Fungal and vascular plant polysaccharide digestion by larvae of *Aenetus virescens* (Lepidoptera: Hepialidae)

J. R. GREHAN
Zoology Department, Victoria University of Wellington, Private Bag, Wellington, New Zealand

ABSTRACT

Larval feeding by the wood borer *Aenetus virescens* is characterised by an initial diet of dead wood and fungal fruiting bodies ("litter-phase") followed by a transition to callus tissue of trees and shrubs for the main period of larval development ("tree-phase). Larvae of both stages exhibit gut activity to laminarin and starch, showing the potential to digest β-(1,3) and α-(1,4) glucans respectively. Although β-(1,3) glucans are rare in vascular plants it is suggested that they may be present in the diet of *A. virescens* as callose. Gut activity to laminarin and starch was also confirmed for the foliage feeding *Wiseana*, indicating that the ability to digest β-(1,3) glucans may be widespread in the Hepialidae. Tree-phase gut activity was recorded for galactomannan, very weak activity to carboxymethyl cellulose and no activity to chitin. The litter-phase exhibited very weak activity to chitin, but no activity to carboxymethyl cellulose.

Keywords: Hepialidae, polysaccharide digestion, *Aenetus virescens*, Lepidoptera, Mycophagy, fungivory, larvae, fungi.

INTRODUCTION

The larvae of most Lepidoptera are specialist feeders on live vascular plants (phytophagy) or fungi (fungivory), but some species have a combined diet of fungi and dead plant tissue (detritophagy) or fungi and algae (Powell 1980; Rawlins 1984). The

feeding associations of many larval Hepialidae, in particular, may include both fungal and live vascular plant hosts (Crehan 1979). Two species with confirmed records of fungivory and phytophagy are *Zenophassus schamyl* (Chr.) and *Aenetus virescens* (Doubleday) (Slashchevskii 1929; Zagainyi & lurchenko 1955; Crehan 1979, 1981, 1984) where diet changes with development. Fungivory occurs only in young instars and is followed by a change to vascular plants in the form of roots *(Z schamyl)* or stems *(A. virescens).* In *A. virescens* the dietary change is also accompanied by a temporary "transfer" morph (Crehan 1981, 1983a).

The 2 feeding stages of *A. virescens* larvae are the "litter-phase" consisting of detritophagy and fungivory, and the succeeding "tree-phase" where the larvae are phytophagous and feed on callus tissue. The litter-phase lasts only 2-3 months while the tree-phase occupies the bulk of development ranging from about 1 to 4 years or longer (Crehan 1988a).

Litter-phase larvae are found on the underside of dead twigs, branches or logs on the forest floor and often congregate in large numbers under a webbing of silk, faecal pellets and other debris (Fig. 1a). Larvae may reside in tunnels excavated in the wood. Some wood may be ingested during tunnel construction, but feeding is mainly on surface tissue around the tunnel entrance. Tunnels appear to be constructed only after the 1st instar although older litter-phase larvae are also found directly under the web.

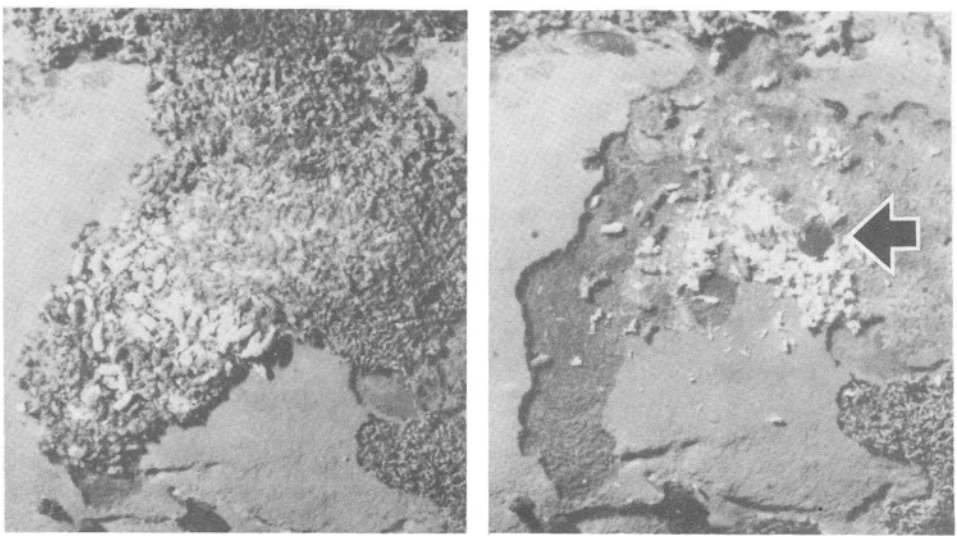

Fig. 1: Fungivory by litter-phase larva *of Aenetus virescens.* (A) Silk/faecal pellet web over feeding surface on polypore (bracket) fungus growing as a surface encrustation on dead wood. (B) Feeding surface exposed by removal of web. Tunnel entrance visible as a dark circle slightly to right of centre (arrow).

Fungivory occurs when larvae feed on fungal fruiting bodies (principally Basidiomycetes) growing from dead wood as surface encrustations or horizontally protruding brackets. Encrusting fungi grow on the underside of dead wood and the larvae "graze" the surface (Fig. 1b). It is also possible that sometimes vegetative fungal hyphae only are eaten when the larva grazes the surface of dead wood. Larvae feeding on brackets are located on the spore bearing hymenial layer on the underside of the bracket (Fig. 2). Tunnels of fungal feeding larvae are constructed in the fruiting body or the dead wood substrate, depending on the size and location of the larva. Where larvae feed on encrusting fungi, the tunnels of larger larvae usually extend into the wood.

The tree-phase diet involves a similar feeding mode to the litter-phase. The larva constructs a tunnel which functions as a refuge while feeding is limited mainly to the tunnel entrance where callus tissue is grazed (Fig. 3). The callus grows as a wound response following removal of bark and other tissue by the larva (Hewitt 1977). Tunnels of tree-

phase larvae have been implicated as a means for the entry of wood-rot fungi into live trees (Milligan 1974; Alma 1977) and litter-phase fungal hosts include, or are related to known plant pathogens (c.f. Gilmour 1966; Grehan 1984). Larvae transferring from fungi to trees could, therefore, act as vectors of fungal spores as might occur with disease fungi (c.f. Grehan 1982; Grehan & Wigley 1984) and particularly in view of litter-phase feeding on the spore bearing surface of fungi. A vector role could be particularly significant for hosts such as *Nothofagus* where the outer sapwood has been described as a barrier to entry of fungi (Milligan 1972).

There are major differences between the polysaccharide composition of fungi and vascular plants. Both groups share starch (an $\alpha-(1,4)-$glucan) as a major storage polysaccharide, but there is relatively little similarity in the cell wall components. Fungal cell walls are composed primarily of the $\beta-(1,4)-$ linked N-acetyl-D-glucosamine polymer chitin, and a mixture of $\beta-D-(1,3)-$ and $\beta-D-(1,6)-$glucans. These compounds are absent or rare in higher plants and conversely, the major vascular plant structural polysaccharides such as cellulose are absent from the tissues of basidiomycete macrofungi (Martin 1979; Martin *et al.* 1981b,c).

Differences in polysaccharide structure may be reflected in the general taxonomic separation between fungivory and phytophagy (Gilbertson 1984: 146). Martin (1979) described mycophagy (fungal feeding, alone or in combination with another substrate) as a feeding strategy involving a distinct set of digestive and metabolic capabilities. Differences between fungivory and phytophagy appear to be both diminished and highlighted by the feeding pattern of *A. virescens*. This paper examines the relationship between digestive capacity and changes in diet with particular attention to the degradation of the $\alpha-(1,4)$ glucan, starch, characteristic of higher plants and the $\beta-(1,3)$, $\beta-(1,6)$ linked glucan laminarin used here as being representative of the type of polysaccharide digested during fungal feeding.

MATERIALS AND METHODS

Litter and tree-phase larvae were collected from the field and the tissues were prepared for enzyme assay within 48 and 12 h respectively. Tree-phase larvae were extracted from the host tree by injecting, into the tunnel, a 5% formalin solution containing 2 drops of detergent. As the larva emerged from the tunnel entrance, it was pulled out and rinsed in water. Larvae were weighed and measured before preparation. After removal of gut contents, the entire gut was rinsed in distilled water and macerated in a homogenising tube. The extract was centrifuged at 4°C for 20 min at 27,000 g. Five tree-phase larvae were analysed for each substrate, but because of their comparatively small size, litter-phase larave were grouped to a total weight of 0.5 g. *Wiseana* larvae were tested in 2 assays of 5 larvae.

Enzyme assay

Larval digestion was assessed by determining the conversion of several polysaccharide substrates to reducing sugars in the presence of a gut extract. The reducing sugars were assayed as "maltose equivalents". Substrates tested were chitin (Sigma C-3641), laminarin (Calbiochem 428001), carboxymethyl cellulose (Sigma C-8758), pectin (Sigma P-9135), larchwood xylan (Sigma X-3875), locust bean gum galactomannan (Sigma G-0753), and starch at a concentration of 5 g/ml. Carboxymethyl cellulose, pectin, and xylan were soluble by use of a magnetic stirrer. Locust bean gum was only partially dissolved by stirring, but formed a homogeneous suspension. Chitin was only partially soluble and was filtered at the conclusion of the assay. Starch was made soluble by stirring and slight warming.

The test for enzyme activity is modified after Bernfield (1955). 1 ml aliquots of gut supernatant were incubated with 1 ml aliquots of a pH 7.4 phosphate buffered substrate solution at 20°C for 0, 30, 60, 120, and 240 min. Incubation was terminated by the addition of 2 ml of 3,5-dinitrosalicylic acid reagent. The solutions were then heated in boiling water for 8 min, cooled with a uniform amount of water and the absorbance immediately recorded at 540 nm. Absorbance was converted to mg of maltose liberated by use of a maltose calibration curve. Control blanks included the reaction system which

was terminated immediately after the addition of the gut extract and the incubation of gut extract or polysaccharide substrate alone. No increase in absorbance was recorded in the controls.

To examine the ability of tree-phase larvae to hydrolyse callus tissue polysaccharides, callus was scraped from the feeding surface on *Carpodetus serratus*, frozen at −40°C, macerated and sedimented at 2,500 g for 15 min to remove simple sugars. Gut homogenates were prepared from 2 larvae and incubated with the callus tissue at 20°C.

RESULTS

Litter and tree-phase larvae exhibit gut activity to both laminarin and starch. Activity toward galactomannan was found in the tree-phase but was not investigated for the litter-phase. The rates of substrate hydrolysis by *A. virescens* gut extracts were expressed as a linear relationship over a 4 h assay period for 5 replicates (Fig. 4). Individual or group variation in rates is reflected in the large standard deviations.

Activity toward chitin and carboxymethyl cellulose was less than 0.03 and 0.06 μmol/larva/h in the litter and tree-phase respectively while no confirmed activity could be detected for xylan and pectin. Although some activity was recorded for chitin and carboxymethyl cellulose, no consistent trend could be clearly distinguished from experimental error and the activities are therefore regarded as inconclusive. In the two experiments on tree-phase hydrolysis of callus tissue the rate was 0.064 and 0.086 μmol/larva/h respectively. This demonstrates activity towards polysaccharides present in the callus and is similar to the rate of laminarin degradation (Table 1), but much lower than for starch or galactomannan.

Table 1: Metabolism of potential nutrient polysaccharides (μmol/larva/hour) by *Aenetus virescens* larvae (rep = replicates. n = number of larvae in each replicate). + = standard error. nd = not determined.

	litter-phase	rep.	n	tree-phase	rep.	n
Laminarin	0.06 + 0.01	5	12	0.20 ± 0.21	5	1
Starch	0.10 + 0.03	5	12	0.75 ± 0.39	5	1
Galactomannan	nd	—	—	0.92 ± 0.13	5	1

When starch and laminarin degrading activity is expressed in terms of rate per larva (Table 1) a higher level of activity is found in the tree-phase. However, when activity is expressed as rate/g of insect tissue (Table 2) the smaller litter-phase larvae exhibit about 16 times the polysaccharide degrading capacity of tree-phase forms. These results suggest a partial loss of starch and laminarin degrading activity relative to increased body weight of individual larvae from litter to tree-phase. Gut activity by *Wiseana* larvae in terms of activity per larva (Table 3) was less than *A. virescens* for laminarin but similar for starch.

Table 2: Metabolism of potential nutrient polysaccharides (μmol/g/hour) by *Aenetus virescens* larvae

	litter-phase	rep.	n	tree-phase	rep.	n
Laminarin	1.14 + 0.01	5	12	0.07 ± 0.07	5	1
Starch	2.58 + 0.72	5	12	0.16 ± 0.09	5	1
Galactomannan	nd	—	—	0.40 ± 0.06	5	1

Table 3: Metabolism of potential nutrient polysaccharides by *Wiseana* sp. larvae.

	μmol/larva/hour	μmol/g/hour	rep.	n
Laminarin	0.02	0.04	1	5
Starch	0.74	1.03	1	5

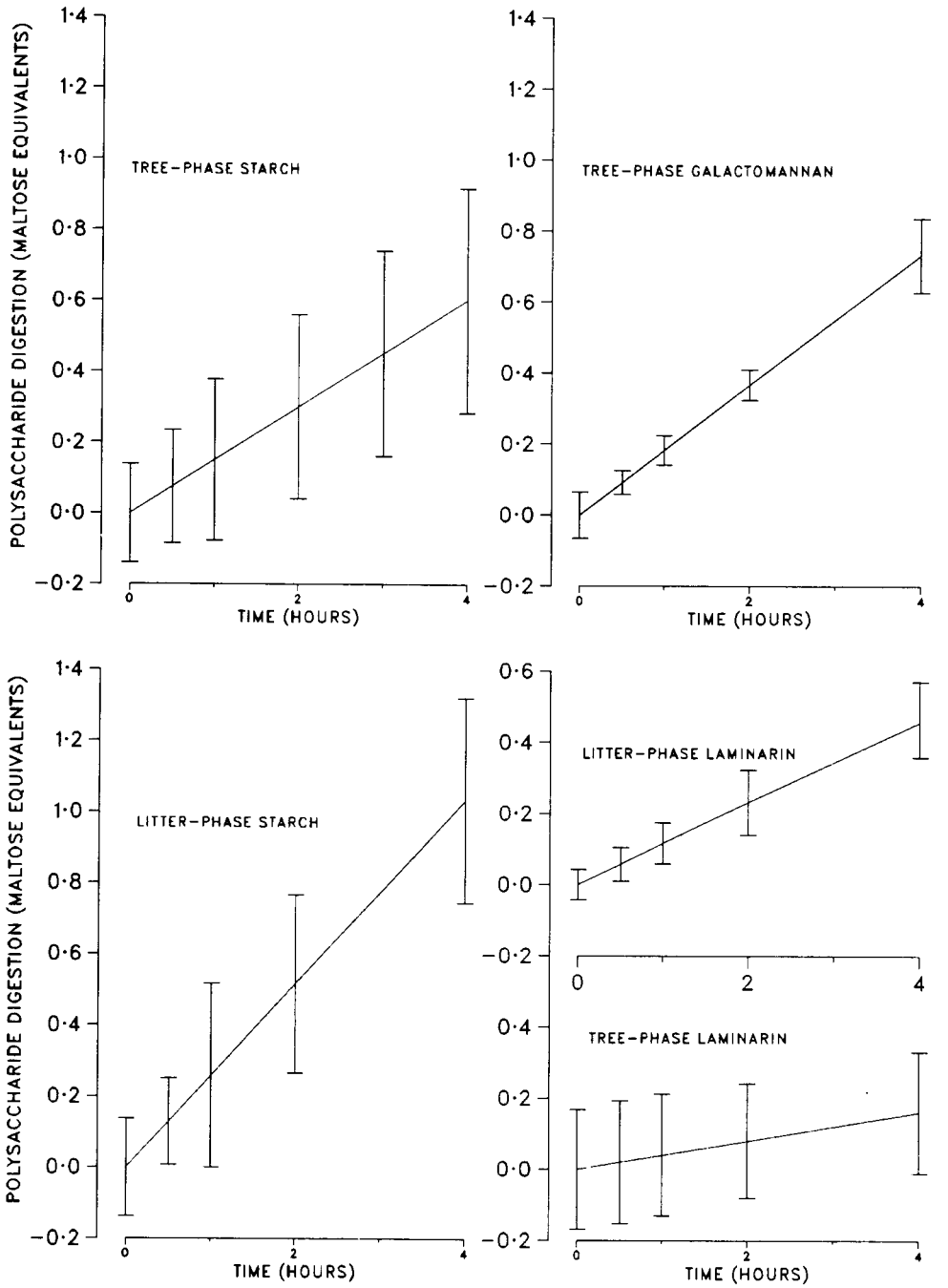

Fig. 4: Hydrolysis of potential nutrient polysaccharides by litter-phase and tree-phase *Aenetus virescens* larvae. Rates of polysaccharide hydrolysis are expressed as a mean with standard deviation (vertical bars) for 5 replicates, each comprising 12 (litter-phase) or single (tree-phase) specimens.

DISCUSSION

The polysaccharide-degrading abilities of litter and tree-phase larvae of *A. virescens* share similarities with both specialist mycophagous and phytophagous Lepidoptera. The presence of $\beta-(1,3)-$ and $\alpha-(1,4)-$ glucanases in the litter-phase is a digestive characteristic found in a diverse range of other obligate fungivorus and detritophagous insects (Nielsen 1962, 1963; Bjarnov 1972; Martin 1979; Martin *et al.* 1980, 1981, a, b, c). Since $\beta-(1,3)-$ and $\alpha-(1,4)-$ glucans are not major components of dead vascular plant tissue (Martin *et al.* 1981b) a primarily mycophagous mode of feeding may be inferred for the *A. virescens* litter-phase (c.f. Martin *et al.* 1980; Rawlins 1984). In some fungivorus insects maceration of fungal tissue releases fungal degrading enzymes (Martin 1979) and it is possible that this contributes to litter-phase digestion of fungi. However, the continued presence of $\beta-(1,3)-$glucanases in the tree-phase may suggest that they are also produced by the insect. Chitin is a major fungal cell wall component, but the lack of chitinase activity in *A. virescens* is not an exceptional feature for obligate fungivores (Martin *et al.* 1981c) and detritovores (Bjarnov 1972; Martin *et al.* 1980).

Tests of lepidopteran digestion have rarely included $\beta-(1,3)-$glucans such as laminarin (Martin *et al.* 1981c), possibly because of their rarity in vascular plants. Inability to hydrolyse laminarin has been demonstrated for the foliage feeding larvae of *Alsophila pometaria* Harr. (Geometridae) and *Anisota senatoria* (J. E. Smith) (Citheroniidae) (Lawson *et al.* 1984) while a study of 2 "xylophagous" Lepidoptera (Cossidae) by Chararas & Koutroumpas (1977) recorded gut activity to laminarin in the larvae of *Cossus cossus* L., but not *Zeuzera pyrina* L. In contrast to laminarin, the ability to hydrolise starch has been widely tested and confirmed for phytophagous Lepidoptera (Agarwal, 1976a, b; Babers & Woke 1937; Pant *et al.* 1958; Hocking & Depner 1961; Applebaum *et al.* 1964; Mathur 1966; Chattoraj & Mall 1969; Khan & Kasting 1969; Verma & Balyan 1972; Saxena 1972; Balyan 1973, 1975; Khan & Bhatti 1973; Burton *et al.* 1977; Dixit & Mall 1977; Somadder & Shrivastava 1980; Lawson *et al.* 1984).

Degradation of $\beta-(1,3)-$glucans by foliage feeding Orthoptera (Morgan 1975, 1976) has been described by Martin *et al.* (1981c) as an "enigma" although they suggested that the enzymes could be involved with digestion of fungi growing on the foliage. A further alternative considered by Martin *et al.* (1981c) was digestion of callose, a $\beta-(1,3)-$ glucan present in vascular plants (Northcote 1969). Although Martin *et al.* (1981c) did not regard callose to be present in sufficient quantities to play a major role in orthopteran diet, grasses often include significant amounts of other glucans containing a mixture of $\beta-(1,3)-$ and $\beta-(1,4)-$ linkages (M. M. Martin pers. comm.). Callose has been recorded from phloem and differentiated callus tissue (Northcote & Wooding 1968) and physical damage to plant tissue can result in the deposition of callose (Eschrich 1975). Callose may be a component of the larval diet of *A. virescens*, but this possibility was not tested other than to show that there was gut activity to the polysaccharide component of callus.

Most phytophagous Lepidoptera show little potential for digestion of the major structural components of vascular plants (Lawson *et al.* 1984) as found in this study for the low activities to carboxymethyl cellulose, pectin, and xylan. However, galactomannan was the polysaccharide most readily digested by tree-phase larvae. Galactomannans are recorded mostly from seed endosperm in the Leguminosae (Dey 1978), but hydrolysis of this polysaccharide by *A. virescens* may indicate a digestive ability for other hemicelluloses. Some wood feeding insects acquire enzymes active against vascular plant cell wall polysaccharides by consuming and macerating fungal tissue in the wood (Kukor & Martin 1983; Martin 1984). The lack of wood feeding and low level of gut activity towards major structural polysaccharides suggests that this mode of digestion is unlikely for larvae of *A. virescens*.

Hydrolysis of laminarin and starch by *A. virescens* and *Wiseana* sp. suggests that these digestive abilities are widespread in the Hepialidae since the 2 species belong to separate subfamilies (Hepialinae and Oxycaninae respectively [Dumbleton 1966]) and have contrasting modes of phytophagy. Larvae of *Wiseana* spp. feed on leaves of grasses and herbaceous dicotyledons and occasionally herbaceous roots (Cockayne 1915; Perrott 1974; Grehan 1983b) while *A. virescens* larvae feed on woody callus tissue. Laminarin activity was also found in a single test on a larva of *Trioxycanus enysii* (Butler *sensu* Meyrick 1890;

Oxycaninae) extracted from soil. This species is a forest insect believed to feed on dead plant tissue throughout larval development (Grehan *et al.* 1983). Digestion of $\beta-(1,3)-$ glucans may be important in early instars of leaf feeding Hepialidae which are present at or near the ground surface (e.g. *Wiseana*, Dumbleton 1945; Taylor 1964) and may feed on dead plant tissue (Evans 1941; Madge 1954; Martyn 1960; Dugdale 1975; Joubert 1975). Older instars of *Oncopera brachyphylla* Turner and *O. parva* Tindale larvae feed on live and dead leaves (Elder 1970; 1978) and this is probably true also for many other species.

Other Lepidoptera with fully generalised diets which include a mixture of fungal and vascular plant tissue appear to be relatively uncommon. Examples include *Hyposmocoma* (Ditrysia: Cosmopteryginae), *Mnesarchaea* (Exoporia: Mnesarchaeidae) and Zeugloptera (Zimmermann 1978; Gibbs 1979; Carter & Dugdale 1982). The relative importance of fungal and vascular plant tissue in the diet of these insects has not been determined and it is possible that some of the host range is facultative rather than obligate. In terms of a capacity to digest starch, the early development of *A. virescens* would not be restricted to fungi and the litter-phase can be "induced" to feed on vascular plant tissue (*Nothofagus* seedings) in the absence of fungi or dead plant tissue (c.f. Alma 1977) but larvae did not survive beyond the 2nd or 3rd instar (Grehan 1988b). Slashchevskii (1929) was able to feed young larvae (equivalent to litter-phase) of *Zenophassus schamyl* on live vascular-plant foliage even though the normal diet consisted of dead plant material.

It is not possible at present to suggest how widespread digestive abilities to $\beta-(1,3)-$ glucans may be in the Lepidoptera. One research possibility to explore is the digestive characteristics of phytophagous families in which callus feeding is prevalent such as the Oecophoridae, Cossidae (Common 1970) and Nepticulidae (Wilkinson & Scoble 1979). Alternatively, it may be found that the digestive ability is largely associated with particular "types of organisation" such as the Hepialidae. In this context, the functional role of these and other digestive abilities of the Hepialidae may be as much a part of the phylogenetic origin of a group as it is of current ecology (c.f. Croizat 1964; Grehan & Ainsworth 1985; Heads 1985).

ACKNOWLEDGEMENTS

I am indebted to Dr T. W. Jordan (Biochemistry Department, Victoria University of Wellington) for his advice and direction in the preparation and analysis of this study and to Professor M. M. Martin (School of Biological Sciences, University of Michigan) for helpful comments, literature and encouragement. I thank Dr G. W. Gibbs, Ms R. Yeoman (Zoology Department, Victoria University of Wellington) and Professor D. H. Janzen (Department of Biology University of Pennsylvania) for criticism of the manuscript. I also thank Mr and Mrs R. A. Page, Mr and Mrs D. Gilliver and Mr and Mrs D. B. A. Innes for permission to collect larvae on their respective properties. This project was supported by the Wellington Regional Council and the Wainuiomata Community Council who allowed access to restricted areas for the collection of larvae.

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