Ecology of *Paropsis charybdis* Stål (Coleoptera: Chrysomelidae): A *Eucalyptus* defoliator in New Zealand

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Peter Gerard McGregor

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Abstract

Paropsis charybdis Stål (Coleoptera: Chrysomelidae), misidentified as *P.dilatata* Er. before 1963, seriously defoliates some *Eucalyptus* species in New Zealand. This study investigated the life history and phenology, behaviour, growth and development, population dynamics and host-plant interactions of *P.charybdis*, emphasising those aspects likely to affect biological control programmes.

P.charybdis in the central North Island had a bivoltine life history. Adults diapaused during winter. Low temperatures and probably changes in foliage characteristics were sufficient for induction of diapause but no single factor was necessary. Adults emerged in spring to lay eggs. Young *Eucalyptus* foliage was necessary for oviposition. Activity of adults was increased in the presence of volatiles from *E.viminalis* leaves but did not differ significantly between mature and young leaf treatments. Egg-laying ceased in midsummer but this was not caused by lack of new growth, nor by high defoliation. The pattern of abundance in summer was driven by oviposition and temperature and enhanced by intra-specific competition among early instars.

Density-dependent mortality occurred between eggs and 4th instars during the second generation and appeared to result from intra-specific competition for new growth. This was also the key stage that caused variation in summer mortality. Egg survival rates were 93-99%. Survival between eclosion and establishment of 1st instars on new foliage averaged 45% and was independent of density in an experiment where food was in excess. Mortality of the pre-pupal to teneral adult stages in the soil was 90%, but was independent of density in both generations.

Development rate-temperature relationships were described for larvae fed *E.viminalis* young adult foliage, and for eggs and pupae. A method was developed for minimising differences in duration among larvae grown at different temperatures, so that the effects of changes in food quality were obviated. The method appears widely applicable for development-rate studies.

Defoliation in a five-year old plantation of *Eucalyptus nitens* was almost twice as severe in the second *P.charybdis* generation as in the first. Shoot growth was significantly correlated with rainfall and defoliation intensity. There was a strong inverse relationship between defoliation intensity and deviations from the seasonal trend in rainfall.

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Liz Grant. Liz kept me going. She still does! She also translated my ideas into the line drawings in this thesis.

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List of Figures

Erratum: Due to an error in numbering, Fig. 4.19 follows Fig. 4.17. However, all figures referred to in the text are present.

Chapter 1. Introduction

1. INTRODUCTION

1.1 Taxonomy

The eucalypt tortoise beetle, *Paropsis charybdis* Stål, is a chrysomelid defoliator of some eucalypts. The Australian genus *Paropsis* includes about 500 species (Hawkeswood 1977) within the Tribe Paropsini, subfamily Chrysomelinae. Following the beetle's discovery in New Zealand in 1916 (Thomson 1922) it was determined as *P.dilatata* Erichson by Clark (1930) who sent specimens to Dr G.A.K. Marshall at the then Imperial Bureau of Entomology, London. There, the Rev. T. Blackburn confirmed the identification (Selman 1963a) and the species was known as *P.dilatata* until Selman (1963a, 1963b) showed that it was *P.charybdis* Stål (1860). *P.charybdis* had formerly been synonymised with *P.obsoleta* (Marsh.) (1808) and *P.obsoleta* Ol. (1807), with the latter having priority, but Selman demonstrated that *P.charybdis* was a different species from these two. He synonymized *P.charybdis* Stål (1860) and *P.atomaria* (Marsh.) (1808), but because *P.atomaria* was a preoccupied name the species became *P.charybdis* Stål (1860). Selman confirmed that *P.dilatata* Er. (1842) was a valid species, but showed that the New Zealand species was *P.charybdis*, not *P.dilatata.* The confusion has persisted at least until 1981, when Hinton (1981), in his definitive treatise on insect eggs, quoted Clark's (1930) description of the egg-laying behaviour of "*P.dilatata*" without realising that it was actually *P.charybdis.* To avoid further confusion, the practice of referring to "*P.charybdis* (= *P.dilatata* Er.)" (e.g. Forest Research Institute (F.R.I.) Annual Report 1963; Styles 1970) should be discontinued, because it suggests a synonymy, whereas it was a misidentification and the two were never taxonomically synonymous.

1.2 Establishment and Dispersal

The first published record of *P.charybdis* in New Zealand was Thomson's (1922) note that a Mr Drummond had found specimens of *Paropsis* sp. at 1800 feet (548m) on Cooper's Knob on Bank's Peninsula's Port Hills in November 1916. Clark (1930) referred to this and added that E.S. Gourlay had found *P.charybdis* adults under bark of fallen *Eucalyptus globulus* trees, also on the Port Hills, earlier in 1916. Dugdale (1965a) mentioned a doubtful observation from the Port Hills in 1912, listing Gourlay as the "reputed source". Steven (1973) concluded that Dugdale had confused Gourlay's 1916 record, but this is questionable, because Dugdale distinguished Gourlay's 1916 record from the earlier 1912 report.

Clark (1938) suggested that *P.charybdis* had entered New Zealand probably as eggs or larvae on young plants, or possibly under the bark of imported Australian hardwoods. Steven (1973) stated that Miller's (1925) view was that *P.charybdis* had arrived under bark of hardwoods, but I could not find this claim in Miller's original paper. However, Miller's concluding paragraph on *Gonipterus scutellatus* (Gyllenhal) (Coleoptera: Curculionidae) which immediately precedes the section on "*P.dilatata*" includes the comment that: "...it seems very probable that the pest (*Gonipterus*) reached the Dominion on Australian hardwood". Steven may have inadvertently read this as part of the *P.charybdis* section.

P.charybdis spread gradually throughout the South Island and became established in the North Island around the Wellington-Manawatu region about 1956 (Dugdale 1965a; Gurr 1957; Steven 1973; White 1962, 1973). Less than 10 years later it had colonised all but the extreme North of the North Island.

White (1973) summarised the dispersal of *P.charybdis* as occurring in "two brief, approximately ten-year 'bursts' - one in the South Island .. the other in the North Island". This is questionable, because *P.charybdis* was not noticeably abundant in Nelson until 1932,

and was only infrequently recorded from Southland before 1938. Therefore, colonisation of the Eastern South Island took at least twice as long as colonisation of the whole North Island except for the extreme North.

There is doubt whether Steven's (1973) accrediting of a 1943 record of *P.charybdis* on Mt Peel in South Canterbury is correct, as there is also a Mt Peel near Mt Arthur, Nelson region, and this locality is at least as consistent with *P.charybdis*' spread as a South Canterbury record. The altitude (1739m) attributed to the "Mt Peel" by White (1973) is that of Mt Peel in Nelson and not South Canterbury.

White (1973) suggested that the pattern of dispersal and abundance of *P.charybdis* was caused by changes in food quality of the host plants. His hypothesis was that a series of unusually wet winters accompanied by a series of unusually dry summers stresses the plant, and this causes an increase in leaf nitrogen content which improves its quality as food. He suggested that *P.charybdis* did not colonise the North Island until about 1956 because only then were the trees in the Manawatu stressed. However, he did not sufficiently substantiate this conclusion and his account contained some inconsistencies. Early in his paper he stated that *P.charybdis* was not abundant in Nelson until 1932 but later he implied that it was "still quite abundant in Nelson" in 1925. More importantly, he did not show the stress indices for the period 1932-1952, when *P.charybdis* was abundant in Nelson but did not establish in the North Island.

Steven (1973) briefly discussed the method of dispersal of *P.charybdis*. He used observations by Clark (1930) and Gibbs and Ramsay (1962) as well as records of specimens from mountain tops to show that it may have been transported by man because its initial spread in the North Island followed main transportation routes. He noted Clark's (1930) observation of beetles clinging to a moving car as support for this theory. White (1973) also accepted that this was possible, but his view favoured dispersal of strongly-flying adults by wind. He

suggested that dispersal by man could not have accounted for the rapid spread of *P.charybdis*, and he added new records that further verified the insect's ability to fly strongly.

1.3 General Biology

Most published literature on the biology of *P.charybdis* has comprised reiteration of already-published work, in some cases with little change to the original phrasing (e.g. Manson 1960; Miller 1944, 1971; Penfold and Willis 1961). The first substantial account was by Miller (1925). He described the eggs, adults and larvae, added brief notes on behaviour and life history, and included photographs of the adult, eggs, "late" larva and pupa. In 1944 he summarised his 1925 publication and included the same photographs of the eggs, adult and "larva". He re-used the adult and larva photographs with new photographs of an egg mass and of newly-hatched larvae in 1971 to illustrate another brief review. The photograph of the "larva", captioned "late larva" in 1925, "larva" in 1944 and "mature larva" in 1971 shows the dorsal aspect of a prepupa. Miller did not mention the existence of a prepupal stage in any of his accounts and only his 1925 article contains new information.

Clark's (1930) paper is still one of the best information sources on *P.charybdis*' biology. He discussed the economic significance of *P.charybdis* and was the first to draw attention to its possible status as a serious pest. Clark also described in detail the life history and the morphology and behaviour of all life history stages, documented the the extent of infestation to 1930 and discussed modes of dispersal and possible control measures. He figured the adult, first, third and final instars, prepupa, pupa, egg and larval repulsive glands. I agree with Steven's (1973) comment that Clark's drawing of the egg appears unfinished, and as Dugdale (1965a) noted, Clark's assessment of damage was weakened because he did not define what he called an "infested" tree. Later, Clark (1938) summarised his 1930 paper and

described attempts at biological control. His view of *P.charybdis* as a serious pest was stronger in this paper than in his 1930 paper.

Other brief accounts of *P.charybdis* were given by Atkinson, Brien, Chamberlain, Cottier, Dingley, Jacks, Reid and Taylor (1956), Manson (1960) and Penfold and Willis (1961) but none added new information. All are short notes; Manson's covers dispersal, morphology and behaviour, that of Penfold and Willis includes dispersal, host range and control, and that of Atkinson *et al.* describes distribution, economic significance, morphology, life history, behaviour and control. The drawing of an adult *P.charybdis* shown as Fig. 14 in Penfold and Willis (1961) appears to be a copy of Clark's (1930) drawing.

Other publications that emphasise the biology of *P.charybdis* include papers by Alma (1980), Bain (1977), Edwards and Suckling (1980), Edwards and Wightman (1984) and Styles (1970). Edwards and Suckling's paper comprised an experimental study of predation of *P.charybdis* larvae by *Cermatulus nasalis* (Westwood) and *Oechalia schellembergii* (Guérin-Méneville) (both Hemiptera: Pentatomidae) under laboratory conditions. Edwards and Wightman (1984) published energy and nitrogen budgets for *P.charybdis.* They gave some morphometric data for larvae and eggs and development times for the four larval instars at 20°C. The results of this work had been summarised in two abstracts a year earlier (Edwards and Wightman 1983; Wightman and Edwards 1983).

Alma (1980) showed that four out of six species of Coccinellidae found in New Zealand eat *P.charybdis* eggs in the laboratory. Styles (1970) described experiments on larval mortality, and he included observations on oviposition, sex ratio, identification of the sexes and host preference, discussing these and possible control methods. Steven (1973) later showed that Styles' method of sexing adults by the orange testes in males was unreliable. From two of his experiments, Styles concluded that very little mortality while *P.charybdis* was in the soil was caused by predation, although he admitted that predation may have been higher in an

infested eucalypt stand. His conclusion was further weakened because he did not adequately explain his methods. The estimate of predation was the difference in survival between mature larvae placed in containers from which predators had been excluded and those to which predators were allowed access. However, instead of placing pairs of containers of each type together, he apparently sited them in different areas, and it is not clear whether they were established at the same time. Consequently, the two treatments seem not to be directly comparable.

The biology of *P.charybdis* was summarised by Bain (1977) and Emberson (1976), both of whom emphasised its economic significance but added no significant new information. Much of the information on the general biology of *P.charybdis* is contained in unpublished New Zealand Forest Service reports. Some of this has been collated and published (e.g. White 1973), while Styles' (1970) paper follows his unpublished Forest Service report (Styles 1969), but some information still remains unpublished and difficult to retrieve. Unpublished reports known to me include those of Dugdale (1963, 1965a, 1965b, 1966), Styles (1966, 1969) and White (1962).

Dugdale's (1965a) report was the most comprehensive of these and in nine pages covered colonisation, defoliation, biology and biological and chemical control. This report summarised what was known of *P.charybdis*' biology by 1965 and presented useful new information on fecundity, incubation times, mating behaviour, overwintering, flight and rearing methods. His claim that "U.V. light is necessary or, rather, normal closed laboratory conditions are not suitable" for maintaining *P.charybdis* cultures was disputed by Steven (1973) and I also found that *P.charybdis* was easy to maintain in the laboratory if fresh, palatable foliage was provided. His classification of eucalypts common in New Zealand into three groups according to their susceptibility to defoliation was later used by Bain (1977). Dugdale's classification was not completely reliable. For example he placed *E.nitens* in group III (infrequent and light defoliation) whereas it should be in group I (severe defoliation in

one-two seasons out of three). This discrepancy was noted by Bain (1977) who acknowledged that locality, seed source and silviculture could affect susceptibility. Dugdale (1965a) presented a useful perspective by illustrating the various interpretations of the extent of defoliation and by noting that severe defoliation did not inevitably cause lasting damage. He implied that the effect of *P.charybdis* on *Eucalyptus* may not be as serious as it first appeared.

Annual reports of the New Zealand Forest Research Institute usually mention *P.charybdis* and provide useful documentation of the progress of biological control attempts.

Population regulation of *P.charybdis* in New Zealand was investigated in 1967 by Dr P.B. Carne from C.S.I.R.O. (Australia). He was based at Entomology Division, D.S.I.R. in Nelson, but travelled extensively throughout New Zealand to survey *P.charybdis* damage. His experimental work was parallelled during his stay by that of J.H. Styles at the Forest Research Institute in Rotorua (Styles 1969, 1970). Carne had previously studied *P.atomaria* Ol. in Australia, and his report on the New Zealand study compared this species' biology with that of *P.charybdis* (Carne 1967). He reviewed current knowledge, and discussed the results of his experiments and field observations. Carne's knowledge of Australian Paropsini led him to suggest that *P.charybdis* was able to reach high numbers each season because the first generation larvae were not subject to the high parasitism that occurred in typical Australian Paropsines. He speculated that the major check to *P.charybdis* in New Zealand was during the second generation, possibly due to the disease that he, Dugdale (F.R.I. report for 1963) and Styles (1969, 1970) had investigated. In his suggestions for ameliorating the *P.charybdis* problem, Carne assessed chemical control, selecting resistant species of *Eucalyptus*, eliminating susceptible species, using mixed-species stands, biological control and selecting trees suitable for particular environments.

Steven's (1973) thesis is the most comprehensive presentation of *P.charybdis*' biology. His primary aim was to investigate the effect of different *Eucalyptus* foliages on *P.charybdis* and to test the insect's ability to distinguish foliages, but he also discussed the available literature on all aspects of the biology of this insect, adding new information on behaviour, sexual dimorphism and adult diapause. He performed selection trials and feeding response experiments on larvae, and tested 17 leaf types from 12 *Eucalyptus* species, measuring their effect on larval survival and development time and pupal size. For some species of eucalypts he tested both adult and juvenile forms of foliage. These are morphologically and physiologically distinct leaf types. Juvenile leaves are usually produced when the tree is young, but may also arise after severe defoliation or other stress (Pryor 1976). Individual leaves do not change from one form to the other as they age. Steven did not compare the effects of young and old foliage of either adult or juvenile forms. He tested adult females on eight foliage types for reproductive ability from 12.5°C to 29.5°C and for longevity. He showed clearly that foliage characteristics had a profound effect on *P.charybdis*' biology and that larvae could distinguish different foliages. He could not attract adults to any of 53 chemicals or four *E.globulus* extracts, but these results were inconclusive because he did not try the tests during the whole of the flight season. Summaries of his current progress were given in the Forest Research Institute's Annual Reports for 1970, 1971 and 1973. None of his work has yet been published elsewhere.

1.4 Economic Significance

P.charybdis is a serious pest in New Zealand because it can severely defoliate some eucalypts, to the extent that it may cause the tree's death. Eucalypts were recognised as a potentially important source of hardwoods soon after the establishment of New Zealand's successful softwoods afforestation programmes during the early 1900's (Clark 1930, 1938).

Unlike the exotic conifers, eucalypts were not very successful, partly because unsuitable species were planted, and also because of insect damage.

Zondag (1979) recently published a checklist of insects attacking eucalypts in New Zealand. Two of the worst insect pests, *Gonipterus scutellatus* (Gyllenhal) and *Eriococcus coriaceus* Maskell (Hemiptera: Eriococcidae) (respectively, the gum-tree weevil and gum-tree scale) were controlled by artificially-introduced parasites or predators, but *P.charybdis* is still abundant. Since the early 1970's there has been a renewed interest in eucalypts both as durable and decorative timbers and veneers to replace the depleted indigenous podocarps, and to replace native tawa (*Bielschmeidia tawa*) as short-fibre pulp for high-quality papers (B.Poole *pers. comm.*; Lembke 1977; F.R.I. report for 1977). Eucalypts are also being planted for firewood, wind shelter and soil conservation (Hathaway and King 1988, Mortimer and Mortimer 1984).

Hathaway and King (1988) recorded the amount of *P.charybdis* damage on 119 provenances of 56 species of *Eucalyptus* that they investigated for soil conservation purposes. At both trial sites in the Wairarapa, south-eastern North Island, they found that *P.charybdis* damage differed significantly among eucalypt species. They also found that, at both sites, *P.charybdis* damaged eucalypts in the subgenus Symphyomyrtus more severely than those in the subgenus Monocalyptus. *P.charybdis* damage also varied significantly among provenances of seven of the *Eucalyptus* spp. Hathaway and King recommended that although *E.brookerana* and *E.nitens* were otherwise suitable for soil conservation purposes, they should be treated with caution because of their susceptibility to *P.charybdis* damage.

Some potentially-valuable *Eucalyptus* species may be unsuitable for commercial production because they are defoliated by *P.charybdis*. For example, *E.nitens* in New Zealand Forest Products Ltd's plantations near Tokoroa has grown three metres in a season and has cold-tolerance superior to the slower-growing *E.delegatensis* (Lembke 1977), but is severely

defoliated by *P.charybdis* when it reaches the adult stage. Consequently, the New Zealand Forest Service recommends *E.delegatensis* but not *E.nitens* for planting in areas too cold for *E.regnans* (F.R.I. report for 1977), a species with growth rates comparable to *E.nitens* (B. Poole *pers. comm.*). The implication is that continually-defoliated *E.nitens* will take longer to reach millable size than the normally slower-growing *E.delegatensis*. A common and related assumption is that defoliation must *ipso facto* slow the tree's growth. Both assumptions may be true under some circumstances, but *E.nitens* near Tokoroa has sustained good growth even during severe defoliation. Rafes (1971) suggested that loss of growth increment is negligible unless more than 75% of the photosynthetic biomass is removed. Eucalypts in Australia are apparently adapted to sustain high defoliation losses (Journet 1981, Morrow 1977, Springett 1978; but see Ohmart, Stewart and Thomas 1983) and eucalypt-defoliating insects may be important rate regulators, especially of nutrient cycling (Springett 1978). Carne, Greaves and McInnes (1974) showed that eucalypts, particularly *E.grandis*, could sustain up to 50% defoliation by the Christmas beetles *Anoplognathus chloropyrus* and *A.porosus* (both Coleoptera: Scarabaeidae) with no reduction in growth rate, because of the insects' feeding preferences and the timing of their life histories. Experimental evidence is, however, inconclusive: studies of defoliation in Australian forests suggest some loss of growth increment due to defoliation (e.g. Cremer 1972, 1973; Greaves 1967; Mazanec 1966, 1967, 1968, 1974) and Morrow and La Marche (1978) found that severe defoliation strongly suppressed productivity in *E.pauciflora* and *E.stellulata*; while unpublished work by Edwards (*pers. comm.*) on artificial defoliation and the paper of Edwards and Wightman (1984) on *P.charybdis* energy and nitrogen budgets supports Springett's (1978) suggestion that insects are important rate regulators in Australian forests. The current situation is that few susceptible species are planted; chemical control has been discounted mainly because of expense and rapid pest reinvasion (Bain 1977; Baker and DeLatour 1962; Styles 1969, 1970) and the possibility of biological control first mooted by Clark (1930) is still seen as the ideal solution to the *P.charybdis* problem.

1.5 Attempts at Control

Attempts at biological control started in the 1930's when parasitised larvae and eggs were sent to New Zealand from Australia in 1934 and 1935. Some Tachinidae (Diptera) and an ichneumonid (Hymenoptera) emerged from these (Anon. 1935). Clarke (1936) very briefly mentioned this work and later implied (Clark 1938) that *P.charybdis* (misidentified then as *P.dilatata)* was the species sent to New Zealand by Tonnoir, but this is doubtful. Both Carne (1967) and McInnes, Albert and Alma (1977) had access to New Zealand Forest Service records, and they stated that these eggs and larvae were *P.atomaria* Olivier. Steven maintained that the species was *P.atomaria* - presumably *P.atomaria* Ol., while the 1961 Forest Research Institute report for 1961 referred to the species as *P.reticularis* (=*P.reticulata*?), which Selman (1963b) later synonymised with *P.atomaria* Ol. This suggests that the species was that which is now known as *P.atomaria* Ol., not Clark's (1938) "*P.dilatata*" (later identified as *P.charybdis).* According to Dugdale (1965a) Clark's attempts at biological control used *Froggatimyia tillyardi* Malloch (Diptera: Tachinidae), *Neopolycystus insectifurax* Girault (Hymenoptera: Pteromalidae) and *Meteorus* sp. (Hymenoptera: Braconidae).

In the 1960's, the New Zealand Forest Service investigated several possible parasites, and concluded that a braconid parasite (*Aridelus* =*Westwoodiella* = *Meteorus* sp.) that attacked larvae was the most suitable (Dugdale 1965a, Steven 1973). However, the attempt to introduce this braconid was unsuccessful (Steven 1973; Styles 1970). Attempts to introduce the tachinids *Froggatimyia* sp. and *Paropsivora* sp., which are parasitic on *P.charybdis,* were made from 1973 to 1975. These culminated in the release of *F.tillyardi* in 1975, but this was apparently unsuccessful (Bain 1977).

In 1977, work at the Forest Research Institute in Rotorua began on the introduction of *Cleobora mellyi* Mulsant (Coleoptera: Coccinellidae), a predator of paropsine eggs and larvae, from Tasmania. Laboratory experiments suggested that *C.mellyi* was the most promising biological control agent for *P.charybdis* so far investigated, and in the summers following the 1978-1979 season, several thousand *C.mellyi* were released near Tokoroa in plantations infested by *P.charybdis*. Results so far have been disappointing, with the major problem being the failure of *C.mellyi* adults to overwinter successfully. Attempts were made to surmount this by providing shelters in which large aggregations of adults could overwinter, but this has been unsuccessful. In the summer of 1982-83, *C.mellyi* was released in the Nelson-Marlborough area, where the milder climate may have allowed successful overwintering. This has apparently been successful, and *C.mellyi* is considered to have established there, but not in the central North Island (J.Bain, *pers. comm.*). A method for laboratory rearing of *C.mellyi* was discussed by Bain, Singh, Ashby and Van Boven (1984).

Parasitoids were again the focus of attention in 1987, when two pteromalid egg parasites were obtained from Australia. These were *Neopolycystus insectifurax* Girault and *Enoggera nassaui* (Girault) (both Hymenoptera: Pteromalidae). Both species parasitised *P.charybdis* eggs under insectary conditions. During the 1987-88 and 1988-89 summers, several thousand of these parasitoids were released at sites throughout the North and South Islands. *E.nassaui* has since been recovered in many areas and appears to be spreading rapidly (G. Hosking, M.Kay, *pers. comm.*). In January 1989 I recovered *E.nassaui* from *P.charybdis* eggs field-collected from Massey University, Palmerston North.

1.6 Aims of the Study

When this study began there was much information on the biology of *P.charybdis* in New Zealand, but many observations were poorly detailed and the reliability of some work was questionable. Much of the literature comprised restatement of earlier results. Other fundamental information, such as the relationship between temperature and development rates, had never been investigated. My primary objective therefore, was to provide a reliable foundation of knowledge concerning the biology of *P.charybdis*. From this basis I could then investigate in more detail the processes that influence the life history, behaviour and population dynamics of *P.charybdis*, particularly those aspects likely to affect the success of biological control programmes.

GOAL:

To improve understanding of the biology of *P.charybdis* in order to assist screening of prospective biological control agents and so facilitate the successful introduction of these agents.

AIMS:

1. Investigate the phenology of field populations of *P.charybdis.*

2. Determine the relationships between temperature and rates of development of eggs, larvae and pupae of *P.charybdis.*

3. Investigate the relationship between characteristics of the host tree and the behaviour, phenology and population dynamics of *P.charybdis*.

4. Investigate processes that affect the population dynamics of *P.charybdis*, with special emphasis on first instar larvae.

Chapter 2. Study Areas

2. STUDY AREAS

I used three main sites for field sampling of *P.charybdis* populations. During the 1978-79 summer, two areas near Palmerston North were used for developing sampling methods. These were at Ferguson Hall near Massey University, Palmerston North, and at Aokautere, near Palmerston North. The following summer, a third, much larger area at Kāhu Farms near Whakamaru, about 180km North of Palmerston North was used to collect substantive data, while the first two sites were no longer used. Kāhu Farms was also visited during the 1980-81 summer to check the development of the *P.charybdis* population in that season. The characteristics of these three sites are summarised in Table 2.1 and their location is shown in Fig. 2.1.

Site One

The Ferguson Hall site comprised a stand of young *Eucalyptus viminalis* trees between two and three metres high, growing on the North-facing aspect of a small gully. They were entering adult foliage and had presumably grown from seed shed from a row of 10-metre high trees above the gully. The stand was well sheltered from wind, and I noticed over the three seasons 1978-81 that these eucalypts began growing slightly earlier in the season than other eucalypts and mature *E.viminalis* elsewhere. The site had a dense understorey of rank grasses and gorse (*Ulex europaeus*). The trees were heavily infested with *P.charybdis* at some periods of the summer and adults could usually be found in large numbers until late autumn. Gum tree weevil (*Gonipterus scutellatus* (Gyllenhal)) and gum emperor moth (*Antherea eucalypti* Scott; Lepidoptera: Saturniidae) were both present in low numbers, together with the following known or possible predators of *P.charybdis*: the bugs *Oechalia schellembergii* (Guérin-Méneville) and *Cermatulus nasalis* (Westwood); german wasps (*Vespula germanica* (F.); Hymenoptera: Vespidae) and mantids (*Orthodera ministralis* (F.); Mantodea: Mantidae).

Site two

At Aokautere, I sampled a single four-metre high *E.leucoxylon* tree growing a few metres from the south side of a shed, in a grazed paddock. Some juvenile foliage was still present. The main trunk had been split so the tree had two distinct crowns, one lower and about half the size of the other. The damage was probably wind-caused, reflecting the tree's exposure to the south, west and, to a lesser extent, the east. The tree supported a moderate population of *P.charybdis*, but numbers were never high enough to cause conspicuous damage. Some *A.eucalypti* and Tortricidae (Lepidoptera) were also present at low densities.

Site three

Site three was the New Zealand Forest Products Ltd (NZFP) plantation at Kāhu Farms, 10 kilometres south of Whakamaru. It was chosen as the major sampling area for the following reasons: it was the largest accessible plantation containing a susceptible species of eucalypt; the trees were small enough and of a form suitable for sampling; the history of the plantation was well known by NZFP staff; and accommodation was available with NZFP at Tokoroa. The plantation is mapped in Fig. 2.2. Of the 170ha plantation, 19ha was *E.nitens*, the remainder being *Pinus radiata* and other *Eucalyptus* spp., predominantly *E.regnans, E.fastigata* and *E.delegatensis*. These last three species were not attacked by *P.charybdis* larvae and suffered only slight damage by adults. In contrast, the *E.nitens* had been severely defoliated during every season when it had produced a significant amount of adult foliage.

The climate at Kāhu Farms was more continental than at Palmerston North, which was still moderated by the nearby coast. Frosts were more numerous at Kāhu Farms and daily extremes of temperature were greater here than at the lower, more southern sites.

This site had been farmland until 1974, when it was sprayed with a low rate of hormonal weedkiller to kill nodding thistle (*Carduus nutans*). A few months later it was planted with pines and eucalypts from a NZFP nursery. The spray was not completely effective and when

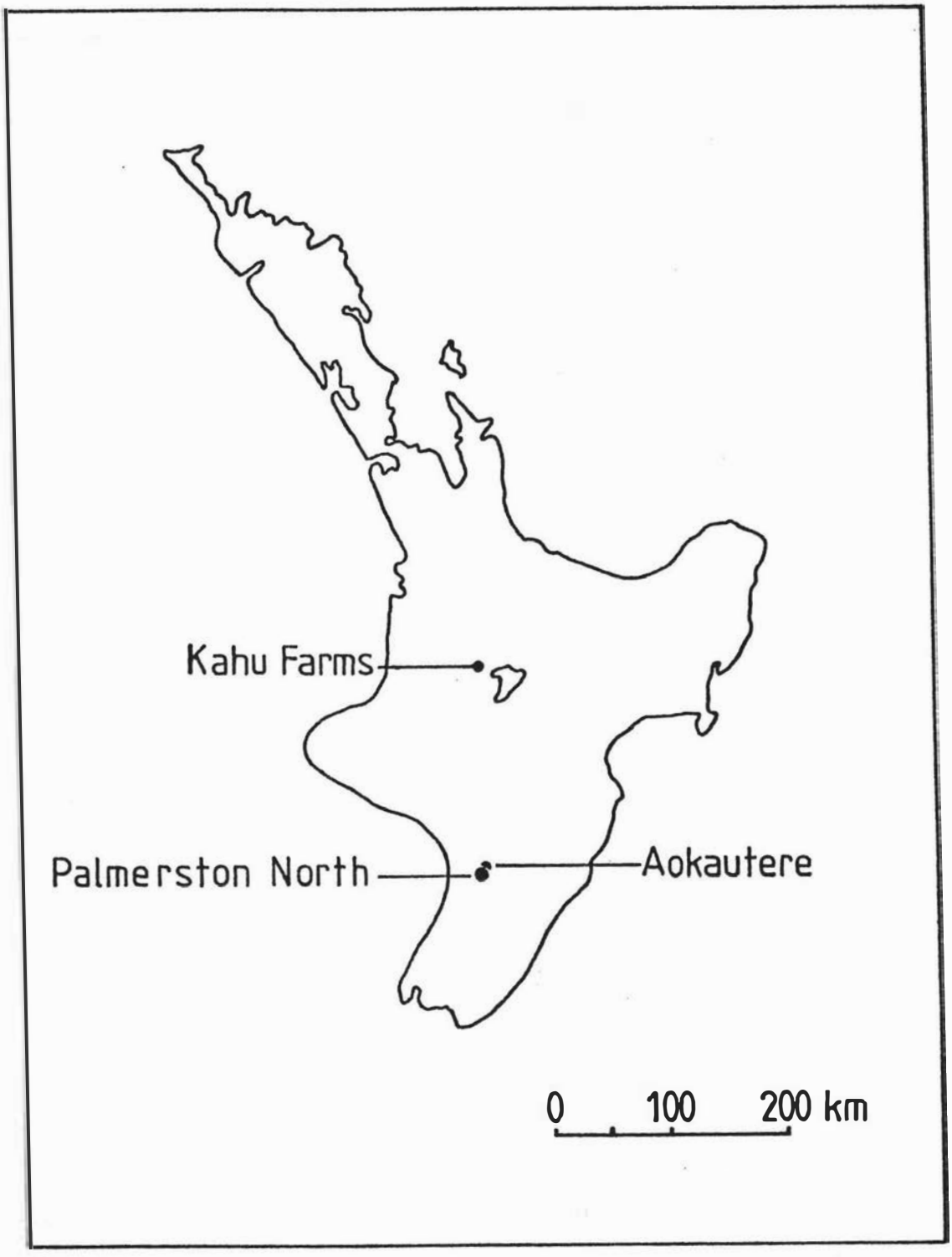


Fig. 2.1 Location of study areas in the North Island, New Zealand.

the nodding thistle reappeared, the plantation was resprayed with the same herbicide. This killed the thistles but caused deformities in many of the trees and retarded the growth of the eucalypts. By 1978 the *E.nitens* were growing normally, but many had multiple leaders and few showed the normal, straight-boled growth form typical of *E.nitens* in other healthy NZFP plantations.

The only marked heterogeneity within the Kāhu Farms *E.nitens* was caused by soil variations. Trees planted where soil had been compacted or where topsoil was removed for roadfill were noticeably smaller and less thrifty than elsewhere. The pumice soils here were quite different from sites one and two, near Palmerston North, and were much less fertile. Nevertheless, this plantation was not fertilised after planting, but may perhaps have received drifts from aerial topdressing of adjacent farmland. Small numbers of sheep were sometimes grazed in the plantation. Consequently the understorey was never dense, although the grass was occasionally rank in parts and dense thistles grew seasonally in open areas.

Kāhu Farms supported a diverse insect population. *P.charybdis* was the major *Eucalyptus* feeder, with Psyllidae (Homoptera), Tortricidae and a few *A.eucalypti* and other Lepidoptera present on *E.nitens*. Manuka beetles (*Pyronota* sp.) and grass grub beetles (*Costelytra zealandica* (White)) (both Coleoptera: Scarabaeidae) were abundant around November, but I could not determine whether they ate *E.nitens*. Diptera and Hymenoptera were conspicuous during the day and included some known predators of other insects. A robber fly, *Neoitamus* sp. (Diptera: Asilidae) was a probable predator of adult *P.charybdis*. German wasps (*V.germanica*) were common and are known to prey on *P.charybdis* (Valentine 1969, Styles 1970). The pentatomid bugs *O.schellembergii* and *C. nasalis* were occasionally seen and both eat *P.charybdis* (Ramsay 1963, Edwards and Suckling 1980).

During the 1980-81 summer several thousand *Cleobora mellyi* adults were released at Kāhu Farms by Forest Research Institute entomologists in an attempt to establish this predator of

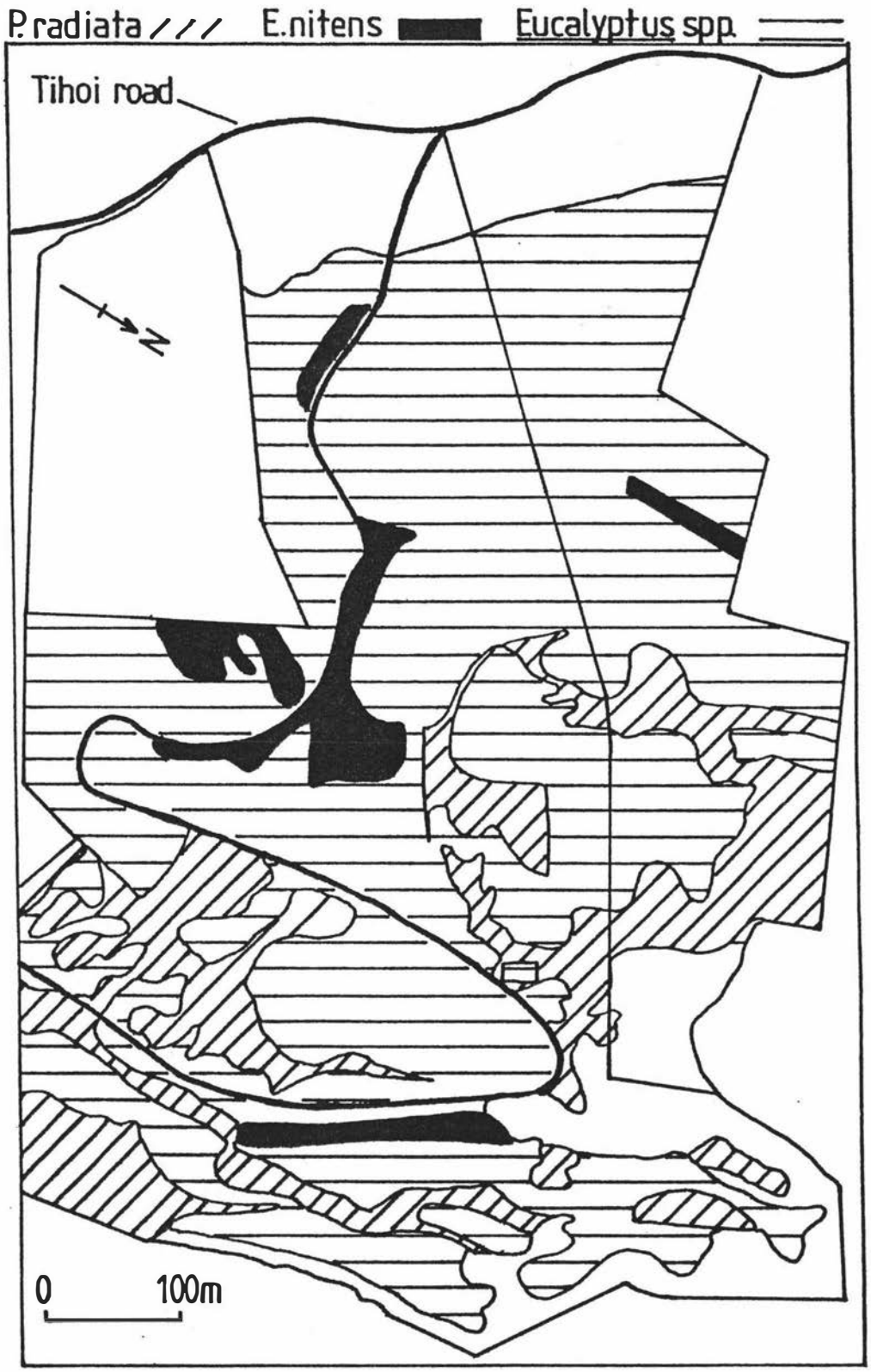


Fig. 2.2 Map of the study area at Kāhu Farms, near Whakamaru.

P.charybdis eggs and larvae as a biological control agent. This attempt was unsuccessful, and *C.mellyi* appears not to have established in the North Island.

Birds that may have eaten foliage-dwelling or flying *P.charybdis* included tuis (*Prosthemadera novaeseelandiae*), fantails (*Rhipidura fuliginosa*), silvereyes (*Zosterops lateralis*) and shining cuckoos (*Chalcites lucidus*). Possible predators on the ground-dwelling stages of *P.charybdis* were blackbirds (*Turdus merula*), mynas (*Acridotheres tristis*), californian quail (*Lophortyx californica*), pheasants (*Phasianus colchicus*), rats (*Rattus norvegicus* and possibly *Rattus rattus*), mice (*Mus musculus*) and hedgehogs (*Erinaceus europaeus*).

Other areas

During the course of the study many trees in different areas were examined, primarily in the southern North Island. Near Palmerston North, several trees or groups of trees were used regularly to obtain foliage for maintaining lab. populations of *P.charybdis*; as a source of *P.charybdis* adults, eggs and larvae; for observational and experimental studies; and to test sampling methods.

The most important of these trees were a row of 20-metre high *E.viminalis* adjoining Best's Estate on the Massey University campus. Each year these trees supported high densities of *P.charybdis*, but only a few low branches and some epicormic growth was readily accessible. This precluded their use for detailed population studies. These trees provided the young adult foliage for the experiments on development rate, fecundity and activity as affected by foliage volatiles. For each of these experiments, foliage was taken from low branches of normal form rather than from epicormic growth.

Sampling methods were also tested on 15 two-metre high *E.nichollii* near Palmerston North Teachers' College; on a four-metre high *E.nichollii* in a Palmerston North garden; and on a six-metre high *E.leucoxylon* along Tiritea road between Massey University and the Northern

Tararua range. *P.charybdis* were also collected from these trees to initiate or supplement lab. cultures. Adults, eggs and larvae were also collected from *E.viminalis*, including the trees mentioned above, and *E.globulus* trees near Palmerston North.

Chapter 3. Development of the Sampling Programme

3. DEVELOPMENT OF THE SAMPLING PROGRAMME

3.1 Introduction

The three major questions I wished to answer from field studies were:

1.) How many generations does *P.charybdis* have each year, and by how much do they overlap (if at all)?
2.) What pattern of growth is shown by the host tree during the summer?
3.) What pattern of defoliation occurs during the summer?

The answers to these questions, in conjunction with laboratory studies, would clarify the interrelationships between *P.charybdis* and *Eucalyptus* and would also provide new knowledge of the individual biologies of both insect and plant. To answer these questions some method of sampling infested trees was necessary.

The second question posed the most problems. I chose to record "growth" as the change in the amount of leaf material on the tree, because this is what is directly affected by *P.charybdis* and it apparently affects *P.charybdis* populations more directly than any other growth characteristic except changes in nutritional quality.

The other obvious alternative, *viz.* girth increment, has several disadvantages. Significant changes in girth increment can only be detected over relatively long periods and I needed a parameter that would show significant changes over a fortnightly interval. Also, girth measurements that have been used to estimate crown biomass have been based on costly regression techniques on single sampling occasions, not fortnightly periods. For example, Attiwill (1966) felled 75 mature *E.obliqua* and Allen (1976) felled 55 *Acer saccharum* for regression studies.

The abundance of *P.charybdis* and the extent of defoliation can be estimated simultaneously from a sample of leaves or shoots. Both variables can also be estimated independently. During this study I estimated *P.charybdis* abundance by counting eggs and larvae on tagged shoots. I estimated defoliation by visually rating the amount of defoliation on each of these same shoots. I obtained independent estimates of *P.charybdis* population density by trapping fourth instar larvae when they dropped from the tree and adults when they emerged from the soil. The methods are detailed in the following section.

3.2 Sampling *E.nitens* at Kahu Farms

The sampled population comprised a large collection of shoots arranged as a smaller number of clusters (trees). Each shoot had certain properties such as a number of leaves, a number of *P.charybdis* of various instars, and an amount of defoliation. I assumed that while these properties changed, the total population size remained constant i.e. there was no change in the number of shoots present. This assumption is valid if, during the sampling period, the number of shoots added to the population by growth was not significantly greater nor less than the number lost through crown development processes (Jacobs 1955) or through wind or other damage. Over several years this assumption is incorrect, because the eucalypt crown changes considerably from juvenile through sapling to mature forms (Jacobs 1955), but during one summer the assumption of no significant change is reasonable. This is supported by the observation that while several sample shoots were suppressed or showed signs of possible suppression, other shoots grew considerably and by season's end comprised the equivalent of several shoots.

The sampling method used to estimate the variables has been described as two-level nested sampling (Southwood 1978), cluster sampling with sub-sampling (Cochran 1977), or two-stage sampling (e.g. Tanner 1978). I will use the last term, with Cochran's (1977) terminology.

From within the main plot of *E.nitens*, 10 trees were chosen as primary sampling units, and a total of 70 shoots was tagged, using numbered nurseryman's labels. A shoot was defined as the collection of leaves on the end of a branch, such that the number of old leaves was less than 50 and the inclusion of the next twig or branch down towards the trunk would have raised the number of old leaves to more than 50. The median number of old leaves per shoot on the first sampling date was 31, and the quartiles were 37 and 26. These shoots were the second stage units. Selection of both trees and shoots was carried out in August, before *P.charybdis* adults emerged, and before the spring flush of foliage. I chose to sample the same shoots on each sampling date for the following reasons:

1. For repeated samples in which the main aim is to estimate changes, it is best to retain the same sample throughout all occasions. This can be seen by comparing variances of estimated change between two sampling occasions as follows:

For estimates drawn from the same unit on two successive occasions;

$$\text{Variance of estimated change} = S^2 + S^2 - 2rS^2_1S^2_2$$

For different units on each occasion;

$$\text{Variance of estimated change} = S^2_1 + S^2_2$$

where S^2_i = variance of estimate on occasion i, and r = correlation coefficient between estimates on first and second occasions (Cochran 1977).

2. Selecting new samples each time would have introduced the possibility of bias related to shoot growth, defoliation and/or *P.charybdis* numbers. This bias was eliminated by selecting shoots before these factors were apparent, assuming that the only possible biases at the time

of selection were for variables which do not significantly correlate with those being investigated. Allen (1976) used this logic when selecting a sample of sugar maple (*Acer saccharum*) trees for developing regressions of number of leaf clusters on such related variables as branch diameter.

3. As new foliage ages and toughens, it becomes progressively harder to distinguish the original shoot. Experienced foresters can age leaves quite accurately, but a burst of growth usually brings an increase in leaf fall (Jacobs 1955) which further obscures the extent of the original shoot. Journet (1981) managed to group *E.blakelyi* leaves into three age classes by counting leaf scars, but I was unable to do this with *E.nitens*. If I could not identify the original shoots I would have lost information about shoot growth and absolute numbers of *P.charybdis*.

A corollary of repeated sampling of the same shoots was that destructive sampling was precluded. Consequently, shoots very high in the crown were inaccesssible because pole-pruning was the only practical way of obtaining samples. Shooting (e.g. Mazanec 1978) was excluded because it was a destructive sampling method and because of the plantation's proximity to a highway, farmhouses and livestock. I tried using a ladder, but the tips of the shoots remained inaccessible, and the time taken to move between trees made sampling inefficient. Destructive sampling was also rejected because of the possible deleterious effects of branch removal on the trees, which were part of a commercial plantation. In practice, I found that I could sample all but the top quarter of the trees by bending and tying down branches during sampling, or by standing on the Landrover. The former method had no apparent deleterious effects on the branch, and did not dislodge *P.charybdis* larvae.

Trees were chosen to include areas that covered the overall range of aspect, water and air drainage, and elevation, but otherwise they were chosen without conscious bias. On each tree the only deliberate bias was to exclude shoots that were obviously being suppressed, and

where possible to include sample shoots from each of the four aspects. The number of sample shoots per tree varied, and was approximately proportional to the number of shoots on the tree.

To check whether shoots were selected randomly, the numbers of leaves per shoot on the first sampling date (27 September 1979) were arranged in their order of selection (from one to 70), and a runs test above and below the median was performed (Sokal and Rohlf 1981). The result (z = -0.2408; P = 0.8104 n.s.) indicated that the total number of leaves on any shoot was independent of the order in which the shoot was selected.

The selection of both primary and second-stage units can be regarded as a form of stratified sampling, with allocation of shoots within secondary strata being approximately proportional to size of strata. Primary strata were based on physical characteristics of the site, and secondary strata were defined as aspects. This stratification was used to decrease the chance of a random sample of trees or shoots falling into one non-representative area. For computation purposes, the formulae for stratified sampling could not be used because the total number of units per stratum was unknown. The procedure therefore gave a probable increase in accuracy but not in precision.

On each sampling occasion, I recorded the following data for each shoot:

1. Number of old leaves (previous season's growth)
2. Amount of new growth. This was expressed as an estimate of the area of new leaf present, in units which were the equivalent of a typical old leaf
3. From 31 December 1979 the distinction between previous years' growth and hardening current season's growth was not clear, so only the total number of

leaves (including the estimate of new growth as "old-leaf equivalents") was recorded

4. Defoliation was rated on a scale of zero (no defoliation) to five (80 - 100% defoliation)
5. The number of eggs, egg masses, first, second, third and fourth instar larvae was recorded separately for both old and new growth until 31 December 1979 and as a shoot total thereafter, for the reasons given in (3)

Samples were taken at fortnightly intervals between 27 September 1979 and 10 April 1980. No samples were taken on 22 November 1979 and 27 February 1980, because of transportation failure.

To estimate the number of *P.charybdis* larvae leaving the tree to pupate, I erected a cone-shaped trap beneath the canopy of each of 12 trees. These traps opened upwards so that falling larvae were funnelled into a plastic collecting bottle attached to the bottom of the trap. The collecting bottles were partly filled with water to drown the larvae, while a row of holes punched two thirds of the way up the bottle ensured that the bottle did not overflow during rain. A diagram of the trap is given in Fig. 3.1 Each trap had a collecting area of 0.2 square metres.

To estimate the number of adults emerging from the litter and soil, traps of the same design as above were used, but the trap was inverted and pegged down and the collecting bottle was fitted with a plastic entrance funnel which allowed adults to enter but prevented exit. The plastic bottles admitted light, which encouraged emerging adults to enter the bottle, and the junction between the soil and the trap was sealed with soil to prevent adults from leaving sideways rather than climbing the walls to the bottle. 12 of these traps were erected, one near each of the fourth instar traps. I checked the traps at fortnightly intervals, and moved the

emergence traps to a fresh area of ground. N.Z. Forest Products personnel checked the traps between my visits to Kahu Farms, so weekly records were taken, until 24 April 1980.

The following season (1980-81) the development of the *P.charybdis* population at Kahu Farms was recorded on seven occasions from 23 September 1980 to 26 March 1981. Time available for sampling was very restricted and only the numbers of eggs and each instar were recorded. Where possible, shoots from the previous year's sampling were used. Any shoots showing obvious signs of suppression were replaced with shoots defined in the same way as for the previous year. The number of shoots inspected varied among sampling occasions, with fewer shoots being examined when *P.charybdis* was abundant.

3.3 Conclusion

The main disadvantage of the sampling scheme I finally decided to use was that most of the foliage sampled was in the lower half to two thirds of the tree. However, this was the optimum sampling scheme as no other method or combination of methods could provide as much information for a comparable cost or effort. More and/or better information could not have been gathered with the limited time available and lack of assistance.

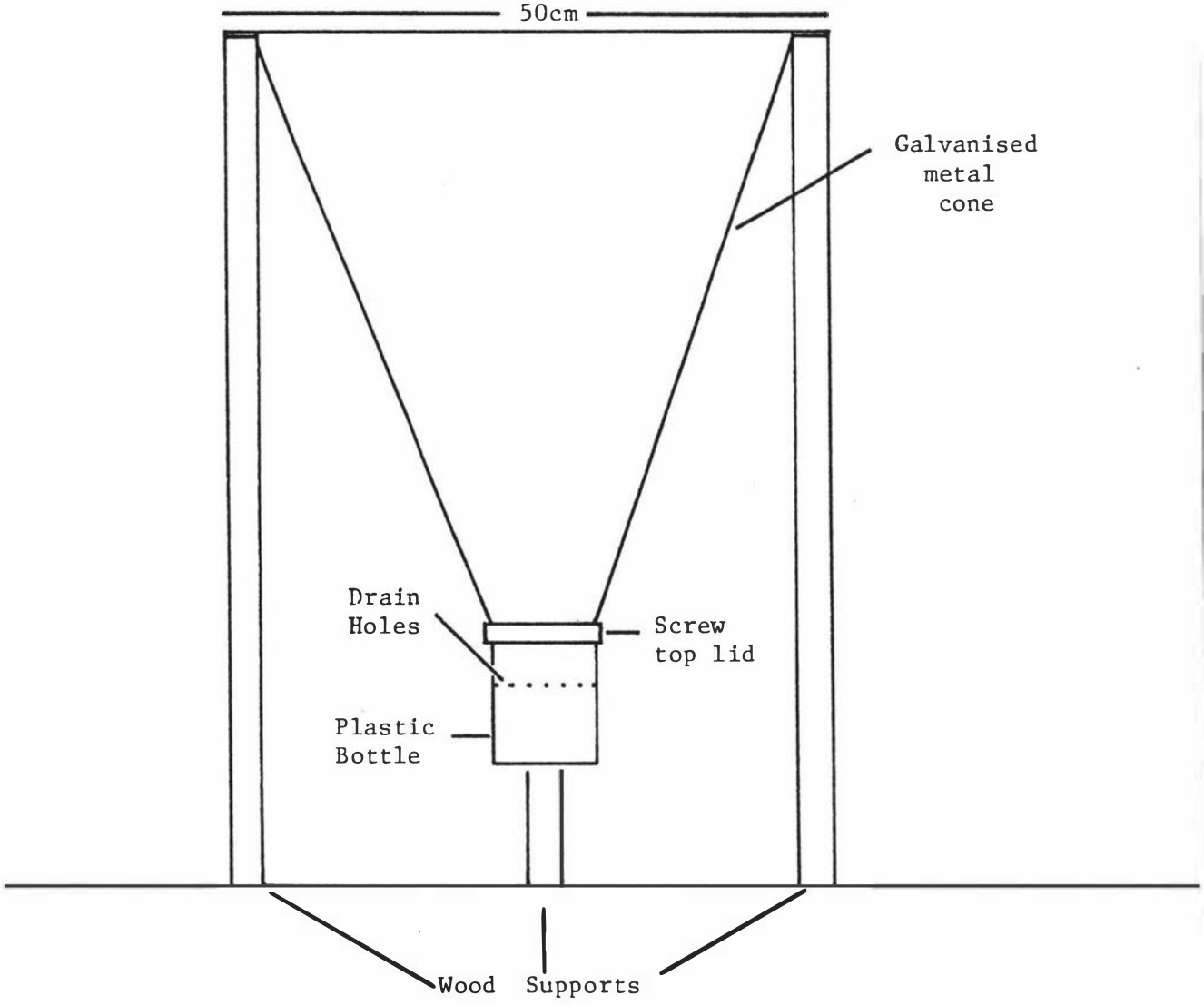


Fig. 3.1 Diagram of the trap used to collect 4th instars falling from the tree.

Chapter 4. Biology of *P.charybdis*

4. BIOLOGY OF P.CHARYBDIS

4.1 Introduction

4.1.1 Life History and General Biology

The life history of *P.charybdis* was first described by Miller (1925). Clark (1930) produced a detailed description of the life history and suggested that *P.charybdis* may complete two or more generations per year. A series of unpublished F.R.I. reports by Dugdale (1965a, 1965b, 1966) showed that while the basic life history of egg, four larval instars including a prepupa, pupa and adult was by then well known, it was still uncertain whether *P.charybdis* completed two generations each summer. Carne (1967) was confident that *P.charybdis* had two generations per year, and Styles (1969, 1970) reported that two complete generations are produced annually, but Miller's (1971) statement that "there are apparently at least two generations a year" showed that the issue was still contentious. Many authors have given brief accounts of the life history of *P.charybdis*, but most contained only reiterations of previously-published knowledge, although new information on other aspects of *P.charybdis*' biology was sometimes included (Atkinson et al. 1956, Bain 1977, Clark 1938, Emberson 1976, Manson 1960, Miller 1971, Styles 1966, 1969, 1970). Steven's (1973) unpublished thesis includes a comprehensive review of literature on the life history and added information on development rates for larvae fed different foliages and for eggs. Much of the uncertainty that existed about the number of generations stemmed from a discrepancy between field observations of abundance and laboratory records of fecundity. Field observations suggested two fairly distinct generations in most areas, with Dugdale (1966) dubiously claiming three, but Dugdale (1965a), Steven (1973) and Styles (1969, 1970) found that adult females under insectary conditions could produce egg clusters at intervals of a few days for many months. This egg-laying pattern should have produced a progressive build-up in *P.charybdis* numbers during the summer, with an increasing overlap of generations, and so it was clearly inconsistent with field observations.

A major event in the yearly life cycle of *P.charybdis* is the resumption of activity in spring by adults which have spent the winter under bark or in forest litter. This spring emergence follows soon after a similarly major event in the yearly life cycle of many eucalypts viz. the resumption of active foliage growth. Spring growth of eucalypts in New Zealand is probably regulated largely by temperature, since the yearly variation in onset of foliage growth is too large for photoperiod to be an acceptable explanation. Temperature is also strongly implicated in the spring emergence of overwintered *P.charybdis* adults, but other factors cannot be easily discounted, particularly if they are also temperature-dependent. The timing of eucalypt new growth in spring determines the earliest date at which *P.charybdis* can begin its spring life cycle, since newly-hatched larvae cannot eat old, toughened leaves. There is therefore very strong selection against adults that oviposit too early in spring so that eggs hatch before sufficient new foliage is available to sustain larvae. Conversely, adults that delay oviposition too long risk losing progeny because of new foliage shortages caused either by foliage depletion via intra- or inter-specific competition or by the common mid-summer slowing of foliage growth and leaf toughening. There is therefore an optimum period for resuming activity in spring. Experiments were designed to investigate the relationship between eucalypt flushing and the onset of the spring generation of *P.charybdis*.

4.1.2 Growth rates

Few reports on development times of *P.charybdis* exist, and these are usually based on casual field observations. Clark (1930) gave estimates of development times for all stages, stating that his data and observations were from specimens in quarantine in Nelson. He did not describe rearing methods, nor foliage type, nor the temperature range, all of which greatly affect development. Dugdale (1965a) reiterated Clark's (1930) observations, and added that the first egg batches in spring took longer to hatch than later batches. Emberson (1976) noted that, in the field, pupation takes up to two months, while Miller (1971) gave egg

hatching times identical to those of Clark (1930) and noted an egg to adult duration of six to eight weeks.

Steven (1973) gave detailed information on the duration of the pupal period and the number of days from unfed first instar until pupation. He found that larvae fed different foliage types had markedly different development times, but the type of foliage eaten by larvae did not affect the duration of the pupal stadium. Sexual differences in development times of both larvae and pupae were also apparent, but these differences were reversed between the two stages. Thus there was no difference in total development from first instar to teneral adult between the sexes.

Styles (1966) gave durations in days for total larval development, prepupae and pupae. He noted that development depended on temperature, but did not say what temperature range his estimates covered. His later papers (Styles 1969, 1970) included field estimates of incubation time (14-21 days) that were twice as long as those recorded by Clark (1930) (7-10 days), and Styles found that the approximate field duration from egg to adult was seven to nine weeks. He again noted that development time was temperature-dependent, and he also stated that the species of eucalypt eaten by larvae affected development.

Steven's (1973) work is particularly useful for comparing development rates, because he gave detailed information on temperature, humidity, lighting and foliage types. However, he used only one temperature (25.6°C) and he did not record the development rates for each instar.

Edwards and Wightman (1984) also described the growth of larvae and eggs at 20°C.

4.1.3 Population Regulation

No reliable quantitative field studies of population processes for *P.charybdis* have been published. Styles' (1970) experiment to investigate predation of the ground-dwelling stages of *P.charybdis* was unsatisfactory because his predation treatments were confounded with site differences. The sites he used for his experiment were not within a natural *P.charybdis* population and, as Styles recognised, this may have contributed to the very high survival rates he recorded.

Carne (1967) spent four months in New Zealand studying *P.charybdis*. Much of his report centred on the differences between *P.charybdis* and *P.atomaria*. His observations and experiments indicated that predation was low on *P.charybdis*, and that the major check to *P.charybdis* population growth in New Zealand occurred during the second summer generation. He speculated that this may have been due to a pathogen that he, Dugdale and Styles observed. Nothing concerning this disease has been published, the disease has apparently not been observed since Carne's visit and, apparently, no further investigations have been carried out.

Several authors have noted predation on *P.charybdis* by various insects or quantified predation, but none of these studies investigated the effects of predators on field populations of *P.charybdis* (Alma 1980, Edwards and Suckling 1980, Ramsay 1963, Steven 1973).

4.1.4 Diapause

The inactivity of *P.charybdis* adults during winter was first recognised by Miller (1925), although Clark (1930) attributed the first record of *P.charybdis* in New Zealand to E.S. Gourlay, who found adults "...hibernating beneath the bark of fallen *Eucalyptus globulus*..." Clark (1930) noted that hibernation depended on local weather conditions, and he commented that it "...takes place generally in May-June..." which may mean either that this was when the

dormancy began, or that adults were dormant during this period. Dugdale (1965a) reported that in exceptionally warm areas such as Port Motueka or parts of North Auckland, adults fed during winter, and larvae were seen very early in spring. Steven (1973) found that some adult *P.charybdis* apparently diapaused under his laboratory conditions, and he attributed this to a brief period of chilling in a freezer to anaesthetise them for examination.

The adult dormancy during winter may have been a quiescence rather than a true diapause, particularly in view of the records of adult activity during winter. If the dormancy was a diapause, then it was facultative, since Steven (1973) and P.B. Edwards (*pers. comm.*) could rear *P.charybdis* continuously if fresh foliage was available, and W.Faulds (*pers. comm.*) found all stages of *P.charybdis* on *E.viminalis* at various times during winter at Maketu in the Bay of Plenty.

4.1.5 Aims

The overall aims of this part of my research were:

1. To obtain a detailed picture of *P.charybdis*' life history and clarify the annual number of generations;

2. To investigate the relationship between eucalypt flushing and the onset of the spring generation of *P.charybdis*;

3. To identify key times of mortality in the life history of *P.charybdis* during the summer generations;

4. To identify any density-dependence in mortality during the summer generations;

5. To investigate the relationship between temperature and the rates of growth and development of eggs, larvae and pupae;

6. To determine whether dormancy is merely a quiescence or a true diapause, and if the latter, to determine when it was induced in the Kāhu Farms population.

4.2 Methods

4.2.1 Life History and General Biology

The sampling programme is discussed in chapter three, because the methods are also those used for estimating the growth and defoliation of *E.nitens* at Kāhu Farms in 1979-80. Life history records thus obtained comprised counts of:

1. Eggs, egg masses and each of the four larval instars on 70 individual shoots from 10 trees;

2. Fourth instar larvae collected in 12 fall traps;

3. Emerging adults caught in 12 emergence traps.

Twice during the 1978-79 season and seven times during 1980-81 at Kāhu Farms the numbers of eggs, egg masses and each instar were recorded on a varying number of shoots. These data were used to estimate the age structure of the *P.charybdis* population on each occasion. Site one was sampled 20 times between 20 october 1978 and 13 March 1979, while site two was sampled 23 times between 9 October 1978 and 13 March 1979. These samplings were used primarily to test and become familiar with the shoot sampling method, because many of the shoots initially selected were suppressed during the sampling period as a

consequence of the trees' natural development. Bias was therefore introduced because few healthy shoots were left in the second half of the summer. This was not a significant problem at Kāhu Farms the following summer. Data from sites one and two were combined because both sites showed similar patterns of development of the *P.charybdis* population and the larger sample size reduced the binomial confidence limits.

4.2.2 Effect of foliage volatiles on spring activity of adult *P.charybdis*

9 adult *P.charybdis* (five males and four females), collected when adults first appeared on foliage in spring, were allocated strictly randomly to each of 18 airtight containers. Each container was enclosed in a tightly-closed clear plastic bag. The cages were placed in a controlled-environment room at 10C +/- 0.5C, 79%RH +/-10%RH and constant light. The cages were numbered sequentially then randomly assigned a treatment number to divide the experiment into three treatments replicated six times. After 24h to allow the animals to adjust to their environment, the following treatments were applied to their respective containers:

1. The lid of the the container was carefully opened. A black plastic 35mm film canister containing 25g of freshly-chopped *E.viminalis* newly-growing adult leaves, closed at the top with fine nylon gauze to prevent adults contacting the leaves or any non-volatile exudate, was gently placed in the cage. The lid was replaced carefully and the plastic bag sealed tight with a wire twist.

2. As for (1), but with newly-growing leaves replaced with freshly-chopped adult leaves taken from a position on the shoot at least 5 leaves back from the start of the current season's growth.

3. As for (1) and (2), but with a wad of cotton wool dampened with distilled water replacing the leaves.

All leaves were collected at the same time and prepared within one hour of collection. The treatments were applied in the order 3, 2, 1, to minimise the risk of cross-contamination with air-borne volatiles. The cage numbers were then hidden and the cages rearranged randomly by an assistant. At the end of the experiment cage numbers were revealed and treatments noted. Handling of the cages was done as little and as gently as possible, to minimise disturbance of the adults.

Each cage was checked at 20-minute intervals for the first two hours after treatment and thereafter at hours 4, 8, 20 and 24 after treatment. Each adult was assigned a score on the following scale:

0 = completely motionless

1 = antennae distinctly moving but adult stationary

2 = walking or flying

Thus the possible score for a cage ranged from 0 (all completely motionless) to 18 (all adults walking or flying).

The number of insects touching the gauze cover of the pottle was also recorded, giving a range of scores from 0 to 6 for each cage.

4.2.3 Effect of foliage age on mating and fecundity

Adult *P.charybdis* were collected from beneath bark and on foliage early in spring before the peak of oviposition and starved for at least two days at 10°C. Five females and three males were allocated randomly to each of 10 cages, which were then transferred to a controlled-environment room at 24+/- 0.5°C, 70+/- 10% RH and 16 hours light. The cages were arranged sequentially and random numbers were used to allocate one of the treatments to each of five cages. The alternative treatment was assigned to the remaining cages. Each day actively-growing shoots were collected from known *E.viminalis* hosts of *P.charybdis* and immediately taken to the lab. for processing. There, each shoot was severed at the junction of old and new growth. A section of old growth plus the new growth section of a shoot was added to each cage in one treatment ("Young + mature") and a similar amount of foliage, but old growth only, was added to each cage in the alternate treatment ("mature only"). The previous day's foliage was removed before adding fresh food and any eggs laid on the container were recorded and removed. After changing the foliage, the number of egg masses and number of eggs per mass were recorded for each cage. Each cage was scrutinised just before the foliage was changed; and the number of adults copulating or actively moving about the foliage or cage was recorded.

4.2.4 Growth rates

4.2.4.1 Morphometrics

Larvae used for measurements of body length, head capsule width and weight were collected from *E.globulus*, *E.leucoxylon*, *E.nitens*, *E.viminalis*, and lab. populations that had been reared from eggs and fed *E.viminalis*. Length measurements were recorded at various magnifications using a Wild M8APO stereo microscope with a graduated eyepiece graticule that was calibrated at each magnification with a stage micrometer. Eyepiece units were transferred to mm for analysis. Weighing was carried out on a Mettler digital balance accurate to 0.1mg.

4.2.4.2 Egg development

Egg development rates were recorded at five different temperatures, using eggs obtained from the leaf age/adult fecundity experiment. The range of temperatures was provided by four controlled-environment rooms at 12, 18, 24 and 30°C and a "Contherm" cabinet at 6°C. None of these temperatures fluctuated more than +/- 1°C and generally stayed within +/- 0.5°C. Relative humidity was maintained within the range 60-80%RH, although this may have been higher and more constant within the containers used to hold the eggs.

Sections of leaf supporting egg masses were excised, leaving about one cm margin around each egg mass. Eggs laid on container walls were not used for this experiment. On each day all egg masses from each of the 10 cages in the leaf age/fecundity experiment were placed in a plastic petri dish. Consecutive random sequences of the digits from one to five were used to assign egg masses to five dishes. Each random digit was uncovered after the leaf section had been picked up with forceps, so that selection of any egg mass occurred before I knew which container it would be assigned to. The egg masses for each temperature were placed all together in a plastic petri dish on two layers of Toyo No. 2 filter paper that had been moistened with distilled water. After the date was written on the outside lid of each of the five dishes, random numbers were used to allocate the dishes to temperatures.

Twice every day the development of each egg mass was recorded. The following categories were recognised:

G = eggs still green-grey

Y = eggs yellow

EBV = egg bursters visible

ES = eclosion starting; defined as the appearance of at least one cut in the chorion of any egg in the mass.

4.2.4.3 Larval development

The relationship between larval development and temperature was determined in two stages. An initial experiment gave approximate estimates of the thermal threshold and the thermal sum required for complete development. A second experiment used this initial information to determine more accurately the parameters of the temperature / development rate relationship. This second experiment was designed primarily to obviate the confounding effects of changes in food quality for larvae grown for long periods at low temperature.

In the preliminary experiment, durations of each larval instar from newly-hatched larva to prepupa were determined at four temperatures. Three controlled-temperature cabinets at the Ministry of Agriculture and Fisheries' (M.A.F.) laboratory at Batchelar Farm near Palmerston North were used for temperatures at 8, 15, and 20°C. All fluctuated up to 1°C either side of the indicated temperature. A walk-in indoor insectary at Massey University's Department of Botany and Zoology was used to provide a temperature of 25°C with a maximum fluctuation of 2°C either side. This insectary and the M.A.F. cabinets fluctuated much less than the indicated range if they were not disturbed unnecessarily. The photoperiod in all cases was 18 hours light, six hours dark. A fifth temperature of about 30°C was tried, using a small particle board cabinet fitted with a double window, fan and thermostatically controlled heating unit. This cabinet could not be used at lower temperatures because it had no cooling system.

I collected egg clusters from *E.nitens* at Kāhu Farms in early February and kept them at 25°C until they hatched. Larvae were distributed randomly among each of the four temperatures by assigning them to holding dishes according to 25 consecutive random sequences of the digits from one to four. The 25 larvae at each temperature were then divided randomly into five replicates using five consecutive random sequences of the digits

from one to five. During both randomisations, larvae were picked up individually with a fine artist's paint brush before an assistant called the cage number.

Larvae were fed fresh young *E.viminalis* foliage because *E.nitens* was not available near Palmerston North. The were kept in clear plastic containers eight cm diameter x ten cm deep, closed at the top with gauze and at the bottom by a plastic lid. The lid was pierced by a small hole through which the stem of the foliage protruded into a container of water. Foliage was changed every two or three days, before it wilted or was completely eaten and all replicates at all temperatures were changed on the same day. Larvae were examined each morning and records taken of the number of larvae in each instar and the numbers ecdysing. I considered a stadium complete when half the larvae had moulted or were moulting to the next stage.

At 8°C, larvae took up to two months to develop from hatching to pupae. Artificial diets have not been developed for *P.charybdis*, so the larvae had to be fed field-collected foliage. An obvious source of inaccuracy in this initial experiment was that food quality may have changed during the course of the experiment. Thus, larvae that grew at high temperatures may have eaten food of different quality than larvae at low temperatures. The second experiment minimised this error by growing all larvae for at least part of their development at an optimum temperature.

Eggs were collected twice daily for two days from adults that were held at 24±1°C and fed *E.viminalis* young adult foliage. These egg batches were held at 24±1°C on damp filter paper in plastic petri dishes until they hatched. Larvae were allowed to feed on their egg shells and collected when they walked away. They were placed in individual 9cm-diameter plastic petri dishes on a single layer of Toyo No. 2 filter paper that was moistened with distilled water. A section of *E.viminalis* adult foliage from the tip of an actively-growing shoot was supplied for each larva. This leaf was chosen from within the shoot section

defined by the first fully-open leaf and the 4th leaf adaxial to this. Larvae were numbered consecutively by labelling the lids of the petri dishes. As larvae became available they were assigned a temperature treatment according to pre-determined random sequences of the numbers 1 - 5, corresponding to temperatures = 8, 12, 20, 24 and 28°C.

Temperature treatments 8 - 24°C were supplied by four, purpose-built, walk-in rooms at Entomology Division, DSIR, Palmerston North. Temperature fluctuations in these rooms were ±1°C. The 28°C treatment was a "Contherm" micro-processor controlled cabinet with a temperature range of ±0.1°C either side of the indicated temperature.

Larvae assigned to the 24°C treatment were held at that temperature throughout their entire development. For the 28°C treatment, larvae developed entirely at that temperature save for the initial few hours of the first instar, at 24°C. For other temperature treatments, larvae were held for varying periods at 24°C for each instar, before being transferred to the test temperature to complete the stadium. After ecdysis, larvae were returned to 24°C and the procedure was repeated for subsequent instars. During the 4th stadium, the start of the pre-pupal stage was estimated as the time when larvae stopped feeding and moved away from the leaf.

Larvae were checked two to four times daily. Each day fresh *E.viminalis* adult leaf, taken from the region of shoots described above, was supplied to each larva. The filter paper was moistened with distilled water as necessary. Every two to four days, depending on instar, larvae were transferred to a clean petri dish and filter paper.

4.2.4.4 Pupal development

4th instars were field-collected from a first-generation population on *E.viminalis* at Massey University. They were held in two cages similar to those used for the preliminary larval development experiment, except that the cages were larger (25cm high x 25cm diameter). They were fed sprigs of fresh, young, adult *E.viminalis* foliage. The floor of the cage was covered with a 1cm layer of damp Vermiculite™. Larvae were removed from the cage each day after they had left the sprig of foliage and attempted to burrow into the Vermiculite™. They were transferred to individual 5cm high x 6cm diameter clear plastic pottles 1/3 full of Vermiculite™ that had been moistened with distilled water, and a sequentially-numbered snap-fit lid was fitted. Larvae in their containers were then assigned to one of four temperature treatments, using consecutive sequences of the random digits 1-4.

Temperature treatments were provided by the same facilities described for the second experiment on larval development, and comprised 12, 18.2, 20 and 24 C. Larvae were checked 2-4 times daily throughout the experiment and the Vermiculite™ moistened lightly with distilled water if it became dry. This was only necessary occasionally, and at the two higher temperatures. Larvae that could not be seen clearly through the walls of the container were gently manoeuvred into a position where this was possible.

4.2.5 Population dynamics

4.2.5.1 Field populations

Methods for the study of field populations are described in chapter 3. Key factor analysis and methods of identifying density-dependent mortality are elaborated in the appropriate *Results and analysis* sections.

4.2.5.2 Experimental study of 1st instar mortality

Six similar, actively growing shoots of *E. viminalis* were selected on one of a row of trees growing at Massey University, Palmerston North. Adjacent shoots were tied back so that their foliage could not brush against the selected shoots. Where necessary, a stick was tied to the base of the shoot and to nearby shoots to separate these. A band of Tangle-Trap™ between 5 and 10cm long was sprayed around the stem of the shoot at least 20cm adaxially from the oldest leaf. A deterrent barrier 4cm long of non-stick aluminium cooking foil was wrapped tightly around the stem just abaxial to the Tangle-Trap™ band. Although first instars could walk across this with the aid of their abdominal adhesive organs, field and lab. observations showed that larvae generally turned back after encountering the band. Any larvae that did persist and crossed the foil were trapped by the adjacent sticky section of stem.

Shoots were selected to have similar amounts of new foliage and numbers of apices. Any *P.charybdis* stages on the shoots were removed daily until the experimental egg batches hatched.

Egg clusters were obtained from adults caged in the lab. The section of leaf supporting each batch was excised, leaving a 1cm margin of leaf around the eggs where possible. Eggs were collected over three days and held at 8°C for varying durations before being transferred to 24°C, so that all egg batches were due to hatch at about the same time. Batches were numbered consecutively and sorted randomly into six groups. These groups were intended to approximate a range of densities

$$D_i = 15(1.5^n)$$

where $n \in \{0,1,2,3,4,5\}$.

(This is a simple growth function with starting value = 15 = an approximate average egg cluster size, and growth factor of 1.5.)

Because egg clusters varied in size and were assigned to densities randomly, the actual densities only approximated this largely arbitrary distribution.

When egg bursters were visible, the number of fertile eggs in each batch was recorded. The groups (D_1 - D_6) comprised 11, 22, 49, 64, 80 and 117 eggs, respectively.

Egg batches were then taken to the field in a cool polystyrene container. Each shoot was randomly assigned one of the densities of eggs. The eggs were stapled to old leaves between the youngest old leaf of the previous season's growth and four leaves adaxial to that leaf. Single batches were attached to each old leaf in this region starting from the youngest leaf and adding one batch per leaf down the shoot and starting again at the youngest leaf when the number of batches to be allocated was greater than four.

Shoots were checked twice daily and the number and position of larvae was recorded until no 1st instars remained on the shoot. The Tangle-Trap™ band was checked at each inspection in order to record any *P.charybdis* stages. The extent of defoliation of each shoot was recorded on a 0 (no defoliation) to 5 (no young lamina remaining) scale.

4.2.6 Diapause

Every fortnight from 4 January to 13 March 1980, adult *P.charybdis* were transferred from Kāhu Farms to an insectary at Palmerston North. There they were held at 25+/-2°C and 18 hours light: 6 hours dark. The samples from each date were kept separate from other samples and all adults in a sample were kept in a single cage. Cages were inverted clear plastic containers 25cm deep x 18cm diameter with a close-fitting lid, through which the

stems of a small bunch of *E.viminalis* foliage protruded into a container of water. Foliage was changed every two or three days and accumulated frass was cleaned out. Samples were assessed daily on the basis of presence or absence of eggs, activity of adults and amount of foliage consumed. These activity estimates were later converted to scores on the following scale:

0 = quiescent
1 = sporadic activity
2 = fully active

From the end of April until early August, any new growth on palatable species of eucalypt was collected and offered to diapausing adults. Because new growth was rare during winter, this opportunity arose only sporadically and cages were chosen at random because there was never enough for all to be tested.

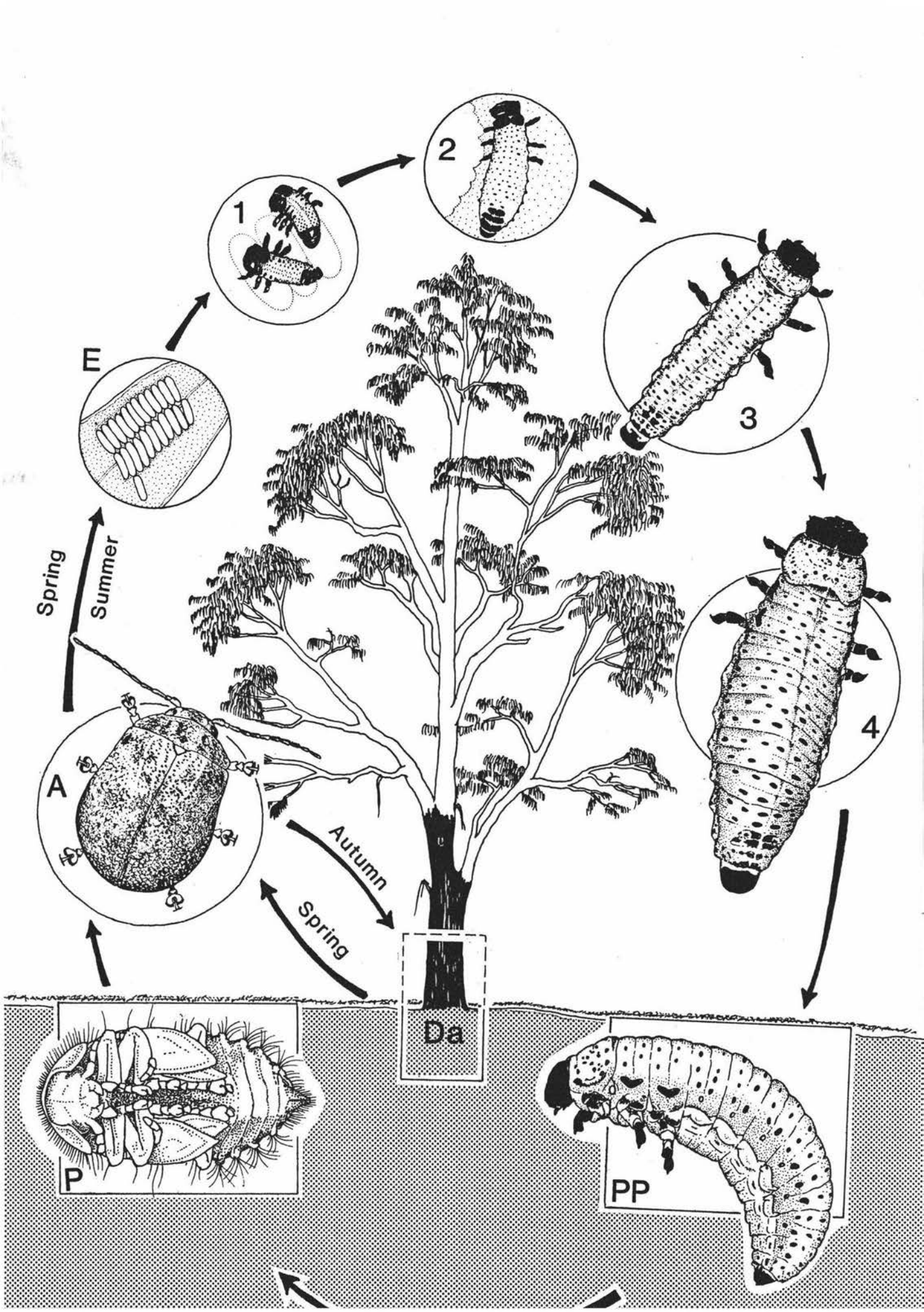
4.3 Results and analysis

4.3.1 Life History and General Biology

The life history of *P.charybdis* is summarised in Fig. 4.1.

Mating

Adult *P.charybdis* (Fig. 4.2) emerge in spring to feed, mate and lay eggs. Mating takes place on the foliage, apparently without elaborate courtship behaviour. Males can often be seen pursuing females and when this leads to copulation the male climbs onto the female's back before inserting his aedeagus between the posterior tergite and sternite of the female's abdomen. The male maintains his position by clinging to the female's elytra with his fore- and mid-tarsi. The hind legs are not used to cling to the female but either trail in mid-air or are in contact with the substrate. The first segments of the fore- and mid-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tarsi of males are equipped with "pads" consisting of very fine hairs. These pads are not present on the tarsi of females (Fig. 4.3) and they presumably allow males to grip the female's elytra. The male's tarsal claws are usually hooked under the lateral edges of the female's elytra. During copulation, the male's position effectively prevents the female from opening her elytra and therefore from flying. Females often attempt to dislodge copulating males by wiping their hind legs over the back of their elytra. This is often unsuccessful, although males sometimes respond by withdrawing the aedeagus. Peristaltic movements within the aedeagus can be seen at intervals of a few seconds and this may continue even while the female is actively walking and carrying the male. After withdrawing the aedeagus and releasing the female, males sometimes attempt to copulate immediately with the same female, even if copulation has persisted for more than an hour.

Oviposition

Eggs are usually laid in clusters on the adaxial surface of old leaves back from the growing tip. I never found eggs on young foliage of any eucalypt in the field. However in the laboratory experiment testing the effect of foliage age on fecundity, eggs were often laid on the sprig of new growth rather than the old foliage sprig. This suggests that females locate new foliage, then walk along that shoot to select adjacent leaves for ovipostion, rather than moving to another shoot to oviposit.

On 7 October 1978 I found many *P.charybdis* eggs scattered singly along the small twigs of a two-metre high *E.viminalis* growing between rows of infested *E.nitens* at Kāhu Farms. This *E.viminalis* was severely defoliated, with only vestiges of young foliage remaining and a few older leaves which supported many egg clusters. A similar scattering of eggs was often observed in crowded laboratory cages and when females were temporarily kept in cages without foliage.

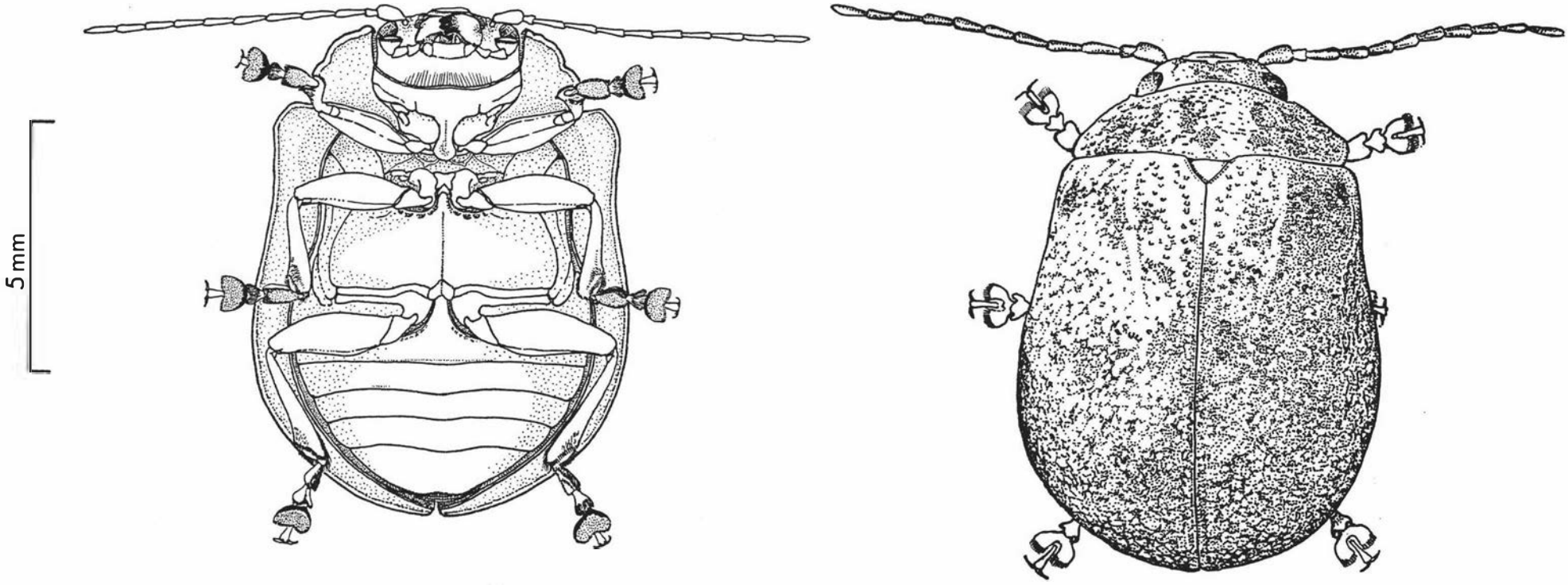


Fig. 4.2 Habitus illustrations of adult *P.charybdis*

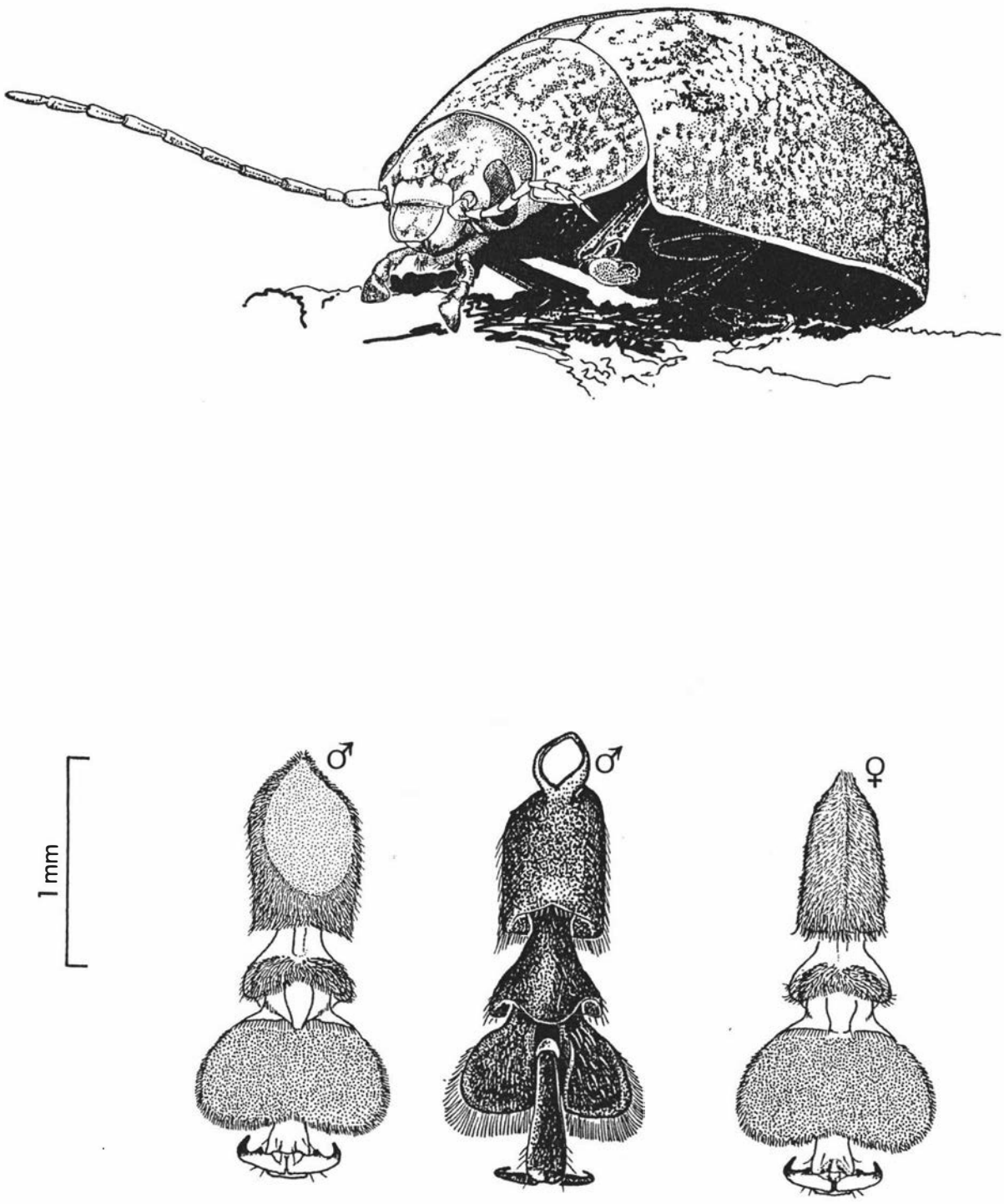


Fig. 4.3 Sexual dimorphism: mid-tarsi of male and female adult *P.charybdis*

Eggs are usually laid near the tip of a leaf or near a constriction such as that caused by feeding damage. This probably relates to the female's need for purchase while ovipositing. During egg-laying, females were often seen gripping opposite edges of the leaf, which suggests that the location of the egg mass is often determined by the female's body width.

Data from the substantive shoot sampling programme at Kāhu Farms in 1979-80 are presented in Appendix 1. Figure 4.4 shows the frequency distribution for the number of eggs per cluster and Table 4.1 lists descriptive statistics for egg clusters at Kāhu Farms on each sampling date when eggs were present. Fig. 4.4 shows slight negative skewness, due in part to the difference between the two generations, as discussed later. In general, values of the mean and median were similar, the median over all sampling occasions ranging from 7.5 to 20, while the mean varied from 7.25 to 20.1. However, upper limits of the mean and median occurred on different dates. Egg cluster sizes recorded during the leaf age/fecundity experiment showed a similar pattern (Fig. 4.5).

TABLE 4.1 Egg cluster statistics for sampling dates when eggs were present; 1979-80, Kāhu Farms

Date	Median	Range	Mean	s.e.	No. clusters
27 Sep 79	14	6-28	15.6	3.75	5
11 Oct 79	20	4-33	18.3	1.65	26
25 Oct 79	16	1-30	14.3	2.16	17
8 Nov 79	14	4-30	14.4	2.93	8
6 Dec 79	19	10-40	20.1	2.36	13
20 Dec 79	12	8-15	11.7	2.03	3
17 Jan 80	7.5	1-13	7.25	3.33	4
31 Jan 80	12	1-26	12.8	1.92	13
13 Feb 80	15	9-20	14.7	1.17	10

Fig. 4.4 Frequency distribution of egg cluster sizes at Kāhu Farms, 1979-80

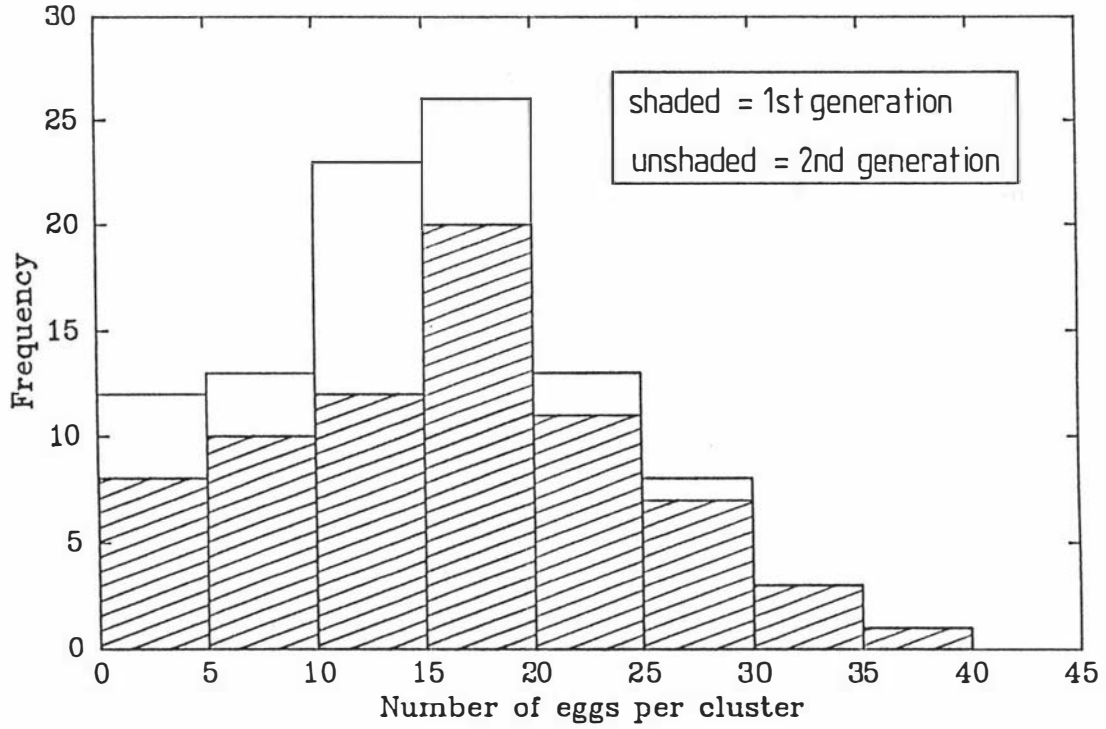
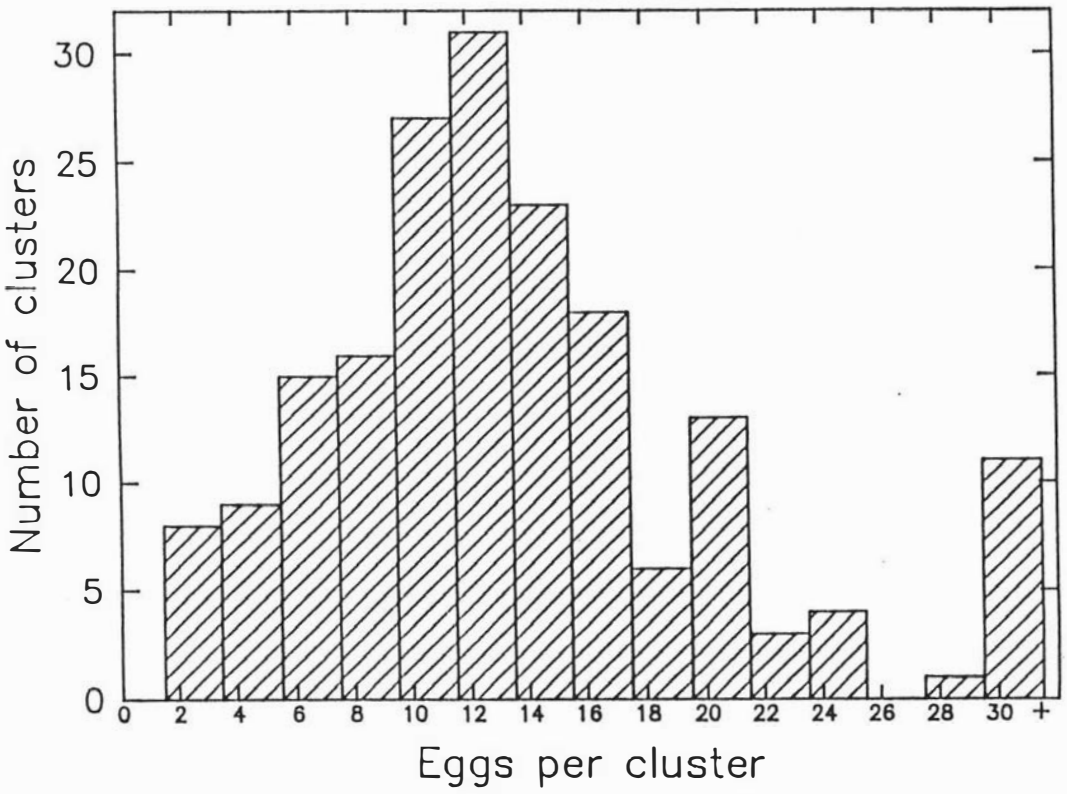


Fig. 4.5 Frequency distribution of egg cluster sizes, recorded during the leaf age/fecundity



P.charybdis clearly had two generations per summer on *E.nitens* at Kāhu Farms. The median cluster sizes were 16.5 eggs for the first generation (sampling dates 27 September 1979 - 20 December 1979) and 13.0 for the second generation (sampling dates 17 January 1980 - 13 February 1980). A Mann-Whitney U-test showed that these were significantly different (U = 3866*; P = 0.037). This trend seemed apparent the following season at Kāhu Farms (Table 4.2), with first and second generation medians of 17 and 10.5 respectively, but the difference was not significant (U = 250.5ns; P = 0.222).

TABLE 4.2 Egg cluster statistics for 1980-81 season; Kāhu Farms

Date	Median	Range	Mean	s.e.	No. clusters
13 Nov 80	17	3-28	15.4	2.73	9
28 Jan 81	11	6-21	11.8	1.33	11
11 Feb 80	10	2-33	11.7	1.66	25

Eclosion

At eclosion, the egg bursters move longitudinally to make two parallel cuts in the chorion. The larva then rotates within the egg as it makes two further parallel cuts which meet the first slits at about 30 degrees. Consequently several V-shaped flaps are formed in the chorion. The larva emerges head first through these, assisted by a series of peristaltic waves and rocking movements. When enough of the body is free, the larva uses its legs to grip the egg shell and surrounding eggs and pulls itself free. Pigmentation at eclosion is restricted to the egg bursters, tarsal claws, the six ocelli, bases of setae, spiracles and some mouthparts. The rest of the body is pale but tanning occurs rapidly and is complete within 45 minutes. As tanning nears completion the larva eats the remains of its egg and soon after crawls away from the egg mass (Fig. 4.6).

Fig. 4.6 A partially-hatched egg mass of *P.charybdis*. Egg bursters are clearly visible as paired dark objects within the eggs and on the meso- and meta-thorax and first abdominal tergite of the emerging, untanned larva in the centre.

Larval aggregation

Larvae are not usually gregarious, and distinct groups usually comprised very young larvae, often in association with the remains of a egg cluster. This suggests that such groups are a consequence of eggs being laid in clusters rather than aggregation behaviour. On a whole-tree scale, larvae are aggregated because of their preference for young foliage. Field observations illustrate this clearly: stationary larvae are seldom found other than on young foliage unless this new growth has been severely defoliated. In a 1m x 0.75m x 0.5 cage containing 14 *E.viminalis* shoots with a total of 139 leaves, 116 larvae of various stages were found predominantly on the outer tips of the shoots. In terms of numbers of larvae per leaf, these larvae were highly aggregated, with a variance: mean ratio of 2.57.

Food location by newly emerged larvae

To investigate larval food location behaviour, field observations were supplemented with simple laboratory experiments. 30 first instar larvae were collected as they moved away from their egg masses. Each larva was placed alone in a closed 8cm diameter plastic petri dish, on 2 layers of Toyo No. 2 filter paper moistened with distilled water. The larva was placed equidistant from two freshly-cut 1.7cm diameter discs of *E.viminalis* foliage. One disc was cut from young, adult foliage, the other from old, adult foliage. The larva's movements were traced on the lid of the dish with a felt-tip pen.

Five of the 30 larvae moved rapidly towards the discs and four of these located the young leaf disc without first touching the old disc. The other larva grasped the old disc briefly but did not attempt to bite it, then turned away and moved directly towards the young disc. Within 30 minutes, three more larvae located the young leaf disc apparently haphazardly. All eight larvae fed briefly and seven remained on the disc while one moved away. After 24

Fig. 4.7 *P.charybdis* feeding damage on *Eucalyptus viminalis*. The scalloping on the lower leaves is typical of adults, while the less regular damage on the two terminal leaves is characteristic of larger larvae. (Beetles not to same scale)

hours, feeding was apparent on 12 young leaf discs, but only three larvae were still in contact with the young leaf disc. No old leaf discs showed signs of feeding. Two larvae were on old discs and the remainder were elsewhere in their dishes, predominantly between the filter paper and the sides or bottom of the dish.

Dispersing, newly-emerged larvae placed on sprigs of foliage that had both old and new growth had little difficulty finding the new growth if the sprig did not fork. If, however, the sprig had one or more bifurcations that led to only old leaves, then larvae often became "trapped" on these side branches. Larvae walking onto these branches often investigated the entire branch and its leaves several times before either stopping or eventually wandering onto the main stem again. While walking, larvae constantly palpate the substrate with their maxillary palps. Occasionally they stop and attempt to bite leaves or petioles, but I did not see them try to bite stems other than the very thin and tender internodes between newly-flushing leaves.

Larvae in the field behaved in a similar way to those on shoots in the laboratory. Because of the proximity of egg masses to young foliage, larvae rarely encountered bifurcations that led to old growth only. I transfered first instar larvae in the field to positions on the stem where they would encounter forks that could lead to old growth only. 71% of encounters with such forks resulted in larvae choosing the alternative that led to new growth, indicating that larvae could distinguish these alternatives (binomial test, $z=-3.464$, $P < 0.001$; $n=75$). However, when larvae encountered forks where both branches were of similar diameters, the choice of branch was apparently random, with 60% taking one branch (binomial test, $z=-1.389$; $P=0.165$n.s; $n=42$). This situation was more common near the growing tips of branches, where both choices were likely to lead to new growth.

Pupation

Larvae feed on young eucalypt foliage (Fig. 4.7) throughout the four instars. During the fourth instar, larvae drop from the tree and tunnel into surface litter or the soil, where they enter a motionless prepupal stage. Although the prepupa is morphologically and behaviourally distinct from the foliage-inhabiting stages, no moult occurs between the fourth instar and prepupa. Larvae construct pupation chambers, consisting of smooth-walled earthern cells, up to 10cm below the surface when soil is loose. Some larvae pupate without constructing such a cell if the litter is deep and the soil surface hard. Prepupae and pupae were also commonly found in cells under large stones and other objects, beneath infested trees.

After emerging, adults feed and mate, so beginning another generation. At Kāhu Farms, adults that emerged at the end of this second generation did not mate nor lay eggs, but fed vigorously before diapausing. All adults dissected at this time showed gonotrophic dissociation, with extensive fat body and poorly-developed gonads. The diapause is discussed in sections 4.1.4, 4.2.6 and 4.3.6.

Seasonal abundance

Fig. 4.8 shows the observed frequency of eggs and instars one to four, plotted on a logarithmic scale to emphasise changes in relative abundance. The progressive ageing of the *P.charybdis* population is clear, although the low number of fourth instars and fortnightly sampling intervals means that the peak abundance of this stage cannot easily be distinguished from that of the combined second and third instars. The results of the fourth instar and emergence trapping are not directly comparable with the shoot-sampling results, because of the different methods used. These trapping results have been presented separately in Fig. 4.9 where instar frequency is expressed for each date as a percentage of the total recorded for

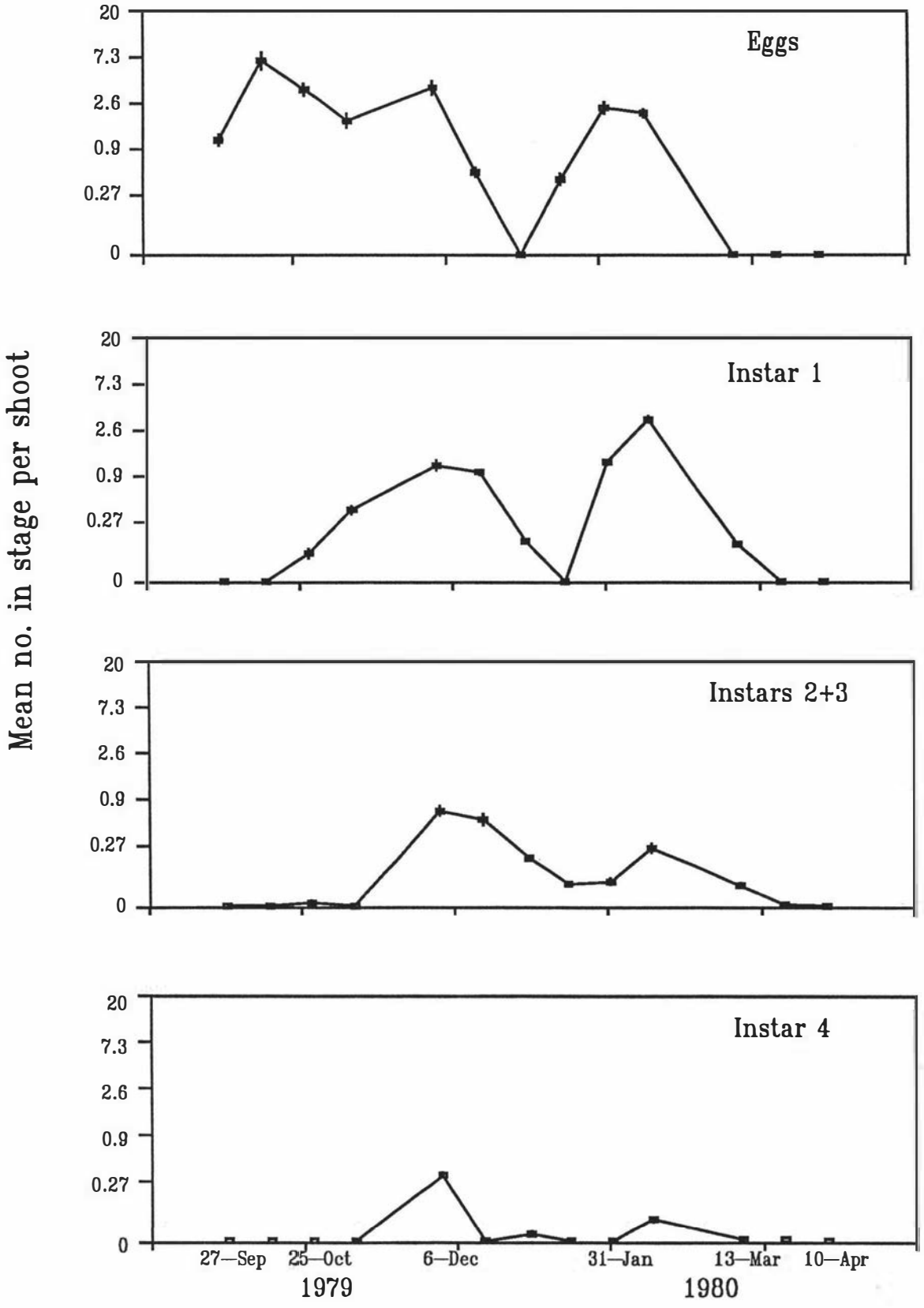


Fig. 4.8 Abundance of *P.charybdis* stages at Kāhu Farms 1979-80, shoot samples.

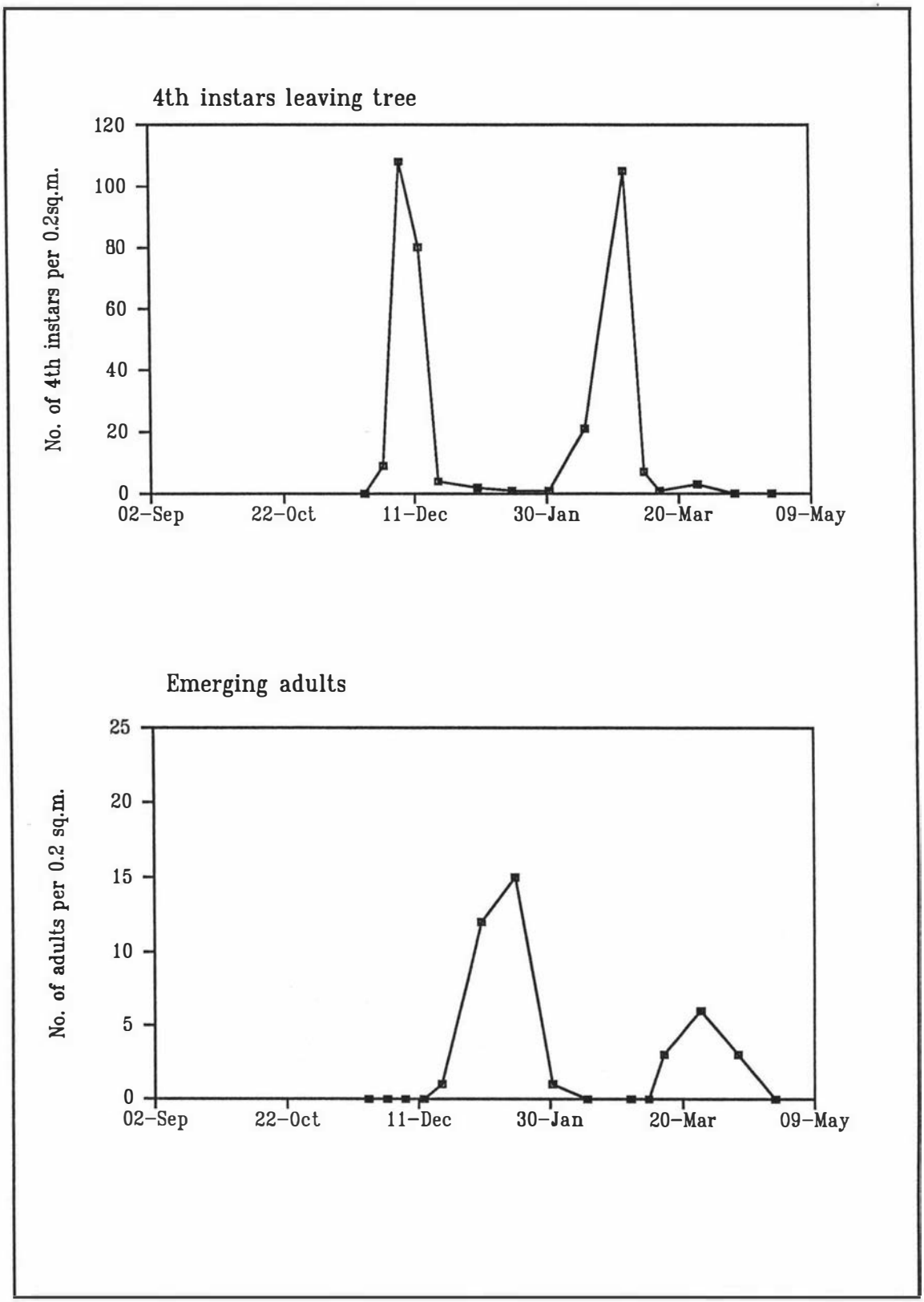


Fig. 4.9 Number of 4th instars leaving tree, and adults emerging, Kāhu Farms 1979-80.

that instar over all dates. In all cases in both Fig. 4.8 and 4.9 the separation into two generations is clearly apparent.

The smaller number of samples from Kāhu Farms in 1980-81 partly obscured the pattern of instar progression, but in this season a relatively normal progression was observed (Fig.4.10). The two samples from Kāhu Farms in 1978 showed age structures consistent with the development of at least a discrete first generation (Fig.4.11). In this season however, the population seemed more advanced than in subsequent years, with significant numbers of first instar larvae present in early October. At this time on trees at sites one and two a very similar age structure was evident. By the second week of December, however, the Palmerston North sites were free of eggs and the population was dominated by third instars (Fig. 4.12), whereas at Kāhu Farms eggs were still abundant and second instar larvae were the dominant larval stage.

Oviposition, which initiates the pattern of population abundance on eucalypt foliage, commences before defoliation becomes severe and while the tree is growing vigorously. It declines when, or soon after defoliation peaks, but new growth is still available (see Fig. 5.1, 5.4). This suggests that females were responding to defoliation rather than to availability of new leaves. To examine this more closely, I regressed the number of eggs per shoot, for shoots that had eggs, on defoliation score and on the number of new leaves. This was done for each sample date between 11 October 1979 and 6 December 1980, as the 27 September sample had only four shoots with eggs and the 20 December sample had only two shoots with eggs.

None of the regressions before 6 December were significant at $\alpha = 0.05$, but the regressions on this date were both significant at $\alpha = 0.01$ (Table 4.3)

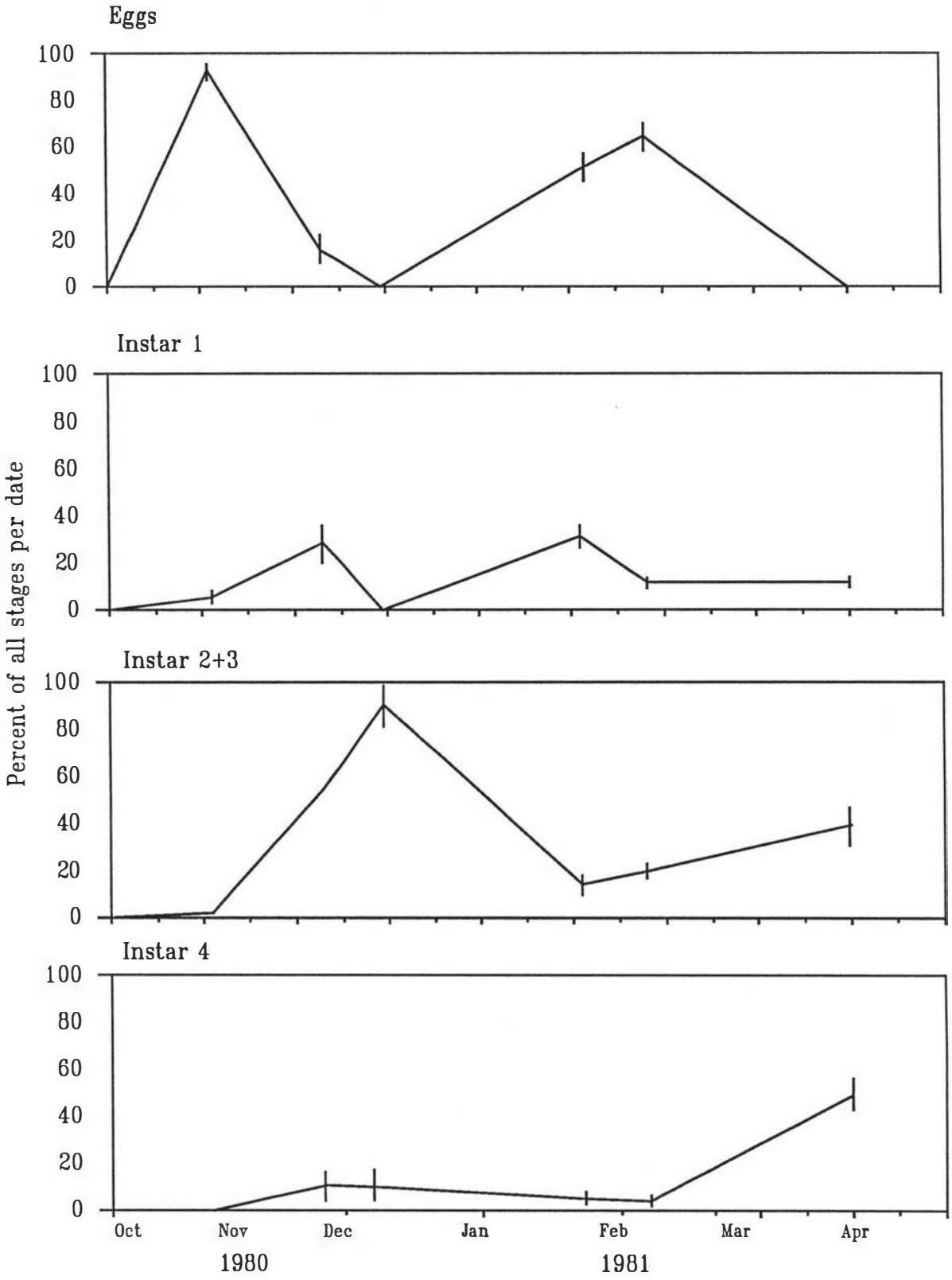


Fig. 4.10 Abundance of *P.charybdis* stages at Kāhu Farms 1980-81, shoot samples. Mean number per shoot expressed as % of all stages, for each date. Vertical bars = 95% binomial confidence limits.

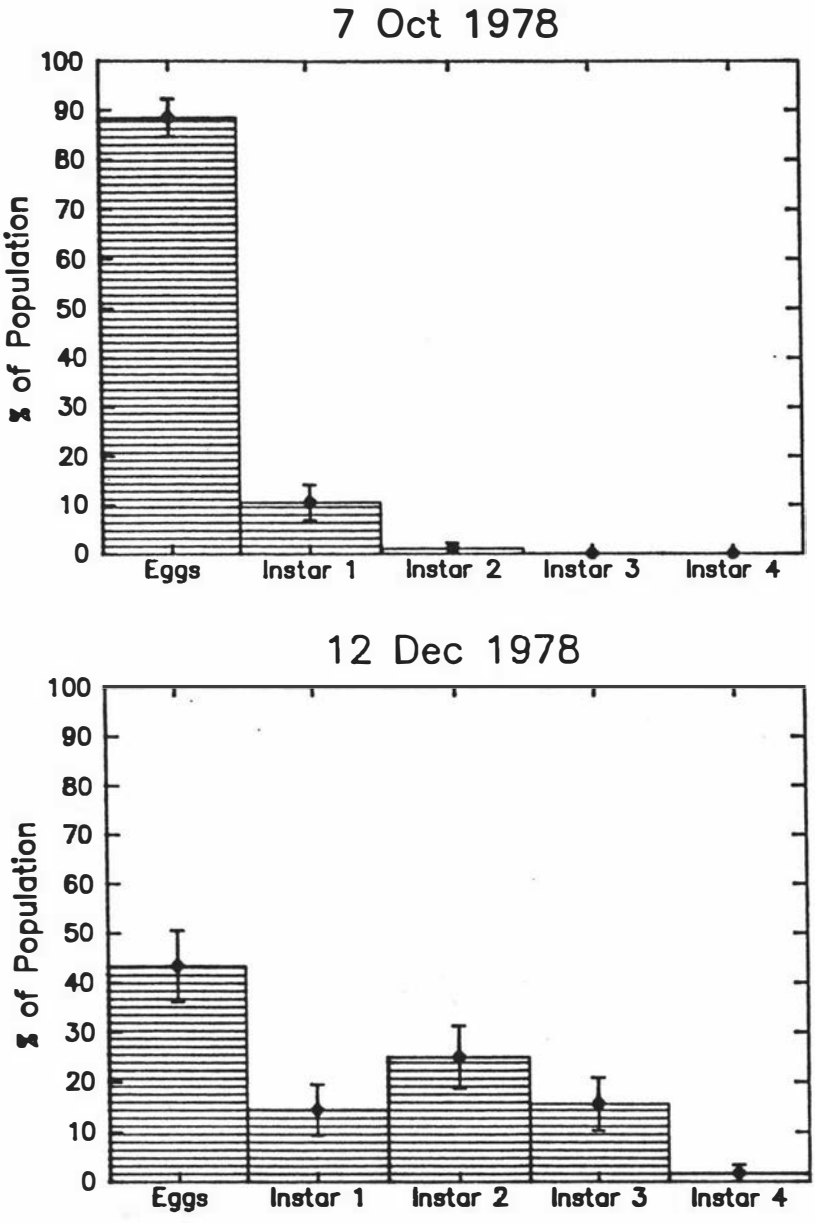


Fig. 4.11 Age structure of the *P.charybdis* population at Kāhu Farms on two dates during 1978. Vertical bars = 95% binomial confidence limits.

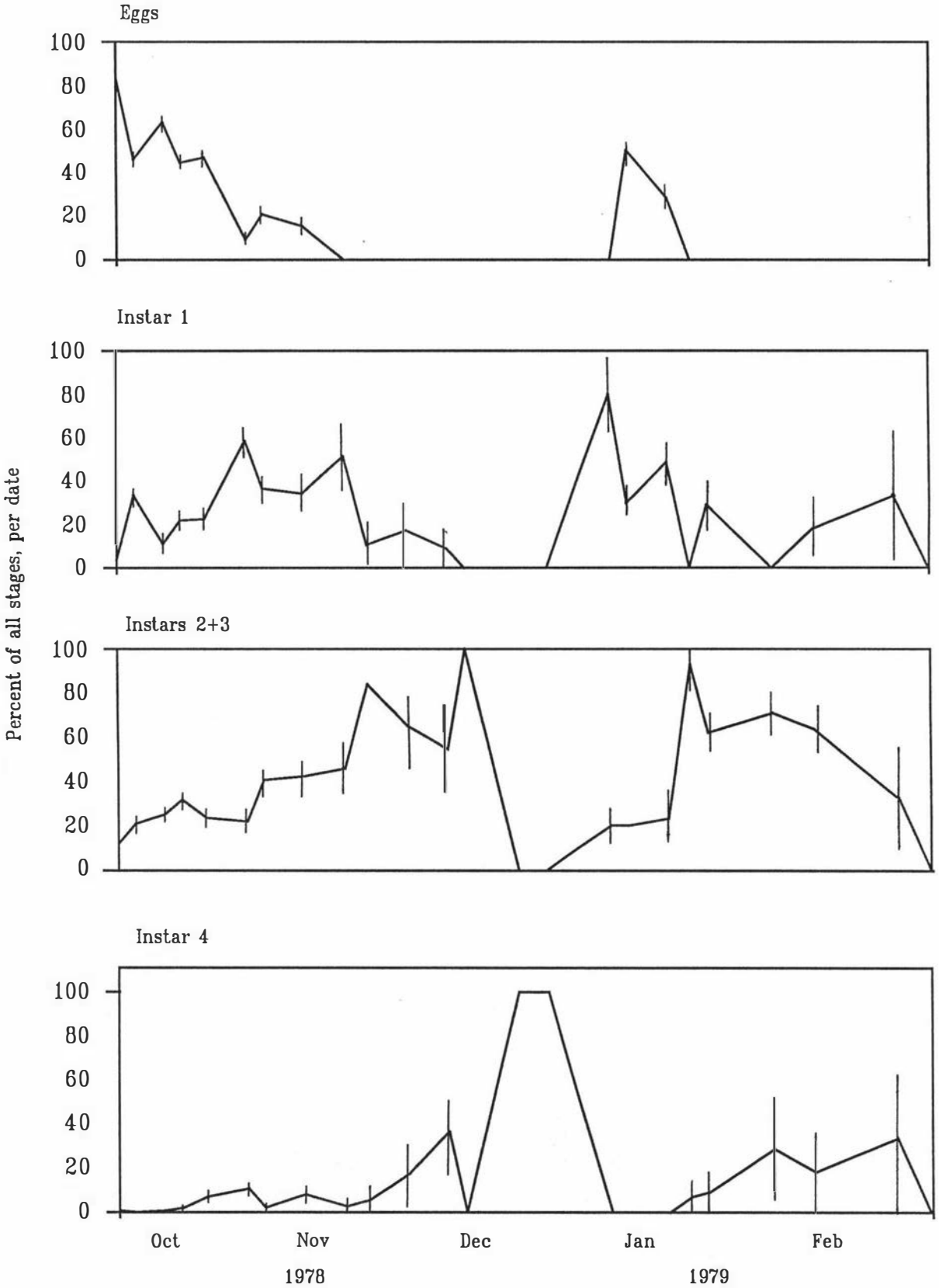


Fig. 4.12 Abundance of *P.charybdis* stages at sites 1 & 2, 1978-79. Mean number per shoot expressed as % of all stages, for each date. Vertical bars = 95% binomial confidence limits.

TABLE 4.3 Regression of eggs per shoot on defoliation and on number of new leaves; 6 December 1979

Carrier	Regression coefficient	Intercept	R^2	*p*
Defoliation	21.9	13.36	57.6%	0.004**
New Leaves	16.82	17.41	54.7%	0.006**

Both regressions were, however, strongly influenced by two points with high leverage values. This, together with the apparent lack of any significant effects of these two factors before the 6 December sample suggests that in fact no discernible trend was evident.

The relationships between leaf growth, defoliation and insect numbers are also discussed in chapter 5.

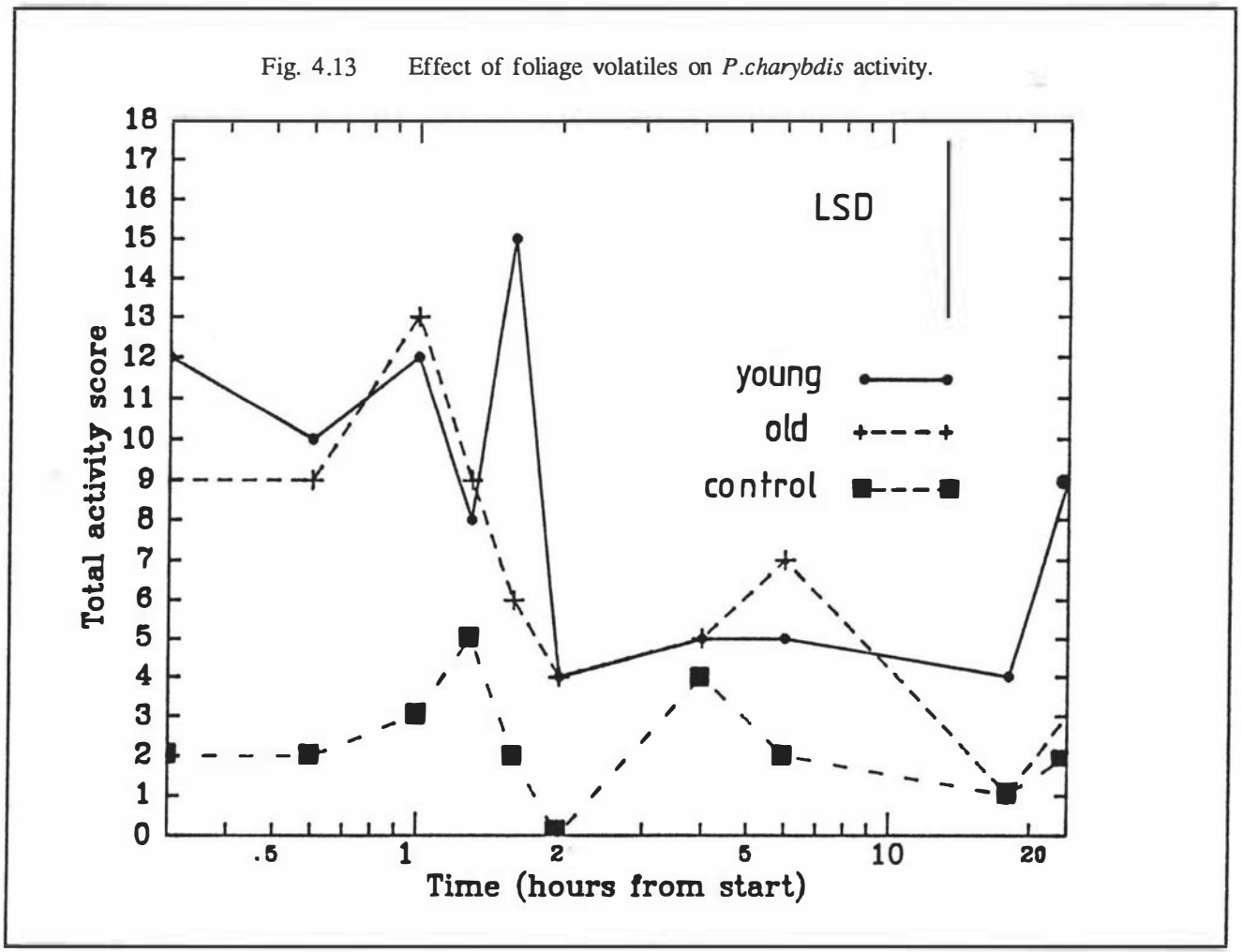
Dispersal of adults

After feeding to build fat body, adults locate overwintering sites. How this occurs and what triggers it are not known. I successfully light-trapped adults only once in autumn and never at any other time of year. On 20th March 1979, 131 adults were caught in a light trap at Massey University, near a row of large *E.viminalis* trees. No adults were caught before this and although a few adults were trapped until 26th March 1979, none were caught after this. This flight occurred when adults would have emerged in autumn of 1979 (Fig. 4.9).

4.3.2 Effect of foliage volatiles on spring activity of adult *P.charybdis*

Both the "young" and "mature" foliage treatments clearly elicited much greater activity than did the control and activity also changed as the experiment progressed (Fig. 4.13). Activity was greatest early in the experiment, peaking in all three treatments between 60 and 100 minutes, but all three treatments peaked at different times. Activity of adults exposed to young foliage volatiles was slightly more prolonged than for those given old foliage volatiles,

Fig. 4.13 Effect of foliage volatiles on *P.charybdis* activity.



with activity in the "young" treatment peaking after 100 minutes. The peak of activity in the "old" treatment occurred 40 minutes earlier.

The observations of activity at each successive sample time are likely to be correlated, so the time series of activity cannot be included as a second factor in an analysis of variance. This holds equally for non-parametric tests, which are unreliable if the observations are not independent (Bhattacharyya and Johnson 1977). Therefore, activity scores for each cage were summed over the entire duration of the experiment for analysis.

Boxplots (Velleman and Hoaglin 1981) of total activity score for the three treatments suggested that the variances were markedly different for each treatment. This was confirmed by a plot of the midspreads against medians, which suggested a relationship of the form

$$\text{midspread} = a(\text{median})^b$$

A variance-stabilising transformation was determined by estimating b as the slope of the regression of log(midspread) on log(median). The appropriate transformation is:

$$y^* = y^{1-b}$$

In this case b = 1.947, so the reciprocal transformation was used. This did stabilise the midspreads, but the transformed observations were very markedly non-normal (Shapiro-Wilk statistic = 0.64). Consequently, the non-parametric Kruskal-Wallis test was used, by carrying out a standard one-way analysis of variance on the ranks of the total activity scores. Treatments differed very significantly (Table 4.4), but a Least Significant Difference plot (Andrews, Snee and Sarner 1980) showed that the young and old treatments did not differ significantly.

TABLE 4.4. Analysis of Variance of ranks of total activity scores (= Kruskal-Wallis test)

Source of variation	Degrees of freedom	Sum of Squares	Mean Square	F	P
Treatments	2	294.33	147.67	11.89	0.001
Error	15	185.67	12.38		
TOTAL	17	480			

Numbers of adults on the pottles gradually increased as the experiment progressed until after 24 hours there were nine on the canisters in the "young" treatment and three on "mature" and control canisters. This difference was not significant (Chi squared = 3.35; $0.1<P<0.2$)

4.3.3 Effect of foliage age on mating and fecundity

The necessity of young foliage for oviposition was shown unequivocally in this experiment (Fig. 4.14). Adults began laying eggs during the third day after being fed young *E.viminalis* foliage and oviposition peaked after the fifth day. When the treatments were reversed, egg-laying was severely inhibited immediately and it ceased two days later in those cages now receiving only old foliage. Conversely, in the cages now receiving new growth, egg-laying began on the third day after reversal and increased rapidly at a similar rate to that observed during the first half of the experiment.

87% of copulating pairs were seen in cages that contained young foliage ($n=30$; $P < 0.001$). The only copulating pairs seen in cages that contained only old foliage were those that had previously eaten young foliage, but this was not statistically testable because only four such pairs were seen. However, when the first and last five days of the experiment were

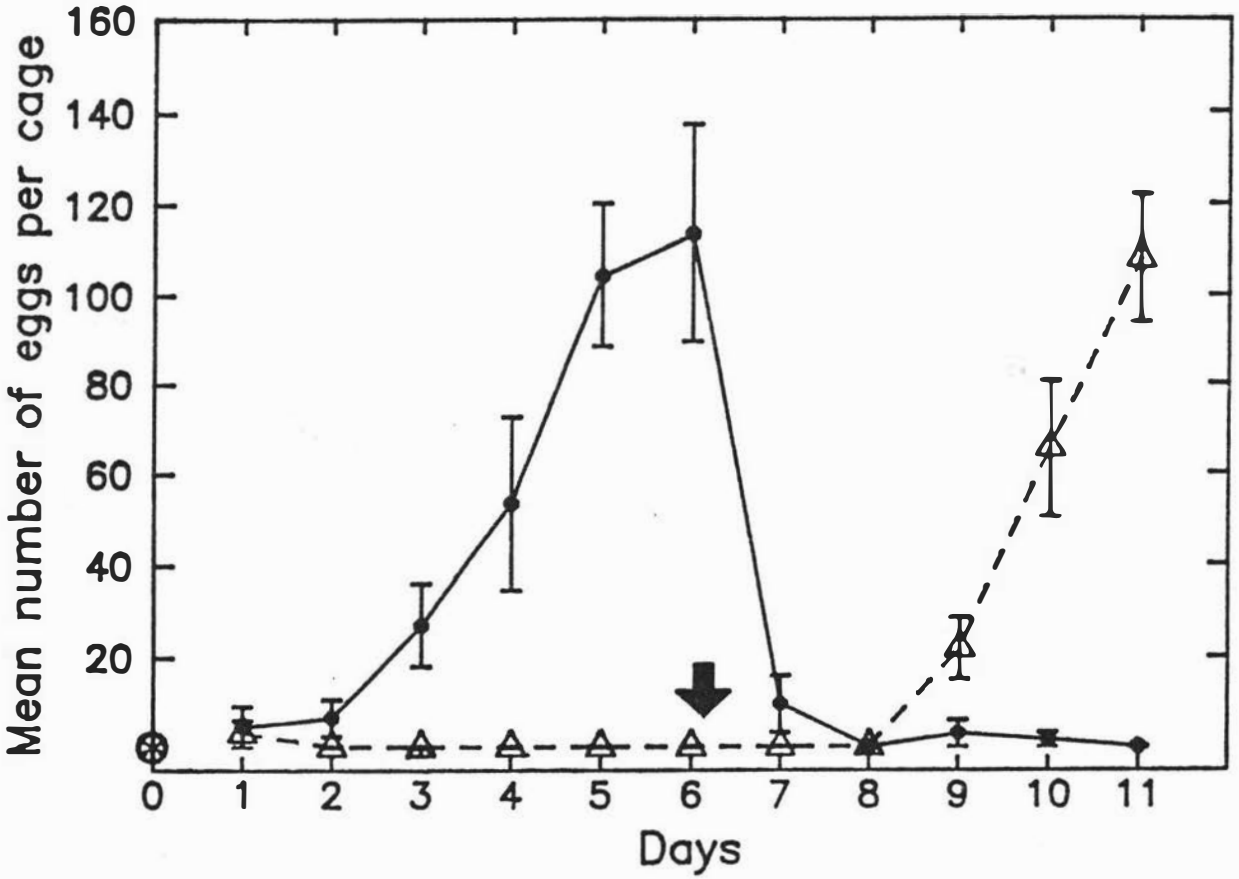


Fig. 4.14 Effect of leaf age on fecundity. Solid line = adults fed young and old foliage first; dashed line = adults fed old foliage first. Vertical bars = 95% confidence limits; arrow marks time of treatment reversal.

compared, removal of new growth did not significantly reduce copulation (Binomial test; P=0.274 n.s.)

The presence of young foliage greatly reduced activity, with 185 of 263 observations of adults moving about the cages occurring in cages without young foliage (Chi squared = 43.53 ***). This effect was still present when the number of copulating adults was added to the total for each cage (Chi squared = 9.365 **; n=323)

4.3.4 Growth rates

4.3.4.1 Morphometrics

Larval weight was a function of body length (Fig.4.15). Logarithmic transformations of both body weight and length removed the heteroscedasticity of the untransformed data and allowed valid statistical inferences about a least squares regression. 94.9% of the variance of larval weight was explained by the power function:

$$\text{weight} = 1.066\text{length}^{2.45}$$

Head capsule width of *P.charybdis* larvae grew by a factor of just over 1.4 between instars (Fig. 4.16), while larval body length was also a power function of head capsule width (= HW) (Fig.4.17). Logarithmic transformation of length and HW stabilised the variance and the relationship was determined by a least squares regression as

$$\text{length} = 2.716\text{HW}^{1.51}$$

92.1% of the variance of larval body length was explained by this relationship.

Fig. 4.15 Weight/body length relationship of *P.charybdis* larvae.

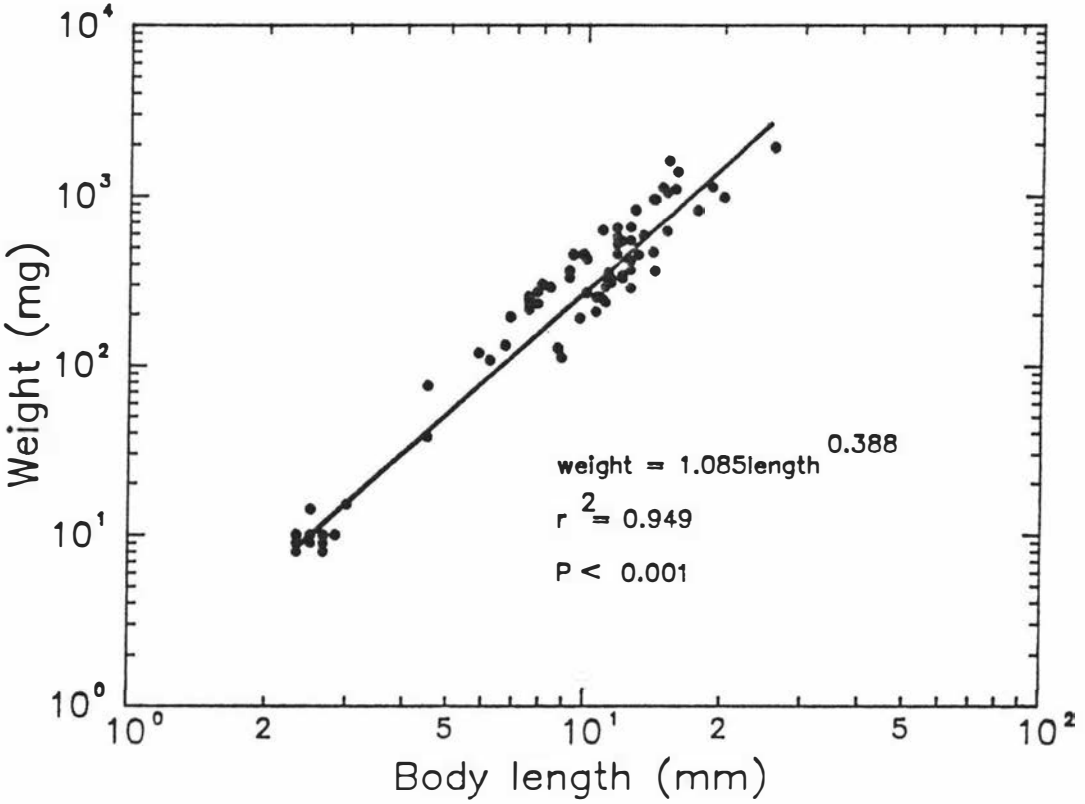


Fig. 4.16 Head capsule width in relation to larval instar.

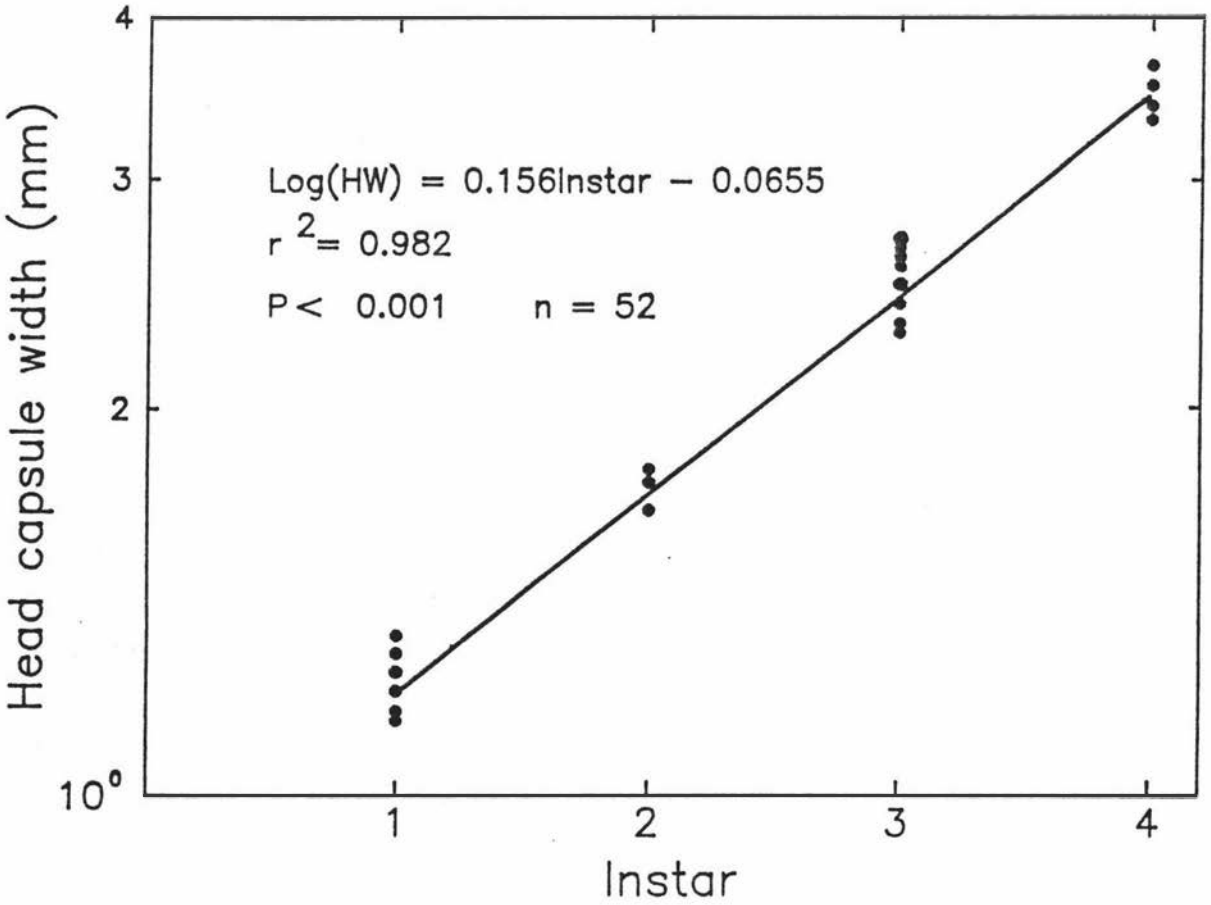
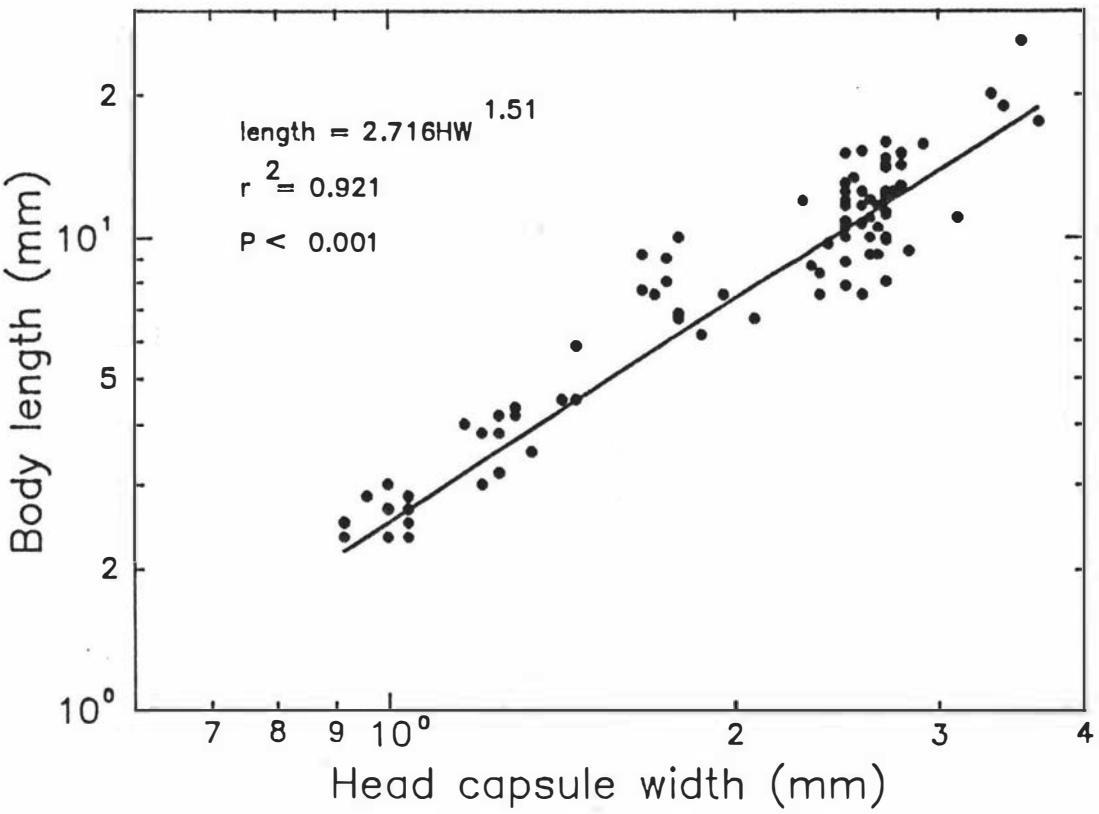


Fig. 4.17 Body length *vs* head capsule width; *P.charybdis* larvae.



4.3.4.2 Egg development

I soon found that the distinction between green-grey and yellow eggs did not accurately indicate development. Some eggs were yellow from a very early stage, while others in the same replicate were green-grey almost up to the time when egg bursters became visible. Occasionally, yellow eggs were even seen laid on top of a green-grey cluster. The colour of eggs was, however, consistent within a mass.

Development time at each temperature was defined as the time taken for 50% of the egg masses to show egg bursters or to hatch. This was estimated from the straight line fitted by eye to a plot of cumulative percent of egg masses hatched or showing egg bursters vs time. All non-zero observations and all replicates were used to obtain the single estimate for each temperature. This was termed the "Nominal Development Time", D_n. On average, eggs at each temperature spent 0.5 days in the 24 C room before being distributed among various temperatures. Therefore, eggs had completed an average of

$$[(100 \times 0.5)/(D_n \text{ at } 24°C)] \ \%$$

of their development before being transferred. This "Pre-Transfer Development", PD, was used to calculate the actual development time, D_a, thus:

$$D_a = [100/(100-PD)](D_n-0.5)$$

Formulae expressing the relationships between temperature/hatching and temperature/egg burster development were calculated using least squares linear regression techniques after reciprocal transformation of development times (Fig. 4.19). In both cases close to 99% of the variation in development rates was explained by the regression on temperature. Developmental thresholds, determined as the x-intercept of each regression, did not differ significantly (Table 4.8).

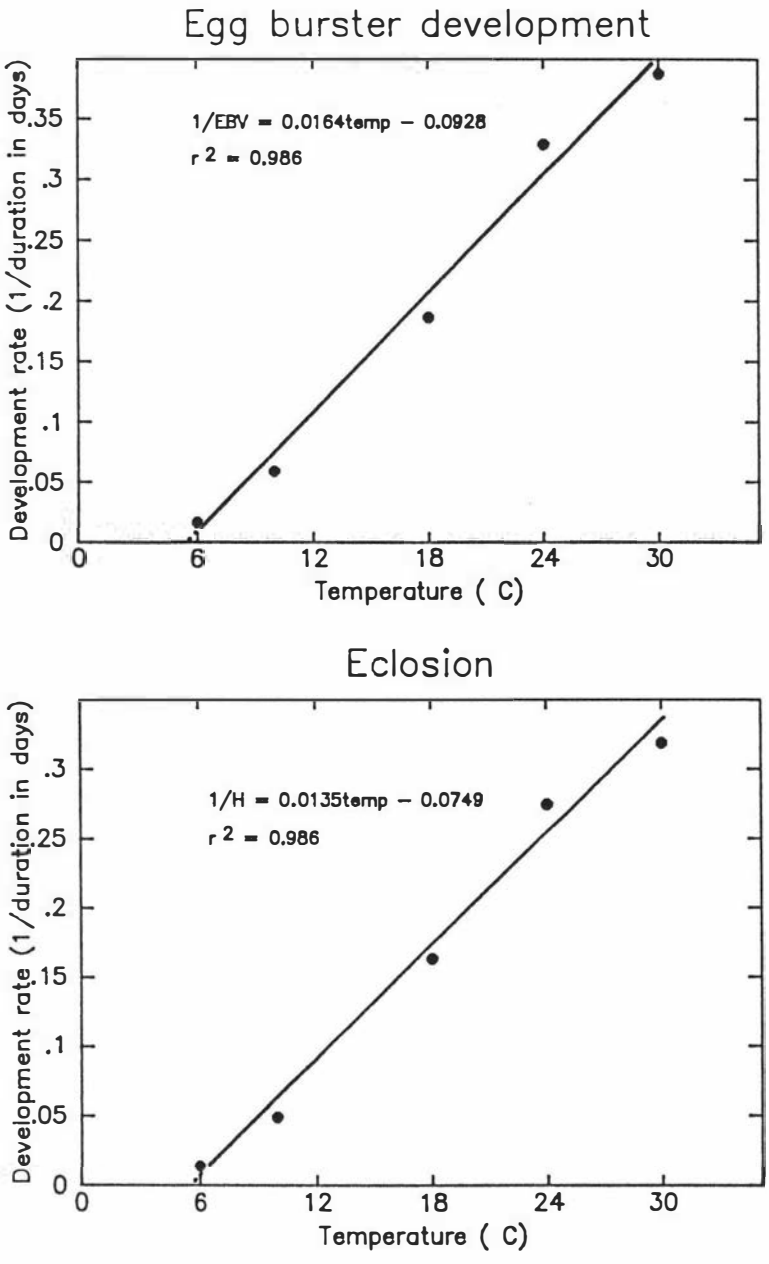


Fig. 4.19 Development rate of eggs in relation to temperature.

TABLE 4.8 Egg development: Developmental thresholds, °Days for development, and coefficients of variation of regressions.

Stage	Developmental threshold (°C)	°Days	R^2
Egg bursters visible	5.66	61	98.6%
Eclosion	5.53	74.1	98.6%

Egg bursters develop on the dorsal surfaces of the second and third thoracic and first abdominal segments and are visible after about 80% of development is completed (Fig. 4.19).

Mortality of unfed first instar larvae is also temperature dependent, although only three estimates were made because of the difficulties of distinguishing moribund and dead larvae and the already-variable time of eclosion at the two lower temperatures. Larvae survived for 3.3 days without foliage at 18°C and 2.0 days at 30°C.

4.3.4.3 Larval development

First instar larvae never survived more than about a day in the 30°C cabinet, despite varying the humidity. The durations of each stadium in each rearing cage at the other four temperatures are tabulated in Appendix 2.

A preliminary two-way analysis of variance was carried out on the untransformed data. The normal plot of residuals from this initial analysis showed that the data were markedly non-normal, while a plot of residuals against fitted values indicated a strong relationship between means and variances. The regression of log(standard deviation) on log(mean) had slope = 0.79, so the data were transformed by raising each observation to the power of 1 - 0.79 = 0.21. The model was then refitted. Examination of the new residuals indicated that

variances were not related to the means. The Shapiro-Wilk statistic ($W = 0.962$, $p > 0.05$) also suggested that the data could be considered normal so the model was accepted.

The ANOVA (Table 4.5) showed not only that the expected effect of temperature was present, but also that the duration of the instars differed and that significant interaction between the effects of temperature and instar was present.

TABLE 4.5 Analysis of variance of durations of stadia in preliminary larval development experiment.

Source of variation	Degrees of freedom	Sum of Squares	Mean Square	F	P
Temperature	3	0.0596	0.0199	11.203	0.000
Instar	3	4.12	1.373	774.345	0.000
Interaction	9	0.115	0.0128	7.216	0.000
Error	64	0.113			
TOTAL	17	480			

To calculate temperature thresholds for development, the duration of each stadium (S) was converted to development rate (DR) thus:

$$DR = 100/S$$

which is the percent development per day. Plots of DR vs temperature are given in Fig. 4.20a-d. Straight lines were fitted by the least squares method and were extrapolated to zero

Fig. 4.20a Preliminary development rate experiment. Stadium 1.

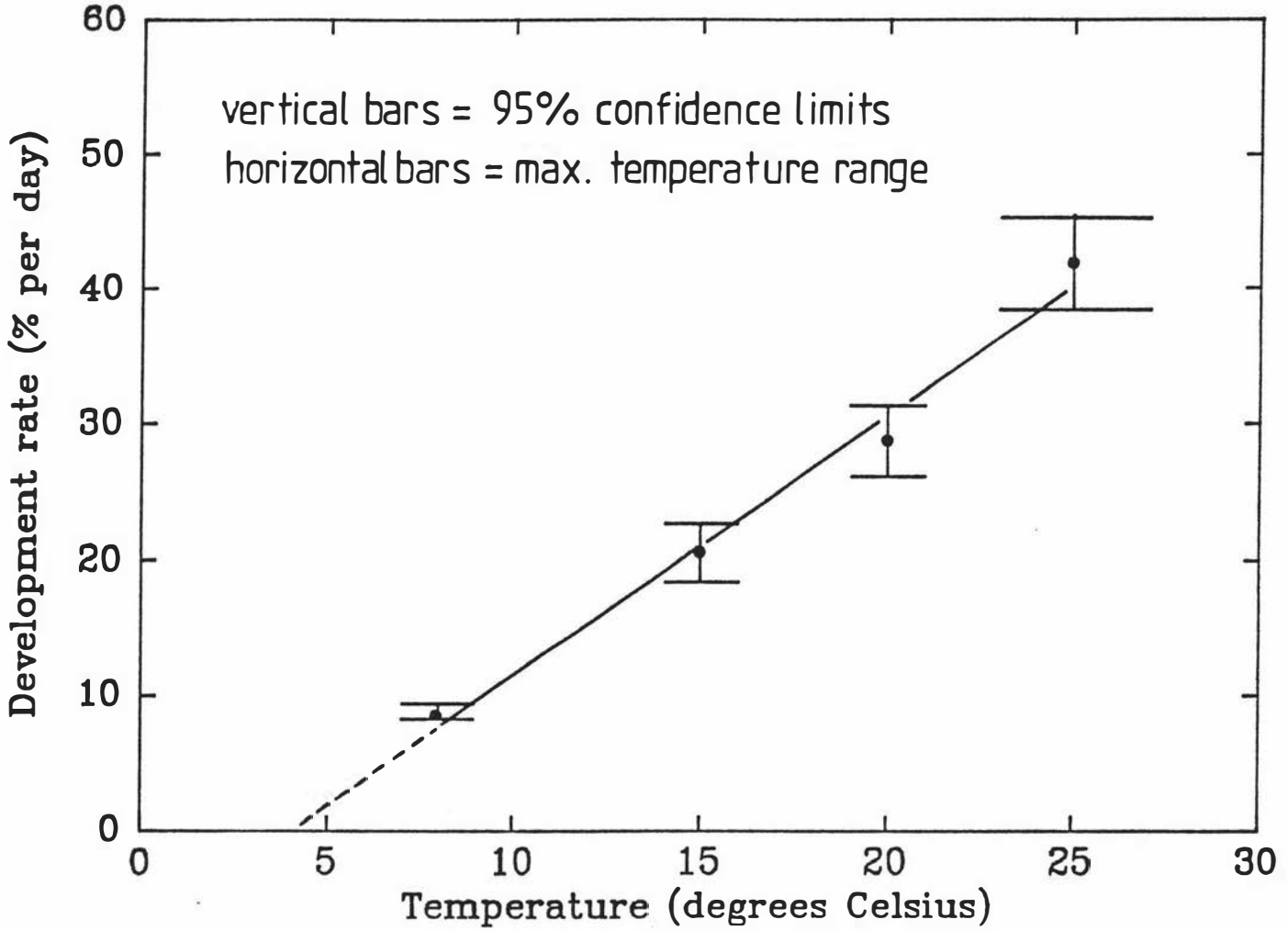


Fig. 4.20b Preliminary development rate experiment. Stadium 2.

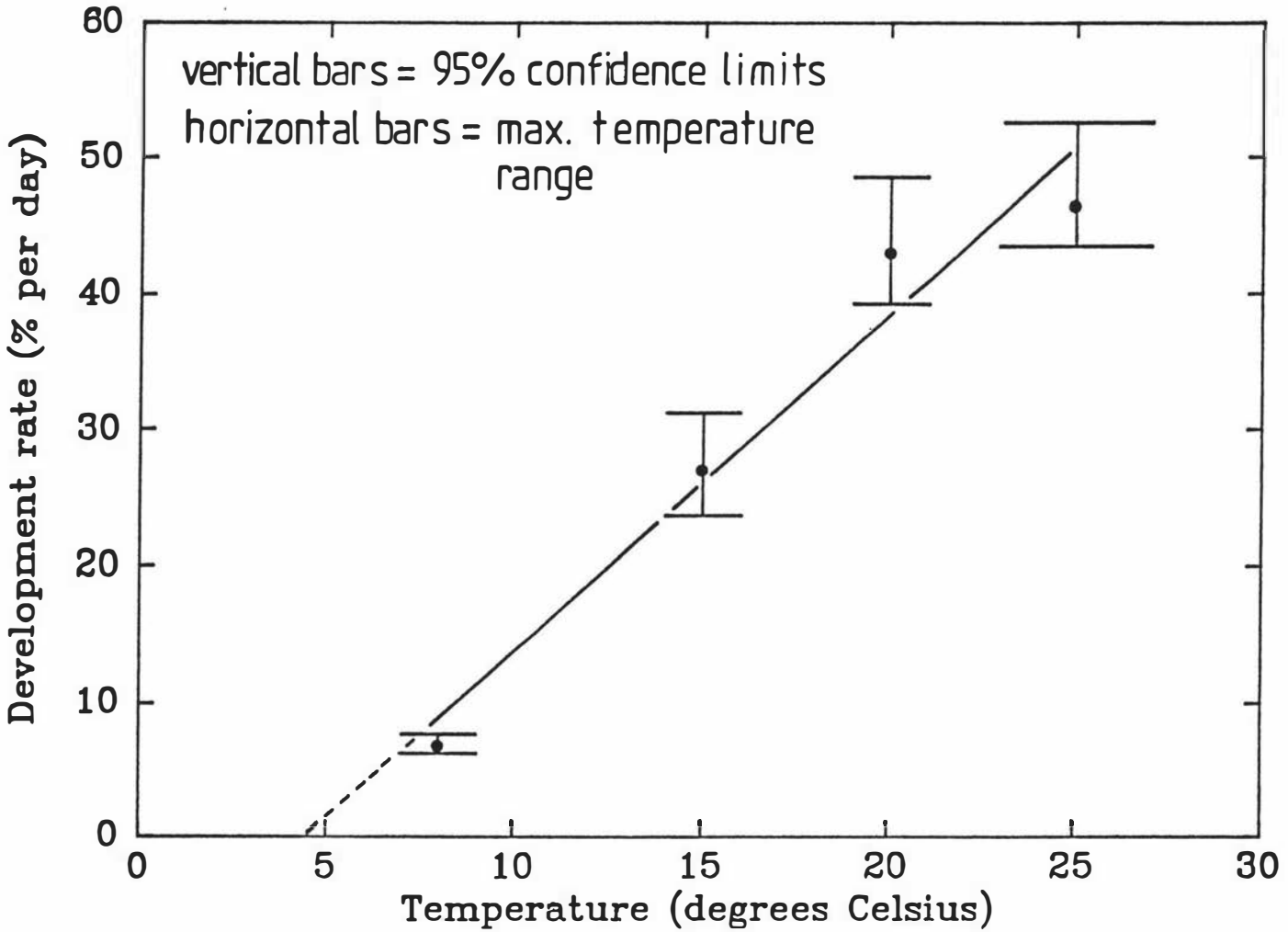


Fig. 4.20c Preliminary development rate experiment. Stadium 3.

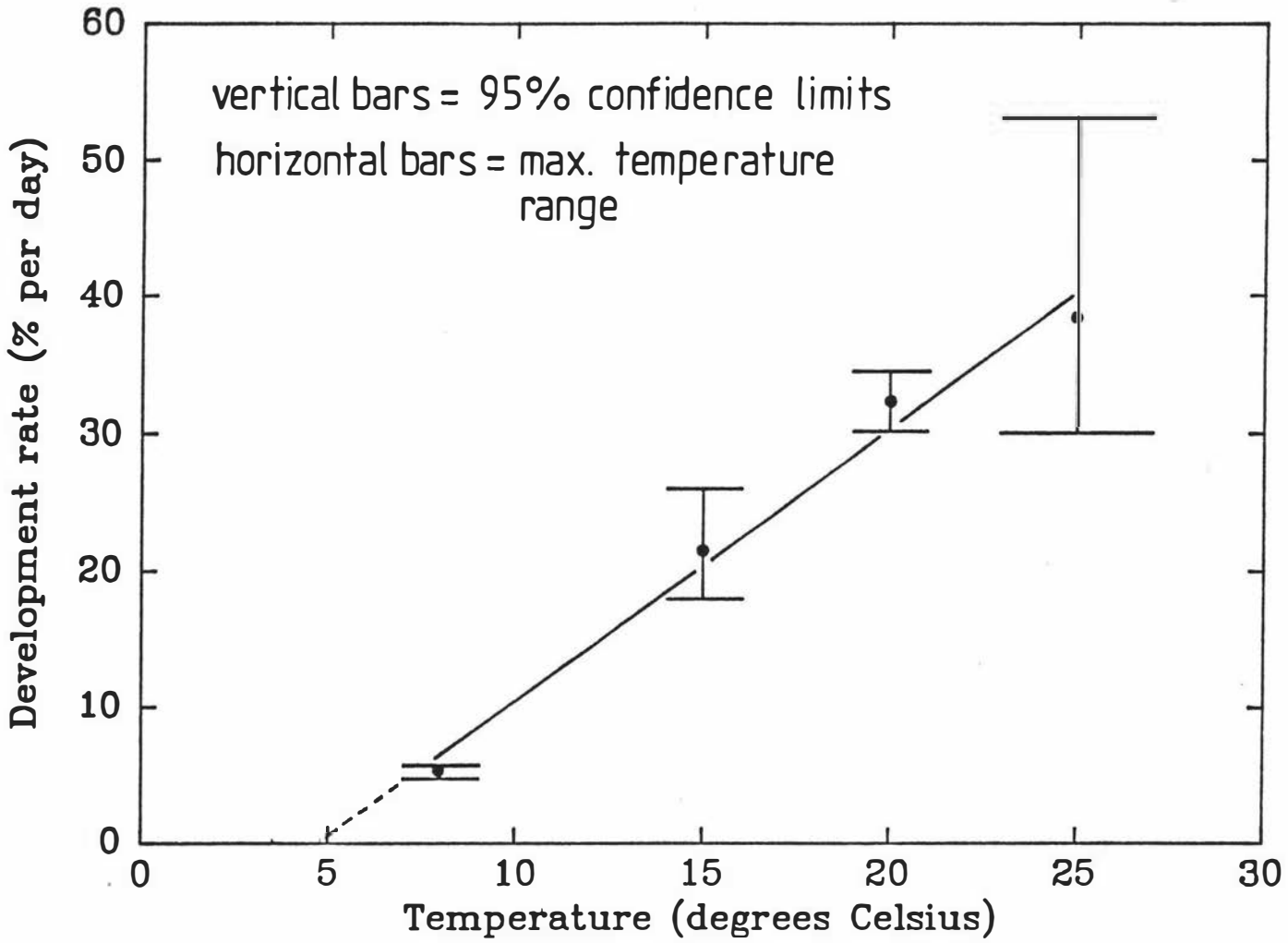
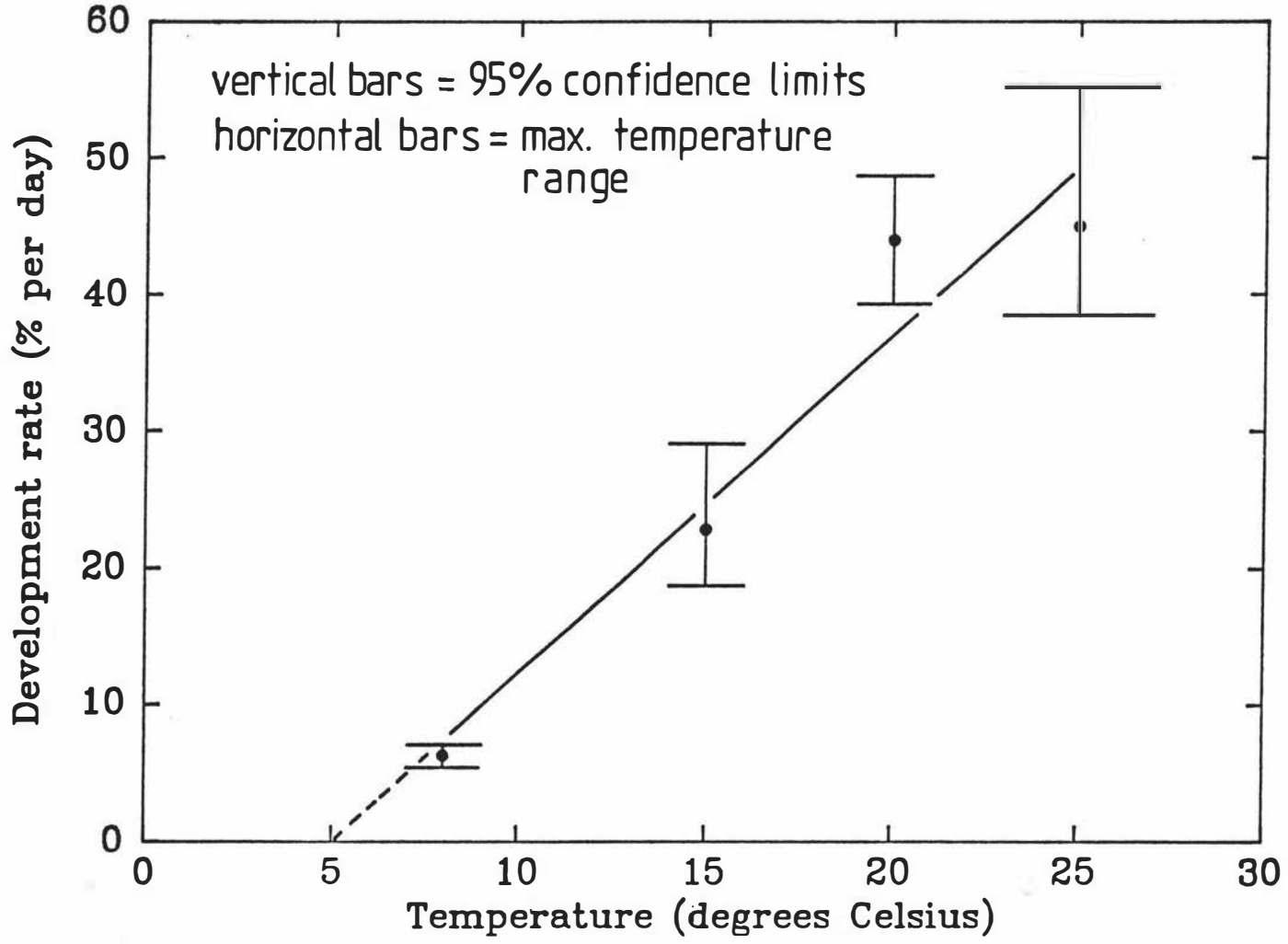


Fig. 4.20d Preliminary development rate experiment. Stadium 4.



development to locate the threshold temperature for development. The estimated temperature thresholds are listed in Table 4.6.

TABLE 4.6 Developmental thresholds for larvae; 1st experiment.

Stage	Estimated threshold (°C)
Instar 1	3.8
Instar 2	4.5
Instar 3	4.8
Instar 4	5.1

Estimates of development rates obtained from this preliminary experiment were used as explained in section 4.2.2.2 to determine development rates without the confounding effects of changes in food quality.

For each larva, the development rate at the alternative temperature, $d \in \{8, 12, 18.2, 28\}$, was calculated from the formula:

$$d_{ij} = (1-d_{24\,j}h_{24\,j})/h_{ij}$$

where d_{ij} = the development rate of larva j at temperature i and h_{ij} = time (in hours) spent by larva j at temperature i. The threshold for development and the thermal sum needed for complete development were estimated by regressing the median development rate at each temperature against that temperature. Each regression was followed by an examination of residuals to test the assumptions of normal distribution of errors and homogeneity of variances. In all cases these assumptions were clearly valid. Development thresholds were calculated by solving the regression equations for temperature with development rate = 0. The thermal requirement for complete development of the stage is simply the reciprocal of the coefficient of temperature in the regression equation.

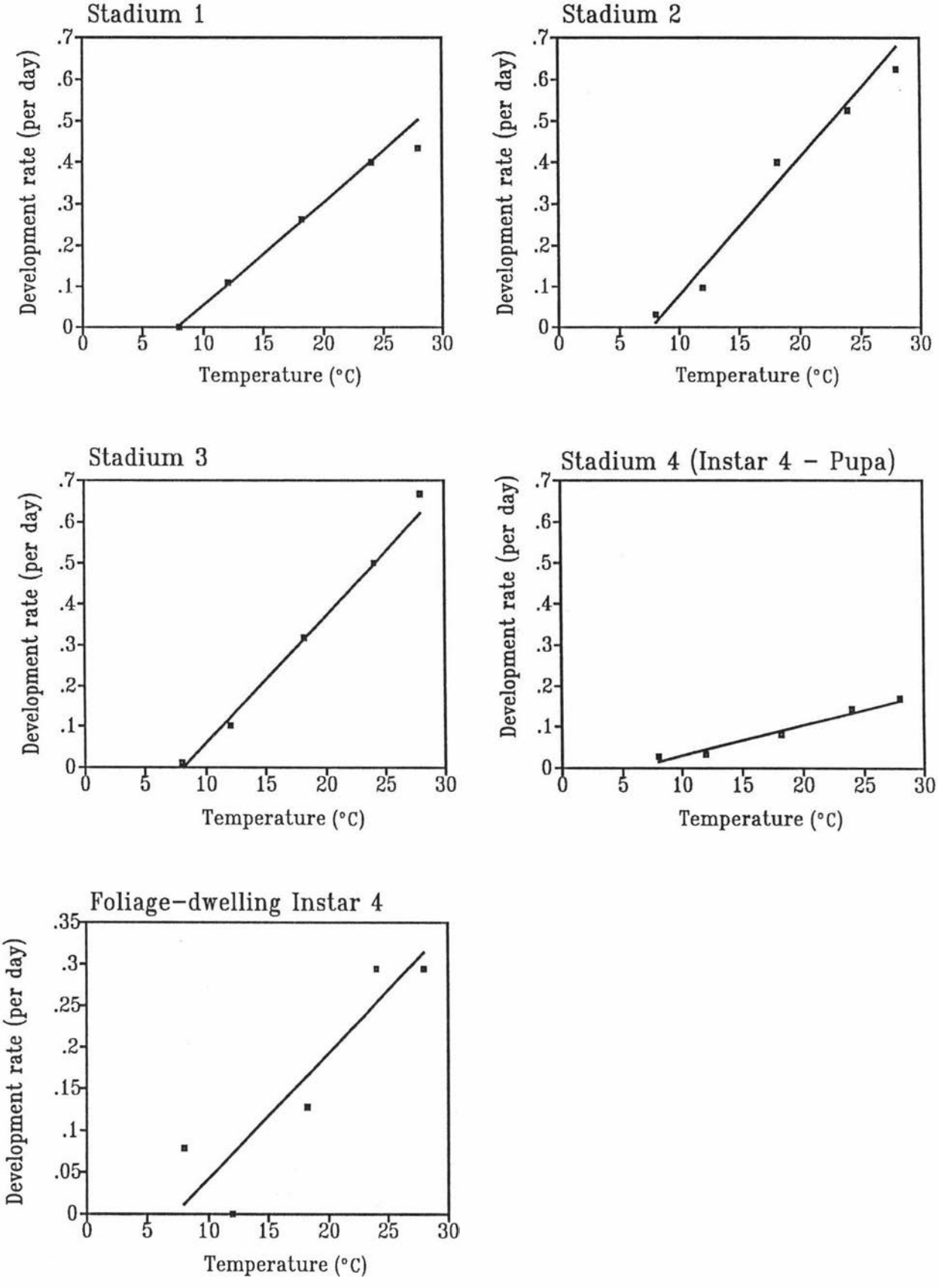


Fig. 4.21 Development rates of larvae in relation to temperature; definitive experiment designed to minimise effects of time-dependent foliage changes.

The estimated development rates at 28°C for instars 1 and 2 were lower than expected, suggesting that this temperature was above the optimum for development (Fig 4.21). This temperature was therefore excluded from the calculation of regressions for all stages. The estimated thresholds were within one degree for the foliage-dwelling stages, with the stage from 4th instar to pupa having a noticeably lower threshold (Table 4.7).

TABLE 4.7 Developmental thresholds, °Days for development, and coefficients of variation of regressions; 2nd experiment.

Stage	Developmental threshold	°Days	R^2
Stadium 1	7.8	40	99.9%
Stadium 2	7.6	30	96.8%
Stadium 3	8.1	32	99.6%
I4 - prepupa	7.2	66	72.5%
I4 - pupa	6.0	135	94.5%

4.3.4.4 Pupal development

The daily development rate (DR) of each pupa was calculated as the reciprocal of the duration of the period from pupa to adult. Standard linear least-squares regression techniques were used to fit an initial regression, which was accepted as a valid model after examination of residuals indicated that the assumptions of the method had been satisfied. The fitted line (Fig. 4.22) was:

$$DR = 0.0089797°C - 0.07197$$

($t = 25.869$; $p < 0.001$; $R^2 = 97.0\%$).

The developmental threshold = 8.0°C; and 111 °Days above this threshold were needed for development. There was no indication that the 24°C temperature was beyond the optimum for development.

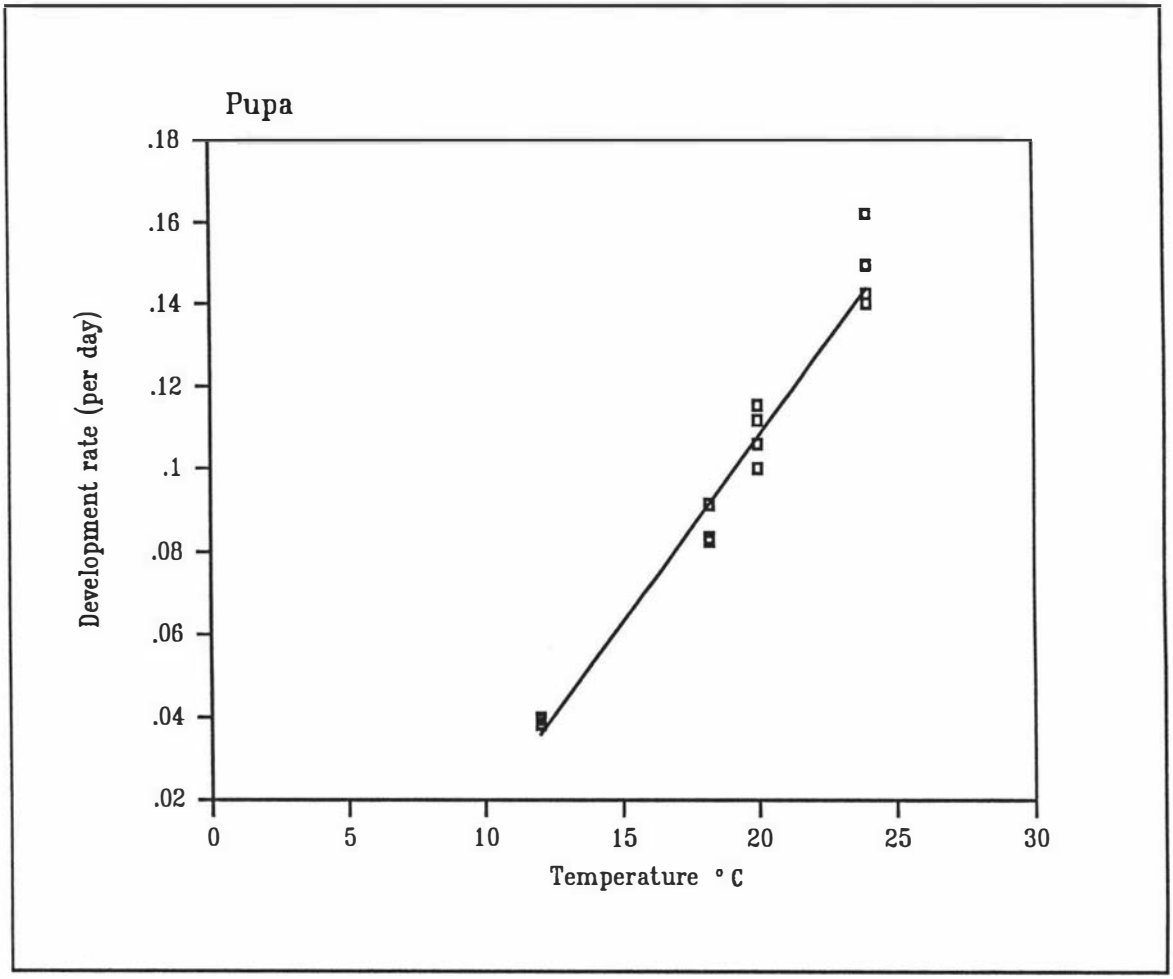


Fig. 4.22 Development rates for the pupal stadium in relation to temperature.

4.3.5 Population dynamics

4.3.5.1 Analysis of field population samples

4.3.5.1.1 Survival of eggs

Egg mortality is low, with records of marked egg masses at sites one and two during the 1978-79 summer showing survival rates of 93.4% at the Aokautere site (n=17 egg clusters) and 94% at the Ferguson Hall site (n=9 egg clusters). This is consistent with laboratory observations that 98.3% of eggs laid in clusters of more than three eggs hatched successfully at temperatures at or above 10°C (n=2518). Single eggs, or those laid in twos or threes, seldom showed signs of development. At 6°C many eggs eventually became infected with a fungus presumably as a consequence of the prolonged very high humidity.

4.3.5.1.2 Mortality of soil-dwelling stages

Once on the ground, *P.charybdis* experienced very high mortality at Kāhu Farms. Table 4.9 presents the numbers of fourth instars and adults trapped during each generation. Mortality is the difference between fourth instars and adults, expressed as a proportion of the number of fourth instars caught.

TABLE 4.9 Mortality of *P.charybdis* during ground-dwelling stages; Kāhu Farms, 1979-80

	No. m^{-2}		
	4th Instars	Adults	Mortality rate
Generation 1	205	23	0.89
Generation 2	126	12	0.91
Total	331	35	0.89

4.3.5.1.3 Stage-frequency Analysis

The method of Southwood and Jepson (1962), which was elaborated by Southwood (1978) and attributed to him by Sawyer and Haynes (1984), was slightly modified to estimate the number of *P.charybdis* in each instar for each of the 10 trees x 2 generations at Kāhu Farms during 1979-80. In the original method, the area under the stage-frequency curve, estimated graphically, is divided by the mean development time for that instar. The area under the stage-frequency curve can, however, easily be calculated numerically using the trapezoidal rule. Thus, if there are k sampling occasions with h_i being the ith of these, and n_i animals are recorded in the instar on occasion i, then the area under the stage-frequency curve is

$$A = 0.5\sum_{i=1}^{k}\{(h_i-h_{i-1})(n_i+n_{i-1})\}$$

If the mean development time for the stage = d, then the number of individuals passing through the stage is

$$N = A/d$$

To allow for the dependence of development rates on temperature Sawyer and Haynes (1984) suggested that stage-frequency curves be plotted against a physiological time scale, expressed in degree days, rather than calendar time. Although not stated by Sawyer and Haynes, this implies that d must also be expressed in physiological time, (d_p).

Now d_p is the temperature requirement for complete development and is the reciprocal of the slope of the regression of development rate on temperature, *b*, i.e.

$$d_p = 1/b$$

If h_{p_i} is the time in degree days at which the *i*th sample was observed, then from the above Eqns, the estimated number of animals passing through the stage is

$$N = 0.5d_p\sum_{i=1}^{k}\{(h_{p_i}-h_{p_{i-1}})(n_i+n_{i-1})\}$$

This Eqn was used to estimate the number of *P.charybdis* entering the egg stage, first instar, combined second and third instars and the foliage-dwelling fourth instar, for each generation. These results are presented in Table 4.10.

TABLE 4.10 Mean number of each stage per shoot per tree, for the 2 generations at Kāhu Farms, 1979-80; estimated by Southwood's method (Sawyer and Haynes 1984).

TREE	1	2	3	4	5	6	7	8	9	10	Total	s.e
						Generation 1						
Eggs	23.5	16.2	38.5	22.3	22.7	27.9	13.6	27.3	29	40.1	28.0	2.55
I1	10.1	2.2	11.4	4.1	24.1	1.9	1.0	10.9	17.4	8.0	9.0	2.23
I2+3	15.3	17.6	11.6	18.7	8.9	16.7	21.6	24.9	27.0	25.8	18.3	1.85
I4	1.4	1.3	2.7	2.4	0.5	6.3	2.0	3.4	4.4	3.5	2.8	0.51
						Generation 2						
Eggs	18.6	6.2	14.7	17.6	0	18.8	11.5	0	0	25.1	12.1	2.76
I1	80.7	13.6	14.9	30.5	9.9	22.8	4.6	0.7	7.9	28.9	21.9	6.9
I2+3	44.2	2.2	7.4	5.1	10.4	6.1	2.8	13.2	3.0	25.4	11.3	3.98
I4	3.6	0	3	0	0	1.8	1.0	2.4	0	4.6	1.3	0.5

4.3.5.1.4 Key factor analysis

To test for overall density-dependent effects and identify key stage mortalities, the trap samples were used to compare changes in the numbers of adults and 4th instars between generations. The fall traps, which caught 4th instars as they left the tree to pupate, remained in fixed positions, so they clearly sampled populations on the same trees in both generations. The adult-emergence traps, although moved every fortnight, remained beneath the same trees as the corresponding fall trap, so they too could be considered to have sampled the same trees' populations in both generations.

Key stage mortalities were investigated first for trap samples, using the method of Barlow, French and Pearson (1986), which is a minor modification of the method developed by Manly (1977, 1979). This attributes the key stage to that with the greatest value of:

$$A_i = b^2_{i+1}b^2_{i+2}...b^2_nMSD_i$$

where there are n stages (i), b_i = the coefficient of the regression of log density in stage i on log density of stage i-1 and MSD_i = mean square deviation from that regression.

This clearly identified the stage from adults emerging in midsummer (G1 adults) to autumn 4th instars (G2 4th) as that which contributed most to seasonal mortality (Table 4.11)

TABLE 4.11 Key factor analysis for Kāhu Farms population 1979-80, based on trap samples

Interval	*b*	MSD	A
G1 4th - G1 Adult	0.0354	0.4801	0
G1 Adult - G2 4th	1.6667	2.6794	0.327
G2 4th - G2 Adult	-0.2096	0.5074	0.022

The estimates of egg numbers per shoot per tree in the two generations, obtained by the modified Southwood method, were obviously not affected by foliage characteristics. Moreover, the larval stages that were suspected to have had unusually long development times were, in both generations, earlier than the fourth instar. This effect occurred mainly in the first instar during the second generation, so 4th instars may have had relatively normal durations. Even allowing a possible increase in duration, this would still have been similar for larvae on the 10 trees. Similar arguments apply for first generation 4th instars. However, some variation in 4th instar duration among trees may have been present, because in this generation, combined second and third instars appeared to have unduly long durations and this effect may have carried into the 4th instar on some trees.

With this proviso in mind, *A*-values were calculated using the estimates of eggs and 4th instars per shoot for each tree. The results of this analysis (Table 4.12) supported the contention that the key stage occured while *P.charybdis* was on the tree in the second generation. In conjunction with the analysis of trap samples, this result indicated that fluctuations in number of eggs laid by adults that emerged in midsummer were not the primary contributor to overall between-tree variation. Rather, this was a result of mortality between eggs and 4th instars in the second generation.

TABLE 4.12 Key factor analysis for Kāhu Farms population 1979-80, based on shoot samples

Interval	*b*	MSD	A
G1 Egg - G1 4th	0.2144	0.1021	0
G1 4th - G2 Egg	0.0402	0.5748	0
G2 Egg - G2 4th	0.4898	11.3407	2.72

4.3.5.1.5 Density-dependence

k-values were calculated for each trap according to the usual formula (Varley and Gradwell 1960). A constant = 0.1 was added to the counts of adults in both generations to avoid problems with zero counts. *k*-values for each tree were then plotted against their corresponding first-generation densities (Fig. 4.23) and the regressions of *k*-value on first-generation density were fitted (Fig. 4.23; Table 4.13). In view of the conservativism of this test (Smith 1973), the significance level was set at $\alpha = 0.1$.

TABLE 4.13 Regressions of *k*-value on number of animals trapped in first generation

	Regression coefficient	Intercept	*t*	*p*	R^2
Adults	1.0284	-1.5072	4.956	<0.001	71.1%
4th instars	0.0618	-0.577	2.443	0.035	37.4%
4th instars*	0.0596	-0.7536	5.063	<0.001	74%

* excluding trap 11

Significant density-dependent relationships were found for both adult-adult and 4th instar-4th instar intervals. The low R^2 of the 4th instar regression compared with that of adults was caused by the trap 11 result; when this was excluded from the regression of *k*-value on first generation 4th instars, R^2 increased to 74% (Table 4.13).

The discovery of this density-dependence justified a closer examination of the timing of density-dependent mortality. *k*-values were calculated for the three consecutive intervals from first generation adults to second generation 4th instars. The *k*-values were plotted against initial numbers per trap, as above, and the corresponding regressions fitted (Table 4.14).

TABLE 4.14 Regressions of *k*-value on initial number of animals trapped

Interval	Regression coefficient	Intercept	*t*	*p*	R^2
G1 4th - G1 Adult	0.0349	2.3913	0.628	0.544	3.8%
G1 Adult - G2 4th	0.5786	-3.6895	4.629	<0.001	68.2%
G2 4th - G2 Adult	-0.0792	1.9461	-1.343	0.209	15.3%

Clearly, density-dependence resides in the foliage-dwelling stages, at least in the second generation. Mortality of soil-inhabiting stages, although very high, is independent of density. Consequently, trees that supported high populations of 4th instars during the first generation could be expected to give rise to relatively high numbers of adults. However, these same trees produced fewer than average autumn-emerging adults.

Two hypotheses could explain this density-dependent mortality. Firstly, females may have preferentially oviposited on trees that supported lower numbers of larvae in the first generation. Secondly, mortality between second generation eggs and 4th instars may have been density-dependent. Both of these may also have operated. A further question concerns the nature of first generation mortality between eggs and 4th instars. To investigate these queries, the results of the shoot sampling programme were analysed.

Estimates of the mean number of eggs and 4th instars per shoot for each tree in each generation were used to investigate possible density-dependence, using the method described above *viz.* regression of *k*-value on untransformed initial density. These independently-derived results (Table 4.15) confirm that density-dependent mortality occurs during the foliage-dwelling stages of the second generation, and that mortality in the soil during mid-summer is independent of density.

These results also suggest that no density-dependent mortality occurs between eggs and soil-dwelling stages in the first generation.

TABLE 4.15 Regressions of *k*-value on initial number of *P.charybdis* per shoot

Interval	Regression coefficient	Intercept	*t*	*p*	R^2
G1 Egg - G1 4th	-0.0003	2.3943	-0.0122	0.991	0.0%
G1 4th - G2 egg	0.2705	-1.105	0.548	0.598	3.6%
G2 Egg - G2 4th	0.1403	-0.0305	1.885	0.096	30.8%

4.3.5.2 Experimental study of first instar mortality

The shoots chosen for this experiment had similar amounts of new foliage (Table 4.11). All shoots had negligible defoliation at the start of the experiment and suffered less than 20% defoliation during its course (Table 4.11). Mortality was not caused by lack of food.

Larvae stayed on the remains of their egg mass for up to 24 hours before moving to new foliage at the tip of the shoot, although most larvae moved away from their egg mass much sooner. Throughout the experiment, larvae clustered almost exclusively on the apical two leaves of each shoot. No larvae were caught on any of the Tangle-Trap™ bands.

Smith's (1973) method of analysis was chosen for the analysis of these results, for reasons presented in the discussion (section 4.4.5). *k*-values were calculated for each density treatment from the usual formula:

$$k = \ln(\text{initial density}/\text{final density})$$

These were plotted against initial density, which was estimated as the number of eggs that developed at least until egg bursters were clearly visible (Fig. 4.23). No relationship between *k*-value and initial density was apparent. The regression of *k*-value on initial density confirmed the lack of a linear relationship. As a further check that no curvilinear relationship was present, the ranks of the *k*-values were plotted and regressed against the ranks of initial density. This also showed that survival was not related to density. Fig. 4.23 suggests that the D_2 treatment may have unduly influenced the analyses, so the tests were repeated, omitting D_2. This did not affect the conclusion of lack of density-dependent mortality.

TABLE 4.11 Summary of results of experiment on 1st-instar mortality

Shoot	Initial no. new leaves	Final defoliation	Initial density	Final density	*k*-value	Survival rate
1	37	1	11	4	1.012	0.364
2	29	1	22	3	1.992	0.136
3	41	1	49	26	0.634	0.531
4	33	1	64	18	1.268	0.281
5	33	1	80	34	0.856	0.265
6	35	1	117	31	1.328	0.265

The major time of mortality of first instars was between hatching and their arrival on new foliage (Table 4.11). Mean survival rate averaged 45% during this period. 154 larvae reached new growth and 118 of these successfully moulted to second instars, giving an average survival rate of 77%.

To convert these stage-specific survival rates to daily rates, estimates of duration of the stages were needed. Since 343 eggs were assumed to have hatched successfully, the time of

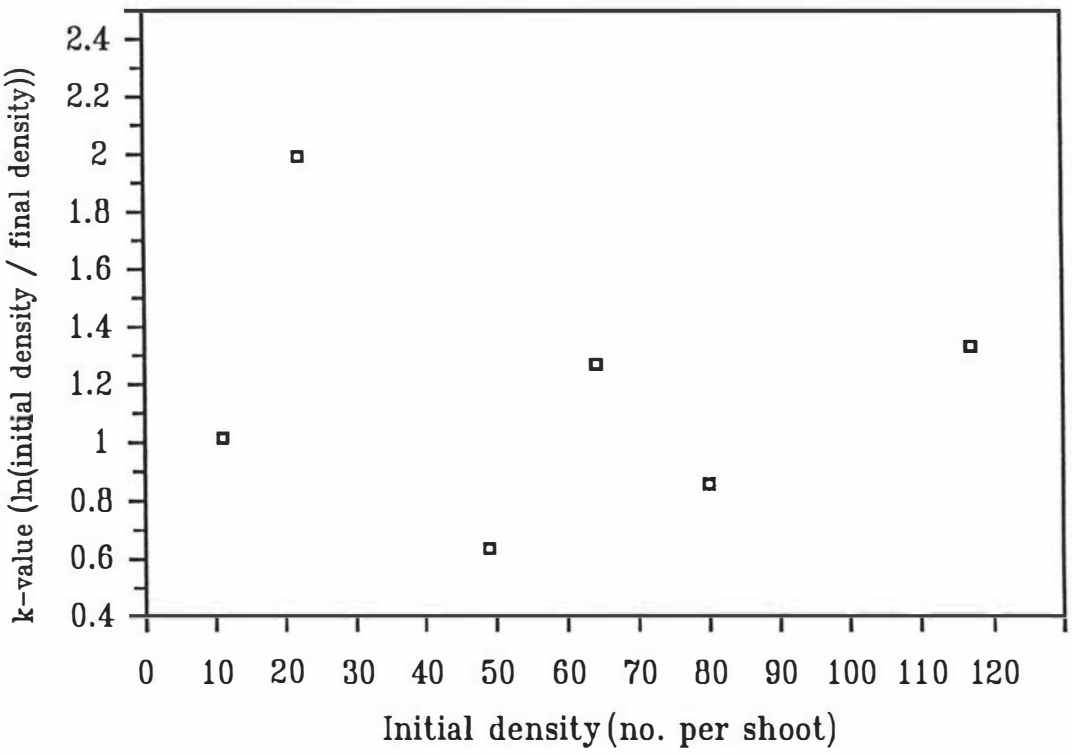


Fig. 4.23 Regression of *k*-value on initial density as a test for density-dependent mortality in the experimental study of 1st-instar mortality.

hatching of the egg that hatched in position 171.5 represents the median egg hatch time. Likewise, the larva that arrived on new foliage in position 77 should have arrived at about the half-way point of these arrival times. The difference between the median egg hatch time and the median arrival time of first instars on new foliage is an estimate of the average time that larvae took to locate new foliage after eclosion. These median times were calculated by linear interpolation between the start and end times of the interval in which the event took place. Thus at 23.75 h after the start of the experiment, 325 eggs were still unhatched, and at 38 h after the start, only 25 eggs were still unhatched. Simple algebra gives the equation of the straight line joining the points (23.75, 325) and (38, 25) as

$$y = 825 - 21.05x$$

so that

$$x = (y - 825)/-21.05$$

and substituting $y = 171.5$ gives an estimate of the median egg hatch time, $x = 31$ h.

Similarly, estimates of the median arrival time on new foliage and median time of moult to second instar can be calculated as 29.9 h and 119 h respectively.

The obvious anomaly is the apparently negative time taken for larvae to reach new foliage. In fact this suggests that the linear interpolation method was inaccurate for either the median time of egg hatching, or the median time of arrival on new foliage, or for both. If, during the sampling interval that included the median time of egg hatch, later eggs took longer to hatch, i.e. the frequency distribution of recruitment to 1st instars was skewed to the right, then linear interpolation would predict a later median egg hatch time than the actual time. Similarly, if more than half the larvae that arrived on new foliage during the interval containing the median arrival time arrived during the second half of this interval, then the predicted median would be earlier than the actual.

In any event, the time larvae took to reach new foliage after hatching was very short compared to the remainder of the first stadium. Median hatching and median arrival on new foliage both occurred between 23.75 h and 38 h, a 14.25h interval. Assuming an arbitrary and probably conservative time of 7h between hatching and location of new foliage gives an estimate of the hourly survival rate for this stage as

$$s_1 = e^{\ln(154/343)/7}$$
$$= 0.8919$$

Similarly, the hourly survival rate between location of new foliage and start of the second stadium is

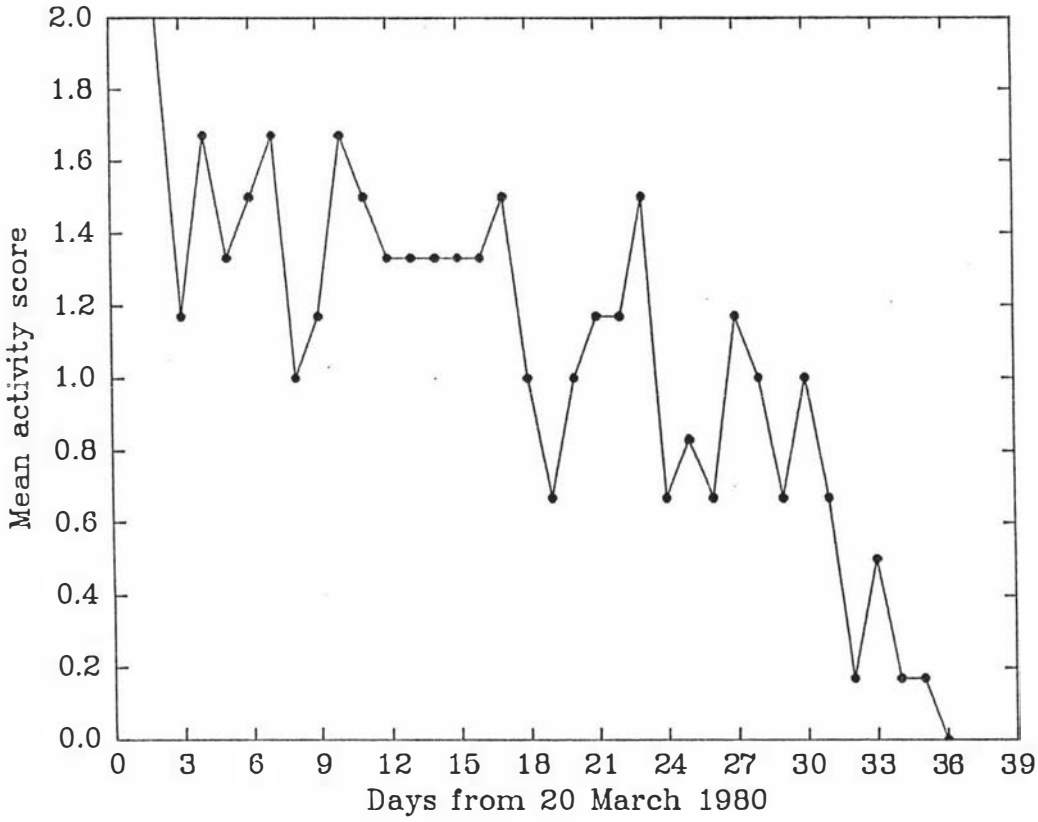
$$s_2 = 0.9978$$

4.3.6 Diapause

All field-collected adults diapaused in the insectary. Because conditions in the insectary were known not to cause dormancy, diapause had already been induced in the field before their transfer. The number of adults transferred from Kāhu Farms to Palmerston North varied, being ten for the first four samples, eight for the fifth and seven for the sixth.

There was apparently an overlap of up to two weeks in diapause onset among adults in a cage. Observations on adults kept separately suggested that diapause did not commence suddenly, because these adults gradually became less active and fed less frequently until they were completely inactive. This process took between three and ten days. Isolated females were easier to assess than males, because the interval between egg clusters increases and the date when eggs were last laid could be used as an approximate estimate of diapause onset.

Fig. 4.24 Diapause induction in adult *P.charybdis*.



Diapause began for all samples within a four week period from late March until 24 April. During May and early June, when newly-flushing *E.viminalis* foliage could occasionally be found, adults did not feed when offered young foliage. Despite the high temperature and long photoperiod in the insectary, all the adults were inactive at this time.

Diapausing adults began to feed again in late July, but the last new growth available before this was in early June. Thus there was a period of about seven weeks when the adults may have eaten suitable foliage had it been available.

4.4 Discussion

4.4.1 Life History

Crowson (1981) suggested that elaborate courtship is necessary to avoid interspecific matings among closely related species of Coleoptera. *P.charybdis* belongs to an enormous genus with over 500 morphologically similar species (Hawkeswood 1977), yet courtship appears to be quite unspecialised. In Paropsis, highly developed host specificity may replace elaborate courtship for this purpose. The choice of only certain trees for adult feeding, mating and oviposition may isolate apparently sympatric species.

Mating behaviour may also be more complex than it superficially appears. The apparent reluctance of females to mate, and their repeated attempts to disengage copulating males, may allow females to distinguish persistent and therefore successful males (Thornhill and Alcock 1983).

Since *P.charybdis* larvae eat new foliage, it is clear that the adult females' avoidance of new growth for egg-laying minimises the risk of egg losses by being eaten or by falling from the tree on leaf fragments excised but not eaten by *P.charybdis* or similar leaf-feeding insects.

Dugdale (1966) also reported that while mature *E.resinifera* was "plastered with eggs", young foliage showed "practically negative" egg counts. Faulds (pers. comm.) did occasionally find eggs on new foliage of *E.viminalis* at Maketu (Bay of Plenty region). The older, hardened leaves which are favoured oviposition sites are much less attractive to herbivores, a characteristic noted for *E.blakelyi* by Journet (1980) and for some other trees by Coley (1980), Feeny (1970) and Watanabe (1982).

The apparent preference of females for young rather than old foliage in the experiment that tested the effect of foliage age on fecundity may be best explained by the females' oviposition behaviour. The result suggests that females select a site by walking along a shoot away from young foliage. This ensures that newly hatched larvae will probably be close to new growth. This is particularly important since the further larvae are from new foliage, the greater the chance that they will encounter forks that lead only to old growth. Also, if eggs are laid on leaves too far down the stem there is a high likelihood that eggs will be lost as those leaves senesce and drop from the tree.

Oviposition on twigs has been previously recorded by Dugdale (1965b), and by Styles (1966), who reared the eggs and found that resulting stages were indistinguishable from normal *P.charybdis* both morphologically and in oviposition behaviour. My findings and those of Dugdale (1965b) and Styles (1966) suggest that twig-laying is an occasional expediency when oviposition sites are scarce. Dugdale's (1965b) comments are somewhat vague, since he wrote that twig-laying persisted even when there was no shortage of "suitable foliage", but offered no explanation of what he considered to be suitable foliage. However, he noted that eggs were "plentiful", which suggests that oviposition sites suitable from *P.charybdis*' viewpoint, rather than that of a human observer, may have been scarce. The scattering of eggs under crowded laboratory conditions supports the hypothesis that laying of unclustered eggs on twigs is a behavioural expediency caused by crowding. An alternative hypothesis is that the behaviour is peculiar to a certain proportion of individuals. This explains why twig-

laying has only been recorded in dense populations, as does the previous hypothesis, but it cannot explain the frequent egg-scattering in artificially-crowded groups taken from low-density field populations. Furthermore, adult females that scattered eggs in empty containers in the laboratory laid normally in clusters when supplied with fresh young foliage with a few older leaves.

The location of egg clusters on the adaxial leaf surface may provide protection from direct sunlight and consequent desiccation, but is probably of little use as a defence against predators or specific parasites. Most non-specific predators of defoliating insects are well-accustomed to searching the undersides of leaves and any specific parasite would, almost by definition, search this location.

The range of egg cluster sizes described in Tables 4.1 and 4.2 and shown in Fig 4.4 and 4.5 is in accord with other authors' findings. Clark suggested a usual range of 12-24 eggs per cluster, while Dugdale (1966) quoted two ranges: 10-45 and 14-18 eggs per cluster. Dugdale's meaning is obscure: it appears that the estimate "14-18" is an informal interval estimate of the average number of eggs per cluster while the first (10-45) range is apparently an estimate of the usual range. For adult females fed *E.globulus* foliage, Steven (1973) recorded a mean egg cluster size of 12.38, with the mean for individual females ranging from 7.94 to 17.39. He found that adults collected from the field in early spring, apparently before oviposition began and kept at 25.6 C laid egg clusters with a mean of "over 20 eggs, with a range from 12 to 25". Styles' (1969,1970) graph showed a range from nine to 53 eggs per cluster and a mean of 24.2 for a single female during 123 days, but he did not indicate the type of foliage used, nor the temperature range.

Fig 4.5 shows that egg clusters of more than about 26 eggs are probably multiple clusters, laid by several females or one female on several occasions. The clusters of 28 or more eggs shown in Fig.4.5 were all laid when females were laying freely and oviposition sites were

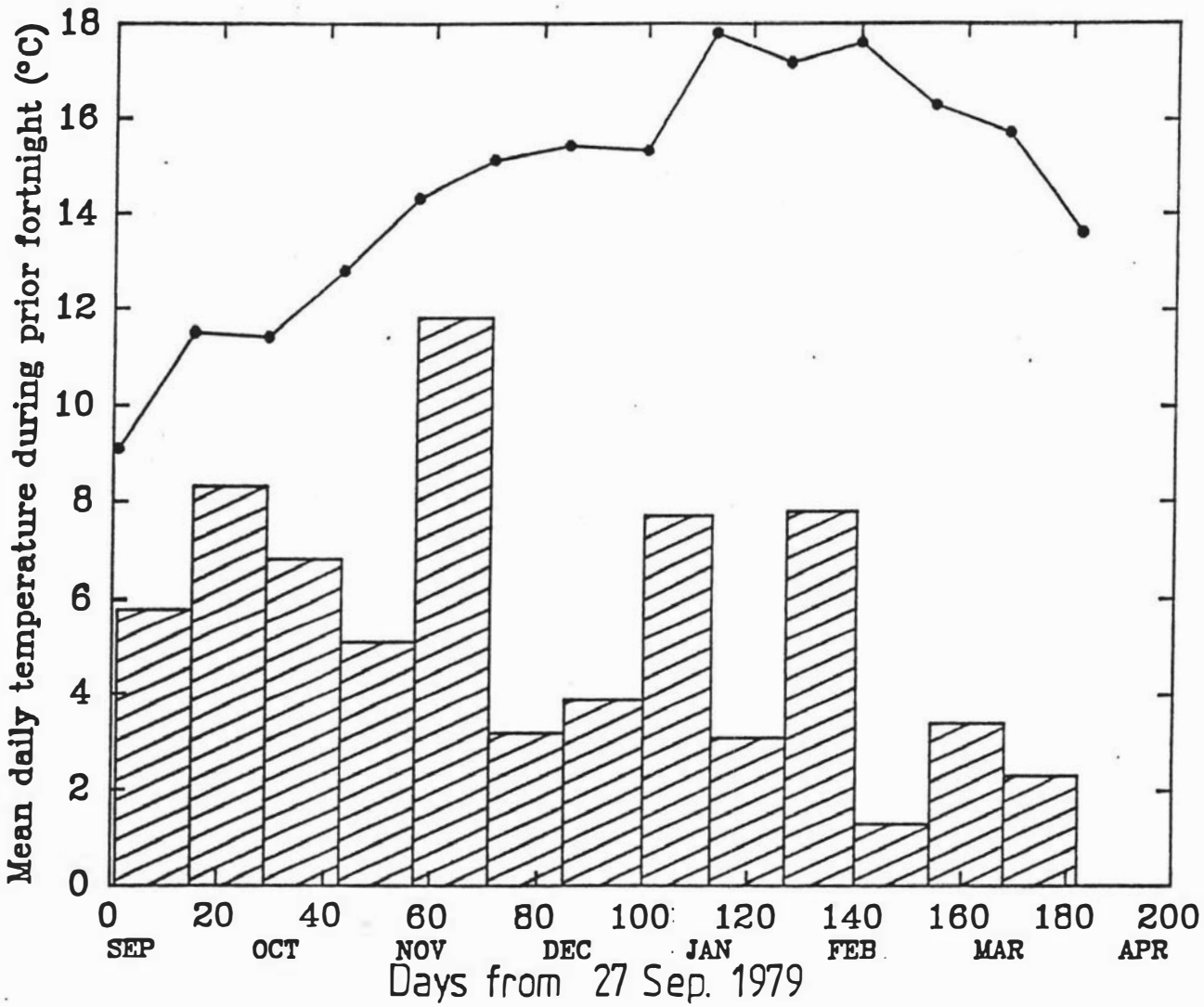
scarce. Morphological differences during development later proved that most of these were indeed multiple clusters.

Several hypotheses could explain the significant difference in cluster sizes between the first and second generations. The size of egg clusters is weakly correlated with fecundity and also with body size (Steven 1973). The relationship between fecundity and body-weight has been well documented for many other insects (Southwood 1978; pp.302-303). First generation (spring-emerging) adults may on average have had higher body-weights because of differential mortality during winter and this would be reflected in increased fecundity, manifested partly as an increase in egg-cluster size during the first generation. This pattern would not hold true for the second generation unless the differences in body-weight of overwintering adults were primarily genetically determined.

A more likely hypothesis is that the different cluster sizes were caused by nutritional differences. Steven (1973) showed that the foliage of different *Eucalyptus* species and its type (juvenile or mature) caused significant differences in fecundity of *P.charybdis*, while adult body weight, and therefore fecundity, was greatly affected by larval nutrition. I found that oviposition was dependent on the availability of newly-flushing foliage. Because the availability of *E.nitens* young foliage at Kāhu Farms changed during the season, some change in adult fecundity would therefore be expected.

Other environmental factors such as temperature or humidity could have caused the difference in cluster size and again these factors could have acted directly on the adults, or affected larval performance to produce smaller adults. Steven (1973) found that as temperature increased, the number of eggs per cluster decreased, although this was offset by an increased oviposition rate so that the total number of eggs laid increased. In my study during the second generation at Kāhu Farms in 1979-80, mean fortnightly temperatures were higher than during the first generation (Figure 4.25) and this may have caused the difference in egg

Fig. 4.25 Weather records for Kāhu Farms, 1979-80 summer



cluster sizes between generations. However, there was no significant correlation between mean fortnightly temperatures and corresponding median egg cluster sizes for dates when eggs were present (Spearman's rank correlation coefficient r_s= -0.479 corrected for ties; n.s.). No significant correlations were found between either the median or mean and mean fortnightly temperatures when the two generations were examined separately.

Lastly, the change in average cluster size may be a behavioural response by adults. Carne (1966) showed that *P.atomaria* oviposited preferentially on less defoliated trees and the high levels of defoliation during the second generation at Kāhu Farms (Chapter 5) may similarly have altered *P.charybdis*' oviposition behaviour. However, Spearman's rank correlation coefficient indicated no significant correlation between the amount of defoliation and the median (r_s=0.277; n.s.) or mean (r_s = 0.15; n.s.) egg cluster size for dates when eggs were present. The relationship between defoliation and oviposition is treated in more detail in chapter 5.

Construction of a pupal cell is not essential, as pupation can be completed successfully in loose leaf litter. Under laboratory conditions they frequently pupated on damp filter paper in petri dishes or on the bottom of their rearing cage. However, in the latter situation deformities were common. This may have been caused by desiccation since prepupae transferred to small containers or individual cells in a polystyrene block had a much lower rate of deformity. In the pupal development experiment, two adults with minor deformities were noted at 28°C, but at the other temperatures all adults were normal.

I have found no records of *P.charybdis* having been light-trapped in large numbers. Large, mass flights of *P.charybdis* have been recorded on a few occasions (White 1973) but whether this is a regular form of dispersal before overwintering is not clear.

4.4.2 Effect of foliage volatiles on spring activity of adult *P.charybdis*

The approximate temporal correspondence of activity among the three treatments suggests either that the animals were disturbed more during some observations than others, or that a circadian rhythm affected the animals. The former is unlikely because the activity peaks were slightly different among treatments. Adults caged under normal laboratory conditions were much more active during the late afternoon than at other times, but this effect of time of day was always confounded with changes in temperature and lighting. This experiment, under constant light and temperature, suggests afternoon and evening activity.

Mean daily temperatures at Kāhu Farms during the peak of oviposition were around 8-10°C (Fig. 4.25). The 10°C of this experiment therefore corresponds well with the temperature at which adults become active in spring. This experiment suggests that under these conditions, eucalypt foliage volatiles can stimulate activity. The smell of bruised *E.viminalis* old foliage and young foliage (both adult) differ quite noticeably. It is therefore surprising that no significant differences in activity were caused by these two treatments. Because adults need young foliage to oviposit, adults that become active only when it is present waste less time and energy searching.

Foliage volatiles are unlikely to be the main factor that induces adults to emerge in spring, but instead may act to "fine-tune" emergence, ensuring that adults emerge near the optimum time to exploit a resource of limited duration. The resulting increased synchrony of emergence also ensures that individuals are very likely to find mates.

4.4.3 Effect of foliage age on mating and fecundity

New foliage is necessary for oviposition and perhaps also for mating. When young growth is not available, oviposition ceases but copulation may continue. Mating seems therefore to be less closely related than egg-laying to the availability of new growth. Either accurate synchronisation of mating and oviposition is not necessary, implying that females can store sperm for long periods and sperm from more recent matings are not more likely than older sperm to fertilize eggs, or sperm are short-lived and competition among males for matings is intense. Both males and females mate frequently, suggesting that the latter alternative is the case, or that the viability of sperm decreases with time from mating.

The reduced activity in cages containing new foliage was presumably a result of adults' successfully finding and eating this foliage. Adults without new growth but exposed to the smell of old leaves would have spent more time searching unsuccessfully. In fact, the simple distinction between adults that were stationary and those that were moving underrated the difference between treatments. Adults in old-foliage-only treatments were very obviously more active than other adults, particularly in the first two to three days after being offered only old foliage. This also confirms the result that copulation was not the cause of the reduced activity.

4.4.4 Growth of larvae, eggs and pupae

Morphometric analyses showed that larval development followed a pattern typical for many insects. The observed increase of about 1.4x in head capsule width at each moult is usual for many insects (Wigglesworth 1972) and follows Brooks' rule, usually known as Dyar's rule (Crosby 1973). The allometric growth of weight, length and head capsule width is also generally true of insects as well as other animals (Wigglesworth 1972). Edwards and Wightman (1984) found that dry weight of *P.charybdis* larvae was related to the cube of body length, as expected for a volume/linear relationship. I found that live weight increased

more rapidly than this in relation to length, suggesting that larger larvae contained proportionally more water than small larvae. This is consistent with the greater surface area:volume ratio, and hence greater susceptibility to desiccation, of small larvae. This may be particularly significant for larval mortality during the higher temperatures and lower rainfall experienced by second generation larvae (Fig. 4.8).

The method I developed for obviating the effects of time-dependent food quality in development-rate experiments is widely applicable. The results I obtained predicted well the observed phenology of *P.charybdis* at Kāhu Farms in 1979-80. Many foliage-feeding insects are sensitive to nutrient status, secondary compounds or physical characteristics of their host plants. If artificial diets or other means of standardising food quality are not available, then insects that develop at widely varying temperatures may also be influenced by changes in food quality.

The very low estimates of temperature thresholds given by the first experiment on larval development rates are perplexing. The 25°C treatment appeared, in retrospect to show a slightly lower than expected development rate. When this was excluded all threshold estimates except that of the 1st instar increased to around 5-6°C. This is still low compared to the much more rigorous second experiment. Possible explanations include incorrect calibration of the cabinets, error caused by the less frequent checking of insects, or foliage effects of the type discussed above, which the second experiment removed. Nevertheless, this experiment served its primary purpose, which was to get preliminary estimates of development rates for use in the definitive experiment.

In view of the imprecise nature of the results from the first development rate experiment, not a lot of practical significance should be attached to the statistically significant interaction between development rate and instar. No such effect was noted in the second experiment, but if the effect is real, then it both complicates and adds interest to future investigations.

Extrapolation of results from the rearing experiments to field situations depends on several assumptions. Firstly, larvae were fed *E.viminalis*, not *E.nitens*, so care must be taken if these laboratory results are used to interpret population dynamics on *E.nitens*, as at Kāhu Farms. Dugdale (1965a) included *E.viminalis* with those eucalypts liable to severe defoliation by *P.charybdis* over one or two seasons in any three year period, while he grouped *E.nitens* with the least susceptible eucalypt species. Bain (1977) noted that, locally *E.nitens* was sometimes more severely defoliated than Dugdale's classification indicated and Emberson (1984) commented that *E.nitens* was subject to serious attack. At Kāhu Farms, *E.nitens* has always suffered extensive defoliation of adult foliage and clearly should be included with the most susceptible eucalypts such as *E.viminalis*. Furthermore, female *P.charybdis* transferred from Kāhu Farms, where they had fed on *E.nitens*, to Palmerston North where they were fed *E.viminalis* did not show the sudden change in oviposition behaviour that Steven (1973) recorded as common when foliage types were changed and this further supports the contention that *E.viminalis* and *E.nitens* have similar nutritive qualities for *P.charybdis*. Growth rates of *P.charybdis* larvae may be similar on *E.viminalis* and *E.nitens* especially when compared with, for example, *E.linearis* or *E.obliqua*, which Steven (1973) found were poor foliages for *P.charybdis*. My results are useful for comparison with future studies, and it would be constructive to compare development rates on *E.viminalis*, *E.nitens* and some other commercially important species such as *E.globulus*, particularly to see if any interaction between instar duration and foliage was present.

A second premise is that development rates at a constant temperature were the same as development rates under a fluctuating temperature with the same mean as the constant temperature. Kitching (1977) showed that there was wide variation among insect species for development rates under constant vs fluctuating temperatures. Without specifically testing this premise for *P.charybdis*, one cannot say to what extent the results are affected, although for laboratory comparisons the assumption is irrelevant.

For calculating temperature thresholds for development, the last premise is that development rate is a linear function of temperature. Stinner, Gutierrez and Butler (1974) showed that a sigmoid function predicted development rates much more accurately than a linear model. However, Kitching (1977) stated that neither a sigmoid nor a polynomial function gave any appreciable improvement over a linear function for sheep blowfly (*Lucilia cuprina*; *Diptera: Calliphoridae*) adults, and he considered that for most temperatures encountered by an insect in the field, the linear function adequately described the development/temperature relationship. Harcourt and Yee (1982) developed a polynomial algorithm for predicting the duration of insect life history stages and they found that this algorithm as well as the sigmoid model of Stinner *et al.* (1974) and a thermal summation based on a simple linear model all gave a good fit to observed data. For field extrapolations the linear model should prove adequate considering the difficulty of measuring the temperatures actually experienced by larvae on leaves.

At Kāhu Farms in 1979-80, the first eggs to have been laid in spring should have hatched after about 74 °$Days_{5.6}$, at the beginning of October. These first larvae would have left the trees during the first half of November and emerged as adults 240 °$Days_{7.7}$ after hatching; around the beginning of December. This fits very well with the observed pattern. Eggs that hatched late in the first generation could have given rise to adults by mid January. It seems potentially possible for early-hatching eggs in spring to give rise to more than two generations. However, the trap samples and stage-frequency analysis suggest that later larvae in the first generation and early-hatching larvae in the second have a much lower probability of survival and this serves to keep the two generations relatively discrete.

The time taken for unfed first instars to die at different temperatures probably overestimates survival in the field at corresponding temperatures. Larvae in the field will be much more exposed to potentially desiccating conditions compared to those in the humid lab.

environment. Also, although larvae in the lab. survive longer at lower temperatures, their reduced activity and slower development exposes them to greater risks of predation and/or unfavourable weather under field conditions.

None of the development times given by authors listed in the introduction to this section (Section 4.1.2) conflict with my results. Clark's (1930) estimates of five to seven days each for the first and second stadia, four to six days for the third stadium and four to five days for the actively-feeding stage of the fourth stadium agree with my result that there are only small differences in larval duration. Styles' (1966) estimate of 10-15 days for larval development suggests a high mean temperature of about 20-25°C or a better food source than the *E.viminalis* I used. Finally, it must be remembered that development rates are affected greatly by foliage characteristics. Steven (1973) found that at 25.6°C the time from unfed first instar to pupa varied from 12.2 to 17.3 days, depending on the type of foliage used. This amounts to a 91 $°Day_{7.7}$ range, or about a 40% increase in duration from poor to good quality foliage. When Steven's results are considered in relation to my temperature vs development rate results, one can see that the potential duration of the larval stage varies enormously. The opportunity is present for an interesting and useful analysis of the relationship between growth rates, temperature and foliage.

4.4.5 Population dynamics

The cause of the high mortality of newly-hatched larvae, in the absence of any significant predators, appears to be failure to find suitable foliage for feeding. Actual death may be caused by being dislodged, starvation or desiccation. The failure to find food is probably a function of two factors: the availability of suitable foliage and the distance a larva has to travel to find it. First instar larvae are physically incapable of biting through the cuticle of even moderately-toughened leaves, so they must find newly-flushed leaves. Scarcity of foliage is caused by defoliation or the abscission of damaged foliage which often follows severe attacks (Jacobs 1955); by a lower rate of foliage production; or by a faster rate of leaf

toughening. The distance a larva must travel is also affected by several factors. If egg numbers are high, clusters may be laid further back from young foliage because of the consequent competition for oviposition sites. Furthermore, if the ratio of incubation time to the rate of foliage toughening increases, then there will be more toughened leaves and a greater distance to travel between the egg cluster and new foliage.

Larval food location seems to depend largely on gustation or mechanoreception. Simple tests suggested that olfaction may occur over distances of a few centimetres. This is unlikely to provide any directional guidance under field conditions. My observations suggest that larvae may distinguish petioles of old and young leaves by any or all of their diameters, taste or resistance to biting. Larsson and Ohmart (1988) noted that *P.atomaria* larvae were also able rapidly to distinguish young and old leaves by palpating the base of the leaf. Bifurcations in the stem create more difficult problems for the larvae, particularly if both branches are similar in diameter. The female's oviposition behaviour minimises the chance of larvae encountering a bifurcation and even if shoot growth presents larvae with such a choice, both branches will probably carry newly-flushing foliage.

It is apparent, therefore, that mortality in the first larval stadium could be a result of both density-dependent and density-independent factors. Scarcity of foliage caused by defoliation clearly results in density-dependent mortality, but larval density probably does not significantly affect the rate of leaf toughening. The results of the experimental study of 1st instar mortality suggest that when new leaves are abundant, mortality is independent of density.

The estimate of high mortality of *P.charybdis* on the ground at Kāhu Farms is subject to possible error if fourth instar larvae dropped from the tree before they were capable of successfully pupating. Factors like wind gusts or death of larvae on the tree could have caused this effect. These would increase the apparent number of larvae surviving the fourth

instar on the tree. There are two reasons why this error is likely to be small. Firstly, the larvae have well-developed abdominal adhesive organs which make them very difficult to dislodge. The most probable way in which larvae could be dislodged from the tree is if the leaf or branch itself is blown down and because larvae are found predominantly on young foliage rather than the older leaves which are far more prone to abscission, then loss of larvae this way is probably small. However, larvae feeding on new foliage are at risk because other larvae feeding on the same leaf may excise leaf sections that support larvae. Secondly, diseases that may have cause moribund larvae to fall were not observed. The only pathogen I found was a fungus which attacked occasional late second-generation larvae, but in this case the cadaver was stuck firmly to the leaf by fungal hyphae. The number of larvae caught, then, may slightly overestimate the actual number leaving the tree to pupate.

The very high apparent mortality at Kāhu Farms during this stage of the life history contrasts with Styles' (1969,1970) results. He found a maximum mortality of only 36.6% for mature larvae placed in 203 mm diameter x 127mm deep metal cylinders sited in grass turf near his laboratory. Each contained 76mm of sieved soil covered with a layer of sphagnum moss and was closed at both ends with fine nylon mesh. Maximum mortality was 34.6% in similar containers open at one end to admit soil-inhabiting predators. However, Styles' experiments were predisposed in several ways to underestimate mortality. Larvae in his containers had an excellent medium in which to pupate and this would protect them from physical hazards such as desiccation or from predation while searching for a site. Predators had only restricted access to the containers which were left open at the base, and this may have caused similarity between the two treatments (open vs closed containers), rather than Styles' interpretation that predation was unimportant in field populations. The loose pumice soils at Kāhu Farms are generally free-draining and pupae may often have encountered very dry soil conditions. Unlike Styles' larvae, fourth instars at Kāhu Farms were exposed to predation by such litter-searching birds as thrushes, blackbirds, californian quail and pheasants and in some places suitable pupation sites may have been scarce, particularly where the ground was very dry and

hard with little surface litter. These birds and possibly hedgehogs (*Erinaceus europaeus*), mice (*Mus musculus*) and rats (*Rattus* spp.) may eat significant numbers of larvae, prepupae, pupae and teneral adults. These vertebrate predators were denied access to Styles' larvae. Finally, it is probable that some mortality resulted from trampling by sheep, which were occasionally grazed at Kāhu Farms. During some periods the number of *P.charybdis* in the soil or litter was quite high; over 20 m^{-2} in early December 1979, for example and the larvae were therefore at risk.

Insectivorous birds of several species were active at Kāhu Farms during December 1979 and I saw silvereyes and a shining cuckoo eating larvae that may have been *P.charybdis*. Nevertheless it is doubtful whether the intensity of predation was great enough to affect larval *P.charybdis* populations. P.A. Morrow, who has studied eucalypt/insect herbivore systems extensively, has suggested that the putative high levels of herbivory in Australian eucalypt forests may result from the relative scarcity of insectivorous birds there, compared with many other continents (Strong, Lawton and Southwood 1984: p154). If this is so for Australian forests then it appears very improbable that birds could significantly affect *P.charybdis* populations in New Zealand.

4.4.5.1 Stage-frequency analysis

The striking feature of the results of the stage-frequency analysis is the apparently anomalous estimates of instars 2+3 in the first generation and instar 1 in the second generation. The estimated number of individuals in these stages exceeded the number in the preceding stage. The obvious reason for this is that the durations of these stages in the field exceeded the durations used in Southwood's method of stage-frequency analysis. Comparison of the stage-frequency graphs for these stages (Fig. 4.8) with the time-series of defoliation (Fig. 5.4) shows that the apparent lengthened development times coincided exactly with peaks in defoliation. Thus, the peak of defoliation in the first generation coincided with peak numbers of 2nd+3rd instars, while second generation defoliation coincided with peak first instars in

that generation. This strongly suggests that at these times there was a significant shortage of high-quality food, caused by intra-specific competition and resulting in protracted development. As previously discussed, Steven (1973) found that at constant 25.6°C, larvae fed different foliage species had development times that ranged from 12.3 to 17.3 days. Larsson and Ohmart (1988) also showed that development times of P.atomaria larvae fed old foliage from the 4th instar averaged 29% longer than those for larvae fed young foliage from the same tree. They found that all larvae forced in instars 1 and 2 to feed on mature leaves, died. Grabstein and Scriber (1982) found that larvae of *Hyalophora cecropia* (Lepidoptera: Saturniidae) had lower growth rates and food utilization efficiencies when food was restricted and they quoted other examples where food deprivation affected the post-ingestive utilization of food. Watanabe (1982) found that the tough, thick leaves from tall *Zanthoxylum ailanthoides* trees (Rutales: Rutaceae) caused greater mortality and slower development of *Papilio xuthus* larvae (Lepidoptera:Papilionidae) than tender, thin leaves from short young trees of this species. He attributed the difference to diarrhoea, starvation or failure to ecdyse.

4.4.5.2 Key factor analysis

Methods for identifying the key stage mortalities that most influence the variation in total generation mortality were reviewed recently by Barlow, French and Pearson (1986). The method used here, that of Manly (1977, 1979) was considered by Barlow *et al.* to be the only one of numerous methods that considers the effects of density-dependent mortality factors. All other methods, including the widely-used techniques of Varley and Gradwell (1960) and Podoler and Rogers (1975) can suggest misleading conclusions if a factor unrelated to total mortality varies by chance in a similar way to total mortality *and* if that factor precedes a strongly density-dependent factor. Manly's method deserves wider acceptance, yet a recent paper (Stiling 1988) reviewing key factor studies did not mention it and implied that Podoler and Rogers' (1975) method was the latest refinement.

The actual causes of variation in mortality among populations on different trees in the second generation are not clear. Predation by birds is unlikely, as I never saw birds definitely taking *P.charybdis* larvae. Bird density appeared to be well below that which could be considered sufficient to affect *P.charybdis* populations. Likewise, invertebrate predators were seldom observed on sample shoots, nor adjacent foliage. The disease noted by Carne (1967) during the second generation was never observed by me, nor do I know of any records of its reoccurrence since Carne's observations.

Morrow (1977) argued that the effects of defoliation of eucalypts were more severe after trees had begun to grow vigorously, thus mobilising food reserves. If this is the case, then defoliation during the first generation may leave some trees better able than others to recover after midsummer. Consequently, these trees may be able to support higher survival rates of *P.charybdis* larvae.

4.4.5.3 Density dependence

Detection of density-dependence in un-manipulated populations is problematic. The statistical problems can be at least partly surmounted by the method used here, i.e. that of Smith (1973). However, theoretical considerations suggest that even when density-dependence does occur, it may be impossible to detect because of stochastic effects and undersampling (Hassell 1985, 1987; Hassell, Southwood and Reader 1987).

The regression of k-value on untransformed initial density as a test of density-dependence was suggested by Smith (1973), for two reasons. Firstly, the test proposed by Varley and Gradwell (1968), *viz* that of regressing k-value on log(initial density), is statistically suspect because log(initial density) occurs on both sides of the regression equation. Sampling errors in initial density will appear also in the k-value, thus exaggerating density-dependent effects.

Significance tests are invalid. However in Smith's (1973) method, *k*, which incorporates log(initial density), is regressed against untransformed initial density. The relationship between initial density and its log-transformed value is not close, so sampling errors do not significantly affect the independence of the carrier and response variables. The effect of sampling errors in the carrier variable when the axes are independent is to underestimate the least squares regression coefficient. Thus, the test is conservative (Kuno 1971, Smith 1973) and the choice of a significance level $\alpha = 0.1$ rather than the conventional but arbitrary $\alpha = 0.05$ seems reasonable.

An alternative and widely-used test is to consider the regression of log(initial density) on log(final density) and vice versa. If both regressions have slopes significantly different from 1.0 and both have the same sign, then density-dependent effects exist (Varley and Gradwell 1968). This is highly conservative and therefore will often lead to incorrect conclusions (Smith 1973).

The second reason advanced by Smith (1973) for the use of his method is that it is founded on a biologically more realistic model. Smith's method is based on the Ricker model (Barlow, French and Pearson 1986), which incorporates the effects of resource limitation and allows for overcompensation. Smith apparently did not recognise the last effect as he incorrectly stated that his method was based on the logistic model, which must approach equilibrium asymptotically. The alternative tests for density-dependence described above assume a simple model of the form

$$N_{t+1} = e^{rm} N_t^{1-b}$$

This has been criticised as a model for detecting density-dependence (Smith 1973, Huffaker, Berryman and Laing 1984).

The apparent absence of density-dependence during the soil-dwelling stages is not proof that it does not occur (Hassell 1985, 1987; Hassell *et al.* 1987; Southwood 1978). Nevertheless, there seems to be sufficient evidence to accept that survival in the pre-pupal to adult stage is not affected by density. *P.charybdis* in New Zealand lacks specific predators or parasites at this stage. It seems unlikely that generalist vertebrate predators like birds, rats, mice or hedgehogs could exert sufficient pressure on soil-dwelling *P.charybdis* unless pre-pupae stayed within the surface litter instead of burrowing. Even then, the functional response of these predators would have to follow a sigmoid model (Hassell, Lawton and Beddington 1977), with the most probable cause of this pattern being a change in searching behaviour to concentrate on *P.charybdis* at high densities. Studies on density-dependent predation of the winter moth (*Operophtera brumata* (L.)) showed that beetles were more important than vertebrate predators (East 1974, Kowalski 1976, Varley, Gradwell and Hassell 1973). Styles' (1970) experiments suggested that invertebrate predation is not important for *P.charybdis* on the ground, although his methods were flawed.

A recent review of population dynamics studies (Stiling 1988) found only three cases of density-dependent mortality in pupae of non-parasitic insects, out of 49 studies that investigated density-dependence. Only one of the three insects was free-living: this was *O.brumata* (Varley *et al.* 1973). Density-dependent mortality during the pupal stage of free-living insects does not appear to be common.

Ten studies reported density-dependence in larval/nymphal stages. Nine of these insects were free-living, like *P.charybdis*. Starvation, suggested as the probable cause of density-dependent mortality of second-generation, foliage-dwelling *P.charybdis* at Kāhu Farms, was identified as the source of density-dependence in four of these nine studies.

4.4.6 Diapause

The results of the diapause experiment show that diapause was induced in the Kāhu Farms population before 4 January. To be consistent with the observations of an apparently continuous life history in very warm parts of New Zealand, diapause must be induced by some factor that occurs before mid-summer and is positively correlated with environmental conditions the following winter. Photoperiod is obviously precluded and the most likely factors are maximum temperature or the rate of temperature accumulation.

Steven's (1973) results suggest that the adult is both the sensitive stage and the diapausing stage. My samples contained adults from two generations: those that emerged in mid summer and some that emerged in autumn, yet the summer-emerging adults reproduced before diapausing while the autumn adults did not. Therefore a period of post-induction development seems necessary for diapause onset. This may proceed much more rapidly in the autumn-emerging adults, thereby preventing them from reproducing. In many insects diapause development (after onset) proceeds faster at lower temperatures, although the converse is true in other insects (Tauber and Tauber 1973). During the period 4 January to 13 March 1980, mean fortnightly temperatures near Kāhu Farms were well below the constant 25°C of the experiment and this post-induction development may therefore have proceeded faster in the field. Since low temperatures generally herald the approach of winter, faster post-induction development before diapause onset increases the insects' likelihood of survival during winter. This effect compensates for the younger age of later-collected adults and therefore tends to synchronise diapause onset. Alternatively, the development may have been inversely correlated with photoperiod and a similar process would have caused the approximate synchronisation of diapause onset.

Diapause may have been induced by stress associated with the transfer to Palmerston North and in particular with the change of foliage from *E.nitens* to *E.viminalis*. I found that females sometimes ceased laying or scattered single eggs during their half-day transit from

Kāhu Farms to Palmerston North, but they readily ate *E.viminalis* and oviposited normally when released in the insectary. Thus the transfer appeared to cause minimal stress.

A complicating factor in the experiment was that food quality could not be controlled and although care was taken to ensure that all cages were treated identically, the *E.viminalis* foliage had to be collected from the field every few days. Diapause may therefore have been induced by changes in foliage characteristics. Tauber and Tauber (1973) reviewed the effects of nutrition on diapause induction in several arthropods, and they specifically mentioned four examples where diapause induction was caused by increasing age of the host plant's leaves. This hypothesis is a simpler explanation of my results, but until a suitable artificial diet can be developed or food quality otherwise controlled, it remains uncertain whether diapause was induced before mid summer at Kāhu Farms or by changes in food quality after adults were transferred to Palmerston North.

No single factor appears necessary for diapause induction. Low temperature treatment alone is apparently sufficient (Steven 1973), photoperiodic effects are both unnecessary and apparently insufficient, but changes in foliage characteristics may be sufficient without interaction from temperature. Temperature and foliage effects may interact in the field to alter the critical threshold for diapause induction and other factors such as photoperiod may also modify the insects' responses.

As indicated by the acceptance of food in late July, diapause was completed some time during the previous seven weeks. Diapause development therefore required six to 16 weeks for completion, allowing a three week error in estimating the time of diapause onset. Steven (1973) found that his diapausing adults accepted foliage after six weeks' inactivity at temperatures from 21°C to 25.6°C and day lengths of 12-15 hours and this agrees well with my results.

These results suggest that diapause in *P.charybdis* is a Category 1 type, of Tauber and Tauber (1976). Typically this comprises a true autumnal-hibernal diapause that does not require a specific stimulus for completion of diapause development. Diapause ends some time after mid-winter and is replaced with a quiescence, as temperatures are usually well below the threshold for post-diapause activity. Emergence in spring is regulated by temperature accumulation and this pattern serves to synchronise the emergence of the over-wintering population. If this type of diapause includes that of *P.charybdis*, then it allows this insect to respond to spring weather to optimise the use of newly-flushing eucalypt growth.

4.5 Conclusion

P.charybdis is an Australian insect, which inhabits a very different environment in New Zealand. The high levels of defoliation in Australian eucalypt forests (Journet 1981, Morrow 1977a, Springett 1978) imply intense interspecific competition among a plethora of defoliating insects. New Zealand has relatively few important defoliators of eucalypts, and *P.charybdis* in New Zealand lacked significant predators or parasites until the recent establishment of *E.nassaui*. At Kāhu Farms, the key stage in the summer generations was the foliage-dwelling second generation. This was also the stage at which density-dependent mortality operated. High mortality does occur between egg hatching and the establishment of first instars on new leaves, and during the soil-dwelling stages, but for both these stages mortality is apparently independent of density. The probable cause of density-dependent mortality in the second generation is intra-specific competition caused by starvation. Increasing shoot complexity makes it harder for larvae to locate food and this may also increase mortality.

The separation of the two generations around mid-summer was caused primarily by the pattern of oviposition and was accentuated by the relatively high threshold temperature for development of the larvae and pupae. My laboratory studies showed that new foliage affects

adult activity and oviposition but this is not sufficient to explain the decline and eventual cessation of oviposition in midsummer.

The two-generation life history at Kāhu Farms is probably typical over much of New Zealand. Only in very warm climates where eucalypts can produce new foliage all year round is it possible for *P.charybdis* to breed continuously, as at Maketu in the Bay of Plenty, where new *E.viminalis* foliage and all stages of *P.charybdis* can be found at any time of year (W. Faulds *pers. comm.*). Conversely, in regions that allow only a single, short growing season, temperature accumulation will be inadequate for completion of two generations and only one may occur.

The temperature vs development relationships I described provide the essential background for many sorts of future studies. The data for egg and pupal development times have particularly wide application, because these stages are least affected by foliage characteristics. For the larval stages, the developmental threshold can be expected to stay constant irrespective of foliage characteristics. This allows the simple linear degree-day model of development for these stages to be fitted easily to developmental data obtained for various foliages.

Like many insects in a seasonal environment, *P.charybdis* avoids unfavourable conditions by diapausing and therefore must recolonise its habitat each spring. New growth at Kāhu Farms and most other areas of New Zealand is a resource of limited duration. These studies have shown how the biology of *P.charybdis* ensures that these problems are successfully surmounted.

Chapter 5. Growth and Defoliation of *Eucalyptus nitens*

5. GROWTH and DEFOLIATION of *Eucalyptus nitens*

5.1 Introduction

The host plant's phenology is clearly very important for *P.charybdis*. This has been discussed briefly in chapter four, and will also be considered in chapter six. Both the amount and nutritive value of eucalypt foliage change as the summer progresses, and this affects behaviour, survival, larval growth rates and fecundity of *P.charybdis*.

For this reason, I needed to know the growth pattern of *E.nitens* at Kāhu Farms. Because eucalypts are evergreen, patterns of growth are not as immediately obvious as for deciduous trees. Leaves may be retained for up to three years, or shed after six months, and leaf fall is affected by flowering, fruiting, insect attack, growth flushes and weather (Morrow 1977). Information on *E.nitens* is restricted (Pederick 1979, Pederick and Lennox 1980) and the results of silvicultural trials under New Zealand conditions have only recently become available (Frederick, Madgwick, Oliver and Jurgensen 1984, Frederick, Madgwick, Jurgensen and Oliver 1986, Wilcox 1980). Local variation in soil structure, planting method and micro-climates clearly influences growth and vigour of *E.nitens* in the central North Island (Frederick *et al.* 1984, 1986; B.Poole *pers. comm.*), and this confirmed the necessity for recording the growth pattern of *E.nitens* at the Kāhu Farms site.

Defoliation resulting from insect attack is an obvious cause of changing leaf numbers, and one that is of considerable practical importance. To interpret changes in leaf numbers I had to determine the contribution made by defoliation, and I also wished to investigate the pattern of defoliation of *E.nitens* at Kāhu Farms because of its importance in forest production.

5.2 Methods

Shoot sampling is described in chapter three. The data collected comprised counts of the number of old leaves on each shoot and an estimate of the amount of new growth until 17 January 1980, inclusive, and thereafter comprised counts of the total number of leaves. Old and new growth records were later pooled and recorded as total leaves for each of the first four samplings.

Methods for studying defoliation are also described in chapter three. Strictly, the estimates of defoliation were ranks from zero to five, but they can also be considered as percentage classes, with zero being the absence of defoliation. Thus:

0 = no defoliation
1 = 1-19% defoliation
2 = 20-39% defoliation
3 = 40-59% defoliation
4 = 60-79% defoliation
5 = 80-100% defoliation

This scale can be considered as an imprecise interval scale rather than an ordinal scale.

5.3 Results and analysis

Raw data are tabled in Appendix three. A plot of the mean number of leaves per shoot on each sampling date is shown in Fig. 5.1. A small amount of new growth was already present when sampling began, and on this first sampling date (27 September 1979), 1935 old leaves were recorded. By the final sampling date (10 April 1980) 2554 leaves were present on the 70 shoots. There was no recently-flushed foliage present on this final date, and most foliage

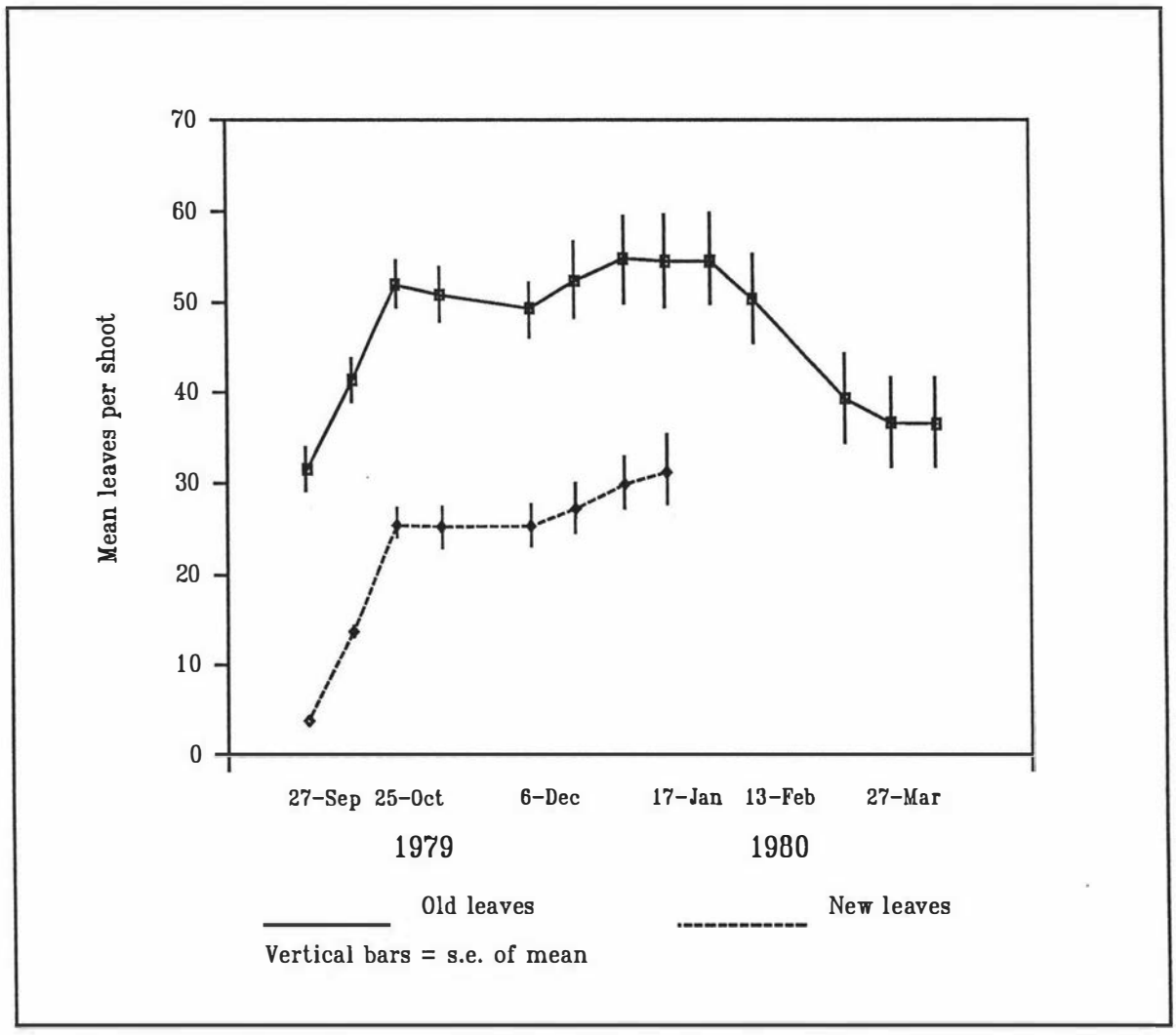


Fig. 5.1 *E.nitens* shoot growth at Kāhu Farms, 1979-80 summer.

was well-hardened. Therefore, the nett increase in number of leaves during the 1979-80 summer was roughly 32% . Fig. 5.1 shows an unsurprising pattern of rapid increase in spring followed by relatively stable leaf numbers during summer, and a steady decline in total leaves from February, presumably due to the combined effects of late summer-autumn abscission and defoliation during the second *P.charybdis* generation.

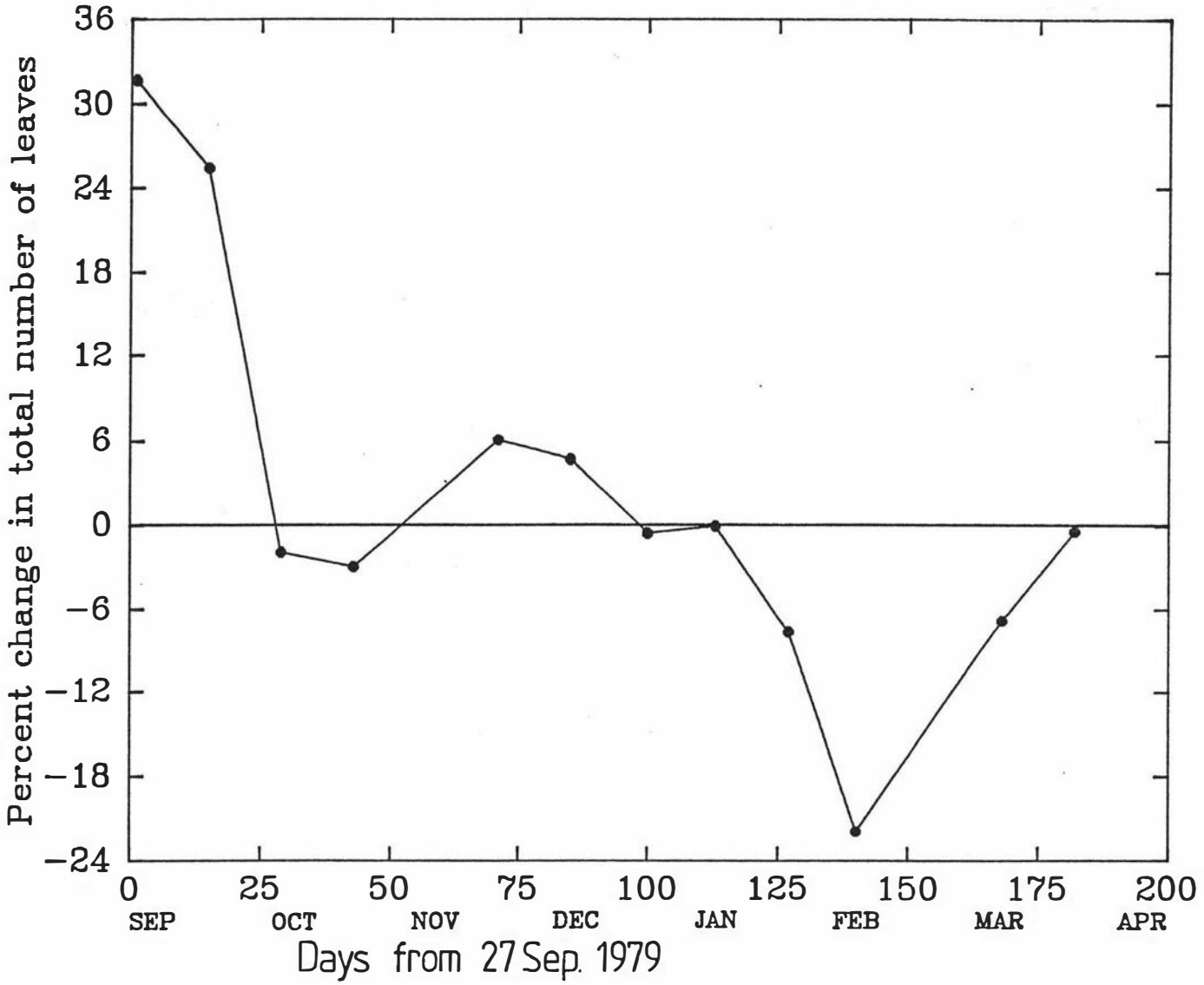
Extrapolation by eye of the plot of new growth suggested that the trees began producing new foliage in mid-September. During this September-October initial period of rapid leaf production new foliage accumulated at about one leaf per day. However, after 25 October, the nett amount of new growth stayed constant and only began to increase again slowly after the peak of first generation defoliation. The increase in total leaf numbers paralleled that of new foliage. After 17 January 1980, when second generation defoliation peaked at very high scores corresponding to over 80% defoliation, total leaf numbers declined and gradually stabilised towards winter.

To examine more closely the changes in leaf numbers, data were expressed as the percent change in leaf numbers between sampling dates (Fig. 5.2). Nett growth was positive only in spring and, perhaps surprisingly, in the second half of the *P.charybdis* generation. Nett growth was negative throughout the entire second *P.charybdis* generation.

For the period 27 September 1979 to 17 January 1980, information on the total number of new leaves was combined with defoliation records to estimate the rate of production of new leaves, as follows:

At any time, the total number of new leaves is equal to the number of leaves produced minus losses. Assuming that losses caused by factors other than defoliation are insignificant, the potential standing crop of new leaves at time t (PSC_t) differs from the actual standing crop at that time (SC_t) by the amount lost to defoliation. Actual standing crop is the amount remaining after defoliation has reduced potential standing crop:

Fig. 5.2 Percent change in total leaf numbers at Kāhu Farms, 1979-80 summer.



$$SC_t = PSC_t(1-D/100)$$

where D_t = percent defoliation at time *t*, so:

$$PSC_t = SC/(1-D/100)$$

Thus, T, the total production of leaves during the interval *t-1* to *t* is defined by the formula:

$$T = SC/(1-D_t/100) - SC_{t-1}$$

and if there are n days in the period *t-1* to *t*, then the daily rate of production of new leaves is simply T/n.

Rate of production of new leaves declined markedly after 25 October 1979, despite low defoliation (Fig. 5.3). Through November and December, trees produced leaves at a rate that compensated for the increasing rate of defoliation (Fig. 5.1, 5.3)

Defoliation estimates for each shoot during the 13 sampling dates are presented in Appendix Four. A Plot of the sum of defoliation scores against date is shown in Fig. 5.4. Two peaks of defoliation were apparent; the first in early December and the second in mid February.

The two major weather factors, temperature and rainfall, could *a priori* be expected to affect leaf growth, and these probably interact with defoliation. Spearman's rank correlation coefficient (r_s)indicated significant associations between the percent change in leaf numbers and rainfall (r_s = 0.755*) and percent change in leaf numbers and defoliation (r_s = -0.645*) but not between the remaining four combinations. After ranking the data I calculated partial correlation coefficients for the ranks, using the standard formula (Snedecor and Cochran

Fig. 5.3 Estimated rate of production of new leaves, Kāhu Farms 1979-80. See text for method of estimation.

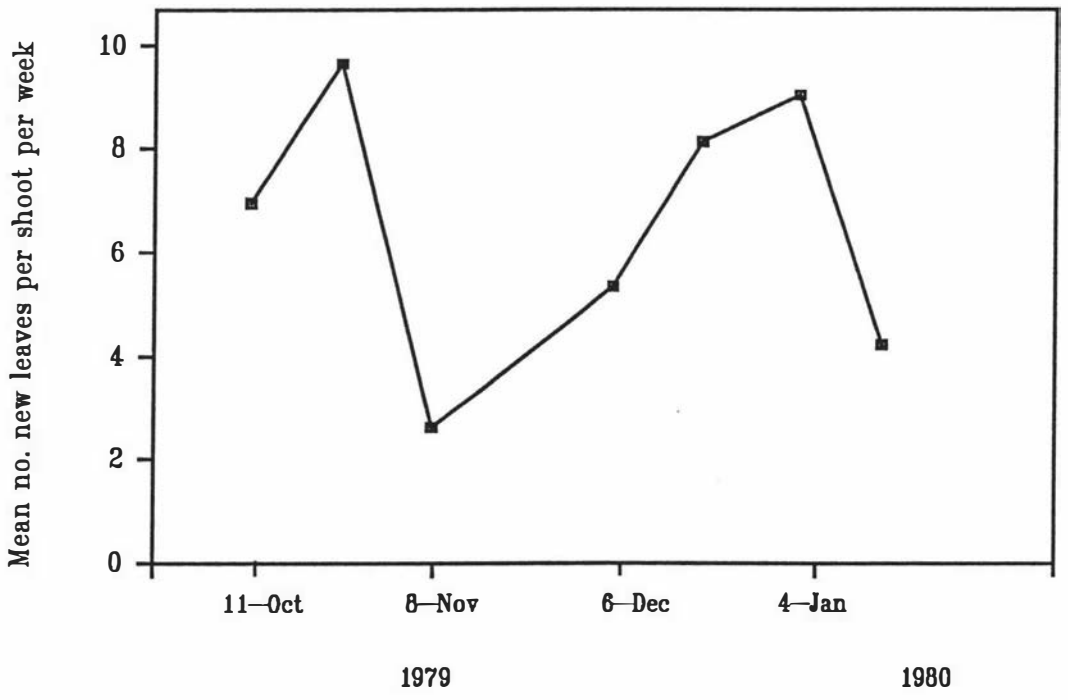
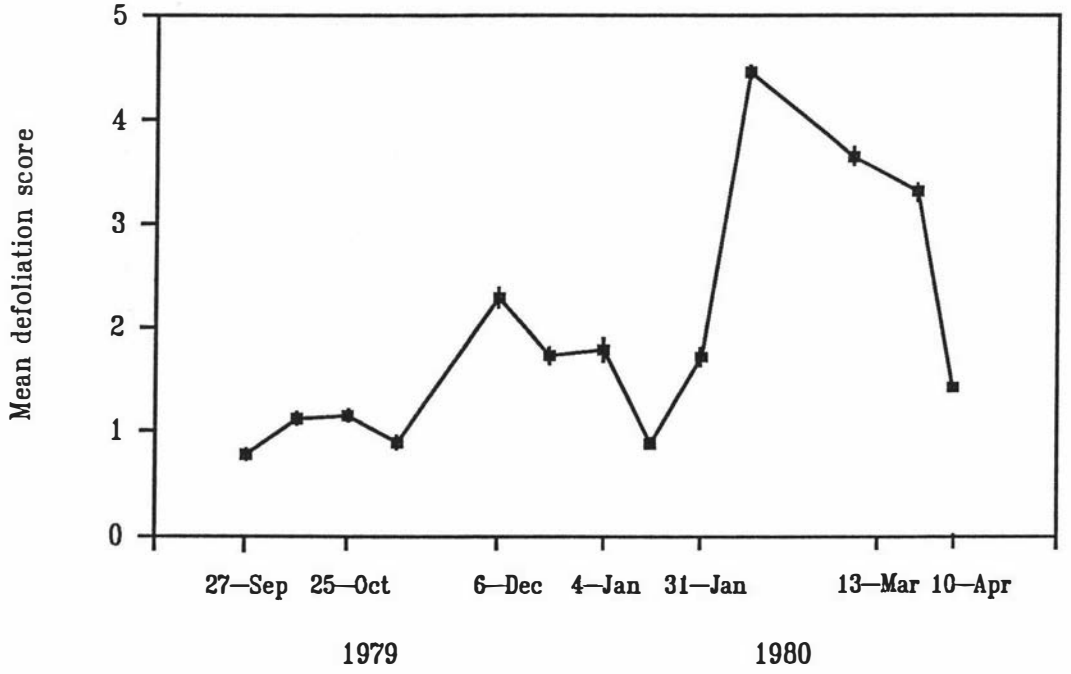


Fig. 5.4 Progression of defoliation at Kāhu Farms during the 1979-80 summer. Vertical bars = s.e. of mean



1980). This was equivalent to using Spearman's r_s in the usual formula, so the partial correlation coefficients were compared with critical values of r_s for n-4 = 7 degrees of freedom. No significant associations were found. When the same method was used to calculate partial correlation coefficients for pairs of variables considering only three at a time, the association between percent change in leaf numbers and rainfall holding defoliation constant was significant at the 5% level (r_s = 0.658*), but none of the remaining 11 combinations were significant.

Plots of percent change in leaf numbers against rainfall and against defoliation are shown in Fig. 5.5 and Fig. 5.6. Theil's non-parametric method was used to fit regression lines to both plots. The significance of the regressions was tested used Theil's method, based on Kendall's τ (Daniel 1978), with α = 0.5. Both regressions were significant (Table 5.1).

TABLE 5.1 Regression statistics for % change in leaf numbers on total defoliation and on rainfall (mm) during preceding fortnight. (Theil's non-parametric method (Daniel 1978)).

Carrier	Regression coefficient	Intercept	τ
Defoliation sum	-0.0695	11.964	-0.455**
Rainfall	-17.257	4.0	0.564**

Defoliation intensity is a function of the number of defoliators present and the rate at which they eat. Since larger larvae have higher consumption rates per individual, the relationship between defoliation and larval stage was examined more closely.

The association between defoliation and larval stage was examined for both generations by comparing defoliation per tree with larval numbers per tree at the time of peak defoliation (Fig. 5.7). Sample correlation coefficients were calculated for each combination of *P.charybdis* generation and larval stage. For both generations all trees were considered and

Fig. 5.5 Regression of % change in leaf numbers on rainfall during prior fortnight. Kāhu Farms, 1979-80.

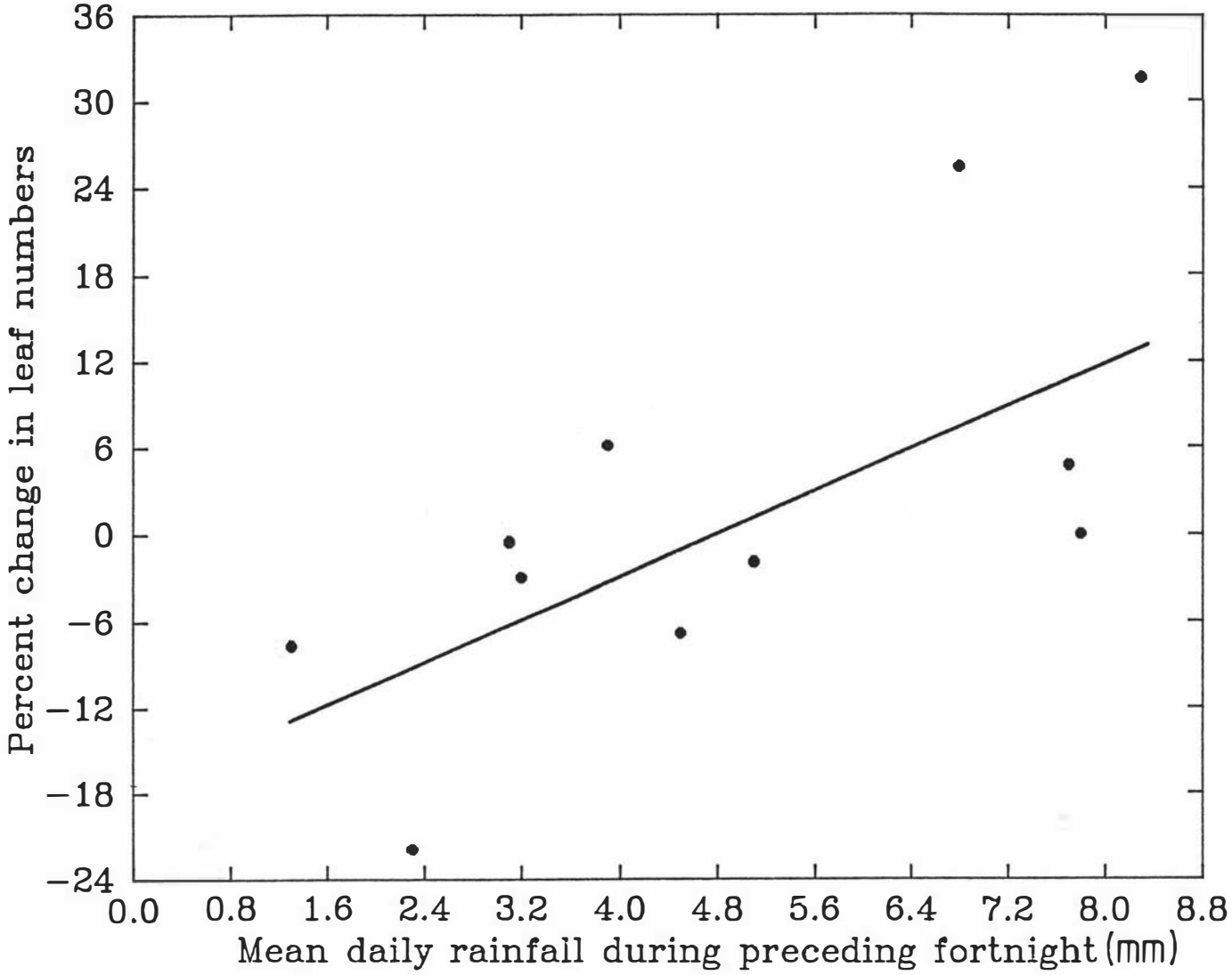
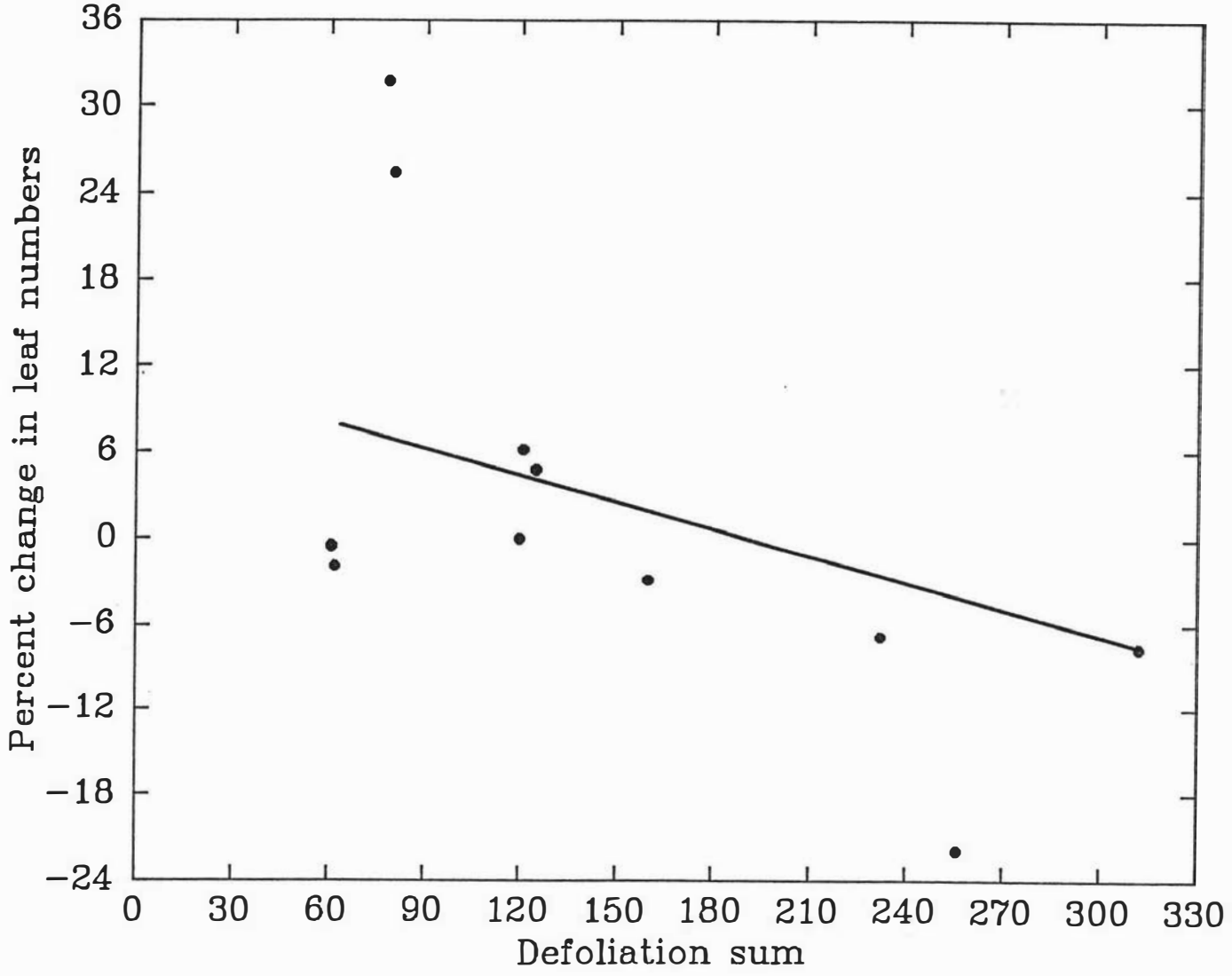


Fig. 5.6 Regression of % change in leaf numbers on defoliation. Kāhu Farms, 1979-80.



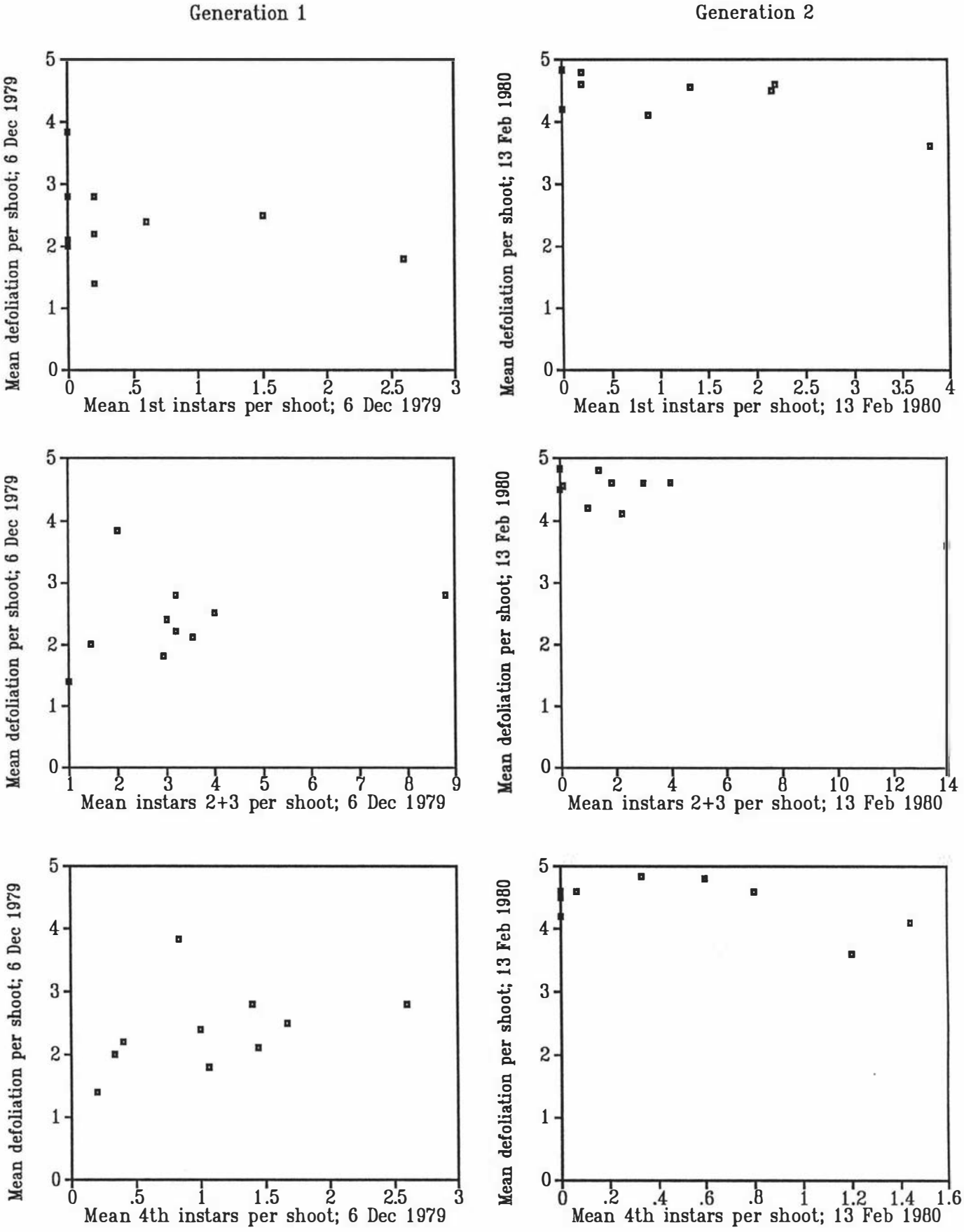


Fig. 5.7 Defoliation *vs* density of *P.charybdis* larvae: analysis by larval stage and generation. Kāhu Farms, 1979-80 summer.

for the first generation the analysis was repeated with tree 7 excluded as a likely outlier (Table 5.2).

TABLE 5.2 Sample correlation coefficients (r) for association of defoliation with *P.charybdis* larval stage.

	Instar 1	Instars 2+3	Instar 4
Generation 1 (6 Dec 1980)			
All trees	-0.2855ns	0.2833ns	0.3841ns
Tree 7 excluded	-0.191ns	0.6949*	0.7838*
Generation 2 (13 Feb 1980)			
All trees	-0.6348*	-0.7731**	-0.548ns

Facultative plant defences have been noted for other plants, and may be relatively common (Carroll and Hoffmann 1980, Edwards and Wratten 1983, Haukioja 1980,1982, Haukioja and Hakala 1975, Janzen 1979, Karban 1983, McIntyre, Dodds and Hare 1980, Maugh 1982, Russell, Sutherland, Hutchins and Christmas 1978, Schultz and Baldwin 1982, Valentine, Wallner and Wargo 1983). To test whether this effect was present in the *E.nitens/P.charybdis* system at Kāhu Farms, first- and second-order serial correlation coefficients, based on both Spearman's and Pearson's methods, were calculated for the defoliation samples (Bhattacharyya and Johnson 1977). No significant correlations were found.

An exploratory plot of defoliation sum against rainfall during the prior fortnight indicated a strong inverse relationship between these two variables. The relationship was investigated further by examining the effect of deviations from the seasonal trend in rainfall on defoliation. The time series of fortnightly rainfall was smoothed using the sequence **4253H**

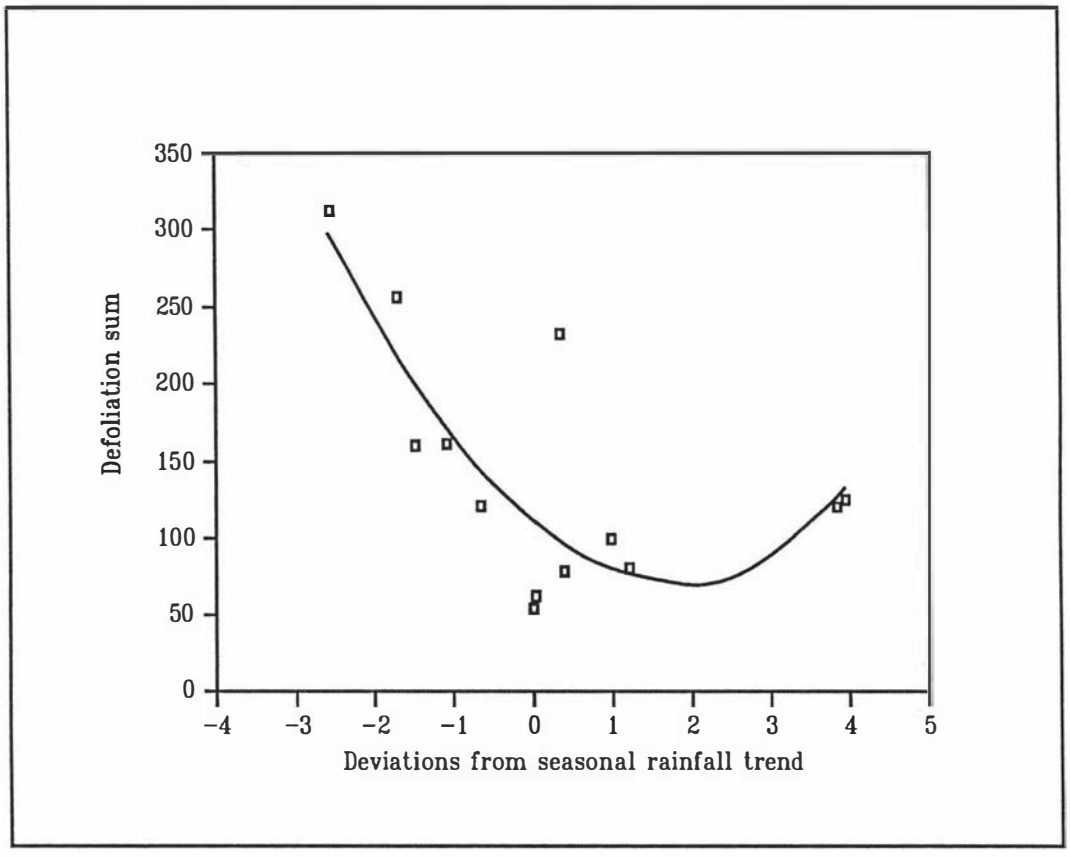


Fig. 5.8 Defoliation *vs* rainfall during prior fortnight. Kāhu Farms 1979-80 summer.

(Velleman and Hoaglin 1981) and the residuals used as indicators of deviations from the seasonal trend. When defoliation sum was plotted against residual rainfall, the inverse relationship was still apparent (Fig. 5.8). A least squares linear regression model was fitted and residual plots examined. The residual *vs* predicted plot suggested that the curve was non-linear, confirrning the impression given by Fig. 5.8. A second model was fitted, incorporating the square of residual rainfall as a second carrier. This described the relationship very well (Table 5.3). Defoliation sum on 27 March 1980 was markedly higher than the expected value, so the regression was re-fitted, omitting this point. More than 88% of the variation in defoliation was explained by this model, compared with about 63% for the quadratic that included the 27 March data.

TABLE 5.3 Parameters of the quadratic model describing the relationship between defoliation and deviations from the seasonal trend in rainfall. (27 March 1980 data included)

Carrier	Coefficient	*t*	P
Constant	111.86	5.963	<0.001***
Residual rain	-42.1	-4.048	0.002**
(Residual rain)^2	11.8	3.185	0.010**

There was no significant correlation between defoliation and temperature.

5.4 Discussion

The correspondence between changes in total leaf number and changes in nett amount of new growth suggests that old leaves were shed at a roughly constant rate. This is consistent with the results of Frederick *et al.* (1986), who concluded that leaf production and abscission followed similar seasonal trends in a 5-year-old stand of *E.nitens* in the central North Island.

Changes in total leaf numbers represent the difference between the amount of leaf produced and the amount lost. Increases can arise directly only from growth, but losses have two major causes; insect defoliation and abscission. Defoliation by insects contributes directly to leaf losses, and it has indirect effects on subsequent plant growth and abscission. If growing conditions are favourable, insect attack on eucalypts stimulates a vigorous development of "accessory" growth arising from pads of meristematic tissue in the leaf axils, or of "epicormic" growth derived from the axillary meristematic tissue that lies beneath the most recently-formed wood of the trunk and branches. This burst of growth is likely to be associated with substantial abscission of leaves damaged previously by insect chewing (Jacobs 1955, Morrow 1977). Although no distinction between primary-bud and accessory growth was recorded, much of the foliage growth after midsummer was accessory growth, reflecting the effects of defoliation in the first *P.charybdis* generation.

Litterfall patterns recorded by Frederick *et al.* (1986) showed two peaks of loss of foliage: the first in late spring, around November-December; the second in mid- to late summer (late January-February). These seem to correspond more closely with actual peaks of defoliation than with peak growth periods, suggesting that the abscission effect noted by Jacobs (1955) and Morrow (1977) is either insignificant in New Zealand or is masked by the heavy rain of excised leaf material during periods of high defoliation.

The relationship between defoliation and shoot growth is potentially complex, so it is not surprising that the simple correlation between density of larvae in each stage and defoliation was not strong. An apparent inverse trend between larval density and defoliation in the second generation implies that the effects of defoliation were already becoming apparent by 13 February. Trees with higher levels of defoliation supported lower densities of instars 1-3, suggesting increased mortality on these trees. Migration to less severely defoliated trees is a much less plausible explanation, although I did see larvae the previous summer crawling

down the trunks of some trees that had been completely stripped of new foliage. Larsson and Ohmart (1988) reported finding larvae of *P.atomaria* beneath *E.blakelyi* trees that had been completely stripped of new foliage, but not below trees that still had new foliage. They concluded that larvae left trees that lacked high quality foliage to search for food elsewhere. So far there are no published estimates of mortality of larvae in this situation. The probability seems small in situations like that at Kāhu Farms that larvae transferring between trees will locate a tree that has sufficient new foliage to allow the larva to complete its development.

There is little doubt that at some periods during the summer larvae face severe food shortages and consequent intra-specific competition. On 12 December 1978 at Kāhu Farms, severe defoliation of all adult *E.nitens* foliage was evident, with only vestiges of new growth remaining. First, second and third instars were all abundant, with occasional fourth instars. Very few adults were observed and none were actively feeding, copulating, ovipositing nor flying, although eggs were still numerous. Larvae could be seen in large numbers crawling along stems and also on the main trunk of many trees. This was unusual, since larvae were seldom seen well down branches near the trunk. Many of these trees had abundant juvenile foliage, yet this was virtually untouched and only occasional larvae were seen on this foliage. Adult foliage from previous years or that which had begun to develop the leathery characteristics of older foliage was moderately infested by larvae. This foliage showed signs of chewing but on close inspection this damage appeared to be old and had presumably occurred when the leaf was young. A few fourth instars were seen gnawing this old adult foliage, but consumption rates were obviously far lower than those observed on tender new foliage. Recent feeding damage on these older leaves showed a markedly "whiskery" appearance, caused by small leaf veins protruding beyond the damaged edge. This same effect was noted in the lab. when adults were fed old adult *E.viminalis*. First and second instars were seen attempting to bite old leaves but none managed to penetrate the cuticle. No larvae were ever seen to bite juvenile *E.nitens* foliage in the field. Under these circumstances

the likelihood of small larvae surviving was very low and larger larvae would also have had difficulty finding sufficient food to reach the prepupal stage.

The serial correlation coefficients calculated for defoliation records provide no evidence to suggest that plant defensive compounds were increased by *P.charybdis* attack. The result also suggests that a possible error in estimating defoliation, namely the inclusion of a prior fortnight's damage in a current estimate, was insignificant.

There is little doubt that the intensity of defoliation is affected by rainfall. The effect seems to be non-linear, although the data for 4 and 13 January 1980 alone determine this. A non-linear relationship does have biological meaning: it suggests that there is an optimum level of rainfall, above and below which trees are less able either to compensate for or resist defoliation. Several hypotheses could explain the relationship between rainfall and defoliation.

Firstly, rainfall may have affected the trees' ability to replace defoliation losses. This is supported by the previously-mentioned finding of a significant partial correlation coefficient between rainfall and the percent change in leaf numbers when defoliation was held constant. Larsson and Ohmart (1988) suggested that drought stress in *E.blakelyi* may reduce the abundance of new leaves and they proposed this as a factor that could reduce the life history performance of insects that feed predominantly on new leaves. The dependence on suitable growing conditions of accessory or epicormic growth in eucalypts in general following insect attack has been noted by Jacobs (1955) and Morrow (1977).

Another explanation may be found in the alternative hypotheses of White (1969, 1973, 1974, 1976, 1978) and of Haukioja and Hakala (1975). White proposed that moisture stress results in an increase in soluble nitrogen content of leaves, which causes an increase in food quality of the plant for the insect. Haukioja and Hakala (1975) also suggested that moisture stress increases the food quality of the plant, but they maintained that the probable reason for this

was that the plant was less able to maintain defences such as secondary plant compounds. Some support is given to White's hypothesis by Fox and Macauley (1977), who showed that increasing nitrogen content of *Eucalyptus* leaves was associated with increased growth rates, nitrogen gains and nitrogen use efficiencies of *P.atomaria* larvae. Absolute feeding rate was constant, but because larvae eating leaves with a higher nitrogen content grew faster, and larger larvae eat more, these larvae had greater average feeding rates. These results predict an increase in defoliation following an increase in leaf nitrogen content. Schönau (1983) also found that changes in foliar nutrients of *E.grandis* varied with rainfall. However, Miles, Aspinall and Correll (1982) found no relationship between nitrogen content of *E.camaldulensis* leaves and growth and development of *P.atomaria* larvae. They proposed that the nitrogen effects found by Fox and Macauley (1977) were caused by sub-optimal nitrogen levels, and they showed that Fox and Macauley's leaf nitrogen levels were much lower than those they (Miles *et al.*) recorded. Ohmart, Stewart and Thomas (1985) found that low leaf nitrogen levels limited the growth of larval *P.atomaria*, but Larsson and Ohmart (1988) rejected White's hypothesis as a possible influence on the performance of larval *P.atomaria*, claiming that any increase in soluble nitrogen would occur only in mature leaves that were too tough for larvae to bite. Instead, they proposed that the effects of drought stress act by limiting the availability of new growth.

In conclusion, there appears to be a close relationship between rainfall, defoliation by *P.charybdis*, and leaf production in *E.nitens*. Rainfall and defoliation affect leaf growth, while rainfall may affect the intensity of defoliation by altering either the amount of new growth or the quality of *E.nitens* leaf tissue as food for *P.charybdis*. Defoliation is also a function of *P.charybdis* population intensity, and the relationship is affected by larval competition at some stages of the life history. Facultative plant defences induced by *P.charybdis* attack are unlikely to be present, and do not have significant effects, at least in the short or medium term.

Chapter 6. Conclusions

CONCLUSIONS

P.charybdis is an Australian insect that has only recently arrived in New Zealand. It faces the problems of a seasonal environment and food resources of limited duration. Problems not encountered in New Zealand but present in Australia where it evolved, are intense interspecific competition with many other *Eucalyptus*-feeding insects (Journet 1981, Morrow 1977, Springett 1978) and predation and parasitism (Carne 1967). These factors may explain the apparently low abundance of *P.charybdis* in Australia (D. deLittle*, *in litt.* to B.Poole). Attempts to control *P.charybdis* by introducing parasites and predators such as *Enoggerra nassaui*, *Neopolycystus insectifurax* and *Cleobora mellyi*, assume that these, not competition, regulate *P.charybdis* populations. This study showed that intra-specific competition occurred during both summer generations. Mortality of second-generation *P.charybdis* during the foliage-dwelling stages was density-dependent. The duration of the first stadium in the second generation at Kāhu Farms was greater than expected from lab. studies. This appears to have been caused by poor nutrition, resulting from the intense defoliation early in this generation. Mortality of foliage-dwelling stages in the second generation was also identified as the main cause of variation in summer mortality. The effects of *E.nassaui* and other parasites or predators cannot be accurately predicted, although mortality caused by these natural enemies during the early part of the second generation may remove the regulatory effects of intra-specific competition in this generation. If this does happen, then the programme is likely to have achieved its ultimate aim, i.e. the reduction of injury to the tree.

The study has also shown how *P.charybdis* is adapted to cope with its seasonal environment. Diapause is induced apparently before mid-summer, begins in autumn and takes place during winter so that the scarcity or lack of food during winter is avoided.

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* Dr D. deLittle investigated the biology of the paropsine chrysomelid *Chrysophtharta bimaculata* (Olivier) in Tasmania and was instrumental in the introduction of *Cleobora mellyi* to New Zealand

Adults that emerge in spring face the problem of when to lay eggs. Premature oviposition has disastrous consequences since newly-emerged larvae cannot eat leaves that hardened the previous autumn. Premature oviposition does not occur because adults must eat young foliage to oviposit, thus ensuring that new growth is present when eggs hatch. Conversely, adults that lay later in spring risk leaving progeny that will encounter high intra-specific competition for food.

Mortality of first instars is high between eclosion and establishment on new leaves, but is independent of density, at least when food is not limiting. The oviposition behaviour of females maximises the likelihood that newly-hatched larvae will locate new foliage and represents a compromise that accommodates several factors. The effect of laying eggs on old rather than new foliage is to minimise the risk of eggs' being eaten by herbivores that favour new foliage, including *P.charybdis*. However, larvae that hatch some distance from new foliage have a greater chance of choosing shoots that do not support new growth. Females oviposit on old leaves close to new foliage, avoiding this problem and also reducing the chance that eggs will be lost on senescing leaves.

In at least some years, egg clusters comprised more eggs in the first than the second generation. This may reflect females' body weight or nutritional differences, but temperature was not correlated with egg cluster size in this study. Differences in egg cluster size may affect the efficiency of predation or parasitism by recently-introduced biological control agents. *C.mellyi* may also be affected by the aggregation of larvae. This aggregation is caused by the preference of larvae for new growth and has as a corollary the predator-avoidance benefits of group living (Bertram 1978).

Very high mortality of *P.charybdis* occurred among *P.charybdis* stages on the ground, but this was not related to the density of 4th instars that initiate this stage. Variation in mortality

during this stage at the end of the first generation at Kāhu Farms did not influence variation in total seasonal mortality.

The putative two-generation life history occurred both at Kāhu Farms and near Palmerston North each year. The discrepancy between this pattern and that predicted by previous laboratory records of oviposition (Dugdale 1965a, Steven 1973, Styles 1969, 1970) was caused by several factors. First, oviposition declined rapidly to zero in midsummer. This was apparently unrelated to either nett availability of new growth or defoliation intensity. Consequently, the decline was most likely caused by death of adults much earlier than has been suggested from lab. studies. Secondly, development rates measured on high-quality food suggested that even if freshly-flushing new growth was continuously available at Kāhu Farms, a second *P.charybdis* generation, starting from teneral adults, could not have begun before early December. Even at 20°C, several degrees higher than the midsummer mean at Kāhu Farms, the pre-reproductive period of *P.charybdis* is about a fortnight (Edwards and Wightman 1984). Thus, the predicted appearance of second generation eggs was around late December - early January; very close to the observed pattern.

Third, intra-specific competition for the youngest foliage in both generations resulted in depletion of this resource, forcing larvae to feed on older age classes. Consequently, development was protracted and mortality of later-hatching larvae would have been increased, truncating recruitment to later stages and enhancing the separation of generations.

Adults were light-trapped one autumn. This seldom-recorded event suggests that dispersal flights take place before adults overwinter, but it is not clear how regular this is.

Growth and development of eggs, larvae and pupae follows a typical pattern (Wigglesworth 1972), with larvae and pupae having a higher temperature threshold for development than the egg. The marked effect of foliage species on development (Steven 1973) has made accurate

prediction of stage durations difficult in the field. This study enables linear estimates of temperature-development relationships for any foliage type to be obtained easily by growing larvae at a single temperature. Since the developmental threshold is a stage characteristic, it can be expected to remain constant irrespective of foliage type. This provides a point on the temperature-development rate line. The slope of the line is the reciprocal of the stage duration in day-degrees, and this can be obtained from experiments at a single temperature provided that it lies on the linear part of the graph. These two items of information are sufficient to describe the line.

The development rate information described here for eggs and pupae does not depend on foliage characteristics and can be used directly in population or phenological models.

The method developed in this study to estimate development rates without the confounding effects of changing food quality is widely applicable for this purpose. It can also be used for developmental studies whenever it is desirable to minimise the difference in duration between low and high temperature treatments.

Despite severe defoliation, particularly in the second *P.charybdis* generation, *E.nitens* at Kāhu Farms showed about a 30% increase in leaf numbers, i.e. photosynthetic tissue, during 1979-80. Changes in leaf numbers were significantly correlated with rainfall and with defoliation.

Defoliation is significantly and inversely related to rainfall, confirming White's (1973) observation that moisture-stressed trees are particularly liable to defoliation. This supports the argument that damage to susceptible eucalypts may be reduced by good silvicultural practices, especially the careful matching of plantation sites with *Eucalyptus* species and provenances suitable for those sites.

This study has provided new information on the life-history, behaviour, growth and development, population dynamics and host-plant interactions of *P.charybdis*. With this knowledge, the progress of biological control programmes can be better assessed, thus increasing the likelihood that *P.charybdis* can be successfully controlled at low levels.

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APPENDIX 1a. SHOOT SAMPLING DATA FOR TOTAL NUMBER OF EGGS PER SHOOT

DATE														
27 SEP	11 OCT	25 OCT	8 NOV	6 DEC	20 DEC	4 JAN	17 JAN	31 JAN	13 FEB	13 MAR	27 MAR	11 APR	SHOOT	TREE
0	0	0	19	0	0	0	0	0	0	0	0	0	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
0	0	0	0	0	0	0	0	10	0	0	0	0	3	1
0	0	0	19	0	0	0	0	0	0	0	0	0	4	1
0	24	0	0	0	0	0	0	33	0	0	0	0	5	1
0	0	0	0	0	0	0	0	0	0	0	0	0	6	2
0	0	0	0	0	0	0	0	0	0	0	0	0	7	2
0	0	0	0	0	0	0	0	0	0	0	0	0	8	2
0	60	0	0	0	0	0	0	0	0	0	0	0	9	2
0	0	0	0	0	0	0	0	0	0	0	0	0	10	2
0	12	0	0	0	0	0	0	0	0	0	0	0	11	2
0	0	0	0	0	0	0	0	26	0	0	0	0	12	2
0	0	0	0	25	0	0	0	0	0	0	0	0	13	2
0	0	0	0	0	0	0	0	0	0	0	0	0	14	2
0	0	9	0	33	0	0	0	0	27	0	0	0	15	3
0	19	0	0	0	0	0	0	0	0	0	0	0	16	3
0	8	0	0	20	0	0	0	1	0	0	0	0	17	3
0	8	0	0	21	0	0	0	6	0	0	0	0	18	3
0	4	23	0	16	0	0	0	0	0	0	0	0	19	3
0	33	0	0	0	0	0	0	0	0	0	0	0	20	3
0	0	10	0	0	0	0	0	0	0	0	0	0	21	3
0	0	0	0	0	0	0	0	0	0	0	0	0	22	3
0	8	21	0	0	15	0	0	0	0	0	0	0	23	3
0	0	0	0	0	0	0	0	0	0	0	0	0	24	3
0	0	0	0	40	0	0	0	0	0	0	0	0	25	3
0	0	0	0	0	0	0	0	26	0	0	0	0	26	3
0	0	0	16	0	0	0	0	12	0	0	0	0	27	3
0	0	1	0	0	0	0	0	0	0	0	0	0	28	3
0	0	0	0	0	0	0	0	18	0	0	0	0	29	3
0	0	5	0	0	0	0	13	0	0	0	0	0	30	4
0	31	0	0	0	0	0	0	0	0	0	0	0	31	4
0	0	0	6	0	0	0	0	22	0	0	0	0	32	4
0	0	0	0	0	0	0	0	0	0	0	0	0	33	4
0	39	0	0	0	0	0	0	12	0	0	0	0	34	4
0	0	0	0	0	0	0	0	0	0	0	0	0	35	5
0	0	0	0	0	0	0	0	0	0	0	0	0	36	5
0	26	0	0	0	0	0	0	0	0	0	0	0	37	5
0	9	12	0	0	20	0	0	0	0	0	0	0	38	5
0	20	0	0	0	0	0	0	0	0	0	0	0	39	5
28	0	17	0	0	0	0	0	0	0	0	0	0	40	6
0	29	0	0	0	0	0	0	0	0	0	0	0	41	6
0	0	0	0	0	0	0	13	0	0	0	0	0	42	6
0	24	0	12	0	0	0	0	0	0	0	0	0	43	6
0	0	0	0	0	0	0	2	0	25	0	0	0	44	6
0	8	0	0	0	0	0	0	0	0	0	0	0	45	7
0	0	0	0	0	0	0	0	0	0	0	0	0	46	7
0	0	0	30	0	0	0	1	0	0	0	0	0	47	7
0	0	0	0	0	0	0	0	0	22	0	0	0	48	7
0	0	0	0	0	0	0	0	0	0	0	0	0	49	7
0	0	0	0	0	0	0	0	0	0	0	0	0	50	7

0	25	4	0	0	0	0	0	0	0	0	0	0	51	8
0	0	0	4	0	0	0	0	0	0	0	0	0	52	8
0	20	0	0	0	0	0	0	0	0	0	0	0	53	8
0	0	0	0	0	0	0	0	0	0	0	0	0	54	8
0	0	16	0	20	0	0	0	0	0	0	0	0	55	8
25	0	0	0	0	0	0	0	0	0	0	0	0	56	9
0	0	0	0	0	0	0	0	0	0	0	0	0	57	9
0	0	0	0	0	0	0	0	0	0	0	0	0	58	9
14	0	16	0	27	0	0	0	0	0	0	0	0	59	9
0	0	20	0	0	0	0	0	0	0	0	0	0	60	9
0	0	30	0	0	0	0	0	0	0	0	0	0	61	9
0	0	20	17	0	0	0	0	0	16	0	0	0	62	10
11	44	21	0	17	0	0	0	0	0	0	0	0	63	10
0	25	26	0	13	0	0	0	0	0	0	0	0	64	10
0	0	0	0	19	0	0	0	0	0	0	0	0	65	10
0	0	0	0	0	0	0	0	0	0	0	0	0	66	10
0	0	0	0	10	0	0	0	0	0	0	0	0	67	10
0	0	0	0	0	0	0	0	0	0	0	0	0	68	10
0	0	0	0	0	0	0	0	0	37	0	0	0	69	10
0	0	0	0	0	0	0	0	0	20	0	0	0	70	10

APPENDIX 1b. SHOOT SAMPLING DATA FOR FIRST INSTARS

DATE													
27 SEP	11 OCT	25 OCT	8 NOV	6 DEC	20 DEC	4 JAN	17 JAN	31 JAN	13 FEB	13 MAR	27 MAR	11 APR	TREE
0	0	0	1	0	12	0	0	14	8	0	0	0	1
0	0	0	3	0	0	0	0	0	0	0	0	0	1
0	0	0	0	1	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	5	2	0	0	0	1
0	0	0	0	0	0	0	0	0	63	1	0	0	1
0	0	0	0	0	2	0	0	0	18	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	1	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	1	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	1	0	0	0	0	1	0	0	0	0	2
0	0	0	0	0	0	1	0	11	0	0	0	0	2
0	0	1	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	1	4	0	3	0	3	3	1	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	1	0	0	0	0	0	0	6	0	0	0	3
0	0	0	2	0	0	0	0	0	11	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	1	34	0	0	0	0	1	0	0	0	3
0	0	0	0	0	0	0	0	6	0	1	0	0	3
0	0	0	0	1	0	0	0	21	1	0	0	0	3
0	0	0	0	0	0	0	0	3	1	0	0	0	3
0	0	0	0	0	1	1	0	0	24	0	0	0	4
0	0	0	1	2	0	0	0	0	9	0	0	0	4
0	0	0	0	0	0	0	0	0	0	0	0	0	4
0	0	0	0	0	0	0	0	0	0	0	0	0	4
0	0	0	0	1	0	0	0	0	0	0	0	0	4
0	0	0	0	0	0	0	0	0	0	0	0	0	5
0	0	0	0	1	0	0	0	1	0	0	0	0	5
0	0	0	0	0	12	0	0	0	10	0	0	0	5
0	0	0	6	0	0	0	0	0	0	0	0	0	5
0	0	0	0	0	23	0	0	0	0	0	0	0	5
0	0	1	0	0	0	0	0	0	4	0	0	0	6
0	0	0	0	0	1	0	0	0	0	0	0	0	6
0	0	1	0	0	0	0	0	0	20	0	0	0	6
0	0	0	0	0	0	0	0	1	0	0	0	0	6
0	0	0	0	1	0	0	0	0	0	0	0	0	6
0	0	0	0	0	0	0	0	0	6	0	0	0	7
0	0	0	0	0	0	0	0	0	0	0	0	0	7
0	0	0	0	0	0	0	0	0	0	0	0	0	7
0	0	0	0	0	0	0	0	0	0	0	0	0	7
0	0	0	1	0	0	0	0	0	0	0	0	0	7
0	0	0	0	0	1	0	0	0	0	0	0	0	7

0	0	0	0	2	0	0	0	0	0	0	0	0	8
0	0	0	0	3	0	1	0	0	0	0	0	0	8
0	0	0	0	1	0	0	0	0	0	0	0	0	8
0	0	1	0	2	0	1	0	1	0	0	0	0	8
0	0	1	0	4	0	0	0	0	0	0	0	0	8
0	0	0	1	5	0	0	0	11	0	0	0	0	9
0	0	0	0	3	0	0	0	1	0	0	0	0	9
0	0	0	0	4	0	0	0	0	0	0	0	0	9
0	0	0	5	3	0	0	0	0	0	0	0	0	9
0	0	0	3	2	0	0	0	1	0	0	0	0	9
0	0	0	0	2	1	0	0	0	1	0	0	0	9
0	0	0	0	0	0	0	0	0	9	0	0	0	10
0	0	0	0	0	0	0	0	0	0	0	0	0	10
0	0	0	0	0	0	0	0	0	0	0	0	0	10
0	0	0	0	0	8	0	0	7	14	0	0	0	10
0	0	0	0	0	0	0	0	0	0	0	0	0	10
0	0	0	0	0	0	0	0	0	6	0	0	0	10
0	0	0	0	2	3	0	0	0	1	5	0	0	10
0	0	0	0	3	0	0	0	0	0	0	0	0	10
0	0	0	0	0	6	2	0	1	15	1	0	0	10

APPENDIX 1c. SHOOT SAMPLING DATA FOR SECOND INSTARS

DATE													
27 SEP	11 OCT	25 OCT	8 NOV	6 DEC	20 DEC	4 JAN	17 JAN	31 JAN	13 FEB	13 MAR	27 MAR	11 APR	TREE
0	0	0	0	0	12	2	0	1	3	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	1	0	3	0	0	0	0	0	0	1
0	0	0	0	0	1	0	3	1	3	0	0	0	1
0	0	0	0	7	0	0	0	0	15	0	0	0	1
0	0	0	0	0	13	0	0	0	0	0	0	0	2
0	0	0	0	0	1	3	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	8	0	6	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	1	40	0	2	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	2	0	0	0	0	0	0	0	3
0	0	0	0	1	2	0	0	0	2	5	0	0	3
0	0	0	0	0	0	1	0	0	0	0	0	0	3
0	0	0	0	0	0	1	0	0	0	0	0	0	3
0	0	0	0	0	0	6	0	0	4	0	0	0	3
0	0	0	0	0	2	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	1	0	0	0	3
0	0	0	0	0	2	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	17	4	0	0	0	1	0	0	0	3
0	0	0	0	0	1	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	15	0	10	0	5	0	0	0	4
0	0	0	0	2	0	0	0	0	0	0	0	0	4
0	0	0	0	1	0	0	0	1	0	0	0	0	4
0	0	0	0	1	0	4	0	0	0	0	0	0	4
0	0	0	0	2	0	4	0	0	0	0	0	0	4
0	0	0	0	0	0	2	1	0	1	0	0	0	5
0	0	0	0	1	0	1	0	0	0	0	0	0	5
0	0	0	0	0	7	0	0	0	0	0	0	0	5
0	0	0	0	0	0	0	0	0	0	0	0	0	5
0	0	0	0	0	6	0	0	2	0	0	0	0	5
0	0	0	0	0	0	0	0	0	0	0	0	0	6
0	0	0	0	0	17	0	0	0	0	0	0	0	6
0	0	0	0	0	0	1	0	0	0	0	0	0	6
0	0	0	0	1	0	0	0	0	0	0	0	0	6
0	0	0	0	10	4	0	0	0	0	0	0	0	6
0	0	0	0	0	0	8	0	0	0	0	0	0	7
0	0	0	0	1	0	0	0	0	0	0	0	0	7
0	0	0	0	0	3	0	0	0	0	0	0	0	7
0	0	0	0	0	0	0	0	0	0	0	0	0	7
0	0	0	0	1	1	5	0	0	0	0	0	0	7
0	0	0	0	1	33	0	0	0	0	0	0	0	7

0	0	0	0	7	0	0	0	0	0	0	0	0	8
0	0	0	0	4	0	0	3	0	3	0	0	0	8
0	0	0	0	2	3	0	0	0	0	0	0	0	8
0	0	0	0	2	1	0	0	3	0	1	0	0	8
0	0	0	0	5	0	0	0	0	0	0	0	0	8
0	0	1	0	0	29	2	0	1	0	0	0	0	9
0	0	0	0	0	0	1	0	2	0	0	0	0	9
0	0	0	0	3	0	0	0	0	0	0	0	0	9
0	0	0	0	7	1	0	0	0	0	0	0	0	9
0	0	0	0	0	0	1	0	0	0	0	0	0	9
0	0	0	0	5	17	1	0	0	0	0	0	0	9
0	0	0	0	9	0	0	6	0	6	0	0	0	10
0	0	0	0	2	0	0	0	0	0	0	0	0	10
0	0	0	0	7	0	0	0	0	0	0	0	0	10
0	0	0	0	1	9	0	1	0	1	1	0	0	10
0	0	0	0	0	0	0	0	0	0	0	0	0	10
0	0	0	0	1	5	0	1	0	1	0	0	0	10
0	0	0	0	0	13	0	0	2	0	3	0	0	10
0	0	0	0	3	0	0	0	0	0	3	0	0	10
0	0	0	0	4	33	13	1	7	1	12	0	0	10

APPENDIX 1d. SHOOT SAMPLING DATA FOR THIRD INSTARS

DATE													
27 SEP	11 OCT	25 OCT	8 NOV	6 DEC	20 DEC	4 JAN	17 JAN	31 JAN	13 FEB	13 MAR	27 MAR	11 APR	TREE
0	0	0	0	2	0	0	0	0	5	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	1	0	0	0	1
0	0	0	0	1	0	0	0	0	10	1	0	0	1
0	0	0	0	5	0	0	1	0	33	0	0	0	1
0	0	0	0	0	0	0	0	0	1	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	3	0	0	0	0	0	0	2
0	0	0	0	1	0	0	0	0	0	0	0	0	2
0	0	0	0	1	0	0	0	0	0	0	0	0	2
0	0	0	0	1	0	0	0	1	0	0	0	0	2
0	0	0	0	1	0	2	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	4	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	4	0	0	0	0	7	1	0	0	3
0	0	0	0	0	1	0	0	0	1	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	3	11	1	0	0	5	0	0	0	3
0	0	0	0	0	1	1	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	19	0	0	0	0	1	0	0	0	3
0	0	0	0	0	0	0	0	0	2	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	4
0	0	0	0	1	0	0	0	0	0	0	0	0	4
0	0	0	0	3	2	0	0	0	0	0	0	0	4
0	0	0	0	1	0	0	0	0	0	0	0	0	4
0	0	0	0	4	0	0	0	0	0	0	0	0	4
0	0	0	0	0	0	0	0	0	0	0	0	0	5
0	0	0	0	3	0	0	0	0	1	0	0	0	5
0	0	0	0	0	0	0	0	0	13	0	0	0	5
0	0	0	0	0	1	0	0	0	0	0	0	0	5
0	0	0	0	1	0	0	0	0	0	0	0	0	5
0	0	0	0	0	0	0	0	3	0	0	0	0	6
0	0	0	0	0	0	0	1	0	0	0	0	0	6
0	0	0	0	0	0	0	0	0	0	0	0	0	6
0	0	0	0	0	2	0	0	0	0	0	0	0	6
0	0	0	0	5	0	0	0	1	7	0	0	0	6
0	0	0	0	0	0	3	0	0	0	0	0	0	7
0	0	0	0	2	0	0	0	0	0	0	0	0	7
0	0	0	0	4	0	0	0	0	0	0	0	0	7
0	0	0	0	0	0	0	0	0	0	0	0	0	7
0	0	0	0	2	1	0	0	0	0	0	0	0	7
0	0	0	0	1	0	0	0	0	0	0	0	0	7

0	0	0	0	5	0	0	0	0	2	0	0	0	8
0	0	0	0	12	1	0	0	0	6	0	0	0	8
0	0	0	0	0	2	0	0	0	0	0	0	0	8
0	0	0	0	7	0	0	0	0	0	0	0	0	8
0	0	1	0	0	0	0	0	0	9	0	0	0	8
0	0	0	0	0	0	0	0	1	0	0	0	0	9
0	0	0	0	0	0	4	0	0	0	0	0	0	9
0	0	0	0	0	0	0	0	0	0	0	0	0	9
0	0	0	0	0	0	0	0	0	0	0	0	0	9
0	0	0	0	2	0	0	0	0	0	0	0	0	9
0	0	0	0	7	0	0	0	0	0	0	0	0	9
0	0	0	0	2	0	0	0	0	1	1	0	0	10
0	0	0	0	1	0	0	0	0	0	0	0	0	10
0	0	0	0	0	0	0	0	0	0	0	0	0	10
0	0	0	0	0	1	0	0	0	1	3	1	0	10
0	0	0	0	0	0	0	0	0	0	0	0	0	10
0	0	0	0	0	1	0	0	0	0	1	0	0	10
0	0	0	0	1	0	0	0	2	0	0	0	0	10
0	0	0	0	1	0	0	0	0	0	4	0	0	10
0	0	0	0	0	3	1	0	4	9	15	0	0	10

APPENDIX 1e. SHOOT SAMPLING DATA FOR FOURTH INSTARS

DATE													
27 SEP	11 OCT	25 OCT	8 NOV	6 DEC	20 DEC	4 JAN	17 JAN	31 JAN	13 FEB	13 MAR	27 MAR	11 APR	TREE
0	0	0	0	0	0	1	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	1
0	0	0	0	1	0	0	0	0	1	0	0	0	1
0	0	0	0	1	0	0	0	0	5	0	0	0	1
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	3	0	2	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	2	0	0	0	0	1	0	1	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	4	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	9	0	0	0	0	0	0	0	0	3
0	0	0	0	1	0	1	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	3
0	0	0	0	0	0	0	0	0	0	0	0	0	4
0	0	0	0	1	0	0	0	0	0	0	0	0	4
0	0	0	0	0	0	0	0	0	0	0	0	0	4
0	0	0	0	1	0	0	0	0	0	0	0	0	4
0	0	0	0	3	0	0	0	0	0	0	0	0	4
0	0	0	0	0	0	0	0	0	0	0	0	0	5
0	0	0	0	0	0	0	0	0	0	0	0	0	5
0	0	0	0	0	0	0	0	0	0	0	0	0	5
0	0	0	0	0	0	0	0	0	0	0	0	0	5
0	0	0	0	1	0	0	0	0	0	0	0	0	5
0	0	0	0	1	0	0	0	0	0	0	0	0	6
0	0	0	0	0	0	0	0	0	0	0	0	0	6
0	0	0	0	1	0	0	0	0	0	0	0	0	6
0	0	0	0	7	0	0	0	0	3	0	0	0	6
0	0	0	0	4	0	0	0	0	0	0	0	0	6
0	0	0	0	1	0	0	0	0	0	0	0	0	7
0	0	0	0	1	0	0	0	0	2	0	0	0	7
0	0	0	0	1	0	0	0	0	0	0	0	0	7
0	0	0	0	0	0	0	0	0	0	0	0	0	7
0	0	0	0	1	0	0	0	0	0	0	0	0	7
0	0	0	0	1	0	0	0	0	0	0	0	0	7

0	0	0	0	3	0	0	0	0	0	0	0	0	8
0	0	0	0	1	0	0	0	0	0	0	0	0	8
0	0	0	0	1	0	0	0	0	0	0	0	0	8
0	0	0	0	2	0	0	0	0	0	0	0	0	8
0	0	0	0	0	0	0	0	0	4	0	0	0	8
0	0	0	0	1	0	0	0	0	0	0	0	0	9
0	0	0	0	3	0	0	0	0	0	0	0	0	9
0	0	0	0	0	0	0	0	0	0	0	0	0	9
0	0	0	0	0	0	0	0	0	0	0	0	0	9
0	0	0	0	3	0	1	0	0	0	0	0	0	9
0	0	0	0	3	0	0	0	0	0	0	0	0	9
0	0	0	0	2	0	0	0	0	6	0	0	0	10
0	0	0	0	0	0	0	0	0	0	0	0	0	10
0	0	0	0	0	0	0	0	0	0	0	0	0	10
0	0	0	0	0	0	0	0	0	2	1	0	0	10
0	0	0	0	1	0	0	0	0	0	0	0	0	10
0	0	0	0	1	0	0	0	0	0	0	0	0	10
0	0	0	0	5	0	0	0	0	1	0	0	0	10
0	0	0	0	2	0	0	0	0	0	0	0	0	10
0	0	0	0	2	0	0	0	0	4	0	0	0	10

APPENDIX 2: Durations, in days, of each instar at four temperatures, in five replicates

TEMPERATURE = 8 ± 1°C

	CAGE NUMBER				
Instar	1	2	3	4	5
1st	10.5	11	12	11.5	11.5
2nd	14	17	13.5	14.5	13
3rd	21	17	19	19.5	20
4th	16	14	15.5	15.5	20

TEMPERATURE = 15 ± 1°C

	CAGE NUMBER				
Instar	1	2	3	4	5
1st	5	5	4	5	5.5
2nd	4	3	3.5	3.5	4.5
3rd	3.5	4	5	5	6
4th	3.5	6	5	4	3.5

TEMPERATURE = 20 ± 1°C

	CAGE NUMBER				
Instar	1	2	3	4	5
1st	4	3.5	3.5	3	3.5
2nd	2	2	2.5	2.5	2.5
3rd	3	3.5	3	3	3
4th	2.5	2	2	2.5	2.5

TEMPERATURE = 25 ± 2°C

	CAGE NUMBER				
Instar	1	2	3	4	5
1st	2.5	2	2.5	2.5	2.5
2nd	2	2	2	2.5	2
3rd	2.5	4	2.5	2	2
4th	2	2	2	2	3

APPENDIX 3. SHOOT SAMPLING DATA FOR TOTAL NUMBER OF LEAVES PER SHOOT

DATE														
27 SEP	11 OCT	25 OCT	8 NOV	6 DEC	20 DEC	4 JAN	17 JAN	31 JAN	13 FEB	13 MAR	27 MAR	11 APR	SHOOT	TREE
39	57	72	81	61	81	97	80	68	53	34	39	31	1	1
20	18	21	16	15	15	12	10	8	0	0	0	0	2	1
27	35	31	24	27	26	25	20	20	21	8	2	0	3	1
23	36	66	56	48	52	74	70	64	45	33	32	31	4	1
36	47	87	92	122	142	170	197	233	249	171	172	180	5	1
35	38	36	34	29	37	44	53	52	59	40	43	40	6	2
30	35	42	34	33	32	37	40	31	29	22	18	20	7	2
49	50	59	48	37	36	43	35	24	20	15	5	5	8	2
31	28	37	32	27	25	16	15	15	9	2	1	0	9	2
34	47	33	38	27	31	34	34	23	17	15	5	3	10	2
35	37	40	40	34	36	48	35	26	13	0	0	0	11	2
27	41	57	41	35	29	27	24	25	10	9	7	6	12	2
38	47	65	57	50	54	61	61	41	33	26	18	15	13	2
30	31	36	32	29	27	35	29	27	23	0	0	0	14	2
21	39	29	20	15	25	25	27	18	18	5	3	1	15	3
30	32	32	27	19	20	26	15	13	12	3	0	0	16	3
43	56	47	39	50	55	60	56	40	69	35	32	28	17	3
39	52	44	34	34	40	40	30	25	18	13	5	2	18	3
29	48	21	26	22	26	35	28	18	14	7	7	5	19	3
26	31	34	40	33	29	20	27	46	13	5	4	3	20	3
32	49	37	26	20	17	16	35	54	13	4	3	1	21	3
32	33	23	27	20	21	24	10	13	9	3	1	0	22	3
38	36	29	29	21	23	24	11	9	6	0	0	0	23	3
28	34	31	26	23	23	21	20	19	13	5	1	1	24	3
33	37	28	27	23	23	23	27	20	19	7	5	2	25	3
28	46	51	61	35	27	20	50	79	16	1	5	19	26	3
37	45	49	35	27	26	28	30	43	27	17	12	27	27	3
20	26	22	18	17	15	15	15	18	12	9	4	0	28	3
28	28	32	26	22	22	21	22	27	17	10	2	1	29	3
32	32	38	30	26	32	34	26	23	18	7	6	4	30	4
26	32	40	47	47	48	58	49	45	40	28	25	27	31	4
26	65	79	71	74	97	121	132	136	120	87	81	90	32	4
26	37	43	43	54	54	61	59	56	60	44	40	40	33	4
28	41	59	58	46	55	60	53	49	44	38	37	35	34	4
14	22	34	31	28	32	48	60	62	50	43	43	43	35	5
30	40	66	42	34	31	44	42	41	41	40	38	38	36	5
17	23	17	23	15	21	13	11	9	6	6	4	2	37	5
27	42	36	32	22	28	29	25	18	17	17	16	12	38	5
29	58	90	87	70	68	70	74	71	70	59	62	57	39	5
42	48	53	40	21	26	31	31	22	22	24	34	30	40	6
36	34	54	20	24	24	26	22	18	16	13	14	13	41	6
17	26	28	11	21	25	20	22	15	15	8	12	10	42	6
12	22	22	20	33	35	32	35	32	33	32	35	35	43	6
47	53	90	104	85	101	120	115	116	87	57	64	60	44	6
16	23	37	27	12	14	12	8	3	3	1	0	0	45	7
36	40	53	33	26	25	31	38	35	34	24	24	20	46	7
20	31	37	21	21	25	22	17	12	10	3	4	0	47	7
21	28	37	19	17	26	22	13	9	5	3	3	1	48	7
35	48	53	43	33	21	20	18	18	6	5	2	2	49	7
12	14	19	10	17	18	20	14	10	9	3	1	0	50	7

49	63	88	84	86	93	97	73	64	61	53	38	40	51	8
53	59	56	58	66	76	87	59	46	38	36	36	37	52	8
32	40	39	47	38	40	45	41	35	24	21	17	18	53	8
37	56	86	90	98	108	115	87	76	49	50	60	58	54	8
47	78	83	94	90	93	97	102	88	73	67	58	60	55	8
42	62	79	82	85	78	69	60	53	41	10	8	40	56	9
27	30	45	55	93	82	67	23	20	15	14	8	6	57	9
43	49	44	48	61	74	45	26	17	7	5	2	0	58	9
45	39	41	49	55	53	66	45	34	26	15	15	10	59	9
24	31	28	50	62	83	76	75	61	63	53	55	58	60	9
27	32	34	32	40	51	51	32	20	18	16	17	15	61	9
46	51	81	91	91	95	96	106	102	130	87	91	91	62	10
31	49	49	51	57	37	31	44	46	46	29	28	27	63	10
37	37	40	43	34	28	21	16	14	13	2	2	2	64	10
36	64	106	138	149	135	134	147	177	261	205	105	99	65	10
27	31	45	42	42	38	38	38	37	34	27	27	25	66	10
18	34	66	61	64	51	56	56	62	59	46	47	50	67	10
35	61	110	121	117	120	119	126	193	226	155	183	170	68	10
36	61	135	137	142	150	152	160	167	164	185	162	168	69	10
38	68	197	288	304	360	362	533	607	614	635	634	640	70	10

APPENDIX 4. SHOOT SAMPLING DATA FOR DEFOLIATION SCORES

DATE														
27 SEP	11 OCT	25 OCT	8 NOV	6 DEC	20 DEC	4 JAN	17 JAN	31 JAN	13 FEB	13 MAR	27 MAR	11 APR	SHOOT	TREE
1	2	1	0	2	1	4	0	3	3	5	3	3	1	1
0	0	0	0	0	2	0	1	0	0	0	0	0	2	1
1	2	1	0	4	0	0	2	0	5	5	5	1	3	1
0	1	0	2	3	0	4	2	2	5	4	4	3	4	1
0	1	0	2	2	0	0	1	1	5	4	3	2	5	1
1	1	1	0	3	2	0	0	1	4	2	2	1	6	2
1	1	1	3	0	0	4	1	1	5	1	2	1	7	2
1	1	1	3	1	3	4	3	4	4	3	4	2	8	2
0	0	0	0	0	1	2	0	0	5	5	5	3	9	2
0	1	1	0	4	0	4	0	4	5	5	5	1	10	2
1	1	1	1	4	2	0	2	0	5	4	4	2	11	2
0	1	1	1	3	0	0	0	0	4	5	4	4	12	2
0	1	0	0	3	0	1	0	0	4	4	3	1	13	2
1	1	1	0	0	3	4	1	5	5	4	3	1	14	2
0	1	1	0	0	3	1	1	1	4	4	5	2	15	3
1	0	1	0	3	3	1	1	1	5	5	5	2	16	3
0	1	0	0	3	0	4	0	3	5	3	3	1	17	3
1	2	2	0	4	0	0	1	2	5	4	5	2	18	3
0	1	1	0	4	3	3	1	1	4	4	3	1	19	3
1	1	1	2	3	3	4	0	3	4	5	4	4	20	3
0	1	1	0	0	1	3	0	1	4	4	4	4	21	3
0	1	1	0	0	0	0	0	0	5	5	4	2	22	3
0	0	0	0	0	0	0	2	0	5	4	3	1	23	3
0	1	1	0	0	0	0	0	1	5	5	4	1	24	3
0	1	0	0	0	3	0	0	0	5	4	3	0	25	3
1	2	1	1	4	1	4	0	3	5	5	4	0	26	3
1	2	1	3	0	1	4	0	2	5	4	3	1	27	3
0	1	0	0	3	0	0	1	1	4	5	5	5	28	3
1	0	0	2	3	3	4	2	2	4	5	5	0	29	3
1	1	1	0	3	2	2	1	5	3	5	5	1	30	4
1	1	1	2	3	1	1	0	3	5	4	3	1	31	4
1	2	1	1	2	1	4	1	1	4	3	2	1	32	4
0	1	1	1	2	1	0	1	1	5	3	3	1	33	4
1	1	1	3	2	3	3	2	1	4	3	3	1	34	4
1	1	1	0	1	3	3	0	1	5	1	3	0	35	5
1	1	1	0	0	4	4	1	1	5	1	1	0	36	5
1	1	1	0	2	1	1	1	1	4	5	4	1	37	5
1	2	1	1	1	1	1	0	1	4	2	2	0	38	5
1	2	1	0	3	0	2	1	1	5	1	1	0	39	5
1	2	1	3	4	2	0	0	3	5	4	4	1	40	6
1	1	2	0	4	3	4	1	3	5	5	4	2	41	6
1	1	2	3	1	2	3	2	4	5	4	3	1	42	6
1	1	3	0	1	2	4	0	0	5	2	2	1	43	6
1	2	1	2	4	1	1	0	1	4	3	3	1	44	6
1	1	2	0	4	2	3	2	4	5	5	2	1	45	7
1	1	2	2	4	1	0	2	1	4	3	3	1	46	7
1	2	3	2	4	3	2	0	4	5	5	4	2	47	7
1	1	3	0	4	4	4	0	4	5	5	4	2	48	7
1	2	2	2	3	3	4	1	3	5	5	4	1	49	7
1	1	2	0	4	5	1	2	3	5	5	4	2	50	7

1	1	1	2	4	0	4	0	3	5	3	1	1	51	8
1	1	1	1	3	2	0	1	4	3	4	2	1	52	8
1	1	1	0	4	1	4	2	3	5	3	3	1	53	8
1	1	2	1	0	4	0	1	1	5	4	3	2	54	8
1	2	2	0	3	1	0	1	1	5	4	4	1	55	8
1	2	3	1	3	1	3	1	3	4	4	4	1	56	9
1	1	1	0	0	3	4	2	3	4	5	4	4	57	9
2	1	0	3	0	3	0	1	0	5	5	5	2	58	9
1	0	0	2	4	1	1	1	4	5	5	4	1	59	9
1	1	1	2	4	4	0	1	1	4	1	1	0	60	9
1	1	1	0	4	1	0	2	2	5	4	4	1	61	9
1	1	3	0	2	3	0	1	1	4	3	2	1	62	10
1	1	4	1	2	1	1	0	1	5	2	5	1	63	10
1	1	1	0	3	1	0	0	0	5	5	5	4	64	10
1	1	1	2	2	3	1	1	1	4	2	3	1	65	10
1	1	1	1	2	0	1	0	0	5	1	3	1	66	10
1	1	1	0	2	3	2	2	1	4	2	3	1	67	10
1	1	1	0	3	4	1	1	1	2	3	2	1	68	10
1	1	1	2	2	3	0	2	1	5	2	2	1	69	10
1	1	1	2	1	2	1	1	1	3	3	1	1	70	10