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**Aspects of the Ecology of *Trachymela catenata* Chapuis  
(Coleoptera : Chrysomelidae) in New Zealand.**

A thesis presented in partial fulfilment  
of the requirements for the degree

of

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## Table of Contents

Abstract	iv
Acknowledgements	v
<b>Chapter 1. Introduction</b>	1
The Eucalypts	1
Eucalypt forest insects	3
Eucalypt plantation forest pests and biocontrol	7
The Paropsina	14
History of <i>Trachymela catenata</i> in New Zealand	17
Study sites	18
References	22
<b>Chapter 2. Ecology of the recently established paropsine <i>Trachymela catenata</i> Chapuis (Coleoptera : Chrysomelidae) in New Zealand: Life history, Phenology, Mortality and Development.</b>	32
Abstract	32
Introduction	33
Methods	36
Results	45
Discussion	56
References	68
<b>Chapter 3. Larval performance and oviposition preferences of <i>Trachymela catenata</i> Chapuis (Coleoptera : Chrysomelidae) on eight species of <i>Eucalyptus</i>.</b>	73
Abstract	73
Introduction	74
Methods	77
Results	81
Discussion	87
References	93
<b>Chapter 4. Comparative studies of the effects of the parasitoid <i>Enoggera nassau</i> Girault (Hymenoptera : Pteromalidae) on <i>Trachymela catenata</i> Chapuis and <i>Paropsis charybdis</i> Ståhl (Coleoptera : Chrysomelidae).</b>	98
Abstract	98
Introduction	99
Methods	104
Results	106
Discussion	109
References	113
<b>Chapter 5. Conclusions</b>	117



### Location of Plates

Plates 1, 2, 3 and 4	between pages	21-22
“ “ 5 and 6	“ “ “ “ “	38-39

## Abstract

A member of the *Eucalyptus* defoliating Australian tortoise beetles *Trachymela catenata* was recorded in New Zealand in December 1992. To date *Eucalyptus viminalis*, *E. macarthurii* and *E. macarthurii* × *botryoides* are known hosts. Investigations of a range of ecological parameters for *T. catenata* are presented in order to provide information with which to assess the potential status of this recent introduction. Life history as for all other paropsina comprises eggs, four larval instars, prepupa, pupa and adult. Adults overwinter and emerge during October to lay first generation eggs in November/December. An estimated generation time of 50 days means a second generation lays eggs during February, indicating a bivoltine life history. Females are as fecund as some other paropsines which erupt to pest levels in other countries. Larval mortality is highest during the first instar and 45.8% mortality occurred during pupation. Developmental thresholds and development times indicate that thermal requirements for completion of two generations will be met throughout most of New Zealand. Laboratory trials to determine female oviposition preference and larval performance on eight potential host eucalypts indicate *E. nitens* (an important commercial species) and *E. coccifera* to be equally as suitable hosts as those currently utilised. *Trachymela catenata* is therefore polyphagous and field monitoring of these two potential hosts is needed. The hymenopteran pteromalid egg parasitoid *Enoggera nassauii* was trialed in a study comparing parasitization of *T. catenata* eggs with those of *Paropsis charybdis*, a known host. The parasitoid had no apparent effect on *T. catenata* eggs and offers no potential control of *T. catenata* populations.

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This thesis is for Claire, Lorelle and Brad  
and my parents.

“ the field of forest entomology must in its very nature rest upon an ecological foundation”

Samuel A. Graham, 1956,

*Annu. Rev. Entomol.*, Vol. 1.

# *Chapter 1*

## **Introduction**





## Introduction

### *The Eucalypts*

The genus *Eucalyptus* contains approximately 700 species (Brooker and Kleinig 1990) of which almost all occur in forests and woodlands in Australia. Ten species occur only in northern Australia and New Guinea and four tropical species are endemic to northern New Guinea and southern Indonesia. In Australia, eucalypts dominate the coastal forests (often categorised as tall open-forests and open-forests) and woodland regions (trees 10-25 m in height often associated with grassland). They also comprise vast areas of mallee shrublands of dry inland southern regions of the continent (Williams and Brooker 1997). It is however, the tall open-forests of coastal Australia (known also as wet sclerophyll forests due to the usually mesic sites on which they grow) where large single stemmed eucalypts dominate the forest canopy and form tall majestic stands, with tree heights in excess of 30 m and some that exceed 90-100 m. These forests occur in a large coastal strip on the eastern side of the continent from the higher rainfall regions of northern Queensland (lat. 17° S) to Tasmania (lat. 42° S), are absent across the southern xeric regions of South and Western Australia, but also occur in the south west of Western Australia (lat. 35° S) (Ashton and Attiwill 1994). A floristic discontinuity

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exists between these major areas which can be divided into three broad groups. In southwest Western Australia *Eucalyptus diversicolor*, *E. calophylla*, *E. guilfoylei* and *E. jacksonii* are the predominant species. Predominant species associated with Tasmania, Victoria and parts of the northern New South Wales highlands, comprise one major east coast group consisting of *E. regnans*, *E. viminalis*, *E. obliqua*, *E. globulus*, *E. fastigata*, *E. delagatensis*, *E. cypellocarpa*, *E. dalrympleana*, and *E. nitens*. The remaining group, occurring from central New South Wales to southern Queensland, consists of *E. cloeziana*, *E. microcorys*, *E. pilularis*, *E. saligna* and *E. grandis* (Ashton and Attiwill 1994).

Many of the tall open-forest eucalypt species have long been recognised as a valuable timber resource. Uses for eucalypts include saw logs for construction, furniture and joinery timber; pulpwood for paper manufacture; shade, shelter and fuel wood; and volatile oils for pharmaceutical and industrial use (Eldridge *et al.* 1993). Eucalypts (particularly those in the sub-genus *Symphyomyrtus*) are now the most widely propagated tree genus in the world and can be grown in most tropical and temperate climatic regions between lat. 45° S and lat. 40° N. Plantation eucalypts in Brazil cover a huge 3x10<sup>6</sup> ha. India, Spain, Portugal, South Africa, Angola and China, all have between 3-4x10<sup>5</sup> ha while Ethiopia and Argentina each have 2.4x10<sup>5</sup> ha. These plantations, along with more than 5x10<sup>5</sup> ha, in 60 other countries attest to the tremendous adaptability of this genus (Eldridge *et al.* 1993).

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The success of eucalypts to adapt to new environments in both Australia and exotic sites is suggested to have evolved from the extremely variable environmental conditions of the past. Through millions of years of cyclic wet fertile to dry infertile and more recently (mid miocene), marked seasonal dry periods with increased frequency of fire, eucalypts have developed strategies which allow them to take advantage of periods of favourable growth between periods unfavourable for growth (Eldridge *et al.* 1993; Wardell-Johnson *et al.* 1997).

Rapid growth of plantation eucalypts in exotic locations is often attributed to the lack of environmental constraints that these species experience in Australia. Conditions of higher fertility, more reliable and higher rainfall, well prepared weed free sites and absence of fire may all contribute to such accelerated growth (Eldridge *et al.* 1993). Pryor 1976 (Eldridge *et al.* 1993) postulated that eucalypts grow better in exotic sites due to lack of naturally occurring insect pests and diseases. Ohmart (1984) however maintains that insufficient data exist to validate these claims and cites a number of examples where eucalypt growth trials in Australia, unprotected from insect pests, produce similar results to comparable overseas trials.

### *Eucalypt forest insects*

A large and diverse suite of insects belonging to a number of functional or feeding guilds (e.g. sap-suckers, seed-eaters, Gall-formers, leaf-miners, leaf-chewers and wood-borers) are associated with eucalypt forests in Australia



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(Majer *et al.* 1997; Ohmart and Edwards 1991). Sap-feeders include the hemipteran psyllids (Psyllidae), leafhoppers (Eurymelidae), scale insects (Eriococcidae) and coreid bugs (Coreidae). These species have a range of feeding strategies utilising the phloem of leaves and shoots and many produce large quantities of honeydew. Psyllid species, most notably the White Lace Lerp (*Cardiaspina albitextura*) and the scale insect *Eriococcus coriaceus*, during eruptive outbreaks can cause considerable damage to trees (Landsberg and Cork 1997).

Seed-eaters associated with eucalypts are mainly small beetles and wasps and seed exclusion experiments have shown they can cause up to 66% seed loss. Ants are also responsible for consuming a large amount of the seed that falls to ground (Landsberg and Cork 1997).

Gall-formers belong mostly to the hymenopteran Chalcid wasps, hemipteran coccoid scale insects, dipteran cecidomyiid gall-forming flies and a number of psyllids. Galls resulting from these insects however are not considered to be particularly harmful to eucalypts (Elliott and de Little 1984; Landsberg and Cork 1997).

Eucalypt leaf-miners belong primarily to the Lepidoptera (including families Cosmopterygidae, Incurvariidae, Nepticulidae, Gracillariidae and Nolidae) as well as sawfly larvae (Hymenoptera : Perigidae) (Landsberg and Cork 1997). One species which causes considerable damage to *E. camaldulensis* is the gum

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leaf skeletonizer *Uraba lugens* (Lepidoptera : Nolidae) (Campbell 1962) and it is known to erupt to outbreak levels on eight other eucalypt species from many areas of Australia (Ohmart and Edwards 1991).

A guild of considerable importance is the leaf-chewers (defoliators) and of these the Coleoptera comprising mainly Scarabaeidae, Chrysomelidae and Curculionidae are the most important groups (Ohmart and Edwards 1991). These beetles, particularly the chrysomelids are implicated in many outbreaks causing serious defoliation of eucalypts throughout Australia (Bashford 1993; de Little 1983; Elliott and de Little 1984; Greaves 1966; Ohmart 1990; Ohmart and Edwards 1991). Stick insects (Phasmatodea : Phasmatidae), sawflies (Hymenoptera : Pergidae) and many lepidopteran caterpillars from the families Geometridae, Nolidae, Anthelidae, Limacodidae, Saturniidae, and Lasiocampidae are also notable defoliators (Landsberg and Cork 1997; Ohmart and Edwards 1991).

Termites (Isoptera) are the most important group of wood-boring insects associated with eucalypts. According to Perry *et al.* 1985 (Landsberg and Cork 1997) these insects are not usually associated with healthy living trees but invade trees that have suffered fire or physical damage to the trunk. They can, after invasion, cause the death of burnt or damaged trees. Many weevils (Coleoptera : Curculionidae) feed on the bark, roots and shoots of eucalypts but damage is often only restricted to young plantation trees (Landsberg and Cork 1997).

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Studies of the impact of insect defoliation on tree growth have shown eucalypt species to be highly variable. According to Carne *et al.* (1974), defoliation levels of less than 50% are unlikely to impair subsequent growth of *E. grandis*. Studies by Readshaw and Mazanec 1969 however, (reported in Landsberg and Cork 1997) showed that defoliation by the phasmatid *Didymuria violescens* over a 16 year outbreak caused a reduction in radial growth increment of *E. pauciflora* and *E. stellulata* of 20%. Further, using the same eucalypt species during a non outbreak year, foliage insecticide treatment of branches produced a growth ring increment of more than double that of the untreated branches (Landsberg and Cork 1997).

These naturally occurring insect communities and guilds have been implicated in important eucalypt forest ecological and population processes. Processes such as nutrient cycling (Springett 1978; Ohmart *et al.* 1983), eucalypt species geographic distribution limits and influences on the coexistence of eucalypt species in mixed forest stands (Burdon and Chilvers 1974a, 1974b; Landsberg & Cork 1997; Morrow 1977; Williams 1990) have all been postulated. Evidence for many of these processes in the Australian eucalyptus open-forests is equivocal, but in disturbed regrowth or plantation forests, insect outbreaks are known to cause severe economic damage to tree growth (Bashford 1993; Elliott and de Little 1984; Ohmart 1990).

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### *Eucalypt plantation forest pests and biocontrol*

In Australian tall-open forests, the suite of insects associated with this habitat are almost without exception subject to many constraining factors of naturally occurring predators, parasitoids and pathogens (Landsberg and Cork 1997). In countries (including Australia) where eucalypts are established as monospecific plantation forests, reduction of biodiversity and alteration of the ecosystem mean that such constraints are often no longer present. Such habitats can allow an introduced insect species population to establish unimpeded and often reach eruptive and sometimes pest status.

One of the most serious and economically important insect guilds associated with loss of production from, or the death, of plantation eucalypts are the defoliators, especially leaf chewers (Bashford 1993; de Little 1989; Elliott and de Little 1984; Elliott *et al.* 1993; Ohmart 1990). It is this guild we will focus on for the rest of this section.

In Tasmania, the most notable of the leaf chewing defoliators are the paropsine leaf beetles (Coleoptera : Chrysomelidae). The paropsine *Chrysophtharta bimaculata* is capable of serious defoliation to plantation eucalypts especially *E. delegatensis*, *E. regnans* and *E. obliqua* (Bashford 1993; de Little 1989; Elliott and de Little 1984; Elliott *et al.* 1993; Ohmart 1990) with *C. agricola*, and *C. variicollis* also capable of notable damage (Elliott and de Little 1984). *Paropsis porosa* is known to cause damage to young eucalypt seedlings (Elliott and deLittle 1984).



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Other defoliating coleoptera are the eucalyptus weevil *Gonipterus scutellatus* (Curculionidae), scarabaeid beetles (many of the genus *heteronyx*), the green scarab *Diphucephala colaspidoides* (Elliott and de Little 1984) and in mainland Australia Christmas beetles *Anoplognathus* spp. (Carne *et al.* 1974; Elliott and de Little 1984; Ohmart 1990). Lepidopteran caterpillars particularly the gum leaf skeletonizer *Urba lugens*, the autumn gum moth *Mnesampela privata* and the helena gum moth *Antheraea helena* are all common defoliators of a variety of eucalypts. Leaf miner species include Lepidoptera of the *Acrocercops* spp. and hymenopteran sawfly larvae *Phylacteophaga eucalypti* (Elliott and de Little 1984; Ohmart 1990).

While many of these eucalypt pests in Australian plantations are controlled to a large extent by natural enemies, many of the controlling parasitoid and predator species are themselves suppressed by their natural enemies (e.g. parasitoids such as *Aridelus* sp. (Hymenoptera : Braconidae) being suppressed by a hyperparasitoid such as *Perilampus* sp. (Hymenoptera : Pteromalidae). Known predators of chrysomelid eggs and young larvae are the cantharid *Chauliognathus lugubris* (Coleoptera : Cantharidae) (Shohet and Clarke 1997) and the ladybirds *Cleobora mellyi* and *Harmonia conformis* (Coleoptera : Coccinellidae). Both adult and larval ladybirds are predatory and laboratory studies have shown that adults of both species can consume up to 25 *C. bimaculata* eggs per day. Field studies have shown that these coccinellids can achieve up to 80% predation of *C. bimaculata* eggs (Elliott and de Little 1984).

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In some seasons such controls can break down (Landsberg and Cork 1997) and eruptive pest outbreaks occur. Integrated Pest Management (IPM) strategies are being developed (particularly for *C. bimaculata*) not only to take advantage of naturally occurring biocontrol agents but to also enhance pest control using various insecticidal compounds such as *Bacillus thuringiensis* (Greener and Candy 1994) at strategic times (Clarke *et al.* 1997; Elliott *et al.* 1992; Harcourt *et al.* 1996).

Eucalypt plantations in other countries also experience insect pest problems. Most, but not all problems are associated with specialist eucalypt insects that have accidentally been introduced from Australia. A paropsine leaf chewing defoliator *Trachymela tincticollis* Blackburn has established and reached pest status on commercially grown eucalypts in South Africa. *Trachymela tincticollis* was first recorded in the Cape Town region in 1982 and had by 1985 spread up the east coast to Piketberg and West as far as East London (Cillié 1981; Tribe and Cillié 1985a, 1989). *Trachymela tincticollis* has been found on a number of *Symphyomyrtus* eucalypts in that country but the most severe defoliation occurs on *E. gomphocephala*.

Four hymenopteran egg parasitoids were released in South Africa in 1986 as possible biocontrol agents for *T. tincticollis*. Those released were the three

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pteromalids, *Enoggera reticulata*, *E. nassaui* and *Neopolycystus insectifurax* and an encyrtid species *Procheiloneurus* (G. Tribe pers. comm.). *Enoggera reticulata* (initially reported as *E. polita*) was the only species to establish. Establishment and spread was rapid, with consequent high rates of parasitism (97% of egg batches, 93% of eggs per batch) of that target species. The success of *E. reticulata* is attributed to the absence of hyperparasitoids and the initial high density of the target species (Tribe and Cillié 1989; Urban, *et al.* 1987).

The eucalyptus weevil *Gonipterus scutellatus* Gyllenhal was first recorded in South Africa in 1916 and caused considerable damage wherever eucalypts were grown. It has subsequently spread throughout Africa, Madagascar and Mauritius (Majer *et al.* 1997) and into Italy and France making eucalypt plantations in all these areas almost non-viable (Ohmart and Edwards 1991). This species also established in New Zealand with similar consequences to its host eucalypts. Successful biological control of *G. scutellatus* in South Africa (Ohmart and Edwards 1991) and New Zealand (Flux *et al.* 1993; Miller 1984) was achieved with the egg parasitoid *Patasson nitens* (Hymenoptera : Myrmaridae).

The most pervasive and wide spread eucalypt pest globally is *Phoracantha semipunctata* (Coleoptera : Cerambycidae). This Australian beetle has established in all major eucalypt growing areas of the world except India. First recorded in South Africa, it has spread throughout Africa, the middle east, the

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Mediterranean, South America, California (Ohmart and Edwards 1991) and Madagascar (Majer *et al.* 1997). In Australia, *P. semipunctata* is associated with debilitated trees particularly during drought conditions and indeed this species has its greatest ecological and economic impact in countries such as the xeric Mediterranean regions of Spain and Portugal where droughts are relatively common (Ohmart and Edwards 1991).

New Zealand eucalypt plantations have and continue still to suffer from introduced insect pests. It appears relatively easy for Australian eucalypt insect fauna to invade and establish in this country. Up until 1993, 15 species from 4 orders (Lepidoptera, Coleoptera, Hemiptera and Hymenoptera) have established and become pests of New Zealand's eucalypt forest industry (Flux *et al.* 1993).

The eucalypt leaf chewing defoliators established in New Zealand other than *Opodiphthera eucalypti* (Lepidoptera : Saturniidae), *Strepsicrates macropetana* (Lepidoptera : Tortricidae) *G. scutellatus* and the leaf miner *Phylacteophaga froggatti* (Hymenoptera : Pergidae) belong to the paropsina (Flux *et al.* 1993). *Paropsis charybdis* Ståhl was the first, recorded at Banks Peninsula in 1916 (Clark 1930) and by 1955 it had colonised the entire South Island (Bain 1977; Bain and Kay 1989). In 1956 *P. charybdis* was recorded in the North Island and is now present over the entire island wherever eucalypts are grown. *Paropsis charybdis* reached serious pest status causing severe defoliation and tree loss to eucalypt



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farm shelter and plantation forestry. The species *E. globulus*, *E. viminalis*, *E. macarthurii*, *E. nitens*, and *E. ovata*, all of which belong to the sub-genus *Symphomyrtus* (section *Maidenaria*), all suffered damage.

Attempts at biological control of *P. charybdis* were undertaken as early as 1932 (Bain and Kay 1989) when seven cocoons of the larval parasitoid *Meteorus* sp. (Hymenoptera : Braconidae) were sent from Canberra. What happened to these is unknown. During 1934-35 importations of *Aridelus* (Hymenoptera : Braconidae), *Froggattimyia tillyardi*, *Froggattimyia* spp. and a *Paropsivora* sp. (Diptera : Tachinidae) were of no use as they contained hyperparasitoids and sufficient numbers could not be reared for release (Bain and Kay 1989). In 1975 the first release of a biocontrol agent was the larval parasitoid *F. tillyardi* but the species has not been recorded since. In 1979 *Cleobora mellyi* the egg and larval predator of paropsines alluded to earlier was released, but it has only established in the Marlborough region (Bain and Kay 1989). *Cleobora mellyi* appears to need additional prey in the form of psyllids to enable it to achieve the fecundity and survival necessary to maintain an adequate population (Bain *et al.* 1984; Bain and Kay 1989). It was not until 1988 with the release of the egg parasitoid *Enoggera nassau* Girault (Hymenoptera : Pteromalidae) that *P. charybdis* was brought under control (Kay 1990). *Enoggera nassau* was released together with another pteromalid *Neopolycystus insectifurax* during the same season, but *N. insectifurax* has not been recorded since. In contrast *E. nassau*

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has established over almost the entire country, causing a dramatic decline in *P. charybdis* populations (Kay 1990).

In 1976 the paropsine, *Trachymela sloanei* was recorded in the Auckland region (Bain 1977) but this species has been slow to spread and has not reached pest status. Its southernmost population occurs in the Gisborne region (M. Kay pers. comm.). This species has established in California where its impact is causing much concern to foresters and biological control strategies are being considered (J. Millar pers. comm.).

*Trachymela catenata* was first recorded in the Gisborne/northern Hawkes Bay region in December 1992. This paropsine was recorded from a number of the *Symphyomyrtus* eucalypts that *P. charybdis* also favours i.e. *E. viminalis*, *E. macarthurii*; and *E. macarthurii* x *botryoides* (M. Kay pers. comm.). The current range of this species is still within the original area recorded. The future status of *T. catenata* in relation to its impact on plantation eucalypts was unknown and was the prime reason for the present investigation.

The most recent paropsine introduction, *Dicranosterna semipunctata*, was recorded in from Auckland in 1997 and has spread rapidly into other regions. This species, and a *Pyrgoides* sp. recorded from Auckland in 1976 (and which still occurs only in that region), are associated with *Acacia* (M. Kay pers. comm.) and will not be considered further. They do however serve to

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illustrate the variable pervasiveness of the paropsina genera and that the biology and ecology of any of this taxon that establish in New Zealand needs to be understood.

### *The Paropsina*

In Australia, the term paropsine refers to members of the two subtribes Paropsina and Dicranosternina within the tribe Chrysomelini (Reid 1992). This tribe is in the largest Australian sub family Chrysomelinae (with over 50 described genera and more than 600 species) of the family Chrysomelidae (Lawrence and Britton 1991). The Chrysomelidae (leaf beetles) comprise one of the biggest families (with about 3000 species) of the Australian Coleoptera (Lawrence and Britton 1991). The paropsina form the most important group of Australian Chrysomelinae as it contains several large genera including *Paropsis*, *Trachymela*, *Chrysophtharta*, *Paropsisterna*, and *Stethomela* (together comprising 138 species) with the majority of them feeding on eucalypts (Lawrence and Britton 1991; Majer *et al.* 1997).

In their Australian habitat, the majority of paropsina are relatively rare and are often treated as a functional group when considering their role in native forest ecosystems. This possibly explains the general paucity of literature available (given the size of the taxon) for this group. A few species however, because of their pest status in plantation forests or availability for laboratory studies, have received much attention.

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Considerable literature is available on the biology and ecology of *Paropsis atomaria* Ol. The ecology (Carne 1966a; Tanton and Khan 1978) and food utilisation efficiency (Carne 1966b; Fox and Macauley 1977; Morrow and Fox 1980) of *P. atomaria* has been extensively studied. The effects of leaf age/toughness and nitrogen concentration on larval performance (Larsson and Ohmart 1988; Ohmart *et al.* 1985a), population dynamics (Ohmart *et al.* 1987; Ohmart 1991), and fecundity (Ohmart *et al.* 1985b) as well as the effects of parasitization (Tanton and Epila 1984) on *P. atomaria* have all received attention.

The establishment of *Paropsis charybdis* in New Zealand in 1916 is documented in several publications since that time (Bain 1977; Clark 1930, 1938; Gurr 1957; Thompson 1922). The impact of *P. charybdis* on eucalypts since then has prompted much research effort into the biology and ecology of this species. Life history and ecology (McGregor 1989), and host plant relationships (Steven 1973) were the subject of two Ph. D. theses and experimental work on energy and nitrogen budgets using *P. charybdis* were conducted by Edwards and Wightman (1984). Much work in relation to pest management of *P. charybdis* in New Zealand has accrued since the 1970s. Larval mortality and pest control (Styles 1970), susceptibility to *Bacillus thuringiensis* (Jackson and Poinar 1989), a historical review of biological control (Bain and Kay 1989), a report of successful biocontrol (Kay 1990) and predation by hemipteran pentatomids (Edwards and Suckling 1980) of *P. charybdis* have all been produced.

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Biology of *Chrysophtharta bimaculata* Olivier has received attention from de Little (1979, 1983), de Little *et al.* (1990). Oviposition/host preference work has been done by de Little and Madden (1975) and Steinbauer *et al.* (1998) while Patterson *et al.* (1996) have considered larval performance on a range of eucalypt species. Dispersal behaviour of *C. bimaculata* and its implication for IPM strategies has been provided by Clarke *et al.* (1997) while Clarke *et al.* (1998) has provided information on adult overwintering sites. Defoliation and the pest status of *C. bimaculata* has been alluded to in a previous section. Life cycle, development and fecundity estimates of *C. agricola* have now been provided by Ramsden and Elek (1998) and one of the few studies of West Australian paropsina focuses on host preference studies of *C. debilis* and *P. elytrura* (Hall 1992).

The only literature available on the *Trachymela* genus consists of a brief descriptive bulletin of *T. tincticollis* (Cillié 1981), some short scientific notes (Tribe and Cillié 1985a, 1985b, 1989; Urban *et al.* 1987) and recently an ecological paper on *T. tincticollis* in South Africa (Tribe and Cillié, 1997). No information is available on the biology or ecology of *T. catenata*.



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### *History of Trachymela catenata in New Zealand*

During routine port inspections of Gisborne city in December 1992, Ministry of Forestry staff collected an unidentified paropsine beetle in eucalypt plantations from Hospital Hill and Kaiti Hill reserves. New Zealand Forest Research Institute (NZFRI) sent adult and larval specimens to Dr. Chris Reid at the Australian National University, Canberra, for identification. Dr. Reid identified the species as *Trachymela catenata* Chapuis. Past history of the closely related *P. charybdis* in New Zealand provided knowledge of possible pest status and prompted a wider search for this new introduction. In addition to the original collection of specimens around Gisborne, *T. catenata* was also recorded from sites 40 km west of Gisborne at Otoko and 40 km to the south at Morere. Both adults and larvae were recorded from *E. viminalis*, *E. macarthurii* and *E. macarthurii* x *botryoides* in these regions. Feeding damage on young eucalypt foliage by *T. catenata* is typical of the paropsine group. The margin of the leaves become deeply scalloped suffering severe damage and some are completely consumed, leaving many of the growing tips of the sprig totally denuded (Plate 3). The actual year of establishment of *T. catenata* is unclear. Discussion with Messrs Crawshaw and Alexander at Morere indicate that the species may have been established at the Morere sites for two seasons previous to discovery by Ministry of Forestry/NZFRI. As the time of establishment is unclear, the idea was also postulated that the *T. catenata* population had been established for some years but remained latent due to interspecific competition

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with *P. charybdis*. The decline of *P. charybdis* populations since the release of the parasitoid *E. nassau*, could have freed *T. catenata* from that competitive restraint and allowed the species to increase in numbers.

Before assessment of the future status of *T. catenata* in New Zealand could be evaluated specific areas of ecological information on the species were necessary. To this end I focussed on the areas of life history, phenology, mortality of larval and pupal stages and development as a function of temperature which comprise chapter two of this thesis. Chapter three focuses on the potential of eight eucalypt species as hosts which included assessing larval performance and female ovipositional preferences on each. In chapter four, by comparison with *P. charybdis*, the potential impact of the solitary egg parasitoid *E. nassau* on *T. catenata* was evaluated. Finally chapter five synthesises the information from the preceding chapters.

### *Study sites*

Field studies were conducted on the southern most population at Morere (177° 47' E, 38° 59' S) in northern Hawkes Bay New Zealand (Fig. 1.1). Two sites were maintained over the period 1 August 1993 to 4 March 1995. Site AL was at Alexanders farm, and site CR at Crawshaws farm, Morere. Site AL comprised a fenced area  $\approx 350 \text{ m}^2$  under five mature *E. viminalis* on the north facing edge of a small woodlot. Inspection of this site during April 1993 revealed numbers of *T. catenata* adults greater than we had observed at any

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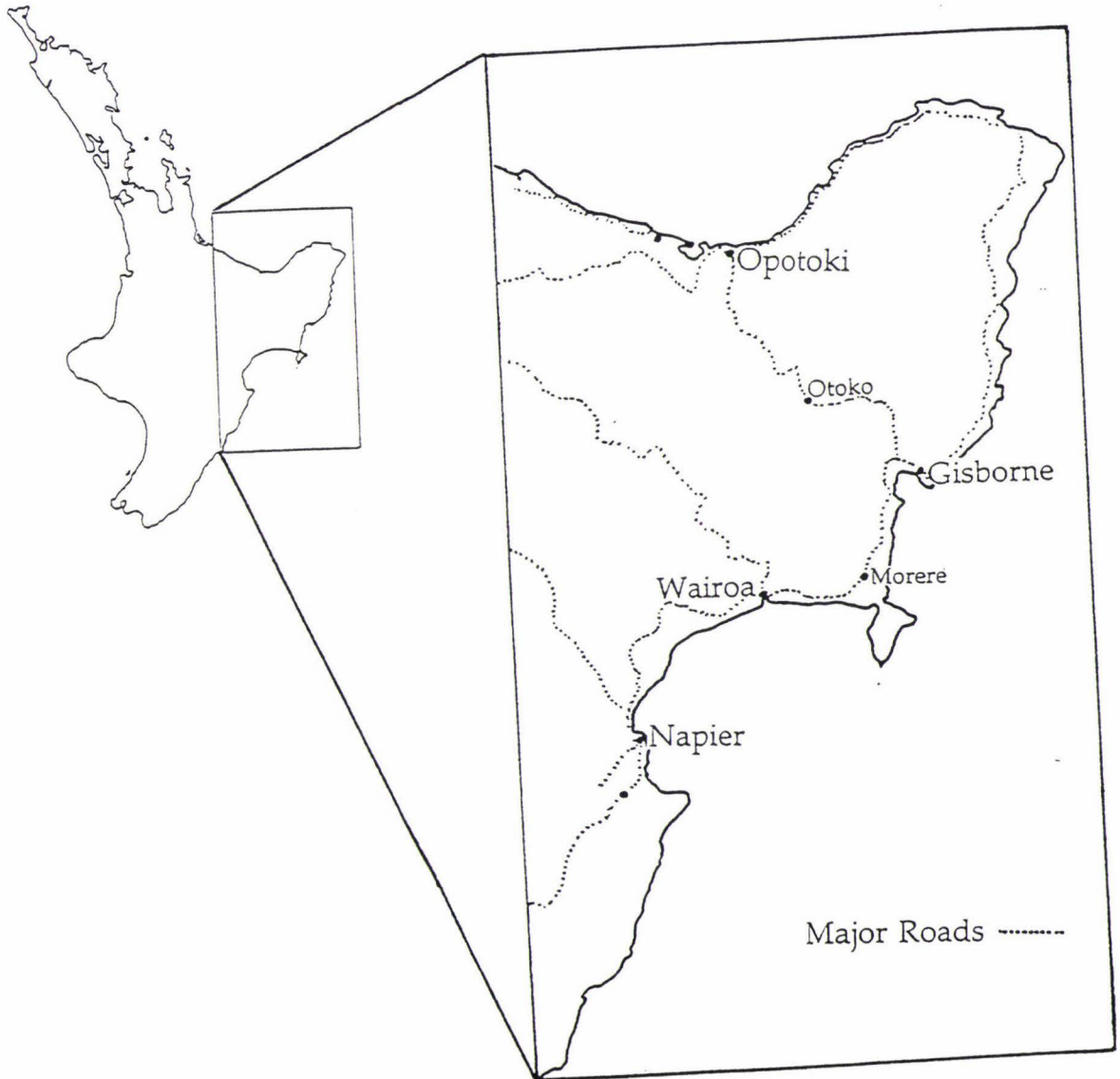
other site in the Gisborne/northern Hawkes Bay region. Overwintering adults were readily observed in the leaf litter on 24 July. Fencing of the site, installation of a continuous recording thermograph and erection of five cone traps (to monitor prepupa fall into the leaf litter) was completed on 1 August 1993 (Plate 1). Site CR comprised a fenced area  $\approx 550 \text{ m}^2$  under a mixed stand of *E. viminalis* and *E. macarthurii* also on the north facing edge of a woodlot approximately three times larger than that at site AL. Smaller numbers of adult *T. catenata* were observed at site CR in April and overwintering adults were observed on 25 July. Fencing, installation of a continuous recording thermograph and erection of five cone traps (under *E. viminalis* only) was completed 29 August 1993 (Plate 2).

Cone traps were constructed from moulded polythene, and were erected on four 1 m long timber stakes driven into the ground. The top diameter of each cone was 600 mm ( $2827 \text{ cm}^2$ ) with a tapered bottom opening of 75 mm reducing to 70 mm (outside diameter). Over the bottom opening was placed a PVC pipe "catch" cup 73 mm (inside diameter)  $\times$  180 mm long. The cup had a fine gauze bottom inserted to allow water to pass through but retain all leaf material and larvae that dropped from the foliage.

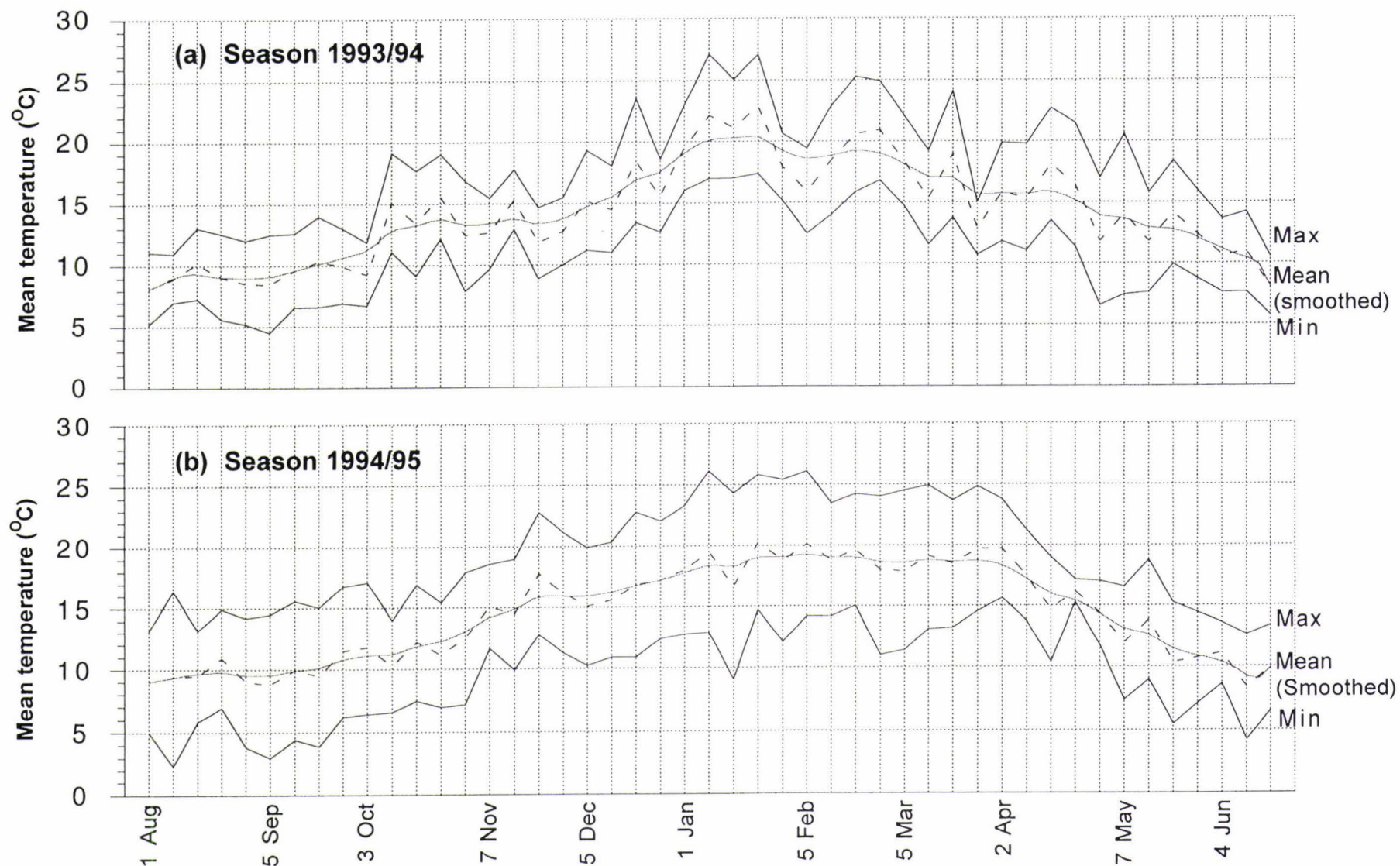
Thermograph records were obtained continuously from 1 August to 11 June for both 1993/94 and 1994/95 seasons. Graphical presentation of these data includes the mean, rounded mean, maximum and minimum based on the mean reading of each, for each 7 day interval (Fig. 1.2a&b). Dates included for these



data are the end date for each interval and give a guide to the monthly temperature changes.



**Fig. 1.1** Gisborne/Hawkes Bay region of North Island New Zealand. Current range of *T. catenata* is within a 40 km radius (west to Otoko and south to Morere) of Gisborne city.



**Fig. 1.2a&b** Seasonal temperatures in 7 day intervals at site AL for seasons 1993/94 and 1994/95.

Notes: Dates are the last day of each interval - Dotted line = actual mean temp.





**Plate 1.** Site AL, at Alexanders farm, Moreere.



**Plate 2.** Site CR, at Crawshaws farm, Moreere.





**Plate 3.** Leaf damage by *T. catenata*, typical of the paropsine group.



**Plate 4.** Typical emergence and cone trap set up for pupal mortality experiments.

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## *Chapter 2*

# *Ecology of Trachymela catenata*

**Ecology of the recently established paropsine *Trachymela catenata*  
Chapuis (Coleoptera : Chrysomelidae) in New Zealand: Life  
history, Phenology, Mortality and Development.**

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***Abstract:***

Many members of the Australian paropsina (Coleoptera : Chrysomelidae) are serious defoliating pests of *Eucalyptus* trees. Knowledge of the ecology of *Trachymela catenata*, was necessary to assess the potential pest status of this recent introduction to New Zealand. Field records show *T. catenata* to be feeding on members of the *Symphomyrtus* sub-genus of eucalypts, especially *E. viminalis*. *Trachymela catenata* life stages are similar to other paropsina and females are as fecund as many of those which have reached pest status in other countries. Phenological data shows overwintering *T. catenata* adults emerge and lay eggs in spring with resulting larvae and adults grazing *Eucalyptus* foliage over spring/summer causing damage to host trees. Similar to other paropsina, evidence points to *T. catenata* being bivoltine. Over a season, mortality is highest in first instar larvae (60%) compared 14% and 13% for second and third instars respectively. Using a simultaneous combination of cone and emergence traps, mortality of the prepupal/pupal stage was



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estimated at 45.8%. Using a range of six temperatures (range 7 - 32 °C) developmental thresholds were determined for each life stage. Thresholds were 4.95 °C for eggs, 6-7 °C for larval stages and 8-9.5 °C for pupal stages. Development rates were shown to be similar (including a decline in rate at the highest temperature trialed) to results obtained for other paropsina. Thermal conditions in the current geographical range and that of all (except colder alpine areas) of New Zealand should not be limiting to *T. catenata's* potential spread.

### *Introduction:*

In December 1992, a tortoise beetle (Coleoptera : Chrysomelidae) previously unrecorded in New Zealand, was found in the Gisborne and Northern Hawkes Bay areas. Specimens of this *Eucalyptus* defoliator were identified by Dr. Chris Reid (Australian National University) as *Trachymela catenata* Chapuis. *Trachymela catenata* is one of a large group of species belonging to the Australian paropsine group comprising members of the subtribes Paropsina and Dicranosternina (Reid, 1992).

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Both paropsine larvae and adults feed on leaves of the host tree and can be serious pests of plantation eucalypts in Australia (Bashford 1993; de Little 1989; Elliott *et al.* 1992, 1993; Greaves 1966; Kile 1974; Ohmart and Edwards 1991), South Africa (Cillié 1981; Tribe and Cillié 1985, 1997) and New Zealand (Styles 1970; Steven 1973; Bain 1977; Bain and Kay 1989; McGregor 1989) as well as acacias throughout Australia (Elliott and de Little 1984).

Five members of the Paropsina have been recorded in New Zealand since early this century (see Chapter 1) and all appear to have established stable populations (M. Kay, pers. comm.). The most notable of these was *Paropsis charybdis* Ståhl first recorded at Banks Peninsula in 1916 (Clark 1930, 1938; Bain 1977; Bain and Kay 1989). *Paropsis charybdis* reached serious pest status and caused severe defoliation and tree loss, wherever establishment of farm and commercial forestry plantings of eucalypts (particularly those belonging the *Symphyomyrtus* sub-genus - Section Maidenaria) were attempted (Bain 1977; Bain and Kay 1989; McGregor 1989; Steven 1973; Kay 1990). These host species include *E. globulus*, *E. viminalis*, *E. macarthurii*, *E. nitens*, and *E. ovata*.

*Paropsis charybdis* is relatively rare in Australia and little information about its biology exists from that country. A number of studies conducted since the 1970s however, provide considerable information on the biology of *P. charybdis* in New Zealand (Edwards 1982; Edwards and Suckling 1980; Edwards and Wightman 1984; McGregor 1989; Steven 1973; Styles 1970). The pest status of *P. charybdis*, was such that since 1933 a series of attempts at finding a suitable

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biocontrol agent for this species were undertaken (Bain and Kay 1989). In 1988, release of the egg parasitoid *Enoggera nassau* (Hymenoptera : Pteromalidae) resulted in a severe decline in *P. charybdis* numbers (Kay 1990) throughout most areas of New Zealand.

*Trachymela sloanei*, first recorded in New Zealand in 1976 (Bain 1977), utilises similar eucalypt host species to *P. charybdis* (i.e. *E. macarthurii*, *E. viminalis*, *E. globulus*, *E. pulchella*, *E. ovata* and *E. botryoides*) (M. Kay pers. comm.) but has not reached pest proportions, has been slow to disperse, and is presently limited to the northern half of the North Island.

Like *P. charybdis*, *T. catenata* is relatively rare in Australia and little information is available on the biology or ecology of this species. This work is intended to provide information on the biology and ecology of *T. catenata* which can be compared to what is known of *P. charybdis* and other paropsina in an effort to help evaluate the future status of this species in New Zealand.

Key areas of investigation were to : 1, determine the life stages and seasonal population patterns and the habitats used for these; 2, investigate temperature requirements (a well documented correlate associated with development and important when predicting population growth and potential geographic distribution); and 3, investigate reproductive capacity and mortality (important when considering potential population growth).

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This paper gives a brief morphological description, reports on life history, habitat use, phenology, development, fecundity and mortality of *T. catenata* using both field and laboratory data and observations.

## **Methods:**

### *Adult Identification*

Adult *Trachymela catenata* and *T. sloanei* are morphologically very similar and the two species coexist in localised areas of the Gisborne region. Dorsally the pronotum of both species extends well over and around the head, obscuring the posterior margin of the eyes and leaving only the anterior portion of the head, the filiform antennae and labrum visible. The elytra are oval and convex with the elytral suture extending to the tips. The elytra also have a large epiplural area, with a heavily reinforced margin that extends well below the level of the abdominal sternites thus covering the entire abdomen and completely obscuring the legs (when at rest) giving the beetle its typical "tortoise-like" appearance. Unlike *T. sloanei*, the adult head, pronotum and elytra of *T. catenata* are shiny dark to rusty brown with black marking varying in size between individuals. The black markings of the pronotum usually form a central, anteriorly oriented "W" shape (Fig. 2.1a). with a black area also

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at the lateral margin. The elytral markings generally consist of two semicircular black areas at the apex of the suture which form a central 'spot' and anterior to this are similarly sized black areas sometimes extending to the anterior margin (humeri) (Fig. 2.1a). Black areas of varying size also extend from the humeri to approximately halfway along the epiplurae (Fig. 2.1b) and around the posterior epiplural margin. *Trachymela sloanei* are of a similar base colour but have small, uniformly distributed black specks over the entire surface of the elytra.

A number of *Trachymela* species (including *T. catenata* and *T. sloanei*) have secretory glands on the pronotum and elytra which (in mature adults) produces a waxy secretion (Selman 1988) that covers the entire pronotum and elytral surface giving the animal a dusty flour like appearance (Plates 5 & 6). For both these species this masks many of the features described above and identification is only possible when the secretion is removed.

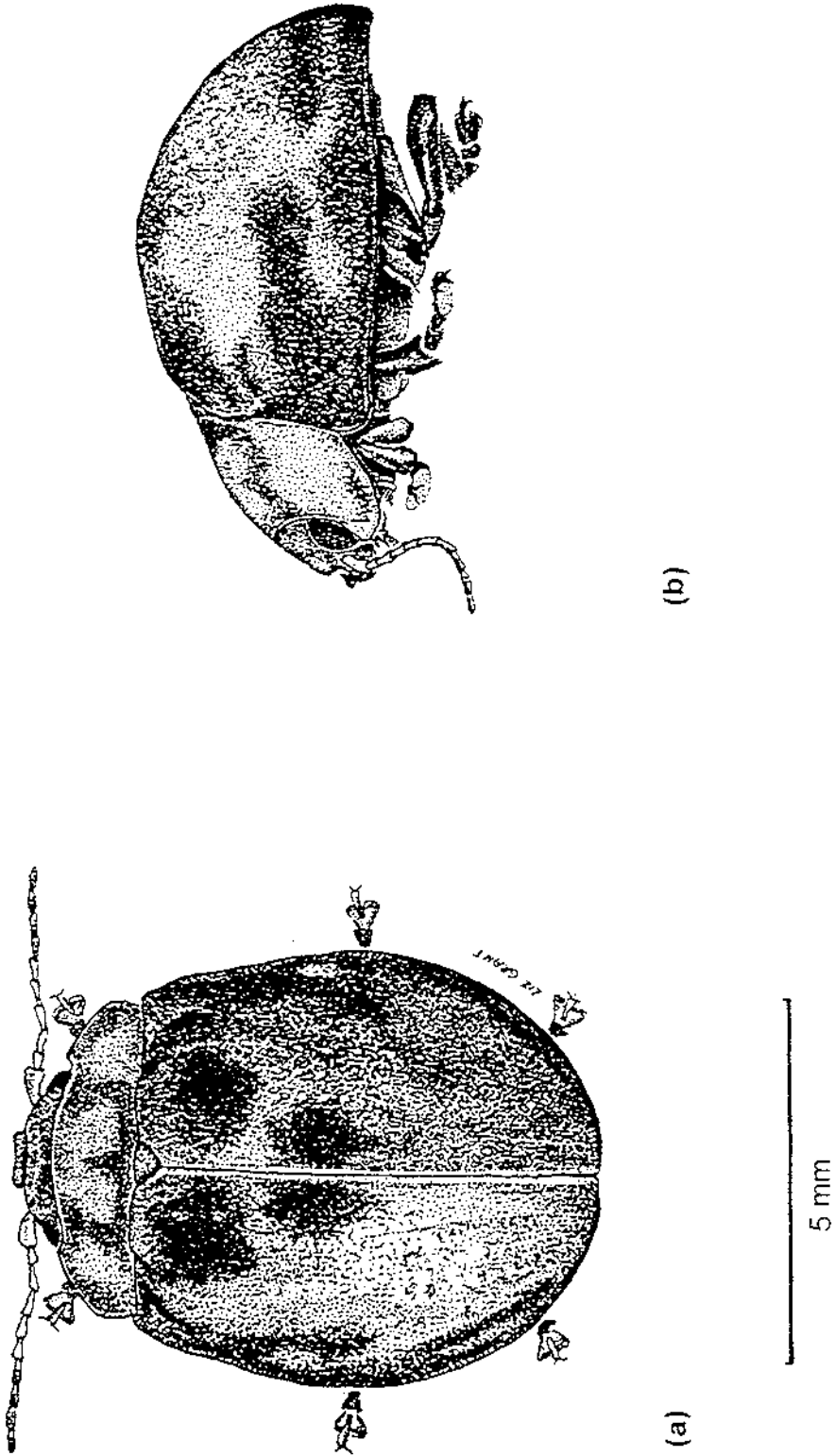


Fig. 2.1a&b Adult *T. catenata* dorsal (a) and lateral (b) views.





**Plate 5.** Teneral adult *T. catenata* showing no wax secretion on pronotum or elytra.



**Plate 6.** Mature adult *T. catenata* with wax secretion covering pronotum and elytra.

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*Life history and Phenology:*

Field studies were conducted at Morere (177° 47' E, 38° 59' S) in northern Hawkes Bay, New Zealand, from 1 August 1993 to 4 March 1995. Two sites were maintained. One, site AL (Alexanders) comprised of a fenced area of  $\approx 350 \text{ m}^2$  under five mature *E. viminalis* trees on the north facing edge of a small woodlot. The second, CR (Crawshaws), comprised a fenced area of  $\approx 550 \text{ m}^2$  under mature *E. viminalis* on the north facing edge of a much larger woodlot. This woodlot also included a small number of *E. macarthurii*. Continuous thermograph records were maintained at both sites.

Life history of *T. catenata* was determined from field observations and cultures reared in the laboratory. The number of larval stages was determined from these cultures by observation and by recording head capsule measurements. Similarly, pupal characteristics were determined from laboratory and field observations.

Phenological data were obtained from site AL at Morere from April 1994 to March 1995. Data on overwintering adults were obtained from monthly leaf litter searches over the period June to November 1994. These searches consisted of recording all live adult *T. catenata* from five  $50 \text{ cm}^2$  quadrats each consisting of the leaf litter layer and the top 2 cm of soil. Estimates of the mean number of live adults per quadrat were used to assess the overwintering population over time.

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Foliage surveys provided data on oviposition patterns, with the number of eggs per batch and the age class of leaf upon which each batch was laid being recorded. Age classes of leaves were determined as: class 1 the terminal leaf, class 2 the first pair of leaves below the terminal leaf, class 3 the second pair below the terminal leaf (adjacent to leaf class 2) and so on down to the leaf class 7. All classes after that pair were termed >7. Mean egg batch size was then calculated along with the proportion of egg batches laid on each leaf-class over the season.

Foliage surveys also provided data on adult phenology during spring/summer 1994/95. These surveys comprised of recording adult numbers from each of 10 permanently tagged branches, thus standardising the leaf area surveyed. The mean number of adults from the 10 branches for each survey was calculated and then expressed as the proportion of total mean number over the season to give relative seasonal abundance (RSA).

Prepupae fall from the foliage to pupate in the leaf litter. This allowed prepupal numbers to be recorded from five cone (litter-fall) traps (600 mm top opening tapering to a 73 mm diameter gauze bottomed "catch cup" (see Chapter 1)) erected under *E. viminalis* foliage. Dead fourth instar larvae are morphologically similar to the prepupal form and difficult to tell apart. All specimens of this size and morphology were therefore considered prepupae.

Dead larvae representing all instars were also obtained from these traps and together with the prepupal catch provided phenological data. Trap samples containing the larvae and prepupal fall were collected monthly from September 1993 to April 1994 and every 7 - 10 days from September 1994 to April 1995 and preserved in 70% EtOH until counting. The 1994 to 1995 samples for 1st instar and prepupae were used to provide data on larval phenology. As for the RSA for adults (above), the mean number of larvae from the five traps for each sample period was obtained and expressed as the proportion of total mean number over the season to give relative seasonal abundance (RSA).

$$RSA = Sp_x = \frac{\sum T_{1-5}}{T_n} \Rightarrow \frac{Sp_x}{\sum Sp_{x,1-12}}$$

Where  $Sp_x$  = Mean number trapped for sample period x  
 $T_{1-5}$  = Trap numbers 1-5  
 $T_n$  = Total number of traps  
 $Sp_{x,1-12}$  = Mean number trapped for sample periods 1 - 12

#### *Development trials:*

Trials testing larval and pupal development time as a function of temperature were initiated using newly hatched *T. catenata* larvae which had had the opportunity to consume the egg chorion i.e.  $\leq 24$  h old. One larva was placed in a petri dish containing a layer of damp paper towelling (to maintain humidity) and a small adult leaf of flush *E. viminalis*. One replicate comprised ten such dishes. Three replicates were run at each of six temperatures i.e. 7, 12, 17, 22, 27 and 32 °C ( $\pm 1.5$  °C) and 16 h photoperiod. During the larval growth stages (1st - 4th instars) leaves were replaced every 48 h and enough moisture replaced to keep the towelling damp but not "wet". Pupal stages

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continued to be maintained in the damp dish conditions. Each individual was monitored twice daily, assessed as live or dead, and its instar recorded. Monitoring was maintained until adult emergence.

Development time ( $t$ ) for each of the six instars, at each temperature, was established (to the nearest half day) using the value at which  $\geq 50\%$  of larvae/pupae had moulted to the next instar. Using the three replicates, mean and standard errors (S.E.) were obtained. To test the validity of the effect of temperature and instar on development time, two way ANOVA including an interaction term was run on the development times for each of the six instars. Results from trials at 7 °C were not included as complete development did not occur for any instar at this temperature.

Trials to determine development time of eggs (laying to eclosion) were conducted over the same range of temperatures as that used for larval/pupal development. Three replicates with 30 - 68 eggs in each, were set up for each temperature using eggs of a known age (range 8 - 24 h). Eggs were monitored twice daily until eclosion. The number of days on which the greatest number of eggs eclosed was adjusted for age at beginning of trial and the mean and standard error of the three replicates obtained. Total development time (egg lay to adult emergence) was obtained from the combined times of egg, larval and pupal development trials.

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To determine developmental thresholds for each instar, development rate needs to be determined from development time at each temperature. Development rate was determined as the inverse of those values obtained for development time

$$\text{Development rate} = 1/t$$

and the mean and standard errors (S.E.) established over the three replicates.

These data were subjected to regression analysis of the four linear points i.e. 12, 17, 22 and 27 C° and developmental thresholds taken as the x intercept of the regression line (Harman *et al.* 1989; Petitt *et al.* 1991) The mean developmental threshold over all stages was then obtained.

#### *Mortality and infertility:*

Egg infertility data were gained from the various trials where 1st instar larvae were required. The number of larvae hatched in relation to the number of eggs collected from cultures provided these estimates. Unhatched eggs were checked under the microscope to confirm that no development had occurred. Leaves with eggs intact were recovered from cone trap samples. These provided an estimate of egg losses (mortality) in the field due to infertility and leaf abscission.

Stage-specific mortality rates were not recorded for larvae in the field, but cone trap samples provided a measure of larval mortality (again expressed as RSA) and estimates of the mean percent mortality for 1st, 2nd and 3rd instar larvae



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were gained over the entire sampling period. To investigate differential mortality between 1st and 2nd (spring and summer) generations these data were divided into two appropriate groups. 1st generation data were taken from 3 December to 31 December and 2nd generation from 18 January to 4 March. This removed 26 November data which contained no 3rd instars, removed 7 January to reduce overlap of the two generations and provided an equal number of sample periods in each group. The sum of the mean frequency of mortality for each generation for each instar was obtained and subjected to Chi-square analysis to test the null hypothesis that there was no difference in the frequency of mortality for each instar between generations.

Mortality data for the prepupal/pupal stage were obtained from the addition of emergence traps, (prior to spring 1995) to the five cone traps (each 600 mm diameter (2827 cm<sup>2</sup>) previously alluded to. This set up consisted of a series of five gauze-covered emergence traps being placed around each of the above cone traps (Plate 4). Emergence traps were 50 x 50 cm (2500 cm<sup>2</sup>) at the base tapering to a 12 mm funnel hole, opening into a glass jar (Plate 4). At the beginning of prepupal fall, one emergence trap was shifted back, thus exposing the quadrat it previously covered, and allowing prepupa to fall into that area. At seven-day intervals, the adjacent traps were sequentially shifted back to cover the previously exposed quadrat and expose the next one for prepupa to fall into. In this manner cone traps provided a weekly estimate of the number of prepupae falling into the leaf litter and emergence traps an estimate of live adults emerging. Numbers in the 2500 cm<sup>2</sup> emergence trap were reduced by

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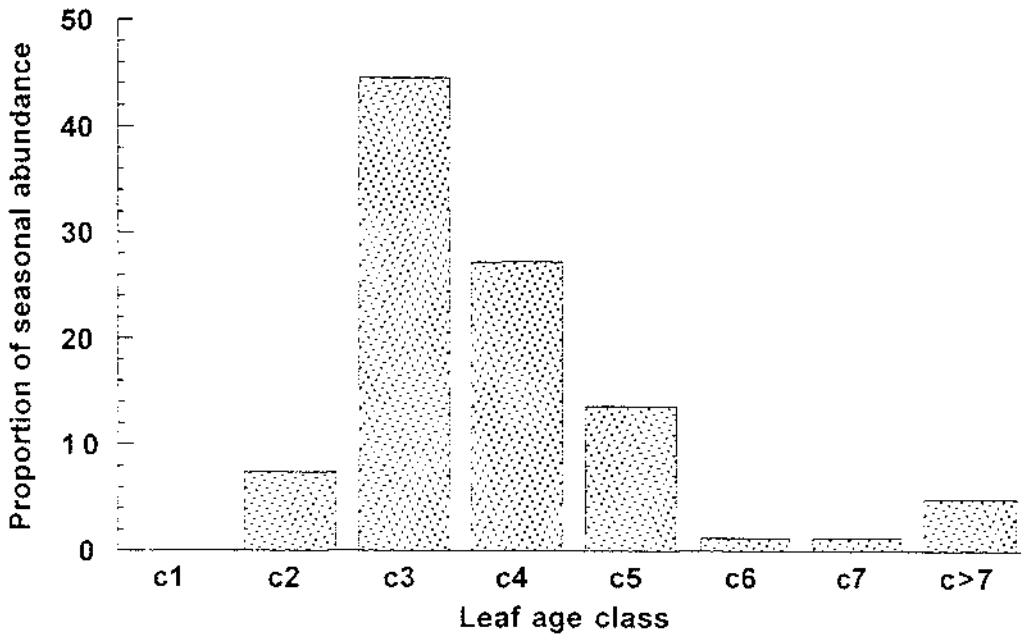
the ratio of the difference in cm<sup>2</sup> of the larger cone trap, then percent mortality gained for the five replicates. Mean mortality over the five data sets was then estimated.

### *Results:*

Considerable variation in *T. catenata* population sizes existed between the two study sites despite them comprising the same dominant mature *E. viminalis* species, being just 7 km apart and at the same elevation. Site AL, while small, maintained a much larger population of *T. catenata* than site CR. Site CR, (set up as a replicate of AL), provided very little information from litter surveys or cone trap samples.

### *Life History:*

Eggs, once hatched, progress through four larval instars. A prepupal stage precedes the pupal form, which then emerges into an adult. Egg batches (mean number of eggs =  $7.67 \pm 0.27$  S.E.,  $n = 76$ , range 3 - 16) are laid in a single row and with an apparent preference for the flush 3rd and 4th leaf pair (age class) of the sprig (Fig 2.2).



**Fig. 2.2** Proportion of egg batches laid on each leaf class. (Classes 1-7 = current seasons growth and class >7 = previous seasons growth). Leaf age classes are described in methods.

Observations showed that newly hatched larvae consume the egg chorion and then feed exclusively on flush foliage showing a strong preference for the soft tissue of the terminal, and 2nd and 3rd leaf pairs during the 1st and 2nd instars. Third and 4th instars also feed on softer leaves but as the larvae mature, they shift to older, thicker, less succulent leaves.

An indication of female fecundity was determined from cultures of *T. catenata* maintained at  $27 \pm 1.5$  °C. Three females produced mean egg batch sizes of  $7.63 \pm 0.19$  S.E. eggs batch<sup>-1</sup> (n = 205). The mean number of eggs laid per female per day was 6.9 with oviposition periods ranging from 65 - 88 days. This provided an estimate of  $522 \pm 89$  S.E. eggs per female over the laying period and indicates they lay approximately one batch per day.

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The prepupal stage was characterised by the larvae ceasing to feed, taking on a curved semi-circular appearance and dropping to the leaf litter. The pupal form then develops a few days later within the leaf litter. No evidence was found of pupae forming pupal cells in the litter or soil as has been described for *P. atomaria* (Carne 1966) and *T. tincticollis* (Tribe and Cillié 1997).

Adults emerged from the leaf litter to then feed on the foliage. Observations from the laboratory cultures (at 22 °C) showed that mating and oviposition can occur in 7 - 12 days. Duration from adult emergence to mating and oviposition under variable field temperatures however is not known.

*Phenology:*

At the AL site Morere, observations from monthly leaf litter surveys for overwintering adults suggests a preference for deeper moist conditions (presumably to avoid desiccation) and quantified a progressive decline in mean number per quadrat from 24 July 1994 (mean = 4.12) until 30 October 1994 when no more were found (Fig. 2.3 c). October 30 foliage surveys however, revealed a substantial increase in the Relative Seasonal Abundance (RSA) of adults (RSA = 10.1, Fig. 2.3 c). This shift of habitat coincided with a progressive increase in mean daily temperatures (as determined from the thermograph records) from 9 °C (winter) to 13 °C in October - November (spring) (see Chapter 1).

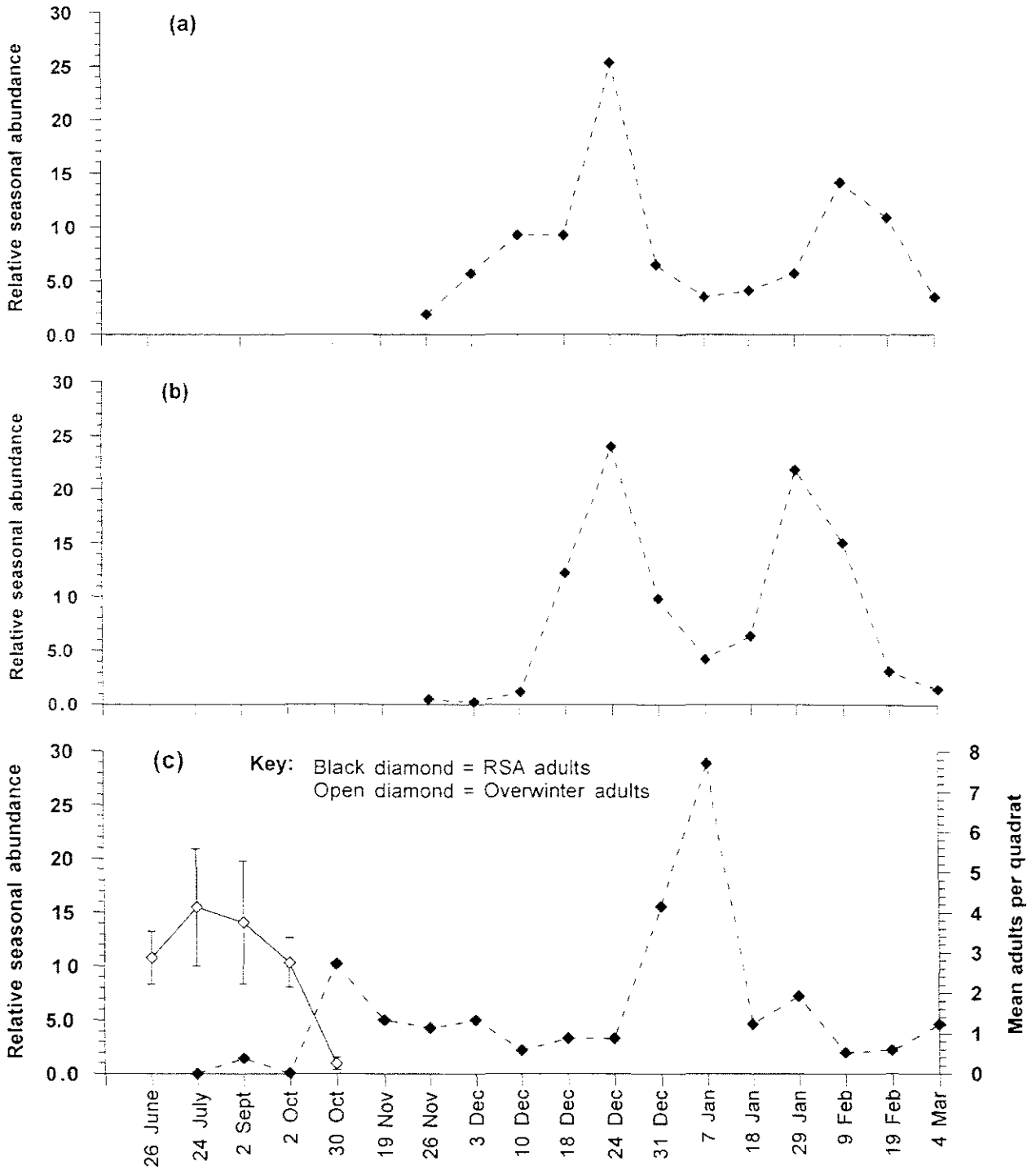


Fig. 2.3 a,b,c. Relative seasonal abundance of 1st instar (a), 4th instar/pre pupa (b) and adult (c) *T. catenata* over 1994/95 season. Overwintering adults equal mean number (including S.E.) per quadrat

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Egg batches for the 1994/95 season were first recorded on 30 October. Batch numbers then increased steadily over time with a substantial increase recorded on 10 December.

First and 2nd instar larvae were observed during the above period with 3rd and 4th instars becoming more readily seen as time progressed. These observations are supported by RSA from the cone trap samples for the period 26 November - 10 December (Figs. 2.3 a&b) where a number of 1st instar larvae were caught but few 4th instar/prepupa stages are evident. Numbers for 1st instars peaked on 24 December with a second, though smaller peak evident 47 days later on 9 February (Fig. 2.3 a). No egg batches were observed from 18 December through to 18 January. Egg batches were readily observed again on 29 January some 11 days before the second 1st instar peak.

Adult numbers (from standard branch counts) revealed a substantial increase on 7 January (Fig. 2.3 c) some 14 days after the peak in 4th instar/prepupa fall. An increase in adult numbers is evident on the 4 March when sampling ceased. The foliage of the tagged standard count branches at this time held very little flush foliage, however increased adult numbers were observed on many small areas of flush not included in these tagged areas. These individuals had not yet developed the waxy elytral secretion indicating they had only recently emerged so are taken to be teneral second generation adults.



*Development:*

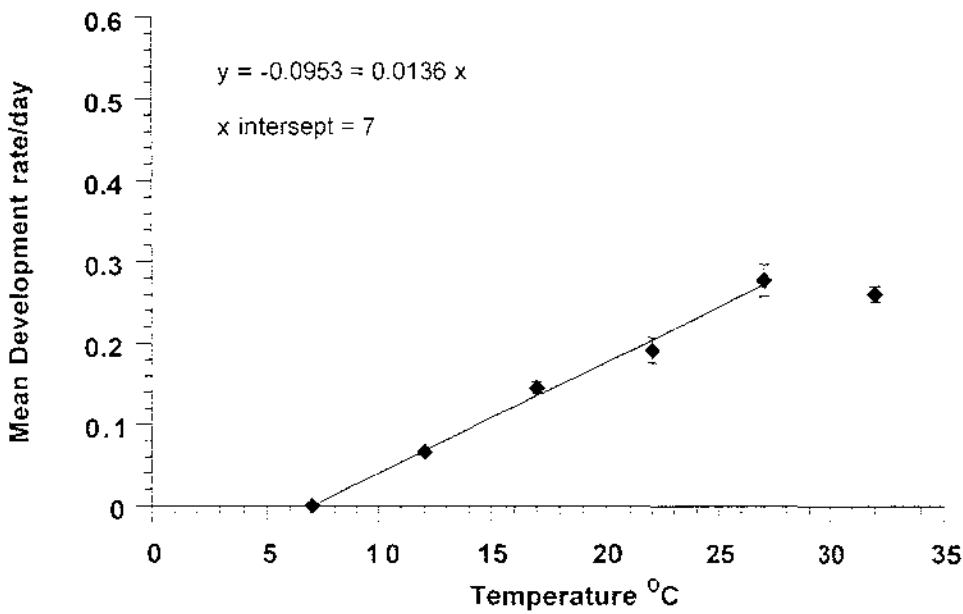
Mean total development time decreased as temperature increased until a temperature of 32 °C was attained, after which development time increased (egg to adult time Table 2.1). No development occurred in any life stage at 7 °C and development did not proceed past the prepupal stage at 12 °C (Table 2.1). First instar development time continued to decrease over all temperatures. The 2nd and 3rd instars were consistently the shortest life stages except at 32 °C. A marked slowing of development occurred in the 4th instar also at 32 °C which accounts for the greatest part of the increase in development time as compared to 27 °C.

Two way ANOVA indicated highly significant effects of temperature ( $F_{3, 71} = 32.71$ ,  $P = 0.0001$ ), instar ( $F_{5, 71} = 42.05$ ,  $P = 0.0001$ ) and temperature  $\times$  instar interaction ( $F_{15, 71} = 7.22$ ,  $P = 0.0001$ ) on development time.

A significant effect for trial was also obtained, seemingly due to the high mortality and slow growth rates at 12 °C, which for later instars in some trials produced highly variable data with zero data points and/or very high single values. Exclusion of the 12 °C data set then, produced a non significant result ( $F_{2, 71} = 0.40$ ,  $P = 0.6742$ ) for the effect of trial. ANOVA grouped 2nd and 3rd instars together as not being significantly different.

**Table 2.1** Mean number of days ( $\pm$ S.E.) required for each instar of *Trachymela catenata* to develop under a range of constant temperatures.

Stage	Temperature ( $^{\circ}$ C)					
	7	12	17	22	27	32
Eggs	-	15.2 $\pm$ 0.59	6.86 $\pm$ 0.35	5.28 $\pm$ 0.39	3.63 $\pm$ 0.27	3.86 $\pm$ 0.14
Instar						
1st	-	12.83 $\pm$ 2.16	5.42 $\pm$ 0.55	5.16 $\pm$ 0.44	3.50 $\pm$ 0.5	2.16 $\pm$ 0.16
2nd	-	10.33 $\pm$ 1.45	4.00 $\pm$ 0.57	3.00 $\pm$ 0.28	2.16 $\pm$ 0.17	2.33 $\pm$ 0.16
3rd	-	10.75 $\pm$ 0.2	4.00 $\pm$ 0.0	3.16 $\pm$ 0.58	2.33 $\pm$ 0.17	2.66 $\pm$ 0.33
4th	-	15.00 $\pm$ 2.0	5.50 $\pm$ 0.5	4.83 $\pm$ 0.17	3.50 $\pm$ 0.29	7.83 $\pm$ 0.83
Pre pupa	-	42.00 $\pm$ 0.0	6.66 $\pm$ 0.17	4.50 $\pm$ 0.29	3.50 $\pm$ 0.29	2.83 $\pm$ 0.16
Pupa	-	-	12.66 $\pm$ 1.92	7.50 $\pm$ 0.5	5.66 $\pm$ 0.33	5.16 $\pm$ 0.66
Egg to adult	-	-	45.10 $\pm$ 2.18	33.43 $\pm$ 0.33	24.28 $\pm$ 0.43	26.83 $\pm$ 0.77
Total rate			0.022	0.029	0.041	0.037

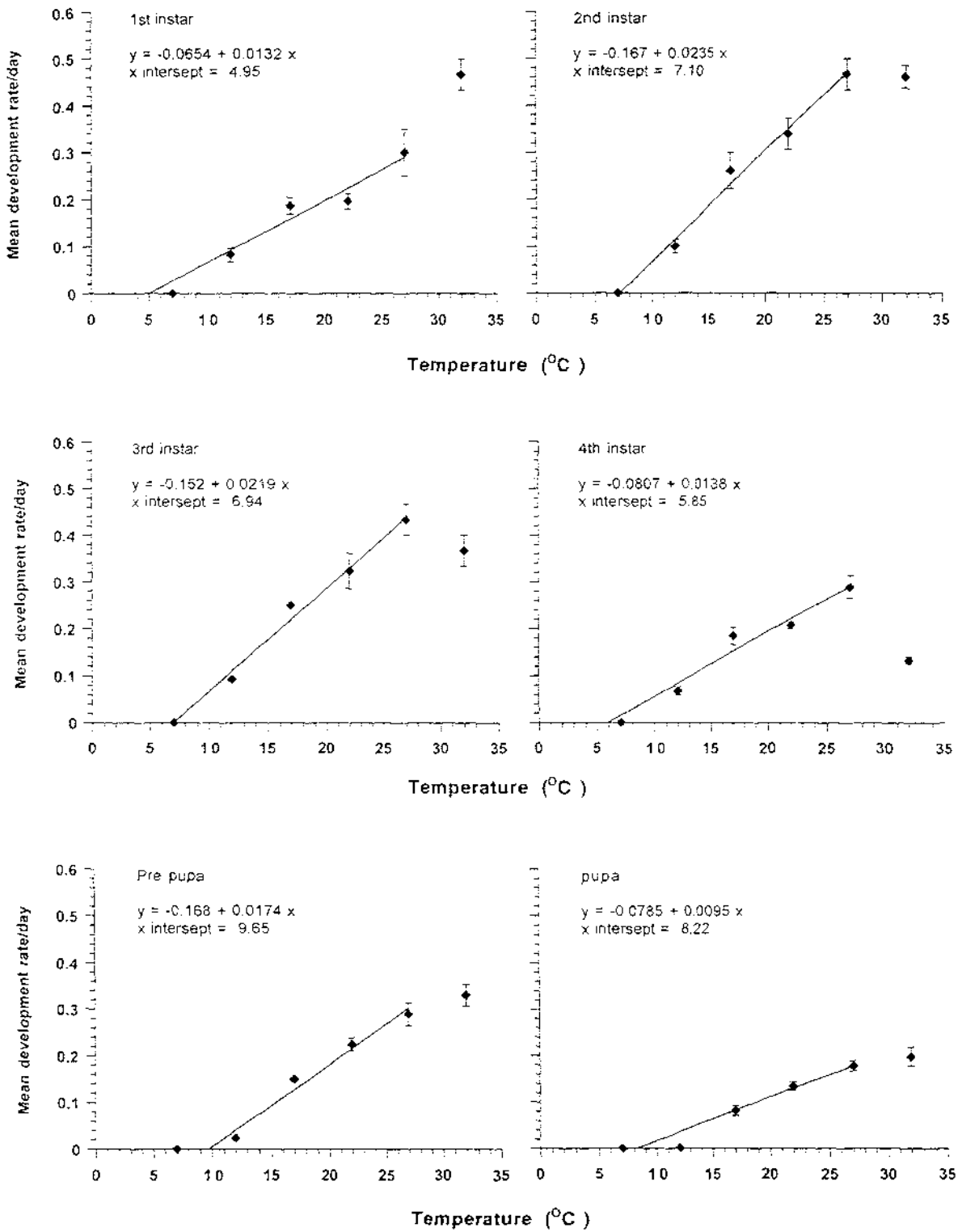


**Fig. 2.4** Egg development rate as a function of temperature and developmental threshold for *T. catenata*. (vertical bars = S.E.)

Development rate regression analysis reveals a developmental threshold of 7 °C for eggs (Fig. 2.4) and 2nd 3rd and 4th instar larvae (Fig. 2.5) of 6 - 7 °C but only 4.95 °C for the 1st instar stage (Fig. 2.5). Thresholds for prepupa and pupa are slightly higher at 8 - 9.5 °C. The mean development threshold for the species is 7.10 °C  $\pm$  0.57 (S.E.). Development rate is linear for eggs and all instars from 12 to 27 °C but a general decline in rate occurs (with the exception of 1 instar) at 32 °C (Fig. 2.5).

#### *Mortality and infertility:*

Estimates of losses during the egg stage were based on two factors: One; - egg infertility; and two - losses due to abscission of leaves upon which eggs were laid. Percentage of infertile eggs from laboratory trials was 1.5 - 2% (98% fertility).



**Fig 2.5** Larval and pupal development rate as a function of temperature and developmental thresholds for *T. catenata*. (vertical bars = S.E.)

Leaves with eggs intact occurred in four of the larval cone trap samples, thus some loss due to leaf abscission was evident. Total egg loss due to these two factors is estimated at 5%.

Mean number (per trap) of dead larvae for each instar for each sampling period over the season is shown in Fig. 2.6. These samples provided estimates of percent mortality for each instar. Mean seasonal percentage was 60%, 14% and 13% for 1st, 2nd and 3rd instars respectively. An estimate of 4th instar mortality from the development trials however suggest that this stage suffers a similar mortality rate as the 3rd instar.

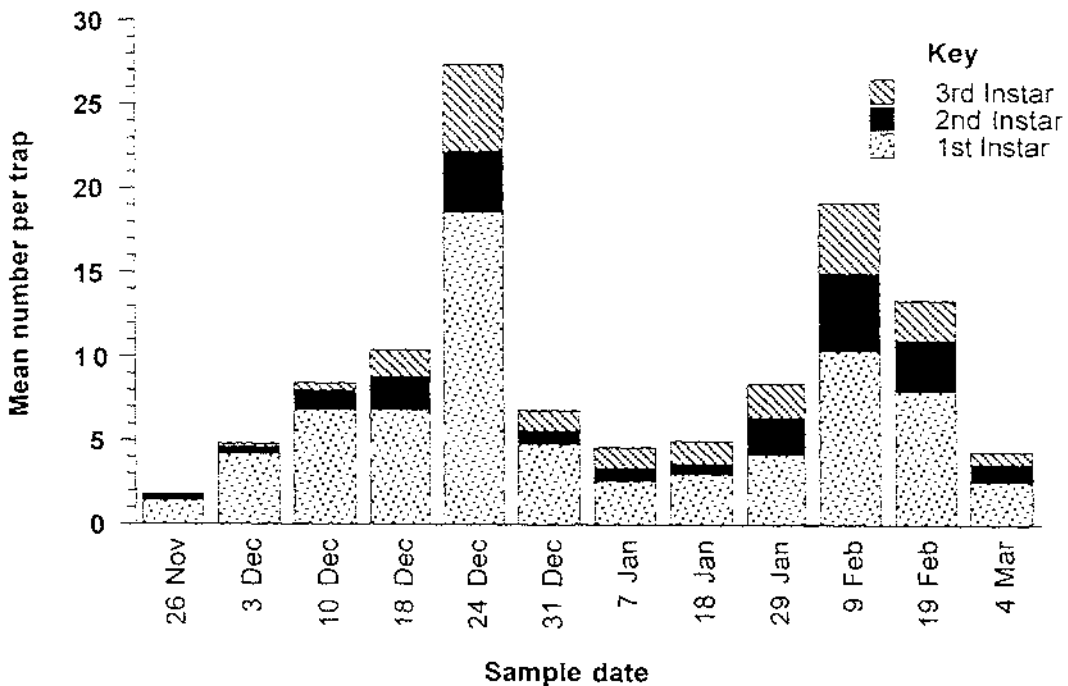


Fig. 2.6 Mean number of dead 1st, 2nd and 3rd instar *T. catenata* larvae from traps over season 1994/95

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The sum of the mean frequency of mortality for 1st, 2nd, and 3rd instar respectively was, for spring generation 41, 8 and 9 and summer generation 28, 11, 11. Chi-square analysis revealed no significant difference between these data ( $\chi^2 = 2.54$ , d.f. = 2, 0.05, = 5.99) and we failed to reject the null hypothesis that there was no significant difference between the frequency of mortality for each instar between generations.

From the five replicates of sequential emergence trap data, mean mortality rate for the prepupa/pupal stage was 45.8%.



**Discussion:**

The number of life stages of *Trachymela catenata* follows the pattern (i.e. four larval instars, a prepupal stage, pupa and adult) established by previous workers for other paropsina (Carne 1966; Cumpston 1939; de Little 1979a; McGregor 1989; Ramsden and Elek 1998; Tribe and Cillié 1997).

The mean (first generation) egg batch size from field data ( $7.67 \pm 0.27$  S.E.) is considerably less than that reported for first generation *Paropsis charybdis* (median 16.5 & 17.0 per batch over two seasons respectively) by McGregor (1989). Tribe and Cillié, (1997) reported a mean egg batch number of 9.8 for *T. tincticollis* under 27 °C laboratory conditions.

The population growth potential of *T. catenata* (based on female fecundity) appears similar to some of the other paropsina which have reached pest status thus far. The estimate of fecundity (6.9 eggs per day) obtained from the small number of *T. catenata* females is considerably less than the 11.4 eggs per day reported for *T. tincticollis* (Tribe and Cillié, 1997) and an estimated mean seasonal fecundity of 1463 eggs (G. Tribe pers. comm.). However, estimated seasonal fecundity for *T. catenata* of  $522 \pm 89$  S.E. is more closely aligned with Carne's (1966) estimate for *P. atomaria* which lays fewer but larger egg batches than *T. catenata* and has an estimated seasonal fecundity under favourable

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conditions of  $\approx 640$  eggs. A laboratory estimate of total fecundity for *C. agricola* is  $477 \pm 333$  eggs per female (Ramsden and Elek 1998). Similarly, laboratory estimates of fecundity from 13 *C. bimaculata* females was  $674 \pm 127$  eggs per female (de Little 1979a).

Choice of oviposition site is highly variable between paropsines. *T. catenata* oviposits a single row preferentially on the third or fourth leaf pair of rapidly expanding flush foliage. This contrasts with *T. tincticollis* which oviposits in narrow crevices of the bark or under bark curls of the tree (Tribe and Cillié, 1997). Similarly, *T. sloanei* oviposits in crevices of the bark (M. Kay, pers. comm). *Paropsis atomaria* females oviposit eggs in successive circular collars around terminal shoots of its host tree (Carne 1966, Tanton and Khan 1978) whereas *P. charybdis* oviposits on the tips of old sclerophyllous foliage adjacent to flush growth (de Little 1979b; McGregor, 1989). *Trachymela catenata* eggs were only occasionally recorded from this class of foliage i.e. class >7. *Chrysophtharta bimaculata* oviposits rows of eggs (de Little 1979a) while *C. agricola* deposits eggs in an untidy clump (Ramsden and Elek 1998) on flush foliage, similar to *T. catenata*.

Aggregation behaviour of *T. catenata* larvae appears not to be as strongly developed as it is for some of the other paropsina. First instar larvae aggregate on and consume the chorion of the egg cluster before they begin feeding on foliage. During this instar, they were often observed in small aggregations but from the 2nd instar onwards, appeared to disperse singly

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through out the foliage, and are only occasionally observed in two's or three's. *T. tincticollis* larvae are reported to conceal themselves in bark crevices during the day, only emerging at dusk or before dawn to feed (Tribe and Cillié, 1997). Conversely *T. catenata* larvae of all stages can be observed on the foliage at any time of the day. Carne (1966) reported that several *P. atomaria* larvae of the same or different instars aggregate whenever they are not feeding. *Paropsis charybdis* is similar in habit to *T. catenata* in forming loose aggregations while consuming the egg chorion and thereafter dispersing (McGregor 1989). I have however observed, in very windy conditions, small ( $n \approx 3$ ) tightly aggregated groups of 3rd and 4th instar *P. charybdis* larvae in the axis of stems and small branches.

*Phenology:*

*Trachymela catenata* adults overwinter in the leaf litter apparently maintaining metabolic processes from the extensive fat bodies laid down from the previous autumns feeding (Carne 1966). Like *P. atomaria* (Carne 1966) adult *T. catenata* may be observed in late winter - early spring on unseasonally warm days, attached to logs or foliage but feeding was never observed at this time. Spring emergence appears to be temperature related (Carne 1966, McGregor 1989). Thermograph records from site AL for 1993 and 1994 indicated an increase in mean ambient temperature during October of about 3-4 °C. During October 1994, a marked decline in overwintering adult numbers in the leaf litter was matched by a definite increase in adult numbers on the foliage (Fig. 2.3 c).

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Eggs (2 small batches) were first observed on 30 October and the number of egg batches increased slowly through to December. On 10 December 1994 a substantial increase in egg batch numbers was observed and numbers decreased rapidly thereafter. At 17 °C (the mean weekly temperature at that time) we expect egg development to take 6.9 days thus producing an increase in 1st instar numbers the following week. Field data support this expectation with the peak in 1st instar mortality being recorded for the 24 December sample period (Fig. 2.3 a). The second increase in egg numbers was recorded on 29 January. Using similar assumptions as for the first generation (but with a slightly shorter development time allowing for mean weekly temperature now at 19 °C) we would expect the increase in 1st instar mortality around 9 February (Fig. 2.3 a). The new egg batches observed on 29 January is 50 days after 10 December and along with the duration between 1st instar peaks may be indicative of the field generation time. From the temperature vs development trials, the estimated development time for a complete generation (at 17 to 19 °C) is  $\approx$  41 days for eggs to adult plus 10 days adult emergence to oviposition time totals 51 days. Given sampling intervals of 7 to 11 days in my field data, variation of  $\pm \geq 7$  days may exist in the 50 day duration between the two egg/1st instar seasonal peaks. The estimated generation time of 51 days however, lies within this possible variation and is as close as these data allow.

First generation adult numbers peak approximately 13 days after the maximum fall of 4th instar/prepupa (Fig. 2.3 b&c). The subsequent sampling period revealed a substantial decrease in adult numbers (Fig. 2.3 c). In Tasmania,

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major dispersal events are characteristic of *C. bimaculata* (Clarke *et al.* 1997; de Little 1979a). Clarke *et al.* (1997) showed that *C. bimaculata* adults will disperse after egg laying and as well, found a negative correlation between adult numbers and tree damage or larvae present. During this time at site AL, very little flush foliage remained available as female oviposition sites but whether a similar dispersal event could explain this decline in numbers is not known.

The second peak of 4th instar/prepupa appears only 22 days after the peak of the first generation adults. At 19 °C, adult maturation to oviposition, egg and larval development are estimated to take  $\approx$  33.5 days. Assuming those adults were responsible for the second peak in egg laying and hence the second generation, the estimated duration is unacceptably reduced. If the time is taken from the 31 December however (when adult numbers were increasing rapidly), that date provides a more acceptable 29 day period. The 4th instar/prepupa peak appearing earlier than predicted may well be error due to the problem of sampling interval previously alluded to.

Adult numbers resulting from the second generation fall of 4th instar/prepupa were difficult to quantify because the standard branch count areas at that time (4 March) were no longer in a flushed state and few adults remained at these sample points. However, on 4 March, increased numbers of teneral adults were readily observed on adjacent small areas of flush foliage but obviously could not be included in these data.

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This phenological pattern indicates that *T. catenata* appears to follow the bivoltine life history established for many of the other paropsine taxa.

A single trial to mimic adult overwintering and the importance of flush feed for the initiation of spring oviposition was conducted in the laboratory. Overwintering adult *T. catenata* were collected and maintained in leaf litter for three months at a constant 12 °C. Over a period of 10 days (commencing on 15 October) the temperature was progressively increased to 22 °C. Once emerged from the overwintering state these adults, survived for > 8 weeks while being fed only last seasons sclerophyllous foliage. While on this diet both male and female adults formed waxy elytral secretions, appeared active and healthy but did not mate or oviposit. After five weeks this culture was divided. One group of males and females was fed flush foliage while the other (males and females) remained on sclerophyllous feed. Adults on flush feed mated and commenced egg laying in 10 - 11 days whilst those remaining on old foliage layed no eggs at all.

Carne (1966) suggests, that emergence from overwintering is initiated by the depletion of fat bodies, and my observation of fat bodies from dissected overwintering adults supports this hypothesis. The result of this trial may indicate that overwintering *T. catenata* adults can emerge in the spring and await the onset of flush foliage. The precise timing of emergence to coincide with eucalypt flush growth therefore, may not be an obligate requirement. Such a strategy may confer considerable adaptive advantage to a phytophage

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that is constrained to a single or a small range of host eucalypts that display considerable seasonal variability in the onset of spring growth.

### *Development*

Results of the development versus temperature trials (where development time progressively decreased with increasing temperature), are comparable with development rates attained for *C. bimaculata* (de Little and Madden 1975) and *C. agricola* (Ramsden and Elek 1998) and produced a useful working model which will assist in estimating field based generation times. The major assumption with the working model is that growth rates and hence development time under constant temperatures are similar to those obtained in fluctuating field temperatures. Developmental studies on various insect species using constant temperatures have, when exposed to fluctuating temperatures, resulted in a decrease (Hagstrum and Leach 1973) or in some cases no difference (Kitching 1977) in development time. The effect of fluctuating temperatures on development time for *T. catenata* are unknown.

Fourth instar development time at 32 °C increased markedly when compared to the development time of other stages at that temperature. Like many insects the greatest increase in volume and mass occurs during the final instar and any impairment to metabolic rate will presumably have its greatest effect at that stage. Interestingly, at 32 °C the development time of prepupa and pupa



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continues to decrease which is reflected in an almost continual linear trend in development rate (Fig 2.5).

Over all, egg and larval development rate increased as a function of temperature up to 27 °C with a decline (particularly during the larval stages) thereafter. The decline in development rate at 32 °C is in line with similar experimental work on *P. atomaria* (Carne, 1966). Maximum intrinsic growth rate for *T. catenata* therefore is at temperatures approaching the high 20s. In its present northern Hawkes Bay/Gisborne range, daily maximum temperatures can reach in excess of 27 °C but sustained mean temperatures at that level are unlikely. In terms of thermal requirement, population growth for *T. catenata* will most likely remain below its maximum potential.

The relatively low developmental threshold (4.95 °C) for 1st instar larvae seems to arise from the relatively low development rates at 22 °C and 27 °C which affects the slope of the regression line. Explanation of these values is difficult. Controlled temperature conditions as for all other trials, remained within  $\pm 1.5$  °C, humidity was maintained with damp paper towelling and foliage was carefully selected to maintain similar quality and sourced from the same tree for all trials in each replicate. However, with increased development rates at 22 °C and 27 °C the threshold rate is unlikely to increase to greater than 7 °C and given that all other larval thresholds are around 7 °C, 100% mortality of 1st instar larvae under controlled conditions is to be expected.

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The overall developmental threshold for larval stages is similar to that reported by McGregor (1989) for *P. charybdis*.

Results from the development trials indicate *T. catenata* larvae at 12 °C fail to complete development and need temperatures greater than 12 °C to persist. By the time 1st generation larvae are developing at site AL, mean daily temperatures are 15 - 17 °C and larvae are able to develop well. With an almost exponential rate of increase in development time from 17 down to 12 °C, *T. catenata* is not likely to persist at mean weekly temperatures in the low teens because the generation time would be such that completion of one generation per season is unlikely. The mean spring/summer temperatures (17 - 19 °C) of the current range of *T. catenata* are not dissimilar to those in a large portion of New Zealand. The potential therefore for *T. catenata* to establish over a wide area of the country should not be limited by thermal requirement.

The developmental threshold for prepupa/pupal development was the highest of any stage at about 9.6 °C and development was not completed at 12 °C in the trials. Conceivably, these instars may be vulnerable to a period of very cold temperatures (perhaps not likely in its current range) which could have an important impact on the number of pupa entering adulthood. Further work is required to investigate how long *T. catenata* prepupa/pupa can sustain temperatures around this threshold level and survive to continue development when conditions are again favourable.

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*Mortality:*

The laboratory results for egg fertility for *T. catenata* of 98% are similar to those reported for other paropsina (e.g. Ramsden and Elek 1998) and my estimate of an overall 5.0% loss due to infertility and leaf abscission may well be conservative. McGregor (1989), using marked egg masses, reported egg survival for *P. charybdis* of  $\approx 94\%$  in the field and 98.5% from laboratory trials. His field estimate does not appear to account for losses due to leaf abscission, so the survival rate of total eggs laid may be less than his estimate suggests.

The very low mortality for 2nd and 3rd instar larvae early in the season (26 November - 10 December) is expected due to the time lag for the population to attain those stages. This is in line with the phenology data. Thereafter, 1st instar mortality appears relatively high (as compared to 2nd and 3rd instars) in the spring. Flush foliage appears abundant at this time, however minimum daily temperatures do drop to around 5 °C. It is conceivable that such temperatures could reduce the larvae's metabolic rate and impair their ability to maintain a hold on the leaf and be instrumental in the high mortality rate for this stage.

The Chi-square analysis comparing larval mortality between instars of the 1st and 2nd generations was not significant. The test was justified on the basis that for the second generation, mortality rate of 2nd and 3rd instar larvae appeared high compared to 1st instars when compared with the 1st (spring) generation. During the second generation period 18 January to 4 March

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considerable defoliation from 1st generation larvae and newly emerged teneral adults was apparent. This appeared to result in much poorer feed quality and quantity (this however was not quantified) which may conceivably manifest itself in increased mortality, especially for the larger instars which require ever increasing quantities of quality food.

Mortality during the pupation stage of 45.8% is considerably less than that reported for *P. charybdis* at  $\approx 89\%$  (McGregor, 1989). McGregor (1989) shifted his emergence traps every fortnight. My method of emergence trapping may have caught a greater number of emerging adults (thereby decreasing the estimated mortality rate) due to the traps being left in place through out the entire sampling period. Sixty five percent of emerging *T. catenata* adults caught were in the period  $31 (\pm 7)$  days after the emergence traps were placed and sealed down. This time span is considerably longer than that recorded from the development trials and does not fit well with the peak in adult numbers recorded from the standard branch counts. One explanation may be that adults after that peak were dispersing in search of oviposition sites (hence not being counted) and at a rate faster than that of emergence from the leaf litter. It also suggests that pupation in the leaf litter may be influenced by environmental parameters (e.g. temperature) and is an area that would benefit from further investigation.

The differences in population size between the two sites is not easy to explain.

I assumed the sites to be the same as both contained stands of mature trees

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which were well synchronised in the onset of flush foliage. Temperature conditions were well synchronised between the two sites and both would be exposed to similar rainfall patterns and available moisture. It is therefore difficult to conclude that tree growth conditions are not similar between the two. The small number of *E. macarthurii* mixed with *E. viminalis* at site CR presumably should not have any detrimental effect on the *T. catenata* population since these two eucalypt species are closely related (Pryor and Johnson 1971) and all life stages were observed on *E. macarthurii* over the sampling period. At site CR traps were placed only under *E. viminalis*. Disturbance and trampling of leaf litter from farm stock (thus perhaps destroying overwintering adult *T. catenata*) at site CR occurred during winter of 1993 and may have explained low *T. catenata* numbers for the summer of 1993/94 but such disturbance did not occur for season 1994/95. The population numbers at CR therefore may be indicative of the pervasiveness of *T. catenata* in that habitat conditions which for some reason are not totally suitable (or highly variable) will not allow the population to erupt. The corollary of this, is that overall, *T. catenata* populations and consequent damage to eucalypt trees may be highly variable between sites and or regions.

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# *Chapter 3*

## Host Preferences



**Larval performance and female oviposition preferences of  
*Trachymela catenata* Chapuis (Coleoptera : Chrysomelidae)  
on eight species of *Eucalyptus*.**

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***Abstract:***

The potential of eight *Eucalyptus* species were assessed as hosts to the paropsine *Trachymela catenata* (Coleoptera : Chrysomelidae). Performance of *T. catenata* larval growth rates, mortality and adult live weights were quantified for each eucalypt species. Only three species, *Eucalyptus viminalis* and *E. nitens* (from the sub-genus *Symphyomyrtus*) and *E. coccifera* (sub-genus *Monocalyptus*) proved suitable hosts for complete larval development and adult maturation. Using the potential host tree species, caged trials designed to assess female ovipositional preference were performed. Foliage in all possible combinations was presented and results show that *T. catenata* females do not discriminate between the three potential host species. These results suggest that *T. catenata* may be relatively polyphagous and capable of sustaining viable populations, on hitherto unrecorded eucalypt host species.

## *Introduction*

Reliance on a small number of *Eucalyptus* species for commercial forestry and farm shelter planting in New Zealand has prompted a need to identify the eucalypts which may be attacked by the paropsine defoliator *Trachymela catenata* (Coleoptera : Chrysomelidae) recently established in this country. Past experience with paropsine defoliators such as *Paropsis charybdis* in New Zealand (Styles 1970; Steven 1973; Bain 1977; Bain and Kay 1989; McGregor 1989), *T. tincticollis* in South Africa (Cillié 1981; Tribe and Cillié 1985, 1997) and *Chrysophtharta bimaculata* in Tasmania (Bashford 1993; de Little 1979, 1983, 1989; Elliott *et al.* 1992; Greaves 1966; Kile 1974; Ohmart 1990) has shown that members of this group of insects are capable of causing severe defoliation to plantation eucalypts.

Some 30,000 ha of eucalypts are established in New Zealand for commercial forest purposes. Species grown as specialty timbers include *E. saligna*, and *E. botryoides*; for pulpwood and veneer *E. fastigata* *E. regnans*, *E. delegatensis* *E. obliqua*; and pulpwood only *E. nitens* (T. Withers and M. Kay pers. comm.). To date, in New Zealand *T. catenata* eggs, larvae and adults have been recorded on three members of the *Symphyomyrtus* sub-genus (Section Maidenaria) (after Pryor and Johnson 1971) i.e. *E. viminalis*, *E. macarthurii* and *E. macarthurii* × *botryoides* (M. Kay pers. comm.) which are planted widely as farm shelter and woodlots.

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In Australia, *T. catenata* has been collected from *E. polyanthemos* (a member of the *Symphyomyrtus* sub-genus) and *E. robertsonii* (a member of the *Monocalyptus* sub-genus (Reid pers. comm.)) but plantings of these species are not common in New Zealand. This range of known hosts covering two sub-genera, suggests that *T. catenata* may display a relatively polyphagous feeding association.

Two areas of investigation are important in providing assessments of potential host plant use; firstly, investigations of larval growth rates and survival (performance, see Thompson 1988) on a range of possible host species; and secondly, investigations concerning host plant selection by females (oviposition preference, see Singer 1986). Work on a wide range of insect species has led to the formulation of the preference - performance hypothesis and its relationship to various plant species and or properties (Courtney and Kubota 1990; Leather 1994; Price 1994; Singer 1986; Thompson 1988; Valladares and Lawton 1991). The preference - performance hypothesis suggests that ovipositing females should select host plants or a particular part of a host plant on which the phytophagous larvae will gain the greatest survival and subsequent reproduction (Dodge *et al.* 1990; Valladares and Lawton 1991). However, investigations on a wide range of insect species on which larval growth and development is entirely dependent on the female's choice of host and/or quality of site, has shown such preference performance relationships to range from very strong to very poor (Price 1994; Valladares and Lawton 1991).



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For the paropsina, investigations of larval performance and caged trials of female host preference of *C. bimaculata* (de Little and Madden 1975) as well as *C. debilis* and *P. elytrura* (Hall 1992), have shown that these species have “preferred” hosts but the linkage of preferences to larval performance is less clear. de Little and Madden’s (1975) work showed that both *C. bimaculata* and *C. agricola* larvae developed faster on *E. delegatensis* than on *E. dalrympleana*, while caged *C. bimaculata* females preferred to oviposit on *E. delegatensis* and *C. agricola* females preferred to oviposit (also in line with field observations) on *E. dalrympleana*. Apparently then, *C. agricola* cannot compete successfully with *C. bimaculata* and accepts a host which is less favourable to its offspring (de Little and Madden 1975). Hall (1992) showed that *C. debilis* and *P. elytrura* larvae had significantly higher survival rates on eucalypts that females selected as preferred hosts (*E. rudis* and *E. calophylla* respectively). However, *C. debilis* achieved no significant difference in liveweight on both hosts while *P. elytrura* achieved significantly higher liveweights on its preferred host. Similarly, Carne (1966) showed in laboratory trials, that although *P. atomaria* females preferred to oviposit on *E. blakelyi* in the field, the larvae grew faster and achieved greater weights on *E. bicostata* and *E. machrorhyncha*. Neither of these latter two eucalypts are utilised as hosts in the field.

The objectives of the present study were to quantify two aspects of the ecology of *T. catenata*. Firstly, performance, by measuring larval development rate and mortality as well as adult liveweights (as an indicator of potential adult fecundity) using eight species of eucalypt (including *E. viminalis* as a benchmark). Secondly, oviposition preference, focusing on those host species

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on which *T. catenata* completed development. The overall aim was to identify the most likely eucalypt species (other than *E. viminalis* and *E. macarthurii*) that *T. catenata* could utilise as hosts in New Zealand.

### *Methods:*

Investigations of *Trachymela catenata* larval performance over a range of hosts were conducted using eight different eucalypt species. The host species selected for trial belong to two major sub-genera of the *Eucalyptus* genus. Those from the *Symphyomyrtus* sub-genus, section Maidenaria were *E. ovata*, *E. globulus*, *E. viminalis*, *E. nitens* and section - Equatoria *E. saligna*. Those selected from the *Monocalyptus* sub-genus were *E. fastigata*, *E. regnans* and *E. coccifera* (after Pryor and Johnson 1971).

Species were selected for trial which are already widely distributed and could therefore potentially act as vector hosts i.e. *E. ovata*, *E. globulus* and *E. viminalis* (also a known host) or are valuable or potentially valuable commercial species i.e. *E. saligna*, *E. nitens*, *E. fastigata* and *E. regnans*. *E. coccifera* was added as an additional example of the *Monocalypt* sub-genera and it is also a close relative of *E. robertsonii* (both belong to the Super-species *Amygdalina* (Pryor and Johnson 1971)) a known host in Australia.

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Eucalypt foliage is often produced in a juvenile or an adult form which can have considerable physical and chemical differences and can influence the grazing patterns of leaf-eaters. All foliage used in these trials was the adult form. All foliage, except *E. viminalis*, in these growth trials was collected every 3 - 4 days from the Forest Research Institute campus at Rotorua, wrapped in damp paper, placed in a plastic bag and immediately transported to Massey University. On arrival, foliage was placed in water and maintained in the dark at 4 °C until used. *Eucalyptus viminalis* foliage was collected at similar intervals from Massey University, Palmerston North and stored in the same manner. Foliage collected and stored for more than four days was discarded.

Trials testing larval growth as a function of host species were conducted using neonate *T. catenata* larvae (sourced from cultures reared entirely on *E. viminalis*) which had completed consumption of the egg chorion i.e.  $\leq 24$  h old. One larva was placed in a petri dish containing a layer of damp paper towelling (to maintain humidity) and a leaf of flush foliage. Ten such dishes comprised one replicate, with three replicates for each eucalypt species trialed. All trials were conducted using the same controlled temperature facility, at  $22 \pm 2.5$  °C. During the larval growth stages, leaves were replaced every 48 h and enough moisture replaced to maintain the humidity but not keep conditions "wet". The individual larvae were monitored twice daily, assessed as live or dead and its instar recorded. In this way the number of instars attained for each individual and the mortality rates on each host species was gained. Prepupa and pupal stages were also maintained in the damp petri dish conditions. Monitoring continued until adult emergence.

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Development time (t) for each instar, for each host species was established (to the nearest half day) using the value at which  $\geq 50\%$  of larvae had moulted to the next instar.

Development rates were determined as the inverse of those values obtained for development time

$$\text{Development rate} = 1/t$$

Mean development rate and standard errors (S.E.) were obtained from the three data sets for each host species trialed. These data (for each host species on which development occurred) were then added together to produce a life time cumulative development profile. The trials were not conducted concurrently because flush foliage of some species was temporally variable. The development rates for each host eucalypt therefore were not tested for significance.

When adults emerged, they were provided with flush foliage on which to feed. At no more than two days after emergence, their live weights were recorded. From the host species which successfully produced adult *T. catenata*, one way ANOVA was used to test the null hypothesis that adult live weights are the same over all replicates within each host species trialed. Once satisfied that no significant difference existed, live weights for the three replicates were combined. This allowed a one way ANOVA test of the null hypothesis that adult live weights attained on each host species were the same.

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Having established the eucalypts (from those trialed) that *T. catenata* could complete development on, investigations using those species were conducted to assess ovipositional preferences of *T. catenata* females.

Three each of teneral female and male adults, were taken from cultures reared on *E. viminalis* and combined until they (as a group) regularly oviposited each day. Six such groups were used in a series of caged, oviposition choice trials. Choice of host foliage was limited to the three eucalypt species alluded to above i.e. *E. viminalis*, *E. nitens* and *E. coccifera*. For each of these, a sprig of flush foliage was mounted in a conical flask, provided with water and secured with a cotton wool plug. One each of these flasks was placed in a gauze covered cage (50 cm wide X 60 cm deep x 50 cm high) and set out  $\approx$  35cm apart. Trials were run in pairs i.e. 2 x 3 cages and for the first four days females in all six cages were provided with all three host choices. Thereafter, the females in each pair of cages were provided with only two choices (each pair initially receiving a different combination) which were changed every four days until all pairs/cages had been exposed to all possible host choice combinations. Further, the placement of each host species were alternated in the cages such that the same species did not occur in the same position of the cage on sequential trials.

Two such trials were run. Trial one was initiated on 15 April 1995 and trial two on 1 February 1996. Number of egg batches and egg-batch size on each host species were recorded every day.

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Both trials one and two for oviposition preference were analysed in two stages. Stage 1 was the first four days of each trial where *T. catenata* females had a choice of all three host foliage species. Chi-square analysis was used to test the null hypothesis that there was no difference between egg-batch numbers of stage 1 of trials one and two. A non-significant result from this test then allowed combination of trials one and two to allow a test of the null hypothesis that there is no difference between the egg-batch numbers laid on each species. These tests excluded eggs laid on the cage wall. For stage two, females had a choice of only two foliage species and egg-batch counts for each trial are shown in the absence of one host species.

### **Results:**

Mortality of *Trachymela catenata* larvae was greatest on the host species *Eucalyptus saligna*, *E. ovata* and *E. fastigata* where all 1st instar larvae ( $n = 30$ ) died (Table 3.1). Only 10 larvae attained the 2nd instar stage on *E. globulus* and of those, only two survived to enter the 3rd instar during which they also died. *E. regnans* produced the next highest overall mortality rate ( $n = 26$  in the 1st instar) but two larvae did reach the 4th instar (Table 3.1).

**Table 3.1.** Number of *T. catenata* larvae attaining each life stage and mortality per stage (in parenthesis) as a function of host species.

Life Stage Attained	<i>E. saligna</i>	<i>E. ovata</i>	<i>E. globulus</i>	<i>E. viminialis</i>	<i>E. nitens</i>	<i>E. fastigata</i>	<i>E. regnans</i>	<i>E. coccifera</i>
1st instar at start	30 (30)	30 (30)	30 (20)	30 (1)	30 (1)	30 (30)	30 (26)	30 (2)
2nd instar	-	-	10 (8)	29 (0)	29 (1)	-	4 (2)	28 (0)
3rd " "	-	-	2 (2)	29 (1)	28 (0)	-	2 (0)	28 (3)
4th " "	-	-	-	28 (0)	28 (1)	-	2 (2)	25 (1)
Prepupa	-	-	-	28 (3)	27 (2)	-	-	24 (2)
Pupa	-	-	-	25 (2)	25 (0)	-	-	22 (1)
Adult	-	-	-	23	25	-	-	21

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Of the *Symphomyrtus* sub-genus only *E. viminalis* and *E. nitens* produced adult *T. catenata*. Mortality was very low on both hosts with 23 and 25 adults produced from *E. viminalis* and *E. nitens* respectively (Table 3.1). *E. coccifera* was the only monocalypt species to produce adults ( $n = 21$ ) and is comparable in terms of overall mortality rate with both *E. viminalis* and *E. nitens*.

Development rates were slowest on the host species where mortality was highest i.e. on *E. regnans* and *E. globulus* (Fig. 3.1). Means and standard errors for *E. regnans* and *E. globulus* should be treated with caution since the number of data points are few, due to high mortality in these trials. For the three host eucalypt species which successfully produced adult *T. catenata* (i.e. *E. viminalis*, *E. nitens* and *E. coccifera*) the means for development rates (and therefore growth and development pattern) were remarkably similar and standard errors very small (Fig. 3.1).

Since development rate is the inverse of time ( $t$ ), *T. catenata* developed from 1st instar to adulthood in  $22.64 \pm 0.16$ ,  $21.32 \pm 0.33$  and  $22.15 \pm 0.72$  (S.E.) days on *E. viminalis*, *E. nitens* and *E. coccifera* respectively.

From the three trials that successfully produced adult *T. catenata*, i.e. *E. viminalis*, *E. coccifera* and *E. nitens* one way ANOVA revealed no significant difference ( $F_{2, 20} = 1.73$ ,  $P = 0.205$ ;  $F_{2, 17} = 1.13$ ,  $P = 0.349$  and  $F_{2, 23} = 0.91$ ,  $P = 0.471$  respectively) in the adult live weights attained between the three replicates within each host species.



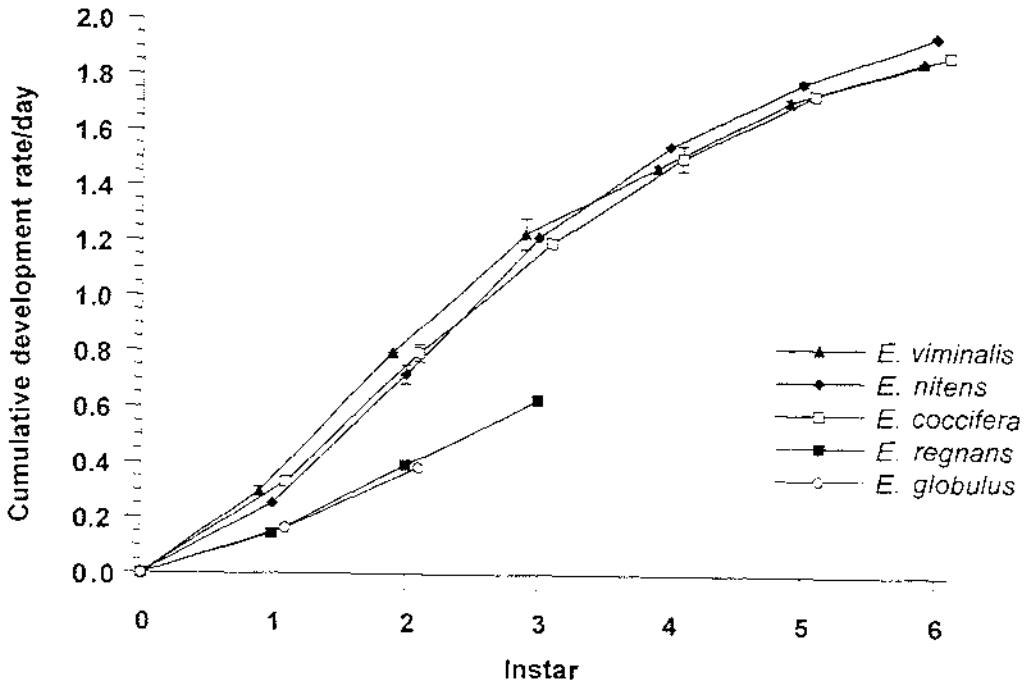


Fig. 3.1 Cumulative development rates (with S.E.) of *T. catenata* larvae as a function of host species

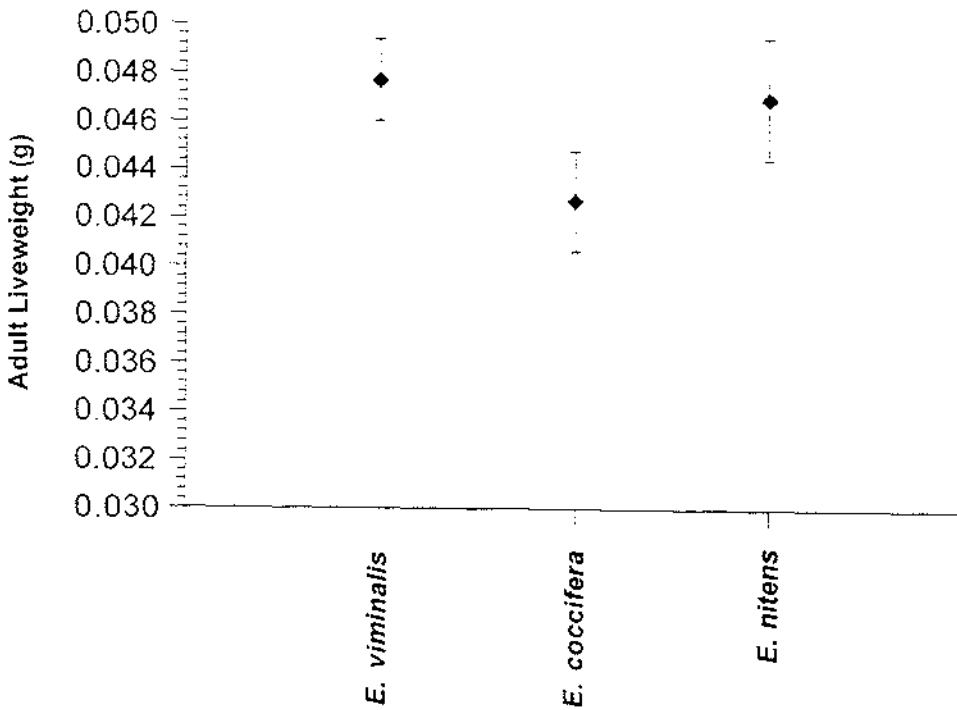


Fig. 3.2 Adult *T. catenata* liveweights (with S.E.) attained on three host species of eucalypt.

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Combination of the replicates, thus allowing one way ANOVA to be conducted for the mean live weight attained between the above three host species, showed no significant difference ( $F_{2,62} = 0.68, P = 0.51$ ). Mean weights were  $0.0476 \pm 0.0016$  g,  $0.0427 \pm 0.0020$  g and  $0.0469 \pm 0.0025$  g for *E. viminalis*, *E. coccifera* and *E. nitens* respectively (Fig. 3.2).

During the first four days (stage 1) of each trial, numbers of egg batches laid on the three species available were variable. *E. viminalis* received 4 and 9 batches for trials one and two respectively (Fig. 3.3 a.). *E. nitens* and *E. coccifera* appeared the most favoured hosts with 7 and 14 batches and 13 and 7 batches respectively. Eggs laid on the cage wall or glass flask also accounted for 3 and 2 batches.

Chi-square analysis of stage 1 between trials one and two revealed no significant difference ( $\chi^2 = 4.63, \text{d.f.} = 2, 0.05, = 5.99$ ) thus we failed to reject the null hypothesis that there was no difference between egg batch numbers of stage 1 of trials one and two. This however, allowed us to combine these data and perform a further analysis using the G-test goodness of fit for single classification frequency distributions (Sokal and Rohlf 1981) and the assumption that all expected values are equal. Chi-square analysis of these combined data revealed no significant difference ( $\chi^2 = 1.268, \text{d.f.} = 2, 0.05, = 5.99$ ) and we failed to reject the null hypothesis that there is no significant difference in the number of batches laid on each host species were females had a choice of all three. These analyses exclude the eggs laid on the cage wall since the chi-square expected values for that group were less than five.

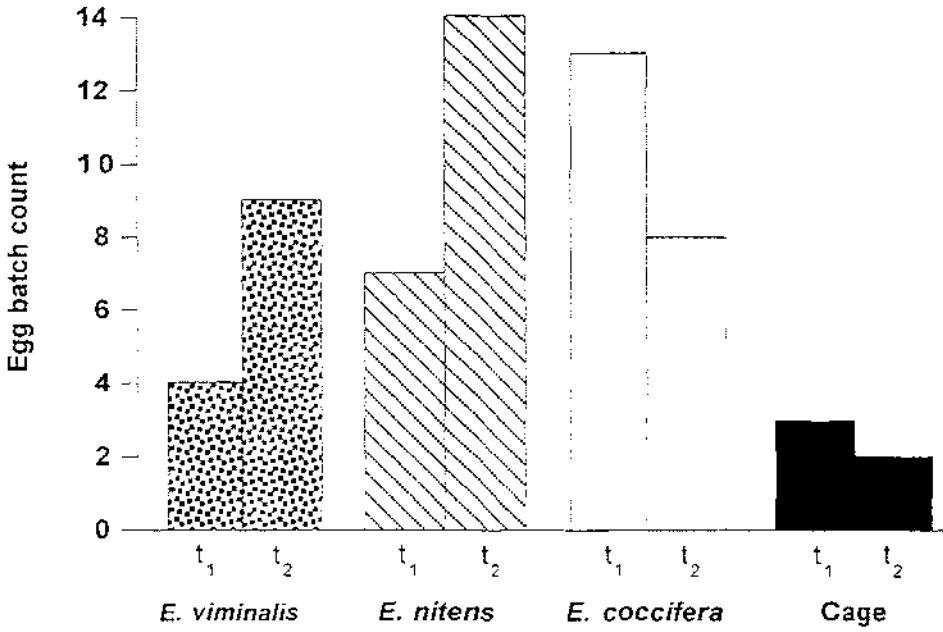


Fig. 3.3a Number of *T. catenata* egg batches on each host species with all three choices available for trails  $t_1$  and  $t_2$ .

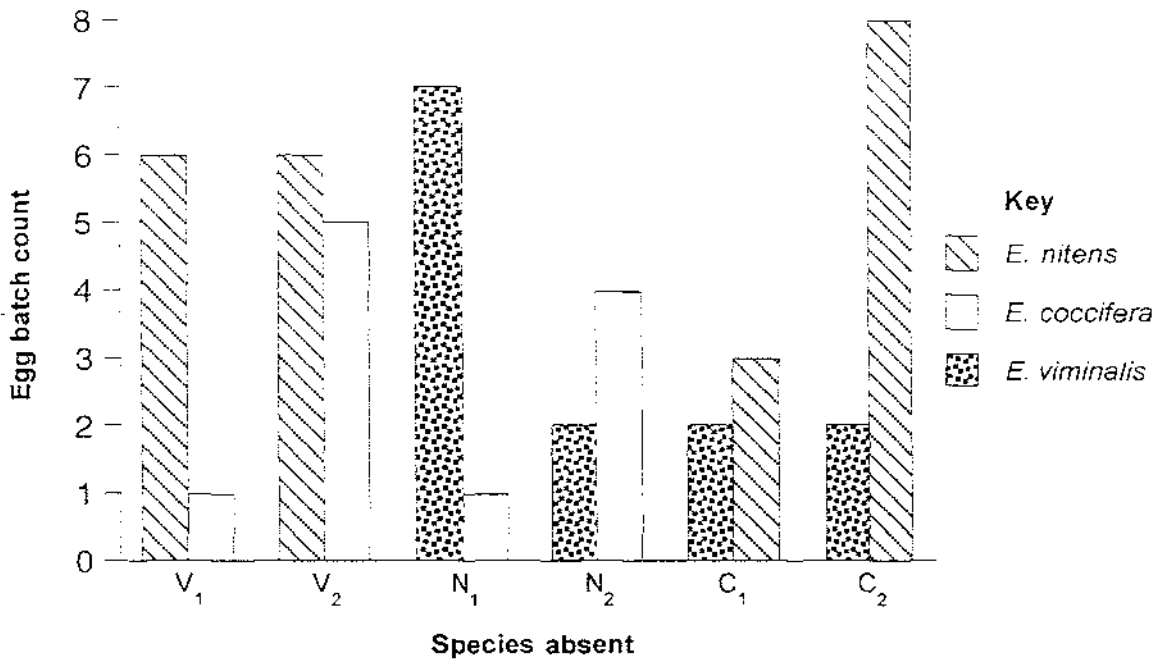


Fig. 3.3b Number of *T. catenata* egg batches on each host species where only two choices are available.

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Where choice was restricted to two eucalypts and in the absence of *E. viminalis*, *E. nitens* appeared to be favoured more than *E. coccifera* over both trials (v1& v2 Fig. 3.3b). The results were variable however, where *E. nitens* was absent (n1 & n2). *E. viminalis* was favoured in trial 1 but *E. coccifera* was favoured in trial 2. Where *E. coccifera* was absent (c1 & c2) *E. nitens* was favoured in both trials.

### *Discussion*

Results of *Trachymela catenata* larval performance (development rates) and survival on the range of *Eucalyptus* host species trialed are unequivocal. Under the laboratory conditions that these trials were conducted *T. catenata* larvae will not survive on *E. saligna*, *E. ovata* or *E. fastigata*.

The very slow development rate and low survival rate achieved on *E. globulus* and *E. regnans* also rules them out as likely host species. Even if *T. catenata* females did oviposit eggs on these two host species, the poor performance (and consequent long generation time) and low survival are such that establishment of viable populations is unlikely.

Probable explanations for these results may be associated with leaf toughness, nitrogen concentration, plant secondary compounds or a combination of these. Leaf toughness and nitrogen concentrations have been shown to be important

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factors in larval performance of *Paropsis atomaria* (Fox and Macauley 1977; Larsson and Ohmart 1988; Ohmart 1991; Ohmart *et al.* 1985a, 1987). Research conducted on the effects of plant secondary compounds e.g. phenolics (Fox and Macauley, 1977) and essential oils (Morrow and Fox, 1980) have shown that these compounds do not usually affect nitrogen use efficiency and therefore performance of *P. atomaria* larvae. The work of Fox and Macauley (1977) and Morrow and Fox, (1980) however did not include any of the eucalypt species that were used in this study. The overriding factor of the above reports is that nitrogen, when below a threshold level of  $\approx 1.7\%$ , has the most important detrimental effect on larval performance. Leaf toughness also increases significantly when nitrogen levels reached  $\leq 1.7\%$  (Ohmart, 1991) and so may be intercorrelated with low nitrogen levels.

How these factors may have affected my results is not known, but given that foliage was stored for up to three days before feeding, possible differential declines in foliage quality between all eight species trialed, especially leaf nitrogen and leaf toughness may be factors worthy of consideration.

*Eucalyptus viminalis*, *E. nitens* and *E. coccifera* all successfully reared *T. catenata* through to adults. Survival on each host species followed a very similar pattern with similar numbers reaching adulthood. Likewise, the development rates of *T. catenata* on each of these hosts was remarkably similar with all three groups attaining each life stage at similar rates and therefore similar time intervals.

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The time elapsed to adult emergence on *E. viminalis* in these trials ( $22.64 \pm 0.16$  S.E. days) is noticeably shorter than the  $28.16 \pm 0.33$  S.E. days obtained for *E. viminalis* at 22 °C in the larval growth rate versus temperature development trials (see Chapter 2.). This may be due in part, to the controlled temperature conditions under which each experiment was run. The temperature during these experiments was  $22.0 \pm 2.5$  °C and fluctuated on a regular diurnal basis, thus sustaining temperatures at up to 24.5 °C during the day for several hours. The facility used for the temperature development trials in chapter 2 maintained a tighter control ( $22.0 \pm 1.5$  °C).

As adult live weights attained on *E. viminalis*, *E. nitens* and *E. coccifera* were not significantly different we would predict that adult fecundity for populations reared or established on these species would be similar. Trials investigating fecundity as a function of live weight have produced varying results. Carne (1966), showed that fecundity was greatest in the heaviest live weight groups of *P. atomaria*, and that fecundity decreased as liveweight decreased. This was due to egg number per batch decreasing rather than number of batches decreasing. Work by de Little (1983) however reported no correlation between fecundity and live weight in *Chrysophtharta bimaculata*. Foliar nitrogen concentrations are also known to affect fecundity of *P. atomaria* (Ohmart *et al.*, 1985b; Ohmart, 1991). Investigations looking at interactions between *Eucalyptus* species (including possible effects of nitrogen concentrations) and the fecundity of *T. catenata* may therefore be beneficial in assessing the potential status of this species in New Zealand.

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From the oviposition choice trials, it is difficult to determine any pattern of choice. Females had, until the beginning of the trials, been confined to ovipositing entirely on *E. viminalis* but immediately they were introduced to the choice situation, began ovipositing on any one of the three possible host choices. Chi-square analysis revealed no significant difference in the number of egg batches on each host species during the first four day period when all three choices were available. This suggests that *T. catenata* females under laboratory conditions, appear not to discriminate between these three host choices. Where two choices were available and where one species was excluded after having it available in the previous four days, no pattern of switching from one particular species to another was evident. Further, where one species was used in a two choice situation and that same species was available but paired with a different species in the next four day period, oviposition was just as likely to occur on that different species. Such indiscriminate patterns may be confounded by the fact that a change in foliage choice also coincided with the introduction of fresh foliage.

Trials which recorded and removed egg batches perhaps twice daily may have produced a clearer picture of ovipositional preferences i.e. choices may have been more positive when foliage was fresher and had no or low numbers of eggs already laid on the leaf surface. Singer (1986) cites a number of insects species which are deterred from ovipositing where conspecific eggs have already been laid and in such cases, preference trials where egg numbers are allowed to build up before counting may produce confounding results. *Trachymela catenata* females like *P. charybdis* females in these cages will

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occasionally oviposit on the cage wall which may indicate less than optimal conditions for oviposition. Again regular clearance of eggs may deter that behaviour and provide more reliable data. Trials to assess rejection or not, of possible hosts from previously laid egg batches in paropsina may prove to be a valuable prelude to any further preference investigations on this group. Further, trials designed to assess host acceptance by ovipositing *T. catenata* females where eggs of either conspecific or competing (e.g. *P. charybdis*) females are laid especially on *E. nitens* could be important for assessment of possible host selection for that eucalypt species.

The sprigs of foliage for all these trials composed of approximately equal numbers of old and flush adult leaves. Caged trials conducted by Steinbauer *et al.* (1998) have shown that ovipositional choice of *C. bimaculata* is influenced by the presence of older fully expanded foliage when presented along-side flush foliage. *C. bimaculata* would discriminate against *E. nitens* and prefer to oviposit on *E. regnans* when both host choices included older fully expanded leaves but no such discrimination occurred when *E. nitens* was presented with the older leaves removed. Steinbauer *et al.* (1998) suggest that leaf age may be more important in female host choice than the physical or chemical composition of possible host species. Again, trials that manipulate the quality of foliage between the host species used may also prove useful in clarifying or better predicting potential host use by *T. catenata*.

Ultimately host use or preference by *T. catenata* females will be best assessed from field observations. There are currently no records of *T. catenata* ovipositing in the field on anything other than *E. viminalis*, *E. macarthurii* and *E.*



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*macarthurii* x *botryoides*. Currently, the Gisborne/Northern Hawkes Bay area has a limited range of *Eucalyptus* plantings but these need to be monitored to assess any other possible hosts. *Eucalyptus nitens* and *E. coccifera* in this region are notably absent or occur as single isolated specimens making field verification of the laboratory trials difficult.

Use of all eight eucalypt species for the preference trials may have been beneficial as the literature shows performance - preference links including those of the paropsina can be very obscure. If egg laying had occurred on some of the species on which larvae grew and/or survived poorly it may provide an indication of the degree of the specificity of this species or it may give an indication as to the reliability of the caged experimental methods used (see Singer 1986; Steinbauer *et al.* 1998). Alternatively these trials may indicate that the search for possible hosts needs to be much wider in order to provide a better indication of the possible range and pervasiveness of *T. catenata*.

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## *Chapter 4*



## **Parasitoid Interactions**

Comparative studies of the effects of the Parasitoid *Enoggera nassau* Girault (Hymenoptera : Pteromalidae) on *Trachymela catenata* Chapuis and *Paropsis charybdis* Ståhl  
(Coleoptera : Chrysomelidae).

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*Abstract:*

The paropsine *Paropsis charybdis* Ståhl (Coleoptera : Chrysomelidae), a serious pest of *Eucalyptus* species in New Zealand, is now well controlled by the introduced egg parasitoid *Enoggera nassau* (Hymenoptera : Pteromalidae). Eggs of another recently introduced paropsine *Trachymela catenata* Chapuis, as well as eggs of *P. charybdis* were exposed to *E. nassau* in a series of choice trials. The results indicate that *E. nassau* displays an unequivocal specificity toward parasitization of *P. charybdis*. Ovipositional experience on *P. charybdis* did not induce the parasitoid to oviposit into *T. catenata* eggs. It is unlikely that *E. nassau* will have any controlling effect on *T. catenata* populations.



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### *Introduction:*

*Trachymela catenata* Chapuis (Coleoptera : Chrysomelidae), first recorded in the Gisborne/Northern Hawkes Bay of New Zealand in December 1992, appears to have established a permanent population. Larvae and to a lesser extent adults, cause considerable leaf damage to some eucalyptus trees. In this region, the eucalypt species *Eucalyptus viminalis*, *E. macarthurii* and *E. macarthurii* x *botryoides* all belonging to the *Symphyomyrtus* sub-genus, section Maidenaria), (Pryor and Johnson 1971) are known to be attacked.

The pest status of an earlier paropsine introduction into New Zealand, *Paropsis charybdis*, was recognised early this century when it caused extensive defoliation of young *Symphyomyrtus* eucalypts, most notably *E. viminalis* and *E. globulus*, that were established as shelter or as small woodlots on farms (Bain & Kay 1989). Later, plantings of *E. nitens*, established for production forest purposes, were also extensively defoliated. Programmes investigating biological control agents for *P. charybdis* were initiated as early as 1934 (Bain and Kay 1989).

Early importations from Australia in the 1930s of *Aridelus* (Hymenoptera : Braconidae), *Froggattimyia tillyardi*, *Froggattimyia* spp. and a *Paropsivora* spp. (Diptera : Tachinidae) were of little use, as most importations contained

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hyperparasitoids and sufficient numbers could not be reared for release (Bain and Kay 1989). Interestingly, none of these imported species were reared or derived from *Paropsis charybdis* but from other paropsines more common in Australia.

The first biocontrol agent to be released, was the larval parasitoid *F. tillyardi* in January 1975, but this species has never been recovered since. The egg and larval predator *Cleobora mellyi* (Coleoptera : Coccinellidae) was released in 1979. This species established a stable population only in the Marlborough region and has little effect on the *P. charybdis* population (Bain and Kay 1989).

During a screening programme in 1987 for possible parasitoids of *Trachymela tincticollis* (which had established on eucalypts in South Africa), two parasitoid species were identified as having potential for use on *Paropsis charybdis*. The two species, *Neopolycystus insectifurax* Girault and *Enoggera nassau* Girault (Hymenoptera : Pteromalidae) are solitary egg parasitoids of several paropsine species. [In accordance with the accepted definition of a parasitoid, these pteromalids kill the host, complete their life cycle within the host and emerge as adults]. *Neopolycystus insectifurax*, collected from *P. atomaria* was the subject of an earlier biocontrol attempt on *P. charybdis* (Bain and Kay 1989). *Enoggera nassau* has been reared from eight paropsine species covering four genera (*Chrysophtharta*, *Trachymela*, *Paropsis*, and *Paropsisterna*) and is recorded from a *Creiis* sp. and what was thought to be *P. geographica* Baly (Naumann 1991). *Enoggera nassau* then, was considered "moderately polyphagous" (Naumann

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1991) and a generalist paropsine parasitoid which could potentially control *P. charybdis* as well as a number of possible future paropsine introductions. Both species were subsequently imported into New Zealand by the New Zealand Forest Research Institute (NZFRI), screened for hyperparasitoids and evaluated for possible release.

Evaluation of these two pteromalids in the laboratory, revealed that both could achieve 100% parasitism of *P. charybdis* eggs. *Enoggera nassau*i averaged 90% survival to eclosion, has a female to male sex ratio of 7 : 1 and has a developmental threshold of 9 °C. *Neopolycystus insectifurax* averaged 60% survival to eclosion, has a female to male sex ratio of 3 : 1 and has a developmental threshold of 12.5 °C. Competitive exclusion experiments however, revealed that *N. insectifurax* dominated and produced more offspring especially at warmer temperatures (Bain and Kay 1989; Kay 1990).

Both *E. nassau*i and *N. insectifurax* were released at several sites in New Zealand during the summer of 1987/88. It was expected that *E. nassau*i would predominate earlier in the season and *N. insectifurax* would then complement *E. nassau*i as temperatures increased. *Neopolycystus insectifurax* however, was never recovered, while *E. nassau*i established rapidly throughout the entire country resulting in a dramatic decline in *P. charybdis* populations (Kay 1990).

*Enoggera nassau*i in New Zealand appears to be free of hyperparasitoids and can produce several generations each season. This, coupled with the species 7 : 1

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female sex ratio, an initial high density and relatively contiguous population of its target species and favourable environmental conditions, probably contributes to the successful establishment and control of *P. charybdis* populations.

Similar success was achieved with the release of *E. reticulata* to control *T. tincticollis* in South Africa. The success of that programme is largely attributed to the absence of hyperparasitoids and the initial high density of the target species (Urban, *et al.* 1987, Tribe & Cillie 1989).

*Enoggera nassau*, as alluded to earlier, was considered a relatively polyphagous species (Naumann 1991), however field observations by myself and NZFRI staff, had revealed no evidence of parasitism of *E. nassau* on *T. catenata*. Both *P. charybdis* and *T. catenata* oviposit their eggs on the upper leaf surface of the same and/or closely related *Eucalyptus* species. *Paropsis charybdis* tends to oviposit on older sclerophyllous foliage (McGregor 1989) while *T. catenata* utilises young expanding foliage, so the areas that parasitoids would search for potential hosts are therefore adjacent to one another. With *E. nassau* already well established in New Zealand, experimental work investigating possible parasitism of *T. catenata* eggs by *E. nassau* could prove useful in evaluating the future status of this recent paropsine introduction.

Investigations designed to assess host preferences were required to evaluate the potential impact of *E. nassau* on *T. catenata*.

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Egg parasitoids which have received most attention regarding host preferences to date are members of the polyphagous *Trichogramma* genus (Hymenoptera : Trichogrammatidae), many of which have been assessed and applied in biological control programmes through out the world. Host preference studies on *Trichogramma* species have often focused on ovipositional experience and learning and usually include choice ( Bjorksten and Hoffmann 1995; Pak *et al.* 1986, 1990; Stevens 1995) and/or no choice (Bjorksten and Hoffmann 1995, 1998; Gross *et al.* 1981; Kaiser *et al.* 1989; Stevens 1995) host exposure. Analysis is usually based on the number of parasitised hosts or quantification of ovipositional behaviour.

Controlled choice experiments were conducted to investigate whether *E. nassau* parasitises *T. catenata* and to assess whether ovipositional experience with *P. charybdis* (a known host) would induce *E. nassau* to parasitise *T. catenata*.

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### *Methods:*

A culture of *Enoggera nassaui* was established from adults obtained during January 1995, from parasitised *Paropsis charybdis* eggs collected from *Eucalyptus viminalis* at Massey University. The *E. nassaui* cultures were maintained in petri dishes at room temperature and adults provided with 50 : 50 honey and water from a paper wick. Reproduction was achieved by periodically exposing adults to < 24 h old *P. charybdis* eggs.

Reproducing cultures of *T. catenata* and *P. charybdis* were fed exclusively on *E. viminalis* and maintained in a constant temperature of  $27 \pm 1.5$  °C. Sufficient *P. charybdis* eggs (< 24 h old) were collected periodically to maintain the *E. nassaui* cultures.

To test the null hypothesis that *E. nassaui* would parasitize *P. charybdis* and *T. catenata* equally, trials were carried out using three different exposures with two different treatments in paired replicates. Two petri dishes (forming one pair) each containing four (to ensure females would be present) newly eclosed *E. nassaui* adults were maintained at room temperature and to enhance longevity and fecundity, supplied with 50 : 50 honey and water on a wick as a food source (Ashley and Gonzalez 1974; Hoffmann *et al.* 1995). One of the pair of each replicate, was for its treatment, first exposed to  $\approx 20$  fresh ( $\leq 24$  h old) *P. charybdis* eggs for 48 h, then for the second exposure to  $\approx 20$  fresh ( $\leq 24$  h old)

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*T. catenata* eggs for the same duration. Treatment for the other pair of the replicate was the converse of this i.e. using a similar number of eggs and the same duration, first exposed to *T. catenata* and then to *P. charybdis*. The third exposure (treatment) for both pairs was to  $\approx 20$  eggs of both *P. charybdis* and *T. catenata* simultaneously, again for 48 h. In an effort to present the eggs in as natural a state as possible, all egg batches were still attached to the *E. viminalis* leaf on which they were laid. Using new batches of *E. nassau*i, each of the three paired exposures were replicated five times.

Exposed eggs were observed until either 1st instar paropsine larvae or adult parasitoids emerged. The number of larvae, parasitoids and non-viable eggs in each trial were recorded and the percentage of non-viable eggs calculated. Differences in the number of non-viable eggs for *T. catenata* from choice and no choice exposures were subjected to Chi-square analysis.

A second trial was designed to investigate the possible effect of *E. nassau*i on the viability of *T. catenata* eggs. The null hypothesis that *E. nassau*i has no effect on the viability of *T. catenata* eggs was tested by placing fresh ( $\leq 24$  h old and attached to *E. viminalis* leaf) *T. catenata* eggs into each of two petri dishes. One of the two dishes contained a wick of 50 : 50 honey and water and four newly eclosed adult *E. nassau*i and the other as a control with a wick of 50 : 50 honey and water only. Five replicates were maintained at room temperature, the *E. nassau*i removed after 48 h and the eggs allowed to develop. Egg hatch was recorded until all larvae had hatched or the egg determined as non-viable

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by way of colour and content under a dissecting microscope. The number of viable and non-viable eggs was determined and subjected to Chi-square analysis.

### *Results:*

Trials which exposed *P. charybdis* to *E.nassau*i first, resulted in adult *E. nassau*i emerging from 100% of the 99 eggs exposed whereas no *E. nassau*i emerged from the 99 *T. catenata* eggs subsequently exposed. Of the *T. catenata* eggs, 84.8% hatched into 1st instar larvae.

Results of the choice exposures revealed 97% *E. nassau*i emerging from the 100 *P. charybdis* eggs exposed and as before, none of the 101 *T. catenata* eggs. The 1st instar emergence rate from these 101 eggs was 94.1% (see above shaded line Table 4.1).

Similar results were obtained from the exposures in which *T. catenata* were trialed with *E. nassau*i first (below shaded line Table 4.1). No *E. nassau*i emerged from the 99 *T. catenata* eggs and 83.8% of these eggs hatched as 1st instar larvae. Adult *E. nassau*i emerged from 99% of the 100 *P. charybdis* exposed after the initial *T. catenata* exposure. As before, the choice exposures



**Table 4.1. Comparative parasitization of *P. charybdis* and *T. catenata* by the parasitoid *E. nassaui*.**

	n	<i>E. nassaui</i> % emerged	<i>P. charybdis</i> % emerged	<i>T. catenata</i> % emerged	Non- viable	<i>P. charybdis</i> non-viable %	<i>T. catenata</i> non-viable %
<i>P. charybdis</i> 1st	99	100	0	-	0	0	-
<i>T. catenata</i> 2nd	99	0	-	84.8	15	-	15.2
Choice - <i>P. charybdis</i>	100	97	0	-	3	3	-
Choice - <i>T. catenata</i>	101	0	-	94.1	6	-	5.9
<i>T. catenata</i> 1st	99	0	-	83.8	16	-	16.2
<i>P. charybdis</i> 2nd	100	99	0	-	1	1	-
Choice - <i>P. charybdis</i>	99	98.9	0	-	1	1	-
Choice - <i>T. catenata</i>	101	0	-	92.1	8	-	7.9
Mean		98.75		88.7		1.25	11.3

**Table 4.2. Effect on the viability of *T. catenata* eggs by the parasitoid *E. nassaui*.**

Chi-square analysis of viable and non-viable eggs (grey block) gives  $\chi^2 = 2.79$   
(d.f. = 1, p 0.05, = 3.84)  $\therefore$  no significant difference between treatments.

	n	<i>E. nassaui</i>	<i>T. catenata</i> (viable)	Non- viable	Non- viable (%)
<i>T. catenata</i> + <i>E. nassaui</i>	136	0	111	25	18.38
<i>T. catenata</i> (control)	142	-	126	16	11.26

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revealed complete specificity toward parasitization of *P. charybdis* with *E. nassau* emerging from 98.9% of exposed *P. charybdis* eggs and none from the *T. catenata* eggs. Of the 101 *T. catenata* eggs exposed, 92.1% emerged as first instar larvae.

The overall means from these trials revealed a total of 98.75% of all the *P. charybdis* eggs exposed to *E. nassau* produced adult parasitoids and 88.7% of all *T. catenata* eggs exposed to *E. nassau* produced *T. catenata* larvae (Table 4.1).

There were apparent differences in the percentage of non-viable eggs with a mean value of 1.25% for *P. charybdis* compared to 11.3% for *T. catenata* (Table 4.1). Further, Chi-square analysis revealed a significant difference ( $\chi^2 = 7.62$ , d.f. = 1,  $p < 0.05 = 3.84$ ) in percent of non-viable *T. catenata* eggs where *T. catenata* were trialed in the no choice exposures (15.2 and 16.2%) compared to eggs trialed in the choice exposures (5.9 and 7.9%).

However further trials investigating the effect of *E. nassau* on the viability of *T. catenata* eggs revealed no significant difference between treatments. From 136 eggs exposed to *E. nassau*, 111 1st instar larvae were produced (18.38% non-viable) and from the control group of 142 eggs not exposed, 126 (11.26% non-viable) (Table 4.2). This difference was not significant ( $\chi^2 = 2.79$ , d.f. = 1,  $p < 0.05 = 3.84$ ) so we failed to reject the null hypothesis that *E. nassau* has no effect on the viability of *T. catenata* eggs.

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### *Discussion:*

These results show that under laboratory conditions, *Enoggera nassau* has an absolute preference for parasitising *Paropsis charybdis* eggs rather than those of *Trachymela catenata*. The high rate of parasitism (mean 98.75%) of *P. charybdis* is consistent with the high rate of parasitism observed in the field and reports of a dramatic decline in the *P. charybdis* population since the introduction of this parasitoid (Kay 1990). The absence of any parasitism of *T. catenata* eggs in the laboratory suggests that *E. nassau* will not utilise this species as a host and is therefore unlikely to provide any form of control on *T. catenata* populations. These results support the field observations that no parasitised *T. catenata* eggs have been recorded by the author or other workers.

There may be a number of explanations for this lack of parasitism. Inappropriate cues for host acceptance and suitability are likely to be the most important. Host size, shape or surface texture (Salt 1935; Schmidt and Smith 1985; Strand 1986; Strand and Vinson 1983a, 1983b; Vinson 1976), as well as age (Pak *et al.* 1986; Stevens 1995), appear to be important cues used by egg parasitoids to determine acceptability and suitability of host eggs. There are obvious differences between the size and surface of the two egg types trialed. *Paropsis charybdis* eggs are smooth and appear relatively dry while *T. catenata*

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have a rough spiked surface and a sticky mucus-like coating. *Paropsis charybdis* eggs are also larger ( $\approx 3 \times 1$  mm) than eggs of *T. catenata* ( $\approx 2.2 \times 0.8$  mm). Size of the potential host eggs would be of paramount importance for a solitary egg parasitoid for two reasons. One, the egg may not be physically large enough to contain the developing or mature individual; and two, a small egg might contain insufficient food resources to enable complete development of the parasitoid. Observations from *E. nassaui* cultures being reared from *P. charybdis* in the laboratory indicate strong discrimination against older eggs (3-4 days) when exposed alongside fresh (< 24 h) eggs. Chemical cues, such as kairomones associated with the egg chorion or contents can also be important in host recognition (Drost and Cardé 1992; Strand and Vinson 1982, 1983b) but what influence such cues had on these trials is unknown.

Investigations using *Muscidifurax zaraptor* (Hymenoptera : Pteromalidae), a larval parasitoid of muscoid Diptera, ovipositional experience (learning) has been shown to bias host preference towards the host with which the parasitoid had previous experience (Mandeville and Mullens 1990). Similarly, egg parasitoids in the *Trichogramma* genus have revealed a number of examples where ovipositional experience has biased subsequent host preference in experimental situations (Bjorksten and Hoffmann 1995, 1998; Kaiser *et al.* 1989). Previous oviposition experience with *P. charybdis* however, did not induce *E. nassaui* to parasitize *T. catenata* which, given the physical and structural differences was not unexpected.

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These results indicated a possible negative effect on the viability of *T. catenata* eggs by *E. nassau*. The percent of non-viable eggs for *P. charybdis* was 1.25 while that of *T. catenata* was 11.3%. Further there was a significant difference in the number of non-viable eggs between the choice and no choice trials. Conceivably, these differences may have been due to a more intense effort (i.e. increased ovipositor probing and drilling) or the possibility of feeding at drilling sites by *E. nassau* while assessing the suitability of *T. catenata* eggs when there was no choice than when there was some choice. Unlike *P. charybdis*, evidence of drilling sites on *T. catenata* eggs is difficult to determine due to the rough mucous covered surface of the egg and possible reasons for the non-viability remain unknown.

The trials done to determine any possible negative effect on egg viability by *T. catenata* showed no significant difference between eggs exposed to the parasitoid and eggs in the control. Under laboratory rearing conditions then, 11.25 - 18.3% non-viability seems to be the norm. How this compares to non-viability rates under field conditions is not known.

The very high parasitism rate of *P. charybdis* eggs in these trials gives some insight into the reason for the dramatic decline of this species in New Zealand since the release of *E. nassau* in 1987/1988. Unparasitized *P. charybdis* egg batches are very difficult to find in the field in most regions of New Zealand. Obviously environmental conditions for the spread and pervasiveness of this parasitoid are highly favourable. It also indicates a lack of hyperparasitoids

for *E. nassaui* as has been indicated for *E. reticulata* in South Africa (Urban, *et al.* 1987, Tribe & Cillié 1989).

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## *Chapter 5*



## **Conclusions**

## Conclusions

*Trachymela catenata* is the third Australian defoliating paropsine beetle associated with eucalypts to have established in New Zealand this century. *Paropsis charybdis* proved to be a serious pest of plantation eucalypts while *T. sloanei* has been slow to spread and has never achieved pest proportions. Time of establishment of *T. catenata* is unclear and to date the current population appears to be spreading only slowly and is still confined to the original known range. This slow rate of spread may in part be due to suitable host eucalypts not being contiguous which may impair the spread and establishment of new *T. catenata* populations.

As expected *T. catenata* completes its life cycle with the same number of larval and pupal stages as other paropsina, follows similar phenological patterns and in its present range appears to be bivoltine. The preferred oviposition site of *T. catenata* on young expanding foliage does not overlap with *P. charybdis* which prefers older sclerophyllous leaves for oviposition and competition for this resource should not occur between the two species on the same host eucalypts. Whether interspecific competition occurs between *T. catenata* and *P. charybdis* for other resources such as flush foliage for feeding is not known

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and is deserving of further investigation. If in fact these two species have coexisted for a number of seasons, such investigations would provide some insight as to whether *P. charybdis* (before control by the parasitoid *Enoggera nassau*) did suppress *T. catenata* populations in its current range. Seasonal female fecundity of *T. catenata* is similar to *Chrysophtharta bimaculata* (a serious plantation eucalypt pest during some seasons in Tasmania) but only half that of *T. tincticollis*, a pest of eucalypts in South Africa or *Paropsis charybdis*, in New Zealand. Mortality rates for *T. catenata* larvae is highest during the 1st instar and like *P. charybdis* considerable losses occur during pupation in the leaf litter. Thermal requirements for establishment and growth of *T. catenata* appears similar to *P. charybdis* and therefore provided suitable host species are available, *T. catenata* populations should extend over the same geographical regions of New Zealand. While *T. catenata* appears to be more *r* selected by comparison with *P. charybdis* or *T. tincticollis* (the potential for population growth therefore being smaller), *T. catenata* is likely to pervade, spread and become relatively common, but whether it reaches pest status may depend on factors which are not yet clear. Factors such as host range, dispersal behaviour, inter-seasonal influence (e.g. temperature, wind, rainfall, flush growth) on variability of female fecundity, population numbers/dynamics and mortality of all stages will all influence the potential for population increase.

The host preference investigations and the known host associations from Australia indicate that *T. catenata* may be a relatively polyphagous species. My

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host preference trials indicate *Eucalyptus nitens* to be a possible host while *E. viminalis*, *E. macarthurii*, *E. macarthurii* x *botryoides*, in New Zealand, and *E. polyanthemos*, in Australia, all belonging the *Symphomyrtus* sub-genus, are known host species. *Eucalyptus robertsonii* in Australia is a known host and the host preference trials indicate *E. coccifera* (both from the *Monocalyptus* sub-genus) to be a potential host. Larvae reared on *E. viminalis*, *E. nitens* and *E. coccifera* all developed and survived at remarkably similar rates indicating generation times and mortality would be comparable to what is currently achieved at Morere. Adult live weights achieved on the same three species indicate that females would be as fecund and capable of establishing viable populations on any one of those hosts. If *T. catenata* is as polyphagous as my trials suggest, such capability may confer considerable advantage to this species to colonise new regions. While *T. catenata* has, to date, not been recorded from *E. nitens* or *E. coccifera*, any plantations of these eucalypt species will need careful monitoring in the future.

Currently, other than natural attrition, there appears to be little regulatory control on the *T. catenata* population in New Zealand. Trials investigating the potential effect of the egg parasitoid *E. nassau* on *T. catenata* indicate that *E. nassau* will have no effect on *T. catenata* populations. If *T. catenata* reaches pest status and is found to attack *E. nitens* or any other commercially viable eucalypt species so that control is deemed necessary then a specific biocontrol agent will need to be found.