47 Xylocarpae

Species:

* 1 E. ficifolia F. Muell.

2 E. camaldulensis Dehnh.

3 E. globulus Labill.

4 E. cordata Labill.

5 E. perriniana F. Muell. ex Rodway.

6 E. macarthuri Deane and Maiden.

7 E. viminalis Labill.

8 E. alpina Lindl.

9 E. fastigata Deane and Maiden.

10 E. obliqua L'Hérit.

11 E. delegatensis R.T. Baker (= E. gigantea Hook.f.) hybrid tree used

12 E. linearis Dehnh.

13 E. andreana Naudin.

14 E. amygdalina Labill.

15 E. sideroxylon A. Cunn. ex Bentham.

16 E. leucoxylon F. Muell.

Of the 16 species used 8 (linearis, amygdalina, cordata, delegatensis, globulus, viminalis, obliqua and perriniana) occur in Tasmania. The first three mentioned of

these are found only in Tasmania and the very first is apparently of restricted distribution on this island. The remainder also grow in south-eastern Australia. E. andreana, E. fastigata, E. macarthuri and E. sideroxylon also occur in this area of Australia while E. leucoxylon is found from Adelaide to Melbourne and on Kangaroo Island, and E. ficifolia grows naturally along the south coast of Western Australia. E. camaldulensis is the most widespread of all eucalypts and the range is given as widespread east of the Western Australia-South Australia border. Thus it appears that most of these gum trees could be found within the area where Paropsis charybdis is thought to occur - Tasmania and the south-eastern corner of mainland Australia. However because of the existence of different varieties and physiological forms of some of these eucalypt species the exposure of evolving populations of P. charybdis to specific foliages cannot be estimated more definitely.

Leaves and seed capsules of five of the Eucalyptus species used are shown in figures 4 - 8. These give some idea of the variety of leaf size and shape encountered. Figure 4 depicts E. globulus and shows old mature leaves and a shoot of juvenile leaves. The glaucous nature of this juvenile foliage is not marked in this photograph. The damage to the mature leaves is typical of Paropsis. E. linearis, a narrow-leafed variety of gum tree is shown in figure 5 and E. ficifolia, an ornamental variety, appears in figure 6. The two closely related species E. fastigata and E. obliqua are in figures 7 and 8 respectively.

Physiological varieties of eucalypts have been recognised largely because the trees of this genus provide

Figure 4. Eucalyptus globulus, showing both mature and juvenile (upper) leaves, and a seed capsule with opercula. Note the feeding damage.

Figure 5. Eucalyptus linearis, a small-leafed gum.

Figure 6. Eucalyptus ficifolia, showing the leaves, flower buds and seed capsules.

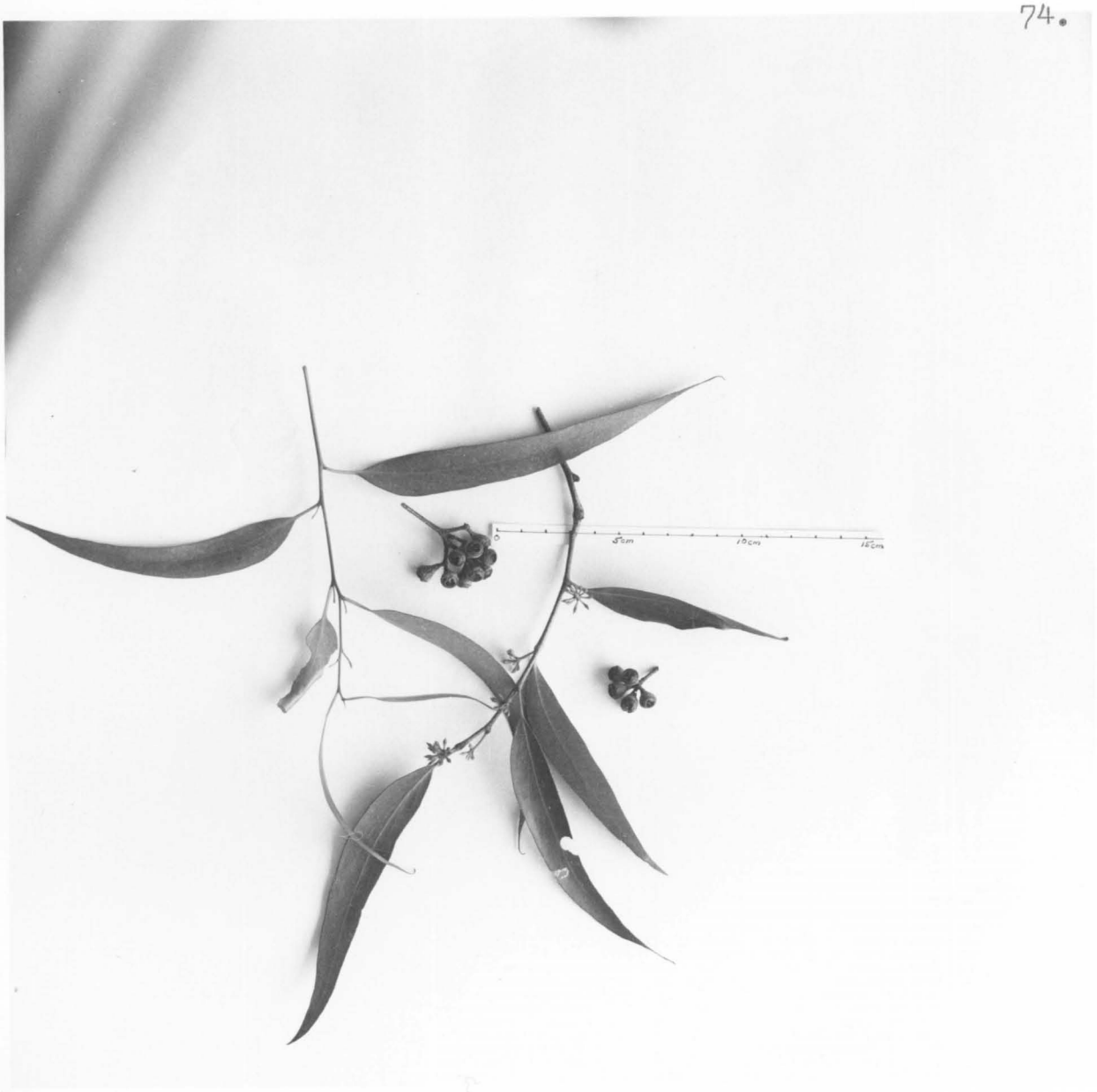


Figure 7. Eucalyptus fastigata specimens from the Botanic Gardens, Christchurch.

Figure 8. Eucalyptus obliqua specimens from the Chamberlain Farm. Some of the leaves have been extensively damaged.

essential oils which have been commercially distilled since the 1850s for medicinal, industrial and perfumery uses. Many species have been surveyed for oil yield and the content of desirable chemicals, which are cineole, phellandrene, piperitone, geranyl acetate, citronellal and citral. Many other chemicals have been recognised in the oils (Penfold and Willis, 1961, Chapter 12); recently there have also been investigations into other aspects of the chemistry of these plants such as the series of papers on leaf polyphenols by Hillis and collaborators (Hillis, 1966 a and b, 1967 a, b, c, d ; Hillis and Isoi, 1965 a and b; Hasegawa and Hillis, 1966; Banks and Hillis, 1969).

In the brief literature survey that follows some of the references have not been sighted because sufficient information was obtained from the abstract appearing in Chemical Abstracts; these are so indicated in the bibliography. The information available about the chemical composition of the foliage of each species used is presented in turn.

Nothing is known about the chemicals occurring in E. ficifolia.

The oil distilled from E. camaldulensis (previously E. rostrata) contains only 8-10% cineole but also p-cymene, phellandrene, cuminal, phellandral and geraniol (Baker and Smith, 1920; Gandini, 1936). This species has been extensively sampled by Hillis who found the following polyphenols; aromadendrin, kaempferol, a pelargonidin - like compound, quercetin, delphinidin, macrantherin and the acids ellagic, gallic, caffeic, sinapic, ferulic, gentisic, p-coumaric and chlorogenic (Hillis, 1966; Banks and Hillis,

1969). Hillis (1966) mentioned five unidentified glycosides while the second paper (Banks and Hillis, 1969) stated that a total of 47 polyphenols were characterised from the leaves, seeds and seedling leaves of E. camaldulensis. The leaves also contained five sugars - xylose, arabinose, galactose, mannose and lyxose (Fumasoni, 1959) and two isomers of ursolic acid while lacking oleanolic acid (Theodossiou, 1962). Karschon (1959, 1960) and Bhimaya and Kaul (1966) analysed the levels of macro- and micro-elements including nitrogen, potassium, phosphorous, iron and manganese. There is also an unidentified germination inhibitor present (Lerner and Evanari, 1961). The essential oil produced from E. camaldulensis in Egypt was reported on by Elkeiy, Darwish, Hashim and Assem (1964a) and that distilled in Albania by Angeli (1963).

E. globulus has been used as a commercial source of Eucalyptus oil mainly outside Australia, because the oil although not in heavy yield contains mainly cineole, with some d-α-pinene, globulol, volatile aldehydes and sesquiterpenes (Baker and Smith, 1920). Hillis (1966a) in his survey of the taxonomic usefulness of leaf polyphenols identified as present quercetin, macrantherin and caffeic, chlorogenic, p-coumarylquinic, ellagic, ferulic, gallic, and gentistic acids. Elkeiy et al (1964b) found that the leaves contained eucalyptin which they suggested as a γ-lactone derived from coumarin. The same authors in an earlier paper (1964a) found terpineol, geraniol, cuminaldehyde and chloramine in the oil of E. globulus grown in Egypt. Angeli (1963) and Raoul (1956) commented on the properties of the oil derived in Albania and Brazil respectively. An unidentified

growth inhibitor was present although this was attributed to cineole (Baker, 1966). Horn, Kranz and Lamberton (1964) worked on the composition of the wax and noted that the alcohols incorporated were long chain alkan-1-ols with an even number of carbon atoms. The persistance of the waxy-glaucous juvenile leaves for some time is noted in the whole Globulares Series. Johnson (1926) examined physical differences (such as the arrangement of stomata) between adult and juvenile leaves of E. globulus.

E. cordata and E. perriniana belong to the same Series as E. globulus but less is known about them. The oil of both contains mainly cineole, d-α-pinene and volatile aldehydes, with butyl butyrate also identified in E. perriniana oil (Baker and Smith, 1920). The polyphenol composition of these species differs from E. globulus in that E. cordata lacks caffeic and ferulic acids while E. perriniana contains kaempferol and leucocyanidins in addition to those compounds known from E. globulus (Hillis, 1966b).

E. macarthuri differs from most other species in that the oil distilled from it contains mainly geranyl acetate with geraniol, eudesmol and d-α-pinene also present (Baker and Smith, 1920; Penfold and Willis, 1961). The number of polyphenols present is low with only macrantherin, quercetin, ellagic acid, gallic acid and gentisic acid, leucocyanidins and leucodelphinidins (Hillis, 1966c).

E. viminalis is included in the same Series as E. macarthuri but its oil contains cineole, d-α-pinene, α-phellandrene, and sesquiterpenes (Baker and Smith, 1920). These authors also recognised a physiological variety of E. viminalis yielding an oil with a higher proportion of cineole, and with

benzaldehyde and not α-phellandrene. Hillis (1966a, 1967b) mentioned that E. viminalis contained 6 of the 7 polyphenols found in E. macarthuri, lacking only leucodelphinidin. In addition the three acids - sinapic, chlorogenic, and p-coumarylquinic - were noted as present.

E. alpina, E. fastigata and E. obliqua are all placed in the Pachyphloiae Series. The oils from all these species contain some cineole; that from E. alpina also has α-pinene, from E. fastigata pinene and phellandrene, and that from E. obliqua phellandrene, aldehydes and p-cymene as well as cineole (Baker and Smith, 1920). The polyphenol content of each of these eucalypts has also been worked on (Hillis, 1967a). Leucocyanidins, quercetin and ellagic, gallic and gentisic acids are common to all three while renantherin is present in E. alpina, leucodelphinidins and chlorogenic acid in E. fastigata and leucodelphinidins and myricetin in E. obliqua. Hillis (1966a) had previously also listed kaempferol, ferulic acid and p-coumarylquinic acid as present in E. obliqua. E. alpina contains rutin (Humphreys, 1964).

E. delegatensis has not been extensively investigated. The oil contains l-α-phellandrene and piperitone (Baker and Smith, 1920) and the leaves rutin (Humphreys, 1958) and the polyphenols quercetin, ellagic acid, gallic acid and gentisic acid. (Hillis, 1967a).

The three species from the Series Piperitales used - E. linearis, E. andreana and E. amygdalina - have similar compositions. The oil from these also contains l-α-phellandrene and piperitone with cineole in most varieties, eudesmol in E. linearis, and piperitol in some forms of E. andreana (Baker and Smith, 1920; Penfold and

Willis, 1961). All three species contain the four polyphenols identified from E. delegatensis and also leucodelphinidins and myricetin. E. amygdalina has as well chlorogenic and p-coumarylquinic acids (Hillis, 1967a).

The final two species, E. sideroxylon and E. leucoxylon, are closely related. Cineole predominates in the oil of both, with α-pinene also present and sesquiterpenes from E. sideroxylon, and limonene from E. leucoxylon (Baker and Smith, 1920). The polyphenols present in the leaves of both consist of seven of the nine present in E. globulus (the exceptions being caffeic and ferulic acids) and also of myricetin, leucodelphinidins and leucocyanidins (Hillis, 1966a, 1967c). Hillis and Isoi (1965a) reported on the variability of the chemical composition of E. sideroxylon. They found that the sugar moiety of glycosides varied with the location of the plant sampled, for example quercetin was found as a 3-glucoside, a 3-rhamnoside or a 3-rutinoside. They also mention finding kaempferol, catechin, epicatechin, shikimic acid, engelitin, nicotiflorin, the flavonoids sideroxylin and eucalyptin and the stilbenes, piceid, rhapontin and astringin.

CHAPTER III

LABORATORY REARING - LARVAE

The present research was initiated to investigate the reports of differential damage to the various types of eucalypt which have been recounted in the preceding chapter. The approach adopted was to establish a population of Paropsis charybdis in the laboratory to provide material for experiments into several aspects of the insect host-plant interaction. Firstly several series of rearings were carried out under controlled conditions in order to establish whether or not the damage levels could be explained as an antibiotic or antifeeding effect of the different species of Eucalyptus. This chapter describes that portion of this work done using the larvae of P. charybdis.

1. MATERIALS AND METHODS

A laboratory population of Paropsis charybdis was established with beetles taken from a row of mixed eucalypt seedlings (principally Eucalyptus ovata with some E. macarthuri) in the Head-of-the-Harbour region of Lyttleton Harbour, 21 September 1969. Beetles in a sheltered area such as this were active several weeks before those on the Canterbury plains had emerged from diapause. The growth cycle of the Eucalyptus trees was similarly advanced.

The colony initially consisted of 74 beetles (42 females and 32 males) in two large cages. Numbers, especially of males, declined slowly but steadily from the beginning, partly due to a poor local supply of succulent leaves for feeding. This resulted in an imbalance in the sex ratio (36 females to 15 males and a further 23 males were caught from the same site. Numbers

continued to drop and six weeks after the beginning the two groups of beetles were combined into a single cage.

The larval rearings done in open laboratory conditions were carried out using unfed first-instar larvae derived from this initial laboratory colony. However the supply from this source although at first very large, declined sharply and became somewhat erratic. Ten pairs of adults were selected from a control rearing, that is from one where the larvae had been fed on the mature leaves of E. globulus, to become the parent generation for the next cycle of larval rearings. This took place in late autumn 1970. Thus these subsequent rearings were carried out in controlled conditions using larvae from a colony which for a complete generation had fed solely on the mature leaf-form of E. globulus. In this way any possible differences in response induced by the exposure of the larvae or the parent beetles to various host plants should have been obviated. Similarly a second parent generation was derived from larvae fed on E. globulus foliage during this first season of controlled rearings. This provided eggs for a further season of larval rearings, from December 1970 to April 1971.

In early spring 1971 adults were caught in the field as soon as they become active. This was done partly to check on the fecundity and longevity of adults which had overwintered naturally, and partly to provide a number of fresh, actively-laying females. This new colony provided the larvae for rearing experiments in the final season.

(1) Cages

The large cages used for the initial population were based on a design used successfully for P. charybdis at the Forest Research Institute, Rotorua (J. Styles - pers. comm.)

(Figure 9). The cages were approximately 31cm wide, 38 cm deep and 50 cm high. Each unit consisted of two such cages in a common frame and five units were constructed. The framework of each was of untreated rimu timber which supported nylon netting (mesh 8-9 per cm) sewn together to form the roof and three sides. The lower edge of the netting was held between the hardboard floor of the unit and the lower framework to which the floor was nailed. The front edges including that of the central netting partition was held by battens nailed to the framework in four of the units. In these units the battens provided a recessed frame into which the removable door to each cage (the whole front) was tightly fitted. These doors consisted of hardboard with the centres (18 x 24cm) removed and replaced by nylon netting attached by Davis PVA Resin Glue. They were held firmly in place by simple turning catches. The lower edge of the sewn-in netting partition was glued to the hardboard base.

The fifth unit differed in that, except for the hardboard floor, the beetles were only exposed to direct contact with netting. The front sides had 28cm nylon zips sewn along the upper and lower edges. A strip of material along the inner edge of each door section could be reversibly sealed by contact with a matching strip glued to the central strut of the cage unit ('Touch-and-Close' made by Velcro N.Z. Ltd.) This unit had some advantages during the handling of the beetles but took longer to construct and was more expensive.

In use the floor of each cage was covered with two layers of either paper towels or newspaper cut to size. Four holes (0.7 to 1.0cm diameter) were drilled through the floor of each cage - one midway between the centre and the corner along each diagonal - to enable branchlets of eucalypt foliage

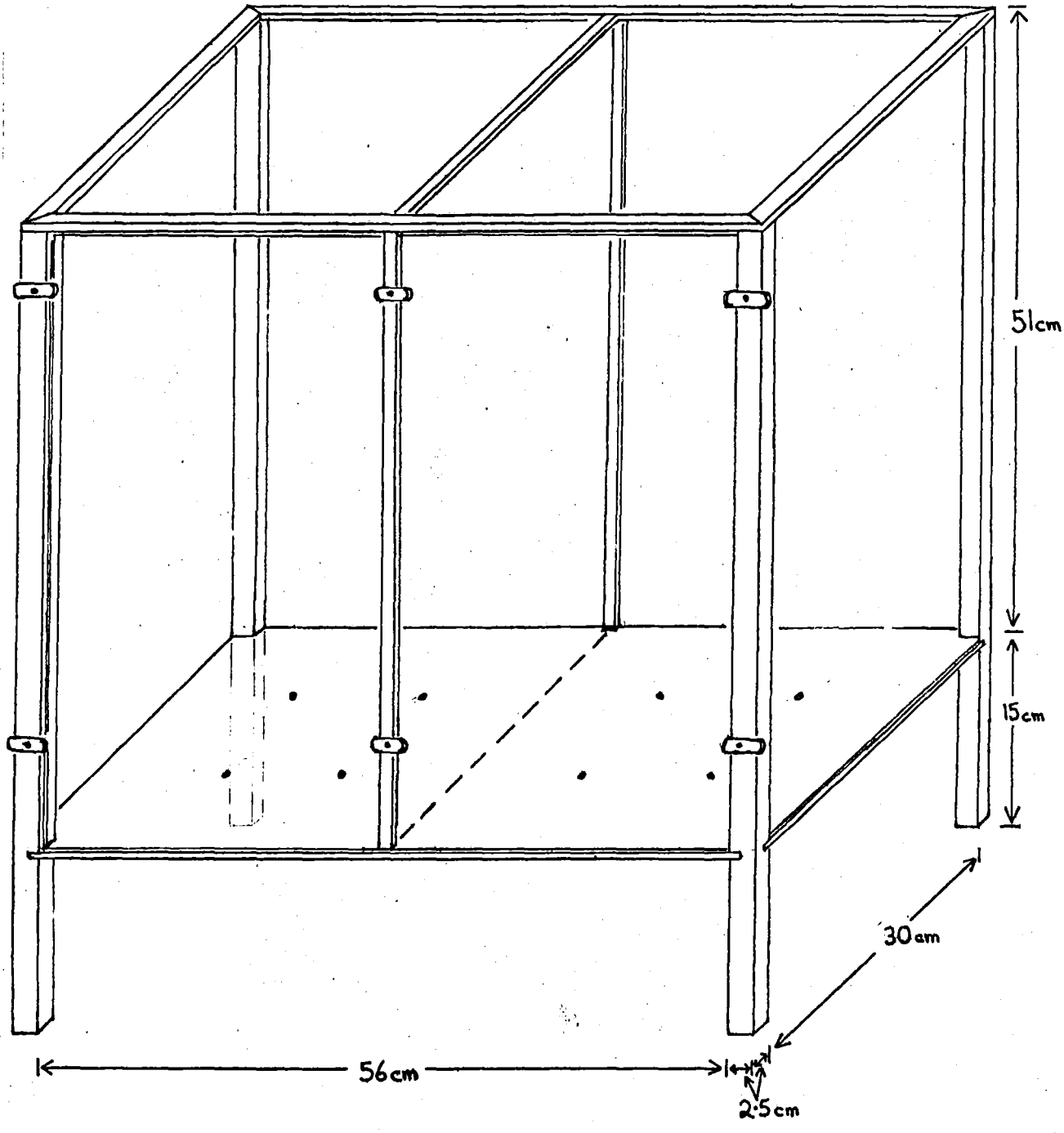


Figure 9. The design of the large cage used for rearing P. charybdis; netting was glued behind beading around doorways and stapled to other posts.

to pass through into containers of water. To prevent larvae escaping any gaps around the stems were plugged with cotton-wool. When the cages were used for adult rearing after the first season these holes were sealed off and foliage in 250ml conical flasks was placed inside each cage.

However these large cages proved unwieldy for detailed laboratory rearings although well suited to bulk rearings such as to supply Paropsis at any instar for other laboratory work. A large number of larvae were required for each experiment in order to fully use the large size of these cages and this in turn needed both an abundant source of eggs and a plentiful supply of young growth of each foliage being tested. This latter in particular was a major problem.

To avoid these difficulties, to increase the accuracy of the measurements taken and to reduce the time and error taken to check the number of larvae and the state of development of each, it was decided to change to smaller cages that could be checked every 24 hours. Such a system would be considerably more flexible. A trial was carried out using cylindrical plastic cages containing 30 larvae and this number proved much more convenient for handling. However these particular cages did not have adequate lids and so were replaced by plastic containers (Table Talk Canisters made by Consolidated Plastic Industries Ltd., Auckland). These were available in two sizes. The larger enclosed 1150cm^3 (11.3cm high and 9.2cm square tapering to 10.2cm square) and were used for larval rearing; the smaller enclosed 660cm^3 (10.8cm high and 7.2cm square tapering to 8.0cm square) and were used for rearing adults individually or in pairs. They were used inverted with a 0.5cm - 0.8cm diameter hole in each lid to allow the stems of eucalypt sprigs to pass outside the cage into water.

The larger cages had up to three holes per lid. In the top of each inverted container a hole was cut for ventilation (7.7cm diameter in large cages, 5.7cm in small). This was covered with nylon netting (mesh 8-9 per cm) in the small cages and about half the large cages; in the remaining large containers a fine nylon gauze (mesh about 20 per cm) was used. The latter was to contain the small first-instar larvae which could escape from the coarser netting. Similarly to ensure that no young larvae escaped, removable lids for the larval rearing containers were sealed with adhesive tape until all larvae had reached the second instar.

The containers were placed on a stand containing a 61cm length of plastic guttering as a water trough. Although this gave the possibility that chemicals might diffuse from one species of Eucalyptus and be translocated into other species sharing the common water supply, this was not considered probable. The trough was considerably easier to maintain than individual water jars. Foliage was renewed daily in larval rearing and every two days in adult rearing. The water troughs were kept full and were cleaned when noticeable algal slime formed, at 8 to 14 day intervals. Cages were wiped out at every foliage change and replaced with clean containers every third change or more frequently if required. This cage hygiene was required in order to prevent a fungal growth which flourished if the frass was not removed.

(2) Experimental conditions

Many of the initial colony of P. charybdis had been captured in copulation and laying began within 24 hours of the beetles being returned to the laboratory. The beetles were fed E. ovata from the site where they had been captured for the

first four days, and from then on were fed E. globulus using young foliage of the mature leaf-type. The cages were kept in a large laboratory close to a window, with artificial lighting (Osram 'White' fluorescent tubes) erratically supplementing natural illumination and with room heating during the day until summer. Foliage was renewed every one or two days and all eggs were also removed at these times.

Beginning 29 September 1969 larval rearings were carried out using more of these large cages. Eggs were incubated on moist filter paper in plastic petri dishes kept in the dark in a cupboard. Actively moving, unfed, first-instar larvae were placed on the foliage in each cage using a moistened fine brush. Larvae were distributed singly to each cage using the sequence of cages ABCDDCBA..... until there were 110 larvae per cage. This distribution pattern was chosen to avoid biassing the results through the use of successively weaker individuals as the supply of active larvae decreased. The foliage in these cages was renewed as required and the number and instar of all larvae dead or alive together with the total live weight was recorded at 4 day intervals. This frequency of checking was chosen because initial small-scale rearings had shown this to approximate the duration of the larval stadia under the prevailing conditions. Each instar could be readily determined visually by the overall size, the size of the head capsule, the colour and the pattern of cuticular ornamentation. Larvae which had reached the prepupal stage, ceased feeding and generally accumulated on the floor of the cages from where they were removed and placed individually in pupal cells. These were holes made in a 1.3cm thick layer of foam plastic using a No.10 corkborer (1.5cm diameter). The foam plastic was cut into blocks approximately 30cm by 25cm, a sheet of blotting paper

was lightly glued to the lower side to give a floor to the pupal cells (using Davis PVA Resin Glue) and the whole was sandwiched between two sheets of glass. (Figure 10). Each cell was individually numbered. Cell blocks were kept in the laboratory in darkness in a cupboard. These were checked every 24 hours and the dates each individual beetle entered the pupal and adult stadia recorded. Pupae were weighed when they were first observed, i.e. within 24 hours of pupation.

In all rearings of both larvae and adults, the results obtained for the various Eucalyptus species from each experimental series were compared with results obtained simultaneously using E. globulus foliage of the mature leaf-form as the food. This particular species was chosen as the reference point both because it has been recognised as a favoured host from damage reports and because it is one of the most common eucalypts grown throughout New Zealand. It is prevalent in the Christchurch region, particularly in older plantings of gum trees. A similar approach to any investigation into the apparent host specificity of P. charybdis had been urged by White (1962).

In spite of the use of this control, the initial larval rearings in larger cages suffered from the drawback that they were carried out in an unregulated laboratory under variably fluctuating environmental conditions of temperature, humidity and light. This was felt to provide unnecessary heterogeneity in the results of simultaneous rearings and to prevent valid comparisons between experiments done at different times. Not only the absolute values but also the relative values of any measurements taken could be affected, for example by temperature changes. Cram (1970) who compared three temperature regimes on the effective use as food of different blueberry cultivars by adult weevils (Otiorhynchus sulcatus F.) showed great apparent changes in fecundity - both absolute and

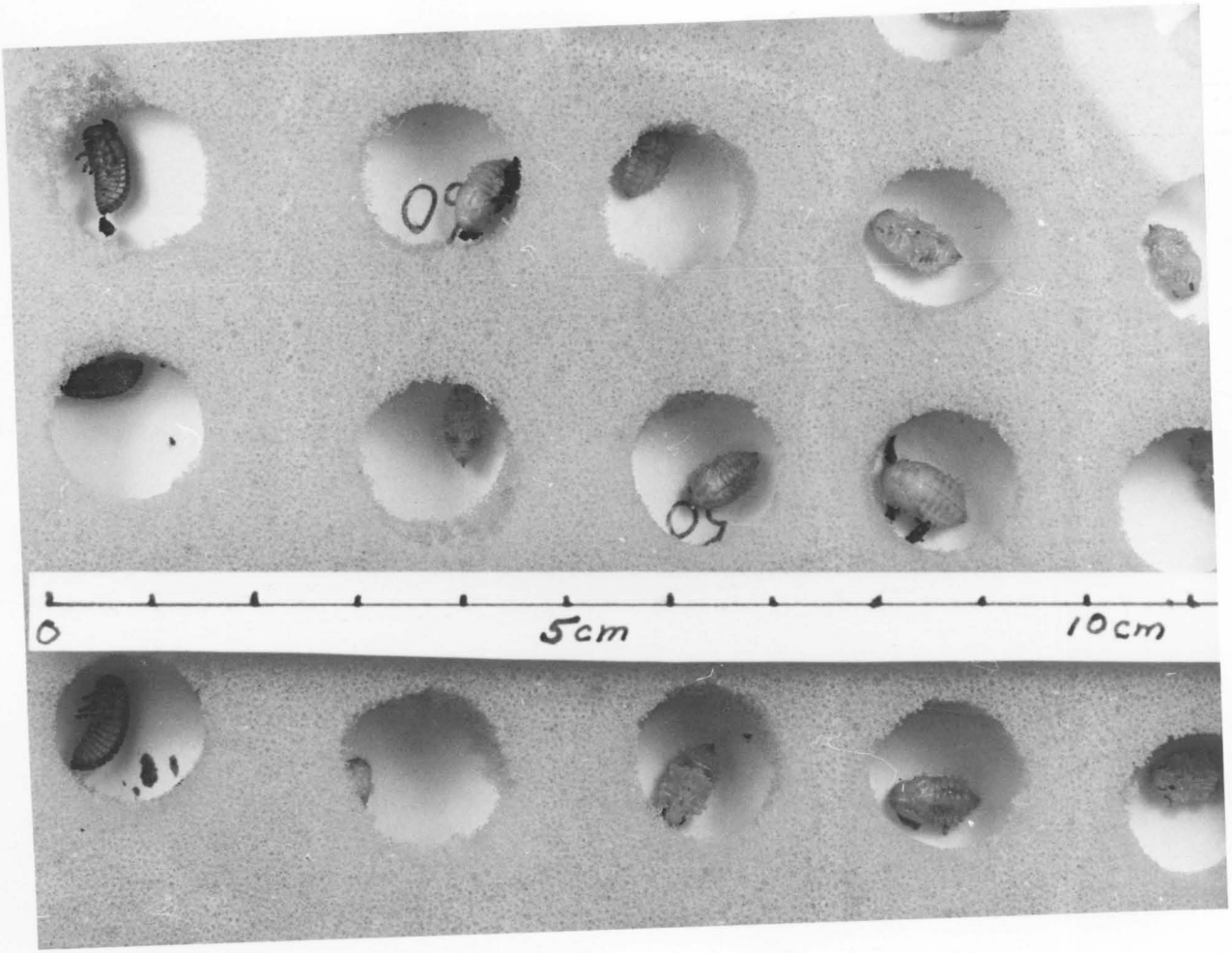


Figure 10. Some pupal cells showing both prepupae and pupae and the way in which they were reared (isolated from one another).

relative. The latter was due to the fecundities on each plant changing at differing rates in response to temperature increases. Hagstrum and Hagstrum (1970) in a short review of the ecological significance of fluctuating temperatures recorded that the generally beneficial effect of a fluctuating temperature when compared to a constant temperature was altered when the amplitude of fluctuation was changed.

To standardise rearing conditions all experimental work was transferred to a walk-in chamber with temperature and humidity controls. All larval rearings in this were carried out at 25.6 ± 1.2'C and at 65 ± 10%R.H. with a lighting regime of 15 hours on and 9 hours off. The bank of lights (4 x 65 watt fluorescent tubes positioned 1m above the bench top) caused a small cyclic variation in the temperature and humidity as was shown by recordings from a thermohygrograph placed on the bench (Figure 11).

Eggs taken from adult cages were incubated in plastic petri dishes in the controlled temperature room. No shading was given. At the start the petri dishes contained filter paper moistened with distilled water containing about .01% of choline chloride as an antimicrobial agent. This additive proved unnecessary provided that the eggs were removed from the leaf and were free from contamination by frass, and the use of it was stopped. Later it was found that the amount of moisture affected the vitality of the larvae after hatching. If the petri dishes were too damp some of the larvae once they had left the egg shells moved sluggishly and became a little bloated in appearance. These larvae did not establish well when transferred to foliage and many died without feeding. To prevent this mortality either only enough water was added to

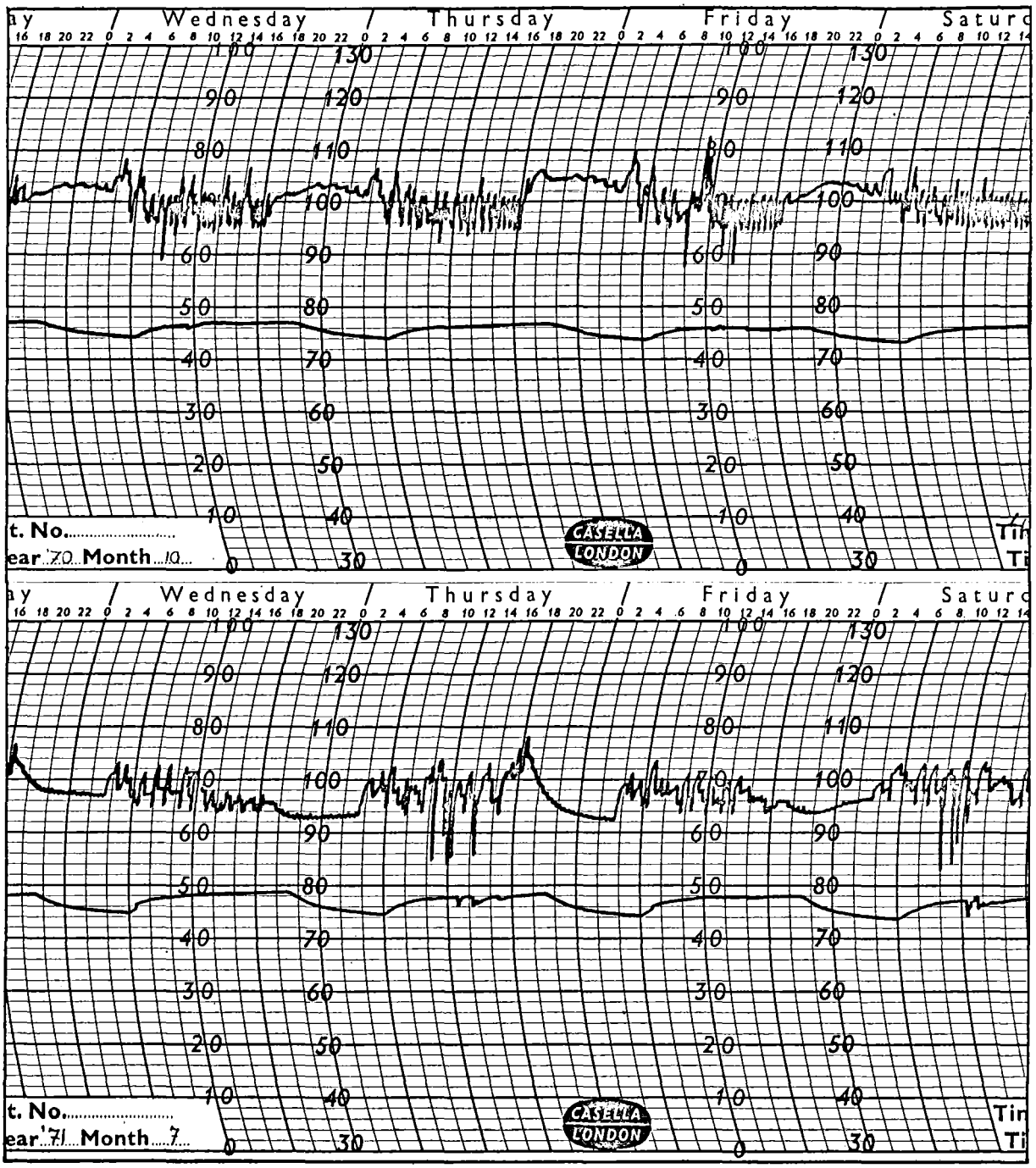


Figure 11. Thermohygrograph recordings showing the slightly cyclic fluctuations provided by the controlled-environment room. The upper trace in each recording gives the relative humidity and the lower the temperature.

keep the dishes moist during the start of incubation, or the petri dishes were kept dry. This lowered vitality could possibly have been due to a physiological disorder induced by moisture stress, or to a fungal infection. In a later experiment when some eggs were deliberately exposed to a suspected fungal pathogen a similar lowered motility was observed among emerging larvae.

Larvae were distributed to the foliages in all trials in the ascending - descending order of cages described earlier. The cages in each experiment were not arranged in any particular order unless, as happened during the final season, each foliage was duplicated within a trial. In this case the foliages were placed haphazardly within each of two sequences of cages within the overall arrangement. Twenty-five active first-instar larvae were distributed to each eucalypt being tested. These were recounted once all larvae had been shared out and the containers then closed and sealed until the first 24-hourly count. This method resulted in 25 larvae of comparable vitality being distributed to each foliage.

(3) The foliages used

The overall design of the complete series of experiments was complicated by several factors. The two most important of these were the variable supply of eggs from the parent colony in use at the time and the availability of fresh young leaves for each species. The egg supply limited the number of cages which could be included in any particular experimental trial and the foliage supply limited the number of replications which could be attempted using any one species of eucalypt. The problem involving the egg supply could have

been overcome by maintaining a sufficiently large population of beetles to ensure an adequate production of eggs at all times. However this would have severely overtaxed the amount of succulent leaf material available as food as well as necessitating more space and maintenance than was available.

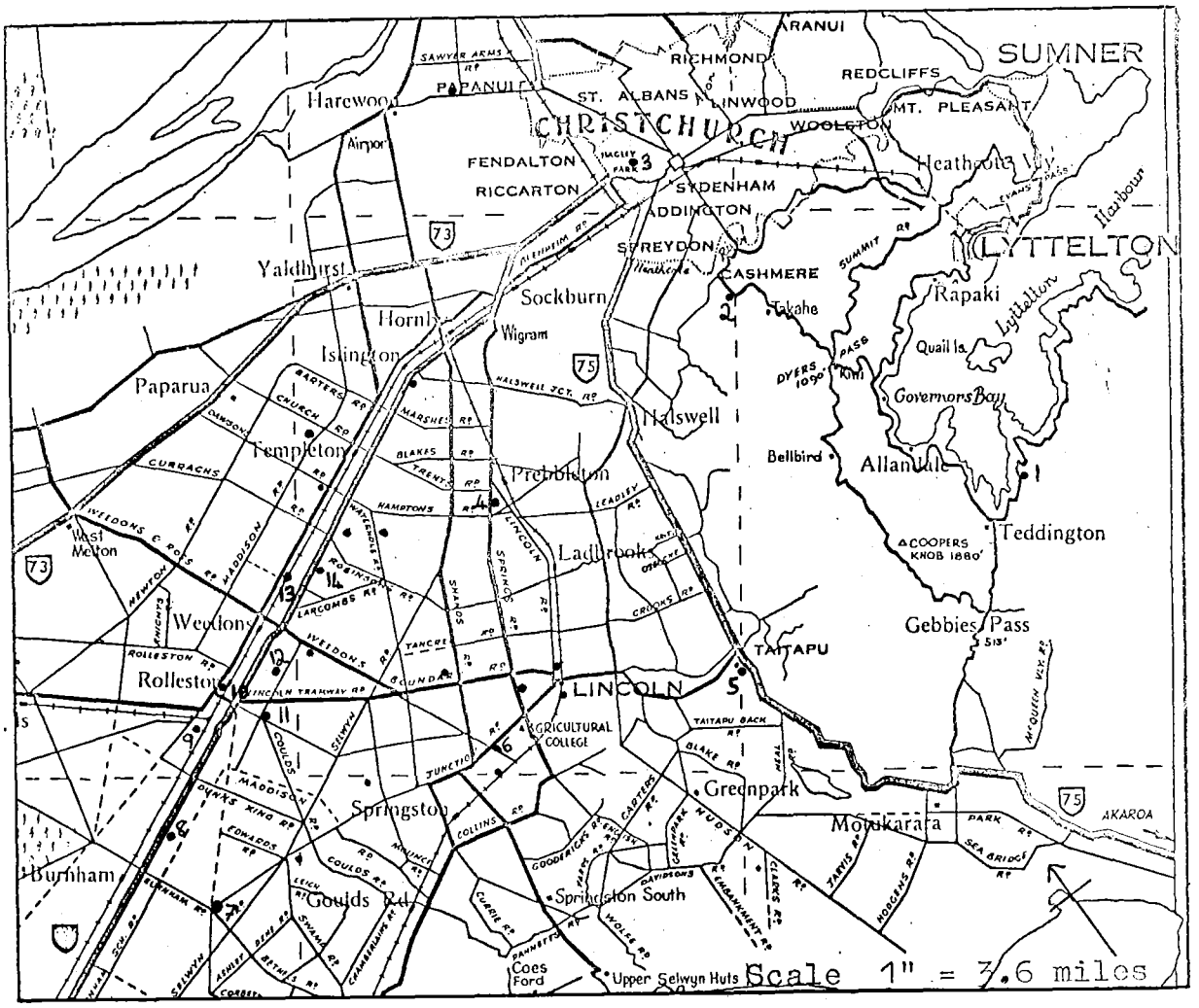
The foliage supply varied with the number, size and shape of trees available, and the state of growth of these. P. charybdis, at all stages in the life cycle, is restricted to feeding on fresh young leaves. Thus the growth pattern restricted both the number of replications possible at any particular time and the time period during which each foliage could be used. Some eucalypts produced new growth during only a short period while others had a more extensive growing period. Most gums in the Christchurch region grew in both the spring and autumn and not during either the hot dry period of summer or the cold of winter (for example E. andreana and E. viminalis). Others appeared to have a more restricted seasonal flush of growth (for example E. ficifolia was found to grow mainly in the spring and E. fastigata in late summer and autumn). Some new growth could be found on E. globulus trees throughout the year although such fresh foliage was rare in mid-summer and winter and a supply could only be maintained during these spells by extending the area collected from. Growth at times other than spring and autumn was largely confined to epicormic shoots or to branches originating from such shoots and varied markedly from tree to tree. The appearance and duration of succulent young growth is obviously dependent on site and climatic conditions and, as the above remarks are based on personal observations over only three or fewer years with dry summers and mild winters, they may not relate to the growth patterns of each Eucalyptus species in other areas or during

other years.

This variation in the seasonal timing of the growth of the various eucalypt species both increased the need for the use of a control foliage (to enable possible temporal variations in the supply of first-instar larvae - such as vitality and rate of growth - to be monitored) and yet also increased the problems in using the control. This difficulty arose because it would be possible for the quality of the control foliage to vary during its growth cycle. It was fortunate that it did prove possible to maintain a supply of foliage from E. globulus without any apparent variation in food quality.

The area from which samples of eucalypts were drawn in the present study is shown in figure 12. As far as was possible all the leaves of each type were taken from a single tree. This was achieved for 10 of the 17 foliages used. However when the amount of suitable leaf material was limited other trees which were known or appeared to result from the same planting (and hence seed source) were used. There were two exceptions to this; mature leaves of E. globulus were collected from a wider area because of the large quantities used, and the juvenile foliage of this species was taken when required from the trees being used at that time.

Because of the difficulties in eucalypt identification, leaves from trees growing at different sites were classified separately until their identity was positively established. This practice soon showed that leaves of the same species of gum tree collected from different sites could produce very different results when fed to P. charybdis. The abbreviations used to identify each source are shown in figure 12.



KEY:

1 Head-of-the-harbour - initial P. charybdis from here.
2 E. ficifolia - several trees.
3 BG = The Botanic Gardens, Christchurch - E. alpina, E. cordata, E. delegatensis-hybrid, E. fastigata, E. leucoxylon, E. linearis, E. obliqua.
4 E. andreana.
5 T = Tai Tapu - 2 trees of E. linearis; N = north; S = south.
6 E. viminalis.
7 Ch = The Chamberlain Farm near Burnham - E. globulus, E. linearis, E. obliqua.
8 SP = The Selwyn Plantation Board - E. linearis.
9 Cl = Mr Clarke's Farmlet - E. camaldulensis, E. fastigata, E. globulus.
10 RS = Rolleston Station - E. obliqua.
11 E. amygdalina.
12 Rf = Rolleston farmyard - E. obliqua.
13 E. sideroxylon.
14 E. macarthuri.

Sites not numbered indicate sources of E. globulus.

Figure 12. Map of the Christchurch area showing the sites used in this study.

(4) The experimental design

The limitations imposed by the characteristics of the study material outlined above clearly prevented the use of a balanced overall experimental design. Instead a flexible programme was followed which it was hoped would allow maximum use of the egg supply produced at any one time as well as permitting valid comparisons between foliages with a temporal separation of their growth seasons. Its flexibility had the added advantage that improvements in handling techniques could be incorporated. This meant that the number of cages in each trial could be increased as time progressed, provided the egg supply was not limiting.

The first season of controlled rearings was begun using trials of three or four cages each containing foliage from a different species of eucalypt, but with one cage in each trial containing the reference foliage. This control was the mature leaf-form of E. globulus. Reproducability had been found to be high during the preliminary large-cage rearings but a check on this was made by including a trial of four cages all reared on the control foliage. The results of this indicated that unreplicated cages should prove adequate provided that all foliages showed the same high degree of consistency. The last two trials in the first season consisted of five cages one of which was the control, and with duplicated cages of the other foliages being tried. Triplicate replication of the experimental foliages was used for the first three trials of the second season and the within-trial variation in the measurements taken appeared only little greater than for the control. However the supply of eggs then decreased and for the remainder of the second season each trial consisted of a control cage plus a single experimental cage.

During the third season all cages including the control were duplicated and the number of cages per trial varied from 10 to 16. The replication of all foliages including the control was felt necessary because in the last trial of the second season the control cage was seriously affected late in the rearing by an unknown influence. This cage has been ignored in the calculations that follow. In the second trial of the final season two cages on different experimental foliages suffered complete mortality while the other member of these pairs underwent only the small mortalities normal to the particular test species of Eucalyptus. Check rearings were carried out to determine the cause of the mortality. Crude suspensions of the corpses from each affected cage smeared onto young mature leaves of E. globulus and fed to first-instar larvae did not cause any excess mortality. However larvae reared on untreated leaves of this control foliage in the original plastic cages died, even after the cages had been washed thoroughly using soap and water. Thus it was felt that the mortality was caused, not by a viral infection, but by an unknown chemical contamination of the plastic. These containers were destroyed.

During each of the three seasons of research a trial was found which within 48 hours of initiation had such high mortalities on all foliages including the control that it was abandoned. In each case the number of active unfed larvae available had been limited and some slightly less active larvae had also been used. Thus the control foliage also served to monitor the vitality of the first-instar larvae used.

2. RESULTS AND ANALYSIS - INTRODUCTION

The parameters chosen to assess the ability of the larvae of Paropsis charybdis to use each eucalypt species as food were the survival of the larvae, the duration of the larval and pupal stages, and the weight of the pupae produced. During the preliminary rearings in large cages an attempt was made to assess the progressive weight gain of the larvae by weighing them in groups of ten every 4 days. This method was discontinued because it was inaccurate and involved too much effort for the small amount of information that could be obtained. Subsequently, in the controlled rearings, only the pupae were weighed and these measurements were used as an indication of the size reached by larvae fed exclusively on any foliage.

For each species of Eucalyptus used in rearing the larvae of Paropsis charybdis the following information has been obtained; the survival rate from unfed first-instar larvae to imagos, the length of time taken for these unfed, just-hatched larvae to become pupae and then adults, and finally the size of pupae attained. These three measurements reflect in an interdependent way the combination of both the food quality of the foliage and the ability of P. charybdis larvae to convert the food into their own requirements. Therefore these involve the sum of the nutritional value of the foliage when compared with the insects' requirements, the presence of any antibiotic or antifeeding factors, and the ability of the larvae to recognise the foliage as a source of food and consume it. The three parameters measured are considered separately below.

3. SURVIVAL

In this section the overall survival rate from unfed

first-instar larvae to adulthood will be considered and then this survival will be broken down to show the mortality occurring during each separate stage of preadult life.

(1) Analysis

The analytical scheme used to allow for the flexibility of the experimental design, was evolved with the help of Mr A. Wallace of the Applied Mathematics Section of the Department of Scientific and Industrial Research.

The survival rate is a group characteristic and so a single value resulted from each cage. For the purpose of analysis the result from each cage was considered as an independent measurement of survival rate regardless of whether the cages were from different experimental runs or were replicates within a run, and the results for each foliage were grouped. This was done after preliminary analyses were carried out on the results from the mature foliage of E. globulus. There was a total of 27 rearings using this, the control foliage, and the next largest total was 12 for the juvenile leaf-form of E. globulus. The remaining foliages ranged from a total of nine rearings down to one. The overall number of foliages used was 17, involving 12 distinct species of Eucalyptus. The remainder comprised the two very distinct leaf-forms of E. globulus, two species sampled at two different sites and one species sampled at three sites. Wherever possible all the leaf material of each foliage type was taken from a single tree.

The first step of the preliminary analyses was a graphical check on normality. The cumulative frequency expressed as a percentage of total cumulative frequency was plotted on linear probability paper against the survival rate expressed as a percentage of the initial number of larvae per cage and

also plotted against the survival rate when this percentage had undergone the angular transformation (Appendix, figure 1). Only the transformed data approximated a straight line when so graphed demonstrating that this transformation was needed to convert the distribution of the data into a pattern that closely approached the normal distribution. All subsequent analyses of the survival rate have been performed on data so transformed.

Next a check was made to determine whether or not the survival values when placed in a time sequence could be considered to follow a random order. If the progression could not be considered random, such as would occur if handling improvements due to, say experience, altered the survival rate in a regular way, one of the basic assumptions of an analysis of variance (that of the independence and randomness of the error terms) would not hold. This was simply checked using a sign test about the median, in which the survival values, in time sequence, were assigned plus or minus signs depending on whether they were greater or less than the median value. The number of sequences of like signs was compared with tabulated critical values (Rohlf and Sokal, 1969, Table BB). In this case the number did not deviate from that which could be expected in a series of randomly arranged objects at a 5% probability level (Appendix, Table 1).

The results from rearings on the mature foliage of E. globulus were next subjected to an analysis of variance using the design of a two-level nested analysis with unequal sample size (Appendix, Table 2). This verified the simple sign test about the median recorded above, but primarily showed that even at the 5% probability level there was no significant increase in variance either between the three seasons or

between the trials within each season compared to the variance between replicated cages within single trials. Thus the survival rates obtained from trials at different times and even at different seasons could be validly compared.

The final stage in the preliminary analysis was to check that the variances of the grouped data for each foliage were comparable. For this, a Bartlett's Test of the Variances was carried out on all foliages having five or more results. This showed that there was no significant difference between foliages (Appendix, Table 3).

(2) Results

Table 10. The survival data obtained for each foliage

Foliage	Sample Size	Mean (Transformed Data i.e. degrees)	Mean back-transformed to %
E. obliqua(Rf)	2	90.00	100.0
E. camaldulensis	2	84.23	99.0
E. amygdalina	6	81.91	98.0
E. perriniana	2	81.79	98.0
E. macarthuri	1	78.46	96.0
E. globulus-mature	27	77.69	95.5
E. andreana	5	74.34	92.7
E. linearis(Ch)	8	71.16	89.6
E. sideroxylon	5	70.30	88.6
E. delegatensis-hybrid	7	69.96	88.3
E. globulus-juvenile	12	63.38	79.9
E. obliqua(Ch)	7	58.74	73.1
E. obliqua(BG)	9	44.00	48.3
E. linearis(BG)	6	36.17	34.8
E. ficifolia	9	2.56	0.2
E. fastigata(BG)	4	0	0
E. fastigata(Cl)	4	0	0

Having thus showed that the assumptions implicit in an analysis of variance held, the final analysis was performed as a one-way analysis of variance with unequal sample sizes. The analysis involved a total of 116 rearings spread amongst the 17 different foliage types. Table 10 gives sample size (number of rearings) and the mean survival rate for each foliage. It shows that although survival was very good on the majority of Eucalyptus foliages used, some types gave markedly inferior results. The complete range from all to none surviving was represented. One feature of interest is the marked variation between different trees of the same eucalypt species as shown by the two trees of E. linearis and the three of E. obliqua included in the rearings.

Table 11 summarises the result of the main analysis which showed a highly significant increase in variance when larvae were reared on the foliage of different eucalypt species.

Table 11. Analysis of variance table of survival data (transformed data).*

Source of variation	df	SS	MS	Fs
Among foliage types	16	82886.7	5180.4	61.9***
Among cages	99	8280.9	83.7	
Total	115	91167.6		

$F_{.01(16,80)} = 2.24$

(* In this and all other analysis of variance tables the following abreviations have been used - df = dgrees of freedom, SS = sum of squares, MS = mean square, Fs = variance ratio; similarly, ns, *, **, ***, refer to the significance of the result obtained and mean not significant, and significant at the 5%, 1% and 0.1% probability levels respectively.)

To investigate this variation further several comparisons of the foliage mean survival rates were made on the basis of 'a priori' considerations, and then an 'a posteriori' test was carried out comparing each foliage mean with every other one.

The 'a priori' tests are summarised in Table 12. The method followed was that given by Sokal and Rohlf (1969, p233). The tests are not orthogonal because of the inclusion of number 5 which is a logical alternative to 4. Also number 5 does not cover all the species of Eucalyptus used.

Table 12. 'A priori' comparisons of the survival rates of larval P. charybdis reared on different Eucalyptus species.

Test	Comparison	Significance level of differences between means
1	E. globulus, mature v juvenile leaf-forms	***
2	Among the three trees of E. obliqua	***
3	Between the trees of E. linearis	***
4	Among botanical groupings of the trees	***
5	Among Dugdale's groupings of the trees on the basis of damage done:- Group 1 E. globulus; E. macarthuri Group 2 E. fastigata; E. obliqua Group 3 E. camaldulensis; E. ficifolia; E. linearis	***

The first of these tests showed that there was a highly significant difference between the survival of larvae of P. charybdis reared on the two foliage types (adult and juvenile) of E. globulus. This is not surprising considering the very obvious physical differences between these leaf forms, in particular the highly glaucous nature of the juvenile foliage.

The tests also verified that different trees of the same species could show highly significant differences. This result is interpreted as reflecting the variable genetic material constituting the species involved. This is in line with clonal variations in insect resistance and other characteristics of gum trees. The genetic nature of at least some of this varia-

tion is clearly shown by the research of Green (1971) into the differences within E. obliqua.

The last two tests were carried out to see whether or not either grouping scheme - taxonomic or on the basis of damage susceptibility - related to the pattern of survival rates observed. The highly significant differences between the groups of means so arranged indicates that nether scheme is superior to the other in explaining the differences and that both segregate the means to some extent. Closer examination of the means themselves however shows that there is considerable variation within the derived groupings. This severely limits the usefulness of either scheme in explaining or predicting differences in mean survival for P. charybdis fed any particular species.

This section of analysis was completed by performing a set of 'a posteriori' tests to compare the mean for each foliage type with every other mean (the two identical results for E. fastigata were grouped for this). The method used was the sum of the squares simultaneous test procedure (Sokal and Rohlf, 1969, p237) attributed to Gabriel (1964). This method is exact for equal and unequal sample sizes. The allowance incorporated because the tests are 'a posteriori' and serving to minimise the error of declaring samples from similar populations different, appears in this case to overcompensate and in fact to greatly increase the error of declaring samples from different populations 'not different'. For instance, the comparison of the results from the two leaf-forms of E. globulus is now not significant whereas in the 'a priori' test the difference between these two means was highly significant. For this reason the results in Table 13 show the actual test value calculated as well as indicating the significance of

Eucalyptus species														
	obliqua(RF)													
camaldulensis	33	camaldulensis												
amygdalina	368	8	amygdalina											
perriniana	67	6	0	perriniana										
macarthuri	89	22	10	7	macarthuri									
globulus-mature	287	82	92	33	1	globulus-mature								
andreana	351	140	426	79	14	44	andreana							
linearis(Ch)	568	274	667	181	47	254	31	linearis(Ch)						
sideroxylon	555	277	638	189	56	224	41	2	sideroxylon					
delegatensis-hybrid	625	317	732	218	63	323	56	5	0	delegatensis-hybrid				
globulus-juvenile	1215	745	1374	581	210	1674	424	290	169	191	globulus-juvenile			
obliqua(Ch)	1520	1010	2004	826	340	1971	709	575	389	440	95	obliqua(Ch)		
obliqua(BG)	3416**	2607*	5359***	2298	1049	7468***	2897*	3052**	2170	2590*	1870	820	obliqua(BG)	
linearis(BG)	4347***	3465**	6548***	3122**	1533	8418***	3974***	4198***	3177**	3689**	2963**	1647	239	linearis(BG)
ficifolia	12510***	10913***	22936***	10270***	5184***	37980***	16557***	19926***	14746***	17883***	19021***	12427***	7843***	4064***	ficifolia
fastigata	12960***	11352***	23274***	10702***	5472***	37139***	17003***	20252***	15205***	18271***	19282***	12883***	8316***	4484***	28

(* = P .05, ** = P .01, *** = P .001; Critical values $P_{.05}$ = 2342, $P_{.01}$ = 2931, $P_{.001}$ = 3717)

Table 13. The results of comparing the mean survival rate of each foliage with the mean of every other foliage using the sum of squares simultaneous test procedure.

such a value. In this way those that approach the significance levels set can be seen as well as those that exceed them.

Table 13 shows that between most of the foliages tested the survival rates were not significantly different. However in view of the small sample size for many foliages this is not surprising. (Only two means are based on more than ten measurements of survival rate.) The two foliages, E. fastigata and E. ficifolia, on which an almost complete mortality was obtained, were highly significantly different from all other foliages but not different from each other. The foliages giving the next two lowest survival rates, E. linearis(BG) and E. obliqua (BG), differ at various levels of significance from most other means. The exceptions are either high value means based on very small sample size (E. macarthuri, E. perriniana) or lower values closer to the two means under consideration.

4. AN ANALYSIS OF MORTALITY BY INSTARS.

In this section the overall mortality will be broken down into the components occurring at each phase in the life cycle of Paropsis charybdis in an attempt to discover whether mortality is more likely to occur in any particular stage.

Table 14 shows the number of individuals surviving on each type of leaves at the beginning of each stadium. Insects that died once a moult had been initiated were considered to have survived the stadium preceding the moult and so were included in the mortality figures for the stadium after the moult. In Table 14 the number of these insects dying during ecdysis is also shown separately in brackets between the stages of the life cycle involved. There is one exception to this. Adults dying during emergence are recorded in a distinct category.

Table 14 shows the actual number of larvae used in

	Eucalyptus species															
	obliqua(Rf)	camaldulensis	amygdalina	perriniana	macarthuri	globulus-mature	andreana	linearis(Ch)	sideroxylon	delegatensis-hybrid	globulus-juvenile	obliqua(Ch)	obliqua(BG)	linearis(BG)	ficifolia	fastigata
Initial number	50	50	149	50	25	675	123	199	125	175	302	175	225	150	225	200
No. to instar 2	50	50	(1) 146	(1) 50	25	(3) 654	(1) 116	(1) 186	122	(1) 161	(3) 256	(2) 146	(4) 124	(9) 134	14	0
No. to instar 3	50	50	145	49	25	647	114	183	119	159	251	(1) 134	118	(2) 64	8	0
No. to instar 4	50	50	145	48	(1) 25	645	113	182	115	157	247	131	116	57	2	0
No. to prepupa	50	50	145	48	24	644	111	182	115	157	247	131	115	57	2	0
No. to pupa	50	50	(1) 145	48	24	635	109	181	113	155	242	131	(1) 114	56	2	0
No. to emergence	50	50	144	48	24	635	109	180	110	153	240	128	112	55	2	0
No. to imago	50	49	143	48	24	629	108	173	107	153	238	127	110	54	2	0

Table 14. The survivorship of Paropsis charybdis larvae reared on different species of Eucalyptus (numbers in parentheses show mortalities at ecdysis).

rearing. A greater portion of the mortality occurred during the first instar than at all other stages combined. Excluding E. ficifolia and E. fastigata, 460 larvae died out of the total of 2473 first-instar larvae used in rearing. Of these 253 died during the first instar, 112 during the second, 27 during the third, 26 during adult emergence, 22 as prepupae, 15 as pupae and only 5 during the active phase of the fourth instar. The behaviour underlying this mortality of the very young larvae was shown by the observation that many first-instar larvae did not settle properly when placed on the foliage and that frequently they left the leaves and could be seen on the sides or roof of the cages as in figures 14 and 15. (p 115, 116).

A further point arising from the results shown in Table 14 and also apparent during rearing was the number of larvae fed E. linearis (BG) that died during ecdysis. Nine died during the moult between the first and the second instar and two more between the second and third instar. Excluding this foliage, E. ficifolia and E. fastigata, 19 Paropsis died during larval ecdyses (before pupation) out of a total of 323 deaths. Using this fraction (19/323) as the population parameter delimiting the proportion of larval deaths occurring during ecdysis it would be expected that 5.53 of the 94 larvae dying on E. linearis (BG) would die in ecdysis and 88.47 between moults. Comparing these anticipated results with those actually observed using a chi-squared test gives a probability between 0.01 and 0.025 that the result for E. linearis (BG) could be a random deviation from the population parameter. Thus the number of deaths during ecdysis on E. linearis (BG) can be considered significantly different from the number for other foliages at the 5% level. An explanation for this mortality during moulting might be that the level of available nutrients

in this particular foliage is close to the minimal levels for growth and development with respect to one or more factors. Thus those larvae commencing ecdysis in less than optimal condition would find that bodily reserves of these particular factors are insufficient to cope with the additional stresses at this time of active metabolism when feeding is not possible. A further observation supporting this idea of inadequate nutrition was that the feeding period of the larvae on E. linearis(BG) was very protracted.

The numbers of P. charybdis surviving at each step in the life cycle when the larvae were reared on the different Eucalyptus species were shown in Table 14. However because of the differing number of replicates and varying mortality rates the actual numbers of each stage present on each foliage varied widely. To express these stadial mortalities with respect to a common base, they have been recalculated as the percentage of the number of insects alive at the beginning of each phase in the life cycle. The low mortality of many of the foliages and the very similar mortality patterns for these has been used as a basis for grouping the results (Table 15).

The two most interesting features of the mortality patterns shown in this table are, the importance of the first instar in determining overall survival on any particular foliage, and the pattern obtained for E. linearis(BG) in which the mortality during the second instar made the greatest contribution to the overall mortality. The situation on this foliage is the only real exception to the deciding importance of the first instar in determining the overall mortality because the high mortalities recorded for the second and third instars of E. ficifolia reflect the low number and sickly

nature of larvae surviving beyond the first instar on this foliage. The mortality during the first instar for insects fed E. linearis(BG) was lower than that for several foliages having much lower overall mortalities. Larvae fed E. linearis(BG) developed very slowly and did not appear particularly vigorous. However a fair proportion still did manage to complete development.

Table 15. The patterns of larval and pupal mortality for Paropsis charybdis fed various Eucalyptus species (mortality expressed as a percentage of the number alive at the start of each stage in the life cycle).

Eucalyptus foliage groups	Stage of the life cycle						
	Instar				Pre-pupa	Pupa	Emergence
	1	2	3	4			
amygdalina andreana camaldulensis delegatensis-hybrid globulus-mature linearis(Ch) macarthuri obliqua(Rf) sideroxylon	3.76	1.22	0.71	0.26	0.98	0.46	1.32
globulus-juvenile obliqua(Ch)	15.72	4.23	1.82	0	1.32	1.34	0.82
obliqua(BG)	44.89	4.84	1.69	0.86	0.87	1.75	1.79
linearis(BG)	10.67	52.24	10.94	0	1.75	1.79	1.82
ficifolia	93.78	42.86	75.00	0	0	0	0
fastigata	100.00	0	0	0	0	0	0

The mortality that occurred during the transition from an actively feeding fourth-instar larva to an adult was looked at more deeply because the stresses of metamorphosis would tax the food reserves available at this time and possibly point out any inadequacies in the foliages eaten. Table 16 gives the mortality of different stages of this transition.

Some explanation is called for to define how the steps in this segment of the life cycle were recognised. The prepupa was recognised by its shape and behaviour. Larvae were considered to have died during this stage if no sign of ecdysis could be observed. However if the prepupal period had been unusually prolonged the body was dissected to check for the formation of a pupa within the prepupal skin. The insect was considered to have died in moult if the prepupal skin had not been shed and if the pupa inside showed no sign of development. If the pupa had begun to develop while still encased in the prepupal skin, the insect was classed as a 'pupa within a prepupa'. The next two categories - mortality during the pupal period and during the emergence of the adult - are self explanatory, except that those adults emerging so malformed as to be considered unviable were included as mortalities during emergence. Other individuals counted as dying during emergence were the six which developed through the pupal phase still encased in the larval head capsule. These are shown by a 'c' superscript in the appropriate column in Table 16.

Table 16. The mortality associated with the prepupal and pupal stadia for P. charybdis reared on different eucalypt foliages.

Stage at which mortality occurred	Eucalyptus species											Total
	camaldulensis	amygdalina	globulus-mature	andreana	linearis (Ch)	siderоxylon	delegatensis-hybrid	globulus-juvenile	obliqua (Ch)	obliqua (BG)	linearis (BG)	
Prepupa	-	-	9	2	1	1	2	5	-	1	1	22
Prepupa/pupal moult	-	1*	-	-	1	2*	-	1*	-	1	1*	7
Pupa within prepupa	-	-	-	-	-	1*	-	-	-	1	-	2
Pupa	-	-	-	-	-	1	2*	1	3	1	-	8
Emergence	1*	1[c]	6[cc]	1	7*	3*	-	2[c]	1[c]	1	1[c]	24
Total	1	2	15	3	9	8	4	9	4	5	3	63

(* = fluid lost during pupation,

c = larval head capsule not shed.)

An asterisk indicates a mortality which had lost sufficient fluid during pupation to markedly stain the floor of the pupal cell involved. Several insects that lost fluid at this occassion survived successfully but most died or developed into malformed adults.

The table shows that more P. charybdis died during the emergence of the adult from the pupa than at any other of these stages. The cause of this is unknown, but the conditions under which the pupae were kept probably had as much, if not more, influence on the results than the foliage that they had been reared on as larvae. However the results of those raised on the foliage of E. linearis (Ch) do appear to have been affected by the larval food. A chi-squared test

showed that the number reared on this foliage and dying during emergence differed significantly from the accumulated results for the other foliages ($\chi^2 = 8.96$, df = 1, $p < .005$).

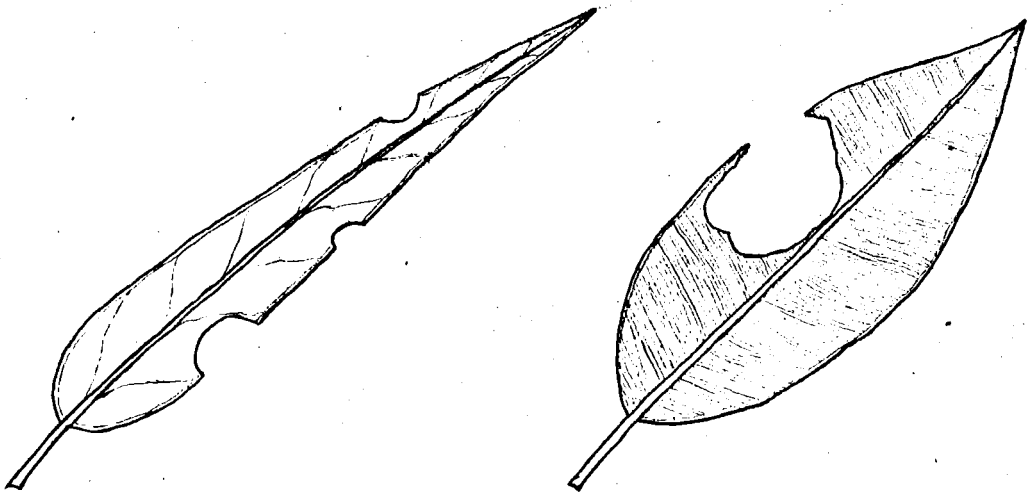
The results for the mature foliage type of E. globulus were also tested from the remaining foliages and found to be significantly different from what could be expected on the basis of the combined other results ($\chi^2 = 11.36$, df = 4, $p < .025$). It is possible that foliages which formed a moist frass would increase the mortality of prepupae, because the larvae at this stage left the foliage and were to be found amongst the frass. The frass from both E. globulus foliage types was noticeably damper than others, but that of the juvenile E. globulus foliage was the more moist. The total number of mortalities on each foliage is low however, and so conclusions must be drawn warily. Similar caution must be made in examining the significant difference found for E. linearis (Ch). It is interesting to note that E. linearis (Ch) is the same species, although from a different tree, that was found to give an unusually heavy mortality during larval moulting. It is possible that both these variations from the typical pattern are due to the same factor in the nutrition of P. charybdis.

5. E. FICIFOLIA AND E. FASTIGATA

These two eucalypt species were examined more closely in an attempt to determine what caused the high mortality noted in larval rearings.

E. ficifolia was, as was described earlier in the section on the genus Eucalyptus, the sole member of the eucalypt series Corymbosae-Peltate used in rearing; in fact

it was the only representative of the subsection Longiores used. The young growing leaves of this gum tree are not particularly tough or fibrous but they are covered on both surfaces by a thick elastic cuticle. The feeding notches made in these leaves by young Paropsis larvae frequently showed a small white fringe along the eaten edge where the cuticle extended beyond the inner layers of leaf tissue. It was felt that this was more probably due to the larvae being unable to bite as easily through the cuticle as through the inner tissue rather than a retreat of these inner layers due to rapid evaporation following damage to the cuticle. On some leaves the edges were marked as if they had been bitten without penetration through the cuticle. It was also noticeable on this foliage that the feeding notches were fewer but larger than on others, and that there often were more than one larva feeding at the one site. Multiple use of the one feeding site was not uncommon for young larvae on any foliage but appeared more marked on E. ficifolia. Another feature of the feeding damage not confined to E. ficifolia but more prevalent on this eucalypt, was that once a break had been made in the leaf edge, the feeding notch was increased in width without removing much more of the leaf edge. This left a narrow strip of leaf rim projecting along the outer limits of the eaten area, which is depicted in figure 13 and can also be seen in figure 14. Thus the mortality resulting from feeding young larvae on E. ficifolia was considered primarily due to the physical characteristics of the leaves.



E. globulus-mature E. ficifolia

Figure 13. The different patterns of feeding damage on E. ficifolia and E. globulus-mature leaves.

The other species giving a very high mortality, E. fastigata, belongs in the series Pachyphloiae. Two other species from this same series were used in the present work, E. alpina and E. obliqua, and the latter is particularly closely related to E. fastigata. The foliage of three different trees of E. obliqua was used in larval rearing and each produced different results ranging from very good to very poor. Survival on the worst was very low approaching that of E. fastigata. It is highly possible that the same factor was operative in both these eucalypts.

All first instar larvae died when placed on E. fastigata foliage. Many of the larvae fed before dying but none appeared to increase in size, and very little frass was produced. Over half the larvae died while they were still on the foliage so that larvae of P. charybdis could not be said to be repelled by E. fastigata. There was no obvious physical factor impeding feeding and so the mortality was considered due to chemical factors. The lack of continued feeding followed rapidly by death

would also appear to indicate the presence of a toxic substance. It is possible that the damage could have been caused by the larvae biting the leaves without actually consuming any, because starved first-instar larvae died almost as rapidly. If so such starvation could be brought about by the presence of a feeding inhibitor or the lack of a specific feeding stimulant, if the larvae fed using a behaviour pattern based on the taking of trial bites (as for example is common in grasshoppers (Mulkern, 1967)). However neither of these two alternatives appears likely in view of the fact that some young larvae placed on E. fastigata were noticed to take a whole series of bites without pause. As well the damage indicated that the feeding, although not protracted, was not by single bites (Figures 14 and 15).

Figure 14 shows massed rearings of about a hundred first-instar larvae per cage on E. fastigata and E. ficifolia. Figure 15 is a close-up view of the larvae on E. fastigata foliage. Note the shallow feeding along the edge of the leaves of E. fastigata compared to the deeply indented feeding notches on E. ficifolia. In this short observation trial the greater density of first-instar larvae gave a higher survival to the second instar for the larvae fed on E. ficifolia leaves.

This lethal effect of E. fastigata on P. charybdis was first reported by Styles (1969, 1970) and Carne (1967) using the leaves from young regeneration trees. In the present work four rearings used young mature leaves from one tree, while the remaining four used a mixture of semi-epicormic and mature growth taken from a second site. There was no apparent difference between the mature and epicormic foliages

Figure 14. Massed rearings of first-instar larvae on E. fastigata(BG) and E. ficifolia on the left and right respectively. Note the cages used.

Figure 15. A closer view of first-instar larvae on E. fastigata.

in the latter case.

The short period of active growth of E. fastigata hindered a more detailed study into the cause of the larval mortality on this foliage. That the factor is relatively specific and does not cause general mortality to a wider group of insects is shown by the fact that the trees of E. fastigata(C1) were attacked by an unidentified psyllid which at the same site was also common on E. globulus and E. camaldulensis; while the single tree of E. fastigata in the Botanic Gardens, Christchurch, was attacked by the scale Eriococcus coriaceus Maskell which shares several hosts with P. charybdis, in particular E. globulus, E. obliqua and E. linearis.

After the original discovery of the effect, Styles (1969, 1970) stated that;

"Larvae would not feed on the foliage of Eucalyptus fastigata Deane et Maiden collected locally from natural regeneration trees..... First instar larvae died without feeding and larger larvae, although they sometimes fed, died within 48 hours."

This referred to laboratory rearings. In Carne (1967) the following comment was made, presumably referring to the same work;

"Under field conditions, all first-instar larvae placed on E. fastigata foliage died within 6 days, none moulting to the second instar. In the laboratory, larvae of all instars were offered foliage of E. fastigata: all fed to some extent but became sickly in appearance and none survived to pupate."

Several short trials carried out during the present work in which larvae were fed for some time on the control foliage and then transferred on to E. fastigata or E. ficifolia gave very variable results. In the first of these three cages were set up each of which contained 10 small second-instar larvae of between 8.5 and 11.0 mg in weight.

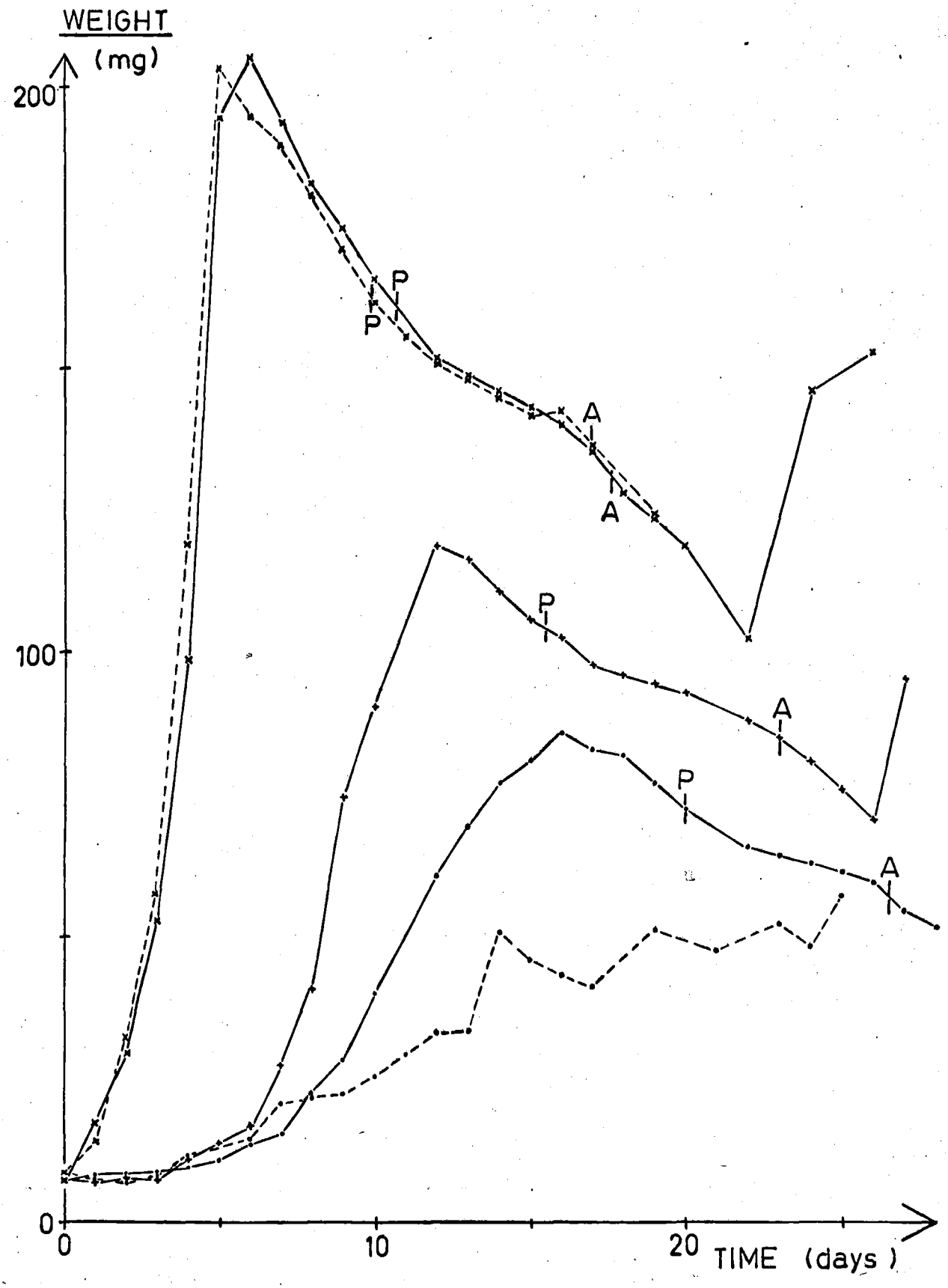
(For comparison, unfed first-instar larvae weighed about 1 mg each - 15 weighed individually averaged 0.96 mg.) One cage was reared on the control (the mature leaves of E. globulus), one on E. ficifolia and one on E. fastigata (C1). All the larvae survived through the subsequent moult and nine reared on the control, nine on E. ficifolia and six on E. fastigata (C1) eventually pupated. Those fed the two experimental foliages took longer to reach maturity, pupating on average 1.9 days and 2.4 days after the control (for E. ficifolia and E. fastigata (C1) respectively).

A second trial was carried out beginning with slightly smaller larvae (between 7.0 and 8.0 mg in weight) and using E. fastigata (BG) as well as the three foliages included in the first trial. None of those fed E. fastigata (C1) managed to reach the third instar; three died within 24 hours of the start of the experiment, six more within a further 24 hours and the last between 72 and 96 hours. Again nine of the control larvae but only two of those fed E. ficifolia and one fed E. fastigata (BG) managed to attain adulthood. The average time to adult emergence (and also to pupation) on these two trial foliages was respectively 4.8 and 8.8 days longer than for the control. This trial was repeated using second-instar larvae initially weighing between 8.1 and 9.0 mg. Nine of the control larvae became pupae and eight emerged as adults. All ten fed E. fastigata (C1) and E. ficifolia died within 4 days, whereas two reared on E. fastigata (BG) reached the fourth instar. However they were still larvae after 21 days by which time the control insects were all adults. Neither were large enough to be able to attempt pupation, and because of a decreasing supply of young leaves the trial had to be terminated.

These two larvae were then fed on the control foliage for 11 days. Neither pupated; one died after 6 days and the other increased its weight from 55 to 80 mg and then decreased this again to 50.2 mg. This last trial was unfortunately complicated by a scarcity of the most succulent leaves of all the experimental foliages.

The larvae in both the second and third trials were weighed individually every 24 hours. The daily mean results for those foliages on which the larvae survived for more than 4 days are presented in figure 16. Results from the second trial are shown using solid lines; those from the third using dashed lines. Larvae fed on the control foliage - the mature leaves of E. globulus - gave very similar results in both trials. They underwent a very rapid exponential growth during the short feeding period followed by a gradual decline which was slightly more rapid before and after than during the pupal stadium. The final rise in the results from the control foliage and from E. ficifolia in the second trial occurred when the adults were fed.

The two cages fed E. fastigata(BG) and one fed E. ficifolia gave very similar results for the first 6 days with a negligible change in weight over the first 3 days and than a slight increase. After this the results diverged with those from E. ficifolia increasing the most rapidly although much less steeply than those from the control. The maximum mean weight for E. ficifolia was also only just over half the highest attained on the control. The results from E. fastigata(BG) during the second trial conformed to the basic pattern shown by all foliages but with an even slower increase to a smaller maximum. The cage in the third trial which was fed on this foliage produced erratic results.



KEY: -x—x- E. globulus-mature — — Trial 2 P = pupation
-+—+- E. ficifolia ----- Trial 3 A = adult emergence
-•—•- E. fastigata

Figure 16. The mean weight of P. charybdis when reared from the second instar on E. fastigata(BG) E. ficifolia and E. globulus-mature.

As was described earlier neither of the two larvae which survived beyond the third instar in this cage pupated, although both had reached the fourth instar by day 12. The final period (from day 21) during which the larvae were fed on the control foliage did not produce a regular but a fluctuating weight change. The greatest increase in the average weight of these larvae occurred between the thirteenth and fourteenth days and was suspected to be brought about by the accidental inclusion of some leaves of E. viminalis among the foliage used.

The average times of pupation and of adult emergence for each cage are shown in figure 16 by the letters 'P' and 'A' respectively.

Thus it can be seen that the absolute mortality experienced with E. fastigata in rearings beginning with unfed first-instar larvae was not repeated when older larvae were placed on leaves of this species, contrary to what has been recorded in the literature. The variability of the few results obtained precludes a definite interpretation but they appeared to indicate the presence of a toxin in marginal quantities so that the more vulnerable first-instar larvae were the most adversely affected. An attempt to develop a bioassay using extracts of E. fastigata impregnated into discs of E. globulus failed to give any definite results.

The results for E. ficifolia show that even the larger second-instar larvae were affected but to a lesser extent than first-instar larvae.

6. THE DURATION OF THE LARVAL AND PUPAL PERIODS.

As was described in section 1 of this chapter, larvae which had finished feeding and entered the prepupal phase were

removed from the larval rearing cages and placed individually in pupal cells. These were checked every 24 hours and the dates by which each larva had become a pupa and then an adult were recorded. This gave for every surviving insect a measure of the total time taken from unfed first-instar larva to adulthood, and also of the two stages comprising this total, the larval and pupal periods. Each of these three measurements will be considered separately.

(1) The Total Duration of Larval plus Pupal Stadia

(a) Preliminary Analysis. An analysis of variance was carried out on the individual data grouped for each foliage. This was as a two-way analysis separating the sexes, with unequal but approximately proportional numbers. An adjustment to give exactly proportional subclass numbers was involved.

Table 17. Analysis of variance for the total duration of larval plus pupal stadia.

Source of variation	df	SS	MS	Fs
Subgroups - total	29	11194.00	386.00	
- sex	1	14.09	14.09	2.90^{ns}
- foliage	14	11100.66	792.90	163.15^{***}
-interaction	14	79.25	5.66	1.16^{ns}
Within = error	2027	9854.71	4.86	

($F_{.05(14,1000)} = 1.70$; $F_{.01(14,1000)} = 2.09$;

$F_{.05(1,1000)} = 3.85$)

This method, because it was based on measurements from individual insects and not cage means, involved large sample sizes. It was hoped that this would help to discriminate between results from different foliages. The analysis showed that there was no significant interaction between the sex and the measurement of the total period and no significant differences

between the sexes, but that there was an extremely significant difference between results from the different foliages (Table 17). Because this was a Model 1 analysis the various subgroup mean squares were tested over the error mean square.

The only preliminary analysis that had been used on this data had been a graphical check on normality (a plot of the cumulative percent frequency on probability paper) which had shown that a logarithmic transformation was needed to improve normality. Before an in depth comparison of foliages was attempted further checks concerning the assumptions underlining the analysis of variance were carried out. The first of these was the sign test about the median outlined previously, which checked for the independence and the randomness of the results. When this check was performed on the results for the control foliage, the mature leaf-form of E. globulus, it unfortunately showed that this important condition did not hold. This meant that data obtained from rearings at different times could not validly be compared in the form in which they had been originally obtained. To correct for this variation a scheme of analysis was devised which would adjust the results for each run with respect to the control results for that run. Alternative schemes which involved recalculating the data as either the number or percentage of insects in each cage attaining maturity by the day on which a set percentage of those on the control foliage had also become adult were tried for three foliages (the juvenile form of E. globulus, E. obliqua (BG) and E. sideroxylon) but abandoned as impracticable because the results so obtained were extremely variable within each foliage or else consisted mainly of zeros. A similar approach - calculating the number or percentage of insects mature in each cage after a fixed time - was tried on

the control foliage. It too gave such a wide range of results (from 0 to 100%) that it was not continued with.

The first step in the approach eventually adopted was an analysis of variance of the results from the control foliage, the mature leaf-form of E. globulus, in order to determine the relative components of variation among and within experimental runs. This analysis was carried out on the results for each individual. Although the previous (but now discarded) analysis had shown no significant variation between the combined duration of larval plus pupal periods for each sex, the results for the component intervals - the larval and pupal periods - appeared to show distinct differences with regard to sex, and so this division was maintained in the present analysis. This separation was also necessitated by the unequal numbers of males and females in each cage. The logarithmic transformation was maintained to normalise the distribution which, before transformation, had been skewed to the right.

The results for the analyses for both sexes are given in Table 18.

Table 18. The results of a three-level nested analysis of variance on the data for the total duration of the larval and pupal periods for insects of each sex reared on the control foliage.

Source of variation	Male			Female		
	df	MS	Fs	df	MS	Fs
Among seasons	2	107.5	$(3.0)^{ns}$	2	98.8	$(2.8)^{ns}$
Among trials	16	36.4	$(11.4)^{***}$	16	35.2	$(18.8)^{***}$
Among cages	8	3.2	1.88^{ns}	8	1.9	1.04^{ns}
Error = within cages	279	1.7		294	1.8	

($F_{.05(2,16)} = 3.63$; $F_{.001(16,8)} = 10.8$; $F_{.05(8,\infty)} = 1.94$;

F results in parentheses are approximate values.)

Table 18 shows that there was no significant additional variance due to either variation among cages within trials or variation between results at the different seasons. However there was highly significant added variance among results from the different trials. This meant that the trial became the unit around which adjustments had to be made in order to reduce the results from separate trials to a common basis. Because the analysis involved unequal sample sizes, 'F'-values calculated directly from the mean squares were not exact and are shown in Table 18 in parentheses. The results are sufficient though to indicate very clearly the significance levels, and to demonstrate that the greatest source of variance was among the experimental trials.

The second stage in this analytical procedure was to check that the variance among cages within trials was similar for foliages other than the control. This was done for each sex by taking the differences between the mean results from

paired cages, that is from two cages of one foliage included in a single trial. These differences were pooled without regard to the foliage type involved to obtain samples of meaningful size, resulting in comparisons of results from all other foliages against those from the control. Variances for the control rearings were calculated in the same way for each sex. Results are shown in Table 19.

Table 19. Comparison of the variances of the differences between paired cages.

		Female	Male
Control foliage	df = 7	.26	.79
All other foliages	df = 28	1.44	1.44
Fs		5.5*	1.8^{ns}

($F_{.05(30,7)} = 4.4$; $F_{.01(30,7)} = 7.5$)

The comparison of variances was by a two-tailed test, dividing the greater by the smaller, which has been allowed for in determing the critical F-values. The variances from the data for males were not significantly different, but those from the females did exceed the critical value at the 5% level. This was not because the results for females reared on foliages other than the control were more variable than those for the males from these foliages, but because the variance for the females on the control foliage was considerably lower than the similar value for the males. However after considering the low level of significance obtained, the fact that only one of two tests was significant and the nature of the data used in the tests, it was felt that this slight increase in variance was not symptomatic of a major fault likely to invalidate the analytical approach adopted.

The third stage in the procedure was to adjust every cage mean for each treatment foliage with respect to the mean of the control foliage for that experimental run. Both additive and proportional adjustments were considered. Either could be supported by a consideration of the type of response of the insect, because the nature of the underlying cause of the fluctuations among the separate trials was unknown. However a plot of the grand mean versus the variance for each foliage and for each sex showed an apparent positive relationship, and on this ground a proportional change was chosen. This involved multiplying each cage mean by the ratio resulting from dividing the control grand mean (i.e. the mean of all control-cage means) by the mean from the cage in that particular trial reared on the control foliage. When there was more than one contol cage in a trial the average of the means of these cages was used as the basis for adjusting. This was carried out independently for the results for males and for females. The effect of the adjustment was tested by examining one aspect of the final analysis of variance. This verified that the added variance among trials was now non-significant for the foliages other than the control. After adjustment, the cage means which were based on fewer than five individual measurements were inspected, and discarded if they were the outlying values for that foliage.

(b) Final Analysis. The final analysis, testing for differences among foliages, was carried out as a two-part, one-way, unbalanced, nested design for each sex (Table 20). It was a two-part analysis because it was performed both with and without including the results on the control foliage. This

was because it was desired to compare the results from control rearings with those from every other foliage, and also the results from these other foliages each with every other. The decision to segregrate the tests in this manner was made because of the adjustment made to the results from all foliages other than the control, and because the results from the control - the mature leaf-form of E. globulus - were obtained from a greater number of trials carried out over a longer period of time than those from any other foliage.

Once again although the upper-level F-tests calculated in Table 20 are inexact their values are so high that they are very clearly highly significant, showing that the foliages do markedly affect the total duration of the larval plus pupal periods of Paropsis charybdis.

Table 20. The results of the analyses of variance comparing the duration of the combined larval and pupal stadia for insects of each sex reared on different Eucalyptus species.

Sex	Source of variation	All foliages			Excluding control		
		df	MS	Fs	df	MS	Fs
Female	Among foliages	13	55.4	(25)***	12	43.7	(28)***
	Among trials	59	2.22	1.8ns	40	1.56	0.96ns
	Among cages=error	22	1.16		15	1.62	
Male	Among foliages	13	58.1	(24)***	12	45.5	(23)***
	Among trials	59	2.42	2.12*	40	1.95	1.38ns
	Among cages	24	1.14		17	1.42	

($F_{.01(12,40)} = 2.66$; $F_{.05(40,15)} = 2.20$; $F_{.05(60,22)} = 1.89$

$F_{.01(14,60)} = 2.40$; $F_{.05(40,17)} = 2.11$; $F_{.05(60,24)} = 1.84$

$F_{.01(60,24)} = 2.40$)

The comparison of grand means of the foliages, each with every other, was carried out using Scheffe's method which applies to means based on unequal as well as equal sample

sizes. The general formula for this is a comparison of L/s_L against ..... $\sqrt{(a-1)F_{.05}}$, where L is any linear combination of the treatment means (i.e. $L = \sum_{i=1}^{a} \lambda_i \bar{X}_i$), S_L is the standard deviation of L, a is the number of treatments and $F_{.05}$ is the tabulated F value at the 5% level with the degrees of freedom (a-1), (Σn-a). In this particular case L reduces to the difference between the means for two foliages, $\bar{X}_i - \bar{X}_j$. For ease of calculation each side was squared and the final comparison was of

$$(\bar{X}_i - \bar{X}_j)^2 \text{ against } (a-1)\, F_{.05(a-1, \Sigma n-a)} S_L^2 \left(\frac{1}{n_i} + \frac{1}{n_j}\right)$$

(The final term $(\frac{1}{n_i} + \frac{1}{n_j})$ is a correction for unequal sample size. An estimate of the variance of L (S_L^2) is provided by the among-trials mean square from the appropriate analysis of variance.) This method is exactly equivalent to the sum of squares simultaeous test procedure used previously when comparing the survival rates on each foliage. However it is more easily calculated. The foliage means and sample size are given in Table 21 and the comparisons of means in Table 22.

(c) Discussion. The results in Table 21 show that even under constant conditions of temperature and humidity the duration of the preadult stages of P. charybdis could vary depending on the species of Eucalyptus on which the larvae feed. At 25.6 $\pm$ 1.2'C and approximately 65% relative humidity there was a range of mean values the equivalent of from 19.89 to 24.08 days for females and from 19.48 to 23.80 days for males. The transformation and coding of data has intensified the differences between the results from the various foliages. Most of the eucalyptus gave very similar results, between 19.9 and 21.0 days. Two species, E. amygdalina and E. sideroxylon, were noteworthy in that whereas most foliages having high

survival rates gave rapid development, these two had a good survival with slow development. This suggests that these provided nutrients of adequate quality but in marginally unsatisfactory quantities; either because the nutrient level of these leaves was low, or because the larvae were not eating enough of an adequate food to obtain sufficient nutrients as could occur with a deficiency of a feeding stimulant.

Table 21. The mean duration of the larval plus pupal period for each sex and for each foliage.

Foliage type	No. of cages	Mean span of the larval plus pupal period			
		Females		Males	
		Coded values	Days	Coded values	Days
globulus-mature	27	30.275	20.08	30.075	19.99
camaldulensis	2	29.855	19.89	29.990	19.95
perriniana	2	29.960	19.93	28.970	19.48
macarthuri	1	30.460	20.15	30.970	20.40
andreana	5	30.478	20.17	30.302	20.09
delegatensis-hybrid	7	30.504	20.19	30.080	19.99
linearis (Ch)	8	31.598	20.70	31.739	20.77
globulus-juvenile	12	32.042	20.91	31.896	20.84
obliqua (Rf)	2	32.130	20.96	31.515	20.66
obliqua (Ch)	7	34.937	22.35	34.874	22.32
amygdalina	6	35.705	22.75	35.062	22.42
sideroxylon	5	36.428	23.14	36.044	22.93
obliqua (BG)	7*	37.707	23.83	37.651	23.80
linearis (BG)	4	38.158	24.08	37.505	23.72

(* for males n = 9)

Table 22 gives the significance level of each test of the results from one foliage against another. Once again the small size of many samples limits the probability of discriminating statistically between the mean results for the various eucalypts. In this respect it is unfortunate that the unbalanced design, resulting from the elimination of outlaying cage means based on fewer than five individuals, does not permit the results to be analysed as a two-factorial experiment. This would have resulted in doubling the sample size for each foliage and hence would have raised the discriminatory power of the analysis. Table 22 simply verified what was apparent from the means listed in Table 21 - that there was a group of nine foliages giving rapid development that cannot be differentiated, and a group of five poorer foliages differing from the others almost entirely. This last group showed no distinct differences within itself except that the best differed significantly from the worst two. In general the results for females were more distinct than those for males, presumably due to a smaller variance among the results from each eucalypt for the females.

(2) The Duration of the Pupal Stadium

The measurements of the larval plus pupal period analysed in the preceding section could be separated into two distinct portions, that is into the period of larval life and the period passed as pupae. These stages are not only distinctly different with respect to the behaviour and physiology of the insect, but also as had been apparent during rearing, with respect to the way that they were affected by the foliage on which the larvae had been reared. The duration of the pupal

Eucalyptus species		camaldulensis	perriniana	macarthuri	andreana	delegatensis-hybrid	linearis(Ch)	globulus-juvenile	obliqua(Rf)	obliqua(Ch)	amygdalina	sideroxylon	obliqua(BG)	linearis(BG)
globulus-mature	male	ns	ns	ns	ns	ns	ns	ns	ns	***	***	***	***	***
	female	ns	ns	ns	ns	ns	ns	ns	ns	***	***	***	***	***

MALES

FEMALES	camaldulensis	perriniana	macarthuri	andreana	delegatensis-hybrid	linearis(Ch)	globulus-juvenile	obliqua(Rf)	obliqua(Ch)	amygdalina	sideroxylon	obliqua(BG)	linearis(BG)	MALES
camaldulensis		ns	ns	ns	ns	ns	ns	ns	ns	ns	*	***	**	
perriniana	ns		ns	ns	ns	ns	ns	ns	*	*	**	***	***	
macarthuri	ns	ns		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
andreana	ns	ns	ns		ns	ns	ns	ns	*	*	**	***	***	
delegatensis-hybrid	ns	ns	ns	ns		ns	ns	ns	**	**	***	***	***	
linearis(Ch)	ns	ns	ns	ns	ns		ns	ns	ns	ns	*	***	***	
globulus-juvenile	ns	ns	ns	ns	ns	ns		ns	ns	ns	*	***	***	
obliqua(Rf)	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	*	*	
obliqua(Ch)	*	*	ns	**	***	*	*	ns		ns	ns	ns	ns	
amygdalina	**	**	ns	***	***	**	**	ns	ns		ns	ns	ns	
sideroxylon	**	**	ns	***	***	***	***	ns	ns	ns		ns	ns	
obliqua(BG)	***	***	*	***	***	***	***	**	ns	ns	ns		ns	
linearis(BG)	***	***	*	***	***	***	***	**	ns	ns	ns	ns		

FEMALES

Table 22. The total duration of larval plus pupal stadia - the comparison of foliage means one with every other for each sex (Scheffe's Method).

period appeared comparatively independent of foliage type, while the duration of the larval stadia was markedly affected by the larval food. These stages in the life-cycle will now be considered separately beginning with the pupal period.

(a) Preliminary Analysis. The individual results from the control foliage, the mature leaf-form of E. globulus, and the juvenile form of this species were first analysed for normality by plotting them as cumulative percentages on probability graph paper. However as the pupal period for these eucalypts only ranged from 6 to 8 days the results were too tightly grouped for this analysis to be accurate. A logarithmic transformation was tried, but it gave a marginally greater deviation of the central point from a line connecting the two extremes. On this basis the data was left untransformed.

The cage means for the control foliage were then checked for randomness and independence of the error term by a sign test about the grand mean as described earlier. It had been on this basic assumption underlying the analysis of variance that the data for the total length of development had foundered. Unfortunately these data too were found to lack randomness. The number of runs of like sign (plus or minus) was significantly less than could be expected by chance alone at the 5% probability level, showing that the duration of the pupal period had decreased regularly throughout the course of the research. The results for each sex were analysed separately and found to give identical conclusions.

Because of this non-randomness the same approach adopted above had to be repeated for this set of data. This time explanation of the sequence of tests will be minimal. The first

step was to check that the trial was the unit around which to make adjustments by showing that there was no added variance among cages within trials but significant added variance among the different trials. This was so, as can be seen from Table 23 which gives the results of two analyses of variance of data from the control foliage, one for each section of the work during which there was more than one control cage per trial.

Table 23. Analyses of the length of the pupal period obtained from the control foliage.

A. Analyses of variance for the results from trial 6, season 1, for each sex (one-level nested analyses).

Source of variance	Female			Male		
	df	MS	Fs	df	MS	Fs
Among cages	3	.07	$.33^{ns}$	3	.27	1.17^{ns}
Error	49	.21		38	.23	

($F_{.05(3,40)} = 2.84$)

B. Analyses of variance for the results from season 3 for each sex (two-level analyses).

Source of variance	Female			Male		
	df	MS	Fs	df	MS	Fs
Among trials	4	1.98	$(19)^{**}$	4	0.98	$(13)^{**}$
Among cages	5	0.10	$.63^{ns}$	5	0.08	$.80^{ns}$
Error	116	0.16		106	0.10	

($F_{.05(5,120)} = 2.29$; $F_{.01(4,5)} = 11.4$)

The differences between paired cages for both sexes combined had a variance of 0.0344 (n=16) for the control foliage, and a variance of 0.0321 (n=58) for all other foliages. These results are not significant at the 5% probability level (Fs = 1.07; $F_{.05(15,60)}=2.06$ (Two-tailed test)). This confirmed that the variance between cages within runs was similar for foliages other than the control. The cage means

were then adjusted with respect to the mean of the control foliage of that particular trial. The proportional change was used to be consistent with the adjustment made for the total duration of larval plus pupal stadia because a graph for pupae of foliage means against variances showed no clear trends.

(b) Final Analysis. Table 24 shows the final analysis of variance comparing the results from the various foliages. This indicated that when the comparison was between all foliages including the control there was highly significant added variance between trials, but no additional variance between foliages. Eliminating the control foliage from the analysis removed almost all the additional variance between trials and slightly increased the variance between foliages. This latter was still clearly below the values set for significance at the 5% level.

Table 24. The final analyses of the results for the duration of the pupal period for each sex; one-way, unbalanced, nested analyses of variance both including and excluding the results from the control foliage.

Sex	Source of variation	All foliages			All foliages except *globulus*-mature		
		df	MS	Fs	df	MS	Fs
Female	Among foliages	13	.116	$(.77)^{ns}$	12	.098	$(1.36)^{ns}$
	Among trials	59	.150	5.17^{***}	40	.072	1.85^{ns}
	Among cages=error	24	.029		17	.039	
Male	Among foliages	13	.122	$(.95)^{ns}$	12	.131	$(1.77)^{ns}$
	Among trials	59	.128	5.12^{***}	40	.074	2.85^{*}
	Among cages=error	24	.025		17	.026	

($F_{.05(12,60)} = 1.92$ $F_{.05(12,40)} = 2.04$ $F_{.05(40,17)} = 2.10$

$F_{.001(60,24)} = 3.29$ $F_{.01(40,17)} = 2.92$)

The next table (Table 25) lists the sample size and mean pupation time for each sex for each foliage and demonstrates the uniformity of this period. The foliages in this table are ranked for mean female pupal period, and the lack of similar order in the results for males once again indicates the constancy of this stadium and its independence from the type of larval foodstuff.

Table 25. The means and sample sizes (number of cages) for the data on the duration of the pupal period for each foliage.

Eucalyptus foliage	Sample size	Mean (days)	
		Female	Male
E. andreana	5	7.150	7.270
E. perriniana	2	7.210	7.455
E. globulus-mature	27	7.243	7.392
E. globulus-juvenile	12	7.291	7.275
E. amygdalina	6	7.312	7.373
E. linearis(BG)	4	7.325	7.363
E. delegatensis-hybrid	7	7.331	7.410
E. linearis(Ch)	8	7.343	7.508
E. obliqua(Ch)	7	7.356	7.333
E. macarthuri	1	7.470	7.620
E. obliqua(BG)	9	7.516	7.639
E. obliqua(Rf)	2	7.580	7.280
E. camaldulensis	2	7.600	7.210
E. sideroxylon	5	7.642	7.724

(c) Discussion. No further analytical comparison of these means was warranted. This independence of the length of the pupal period and its constancy are not unexpected because this is a non-feeding metamorphic phase. During this the insect

exists on stored nutrients, the quality of which will not vary greatly between individuals of the one species of insect. Differing quantities of any stored nutrient would be more likely to be reflected in the size of the resulting adults, or in increased mortality during the pupal interval if below minimal requirements. In other words while the insect is largely quiescent its rate of development cannot be affected through some behavioural modification but will depend on the rate of the chemical reactions effecting metamorphosis.
These reaction rates in turn will vary but little except under the influence of variable temperature. However the present research has been carried out under a controlled temperature, so that the lack of variability of the pupal period is not unexpected.

(3) The Duration of the Larval Period

The remaining measurement to be examined is the duration of the larval period which in this case has been taken as the number of days from an unfed first-instar larva until pupation.

(a) Preliminary Analysis. When the results for this parameter for the mature foliage of E. globulus were subjected to the initial analyses as reported above for the results for the total larval plus pupal period, it was found that the assumption of a random distribution of the error term did not break down. Considering each sex reared on the control foliage in turn, the cage means for this measurement after transformation were more variable than for other lifespan measurements and their variation from the grand means did not indicate any significant non-random trend or pattern in a sign test about the mean although approaching the lower limit ($r = 10$, $n_1 = n_2 =$

13, 5% limits: 8 - 20; Sokal and Rohlf, 1969, Table BB). The benefit of the logarithmic transformation had been shown by graphical analysis using linear and logarithmic probability paper.

However analysis of this data for males from all seasons (as a three-level, unbalanced, nested analysis of variance) showed a significant increase in variance at the level of experimental trials. The increase in variance among cages compared to that within cages and that among seasons compared to among trials was not significant (Table 26A). The second part of the table records the results for females from rearings on the control foliage during the third season when most within-trial replication was carried out. This short analysis indicated that for females too the variance among trials was significantly greater than that among cages.

Table 26. Preliminary analyses of the duration of the larval period on the control foliage.

A: Males - data from all rearings.

Source of variation	df	MS	Fs
Among seasons	2	121.7	$(2.8)^{ns}$
Among trials	16	43.0	$(6.4)^{**}$
Among cages	8	6.7	1.4^{ns}
Error	279	4.9	

($F_{.05(15,8)} = 3.22$ $F_{.05(2,16)} = 3.63$

$F_{.01(15,8)} = 5.52$ $F_{.05(8,\infty)} = 1.94$)

B: Females - data from rearing during the third season.

Source of variation	df	MS	Fs
Among trials	4	103.2	$(19.5)^{**}$
Among cages	5	5.3	1.4^{ns}
Error	116	3.8	

($F_{.05(5,120)} = 2.29$ $F_{.01(4,5)} = 11.4$)

These results showed that although no significant trend had been indicated by the runs test, there was such a marked increase in variance at the level of experimental trials that grouping the results of all individuals reared on each foliage would be invalid. Instead the trial became the unit to work with and the cage means for each sex were adjusted proportionally with respect to the particular mean result obtained from the control for that trial. Thus the same approach as previously described for larval plus pupal measurements was eventually followed. After combining the derived data for each sex the variance of the differences between means of replicated cages within trials was calculated for the control foliage, and for all other foliages as one. These variances were compared by a two-tailed F-test. The result was significant at the 5% level but not at the 1% level ($F_{s(57,15)} = 3.2$; $F_{.05(60,15)} = 2.52$; $F_{.01(60,15)} = 3.48$). This difference would be unlikely to markedly affect the final analysis. The proportional adjustment of the cage means of treatment foliages (multiplying these by the ratio of control grand mean to control cage mean for that particular trial) was indicated by an apparent positive graphical correlation between treatment grand means and variances.

(b) Final Analysis. The final analyses compared the transformed and adjusted results obtained from the different foliages in one-way, unbalanced, nested analyses of variance. As previously one was done for each sex excluding, and one including the results from the control foliage (Table 27).

Table 27. The analyses of variance of the duration of the larval stage from each foliage for each sex; analyses both with and without the results from the control foliage.

		Data for all foliages			Data excluding E. globulus-mature		
Sex	Source of variance	df	MS	Fs	df	MS	Fs
Female	Among foliages	13	121.3	$(25.8)^{***}$	12	96.1	$(19.6)^{***}$
	Among trials	58	4.7	1.8^{ns}	39	4.9	1.4^{ns}
	Among cages =error	22	2.6		15	3.4	
Male	Among foliages	13	135.6	$(25.6)^{***}$	12	100.8	$(17.7)^{***}$
	Among trials	59	5.3	2.1^{*}	40	5.7	1.7^{ns}
	Among cages=error	24	2.5		17	3.4	

($F_{.05(60,22)}=1.89$; $F_{.05(60,24)}=1.84$; $F_{.001(12,60)}=3.31$; $F_{.05(40,15)}=2.20$; $F_{.01(60,24)}=2.40$; $F_{.001(12,40)}=3.64$; $F_{.05(40,17)}=2.10$.)

The analyses including the mature foliage of E. globulus were to be used to compare this the control foliage with each treatment foliage in turn, and the analyses excluding this were to be used to compare the treatment foliages each with every other one. All analyses showed a very highly significant increase in variance between results from rearings on different foliages. The analysis of all the results including those from the control foliage for the length of the larval stage of the males showed a slightly significant increase in variance between trials (at the 5% but not the 1% level) but this was very minor compared with the increase between foliages.

The mean values of the duration of the larval period for each foliage and for each sex were compared each to every other using Scheffe's Method. Table 28 lists these mean values and the sample sizes from which they have been derived,

while the results of the Scheffe's test comparisons are given in Table 29.

Table 28. The mean duration of the larval period for Paropsis charybdis reared on various Eucalyptus species, and the number of cage means involved in each foliage mean.

Foliage	Number of cages	Mean			
		Females		Males	
		Coded values	Days	Coded values	Days
camaldulensis	2	8.940	12.29	10.485	12.73
perriniana	2	10.910	12.86	8.515	12.17
globulus-mature	27	11.343	12.98	10.439	12.72
delegatensis-hybrid	7	11.347	12.99	10.387	12.70
macarthuri	1	11.810	13.13	11.450	13.03
andreana	5	11.924	13.16	11.094	12.91
linearis(Ch)	8	13.486	13.64	13.315	13.59
obliqua(Rf)	2	13.960	13.79	13.415	13.62
globulus-juvenile	12	14.383	13.93	14.333	13.91
obliqua(Ch)	7	18.536	15.32	18.874	15.44
sideroxylon	5	20.014	15.85	18.460	15.30
amygdalina	6	20.110	15.89	19.298	15.60
obliqua(BG)	6*	21.675	16.47	20.921	16.19
linearis(BG)	4	23.728	17.27	23.120	17.03

(* males n = 9)

As had been done for data pertaining to the total span from unfed first-instar larvae to adult emergence, these comparisons were intended to be done as two separate series of tests. In the earlier comparisons this approach appeared to be justified because the variance among trials was lessened when E. globulus-mature results were excluded. However in the present case Table 27 shows that there is negligible difference between these variances. Similarly there was no difference be-

tween the critical F-values calculated from the corresponding degrees of freedom. Thus to simplify the calculations for Scheffe's Tests no distinction has been made and all comparisons are based on the data from the analysis of variance that included all the different foliages.

(c) Discussion. The range of the means obtained from each type of leaf was considerable; the larvae of P. charybdis that fed on the foliage giving the slowest development (E. linearis(BG)) took almost one and a half times as long as larvae on the foliage giving the most rapid development (E. perriniana). The results are, naturally enough, very similar to those already reported for the duration of the combined larval plus pupal stadia. The most noteable feature is the relatively slow rate of development of larvae fed E. sideroxylon and E. amygdalina; foliages on which, unlike on E. linearis(BG) or E. obliqua(BG), the survival rate was high.

Table 29 shows that the pattern of significance between foliage means is very much dependent on sample size, and is confused especially by those of very small sample size such as E. macarthuri and E. obliqua(Rf). The values for E. camaldulensis and E. perriniana are also both derived from very small samples and demonstrate the error factor incorporated into such data. Scheffe's Method involves the use of a correction factor for sample size (in this case $\frac{1}{n_i} + \frac{1}{n_j}$) which is combined with the variance derived from the total number of samples, but besides the accuracy of the variance the sample size also affects the accuracy of the means. In table 28 the means are ranked with respect to the means for females. The results for males generally follow the

Eucalyptus foliage		globulus-mature	camaldulensis	perriniana	delegatensis-hybrid	macarthuri	andreana	linearis(Ch)	obliqua(Rf)	globulus-juvenile	obliqua(Ch)	sideroxylon	amygdalina	obliqua(BG)	linearis(BG)
		MALE													
globulus-mature			ns	ns	ns	ns	ns	ns	ns	ns	***	***	***	***	***
camaldulensis		ns		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	**
perriniana		ns	ns		ns	ns	ns	ns	ns	ns	*	*	**	***	***
delegatensis-hybrid		ns	ns	ns		ns	ns	ns	ns	ns	***	**	***	***	***
macarthuri		ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns	ns	ns
andreana		ns	ns	ns	ns	ns		ns	ns	ns	**	*	**	***	***
linearis(Ch)	FEMALE	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	***	***
obliqua(Rf)		ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns
globulus-juvenile		ns	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	**	***
obliqua(Ch)		***	*	ns	**	ns	*	ns	ns	ns		ns	ns	ns	ns
sideroxylon		***	**	ns	***	ns	**	*	ns	ns	ns		ns	ns	ns
amygdalina		***	**	*	***	ns	**	*	ns	*	ns	ns		ns	ns
obliqua(BG)		***	***	**	***	ns	***	***	ns	***	ns	ns	ns		ns
linearis(BG)		***	***	***	***	ns	***	***	*	***	ns	ns	ns	ns	
		FEMALE													MALE

Table 29. Comparisons of the mean length of the larval stages for each foliage using Scheffe's Test.

same order but are slightly lower. However the results for E. camaldulensis and E. perriniana show a much greater difference between the sexes and for the former the males actually took markedly longer to develop, so that the order of these foliages is reversed. Much of this variability is probably due to this inherent inaccuracy of means calculated from so few measurements.

(4) Other Facets of the Developmental Period

Before leaving this section on the duration of the stages of the life cycle there are three minor points to be made.

The first of these refers to the differences between the sexes with regard to the measurements taken. Table 30 records the number of cages for each foliage in which the mean value for females varied from that for males for each separate measurement. The significance levels attained by the larger samples and by the accumulated totals are also shown. As can be seen from the table there is no significant sexual variation between the accumulated totals for the combined larval plus pupal period and, of the results for individual foliages, only one is significant. However the separate pupal and larval figures show sexual differences in both the accumulated totals and in several of the foliages. Because this test simply compares the number of differences between means which were either positive or negative and ignores the actual size of the differences, it has a low sensitivity and hence cannot be used on small samples. In the present case however the accumulated totals are large enough to very clearly demonstrate that sexual differences were present.

Table 30. The number of differences between cage means (female minus male) of each sign for each measurement taken.

Eucalyptus foliage	Developmental period								
	Total			Pupal			Larval		
	+		-	+		-	+		-
globulus-mature	17	ns	10	6	*	19	21	**	6
globulus-juvenile	6	ns	6	2	*	8	9	ns	3
obliqua(BG)	0	**	7	3	ns	6	4	ns	2
linearis(Ch)	3	ns	3	1	ns	6	6	ns	2
obliqua(Ch)	5	ns	2	3		2	4	ns	3
delegatensis-hybrid	5	ns	2	0	*	6	5	ns	2
amygdalina	5	ns	1	1	ns	5	6	*	0
sideroxylon	2		3	2		3	3		2
andreana	3		2	1		4	4		1
linearis(BG)	2		2	3		2	2		2
obliqua(Rf)	1		1	0		1	1		1
perriniana	2		0	0		2	2		0
camaldulensis	0		2	1		1	0		2
macarthuri	0		1	0		1	0		1
Total	51	ns	42	23	**	66	67	**	27
Total as %	54.8		45.2	25.8		74.2	71.3		28.7

(From Table W, Sokal and Rohlf, 1969, for n = 90 p = 0.5;
95% confidence limits = 39.4% - 60.6%
99% confidence limits = 36.3% - 63.7%)

The total development period from egg-hatch to adult did not vary between sexes, but only because the independent sexual differences in the duration of both larval and pupal stadia cancelled out when these two measurements were combined. Females on the average took longer than males to complete larval development, but then spent less time as pupae. These sexual differences in the duration of stages of develop-

ment appeared to be independent of the foliage on which the larvae had been raised. Most foliages had too few rearings for any difference to be determined by the test used, but even so appeared to conform to the pattern.

It would have been informative if more exact measurements of the duration of each larval stadium for individual insects could have been obtained in order to determine whether such sexual dimorphism occurred throughout the life or was associated with the development and elaboration of the gonads late in development. The present example of small but significant sexual differences of development rate cancelling out when total preadult development is considered is not known to have been recorded before.

The second small point arose when it was discovered that the among-trials variance for the control foliage showed a significant increase over the lower level variance. A graph was drawn plotting the cage mean of the control against the date on which each trial run began for each series of measurements (Appendix, figure 2). The plots for larval and total data did not show any regular pattern of variation, but a pattern was apparent in the pupal data. For this parameter there appeared to be an approximate linear correlation with the highest results (i.e. longest pupal periods) in March to May and the lowest in December and January. Unfortunately although the research was carried out over a period of over two years involving three summer seasons there was not much overlap of results. In the first season most rearings were done in the autumn-early winter period, in the second some were in early summer and some in late summer-autumn, while only

the spring-early summer period of the last season were used. Thus the trend could possibly reflect for example a change in handling technique, or a drift in the setting of the temperature or humidity controls of the controlled - environment room over the years. However the overlap of results from the beginning of the second season with those of the third while the fact that those done later in the second season are closely similar to those from the first, tend to negate such ideas.

The cause of the observed effect is unknown; but it is likely to be truly a seasonal effect mediated indirectly through a modification of the immediate environment or of the food. Although rearings were done under a controlled regime of temperature, humidity and daylength there is the possibility that the external environment affected the regulatory systems of the controlled-environment room. The refrigeration units in particular had difficulty in maintaining a set low temperature during hot spells in summer, although during the research reported here no breakdown occurred. However the observed trend did not reflect closely the external changes in temperature or any other factor so that these are most unlikely to be operating.

This leaves open only the possibility that the change was brought about through changes in the food quality. The graphical analysis had been performed initially in order to see whether there would be any correlation of the measurements with the changing abundance of fresh young leaves. The amount of growth would itself reflect seasonal changes in the flow of nutrients and hence alterations in the quality of the foliage as a food for P. charybdis. However the food quality would be expected to have more effect on the period of larval development than on the pupal. Also the observed growth cycle

for E. globulus was not a single, once-yearly cycle as shown by the graph of the duration of the pupal stadium, but a twice-yearly one with growth peaks in spring and also in autumn. It is possible that the observed change related to the availability of some nutrient that varied in a single seasonal cycle but this is very unlikely in view of the fact that the duration of the pupal period was independent of the foliage that the larvae were reared on, and, given the marked effect of foliage type on the survival rate and on the length of the larval stadia, the nutrient quality could be expected to vary much more among the different kinds of foliage than among samplings of one foliage-type taken at different times during the year.

The lack of a seasonal pattern for the results of larval development also makes it very difficult to explain the observed change with time of the pupal development. It is probable that this does not reflect an independence of the larval measurement from the unknown factor causing the observed progression but rather that these results are subjected to over-riding effects of a random nature. One such could be the effect of competition for food especially if more than a few larvae are eating the one leaf. This last situation could result in localised food shortages and increased larval-larval contact in spite of an adequate supply of food considering the cage as a whole. The larvae do tolerate crowding and the younger instars tend to be gregarious, but food shortages can result in cannibalism and at high densities some irritability of larvae to contact by others was noted. This took the form of the alarm reaction - raising the abdomen in a jerky motion - with or without the eversion of the defense glands. The

larvae in a cage are all of the same age group so that competition could result in a slower development and decreased weight gain of all or most individuals. The alternative would be the normal development of most at the expense of a few. This would be very likely to occur in a group of mixed ages and hence sizes. Such effects as these could increase the variances both among cages of the one foliage and among the various foliages; the latter because the foliages differed markedly in leaf size and shape. These characteristics of the leaves would affect the ability of larvae to avoid competition and to move between the most succulent young leaves.

Thus the lack of detection of any change in the duration of the larval stadia with time may be due to such factors affecting the time the larvae take to reach full development. This would help explain the wide variation in this measurement within foliages, and also any differences in the variances for each foliage. It could also be one further reason why the results for each foliage and sex for the duration of the pupal stadium varied so little, as the pupae are inactive and were also kept isolated from one another. However it contributes nothing towards a possible understanding of the seasonal variation of the length of pupal development. This remains unexplained.

The third and last minor point concerning these measurements of stadial length are the results for E. ficifolia. Only two larvae, one male and one female, survived to adulthood on this foliage and so the results were not included in the analyses above. The female spent 14 days as a larva, and 7 days as a pupa, while the corresponding figures for the male were 19 and 7 days. Thus the female was only marginally slower than the mean value for the control foliage for the

length of the larval period while the male was very much slower being among the slowest individuals recorded. Neither pupal result could be distinguished from the control as would be expected from the marked constancy of this measurement. The extreme variation between the larval results for male and female hinder prediction of the foliage means, but it can be said that in spite of the very high mortality rate recorded on this eucalypt it can provide adequate nutrition to enable development to proceed as rapidly as on the mature foliage of E. globulus. Not all larvae would develop at this optimum rate though.

7. PUPAL WEIGHTS

As outlined in the introductory section of this chapter weight attainment was used as a third indicator of how well the larvae of P. charybdis fared when reared on various types of Eucalyptus foliage. The prepupae were confined individually and observed at 24 hour intervals until they had transformed into pupae at which time each was weighed. Later, when the adult beetles emerged, the sex of each was recorded. Initial rearings had indicated a marked sexual dimorphism in size with the females on the average heavier than the males from the same rearing.

(1) Preliminary Experimentation

The gain in accuracy from restricting the age-class that was weighed to a fixed interval of 24 hours was demonstrated when on one occasion six individuals of each sex were weighed at pupation and also 24 hours later. Under the constant conditions used, 25.6 ± 1.2'C and 65 ± 10% R.H., these pupae had lost an average of 1.69% of their initial weight during

the 24 hours after first having been weighed (Table 31). Unless the weight change was expressed as a percentage of the initial weight, the sexual differences in the basic size of the adults were also reflected in the losses. There was no apparent sexual differences when the weight loss was given as a percentage.

Table 31. Weight changes of pupae of P. charybdis over 24 hours at a temperature of 25.6 ± 1.2°C.

Sex	Initial Weight (mg)	Final Weight (mg)	Decrease	
			mg	%
Female	202.42	199.31	3.11	1.54
	178.53	175.01	3.52	1.97
	172.42	169.33	3.09	1.79
	159.49	156.32	3.17	1.99
	158.58	156.15	2.43	1.53
	157.58	154.69	2.89	1.83
Male	153.39	150.65	2.74	1.79
	149.03	146.04	2.99	2.01
	148.99	146.11	2.88	1.93
	127.94	126.00	1.94	1.52
	125.98	124.59	1.39	1.10
	122.38	120.81	1.57	1.28
Mean			2.64	1.69

Table 32. The average daily weight loss (as a percentage of the initial weight) of pupae kept under uncontrolled room conditions.

Sex	Weight at pupation (mg)	Average daily weight loss after x days (%) x=1	2	3	4	5	6	7	8	10
Female	139.8	1.22	1.11	1.07	0.98	0.93	0.97	0.95	0.98	(1.42)
	138.5	1.16	1.16	1.08	0.99	0.95	0.95	0.94	0.97	(1.36)
	123.3	1.62	1.54	1.43	1.30	1.24	1.24	1.23	1.26	(1.72)
	106.7	1.31	1.50	1.41	1.36	1.29	1.28	1.29	1.29	(1.78)
	103.9	1.35	1.20	1.12	1.03	0.96	0.95	0.95	0.99	1.33
	90.4	1.33	1.33	1.22	1.22	1.17	1.14	1.14	1.16	(1.59)
	Mean	1.33	1.31	1.22	1.15	1.09	1.09	1.08	1.11	1.53
Male	99.6	1.31	1.15	1.17	1.07	1.00	1.00	1.03	1.02	1.13
	96.7	1.34	1.34	1.21	1.14	1.12	1.10	1.09	1.12	(1.47)
	95.3	0.94	1.15	0.94	0.97	0.92	0.87	0.93	0.96	(1.47)
	89.8	1.89	1.65	1.56	1.34	1.14	1.24	1.22	1.21	1.27
	Mean	1.37	1.32	1.22	1.13	1.05	1.05	1.07	1.08	1.34

(Results in parentheses are after adult emergence.)

The necessity to weigh the insects at a set point of development had been first indicated by results obtained during the initial large-cage rearings which were not carried out under controlled conditions but simply in an ordinary laboratory (Table 32 and Table 33). No record had been kept of temperature variations but the blocks of pupal cells were placed in a cupboard and hence insulated against extremes of temperature fluctuations. The first of these two tables shows the progressive daily weight loss during the pupal period for six females and four males. The larvae in these uncontrolled rearings took 10 or 11 days to complete pupal development, as compared to the 7 taken by the majority in the controlled rearings. This slower development indicates a lower tempera-

ture and is also reflected in the lower percentage decrease of weight in Table 32 as compared to Table 31. A lower temperature would decrease both metabolic and evaporative losses. These insects which were small compared to those reared later on this, the control foliage, appeared to loose weight at an almost constant but slowly lessening rate until emergence with its associated losses of fluids and consumption of energy. However the lack of assured environmental constancy could have affected the pattern of results.

Table 33. The weight change of immature P. charybdis under uncontrolled conditions measured over an 8-day period during which pupation had occurred.

	Female			Male		
	Weight of prepupae (mg)	Weight after 8 days-pupae (mg)	% loss per day	Weight of prepupae (mg)	Weight after 8 days-pupae (mg)	% loss per day
1	152.9	123.0	2.44	118.8	95.0	2.50
2	152.0	124.5	2.26	113.1	90.8	2.46
3	147.2	121.7	2.17	110.6	90.2	2.31
4	146.2	117.8	2.43	110.2	82.7	3.12
5	137.4	113.0	2.22	107.4	88.1	2.25
6	136.9	113.0	2.18	105.1	84.3	2.47
7	136.8	108.5	2.59	96.4	76.4	2.59
8	133.9	105.8	2.62	93.9	77.1	2.24
9	123.1	93.7	2.99	92.7	72.6	2.71
10	122.0	100.1	2.24	89.0	72.4	2.33
Mean	138.84	112.11	2.41	103.72	82.96	2.50

Table 33 gives some results from weighings carried out even earlier in the first season when some larvae from a large cage were weighed individually as prepupae and then again 8

days later. (The larvae had been weighed collectively at intervals of 8 days throughout development.) The prepupae were kept separately and had pupated by either the fourth or fifth day during this period. The greatly increased daily weight loss compared to the results in Table 32 above reflected two main factors - the fact that prepupae take several days to completely void all solid matter from their gut and so some would not have completely defaecated when first weighed, and the loss of fluid and energy during pupation. The shed pupal skin does not contribute a significant proportion of the loss at this time as was shown when 10 were weighed separately at an average of 1.08 mg (range 0.81 - 1.32 mg). Fluid losses are felt to be more important because many lost small quantities during the change. These amounts were generally small enough not to affect future development but were sufficient to just mark the blotting paper base of the cells in which such pupae were confined.

Because of this relatively rapid change in weight, two decisions were made before the main larval rearing trials commenced. The first was to reduce variability in weighings by taking all measurements on insects at the same stage of development. The duration of these experiments meant that the shortest interval at which regular equally-spaced checks could be carried out was 24 hours. Thus all P. charybdis were weighed within 24 hours of the same point in their development - the chosen point being the metamorphosis from larva to pupa.

The second decision related to the first in that the accuracy of the measurement taken depended on the variability of the time of development of individual insects when weighed and also on the exactness of the weight determination. The

balance used was a Mettler H6 capable of weighing to two decimals of a milligram. However because of the rapidity of weight loss all results were rounded to the nearest milligram. Greater exactness would have been meaningless.

(2) Analysis

The analytical approach used on the main results comparing the different foliages was essentially the same as was used above with the measurements of the duration of the developmental stages. The combined results for all individuals reared on the control foliage - the mature leaf-type of E. globulus - were graphed for each sex as frequency and then also as cumulative-percentage frequency distributions. The latter plot on probability paper confirmed the impression gained from the former - that the data conformed to a normal distribution without transformation. The cage means for each sex from this foliage were quickly checked for randomness and independence of their error terms by a sign test about the median. Neither sex showed any regular progession. (In the males the median was 140.54 mg and there were 12 runs of the same sign in a series of 26; in the females the median was 179.11mg and there were 10 runs. From Rohlf and Sokal (1969, Table BB) the 5% limits on the number of runs for $n_1 = n_2 = 13$ are 8 and 20 so that neither result is significantly different from what could be expected with random selection from a normal distribution).

This independence of results from different trials was confirmed by an unbalanced nested analysis of variance of the individual weights for each sex from control foliage rearings (Table 34).

Table 34. The results of an analysis of variance of the weights of individual pupae of each sex reared on the control foliage.

	Female			Male		
Source of variation	df	MS	Fs	df	MS	Fs
Among seasons	2	3426	$(2.9)^{ns}$	2	4117	$(4.8)^{*}$
Among trials	16	1194	$(2.2)^{ns}$	16	858	$(2.3)^{ns}$
Among cages	8	555	3.3^{***}	8	378	1.3^{ns}
Error	294	167		279	285	

($F_{.05(2,16)}=3.63$; $F_{.05(16,8)}=3.22$; $F_{.01(8,\infty)}=2.51$;

$F_{.01(2,16)}=6.23$; $F_{.05(8,\infty)}=1.94$; $F_{.001(8,\infty)}=3.27$)

These analyses showed that for both sexes the variances among trials were not significantly different from those among cages, and that for the females the among seasons variances was not significantly different from that among trials. For the males the among seasons variance showed an increase significant at the 5% level but not at the 1% level. This slight increase meant that the results should be interpreted with care because of possible complications in comparing the results of trials done during different years. However the low level of significance attained, and that for only one sex, indicated that the breakdown in randomness was not severe.

The cage results for females showed a highly significant increase in variance over the results for individuals within cages, whereas for males the increase was not significant. In this case although the discrepancy occurred for only one sex, the significance level of the increase was very high, so that measures have had to be taken to counter this inconsistency. This meant that once again the individual measurements could not be used in comparing

results but only the cage means, with their lower sample size and hence reduced powers of discrimination. However because the far greater variation in this parameter was at the cage level and not higher, no adjustments were required and the final comparison was done as an unequal but proportional two-way analysis of variance incorporating the mean weights for each cage grouped for each foliage with sex as the second dimension.

Table 35. The results of a Model 1 two-way analysis of variance of the pupal weight data for various Eucalyptus foliages.

Source of variation	df	MS	Fs
Among subgroups	27	3966	13.6^{***}
- Sex	1	31736	109.0^{***}
- Foliage	13	5728	19.7^{***}
- Interaction	13	69	0.2^{ns}
Within subgroups(error)	166	291	

($F_{.001(24,120)}=2.40$; $F_{.001(1,120)}=11.4$; $F_{.001(12,120)}=3.02$)

The results of the two-way analysis showed that there was both a highly significant difference between the sexes and among the different foliages (Table 35). (The F-values here are all exact because both foliage type and sex are fixed treatment effects so that all other mean squares are compared directly with the error mean square.) The mean pupal weights attained by larvae feeding on each foliage are shown in Table 36.

Table 36. The mean pupal weights of P. charybdis for each sex on each foliage type, and the number of cage means each foliage mean is derived from.

Foliage type	Sample size	Mean weights of pupae (mg)	
		Female	Male
globulus-mature	27	179.22	135.51
sideroxylon	5	198.95	163.57
obliqua (Rf)	2	192.48	148.61
camaldulensis	2	191.23	152.05
delegatensis-hybrid	8	188.26	145.90
linearis(BG)	3	170.28	136.09
globulus-juvenile	12	170.06	132.27
linearis(Ch)	8	166.88	131.16
obliqua (BG)	8	163.72	123.48
andreana	6	159.12	123.79
obliqua(Ch)	7	152.83	110.89
amygdalina	6	150.29	117.49
perriniana	2	145.25	122.82
macarthuri	1	135.67	112.93

The analysis was extended to a comparison of the foliage means for each sex using Scheffe's Method. However in spite of the very significant increases in variance among all foliages shown by the analysis of variance, none of the individual paired comparisons of foliage means was significant. This illustrated the extensive margin of safety against wrongly declaring means sampled from the same population different (a type one error) incorporated into 'a posteriori' tests. In this case where all the comparisons involve only two treatments the square of the differences has to be 13 times greater than in an 'a priori' test to be declared significant.

It is apparent that with so many treatments and yet limited (paired) comparisons the chances of not showing up significant differences (and so making a type two error) have been greatly exacerbated by the inclusion of the term(a-1) in Scheffe's Test (p128). It is only because of the very great variation between foliage effects with regards to the survival and duration of development data that such insensitive testing has resulted in significant differences being demonstrated on the previous occasions when this 'a posteriori' test has been used. Unfortunately similar if not identical measures are incorporated into all rigorous 'a posteriori' tests applicable to unequally sized samples.

In the present case the mean pupal weights for each sex clearly vary markedly in absolute terms (i.e. in mg) among foliages. The relative range from smallest to largest was less than for some of the measurements discussed earlier but greater than for the section on larval development. Thus the reason that significance between individual pairs of foliages was not found here using Scheffe's Test must relate to both the conservative nature of this particular test and to the greater variability among individual readings for each foliage for the weight data. The incorporation of the degrees of freedom of treatments (here 13) in place of the degrees of freedom of the particular comparison (here 1) that gives Scheffe's Test its safety factor is an arbitrary measure designed so that even the best and worst treatments can be compared without exceeding a 5% error probability. If this conservative factor is ignored the following table of significance can be derived (Table 37).

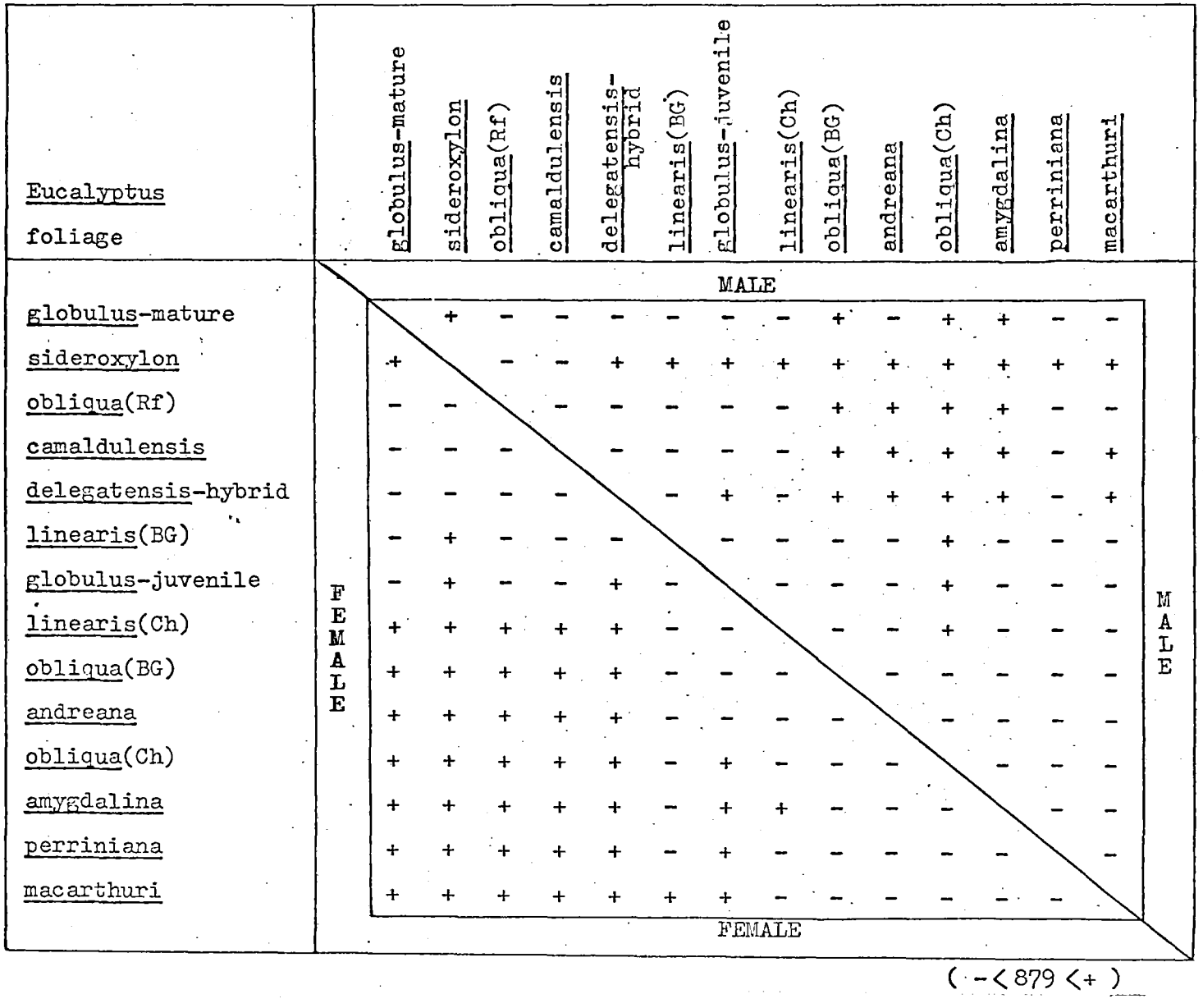


Table 37. A modified test comparing the foliage effect on the pupal weight of P. charybdis for each sex (Scheffe's Test with (a-1) = 1).

(3) Discussion.

The results of these tests indicate that for both sexes there is a strong probability that there are significant treatment effects between foliages when the comparison involves one foliage better than E. linearis(BG) and one worse than the juvenile foliage of E. globulus. Few differences within these groups of good and poor foods are apparent from the present research.

The interesting feature shown up by this parameter of larval success is the complete disparity between foliages ranked for this and for the other two parameters - larval survival and length of development. E. perriniana and E. macarthuri which had high survival rates and relatively rapid development produced the smallest pupae. E. linearis (BG) gave consistently poor results for survival and rate of development and yet produced fairly large pupae. However E. camaldulensis performed well with regards to all three factors. This serves to emphasise once again that the interaction between P. charybdis and its host plants is complex and multifactorial in nature.

CHAPTER IV

LABORATORY REARING ADULTS OF PAROPSIS CHARYBDIS

Concurrently with the larval rearing described in the previous chapter, adults were maintained to provide information about the longevity, fecundity and fertility of Paropsis charybdis imagos fed on the various types of Eucalyptus foliage. They also served as the source of eggs required for larval rearing experiments.

1. MATERIALS AND METHODS

(1) The Initial Selection

The beetles captured in the spring of 1969 which formed the initial colony established in the laboratory had already begun oviposition in the field, and had fed on unknown foliages. Once set up in the laboratory these beetles were fed solely on the control foliage - the mature leaf-form of E. globulus. At the start this colony provided many eggs in accord with the previously recorded observations on fecundity (Dugdale, 1965; Carne, 1967; Styles, 1969, 1970). Because of this only 10 pairs of beetles were selected from those resulting from the first large-cage larval rearings to begin a detailed study of the adults. The choice was restricted to adults reared as larvae on the control foliage in order to avoid any variation among the larvae to be used in subsequent rearings caused by a longterm effect of any foliage fed to the adult beetles, and also to provide basic information about the reproductive behaviour and the longevity when P. charybdis was fed this, the

reference foliage.

The 10 females were chosen on the basis of their pupal weights to represent the weight classes present in the population from which they had been selected. Each weight class covered a 10mg range and there were two females representing each group between 121 and 160mg with one female from beyond each of these extreme values. After being set up in pairs in small cages they were kept in the unregulated laboratory for a further 40 days and then shifted into a chamber with controlled temperature, humidity and photoperiod. The large-cage rearings had been carried out during early summer (November) close to windows giving good lighting. At this time of year in the field the newly emerging adults are recorded as giving rise to a second generation of larvae (Styles, 1969). However, the adults placed in the controlled-environment room did not begin laying immediately.

(2) The Problem Encountered

After a further period without general oviposition the daylength to which these beetles were exposed was increased from 12 to 15 hours and the temperature from 21.0'C to 25.6'C. These changes did not initiate egg production, but no further interference with the environmental conditions was attempted. Before this change was made three of the 10 pairs had oviposited for a short time, laying 4, 19 and 21 egg batches respectively before stopping. Eventually after about 6 weeks several beetles began to lay continually in March. The last female did not begin laying until the middle of April. During this pre-ovipositional period the beetles fed but were only sluggishly active and mating was not seen. It had been reported that adults emerging in late summer and autumn fed

and then over-wintered in a state of diapause. The diapause was not absolute as feeding had been recorded from warmer areas, but it did apparently completely prevent reproduction (Dugdale, 1965; Styles, 1970). The length of time that passed before laying commenced in the present case suggests that these laboratory-reared adults entered diapause, especially as this long pre-laying interval was not experienced with later rearings carried out completely from egg to adult under the warm, long-day conditions that remained enforced in the controlled-environment room.

The factor, or factors, that would have been operating under ordinary room conditions to initiate diapause were unknown. Photoperiod has frequently been shown to be the prime factor triggering diapause (Danilevsky, Goryshin and Tyshchenko, 1970; Beck, 1968; de Wilde, 1962). It is possible that larvae in the open laboratory were not exposed to light of sufficient intensity for long enough each day because the room did not catch the late afternoon sun. However the room was in use frequently in the evenings which would have broken the dark phase with a period of fluorescent lighting. The threshold intensity of white light necessary to activate biological trigger systems has been found to be as low as 0.1 lux in adult Leptinotarsa decemlineata Say (de Wilde, 1962) and even the indirect natural lighting experienced by P. charybdis in this case would have exceeded this level. Light quality has also been indicated as important (e.g. Hayes, Schechter and Sullivan, 1968) but it appears that this is minor compared to the actual period of exposure (Danilevsky et al, 1970). Although most insects that have been investigated have shown some reactive select-

ivity to light of different wavelengths, the sensitivity has been to relatively broad bands and often at more than one point in the spectrum, so that the fluorescent lighting should have been an effective trigger. Another point arguing against a possible photoperiodic induction of diapause in the present case is the generalisation made by Danilevsky et al (1970) that in all polyvoltine insects investigated to that time, diapause induction had been of a 'long-day' type, where a long photoperiod (such as would have been present at that time both in nature and the laboratory) prevented the onset of diapause.

Food quality is unlikely to be involved because diapause did not affect later rearings and the insects were always fed on fresh young foliage which was not in short supply at this time of year (early summer). This leaves only the question of the influence of temperature on diapause initiation.

Difficulty had been experienced initially in sexing adult P. charybdis because the method recommended was to examine the beetles for the orange colour of the testes (Styles, 1969). In mature males these organs are clearly visible ventrally through the cuticle of the anterior abdomen, but in immature adults the colour is not fully developed and there is also much yellow-orange fatty material obscuring the distinctiveness of this test. To try to avoid these complications the adults were held for as long as possible in their pupal cells before sexing. Unfortunately 3 or 4 days after becoming adults, the beetles became restless and attempted to escape from their pupal cells. They had little difficulty in chewing through the foam plastic

of the cell blocks, limiting the time for which they could be left to mature. To immobilise such active adults to enable them to be readily handled and sexed the pupal cells were placed in a deep freeze for a short period of up to one hour. This short exposure to low temperature had been inflicted on the adults which later became the first parent generation in the controlled-environment room. It could possibly have triggered the diapause of these, although low temperature is more usually associated with the termination of diapause rather than its initiation, and the exposure time was very brief.

Short periods of low temperature inactivation have been used to ease the handling of adult Diptera and Lepidoptera without affecting the fecundity and fertility of these (Hightower and Garcia, 1972; Richmond, Graham, Perez and Llanes, 1971), but there is no report of it being used on any adult insect with an imaginal diapause similar to P. charybdis.

(3) Alternative Methods of Sex Determination

In subsequent rearings the problems encountered during this early work in attempting to sex the adults were avoided because an alternative method of sex determination was devised. Immature quiescent adults were examined for features that could be used to differentiate the males and females at this stage while they could still be easily handled. Several such features were found and their use as distinguishing characteristics confirmed by dissecting a series of these immature adults to display the reproductive systems. During this a male was found with completely colourless testes, although in all others of equivalent maturity these organs were an orange colour. The most

distinctive of these secondary sexual characteristics was the shape of the tarsal pad. In females the tarsal pads on all feet were the same shape - an elongated isosceles triangle. In males the pads on the hind legs were also in this form with straight edges, but the pads on the first two pairs of legs were expanded to give a heart-shaped outline. Besides this the males had longer antennae and larger distal segments to the maxillary palps. The size of the elliptical terminal face of the latter was especially noticeable. The shape of the tarsal pad became the prime factor for sex determination; it could be used on insects dying during emergence or late in pupation as well as on normal adults.

(4) Experimental Techniques and Conditions

Several other female beetles from the same rearing as the parent generation were also kept. These were retained individually in small cages in case of the premature or accidental death of one or more of the 10 females of the parent generation. A supply of males was kept as well. When the parent generation females did not begin reproduction, two males were added to four of these cages and one to each of the remaining five. Some of these females had deposited unfertile eggs during the time that they remained unmated but none began to lay fertile eggs until several months later, about the same time as did the parent generation pairs. The presence of an extra male did not appear to have any effect.

After the initial adjustment all beetles were kept under the same environmental conditions as has been described for the larvae - 25.6 $\pm$ 1.2'C, 65 $\pm$ 10% R.H., with a light regime of 15 hours of light and 9 hours of darkness. They were kept in inverted small plastic canisters with a 20-mesh

netting vent forming the roof. Stems of eucalypt twigs passed through a hole in the canister lid (the floor of the cage) into a trough of water. The foliage was renewed every 2 days when the cages were wiped out and all eggs removed. At 4 or 6 day intervals the cages were replaced, and washed. The troughs were similarly replaced and cleaned at approximately 10 day intervals.

The first and second generations began with equal numbers of males and females but this ratio was upset when the insects began dying. However shortly after adult rearing commenced a system was begun where the males were circulated amongst the females. Every 4 days each male was moved from one cage to the next in a regular sequence. In this way it was hoped to avoid problems of male infertility, and possible reproductive incompatibility between particular pairs of beetles. During the later stages of the research an excess or scarcity of males was similarly shared between all the females in turn. This was another advantage of this 'pooling' of males. The male reproductive disorders mentioned above were regarded as factors that would complicate the discrimination of the effect of the different foliages on reproduction. The female with the greater nutritional demands of ova production would be more likely to show up any variance in the food quality provided by the different eucalypts. Also any effect on the males would only be discerned by its effect in turn on the females unless smears and sperm counts were made so that it was of prime concern to first discover the normal pattern of reproduction exhibited by the females.

The eggs taken from each cage for every two-day

period were counted and kept until it could be determined whether or not they were fertile. Those eggs that developed sufficiently so that an embryo could be recognised within them were considered fertile. Many eggs were kept up to the time they hatched but the proportion of larvae emerging successfully was not calculated because at this point there was often a high mortality resulting from cannabalism by earlier emerging larvae. Much of this was due to the unnaturally crowded conditions in which the eggs were kept - up to several hundred in a 7cm-diameter petri-dish. The eggs were removed from the leaf if this was possible without damaging them, or a small area of the surrounding leaf was excised with each egg batch.

Initially the cages were marked on the outside but because of the frequent cage changes necessary to prevent contamination by fungi attacking the frass, this was not practical. Instead a numbered piece of light cardboard was included in each cage. At the beginning these were almost square but when it was found that the females would lay freely on them, the shape was changed to a long thin strip (about 20 x 1 cm). This could be forced among the leaves of the foliage to provide ready access to it for the beetles. The narrower shape also suited the adults in that they could move freely along the strip, as if it were a twig or the margin of a leaf. The beetles were encouraged in this way to lay on the cardboard rather than the leaves and some beetles deposited more than half their egg batches there. The advantages in this were twofold. Firstly it avoided accidental consumption of the eggs by the adults as could occur when a batch had been laid on succulent young foliage,

and secondly eggs laid on paper could be excised and handled readily without the problem of rot. Pieces of leaf that rotted during the incubation of attached eggs could interfere with the development of the eggs. This characteristically resulted in malformed, unviable embryos, or in embryos that developed in only a portion of the egg with the rest of the contents wasted. If development was confined to half or less of the egg contents the embryo generally failed to survive to hatching.

Recordings were kept for each female of the total number of eggs per batch, the order of hatching of batches (and hence the order in which they had been laid), and the number in each batch fertile, infertile or indeterminant. The last category included those eaten by adults, or by the earliest hatching larvae, as well as those few damaged in handling.

The first parent (or 'P_1') generation gave rise to the first series of larval rearings carried out under controlled conditions; these larval rearings in turn gave rise to the next (the 'F_1') generation of adults. The F_1 generation began with 51 female beetles and 65 males, representing eight different foliages. Each beetle was fed only on the foliage on which it had been raised as a larva in order to measure any effect on fecundity or fertility resulting from confining P. charybdis to a single foliage throughout a complete life-cycle. From each cage of larvae reared during the first season two females were selected that lacked any wing or other deformity and also were closest to the mean and median pupal weights for that cage. The only exceptions were the first cage reared on E. delegatensis-hybrid and the last cage

on the control foliage. In the former case the selected females were killed along with most of the other females and the mistake not realised until only the two female beetles from the heaviest pupae were left. These were used. In the latter case only the single female closest in pupal weight to the cage mean was chosen because of the greater number of beetles fed on the control foliage which had been included in the F_1 generation by this time. Males were selected less rigorously with the emphasis only on their apparent health.

The rearing equipment and conditions were the same as for the parent generation, and the males were similarly pooled, but only within each type of foliage. The slight excess of males mentioned above was held because it appeared that males might not live as long as females but mainly because a statement in Dugdale (1965) appeared to imply that this was necessary to maintain oviposition (p6 " .... a female left unmolested and given a supply of males can produce up to 800 eggs in 45 days .... ").

No problems occurred at any stage during the present work that could be attributed to male infertility or lack of vigour. In fact when it became obvious that the 'extra' females of the parent generation had not derived any impetus to initiate laying from being confined with two males instead of one, some of these females were used for investigating other aspects of oviposition. One of these trials was an attempt to discover how frequently mating was required in order to sustain fertile oviposition at normal rates. This arose out of the observation that in the confined conditions of the small cages mating occurred frequently, even at intervals of only a few days. In the field on mature

eucalypt trees meetings of beetles which would result in matings would be unlikely to occur this often, unless powerful sex or aggregating pheromones existed. It was found that the females could store enough sperm to enable normal oviposition to continue without an increase in infertility for up to 50 days during which time 1200 eggs could be laid.

The longevity of the adult beetles severely restricted the amount of research which could be carried out on the interactions between this stage in the lifecycle of P. charybdis and those Eucalyptus foliages on which it feeds and lives. This long span had not been anticipated when adult rearing began and it resulted in an increased complication in that the initial inbalance in the numbers reared on each foliage could not be subsequently corrected. This inability to achieve balanced results stemmed partly from physical limitations on the amount of space and time available, but mainly because of limited amounts of suitable foliage. The trees sampled not only had to provide sufficient young growth at any one time but the supply had to be continuous for an extended period. This latter condition was extremely hard to fulfil even with the most plentiful foliage, the mature leaf-type of E. globulus, and although the young growth necessary for feeding was neither as abundant nor as succulent during the winter or in late summer as during the main growing seasons, sufficient was collected. Two mild winters in succession failed to terminate the autumn growth in all trees, and so enabled research to continue.

In early and late summer of 1970-71 eggs from the F_1 generation of adults were used in the F_2 series of controlled

larval rearings. The F_2 adults so obtained were not kept for full-term studies of the factors affecting oviposition. Instead an attempt was made to assess the effect of exposing females to short periods of feeding on various foliages. The 23 pairs with which this trial began all came from the two control cages of successive trials. The experimental details of rearing were, as has been described previously, with a pool of males circulating among the females. The basic or control food used was again the mature foliage of E. globulus. Once each female had begun laying it was allowed to continue until at least 10 periods of oviposition had been obtained. Then it was fed on a trial foliage for five periods (i.e. 10 days), or in one case for 10 periods; or it was starved for one or two periods. Following this each was again fed on the control foliage until after a further interval of at least 10 periods during which oviposition had occurred. The feeding of a trial foliage could then be repeated. Four of the beetles were kept on the control foliage throughout the trial as an additional control measure.

When this short-term feeding trial was abandoned the remaining beetles were used in a trial to assess the effect of temperature on oviposition. All other factors were kept constant and the beetles were all fed on the control foliage.

To provide eggs for the final series of larval rearings a new parent generation (the 'P_2') was established in the controlled-environment room using adults captured in the field in early spring 1971. These beetles were taken while actively feeding but were among the first seen that

season and so had not been long emerged from their over-wintering diapause. No eggs were found in the field until several days later than the date of capture, and batches were not commonly found until several weeks after that. However the females transferred from the field into the warm long-day conditions of the controlled-environment room began laying immediately. Only one did not oviposit during the first 48 hours after capture, although two others produced only a single egg each, and a further three produced markedly fewer than during subsequent periods. During the second two-day period all the females laid considerable numbers of eggs. Thus it was highly probable that some of these beetles had already begun laying when captured, but the number of egg batches involved in this would have been small. These beetles were taken at three different sites, at two of which E. globulus was the predominant host (12 females) while E. viminalis predominated at the third.

One of the reasons for using field-collected adults as the source of eggs for the final generation was to check whether these would also show the very high fecundities (total egg production) found among laboratory reared individuals. This had been several times greater than the results reported in the literature. Another reason was to safeguard against any possible decrease in larval vigour or other consequence of the selection pressures resulting from a sequence of laboratory rearings. Such an effect was unlikely because of the few generations of rearing involved but could be implicated by the more rapid rate of egg-laying demonstrated by these field-collected females compared to earlier laboratory-reared ones.

Similarly the larval rearings during the final season generally appeared to give better performances than during previous seasons, although the analyses of the various and separate measurements of larval success demonstrated that there was not a definite trend in the results. However, because larval rearings of P. charybdis derived from wild and laboratory populations were not carried out simultaneously a minor change in rearing technique, or a drift in the regulatory mechanisms controlling the constant-environment room, cannot be discounted. The lifespan of adult beetles was so long that the last survivors of the F_1 generation - two females and five males - were still alive when this final adult generation of field-collected beetles was established, together with many F_2 adults, but the effect of the age of the beetles on oviposition precluded a comparison of the results obtained simultaneously. Most individuals of the final (or 'P_2') adult generation were not kept for the complete natural span of their adult life because the time available was limited. After all larval rearings had been finished, and since egg production of most females had already exceeded the values reported in the literature, these adults were also used in a second trial on the effect of various temperature regimes on egg production.

The survivors were eventually killed.

2. RESULTS AND DISCUSSION

(1) The Parent Generation - Rearings on the Control Foliage

(a) Results. A summary of the data obtained from the ten females of the P_1 generation is presented in

Table 37.

Table 37. Adult rearing data from the ten females of the parent generation giving the results for each individual and the group means.

Pupal Weight (mg)	Time Alive (days)	No. of laying periods (48 hrs)	Egg production					
			Total No.	% Fertile	No. of Batches	Eggs/ Batch	Rate per laying period Eggs	Batches
120	488	129	4809	91.33	460	10.45	37.28	3.57
122	261	63	2106	97.92	177	11.90	33.43	2.81
130	518	127	4563	93.57	433	10.54	35.93	3.41
133	369	112	4757	97.94	384	12.39	42.47	3.43
136	399	127	5392	93.51	351	15.36	42.46	2.76
143	442	101	2422	91.56	231	10.48	23.98	2.29
143	516	128	4880	98.20	333	14.65	38.13	2.60
151	466	132	5826	79.79	378	15.41	44.14	2.86
155	577	113	2156	90.56	267	8.07	19.08	2.36
177	408	140	5165	98.03	303	17.05	36.89	2.16
141.0	444.4	117.2	4207.6	93.24	331.7	12.63	35.38	2.83

Table 38 records ancillary information from the additional females kept from the same larval rearing. It must be remembered that these latter were not kept under exactly the same conditions as the former with regard to sex ratio or mating pressure although the general environmental factors of temperature, humidity and photoperiod were identical once the second group had been placed in the controlled-environment room. In spite of these slight differences in background the means for each group show remarkedly close accord. These means are given in the bottom line of each table.

Table 38. Adult rearing data from the nine females kept with the parent generation. The first three of these were accidentally killed during the research period and have been excluded from the group means unless indicated.

Pupal Weight (mg)	Time alive (days)	No. of laying periods (48 hrs)	Egg production					
			Total No.	% Fertile	No. of batches	Eggs/ batch	Rate per Laying period Eggs	Batches
134	162	6	213	93.98	18	11.83	35.50	3.00
155	130	12	389	97.31	49	7.94	32.42	4.08
164	260	48	2241	97.98	160	14.01	46.69	3.33
134	562	121	3493	93.35	305	11.45	28.87	2.52
134	416	67	3122	97.57	217	14.39	46.60	3.24
139	579	161	4590	94.68	264	17.39	28.51	1.64
147	422	90	2489	94.85	225	11.06	27.66	2.50
150	445	127	4304	87.48	387	11.12	33.89	3.05
153	533	133	4032	93.57	412	9.79	30.32	3.10
142.8	492.8	116.5	3671.7	94.53*	301.7	12.11*	34.50*	2.94*

(* mean of 9)

The greatest disparities between the two groups (Tables 37 and 38) are in the figures for the average total egg production and also the average number of batches per female. With respect to both of these factors the second group - the ancillary females - had slightly lower results. These two measurements are obviously linked but as the rate of oviposition (expressed as either the number of eggs or the number of batches per 48 hour period in which laying occurred) and duration of oviposition were very similar in both, this slight difference is probably not meaningful. The average life of the second group of adults was also slightly longer.

The results given in these two tables emphasise the

extreme longevity and fecundity of females of P. charybdis, with the maximum values recorded here for these parameters being 579 days and 5826 eggs respectively. These figures must be regarded as showing the potential of this insect species rather than the actual performance because this research was conducted under conditions which must approach the optimum for this beetle. In particular the scarcity of suitably succulent foliage during mid-winter and mid-summer and the temperature extremes also experienced at these times would tend to limit the life and reproductive capacity of P. charybdis adults. The proportion of the lifespan of each of these 19 females during which eggs were produced is depicted in figure 17. This shows clearly the long delay before oviposition began and then the almost continuous period of laying for each individual. The additional females had a more broken period of oviposition than those caged continuously with a male. Females stopped laying only shortly before their death except for one that lived just over 100 days after she had laid her last batch.

Graphical methods were used to check for correlation between the various measurements taken for each adult. Carne (1966) working on the biology of Paropsis atomaria in Australia found that the adult size as measured by weight strongly influenced the size of egg batches. Four females representing each of the three weight classes 120 - 140mg, 170 - 190mg and 210 - 250mg gave average batch sizes of 49.5, 69.4 and 75.2 eggs respectively. As the number of batches per female was independent of female size this influence of body size on batch size was reflected in the overall fecundity. Carne also showed a strong relationship

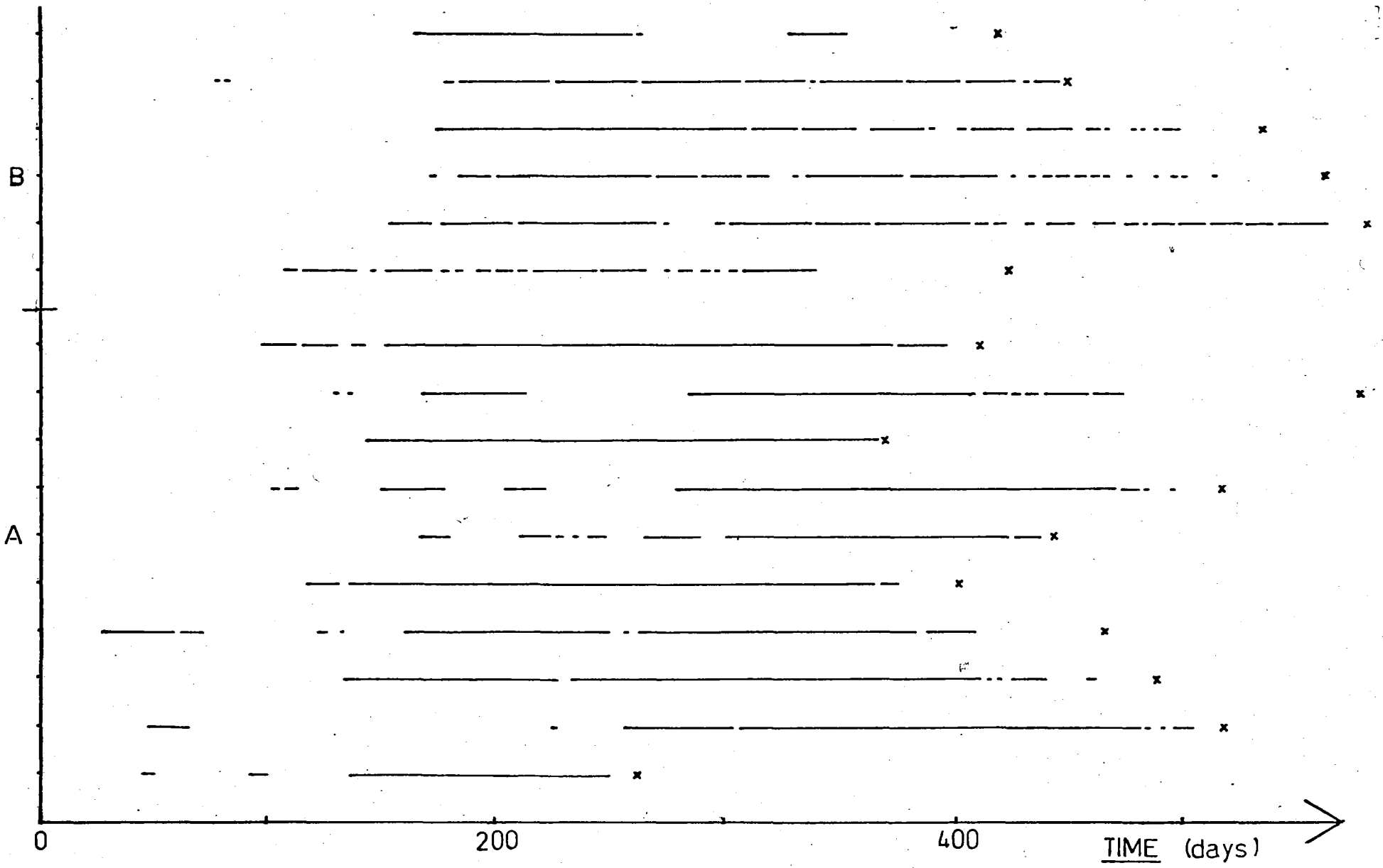


Figure 17. The ovipositional pattern of females of the P1 generation (A) and of the additional females (B); a solid line indicating oviposition during a sample period.

between adult and prepupal weights enabling him to draw the conclusion that fecundity ultimately depended on the favourability of the environmental conditions in which the larvae had lived.

In the present case no correlation between fecundity and size was apparent, and if batch size did correlate with body size the relationship was not strong and showed only slight variation from an approximately constant batch size. Similarly fecundity showed no apparent relationship to total lifespan. However fairly strong correlations were apparent between fecundity and the number of batches laid by an individual beetle and between fecundity and the duration of laying. Neither these parameters nor the length of adult life related to pupal weight. The rate of oviposition measured by the number of eggs or the number of batches per two-day laying period was also not related to body size. The number of eggs per batch and the rate expressed as total eggs per period both showed some correlation to fecundity, while the number of batches laid per period appeared more or less independent of fecundity.

Thus it appeared that in this case the fecundity depended primarily on the number of batches laid by each female, which in turn depended on the length of time spent in a laying state. Fecundity was also influenced to a small extent by the batch size.

(b) Discussion. Previous work on the reproductive behaviour of P. charybdis, as detailed in the literature review, was limited to the comment by Clark (1930) that the size of egg batches varied between 12 and 24, and to the observations on fecundity by Dugdale (1965, and unpublished

data 1963-64) and Styles (1969, 1970) which are summarised in Table 39.

Table 39. Data from the literature on the reproduction of P. charybdis.

	Fecundity	Ovipositional span	Average rate: Eggs/day	Source
	800	45 days	17.8	Dugdale (1965)
	>1700	3 months	c.18.5	
	1397	110 days*	12.7	unpublished results of Dugdale (1963-4)
	1318	65 days	20.3	
	1959	125 days	15.7	
	1738	140 days	12.4	First generation
	2102	140 days	15.0	
	1944	135 days	14.4	
Mean	1743	120 days	15.1	
	386	60 days	6.4	
	374	40 days	9.4	Second generation
	959	65 days	14.8	
Mean	573	55 days	10.2	
	1791**	123 days	14.6	Styles (1969)

(* Time unit used by Dugdale = 5 days)

(** in 74 batches)

(The statements in Dugdale's 1965 unpublished report presumably are derived from the rearings carried out by him in the 1963-64 season; a table from the latter showing the number of eggs laid by each female per 5 day period is marked after nine periods (i.e. 45 days) by the label 'Le50'. At this point the total oviposition from the accumulated average rate was 819. These figures tally with those given in the

later report. No written comments are attached to the earlier data.)

Dugdale's unpublished data also showed graphically the daily total of eggs laid for each of the first five females of the spring generation together with their weights measured at 2 or 3 day intervals. These graphs showed a fairly rapid increase in weight during the initial pre-ovipositional period, a period with fluctuations of up to 40 mg about relatively constant weight levels of from 200 mg to 250 mg depending on the individual, and a final period as the oviposition tailed to an end in which the weight dropped slowly but only slightly. The daily rate of oviposition pictured in these graphs showed severe fluctuations even during the period of laying of from zero to over 40. The maximum reached on any one day was 69 eggs.

However as the eggs are laid in batches the distribution of them with respect to time is clumped, so that they would be expected to show such a variable pattern when the sampling unit is smaller than or of the same order as their separation. Thus if the environmental factors were constant an increase in the size of sampling unit, in this case an increase in the time over which the ovipositional rate was measured, would have eliminated much of the variability shown in this statistic. Unfortunately as the rearings were not carried out in controlled but in semi-natural conditions, with no records to show that even the foliage was kept constant, the results obtained will be complicated by changes in the external environment.

Measurements of the rate of oviposition related to the number of batches per day should be less variable because

batches should not show as clumped a temporal distribution as eggs, but Styles is the only one of the earlier authors to record the number of batches as well as the number of eggs. However, Styles too presented the bulk of his results for the single female studied in the form of a graph showing the number of eggs per batch plotted against the date of laying. As there was an overall average of only 0.60 batches laid per day the resultant graph is very similar to that shown by Dugdale. When these data were rearranged by accumulating the number of eggs laid over 2, 3, 4, 5, 6, and 10-day intervals and graphing each result against time, the fluctuations in ovipositional rate evened out as the sample time increased revealing a basic pattern with an initial peak, a decrease, and a final lower peak. Approximately threequarters of all eggs were laid during the main period of activity with the rate of production reaching a maximum of nearly 27 eggs per day a quarter of the way through the total period of laying. The rate fell to about 5.6 eggs per day when the laying period was three-quarters spent and then rose again to 15.3 eggs per day in the final peak.

The most startling difference between these earlier reports and the results presented from this study is the great disparity in the total egg production or fecundity. Under the controlled conditions used and fed on the mature foliage of *E. globulus*, female *P. charybdis* on the average were laying more than twice as many eggs as had been recorded previously. The rate of laying in both present and past work was comparable (remembering that in the present case the rate is expressed per 48 hours), so that this difference in total production must have been due to a greatly prolonged laying

life in the present research. This was so. However these beetles had also spent much time in a non-laying state to give even greater differences in the total length of the adult life.

The females used in the earlier research at Rotorua presumably came from the over-wintering population in the field when they first emerged from diapause. The extreme difference in Dugdale's unpublished data between the spring and autumn generation of adults would appear to be caused by some severe restriction of the potential by external factors, such as temperature, or more probably food. Styles (pers. comm.) recalled that his beetle was fed on E. viminalis while those of Dugdale were probably reared on this eucalypt plus E. macarthuri. That the foliage fed on is important in determining the reproductive potential will become apparent when the results of the F_1 generation are discussed.

Carne (1966) worked on the closely related Paropsis atomaria in Australia and recorded an average fecundity of 640 eggs for 12 females. However the laying pattern of this species contrasts markedly with that of P. charybdis and consists of a few, very large batches (up to 80 eggs) well separated in time (an average interval of 7.6 days).

The average size of each batch laid by the female kept by Styles was 24.2 eggs, and batches were laid at the average rate of 0.60 per day. Thus although the overall rate of oviposition recorded by this author was close to that found in the present work, it resulted from batches twice as large but laid only half as frequently. The average batch size in the present study was calculated over the complete life and was affected by the occurrence of smaller batches, frequently of only a single egg, at either end of

the period of active laying. However the size was quite variable throughout oviposition. The average size of batches found was within the general range given by Clark (1930) (12 - 24 eggs) and close to but slightly lower than the figures given by Dugdale (1966) from field observations (14 - 18 eggs). In this same report Dugdale mentioned that "occasional batches" contained up to 45 eggs, while Styles' graph showed a maximum batch size of 53 eggs. The largest batch recorded for any of the 19 females in this first laboratory-reared generation was 40 and the next largest 38 (two batches). However only four females gave batches of more than 30 eggs and there were only 13 out of a total of 5354 batches that exceeded this number.

(c) The Pattern of Oviposition. A confusing factor in any determination of batch size is the habit of beetles of clumping batches. In cages where there were more than one female it was very difficult at times to tell whether a large group of eggs was one or more batches because females would add to the first batch often following exactly the same pattern. When the time interval between the laying of one batch and the next was greater than in this case, as occurred in those cages where both egg masses were laid by the one beetle, the eggs of each batch could be distinguished by the different states of development after a period of incubation (figure 18). Separation during the initial stages of embryogenesis was still difficult. This over-clumping of eggs has also been found in the field in trees supporting high populations of beetles. In the laboratory the incidence of this was noted to increase as the density of beetles increased and, because such over-clumping caused

increased larval mortality, it would tend to act as a density-dependent population-regulating factor.

The mortality was caused by the hatching first-instar larvae. These normally spent several hours after their cuticle had hardened and darkened, feeding on the remains of the egg-shells before moving away to the young foliage. Some of the first larvae hatched would eat not only their own shell remains but those of the eggs nearby. This was observed occasionally to have aided those in the process of hatching to emerge, but far more frequently it caused the death of the embryo or neonate larva. Larvae just prior to hatching were occasionally physically damaged but in most cases their death appeared to be due to dessication. The contents of eggs attacked in an early stage of development, or of infertile eggs, were eaten. In many cases in the incubation dishes there was almost complete annihilation of the later-laid batches in unseparated clumps of eggs. A few cases were seen in the field while this cannibalism was occurring but the remains of many more clumped batches were seen where the dessicated remains of some embryos were interpreted as indicating death by this means.

Figure 18 shows a clump of two egg batches laid at the tip of an old leaf. A batch of 10 eggs has been laid first (hatching spines on the developing larvae are visible in these in the photograph) and subsequently a female has laid a further 24 eggs - one at the extreme tip of the leaf and 23 on the opposite side of the first batch.

On all species of eucalypt the tip of the leaf was a favoured position for oviposition. With large leaves such

Figure 18. Two batches of eggs of P. charybdis laid together at the tip of an old mature leaf; hatching spines are visible in the older batch of 10 eggs towards the tip of the leaf.

Figure 19. Four batches of eggs laid at the end of a paper tag, their colour density showing clearly that they are at different stages of development.

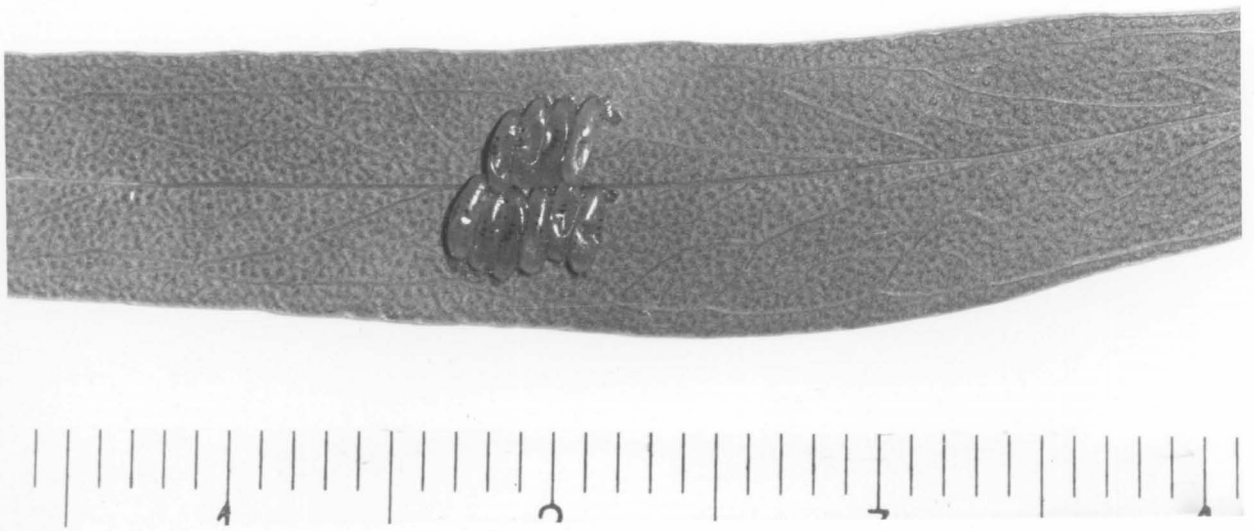


Figure 20. Eggs laid on the middle of a narrow leaf of E. linearis.

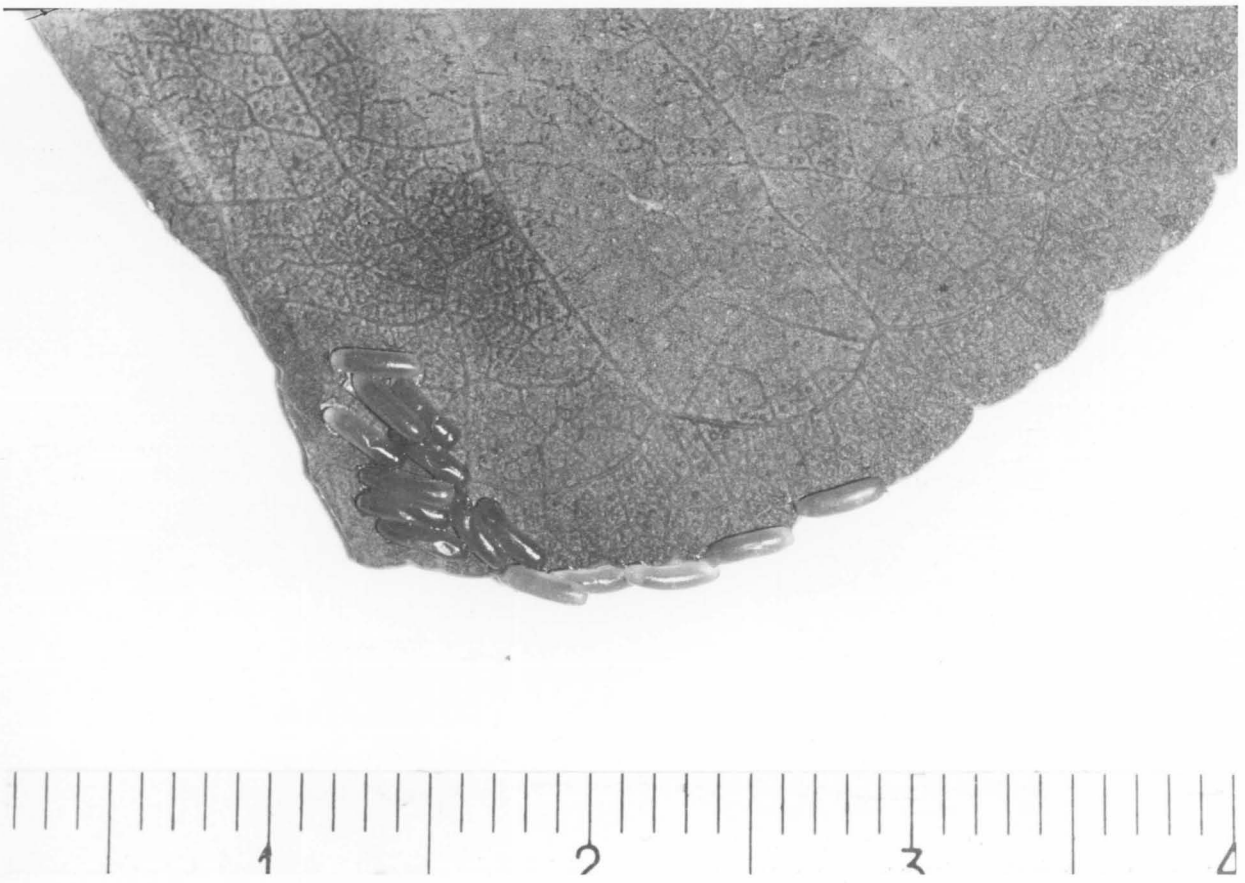


Figure 21. A batch of eggs partly laid along the edge of a juvenile leaf of E. globulus.

as the mature foliage of E. globulus any break in the edge of the leaf was similarly favoured for oviposition. The tips of the heavy paper strip identifying the particular female were also often laid on. Figure 19 shows the tip of a wider strip (2cm) that had been hung in a large cage containing a number of adults. Four different batches can be recognised (one of only a single egg).

On narrow leaves the preference for the tip was less marked and the distribution of eggs more even. In figure 20 an egg-batch is shown in the middle of a narrow leaf of E. linearis. A batch laid away from the tip on a leaf of any shape was most frequently deposited against or close to the edge of the leaf. This habit was exaggerated on the glaucous juvenile foliage of E. globulus when the eggs were often placed in a string along the very edge of a leaf. Figure 21 shows a batch on this foliage where half the eggs have been so arrayed.

The positioning of the eggs in all these cases would appear to relate to the ease with which the females could maintain their footing during the laying process. This would in turn depend on the physical characteristics of the foliage under consideration.

(2) The F_1 Generation - Rearings on Different Foliages.

This next portion of the results presents data obtained from the F_1 generation of adults, which was reared on several different eucalypt foliages. As explained in the section on materials and methods the females were chosen to represent the average of each cage of larval rearing on the basis of pupal weight and were fed the same foliage that they had been reared on as larvae. This method was chosen in preference

to a comparison of the effects of feeding different foliages to beetles of one set size because the effect on pupal weight of the foliage used in larval rearing could make it difficult to find a female of any particular weight among the resulting adults. The selection used was also felt to be more realistic in that it approximated what would happen in the field. As well the P_1 adult rearings on the mature foliage of E. globulus had been used to investigate the possible modifying influence of size on any of the parameters measured here.

A summary of results from the F_1 generation is given in Table 40. (A full table of the mean results for each female is given in the appendix, Table 4.)

It is obvious from Table 40 that the type of foliage fed to the adults markedly affected the reproductive potential of female P. charybdis. The results were too variable and based on too few individuals for a statistical analysis to be attempted for any of these facets of adult rearing but some differences appeared obvious on inspection.

The fecundity or total number of eggs produced was greatest for the control foliage and was even greater than that recorded from this eucalypt for the parent generation. The maximum recorded for an individual female fed on this leaf material increased from 5826 to 6737 eggs and 4 of the 17 females on this food bettered a production of 6000 eggs.

Table 40. Biological data for female P. charybdis of the F_1 generation reared for their entire lifespan on various Eucalyptus foliages.

Foliage		globulus - mature	delegatensis - hybrid	linearis *	obliqua (Ch)	obliqua (BG)	globulus - juvenile	andreana	linearis (Ch)
Number of females		17	3	2	4	1	8	6	4
Size (mg)		175.2	200.0	147.0	158.3	161.0	159.6	162.8	150.3
Fecundity		4957	4262	3257	3155	2991	2460	2447	2308
Longevity (days)		328.8	267.7	250.5	425.5	372.0	196.9	233.7	241.5
Fertility (%)		94.63	81.45	97.20	97.08	88.90	97.44	96.33	96.53
No. of Batches		321.5	313.0	315.0	264.0	270.0	181.4	199.0	229.8
Batch size		15.51	13.30	11.29	12.23	11.08	13.94	11.96	10.80
Laying life(48hrs)		116.0	88.7	86.5	112.3	110.0	69.3	67.5	64.0
Rate of laying	A **	43.63	45.90	38.04	28.76	27.19	36.07	35.43	35.21
	B	2.91	3.50	3.54	2.35	2.45	2.63	2.96	3.39

(* Fed both types of E. linearis - see text

** A = eggs / 48 hrs; B = batches / 48 hrs)

The next best foliage to E. globulus - mature in terms of the mean fecundity achieved was E. delegatensis - hybrid. This mean was based on one very low value (965 eggs) and two very high ones (5855 and 5985 eggs). These females were the largest of any used in the whole generation. Foliages other than these two produced considerably lower mean fecundities, and these results fell into two groups separated by a small gap. The more productive of these groups consisted of E. linearis *, E. obliqua (Ch) and E. obliqua (BG); the

second consisted of the juvenile foliage of E. globulus, E. andreana and E. linearis(Ch). (E. linearis* denotes adults reared as larvae on E. linearis(BG) and fed for a short time only on this before the supply ran out. For the rest of the time they ate E. linearis(Ch).) Even the worst foliage, E. linearis(Ch), was better on average than the results reported in the literature and yet only half as good as the control. However the values for individual females within foliage types were very variable except for those fed on E. globulus-juvenile, and E. linearis*.

The mean size of the females reared on each foliage as indicated by pupal weights ranged from a maximum of 200 mg for those on E. delegatensis-hybrid, down to 150.3 and 147.0 mg for the two groups fed E. linearis. The beetles on the mature leaves of E. globulus averaged 175.2 mg to be the second heaviest, while the remaining four foliage results were all very close to 160 mg. There would appear to be a slight correlation between fecundity and mean body size which would be strengthened by the exclusion of the female on E. delegatensis-hybrid that laid so few eggs. However if the results for both this foliage and the control are ignored those from the six remaining foliage types are not distinct enough to show any definite pattern. Also the size of the individuals fed each type of eucalypt was not widely divergent whereas the fecundity was. Thus any size-fecundity correlation is of doubtful value.

The fertility of most individuals was very high, exceeding 93% in all bar 5 of the 45 females used on all foliages. One of the five was reared on the control foliage and was an extremely aberrant result (59.7% fertile). This

would indicate a fault in the reproductive functioning of the particular female and not a foliage effect. Another of the low fertilities was from the single representative fed E. obliqua (BG) so that this too could be due to the chance selection of a faulty beetle or to a foliage effect. The remaining three low results were from the three females from E. delegatensis - hybrid so that a lowering of the fertility directly caused by the type of food seems probable here. The foliage could have been affecting gamete production in either sex, or the transfer and union of these.

Although this generation had been reared from egg onwards under warm long-day conditions, the commencement of oviposition was erratic and a few adults could possibly have undergone a short diapause. The shortest duration recorded for the pre-ovipositional portion of adult life was 15 days, most took up to twice as long as this and several had not begun laying three months after emerging as adults. On the average many days of the adult life were spent in a non-laying state. This ranged from a quarter to almost a half of the total period of adulthood.

Both the average number of batches per female for each foliage and the average size of these batches showed a slight correlation with mean fecundity in further graphical analyses of the results for each foliage. The mean rate of oviposition expressed as the number of eggs per ovipositional-period also showed a weak positive correlation with fecundity. The alternative expression of laying rate - the mean number of batches per ovipositional-period - did not display a relationship with either fecundity or body size. All these relationships were also apparent from the results of the P_1

generation. The mean body size showed some positive correlation to batch size, a very slight positive correlation to ovipositional rate expressed as the number of eggs per laying-period, and no significant relationship to the number of batches. The first two of these correlations were not shown by the earlier results. Similarly although the results from the parent generation for fecundity had related to the duration of laying no such correlation appeared now. The number of periods during which laying occurred also showed no dependence on the size of the females involved.

The picture which emerged from these graphical analyses was of a relationship between the mean fecundity and the mean size of the adults reared on the different types of Eucalyptus foliage. This relationship was paralleled by dependencies between either of these measurements and both the size of the batches laid and the rate of egg-laying during the period of active oviposition. However as the number of batches that were laid per sampling period was independent of fecundity and also of body size the trends in the results for the number of eggs laid per period simply reflected the changes in batch size with respect to body size. Batch size and the number of batches laid would interact to determine the overall fecundity, but as the number of batches deposited showed no correlation with body size, the dependence of fecundity on body size was primarily due to the relationship between the size of the females (expressed as their weight as pupae) and the number of eggs per batch laid by these.

This is very similar to the conclusion reached by Carne (1966) for P. atomaria. However in the present case it must be remembered that much of the visual correlation

is due to the performances of P. charybdis fed on either E. delegatensis - hybrid or on the control foliage. The females in these groups were large and tended to perform well with respect to all the aspects studied. The mean weights of females reared on other foliages were all very similar, and the mean figures for other parameters obtained from these beetles were variable but also basically similar. A further consideration that must be taken into account in interpreting these results was that within each foliage group most of the values from individual females varied markedly and without any distinguishable pattern. The exceptions were the weight, the percentage of the eggs that were fertile, and the rate of laying expressed as the number of batches per period. The last two of these measurements were relatively constant for all the females in the generation, whereas the weight tended to be constant among females fed on each different foliage because these beetles had been selected to represent the mean results of larval rearings, and larval size was largely determined by the type of leaf eaten.

(3) The F_2 Generation - Short-term Feeding Trials.

This section is concerned with an attempt to assess the effect of short-term feeding on different foliages which was carried out with the F_2 generation of adults. This trial was planned because of the intrinsic value of information on changes in the food of adult P. charybdis, and also because the longevity of the beetles in the laboratory made it difficult to obtain adequate amounts of information using only full-term rearings. Unfortunately at the time this trial was undertaken many of the foliages, especially those required for the F_1 adult rearings, were in

short supply and could not be included. Several of the eucalypt trees sampled during the F_2 adult rearings were not used in any other portion of the study. Although these have been identified by the author as belonging to species used more intensively at other times, the variation already disclosed between trees of the same species makes the extension of the present results tentative.

(a) Results. A summary of the results of these short-term feeding trials is given in Table 41 and the results for individual beetles are presented in the appendix, Table 5. The beetles were reared on the control foliage (E. globulus - the mature leaf-form) until they had laid for at least 10 consecutive sampling periods (i.e. 20 days). They were then fed an experimental foliage throughout five sampling periods (10 days) before being returned to the control foliage until they had laid for a further term of at least 20 days. The only deviations from this test period of 10 days were several trials checking the effect of starving the beetles for 2 or 4 days at a time, and one checking for a longer term foliage effect by using an extended trial period of 20 days. The results for this last experiment are given in the table as two consecutive 10 day trial periods. Standardisation of the period over which measurements were made meant that comparisons of the average total number of eggs or batches laid were also comparisons of the rate of oviposition. It also stabilised as far as possible the test procedure. The variation in sample size for each foliage type means that the average results are not all expressed with the same accuracy. This and the fact that the feeding background of the experimental beetles varied according to the trial foliages

Eucalyptus foliage	Number of trials	Number of eggs			Number of batches			Mean batch size			% Fertility		
		Pre	Test	Post	Pre	Test	Post	Pre	Test	Post	Pre	Test	Post
globulus-mature (control)	10	258	263	250	14	14	14	18.0	18.6	18.7	98	98	98
globulus-juvenile	4	263	236	201	16	13	13	17.0	18.4	15.9	100	99	97
delegatensis-hybrid	3	260	153	216	14	12	13	18.2	11.9	16.1	96	94	96
obliqua(BG)	3	248	8	168*	13	4	10*	18.7	2.6	16.1*	99	97	96*
obliqua(Rf)	1	317	213	220	14	12	11	22.6	17.8	20.0	99	96	95
obliqua(RS)	4	270	42*	243	14	4*	14	19.6	5.7*	17.7	99	95*	96
fastigata(Cl)	2	208	0*	159	12	0*	8	17.4	0*	19.9	96	-*	96
alpina	1	274	0*	-	14	0*	-	19.6	0*	-	99	-*	-
linearis(Ch)	8	254	136	236	13	12	13	19.1	12.0	18.0	98	88	98
linearis(BG)	2	181	0*	178	17	0*	14	10.8	0*	12.7	95	-*	96
linearis(TS)	2	288	78	207	13	7	10	23.1	9.0	21.8	99	68	97
linearis(TN)	1	313	3	147	15	3	10	20.9	1.0	14.7	99	100	97
linearis(SP)	9	256	146*	244*	13	10*	12*	19.5	12.8*	20.0*	94	89*	96*
linearis(SP) a (20 days)	1	362	326 341	152	18	18 23	12	20.1	18.1 14.8	12.7	93	97 94	91
starved (2 days)	3	306	4	284**	15	1	13**	20.4	3.7	21.9**	99	75	97**
starved (4 days)	1	280	16	0*	15	1	0*	18.7	16.0	0*	98	100	-*

(* = beetle died during this period;

a = test period of 20 days shown as two consecutive 10 day periods.)

Table 41. The average effect of feeding adult female P. charybdis on various eucalypt foliages for short intervals. The test period for all except the last three trials was 10 days; the results before and after testing were always for 10 day periods.

previously used on them makes interpretation of the results obtained more difficult.

(b) Discussion. The feeding background could be expected to have a marked influence on subsequent results if exposure to a type of foliage once, caused the beetle to adapt either more or less readily when exposed to the same or a similar foliage later. This was checked by repeat feedings of the same foliages on four separate occasions and pre-adaptation through prior exposure did definitely seem to be indicated. The average production of the four first-feedings to each beetle was 273 eggs in the 10 days immediately preceding the trial, 94 eggs during the test period and 267 eggs in the 10 days immediately following the trial. The corresponding averages for the four subsequent feedings of the same foliages were 254, 153 and 228 eggs respectively. Thus average egg production during the first trial period was 34.4% of the production before the trial and 35.2% of the production on being returned to the control foliage. In the second trial egg production was affected only half as much with laying during the trial being 60.2% of that for the preceding and 67.1% of that for the following periods. Because of this conditioning effect no more than five trials were carried out using any one female beetle.

Table 41 shows that the total number of eggs laid was the most sensitive measurement of the effect of short-term feeding on various foliages because both the number and size of egg batches was reduced. The fertility was not generally altered although it was lowered by E. linearis(Ch), E. linearis(TS) and E. linearis(SP). The low fertility from a two-day starvation period could be significant but the total number of eggs

involved is very small. The average batch size showed a decrease during the test period in all cases except for a constant level in control tests and a slight rise when the test foliage was the juvenile leaf-form of E. globulus. The mean number of batches also decreased in most cases but by a lesser amount. The results for both these measurements were less clear than for average total egg production. Much of the decrease in batch size and yet comparative stability in the number of batches resulted from the frequent occurrence of eggs laid singly during trial periods.

Ten beetles died during the series of trials; seven during or immediately following feeding periods on different foliages and three after being starved for a short time. This high mortality is one reason why few trials were carried out using some foliages. The starvation trials were included when it became obvious that a change in diet was having a major effect and were planned to determine whether this could be attributed to a refusal to eat the new foliage or only to a debilitating effect of the unaccustomed foodstuff (a regular occurrence in medical and veterinary cases). The result of these starvation trials indicated that unacceptability of a novel food could well account for the observed depression in oviposition, but the high level of mortality showed that mature ovipositing females were intolerant of even relatively short periods of starvation. The generally lower level of mortality from experimental foliages when compared with the starvation results indicated that consumption of these trial foliages sufficient to sustain life must have generally occurred.

The single 20-day experimental run using E. linearis

(SP) as the trial foliage gave surprising results in that no great drop in laying resulted although in the nine other cases where this foliage was used for 10 days an average reduction of nearly 50% resulted. The overall oviposition rate for both halves of this 20-day period was very similar although the second result arose from more and smaller batches than the first. The fall in the number of eggs laid on the return to the control foliage in this case was due to the beetle laying well for the first and last 2 days of the 10, not laying for the middle 2-day period and producing only a few eggs on the intermediate periods. Over the next 10 days on the control foliage a total of 323 eggs in 15 batches were produced which is equivalent in number and batch size to the pre-test and test periods. This lessened oviposition post-test may have been due to the beetle becoming used to E. linearis (SP) and reacting unfavourably to the change back to the mature foliage of E. globulus, but the normal laying over the first two-day sampling period and gradual lessening over the next argue against this. In all other cases the effect of a change in foliage was immediately apparent.

There were only 5 cases in the 35 trials in which laying was very markedly altered in which large egg batches were laid, and in these the time of hatching indicated that the batches (only one in each case) had been laid only shortly after the foliage had been changed. Thus the shock effect of a variation in the food resulted in the complete or partial retention and resorption of eggs within, at the maximum, 12 hours of the beetle being exposed to the new foliage. That resorption occurred was apparent from the unchanged or slightly lowered oviposition immediately after

the test period when compared with that prior to the test.

The similarity shown in the table for the mean number of eggs laid in the pre-test period for all samples based on reasonable numbers has been taken as indicating that the chosen minimum interval between tests (10 consecutive periods of laying) was adequate to allow the beetles to readjust to feeding on the control foliage.

The results of all the 10-day trials, excepting those on the juvenile form of E. globulus and on E. obliqua (Rf), showed a reduction in total oviposition brought about by both reduced batch size and generally fewer batches. E. delegatensis - hybrid, E. linearis (Ch) and E. linearis (SP) had the total number approximately halved and all the rest were even more affected with E. fastigata (Cl), E. linearis (BG) and E. alpina giving no oviposition and some mortality and E. obliqua (BG) and E. linearis (TN) giving negligible laying.

Once again these results showed the spectrum of effectiveness of different trees within the species of E. linearis and E. obliqua. The harsh effect of E. fastigata (Cl), E. linearis (BG) and E. obliqua (BG) agreed with the low value of these foliages as larval food. E. alpina was a eucalypt with very thick leathery leaves from the time that they first unfolded and the result from this was thought to be caused by a physical interference with feeding similar to that of E. ficifolia with young larvae. E. obliqua (Rf) gave a result similar to its good performance as a larval food. E. globulus - juvenile, E. linearis (Ch) and E. delegatensis - hybrid all gave intermediate results in larval rearing and the first two also returned low results in the

long-term adult rearings of the F_1 generation. The last was very successful as an adult food. However in these short-term trials the juvenile foliage of E. globulus was almost indistinguishable from the control (marginally fewer eggs in slightly larger batches) whereas the other two showed a considerable reduction in effectiveness compared to the control. This would reflect the chemical similarity of the two types of leaf from E. globulus lessening the shock of the change. In other words beetles conditioned by feeding on the mature leaf-form of E. globulus would be largely preconditioned to accept also the juvenile leaves of this species. To eliminate such effects in short-term feeding experiments it would be necessary to have several series of trials each using a different foliage as the control.

(4) The P_2 Generation - The Effect of Temperature on Oviposition.

(a) The Initial Period at 25.6°C. The field collected beetles which constituted the last group of adults reared proved to be very active reproductively. Only 4 of the 20 females failed to lay more than 2000 eggs in the 74 days that elapsed between the time they were taken into the controlled-environment room and the first change of temperature. One of these had died after 45 days and another had been accidentally killed after only 27 days but both had produced more than 1200 eggs before death. During the 74 days the overall average fertility was greater than 98% and no female had more than 5% of infertile eggs. The average of the mean batch sizes was over 20 eggs, with a range from 12 to 25. The average total number of batches exceeded 100 which represents a rate of more than 1.6 per day. These results

are given in more detail in Table 42.

The most obvious features in this table were the very rapid rate of laying compared to the results on this, the control foliage, in previous trials and the large average batch size. The oviposition rate was twice as rapid as those calculated for P_1 and F_1 adults and the batch size greater by the same margin, although the latter was equivalent to the figure obtained by Styles (1969). Both values were also greater than the corresponding figures from the F_2 females. Graphical analyses showed no correlation between live weight and the size of batches but definite correlation between live weight and the rate of egg-laying.

A check carried out on females of the F_2 generation attempted to find a relationship between pupal weights and adult live weights. Unfortunately only a very slight positive correlation was apparent when these results were graphed. The rate of oviposition and mean batch size plotted against either pupal or live weight showed no definite relationship in either case.

Table 42. Results from the P_2 generation of adult P. charybdis after 74 days.

Number of eggs	Number of batches	Mean batch size	Fertility (%)	Ovipositional rate per 48 hours		Weight (mg)
				Eggs	Batches	
1254*	54	23.22	99.12	92.88	4.0	-
1385	62	22.34	99.18	37.44	1.68	-
1629	131	12.44	98.25	44.02	3.54	102
1689**	67	25.21	95.62	75.06	2.98	-
2043	130	15.72	96.15	55.62	3.52	215
2151	102	21.09	97.61	58.14	2.76	145
2321	97	23.93	99.27	62.72	2.62	197
2325	141	16.54	99.10	62.84	3.82	175
2427	114	21.29	99.33	65.60	3.08	233
2431	127	19.08	98.72	65.70	3.44	199
2447	133	18.40	95.46	66.14	3.60	203
2520	127	19.84	99.14	68.10	3.44	218
2529	105	24.09	98.80	68.36	2.84	196
2577	105	24.54	99.40	69.64	2.84	203
2620	142	18.40	99.07	70.82	3.84	203
2654	104	25.52	99.45	71.72	2.82	-
2690	130	20.69	98.53	72.70	3.52	184
2813	126	22.32	99.29	76.02	3.40	245
2969	134	22.16	96.53	80.24	3.62	215
3164	147	21.52	96.61	85.52	3.98	247
2427.50	119.83	20.92	98.23	67.44	3.26	198.75

(* Killed after 27 days; ** Died after 45 days)

(b) The Effect of Various Temperatures. The final aspect of adult rearing examined did not relate directly to host-plant relations but was necessary to interpret and extend results from laboratory rearings to field conditions. This was the effect of temperature. The foliage eaten had been shown to affect the fecundity of P. charybdis and also the rate of oviposition expressed as the number of eggs per unit of time. Temperature too could well be expected to influence both the overall number of eggs produced and the rate at which they were laid. It is possible that there would be interaction between foliage type and temperature regime, so that foliages would be ranked with regards to suitability in a different order at different temperatures. Little has been done in this field but Cram (1970) has demonstrated such an interaction between host variety and temperature for Otiorhynchus sulcatus, the black vine weevil, attacking blueberry cultivars. In the present research the availability of time precluded studying the effect of temperature on total egg production and the limited number of adults that could be reared simultaneously prevented a study of possible temperature-foliage interaction. Instead base information was sought on the effect of several different temperature regimes on the oviposition of P. charybdis fed on only one type of eucalypt foliage - the mature leaf-type of E. globulus.

The experiments were performed using a similar procedure to that in the short-term feeding trials described above. Beetles that had been reared and begun ovipositing at one temperature (25.6'C) were kept for about 20 days at another temperature level and then returned to the initial

temperature. Two different groups of adult beetles were used. The first consisted of seven females of the F_2 generation after short-term feeding trials had been finished and the second was made up from the field-caught P_2 generation once they were no longer required to supply eggs for the last series of larval rearings.

The F_2 females were held for nine sampling periods (18 days) at the reference temperature of 25.6'C and then moved to a second controlled-environment room maintained at 20.0 $\pm$ 1.0'C for 36 days before being transferred back to 25.6'C for a further 18 days. The time spent at 20.0'C was treated as two 18-day periods. The number of eggs and batches, the size of batches and the fertility of the eggs were checked for any change. The average values of these measurements are given in the first section of Table 43, and the results for each individual female in the appendix (Appendix Table 6). (In order to facilitate comparisons with the subsequent experiment which was based on the use of 20-day periods, the total numbers of eggs and batches have been corrected to give results equivalent to those from 20 days of sampling.)

The females in the second experiment were held at 25.6'C for 20 days, transferred to 15.0'C in a growth cabinet with a 16 hour light : 8 hour dark photo-cycle for 20 days, returned to the reference temperature for 20 days, to another growth cabinet held at 12.5'C for a further 20 days and then back to the reference temperature again. Finally the females were placed in a controlled-environment room at 29.5'C and then returned to the standard temperature. The last two changes were not for a full 20 days; the warm

temperature was maintained for 14 days and the reference for only 12 days, but the tabled results below have been altered to a 20-day time base where required. There were 17 females in the P_2 generation at the start of this experiment but five died during the temperature changes and data from these have not been included. Averages from these 12 beetles for each temperature and period are shown in the second section of Table 43, while results for individual beetles are included in the appendix (Appendix Table 7).

Table 43. The average effect of various temperatures on oviposition of P. charybdis (results based on a 20-day trial period).

Test	Temperature 'C	Number of eggs	Number of batches	Mean batch size	Fertility %
F_2 - 1	25.6	540.9	26.1	20.86	95.8
- 2	20.0	394.0	21.3	18.66	97.8
- 3	20.0	288.3	16.6	17.43	97.2
- 4	25.6	349.3	19.4	17.60	91.8
P_2 - 1	25.6	597.8	29.9	20.44	97.9
- 2*	20.0	(408.4)	(19.2)	(21.54)	(98.3)
- 3	15.0	222.3	10.3	22.15	98.4
- 4	25.6	488.5	25.8	19.25	98.5
- 5	12.5	229.9	9.3	26.15	99.0
- 6	25.6	500.6	25.3	20.10	97.7
- 7	29.5	408.4	25.6	16.26	91.9
- 8	25.6	398.1	24.4	16.92	90.3

(2* - results derived from a 4-day transition period at this intermediate temperature.)

The average values tabulated above showed the expected temperature dependence of oviposition for measurements in the 12.5'C to 25.6'C range. The trial at 29.5'C produced results of lower value than those for 25.6'C indicating that this upper temperature was beyond the optimum for P. charybdis. Another observation confirmed by these mean results was the increase in batch size at the lowest temperatures used. No explanation for this was obvious. It is possible that at the slower rates of development occurring at these lower temperatures localised internal food deficiencies caused by competition among the maturing ova for nutrients may be minimised.

The results from the F_2 trials are not easy to interpret because of the decrease in all measurements between the first and second halves of the time spent at 20.0'C, and because the increase on return to 25.6'C raised these values only partway to their former levels. The fertility decreased fractionally during this last test period. A possible explanation is that because these females were nearing the end of their reproductive life there was a gradually progressive decline in oviposition over which the pattern caused by the temperature changes had been superimposed. Although the females of the P_2 generation had also been laying for some time before the temperature trials began (and all 12 had produced more than 2000 eggs), they should have had at least as much reproductive potential as this still unused at that time. The initial rate of oviposition was not regained during later periods at the same temperature but the second and third periods at 25.6'C gave comparable results. The final check period

at this reference temperature showed a marked decrease in results and this could be due either to a natural reduction in oviposition with advancing age or to a carry-over of the debilitating effects of the previous period at a high temperature. The fertility dropped with the change to a high temperature and did not improve with the return to 25.6'C. However the major contribution to the average fertility during the final check period came from one beetle, the eggs of which were only 62% fertile. Including this only four of the 12 females had a depressed (lower than 91%) fertility during the final trial whereas six were affected at 29.5'C. Only one showed a reduced fertility at any other time.

The results derived from the very short time spent at 20.0'C prior to fully lowering the temperature to 15.0'C have been included in spite of the great difference in duration because they show agreement with those obtained using the F_2 females. It should be noted that these results for 20.0'C give the same overall rate of egg-laying as occurred at 29.5'C, but in line with the apparent inverse relationship between temperature and batch size remarked on earlier, this amount results from fewer but larger batches.

The variation in all aspects of oviposition recorded for each period spent at the reference temperature as the experiment progressed prevented any fine relationship between any aspect of oviposition and temperature being found. However it was clear that increasing the temperature up to 25.6'C increased the number of eggs laid. This resulted from an increase in the number of batches laid because the average number of eggs per batch tended to decrease. At 29.5'C there was a slight decrease in total

oviposition due to the same number of even smaller batches being laid. Fertility did not seem to be affected by any of the temperatures used except for the highest (29.5'C) under which it was slightly lower.

The experimental rearing of adults of P. charybdis under controlled environmental conditions has demonstrated the pronounced longevity and fecundity of this species. The type of Eucalyptus foliage fed on has also been shown to be a major influence in determining both the longevity and the pattern of reproductive behaviour. The latter was affected by either continual or short-term feeding on different types of leaf. Temperature also affected the rate of laying and batch size, but oviposition continued over quite a large range of temperatures.

CHAPTER V

SELECTION EXPERIMENTS AND FEEDING TRIALS WITH LARVAE OF PAROPSIS CHARYBDIS

As a logical extension of the research reported earlier which aimed to determine whether different Eucalyptus foliages varied in their ability to sustain both larval and adult P. charybdis, further series of experiments were performed to find out if the beetle could distinguish between the various foliage types, and so increase the efficiency with which it exploited available foor resources. The present chapter covers those trials carried out using larvae as test individuals, and is divided into three sections; selection experiments, reaction experiments, and feeding trials on non-host plants.

1. SELECTION EXPERIMENTS

These trials were those in which the larvae were confronted with a choice or selection situation. The main series of experiments was based on exposing larvae individually to a pair of discs of the same size but of different foliage types and measuring the response by finding the area of each that was eaten in a set time.

(1) Initial Trials

(a) Method. Initially the selection trials had been based on a multi-disc method running for a variable length of time. Preference in these was not rated by the absolute areas of each foliage eaten but from the ratio of

these. This was because of the variable length of exposure which in turn was instigated so that each trial continued until the subject larva had had contact with both foliages as shown by the pattern of feeding damage. Thus each trial ran until approximately one-fifth of the total area of both foliages (involving four of six discs or six of eight discs), or one-third of each disc of one foliage had been eaten. As the discs of each foliage alternated in sequence around the rim of the trial arena, by the time either of these criteria had been met the larva would have had contact with both trial foliages. Trials in which more than two-thirds of both foliages had been eaten before they were stopped were discarded because it was felt that these could have been affected by starvation forcing a larva which had consumed most of the available palatable foliage to then begin eating the relatively unpalatable leaf material. (This only occurred when trials had to be left overnight because insufficient foliage had been eaten by evening.) The leaf discs were pinned in a vertical position into the edge of a horizontal cardboard disc which constituted the arena of the selection chamber. A larva was confined to this arena because the cardboard disc was supported through a central hole by the stem of an inverted glass filter funnel. The base of the funnel rested in 0.5 to 1.0cm of water in a pint glass preserving jar which had a glass petri-dish as a lid to maintain a humid atmosphere. Glassware was used for ease of cleaning; cardboard discs were disposed of after a single use.

Each jar with the distilled water in the base was set up at least 3 hours before a larva was added to allow

the humidity to develop. Just before the trial began the leaf discs were punched out using a cork borer and pinned to the arena. Initially three 54 mm^2 discs of each foliage were used per test but later four 35 mm^2 discs were used. The cork borer was thoroughly cleaned between uses to prevent contamination of the subsequent samples. Each trial used an early fourth-instar larva reared on the control foliage and then starved for 2 hours prior to testing. The control in this as in previous work was the mature leaf-type of E. globulus; it served not only as the food on which all larvae for selection experiments were reared but also as the control in each trial against which every other foliage was tested.

Consumption was measured as the area eaten. Prior to the trials a recording sheet was set up using the appropriate cork borer, an ink stamp-pad and graph paper divided into 1mm squares. Then when the trial was stopped the remains of each disc was placed in a recording circle and the area eaten traced out. Later this area was measured by counting all the 1mm squares more than half in the eaten area. Counting was found to be more reliable than weighing cutouts of the eaten area, and weighing the actual leaf remains would have required all the work to be done as soon as each trial was terminated and so would have restricted the rate at which these trials could have been run. (Cutouts of 10 uneaten discs had weights ranging from 388 to 435 mg - average 411 mg - whereas the area by counting ranged from 53 to 56 mm^2 - average 54.5 mm^2 .) The trials were carried out in the controlled-environment room at 25.6 $\pm$ 1.2'C. After the first which was done in indirect but

full light all the jars were covered with black polythene covers during each trial. In the first trial the larvae had spent most of their time sheltering on the lower face of the arena and had been slow to feed.

The foliages used in the initial series of larval selection trials represented several different situations. E. delegatensis - hybrid had given results in larval rearings only slightly inferior to those from the mature foliage of E. globulus, while E. fastigata (BG) had caused the deaths of all first-instar larvae placed on it. There was also E. obliqua from two different sites. On both types of E. obliqua leaves the larvae had performed poorly, but these results had been distinct and the question was whether or not the larvae could discriminate between these two. Several trials were also set up with all discs of the control foliage to check on the method used. (The sites used and their abbreviations have been given on p94.)

(b) Results. The valid results obtained from these trials are shown in Table 44. The tabled results show how great the variation was between samples of the same foliage, and the single "dummy" trial using all discs of the control was markedly different from the expected 1;1 ratio. What the table cannot show is the number of runs not realising valid results. This and the time consuming task of setting up each arena were the reasons why this method was not persisted with. The results do show a definite, but not absolute, slight preference for E. delegatensis - hybrid over the control, and preferences for control rather than E. obliqua (Ch), E. obliqua (BG) and E. fastigata (BG). The preference relative to the control against the latter two

foliages is stronger than that for E. obliqua (Ch). The observed discrimination in this preliminary work would appear to correlate roughly with the relative suitabilities of these foliages as larval and adult foods.

Table 44. The ratio of the amount of a test foliage eaten to the amount of the control eaten when the control value was adjusted to 1.00

	Test Foliage				
	delegatensis - hybrid	obliqua (Ch)	obliqua (BG)	fastigata (BG)	globulus - mature
Ratio globulus-mature : X = 1.00 :	1.34	0.99	0.07	0.42	0.61
	1.08	0.32	0.34	0.16	
	0.70	0.98		0.23	
	1.19	0.64			
	1.47	0.59			
	1.19				
	1.40				
	0.41				
Average	1.10	0.70	0.21	0.27	0.61

(2) Determining the Final Methods

After a period of experimenting with various methods for selection experiments a similar but much simpler technique was adopted. This involved the use of two larger leaf discs (176.2 mm^2 in area, 15 mm diameter) placed on a moist 55 mm diameter filter paper in a 7cm diameter glass petri-dish. One disc was of the control and the other of the experimental foliage. The moist filter paper was

necessary to maintain humidity and prevent the leaf discs from wilting. In place of fourth-instar larvae, third instars were used. This change was brought about because although the larger later instars might be expected to eat more rapidly and hence allow a shorter testing period it was not always easy to distinguish early actively-feeding fourth instars from those about to cease feeding and become prepupae. These latter were obviously unsuitable as test individuals. The trial was run for a fixed time (24 hours) in a controlled-environment room held at 20.0'C. The same system of recording as previously described was used. An example of the results obtained using this method is shown in figure 22.

The length of the test period, the temperature regime used (20.0'C or 25.6'C) and the duration of a starvation period for the larvae before testing were decided on the basis of preliminary tests and of convenience in operation. Twenty larvae were used in these preliminary tests which were not selection trials because only one leaf disc - a sample of the mature foliage of E. globulus - was included in each dish. As well as the area that was eaten, the number of frass pellets produced by each larva during the experimental period was counted to give another measure of consumption. The different treatments used in this techniques trial were a 2 hour starvation with a 6 hour feeding period and a 2, 16 and 24 hour starvation period followed by a 24 hour feeding period at 25.6'C, and 2, 6, 16 and 24 hours starvation followed by 24 hours of feeding at 20.0'C. The treatments were unreplicated except for the trials involving 2 and 6 hour periods of starvation at 20.0'C which were duplicated. The summed total area eaten

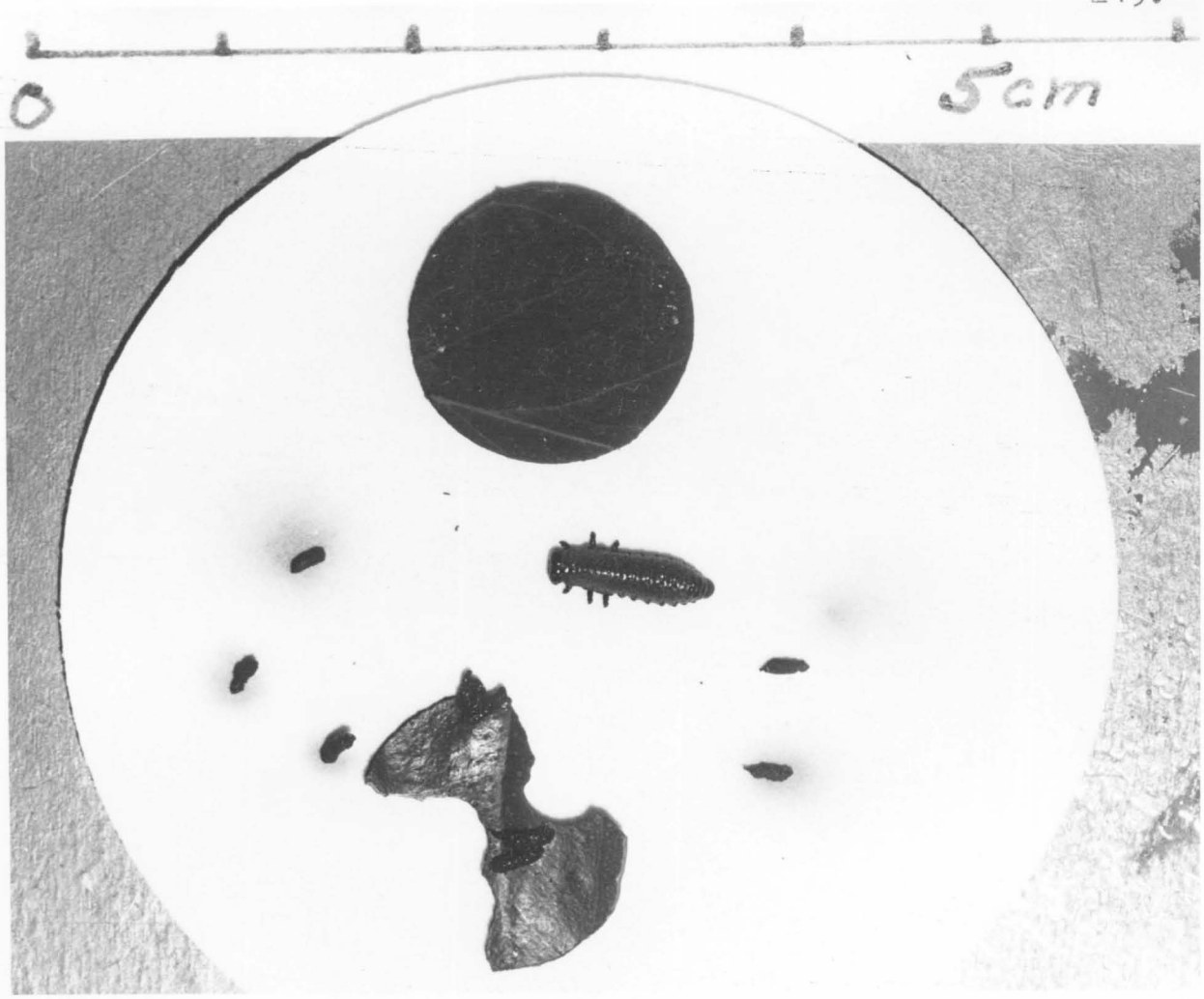


Figure 22. A photograph showing the result of a larval selection experiment comparing discs of E. globulus-mature (lower) and E. fastigata(Cl) (upper).

and the number of frass pellets produced for each treatment are given in Table 45.

Table 45. The effect of different periods of starvation at two temperatures on two measurements of foliage consumption.

Test conditions			Area eaten(mm^2)	Frass pellets produced
Temperature ('C)	Starvation period (hr)	Feeding period (hr)	Mean ± Standard deviation	Mean ± Standard deviation
25.6	2	6	29.2 ± 19.7	3.2 ± 2.0
	2	24	54.7 ± 30.0	7.8 ± 4.7
	16	24	41.8 ± 38.8	7.0 ± 5.6
	24	24	35.0 ± 32.3	6.3 ± 5.5
20.0	2	24	60.9 ± 38.3	9.7 ± 4.6
	2	24	69.5 ± 42.1	11.0 ± 5.4
	6	24	68.1 ± 52.0	9.5 ± 6.8
	6	24	70.2 -	- -
	16	24	46.6 ± 41.4	7.0 ± 5.2
	24	24	66.7 ± 41.5	9.2 ± 5.4

It is obvious that the larvae ate more at the lower temperature and so this was chosen. This could have been due to heat stress acting directly on the larvae or on the leaf discs to make these less palatable; the discs did tend to wilt more readily at the higher temperature. It was also obvious at 25.6'C although less apparent at 20.0'C that the longer periods of starvation decreased rather than increased consumption. Starvation is frequently used in order to prime subjects before a feeding trial to ensure a low threshold for those stimuli that trigger feeding. Excessive

food deprivation might result in lowered consumption due to weakening the larvae, or it may upset the intake-regulating mechanism, or even cause damage to the larvae if the threshold is lowered so far that non-edible material is eaten. Some effect caused in the latter way is possibly indicated by the consumption of filter paper by larvae during starvation and also during feeding. Some larvae produced faecal pellets which were entirely composed of cellulose fibres from the paper, and many had some of this cellulose in a few pellets. These have been allowed for in the count of frass pellets by deducting one for each pellet entirely derived from paper and half for each containing some of this material.

Once the method had been established the main series of experiments was begun. It had been hoped to cover all the foliage types used during other sections of this research but this was not possible because there was a very short growing season for E. camaldulensis in the 1971-1972 season when this work was undertaken. The shape and size of young leaves of E. linearis also prevented the inclusion of foliage of this eucalypt species. One modification to the method was made during the trials to allow most of the narrow-leafed eucalypts to be included. Instead of 15mm diameter leaf discs, 7 x 25mm rectangles were used. These were of equivalent area and because both trial and control foliages were always presented in the same form no effect on consumption should have resulted. A trial on the control foliage compared the two shapes and although the average value of the difference between the two showed that more was eaten from the discs than the strips this was not

significant (t - test of the difference i.e. area of circle eaten less area of rectangle eaten; $n = 25$, $\bar{Y} = 18.8$, $t_s = 1.64$, $t_{.05(24)} = 2.06$). Strips were cut using a template marked on a clear plastic ruler and a sharp disposable scalpel. This presentation was more time consuming and so was avoided where possible.

(3) Results and Analysis

The main series of experiments was divided into two sections depending on the number of replicates for each foliage because a more intensive study was made to check for discrimination by P. charybdis between the three different trees of E. obliqua used in rearing experiments where they returned such markedly different results. For these foliages 60 replications were undertaken whereas for all other foliages there were only 25 replications. Trials in which no feeding occurred on either disc were discarded on the grounds that the larvae involved were unsuitable because they had been damaged in handling or were in a pre-moulting state. The first was the more probable because the larvae selected for use were those appearing to be at the start of the third instar.

(a) Varieties of E. obliqua. The results from the selection trials involving the three types of E. obliqua foliage were examined first to determine the analytical approach to be used on both groups of selection experiments. Frequency distributions of the amount eaten for both control and trial discs considered independently for each trial foliage showed different patterns of distribution for control and experimental foliages (Figure 23). In each case the results from the experimental foliage formed an apparently

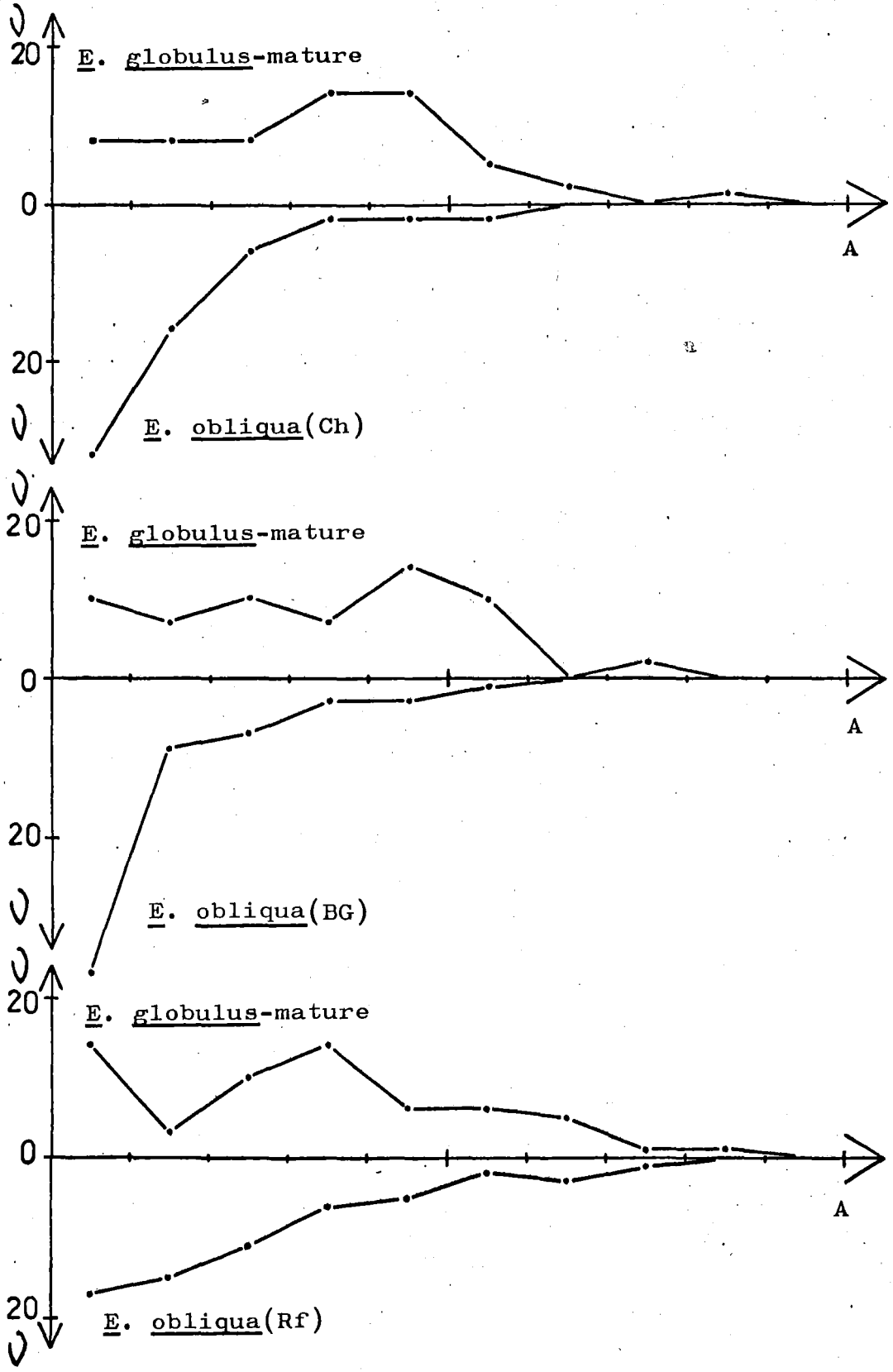
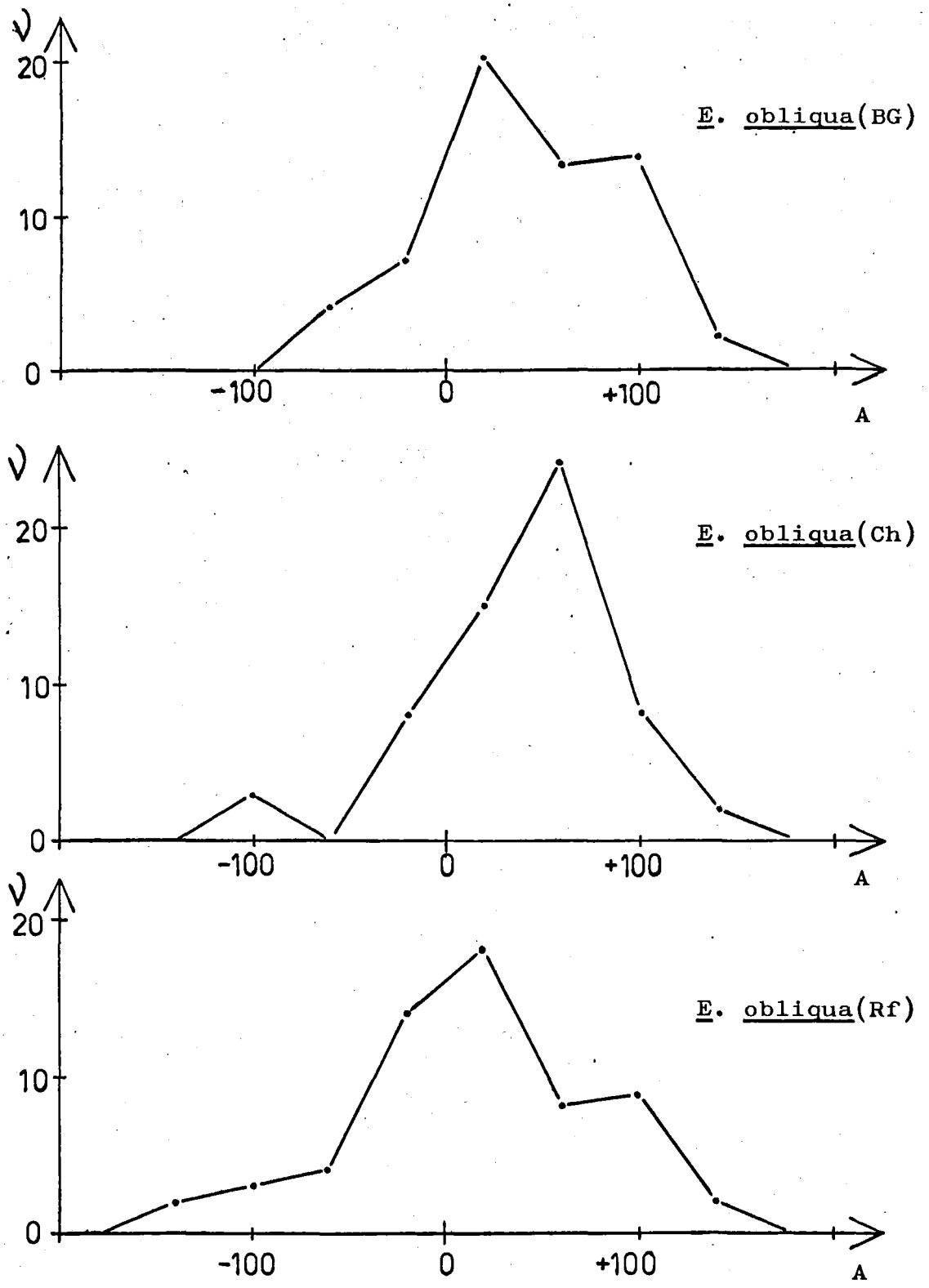


Figure 23. Frequency polygons derived from the first section of larval selection experiments considering the amounts of each foliage eaten as independent variables.



(ν = frequency A = area of control eaten minus area of trial foliage in mm^2).

Figure 24. Frequency polygons for the difference between the amounts of control and trial foliages eaten in larval selection experiments.

regular declining curve while the control foliage results did not conform to any regular pattern.

For Figure 23 the results for each foliage had been taken as being independent. However it is obvious that the results are linked since a pair of discs was exposed to each larva. To determine whether this pairing incorporated into the experimental design caused the results to be mutually dependent, and if so in what way, graphs were prepared from the results from trials using E. obliqua (Rf) (a favourable host) and E. obliqua (BG) (an unfavourable host). For every larva in each trial the area of the experimental foliage eaten was plotted against the area of control foliage eaten, the difference between these results was plotted against their sum, and the square of the difference against the sum. In all cases the graph showed such a scatter of points that no relationship was indicated. However frequency distributions of the differences between paired results for all three foliages showed a fairly regular unimodal curve in each case (Figure 24). No further preliminary testing was performed but on the basis of these graphs a parametric analysis was carried out on the differences between the amount of the control and experimental discs eaten.

The results of this analysis of variance for the three types of E. obliqua resulting from sampling trees at different sites (p 94) are shown in Table 46. This revealed that there was an increase in the variance among these groups that was significant at the 5% level of probability but not at higher levels. Although this experiment had been set up specifically to test for differences between all three foliages it was not statistically proper to test all these means separately by

'a priori' tests (Sokal and Rohlf, 1969, p 230); instead less sensitive 'a posteriori' tests had to be used. The particular test chosen here was the Student - Newman - Keuls (SNK) test for multiple comparisons among means based on equal sample sizes (Sokal and Rohlf, 1969, p 240). The results of this are given in the second part of Table 46, and show that the results for E. obliqua (Ch) and E. obliqua (BG) were indistinguishable while both differed significantly from E. obliqua (Rf). The last named foliage was eaten almost as readily as the control whereas the other two were more greatly discriminated against. (The result for E. obliqua (Rf) was not significantly different from zero - the theoretical value for the control foliage compared to itself.)

Table 46. Analysis of the results of selection experiments using three different foliages of E. obliqua each compared with the control (the mature foliage of E. globulus).

A: Analysis of variance of the differences in consumption between the control and trial foliages.

Source of variance	df	MS	Fs
Among foliages	2	11,109	3.69*
Within foliages	177	3,013	

($F_{.05(2,\infty)} = 3.00$; $F_{.01(2,\infty)} = 4.61$)

B: SNK test of foliage mean differences from control (Means annoted with the same letter are not significantly different at the 5% level).

Foliage	Mean	
E. obliqua (Rf)	17.28	a
E. obliqua (Ch)	40.75	b
E. obliqua (BG)	40.95	b

($LSR_{(.05)} = 19.71$; $LSR_{(.01)} = 25.88$

LSR = least significant range)

(b) Other eucalypt species. The main series of selection experiments involved the comparison of 13 different eucalypt foliages with 25 replicates per foliage. An analysis of variance and subsequent SNK test of foliage means were carried out as described above. The results are shown in the two sections of Table 47.

Table 47. Analysis of the results of the main series of selection experiments in which 13 different Eucalyptus foliages were each tested against a control.

A: Analysis of variance of the differences in consumption

Source of variance	df	MS	Fs
Among foliages	12	15,256	4.10^{***}
Within foliages	312	3,719	

($F_{.001(12,\infty)} = 2.74$)

B: SNK test of foliage mean differences from control (Groups linked by a continuous line are not significantly different at the indicated level).

Foliage	Mean	5% Significance	1% Significance
viminalis-juvenile	-20.08		
globulus-juvenile	-10.84		
viminalis-mature	- 1.12		
fastigata (Cl)	2.32		
perriniana	5.12		
ficifolia	14.04		
cordata	23.12		
delegatensis-hybrid	23.92		
amygdalina	24.84		
leucoxylon	31.24		
sideroxylon	37.28		
andreana	41.16		
fastigata (BG)	73.36		

The discrimination of results achieved was rather disappointing but not unexpected in view of the great range of differences in the amount eaten among replicates for each foliage type. The analysis of variance showed that the added variation among foliages was very highly significant. The SNK test of the means showed that at the 5% probability level the most favoured foliage (the juvenile form of E. viminalis) differed significantly from the three least liked foliages (E. sideroxylon, E. andreana and E. fastigata (BG)) and also that E. fastigata (BG) differed from all but the next three lowest foliages. The only significant differences at the 1% level were between E. fastigata (BG) and the five most favoured foliages. However the series did prove that larval feeding preferences do exist.

The most outstanding feature in Table 47 is the relative positions of the two foliage types of E. fastigata which are significantly different even at the 1% level, when both caused complete mortality in unfed first-instar larvae confined to them. E. fastigata (Cl) was also used in the short-term adult rearing trials and found to completely inhibit egg production. Yet third-instar larvae ate almost as much of this as they did of the control foliage, while very little of E. fastigata (BG) was eaten. E. viminalis was not used in rearing experiments but its high ranking (the most and third to most preferred for juvenile and mature leaf types respectively) could be expected in view of the fact that this species has been reported as a favoured host (Clark, 1930; Dugdale, 1965). Although not significantly different from a zero value (the theoretical position in this ranking of the control - the

mature leaf-type of E. globulus) the slight preference for the juvenile foliage of E. globulus over the control is unexpected in view of the relatively worse performances of this in both larval and adult rearing.

The slight preference for control relative to E. perriniana and E. delegatensis-hybrid fits with the poorer value of these as larval food but not with the excellent performance of adults fed the latter. In contrast the poor rankings of E. sideroxylon and E. andreana do not correlate with the usefulness of these foliages in larval rearing. E. andreana, however, did not show up well as an adult food. E. ficifolia and E. cordata were only slightly less preferred than control and yet both are unsuitable for larval rearing (E. cordata is a glaucous species used in the initial large-cage rearings with little success). Trees of both of these species in the Christchurch area were not damaged whereas some of E. leucoxylon were badly attacked. E. leucoxylon var.'rosea' was the variety of this ornamental gum used in the present trials and it was not much liked by the beetle. Thus it can be seen that whereas some correlation exists between the results of these larval selection feeding trials and those from larval rearing, there are more discrepancies.

(4) Experiments Using First-Instar Larvae

Attempts were also made to devise a selection technique which could be used on smaller larvae, and in particular on the unfed first instars. Due to the habit of female beetles of ovipositing on mature leaves the newly hatched larvae frequently would have to travel relatively far to find succulent new growth suitable for food. It is

likely that chemical attractants play a part in the search for food by these larvae; but it is possible that thigmotactic and negative-geotropic responses provide sufficient guidance to enable a high proportion of larvae to establish on edible foliage. Newly hatched codling moth larvae do use chemosensory powers to locate apples and many codling eggs are laid on foliage close to fruit rather than on the fruit itself (C.H. Wearing, pers. comm.), but unlike the situation with P. charybdis on eucalypts where new growth is normally terminal, apple-tree fruiting spurs are not so regularly positioned. Thus for codling larvae a direct method of orienting towards the host - the fruit - would be far more efficient than the indirect alternatives mentioned above for P. charybdis which are based on stimuli not originating from the food itself.

First-instar larvae present problems in assessing their response to foods. Their small size means that measurements based on food consumption (such as the area eaten or the number of frass pellets produced) are impractical. Distribution parameters become the most obvious choice for expressing selection; that is the number of individuals from a group exposed to a choice situation that aggregate on each of the alternate foods. This approach was tried using several different arrangements in an attempt to create a balanced choice situation in an arena to which the larvae could be confined. The chief difficulties encountered were in trying to regulate the amount of stimulating leaf material used while still providing an enclosed arena giving equal opportunities of access to either or all foliages used. Adequate moisture had to be

maintained as well to prevent leaf deterioration by wilting. These small larvae appeared to be more dependent than later instars on initiating feeding along a leaf edge which meant that the foliage could not be placed flat on a surface. Also their ability to move freely was greatly affected by the presence of excessive moisture. As well it was noted in setting up larval rearing cages that after handling, first-instar larvae did not immediately settle down and begin feeding but would often move about for some time and then rest for a while on the lower or more sheltered face of a leaf before eating. To avoid this agitation due to handling, eggs close to hatching were used, rather than the larvae themselves. This necessitated a longer trial period.

Two of the arrangements involved 3cm basal lengths of young leaves about 1cm wide along the cut edge and with a short section of stem. Stems from two leaves were wedged with damp cotton wool into the opposite ends of a 1.5cm length of 1mm internal diameter glass tubing. A batch of eggs without any leaf material attached was glued to the glass tubing using PVA resin glue. In one trial this arrangement was suspended by cotton tied about the tubing, with grease on the cotton to prevent larvae climbing away; in the other this arrangement was placed in a petri-dish on damp filter paper. In the former some leaves after 12 hours (overnight) were wilting and several had fallen out of their tubes while in the latter a few of the leaves had begun to rot. Some larvae dropped from the former and many in the petri-dish were not on either foliage but on the dish itself. Several cardboard arenas to which leaf sections were attached were also tried without any proving satisfactory, before this

aspect of the research was terminated.

2. FEEDING - REACTION TRIALS

(1) Method

The feeding-reaction tests were carried out in the same way as the main series of selection experiments described above except that instead of subjecting each larva to a choice situation, the tests involved presenting each with a single disc of a trial foliage under otherwise constant conditions and measuring the average response of the larvae to each type of foliage. All other aspects of the test were standardised and were the same as had been used in the series of selection experiments - an early third-instar larva, a 6 hour starvation and a 24 hour feeding period at 20.0°C, high humidity due to the damp filter paper in each petri-dish, and a leaf disc (or if necessary a rectangle) with an area of 176mm^2.

The assessment of feeding response by measuring the area eaten was carried out as previously but as well the number of frass pellets produced during each trial feeding was counted. Some authors (e.g. Sutherland, 1971) have used frass production as the sole measure of feeding activity but although this method should correlate more exactly with the amount (i.e. mass) consumed and so in this case compensate for variation in the thickness of the different foliages used, the error inherent in the measurement was large due to variation in the size of faecal pellets. Also in some trials pellets were not compact but disperse or even scattered by later movements of the larvae, and the exact process of counting had to be replaced by estimation. This method did

lend itself to a much more rapid assessment of trials, and could possibly have been used more. However after showing by a graphical analysis that there was a band of strong correlation between consumption measured by the area eaten or by the frass pellets produced for the 100 replicates of each of the four foliages used in the first section of feeding-reaction trials, no further analysis of these counts has been carried out.

As in the selection experiments a greater effort was made with these feeding-reaction trials to discriminate among the three types of E. obliqua used and between each of these and the control foliage, than among the remaining foliages. This was done because the ability to distinguish among different types of the one species of eucalypt, or lack of same, could indicate the power of discrimination that larvae of P. charybdis were capable of. Such an ability could also provide a lead for further research into the nature of the factors by which discrimination was achieved. For each of these four leaf types 100 replicates were carried out while for the remaining 13 types there were only 40 replicates each. To include the control in both groups of trials, a selection of 40 was made from the 100 trials on the mature type of E. globulus using the table of random numbers in Snedecor and Cochran (1967, p543).

(2) Analysis

A preliminary graphical test on the frequency distribution of the results from the first group of four trials showed that these did not conform to any regular distribution. The number of zero results made the first step in the frequency plot the largest, but the level then

rose more-or-less to a minor second peak further along the graph. Thus the results were not suitable for parametric analysis and instead were ranked from the frequency versus area-eaten graphs for each of the four foliages and a Kruskal-Wallis non-parametric test carried out (Sokal and Rohlf, 1969, p388). The results of this analogue of a single classification analysis of variance are given in Table 48. The statistic 'H' calculated from the summed rank values for each foliage was very much greater than the tabled chi-squared value for a 1% level of probability, indicating highly significant heterogeneity among the results of the four trial foliages. (No correction for tied values was needed because 'H' is conservative before the correction and so the corrected value would have been significant by an even greater margin.)

Table 48. Analysis of the results of feeding-reaction trials for larval P. charybdis fed the control foliage or one of the three types of E. obliqua.

A: A Kruskal-Wallis non-parametric test of variance.

$$H = \frac{12}{n(\Sigma n)(\Sigma n + 1)} \sum^{a} (\Sigma^{n} R)^2 - 3(\Sigma n + 1)$$

$$= 33.51^{***}$$

$$\chi^2_{.01(3)} = 11.35$$

(Where n = number of trials per foliage = 100
and R is the rank value of each result.)

B: Results of non-parametric simultaneous testing of pairs of foliages - count values.

Foliage	Mean area eaten (mm^2)	globulus-mature	obliqua (Rf)	obliqua (BG)
globulus - mature	69.98			
obliqua (Rf)	45.93	5651		
obliqua (BG)	35.23	6864**	6003	
obliqua (Ch)	30.36	7211**	6277**	5286

(Critical values $U_{.05} = 6051$; $U_{.01} = 6274$)

(3) Results

The results for each foliage were compared to every other result separately using a non-parametric simultaneous test procedure; an 'a posteriori' test for samples of equal size which is based on the Wilcoxon-Mann-Whitney statistic (Sokal and Rohlf, 1969, p396). These U-values were derived from the frequency graphs for each foliage already plotted. The results are depicted in the second section of the table above. These show that E. globulus - mature and E. obliqua (Rf) gave favourable results indistinguishable from each other, but both significantly different from the result for E. obliqua (Ch), the least-liked foliage. E. obliqua (BG) differed significantly from the control foliage but not from either of the other two types of E. obliqua. These results differ from those obtained in the true selection experiments described earlier in that E. obliqua (BG) is now no longer nearly the same as

E. obliqua (Ch) but falls midway between this and E. obliqua (Rf) in preference. Previously it was significantly different from E. obliqua (Rf) as well as the control foliage. This new ranking is of interest because the overall suitability of foliages for larval rearing was in the descending order E. obliqua (Rf), E. obliqua (Ch), E. obliqua (BG) with E. obliqua (BG) of just lower value than E. obliqua (Ch).

The main series of feeding-reaction trials involved 15 different foliages including the control which was used in both disc and rectangular form. The results were analysed using exactly the same tests as have just been described for the series involving the three types of E. obliqua foliage. The results are given in Table 49. The very high value for the statistic 'H' in the Kruskal-Wallis test even without making allowance for the number of rank-tied observations showed that there were very highly significant differences in the reactions of larvae to leaf sections of the various eucalypt foliages. The tests comparing the results from each foliage showed that the larvae ate significantly less of the two E. fastigata foliages than many of the others, the amount of E. fastigata (BG) consumed being significantly less than that for nine of the favoured foliages while the amount of E. fastigata (Cl) eaten was less than eight of the others. The three most-eaten foliages (the rectangular form of E. globulus-mature, E. delegatensis-hybrid, and E. viminalis-mature) gave results also statistically different from E. andreana and E. amygdalina, which were not liked. Two other favoured foliages - E. perriniana and E. leucoxylon - were also significantly different from E. andreana.

Table 49. Analysis of the results of the main series of feeding-reaction trials involving 13 experimental foliages and two different presentations of the control foliage.

A: A Kruskal-Wallis non-parametric test of variance.

$$H = \left(\frac{12\,\Sigma^{a}(\Sigma^{n}R)^{2}}{n(\Sigma n)\,(\Sigma n + 1)}\right) - 3\,(\Sigma n + 1)$$

$$= 110.8^{***}$$

$$\chi^{2}_{.01(14)} = 29.14$$

(n = number of replicates per foliage = 40

R = rank value of each individual result)

B: The average area of each foliage that was eaten.

Foliage	Mean area eaten (mm^2)
delegatensis-hybrid	97.1
viminalis-mature	87.2
viminalis-juvenile	81.0
globulus-mature (rectangles)	79.4
perriniana	72.8
globulus-mature (discs)	70.1
leucoxylon	67.5
ficifolia	58.0
sideroxylon	57.0
cordata	56.8
globulus-juvenile	47.5
amygdalina	40.6
andreana	30.0
fastigata (Cl)	17.9
fastigata (BG)	4.9

Eucalyptus Foliage	delegatensis-hybrid	viminalis-mature	viminalis-juvenile	globulus-mature (rectangles)	perriniana	globulus-mature (discs)	leucoxylon	ficifolia	sideroxylon	cordata	globulus-juvenile	amygdalina	andreana	fastigata(Cl)
delegatensis-hybrid														
viminalis-mature	904													
viminalis-juvenile	904	822												
globulus-mature (rectangles)	983	893	841											
perriniana	1035	935	906	856										
globulus-mature (discs)	1039	963	905	883	827									
leucoxylon	1071	1000	905	929	869	829								
ficifolia	1111	1053	981	1011	943	912	899							
sideroxylon	1093	1066	966	1037	931	910	914	820						
cordata	1092	1055	962	1037	948	933	936	823	847					
globulus-juvenile	1140	1118	1026	1099	1001	988	1011	882	919	872				
amygdalina	1183*	1178*	1065	1177*	1063	1050	1080	932	973	902	838			
andreana	1231**	1241**	1100	1281**	1163*	1141	1193*	1009	1046	961	875	835		
fastigata(Cl)	1317**	1369**	1175*	1435**	1293**	1272**	1347**	1120	1183*	1076	956	935	1053	
fastigata(BG)	1309**	1409**	1171*	1514**	1322**	1304**	1448**	1157*	1252**	1113	1020	1001	875	965

(Critical values $U_{.05(15,600)} = 1152$; $U_{.01(15,600)} = 1200$)

Table 49 C: Results of the count-values obtained for the non-parametric testing of the foliage types by pairs.

(4) Discussion

There are some puzzling differences between the results of these feeding-reaction experiments and the series of selection experiments reported earlier. In particular the high ranking achieved for E. fastigata (Cl) and E. globulus-juvenile in the selection trials contrasted to their positions in the results of the present work. The selection experiments also recorded E. delegatensis-hybrid and E. leucoxylon as relatively unfavoured foods, whereas the feeding-reaction experiments placed them among the more liked foliages. In both these cases the second series of trials, the feeding-reaction trials, gave the results which correlated most closely with those obtained in larval and adult rearing experiments.

The only difference in method between the two series of trials was the absence of a control disc in the reaction trials, and hence the inclusion in these of all zero results, whilst in the selection experiments larvae which fed on neither foliage were rejected. Rejection would not have had a selective effect either for or against any particular foliage. The presence of a second type of foliage however could possibly alter the results in such a way. The feeding habit of larvae of P. charybdis as noted in casual observations and borne out from the repeated checking of the multi-disc selection experiments was to feed for a while, move a short distance and pause for a while before returning to feed. Another point supporting this view was the general occurrence of two or three separate feeding notches in discs even if the total area eaten was not great. If this is the usual sequence in feeding the presence of the control foliage

(a favoured feed) could bring about increased or decreased consumption of the trial foliage.

A decrease in the relative amount of the trial foliage eaten would result simply from the expression of a distinct preference for the control foliage, and would be especially likely to occur if there were either a strong attraction to the control or marked differences in the quality or quantity of stimuli present in the control and trial foliages. More of the trial foliage could be eaten if chemical stimuli diffusing from the control disc saturated the atmosphere in which the selection experiment was conducted. This could result in an increased consumption of any disc (and see the comments in the following section covering experiments using plants other than eucalypts), unless this response was countered by stimuli met with on eating the trial foliage. A similar result would occur if there was a "remembered" carry-over of stimulation when the trial disc was encountered after a period of feeding on the favoured food of the control (similar to the persistence of induced feeding preferences reported for larvae of Manduca sexta by Jermy et al, 1968). This is likely to be involved in the changed results for the juvenile foliage of E. globulus which would be very similar chemically to the control, but is less plausible to explain the change for E. fastigata (Cl). The lethal effect of this latter when used as a food for young larvae, or as a short-term food for adults remains in antithesis to the favoured rating from the selection experiments. A confusion of the foliage sampled would not be at all likely and a seasonal change in foliage quality of this magnitude is also implausible. Thus this variation in results remains

inexplicable.

The depressed ranking of E. delegatensis-hybrid and E. leucoxylon in the feeding-reaction trials compared with the selection experiments could be due to these containing only a weak feeding detterent(s) which could be overcome readily by starvation but which was sufficient in the presence of a more favoured food to prevent much being eaten. However this is simply speculation without supporting evidence.

3. TRIALS INVOLVING PLANTS OTHER THAN EUCALYPTS

This series of feeding trials was carried out to determine just how specific were the host-plant stimuli necessary to initiate and maintain feeding in larvae of P. charybdis.

(1) Methods and Results

A range of plants representing many plant families were fed to early third-instar larvae using the same method as described previously for feeding-reaction trials based on various Eucalyptus foliages. There had been no reports from the field of feeding on any plant outside this genus but, given the very diverse nature of this plant group both chemically and physically (Baker and Smith, 1920; Penfold and Willis, 1961) and the views that have been advanced on the common availability of suitable nutrients in plants (e.g. Fraenkel, 1969) it was felt of interest to examine this question.

Before the large disc feeding-reaction method was adopted several selection trials were carried out using fourth-instar larvae and the multi-disc method described at

the beginning of the chapter. The results of these are given in Table 50 while the results of subsequent trials using the feeding-reaction method are given in Table 51. The latter results are all based on 12 replicates per foliage. Most of the plants used came from the Botanic Gardens, Christchurch, and their identification has been checked by Mr L.J. Metcalf, Assistant Director of Parks and Reserves in Christchurch. Table 51 records the reactions of individual larvae to test foliages in three categories - the foliage untouched, negligible feeding (i.e. less than 1mm^2 at any one point), and definite feeding. The total area eaten in definite feeding is given as well as the number of trials in which such feeding occurred.

Table 50. Feeding trials with P. charybdis and plants other than Eucalyptus species, initial experiments using fourth-instar larvae.

Multi-disc trials				
Family	Test plant	No. of trials	Mean area eaten (mm^2) Control	Test plant
Anacardiaceae	Cotinus americanus	6	63.5	4.8
Betulaceae	Betula pendula	2	69.5	31.0
Fagaceae	Fagus sylvatica var. "Purpurea"	3	43.7	13.3
Fagaceae	Quercus incana	4	70.0	43.8
Leguminosae	Wisteria sp.	3	76.0	22.7
Salicaceae	Populus nigra var. "Italica"	4	83.5	0.9
Tiliaceae	Tilia europaea	2	74.0	B
Single-disc trials				
Fagaceae	Quercus incana	10		11.4*
Salicaceae	Populus nigra var. "Italica"	5		0.0**

(* 5 with feeding, 5 without

** all 5 without feeding

B = negligible feeding)

Table 51. Feeding-reaction trials using non-eucalypts.

Family	Test species	Amount of feeding			
		None	Negligible	Definite	
				Frequency	Total area eaten (mm^2)
Aceraceae	Acer platanoides var. "Goldsworth Purple"	5	7	-	-
Anacardiaceae	Cotinus americanus	9	3	-	-
Berberidaceae	Mahonia aquifolium-hybrid	9	2	1	1
Caprifoliaceae	Lonicera (periclymenum ?)	9	3	-	-
Caprifoliaceae	Sambucus nigra	7	4	1	1
Ericaceae	Arbutus sp.	9	-	3	9
Ericaceae	Rhododendron augustinii	5	1	6	18
Fagaceae	Quercus incana	7	4	1	1
Juglandaceae	Juglans regia	4	4	4	11
Leguminosae	Cercis siliquastrum	11	1	-	-
Leguminosae	Phaseolus vulgaris	11	1	-	-
Leguminosae	Trifolium repens	12	-	-	-
Liliaceae	Ruscus aculeatus	9	3	-	-
Magnoliaceae	Liriodendron tulipifera	8	2	2	4
Monimiaceae	Atherosperma moschatum	12	-	-	-
Oleaceae	Forsythia x intermedia var. "Spectabilis"	11	1	-	-
Oleaceae	Fraxinus excelsior var. "aurea"	10	2	-	-
Pittosporaceae	Pittosporum tenuifolium	2	3	7	47
Rosaceae	Chaenomeles var. "alba"	4	8	-	-
Rosaceae	Crataegus crus-galli var. "salicifolia"	8	4	-	-
Rosaceae	Malus sargentii	10	1	1	2
Rosaceae	Photinia glabra	4	3	5	58
Rosaceae	Prunus (serrulata?)	11	1	-	-
Rosaceae	Rosa cultivar	7*	6*	5*	38*
Salicaceae	Salix caprea	12	-	-	-
Saxifragaceae	Hydrangea macrophylla var. "Coerulea"	10	1	1	2
Styracaceae	Pterostyrax hispida	11	1	-	-
Theaceae	Camellia japonica	10	2	-	-
Tiliaceae	Tilia cordata	6	4	2	3
Tiliaceae	Tilia europaea	11	1	-	-

(* 18 replicates instead of 12)

Plants are arranged within each section of the table in the alphabetical order of the families to which they belong. The nomenclature follows that in Chittenden and Synge (1965) except that Cotinus americanus has been preferred over the synonym Rhus cotinoides (Hay and Synge, 1969; Harrison, 1963).

(2) Discussion

It is immediately apparent from the tabulated results that only in very few cases did the larvae reject the non-eucalypt leaf-discs at a distance, that is without at least biting into the test material. In the multi-disc trials in fact considerable feeding activity occurred on these non-hosts. When the results for those foliages (Cotinus americanus, Tilia europaea, Quercus incana, and Populus nigra var. 'Italica') which were tested using both test methods are compared it can be seen that in three of these four cases considerably more of the trial foliage was eaten when the multi-disc method was used. This could indicate an insensitive continuation of feeding behaviour after an initial stimulation by the control leaf discs, or the stimulatory effect of volatile chemicals originating from the favourable control foliage.

There are also some interesting results from individual plant species, in particular from Pittosporum tenuifolium, and Rhododendron augustinii. Both of these were accepted relatively readily by the larvae and yet both proved toxic to these larvae. Six of the 12 larvae fed P. tenuifolium were dead or moribund at the end of the 24 hour test period, and yet three of these had been seen biting the discs within 5 minutes of being placed in the petri-dishes. This was an

unusually rapid feeding response.

The symptoms of advanced poisoning included a very fluid anal discharge, with abdomen distended and anus and defence glands slightly extruded, muscular spasms affecting especially the extremities (the legs, mouthparts and antennae) and a rapid pulse in the posterior side wall of the abdomen. The frass-and-anal-fluid mixture of at least two of the dead larvae contained small fluid-filled cysts but these were probably undigested oil glands from prior feeding on E. globulus. Similar symptoms were seen among the six larvae dead or dying after feeding on the rhododendron; with the liquid appearance of the body, anal discharge of fluid and eversion of defence glands being noted. This apparent favourability of foliages which are actually lethal is unusual in that there is a breakdown of the defence provided by the discrimination and selection of foodstuffs. In fact not only did these processes fail to exclude harmful material but, in the case of P. tenuifolium at least, there were indications that these poisons or other leaf compounds were very highly acceptable.

Two foliages from the family Rosaceae showed much acceptability to the larvae in the feeding-reaction tests. These were Photinia glabra and a rose cultivar (Rosa hybrid). However four other members of the same family (Chaenomeles var 'alba', Crataegus crus-galli, Malus sargentii, and Prunus sp. (probably P. serrulata)) showed no or very little feeding. Only leaf material from three plant species, Trifolium repens, Salix caprea and Atherosperma moschatum, showed no sign of suffering even trial bites.

CHAPTER VI

ADULT ATTRACTANT TRIALS

This chapter briefly summarises an attempt made to attract adults with various chemicals.

1. MATERIALS AND METHODS

The first of this series of trials was carried out using a small quantity of each chemical in a 4oz. wide-necked jar with a screw top. These were prepared in the laboratory the day prior to being used in the field at the Head-of-the-Harbour site from which the original adults of P. charybdis had come (see figure 12,p94). The jars were placed on the ground at approximately 1.5m intervals in a line midway between a row of young eucalypts planted along the roadside and a parallel belt of older eucalypts about 60m up a hillside. The eucalypts were a mixture of species with E. macarthuri and E. ovata predominating especially among the younger trees.

The chemicals used were exposed on two fine, warm days in spring at a time when the adult beetles had emerged from hibernation and oviposition had begun. On both occasions there was a light north-easterly breeze off the harbour which changed to a fresh cool southerly during mid-afternoon and terminated the trials. Paropsis charybdis adults are reputed to fly most readily in warm sunny conditions (Clark, 1930; Gibbs and Ramsay, 1964). The jars were watched constantly from a distance and inspected more closely every half hour.

Further trials using the same basic arrangement were

conducted at a site closer to Lincoln College later in the spring on days when the weather conditions were favourable. The site was a strip of waste land alongside Weedons Cross Road just south of the intersection with Junction Road (Site 6 on the map, p94). A row of E. viminalis interspersed with oaks grew along the east side of this area and between this and a ditch bordering the road was an area of long grass containing seedlings of E. viminalis. The jars containing the chemicals were placed at about 1m intervals along a mound beside the ditch. Due to the small extent of the area of seedlings only 20 chemicals could be tested here at any one time.

After this arrangement had been used on a number of occasions a change was made to a more permanent trap which could be left in place and inspected at intervals. These traps consisted of a circular cake-tin base surmounted across a diameter by a vane. A 2oz glass jar containing cotton wool impregnated with four drops of the particular test chemical was held in a bracket in the centre of the vane. The basal tin was kept partly full of water to snare any insects attracted to the trap. Traps of this model have been used successfully in research into attractants for the grassgrub beetle, Costelytra zealandica (White). To allow for wind changes the traps were placed in two rows, along the west and east sides of the main area of seedlings. The traps were about 3m apart in each row.

A further site was also used to increase the number of chemicals under test at any one time. This was the area about the haybarn on the Research Unit of the Lincoln College Farm. This fenced-off area had loosely spaced rows of

mature eucalypts along the north, south and west edges, and other mature gum trees scattered throughout. E. globulus predominated although other unidentified species were also present. Various lines of traps were operated through this area depending on the prevailing wind conditions. Generally traps were placed along sections of the boundary fences at the base of every other post or batten but one line crossed the area and one was in a neighbouring field parallel to the western perimeter.

Five of the 4oz jars used earlier were for a fortnight suspended among the foliage of several trees of E. globulus at the site on the Chamberlains' farm (Site 7, see p94) where there was a damaging population of P. charybdis. They were inspected daily. This arrangement was used in case the chemicals only worked at a short range, after the beetles had located a tree by visual means. Dugdale (1963) commented in a report that Paropsis adults had been observed,

"...flying at between 6-20' (? some higher) along the ocean beach on the northern end of Rabbit Island, in a S.E. direction, at right angles to the sea breeze. Adults turned downwind and travelled over a ¾ ? mile area of low P. radiata regeneration to a patch of E. viminalis coppice growth".

From these observations he made the tentative assumption that the beetles had been able to sight the stand of gums from half to three-quarters of a mile away. However he also mentioned the possibility of wind eddies carrying odours from the E. viminalis against the apparent wind direction.

All the chemicals which were available and known to occur in any member of the genus Eucalyptus were screened at some stage of these trials, together with other odorous compounds. In all a total of 53 different compounds were

used. More attention was given to the more volatile and odorous chemicals. With the large number of chemicals used initially a blank control jar was incorporated at every tenth place in the row, but with the more permanently positioned traps the number of controls was increased to one every third place. The chemicals used in the traps were changed and renewed periodically.

2. RESULTS

The 53 chemicals and 4 different preparations derived from young mature leaves of E. globulus used in these trials are recorded in Table 52. Unfortunately none of these succeeded in attracting adults of P. charybdis. The small jars used initially were observed continuously in case the greater quantity of chemical used resulted in attractance at a distance and then repellence as the concentration of the vapour increased. No case attributable to this was seen.

The lack of positive results was not brought about by the absence of beetles but by the absence of the conditions necessary to bring about flight. Adults were present at all sites, and were especially noticeable on the seedlings of E. viminalis. Up to 12 adults were counted on walking through this small area, and many were seen in or initiating copulation. Others were basking in the sun, and walking about on the leaves and stems, or sheltering under the leaves, depending on the conditions. None were seen in flight although the maximum temperatures recorded during the early work were 30.0'C in the sun and 21.4'C in the shade. Temperature conditions were not continuously monitored after early summer.

Table 52. The chemicals tested as lures for adult P. charybdis.

Eucalyptus globulus distillate - 3 fractions

Eucalyptus globulus hot water extract

acetic acid
amyl acetate
anisaldehyde
benzaldehyde
benzoic acid
butyl butyrate
n-butyraldehyde
n-butyric acid
Δ^3-carene
caryophyllene
cedar wood oil
cineole
cinnamaldehyde
citral
citronellal
citronellol
clove oil
cuminal
decanoic acid (capric acid)
farnesol
formaldehyde
formic acid
furfural
geraniol
geranyl acetate
heptanoic acid
hexaldehyde
hexanoic acid (caproic acid)
lactic acid
lauric acid
limonene
linoleic acid
menthol
myristic acid
octanoic acid (caprylic acid)
oleic acid
palmitic acid
pentadecanoic acid
phellandrene
phenol
phenylacetic acid
α-pinene
piperitone
propionic acid
protocatechuic aldehyde
salicylaldehyde
sorbic acid
stearic acid
α-terpineol
p-tolualdehyde
uric acid
n-valeric acid
n-valeraldehyde

Adult P. charybdis were observed to cover considerable distances by walking. At one site one was noticed on an isolated tuft of epicormic growth from which all the young leaves had been eaten. This beetle was observed for 2 hours during which time it walked approximately 4.8m in an apparently aimless wander. At one stage it raised its elytra several times, but it did not fly. After a further interval of 2 hours it was moved to a smaller isolated shoot that still had some succulent foliage. It was still there the following day. The shade temperature at the start of this period was 16.1'C and the maximum for that day 21.2'C.

Carne (1966) found that P. atomaria did not readily disperse from the trees on which they had lived as larvae, although capable of flying substantial distances. It would appear that the P. charybdis in the study area were also largely sedentary. However the rapidity of the spread of this beetle throughout New Zealand would contrast with this finding. Canterbury was the area originally colonised and the population there may now be less mobile, or the main period of migration may be during autumn when this aspect of the ecology was not studied.

During the early part of this work, one clear example of attractance to a trial chemical did occur. The native bee, Lasioglossum sordidum, was strongly attracted to heptanoic acid, and at one stage at least 12 were hovering over the jar containing this or had alighted on the adjacent vegetation. A few were also near the next jar which contained sorbic acid. Specimens of this bee were identified by Dr B. Donovan, Entomology Division, Department of Scientific and Industrial Research, Lincoln.

CHAPTER VII

DISCUSSIONS AND CONCLUSIONS

1. PRACTICAL CONSIDERATIONS

Because of the scattered plantings of eucalypts, and the difficulties and associated costs of applying insecticides to these in order to control defoliators such as Paropsis charybdis, it is necessary to resort to biological and cultural control measures. The results obtained in the present study of the insect-host relationship between Paropsis charybdis and various eucalypt species contribute substantially to the background knowledge required for both methods of control.

Although several attempts to introduce parasites from Australia have been made none have been continued with to the point where any results have been obtained. In fact no releases of parasites have occurred in this country, so that the success or failure of these ventures must be said to remain unproven in either direction. It must be remembered that there are no records of just what parasites and other agents do attack P. charybdis in its natural habitat, and that all introductions have been with parasites of the very closely related P. atomaria. This beetle, as has been pointed out earlier (p45), has distinctly different habits, including a different host range, from its congener in New Zealand. P. atomaria in the Australian Capital Territory most commonly attacked E. blakelyi Maiden, E. melliodora A.Cunn., E. polyanthemos Schau., and E. fastigata F.Muell. (Carne, 1966). The only one of these species which could be

fed to P. charybdis during the present work was E. fastigata and this foliage caused all first-instar larvae placed on it to die. It also inhibited oviposition when fed to female beetles. Perhaps this is a case where two closely related phytophagous species exploit mutually exclusive host ranges. If so this might markedly affect the efficiency with which parasites of one locate and attack the other. The orientation of insect parasites firstly to the food-plant of the host rather than directly to the host has been studied by Thorpe and Candle (1938) and Chandler (1968). Flanders (1942) found that abortive development of parasitic hymenoptera could be induced by the food-plant of the host. Another difference which could also affect the behaviour of parasites or predators is the fact that whereas P. atomaria larvae are strongly gregarious those of P. charybdis are essentially solitary after the first instar, although they tolerate crowding and can even form loose associations.

The brief trials investigating the natural mortality in P. charybdis populations in New Zealand conducted in conjunction with Dr Carne's visit to this country enabled him to report that larval survival in this case, especially in recently colonised regions, was much greater than for P. atomaria in Australia. In Australia parasites caused considerable mortality in the first generation commonly destroying more than 50% of the larvae that had survived to pupation and this could even rise to 80%. (The effect of the common parasites was not felt until the pupal stadium.) During the second generation there was a sharp decline in the level of parasitism. Carne (1967) concluded from the research done during his visit, and in particular from an

increase in the incidence of disease in rearings during March, that almost all larvae survived during the first instar due to the absence of parasites, but that numbers were checked during the second generation possibly by an epizootic of an unidentified disease. During the present work no case of diseased larvae of P. charybdis was found and the impression was gained that abiotic factors accounted for a large proportion of the variable mortality.

Climatic factors affected populations of Paropsis directly, for example by the dessication of eggs and larvae, but in general they appeared to be more important indirectly. This indirect action could be due to a modification of the general environment such as would favour fungal infection of the eggs, but more frequently it appeared to be mediated through the host plant. For instance, the summer drought conditions experienced in 1971-1972 inhibited growth in most trees of E. globulus and caused the young leaves present to harden off more rapidly than normal. This resulted in a severe restriction of the available food. Carne, (1966) in discussing the ecological characteristics of P. atomaria, noted similar population limiting factors.

Although environmental factors could in this way alter the growth cycle of the gums and limit the population of the beetle, a severe setback to the trees, such as very heavy defoliation, was followed by a period of modified growth favouring the beetle. This was brought about because the eucalypts responded by producing epicormic growth even at times when active growth was not normally present. The greater seasonal duration of growth so resulting must have eased the necessity for the beetle to correlate its life

cycle more closely to any growth cycle of the host plants. Seedling plants of some species also grew over a longer period than mature trees. Thus juvenile trees of E. viminalis, and epicormic reversion growth on mature trees, had suitably succulent foliage present for a greater part of the summer season and so extended the period for which this species was a suitable host for P. charybdis.

When P. charybdis was fed only on the juvenile leaves of E. globulus, this foliage was found to be far less capable of supporting large populations of the beetle than the mature type of foliage of this species. Juvenile leaves of this species caused an increase in the larval mortality and a decrease in the fecundity of the resulting adults. As this eucalypt is a prime host plant of P. charybdis this might be said to be an example of co-evolution, or of the conservation of its food resources by the beetle, because as the tree is stressed and becomes more susceptible to insect damage, the insect becomes less able to exploit the foliage produced thus limiting the rate of increase of the populations of the defoliator. This would possibly minimise the risk of the eucalypt being over-exploited and killed.

Tree responses which restrict the time for which favourable foliages are available, and hence limit the expansion of beetle numbers, should be considered in any programme of eucalypt breeding. The same is also true in planning plantings of mixed species of gum trees. In such mixed stands there should not be an unbroken run of new foliage due to successive growth cycles of different species. Also the seasonal timing of growth and the length of time that new leaves remain succulent could well prove amenable to

alteration by selection. Ideally, for resistance, the growth period should be short and timed to follow periods during which beetle numbers would be reduced, such as in early spring or late autumn. The length of time before the leaves hardened should also be short; E. alpina which has leathery leaves even in the opening bud stage would be the extreme case of such a temporal restriction of food.

The present work has demonstrated that for all facets of beetle life the foliages cause different responses both physiological and behavioural. There does not appear to be much correlation between the foliage suitability for beetle growth and multiplication and the taxonomic relationships of the species of Eucalyptus, and hence between plant suitability and the major constituents of the volatile oils derived from each gum (Baker and Smith, 1920). However, analyses of the chemical nature of the foliages and the determination of the underlying agents causing the observed beetle responses remain to be done. The marked variability demonstrated within E. obliqua, E. linearis and E. fastigata indicates that these species might be extremely useful for such analyses because the majority of the constituents should be common to the different foliage types within each species, enabling the effective chemicals to be more readily recognised.

2. THEORETICAL CONSIDERATIONS

As well as these more pragmatic considerations arising out of the present work there are others of a more basic nature. The problem of the differential levels of damage attributed to P. charybdis as presented by previous work was

approached by considering the two extreme cases which could be causing this. The first of these was that P. charybdis females were actively selecting certain species of gums for oviposition so that egg distribution and density would depend on the types of foliage available at the time of oviposition and the relative strengths of the stimuli provided by these. In this hypothesis the response of the insect to the host plant is purely behavioural; thus it depends on the insect receiving stimuli from the plant and reacting according to the quality (type) and quantity of stimulation perceived. It is this idea of insect host-plant interaction that has received most attention in the past and examples of insect-plant interactions based on this model abound in the literature. These range from major pest species investigated extensively (such as the boll weevil, Anthonomus grandis, the Colorado potato beetle, Leptinotarsa decemlineata, and various aphids including Myzus persicae) as well as lesser pests and some of the rarer phytophagous insects. Naturally enough most work has been concerned with insect species of some economic importance, either as pests or those useful as biological control agents of weeds, such as the alligator-weed flea beetle, Agasicles n.sp. (Maddox and Resnik, 1969).

The alternative extreme case postulated was that the ovipositing females showed no discrimination of host so that eggs were deposited more or less at random with a uniform rather than clumped distribution. The number and vigour of surviving larvae and hence of the next generation of adults, controlled the level of plant damage attained. This extreme underlies the 'antibiosis' type of plant resistance emphasised by Painter (1951, 1958, 1967, 1969).

The complimentary nature of the two postulates is best exemplified by the statement that in no known case has either been shown to be the sole regulatory mechanism. Indeed this empirical observation can be supported by a simple logical argument based on the selective advantages of the combined factors. Unless there are differences in the ability and efficiency with which a particular phytophagous insect species can exploit the various plant species which constitute its biotic environment there is no selective advantage in being able to discriminate between the plant species, and so selective ability would never have evolved. Conversely if differences in plants affect the ability of insects to make use of them, then there will be selective advantages conferred on those members of an insect species that are able to recognise and select the most favourable plant and so optimise the efficiency with which they exploit the available resources. The utilisation will be optimal rather than maximal because the ability to seek out and then use a host plant for growth and reproduction must be balanced against such factors as the spatial and temporal availability of host material. Over-exploitation of the host is just as likely to lead to extinction of the phytophage as under-exploitation.

A stable insect-plant relationship requires the simultaneous exploitation and conservation of the resources provided by the host. This is the same principle that underlies all parasite-host situations. Evolutionary pressures have generally modified and refined all these relationships to give a high degree of interaction and over all stability. However the details of each case are individual, reflecting as they do the intrinsic properties of

the particular insect and host involved, as well as the mutual dependencies of these. The extant characteristics of each species depend in turn on the equilibrium achieved during evolution between the forces restricting the increase of the organism and its ability to oppose or circumvent these pressures. For example, the problem caused by a dispersed host could be overcome by a phytophagous insect either evolving ways of locating the host and so preventing a large mortality among the insect migrants, or by increasing its multiplicative powers to compensate for this high mortality.

Characteristics which affect the balance achieved between host exploitation and conservation include the spatial and temporal distribution of the host plant and the distribution, mobility, and searching ability of the phytophagous insect as well as its digestive, assimilative and reproductive powers. Of special importance are factors limiting the population levels of both host and phytophage. In areas where environmental or other conditions prevent the numbers of insects ever reaching levels where the quantity or quality of host available is depleted, selection pressures will not be operating on the insect-plant relationship except perhaps to increase exploitation (Andrewartha and Birch, 1954). Similarly if the ranges of phytophage and host are not completely concurrent exploitation will be high because the areas outside the zone of overlap will function as reservoirs from which emigration will occur in the case of extinction of local populations of both species following host over-exploitation. This latter situation is intermediate between the state where the availability of the host is not limiting and that where it is.

If the numbers of host affect the size of the population of the phytophagous insect (and this will also cover cases when the phytophage affects the numbers of the host plant) mechanisms will have been evolved so that continuation of both species is ensured. Thus the moth Cactoblastis cactorum, although severely attacking its host Opuntia spp., in areas in Australia where the latter was in dense stands, conserved the host when the occurrence of this was sparse (Monro, 1967). The way in which this was achieved was behavioural. Female moths tended to lay their egg batches in clumps on the cacti rather than spreading them more evenly over the plants. Because of this, the hatching larvae on many plants consumed all the available food before they had attained maturity. These larvae then migrated, but, because of their low mobility, their chances of successfully finding a new host were small and many perished. The uneven distribution of the eggs meant that more larvae died of starvation, and more plants escaped with no or only slight infestation than would have occurred if the eggs had been laid at random. The system may appear inefficient in that energy is expended in producing a surplus of eggs and larvae, but this "inefficiency" gives added flexibility to the ability of the moth to exploit the host in situations where the host abounds. By deduction the natural situation of these protagonists would be one where the cactus is very unevenly distributed, and where the abundance of this determines the size of the local moth population.

The egg batches of P. charybdis also tended to be clumped togather. However the main way in which this would

affect the resulting level of the population of the beetle would not be by such food shortages, but by the cannibalism of the remaining eggs by the larvae that hatched first. The regulation of the population in this way before the excess larvae had fed would result in a more efficient utilisation of the available food than that reported for Cactoblastis. This is indicative of the characteristics of the plants serving as hosts in the two cases; Opuntia being more quickly growing and freely seeding than the longer-lasting trees of Eucalyptus.

However, food restrictions will still be involved in determining the final numbers of beetles present. Because the feeding larvae remove the new growth and will even eat the growing tips of the shoots, the amount of food available to later developing larvae and adults will be restricted. This would affect the levels and potential of future generations and probably give rise to a higher incidence of migrating adults. Serious defoliation would however result in epicormic replacement growths enabling low levels of Paropsis to carry through in the original area until later growing periods. In some respects the situation resembles that of another chrysomelid beetle, Leptinotarsa decemlineata (Say). Harcourt (1971) found that this, when living on its principle host, the potato, tended to over-exploit the available food resources with the result that the larvae starved and adults were forced to emigrate in search of new or alternative hosts. When populations were studied on tomato plants the decreased survival and reproductive capabilities meant that the food supply was not endangered (Latheef and Harcourt, 1972).

This same paper reported that larvae of L. decemlineata developed more slowly and yet ate more when fed tomato foliage instead of potato. The digestibility of the tomato foliage eaten was lower than that of potato (e.g. 91.8% versus 95.1% for first-instar larvae using dry weight values) and the weight gain per unit weight digested was also less (e.g. 8.6% versus 15.2% for first instars). These facts indicated that the tomato foliage was being used less efficiently as a food, rather than the consumption of this being blocked by feeding deterrents or a lack of stimulants. Half of the Leptinotarsa adults refused to feed on tomato; this was attributed to the presence of an alkaloid deterrent, tomatin, showing that different aspects of the host dominated the interaction during the larval and the adult stages.

3. THE INTER-RELATIONS OF THE PRESENT RESULTS

In the present research, the different results obtained for each measurement of the success with which P. charybdis could use the various types of Eucalyptus foliage as food showed that many different aspects of the host were involved. The multifactorial nature of the interaction makes it difficult to evaluate the overall suitability of each foliage as food for Paropsis. However an attempt to combine the results of the different facets of larval rearing is presented in Table 53.

Table 53. Rank values of the results of the three parameters of larval rearing measured for each foliage.

Eucalyptus Foliage	Survival (S)	Duration of development (L)				Weight (W)		Totals*	
		Total		Larval					
		F	M	F	M	F	M	A	B
camaldulensis	2	1	2	1	4	3	2	26	34
obliqua (Rf)	1	9	7	8	8	2	3	46	50
globulus-mature	6	3	3	3	3	5	6	58	82
perriniana	4	2	1	2	1	13	11	70	86
delegatensis-hybrid	10	6	4	4	2	4	4	72	112
sideroxylon	9	12	12	11	10	1	1	85	121
andreana	7	5	5	6	5	10	9	87	115
linearis(Ch)	8	7	8	7	7	8	8	93	125
macarthuri	5	4	6	5	6	14	13	95	115
amygdalina	3	11	11	12	12	12	12	106	118
globulus-juvenile	11	8	9	9	9	7	7	107	151
linearis(BG)	14	14	13	14	14	6	5	133	189
obliqua(Ch)	12	10	10	10	11	11	14	139	187
obliqua(BG)	13	13	14	13	13	9	10	143	195

(F = female; M = male;

* $A = 4S + \Sigma L + 2\Sigma W$; $B = 8S + \Sigma L + 2\Sigma W$;

Possible ranges; A = 12 - 168; B = 16 - 224)

This table shows the rank value of each foliage for each measurement taken. A rough index of the overall ability of each foliage to support larval P. charybdis can be obtained by summing these rank values. Two such summations are shown. In the first equal weighting has been given to each type of measurement while in the second twice as much emphasis has been placed on the survival

results as on either alternative parameter. These two cases have been chosen because they represent the situation in which the chosen parameters are independent and equally important measures of the foliage effect on the larvae of P. charybdis, and also a situation more closely akin to reality. Survival will have a more direct and drastic effect on population increase than the other two factors and so the bias towards these results has been increased.

The first summation results in four approximate groups of foliages. The most suitable foliage, E. camaldulensis, forms the first group, while the next consists of E. obliqua (Rf), the mature leaf form of E. globulus, E. perriniana and E. delegatensis-hybrid. The remainder fall into an intermediate class of suitability except for the final three - E. linearis(BG), E. obliqua(Ch) and E. obliqua(BG) - which appear to be considerably worse food sources. When a greater weight is given to the survival figures the sums appear to fall into five groups. E. camaldulensis is still by itself as the best foliage, E. obliqua(Rf) is second best, and E. globulus-mature and E. perriniana make up the next group. The three worst foliages mentioned above still form a group of their own while the total for the juvenile leaf-form of E. globulus stands on its own between these and the remaining six foliages which form a large intermediate group. This comparison could be extended by including rank values obtained from adult rearing.

Another realistic comparison of the overall suitabilities would be obtained by constructing life-tables from the rearings, both larval and adult, and comparing the rate of increase and generation time for each foliage type. However

the extended life of adult beetles when compared with the minimum time required for a generation would complicate this approach. It has not been attempted in the present case because of the large variation in the size of samples used in adult rearings and the variation in ovipositional parameters. It would be extremely difficult to convert such life-tables from laboratory to field conditions and to allow for host selection without many more details concerning the growth cycles and local distribution of eucalypts, and the flight characteristics of the beetles.

The experiments carried out rearing adult P. charybdis on various eucalypt foliages did demonstrate that the host plant profoundly affected various facets of oviposition and also the length of life. Unfortunately many of the leaf-types used in larval rearing could not be included in adult rearing and so the results of this second major section of the work cannot be readily compounded with the results presented above for measurements of larval growth. When females were reared solely on one type of foliage the most obvious effect was on the fecundity or total number of eggs produced. However the rate of oviposition, fertility, average batch size, number of batches, and the duration of the reproductive life also varied. Generally these factors were not interdependent, but were independent aspects of the reproductive behaviour. The length of life of adults and the proportion of this during which oviposition occurred also varied between beetles reared on different foliages.

The results obtained for the control foliage showed that variations in these same factors did not correlate strongly with the size of the female or with each other,

except that both the number of batches and the duration of the reproductive phase of each beetle showed a strong positive correlation with fecundity. However when the results for the F_1 generation reared on different foliages were considered, a correlation between the mean fecundity and mean size of females on each foliage was apparent. Thus in this chrysomelid as in P. atomaria (Carne, 1966) the potential fecundity was ultimately dependent on the suitability of the host plant eaten during larval development, and hence the size of the resulting adults.

The number of batches, size of batches and rate of oviposition expressed as the number of eggs per unit time all showed weak positive correlation with fecundity, and the last two similarly could be related to body size. With all these results, however, it must be remembered that although a pattern of relationships was apparent between group means, the variation in values within each group was too great to demonstrate any relativity between individual measurements.

The short-term feeding trials showed that P. charybdis was sensitive to food changes and could react rapidly to them. Recovery on the original food could also be remarkedly rapid. Active female and male beetles proved intolerant of starvation under the conditions used. In the laboratory the beetles appeared to obtain all their moisture requirements from the foliage eaten, but the ones starved in these trials were given alternative sources of water, which they used. The initial or 'shock' reaction to a change in the food was to cease laying or deposit singly or in very small groups a few eggs that were frequently infertile. The effect of any particular foliage was determined by its inherent food

value and also by the previous exposure of the beetles to either the same or related types of foliage.

Temperature was also shown to influence oviposition affecting the rate directly and the batch size inversely. The rate of laying increased until optimum temperature was exceeded and then decreased. Beyond this point the percentage of infertile eggs also increased. The temperature effect on batch size was less than the effect of foliage on this measurement. P. charybdis females continued to lay at temperatures ranging from 12.5'C to 29.5'C. The possibility of interactions between temperature and foliage type with respect to the suitability of the various types of foodstuffs was not investigated. Neither were the long-term effects of different constant temperatures, of alternating temperatures, or of alternating foliage types examined.

The third major section of the work showed that larvae of Paropsis were capable of distinguishing between the various types of leaf, whether the leaf material was from different species of Eucalyptus or different forms of the one species. This research also brought to light aspects of the behavioural mechanism by which the selection was made. When the results from the selection experiments using plants other than eucalypts were compared with the results of the feeding-reaction experiments involving the same non-host plants, it was apparent that there had been considerably more feeding on non-host material when host material was also present. It was possible either that feeding on the favoured food lowered the threshold for further feeding enabling the contact physical and chemical properties common to all leaf matter to cause subsequent consumption of non-host material, or that

volatile chemical stimuli from favoured plants coupled perhaps with contact stimuli common to all leaves brought about feeding unless countered by strong deterrents.

It must be remembered that with the change in method giving rise to this comparison a change was also made in the stage of larval life tested and, whereas fourth instars were used for the multi-disc selection and two single-disc feeding-reaction trials, all the remaining feeding-reaction trials used third instars. This change in the age of the test larvae could have brought about the change in the results from the two different test methods, because it is possible that the larger larvae were less selective in their feeding. However, as the comparison between the methods made above involved the two reaction trials that used fourth-instar larvae, it would appear that the indicated difference was in fact due to the presence of the discs of control foliage in the earlier experiments.

These speculations as to the mechanism controlling feeding in the larvae should also take into account the very frequent occurrence in the single-disc feeding-reaction trials of the very small feeding notches counted as negligible feeding. The discs were all scored after being checked under a stereomicroscope. In only one test foliage (Trifolium repens) was the edge cut by the cork borer too uneven to be sure of discriminating between bites and mechanical damage in disc preparation, and for this foliage there were only two discs with doubtful feeding damage. In other foliages the damage due to single bites could be clearly seen and estimates made of the number of bites taken to create larger holes where the amount eaten was still

negligible. Some discs were seen where a bite had been begun but not carried through to the point of actually removing a section of leaf. This pattern of damage occurring as it did in the absence of favourable food would suggest in contrast to the above ideas that feeding is regulated by the use of 'trial' bites as is the case in polyphagous insects such as grasshoppers (Mulkern, 1968), and in some more selective feeders, for example the Colorado potato beetle, Leptinotarsa decemlineata (Chin, 1950, in Schoonhoven, 1968).

A compromise idea combining these speculations would be that there was a basic pattern of feeding behaviour utilising food sampling by trial bites and initiated by leaf contact and starvation, but which could be over-ridden or circumvented by volatile stimulants from favourable foods. Thus this combination incorporates the use of both short-range and long-range stimuli originating from the leaf material.

4. RECAPITULATION

To reiterate, the picture that emerged of the interactions between P. charybdis and its host plants, a group of eucalypts, was far from complete, but even so the complexity of the relationship was apparent. A larva after emerging from its egg and finding its way to a suitable succulent leaf by chemotropic or thigmotropic means would be markedly affected by the type of foliage on which it found itself. The foliage would to a large extent determine the larva's chances of successful establishment and survival, the length of time spent as a larva and the size to which

it could grow. The sex of the individual would also affect the last two measurements. As well the variety of leaf shapes and sizes and of tree growth patterns found among the eucalypts would alter the physical environment of the larvae and so affect such things as shelter, and the amount of exposure to predators. If parasites were introduced the searching ability of these would be similarly affected by the physical characteristics of the plant, and as well the chemical characteristics might alter the physiological balance in P. charybdis and hence the ability of the parasite to grow and develop. The efficiency of the parasitism of larvae on different host plants might also vary if the parasite located the insect by first locating the favoured host plants.

The larvae proved capable of discriminating among the different foliages although their opportunities to do so in the field are probably limited. In the mixed wet schlerophyll forest to which Paropsis was probably endemic there might have been more opportunities for such selection. The basic behaviour pattern by which food was recognised appeared to rely more on close range analysis using trial bites than on the more distant olfactory selection. However olfactory stimuli might still be involved. Some of the stimuli used in the feeding sequence appeared to be widely spread in the plant kingdom, although probably of restricted distribution among plants growing in the natural habitat of P. charybdis. The stimuli by which adult beetles locate a host plant remain unknown.

The first larval stadium, involving as it does the location and settling on foliage of suitable quality, was

a crucial one during which much mortality occurred on all types of foliage. A second period during which much mortality occurred in laboratory rearing was during the prepupal and pupal stadia. Some of this may have been due to the food quality of the foliage eaten and so would also occur in the field, while another portion was thought due to the moist, deliquescent nature of the frass produced from certain foliages and hence would be of doubtful importance in the field. In one foliage, E. linearis(BG), there was an increase in the mortality occurring at larval ecdyses. The pupal stadium being a resting phase could be expected to be independent of the foliage previously eaten and yet the duration of this showed a seasonal trend which could possibly be due to food quality changes caused by the metabolic state of the trees altering as the season progressed.

Generally, on those foliages on which P. charybdis had a high survival, the mortality was spread evenly between the various life stages with only a slight preponderance in the first instar. However on the foliages on which survival was moderate to low, there was an increasingly severe mortality rate during the first instar, compared to smaller rises in the mortality rates during other stages. E. linearis (BG) was the exception to this generalisation and here the most critical stage for survival was during the second instar. The mortality among larvae fed E. ficifolia was almost completely confined to the first instar, while all first-instar larvae placed on E. fastigata died within 48 hours.

The larvae as prepupae would leave the foliage that they had fed on and would pupate in the soil, or leaf and bark litter. Thus the newly-emerged adults would have to find

their way a short distance at least to obtain food. The foliage eaten as an adult affected the longevity, and the time spent in a state of active reproduction as well as the fecundity, rate of oviposition and egg-batch size. Fertility might be altered in a few cases by the food-plant. Effects on the adults might be actioned directly or might be residual and due to the foliage fed on as larvae. Even on the poorer foliages tested in the trial in which P. charybdis adults were confined to the foliage on which they had been reared as larvae, the females had both a high reproductive potential and a long life expectancy. If the adult stage was freely mobile, the beetles would more frequently find themselves in situations where the ability to recognise and select a favourable host plant would be an advantage. Selection experiments using adults have not been reported on here, but adults and larvae ate the same foods, and, from field observations, it appeared that much of the adult discrimination of a host plant could have been mediated through feeding, possibly even the selection of a host for oviposition. However, a change in the food fed to adults in the laboratory, even one involving foliages of similar value, often resulted in a shock disruption of oviposition. Prior exposure to the foliage lessened the extent of this reaction, unless the foliage was completely unacceptable. At 25.6'C the adults were intolerant of starvation.

The multiplicity of factors involved in these interactions between insect and plant was well demonstrated by the variations in the relative favourability of the foliages for the different measurements made. Chemical and physical characteristics of the leaves each would have their

role to play. The effects of chemicals were probably seen in the lack of any reaction to the juvenile form of E. globulus in the short-term adult feeding trials, and in the preferred position of this foliage in larval selection experiments. In both these cases the comparison was between the two types of E. globulus foliage - mature and juvenile - which would probably not be greatly different chemically, especially with respect to secondary plant substances. Even here though, the picture was complicated by the marked physical differences in leaf size, shape, thickness and rigidity, and in the glaucous wax layer on the juvenile leaves. A more definite example of chemical effect was provided by the various sources of E. linearis and E. obliqua used which could not be distinguished visually but which gave different results. Physical barriers to feeding were shown for E. alpina (which had a very coarse tough leaf) and E. ficifolia (which had a thick elastic cuticle on young leaves). Physical barriers present in older leaves also restricted all stages in the life cycle of P. charybdis to feeding on the young succulent new growth, and this was probably one of the most important factors limiting the abundance of this insect.

Thus it can be seen that the relationship between plant and insect is determined in a complex series of interactions. Any attempt to classify insect-plant relationships must take this complexity into account because otherwise, as in the nutrients versus secondary plant substances argument, they run the risk of over-emphasising one characteristic at the expense of the rest. Much of the

work currently in print stresses the chemical factors while relegating physical characteristics to a minor role. This is a result of the surge of interest in the means by which a host is located (where chemical factors are the most effective and efficient to use), and in chemical interactions among insects in the quest for new ways to manipulate the population levels of insect pests. Because each insect-plant interaction depends on the characteristics present in both protagonists which in turn depend on the genetic potential and evolutionary paths followed by each member separately and jointly, each interaction is individual, and should be considered against the complete ecological background in which it has evolved. There will be general principles underlying all interactions but no simple formula will retain sufficient flexability to adequately describe all the possible variations. As yet too little is known about too few interactions for these holistic principles to be clearly apparent.

CHAPTER VIII

SUMMARY

The research presented here has been an attempt to understand why differing levels of damage have been reported from different species of Eucalyptus when attacked by Paropsis charybdis, the eucalypt tortoise beetle. This problem was approached both by rearing P. charybdis on various eucalypts and measuring the effect of these host plants on this insect, and by examining the ability of larvae of the beetle to discriminate among these hosts. Experimental work was carried out under controlled conditions of temperature, humidity and photoperiod.

The utilisation of each type of foliage was considered separately for larvae and for adults. Seventeen different types of leaf involving 12 species of Eucalyptus were used in larval rearing and 8 of these foliage types were investigated for their effect when adults were maintained on the foliage on which they had been reared as larvae. The success with which the larvae could feed on the different foliages was gauged by measuring the survival rate, the duration of the larval and pupal stadia together with the total length of the developmental period from unfed first-instar larvae to adults, and the size of the pupae when larvae were reared solely on each particular leaf type. Significant variation among the results for the different types of foliage was demonstrated statistically for all parameters of larval rearing except the duration of the pupal stadium. There were also sexual differences; females taking

longer to complete larval development but then spending less time as pupae. These differing rates of development of males and females were compensatory so that there was no difference between the sexes in the overall time taken to reach adulthood. Female pupae were on the average heavier than the males obtained from the same foliage.

Eucalyptus camaldulensis proved over all to be the most suitable food for larvae of P. charybdis while E. obliqua(BG) was the least suitable of those for which all measurements could be taken. However all first-instar larvae placed on E. fastigata and almost all on E. ficifolia died. This lethal effect was less marked when later larvae were transferred to these foliages from a favourable one.

Adult success was judged by the longevity of female beetles, and their reproductive ability. The aspects of oviposition studied were the fecundity or total egg production, the fertility, the duration of ovipositional life, the number and size of the egg-batches produced and the rate of oviposition expressed as both the number of eggs and the number of batches per unit of time. There were apparent differences among these results for each foliage, although these reaults were not analysed statistically. The most outstanding feature was the large number of eggs laid. Females reared on the mature leaf-form of E. globulus produced the most (4957 per female) and those on E. linearis(Ch) the least (2308). Short-term feeding trials with female beetles and using 13 leaf sources showed that generally a change of diet rapidly disrupted oviposition. The extent of this 'shock' reaction was lessened by previous exposure to the test foliage.

Oviposition occurred throughout the range of temperatures tested (12.5'C to 29.5'C). The greatest rate was recorded at 25.6'C while the size of egg batches appeared to decrease with increasing temperature.

Larval selection experiments and feeding-reaction trials, each with 17 different eucalypt leaf-types, showed that the larvae do have the ability to distinguish the different foliages. However each series of experiments gave slightly different results. E. delegatensis-hybrid was most readily eaten in feeding-reaction trials while the juvenile leaves of E. viminalis were preferred in selection experiments comparing each foliage with the standard (E. globulus-mature). E. fastigata(BG) was the least-liked in both series. Generally the favoured foliages were those which had given the best results in rearing experiments. Under laboratory conditions feeding was not restricted to members of the genus Eucalyptus, and selection appeared to be predominantly carried out at short range rather than at a distance.

No success was had in attempting to attract adult beetles to traps baited with any of the 53 chemicals used.

All the active stages in the life cycle of Paropsis charybdis were affected by the host plant fed on, and each plant used caused a distinct and different pattern of reaction when fed to this beetle. Not only were there differences among the different species of Eucalyptus, but also among different trees of the one species. The breadth of the approach used demonstrated that this insect host-plant relationship was complex and multifactorial.

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APPENDIX

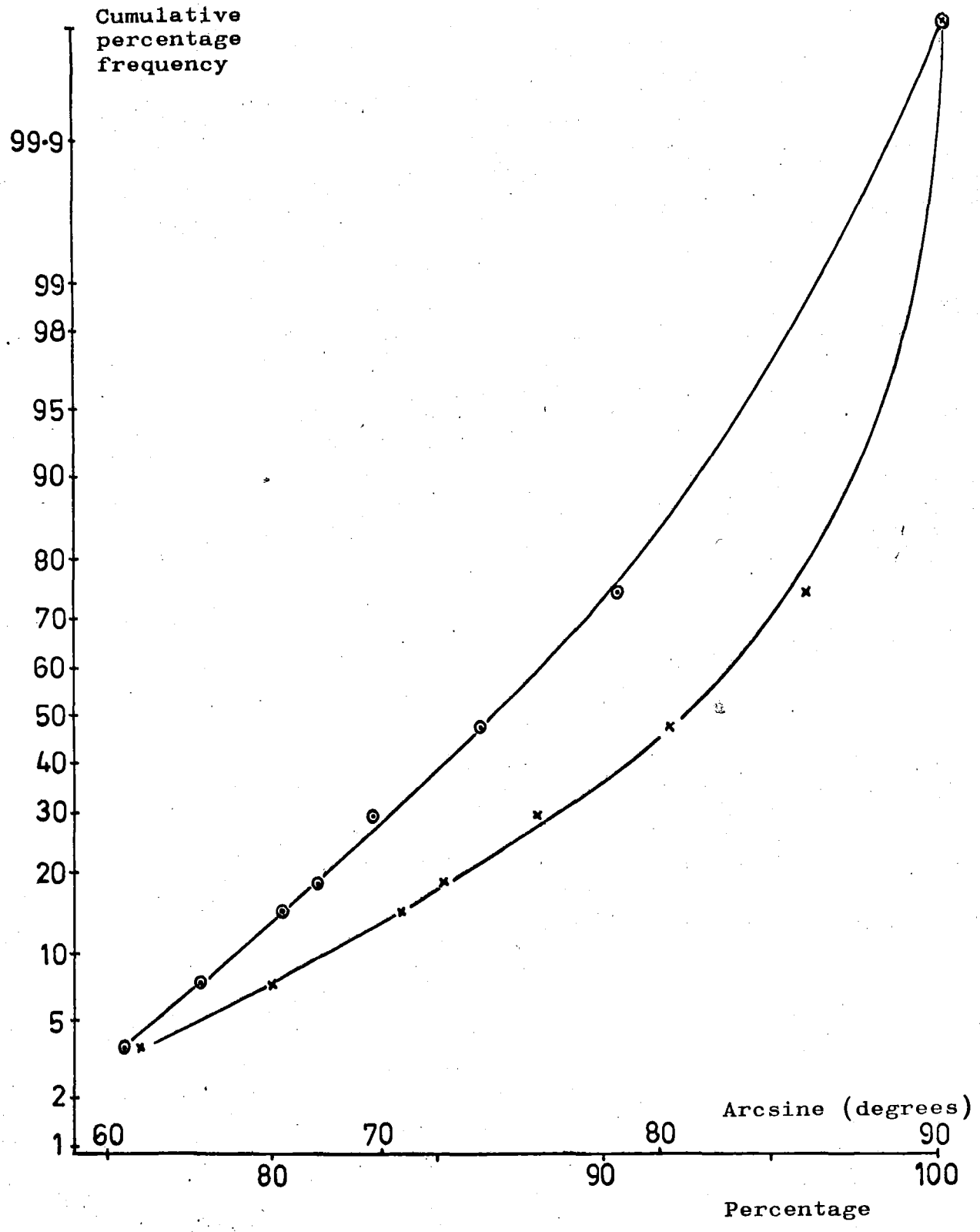

Figure 1. The cumulative frequency of the survival data for E. globulus-mature plotted as percentage and arcsine percentage on probability paper.

Table 1. The survival data for E. globulus-mature; a sign test about the median.

---0+-0--0----00+-000+++++0

$n_+ = 7, \qquad n_- = 11$

r = 6 n.s.

(from Rohlf and Sokal, 1969, Table BB, sequence non-random at 5% probability if $5 \geqslant r \geqslant 14$)

Table 2. The survival data for E. globulus-mature; the results of a two-level, nested analysis of variance.

Source of variation	df	MS	Fs
Among seasons	2	275.6	2.87^{ns}
Among trials	16	82.1	2.30^{ns}
Within trials	8	35.6	

(Corrected df for variance ratio among seasons to among trials is 2, 13

$F_{.05(16,8)} = 3.20$; $F_{.05(2,13)} = 3.80$)

Table 3. The survival data; a Bartlett's Test of Variance for all foliages with five or more results.

Foliage	Variance, s_i^2	df	log variance
E. globulus-mature	82.69	26	1.9175
E. globulus-juvenile	65.80	11	1.8182
E. amygdalina	85.65	5	1.9328
E. obliqua(Ch)	41.98	6	1.6230
E. obliqua(BG)	65.29	8	1.8148
E. linearis(Ch)	131.04	7	2.1174
E. linearis(BG)	249.12	5	2.3964
E. sideroxylon	152.76	4	2.1839
E. delegatensis-hybrid	42.12	6	1.6245
E. andreana	244.07	4	2.3875
E. ficifolia	25.90	8	1.4132

$$\chi^2 = 2.3026((\Sigma(n-1))\ln s_{av}^2 - \Sigma(n-1)\ln s_i^2)$$

$$= 15.776$$

$$\chi^2 \text{ adjusted} = \frac{15.776}{1.058}$$

$$= 14.911 \quad \text{n.s.}$$

$$(\chi^2_{.05(10)} = 18.307)$$

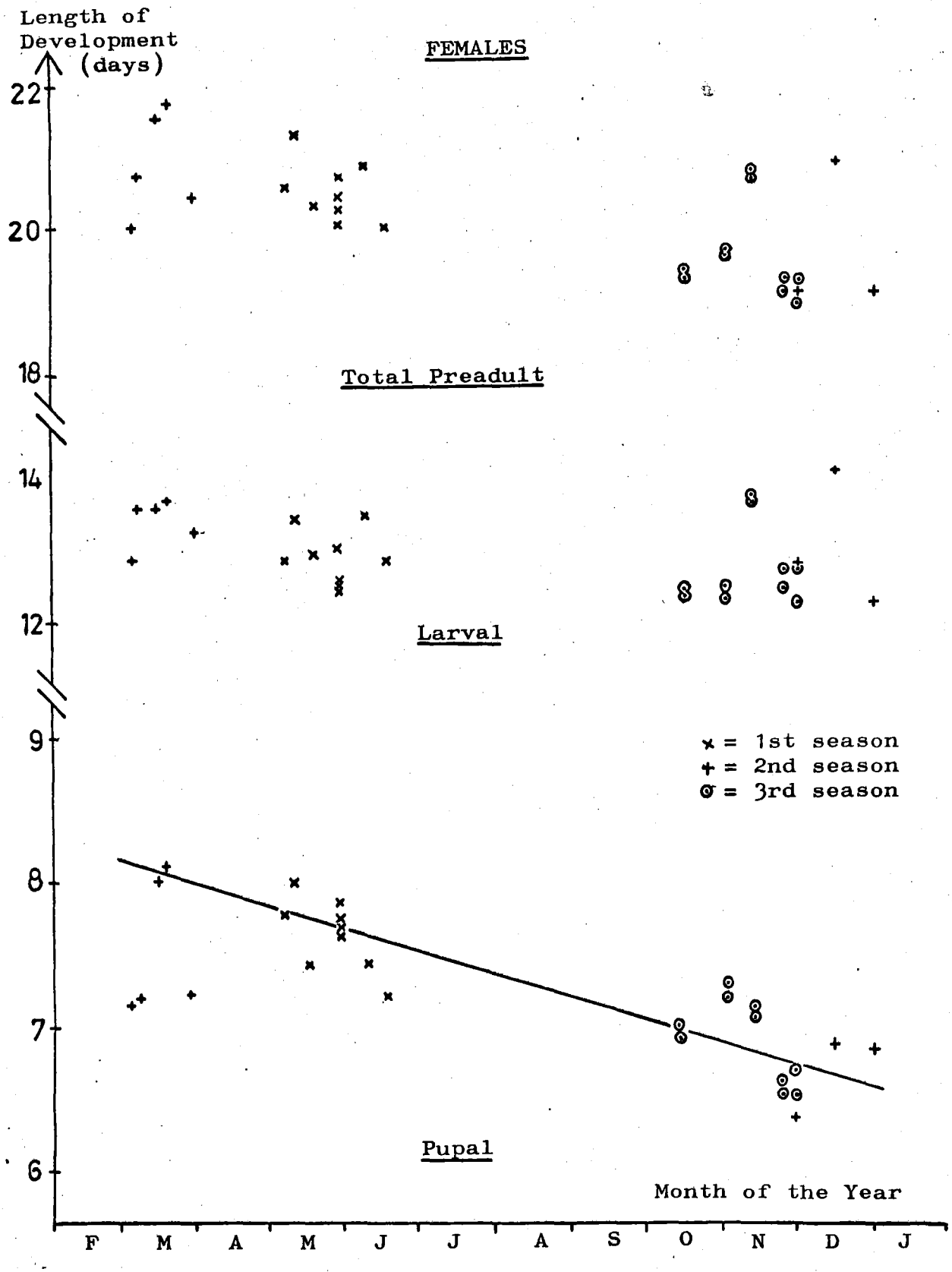

Figure 2A. The seasonal variation in the length of development of female P. charybdis (line fitted by eye).

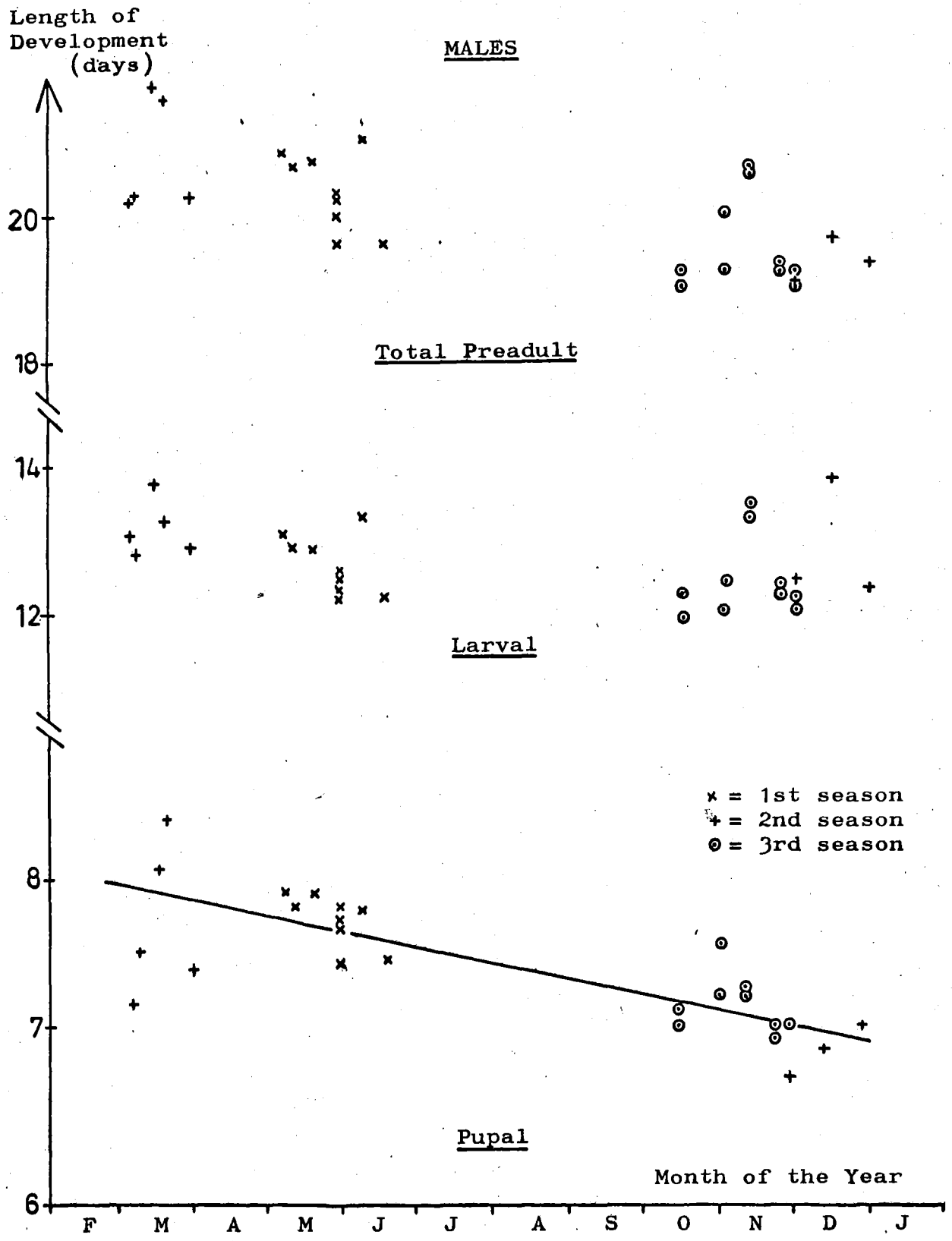

Figure 2B. The seasonal variation in the length of development of male P. charybdis (line fitted by eye).

Foliage	Identity number	Pupal weight (mg)	Adult life (days)	No. of 2-day periods of laying	Egg production					
					Total no. of eggs	% Fertile	No. of batches	Eggs per batch	Eggs per lay-period	Batches per lay-period
globulus-mature	A2	194	234	74	3690	98.14	217	17.00	49.86	2.93
	A10	152	287	114	4220	59.56	474	8.90	37.02	4.16
	A15	187	346	131	6283	98.72	379	16.58	47.96	2.89
	A16	187	296	101	4338	96.54	269	16.13	42.95	2.66
	A19	179	527	148	5260	96.10	316	16.65	35.54	2.14
	A20	178	390	134	5270	96.88	279	18.89	39.33	2.08
	A27	174	207	104	3886	95.98	305	12.74	37.37	2.93
	A28	182	334	138	6528	96.25	371	17.60	47.30	2.69
	A29	173	376	121	5162	97.69	276	18.70	42.66	2.28
	A30	173	340	125	5829	97.86	355	16.42	46.63	2.84
	A32	164	259	115	5837	97.80	368	15.86	50.76	3.20
	A33	174	410	157	6737	95.94	404	16.68	42.91	2.57
	A34	172	378	142	4987	97.32	326	15.30	35.12	2.30
	A35	177	360	129	6131	94.65	411	14.92	47.53	3.19
	A36	180	265	45	1947	93.94	204	9.54	43.27	4.53
	A43	178	430	143	5122	97.33	318	16.11	35.82	2.22
	A9	153	151	51	3048	97.99	194	15.71	59.76	3.80
	Average	175.18	328.83	116.00	4957.35	94.63	321.53	15.51	43.63	2.91
globulus-juvenile	A3	154	250	80	2621	96.68	244	10.74	32.76	3.05
	A4	149	150	51	2366	97.63	126	18.78	46.39	2.47
	A11	158	240	88	2908	95.55	206	14.12	33.05	2.34
	A39	168	218	75	2912	98.65	170	17.13	38.83	2.27
	A40	173	162	72	2293	96.43	187	12.26	31.85	2.60
	A41	156	191	64	2098	98.28	180	11.66	32.78	2.81
	A42	159	167	55	2025	98.86	157	12.90	36.82	2.85
	Average	159.57	196.86	69.29	2460.43	97.44	181.43	13.94	36.07	2.63
andreana	A6	165	250	73	2842	98.02	244	11.65	38.93	3.34
	A18	159	257	74	2429	98.52	175	13.88	32.82	2.36
	A23	161	250	73	3190	93.76	238	13.40	43.70	3.26
	A24	162	249	81	3461	96.37	238	14.54	42.73	2.94
	A37	165	146	48	1697	95.88	174	9.75	35.35	3.63
	A38	165	250	56	1065	95.44	125	8.52	19.02	2.23
	Average	162.83	233.67	67.50	2447.33	96.33	199.00	11.96	35.43	2.96
delegatensis-hybrid	A7	203	335	112	5855	77.22	480	12.20	52.28	4.29
	A21	198	126	24	945	81.64	79	11.96	39.38	3.29
	A22	199	342	130	5985	85.49	380	15.75	46.04	2.92
	Average	200.00	267.67	88.67	4261.67	81.45	313.00	13.30	45.90	3.50
obliqua(BG)	A13	161	372	110	2991	88.90	270	11.08	27.19	2.45
linearis(BG)	A25	140	237	99	3503	95.24	418	8.38	35.38	4.22
	A26	154	264	74	3011	99.16	212	14.20	40.69	2.86
	Average	147.00	250.50	86.50	3257.00	97.20	315.00	11.29	38.04	3.54

Table 4. The results for each female of the F_1 generation of adult rearing.

Foliage	Identity number	Pupal weight (mg)	Adult life (days)	No. of 2-day periods of laying	Egg production					
					Total no. of eggs	% Fertile	No. of batches	Eggs per batch	Eggs per lay-period	Batches per lay-period
linearis(Ch)	A44	144	230	91	3594	93.25	404	8.90	39.49	4.44
	A45	147	310	44	1391	98.89	130	10.70	31.61	2.95
	A46	156	183	69	2515	95.93	261	9.64	36.45	3.78
	A47	154	243	52	1730	98.06	124	13.95	33.27	2.38
	Average	150.25	241.50	64.00	2307.50	96.53	229.75	10.80	35.21	3.39
obliqua(Ch)	A48	154	359	64	1986	97.08	150	13.24	31.03	2.34
	A49	158	405	90	2702	97.58	209	12.93	30.02	2.32
	A50	162	404	140	4097	96.95	360	11.38	29.26	2.57
	A51	159	534	155	3834	96.71	337	11.38	24.74	2.17
	Average	158.25	425.50	112.25	3154.75	97.08	264.00	12.23	28.76	2.35

Table 4 continued

Test foliage	Trial no.	Number of eggs			Number of batches			Mean size of batches			% Fertility		
		Pre	Test	Post	Pre	Test	Post	Pre	Test	Post	Pre	Test	Post
globulus-mature (control)	D1	374	225	227	20	15	13	18.7	15.0	17.5	99	100	100
	D2	277	308	260	17	18	16	16.3	17.1	16.3	100	99	99
	D3	339	354	272	18	16	15	18.8	22.1	18.1	99	97	98
	D4	316	359	346	18	17	15	17.6	21.1	23.1	88	99	94
	E1	199	233	220	13	14	15	15.3	16.6	14.7	97	97	99
	E2	136	100	84	13	12	16	10.5	8.3	5.3	100	93	95
	N1	189	214	255	9	11	12	21.0	19.5	21.3	100	97	99
	S1	185	307	234	10	13	11	18.5	23.6	21.3	100	100	98
	S2	297	256	302	14	12	12	21.2	21.3	25.7	100	98	98
	S3	264	273	303	12	13	13	22.0	21.0	23.3	98	99	99
	Average	258	263	250	14.4	14.1	13.8	18.0	18.6	18.7	98	98	98
globulus-juvenile	J2	300	230	110	21	13	12	14.3	17.7	9.2	100	99	99
	M1	294	311	322	14	14	16	21.0	22.2	20.1	100	98	98
	O2	273	245	194	14	15	10	19.5	16.3	19.4	98	100	97
	V1	186	157	179	14	9	12	13.3	17.4	14.9	100	100	95
	Average	263	236	201	16	13	13	17.0	18.4	15.9	100	99	97
delegatensis-hybrid	C1	195	97	192	11	11	13	17.7	8.8	14.8	92	93	92
	F1	253	76	180	13	7	12	19.5	10.9	15.0	98	90	99
	K1	333	287	276	19	18	15	17.5	15.9	18.4	99	98	98
	Average	260	153	216	14	12	13	18.2	11.9	16.1	96	94	96
obliqua(BG)	L1	320	10	236	13	2	12	24.6	5.0	19.7	99	100	99
	O1	174	2	100	11	2	8	15.8	1.0	12.5	99	100	93
	T1	251	13	*	16	7	*	15.7	1.9	*	99	92	*
	Average	248	8	168*	13	4	10*	18.7	2.6	16.1*	99	97	96*
obliqua(Rf)	R2	317	213	220	14	12	11	22.6	17.8	20.0	99	96	95
obliqua(RS)	F2	203	2*	-	11	1*	-	18.4	2.0*	-	98	50*	-
	I1	278	19	250	13	3	16	21.4	6.3	15.6	99	95	95
	I2	343	108	255	20	10	12	17.2	10.8	21.3	97	95	94
	J1	254	0	225	12	0	14	21.2	0	16.1	100	-	98
	Average	270	42*	243	14	4	14	19.6	5.7*	17.7	99	95*	96
fastigata(Cl)	B1	189	0	159	12	0	8	15.8	0	19.9	92	-	96
	G1	227	28*	-	12	1*	-	18.9	28*	-	99	96*	-
	Average	208	0*	159	12	0*	8	17.4	0*	19.9	96	96*	96
linearis(Ch)	A3	316	40	257	14	9	14	22.6	4.4	18.4	98	40	96
	H3	145	93	169	10	13	12	14.5	7.1	14.1	96	87	96
	J3	205	78	195	14	8	13	14.6	9.8	15.0	98	97	96
	J4	199	122	82	14	17	12	14.2	7.2	6.8	98	95	100
	L3	342	168	295	15	11	12	22.8	15.3	24.6	99	97	99
	L5	335	241	334	15	12	13	22.3	20.1	25.7	97	97	99
	O5	305	249	267	13	11	13	23.5	22.6	20.5	99	98	99
	R3	181	100	287	10	11	15	18.1	9.1	19.1	97	93	99
	Average	254	136	236	13	12	13	19.1	12.0	18.0	98	88	98

Table 5. The results for each of the short-term feeding trials.

Test foliage	Trial no.	Number of eggs			Number of batches			Mean size of batches			% Fertility		
		Pre	Test	Post	Pre	Test	Post	Pre	Test	Post	Pre	Test	Post
linearis(BG)	C2	205	0*	-	20	0*	-	10.3	0*	-	93	*	-
	Q1	157	0	178	14	0	14	11.2	0	12.7	97	-	96
	Average	181	0*	178	17	0*	14	10.8	0*	12.7	95	-*	96
linearis(SP)	A2	305	58	242	15	8	16	20.3	7.3	15.1	92	77	93
	H1	265	109	329	13	8	14	20.4	13.6	23.5	100	99	97
	H2	137	140	241	9	11	12	15.2	12.7	20.1	91	89	98
	I4	341	280	187	14	15	8	28.4	18.7	23.4	98	99	96
	M3	280	108	168	14	13	10	20.0	8.3	16.8	100	85	93
	O4	295	211	228	14	11	12	21.1	19.2	19.0	100	97	97
	R1	161	253	311	16	13	14	10.1	19.5	22.2	66	99	99
	U1	296	12	0*	13	4	0*	22.8	3.0	*	100	67	*
	V2	225	1*	-	13	1*	-	17.3	1.0*	-	100	0*	-
	Average	256	146*	244*	13	10*	12*	19.5	12.8	20.0	94	89*	96*
linearis(TS)	A1	299	5	206	12	4	10	24.9	1.2	20.6	98	40	96
	O3	276	151	207	13	9	9	21.2	16.8	23.0	100	96	98
	Average	288	78	207	13	7	10	23.1	9.0	21.8	99	68	97
linearis(TN)	M2	313	3	147	15	3	10	20.9	1.0	14.7	99	100	97
alpina	K2	274	0*	-	14	0*	-	19.6	0*	-	99	*	-
Starved (2 days)	B2	265	0	0*	14	0	0*	18.9	0	0*	97	-	*
	L2	372	2	284	15	2	13	24.8	1.0	21.9	99	50	97
	Q2	280	10	0*	16	1	0*	17.5	10.0	0*	100	100	*
	Average	306	4	284**	15	1	13**	20.4	3.7	21.9*	99	75	97**
Starved (4 days)	P1	280	16	0*	15	1	0*	18.7	16.0	0*	98	100	*
linearis(SP) 20 day trial	I3	362	326 341	152 323	18	18 23	12 15	20.1	18.1 14.8	12.7	93	97 94	91

(* = death occurred during this period.)

Table 5 continued

Table 6. The effect of temperature on oviposition in P. charybdis; the results for the F_2 females over 18-day test periods.

A: Total number of eggs.

Test period	1	2	3	4
Temp.('C)	25.6	20.0	20.0	25.6
Beetle No.				
1	508	431	231	276
4	541	397	259	277
8	458	384	343	411
9	688	444	350	523
13	712	493	355	473
14	385	234	103	109
19	494	375	377	376
Average	540.9	394.0	288.3	349.3

B: Total number of batches

Test period	1	2	3	4
Temp.('C)	25.6	20.0	20.0	25.6
Beetle No.				
1	23	21	18	15
4	28	23	17	17
8	29	21	18	20
9	30	28	18	25
13	29	24	21	30
14	23	15	10	10
19	21	17	14	19
Average	26.14	21.29	16.57	19.43

Table 6 continued

C: Average batch size per female.

Test period	1	2	3	4
Temp.('C)	25.6	20.0	20.0	25.6
Beetle No.				
1	22.00	20.63	13.13	17.92
4	19.68	17.19	15.67	16.80
8	16.00	18.37	19.50	20.78
9	23.15	16.16	19.88	20.65
13	24.88	20.36	17.00	15.93
14	16.67	15.21	10.44	11.00
19	23.63	22.73	26.38	20.12
Average	20.86	18.66	17.43	17.60

D: Percentage fertility of eggs

Test period	1	2	3	4
Temp.('C)	25.6	20.0	20.0	25.6
Beetle No.				
1	94.31	97.41	92.16	89.92
4	99.80	98.61	98.72	97.21
8	93.48	97.94	99.04	97.55
9	91.94	97.77	98.74	95.93
13	95.65	92.84	93.17	79.72
14	97.13	100.00	98.94	82.83
19	98.41	99.70	99.40	99.38
Average	95.82	97.75	97.17	91.79

Table 7. The effect of different temperatures on oviposition in P. charybdis.

A: Total number of eggs per 20 days per female.

Test period	1	2*	3	4	5	6	7*	8*
Temp.('C)	25.6	20.0	15.0	25.6	12.5	25.6	30.0	25.6
Beetle No.								
4	603	450	183	445	238	521	433	434
5	662	335	193	553	293	479	217	230
6	571	480	255	545	283	600	488	443
9	502	395	193	403	220	438	306	347
10	577	310	198	477	57	458	386	436
12	663	475	258	508	261	564	400	367
13	616	445	251	466	256	566	553	463
14	534	425	214	377	202	320	183	266
15	531	175	178	516	226	483	450	444
17	689	530	275	537	240	475	482	498
18	574	460	198	504	223	551	501	428
19	651	420	272	531	260	552	502	421
Average	597.8	408.4	222.3	488.5	229.9	500.6	408.4	398.1

B: Total number of batches laid per female per 20 days.

Test period	1	2*	3	4	5	6	7*	8*
Temp.('C)	25.6	20.0	15.0	25.6	12.5	25.6	30.0	25.6
Beetle No.								
4	29	20	10	26	12	29	26	20
5	41	20	11	32	12	28	29	30
6	30	20	13	31	14	32	34	27
9	22	15	7	19	7	19	17	20
10	29	15	9	21	1	25	26	25
12	26	20	10	24	9	25	19	18
13	30	25	11	24	10	24	29	22
14	39	25	12	29	11	27	19	30
15	28	10	8	30	9	25	29	27
17	31	25	13	27	10	22	29	30
18	24	20	7	21	8	22	23	22
19	30	15	12	26	9	25	27	22
Average	29.92	19.15	10.25	25.83	9.33	25.25	25.58	24.42

(* period 2 was only 4 days, periods 7 and 8 were 18 days, but the results have been adjusted to a 20-day basis.)

Table 7 continued

C: The average size of egg batches per female per 20 days.

Test period	1	2*	3	4	5	6	7*	8*
Temp.('C)	25.6	20.0	15.0	25.6	12.5	25.6	30.0	25.6
Beetle No.								
4	20.79	22.50	18.33	17.12	19.59	17.97	16.83	21.67
5	16.15	16.75	17.85	17.28	24.12	17.11	7.60	7.67
6	19.03	24.00	19.19	17.58	19.85	18.75	14.21	16.56
9	22.82	26.33	25.78	21.21	30.80	23.05	17.83	17.33
10	19.90	20.67	21.64	22.71	40.00	18.32	15.00	17.46
12	25.50	23.75	25.92	21.17	28.08	22.56	21.54	20.00
13	20.53	17.80	23.23	19.42	25.64	23.58	19.35	21.31
14	13.69	17.00	18.43	13.00	18.87	11.85	9.85	8.83
15	18.96	17.50	21.50	17.20	26.42	19.32	15.75	16.63
17	22.23	21.20	20.69	19.89	24.00	21.59	16.85	16.56
18	23.92	23.00	29.75	24.00	28.45	25.05	21.88	19.69
19	21.70	28.00	23.43	20.42	28.00	22.08	18.47	19.38
Average	20.44	21.54	22.15	19.25	26.15	20.10	16.26	16.92

D: The percentage of fertile eggs laid by each female for each test period.

Test period	1	2*	3	4	5	6	7*	8*
Temp.('C)	25.6	20.0	15.0	25.6	12.5	25.6	30.0	25.6
Beetle No.								
4	97.92	100.0	99.53	99.53	99.40	99.18	98.62	93.82
5	98.61	96.15	98.20	99.28	99.75	97.36	85.27	84.21
6	97.25	100.0	99.65	98.89	99.75	98.38	96.75	95.70
9	99.56	98.65	97.84	99.21	98.70	100.0	87.32	99.48
10	98.08	98.39	99.12	98.74	98.75	92.36	90.67	97.25
12	99.38	100.0	100.0	99.80	98.36	99.44	95.71	96.74
13	99.67	100.0	99.67	98.26	99.72	99.82	97.65	98.56
14	97.73	98.82	99.67	97.61	96.47	93.35	85.16	62.26
15	96.32	100.0	98.59	97.64	100.0	96.05	89.64	80.93
17	92.44	88.68	88.71	93.86	98.51	98.52	94.66	93.96
18	98.40	100.0	99.56	99.18	99.65	99.27	83.72	83.20
19	99.69	98.81	99.70	99.62	99.45	98.91	98.01	97.17
Average	97.92	98.29	98.35	98.47	99.04	97.72	91.93	90.27

(* period 2 was only 4 days, periods 7 and 8 were 18 days but the results have been adjusted to a 20-day basis.)