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Some Aspects of the Ecology and Behaviour of the Australian Red Cedar Tip Moth, *Hypsipyla robusta* Moore

A thesis submitted for the degree of Doctor of Philosophy of the Australian National University

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(April 1996)
Responsibility

The research presented in this thesis is conducted by me, unless otherwise explicitly acknowledged.

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(April 1996)
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Abstract

The primary objective of this study was to provide some basic information about the ecology and behaviour of Hypsipyla robusta Moore, a serous shoot borer of Australian red cedar (Toona australis (F. Muell.) Harmes). First, quantitative analyses were made on the temporal and spatial patterns of infestation. This was followed by artificial rearing of the insect in the laboratory, modelling of temperature-dependent development, studies of the feeding behaviour of larvae, analyses of the diel patterns of reproductive activities, and preliminary investigations of the host selection behaviour of larvae and adults.

The infestation patterns of the insect were investigated with sample data from a red cedar plantation. The temporal pattern of infestation levels was closely correlated to rainfall: the larger the amount the rainfall the higher the infestation levels. Temperature did not affect the general infestation levels, but low daily minimum temperatures in the winter (< 6.5 °C) were always associated with low proportions of attacked trees. Attack by the insect was concentrated on open-grown trees. However, attack on forest-located trees was also observed. Among trees planted in the open, attack was largely a random process, i.e. all trees had similar likelihood of being attacked. The only exception was trees ≤ 1.5 m tall, which were attacked significantly less often than larger trees. For attacked trees, the percentages of shoots attacked per tree decreased as tree size increased. Within an attacked tree, shoots positioned among the upper tree crown were attacked significantly more often than those positioned in the lower tree crown or offshoots.

Damaged shoots are likely to retain the section starting from the position of 1.0 cm diameter to the shoot base. The most significant factors affecting the height increments and changes in tree-form values of trees in the heavily infested open plot were all associated with initial tree size or
tree form: larger and less-well formed trees achieved larger height increments and better-formed trees achieved less gain in tree-form values. For trees with initial height in the same range, the average height increment of trees in the forest during the same period was over twice as much as that in the open plot.

The insect had been successfully reared on an artificial diet for 23 generations. The colony reared in this study compared favourably to other artificially-reared colonies of *H. robusta* in terms of pupal weight and fecundity. Duration of development was comparable to that observed for individuals reared with host tissues. The number of larval instars varied from five to seven, with most larvae completing six instars before pupating. Mating in indoor cages was enhanced by exposing the moths to wind. Mortality due to non-feeding was reduced by confining the neonates to small rearing vials.

Mean rate of development of the combined larval and pupal period in the temperature range of 16.4 °C - 28.7 °C was closely fitted by the linear model. Based on the model parameters and data on duration of egg development, the maximum number of annual generations of the insect around the field study site was estimated at five to six. Variable development rates among individual insects were evident at all the five constant temperatures tested. Distribution of develop times at different temperatures can be modelled by a common 3-parameter Weibull function, which can be incorporated into field phenology models in estimating the proportions of individuals completing development by a certain amount of accumulated DD. A simpler but practical approach to modelling the stochastic process of development was also demonstrated by directly fitting the proportional development data to the logistic phenology model, which can be used to estimate the proportions of individuals in each development stages at a given time.
Feeding bioassays using neutral substrates confirmed the existence of feeding stimulants in the ethanol extracts of young shoots. Larvae fed more intensively on agar-cellulose medium or filter paper treated with the ethanol extracts than on the corresponding plain substrates in both non-choice and choice tests. The biting response could be elicited by the presence of host odour alone. Feeding stimulants for the insect was not confined to red cedar. The larvae readily bored into the shoots of three non-host meliaceous species: Spanish Cedar (*Cedrela odorata*), Chinese toon (*Toona sinensis*), and white cedar (*Melia azadirachta var. australasica*), although those bored into the shoots of the latter two species later died.

Feeding spots with respect to host tissues changed as the larva aged. Feeding by larvae of the first two instars were mostly found in terminal foliage (buds and unexpanded foliage) or damaged tissues (leaf scars or other damaged areas on the surface of shoots or stems). Pith-feeding (tunnelling) started at later 2nd instar. Some larvae came out of the tunnels before pupating. With potted plants, the preferred pupation sites was around the base of the plant close to the soil level. On average, a larva initiated feeding in 5.4 different locations during its life time, with a minimum of three and a maximum of 11. Switching of feeding spots was most frequent during early first instar and much of the 3rd and 4th instar.

Most moths emerged in the early hours of the scotophase (82%), the rest before light-off. Female calling started at 3 hours after light-off and peaked around 5.5-7.5 hours after light-off. There was an apparent trend for earlier calling as the females aged. Mating started 1.5 hours later than calling but reached its peak around the same time as calling. Mating was recorded for females aged 1-6 days and for males aged 0-4 days. Females appeared to be most receptive to males in the 2nd-3rd day following emergence. Egg-laying was observed in all but the first 0.5 hour and the last 1.5 hours of the scotophase. There appears to be no single, dominant peak in the diel pattern of egg-laying. Despite being nocturnal, adults of the insect, irrespective of
their sex and mating history, periodically underwent rest in the dark phase. Virgin females were more active during the early half of the scotophase and remained relatively stationary during the latter half of the scotophase. Average duration of active intervals was significantly longer in mated females than that in virgin females. Males were most active in the first scotophase following emergence, and virgin females in the second scotophase following emergence. The results suggest that mated females may be responsible for host finding.

Volatile from the young foliage of the host plant were attractive to larvae when tested in the larval olfactometer. However, tests in the Y-tube olfactometer and wind tunnel failed to detect apparent directional responses toward host volatiles in virgin and mated females. Hypotheses were proposed to explain the lack of olfactory responses of females to host volatiles.

Implications of results of this study for the management of the insect are discussed.
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Chapter 1

General Introduction

Australian Red Cedar, *Toona australis* (F. Muell.) Harmes* (Swietenioidea: Meliaceae), is a deciduous rainforest species distributed along the eastern coastline of New South Wales and Queensland (Floyd, 1989). Timber of Red Cedar is durable, easy to work, and considered as one of the best furniture materials in Australia (Floyd, 1989). The attractive wood properties of the tree have long been recognised and generated considerable demand for its supply during the early period of European settlement, resulting in its massive destruction (Vader, 1987). As a result, standing mature trees of the once abundant species are now found only in a few isolated locations (Vader, 1987).

Attempts to grow Red Cedar in plantations have so far been unsuccessful due to the attack by the Australian Red Cedar Tip Moth, *Hypsipyla robusta* Moore (Lepidoptera: Pyralidae). Larvae of the insect feed primarily inside the growing shoots of the host tree (Anon., 1918). As a result of larval tunnelling, terminal sections of the shoots eventually die or break off. Secondary shoots are formed, only to be attacked again in the same way as the primary shoots. Repeated destruction of the growing shoots results in the severe deformation of the host tree, as characterised by numerous branches and stunted height growth, rendering them of little commercial value. The severity of the problem is perhaps no more strikingly shown than the fact that the insect is mentioned in almost all forestry publications (scientific or non-scientific) or pamphlets which have devoted some length to the cultivation of Red Cedar. The 'gloomy' situation has even prompted the suggestion for the discouragement of any

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*The name *Toona australis* M. Roemer is used in a recent revision of the genus *Toona* by K.N. Bahdur (1988) [Monograph on the genus *Toona* (Meliaceae), iii+251 pp. Forest Research Institute and Colleges, Dehra Dun, India]. See Chapter 2 for further discussion.
future plantations of Red Cedar (pamphlet, New South Wales Forestry Commission). In addition to Red Cedar, *H. robusta* attacks related Meliaceae species in Asia and Africa, including the mahogany (*Swietenia* spp.) and African mahogany (*Khaya* spp.) (Beeson, 1919; Roberts, 1968). Planting efforts of these valuable timber species are similarly constrained by the insect (Entwistle, 1967).

Despite the devastating effects of *H. robusta*, there have been surprisingly few studies of the insect. Much of what we know about the insect was provided by Beeson (1919) almost 80 years ago. Since then, there have been few advances toward a better understanding of the insect. The ecology and behaviour of the insect remains poorly understood. While a number of reports has addressed the temporal and spatial patterns of attack (eg. Campbell, 1964; Roberts, 1968; Wagner *et al.*, 1991), there have been practically no quantitative studies of the infestation patterns. No attempts have been made to determine the ecological and/or behavioural mechanisms underlying the infestation patterns. Although the composition of female sex pheromones of the insect has been identified (Bosson and Gallois, 1982), behavioural aspects of host selection in larvae and adults are unknown (Newton *et al.*, 1993). Studies of the insect in Australia are particularly lacking, with only four reports published so far, all of which are descriptions of damage characteristics based on casual observations and lack the support of concrete data (eg. Anon., 1918; Froggatt, 1923, 1927; Campbell, 1964). This overall lack of knowledge greatly hinders the development of efficient control strategies against *H. robusta*.

Recognising the acute shortage of information with respect to *H. robusta*, a broad approach has been adopted in studying the insect, rather than focusing on particular aspects. The primary objective of this study was to provide some basic information about the ecology and behaviour of the insect. First, a quantitative approach is made in describing the temporal and spatial patterns of attack of the insect on Red Cedar based on systematic
sampling data from a Red Cedar plantation in New South Wales (Chapter 3). Temporal patterns of attack are analysed in relation to temperature and rainfall. Spatial patterns of attack are analysed at three levels: within-tree variations, inter-tree variations, and habitat differences. Growth of trees in an infested open plot was also investigated. To provide a steady supply of test insects for off-site studies, a rearing programme was initiated using an artificial diet. The performance of the diet in rearing the insect was assessed and a novel solution provided to the long-standing problem of mating in cages (Chapter 4). With the success of artificial rearing, it was possible to study temperature-dependent development of the insect (Chapter 5), larval feeding behaviour with respect to host tissues and host chemicals (Chapter 6), diel patterns of emergence, calling, mating, and physical activities of the moths (Chapter 7), orientation responses of larvae and adults to host odours and host acceptance in egg-laying females (Chapter 8). Results from individual chapters are drawn together and discussed in Chapter 9. Where applicable, implications of the results of this study to the control of the insect are discussed, and recommendations suggested as to the future areas of research. Finally, a technique using frass widths to estimate larval instars in the field is described (Appendix).

It is hoped that the information presented here will better our understanding of *H. robusta* and its infestation of Red Cedar, and serve as a basis for future studies, leading ultimately to the establishment of integrated pest management strategies against the insect.
Chapter 2

A Review of Hypsipyla robusta Moore

2.1 Introduction

Attack by Hypsipyla robusta Moore (Lepidoptera: Pyralidae) on Australian Red Cedar (Toona australis (F. Muell.) Harmes) has been recognised since early this century (Anon., 1918). The insect is also responsible for extensive damage on related Meliaceae species in Asia (Beeson, 1919) and Africa (Roberts, 1966). Within the same genus is another equally notorious shoot borer of important Meliaceae species in Latin America, H. grande//a (Zeller). Collectively, the two shoot borers are known as mahogany shoot borers. Mahogany shoot borers are considered as the overriding factor restricting the establishment and cultivation of many tropical members of the Meliaceae, some of which are the world’s best regarded timber species (Newton et al., 1993).

The devastating effect of damage by H. robusta on its host trees has been well documented (Coventry, 1899; Anon. 1918; Beeson, 1919; Froggatt, 1923; Roberts, 1968; Grijpma, 1976; Newton et al., 1993). As a result of tunnelling by larvae, the infested shoots eventually die or break off above the entrance holes and then branch out below (Beeson, 1919). Killing of host trees is unusual, but, instead of forming long and straight boles, the infested trees resemble orchard trees, losing much of their timber value (Grijpma, 1976). Wherever host trees are planted in groups, attack by H. robusta generally occurs (Coventry, 1899). As a result, attempts to grow the native host trees have largely failed in Australia (Campbell, 1964), in India (Rao and Bennet, 1969) and in Africa (Wagner et al., 1991). The same problem was reported in Latin America (see Newton et al., 1993).

Reviews of the mahogany shoot borers include Tillmans (1964), Entwistle (1967), Grijpma and Styles (1973), Grijpma (1976), and Newton et
al. (1993). Although of recent date, the review by Newton et al. (1993) concentrated on the damage patterns and control of the insects, particularly *H. grandella*, with only brief mention of other aspects. No comprehensive reviews have been done specifically on *H. robusta*. The review below therefore attempts to offer a comprehensive view of existing information on *H. robusta*. Relevant information on *H. grandella* is also discussed, as the two species of *Hypsipyla* appear to behave similarly (Newton et al., 1993).

2.2 Distribution

*H. robusta* has been recorded in 20 countries, scattered around in tropical and subtropical regions of Africa, Asia, and Australia and neighbouring islands (Commonwealth Institute of Entomology, 1983). Countries where the insect has attracted considerable attention are: India, Indonesia, Nigeria, Ghana, and Australia.

2.3 Host Trees

2.3.1 Host range

Long-time isolation of regional populations of *H. robusta* has resulted in different species being used as its host trees in different geographic regions. A list of host plants and the countries in which the attack occurs was compiled by Entwistle (1967) (Table 2.1). Most of the host plants were recorded in Africa and the Indian subcontinent, including the well-known mahogany (*Swietenia* spp.) and African mahogany (*Khaya* spp.). In Australia, only Red Cedar is attacked by the insect. However, the possibility of alternative hosts was suspected since Red Cedar trees planted many miles away from the natural cedar country were similarly attacked (Anon., 1918). Except for four species, all host plants of *H. robusta* fall in the subfamily Swietenioideae of the family Meliaceae (see Grijpma, 1976). It is interesting to note that *Cedrela odorata* L. was also attacked by *H. robusta*, while T.
_australis_ was not attacked by _H. grandella_ and was poisonous to it (Grijpma, 1970, 1976; Grijpma and Roberts, 1975).

There is no agreement as yet as to whether the Red Cedar should be treated as a separate species or as a variety of _T. ciliata_ M. Roem., the major host of the insect in India (Beeson, 1919). This is reflected in the parallel use of the name _T. australis_ and the name _T. ciliata var. australis_ in current literature (Edmonds, 1993). The name _T. australis_ is adopted in this thesis in accordance with the usage in a recent Australian botanical book on rainforest tree species (Floyd, 1989). Also included in the genus _Toona_ are at least three other species: _T. sinensis_ (A. Juss.) M. Roem., _T. fargesii_ A. Chev., and _T. sureni_ (Bl.) Merr. _sensu lato_ (Edmonds, 1993). Only _T. australis_ is native to Australia, the other _Toona_ species occurring in India, Pakistan, South East Asia, China and Malaysia (Edmonds, 1993).

2.3.2 Natural distribution and habitats of Red Cedar

Red cedar is distributed along the coastal rainforests of Australia from Benandrarah, NSW to the McIlwraith Range, Qld, covering a range in latitude from 13 - 35 °S (Floyd, 1989). The main distribution is between Ulladulla, NSW, and Gympie, Qld, with disjunct populations further north and in New Guinea (Boland _et al._, 1984). The mean monthly temperature range in this region is 5 - 31 °C and the mean annual rainfall 1200 - 3800 mm. It is most abundant in moist gullies or along streams and requires alluvial or volcanic soils for its best development (Anon., 1918; Mitchell, 1972; Boland _et al._, 1984). The rainforest types it occurs in range from warm temperate (simple notophyll vine forests) to subtropical (complex notophyll vine forests) and tropical rainforests (mesophyll vine forests), where it is often associated with white and black booyong (_Argyrodendron trifoliolatum_ and _A. actinophyllum_), Red Carabeen (_Geissois benthamiana_), sassafras (_Doryphora sassafras_), Yellow Carabeen (_Sloanea woollsii_) and occasionally Hoop Pine (_Araucaria cunninghamii_) (Boland _et al._, 1984).
2.3.3. Characteristics of Red Cedar

Red cedar is a large deciduous tree attaining a height of 45 m and a breast diameter of 210 cm (Floyd, 1989). The tree crown is very open with large spreading limbs (Boland et al., 1984). In comparison with other related Swietenioideae species, growth of Red Cedar is rapid (Grijpma, 1970). In Costa Rica, Red Cedar was reported to reach a height of 6.8 m in 13 months (Edmonds, 1993). An early report based on tree ring studies of old stumps over 200 years old put the annual girth increment at ca. 2.54 cm (Anon., 1918). Seedlings of Red Cedar have recorded a growth rate of 3 cm/day in growth tubes (Applegate and Bragg, 1989). The mature timber is red, soft, light and durable and considered as one of the best for furniture in Australia (Boland et al., 1984; Floyd, 1989). Current price of the timber is estimated at around A$1000/ m³ (Beutel, P., Forestry Dept., ANU, Australia. pers. comm.). Apart from its attractive timber, Red Cedar is highly regarded as an ornamental, wildlife shelter and shade species (Cremer, 1990).

The timber value of Red Cedar created a large demand in Australia and overseas in the late 19th century, resulting in its rapid destruction (Vader, 1987). The destruction was so severe that this once abundant species is now confined to a few isolated localities (Cremer, 1990; Vader, 1987), with scattered trees from Ulladulla, NSW, to Atherton, Qld (Cremer, 1990).

2.4 Systematic Position

H. robusta belongs to the subfamily Phycitinae of the family Pyralidae (Beeson, 1919). It was first described in 1886 as Magiria robusta Moore in Sri Lanka and later found to be synonymous with Hypsipyla pagodella Ragonot described two years later in India and subsequently renamed as Hypsipyla robusta Moore (Bradley, 1968). The genus Hypsipyla Ragonot contains 11 species, of which four are from the New World and seven from the Old World (Entwistle, 1967). H. robusta and H. grandella (Zeller) are the two most economically important and widely distributed species (Newton et al.,
1993). *H. grandella* closely resembles *H. robusta* in morphology and attacks related Meliaceae species, mainly *Cedrela* spp. and *Swietenia* spp., in the New World from Florida to Brazil (Entwistle, 1967). Other species in the genus are of limited distribution and not well known (Entwistle, 1967). After examination of specimens from a number of geographic areas, Bradley (1968) suggested that *H. robusta* probably comprised several geographical races or subspecies.

### 2.5 Morphological Characteristics

Diagnostic characteristics of *H. robusta*, as shown in the wing venation and genitalia, have been provided by Bradley (1968) and more recently by Sharma and Singh (1980b). Moths of both sexes are dark grey and of medium size, with a wing-span of 18-31 mm (Roberts, 1966). Detailed descriptions of the external morphology of the insect at individual developmental stages are given by Beeson (1919). The most striking feature is shown in the colour of the last instar larvae, changing from reddish brown immediately after moult to light blue just before pupation. Described in India, this feature is shared by *H. robusta* in Africa (Roberts, 1966) and Australia (Froggatt, 1923, 1927). The colour of the eggs is also characteristic, with distinctive red and white patches developing in about one day after egg-laying (Beeson, 1919; Atuahene and Souto, 1983). Sex of the insect can be identified at the pupal stage, with the male genital opening at the 9th abdominal segment and the female at the 8th abdominal segment (Sharma and Singh, 1980a).

### 2.6 Biology

#### 2.6.1 Life history

Considerable regional variations exist in the life history of *H. robusta*. Based on his study in India, Beeson (1918, 1919) reported five annual generations, the first and second generations developing on flower and
fruits, respectively, and the next three generations on growing shoots. Evidence of flower- and fruit-feeding generations are lacking in other parts of Asia (Entwistle, 1967). In Africa, attack on flowers and fruits did occur but seemed to be restricted to certain host plant species and regions. Only shoot-feeding generations have been reported in Australia (Froggatt, 1923, 1927). Reports on the number of larval instars are also inconsistent. Earlier studies indicated 4 larval instars (Beeson, 1919; Roberts, 1968), while later studies showed either 5 instars (Morgan and Suratmo, 1976) or a 5-7 instar pattern with the majority exhibiting 6 instars (Couilloud and Guiol, 1980; Atuahene and Souto, 1983). It is suspected that larvae of certain instars may have been overlooked in early studies (Atuahene and Souto, 1983). This seems likely as the results of those studies were obtained with field observations or samples.

2.6.2 Habits of larvae, pupae and adults

Larvae of *H. robusta* are cryptic, spending most of their life in concealment inside shoot tunnels, fruits, self-constructed silk enclosures (Beeson, 1919), or under the bark (Roberts, 1968). When feeding on flowers, the larvae feed gregariously on all parts of the inflorescence by binding together individual flowers through a loose network of silk threads (Beeson, 1919). Early instar larvae of the fruit generation select the young and soft fruits and feed on the epidermis, while older larvae attack more advanced fruits and feed inside (Beeson, 1919). Tunnelling of shoots usually commences at leaf axils (Ballard, 1914; Coventry, 1899). Late instar larvae may emerge from existing tunnels and construct new ones (Beeson, 1919), as shown by the many empty tunnels found in the field (Roberts, 1968). Occasionally more than one larvae may be found in a single shoot, in which case the larvae either occupy separate tunnels or a single continuous tunnel (Roberts, 1966). As many as 20-40 wounds may occur on a single stem, resulting in heavy sap exudation (Wagner *et al.*, 1991). Feeding on the
Cambium has also been reported (Ballard, 1914; Roberts, 1968). Where both shoot- and bark-feeding occurs, cambium-feeding was more likely to occur on mature trees, whereas shoot-feeding was the primary form of damage on young trees (Ballard, 1914). Cambium-feeding or barking at the bases of the stems may lead to the death of young trees (Menendez and Berrios, 1992). In times of high population density, larvae may disperse in groups, usually during the 3rd instar, to other trees (Beeson, 1919). Larvae of *H. robusta* can be cannibalistic, with the young and weak ones eaten by the more mature and robust ones when confined together (Froggatt, 1923, 1927).

Pupation site differs with the plant tissues used by larvae for feeding, and in relation to regional populations. In India, pupation of the flower- and fruit-feeding generations takes place almost invariably under flakes of barks (Beeson, 1919), while in Nigeria the flower- and fruit-feeding larvae frequently pupate in ground vegetation (Roberts, 1966). Shoot feeding larvae usually pupate inside larval tunnels (Beeson, 1919; Roberts, 1966).

Little is known about the behaviour of the moths of *H. robusta* (Newton et al., 1993). Beeson (1919) noted that the moth is of retiring habit and is rarely seen in the field and that they were inactive most of the time except for wing-fanning in early morning and late evening. Moth emergence concentrates during the early evening (Atuahene and Souto, 1982). Beeson (1919) reported a highly male-biased sex ratio (75%). However, later studies indicate a roughly 1:1 sex ratio (Atuahene and Souto, 1982; Couilloud and Guiol, 1980). The maximum number of eggs laid per female ranges from 228 to 624, with an average of 472 (Beeson, 1919). Egg-laying females exhibit strong preferences for cracks or creased spots as oviposition sites (Atuahene and Souto, 1982) and may be attracted to products from larval feeding activities (Roberts, 1968). Typical oviposition sites are unexpanded young leaves and leaf buds (Beeson, 1919) and shoots (Beeson, 1919; Roberts, 1968). Eggs are laid singly, firmly attached by a fluid secretion from the cement gland of the female moth (Beeson, 1919).
More detailed information of moth behaviour is available on *H. grandella*, which appears to behave similarly to *H. robusta* (Newton et al., 1993). Oviposition of *H. grandella* occurs during evening or early morning (Wilkins, 1972; Holsten, 1977). Eggs are laid singly or occasionally in small clusters (Grijpma, 1971). Usually only 1-3 eggs are laid on each tree. Peak flight activity takes place in the latter half of the night (Gara et al., 1973). Active flight of females concentrates during the first two days after emergence, while that of males last for about four days. One- to two-day-old females fly farther than males of similar ages (Fasoranti et al., 1982). Although they are able to fly over 14 kilometres within one day (Fasoranti et al., 1982), the moths apparently do not disperse readily from areas of active infestation (Grijpma and Gara, 1970a).

### 2.7 Ecology

#### 2.7.1 Damage patterns

**Spatial patterns**

Attack by *H. robusta* is usually more severe in open habitats (Froggatt, 1923; Mitchell, 1972; Wagner et al., 1991). Campbell (1964) indicated that 50% shade is necessary to reduce infestation. Growing host plants with non-host plants seems to lessen the attack (Newton et al., 1993). Roberts (1968) found that host and non-host plants mixed in the same lines, instead of in pure lines, afforded some degree of protection against the insect.

Intensity of attack within a host stand differs with tree age and height. Morgan and Suratmo (1976) reported negative correlations between attack intensities and tree height and age. Trees less than 13 years old were attacked more often, with the heaviest attack on trees 3-6 years old and 2-8 m tall, whereas trees over 15 m high and aged 13 years or more were only slightly attacked or not attacked at all. It is worth noting that large and old trees are by no means immune from attack (Coventry, 1899; Anon., 1958). However, mature trees are more tolerant to, and suffer less from, the attack (Coventry,
Minimal height and age of susceptible trees appears to be low. In India, host trees were attacked when as young as 3-month-old and ca. 30 cm tall (Entwistle, 1967). In Nigeria, most of the host tree species became susceptible at the age of 1-2 years (Roberts, 1966). For trees of similar age and height, vigorously-growing trees are more likely to be attacked than slow-growing trees (Entwistle, 1967; Lamb, 1968; Newton et al., 1993). Such a preference pattern can also be seen temporally. Brunck and Fabre (1974) noted a high correlation between the attack rates by *H. robusta* and the monthly growth rates of its host trees. Tree form also influences the attack rates, with those producing greater quantity of shoots likely to be attacked more often than single-stemmed trees (Entwistle, 1967; Grijpma, 1976). However, this tendency may be counterbalanced by the negative association between shoot size and shoot abundance (Entwistle, 1967). A study on the within-tree distribution of *H. grandella* indicates that the upper main stems of trees are more frequently attacked than offshoots or middle stems (Yamazaki et al., 1992). Attack on the offshoots is mainly the work of 1st-2nd instar larvae (Yamazaki et al., 1992).

Where multiple host species coexist, *H. robusta* exhibits preference for some species over others. Of the 6 genera of Meliaceae attacked in Nigeria, *Khaya* spp., in particular *K. ivorensis*, suffer the heaviest attack, whereas *Entandrophroagma* spp. and *Lovoa trichilioides* are attacked much less severely even when planted in pure lines (Roberts, 1966). In Indonesia, larvae of *H. robusta* demonstrated a distinct preference for *S. macrophylla* over *K. anthotheca* and *T. sureni* (Morgan and Suratmo, 1976). With respect to exotic Meliaceae species, it is of interest to note that *T. australis* is not attacked by *H. grandella*, and *C. odorata*, the major host of *H. grandella*, suffered little attack from *H. robusta* (Grijpma, 1970).
Temporal patterns

In Australia, attack by the insect appears to be more active in mid-summer than in winter (Froggatt, 1923, 1927). In areas where temperature is not a restricting factor, peak infestation coincides with the rainy season and leaf flush (Brunck and Fabre, 1974; Morgan and Suratmo, 1976; Wagner et al., 1991). Two factors may be responsible for the correlation between attack intensity and rainfall. First, egg-laying females are particularly attracted to new foliage (Grijpma and Gara, 1970a), which is most abundant during the rainy season. Secondly, the ample supply of succulent young shoots during the rainy season ensures better growth of larvae and high fertility of adults.

2.7.2 Attack on tree growth

While the devastating effects of Hypsipyla on the growth of their host trees are well known, few studies have attempted to quantify such effects. Existing information on the C. odorata - H. grandella interactions showed that attacked trees produced more branches than un-attacked trees, but loss of tree height and girth was insignificant after initial exposure to the insect (Howard and Meerow, 1993). Apparently the impact of attack varies with tree species. Some trees may possess higher compensation potential than others.

2.7.3 Host selection

Hypsipyla spp. appear to have remarkable host finding capabilities. Irrespective of their locations, plantations of host trees in their native countries seldom escape attack (Newton et al., 1993). Even isolated trees planted a long distance from the infestation sources are sometimes attacked (Anon., 1918). However, little is known about the mechanisms by which the moths locate the host habitats and eventually lay eggs on their preferred host tree species. Current knowledge in this context is fragmentary and speculative and mainly concerned with H. grandella. Studies with field
olfactory cages indicates that the moths are attracted to the odour of new leaves of the host trees (Grijpma and Gara, 1970a). This finding was later supported by similar experiments with the chemical extracts of the new leaves (Gara et al., 1973) and preliminary electro-antennogram investigations (Schoonhoven, 1974). Antennae of H. grandella may function both as the sense organ of host odours and sex pheromones (Callahan, 1973). Although not yet demonstrated experimentally, H. robusta may possess similar olfactory responses, as attack by this insect is positively correlated with the vigour and growth rate of its host trees (Entwistle, 1967). The responsible volatiles are suspected to involve sesquiterpenes (Carruyo, 1973, 1976). Some of the volatile constituents of T. australis and S. macrophylla have been identified (Oliveira et al., 1986), but their relevance to host selection by Hypsipyla is not known. The task of locating host plants is thought to be accomplished by virgin females (Gara et al., 1973; Holsten and Gara, 1977). Males are subsequently attracted to the females by sex pheromones (Holsten and Gara, 1974). The chemical composition of the sex pheromone is not known, but that of H. robusta consists of a mixture of tetradecenyl acetates (Bosson and Gallois, 1982). In addition to olfactory cues, the involvement of light in the host selection process is also raised. Campbell (1964) speculated that H. robusta would not lay eggs in stands where light intensity is less than 50% of normal incident radiation. Grijpma (1971) went further to suggest that long distance orientation of H. grandella is solely guided by infrared light reflected from host leaves. While direct evidence for the above hypotheses are lacking, the overwhelming number of reports of concentrated attacks on open-grown trees (see Entwistle, 1967; Newton et al., 1993) may be linked to the positive response of Hypsipyla to light. Although nocturnal, oviposition of H. grandella occurs primarily during evening and early morning (Wilkins, 1972; Holsten, 1977), when moderate light intensity is to be expected.
2.7.4 Mortality and natural enemies

No life table data are available on *H. robusta*. Mortality during the larval stage appears to be high, with drowning by sap suspected to be the key factor (Roberts, 1968). The production of sap as a host plant defence mechanism against the larvae of *Hypsipyla* has been suggested by a number of authors (Lamb, 1968; Wilkins, 1972). A large number of natural enemies has been found attacking *H. robusta*. Rao (1969) and Rao and Bennet (1969) listed more than 40 species of parasitoids in India, including 17 braconids, 9 chalcids, 1 elasmid, 1 eulophid, 1 eurytomid, 13 ichneumonids, 2 trichogrammids, 2 tachinids, 2 coleopterans and 1 nematode. Roberts (1968) bred out 5 insect parasitoid species and a nematode parasite from field-collected larvae in Nigeria. The level of parasitisation was found to be very low (usually < 1%), with the possible exception of the nematode. Predators recorded so far are found only in small numbers and do not appear to play an important role in the control of *H. robusta* (Singh and Misra, 1988).

2.7.5 Development rate

Development rates differ with food source and climate. Beeson (1919) noted that development was faster for the flower and fruit generations (24-29 d) than that for the shoot generations (64-79 d) and attributed the differences to the higher nutritional values of the flowers and fruits. For the cambium feeders, the poorer nutritional value of the bark as compared with shoots resulted in an approximately two-week lengthening of the generation time (Roberts, 1968). Under favourable conditions, as indicated by ample food supply and adequate temperature and rainfall, the insect can complete up to 10 generations per year on shoots (Roberts, 1968; Morgan and Suratmo, 1976). In places where temperature or rainfall periodically becomes a limiting factor, the last instar larvae may undertake a period of aestivation (Beeson, 1919; Froggatt, 1923, 1927; Roberts, 1968).
Data on temperature-dependent development are not available. The only available information relating development rate to temperature is obtained with studies of artificial rearing and only one temperature was tested (Atuahene and Souto, 1983; Couilloud and Guiol, 1980).

2.8 Artificial Rearing

Early attempts to mate *H. robusta* in indoor cages largely failed (Beeson, 1919; Roberts, 1966). The problem was not rectified by increasing the sizes of, or providing the host plants in, the mating cages (Beeson, 1919). A similar problem was encountered in the rearing of *H. grandella* (Grijpma, 1971). Fertile eggs can be readily obtained, however, in outdoor cages (Grijpma, 1971). It is not known what factors contributed to the difference. Fasoranti (1985) found that mating of *H. grandella* could be significantly enhanced by flying the moths in pairs in a windmill before placing them in mating cages, suggesting the involvement of wind in mating behaviour. Direct application of Fasoranti’s approach toward the mating problem, however, is not practical in mass rearing of the insect, as it is time-consuming and requires sophisticated equipment. Rearing of *H. robusta* on artificial diets has been attempted by a number of authors (Achan, 1968; Couilloud and Guiol, 1980; Atuahene and Souto, 1983). It is surprising to note that mating was not mentioned as a problem in any of the three reports. However, other problems arose with artificial diets, namely lengthened development duration (Achan, 1968) and high larval mortality (Achan, 1968; Atuahene and Souto, 1983). Large percentages of larvae died due to non-feeding in the first instar (Atuahene and Souto, 1983). The rearing technique of Couilloud and Guiol (1980) appears to be satisfactory, as no adverse effects were mentioned.
2.9 Control

2.9.1 Silvicultural control

Control of *H. robusta* infestation through silvicultural methods was stressed when the insect problem was first brought to attention around the turn of this century (Coventry, 1899; Anon., 1918; Beeson, 1919). Suggested approaches included cutting and burning infested shoots, planting young seedlings away from flowering trees, and/or sack-banding of flowering trees. These approaches are either labour-intensive or based on the assumption that the annual infestation by *H. robusta* began with the flower generation, which has so far been found true only in northern India (eg Beeson, 1919). Summarising earlier studies, Entwistle (1967) recommended growing host species a) with some lateral shade, b) with ca. 50% overhead shade, c) with an admixture of a non-host species, preferably within the same row as the host, and d) if possible away from the centres of active infestation. While many reports pointed to lessened attack of host trees planted in shade (Kalshoven, 1926; Beeson, 1941; Campbell, 1964; Lamb, 1968), conflicting reports exist regarding the shade effect. Roberts (1966) found that shade or cover did not reduce attack by *H. robusta*. Studies in Latin America indicated that attack by *H. grandella* is widespread regardless of whether host trees are grown in the open or in heavy shade (Tillmans, 1964; Chable, 1967; Combe and Gewald, 1979). Newton *et al.* (1993) explained the conflicting reports by the contrasting effects of overhead and lateral shade. They suggested that host trees planted in excessive overhead shade are less likely to recover after attack, thus accentuating the infestation problem, whereas lateral shade reduces the growth and production of branches and thereby also reduces the number of sites available for attack. Efforts involving mixed-species planting did achieve some success. Trials in Nigeria showed that when the host and the non-host plants were planted in the same lines, some degree of control was evident (Roberts, 1968). Similar success was reported with regard to the control of *H. grandella* (see Newton...
et al., 1993). However, planting of mahoganies in mixture with non-susceptible species does not guarantee successful shoot borer control (Newton et al., 1993). The partial success may be explained by the possibility that the presence of non-host trees hinders the location of host trees by egg-laying moths (Grijpma, 1976; Morgan and Suratmo, 1976). Growing host trees away from the centres of active infestation is another approach aimed at hindering the host-locating process. The practice is encouraged in India (Beeson, 1941) and Australia (Froggatt, 1923, 1927). However, this approach is unlikely to maintain lasting protection, considering the strong flying capability of Hypsypyla (Fasoranti et al., 1982). In fact, host trees planted a long distance away from their natural centres are similarly attacked (Anon, 1918). Some authors proposed planting the host trees at a large spacing (10 trees/ha on maturity) (Beard, 1942; Holdridge, 1943; Cater, 1945). Low densities of susceptible trees may prevent build-up of insect populations (e.g., Weaver and Bauer, 1986; see also Watt, 1992). The relevance of the hypothesis to Hypsypyla is seen in the notion that moths of H. grandella tend not to disperse away from active infestation sites (Grijpma and Gara, 1970a). Accepting the seemingly inevitability of attack, some workers proposed planting the trees on good sites to increase their tolerance, since sap production, which sometimes drowns the invading larvae (Roberts, 1968), is more vigorous in fast-growing trees than in slow-growing trees (Lamb, 1968) and trees which display higher vigour are also better able to recover after attack (Grijpma, 1976).

As an alternative to traditional silvicultural control methods, Grijpma (1976) and Newton et al. (1993) stressed the need to switch to resistant Meliaceae species or to select races or strains of the meliaceous species that display resistance. This approach appears to be promising, as exotic Meliaceae species often have been less susceptible to attacks by native Hypsypyla spp. (Chable, 1967; Lamb, 1968; Grijpma and Ramalho, 1969; Grijpma, 1970; Akanby, 1973) and some of them are of comparable wood
qualities to the native host trees (Grijpma, 1976). A good example is shown by the fact that *T. australis* and *C. odorata*, the respective principal host of *H. robusta* and *H. grandella*, can be safely grown in pure plantations in Latin America and in Nigeria respectively (Grijpma, 1976). The relative immunity of *C. odorata* in Nigeria was suspected to be the result of non-preference, while that of *T. australis* in Latin America appears to be due to antibiosis (Grijpma, 1976). The toxic effects of *T. australis* to *H. grandella* can be successfully translocated to *C. odorata* shoots by grafting (Grijpma and Roberts, 1975). A major obstacle in adopting this approach is the tendency for the susceptible meliaceous species to be favoured in countries where they are indigenous (Grijpma, 1976). An alternative approach is to select resistant strains or races within the favoured Meliaceae species. The possible existence of such strains or races has been suggested by a number of authors (Lamb, 1966; Roberts, 1966; Grijpma, 1976). A report in Australia claimed to have found a variety of *T. australis* that appeared to be more resistant (Anon., 1958). No follow-up reports are available in support of this claim. However, a number of reports has pointed to the presence of more tolerant provenances (Burley and Nikles, 1973; Nikles *et al.*, 1978; McCarter, 1986, 1988). The tolerance was characterised by the vigorous growth of the young plants and the re-establishment of a new leading shoot with strong apical growth after attack (Newton *et al.*, 1993).

Selection of resistant species or subspecies appears to have a sound chemical basis. Species in the family Meliaceae are characterised by the common occurrence of limonoids (derivatives of triterpenes), which are found also only in two other families (Taylor, 1981). Many of the limonoids exhibit strong insecticidal properties (Kubo and Klocke, 1986). Different species of Meliaceae have involved limonoids of different structures (Taylor, 1981). For un-adapted insects, the presence of particular limonoids may pose an insurmountable chemical barrier in the form of antifeedants or growth inhibitors. This is shown perhaps no more strikingly than the
limonoids from the Neem tree, *Azadirachta indica* A. Juss., which have been found to be effective against over 200 species of insects and mites and have attracted world-wide attention (see Champagne *et al.*, 1989, 1992; Lee *et al.*, 1991). Although found more widespread in the subfamily Melioideae, limonoids possessing insecticidal properties have also been demonstrated in certain species in the subfamily Swietenioideae (Champagne *et al.*, 1989, 1992). In fact, Cedrelone, a limonoid isolated from *T. australis* was the second-most powerful insect growth inhibitor of the 18 limonoids tested by Kubo and Klocke (1986). A recent study has indicated that the toxic effects of *T. australis* to *H. grandella* are due to the presence of A,B-seco limonoids (Agostinho *et al.*, 1994). The fact that *H. robusta* is able to restrict its host exclusively to the otherwise highly toxic *T. australis* is not surprising since co-evolution may have led to the formation of some forms of detoxification mechanism in the insect. Utilisation of plants with potent toxic chemicals by specialist insects have been well documented (see Bell, 1987). These insects may even use the toxic chemicals or their derivatives to aid their host selection processes. It is of interest to see whether the effective components of the volatiles from *T. australis* that have been shown to be toxic to *H. grandella* (Grijpma and Gara, 1970b) are used by *H. robusta* as olfactory cues.

2.9.2 Biological control

Although a large number of natural enemies has been reported, the observed natural mortality rates of *H. robusta* due to these parasites and predators are very low (Roberts, 1968; Singh and Misra, 1988). A fungus was recently isolated from the dead larvae of *H. robusta* in India (Misra, 1993). It achieved an 80% kill of the larvae when sprayed with a spore culture of the fungus in water. Further studies are needed to evaluate the effectiveness of the fungus in natural populations of the insect. No reports on the effects of artificial release of natural enemies against *H. robusta* are available. A
serious attempt was made in the 1960s and 1970s to introduce some parasitoid species of *H. robusta* from India to Trinidad and other parts of the Caribbean to control *H. grandella* (Cock, 1985). Out of the four species released, only the egg parasitoid *Trichogrammatoidea robusta* Nagaraja has been recovered consistently from the field and the parasitisation rates were low. It appears that biological control alone holds little promise in solving the *Hypsipyla* problem.

2.9.3 Chemical Control

Information on chemical control attempts against *H. robusta* is lacking. Control with conventional insecticides does not offer much hope due to the hidden location of the larvae and the widespread distribution of the pest (Roberts, 1968). Only partial success has been achieved with the application of systemic insecticides (Wagner et al., 1991). Experience with *H. grandella* revealed some effective systemic insecticides, of which carbofuran appears to be most effective (Allan et al., 1970; Wilkins et al., 1976). It achieved complete control for 340 days at one of the sites tested. However, the uptake and translocation of insecticides during dry seasons may be ineffective and caution must be exercised against the possible effects of these insecticides on natural enemies and other insecticides (Grijpma, 1974). Due to these considerations, chemical control with systemic insecticides is recommended only as an interim measure and only as part of an overall programme of integrated pest management (Wilkins et al., 1976).

2.9.4 Other control methods

Samaniego and Katiyar (1974) tested the effect of gamma radiation on final instar larvae, pupae and adults of *H. grandella*. Complete sterilisation of male and female moths was obtained in all treated development stages, with the adult as the most effective stage. The prospect of using sex pheromone to control the *Hypsipyla* problem appears to be good, as males of
H. robusta and H. grandella exhibit distinct pheromone-guided sexual behaviour (Holsten and Gara, 1974; Bosson and Gallois, 1982).

2.10 Conclusions

Attack by the mahogany shoot borers on the Swietenioidae species of the Meliaceae has probably existed for thousands of years. The problem was brought to attention when the timber value of these species was realised. Ironically, human efforts to boost the production of these valuable timber trees by establishing them in large scale plantations have aggravated the problem by providing the insects with abundant food sources and an environment unfavourable for the full expression of their natural controlling factors.

Two distinct control strategies have emerged in the quest to solve the Hypsipyla problem. One focuses on the selection of resistant species or strains or races within susceptible species. Avoiding the problem altogether, Grijpma (1976) proposed planting alternative Meliaceae species that are resistant to Hypsipyla attack and yet are of comparable timber qualities (Grijpma, 1976). The feasibility of such an approach has already been demonstrated for H. grandella. As mentioned before, the insect cannot complete its development in T. australis because of antibiosis. Species completely immune to the attack of H. robusta within the subfamily Swietenioideae are yet to be found, but C. odorata is reported to be a less likely recipient of H. robusta eggs, although in this case development of the insect does not appear to be negatively affected (Grijpma, 1976). More studies are needed concerning the non-selection of C. odorata by H. robusta.

Another possible approach is to switch to the subfamily Melioidae, where none of the species has been reported attacked so far. Some Melioidae species, such as the White Cedar, Melia azadarach var. australasica produce high quality timber (Floyd, 1989). Promising as it appears, there may be considerable reluctance to plant alternative species in countries where
susceptible indigenous species are favoured for cultural and economic reasons (Newton et al., 1993). Selection of resistant strains or races within susceptible species is an obvious alternative when such considerations cannot be compromised. Since tree form is the major concern of Hypsipyla attack, any selections based on superior apical growth or recuperative ability should also lead to improvements in tree form as well as tolerance to attack (Newton et al., 1993). Special attention should be paid to the apical growth of young trees, as attack on mature trees has little impact on tree form. Projects investigating this approach have already been initiated to combat the Hypsipyla problem in Latin America (Newton et al., 1993) and in Australia (Spolc, D., University of Queensland, Australia. PhD project). The latter project is of particular interest as it attempts to quantify the relationship between H. robusta attack and losses of tree form in T. australis, with the aid of computer simulations. Finally, there may be scope in the future for manipulating the genetic structure of susceptible species to achieve some degree of protection against the mahogany shoot borers, for example, by inserting genes of Bacillus thuringiensis (Newton et al., 1993; see also Strauss et al., 1991).

Accepting the inevitability of attack, the other strategy seeks to minimise the damage levels to susceptible species by targeting directly the insect populations or by selecting appropriate planting designs. Approaches directly targeted on the insect populations include the utilisation of natural enemies and applications of chemical control agents. Although a considerable number of natural enemies has been recorded attacking H. robusta in the field, their efficiency in containing the insect populations has not been seriously investigated. Existing information is restricted to the form of sample data and points to general lack of effective control. This is not surprising as the insect is exposed to its natural enemies for only brief periods during its life cycles and usually occur in low densities. The cryptic habit of the larvae also denies the prospect of success of conventional
insecticides. Applications of slow-release systemic insecticides may offer some hope in containing the damage, but their effectiveness is constrained by soil and climatic conditions and further studies are needed to assess their effects on other insects and natural enemies (Grijpma, 1974). Selection of appropriate planting designs is the most widely explored approach in combating the Hypsipyla problem. It is also an approach where reports concerning the control of the insects are least consistent, such as mixed-species planting (Newton et al., 1993). The inconsistency can be partly attributed to the different conditions under which individual studies were conducted. Consequently, results obtained in these studies are not strictly comparable. Any hasty inference drawn from such studies is subject to further scrutiny. Newton et al. (1993) explained the contrasting reports of the effectiveness of shade provided by non-host species on Hypsipyla attack observed in the literature, in terms of the different effects of lateral and overhead shading, claiming that overhead shading aggravates the problem as plants growing under such conditions are less able to recover after attack. Lateral shading reduces the growth of branches and thus the number of sites available for attack. Two different attack indices are mixed up in the explanation: the likelihood of attack and tolerance, the relative levels of which are not clear for both of the two shading schemes. What the authors actually explained appears to be the after-attack conditions of host trees. The effects of mixed-species planting are nested with the effects of shade and host tree densities. No data are available for an independent appraisal of its efficiency in reducing damage. More studies are needed to clarify the effects of individual silvicultural methods.

Successful control of insect problems requires in-depth knowledge of the biology and ecology of the insects and their impact on host plants. On the part of the insects, of particular importance are the host selection behaviour, dispersal, and population dynamics. With respect to silvicultural control, knowledge about the host selection behaviour may
help determine the appropriate plant compositions and layout of mixed-species plantations. The same plantation layout may lead to different attack levels under different host-searching behaviour and dispersal patterns (Stanton, 1983). On the other hand, understanding of the population dynamics of the insects is essential for timing control applications, such as insecticides and artificial release of natural enemies. Unfortunately, such knowledge is scarce and fragmentary for H. robusta. Host selection patterns are known to some extent on H. grandella, but the underlying mechanisms remains largely unaccounted for. Practically nothing is known about the population dynamics of either of the two species, except that peak infestation coincides with rainy periods. On the part of the impact of damage on host trees, what is seen in the literature is a profusion of claims of the seriousness of attack on tree form. Very little is known about the quantitative aspects of the impact, such as the extent of loss of tree form or height increment incurred on trees of different age and height under given attack intensities. Yet knowledge of such aspects is essential in determining the acceptable levels of attack and to what age or height young trees should be protected before they can survive the attacks without significant loss of their commercial values.

Previous control attempts against the insects have been carried out largely on a trial-and-error basis and, not surprisingly, resulted in general lack of success. The prospect of integrated pest management (IPM) strategies against the Hypsipyla problem has been highlighted by several authors (Grijpma, 1974; Morgan and Suratmo, 1976; Newton et al., 1993). As it is probably impossible to totally eliminate their damage and the objective is rather to reduce the damage to a tolerable level, an IPM approach appears to be ideally suited to the Hypsipyla problem. However, the economic thresholds of the insects are expected to be very low, considering the nature of their damage (Grijpma, 1974). Until more knowledge on the insect-plant
interactions and the economical impact of the damage is acquired, the full implementation of IPM strategies is not possible.
Table 2.1. Plants on which attack by *Hypsipyla robusta* Moore has been recorded (from Entwistle, 1967). Plants suspected to be attacked are not included.

<table>
<thead>
<tr>
<th>Species Attacked</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Toona australis</em> (F. Muell) Harmes</td>
<td>Australia, Sri Lanka, India, Malawi, Pakistan, Solomon Islands</td>
</tr>
<tr>
<td><em>T. ciliata</em> M. Roem.</td>
<td>India, Burma</td>
</tr>
<tr>
<td><em>T. sureni</em> (Bl.) Men.</td>
<td>Indonesia</td>
</tr>
<tr>
<td><em>T. serrata</em> (Royle) M. Roem.</td>
<td>India, Pakistan</td>
</tr>
<tr>
<td><em>T. macrocarpa</em> (C. DC.) Harmes</td>
<td>India</td>
</tr>
<tr>
<td><em>Cedrela odorata</em> L.</td>
<td>Indonesia</td>
</tr>
<tr>
<td><em>Cedrela</em> spp.</td>
<td>Zambia</td>
</tr>
<tr>
<td><em>Pseudocedrela kotschy</em> (Schwinf.) Harmes</td>
<td>Nigeria</td>
</tr>
<tr>
<td><em>Swietenia macrophylla</em> King</td>
<td>Sri Lanka, India, Indonesia, Papua, Nigeria, Pakistan, Sarawak, Solomon Islands, Uganda</td>
</tr>
<tr>
<td><em>S. mahogani</em> (L.) Jacq.</td>
<td>India</td>
</tr>
<tr>
<td><em>Carapa guianensis</em> Aubl.</td>
<td>Indonesia</td>
</tr>
<tr>
<td><em>C. procera</em> DC.</td>
<td>Malawi, Nigeria, Uganda</td>
</tr>
<tr>
<td><em>Xylocarpus granatum</em> Koen.</td>
<td>Nigeria, Uganda</td>
</tr>
<tr>
<td><em>X. molucensis</em> (Lam.) M.J. Roem.</td>
<td>Malawi, Nigeria, Papua, Zambia</td>
</tr>
<tr>
<td><em>Khaya anthotheca</em> (Welw.) C. DC.</td>
<td>Ivory Coast, Nigeria</td>
</tr>
<tr>
<td><em>K. grandifoliola</em> C. DC.</td>
<td>Ivory Coast, Nigeria, Pakistan</td>
</tr>
<tr>
<td><em>K. senegalensis</em> (Desr.) A. Juss.</td>
<td>Nigeria</td>
</tr>
<tr>
<td><em>K. nyasica</em> Stapf ex Bak. f.</td>
<td>Nigeria</td>
</tr>
<tr>
<td><em>K. ivorensis</em> A. Chev.</td>
<td>Nigeria</td>
</tr>
<tr>
<td><em>Entandrophragma excelsum</em> (Dawe &amp; Sprague) Sprague</td>
<td>Nigeria</td>
</tr>
<tr>
<td><em>E. cylindricum</em> (Sprague) Sprague</td>
<td>Ivory Coast, Nigeria, Pakistan</td>
</tr>
<tr>
<td><em>E. angolense</em> (Welw.) C. DC.</td>
<td>Nigeria</td>
</tr>
<tr>
<td><em>E. candollei</em> Harmes</td>
<td>Uganda</td>
</tr>
<tr>
<td><em>E. utile</em> (Dawe &amp; Sprague) Sprague</td>
<td>India, Pakistan</td>
</tr>
<tr>
<td><em>Chukrasia tabularis</em> A. Juss.</td>
<td>India</td>
</tr>
<tr>
<td><em>Chloroxylon swietenia</em> (Roxb.) A. Juss.</td>
<td>India, Pakistan</td>
</tr>
<tr>
<td><em>Soymida febrifuga</em> (Roxb.) A. Juss.</td>
<td>Nigeria</td>
</tr>
<tr>
<td><em>Lovoa trichilioides</em> Harmes</td>
<td>India</td>
</tr>
<tr>
<td><em>Dracontomelium multif jugum</em> Radek. (Anacardiaceae)</td>
<td>India</td>
</tr>
<tr>
<td><em>Albizzia</em> spp. (Mimosaceae)</td>
<td>Solomon Islands</td>
</tr>
<tr>
<td><em>Pometia pinnata</em> Forst. (Sapindaceae)</td>
<td>Solomon Islands</td>
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<td><em>Tectona grandis</em> L. f. (Verbenaceae)</td>
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Chapter 3

Infestation Patterns of Hypsipyla robusta Moore in a Red Cedar Plantation

3.1 Introduction

Hypsipyla spp. are among the most economically important insect pests in tropical forestry (Newton et al., 1993). Attack by the shoot borers is the overriding factor restricting the establishment and cultivation of many tropical members of the Meliaceae, including the Australian Red Cedar (Toona australis (F. Muell.) Harms), Spanish Cedar (Cedrela odorata L.), African mahogany (Khaya spp.), and mahogany (Swietenia spp.) in countries of their origin (Newton et al., 1993). In Australia, the severe and almost invariable attack by Hypsipyla robusta Moore, locally known as the Red Cedar Tip Moth, has even prompted the decision to discourage any further plantation efforts on Red Cedar (pamphlet, NSW Forestry Service), despite its well recognised timber value (Boland et al., 1984; Floyd, 1989).

Damage by H. robusta is mainly caused by the tunnelling activity of the larvae in the growing shoots (Entwistle, 1967). Although it does not usually result in the death of the whole tree, attack by the shoot borer retards the height growth and destroys the tree form, rendering it of little commercial value (Coventry, 1899; Beeson, 1919; Anon., 1958; Entwistle, 1967). At times of high infestation levels, the attack may completely nullify the season’s growth; not only are main leaders of the current year killed, but also laterals which have made progress on the woody stems of the previous years (Beeson 1919). Height growth of host trees depends on the occasional escape of some shoots before lignification. Tunnelling usually commences from the axil of a leaf (Coventry, 1899; Anon., 1958). Length of the larval tunnels is often over 30 cm (Coventry, 1899). The tunnel may go as far downward as 60 cm into the stem (Beeson, 1919; Anon., 1958) and
sometimes continues down into the stems of the previous year's growth, although the latter do not usually die as a result of the hollowing (Beeson, 1919). As many as 20-40 wounds may occur on a single stem. The larva usually remains in one shoot and pupates inside the tunnel (Beeson, 1919). Construction of secondary tunnels may also occur (Anon., 1958), especially if the original shoot no longer meets the developmental requirement (Beeson, 1919), resulting in many empty tunnels found in the field (Roberts, 1968). Feeding on the bark of tree trunks and branches has also been reported (Roberts, 1968).

Peak infestation by the insect coincides with the rainy season (Brunck and Fabre, 1974; Morgan and Suratmo, 1976; Wagner et al., 1991). The correlation was attributed to the triggering of leaf flush and sprouting of new shoots. The insect population is usually kept at relatively low levels during the dry season. Attack-induced sprouting is suspected to play a major role in maintaining population density of Hypsipyla spp. at a certain level until the beginning of the next rainy season (Yamazaki et al., 1992). Effects of temperature on the temporal patterns of H. robusta have not been studied, but low temperatures probably cause diapause of mature larvae (Beeson, 1919), and hence lower activity levels of the insect during winter months.

Attack levels vary with plot, tree and vertical positions of the shoots or stems. A number of reports has pointed to the effects of mixed-species planting in reducing the attack levels of Hypsipyla spp. (see Newton et al., 1993). The practice was found to be more effective when the non-host trees were planted within the same lines as the host trees (Roberts, 1968). Retention of weeds is also considered effective in reducing infestation (Anon., 1918). In Peru, plantations of Cedrela spp. that were initially heavily attacked by H. grandella recovered after weed control was abandoned (Dourojeanni, 1963). The protective effects of mixed-species planting cannot be entirely attributed to the physical disruption of the host-finding processes by the presence of non-host species. Grijpma (1976) reported that H.
grandella was able to limit its attack on the local Meliaceae species and leave the exotic Meliaceae species intact, although the latter were mixed with the former at an inter-tree distance of only 3 metres. The same study also reported exclusive attack on host Meliaceae species when the host and non-Meliaceae species were mixed at a ratio of only 1 to 11. Newton et al. (1993) attributed the success of mixed-species planting to the combined effects of three factors: (1) shading, (2) low density of susceptible species, and (3) growth rate, with all factors contributing in a synergistic way due to the often interactive nature of their effects. Attack is usually more severe on trees growing in full sunlight than on trees growing under the shade of other tree species (Froggatt, 1923; Mitchell, 1972; Wagner et al., 1991). Campbell (1964) indicated that 50% shade is necessary to reduce infestation. Lateral rather than overhead shade is suggested as protective as it reduces the production and growth of branches and promotes the growth of the leader and thereby reduces the number of sites available for attack (Newton et al., 1993). Low-density planting of susceptible species is considered as effective in preventing the build up of the pest populations, as such a system mimics that in the natural forest (see Newton et al., 1993). The effect of growth rate has been subjected to conflicting viewpoints. Some advocated that slower growing trees were more susceptible to attack (Lamb, 1968), while others reported a positive correlation between growth rates and attack levels (Whitmore, 1978). The latter viewpoint is supported by the finding that the insects select for oviposition sites the newly produced shoots (Gara et al., 1973), which are more abundant on faster growing trees. In addition, higher population densities of natural enemies may also have contributed to the lower attack levels in mixed plantations (Anon., 1958).

Distribution of attack levels within a plot does not seem to be uniform or random. Froggatt (1923, 1927) noted that susceptible trees were often less than 5-6m tall. Roberts (1966) even gave the maximum tree heights of three host species below which attack was observed. The lower
threshold in tree height of susceptible trees appears to be very low. In India, vigorous seedlings of host trees are attacked when as young as 3 months and less than 50 cm high (Beeson, 1941). Morgan and Suratmo (1976) reported that trees less than 13 years old were attacked more often, with the heaviest attack on trees 3-6 years old and 2-8 m tall. Trees over 15 m tall and aged 13 years or more were only slightly attacked or not attacked at all. They fitted linear regressions of attack intensity against tree height and age. This height-related difference in attack levels was suspected to be caused by the flight behaviour of the moths, as most did not exceed an altitude of 10 m (Morgan & Suratmo, 1976). Lower incidence of shoot attack in mature trees was attributed to their seasonally more restricted production and elongation of terminal shoots (see Entwistle, 1967). In addition, the terminal shoots of fruiting trees were suspected to contain chemicals that cause antibiosis in the larvae (Grijpma, 1976). It is also possible that large trees were similarly attacked as small trees but, due to their numerous branches and shoots, the percentages of shoots attacked would be lower and hence they appear to be less heavily attacked. Attack on mature trees by H. robusta has been reported (Coventry, 1899; Anon., 1958). It is even claimed that any infestation that occurs is traceable to mature trees in the neighbourhood (Beeson, 1918). A more consistent hypothesis regarding the effects of tree size is that large trees suffer less from the attack than small trees in terms of height growth and deterioration of tree form (Coventry, 1899; Grijpma, 1976; Newton et al., 1993), as attack in large trees tends to be confined to branches (Coventry, 1899) and therefore poses no threat to the already established straight bole (Grijpma, 1976). Based on the more tolerant response of large trees, Holdridge (1943) suggested planting host trees in full sunlight in favourable sites so that they could pass quickly through the susceptible stage with the chance of establishing a reasonable length of straight bole before attack ensues. In addition to tree size, the inter-tree variations in attack levels may also be influenced by tree vigour (Entwistle, 1967) and attack history of
individual trees. The latter possibility is backed by the suggestion that egg-laying females are attracted by volatiles given out by the activities of feeding larvae (Roberts, 1968).

The within-tree distribution of attack of *H. robusta* has not been separately studied. Coventry (1899) noted that attacked shoots were often over 30 cm in length. Study on *H. grandella* indicates that upper and terminal shoots are more frequently attacked than lateral shoots or middle stems (Yamazaki *et al.*, 1992). The pattern appeared to apply only to the tunnelling activity of mature larvae, as 1st-2nd instar larvae were mostly found in the lateral shoots. Shoot diameter and slenderness may also affect the susceptibility of shoots to attack, as small and slender shoots are less likely to harbour late instar larvae (Grijpma, 1976).

Despite the enormous effort devoted to the study of *Hypsipyla* spp. around the world, current knowledge on the temporal and spatial patterns of the infestation and its impact on the growth of host trees is largely qualitative rather than quantitative. Much of the published information in this field is in the form of anecdotal reports or summaries based on such reports, coupled with the authors' speculations or untested hypotheses. Frequently these reports are subjected to conflicting viewpoints. Concrete data on *H. robusta* are especially lacking. The few reports available are mostly concerned with the Indian and African populations of the insect. Quantitative data on the damage characteristics of *H. robusta* on Australian Red Cedar are therefore urgently needed.

Studies of the temporal and spatial patterns of infestation are important in pest management. Results obtained by such studies can be used directly in timing controls and setting up efficient monitoring programmes. Indirectly, they provide us with valuable information as to the major factors governing the population dynamics of the pest insects, which, in turn, can be used to design better management strategies.
3.2 Methods

3.2.1 Description of the field site

The field site is in a private farm ca. 5 km north of Macksville, a coastal town in mid-north New South Wales (30°42'S, 152°55'E). The region has an annual rainfall of 1492 mm and a minimum monthly temperature of ca. 12°C. Red Cedar is indigenous to this part of New South Wales and mature trees can still be found in scattered locations. These mature trees probably host active H. robusta populations, as an examination of one of the trees revealed a substantial number of live pupae of this insect.

The farm has a total area of 22 ha. The land is a mosaic of pasture, patches of remnant forest, and planted trees. Trees in the remnant forest mainly consist of Sydney Blue Gum (Eucalyptus saligna), Flooded Gum (E. grandis), Tallow Wood (E. microcorys), and some rainforest tree species. Most of the eucalypt trees are over 15 m tall. One of the remnant forest patches, situated along the eastern boundary of the farm, is relatively large, occupying almost one quarter of the land. In the middle of the farm is a 3 ha block planted, until recently, with peach and nectarine.

Since January 1987, Red Cedar trees have been planted at various locations on the farm (Figure 3.1). Except for a few scattered trees, all planting was concentrated in four plots: Open-1, Open-2, Forest-1 and Forest-2. The four plots and the three isolated groups are separated by at least 50 m. Plot Open-1 was established during January 1987 - December 1989 in an open area on the eastern slope. The eastern edge of the plot is ca. 10 m away from the large forest patch. The plot consists of a total of 746 trees, 495 of which are Red Cedar. The rest are Silky Oak (Grevillia robusta Cunn., n=209), White Beech (Gmelina leichhardtii F. Muell., n=14) and Teak (Flindersia australis R. Br., n=28). All trees were planted at a 3 x 3 m spacing. The layout of the plot is shown in Figure 3.2. According to species composition, the plot can be divided into three blocks: block-1 (columns 1-6), pure Red Cedar, block-2 (columns 7-16), a mixture of Red Cedar and other
species, and block-3 (columns 17-26), predominantly Red Cedar with a few Silky Oaks in the north-east corner. Due to their different planting dates, the size of the trees generally decrease from block-1 to block-3. Plot Forest-1 was established during December 1989 - January 1991 in partially-cleared areas inside the large forest patch. Altogether 170 Red Cedar trees were planted. Plot Open-2 and Forest-2 were established after the initiation of sampling in this site. Plots Open-2, consisting of 59 Red Cedar trees, were planted in July 1992 in an open area ca. 50 m from the north west corner of the large forest patch. Surrounding the plot were some young Cedrela odorata and C. fissilis. Plot Forest-2 was established during July 1992 - August 1993 inside another forest patch in the south-western corner of the farm. A total of 85 Red Cedar trees were planted in this plot. The scattered trees were planted during January - March 1987 in three isolated groups: Iso-1 (n=8), Iso-2 (n=5) and Iso-3 (n=3), with an inter-group distance of ≥100 m.

3.2.2 Sampling Procedures

From April 1992 to November 1994, 13 samples were drawn at one to four month intervals from various plots in the field site, referred to as S1-S13 according to the sequence of the sampling. Plot Open-1 was sampled on each of the 13 sampling occasions, as this was the plot where most Red Cedar trees were planted. The other plots were sampled less frequently.

Fifty-two Red Cedar trees from plot Open-1 were selected as sample trees. Care was taken to ensure that the selected trees were representative of different tree height and locations. On each sampling occasion, all trees in the fixed sample were measured for size and checked for damage by H. robusta. The size indices measured were height (Ht), DBH and the number of shoots with fully-expanded leaves (NSL) (ignoring tiny, newly sprouted shoots) were recorded for each sample tree. Damage data were obtained by randomly checking 10 shoots of current growth (non-lignified) from each sample tree. When less than 10 shoots were available all shoots were
checked. Inaccessible shoots were cut from the bases by a pole-pruner. A shoot was considered as damaged by *H. robusta* if it showed masses of larval frass and fragments of the shoot tissue as a result of larval feeding. These shoots were then dissected to check for the presence of larvae. The developmental stages of the larvae were roughly determined as 1st-6th instars according to previous rearing experiences with the insect (see Chapter 4). Any larval tunnels found inside the main stems of the sampled shoots were measured for length and inner diameters at both ends. Shoot-specific data were recorded for all sampled shoots on 5 sampling occasions. The data recorded were shoot height (SHt), measured from the ground to the terminal buds, shoot length (SL), and shoot basal diameters (SBD).

The same sampling procedure was applied to other plots in the field site. A minimum of 60 trees in Forest-1 and all trees in Iso-1 - Iso-3 were sampled on each sampling occasion. Trees sampled in Forest-1 were not fixed with regard to sampling occasions, but care was taken to ensure that they were representative of tree height and locations. Trees in Open-2 and Forest-2 were not sampled until July 21, 1993, due to their late planting dates. These trees were all sampled.

Additional samplings were made in the field site to supplement information on the damage characteristics of *H. robusta* and the impact of damage on tree growth. These included tunnel dimensions and associated larval instars, the branch height of trees (measured from the ground to the bases of the lowest branches), and the diameters of damaged shoots at the positions where the live and dead sections of the shoots separated (boundary diameters). The first data set was randomly taken from Open-1 among Red Cedar trees not included in the fixed sample. Data on the branch height were taken from all Red Cedar trees sampled with the standard procedure. Measurements on the boundary diameters of damaged shoots were taken on the live section sides of the boundaries as the dead sections often showed some degree of shrinkage. Shoots containing live larvae were
excluded from the data as damage in these shoots was probably still in progress. The height and DBH of all Red Cedar trees in Open-1 were recorded on the first and last sampling occasions.

3.2.3 Weather Data

Daily rainfall data were obtained from two neighbouring farms, about 300 and 500 m away from the field site, respectively. The average of the two data sets was used to represent rainfall in the field site. Daily temperatures were estimated as the average of the data from the weather station in Coffs Harbour and that in Kempsey (Australian Weather Bureau). Both weather stations, one in the north and one in the south, are ca. 55 km away from the field site and similarly located in the coastal areas of New South Wales, with little elevation differences.

3.2.4 Data Analysis

Data from Open-1 were used to analyse the temporal and spatial patterns of H. robusta infestation, as well as the damage characteristics. Infestation levels from all plots and isolated groups of trees were then compared to study the habitat differences. Effects of damage on tree growth were analysed with data from Open-1 and Forest-1.

Infestation patterns

1. Temporal patterns

The temporal patterns of tip moth attack levels were analysed in relation to rainfall and minimum daily temperature. Two attack level indices were used: proportions of attacked trees and the mean percentages of attacked shoots per tree. To enable comparisons of temporal patterns, accumulated rainfall 30 days prior to each of the 13 sampling dates was extracted from the daily rainfall data. The reason for such a treatment of data is that the observed attack level in a particular sampling date was the
accumulated expression of continuous *H. robusta* activities in a period before the sample was drawn and any possible influence of rainfall on the attack levels should likewise be exerted through a period of time. Here the length of the influence period of rainfall was arbitrarily taken as 30 days, which was shorter than the length of time between any successive sampling dates and probably long enough to take into account the indirect nature of the influence. The temperature index used in the comparisons was the average daily minimum temperature during the 15 days prior to each sampling dates. The purpose was to see whether *H. robusta* activities were constrained by low temperature during the winter months in the field site. Considering the more direct nature of the influence of temperature, the length of the influence period was taken as half of that of rainfall.

II. Spatial patterns of attack

i. Inter-tree patterns

The attack level of a sample tree can be split into two components: the attack status (attacked or non-attacked) and the attack intensity (the percentage of shoots attacked once the tree is attacked). Effects of five tree-specific factors on the inter-tree variations of attack status and attack intensities were investigated. The tree-specific factors considered were: tree height (Ht), DBH, tree form (TF, =Ht/DBH), the number of shoots with fully-expanded leaves (NSL), and the block from which the tree was located (Blk); the first four were numerical factors and the last a categorical factor. For trees shorter than 1.3 m, the DBH values were extrapolated from the diameters of tree trunks at the height of 0.5 m by assuming a constant ratio of diameter over height in the 0.5 -1.3 m sections of the tree trunks.

Effects of individual tree-specific factors on the attack status of sample trees were first investigated, with the exception of Blk, by comparing the average values of the respective factors between attacked and non-attacked trees. The effect of Blk was analysed directly by comparing the proportions of
attacked trees in the three location blocks. Considering the limited number of trees in individual samples, direct comparisons in the proportions of attacked trees among trees at various ranges of Ht, DBH and TF were performed only on the pooled data. Such comparisons were not attempted on NSL due to its cyclical seasonal variations. The relative importance of these factors was assessed by logistic regressions and associated analysis of deviance (Everitt, 1994), with the attack status of individual trees (1 for attacked, 0 for non-attacked) as the dependent variable and the tree-specific factors as the independent variables. Analyses on the attack status were performed for those samples with the proportion of attacked trees in the range of 20-80%. Samples outside this range were excluded as little information was available concerning the suitability of sample trees when the attack level was too high or too low.

Similar analyses were applied to the effects of tree-specific factors on the attack intensities. In the case of numerical factors, the effects were analysed by comparing the average values of each factor between relatively heavily attacked and relatively lightly attacked trees. A tree was considered as relatively heavily attacked if its percentage of attacked shoots was higher than the sample average. Otherwise it was considered as relatively lightly attacked. The effect of Blk was analysed by comparing the average percentages of attacked shoots per tree of trees in the three location blocks. To ensure adequate group sizes, separate comparisons were made only for those samples with the number of attacked trees ≥20. The relative importance of individual tree-specific factors was analysed by linear regression models and associated ANOVAs (Neter and Wasserman, 1974). As the dependent variable, the percentages of attacked shoots per tree were transformed with the arcsine-squared root transformation to stabilise the variances (Steel and Torrie, 1960). Due to the limited number of attacked trees in individual samples, the regression analysis was applied only to samples with the number of attacked trees ≥20.
ii. Within-tree patterns

Within-tree patterns of attack were investigated with respect to shoot height (SHt), relative shoot height (RHt, = shoot height / tree height), shoot length (SL), shoot basal diameter (SBD), and shoot slenderness (SS, = shoot height / shoot basal diameter). Effects of shoot-specific factors on the attack status of shoots were analysed by comparing the proportions of attacked shoots at various ranges of each factor and by logistic regressions. The analyses were performed on the pooled data of all shoots from attacked trees of all samples with shoot-specific data collected. Shoots from non-attacked trees were excluded as they might not have been subjected to *H. robusta* selections.

iii. Habitat differences

Proportions of attacked trees planted in the open (Open-1 and Open-2) and inside the forest (Forest-1 and Forest-2) at the same sampling occasions were compared to assess the effects of host habitat on attack levels. Comparisons of attack levels were also made between trees in Open-1 and trees in the three isolated groups (Iso-1, Iso-2 and Iso-3).

*Impact of damage*

I. Damage characteristics

Two damage characteristics were investigated with data from Open-1: (a) the number of shoots attacked per larva, (b) damage site, and (c) tunnel size. The number of attacked shoots per larva was estimated from sampled shoots of trees on which larvae were found. Damage site was analysed with respect to the types of host tissues (buds, leaf petioles, shoots, lignified stems or tree trunks) and the diameter of the shoot or stem at the position where the tunnel started. Tunnel volumes were calculated from the median diameters ((upper diameter + lower diameter)/2) of the tunnels and tunnel lengths, by assuming cylindrical shapes of the tunnels. Since tunnel
diameters were only measured for those constructed by mature larvae, the
diameters of tunnels constructed by younger larvae were indirectly
estimated by assuming a constant ratio of tunnel diameter to larval head
capsule width. Head capsule widths of individual larval instars were
extracted from a separate study (Chapter 4).

II. Survival of damaged shoots

The boundary diameter of a shoot marks the lowest position in the
shoot below which the shoot has survived the boring activities of larvae of
_H. robusta_ attack. Since the diameter of a shoot generally increases from tip
to base, the boundary diameter also indicates the minimum diameter in the
live section of the shoot. In other words, the shoot is dead from tip down to
any positions with diameters less than that boundary diameter. Hence, the
probability of a damaged shoot dying from tip up to a given diameter can be
estimated by the percentage of damaged shoots with boundary diameters
greater than the given diameter. Estimation was done for each boundary
diameter in the data set. The estimated probabilities were modelled by the
equation:

\[ P = \frac{c}{a + \exp(b \cdot BD)} \quad BD \geq 0 \]  

(3.1)

where \( P \) is the probability of a shoot dying from tip to a position with
diameter \( BD \), \( a \), \( b \) and \( c \) are model parameters, with \( c = a + 1 \). \( P \) is a monotonic
decrease function of \( BD \), obtaining its maximum value of 1 when \( BD \) is 0
and approaches 0 when \( BD \) approaches \( \infty \).

III. Effects of damage on tree growth

Effects of damage on the growth of tree height and the changes of tree
form during the period between the first and the last samples were
investigated. The height increments of individual trees in Open-1 during
that period were studied in relation to their attack levels (percentages of attacked shoots) in individual samples and the number of times each tree was attacked across the samples, as well as their initial height, DBH and tree-form values as shown in the first sample (S1). ANOVA based on linear models was used to assess the effects of individual factors and multiple factors. The same set of factors was considered in logistic regressions to assess their effects on the directions of changes in tree-form values (increase or decrease) of sample trees in Open-1 during the same period. Damage on the growth of trees in Forest-1 can not be directly assessed in the same way as in Open-1 since the trees sampled on each sampling occasion were randomly taken from the plot and hence the damage history of individual trees was not known. However, as has been pointed out earlier, these Forest-located trees had experienced relatively less attack compared to those planted in the open and in isolated groups. It is of interest to see whether the light attack levels were reflected in better tree growth. To this end, the overall height increment of trees in Forest-1 and Open-1 between the first and the last sample was compared. To reduce the effect of initial tree height, the comparison was restricted to trees with their initial height in the range of 200-300 cm (inclusive at right). Comparison was also made of the tree-form values and the percentages of unbranched trees in the last sample between the two plots.

Statistical tests

All statistical analyses were performed in S-Plus (Everitt, 1994) in the Unix environment. The S-Plus functions and associated test statistics used in testing the significance of attack-related differences, correlations, goodness of fit, and effects of factors in regression models are summarised in Table 3.1.
### 3.3 Results

#### 3.3.1 Temporal patterns

Summary statistics of infestation levels at successive sampling dates are given in Table 3.2. The proportions of attacked trees varied between 0 and 100%. Except for the two samples (S2 and S3) showing no attack, the average percentages of attacked shoots per tree ranged from 2.6 to 74.3%. The relationship between the proportions of attacked trees and the average percentages of attacked shoots per tree can be described by an upper-plateau exponential equation (Figure 3.4).

Variations in the average percentages of attacked shoots per tree among the 13 samples were significantly correlated to the rainfall index ($P < 0.05$). The two variables were closely matched over most of the sampling period, with apparent mismatch shown only in S9 (Figure 3.3). For some successive sample pairs, matching was shown not only in direction but also in magnitude. Temporal variations in proportions of attacked trees were only slightly correlated with the rainfall index ($P < 0.1$). The temperature index was significantly correlated to both of the attack indices ($P < 0.05$), with a better matching shown with the proportions of attacked trees (Figure 3.3). Although peak periods of the temperature index did not always correspond to peak attack proportions, the 3 valleys in their temporal trends all coincided. Of special interest were the two samples (S2, S12) recording no *H. robusta* attack. Both samples corresponded to the times when the average minimum daily temperature was below 6.5°C.

#### 3.3.2 Spatial patterns

**Inter-tree patterns**

1. **Attack status**

Six samples had the proportions of attacked trees in the range of 20-80%, namely S3, S5, S6, S8, S11 and S13 (Table 3.2). All showed higher average values in Ht, DBH and NSL, and lower values in TF among
attacked trees (Figure 3.5). However, significant differences (P < 0.05) in the average values of all the four factors between attacked and non-attacked trees were detected only in S3. The other significant differences detected were Ht in S6 and TF in S5. No significant differences in the proportions of attacked trees among trees in the three location blocks were detected (P > 0.1). Analyses on the pooled data showed that the attack proportions increased with Ht and DBH but decreased with TF (Figure 3.6). The overall changes were significant with respect to each of the three factors (P < 0.05). However, after the exclusion of trees with Ht ≤ 150 cm, or DBH ≤ 0.5 cm, or TF > 100, the attack proportions of trees in the remaining ranges of the respective factors (Figure 3.6) no longer differed significantly (P > 0.1).

Factors included in the final logistic models through stepwise regressions for each of the six samples are shown in Table 3.3. At the significance level of P < 0.05, non-null models were obtained only for S3 and S5, with TF and NSL as the sole independent variables included, respectively. At the significance level of P < 0.1, two more samples yielded non-null models, S6 and S13 (Table 3.3). Again only one variable was selected for each model, Ht in S6 and DBH in S13. Models in S3 and S5 remain the same despite the relaxation of the entry criterion. It needs to be pointed out that in S3, Ht, DBH and NSL also showed significant effects (P < 0.05) on the independent variable when introduced alone, but the addition of any of them did not result in any significant improvements of the model with TF already in. The same was true with DBH, the inclusion of which even made TF redundant. However, the selection of Ht or NSL as the first variables still left room for the introduction of either TF or DBH (P < 0.05). None of the tree-specific factors showed significant effects on the logarithmic odds of attack in S8 and S11 (P > 0.1). The deviance explained by the significant models in individual samples was generally low (< 25%) (Table 3.3).
II. Intensities of attack

Eight samples had the numbers of attacked trees ≥20: S1, S3-S6, S8, S11 and S13. In most samples, the differences in the average values of Ht, DBH, TF and NSL between relatively heavily attacked and relatively lightly attacked trees were not significant (P > 0.1) (Table 3.4). The differences were slightly significant for NSL in S4 and for Ht and DBH in S5 (P < 0.1), with lower average values of NSL and higher average values of Ht and DBH among attacked trees. However, the attack intensities appeared to be influenced by tree locations. Trees mixed with non-host trees (block-2) generally had lower attack intensities than trees planted in pure host tree blocks (block-1 and block-3) (Figure 3.7). Significant differences of comparisons involving block-2 trees were found in 4 samples (P < 0.1 in S1, P < 0.05 in S2 and S13, P < 0.01 in S4), all revealing less intensive attack among block-2 trees, at least in comparison with trees in one of the other two location blocks (P < 0.05).

Linear dependence of the attack intensities on the tree-specific factors was detected by ANOVA in 4 samples, S1, S3, S4 and S6, at the significance level of P < 0.05 (Table 3.5). NSL showed significant effect in all the 4 samples. It was the only significant variable in S3 and S6 and the most significant variable in S4. The identities of other significant variables were not consistent. In S1, all the numeric variables were significant, the most significant of which was DBH, followed by Ht, NSL and TF. In addition to NSL, Blk and TF also contributed significantly to the attack intensities in S4 when introduced alone into the model. When the entrance criterion was relaxed to P < 0.1, two more samples resulted in non-null models, S5 and S13, both including Blk as their significant variables. After the selection of the most significant variables, no other variables could be entered into the models in 3 of the 6 samples. Blk was the only additional variable that could be entered in the 3 remaining samples. When significant, the tree size variables, Ht, DBH and NSL, contributed negatively and tree form (TF)
positively to the attack intensities, as indicated by the signs of their coefficients in the models (Table 3.5).

Within-tree patterns

The pooled data contained a total of 1231 shoots from attacked trees, of which 314 shoots were attacked. There appeared to be a gradual increase in the proportions of attacked shoots as the values of all the shoot-specific factors increased (Figure 3.8). However, the patterns of the increase and their significance (P< 0.05) varied with factors. With regard to shoot vertical positions, the attack proportions were more influenced by relative shoot height than by absolute shoot height. Shoots with their terminal buds positioned at over 80% of tree height suffered significantly more attacks than shoots positioned at ≤70% of tree height, with the top shoots (RHt >0.90) attacked over twice as severely as most of the lower-positioned shoots. Significant effects of absolute shoot height were shown only when the comparisons involved shoots of ≤100 cm high. An almost linear relationship was apparent between the attack proportions and shoot length, with significant differences detected between all non-adjacent columns. The effect of shoot basal diameter was less gradual. Shoots with SBD ≤ 0.4 cm were significantly less frequently attacked than shoots in any of the other SBD ranges. Significant increases in the attack proportions over shoot slenderness was restricted in the range of SS ≤30.

Results of logistic regressions showed that all shoot-specific factors had significant effects on the logarithmic odds of attack (P< 0.05) (Table 3.6). The most significant factor was RHt and the least significant SHt. The final model contained 3 factors: RHt, SBD and SS, and was highly significant (P< 0.0001) (Table 3.6). As with the analyses of the attack status of trees, the model accounted for only a small proportion of the null deviance in the logarithmic odds (<5.81%).
Habitat differences

Trees in Forest-1 were sampled for *H. robusta* damage on 10 of the 13 sampling occasions. The proportions of attacked trees stayed below 21%, with no attack recorded in three samples (Figure 3.9). In five of the seven samples where attack was observed in both plots, the attack proportions in Forest-1 were only about one-tenth to one-third of that in Open-1. Higher attack levels were observed in Forest-1 in the remaining two samples. As with the spatial attack patterns in Open-1, the average height of attacked trees in Forest-1 was consistently greater than that of non-attacked trees, with significant differences detected in most samples (P<0.05) (Table 3.7). The differences appeared to be caused by the lower limit of the height ranges, as the upper limit was very close between attacked and non-attacked trees (Table 3.7). The shortest attacked trees recorded in these samples were 60 cm tall. Analyses of the pooled data revealed a steady increase in the attack proportions as tree height increased (Figure 3.10). Trees over 400 cm tall suffered over 4 times as much attack as trees below or equal to 100 cm tall. Despite the possible effect of tree size, the generally lower attack frequencies among forest-located trees as compared with open-located trees were still evident after the removal of trees ≤ 200 cm tall in both plots (Table 3.8). However, as expected, the proportions of attacked trees increased somewhat in Forest-1 after the exclusion of small trees, reaching a maximum of 43.2%. Statistical tests in the attack proportions between the two plots were performed only for 3 samples due to the requirement of the chi-square test (the number of attacked and non-attacked trees in either plot should be at least 5) (Steel and Torrie, 1960). The only significant differences detected at P<0.05 pointed to higher attack proportion in Open-1.

The effects of habitat on attack levels was more sharply shown in the comparison between Forest-2 and Open-2 (Figure 3.9). Most trees in both plots were planted about 3 months after the initiation of sampling in the field site. One year later attack by *H. robusta* was observed in Open-2.
Within 5 months, the proportion of attacked trees rose to about 70% and this high level was basically maintained, with the exception of S12, where no attack was recorded in all plots, during the rest of the sampling period. On the contrary, attack in Forest-2 was recorded only on one sampling occasion and only on a single tree.

Proportions of attacked trees among isolated trees across the samples were comparable to those in Open-1 (Figure 3.11). The two trends generally agreed, with their correlation significantly different from zero (P < 0.05). The temporal correlation in attack proportions still held when data from isolated trees, Open-1 and Forest-1 were considered together (P < 0.05). However, attack within the isolated trees did not seem to be synchronous (P > 0.1). More persistent infestations were seen in Iso-3 than in the other two isolated groups (Figure 3.11). The discrepancy probably arose as a result of the small number of trees in each group.

3.3.3 Impact of damage

Damage characteristics

A striking feature of *H. robusta* damage noticed during the sampling process was the high number of shoots showing damage symptoms but with no larvae. The percentages of trees attacked but with no larvae (live or dead) often exceeded 25% and could be as high as 100% (Table 3.9). Even among those trees on which larvae were found, not every attacked shoot yielded larvae. The average number of attacked shoots per larva found among attacked trees was 2.6 ± 1.6 (0.5–8) (mean ± SD). Mortality during the larval stage appeared to be high (Table 3.9). In seven of the 10 samples where larvae were found, at least one third of the larvae were dead and most of the dead larvae were already in their late instars (5-6th).

Larval tunnels were found at all above ground non-photosynthetic tissues of the host trees, with the current-growth shoots as the most frequent tunnel construction site (Plate 1). Tunnels constructed by young
larvae were mainly found in leaf petioles and terminal buds and those by older larvae in the stems of the shoots. However, there were some exceptions. Some mature larvae bored into lignified stems for pupation and many young larvae were found inside abandoned tunnels. Some larvae did not construct any tunnels and fed directly on the epidermis of the shoots or stems under the concealment of frass or other existing forms of concealment. Different choices of tunnel construction sites by larvae of different instars were partly reflected in the diameters of the host tissues at tunnel entrances (Figure 3.12). Although there was considerable overlapping, the average entrance diameters differed significantly between larvae of 1st-2nd, 3rd-4th and 5th-6th instars (P< 0.0001). Entrance diameters below 0.30 cm were only associated with larvae of the first two instars and those above 1.00 cm were restricted to larvae of the last two instars. Highly significant differences (P< 0.0001) among larvae of the three age groups were also found in tunnel lengths, the average of which increased from 2.2 cm in the 1st-2nd instars to 6.3 cm in the 5th-6th instars. Tunnels constructed by 1st-2nd instar larvae were not longer than 5 cm whereas those by 5th-6th instar larvae could be as long as 22 cm. However, as with the entrance diameters, clear boundaries did not exist in tunnel lengths between larvae of successive age groups. Tunnels constructed by mature larvae were sometimes well within the length range of tunnels produced by young larvae. These short tunnels of mature larvae were probably of secondary entries and made for pupation rather than feeding, as most of them were found inside lignified tissues. A better distinction between tunnels produced by larvae of the three age groups was seen in tunnel volumes. Tunnels constructed by the last two instar larvae had an average volume of 1.41 cm³, with a maximum of 5.77 cm³, while those made by larvae of 1st-2nd and 3rd-4th instars were 0.02 cm³ and 0.27 cm³, respectively. The relative volumes of the tunnels for larvae of the 3 age groups were ca. 1:14:71.
Survival of damaged shoots

The shoot boundary diameters obtained fell in the range of 0.3-1.5 cm, with the majority distributed between 0.4 cm and 0.8 cm (Figure 3.13). The estimated probabilities that shoots damaged by *H. robusta* would die from tip down to given positions decreased in a 'Z'-shaped fashion as the diameters of the positions increased, approaching zero when the diameters were greater than 1.0 cm (Figure 3.14). The relation was well fitted by the modified logistic equation (Equation 3.1) (Figure 3.14), with 99.36% of the variations in the estimated probabilities explained by the model. Both parameters in the model were significant (t-test, P<0.0001).

Damage to tree growth

The height of the sample trees in Open-1 increased on average by 78.36 cm during the 32-month sampling period. The increment for individual trees ranged from -90 cm to 218 cm, with 4 trees recording negative growth. Except for S5, variations in the height increment (H_{incr}) were not significantly influenced by the attack levels of individual trees (percentages of attacked shoots) recorded in individual samples (P> 0.1) (Table 3.10). Slightly significant effects (P< 0.1) were shown in b5. Compared with the attack levels in individual samples, the number of times individual trees were attacked across the samples exhibited much stronger influence on the height increment, but the effect was positive, i.e. larger height increment was associated with more frequently attacked trees (Table 3.10). However, none of the damage indices were significant (P> 0.1) after the effect of initial tree height was introduced into the linear model. In fact, all the three numerical tree-specific factors considered in the initial sample, H_{t1}, DBH_{1} and TF_{1}, were highly significant (P< 0.001) on the tree height increment, with TF_{1} as the most significant factor (Table 3.10). The final model included 2 initial factors, DBH_{1} and Blk at the significance level of P< 0.05, although the latter was not significant (P> 0.1) on its own:
\[ H_{\text{tree}} = A_{\text{BR}} + 22.71 DBH, \]

where 

\[ A_{\text{BR}} = \begin{cases} -20.36, & \text{Blk} = 1 \\ 41.37, & \text{Blk} = 2 \\ 8.17, & \text{Blk} = 3 \end{cases} \]

\[ (R^2 = 0.39, F = 9.92, d.f. = 3, 46, p = 0.0000) \]

The equation shows that trees planted in block-2 achieved the largest height gain and those in block-3 the lowest height gain. Graphic comparisons show higher growth rate of block-2 trees in the first 310 days since the first sampling occasion, thereafter the growth rates of trees in the 3 location blocks appear to be similar (Figure 3.15).

Unlike tree height, changes in the values of tree form in Open-1 during the sampling period appeared to be highly non-directional. While 23 sample trees had their tree-form values increased, 27 sample trees had their tree-form values decreased. Analysis of logistic regressions indicate that the increase or decrease in the tree-form value of individual trees was not significantly influenced by any of the damage indices considered above \( (P > 0.1) \). The only significant factors detected at the level of \( P < 0.05 \) were the initial tree form and DBH (Table 3.11). When considered together, the logarithmic likelihood of increase in tree-form values can be described as the linear effects of two factors, initial tree form \( (TF_1) \) and the percentages of shoots attacked in S5 \( (b_5) \), both showing negative effects:

\[ \log\left(\frac{P}{1-P}\right) = 6.53 - 0.07TF_1 - 4.32b_5 \]

where \( p \) is the probability of increase in tree form. The effect of \( b_5 \) was apparently imbedded with that of initial tree form as it became significant \( (P < 0.05) \) only after the term \( TF_1 \) had been included. The negative effect of initial tree form was evident as trees that had initial tree-form values initial tree form was evident (Table 3.12). Tree form deteriorated among tree with \( TF_1 \geq 80 \) and improved among other trees.
Similar effects were also evident in initial tree height and DBH except that their effects were positive, i.e., bigger trees tended to increase their tree-form values whereas smaller trees tended to decrease their tree-form values (Table 3.12).

Forest-located trees appeared to have been growing at a faster rate than Open-located trees (Figure 3.16). The overall height increment in plot Forest-1 was over twice as much as that in plot Open-1 for trees with their initial height in the same range (200-400 cm). Better growth of Forest-located trees was more convincingly shown in the tree-form values. For trees tall enough to have DBH records (>1.30 m), the average tree-form values in Forest-1 (189.95 ± 87.22) (mean ± SD) was almost 2.5 times as high as that in Open-1 (78.76 ± 16.47) in the last sample. The difference can be partly explained by the percentages of un-branched trees in the two plots. While each sample tree in Open-1 had branched by the last sampling occasion, 47% of the trees in forest still maintained the single-stem form. Even for trees over 300 cm tall, the percentage was still over 15%.

3.4 Discussion
3.4.1 Temporal Patterns

Comparisons of the temporal trends between the attack levels and the weather data indicate that levels of *H. robusta* infestation were influenced by the amount of rainfall and by the daily minimum temperature. The average percentages of attacked shoots per tree of all trees in Open-1 were closely correlated with the amount of rainfall, with high rainfall enhancing the activities of the insect. Temporal synchrony between rainfall and mahogany shoot borers has been noted by a number of authors (Grijpma and Gara, 1970a; Brunck and Fabre, 1974; Morgan and Suratmo, 1976; Wagner et al., 1991). The positive association can be attributed to the indirect role of rainfall in promoting host tree growth, which in turn attracts the attack of the insect. This explanation was backed by the finding that egg-
laying females are attracted to the newly produced shoots for oviposition (Gara et al., 1973). Effects of daily minimum temperature appeared to be more pronounced on the proportions of attacked trees, with low minimum daily temperature reducing the number of trees attacked. When the average daily minimum temperature was below 6.5°C in the previous 15 days, no fresh attack by H. robusta was recorded. Low temperatures of this level probably induced some degree of winter diapause of this insect (Beeson, 1919). Further evidence is seen in the simultaneous recording of no fresh attack in all the 4 plots in the field site at the time when S12 was taken (Figure 3.9). Dissecting of some previously damaged shoots in Open-1 at that time revealed only mature blue larvae and pupae that could have been in diapause.

3.4.2 Spatial patterns

Inter-tree patterns

Levels of H. robusta attack did not seem to be randomly distributed among trees. Comparisons of the tree-specific factors between attacked and non-attacked trees suggest that larger trees (higher values in Ht, DBH and NSL) tended to be attacked more often than smaller trees, while better shaped trees (lower value in TF) tended to be less often attacked than less well-shaped trees. The same patterns were shown by logistic models describing the logarithmic odds of attack of individual trees as linear combinations of the tree-specific factors. However, the degree of the tendency was not consistent with respect to samples, as indicated by the sporadic detection of significance in the parameter comparisons and in the logistic models. Considering that the attack status of sample trees was determined by checking 10 shoots from each tree, the probability of having the attacked shoots included in the 10-shoot sample (i.e. detecting an attacked tree) would be much lower among large trees than that among small trees. As a result, the significance levels of the tree-size factors might
have been under-estimated. Preference toward larger trees is not surprising, as these trees have higher number of shoots and therefore are more attractive to the shoot borer (Gara et al., 1973). The same factor may be responsible for the preference toward less well-shaped trees, as the latter are often characterised by higher number of branches than their better shaped counterparts. Support for the proposed common basis of the observed attack patterns is seen by the mutual exclusions of some of the significant factors in the logistic models shown in the previous section and by the considerable correlations between the tree-specific factors (Table 3.13). The implication for the negative association between tree form and attack rates is obvious. It highlights the need to protect young seedlings from initial damage as the latter would open up the tree crown (decreasing the tree-form values) and to cultivate varieties of the host trees with strong apical growth. The latter need was recognised by Grijpma (1976) in his review of the H. grandella problem. Morgan and Suratmo (1976) reported a negative linear dependence of the proportions of attacked trees on tree height. However, the heaviest attacked trees were 200 - 800 cm tall in their study, which covers almost the entire height range of sample trees in Open-1. Also, the negative dependence of attack rates on tree height is doubtful, as the detection of attacked shoots within the relatively dense crowns of large trees can be extremely difficult when the percentages of attacked shoots are low.

The fact that significant differences in the attack proportions among trees at different ranges of the tree-specific factors disappeared after the removal of the lowest ranges in Ht and DBH and the highest range in TF suggests that only very small (Ht ≤150 cm) and very slender (TF >100) trees tend to be less often attacked by H. robusta. Once grown to a certain size and stoutness, Red Cedar trees are likely to be attacked with similar risk. The large proportions of similarly attacked trees may have contributed to the high percentages of unexplained residual deviance in the logistic models. The overall results suggest that the selection of host trees for attack by H.
robusta in Open-1 was largely a random process with the likelihood of attack slightly modified by the tree-specific factors. The hypothesis was supported by the finding that the distribution in the number of times individual trees were attacked across the samples was well fitted by the binomial distribution (Preject > 0.1) (Figure 3.17). Partial mixing of host and non-host trees in a plot as shown in Open-1 did not seem to have any effects on the attack status of host trees.

The intensities of attack among attacked trees, as expressed by the percentages of shoots attacked per tree, appeared to be determined primarily by the number of shoots with fully expanded leaves (NSL) and the location of blocks to which the sample trees belonged (Blk). The fewer the NSL the higher the percentages of shoots attacked. An study on *H. grandella* showed that females laid only a few eggs on each tree (Grijpma, 1974). If the same attribute is true with *H. robusta*, then the observed negative density-dependency of the attack intensities can be rightly justified, as the number of attacked shoots per tree would be limited irrespective of the total number of shoots available. The occasional significant effects of Ht, DBH and TF on the attack intensities might have been due to their correlations with NSL (Table 3.13). However, the more frequent detection of the significant effects of Blk was unexpected. It seemed to have been brought about by the lower attack intensities among trees in block-2 (Figure 3.15), where host and non-host trees were mixed. The reason is unclear. It was unlikely to be attributable to differences in tree sizes, as block-2 trees were, on average, bigger than block-3 trees but smaller than block-1 trees. A possible explanation is that the proximity to non-host trees may have somehow decreased the number of eggs laid on the host trees. In some lepidoptera species, volatiles from non-host plants were found to deter egg-laying females (see Renwick and Chew, 1994). As with the attack status of trees, the tree-specific factors showed significant effects on the attack intensities in some samples but not in
others. The discrepancy did not seem to be the result of different attack levels of the samples.

**Within-tree patterns**

For attacked trees the risk at which a particular shoot would be attacked seems to vary with its vertical position, size and slenderness. The most significant factor appears to be the relative shoot height, as indicated by the results of logistic regressions of the pooled data. Shoots positioned at the upper 20% of tree height were more frequently attacked than shoots positioned lower, with those at the upper 10% most severely attacked. The result suggests that once a host tree is attacked the terminal shoots would be among the first to be bored by the larvae, while the lateral shoots may sometimes escape attack. The same vertical pattern has been reported elsewhere both for *H. robusta* (Morgan and Suratmo, 1976) and for *H. grandella* (Yamazaki et al., 1992). The egg-laying behaviour of the females and the within-tree movement of larvae might have contributed to this pattern. It has been reported that females of *H. robusta* tend to avoid laying eggs in areas with excessive shade (Campbell, 1964). As lateral shoots are often subjected to higher degrees of shading, particularly overhead shading, than terminal shoots, they would thus be less likely to attract eggs. With those eggs that are laid on the lateral shoots, the larvae may still move away and choose to construct their tunnels in the often more vigorously-growing and succulent terminal shoots. Frequent within-tree movement by larvae of *H. robusta* has been suggested in a number of reports (Beeson, 1919; Roberts, 1968; also see Newton et al., 1993). In comparison with the effect of relative shoot height, the impact of absolute shoot height on the likelihood of attack of shoots was less strong. Only shoots ≤100 cm high appeared to be less often attacked. Even this may not have been the direct effect of absolute shoot height itself, as a considerable proportion of these low-grown shoots might have been the lateral shoots of trees above 100 cm tall. This possibility was
supported by the results of logistic regressions, which showed that SHt was no longer significant after RHt had been introduced into the models (Table 3.6). The direct role of shoot length and shoot basal diameter in influencing the shoot selection behaviour of the insect is also questionable. Shoots with longer length and bigger basal diameter were exposed to attack for a longer duration than their shorter and smaller counterparts. As a result, they were more likely to be found as attacked shoots at the time of the sampling. The almost linear relationship between shoot length and the attack proportions (Figure 3.8) provides good evidence for the exposure-time effect. Admittedly, behavioural aspects are probably involved in the discrimination against the selection of very small shoots by late-instar larvae as the size of these shoots may not be adequate for the larval tunnelling and development. The observed preference toward more slender shoots was probably because these shoots were still in the process of fast elongation and were thus more vigorous. When considered together, the attack status of individual shoots appears to be influenced by the simultaneous effects of three shoot-specific factors: relative shoot height, shoot basal diameter and shoot slenderness. However, the degree to which the final attack status was determined by these factors was extremely low (<6%), suggesting the largely random nature of the shoot selection process by H. robusta.

III. Habitat differences

In agreement with previous reports (Frogatt, 1923; Mitchell, 1972; Wagner et al., 1991), host trees planted inside forest appeared to have been afforded some degree of protection against the shoot borer attack. Although attack did occur there, forest-located trees were generally less frequently attacked than open-located trees. The proportions of attacked trees of the forest trees never exceeded 21% throughout the sampling period, while that of the trees in the open frequently rose to over 40% and could be as high as
The difference was still evident after the removal of trees \( \leq 200 \text{ cm} \) high in both plots, indicating that the relative immunity exhibited by Forest-located trees was unlikely to be caused solely by the more widespread distribution of small trees (Ht< 200 cm) in Forest-1 than that in Open-1. Nevertheless the frequency of attack did increase with tree height in both plots and trees \( \leq 200 \text{ cm} \) tall suffered only about half as much attack as their taller counterparts. This probably explained the virtual non-existence of attack in the other forest plot (Forest-2), since all but one tree in that plot stayed below 200 cm throughout the sampling period and 53% were still below 150 cm in height on the last sampling occasion. Apart from the possible effects of shade (Campbell, 1964), the presence of non-host trees on host selection behaviour of the moths (Renwick and Chew, 1994), and tree size, the habitat difference appeared to be attributable to tree vigour. Forest-located trees showed much slower growth rates than openly-located trees, especially during the early stage of their establishment, as was convincingly shown by the comparison of trees between Forest-2 and Open-2. Most of the trees in the two plots were planted around the same time. Yet, in contrast to the slow growth rate in Forest-2, the growth rate of Open-2 trees was surprisingly high, with some trees reaching a height of over 300 cm within one year of planting and in another 3 months some had grown to over 400 cm tall. Corresponding to the strong vigour of trees shown in that plot was the unusually high attack levels, even during times when the attack level was relatively low in Open-1.

Trees planted in small groups in isolated locations did not seem to reduce the tip moth infestation. They were discovered and attacked to a similar extent as trees planted in the open. Although there were considerable variations in the attack levels within isolated trees, examination of the data showed each tree was attacked at some stage during the sampling period. While each individual attack within an isolated group did not necessarily originate from dispersing moths from outside, the
observed attack in every isolated group did point to the strong host-finding capability of the insect. Once discovered, trees in an isolated group may be subjected to heavy attack due to the presence of a local population of the insect and the relatively small number of trees available for attack in that location.

Infestation by *H. robusta* at various locations over the field site were, overall, not synchronous. Attack levels in different plots appeared to have been fluctuating independently of each other, as was shown in the lack of correlations in the proportions of attacked trees between Forest-1 and Open-1 and among isolated groups of trees. The observed correlations in the attack proportions between Open-1 and isolated trees as a whole probably reflected more of their habitat similarity (both were relatively exposed in comparison to forest habitat) rather than synchrony between local *H. robusta* populations. The localised infestation pattern of *Hypsipyla* spp. has been noted by Newton *et al.* (1993). According to their review, the females tend to restrict themselves in areas where host trees have already been discovered and few would venture out to search for new resources. This behaviour of the moth probably evolved as a trait to combat the scattered occurrence of their host trees in nature.

3.4.3 Impact of Damage

A *H. robusta* larva may inflict damage on more than one shoot during its development, as revealed by the low percentages of attacked shoots with larvae (including dead larvae) in the sample data. The missing larvae in those damaged shoots cannot be entirely attributed to predation, the level of which is usually low (Roberts, 1968; Singh and Misra, 1988). A more likely explanation is that the larvae sometimes abandoned their established feeding spots and wandered off in search of new feeding spots, as was noted by Beeson (1919) and Roberts (1968). Too vigorous a sap exudation by the damaged tissue has been cited as the probable reason for the
abandonment (Beeson, 1919). In addition, mature larvae often leave the original tunnels and bore in somewhere else for pupation (Beeson, 1919). Dissection of the sampled shoots on some sampling occasions revealed high proportions of dead larvae. The actual mortality rate during the larval stage could have been even higher, as most of the dead larvae found were already in their late instars. The dead larvae were frequently found trapped in the sap. The production of resins or sap as a defense mechanism against larvae of *Hypsipyla* spp. has been suggested by a number of authors, but little information is available concerning its effectiveness (Newton *et al.*, 1993). My observation on the feeding process of larvae showed that lumps of solidified sap were usually associated with abandoned larval tunnels or other feeding spots. This raises the possibility that some of the dead larvae might have already been killed or weakened by other causes such as natural enemies (nematodes, viruses, insect parasites) known to attack this insect (Entwistle, 1967). The positions and sizes of the tunnels differed with larval ages. Tunnels constructed by larvae of the first two instars were mainly found inside the leaf petioles or buds, whereas those produced by older larvae were generally in the stems of the growing shoots. However, there were some deviations from this general rule as seen in the tunnelling in the lignified stems by mature larvae. Some larvae did not construct any tunnels at all and instead resorted to surface feeding or making use of existing tunnels. Irrespective of the feeding modes, one characteristic was shared by the feeding behaviour of all larvae: they all fed under some form of concealment. It seems that the evolving of the tunnelling habit by *H. robusta* was to reduce the impact of its natural enemies rather than out of the requirement for better nutrition. The size of the tunnels, as measured by the volumes, gives a relative measure of food consumption of larvae at different development stages. According to the maximum tunnel sizes, feeding during the last two instars accounted for over 70% of the food
consumption of the entire larval stage. The longest tunnel recorded was 22 cm in length.

As a result of the tunnelling activities of larvae, growth of the infested shoots is stopped and their physical strength weakened. A terminal section of the shoot would later die or break off, leaving a basal section standing. Examination of the upper diameters of the basal sections (boundary diameters) of sampled shoots indicated that less than 5% of damaged shoots had their boundary diameters greater than 1.0 cm (Chapter 3). The percentages were close to zero for boundary diameters greater than 1.2 cm. Assuming a gradual decrease of the diameters of a shoot from tip to base, the results suggest that most damaged shoots are likely to retain the section from the 1.0 cm diameter mark to shoot base, since all shoots with boundary diameters smaller than 1.0 cm had kept extra slender sections. The probability of damaged shoots surviving from tip to any given diameter can be estimated from the fitted logistic equation (Figure 3.14). For an individual shoot, the exact position above which the shoot would die, depends on the location and relative size of the larval tunnel with respect to shoot size. A relatively young and slender shoot would probably have a large section surviving the tip moth attack if damaged by early instar larvae close to the shoot base. On the other hand, an old and stout shoot may lose a large section of it if it is tunnelled by mature larvae close to the base. It also needs to be pointed out that the observed boundaries may deviate from the eventual boundaries, since the latter are determined by the locations of the first replacement shoots immediately below the damaged area. My observations showed that the undamaged section of a shoot located between the bottom of the larval tunnel and the next replacement shoot would eventually die, due to the diversion of nutrients into the new shoot.

None of the attack indices considered, neither the percentages of attacked shoots in individual samples nor the number of times individual trees were attacked across the samples, showed convincing negative impact
on the height growth of trees in plot Open-1. Increment in tree height appeared to be mainly determined by initial tree size and form, with better growth associated with larger and less well-formed trees (lower tree height-DBH ratios) (Table 3.10). The failure to detect any significant effects of the attack indices on the height increment of trees may have arisen because of the dependence of attack levels on tree vigour. Whitmore (1978) indicated that high levels of attack by *H. grandella* tended to be concentrated on vigorously-growing trees. If this is true for *H. robusta*, then the larger gain in tree height among more vigorous-growing trees is likely to be lost due to the disproportionately high levels of attack, which, in turn, would make the attack levels seemingly unrelated to tree growth. In the case of insufficient cancelling effects by the attacking larvae, attack levels may even appear to be positively correlated with height increments, as was shown by the number of times individual trees were attacked across the samples (Table 3.10). It thus seems that the effect of damage can be fully and realistically shown only after the correction of tree vigour. However, the nature of the sample data in this study precluded such an analysis, as the rates of shoot production of individual trees had not been continually monitored throughout the sampling period. The obtained positive and negative dependence of tree height increment on tree size and tree form, respectively, were probably because larger and less-well formed trees had more shoots and thus were more likely to have some shoots escaping attack, as the attack intensity of attacked trees were found to be negatively correlated with shoot abundance (Table 3.5).

The directions in the changes of tree-form values (tree height and DBH ratios) of individual trees (increase or decrease) during the same period could be explained by two factors, the initial tree-form values and the percentages of shoots attacked in 55 (b5), both contributing negatively to the logarithmic likelihood of increase in tree form. The results suggest that damage by *H. robusta* in the open plot tends to make the host trees
converge to similar tree forms, as initially better formed trees were likely to decrease their tree-form values while initially less-well formed trees were likely to increase their tree-form values. Eventually all trees would become badly formed. Such an outcome was already evident for most of the trees in Open-1 at the time of the last sampling.

Finally, it needs to be pointed out that models established through regression analyses of sample data usually do not represent cause-effect relationships (Rawlings, 1988). The true effect of the impact of *H. robusta* damage on tree growth can be uncovered only with carefully designed experiments involving the setting-up of control (non-attacked) and treatment (attacked) groups consisting of otherwise homogeneous trees.

Growth of Forest-located trees during the sampling period was apparently better than that in the open plot, as was reflected not only in the larger height increment but also in better tree forms. This was true even when the comparison was restricted to trees with the same height ranges (Figure 3.9). Although the difference was likely to be mainly habitat-related, the effect of the lower attack levels in the forest plot cannot be ruled out, considering that a large percentage of trees in the forest plot (Forest-1) stayed single-stemmed (free of attack) throughout the sampling period.
Table 3.1. S-Plus functions and associated test statistics used in the statistical analyses of the sampling data in the field site.

<table>
<thead>
<tr>
<th>Parameters or effects to be tested</th>
<th>S-Plus functions</th>
<th>Test statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differences in the proportions of attacked trees or shoots</td>
<td>prop.test</td>
<td>Pearson's chi-square</td>
</tr>
<tr>
<td>Differences in the values of tree-specific or shoot-specific factors</td>
<td>wilcox.test</td>
<td>Wilcoxon rank sum W</td>
</tr>
<tr>
<td>Correlations between weather indices and attack indices</td>
<td>self-written function</td>
<td>Kendall's rank correlation tau</td>
</tr>
<tr>
<td>Goodness of fit in spatial distributions</td>
<td>self-written function</td>
<td>Pearson's chi-square</td>
</tr>
<tr>
<td>Effects of tree or shoot specific factors on attack status (logistic model)</td>
<td>glm (link=binomial) anova (glim object)</td>
<td>Pearson's chi-square</td>
</tr>
<tr>
<td>Effects of tree or shoot specific factors on attack intensity (linear model)</td>
<td>lm anova (lm object)</td>
<td>Fisher's F</td>
</tr>
</tbody>
</table>
Table 3.2. Proportions of attacked trees and mean percentages of attacked shoots per tree in each of the 13 samples (n=52)

| Samples | Date       | Attacked trees% | Attacked shoots% ± S *%
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>03 April 92</td>
<td>100.0</td>
<td>74.3 ± 3.5</td>
</tr>
<tr>
<td>S2</td>
<td>15 July 92</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S3</td>
<td>06 Oct. 92</td>
<td>59.6</td>
<td>10.9 ± 4.0</td>
</tr>
<tr>
<td>S4</td>
<td>01 Dec. 92</td>
<td>86.5</td>
<td>23.2 ± 1.5</td>
</tr>
<tr>
<td>S5</td>
<td>15 Feb. 93</td>
<td>78.9</td>
<td>20.8 ± 2.0</td>
</tr>
<tr>
<td>S6</td>
<td>20 April 93</td>
<td>44.2</td>
<td>16.3 ± 2.0</td>
</tr>
<tr>
<td>S7</td>
<td>21 July 93</td>
<td>5.8</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>S8</td>
<td>20 Oct. 93</td>
<td>42.3</td>
<td>6.2 ± 2.0</td>
</tr>
<tr>
<td>S9</td>
<td>19 Dec. 93</td>
<td>13.5</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>S10</td>
<td>27 Jan. 94</td>
<td>7.7</td>
<td>5.5 ± 2.0</td>
</tr>
<tr>
<td>S11</td>
<td>18 April 94</td>
<td>53.9</td>
<td>21.3 ± 1.5</td>
</tr>
<tr>
<td>S12</td>
<td>27 Aug. 94</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S13</td>
<td>25 Nov. 94</td>
<td>51.9</td>
<td>15.4 ± 2.0</td>
</tr>
</tbody>
</table>
Table 3.3. Tree-specific factors showing significant effect (P< 0.1) on the logarithmic odds of attack (log(p/(1-p)) of trees as determined by analyses of deviance (chi-square test). Only samples with the proportions of attacked trees in the range of 20-80% were considered. Number of trees in each sample was 52. Ht: tree height (cm), DBH: diameter of tree trunk at 1.3 m high (cm), TF: tree form (=Ht/ DBH), NSL: number of shoots with fully-expanded leaves. Data from Open-1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Factor</th>
<th>D.F.</th>
<th>Deviance (= chi. sq.)</th>
<th>P</th>
<th>Deviance reduced(%)</th>
<th>Sign of coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>S3</td>
<td>Ht</td>
<td>1</td>
<td>9.06</td>
<td>0.0026</td>
<td>12.91</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>1</td>
<td>13.00</td>
<td>0.0003</td>
<td>18.53</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>TF*</td>
<td>1</td>
<td>15.13</td>
<td>0.0001</td>
<td>21.57</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>NSL</td>
<td>1</td>
<td>9.06</td>
<td>0.0026</td>
<td>12.92</td>
<td>+</td>
</tr>
<tr>
<td>S5</td>
<td>DBH</td>
<td>1</td>
<td>3.69</td>
<td>0.0546</td>
<td>6.88</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>NSL*</td>
<td>1</td>
<td>6.88</td>
<td>0.0087</td>
<td>12.82</td>
<td>+</td>
</tr>
<tr>
<td>S6</td>
<td>Ht*</td>
<td>1</td>
<td>3.81</td>
<td>0.0509</td>
<td>5.34</td>
<td>+</td>
</tr>
<tr>
<td>S8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>no significant factor</td>
<td></td>
</tr>
<tr>
<td>S11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>no significant factor</td>
<td></td>
</tr>
<tr>
<td>S13</td>
<td>Ht</td>
<td>1</td>
<td>3.29</td>
<td>0.0697</td>
<td>4.57</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>DBH*</td>
<td>1</td>
<td>3.32</td>
<td>0.0683</td>
<td>4.62</td>
<td>+</td>
</tr>
</tbody>
</table>

* factors included in the final model at P<0.1.
Table 3.4. Comparisons of average values of numerical factors between lightly attacked and heavily attacked trees. Ht: tree height (cm), DBH: diameter of tree trunk at 1.3 m high (cm), TF: tree form (=Ht/ DBH), NSL: number of shoots with fully-expanded leaves. Data from Open-1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Factor</th>
<th>mean ± SD (n)</th>
<th>Pw</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lightly attacked</td>
<td>heavily attacked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>Ht</td>
<td>257.5 ± 78.5 (20)</td>
<td>232.2 ± 85.4 (32)</td>
<td>0.1343</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>3.6 ± 1.6 (20)</td>
<td>3.1 ± 1.7 (32)</td>
<td>0.2143</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>76.3 ± 16.2 (20)</td>
<td>84.9 ± 27.0 (32)</td>
<td>0.3715</td>
</tr>
<tr>
<td></td>
<td>NSL</td>
<td>9.8 ± 4.4 (20)</td>
<td>8.8 ± 4.6 (32)</td>
<td>0.2161</td>
</tr>
<tr>
<td>S2</td>
<td>Ht</td>
<td>290.3 ± 78.7 (18)</td>
<td>270.5 ± 86.3 (13)</td>
<td>0.2971</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>4.2 ± 1.5 (18)</td>
<td>1.7 ± 3.9 (13)</td>
<td>0.3167</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>73.9 ± 17.3 (18)</td>
<td>73.3 ± 12.8 (13)</td>
<td>0.7947</td>
</tr>
<tr>
<td></td>
<td>NSL</td>
<td>25.0 ± 9.6 (18)</td>
<td>19.7 ± 8.1 (13)</td>
<td>0.1728</td>
</tr>
<tr>
<td>S4</td>
<td>Ht</td>
<td>290.9 ± 88.4 (20)</td>
<td>251.8 ± 86.3 (25)</td>
<td>0.1703</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>4.0 ± 1.8 (20)</td>
<td>3.4 ± 1.8 (25)</td>
<td>0.3316</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>77.6 ± 14.0 (20)</td>
<td>85.7 ± 29.7 (25)</td>
<td>0.5374</td>
</tr>
<tr>
<td></td>
<td>NSL</td>
<td>24.8 ± 12.7 (20)</td>
<td>18.7 ± 10.1 (25)</td>
<td>0.0928</td>
</tr>
<tr>
<td>S5</td>
<td>Ht</td>
<td>257.8 ± 100.7 (20)</td>
<td>313.3 ± 110.0 (21)</td>
<td>0.0976</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>3.7 ± 2.1 (20)</td>
<td>4.6 ± 2.0 (21)</td>
<td>0.0874</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>81.4 ± 27.1 (20)</td>
<td>71.4 ± 13.5 (21)</td>
<td>0.3411</td>
</tr>
<tr>
<td></td>
<td>NSL</td>
<td>18.4 ± 11.5 (20)</td>
<td>22.2 ± 12.2 (21)</td>
<td>0.2729</td>
</tr>
<tr>
<td>S6</td>
<td>Ht</td>
<td>308.8 ± 90.1 (13)</td>
<td>311.7 ± 121.1 (10)</td>
<td>0.9505</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>4.6 ± 2.3 (13)</td>
<td>4.0 ± 1.7 (10)</td>
<td>0.5348</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>75.0 ± 13.8 (13)</td>
<td>79.4 ± 14.2 (10)</td>
<td>0.3852</td>
</tr>
<tr>
<td></td>
<td>NSL</td>
<td>16.9 ± 19.2 (13)</td>
<td>11.0 ± 7.8 (10)</td>
<td>0.1065</td>
</tr>
<tr>
<td>S8</td>
<td>Ht</td>
<td>315.4 ± 113.3 (16)</td>
<td>363.8 ± 170.9 (6)</td>
<td>0.9689</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>4.4 ± 2.0 (16)</td>
<td>4.7 ± 2.9 (6)</td>
<td>0.7260</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>75.2 ± 14.0 (16)</td>
<td>83.4 ± 12.2 (6)</td>
<td>0.2590</td>
</tr>
<tr>
<td></td>
<td>NSL</td>
<td>24.2 ± 16.8 (16)</td>
<td>20.0 ± 12.7 (6)</td>
<td>0.5073</td>
</tr>
<tr>
<td>S11</td>
<td>Ht</td>
<td>313.9 ± 99.2 (14)</td>
<td>304.6 ± 117.8 (14)</td>
<td>0.7827</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>4.4 ± 1.9 (14)</td>
<td>4.7 ± 2.3 (14)</td>
<td>0.6458</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>75.3 ± 17.0 (14)</td>
<td>69.9 ± 19.5 (14)</td>
<td>0.4763</td>
</tr>
<tr>
<td></td>
<td>NSL</td>
<td>3.5 ± 4.1 (14)</td>
<td>1.7 ± 2.7 (14)</td>
<td>0.2746</td>
</tr>
<tr>
<td>S13</td>
<td>Ht</td>
<td>338.4 ± 103.6 (18)</td>
<td>385.7 ± 114.2 (9)</td>
<td>0.1291</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>4.8 ± 2.2 (18)</td>
<td>5.9 ± 2.1 (9)</td>
<td>0.1427</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>76.0 ± 16.3 (18)</td>
<td>68.0 ± 15.8 (9)</td>
<td>0.2472</td>
</tr>
<tr>
<td></td>
<td>NSL</td>
<td>18.0 ± 9.5 (18)</td>
<td>18.4 ± 5.4 (9)</td>
<td>0.8769</td>
</tr>
</tbody>
</table>
Table 3.5. Tree-specific factors showing significant effect (P < 0.1) on the attack intensities of attacked trees (percentages of shoots attacked per tree) as determined by ANOVA of linear models. Only samples with the number of attacked trees ≥ 20 were considered. Data on the attack intensities were transformed by arcsine $\sqrt{x}$. Ht: tree height, DBH: diameter of tree trunk at 1.3m high, TF: tree form (≡ Ht/DBH), NSL: number of shoots with fully-expanded leaves, Blk: location of trees with respect to the three blocks in Open-1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Factor</th>
<th>D.F.</th>
<th>F</th>
<th>P</th>
<th>Sum of sq. reduced(%)</th>
<th>Sign of coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>Ht</td>
<td>1, 50</td>
<td>4.6050</td>
<td>0.0368</td>
<td>8.43</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>DBH</td>
<td>1, 50</td>
<td>4.8879</td>
<td>0.0316</td>
<td>8.91</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>TF</td>
<td>1, 50</td>
<td>3.0794</td>
<td>0.0854</td>
<td>5.80</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>NSL</td>
<td>1, 50</td>
<td>4.2837</td>
<td>0.0437</td>
<td>7.89</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Factors in the final model: DBH, Blk; F=3.4650, d.f.=3, 48, P=0.0233</td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>NSL</td>
<td>1, 30</td>
<td>10.2293</td>
<td>0.0033</td>
<td>26.08</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Factors in the final model: NSL; F=10.2293, d.f.=1, 30, P=0.0033</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>TF</td>
<td>1, 43</td>
<td>3.4346</td>
<td>0.0707</td>
<td>7.40</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>NSL</td>
<td>1, 43</td>
<td>6.5292</td>
<td>0.0142</td>
<td>13.18</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Blk</td>
<td>2, 42</td>
<td>3.4607</td>
<td>0.0406</td>
<td>14.15</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Factors in the final model: NSL, Blk; F=4.461, d.f.=3, 41, P=0.0084</td>
<td></td>
</tr>
<tr>
<td>S5</td>
<td>Blk</td>
<td>2, 38</td>
<td>2.9333</td>
<td>0.0654</td>
<td>21.41</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Factors in the final model: Blk, Ht; F=3.36, d.f.=3, 37, P=0.0289</td>
<td></td>
</tr>
<tr>
<td>S6</td>
<td>NSL</td>
<td>1, 22</td>
<td>5.2048</td>
<td>0.0331</td>
<td>19.86</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Factors in the final model: NSL; F=5.2048, d.f.=1, 22, P=0.0331</td>
<td></td>
</tr>
<tr>
<td>S8</td>
<td>No significant factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S11</td>
<td>No significant factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S13</td>
<td>TF</td>
<td>1, 25</td>
<td>3.9149</td>
<td>0.0590</td>
<td>13.54</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Blk</td>
<td>2, 24</td>
<td>3.2634</td>
<td>0.0558</td>
<td>21.38</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Factors in the final model: TF, Blk; F=3.5230, d.f.=3, 23, P=0.0310</td>
<td></td>
</tr>
</tbody>
</table>

NA: not applicable, as Blk is a categorical factor.
Table 3.6. Shoot-specific factors showing significant effect (P < 0.1) on the logarithmic odds of attack (log(p/(1-p))) of shoots as determined by analyses of deviance (chi-square test). Pooled data from Open-1 of all shoots from attacked trees. Total number of shoots: 1231. SHt: shoot height, RHt: relative shoot height, SL: shoot length. SBD: shoot basal diameter, SS: shoot slenderness (=SL/SBD).

<table>
<thead>
<tr>
<th>Factor</th>
<th>D.F.</th>
<th>Deviance (=chi. sq.)</th>
<th>P</th>
<th>Deviance reduced(%)</th>
<th>Sign of coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHt</td>
<td>1</td>
<td>8.77</td>
<td>0.0031</td>
<td>0.63</td>
<td>+</td>
</tr>
<tr>
<td>RHt</td>
<td>1</td>
<td>49.83</td>
<td>0.0000</td>
<td>3.56</td>
<td>+</td>
</tr>
<tr>
<td>SL</td>
<td>1</td>
<td>37.56</td>
<td>0.0000</td>
<td>2.69</td>
<td>+</td>
</tr>
<tr>
<td>SBD</td>
<td>1</td>
<td>47.32</td>
<td>0.0000</td>
<td>3.38</td>
<td>+</td>
</tr>
<tr>
<td>SS</td>
<td>1</td>
<td>13.46</td>
<td>0.0002</td>
<td>0.96</td>
<td>+</td>
</tr>
</tbody>
</table>

Factors included in the final model at P < 0.1: RHt, SL, SS

Significance level of the final model: P < 0.0001
Table 3.7. Comparisons of tree height (cm) between attacked and non-attacked trees in Forest-1.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean ± SD (n) / (range)</th>
<th>Attacked</th>
<th>Non-attacked</th>
<th>P_wilcoxon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>144.7 ± 70.0 (19)</td>
<td>104.9 ± 59.1 (83)</td>
<td>0.0009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(76-360)</td>
<td>(34-360)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S3</td>
<td>133.8 ± 74.9 (17)</td>
<td>117.3 ± 71.8 (89)</td>
<td>0.1553</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(60-380)</td>
<td>(36-370)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S6</td>
<td>304.5 ± 130.5 (4)</td>
<td>194.7 ± 90.5 (106)</td>
<td>0.0712</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(144-440)</td>
<td>(24-535)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S7-S8</td>
<td>No attacked trees</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S9</td>
<td>309.7 ± 106.5 (13)</td>
<td>236.7 ± 121.9 (56)</td>
<td>0.0213</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(156-500)</td>
<td>(78-646)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S10</td>
<td>295.3 ± 80.9 (14)</td>
<td>247.5 ± 131.5 (54)</td>
<td>0.0421</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(210-508)</td>
<td>(74-624)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S11</td>
<td>318.2 ± 112.7 (12)</td>
<td>250.2 ± 120.9 (55)</td>
<td>0.0559</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(112-476)</td>
<td>(93-636)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S12</td>
<td>No attacked trees</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S13</td>
<td>506.0 ± 70.4 (9)</td>
<td>244.4 ± 101.8 (59)</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(395-616)</td>
<td>(43-562)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.8. Proportions of attacked trees (%) in Forest-1 and Open-1 after the removal of trees with Ht ≤200 cm. Numerals in the brackets are the total number of trees in that height category. The p-values are obtained from chi-square tests of the differences in the attack proportions. NA's indicate non-test (due to inadequate number of either attacked or un-attacked trees in either plot (see text for detail).

<table>
<thead>
<tr>
<th>Plot</th>
<th>S1</th>
<th>S3</th>
<th>S6</th>
<th>S7</th>
<th>S8</th>
<th>S9</th>
<th>S10</th>
<th>S11</th>
<th>S12</th>
<th>S13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open-1</td>
<td>100.0</td>
<td>67.4</td>
<td>45.0</td>
<td>46.5</td>
<td>4.8</td>
<td>14.0</td>
<td>9.3</td>
<td>55.8</td>
<td>0.0</td>
<td>59.1</td>
</tr>
<tr>
<td></td>
<td>(35)</td>
<td>(43)</td>
<td>(40)</td>
<td>(42)</td>
<td>(43)</td>
<td>(43)</td>
<td>(43)</td>
<td>(43)</td>
<td>(43)</td>
<td>(44)</td>
</tr>
<tr>
<td>Forest-1</td>
<td>33.3</td>
<td>20.0</td>
<td>6.0</td>
<td>0.0</td>
<td>0.0</td>
<td>25.6</td>
<td>31.8</td>
<td>43.2</td>
<td>0.0</td>
<td>19.6</td>
</tr>
<tr>
<td></td>
<td>(9)</td>
<td>(10)</td>
<td>(50)</td>
<td>(42)</td>
<td>(41)</td>
<td>(43)</td>
<td>(44)</td>
<td>(44)</td>
<td>(46)</td>
<td>(46)</td>
</tr>
<tr>
<td>p-value</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.3</td>
<td>NA</td>
<td>0.3</td>
<td>NA</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Table 3.9. Percentages of trees attacked but with no larvae (P\textsubscript{noL}) and percentages of dead larvae (P\textsubscript{dead}) on various sampling occasions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>S1</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
<th>S7</th>
<th>S8</th>
<th>S9</th>
<th>S10</th>
<th>S11</th>
<th>S13</th>
</tr>
</thead>
<tbody>
<tr>
<td>P\textsubscript{noL}</td>
<td>19.2</td>
<td>29.0</td>
<td>18.0</td>
<td>83.3</td>
<td>100.0</td>
<td>66.7</td>
<td>76.7</td>
<td>0.0</td>
<td>80.0</td>
<td>28.6</td>
<td>55.6</td>
</tr>
<tr>
<td>(n)</td>
<td>(52)</td>
<td>(31)</td>
<td>(39)</td>
<td>(48)</td>
<td>(23)</td>
<td>(3)</td>
<td>(30)</td>
<td>(7)</td>
<td>(15)</td>
<td>(28)</td>
<td>(27)</td>
</tr>
<tr>
<td>P\textsubscript{dead}</td>
<td>45.1</td>
<td>38.7</td>
<td>34.6</td>
<td>16.7</td>
<td>NA</td>
<td>33.3</td>
<td>82.4</td>
<td>25.0</td>
<td>33.3</td>
<td>6.7</td>
<td>28.6</td>
</tr>
<tr>
<td>(n)</td>
<td>(122)</td>
<td>(31)</td>
<td>(52)</td>
<td>(12)</td>
<td>(3)</td>
<td>(17)</td>
<td>(8)</td>
<td>(3)</td>
<td>(30)</td>
<td>(14)</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.10. Analysis of variances of linear models with the height increment of trees during the period between the first and the last sampling occasions as the independent variable and attack indices and initial tree specific factors as the independent variables. $b_1$-$b_{13}$: percentages of shoots attacked per tree in individual samples (S1$-$S13); $b_{\text{times}}$: the number of times individual trees were attacked across the samples; $H_{t1}$, $DBH_1$, $TF_1$: height, DBH and tree-form values of trees in the first sampling occasion (S1); Blk: location block (block-1, block-2, or block-3) of trees. Data from Open-1.

<table>
<thead>
<tr>
<th>Factor</th>
<th>D.F.</th>
<th>F-value</th>
<th>P</th>
<th>sign of coef.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b_1$</td>
<td>1, 48</td>
<td>0.45</td>
<td>0.5052</td>
<td>-</td>
</tr>
<tr>
<td>$b_2$</td>
<td>1, 48</td>
<td>0.14</td>
<td>0.7127</td>
<td>+</td>
</tr>
<tr>
<td>$b_4$</td>
<td>1, 48</td>
<td>1.92</td>
<td>0.1718</td>
<td>-</td>
</tr>
<tr>
<td>$b_5$</td>
<td>1, 48</td>
<td>3.61</td>
<td>0.0636</td>
<td>+</td>
</tr>
<tr>
<td>$b_6$</td>
<td>1, 48</td>
<td>1.42</td>
<td>0.2386</td>
<td>+</td>
</tr>
<tr>
<td>$b_7$</td>
<td>1, 48</td>
<td>0.69</td>
<td>0.4094</td>
<td>-</td>
</tr>
<tr>
<td>$b_8$</td>
<td>1, 48</td>
<td>0.27</td>
<td>0.6032</td>
<td>+</td>
</tr>
<tr>
<td>$b_9$</td>
<td>1, 48</td>
<td>0.45</td>
<td>0.5038</td>
<td>+</td>
</tr>
<tr>
<td>$b_{10}$</td>
<td>1, 48</td>
<td>0.19</td>
<td>0.6621</td>
<td>-</td>
</tr>
<tr>
<td>$b_{11}$</td>
<td>1, 48</td>
<td>0.05</td>
<td>0.8281</td>
<td>-</td>
</tr>
<tr>
<td>$b_{13}$</td>
<td>1, 48</td>
<td>0.25</td>
<td>0.6202</td>
<td>+</td>
</tr>
<tr>
<td>$b_{\text{times}}$</td>
<td>1, 48</td>
<td>10.01</td>
<td>0.0027**</td>
<td>+</td>
</tr>
<tr>
<td>$H_{t1}$</td>
<td>1, 48</td>
<td>14.30</td>
<td>0.0004***</td>
<td>+</td>
</tr>
<tr>
<td>$DBH_1$</td>
<td>1, 48</td>
<td>16.04</td>
<td>0.0002***</td>
<td>+</td>
</tr>
<tr>
<td>$TF_1$ (=H_{t1}/DBH_1)</td>
<td>1, 48</td>
<td>16.68</td>
<td>0.0002***</td>
<td>-</td>
</tr>
<tr>
<td>Blk</td>
<td>2, 47</td>
<td>2.37</td>
<td>0.1050</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA: not applicable, as Blk is a categorical factor.

*, **, ***: factors significant at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.
Table 3.11. Analysis of deviances of logistic models with the logarithmic odds of trees recording positive increase in tree-form values (Height/DBH) during the period between the first and the last sampling occasions as the independent variable and attack indices and initial tree specific factors as the independent variables. b1~b13: percentages of shoots attacked per tree in individual samples (S1~S13); btimes: the number of times individual trees were attacked across the samples; Ht1, DBH1, TF1: height, DBH and tree-form values of trees in the first sampling occasion (S1); Blk: location block (block-1, block-2, or block-3) of trees. Data from Open-1.

<table>
<thead>
<tr>
<th>Factor</th>
<th>D.F. reduced</th>
<th>Deviance reduced</th>
<th>D.F. residual</th>
<th>Deviance residual</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NULL</td>
<td>1</td>
<td>1.02</td>
<td>67.98</td>
<td>0.3125</td>
<td></td>
</tr>
<tr>
<td>b1</td>
<td>1</td>
<td>2.28</td>
<td>66.72</td>
<td>0.1311</td>
<td></td>
</tr>
<tr>
<td>b2</td>
<td>1</td>
<td>0.01</td>
<td>68.99</td>
<td>0.9203</td>
<td></td>
</tr>
<tr>
<td>b3</td>
<td>1</td>
<td>1.63</td>
<td>67.36</td>
<td>0.2017</td>
<td></td>
</tr>
<tr>
<td>b4</td>
<td>1</td>
<td>1.03</td>
<td>67.97</td>
<td>0.3102</td>
<td></td>
</tr>
<tr>
<td>b5</td>
<td>1</td>
<td>0.01</td>
<td>68.99</td>
<td>0.9203</td>
<td></td>
</tr>
<tr>
<td>b6</td>
<td>1</td>
<td>0.07</td>
<td>68.93</td>
<td>0.7913</td>
<td></td>
</tr>
<tr>
<td>b7</td>
<td>1</td>
<td>0.00</td>
<td>68.99</td>
<td>1.0000</td>
<td></td>
</tr>
<tr>
<td>b8</td>
<td>1</td>
<td>0.61</td>
<td>68.39</td>
<td>0.4348</td>
<td></td>
</tr>
<tr>
<td>b9</td>
<td>1</td>
<td>0.42</td>
<td>68.57</td>
<td>0.5169</td>
<td></td>
</tr>
<tr>
<td>b10</td>
<td>1</td>
<td>0.75</td>
<td>68.25</td>
<td>0.3865</td>
<td></td>
</tr>
<tr>
<td>Ht1</td>
<td>1</td>
<td>3.25</td>
<td>65.75</td>
<td>0.0714</td>
<td></td>
</tr>
<tr>
<td>DBH1</td>
<td>1</td>
<td>6.03</td>
<td>62.96</td>
<td>0.0141*</td>
<td></td>
</tr>
<tr>
<td>TF1(=Ht1/DBH1)</td>
<td>1</td>
<td>12.92</td>
<td>56.07</td>
<td>0.0003***</td>
<td></td>
</tr>
<tr>
<td>Blk</td>
<td>2</td>
<td>3.20</td>
<td>65.79</td>
<td>0.2019</td>
<td></td>
</tr>
</tbody>
</table>

*, **, ***: factors significant at P < 0.05, P < 0.01, and P < 0.001, respectively.
Table 3.12. Changes in tree-form (Ht/DBH) values between the first and the last sampling occasions for trees with different initial tree sizes and tree-form values

<table>
<thead>
<tr>
<th>Initial tree height (Ht₁)</th>
<th>Initial DBH (DBH₁)</th>
<th>Initial tree form (TF₁)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤200 cm</td>
<td>&gt;200 cm</td>
<td>≤0.3 cm</td>
</tr>
<tr>
<td>-42.3 ± 90.6</td>
<td>2.8 ± 12.5</td>
<td>-28.7 ± 76.3</td>
</tr>
<tr>
<td>-244.4 ~ +51.2</td>
<td>-21.46 ~ +32.2</td>
<td>-244.4 ~ +51.2</td>
</tr>
<tr>
<td>n=16</td>
<td>n=34</td>
<td>n=24</td>
</tr>
<tr>
<td>P_{wilcoxon}=0.0204</td>
<td>P_{wilcoxon}=0.0140</td>
<td>P_{wilcoxon}=0.0003</td>
</tr>
</tbody>
</table>
Table 3.13. Correlation matrix of the four numerical tree-specific factors. Pooled data of all samples from Open-1.

<table>
<thead>
<tr>
<th></th>
<th>Ht</th>
<th>DBH</th>
<th>TF</th>
<th>NSL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ht</td>
<td>1</td>
<td>0.91</td>
<td>-0.44</td>
<td>0.48</td>
</tr>
<tr>
<td>DBH</td>
<td>0.91</td>
<td>1</td>
<td>-0.68</td>
<td>0.51</td>
</tr>
<tr>
<td>TF</td>
<td>-0.44</td>
<td>-0.68</td>
<td>1</td>
<td>-0.35</td>
</tr>
<tr>
<td>NSL</td>
<td>0.48</td>
<td>0.51</td>
<td>-0.35</td>
<td>1</td>
</tr>
</tbody>
</table>
Cedrela odorata and C. fissilis forest patch (mainly Eucalyptus saligna, E. grandis and E. microcorys)

Cedrela odorata and C. fissilis

Eucalyptus saligna, E. grandis and E. microcorys

Orchard (peach and nectarine)

Other planted trees (mainly Grevillia robusta)

Spring or pond

House

Figure 3.1. Relative positions of sample plots and isolated groups of Red Cedars in the field site. Figure not drawn to scale.
C - Red Cedar (*Toona australis* (F. Muell.) Harms), C - sample trees
S - Silky Oak (*Grevillea robusta* Cunn.), T - Teak (*Flindersia australis* R. Br.)
B - White Beech (*Gmelina leichhardtii* F. Muell.)

Figure 3.2. Layout of trees in plot Open-1.
Figure 3.3. Temporal patterns of the attack indices and the rainfall and temperature indices. Rainfall: the total amount of rainfall in the previous 30 days; Minimum temperature: average daily minimum temperature in the previous 15 days. Solid circles: attack indices; empty circle: weather indices. Data from Open-1.
Figure 3.4. Relationship between the proportions of attacked trees and the average percentages of attacked shoots per tree. Data from Open-1.
Figure 3.5. Comparisons of average values in tree height (Ht), diameters at breast height (DBH), tree form (TF, = Ht/DBH) and the number of leaf-bearing shoots per tree between attacked trees (hatched columns) and non-attacked trees (blank columns). Stopped lines on top of the columns indicate half of the standard deviations. Data from Open-1.
Figure 3.6. Proportions of attacked trees at different ranges of Ht, DBH and TF (=Ht/DBH). The lengths and locations of the ranges are shown by the column boundaries and the attack proportions shown by the column heights. The overlaid lines show the relationship between the attack proportions and the average values of the respective factors. Pooled data of S3, S5, S6, S8, S11 and S13 from Open-1
Figure 3.7. Average percentages of attacked shoots per tree among trees planted in the three location blocks of plot Open-1.
Figure 3.8. Proportions of attacked shoots at different ranges of shoot-specific factors. A range denoted by x-y indicates that the value of the shoot-specific factor is greater than x but smaller than or equal to y. Relative shoot height: shoot height/tree height, shoot slenderness: shoot length/ shoot basal diameter. Pooled data of shoots from all attacked trees. Columns in the same graph not sharing common letters are significantly different in height (P <0.05, chi-square test)
Figure 3.9. Comparisons of the proportions of attacked trees between trees planted inside the forest (Forest-1, Forest-2) and trees planted in the open (Open-1, Open-2).
Figure 3.10. Proportions of attacked trees at different tree height ranges (inclusive on the right) in Forest-1. Numerals inside the columns indicate the number of trees. Columns sharing no common letters are significantly different at $P<0.05$ by chi-square test.
Figure 3.11. Comparisons in the proportions of attacked trees between isolated trees and trees in Open-1.
Figure 3.12. Ranges in the diameter of shoots at the tunnel entrances for larvae in different age groups. Each box contains 50% of the data points for the corresponding age group. Solid lines inside the boxes marks the median values. Lines extending from either side of the boxes marks the ranges of lower 25% quartile - 1.5 * box height to upper 25% quartile + 1.5 * box height. Points out side this range are considered as outliers and marked with empty circles. Outliers for 5th-6th instar larvae were not shown as they extended far beyond the limits of the y-axis.
Figure 3.13. Distribution of shoot boundary diameters in the data.
Figure 3.14. Estimated and predicted probabilities of shoot death from tip down to positions with given diameters.
Figure 3.15. Growth of tree height in the three location blocks in Open-1. Data points from left to right corresponds to sample S1, S2, ..., S12, and S13, respectively.
Figure 3.16. Growth of tree height in Open-1 and Forest-1. Only trees with their initial height in the range of 200-400 cm were used. Data points from left to right corresponds to sample S1, S3, and S6, S7, ..., S12, and S13, respectively.
Figure 3.17. Comparison of the frequency distributions of the number of times individual sample trees were attacked and that estimated from the binomial distribution. Pooled data from Open-1 of all samples with the proportions of attacked trees in the range of 20-80%.
Plate 1. A mature larvae (blue) and its tunnel inside a growing shoot
Chapter 4

Artificial Rearing of *Hypsipyla robusta* Moore

4.1 Introduction

With its ability in providing large numbers of uniform insects with known nutrition and quality at desired time and free of restrictions posed by the availability of host materials (Singh and Ashby, 1985), artificial rearing is widely used in researches and management of pest insects (Knipling, 1984; Singh, 1984; Reinecke, 1985; Wagge et al., 1985). A key aspect of artificial rearing is the development of satisfactory diets (Singh and Ashby, 1985). Although numerous artificial diets have been reported (Singh, 1985), the search for a suitable diet for a particular species can be a long and tedious process (Moore, 1985). Apart from meeting the basic nutritional requirement (Dadd, 1985; Reinecke, 1985), the diet must also be made inductive to feeding, as “insects, like fussy children, may not eat what is good for them” (Moore, 1985). Initiation and continuation of feeding rely on the positive feedback from phagostimulants present in the diet (Bernays, 1985). On the other hand, feeding may be disrupted by the presence of deterents (Bernays, 1985). The nature of phagostimulants is complex. They may appear as part of the nutrients such as carbohydrates and protein or secondary plant metabolites (Reinecke, 1985). Where more than one phagostimulant is required, individual phagostimulants may show little or no effect when applied alone (Bernays, 1985). Moreover, the same chemicals may act as phagostimulant, deterrent, or neutral, at different concentrations (Lewis and van Emden, 1986). In addition to its chemical completeness, the diet must have appropriate physical properties, such as hardness, texture, water content, and homogeneity, much of which can be controlled by gelling and bulking agents (agar and cellulose, respectively) (Moore, 1985). After a suitable diet has been found, the performance of the diet and the
quality of the rearing hinges on the actual procedure whereby the rearing is administered, such as microbial control and the choice of rearing environment (Singh, 1985). Infection of the diet by micro-organisms may cause high mortality and delay larval development (Bell et al., 1981). In one case, a particular microbe species was thought to be able to kill larvae of the first instar, slow the development of the second, and have no detectable effects on the later stages (Sikorowski and Goodwin, 1985).

Three artificial diets have been attempted on H. robusta. Achan (1968) was the first to try to rear this insect with an artificial diet. This diet was unsatisfactory, as the length of development time from egg hatching to adult emergence almost doubled from that obtained with a natural diet. The diet of Couilloud and Guiol (1980) (CGD) appeared to be satisfactory as no adverse effects were mentioned by the authors. Atuahene and Souto (1983) tried a diet that had been used in the rearing of H. grandella. The length of development time was reduced to acceptable levels but a high percentage of larvae died due to non-feeding and pupae were apparently smaller than those found in the field. Two diets for the sibling species H. grandella in Latin America have been used, the Hidalgo-Salvatierra Diet (HSD) (Hidalgo-Salvatierra and Berrios, 1972) and the McMorran-Grisdale Diet (MGD) (see Atuahene and Souto, 1983). In comparison to the natural diet consisting of shoots and leaves, the HSD produced individuals with longer lengths of development time from eggs to pupae and from eggs to adults, but resulted in larger and more fecund adults (Grijpma, 1971). The better performance in terms of adult size and fecundity was more likely due to the inadequacy of the natural diet rather than the superiority of the artificial diet, since in the field larvae feed inside the living shoots instead of leaves and shoot cuttings. With MGD, the same problem of high larval mortality due to non-feeding shown by H. robusta was observed on H. grandella (Creasap, 1973).

Another problem commonly experienced with the rearing of Hypsipyla spp. is the high proportion of infertile eggs in indoor cages
(Atuahene and Souto, 1982; Grijpma, 1971), apparently caused by low mating success. Mating of this insect under indoor conditions has been largely unsuccessful (Beeson, 1919; Roberts 1968). Fertile eggs were obtained in substantial quantity only in outdoor cages (Grijpma, 1971). However, the latter requirement denied the artificial rearing of the species all year round in most of the temperate regions where winter temperature can be too low to be tolerated by the moths. Difficulty of mating in laboratory colonies can be caused by a number of factors, among them the size of mating cages, the requirement for the presence of host materials (Raina et al., 1992), humidity (Callahan, 1962), habituation by males to the female pheromone in a confined environment (Colvin et al., 1994), and differences in the rate at which males and females reaches sexual maturity (Colvin et al., 1994). The first two factors have been ruled out as the bottleneck to mating in \( H. \) robusta (Beeson, 1919). Work on \( H. \) grandella (Zeller), with which the same problem was experienced (Grijpma, 1971), showed that mating could be achieved indoors if the moths were previously allowed to fly in pairs in a wind mill for some time (Fasoranti, 1985). The result points to a possible link between wind and the mating behaviour and/or sexual maturity of \( H. \)ipyla spp.

In addition to feeding and mating, the rearing of \( H. \)ipyla spp. can be improved by imposing strict microbial control and reducing the rearing density (Sterringa, 1973). Among other things, low-density rearing has the advantage of minimising cannibalism among larvae, the latter being found to be common in both \( H. \) robusta (Beeson, 1919; Froggatt, 1923) and \( H. \) grandella (Grijpma, 1971).

Artificial rearing of \( H. \) robusta has not been attempted before in Australia. Development of such a technique is highly desirable as it would facilitate studies into various aspects of this insect and its interactions with the Red Cedar, especially considering the scant information available so far about this insect relative to the other regional populations of \( H. \) robusta.
Apart from general considerations, artificial rearing has its special bearing to this PhD project, as the natural host of the insect does not occur in Canberra where the project is based. This chapter reports the results of rearing the insect using CGD as the larval diet. This particular diet was chosen because of its reported success (Couilloud and Guiol, 1980). Emphases are given to the solving of larval feeding and moth mating problems. Also reported and discussed are the performance of CGD and some basic biological attributes of the cedar tip moth as shown by the laboratory colony.

4.2 Methods

4.2.1 Rearing procedure

The CGD was prepared according to Couilloud and Guiol (1980). The diet was cast in glass jars (90 x 60 mm) to a depth of ca. 2 cm and kept at 4°C in a refrigerator for later use. Rearing was conducted in a temperature-controlled room at 24-26°C, 14L:10D. Daylight was simulated by a 250 W mirror-backed globe. Humidity (> 60%) was not strictly controlled but high humidity (>60% RH) was maintained in the room by an evaporator (KAZ Model 76). The laboratory colony was started with final instar larvae, prepupae or pupae collected from infested Red Cedar trees in a field site ca. 5 km north of Macksville, NSW.

Newly-hatched larvae (<24 h old) were transferred with a fine brush into plastic tubes (76 x 10 mm) containing cylindrical cores (40 x 4 mm) of the diet, punched out using a drinking straw. Each tube contained 10-20 larvae and was capped with cotton wool. At the time when most of the larvae reached the 3rd instar (approximately 10 days later) they were transferred to the glass jars where the diet was kept with vertical holes bored (ca. 4 mm diameter) into it to facilitate the burrowing activities of the larvae. The top of the glass jar was covered with black cloth. About 40-60 larvae were introduced into each jar. Another transfer was made at the late 5th instar to reduce the density to 20-30 larvae per jar. Transfers were also
made if the diet was infected with fungi or almost completely consumed. Larvae that had developed blue coloration and had ceased feeding and crawled out of the diets were taken away and put into plastic jars (50 x 40 mm), with the caps of the jars loosely sitting on top. This step was taken as earlier observations showed that the inactive prepupae and pupae were sometimes eaten by younger larvae. A maximum of 10 mature larvae was put into each plastic jar. To facilitate the construction of pupation cells, a small amount of cotton wool was placed inside the jars.

Moths of mixed ages and sexes were put in a Perspex cage (250 x 250 x 820 mm) for mating and egg-laying. The two ends of the cage were covered with cheese cloth and the floor covered with corrugated paper towels. Cotton wool soaked with 5% sugar solution was put in petri dishes and placed inside the cage as the moths' food. Viable eggs (red) laid on the cheese cloth and paper towels were cut out and placed in plastic vials for egg hatching.

4.2.2 Development rate

Duration of the egg stage was recorded for 250 eggs laid on the same night. These eggs were placed in a plastic vial with the opening covered with black cloth. Newly-hatched larvae were removed and counted daily just before 'daybreak' (19:00-20:30 under reversed light cycle) until the third consecutive day recording no new hatchings. For the study of larval and pupal development rate, ninety newly hatched larvae (<24 hour old) were individually placed in the plastic feeding tubes described in the previous section. Development stages of the larvae were checked daily just before dark (9:00-10:30 under reversed light cycle). A larva was considered to have advanced into the next instar if a new head capsule was found inside the rearing tube. The head capsule and the rest of the exuviae were removed with a fine brush after checking and the head capsules measured for width with a stereoscopic microscopic microscope equipped with an eyepiece scale.
Due to frequent rupture of the head capsule during the final moult, the head capsule widths of the last instar were estimated on the basis of the head widths measured from a separate batch of similarly reared larvae. Larvae not moulted were recorded as either live or dead. If the larva was not seen, the diet core was taken out and opened to reveal the status of the larva and a new diet core was supplied. The diet was also replaced if it was nearly consumed or considered no longer fresh. For mature larvae, the dates at which the larvae turned blue, stopped feeding (indicated by the construction of pupation cells) and pupated were also recorded. The sexes of the pupae were determined according to Sharma and Singh (1980a). Checking terminated when the last adult had emerged.

4.2.3 Pupal weight and fecundity

Pupae were weighed to micrograms with a fine balance (GMBH, Sartorius). Mated females of less than 4-day-old were removed from the mating cage immediately after separation from copulation and placed individually in cages made from cardboard tubes (155 x 74 mm), with the two ends covered with black cloth. Balls of cotton wool soaked with 5% sugar solution were placed inside the oviposition cages to serve as food for the gravid females. The number of red eggs laid by each female was checked after the female died and before the hatching of any eggs. Dead females were dissected under a stereoscopic microscope and the number of mature eggs (those with similar sizes as laid eggs) retained in the ovary were counted.

4.2.4 Non-diet factors affecting egg hatching and survival of neonates

Egg hatching rates

Red eggs laid in the same night by different females were cut out from the paper towels and mixed. These eggs were divided into 3 groups each containing 60 eggs and placed separately in plastic vials, two of which were filled with fresh diet to a depth of ca. 1 cm and the other left empty. Eggs in
one of the diet-filled vial were allowed direct contact with the diet while in
the other they were separated from the diet by aluminium foil. The
percentage of eggs hatched in each group was checked 10 days later.

_Tolerance to starving in the neonates_

Four batches of larvae (> 10 larvae each), hatched within the one-hour
period between 9:00-17:00, were individually placed in plastic tubes with
their hatching hours marked and capped with cotton wool. No food was
provided. The survival status of the larvae (live or dead), determined by
lightly poking the larvae with a fine brush, was checked hourly between
9:00-21:00 each day. The length of survival time was estimated as
cumulative hours from the hatching hour to the time of death. Larvae that
died outside the daily checking period were considered to have died at 3:00
the previous night. To study the effect of access to water, the survival time
was similarly estimated for another two batches of larvae with water-soaked
balls of cotton wool placed at the bottom of the plastic tubes. In a separate
study, 80 neonates each were transferred to the diet at 6, 24 and 36 h after
hatching and allowed to feed for 3 days, at the end of which time the
survival rates of the neonates were checked. The experiment was conducted
in plastic tubes (76 x 10 mm) each containing a length of the diet core (ca. 40
x 4 mm). Twenty neonates were introduced into each tube.

_Effect of egg-laying substrates on the feeding readiness of neonates_

The first-3-day survival of neonates in the artificial diet was tested for
those hatched from eggs laid on the paper towels and those on host leaves.
The test was conducted in plastic tubes each containing a length of cylinder
core of the diet (ca. 40 x 4 mm). The neonates were transferred to the diet
within 16 hours of their hatching. Each egg-laying substrate was tested on 60
neonates with 20 neonates in each tube.
Effect of the size of the rearing containers on the survival of neonates

Three types of containers, plastic tubes (76 x 10 mm), plastic vials (50 x 40 mm), and the glass jar (90 x 60 mm) in which the diet was cast, were assessed for their performance in rearing the neonates with artificial diet. For the plastic tubes the diet was supplied in the same way as above. For the plastic vials the diet was filled to a depth of ca. 1 cm. Diet in the both the plastic vials and the glass jar was densely punched with vertical holes (ca. 4 mm in diameter), using a drinking straw. Twenty neonates were introduced into each of the five plastic tubes and each of the five plastic vials. The glass jar was inoculated with 100 neonates. The plastic tubes were capped with cotton wool and the plastic vials and the glass jar by black cloth. Survival of the neonates was checked 3 days later by counting the number of dead larvae outside the diet (inner walls of the containers, caps, and the surface of the diet).

4.2.5 Feeding enhancement

CGD fortified with leaf powder

Healthy Red Cedar leaves were cut off at the base and air-dried in the laboratory. The dried leaves, after being stripped of the petioles and midribs, were ground to fine pieces in a hand grinder. The leaf powder was incorporated into the ready-made diet in a 1:10 weight ratio by thoroughly mixing the two in the grinder. Performance of the fortified diet in attracting feeding by the neonates relative to the plain diet was tested by packing the two diets in the same plastic vial (76 x 10 mm) to a depth of 10 mm, each occupying a little under one half of the floor space, with a narrow gap left between the interfaces. Twenty newly hatched larvae (< 16 h old) were transferred with a fine brush to the surface of each of the two diets. The test was replicated 5 times. The number of larvae in or on each diet was checked one week later. Also recorded were the relative amount of feeding tunnels or other feeding scars.
CGD fortified with the ethanol extract of young shoots and leaves

Preliminary trials indicated that incorporation of methanol extract of young shoots into the artificial diet showed some degree of feeding enhancement in 1st-instar larvae, whereas extract of the non-polar solvent, light petroleum, was less effective. In light of this finding, later experiments on feeding enhancement were carried out on extracts with ethanol, which has similar polarity as methanol but, unlike methanol, is natural to plants. The ethanol extract were obtained by soaking the young shoots (including leaves) in 95% ethanol for 3 days at room temperature (20-25°C). The extract was filtered and reduced to near dryness in a rotatory evaporator at 40°C. Ethanol was re-added to the residue so that the extract reached a concentration of 10 g/ml fresh weight equivalent (FWE) of the host tissue. Incorporation of the extract into the artificial diet was made during the preparation process of the diet in a way similar to that reported by Hsiao and Frankael (1968). The extract was applied to the cellulose constituent of the diet. After evaporation of the solvent, the treated cellulose was mixed with the other constituents as in the original diet preparing process. The control diet was likewise prepared but the cellulose was applied with pure solvent of the same volume. Three concentrations: 1, 5 and 10 g FWE of extract in 50 g of artificial diet, were tested in a non-choice bioassay at 24-26°C and light period of 14L:10D.

Newly hatched larvae were individually transferred into test tubes (10 x 76 mm) each containing a small amount of the diet in the form of cylindrical (5 x 50 mm) and capped with cotton wool. Each treatment and control diet was tested with 30 larvae. Development and mortality of the test larvae was followed daily until the 3rd instar. The feeding stimulating effect of the ethanol extract was evaluated further in a dual-choice test, using the 5 g FWE diet and the control diet. Ten hatchlings were introduced into each tube containing cylindrical (20 x 4 mm) of both treatment and
control diet, with 5 larvae placed on each diet. The two diet cores were separated by a thin plastic panel (ca. 30 x 5 mm) to prevent physical contacts. As the plastic panel was shorter and narrower than the test tube, larvae could move across the two diets. The test was replicated 8 times. The number of larvae on the treatment and control diets was checked daily, just before the start of the dark period, for 3 days starting on the second day and the relative intensities of feeding, as shown by surface tunnels or entry holes, checked at the end of the test.

4.2.6 Mating

In an effort to improve the mating rate of caged moths in the rearing room, the effect of wind on mating success was investigated. Two experimental designs were tried. (a) Mating in wind and windless cages. Two perspex cages of the same dimension (250 x 250 x 820 mm) were placed side by side, with their openings covered by cheese cloth. One cage was blown continually by wind generated from a fan (160 mm diameter) placed at ca. 30 cm from one end of the cage and the other blocked from wind by plastic sheets at both ends. Wind speed inside the cage was low (ca. 0.5 m/s in the centre). Twenty virgin males and twenty virgin females, emerged within the last three days, were placed in the ventilated cage, and the same number in the windless cage. The moths were then observed for mating for three consecutive nights, with the aid of a red florescent light tube (25 W) sitting lengthwise on top of the two cages in the centre. On the 4th night, air was blown through the windless cage to see if there was any change in mating activity. Three experiments of this type were performed. (b) Mating during wind and windless intervals. Virgin males and females of mixed ages were left in one cage and the fan was switched on and off alternately at fixed intervals. Three fan-on and fan-off interval ratios were used: 20:20, 20:40 and 20:60 minutes. During the fan-off phases, plastic sheets were used to cover the cage ends to simulate windless conditions inside the cage. Four
experiments, each running a full night phase of the inverted light cycle, were conducted for each of the three fan-on and fan-off interval ratios. The number of copulations at each interval was recorded. Comparisons were made between the number of copulations per hour during wind and windless intervals. In all experiments, a petri dish containing cotton wool saturated with 5% sugar solution was placed on the floor at either end of the mating cage to serve as food of the moths.

4.3 Results

4.3.1 Performance of the laboratory colony

Egg hatching and development

Newly laid fertile eggs were creamy white. Within 24 hours, distinctive red bands appeared, which did not occur among unfertile eggs. Egg hatching rates, estimated from batches of eggs (n> 50) laid by mated females on the same nights, varied from 56% to 81% (average 68%). Not all eggs in the same batches developed the red bands, indicating some of them may have been unfertilised. Egg period averaged 5.2 days (range 5-6 days).

Development of larvae and pupae

The number of larval instars varied from five to seven, with the majority (69.1%) passing through 6 instars before pupation. The 5-instar-form was more common (21.8%) than the 7-instar form (9.1%). Except for the missing 6th instar, head capsule widths of the 5-instar form were similar to that of the 6-instar form, the mean and ranges of the latter shown in Figure 4.1. Neighbouring larval instars were clearly separated by their head capsule widths except for the last two instars. Overlapping in the head capsule width ranges of the last two instars may have been due in part to the fact that the head capsule widths of the last instar were estimated by using the head widths instead of being measured on the head capsules as in the other instars. Development times of individual larval instars and of pupae
are given in Table 4.1. Total length of larval and pupal period between the 5- and 6-instar-form were close, averaging 33.25 days and 33.53 days, respectively. The development time of the 7-instar form took, on average, an extra time of five days. However, the lengthened development time needs to be verified as the number of larvae showing this form was very low (Table 4.1). Irrespective of development forms, lengths of the last instar were considerably longer than that of any other instars, accounting for over one third of the total larval period. The differences remained apparent after the time spent as prepupae was deducted from the last instar, except in comparison with the 3rd instar of the 5-instar-form. The last instar larvae turned blue in 2-4 days, with about three quarters of the last instar spent as blue larvae. The mature larvae did not stop feeding upon turning blue: they continued to feed for ca. 3 days before constructing the pupation cells. The non-feeding prepupal stage took an average of 3.1-3.8 d (range 2-6 d), accounting for 12.6-13.4% of the total larval period. Within-stage variation in development times was smallest in the pupal stage, with the standard deviation within 7% of the mean for the 5- and 6-instar forms. No significant difference in the means of the combined development time of larvae and pupae between males (averaging 34.9 d) and females (averaging 35.0 d) was detected (P> 0.1, Welch modified t-test).

Sex ratio, pupal weight, adult life span and fecundity

Examination of 319 pupae revealed no significant deviation of sex ratio from 1:1. (P>0.1, Chi-square test). Pupal weight averaged 150.2 mg (± 26.2 mg), ranging from 80 mg to 221.2 mg. Female pupae (154.5±28.6 mg) were significantly heavier than male pupae (145.6 ± 22.5 mg) (P< 0.01, Welch modified t-test). However, the ranges of pupal weight in the two sexes were highly overlapped and it was impossible to tell the sex of an individual pupa based on either its length or maximum width (casual observation). With the provision of 5% sugar solution, females lived for an average of
9.76 days (5-15 d) and males 8.5 days (3-16 d). The difference in the life span between the two sexes was not significant (P > 0.1, Welch modified t-test).

Within females, mated individuals lived significantly longer (11.1 ± 2.3 d) than unmated individuals (8.5 ± 2.9 d) (P < 0.05, Wilcoxon rank sum test).

The number of eggs laid per female, determined from a sample of 28 females, ranged from 24 to 193, with a mean of 93. Dissection of newly-dead gravid females showed large numbers of unlaid eggs in some individuals.

The potential fecundity, as estimated from the sum of the number of eggs laid and the number of mature eggs retained in the ovary, ranged from 112 to 276, with a mean of 168.

**Mortality during the larval and pupal stage**

Total mortality during the larval and pupal stage ranged from 23% to 54%. The mortality was highest during the 1st instar (17-41%) and relatively low in the later stages. A typical mortality-age curve is shown in Figure 4.2, with data taken from the development study. Of the 114 larvae reared individually on the CGD, ca. 36% died during the first instar, accounting for ca. 70% of all larval and pupal deaths. Except for the second instar, which recorded a 12.33% death rate, mortalities during the later larval instars were below 7%. No death was recorded in the pupal stage. Among those that reached the third instar, 91.5% survived to the adult stage.

4.3.2 Non-diet factors on egg hatching and the survival of neonates

**Egg hatching rates**

Only 4 of the 60 eggs (6.7%) placed directly on top of the diet hatched. A hatching rate of 58.3% was achieved for eggs placed in the empty jar, similar to that (63.3%) in the diet jar where larvae were separated from the diet by aluminium foil. Some of those that failed to hatch died after the completion of embryo development, as indicated by the formation of the outlines of the larvae under the egg shells.
Survival of neonates

The neonates lived for a maximum of 60 hours in the absence of food, with a mean of 36 hours. The first-3-day survival rates of neonates decreased as the length of the hunger period increased. Neonates transferred to the diet within 6 hours of their hatching achieved 73.8% survival during the first 3 days while only 12.5% of those transferred after 36 hours survived. The survival rate for those transferred after 24 hours was 51.3%, which was significantly lower than for those transferred within 6 hours (P< 0.05, Chi-square test).

Larvae hatched from eggs laid on paper towels started feeding more readily than those from eggs laid on host leaves, as seen in the significantly higher first-3-day survival rate for the first group (68.3%) in comparison with the latter group (40.0%) (P<0.05, Chi-square test).

Survival rates of the neonates decreased with the size of the rearing containers. Rearing the neonates in the plastic tubes yielded the least dead larvae during the first 3 days (22%), followed by that in the plastic jars (46%) and the glass jar (74%). The differences were all significant (P> 0.05, Chi-square test)

4.3.3 Feeding enhancement

CGD fortified with leaf powder

Feeding preference of young larvae toward the leaf powder fortified diet was overwhelming. A total of 84 live larvae (2nd-3rd instar) were recovered at the end of the experiment. In four of the five plastic vials all larvae were found on or inside the leaf powder diet. In the other plastic vial, 3 larvae were found feeding on the plain diet and 14 on the treatment diet. The feeding preference was supported by the relative densities of feeding scars on the two diets, which were consistently higher on the fortified diet than on the plain diet.
CGD fortified with the ethanol extract of young shoots and leaves

Feeding on the CGD was apparently improved with the addition of ethanol extracts of young shoots (Figure 4.3). Mortalities of the neonates in the first 24 and 48 hours of introduction were reduced to below 14% and 30%, respectively, about half of that on the plain diet. While 73% of those reared on the plain diet died during the first two instars, the majority of the larvae (>62%) reared on the fortified diet successfully developed into the 3rd instar. Among the three extract concentrations tested, the effect of feeding enhancement was most obvious when the extract was incorporated at the concentration of 10 g FWE/50 g diet. The feeding enhancement effect was more convincingly shown when both the plain and fortified diet were placed in the same plastic tubes (Figure 4.4). Daily inspections in the first 3 days after larval introduction consistently showed more larvae on the fortified diet than on the plain diet in all the test tubes. Direct evidence of feeding enhancement was shown in the density of surface tunnels and entry holes at the end of the experiment. These feeding scars were found densely distributed in all the 8 fortified diet cores, whereas only 2 control cores showed apparent feeding symptoms.

4.3.4 Effects of wind on mating success

Mating was not observed in the windless cage in any experiments during the three observation nights when wind was completely blocked, whereas the ventilated cage produced a total of 11, 9, and 6 copulations per experiment. However, on the fourth night, when wind was provided, 3, 5, and 3 females mated in the windless cage in the three experiments, respectively. Under alternating wind and windless conditions, mating did occur during the windless intervals, although it was less frequent. The number of copulations per hour was consistently lower for the windless intervals than that for the wind intervals (Table 4.2).
4.4 Discussion

The study has shown that, *H. robusta* occurring in Australia can be reared successfully on the CGD. The insect had been maintained continuously in the laboratory for 23 generations. The colony reared in this study compared favourably to other colonies of *H. robusta* reared on synthetic diets in terms of pupal weight and the numbers of eggs laid per female but the development time from egg to adult in this study was a little longer (Table 4.3), the latter may be due partly to the different temperature regimes used. Pupal weight in this study was higher than those reported for field individuals by Atuahene and Souto (1983). Duration of development was comparable to that observed for individuals reared with host tissues (see Chapter 5).

Inconsistency exists in the published reports concerning the number of larval instars. While the same pattern of variation in development forms as revealed in this study was seen in the artificial rearing of Atuahene and Souto (1983), only 5- and 6-instar forms were reported by Couilloud and Guiol (1980). Discrepancies between artificially reared and field populations were even greater. In the two classical papers on the biology of *H. robusta*, Beeson (1919) and Roberts (1966) reported only four larval instars. The inconsistency cannot be explained solely by possible differences among regional populations of *H. robusta*, as different development forms were observed in studies of the same regional populations (Atuahene and Souto, 1982 vs Couilloud and Guiol, 1980) and the same number of larval instars was reported for different regional populations (Beeson, 1919; Roberts, 1968). The choice of diet cannot be held solely responsible either, as seen in the agreement between the results of Atuahene and Souto (1983) and those of this study, although different diets were used. While the combined effect of population origin and diet cannot be ruled out, it appears more likely that certain instars were overlooked in some studies, especially those of a field
nature. Due to their cryptic nature and small sizes, larvae of the first and second instars are difficult to find in the field. Dissections of infested tissues mostly revealed larvae already in their third or later instars (casual observation). The number of larval instars does not appear to be controlled by sex, as the three development forms were recorded for larvae of both sexes. Irrespective of the development forms, the longest development time was recorded in the last instar, accounting for about one third of the total larval period. Food consumption rate during the last instar was also higher than that during any of the other instars, as seen in the need for more frequent supply of diet (casual observation). The combined effect of the relative long duration and high food consumption rate would make the last instar the most destructive stage, at least in terms of quantitative damage that would be incurred to the host tree. Except for the relatively rare 7-instar form, the pupal stage was the longest and yet the most stable development stage (Table 4.1), making the onset of pupation an ideal predictor of the timing of adult emergence.

The CGD apparently lacks the feeding stimulants that the insect has become adapted to in the field. Relatively high proportions of larvae died during the first instar, apparently due to non-feeding. Feeding became less of a problem once initial feeding commenced, as reflected in the much lower mortality rates among older larvae. The non-feeding problem was particularly prominent during initial rearing trials, when the neonates were directly transferred to relatively large containers containing bulky diet, as was the technique used by Couilloud and Guiol (1980) in their use of the CGD. The same feeding problem was reported for *H. robusta* populations in Africa (Atuahene and Souto, 1982) and for the sibling species *H. grandella* in Latin America (Creasap, 1973). This is not surprising, as an artificial diet is usually drastically different from the natural diet both in chemical and physical properties (Moore, 1985).
Several non-diet factors influenced the survival of the neonates. The degree of non-feeding of the neonates differed with the substrates on which eggs were deposited. Larvae hatched from eggs laid on paper towels began feeding on the artificial diet more readily than those on host tissues (leaves, leaf petioles or shoot stems). The likely reason is that larvae hatched from eggs on host tissues had already taken their first meal in the host tissues before being transferred to the artificial diet, causing them to avoid any unnatural and less 'tasteful' food. The size of the rearing containers also influenced the mortality rates due to non-feeding. In comparison to rearing in the relative large glass jars and the plastic vials with the diet flatly packed inside, rearing in the small plastic tubes, with the diet supplied as cylindrical cores, resulted in relatively low percentages of neonate deaths. Apart from the size of the rearing containers, the way the diet was presented may have also contributed to the difference, as the cylindrical cores resembles the shape of the shoots, the preferred feeding tissue of the larvae in the field. Rearing the neonates in small containers has the additional advantage of maintaining fresh diet, as the latter can be easily replaced without losing track of the reared larvae. Finally, survival rates of the neonates can be increased by shortening their pre-feeding hunger period, as larvae transferred to the diet shortly after their hatching (< 6 hours) showed significantly higher survival rates than those transferred after 24 hours or later. However, placing the eggs directly on top of the diet is to be avoided, as the practice was found to result in extremely low egg hatching rates, probably due to the high acidity level of the diet (pH 4.8). The best way appears to be placing the eggs inside the rearing containers but separating them from the diet with non-water-absorbent material, as in Couilloud and Guiol (1980).

More significant improvement in the feeding of the neonates lies in the introduction of some form of feeding stimulants that are present in the host tissues into the artificial diet. Evidence for the existence of feeding
stimulants in host tissues was shown in increased survival rates on fortified diets. The nature of the feeding stimulants was not investigated. They were found to be present in dry leaves and in the ethanol extracts of young shoots and leaves. The fortified diets were not used in the normal rearing of the insect in this study as they were less chemically defined and more difficult to standardise. More detailed discussions on the feeding stimulants of the insect are presented in Chapter 6.

Another important aspect in the artificial rearing of the insect is adult mating. As noted elsewhere (Beeson, 1919; Grijpma, 1971; Atuahene and Souto, 1985; Fasoranti, 1985), mating was found to be difficult in indoor cages during the early phase of artificial rearing. Cages of various sizes have been tried, from small wooden cages (35 x 35 x 35 cm) to a large nylon mesh enclosure (195 x 170 x 140 cm) occupying much of the rearing room, but mating rates remained low. Provision of host plants did not seem to help either. The problem was solved by blowing wind through the mating cages.

Wind showed direct and indirect enhancement of mating success. Mating frequencies were significantly higher in the presence of wind, whether applied continuously or alternately. In the wind and windless cage experiments, the result was extreme: no mating occurred in the windless cage during the first three nights when wind was blocked, whereas copulations took place in the ventilated cage each night. The absence of mating in the windless cage did not seem to be the result of accidentally allocating sexually inferior moths to that cage, as mating was recorded on the fourth night when wind was provided. In the alternating wind and windless experiments, the number of matings per hour was consistently higher for the wind intervals than for the windless intervals. Previous exposure to wind appears to enhance the mating activity, as a number of females mated during the windless intervals under alternating wind and windless conditions. The result indicates that current or past exposure to
wind both enhances mating success, but simultaneous presence of wind is not a pre-requisite of the mating process.

The mechanism for the mating-enhancement effect of wind is not clear. It is suspected that the relatively easy locating of calling females by males in the presence of wind may be partly responsible. Other contributing factors may be: (a) wind stimulates flight, which, in turn, speeds up the sexual maturity of the moths, and (b) males and females select their mates before mating and the chances for suitable matching pairs to end up in mating would be much lower in still air than in moving air. Hypothesis (a) is supported by the finding of Fasoranti (1985), who demonstrated that mating of H. grandella could be greatly enhanced by flying the moths in a windmill for some time before subjecting them to mating cages. The mate-selection hypothesis is supported by the observations that some males came into direct contact with calling females without showing any courtship behaviour and some calling females walked away from approaching males. When the number of sexually-active males and females is low, such mate-selection behaviour may result in the absence of mating.

The positive association between wind and mating may be partly responsible for the relatively low infestation levels of host trees planted inside forests as compared to those planted in the open, a pattern widely reported on Hypsipyla spp (see Entwistle 1967; Newton et al. 1993). Wind inside a dense forest may, at times, be so low that the process of locating calling females by males is hampered and the pre-courtship flight of the moths suppressed. It is worth while investigating whether moths emerged from such a habitat may choose to fly out of the forest to engage in their reproductive activities.
Table 4.1. Duration of development (days) of *H. robusta* in individual larval instars and the pupal stage for the 5-, 6-, and 7-instar development forms. Data from larvae that died before reaching the adult stage were not included. Larvae were reared with the CGD at the temperature of 25±2°C and the light period of 14L:10D.

<table>
<thead>
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<th>Stage</th>
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<th>7-instar</th>
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<tr>
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<td>mean</td>
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<tr>
<td></td>
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<td>SD</td>
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</tr>
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<td>3.1</td>
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</tr>
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<td>3.8</td>
</tr>
<tr>
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<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
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</table>

Data from larvae that died before reaching the adult stage were not included. Larvae were reared with the CGD at the temperature of 25±2°C and the light period of 14L:10D.
Table 4.2. Number of females mated per hour under alternating wind and windless conditions. Each experiment lasted the entire 10-hour scotophase and began with the wind interval.

<table>
<thead>
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<th>Number of moths</th>
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<tbody>
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<td>20</td>
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<td>12</td>
<td>20</td>
<td>60</td>
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</tbody>
</table>
Table 4.3. Performance of the laboratory colony in this study relative to other artificially reared colonies, as judged by development rate, pupa weight and adult fecundity

<table>
<thead>
<tr>
<th>Source</th>
<th>Temp. (°C)</th>
<th>Egg-Adult (days)</th>
<th>Pupal weight (mg)</th>
<th>Eggs laid female⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achan (1968)</td>
<td>n.a.*</td>
<td>46-51**</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Atuahene &amp; Souto (1983)</td>
<td>26-28</td>
<td>31-41</td>
<td>89</td>
<td>92</td>
</tr>
<tr>
<td>This study</td>
<td>24-26</td>
<td>35-45</td>
<td>150</td>
<td>155</td>
</tr>
</tbody>
</table>

*n.a.: not available; ** egg hatching to adult emergence
Figure 4.1. Mean and ranges of head capsule widths of 6-instar-form larvae of *H. robusta* reared on the CGD. The head capsule widths of the last instar were estimated with head widths.
Figure 4.2. Stage-specific mortalities of *H. robusta* reared on the CGD.
Figure 4.3. Mortality rates of *H. robusta* larvae reared on the CGD fortified with 95% ethanol extract at various concentrations (fresh weight equivalents (FWE) of host tissues in 50 g of the CGD) and on the plain CGD in non-choice tests in plastic tubes (76 x 10 mm).
Figure 4.4. Relative performance of the fortified and plain CGD in arresting feeding by neonates of *H. robusta* as measured by the number of larvae on the respective diet, provided as cylindrical cores (20 x 4 mm), in the first three days in a dual-choice test in plastic tubes (76 x 10 mm). Larvae not in contact with neither the fortified diet nor the plain diet were recorded as 'not on diet'. The fortified diet was incorporated with 95% ethanol extract at the concentration of 5 g of fresh weight equivalent of host tissue in 50 g plain diet.
Chapter 5

Temperature-dependent Development of *Hypsipyla robusta* Moore

5.1 Introduction

The relationship between insect development and temperature has long been appreciated (see Wagner *et al.*, 1984a). Understanding this relationship is critical for accurate scheduling of census samples and control strategies. For any species, development occurs within a narrow temperature range, characterised by a lower and upper threshold (Lamb, 1992). Two different approaches have been used to describe insect development within this tolerable temperature range, i.e., the linear approach and the non-linear approach. With the linear approach, the development rate (reciprocal of development time) is taken as a linear function of temperature (Campbell *et al.*, 1974; Pruess, 1983). The underlying hypothesis is that the total thermal units, given by the sum of time units (days or hours) with temperature above a certain threshold required to complete the development, is constant. Despite its simplicity, this approach often yields approximately correct values and is widely used (see Wagner *et al.*, 1984a). However, most authors argue that linear development is valid only over intermediate temperatures. Over the entire range of tolerable temperature, the relationship between development rate and temperature often appear as curvilinear, characterised by a lower asymptote of zero in the left region, an approximate linear section in the mid-region, and a falling-off section in the right region (Wagner *et al.*, 1984a). A number of mathematical equations has been proposed to describe the curve nature of the relationship. Some of the widely used models are: the logistic equation of Davidson (1944), modified sigmoid (Stinner *et al.*, 1974), various matched asymptotic curves (Logan *et al.*, 1976; Hilbert and Logan, 1983), and the
biophysical model of Sharpe and DeMichele (1977) modified by Schoolfield et al. (1981). These and other empirical models describe part or all of the response curve. The biophysical model is favoured because of its basis on biophysical laws (Wagner et al., 1984a). Furthermore, the model can be easily reduced to simpler forms to cater for particular temperature ranges. However, insofar as goodness-of-fit is concerned, different non-linear models may appear equally acceptable (see Worner, 1992, Liu et al., 1995). Sometimes simple linear models perform even better than non-linear models in this respect (see Roltsch et al., 1990). Parameters in rate models, linear or non-linear, are estimated with constant temperature data. The rate models can be used to describe or predict the mean duration of development of field populations experiencing variable temperatures. This is achieved by summing up fractions of development rates at discrete intervals until the sum reaches unity, i.e., rate summation (Kaufmann, 1932). Conflicting reports exist concerning the applicability of rate models to variable temperature conditions. Given the same mean temperatures, development under fluctuating temperature regimes can be accelerated, decelerated, or similar to that at constant temperatures (see Worner, 1992). Reasons for such variable reports were attributed to the limited temperature ranges in which the studies were carried out (Worner, 1992; Liu et al., 1995). When a full range of variable temperature range is investigated, development is generally more rapid with lower mean temperatures and greater diurnal fluctuations (Worner, 1992). A recent study by Liu et al. (1995), based on a re-examination of published data, demonstrates that the disparity in development between constant and variable temperature conditions results primarily from the curve nature of the relationship between development rate and temperature and is less likely to be caused by any physiological charges of insects.

Insects held under identical temperature and other experimental conditions often complete development at different times due to inherent
differences among individuals in a population (Wagner et al., 1984b). The stochastic nature of development also may have contributed to the observation that, in field populations, samples drawn at the same dates rarely reveal individuals in the same developmental stages (Dennis et al., 1986). Individuals at different development stages may cause different damage levels to the host plants and may show different susceptibility to particular control methods (Shoemaker, 1979). Consequently, pest managers are concerned with estimating the proportion of the insect population in various stages over time, as well as predicting the times at which the majority of the population is in those stages most receptive to control agents (Stedinger et al., 1985).

Proportions of a population in individual developmental stages can be estimated by ordinal regression models (Candy, 1991). A biologically based, yet easy to use, model in this category is given by Dennis et al. (1986). The model assumes that the development of a given insect is a stochastic process consisting of accumulated small increments of development time (measured by degree-days) starting at a given stage (e.g. egg). As the amount of development increases, the insect passes through successive stages, delimited by a set of signpost values corresponding to the development times necessary for successive moults. The stochastic process takes the logistic distribution as its probability distribution. One of the advantages of the model is that it requires, in essence, no more than the proportions of various stages sampled at discrete intervals and the corresponding accumulated degree-days for its calibration (Candy, 1991; Munholland and Dennis, 1992). Hence it can be used in situations where only the relative abundance of the insect is sampled, e.g. data from sweepnets or traps. Statistical inference of the model is provided by Dennis et al. (1986) and Dennis and Kemp (1988). The model has been successfully applied to a number of insect species (e.g. Kemp et al., 1986; Kemp and Onsager, 1986). This and other similar models (Osawa et al., 1983; Stedinger et al., 1985) are
based on population attributes rather than the progression of individuals through stages over time, which are more biologically appealing (Candy, 1991). The latter are dealt with by a group of models termed by Munholland and Dennis (1992) as microscopic models (Manly, 1974, 1989; Kempton, 1979; Munholland et al., 1989; Munholland and Dennis, 1992). However, they suffer from the limitation that development depends on only a single time-dependent variable (Candy, 1991). Additional drawbacks are difficulty in parameter estimation and convergence problems (Munholland and Dennis, 1992). As an alternative to the ordinal regression approach, Candy (1991) proposed the use of continuation ratios (conditional probabilities) and generalised linear models to model the insect population phenology. These models may perform better than ordinal regression models in predicting stage-specific proportions, but lack a biological basis (Candy, 1991).

Instead of describing directly the stage-specific frequency data on population development, Wagner et al. (1985) present a rate-summation approach for modelling the development times of individuals of a population. This approach integrates a temperature-dependent rate function and a probability distribution into a simulation model, with the rate function determining the median rate of development per day at a given temperature and the distribution function (Wagner et al., 1984b) determining the fraction of cohort development at a given accumulated rate. Their model can be used to predict the cumulative proportions of individuals completing the development of a particular stage through time under variable field temperatures but it appears to be unsuitable to describe proportions of individuals at different stages at given times.

No studies have been attempted to quantify the temperature-dependent development of Hypsipyla robusta Moore. Reports concerning the development of this species are mostly given in the form of the number of generations per year (2-10 generations/year) or generation times (21-79 days, non-aestivation generations) (Beeson, 1919, 1941; Roberts, 1966, 1968;
Entwistle, 1967; Morgan and Suratmo, 1976). Generalisation of the development pattern of this insect based on these reports is impossible, as they were obtained from different hosts and the corresponding temperature regimes were not provided. The only data relating development to temperature were from studies of artificial rearing (Couilloud and Guiol, 1980; Atuahene and Souto, 1983). However, the effect of temperature on development was not investigated as each study used only one temperature. In view of the need for a better understanding of the development of *H. robusta* populations, the development of the insect on an artificial diet was studied at a series of constant temperatures. In addition to the relationship between mean development rates and temperature, an attempt was also made to describe the variations of development among individuals. Parallel studies were carried out on the development of the insect reared on host tissues at certain temperatures. Young and tender shoots, the natural host tissues of the insect in the field, were not used as the standard food as they tend to lose their freshness quickly in the laboratory, either because of fungus infection or desiccation. Furthermore, the abscission shoots may not fully represent the standing shoots as the nutrient and water flow are cut off.

5.2 Materials and Methods

5.2.1 Source of test insects

Test insects used in this study were from a laboratory colony maintained on the artificial diet of Cuilloud and Guiol (1980) since late 1992 (see Chapter 4). Mature larvae or pupae of the insect from the field site at Macksville, NSW, with which the colony was originally established, were introduced into the colony at least twice a year. Part of the colony was sometimes reared on potted Red Cedar trees grown under glasshouse conditions. These insects were pooled with the rest of the colony at the egg or pupal stage.
5.2.2 Test temperatures and other conditions

Development of the test insects was studied at 5 constant temperatures, 16.4, 19.5, 22.3, 26.2 and 28.7°C, with a daily variation ±1°C. Light period was set at 12L:12D. Humidity was not strictly controlled. However, relative humidity inside the rearing container was high, as fresh, wet diet was maintained in the container.

5.2.3 Test procedure and data collection

Newly hatched larvae of ≤12 h old were individually transferred with a fine brush to plastic tubes (76 x 10 mm) containing 2 cylindrical cores (40 x 4 mm) of the artificial diet and capped with cotton wool. Experiments at each temperature started with 60 larvae. Replacement of the diet was made if the old diet was nearly completely consumed or considered no longer fresh, as indicated by the dark brown colour. Development progress of individual test insects was checked daily until moth emergence. The data recorded were the dates of each moult of larvae, pupation, moth emergence, and when the larvae were found dead. The cast skin and head capsules were removed upon detection. An individual was considered to have advanced to the next stage in the last 24 hours if new exuviae, pupa, or moth was discovered on the day of checking. Larvae that were not seen were recorded as 'NS'. If the NSs were recorded for three consecutive days, the corresponding diet cores were dissected on the third day. Events noted on that day, moulting or death, were assumed to have taken place during the second day. Pupae were sexed according to Sharma and Singh (1980) after the hardening of the pupal skin.

To compare the development rates between larvae reared on the artificial diet and those reared on host tissues, a parallel experiment was conducted at 25°C. Sixty larvae each was tested with the artificial diet and Red Cedar shoots. Fresh and tender shoots (<5 mm in diameter) were cut into sections of about 20 mm in length. One such shoot section was
provided to each test larva. The food was replaced every 1-3 days, depending on the rate of consumption of the larva and the freshness of the shoot. The experiment started with newly hatched larvae (≤12 hrs old) and ended with moth emergence. The emergence dates were recorded individually.

Development of eggs was studied separately from the rest of the stages. One hundred eggs laid on paper towels within the last 12 hours were tested at 20 °C and 25 °C. The eggs were placed in groups of 25 in petri dishes (90 mm diam.) lined with moistened filter paper. The number of eggs hatched was checked daily until 2 days after the day when no more hatching was observed. Hatched eggs were identified by their transparent egg shells or fragments of egg shells. The number of un-hatched eggs at the end of the experiments was also recorded to serve as a check on the total number of hatched eggs.

5.2.4 Data analyses

Rate models

As test temperatures in this study fell in the intermediate range, the mean development rates (r) from egg to adult at the five constant temperatures (T) were fitted with linear models. Re-arrangement of the linear equation gives estimates of the development threshold, T₀ (°C), and the thermal constant required to complete the development, K (degree-days, DD):

\[ K = \frac{1}{b}, \quad T_0 = -\frac{a}{b} \]

where a, b are estimates of the intercept and slope of the linear regression, respectively. Theoretically, the potential number of generations per year expected in field populations can be estimated by dividing the annual accumulated DD above the threshold temperature in the field by the thermal constant. Although the rate models in this study were constructed
with data from insects reared on artificial diet, a study by Couilloud and Guiol (1980) indicated similar development rates of *H. robusta* reared with this diet and those observed in field populations. Hence an attempt was made to estimate the number of annual generations of the insect in the field site in Macksville, NSW, where infestation levels of the insect had been sampled systematically. As daily temperatures were not recorded all year round in the field site, temperature data from a weather station in Urunga, about 30 km north of Macksville, were used to calculate the annual accumulated DD above the threshold temperature of $T_0$. The data were obtained from the Australian Weather Bureau and consisted of daily maximum and minimum temperatures covering a period of 3 years from 1992 to 1994. In calculating the annual accumulated DD, hourly temperatures were first estimated from the daily maxima and minima assuming the sinusoidal temperature regime (Hudes and Shoemaker, 1988):

$$T_{ij} = \frac{1}{2} \left( \left[ \text{max}(i) - \text{min}(i) \right] \cdot \sin \left( \frac{2\pi \cdot j}{24} \right) + \left[ \text{max}(i) + \text{min}(i) \right] \right)$$

where $T_{ij}$ is the temperature at hour $j$ at day $i$, and max$(i)$ and min$(i)$ are maximum and minimum temperatures at day $i$, respectively. Based on $T_{ij}$, the annual accumulated DD was calculated as follows:

$$C = \sum_{i=1}^{365} \sum_{j=1}^{24} \frac{(T_{ij} - T_0)}{24}$$

subject to the restriction that

if $T_{ij} > 35^\circ C$ then $T_{ij} = 35^\circ C$

**Distribution of development time**

Relative frequencies of moth emergence were calculated daily to construct the cumulative probability distribution of the post-egg
development. The observed distribution was then fitted with the 3-parameter Weibull function:

\[
P(t) = 1 - \exp\{-(t - \gamma)/\eta\}^\beta
\]

where \( P(t) \) = the probability of completing development at time \( t \), and \( \gamma \), \( \beta \), and \( \eta \) are parameters to be estimated. Due to the temperature effect, distributions at different temperatures are positioned at different places along the time axis. To find a standard distribution representing all temperatures, development time was first normalised by dividing the times by their corresponding median time. The conversion removes the spatial displacement of individual distributions and aligns them to the normalised time of \( t^* = 1 \). Distributions of development time at different temperatures rarely have identical shapes. However, the shapes are usually similar and the 'same shape' property can be used to construct the standard distribution (Wagner et al., 1984b). The normalised times at which 1, 5, 10, ..., 95, 99, 100% of the test insects completed development were calculated using linear interpolation. These standard time or tau (\( \tau \)) values were then pooled across the temperatures to obtain the weighted means. Finally, the weighted means of the normalised development time and their corresponding standard cumulative probabilities were fitted with the Weibull function to generate the standard distribution of development time.

**Stochastic phenology models**

Differences in development rates of individuals results in the overlapping of neighbouring development stages. Stage-specific proportions of individuals at given times at a test temperature were studied with the logistic phenology model proposed by Dennis et al. (1986):
where \( P_i = \text{the proportion of insects in stage } i \text{ at sampling time } j \) \((j = 1, 2, \ldots, q, q = \text{number of sampling times})\), \( t_j = \text{DD accumulated at sampling time } j \), \( a_i = \text{amount of DD needed to complete the } i\text{th stage} \) \((i=1, 2, \ldots, r-1, r = \text{number of development stages})\), and \( b^2 = \text{positive constant measuring the spread in the distribution of development time. For completeness, } a_0 \text{ and } a_r \text{ are assumed as } -\infty \text{ and } \infty, \text{ respectively. The model was originally designed for progressive sampling data on stage-specific proportions (or numbers) of field populations with all individuals in a pre-determined initial stage at time } t_0. \text{ Theoretically, it is equally applicable to data from constant temperature experiments, although by doing so the original appeal of the model is lost. The primary objective of using this model in this study was to assess its appropriateness in describing the stochastic process of development of } H. \text{ robusta, rather than providing a practical phenology model of the insect. Only data from the larval and pupal stages were used to fit the model. Data from the egg stage were excluded as they were studied separately from the rest of the developmental stages.}

Larvae of \( H. \text{ robusta} \text{ exhibit variable development patterns in terms of number of instars, ranging from 4 instars (Beeson, 1919; Roberts, 1968) to a maximum of 7 instars (Atuahene and Souto, 1983). Artificial rearing of the insect by the author indicated a 5-7 instar range, with the majority passing through 6 instars before pupation (see Chapter 3). Considering the limited number of insects reared through at each temperature, only larvae showing the 6-instar-pattern were used to fit the model. Adding the pupal and adult stages, the total number of post-egg development stages was 8 (r = 8). The dates at which experiments at each temperature started were set as day zero. The amount of DD accumulated to the } j\text{th day was given by}

\[ t_j = j(T-T_0) \]
where \( T = \) test temperature, and \( T_0 = \) threshold temperature above which development occurs. \( T_0 \) was estimated from the linear rate model. It should be indicated that, when applying to sample data collected in the field, as the model was originally designed for, \( t_j \) should be calculated as the sum of \((T-T_0)\) measured at short intervals (hourly or shorter) from day zero to the \( j \)th day.

**Statistical analyses**

Parameters of the Weibull function and the logistic phenology models were estimated using the ‘nlin’ (non-linear) procedure of SAS (SAS Institute, 1982). All other statistical analyses were carried out in S-Plus (Everitt, 1994), including the fitting of linear rate models and hypothesis tests.

**5.3 Results**

5.3.1 Development forms and sex ratios

At all temperatures, the number of larval instars varied between 5 and 7 instars. Proportions of larvae of the 6-instar-form were consistently higher than those of the other two development forms, accounting for about 55 - 89\% of the test insects (Table 5.1). Except at the lowest temperature, larvae of the 5-instar-form were commoner than the 7-instar-form. Significant tests were not performed on the differences, due to insufficient numbers of test insects in either development form. Sex ratios were not significantly different from 1:1 at all temperatures except 19.5°C (\( P > 0.05, \chi^2 \) tests), where significantly more females emerged.

5.3.2 Duration of post-egg development

Duration of post-egg development decreased steadily as test temperature increased (Table 5.2). Average duration of larvae and pupae ranged from 28 days at 28.7°C to 81 days at 16.4°C. The effect of temperature
was more drastically shown in the larval period than that in the pupal period (Table 5.2). Within the little over 10°C increase in test temperatures, larval period was shortened by nearly 70%, while pupal period was reduced by about 50%. No further reduction in pupal period was seen after the test temperature was raised beyond 26.2°C.

No significant differences were detected between the development duration of males and females at all temperatures ($P > 0.05$, Wilcoxon rank sum tests) (Table 5.3). Despite having to pass an extra instar, duration of larval and pupal stages of the 6-instar-form was not always longer than those of the 5-instar-form (Table 5.3). However, duration of the 7-instar-form was consistently longer than those of the other two development forms. The size of the differences increased as the temperature decreased (Table 5.3). Again, differences in development duration among the three development forms cannot be compared statistically due to the low numbers of individuals in either the 5-instar-form or the 7-instar-form.

Average duration of post-egg development of larvae reared on host tissues and the artificial diet at 25°C was 35.8 days and 36.4 days, respectively. The difference was not significant ($P > 0.1$, Wilcoxon rank sum test).

5.3.3 Mean rate models

The relationship between mean development rates and temperature was closely fitted by the linear rate model ($r^2 = 0.99$, $F(1, 3)= 561$, $p=0.0000$) (Figure 5.1). Still better fit was achieved when data from the 6-instar-form was analysed separately ($r^2 = 1.00$, $F(1, 3)= 3369$, $p=0.0000$) (Figure 5.1). Based on the regression coefficients (Figure 5.1), the lower threshold temperatures ($T_0$) of the combined larval and pupal period were estimated at 10.0°C and 9.5°C, respectively, for individuals of all development forms and the 6-instar-form, and the thermal constants ($K$) estimated at 526.3 DD and 555.6 DD, respectively.
5.3.4 Number of generations per year

The thermal constants as estimated above show the amount of DD required for the completion of post-egg development. Separate experiments on the development of eggs at 20°C (n=58) and 25°C (n=73) indicated an average egg period of 7.2 and 5.5 days, respectively. Assuming the same threshold temperature as that of the post-egg development (10°C), this gives a weighted mean of 77.9 DD. The total number of DD for the period from egg to adult was thus estimated as 603.2 DD and 633.5 DD for all development forms and for the 6-instar-form, respectively. The annual accumulated DD above the threshold temperatures in Urunga in the period of 1992-1993 are shown in Table 5.4. Assuming similar development rates for the laboratory colony and field populations and continuous generations in the field, *H. robusta* would be able to complete a maximum of 7-8 generations per year in the Urunga region during the three-year period.

5.3.5 Distribution of development times

Variable development rates among individuals were evident at all temperatures (Table 5.2). Emergence of the first and last adults were separated in time by at least 9 days. The gap became wider as temperature decreased, reaching a maximum of 38 days at 16.4°C. The negative association between the variations and temperatures was more clearly seen in the cumulative probability distributions of development time (Figure 5.2, A). The distributions were much more spread out at low temperatures than at high temperatures. Differences in the development rates of individuals caused some overlapping in the development times between neighbouring temperatures. In other words, some individuals subjected to a certain temperature were able to complete the development before all individuals subjected to the next higher temperature had entered into the adult stage. The degree of overlapping was high, with the exception of data from the lowest temperature tested.
Cumulative probability distributions of development times of the combined larval and pupal period at all temperatures were well fitted by the three-parameter Weibull function (Figure 5.2, A). Estimates of the parameters and the overall $r^2$ values at individual temperatures are given in Table 5.5. Despite their seemingly different shapes, the cumulative probability distributions at different temperatures aligned nicely after the normalisation of development time (Figure 5.2, B). Data points from different temperatures generally overlapped or fell within short distances of one another, with no clear systematic deviations with regard to temperature. Hence, a temperature-independent distribution can be used to characterise the distributions of development times at all temperatures. The weighted means of normalised development times when 1, 5, 10, ..., 95, 99, 100% of the test insects had completed development, or tau values, were well fitted by the Weibull distribution ($r^2 = 0.99$) (Figure 5.3), yielding the standard distribution of development times for all temperatures. Parameters of the distribution are given in Table 5.6.

5.3.6 Stage-specific development

Examination of the stage-specific development of the 6-instar-form larvae (Table 5.6) indicated that most of the post-egg development time was spent as the last instar larvae and pupae. Together two periods accounted for 48% - 54% of the total development duration. Duration of the last instar larvae was slightly shorter than that of pupae. Pre-last-instar development of individual instars, i.e. 1st-5th instar, appeared to take similar lengths of time. As temperature increased, mean duration of these decreased, reaching a minimum of 2.0-3.5 days, depending on instar, at 28.7°C. Variable development rates were evident at all stages. As a result, individuals of different stages were simultaneously present at any checking dates except for the first few days of post-egg development.
5.3.7 Logistic phenology models

Proportions of individual development stages of the 6-instar-form larvae at given sampling times were closely fitted by the logistic phenology model (Figures 5.4 - 5.8). Parameters of the models are shown in Table 5.7. Parameter \(a_i\) \((i=1, ..., r-1)\) indicates the amount of development in DD necessary for the insect to undergo the \(i\)th moult. According to the estimated values, development from larval hatching to moth emergence required 545-581 DD, which is in line with the thermal constant of 556 DD of post-egg development estimated from linear models. Parameter \(b^2\) measures the variability of the data and the resulting individual model. The larger the value of \(b^2\), the wider the distribution will be at any given time. The values of \(b^2\) in this study varied between 0.58 and 2.03, with no clear pattern in relation to temperature. The resulting models well described the temporal occurrence of each stage at each temperature, especially with respect to the peak occurrences. Depending on the test temperature, peak occurrences in degree-days (above 9.5 °C) of the 2nd- to 6th-instar and pupa stage took place at 76-101 DD, 127-155 DD, 174-202 DD, 230-253 DD, 322-349 DD, and 470-493 DD, respectively. Timing of the peak occurrence of the 1st-instar could not be estimated as all experiments began with newly hatched larvae, instead of with eggs. The estimated ranges of DD for the peak occurrences of individual stages at different temperatures were quite small, with a maximum of 28 DD, transforming to a real time range of less than one day at 25 °C. This is remarkable, considering the often unavoidable variations in test conditions other than temperature in different experiments. It highlights the strict thermal requirement of the development process of the insect. Visual examination of the figures indicated some deviations of the estimated starting and ending times from the corresponding actual times. Starting times were generally underestimated and ending times generally overestimated. However, the differences were mostly within the range of 40
Final instar larvae were not expected until after 190-240 DD and pupae were not expected until after 310-360 DD, the exact timing differing with test temperatures. Estimated starting times of the emergence of the moths were around 460-500 DD and continued until after 700-800 DD. Irrespective of test temperatures, final instar larvae and pupae were present over much longer periods than previous stages, with a difference of at least 100 DD. Such a pattern was well described by the logistic models. Overlapping of neighbouring instars was evident at all stages, except in the early periods of the 1st-instar, when experiments just began. At times, as many as 4 different stages were present.

5.4 Discussion

Rate of post-egg development of *H. robusta* appears to increase linearly with temperature in the range of 16.4 °C - 28.7 °C. The linear range probably extends to 15 °C - 30 °C. The result in itself, however, does not indicate a linear relationship between development rate and temperature. Non-linear development usually appears as linear in intermediate temperature ranges. Development outside this range is likely to be non-linear, as has been demonstrated with most insects on which development over the entire ranges of tolerable temperatures has been studied (see Wagner et al., 1984a). Fitting with non-linear models was not attempted in this study as the linear models satisfactorily described the relationship.

With linear development, the amount of thermal units, expressed as accumulated degree-days above the threshold temperature required to complete a certain stage, is constant. Such a property can be used to estimate the number of annual generations of field populations. Although development data in this study were obtained with larvae reared on an artificial diet, similar development rates of the insect were reported for laboratory colonies reared with this diet and for field populations (Cuilloud and Guiol, 1980). No significant difference was also noted in the present
study between individuals reared with this diet and those reared with plant host tissues. Based on the linear equations obtained in this study and weather data from the Australian Weather Bureau, the number of annual generations of *H. robusta* in the Urunga region was estimated as 7-8. The actual number of generations is probably lower, as the assumption of continuous development may not hold true in the field. Although the exact length is not known, the insect is suspected to display the habit of winter diapause (Chapter 3). Even without winter diapause, the estimate should be treated as a maximum, as the moths do not immediately mate and lay eggs upon emergence (Chapter 7). Fertile eggs of the species were usually obtained three days after moth emergence. Taking into account the above considerations, a more reasonable estimate of the number of annual generations in that region is probably 5-6. The estimate may also apply to *H. robusta* in the field site, as the latter is located within 30 km of Urunga. Further studies, such as the monitoring of the seasonal abundance of males through the use of traps containing sex pheromones, are needed to verify the estimate.

Individual insects completed development at different times, which increased as temperature decreased. At 16.4 °C, emergence of the first and last moth was separated by as much as 38 days. Cumulative probability distributions of development time were displaced in time by temperature because of its strong effect on development rate and were of slightly different shapes. However, distributions at different temperatures were satisfactorily described by a common cumulative probability curve after the normalisation of development time, in this study by the 3-parameter Weibull distribution. Theoretically, this temperature-independent distribution can be used to predict the development times of individuals in a population under variable temperatures using a rate-summation approach (Curry et al., 1978). Due to the limited range of temperatures
studied, the temperature-independent distribution obtained in this study may not fully represent the development under field conditions. However, the results indicate that the cumulative probability distributions of *H. robusta* at different temperatures share a certain degree of similarity in shape and can be standardised by appropriate normalisation procedures.

Differences in the development rates of individuals invariably result in the overlapping of development stages of an initially homogeneous insect population after some time. In this study overlapping involved as many as 4 consecutive stages. The degree of overlapping decreased somewhat late in the life cycles, when only two neighbouring stages were simultaneously present, e.g., pupa and moth. Proportions of individual stages were well described by the logistic phenology models. The models performed particularly well in estimating the timing of the peak occurrences of individual developmental stages, irrespective of the test temperatures. Pupae and the last instar larvae were present over much longer periods than previous stages, apparently due to the longer duration of the two stages. Such a pattern was also well described by the models.

The logistic phenology model was initially designed to deal with stage-specific frequency (or relative frequency) data of field populations sampled at discrete intervals. No prior knowledge of the insect's development at constant temperatures is needed. The model was tested here with constant temperature data to determine its general applicability in describing the development process of *H. robusta* populations. It is not intended as a practical phenology model of the insect. However, the stochastic characteristics as disclosed by the models may serve as a useful guide in understanding the development process of the insect in field conditions. Assuming similar development patterns of the insect under constant and variable temperature conditions, parameters of the model estimated through constant temperature data should be at least as accurate and stable as those estimated through sample data, since in the latter case
the data itself are often subject to sampling errors. Admittedly, the assumption may not be met and direct studies of the development of the insect in the field or, variable temperature conditions are needed before the establishment of practical phenology models.
Table 5.1 Percentages of individuals of different sex and development forms. Only those that survived to the moth stage were counted.

<table>
<thead>
<tr>
<th></th>
<th>16.4°C (n=35)</th>
<th>19.5°C (n=27)</th>
<th>22.3°C (n=33)</th>
<th>26.2°C (n=55)</th>
<th>28.7°C (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>males</td>
<td>45.7</td>
<td>33.3</td>
<td>51.5</td>
<td>50.9</td>
<td>50.0</td>
</tr>
<tr>
<td>females</td>
<td>54.3</td>
<td>66.7</td>
<td>48.5</td>
<td>49.1</td>
<td>50.0</td>
</tr>
<tr>
<td>5-instar-form</td>
<td>2.9</td>
<td>14.8</td>
<td>24.3</td>
<td>21.8</td>
<td>36.8</td>
</tr>
<tr>
<td>6-instar-form</td>
<td>88.6</td>
<td>81.5</td>
<td>63.6</td>
<td>69.1</td>
<td>55.3</td>
</tr>
<tr>
<td>7-instar-form</td>
<td>8.5</td>
<td>3.7</td>
<td>12.1</td>
<td>9.1</td>
<td>7.9</td>
</tr>
</tbody>
</table>
Table 5.2. Duration of post-egg development of *H. robusta* at 5 constant temperatures (days, mean ± SD (range)).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Larvae (n)</th>
<th>Pupae</th>
<th>Larvae+Pupae</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.4°C (n=35)</td>
<td>60.6±7.6 (52-89)</td>
<td>19.8±2.1 (14-26)</td>
<td>80.7±9.0 (71-109)</td>
</tr>
<tr>
<td>19.5°C (n=27)</td>
<td>41.3±5.2 (34-56)</td>
<td>16.4±3.2 (10-20)</td>
<td>57.7±5.8 (49-72)</td>
</tr>
<tr>
<td>22.3°C (n=33)</td>
<td>32.6±4.5 (26-38)</td>
<td>12.1±1.1 (11-16)</td>
<td>44.7±4.7 (37-55)</td>
</tr>
<tr>
<td>26.2°C (n=55)</td>
<td>25.1±2.6 (21-35)</td>
<td>8.8±0.7 (7-10)</td>
<td>33.9±2.7 (30-44)</td>
</tr>
<tr>
<td>28.7°C (n=38)</td>
<td>18.9±2.0 (16-24)</td>
<td>9.1±0.5 (8-10)</td>
<td>28.0±2.1 (25-34)</td>
</tr>
</tbody>
</table>
Table 5.3 Duration of post-egg development of different sex and development forms (mean ± SD / (range)) at 5 constant temperatures

<table>
<thead>
<tr>
<th></th>
<th>16.4°C</th>
<th>19.5°C</th>
<th>22.3°C</th>
<th>26.2°C</th>
<th>28.7°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>males</td>
<td>79.7±9.4</td>
<td>62.5±5.7</td>
<td>46.2±4.9</td>
<td>34.9±2.0</td>
<td>28.7±2.0</td>
</tr>
<tr>
<td>females</td>
<td>81.6±8.9</td>
<td>57.7±4.9</td>
<td>43.2±4.0</td>
<td>35.0±3.3</td>
<td>29.3±2.2</td>
</tr>
<tr>
<td></td>
<td>(71-109)</td>
<td>(51-67)</td>
<td>(37-50)</td>
<td>(31-44)</td>
<td>(26-30)</td>
</tr>
<tr>
<td>5-instar-form</td>
<td>74.0±NA*</td>
<td>61.0±5.4</td>
<td>42.3±3.5</td>
<td>33.3±2.5</td>
<td>26.6±2.2</td>
</tr>
<tr>
<td></td>
<td>(74-74)</td>
<td>(53-64)</td>
<td>(37-48)</td>
<td>(30-37)</td>
<td>(25-32)</td>
</tr>
<tr>
<td>6-instar-form</td>
<td>78.6±5.0</td>
<td>57.9±6.1</td>
<td>44.1±3.6</td>
<td>33.5±2.0</td>
<td>28.7±1.6</td>
</tr>
<tr>
<td></td>
<td>(71-90)</td>
<td>(50-72)</td>
<td>(38-51)</td>
<td>(30-39)</td>
<td>(27-34)</td>
</tr>
<tr>
<td>7-instar-form</td>
<td>101.7±11.8</td>
<td>66±NA</td>
<td>53.0±2.2</td>
<td>38.8±3.4</td>
<td>29.0±1.7</td>
</tr>
</tbody>
</table>

* NA: Standard deviations cannot be calculated due to unit sample size.
Table 5.4. Maximum number of annual generations of *H. robusta* in regions around Macksville, NSW, estimated from developmental data conducted at constant temperatures, assuming linear relationships between development rate and temperature and continuous generations. Annual accumulated degree days above the threshold temperatures (DD (T<sub>0</sub>)) were calculated from data taken at a weather station in Urunga, NSW, with the maximum development temperature arbitrarily taken as 35°C.

<table>
<thead>
<tr>
<th></th>
<th>6-instar-form</th>
<th>all development forms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DD (T&lt;sub&gt;0&lt;/sub&gt;)</td>
<td>generations</td>
</tr>
<tr>
<td>1992</td>
<td>4605</td>
<td>7.3</td>
</tr>
<tr>
<td>1993</td>
<td>4800</td>
<td>7.6</td>
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<tr>
<td>1994</td>
<td>4622</td>
<td>7.3</td>
</tr>
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</table>
Table 5.5 Parameters of the Weibull function estimated from the cumulative distributions of the development of post-egg development of *Hypsipyla robusta* reared on artificial diet at five constant temperatures and the pooled distributions under normalised time scale (=time/median time). Data from larvae of all development forms (5-, 6-, and 7-instar forms) were combined.

<table>
<thead>
<tr>
<th></th>
<th>16.4°C</th>
<th>19.5°C</th>
<th>22.3°C</th>
<th>26.2°C</th>
<th>28.7°C</th>
<th>pooled</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>71.58</td>
<td>46.21</td>
<td>36.07</td>
<td>28.32</td>
<td>22.94</td>
<td>0.88</td>
</tr>
<tr>
<td>β</td>
<td>8.27</td>
<td>12.24</td>
<td>9.23</td>
<td>5.67</td>
<td>5.06</td>
<td>0.16</td>
</tr>
<tr>
<td>γ</td>
<td>1.09</td>
<td>1.91</td>
<td>1.81</td>
<td>2.31</td>
<td>2.47</td>
<td>1.61</td>
</tr>
<tr>
<td>R²</td>
<td>0.99</td>
<td>0.99</td>
<td>1.00</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
</tr>
</tbody>
</table>
Table 5.6 Duration of individual development stages of the 6-instar-form larvae and pupae of *H. robusta* at 5 constant temperatures (days) (mean ± SD / (range)).

<table>
<thead>
<tr>
<th>Temperature</th>
<th>1st (n=31)</th>
<th>2nd (n=22)</th>
<th>3rd (n=21)</th>
<th>4th (n=38)</th>
<th>5th (n=21)</th>
<th>6th (n=21)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.4°C</td>
<td>8.6±1.8</td>
<td>6.4±1.3</td>
<td>9.0±2.2</td>
<td>8.0±1.7</td>
<td>8.8±1.4</td>
<td>18.2±2.8</td>
<td>19.5±1.8</td>
</tr>
<tr>
<td></td>
<td>(5-13)</td>
<td>(4-9)</td>
<td>(7-14)</td>
<td>(5-12)</td>
<td>(6-12)</td>
<td>(14-29)</td>
<td>(14-23)</td>
</tr>
<tr>
<td>19.5°C</td>
<td>4.9±1.8</td>
<td>5.5±2.3</td>
<td>5.0±1.2</td>
<td>4.7±1.0</td>
<td>6.2±1.3</td>
<td>14.7±2.7</td>
<td>16.9±1.7</td>
</tr>
<tr>
<td></td>
<td>(3-8)</td>
<td>(2-11)</td>
<td>(3-8)</td>
<td>(3-7)</td>
<td>(5-10)</td>
<td>(11-20)</td>
<td>(12-20)</td>
</tr>
<tr>
<td>22.3°C</td>
<td>4.1±1.0</td>
<td>4.6±0.8</td>
<td>4.2±1.4</td>
<td>3.8±1.1</td>
<td>4.5±1.1</td>
<td>10.2±2.2</td>
<td>12.7±1.0</td>
</tr>
<tr>
<td></td>
<td>(2-6)</td>
<td>(2-6)</td>
<td>(2-7)</td>
<td>(2-6)</td>
<td>(2-6)</td>
<td>(7-15)</td>
<td>(11-15)</td>
</tr>
<tr>
<td>26.2°C</td>
<td>3.9±0.7</td>
<td>3.5±0.6</td>
<td>2.9±1.0</td>
<td>2.9±0.9</td>
<td>3.1±0.5</td>
<td>8.4±1.3</td>
<td>8.8±0.6</td>
</tr>
<tr>
<td></td>
<td>(3-6)</td>
<td>(2-4)</td>
<td>(2-6)</td>
<td>(2-6)</td>
<td>(2-4)</td>
<td>(6-12)</td>
<td>(8-10)</td>
</tr>
<tr>
<td>28.7°C</td>
<td>3.5±0.5</td>
<td>2.0±0.6</td>
<td>2.2±0.4</td>
<td>2.5±0.7</td>
<td>3.0±0.5</td>
<td>6.2±1.3</td>
<td>9.2±0.4</td>
</tr>
<tr>
<td></td>
<td>(3-4)</td>
<td>(1-3)</td>
<td>(2-3)</td>
<td>(2-4)</td>
<td>(2-4)</td>
<td>(4-9)</td>
<td>(9-10)</td>
</tr>
</tbody>
</table>
Table 5.7 Estimated values of the parameters of the logistic phenology models established for the 6-instar-form larvae and pupae. The stage delimiters $a_1$-$a_7$ are given in degree-days (DD). $b^2$ is a measure of the variability of development.

<table>
<thead>
<tr>
<th></th>
<th>$a_1$</th>
<th>$a_2$</th>
<th>$a_3$</th>
<th>$a_4$</th>
<th>$a_5$</th>
<th>$a_6$</th>
<th>$a_7$</th>
<th>$b^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.4°C</td>
<td>61.98</td>
<td>105.6</td>
<td>168.4</td>
<td>223.6</td>
<td>284.7</td>
<td>409.9</td>
<td>545.1</td>
<td>0.58</td>
</tr>
<tr>
<td>19.5°C</td>
<td>51.1</td>
<td>105.6</td>
<td>152.7</td>
<td>200.0</td>
<td>264.2</td>
<td>411.7</td>
<td>580.7</td>
<td>2.03</td>
</tr>
<tr>
<td>22.3°C</td>
<td>57.7</td>
<td>116.9</td>
<td>170.5</td>
<td>218.5</td>
<td>275.1</td>
<td>405.4</td>
<td>570.2</td>
<td>1.51</td>
</tr>
<tr>
<td>26.2°C</td>
<td>72.5</td>
<td>131.4</td>
<td>179.6</td>
<td>226.9</td>
<td>280.1</td>
<td>420.7</td>
<td>567.5</td>
<td>0.56</td>
</tr>
<tr>
<td>28.7°C</td>
<td>76.7</td>
<td>114.1</td>
<td>157.0</td>
<td>205.3</td>
<td>262.9</td>
<td>382.7</td>
<td>560.5</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Figure 5.1. Mean development rates of *H. robusta* of the 6-instar-form and all development forms for the combined larval and pupal period at five constant temperatures.
Figure 5.2. Cumulative probability distributions of development time for *H. robusta* in the combined larval and pupal period. A: distributions in real time; B: distributions in normalised time.
Figure 5.3 Weibull function fitted to the weighted means of normalised development times when 1, 5, 10, ..., 95, 99, and 100% individuals completed development (tau values).
Figure 5.4 Stage-specific proportions of *H. robusta* during the progress of post-egg development at 16.4°C. Data fitted with logistic phenology model.
Figure 5.5. Stage-specific proportions of *H. robusta* during the progress of post-egg development at 19.5°C. Data fitted with logistic phenology model.
Figure 5.6 Stage-specific proportions of *H. robusta* during the progress of post-egg development at 22.3°C. Data fitted with logistic phenology model.
Figure 5.7. Stage-specific proportions of *H. robusta* during the progress of development at 26.2°C. Data fitted with logistic phenology model.
Figure 5.8. Stage-specific proportions of *H. robusta* during the progress of development at 28.7°C. Data fitted with logistic phenology model.
Feeding Behaviour of Larvae of *Hypsipyla robusta* Moore

6.1 Introduction

Feeding involves a consecutive sequence of behavioural components that are triggered and regulated by specific stimuli operating through neural pathways (Hsiao, 1985). For insects with chewing mouthparts, the sequence can be characterised by four steps: biting, ingestion (swallowing), continuation of feeding, and cessation of feeding (Dethier, 1966). The initiation and continuation of feeding require some form of positive stimuli, while the existence of negative stimuli terminate the normal feeding process (Bernays, 1985). Chemicals play a major role in regulating the feeding behaviour of insects (Bernays, 1985; Hsiao, 1985). Those that initiate or inhibit feeding are termed feeding stimulants (phagostimulants) and feeding deterrents, respectively. Some authors advocate the use of the terms feeding incitants and gustatory stimuli to refer separately to chemicals that evoke the biting responses and those that stimulate the ingestion phase, respectively (Beck, 1965). However, separation of the two feeding phases is often difficult in practice (Chapman, 1974; Schoonhoven, 1982). Phagostimulants may appear as primary (nutrients) and/or secondary plant metabolites (Thorsteinson, 1960). Often secondary plant metabolites are used by monophagous and oligophagous insects in selecting their food plants (see Fraenkel, 1959, 1969; Hedin et al., 1974). These chemicals are neither utilised nor required by the plant but the presence or absence of them determines whether or not it will be fed upon by the insect and, for this reason, they are sometimes called token stimuli or sign stimuli (Hsiao, 1985). For most phytophagous insects, however, multiple chemicals, either of primary or secondary plant metabolites, are believed to be involved in the selection process (Hsiao, 1985). Even in cases where dominant substances are known,
they alone do not account for host discrimination even for specialised feeders (see Miller and Strickler, 1984). Some chemicals may not elicit feeding by themselves but produce synergistic effects when combined with phagostimulants (Hsiao, 1985). In addition to external stimuli (positive or negative), the process of feeding is also influenced by the internal excitatory or inhibitory status of the insect. A marginally acceptable plant may be accepted if the insect is in a state of hunger (high internal excitatory input and low internal inhibitory input); the same plant may be rejected if internal inhibitory input outweighs the internal excitatory input (Dethier, 1982).

Effects of plant chemicals on the feeding process of insects are studied with various types of bioassays. A treatise on bioassay designs is provided by Lewis and van Emden (1986). Important aspects in the design of the bioassays are selection of feeding substrates, application of the chemicals or plant extracts, conditions of the test, and measurement and observation of the feeding responses. Bioassays on phagostimulants are usually carried out on artificial substrates, such as elder pith, filter paper, styropor, and agar-cellulose medium. The chemicals are applied and tested at a wide range of concentrations to search for response threshold and peak. Apart from the physical environment, such as light, temperature and humidity, the conditions of the test insects, such as age, sex and hunger status, should also be carefully controlled to bring about clear and consistent feeding patterns. For chewing insects, the responses of feeding can be measured directly by area or weight loss of the substrates or indirectly by the amount of faecal production or weight gain of the test insects. The adequacy of these measurements in quantifying insect feeding responses to test chemicals depends on a number of factors, e.g. the distribution of the chemicals in the substrates and the emptiness of the gut before the test. Sometimes the effects of chemicals can be detected by direct observation of the feeding process. This method, although time consuming, is considered as much more
revealing than a simple measurement of the total substrates ingested. Two approaches are used in searching for phagostimulants. One is to screen potential chemicals (primary or secondary plant metabolites) known to occur in the plant. The other approach starts with the crude extracts of host materials. The extracts are then fractionated and each fraction is assayed behaviourally for activity. Since the number of potential chemicals is large, the second approach is more practical (Hanson, 1983).

Four aspects of larval feeding behaviour are reported and discussed in this chapter. (a) Feeding on inert substrates treated with the ethanol extracts of host tissues. It has been shown in Chapter 4 that the incorporation of ethanol extracts of host-plan tissue enhanced larval feeding on the artificial diet. To further confirm the existence of phagostimulants in the extracts, the latter were tested using filter paper and agar-cellulose medium as feeding substrates. These substrates have the advantage of being chemically inert and thus avoid possible synergistic effects between the chemicals tested and chemicals present in the substrates. Chemicals present in the artificial substrates have been shown to interact with test chemicals in feeding bioassays (Reese, 1979). (b) The role of host odour in eliciting the biting response. The involvement of plant volatiles in host-plant finding has been well established (see Ramaswamy, 1988; Renwick and Chew, 1994). In some insects, odour also influences feeding behaviour, such as the elicitation of biting response (see Miller and Strickler, 1984). It is of interest to see whether host odour is involved in the feeding process of H. robusta. Insect response to olfactory stimuli is difficult to isolate from gustatory stimuli during the ingestion phase of feeding in bioassays, as some gustatory receptors of insects can also be stimulated by volatile chemicals (Hsiao, 1985). However, the effect of odour in the initiation of biting can be detected by measuring the relative biting frequencies of test insects in the presence or absence of odour prior to their contacting with the food. Results of this study will help us understand the mechanisms of the larval feeding process. (c) Suitability
of non-host species as larval food. Although no alternative hosts have been reported for *H. robusta* in Australia, the species was able to complete its life cycle on the introduced *C. odorata* in Africa (Roberts, 1966), suggesting the possible suitability of other Meliaceae species as larval food. There may be some other plant species that are acceptable to the larvae when provided artificially although they may be unsuitable for larval development or even be toxic to the larvae. Knowledge of the range of acceptable plants is helpful in understanding the chemical and physical requirement of feeding of the insect. Host range of *Hypsipyla* spp. is reported to be confined to the subfamily Swietenioideae (Grijpma, 1976). The specialisation may be linked to some common chemicals present in all the species in this subfamily. d) Within-tree distribution of feeding sites. Despite being termed a shoot borer, larvae of *H. robusta* have been reported to feed on various tissues of its host plants in addition to the growing shoots, such as flowers, fruits (Beeson, 1919) and bark (Roberts, 1968). Even the leaves were sometimes fed upon (Beeson, 1919). The diverse range of feeding sites prompted the suggestion for the existence of distinct regional species or subspecies (Roberts, 1968). While the possibility of the existence of regional subspecies cannot be rejected, the observed different feeding habits can be explained by some biological and environmental factors. As pointed out by Beeson (1919), fruit and flower generations of *H. robusta* occur at the time when the growing shoots are not widely available. Grijpma (1971) indicated that *H. grandella* would feed on the bark when the shoots had been fully utilised. Variation in feeding sites also differs with larval age. Young larvae tend to feed in buds and leaf petioles and extensive shoot feeding only takes place at later developmental stages (Beeson, 1919). Study of the within-tree distribution of feeding sites is important for the formulation of better sampling and control strategies.
6.2 Methods

6.2.1 Feeding on inert materials

*Agar-cellulose medium*

Agar-cellulose medium (4% of each constituent) was prepared according to Hsiao and Fraenkel (1968). The ethanol extract of young shoots was tested at 2.5, 5 and 10 g fresh weight equivalent (FWE) in 50 g of agar-cellulose medium. The extract was pipetted to the cellulose portion of the medium, which, after evaporation of the solvent, was combined with the agar solution. Control medium was made in the same way as treatment medium but incorporated with only pure ethanol. Control and treatment media were cast in glass bottles to a depth of ca. 0.5 cm. Cylinder cores of the test media (1 cm diameter) were cut out with cork borer. The effect of the ethanol extract in feeding was tested in binary-choice and non-choice experiments. In the binary-choice experiment, two treatment cores and 2 control cores were alternately arranged in the periphery of the petri dish floor with equal distances between neighbouring cores. One 6th-intar larva (non-blue stage) was introduced into the petri dish. Two tests of 20 replicates each were run in this experiment. The tests were conducted in darkness at 25 ± 2°C. The test cores were checked 24 h later and recorded as either chewed or nearly completely chewed. The non-choice test was conducted in a similar way except that only one cylinder core, control or treatment, was placed in the centre of the petri dish (90 mm diam.) floor and the test larvae were starved for 24 hours before being placed into the petri dishes. Apart from scoring the chewing intensities on the test cores as outlined above, the number of faecal pellets produced by each larva was recorded. Ten control and 10 treatment cores were tested for each extract concentration.

*Filter paper*

Filter paper disks (80 mm diam.) were dipped in either ethanol extract (treatment) or pure ethanol (control). After evaporation of the solvent, they
were tightly rolled to cylindrical shapes to resemble the shoot stems. The ethanol extract was tested at 3 concentrations: 0.05, 0.5, and 5 g FWE/ml, in 4-choice tests, with the control as the 4th choice. Four filter paper rolls, each with a particular extract concentration, were inserted through four evenly-spaced holes (10 mm diam.) bored in the periphery of the cap of a plastic jar (50 x 40 mm), with the lower ends of the rolls touching the vial floor. One 5th- or 6th- instar larva (non-blue stage) was introduced into each vial. Three tests, each with 15 replicates, were performed in darkness at 25 ± 2°C. Each test was run for 12 h. Evidence of feeding was recognised by the presence of chewing holes or roughened surface (surface chewing) in the filter paper. The same bioassay technique was used to test the relative strength of the feeding stimulant effect of the ethanol extract of young shoot stems, young leaves and young shoot stems plus young leaves, all tested at the concentration of 0.5 g FWE/ml. Two tests, each consisting of 20 replicates, were performed.

6.2.2 Host odour and initiation of feeding

Cuttings of freshly-collected young leaves or young shoot stems of approximately equal sizes were placed inside 1 cm sections of opaque drinking straws (4 mm diameter, red or blue, but the colour was fixed within individual experiments) and then blocked in the two ends with cotton wool. For controls, the straws were left empty but similarly blocked with cotton wool. Treatment and control straws were individually placed inside gelatine capsules (0.95 ml, transparent). Twelve small and evenly distributed holes of were punched in the top and bottom quarters of each of the capsules with entomological pins (0.6 mm diam.). These loaded gelatine capsules were then placed vertically in the bottom halves of petri dishes (90 mm diam.) by inserting them through pre-punched holes of slightly smaller diameter in the covering paper. The bottoms of the capsules touched the petri dish floor. About three-quarters of the length of the capsule were
inside the petri dish. Four gelatin capsules (two treatments and two controls) were alternately placed in each petri dish. The set-up of the test unit is shown in Figure 6.1. One 4th-6th instar larva was introduced into each petri dish and each test consisted of 15 replicate petri dishes. The experiment was run for 18 h in darkness at 25 ± 2°C. Feeding was considered to have been attempted if the gelatin capsules bore chewing holes. Five such tests were performed. To check for the uniqueness of the plant volatiles that could elicit the biting response, a similar test of 15 replicates was performed on the young leaves of the White Cedar, *Melia azadirachta* var. *australsica* (A. Juss.) C. DC.

6.2.3 Feeding on non-host species

Three non-host Meliaceae species, Spanish Cedar (*Cedrela odorata*), Chinese Toon (*Toona sinensis*) and White Cedar (*Melia azadirachta* var. *australsica*) and one non-Meliaceae species, Southern Blue Gum (*Eucalyptus globulus* ssp. *bicostata*), were tested for feeding by *H. robusta*. The Meliaceae plants were grown in pots in the glasshouse. The Southern Blue Gum grew mature trees outside the glasshouse. Acceptance of the three Meliaceae species by the larvae was first tested by individually placing 4-6th instar larvae onto five plants of each species on two separate occasions and observing their activity for one hour. A plant was considered to have been accepted if the larva completely burrowed into the shoot and did not come out before the end of the observation period. Infested shoots were dissected three days later to check for the feeding progress and the status of the larvae (live or dead, present or gone). The relative acceptance of the four test species as compared with the Red Cedar was assessed in binary-choice tests in plastic jars (50 x 40 mm). Two opposite holes (5 mm diam.) were drilled through the caps of the bottles. Fresh young shoots of the test plants of slightly smaller diameter than these holes, one from the Red Cedar and the other from one of the four non-host species, were inserted through the
holes until the lower ends touched the floor of the bottles. The upper ends of the shoots were ca. 0.5 cm above the caps. One 5-6th instar larva was introduced into each bottle and allowed to feed for 24 hours in darkness, at the end of which time the presence or absence of feeding tunnels and the relative area of epidermis removed were checked. Each plant species pair was tested with 20 larvae. As the leaves are readily fed upon by the larvae in the laboratory (personal observation), another binary-choice experiment was presented in petri dishes (85 mm diam.) using leaf discs as feeding material. Four holes (10 mm diam.) were punched in the periphery of a filter paper (Whatman No. 1, 80 mm diam.), which, after being moistened with distilled water, was placed on the floor of the petri dish. Leaf disks of the same diameter as the filter paper holes were punched from freshly picked young leaves of the test species. Four such disks, two from the Red Cedar and two from one of the non-host species were placed individually into the four holes with leaf disks of the same species opposite each other. A 5-6th instar larva was placed at the centre of the filter paper and the petri dish was covered. The larva was allowed to feed for 24 hours in darkness. The percentage of each leaf disk consumed by the larva was recorded at the end of the experiment. Each species pair was tested with 10 larvae.

6.2.4 Within-tree distribution of feeding spots

Test trees were 2-year-old potted Red Cedar trees maintained under glasshouse conditions. These trees had been artificially infested with *H. robusta* and old stems had been cut to a height of ca. 30 cm in the previous winter. Only one shoot was retained for each tree from the coppices. To standardise the test trees, each shoot was allowed to retain 8 mature compound leaves, the rest being removed manually from the bases. At the start of the experiment, the new shoots had grown to an average height of 122 ± 35 cm and an average basal diameter of 0.65 ± 0.08 cm. Larvae were from a laboratory stock originated from mature larvae collected in a Red
Cedar plantation in Macksville, NSW, and maintained on the artificial diet of Couilloud and Guiol (1980).

Fifty test trees were arranged in a 5 x 10 layout on the glasshouse floor. Spacing was not strictly controlled but care was taken that foliage of neighbouring trees did not touch. One newly-hatched 1st-instar larva was introduced to each tree on the shoot stems with a fine brush. Locations of larval feeding were checked daily between 10.00-12.00 from the next day on. New feeding spots, recognised by the presence of new frass and absence of previously-attached paper labels, were recorded with regard to locations and type of tissues and then marked with tiny paper labels bearing the attack sequences (#1, #2, etc.) which were kept in position by sticky tape. The frass was carefully removed from the feeding spots with a fine brush and transferred into a plastic jar, together with a piece of paper showing the collection date of the frass. Twenty air-dried frass pellets were randomly selected from each day's frass collection and measured for width under a stereo microscope fitted with an eyepiece scale to the nearest 1/40 mm. Feeding tissues were grouped into terminal foliage, pith, damaged tissues and other tissues. Terminal foliage included terminal buds, unexpanded or juvenile leaflets and leaf petioles. Feeding at leaf axils was considered as the start of tunnelling, i.e. pith-feeding. The association was previously noted (Coventry, 1899; Anon., 1958) and supported by personal observations. A larva was considered alive in the tunnel if new frass was found at the tunnel opening on the day of checking or the day before. The latter situation was included to account for the fact that larvae would not feed for some time before and after molting. Damaged tissues included leaf scars on the shoot surface where leaves had been removed and other damaged areas on the shoots or old stems. Checking stopped when no more new frass was found on any of the test trees. One week later, all test trees were searched thoroughly and shoots dissected to check for the developmental stage of larvae present.

Data from
individual trees were pooled to estimate the proportions of feeding in various tissue categories. Assuming the relatively low mortality rate among late instar larvae, trees that had ceased to show any new frass but had been recording new frass at tunnel openings until as late as 30 days after larval introduction were interpreted as containing larvae that had entered into non-feeding stages, i.e. prepupae or pupae, and were still included in the pith-feeding group.

To further qualify the preferred feeding tissues, 40 1st-instar larvae and 20 each of 2nd-3rd instar larvae were individually introduced into a tree at various locations and followed until the initiation of feeding or for a maximum of 30 minutes if no feeding took place before then. The tree was of similar height and form as those used above.

6.3 Results
6.3.1. Feeding on inert substrates

_Agar-cellulose substrate_

The plain agar-cellulose cores were chewed almost as intensively as those incorporated with the ethanol extract at any of the 3 tested concentrations in the binary-choice experiment (Figure 6.2). Like the treatment cores, some of the control cores were completely chewed to pieces. The same situation was found in the non-choice experiment with regard to the chewing intensities (Figure 6.3). However, the number of faecal pellets produced was significantly higher for larvae fed with the treatment cores than that for larvae fed with the control cores ($P < 0.05$, Wilcoxon rank sum test; pooled data of all concentrations) (Figure 6.3), with the highest differences recorded for the highest concentration, e.g. 10 g FWE in 50 g plain medium. Only one of the 10 larvae in the control group produced faeces, while faecal pellets were found for over half of the test larvae in any of the three treatment groups.
Filter paper substrate

The extent of chewing by 6th-instar larvae on filter paper rolls treated with the ethanol extract, as shown by roughened (hairy) surface or chewing holes, was much greater than that on the control paper rolls (Figure 6.4). The differences in the percentages of paper rolls chewed between any of the treatments and the control in all the three tests were highly significant ($P < 0.0001$, chi-square test). Physical removal of part of the filter paper, as shown by chewing holes, was seen only in the treatment paper rolls in two tests. Among the treatments, the intensities of chewing were significantly higher for the extract concentrations of 0.5 and 5 g FWE/ml than that for the extract concentration of 0.05 g FWE/ml ($P < 0.05$, chi-square test, pooled data), while that between the two higher concentrations was not significantly different ($P > 0.1$, chi-square test, pooled data). The extracts of young shoot stems and of young leaves showed apparent stimulating effects in feeding activities, but the greatest effects were seen in the extract of young shoot stems plus young leaves (Figure 6.5). However, the differences in the proportions of paper rolls chewed among the 3 treatments were not significant ($P > 0.1$, chi-square test).

6.3.2 Host odour in feeding initiation

Odour from young leaf and from young stem cuttings of the host showed clear evidence of initiating the biting response (Table 6.1). Although the control capsules were sometimes chewed upon, the majority of those capsules with chewing holes (65-90%) contained host material. There appeared to be no apparent differences between the attractiveness of the leaf and the shoot. The overall preference was found to be highly significant ($P < 0.001$, chi-square test, pooled data). In those test units (petri dishes) where chewing on the control capsules occurred, the treatment capsules were usually chewed as well. Chewing on the control capsules alone was observed only in two test units in two separate tests. Where the treatment
capsules were chewed, the larvae were frequently found inside the capsules, whereas chewed control capsules were often empty, some of which had exit holes on top. Over 88% of all capsules with larvae inside occurred in the treatment. It is striking to note that in several instances the larvae, after entering the gelatin capsules, had pulled off the covering cotton wool and gained access to the host tissues inside the straw cuttings. Odour from the White Cedar leaves appeared to be equally effective in eliciting the biting response (Table 6.1).

6.3.3 Feeding on non-host species

All larvae introduced to the non-host Meliaceae plants began feeding soon after their release (< 5 min.) (Plate 2). Within the 1-hour observation period, all but two larvae had completely bored into the shoots. The latter two larvae, one introduced to Chinese Toon and the other to Spanish Cedar, wandered off the test plants, probably in search of suitable pupation sites as both were in the blue stage (late in the last instar). Dissection of the infested shoots three days after the introduction of the larvae showed that larvae introduced to White Cedar and Chinese Toon had either died or disappeared, while those introduced to Spanish Cedar were still alive. In particular, four of the five dead larvae on White Cedar were found at the entrance of the tunnels. The binary-choice tests comparing feeding on young shoots of the Red Cedar and the 3 non-host Meliaceae species revealed no significant differences in either the frequencies of shoots tunneled or the relative areas of the epidermis removed (Table 6.2). However, no evidence of feeding was found on the shoots of the non-Meliaceae plant, Southern Blue Gum, while the shoots of Red Cedar placed in the same containers invariably bore some types of feeding scars (tunnels or removed epidermis) (Table 6.2). The same pattern was shown in the leaf disc test (Figure 6.6). The leaf discs of the three non-host Meliaceae species were fed upon almost as extensively as the host leaf discs while the leaf discs
of the eucalypt were mostly avoided by the larvae with feeding scars shown only in 3 leaf discs.

6.3.4 Within-tree distribution of feeding spots

Twenty-one out of the 50 larvae introduced survived into the blue larvae or the prepupal stage. The majority of deaths (79%) occurred during the first three days after introduction. The relationship between frass width and sampling time, expressed as the number of days since the introduction of newly-hatched 1st-instar larvae, was significantly fitted by a logistic equation (F=345.65, df=1,36, p=0.0000) (Figure 6.7). Based on this equation, the moultng dates for successive larval instars were estimated by supplanting the inter-instar boundaries in frass widths (Appendix) into the equation and solving for the corresponding dates (Figure 6.7). The estimated moulting dates for 1st-5th instars were 3.4, 11.9, 19.4 26.8 and 35.0 days following larval introduction. These were estimates of population averages. Individual larva may have developed at different rates.

Relative proportions of feeding in terminal foliage, pith, damaged tissues and other tissues varied as larval development progressed (Figure 6.8). Feeding by first instar larvae was exclusively confined to the terminal and damaged tissues, of which damaged tissues were attacked about four times as much as terminal tissues. Among terminal tissues, buds and leaf petioles were most favoured. On only one occasion was a larva found feeding on the surface of a young leaflet. Feeding on damaged tissues was mainly found in leaf scars. The same confinement of feeding activities continued until late second instar, when terminal feeding dropped to zero and feeding in pith through stem tunnelling commenced. No more terminal feeding was observed thereafter. The period from late second instar to late 5th instar revealed a steady decrease of larval proportions in damaged tissues (from about 90% to zero) and a general increase of larval proportions in pith (from zero to about 90%), reflecting a continuing shift
from surface feeding to pith feeding. All pith feeding began at leaf axils. Feeding in other tissues commenced at early third instar and fluctuated around 20% during the rest of the larval period. Typical locations were epidermis (or bark) of shoots or stems, shoot bases (junctions of shoots and tree stems), and bases of tree stems at the soil level. Coinciding with the sudden rise of feeding in other tissues was a sharp drop of larval proportions in pith. Despite the drop, pith-feeding still accounted for most of the feeding activities (60%). Larval proportions in the various tissues stabilised after 48 days of larval development.

Larvae seldom remained at the same feeding locations during their development. On average, a larva initiated feeding in 5.4 different locations during its life time, with a minimum of 3 and a maximum of 11. When plotted against larval development time, two peaks are evident in the distribution of average number of new feeding spots per larva (Figure 6.9). The first peak was recorded within one day of larval introduction, i.e. among newly-hatched 1st-instar larvae, and the second during much of the third and early fourth instar. Thereafter it decreased as larval development progressed.

The length of time that a larva would stay at a particular feeding spot differed with the location of the feeding spot. The average stay time was highest in pith (6.8 ± 7.6 days), followed by damaged tissues (4.3 ± 3.6 days), terminal foliage (3.0 ± 2.1 days) and other tissues (2.6 ± 2.0 days). The difference was significant at P= 0.05 level when pith was compared with any of the other tissue categories (Wilcoxon rank sum test), which is not surprising as *H. robusta* is a shoot borer. However, most feeding spots were abandoned within one day of feeding initiation (66%, pooled data), regardless of the tissue where feeding took place.

Observations on the settlement processes of larvae released individually supported the above-outlined shoot selection patterns (Table 6.3). It is worthwhile to note that feeding by first instar larvae tended to start
in the vicinity of the locations where they were released, whereas that by
older larvae did not show apparent correlations with their release locations.
All 1st-instar larvae released at the terminal buds started feeding in
terminal foliage and those released on shoot stems or old stems ended up in
damaged areas along the stems. Larvae of 4-6th instars walked extensively
on the release trees before settling down to feed, usually at leaf axils on the
upper sections of the shoots. Some even moved away from the release trees.

6.4 Discussion

The feeding process of insects with chewing mouthparts contains two
important phases: biting and ingestion. During the biting phase, the
suitability of the food, especially the chemical structure and composition, is
assessed by the insect through various receptor cells located in the
mouthparts. The ingestion phase, in which the food is swallowed, would
proceed if the necessary signal pattern is received by the central nervous
system. When biting is not followed by ingestion, no faeces would be
produced, assuming the gut is empty before the current feeding process.
With *H. robusta*, separation of the two feeding phases is further facilitated
by the fact that conspicuous piles of food fragments are produced and these
food fragments can be distinguished from the faeces by their less regular
shapes and less smooth surfaces. This criterion was used in analysing the
results of the no-choice test using the agar-cellulose medium as feeding
substrate. As reported previously (Hsiao and Fraenkel, 1968), the plain agar-
cellulose medium was sometimes extensively chewed but no or few faeces
were produced. In contrast, larvae fed with the treatment medium to which
the ethanol extracts were incorporated mostly produced faecal pellets in
addition to the chewing fragments of food. The result suggests that the
ethanol extracts contained at least some of the necessary phagostimulants,
the presence of which was detected by the larvae through the gustatory
receptor cells located in the mouthparts and led to ingestion of the test
medium. Similar discriminatory feeding behaviour against chemicals might have also been exhibited by larvae in the choice tests but the faeces produced could not be ascribed with confidence to the ingestion of either of the two test media, control or treatment. Extensive chewing on the plain medium was probably driven by hunger, which, in turn, drove the larvae to make repetitive sampling of the medium in an attempt to find suitable food somewhere within the test medium. Hence the chewing intensity alone appears to be insufficient to characterise the chemical-mediated feeding behaviour of *H. robusta* larvae when the agar-cellulose medium is used as the feeding substrate. This does not mean, however, that the act of biting or chewing was completely random with respect to food. In fact, the intensity of chewing was found to be different between the control and the treatment when filter paper was used as the feeding substrate. Filter paper rolls loaded with the ethanol extracts were chewed more intensively than the control rolls, suggesting the involvement of contact and/or olfactory stimuli in initiating and sustaining the chewing activity. The involvement of olfactory stimuli in initiating the biting response was convincingly shown in the gelatin capsule tests. Although the test larvae were prevented from having direct contact with the test material, they were able to concentrate their chewing on those gelatin capsules containing sections of fresh young leaves or shoots, apparently as a result of their detecting the host volatile reaching them through the punched holes on the walls of the treatment capsules. The possibility of capsule softening in the treatment by the water vapour emitted from the host material was minimal as the latter were placed in plastic straws blocked at both ends by cotton wool. In summary, the feeding behaviour of *H. robusta* larvae was regulated by chemicals present in the young shoots and leaves of host trees and some of these chemicals could be extracted by ethanol. These chemicals might have functioned as olfactory, contact, or gustatory stimuli. Olfactory stimuli alone could initiate the biting response. Biting could also be initiated by contact stimuli, whether chemical
or physical, or simply by hunger. The reliance of feeding on these chemicals was more strict during the ingestion phase than that during the biting phase. Certain gustatory stimuli had to be present for any significant ingestion of the food.

Although *H. robusta* attacks only Red Cedar in Australia, the stimuli (chemical or physical) necessary for initiating and sustaining larval feeding are likely to be widely distributed among various Meliaceae species. This was seen in the ready burrowing into the shoots of all the three Meliaceae species tested, despite one of them, the White Cedar, being in a different subfamily. Furthermore, the intensities of feeding on these non-host Meliaceae species were comparable to that on the host and the volatiles from the White Cedar appeared to be equally attractive as that from the host tree in elicitng the biting response. It is not known whether this is due to some commonly existing chemicals in this family. Species in the family Meliaceae are characterised by the common occurrence of limonoids (derivatives of triterpenes), which are found also only in two other families (Taylor, 1981). Current knowledge of the biological activities of the limonoids and their derivatives is restricted to the insecticidal properties (Champagne *et al.*, 1992; Agostinho *et al.*, 1994).

Two of the tested non-host Meliaceae species, Chinese Toon and White Cedar, apparently contained chemicals that were toxic or detrimental to the normal development of the larvae, as the test larvae either died or disappeared after some time. Assuming the attractiveness of the two species to egg-laying moths, these trees may be used in controlling the insect by planting them in mixture with the Red Cedar. The prospect looks particularly promising for the White Cedar as feeding on it inevitably resulted in the death of the larvae and the tree did receive some eggs (personal observation). In addition, White Cedar grows naturally within the geographic range of Red Cedar and it also produces good timber (Floyd, 1989).
It is striking to note that more early-instar larvae were found feeding in damaged tissues than in terminal foliage, considering that the latter has been described as the favourite site for initial feeding (Beeson, 1919). The selection of damaged tissues was also observed in the field where young larvae were found inside old, abandoned tunnels or tunnels occupied by mature larvae. Feeding in damaged tissues appears to have some advantages, like more protection sites and possibly weaker antibiosis reactions from host plants. Early locating of suitable feeding sites is of vital importance to newly-hatched larvae. As they did not seem to travel far before starting feeding, the fate of these larvae depends very much on the oviposition sites.

The sudden drop in the proportions of pith-feeding larvae early in the sixth instar indicates that a considerable number of larvae left their original tunnels, probably searching for alternative pupation sites. Abandonment of old feeding spots was common in this study and probably resulted from vigorous sap exudation or shoot size being too small (Beeson, 1919). It seems that larvae had to search and try a number of locations in the plant before settling down to feed.

As feeding began from outside, the variation in the number of new feeding spots per larva per day can be considered as a measure of the changes in the intensities of surface activities of larvae. The pattern of the variations suggests that there was a re-emergence of larvae from previously established feeding sites during much of the third and early fourth instar. If proven, such a period may by used in timing control approaches.
Table 6.1. Frequencies of gelatin capsules with chewing holes and with larvae inside in the treatment (containing young leaf or shoot cuttings) and the control (containing no plant material) as shown in choice tests. Each test consisted of 15 test units and each test unit contained 2 treatment capsules and 2 control capsules arranged alternately in pre-punched holes in the periphery of a petri dish base (see Figure 6.1 for detail). One 4-6th instar larva was introduced in a test unit and allowed to feed for 18 h in darkness. Materials tested in leaf-1 to leaf-3 and shoot-1 to shoot-2 were of the host tree, *T. australis*. Only young leaves were tested with the White Cedar, *M. azadirachta*.

<table>
<thead>
<tr>
<th></th>
<th>leaf-1</th>
<th>leaf-2</th>
<th>leaf-3</th>
<th>shoot-1</th>
<th>shoot-2</th>
<th>total</th>
<th>White Cedar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment with holes (T)</td>
<td>8</td>
<td>13</td>
<td>9</td>
<td>14</td>
<td>10</td>
<td>54</td>
<td>10</td>
</tr>
<tr>
<td>larva in</td>
<td>6</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>7</td>
<td>34</td>
<td>4</td>
</tr>
<tr>
<td>Control with holes (C)</td>
<td>2</td>
<td>7</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>larva in</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>T/(T+C) with holes (%)</td>
<td>80.0</td>
<td>65.0</td>
<td>90.0</td>
<td>73.7</td>
<td>76.9</td>
<td>75.0</td>
<td>83.3</td>
</tr>
<tr>
<td>larva in</td>
<td>100</td>
<td>88.89</td>
<td>100</td>
<td>88.89</td>
<td>100</td>
<td>94.4</td>
<td>100</td>
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</table>
Table 6.2. Relative intensities of feeding of *H. robusta* larvae on the shoots of non-host species as compared with that on the host shoots as revealed by a binary-choice test. Differences in the frequencies of shoots tunnelled were tested by chi-square test and that in relative surface feeding intensities by Wilcoxon sign test.

<table>
<thead>
<tr>
<th>nonhost species</th>
<th>No. of shoots tunnelled</th>
<th>host</th>
<th>nonhost</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. odorata</em></td>
<td>10</td>
<td>8</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td><em>T. sinensis</em></td>
<td>7</td>
<td>6</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td><em>M. azadirachta</em></td>
<td>14</td>
<td>10</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td><em>E. globulus</em></td>
<td>9</td>
<td>0</td>
<td>NA*</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>No. of shoots with greater surface-feeding in</th>
<th>host</th>
<th>nonhost</th>
<th>both p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. odorata</em></td>
<td>7</td>
<td>5</td>
<td>8</td>
<td>0.58</td>
</tr>
<tr>
<td><em>T. sinensis</em></td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>0.38</td>
</tr>
<tr>
<td><em>M. azadirachta</em></td>
<td>3</td>
<td>5</td>
<td>12</td>
<td>0.49</td>
</tr>
<tr>
<td><em>E. globulus</em></td>
<td>18</td>
<td>0</td>
<td>2</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*the number of tunnelled shoots in the non-host was too low to allow a chi-square test.
Table 6.3. Proportions of initial feeding locations of larvae released individually. Feeding at leaf axils signals the start of tunnel excavation and thus pith feeding.

<table>
<thead>
<tr>
<th>instar</th>
<th>number started feeding</th>
<th>initial feeding locations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>terminal foliage</td>
<td>leaf axils</td>
</tr>
<tr>
<td>1st</td>
<td>23</td>
<td>34.8</td>
</tr>
<tr>
<td>2nd</td>
<td>12</td>
<td>50.0</td>
</tr>
<tr>
<td>3rd</td>
<td>18</td>
<td>0.0</td>
</tr>
<tr>
<td>4th</td>
<td>16</td>
<td>0.0</td>
</tr>
<tr>
<td>5th</td>
<td>9</td>
<td>0.0</td>
</tr>
<tr>
<td>6th</td>
<td>6</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Figure 6.1. Design of the gelatin capsule test unit in the study of the role of host odour in eliciting the biting response from the larvae.

- Paper covering
- Gelatin capsule
- Petri dish base
- Control
- Treatment
- Holes
- Cotton wool
- Drinking straw
- Host tissue
Figure 6.2. Intensities of larval chewing on cylindrical cores of the agar-cellulose medium incorporated with the ethanol extract at different concentrations. The concentration of 0 g FWE/50 g medium denotes the control.
Figure 6.3. Feeding responses of larvae to cylindrical cores of the agar-cellulose medium incorporated with the ethanol extract at different concentrations in a non-choice test. The concentration of 0 g FWE/50 g medium denotes the control. Ingestion or swallowing of the test medium was indicated by the production of faeces.
Figure 6.4. Larval chewing on filter paper rolls treated with the ethanol extract at different concentrations in 4-choice tests. The concentration of 0 g FWE/ml denotes the control.
Figure 6.5. Larval chewing on filter paper rolls treated with ethanol extracts of different host tissues.
Figure 6.6. Feeding by larvae of *H. robusta* on the leaf discs of the host, Red Cedar, and four non-host species in pair-wise choice tests. The area in each pie represent 10 test units, each consisting of 2 leaf discs from the host and 2 leaf discs from the non-host. The leaf discs were arranged alternately in the periphery of a petri dish floor. The percentages given in the four categories represent, respectively, the proportions of test units in which feeding was observed on the leaf discs of the host only, non-host only, both the host and the non-host, and neither the host nor the non-host.
Figure 6.7. Average frass widths (mm) of larvae and the estimated larval instars during the course of larval development on potted Red Cedar plants. One newly hatched larva was introduced to each plant. The boundaries separating the frass widths of neighbouring larval instars were taken from Appendix 1.
Figure 6.8. Within-tree distributions of feeding spots with respect to host tissues during the course of larval development on potted Red Cedar plants. One newly hatched larva was introduced to each plant. Terminal tissues: bud and unexpanded foliage; Pith: tissues beneath the epidermis or barks of shoots and stems; Damaged tissues: wounds resulting from previous damage by the insect or other causes; Other tissues: any other tissues of the host tree, such as epidermis or barks.
Figure 6.9. Daily production of new feeding spots during the course of larval development on potted host plant. One newly-hatched larva was introduced to each plant.
Plate 2. A larva of the *H. robusta* in the progress of boring into the shoot of a potted White Cedar (*Melia azadirachta* var. *australis*) tree.
Chapter 7

Diel Patterns of Reproductive Activities of *Hypsipyla robusta* Moore

7.1 Introduction

Reproduction in most lepidopteran species is accomplished through a series of distinct phases of moth activity, e.g. female calling (pheromone release), male activation, copulation, and oviposition. Knowledge about the patterns of these activities is important in establishing successful control strategies aimed at the reproductive stage of the targeted insects, such as the use of sex pheromones in mating disruption (Shorey, 1977; Cardé and Minks, 1995) and mass release of sterile males (Boake *et al.*, 1996). These strategies hold a special place in integrated pest management (IPM), because of their environmentally safe and species-specific properties.

Although the structure of the sex pheromones of *Hypsipyla robusta* Moore (Lepidoptera: Pyralidae) has been worked out (Bosson and Gallois, 1982) for the African race, little is known about the reproductive behaviour of the moths. All reproductive activities probably take place at night, as the moths are inactive during the day (Beeson, 1919). Studies of an artificially reared colony indicated a peak emergence period between 18:00 and 19:00 h (Atuahene and Souto, 1982). The same authors found that females exhibited strong preferences for cracks or creased spots as oviposition sites. Relatively more information is available on the sibling species, *H. grandella* (Zeller) (see Newton *et al.*, 1993). According to Sliwa and Becker (1973), emergence of the moths occurs just before dark, with no significant differences in the emergence times between males and females. Calling started about 6 hours after light-off and peaked about 3 hours thereafter. The length of an average calling period is about one and half hours and that of copulation about 2

* An earlier version of this chapter has been accepted for publication in *Australian Forestry*
hours. Mating takes place at some 8 hours after light-off (Holsten and Gara, 1977a). Females mate only once and stop calling after mating, whereas remating in males is not unusual (Holsten and Gara, 1977a). Males aged 3-4 days are most receptive to calling females (Holsten and Gara, 1974). Males are most responsive to females aged 2-3 days, coinciding with the peak period of sex pheromone production (Holsten and Gara, 1977b). Flight activities of the moths occur between 24:00 and 05:00 h and cease when temperature falls below 15°C and during high precipitation (Gara et al., 1973). Oviposition occurs in the evening or early morning (Wilkins, 1972; Holsten, 1977).

In an effort to understand the reproductive behaviour of *H. robusta*, diel patterns of moth emergence, female calling, mating, egg-laying, and physical activities were studied under laboratory conditions. The effects of age on these reproductive activities were also analysed. Host searching behaviour of the moths will be dealt with in Chapter 8.

7.2 Methods

7.2.1 Source of the moths and observation environment

Moths used in this study were from a laboratory colony reared on the artificial diet of Couilloud and Guiol (1980). Field-collected larvae were periodically introduced into the colony. Temperature was maintained at 26 ± 2°C. From the pupal stage, light period was strictly controlled at 14L:10D, in a reversed cycle. Daylight was simulated by a 250 W mirror-backed globe, and night time observations were facilitated by a 20 W red fluorescent light. Diel patterns of moths of mixed ages were studied in Perspex cages (250 x 250 x 820 mm), the openings of which were covered with cheese cloth. Two petri dish bases containing 10% sugar-saturated cotton wool were placed on the cage floor to serve as food for the moths. Observations of activities of individual moths were made in plastic vials (40 x 50 mm). The caps of the
vials were placed in such a way that a small slit was kept between the cap and the vial opening to maintain basic ventilation.

7.2.2 Moth emergence

Pupae were sexed according to the characteristics given by Sharma and Singh (1980a) and individually placed in plastic vials. Emergence time of the moths in the scotophase was recorded.

7.2.2 Female calling

After emergence, males were put in the Perspex cage and females left in the plastic vials to allow the recording of calling time. The calling posture of a female was recognised by its lowered wings and raised abdomen. The time when individual females first started calling was recorded. Some calling females were immediately transferred to the Perspex cage containing the males, for mating. Initial calling time of the remaining females was recorded daily until they died.

7.2.3 Mating

Males and females of mixed ages were allowed to mate in the Perspex cage. The time of individual copulations was recorded. Mated females were transferred to another Perspex cage upon separation from the males for oviposition. In a separate experiment, females of given ages were placed in a cage of males to study the effect of age on mating success.

7.2.4 Egg-laying

Egg-laying was considered to have been attempted if, while stationary, the abdomen of the female arched down and the tip of the ovipositor touched the cage surface. Active walking, flying, or wing-fanning between successive egg-laying attempts were considered to be part of egg-laying activities. The timing of individual egg-laying bouts was recorded.
7.2.5 Physical activities

A moth was considered as physically active if it was flying, walking or wing-fanning. This definition is based entirely on the moth's physical rather than physiological status. Hence individuals engaged in calling and mating are not considered as active since they rarely move, although they are not really at rest. Diel patterns of physical activities were studied separately for males, virgin females and mated females. Physical activities of the moths were studied both in groups and in solitary confinement.

Group confinement

On each observation night, selected individuals in the Perspex cage were monitored for their activity status, active or inactive. The monitored individuals were so selected that they could be easily distinguished from others by their morphological characteristics, e.g. size, completeness of wings. The starting and ending times of each active interval of the monitored individuals during the night period were recorded. Males and females that mated during the period of observations were not included in the analysis, as their normal activity rhythms would be disrupted by mating.

Solitary confinement

Activity status of individual moths in plastic vials was recorded every 5 minutes during the scotophase. A moth was considered as active in a 5-minute interval if it had walked or fanned its wings sometime during that interval. Physical activities of these moths were monitored nightly until their death or relocation to the mating cages. Data from the night of moth death or relocation were excluded from analyses. For mated females, recording of physical activities commenced on the first night after mating.
7.3 Results

7.3.1 Moth emergence

Most moths emerged in the early hours of the scotophase (82%), the rest before light-off. The frequency distribution of emergence in the scotophase was relatively sharp and narrow (Figure 7.1). Nearly 50% of nightly emergence occurred in the half-hour interval of 1.0-1.5 h after dark. No more emergence was observed 3.5 h after dark. The emergence patterns of males and females were very similar, the only difference being the slightly sharper peak in the males (Figure 7.1).

7.3.2 Female calling

The posture of calling was similar to that observed in *H. grandella* (Sliwa and Becker, 1973). Before engaging in prolonged stationary calling, virgin females were sometimes seen dragging their ovipositors across the cage surface, probably leaving a trail of pheromones to aid in the elicitation of males (Teal *et al*., 1981). Calling of females was observed as early as 3 hours after dark. However, the majority (67%) started calling at 5.5-7.5 h after dark (Figure 7.2). Thereafter, calling recruitment continued at low levels. There was an apparent trend for earlier calling as the females aged (Figure 7.3A) (P< 0.05, Kendall’s correlation test). The mean time of initial calling was shifted forward by about 20 minutes each scotophase (Figure 7.3B). Once initiated, calling would usually continue until after light-up unless disturbed; in the latter case the females would resume calling after a short while. Most females stopped calling after mating. However, some mated females continued to call in the following scotophases.

7.3.3 Mating

Mating began 1.5 hours later than calling but reached its peak around the same time as calling (Figure 7.2). The decline of mating frequencies after the peak was, however, more gradual and mating persisted till the end of
Mating was recorded on females aged 1-6 days and on males aged 0-4 days. Females appeared to be most receptive to males in the 2nd-3rd day following emergence, accounting for 63% of all copulations obtained in this study.

7.3.4 Egg-laying

Unlike the diel patterns of other reproductive activities, egg-laying in the Perspex cage was observed in all but the first 0.5 hour and the last 1.5 hours of the scotophase. There appears to be no single, dominant peak in the egg-laying pattern (Figure 7.2).

7.3.5 Physical activities

Group confinement

Despite being nocturnal, *H. robusta* moths spent considerable proportions of the dark time resting. Individual moths, irrespective of their sex and mating history, periodically underwent rest in the dark phase (Figure 7.4). Virgin females appeared to be more active during the early half of the scotophase and remained relatively stationary during the latter half of the scotophase, apparently as a result of their calling patterns. The actual proportions of time spent in active status by individual moths varied from 0 to 45%. On average, virgin females spent less time in active status than mated females and males (Table 7.1), but the differences were not significant (P>0.1, Mann-Whitney test). Mated females showed the longest average duration of active intervals (Table 7.1), which is significantly higher than that of virgin females (P<0.05, Mann-Whitney test).

Solitary confinement

Mean intensity of physical activities, as measured in the proportions of active 5-minute intervals, was significantly higher in mated females than that in virgin females and males (P< 0.05, Mann-Whitney test) (Figure 7.5).
Similar patterns were detected in the means of nightly maximum durations of active intervals (Figure 7.5). Mated females, on average, stayed in active status much longer than virgin females and males. One mated female was observed to be active continuously for over 2 hours, while the maximum durations were less than 1 hour in virgin females and males. No significant differences were detected between males and virgin females in either the proportions or the maximum durations of active intervals (P > 0.05, Mann-Whitney test). Physical activities of the moths, males or females, were concentrated in the early and middle phases of the scotophase (Figure 7.6). Mated females were especially active in the first 3 hours of the scotophase (Figure 7.6). The intensity of physical activities decreased substantially after 7 hours into the scotophase. Males were most active in the first scotophase following emergence and virgin females the second scotophase following emergence (Figure 7.7). After the peak, activities of both sexes decreased gradually as age increased. Females were relatively inactive in the night of emergence, with an average intensity of activities comparable to those 5 days after emergence. Among females mated within 5 days of their emergence, 53% reached the activity peaks in the first scotophase following mating and 24% in the second scotophase following mating. The rest exhibited peak activities before mating.

7.4 Discussion

The diel emergence pattern of moths of *H. robusta* observed here was similar to that reported by Atuahene and Souto (1983), both indicating the concentration of emergence in the early hours of the dark period. A different emergence pattern was reported for *H. grandella* (Sliwa and Becker, 1973), which showed a concentration of emergence just before dark.

Calling commenced 3 hours after light-off and terminated at the end of the scotophase. Considering the persistent nature of female calling, most females would be available for mating throughout the latter half of the
The mean time of initial calling varied with age, shifting forward by about 20 minutes each scotophase. Earlier calling in older females may be an adaptive trait in the competition for males (Babilis and Mazomenos, 1992). Most females (74%) called daily for about 6 days. Non-calling was observed in some females on the night of their emergence and in some in the last two nights of their life. In the latter case the females were probably too weak to adopt the typical calling postures. Some continued to call after 10 days from their emergence. However, the sex pheromones released by old calling females were probably of inferior quality or quantity, as they were mostly ignored by males.

Male responded to the calling female by flying upwind toward it and landing in the close vicinity, usually immediately below the female, and then swiftly swiping the tip of its abdomen toward that of the female. Upon clasping the female, the male would immediately turn its body to the opposite direction. When mating took place on a vertical surface, the head of the male always pointed downward. The diel pattern of male sexual activity was not directly studied. Mating attempts by males were observed only after females started calling. It is not known whether males were sexually active before this time. The diel pattern of mating reveals that some males were sexually active as early as 4 hours after dark. Mating appeared to take place only between compatible males and females. Both sexes possessed the ability to select their mates. Sexually active males, recognised by their swinging of abdomens toward females, were seen walking past some calling females without showing mating attempts. On the other hand, not every calling female was receptive to males. The unreceptive behaviour consisted of moving away from the approaching male and flexing of the abdomen so that its tip was out of reach of the male’s claspers. In the event of successful grasping by the males, the females would struggle until escape was achieved. It is not clear what criteria were used by males in selecting the females and under what circumstances
females were unreceptive to males. Age may be one of the factors involved, as copulations were observed only in moths of certain age groups, namely aged 0-4 days in males and 1-6 days in females. In addition, unsuccessful copulations often involved females on the night of their emergence, or old females. The involvement of age in the unreceptive behaviour of females has been documented in a number of lepidopteran species (Teal et al., 1981; Colvin et al., 1994). The attractiveness of females to males may depend on the production of sex pheromone of females, which, in turn, varies with age. A study of H. grandella indicates that the pheromone production of virgin females reaches its peak 2-3 days after emergence and females of this age elicited the strongest response (mating attempt) from males (Holsten and Gara, 1977b). It is interesting to note that some females continued to call after mating. It suggests that single copulations did not always result in the full fertilisations of eggs and females might be able to re-mate. Incompatibility of the male partners cannot fully explain the after-mating calling behaviour, since the latter was also observed in one female that had already laid some fertile eggs.

Egg-laying in the Perspex cage appeared to be randomly distributed throughout most of the night period, as indicated by the widespread occurrence and the lack of a single, dominant peak. However, observations of individual mated females in the plastic vials showed a concentration of activities in the first 3 hours of the scotophase. This agrees partly with the finding of the oviposition behaviour of H. grandella, which indicated active egg-laying in the early and late hours of the night (see Newton et al., 1993). Most eggs were probably laid in the first night following mating, as suggested by the physical activities of mated females.

The diel patterns of physical activities of H. robusta appeared to be similar to those of Chilo partellus, also a shoot borer in the family Pyralidae (Päts and Wiktellius, 1992). Compared with mated females, virgin females were much less active whether in group or solitary confinement. This was
shown both in the proportions and maximum durations of active intervals. Assuming similar relative activity intensities of moths in the field, it appears that mated females, rather than virgin females as was reported for *H. grandella* (Holsten and Gara, 1977c), were mainly responsible for dispersal and locating host plants. The priority of females appeared to be attracting the males and getting mated. Under natural conditions, populations of *H. robusta* often occur in low numbers due to the scattered distribution of its host trees. Mating before dispersal would increase the chance of reproduction in sparse populations. The hypothesis was supported by the notion that virgin females were quite inactive on the night of emergence. Their activities peaked 2 days later, one day after the earliest recorded mating age. However, the hypothesis needs to be tested by field studies. If the hypothesis is found to be correct, then control by mating disruption or mass trapping with pheromones should be applied at the site of current infestation rather than in healthy host stands surrounding the infestation site.

As a result of calling in the latter half of the night, physical activities of virgin females were seen mainly in the early and middle phases of the scotophase. Male activities peaked one day earlier but declined more quickly as age increased (Figure 7.2). No successful mating was recorded for males aged over 5 days.

Study of the rhythms of physical activities is important for the understanding of reproductive behaviour. Males at rest were seen to ignore the calling females, even during the peak mating periods when they were ‘supposed’ to be sexually active. In other words, they did not engage in mating behaviour immediately upon the reception of pheromone signals. Sometimes the resting males were physically disturbed by the passing of other moths, including females that had temporarily stopped calling, but they soon returned to their rest after a few trivial movements. Such a rest-inhibited sexual activity of males has also been reported in other Pyralidae.
species and may be the expression of an endogenous circadian rhythm (Unnithan and Saxena, 1989). It suggests that high ratios of males to females are needed to optimise mating in a cage environment. Female responses to host-plant volatiles were also dependent on their activity status. Preliminary olfactory experiments showed that calling virgin females often remained stationary in the test arena (wind tunnel or olfactometer) and did not make any choices for some time with regard to the presence or absence of host-plant volatiles. It thus seems desirable that olfactory experiments be conducted in the first half of the scotophase, when most of the females are still actively walking or flying.
Table 7.1  Mean proportions of time spent in active status by moths in the scotophase and the mean durations of individual active intervals (min.).

<table>
<thead>
<tr>
<th></th>
<th>virgin female</th>
<th>mated female</th>
<th>male</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average (±SD)</strong></td>
<td>13.2 ± 13.9</td>
<td>20.6 ± 17.7</td>
<td>17.3 ± 9.2</td>
</tr>
<tr>
<td><strong>percent of time spent in activity</strong></td>
<td>(n=17)</td>
<td>(n=12)</td>
<td>(n=15)</td>
</tr>
<tr>
<td><strong>Average (±SD)</strong></td>
<td>8.8 ± 7.79</td>
<td>12.3 ± 22.4</td>
<td>9.3 ± 8.2</td>
</tr>
<tr>
<td><strong>duration of active intervals</strong></td>
<td>(n=152)</td>
<td>(n=117)</td>
<td>(n=139)</td>
</tr>
</tbody>
</table>
Figure 7.1 Emergence patterns of moths during the scotophase (11:30 - 21:30).
Figure 7.2  Diel patterns of female calling, mating and egg-laying in the scotophase (11:30 - 21:30).
Figure 7.3 The effect of age on the time of initial calling. A. Raw data; B. Means, bars indicate standard errors.
Figure 7.4 Typical rhythms of physical activities of virgin female, mated female and male in group confinement in the scotophase.
Figure 7.5 Comparisons among males, virgin females and mated females in the intensities of physical activities and maximum durations of nightly activity bouts.
Figure 7.6 Diel patterns in the intensities of physical activities of males, virgin females and mated females.
Figure 7.7 The effect of age on the intensities of physical activities of males and virgin females.
Chapter 8

Effects of Host Volatiles on the Orientation of Larvae and Adults and Host Acceptance by Gravid Females of *Hypsipyla robusta* Moore

8.1 Introduction

Host plants of phytophagous insects are restricted in space, time, and species. At a given time and space, the insect must find the appropriate plant species in the often diversified plant communities to ensure the success of its future generations. The task appears to be challenging for oligophagous insects as their hosts are restricted to a few closely related plant species. A typical example of an oligophagous insect is *Hypsipyla robusta* Moore. In Australia, only one host plant, the Red Cedar (*Toona australis* (F. Muell) Harmes), has been reported. Elsewhere in the world, a few related species are attacked (Entwistle, 1967). In natural ecosystems, Red Cedar is distributed in isolated locations and nowhere forms pure stands. Yet the insect is able to colonise effectively its much-scattered resource. More strikingly, almost any plantations of Red Cedar, regardless of their location, are plagued by the pest. The highly effective host utilisation pattern suggests that *H. robusta* possesses effective host-finding capabilities.

Host finding in phytophagous insects consists of a sequence of behavioural responses to an array of stimuli associated with host and non-host plants, which the insects are equipped with sensory receptors to perceive (Visser, 1986). Plant stimuli involved include, in varying proportions, visual, mechanical, gustatory, and olfactory characteristics. The sequence of host finding is usually categorised into: host-habitat location, host location and host acceptance (Ramaswamy, 1988). However, the boundaries of the successive steps are not always clear. For this reason, some authors favoured the simple division of pre-alighting and post-alighting for
the host-finding process of flying insects (Jones, 1992). Different sets of sensory cues may be involved at different stages.

Although the roles of visual cues cannot be ruled out, host volatiles appear to be the most likely sensory cues involved in the pre-alighting stage of host finding by moths (Ramaswamy, 1988). The plant volatiles involved may be specific or general in nature (Visser, 1986). Species-specific plant volatiles arise from the breakdown of secondary plant substances, as in the formation of isothiocyanates from the non-volatile glucosinolates in Cruciferae (Finch, 1980). Olfactory responses of most oligophagous insects are influenced by specific plant volatiles (Ramaswamy, 1988). General plant volatiles are formed via biosynthetic pathways generally present in the plants, such as leaf alcohols, aldehydes, and derivatives from unsaturated fatty acids (Visser et al., 1979). Ratios of the components of general plant volatiles affect the olfactory responses of a number of insects (Visser, 1988).

Theoretically, three categories of behavioural responses assist an insect in finding an odour source, namely chemotaxis, chemokineses, and odour-mediated anemotaxis (Shorey, 1973). In chemotaxis, the insect aligns its body in the direction of the odour source as a result of being able to sense directly the gradient of odour molecules. In chemokineses, the insect does not detect the direction of the odour gradient but instead is stimulated to move at different rates (orthokinesis) or to turn at different frequencies (klinokinesis) by changes in the local concentration of odorous chemicals. Neither chemotaxis nor chemokinesis alone is sufficient for locating a distant odour source but may be useful in guiding the insect to the nearby host plant, arresting the insect in the vicinity of the host plant, or helping it to track the odour plume (Kennedy, 1977). Odour-mediated anemotaxis, i.e. up-wind flight or walk that is stimulated by host volatiles, appears to be the only mechanism by which an insect can orientate to its distant host plants (Kennedy, 1977). Such an orientation behaviour has been demonstrated in many insects, including a few moth species (Ramaswamy, 1988). The
effective distance within which an insect can detect the responsible host volatiles depends, among other factors, on the rate of emission of volatiles, wind velocity, and the sensitivity of the olfactory receptors of the insect (Wilson, 1970). However, the exact distances remains practically unknown. The only seemingly authentic distance is 15 m recorded by Hawkes (1974) for *Erioischia brassicae*. Despite its importance, odour-mediated anemotaxis is by no means the only mechanism responsible for long-range host finding by nocturnal insects. As pointed out by Thorsteinson (1960), the insect may search randomly and arrive by chance at the vicinity of the host plant and use vision or other olfactory mechanisms, such as chemokinesis or chemotaxis, to land on the plant. This may explain the lack of evidence of upwind movements toward the host plants in some oligophagous moths (Fenemore, 1988).

Olfactory deterrence may also be involved in host finding. For example, Shurr and Holdaway (1970) showed that volatiles emanating from infested plants ensure dispersal of *Ostrinia nubilalis* females from them, while odours from uninjured plants are attractive to the females. Renwick and Radke (1982) also found that larval damage to the host cabbage plant caused reduced landing by Cabbage Looper (*Trichoplusia ni*) females. However, a recent study by Landolt (1993) indicated that the females were actually attracted to damaged plants in wind tunnels but laid fewer eggs on them compared with healthy plants. It thus seems that damage by larvae attracts the Cabbage Looper in the distance but repels them at close range.

Volatile from non-host plants may disrupt the normal host searching behaviour of oligophagous insects (Kareiva, 1983; Stanton, 1983). Tahvanainen and Root (1972) found that tomato plant odour interfered with the ability of flea beetles to locate their crucifer hosts. Similarly, Altieri *et al.* (1977) noted that the ability of *Empoasca kraemeni* to locate hidden bean foliage was inhibited by placing grass cuttings with host plant material. Odour from some non-host species repels approaching potato beetles.
(Leptinotarsa decemlineata) (Visser and Nielson, 1977). However, Finch (1980) cautioned that many of these results are subject to alternative explanations.

After landing on a potential host plant, the gravid moth would evaluate the suitability of the plant or plant parts based on an array of sensory information at the plant surface. Combinations of physical and chemical factors are involved in the evaluation process. The majority of moth species seems to prefer hairy or rough surfaces (Ramaswamy, 1988), possibly because hairy surfaces allow the female to maintain a better footing and retain the eggs better than smooth surface (Callahan, 1957; Porter, 1984). A recent detailed study of leaf surface characteristics affecting oviposition by Heliothis virescens has emphasised the role of growth habit (erect vs procumbent) as well as texture (Navasero and Ramaswamy, 1991). Contact chemoreception is considered as the major and most common sensory modality involved in host acceptance (Ramaswamy, 1988). Chemoreception in Lepidoptera is perhaps best illustrated in the 'drumming' behaviour of butterflies, in which the forelegs move rapidly so that the terminal segments of the tarsi drum against the leaf surface (Feeny et al., 1983). The drumming behaviour is suggested as an act of scraping the leaf surface to release chemicals that subsequently would be picked up by chemoreceptors located on the fore-tarsi. For moth species, the perception of surface chemicals can be performed by any of the following appendages: antennae, tarsi, proboscis, and ovipositors (Ramaswamy, 1988). Ramaswamy et al. (1987) showed that the presence of one pair of intact tarsi is sufficient for host plant discrimination by H. virescens. Rivet and Albert (1990) have shown with ablation experiments that the Spruce Budworm (Choristoneura fumiferana) depends on its proboscis for perception of chemicals in the wax layer. In addition to contact chemicals, volatiles may also be involved in host acceptance, both in the selection of food (Mitchell and McCashin, 1994) and in choosing egg-laying substrates of adults (Yamamoto et al., 1969).
Apart from stimulating chemicals in host plants, host-specificity in phytophagous insects may also arise as a result of the existence of deterrent chemicals in non-host plants. An oviposition deterrent for a Rutaceae-feeding butterfly, *Papilio xuthus*, has been reported from a non-host rutaceous plant (Nishida *et al.*, 1990). The Cabbage Butterfly (*Pieris rapae*) refuses to lay eggs on a plant that contains an oviposition stimulant when a potent deterrent is present (Renwick and Radke, 1987). Sometimes, deterrent chemicals are also found in acceptable plants (Renwick and Radke, 1985). In this situation, the concentration ratios of stimulants to deterrents may affect the acceptability of a plant. Changes in the ratios may occur as a result of different growing conditions and the ratios is likely to vary according to plant age and the vegetative stage of selected foliage (Renwick and Chew, 1994).

Different sensory cues often act synergistically in regulating host selection behaviour of insects. Oviposition of swallowtail butterflies is elicited by the combination of a number of chemical compounds (Feeny, 1990). Individual compounds elicit either weak oviposition responses or no responses at all. Feeny *et al.* (1989) found that females of *Papilio polyxens* laid more eggs on model plants treated with a combination of contact stimulants and volatiles from carrot leaves than they did on plants treated only with contact stimulants.

Olfactory responses of insects are studied with olfactometers, wind tunnels, and electrophysiological bioassays. Various types of olfactometers have been used, such as single-tube olfactometer, Y-tube olfactometer, and small-arena olfactometer (Finch, 1986). Olfactometers have been used successfully in studying the orientation behaviour of a number of moths (Gupta and Thorsteinson, 1960; Thibout *et al.*, 1982; Palaniswamy and Gillott, 1986; Khan *et al.*, 1987; Pivnick *et al.*, 1990). Small-arena olfactometers are commonly used to screen chemicals as larval attractants. However, larval attractants may also attract adults, a behavioural
association thought to be common to many insects (Thorsteinson, 1960). Ovipositional responses of gravid female moths to plant volatiles are usually studied with box-like olfactometers, with a large enough arena to allow the flight of test moths. The latter facilitation is important for moths that need to fly before responding to host odours. Schurr and Holdaway (1970) designed such an olfactometer based on an oviposition cage to study the olfactory responses of female European Corn Borer (*Ostrinia nubilalis*). A more sophisticated but similarly designed olfactometer was used by Mitchell and Heath (1987) in demonstrating an oviposition stimulant of *Heliothis subflexa*. Recognising the importance of anemotaxis in the long range orientation of flying insects, many investigators have advocated the use of wind tunnels (Renwick and Chew, 1994). Wind tunnels have been used successfully with a few moth species (Phelan and Baker, 1987; Landolt, 1989; Tingle *et al*., 1990). In electrophysiology bioassays, the responses of individual olfactory sense organs or receptors to plant volatiles were recorded and amplified by electro-antenograms (EAGs). EAG recordings from a number of volatiles or their fractions are then compared to find the responsible components or combinations. The technique is often used in preliminary screening of potential olfactory stimuli. Since only the olfactory sense organs, usually antennae, are used, the results of EAG recordings need to be confirmed by additional bioassays involving intact insects (Finch, 1986). Recently, the attraction of the Black Cutworm Moth (*Agrotis ipsilon*) to flowers of 25 plant species was found to be correlated with the corresponding EAG response (Zhu *et al*., 1993). In field conditions, olfactory responses of insects are studied with traps of various designs (Finch, 1986). Grijpma and Gara (1970b) used the so-called field olfactometers to study the olfactory response of adult *H. grandella*, a shoot borer of Meliaceae species in Latin America. Data from trapping studies need to be interpreted with caution. The higher number of trapped insects in traps loaded with host volatiles than are trapped in control traps does not always indicate the
attractive potency of the volatiles. In reality, the volatiles may have only arrested the insects in the vicinity of treatment traps, with subsequent trivial movements contributing to the trapping of more insects (Finch, 1986).

Host-finding behaviour of *H. robusta* has never been studied. Host volatiles from the young foliage have been found to be attractive to adults (Grijpma and Gara, 1970a) and larvae (Grijpma and Gara, 1970b) of the closely related *H. grandella*. It is of interest to see whether host odours play a similar role in the orientation of *H. robusta*. In this chapter, this possibility is assessed, as well as host acceptance by gravid females, based on preliminary investigations.

8.2 Methods

8.2.1 Insects and plant materials

Larvae and adults were from a laboratory colony maintained since late 1992 on the artificial diet of Cuilloud and Guiol (1980) (see Chapter 3). Olfactory responses of females were tested only with the foliage of Red Cedar. In addition to host foliage, larval orientation and egg-laying were tested with the foliage of three other Meliaceae species, Spanish Cedar (*Cedrela odorata*), Chinese Toon (*Toona sinensis*), and White Cedar (*Melia azadirachta var. australasica*), one Myrtaceae species, Southern Blue Gum (*Eucalyptus globulus* ssp. *bicostata*), and one Aquifoliaceae species, English Holly (*Ilex aquifolium*). Spanish Cedar and Chinese Toon also belong to the same subfamily as Red Cedar (Swietenioideae). Test foliage of the meliaceous species was obtained from potted trees in the glasshouse. The two non-meliaceous species were picked for convenience, as they were readily available outside the Department of Forestry Building.
8.2.2 Orientation of larvae

A novel 4-choice olfactometer was used to study the effect of plant volatiles in larval orientation. The major components of the olfactometer (Figure 8.1) were constructed with Perspex tubes of three different diameters. In the centre was a unit consisting of a circular arena (32 x 22 mm) with a cover on top, four horizontal choice tubes (42 x 13 mm) attached to the arena wall through holes drilled at the base at equal angular distances (90°), and four odour-source tubes (23 x 8 mm) each inserted vertically in the choice tubes at a distance of 10 mm from the outer ends. The insertion ends of the odour-source tubes were covered with fine metal mesh. The four-arm central unit sat on the floor of a circular plastic container (150 x 144 mm) with a mesh-covered hole (13 mm) in the centre. An air-outlet tube (42 x 13 mm) was attached vertically to the hole from outside. The lower end of the air-outlet tube was connected with a water-driven vacuum pump. The plastic container was fitted with a Perspex cover with an air-inlet tube in the centre. Test materials were placed in the odour-source tubes which were then covered with rubber caps. Powered by the vacuum pump, air from outside was pulled into the plastic container through the air-inlet tube and then into the central arena through the four choice tubes, carrying with it odours from the test materials, and then passed through the outlet tube at the bottom. Test larvae were directly placed on the floor of the central arena.

Larvae of the 4th-6th instar (excluding those in the blue stage) were used to test orientation responses to host and non-host volatiles. Leaves of the six plant species were tested either independently or in groups of four. In independent tests, two oppositely positioned odour-source tubes were packed with plant leaves and the other two left blank. In group tests, leaves of Red Cedar, Spanish Cedar, and Chinese Toon were tested together with leaves of either White Cedar, Southern Blue Gum, or English Holly. In each test the four plant species were randomly assigned to the four odour-source tubes. The amount of plant material loaded was such that each odour-
source tube was fully packed. Each loading of plant materials was tested on a maximum of 10 larvae. Between successive loadings, the odour-source tubes were washed with light petroleum ether (bp 60°C). Larvae were tested individually and observed for a maximum of 5 minutes. Larvae that did not enter any of the four choice tubes within 5 minutes of introduction were removed and replaced with new ones. A minimum of 38 larvae was tested in independent tests of each plant species. In group tests, each combination of four plant species was tested with at least 64 larvae. Two criteria were used to assess the larval responses to plant odours: (a) entering into a choice tube and (b) contacting with the mesh separating the choice tube and the corresponding odour-source tube.

8.2.3 Orientation of adults

**Y-tube olfactometer**

A simple glass Y-tube (internal diameter 2 cm) olfactometer (Figure 8.2) was used to study walking anemotaxis of the females. The length of the stem section was 31 cm and that of the 2 arms 19 cm. The angle between the two arms was 60°. Connected with each of the 3 openings of the Y-tube was a plastic vial (50 x 40 mm) with a hole the size of the Y-tube drilled in the centre of the floor. The plastic vial attached to the stem of the Y-tube was used for moth introduction and that to each of the two arms for moth recovery. A short tube (length 2 cm, internal diameter 0.8 cm) was inserted through the cap of each of the plastic vials and stuck in position. An odour delivery system was connected to the Y-tube through these tubes. The odour delivery system consists of a tank of compressed air, a charcoal filter, and an Erlenmeyer flask used to place test materials. Air from the compressed air tank first passed through the charcoal filter and then divided into two streams, one going directly to one arm of the Y-tube and the other passing through the Erlenmeyer flask before going to the other arm of the Y-tube.
Virgin females of ≤3 day-old and gravid females mated within the last two days were used to study walking anemotaxis. Moths were tested individually. After the introduction of the test moth, the cap of the introduction vial was closed. The host tissues tested were young shoots with leaves, mature leaves, and tree stems. The amount of host tissue placed in the odour-source flask was not fixed but was enough to generate distinctive host odour in the exit air. The test tissues were replaced with fresh ones once the distinctive smell disappeared (after about one hour). Each moth was observed for a maximum of 10 minutes and the arm of the Y-tube into which the moth initially walked was recorded. Moths that did not walk into either of the two arms within 10 minutes of introduction were removed and replaced with new ones. A minimum of 50 virgin females and 30 mated females were tested with each of the three types of host tissues. After each test run, the treatment and control arms were swapped by changing the connection of the air-delivery tubes. All tests were conducted in the first four hours of the scotophase. Observation was facilitated by a 20-watt red fluorescent light. The red light was placed in front of the Y-tube, with each arm receiving a similar amount of light. The speed of the air flow inside the Y-tube was controlled at 0.5-1 m/s through a valve fitted to the compressed air tank.

Wind tunnel

Flight anemotaxis of the females was studied in a wind tunnel housed in an air conditioned room (24 ± 1 °C). The wind tunnel was a clear acrylic box (0.7 x 0.7 x 1.8 m) fitted with a blower fan at one end and an exhaust fan at the other. Exhaust air was vented out of the room housing the wind tunnel. Light intensity inside the tunnel was controlled at about 1 lux and the speed of air flow at about 0.5 m/s. A screened cylindrical cage (70 x 65 mm) containing fresh young leaves and shoots of the Red Cedar was placed on a 30 cm tall metal stand standing at 30 cm from the upwind end in
the centre line. Another metal stand of the same height was similarly placed at the downwind side and used for releasing the test moths. The test moth was transferred to a screened cage before being released on the metal stand. Each moth was observed for a maximum of 30 minutes. A total of 32 virgin females and 15 mated females were tested in the wind tunnel. Data recorded were whether the moth tracked the odour plume (flying toward the odour cage) and/or landed on the odour cage. All tests were conducted at the first 4 hours of the scotophase.

8.3.4 Oviposition

Host selection by gravid females was studied in a modified oviposition cage (Figure 8.3). The body of the cage was constructed with four clear acrylic Perspex panels (500 x 250 mm). Two holes (150 mm diam.), separated by a centre distance of 260 mm, were drilled on both the top and bottom panel. The top two holes were covered with cheese cloth and the bottom two holes left open.

The test foliage was compound leaves, or branchlets in the case of Blue Gum and English Holly, each retaining the top nine leaflets or leaves. They were cut to a length of about 50 cm and inserted into the cage through the two uncovered holes. To slow down water loss, the lower part of the foliage was wrapped with cotton wool and placed in a beaker containing some water. Two hollowed styrofoam boxes, in which the beakers were placed, were used to support the cage, with the uncovered holes facing the open ends of the supporting boxes. The holes were then closed with paper towels.

In each test, the host foliage was paired with the foliage from a non-host plant. The host and non-host foliage, two compound leaves or branchlets from each species, was placed in the same beakers to prevent possible location effects. Five to eight females mated the night before were released into the cage one hour before the start of the scotophase and kept
there for 10 hours in darkness. The number of eggs laid on each species was counted after the test. Three tests each were run comparing the foliage of the host with that of Spanish Cedar and Chinese Toon. Tests involving the foliage of White Cedar, Southern Blue Gum, and English Holly were each replicated 5 times.

A similar bioassay set up was employed to examine the effect of host volatiles in eliciting oviposition. Four compound leaves of the host were placed in one beaker in water-saturated cotton wool. Direct contact with the host foliage by the test moths was prevented by enclosing the foliage with a PVC tube (235 x 160 mm). Holes (55 mm diameter) were drilled around the tube wall to allow the dispersal of host volatiles into the cage arena. The tube was then wrapped with cheese cloth. Another such tube was placed over the other beaker but no plant materials were placed inside. To standardise the conditions of the two tubes, the beaker below the blank tube was also provided with water-saturated cotton wool. Five females mated the night before were released into the cage one hour before the start of the scotophase and allowed to oviposit for 10 hours in darkness. The number of eggs laid on the cheese cloth of each tube was counted. Five replicates were run.

8.3.5 Statistical analyses

Data from the 4-choice larval olfactometer and Y-tube olfactometer were analysed with the 'prop.test' function of S-Plus (Everitt, 1994). The test statistic used was Pearson's chi-square. In assessing the attractiveness of odour from a particular plant species in individual tests against the control (blank) in the 4-choice larval olfactometer, the proportion of larvae entering either of the two treatment choice-tubes (associated with plant material) was compared with that of larvae entering either of the two control choice-tubes. In simultaneous tests of the relative attractiveness of odour from four different plant species, the proportions of larvae entering individual choice-
tubes were tested against the null hypothesis that the four choice-tubes attracted equal numbers of larvae. With data obtained with the Y-tube olfactometer, the proportions of larvae entering the treatment and control arms were compared. Differences in the number of eggs laid on the foliage of different plant species in dual-choice tests in the oviposition cage were not compared statistically, since the total surface areas of the test foliage were not strictly controlled.

8.4 Results

8.4.1 Orientation of larvae

Choice tubes associated with young leaves of the four meliaceous species and Southern Blue Gum attracted significantly more larvae than blank tubes in independent tests (P < 0.05) (Figure 8.4). Over 75% of larvae entering the treatment tubes came into contact with the mesh separating the choice tube and the odour-source tube and often stayed there for some time. The percentages were less than 10% for larvae choosing the blank tubes. However, odour from young leaves of English Holly showed little effect in attracting the larvae, with a similar number of larvae entering treatment tubes (23) and blank tubes (18) (P > 0.05, chi-square test) (Figure 8.4); no larvae were seen staying close to the plant material. The lack of attractance of English Holly was also evident in group tests (Figure 8.5, Group 3). When young leaves of the four meliaceous species were tested together, Red Cedar attracted the highest number of larvae, followed by Spanish Cedar, Chinese Toon, and White Cedar (Figure 8.5, Group 1). However, the distribution in the number of larvae entering the four choice tubes was not significantly different from random (P > 0.05). The preference order changed when the three Swietenioideae species and one of the two non-meliaceous species were tested simultaneously. Group tests involving the Southern Blue Gum showed it was the most attractive species, followed by Spanish Cedar, Red Cedar and Chinese Toon (Figure 8.5, Group 2). In group tests involving the
English Holly, Spanish Cedar was the most attractive species, followed by Red Cedar, Chinese Toon, and English Holly (Figure 8.5, Group 3). In both species combinations, the numbers of larvae entering the four choice tubes were significantly different from random ($P < 0.05$). However, the random distribution hypothesis could not be rejected ($P > 0.05$) with respect to the numbers of larvae entering the choice tubes associated with the three Swietenioideae species.

8.4.2 Orientation of adults

None of the host materials tested, young shoots, mature leaves, and tree stems, elicited apparent orientation responses either of virgin females or mated females in the Y-tube olfactometer. Of those entering the stem of the Y-tube, similar percentages of moths entered the odour arm and the blank arm ($P > 0.05$, chi-square tests) (Figure 8.6).

The lack of direct orientation responses to host volatiles by virgin or mated females was also noticed in the wind tunnel. Of the 47 moths tested (32 virgin females and 15 mated females), none were seen flying directly from the release cage to the odour-source cage and only one virgin female and four mated females landed in the odour-source cage. Tracking of the odour plume, as shown by the alignment of flight direction to the odour source cage, was rarely seen. However, upwind flight was evident in most moths (81%), even after the withdrawal of the host material. Females, virgin or mated, often flew up and back in the wind tunnel for several rounds before landing on the upwind section of the tunnel, mostly on the upwind mesh. For virgin females this was often followed by prolonged calling (release of pheromones). Mated females were more restless and often resumed flight shortly after landing. In any case, the odour-source cage placed in the centre of the upwind section appeared to be ignored by the passing females. The situation was not changed by replacing the odour-source cage with vertically placed leaf-bearing young shoots of the host.
8.4.3 Oviposition

Eggs were readily laid on the young foliage of the three non-host meliaceous species as well as on that of the host in dual-choice tests (Table 8.1). The percentages of eggs laid on the non-host foliage varied between 35 - 56%. Tests involving none of the three non-host meliaceous species showed consistently more eggs on the host foliage. Significance tests on the differences were not attempted as the total surface area of the test foliage was not controlled. Preference toward the host foliage was apparent when the latter was paired with the foliage of either Southern Blue Gum or English Holly (Table 8.1). Of all the eggs laid on the test foliage, over 77% were found on the host foliage. No eggs were found on the non-host foliage in two tests, one involving Southern Blue Gum and one English Holly.

The presence of host volatiles did not always result in an increases in the numbers of eggs laid on the cheese cloth covering the odour source tube (Figure 8.7). Of the five tests conducted, three showed more eggs on the odour tube and two showed more eggs on the blank tube. Most eggs were found along the edges of the oviposition cage rather than on the provided egg-laying substrate.

Discussion

Host volatiles appeared to be involved in larval orientation to host plants. However, the responsible volatile constituents were not restricted to the host plant, as seen in the attractiveness of young leaves of White Cedar and Southern Blue Gum. Larvae of H. robusta may respond to the so-called general green leaf odour as defined by Visser et al. (1979) rather than to species-specific volatiles. The lack of response to young leaves of English Holly could be due to a lower release rate rather than to the absence of the general leaf volatiles in that plant. Young leaves of Southern Blue Gum appeared to be more attractive to the larvae than to those of the host. Eucalypt leaves are generally rich in essential oils (Boland et al., 1991) and
odour from the young leaves of Southern Blue Gum was noticeably stronger than that from the Red Cedar. It is of interest to investigate whether the essential oils of eucalypts contain those volatile components of Red Cedar that are attractive to *H. robusta* larvae.

Entrance to the odour-laden choice tubes from the central arena of the larval olfactometer can be explained by odour-mediated anemotaxis. Most larvae were seen raising their heads to the entrances of the choice tubes to face the air streams while walking around the central arena before climbing into one of them, possibly in an attempt to locate the right odour. The responsible plant volatiles also appeared to have an arresting effect on the passing larvae as they tended to stay in contact with the mesh separating the odour-source tube from the corresponding choice tube.

Neither virgin females nor mated females showed apparent directional responses toward host volatiles in the Y-tube olfactometer, regardless of the odour sources tested, i.e. young leaves, mature leaves, or tree stems. However, upwind walking was evident in moths that had moved from the introduction vial into the stem of the olfactometer. The lack of straightforward response to the source of host volatiles was also found in the wind tunnel, where flight of the test moths was allowed. Although the test moths generally fly upwind, most of them ignored the odour source and chose to land elsewhere in the upwind section.

Oviposition tests indicated some degree of host discrimination in gravid females. Although leaves of the three non-host meliaceous plants were accepted as egg-laying substrates as readily as the host leaves, the females apparently did not like the leaves of the two non-meliaceous plants. Discrimination appeared to be based on contact stimuli, as the tests were conducted in darkness and the presence of host volatiles did not result in significant increases of eggs laid on the test substrate. The role of contact chemical cues was not investigated, but mechanoreceptive cues appear to be involved. Leaves of the meliaceous plants are soft and similar in texture.
whereas those of Southern Blue Gum are waxy and those of English Holly leathery. Further evidence for the involvement of mechanoreceptive cues was shown in the congregation of eggs along the edges of the oviposition cage.

Several factors may explain the lack of direct responses of females to host odour sources. First, female of *H. robusta* may rely on the provision of a combination of host olfactory cues rather than individual olfactory cues presented in this study to orientate to its host. Synergistic effects of chemical sensory cues are well documented in phytophagous insects (Visser, 1986; also see Renwick and Chew, 1994). In the extreme case, the insects respond only to the full blend of necessary stimuli (Visser, 1986). A study of the closely related *H. grandella* indicates that volatiles from acetone extracts of young leaves of *C. odorata* were ineffective in attracting the moths when provided alone, but the addition of potted host plants bearing only mature leaves in the olfactory traps resulted in significantly more catches (Gara et al., 1973).

Secondly, the moths may need a large manoeuvring space to express their normal orientation behaviour. In odour-mediated anemotaxis, moths usually fly upwind in a zig-zag way in approaching the host (Kennedy, 1983). This behaviour is considered as necessary for the moths to keep track of the odour plume. In the Y-tube olfactometer, casting back into the odour plume is prevented by the tube wall once the moth has entered the wrong arm. The only way it can return to the odour plume is by walking back to the stem tube and entering another arm, which was observed in a number of moths. In the wind tunnel, although the moths could fly, their movements were still constrained by the tunnel walls. This artificially-imposed restriction might have influenced the orientation behaviour of this insect. Also, the way host volatiles are provided in the wind tunnel may be quite different from that perceived by the insects in nature. Insect searching behaviour in the field may be very different from that in an
artificial environment. Field observations often reveal phytophagous insects wandering past host plants, departing high-quality plants, or simply wandering about in a seemingly random manner (Morris and Kareiva, 1991). Clearly these variations have to be taken into account before designing an appropriate bioassay.

Thirdly, orientation behaviour of phytophagous insects to host volatiles may be different from that of males to female pheromones. Among moth species, typical odour-mediated anemotaxis with characteristic odour-plume tracking has so far been documented only in *Amyelois transiteel* (Phelan and Baker, 1987), *Heliothis virescens* (Tingle et al., 1990; Tingle and Mitchell, 1992), *H. subflexa* (Tingle et al., 1989, 1990), and *Trichoplusia ni* (Landolt, 1989). It is possible that many more moth species have been subjected to the study of odour-mediated anemotaxis but the negative results were not published. Different searching strategies are needed since plant volatiles are complex in composition and usually are not species-specific. Instead of acting as attractants, host volatiles may function as arrestants to host-seeking females and thus restrict their searching to the immediate vicinity of host plants (Finch, 1986). Douwes (1968) found that females of *Cidaria albulata* showed straighter flights and lower alighting frequencies outside host habitats than inside and oriented back towards the host habitat when out of it. Subsequent locating of host plants may be assisted by visual and contact stimuli. Although gravid females of *H. robusta* did not seem to respond discriminately to host odour, they were able to allocate more eggs on host foliage than on that of some non-host species, such as Southern Blue Gum and English Holly, in dual-choice tests in the oviposition cage. Discrimination in this case was probably achieved through contact evaluation with the possible involvement of mechanoreceptive cues, as discussed above. Furthermore, larval olfactory tests showed that volatiles of Southern Blue Gum were in fact quite attractive to larvae. Volatiles of young foliage were claimed to be attractive.
to females in *H. grandella* (Grijpma and Gara, 1970a; Gara *et al.*, 1973), but the claim was based on data of field trapping. As pointed out by Finch (1986), such data offer no insight into the behavioural aspects of host finding. The trapping of greater numbers of females in cages baited with the young foliage of the host plant may be a result of the arresting effects of the host material rather than of its attractive properties.

Finally, host-specific volatiles may not be involved in the host finding process and host selection is through other sensory cues. One such sensory cue is probably light intensity. Although visual discrimination of host plants appears to be unlikely in night flying moths, changes in light intensity may affect locomotory activity and cause moths to disperse or enter a search mode (Ramaswamy, 1988). In addition, not all nocturnal insects select their hosts in the middle of the night. Gravid females of *H. grandella* concentrate their egg-laying activities in the evening or early morning (Wilkins, 1972; Holsten, 1977). They are probably able to use some visual cues from their host plants. Host selection can also be achieved by the combination of random dispersal and contact evaluation of encountered plants. Saxena (1969) pointed out that host-plant location may be essentially by random, or at least undirected, movement, so that contact with suitable plants is by chance or by orientation in response to some perceived properties of the plant. Females of *H. grandella* do not usually disperse out of existing infestation areas (Grijpma and Gara, 1970a) and only a few eggs were laid in each tree (Grijpma, 1974). If *H. robusta* behave similarly as *H. grandella*, then the high cost involved in random searching may be offset, to some extent, by the tendency of the moths to remain in the discovered host habitat. Furthermore, the chance of a gravid female laying eggs on host plants in a diverse plant community would be enhanced by its scattered egg-laying strategy.

Based on the models of Kogan (1977), Ramaswamy (1988) conceptualised four models of host finding in moths. Classified within
model II are some oligophagous insects that use non-directional cues, such as visual, textural and contact chemoreceptive cues, for host discrimination. For these insects, long range olfactory cues are generally not involved and short range olfactory cues, if used, are not species-specific. *H. robusta* appears to fit into this category. However, this study is only exploratory and many more studies are clearly needed before we can obtain a clearer picture of the host finding process of the insect.
Table 8.1. Number of eggs laid on the host and non-host foliage in dual-choice tests in the oviposition cage.

<table>
<thead>
<tr>
<th>non-host species</th>
<th>test</th>
<th>host</th>
<th>non-host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spanish Cedar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>35 (65%)</td>
<td>29 (35%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>57 (46%)</td>
<td>66 (54%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>54 (56%)</td>
<td>42 (44%)</td>
</tr>
<tr>
<td>Chinese Toon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>26 (44%)</td>
<td>33 (56%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>42 (52%)</td>
<td>38 (48%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>54 (50%)</td>
<td>55 (50%)</td>
</tr>
<tr>
<td>White Cedar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>26 (53%)</td>
<td>23 (47%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>44 (62%)</td>
<td>27 (38%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>38 (47%)</td>
<td>43 (53%)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>14 (58%)</td>
<td>10 (42%)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>62 (61%)</td>
<td>39 (39%)</td>
</tr>
<tr>
<td>Southern Blue Gum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>71 (89%)</td>
<td>9 (11%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>58 (94%)</td>
<td>4 (6%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24 (77%)</td>
<td>7 (23%)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>36 (100%)</td>
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</tr>
<tr>
<td></td>
<td>5</td>
<td>84 (98%)</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>English Holly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>77 (85%)</td>
<td>14 (15%)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25 (100%)</td>
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<td></td>
<td>3</td>
<td>73 (90%)</td>
<td>8 (10%)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>69 (80%)</td>
<td>17 (20%)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>37 (82%)</td>
<td>8 (18%)</td>
</tr>
</tbody>
</table>
Figure 8.1. Illustration of the larval olfactometer. The olfactometer has two chambers, an outer chamber and an inner chamber. Four tubes are horizontally attached to the base of the inner chamber, serving as the choice tubes. Vertically attached to each of the choice tube is a tube for loading plant materials. Air-flow in the olfactometer is generated by a water-driven vacuum pump. Test larva is placed in the inner chamber.
Figure 8.2. Illustration of the Y-tube olfactometer.
Figure 8.3. Illustration of the bioassay set-up for studying the effect of host volatiles in stimulating egg-laying. In studying the acceptance of leaves of different plant species, the PVC enclosures were not used and both beakers were inserted with leaves.
Figure 8.4. Number of larvae entering the treatment tubes (plant odour) and the control tubes (no plant odour) in the 4-choice larval olfactometer.
Figure 8.5. Number of larvae entering tubes supplied with odour of leaves of different species in the 4-choice larval olfactometer.
Figure 8.6. Number of females choosing the odour and non-odour arm of the Y-tube olfactometer. The odour sources tested were young shoots, mature leaves and tree stems of Red Cedar.
Table 8.7. Number of eggs laid on the cheese cloth covering the PVC tube with young Red Cedar leaves in and on that covering the blank PVC tube.
Chapter 9

General Discussion

In this work, a number of important ecological and behavioural aspects of *H. robusta* was investigated. These included temporal and spatial patterns of infestation (Chapter 3), artificial rearing (Chapter 4), temperature-dependent development (Chapter 5), feeding behaviour of larvae (Chapter 6), diel patterns of reproductive activities of adults (Chapter 7), and olfactory responses of larvae and adults and host acceptance by gravid females (Chapter 8). In this concluding discussion, findings from the various chapters are drawn together and discussed with respect to their contributions to our overall understanding of the insect and, where applicable, to their implications for the management of the insect. Limitations of this study are pointed out and directions for future research are suggested.

9.1 Major findings

Until now, published knowledge of *H. robusta* in Australia has been limited to descriptions or speculations based on casual observations (see Chapter 1). For the first time, this study has provided data about the biology, ecology, and behaviour of the insect in the context of its interactions with Australian Red Cedar. Some findings about the insect are new worldwide.

9.1.1 Infestation Patterns

Intensity of attack by the insect on Red Cedar was found to be positively correlated with the amount of rainfall in the previous month. The pattern was also reported for the insect attacking other Meliaceae species: *Untwistleia* (Robinson, 1967; Robert, 1968; Brunck and Fabre, 1974; Wagner et al., 1991). This is not surprising, as larvae of the insect mainly feed in young shoots, the production of which depends on rainfall. The present study also found that temperatures below 6.5 °C may inhibit the activity of the insect, as suggested by the significant annual minimum infestation intensities. Considering that winter is also the time when no young shoots are available, it is suspected that the insect may have a
winter diapause phase in its lifecycle. The diapause stage may be the last instar or pupa, the stages revealed by all dissections of infested sites during the winter. Elsewhere, winter diapause has been suggested only in northern India (Beeson, 1919).

Spatial patterns of attack by the insect were investigated at three levels: habitat, inter-tree, and within-tree. At the habitat level, trees planted inside the forest appeared to have gained some degree of protection against attack. Although attack did occur among trees planted in the forest, the proportions of attacked trees were much lower compared to those planted in the open. Similar patterns have been reported for the insect attacking other host species and for H. grandella (see Newton et al., 1993). Three factors may have contributed to the relatively low attack levels of host trees grown in the forest: shading, low growth rate, and the mechanical and/or physical barriers of non-host trees.

At the inter-tree level, small trees (<1.5 m tall) were attacked less frequently than larger trees. This may have resulted from the flight behaviour of the egg-laying females. Avoidance of short trees has been demonstrated in H. grandella (Holsten and Gara, 1975). Contrary to the likelihood of attack, the intensity of attack among attacked trees was negatively correlated with tree size. This was likely to be due to the higher number of shoots in larger trees and limited number of eggs laid per tree by visiting females, the latter being demonstrated in H. grandella (Grintra, 1974).

Within attacked trees, shoots positioned in the upper tree crowns were more often attacked than those grown at lower positions. The results suggest that once a host tree is attacked the terminal shoots would be among the first to be damaged. Such a pattern has also been reported for H. grandella (Famaazi et al., 1992).

9.1.2 Feeding behaviour

Experiments using neutral substrates demonstrated the role of certain phagostimulants in the feeding process of larvae. Chapter 8 describes how stems of shoots of the host tree appears to contain the phagostimulants. Mortality of neonates due to non-feeding, commonly observed in the field and the insect, could be reduced significantly by incorporating certain stimuli of
young shoots of the host trees into the synthetic diet. Although *H. robusta* attacks only Red Cedar in Australia, the phagostimulants are likely to be widely distributed among Meliaceae species. This was seen in the ready burrowing by larvae into the shoots of Spanish Cedar (*Cedrela odorata*), Chinese Toon (*Toona sinensis*), and White Cedar (*Melia azedarcha var. australasiae*), the last being in a different subfamily. Some components of the phagostimulants may be volatile, as larvae were able to concentrate their biting on test material loaded with host extracts without contact with the test materials.

Although their preferred feeding sites are inside the growing shoots, the larvae feed on almost any tissues of the host plants, including the buds, leaves, and epidermis of shoots or stems. It appears that the habit of tunnelling evolves as a trait to avoid natural enemies, rather than out of a need for better nutrition. There was a gradual change of feeding locations as the development of the larva progressed. Feeding by larvae of the first two instars was mainly found in the terminal foliage (buds, leaf petioles of un-expanded leaves) or in previously damaged areas on the host plant. Pith-feeding, or tunnelling, did not start until late 2nd stadium. When the shoot was small, pith-feeding was replaced by bark feeding, or the larva would come out of the tunnels before pupating, and constructed pupation cells around the bases of the plants.

9.1.3 Development

The number of larval instars ranged from 5 to 7, with most larvae having 6 instars before pupating. Only 4 instars were reported by Beeson et al. and Roberts (1968). It is suspected that the early instars were overlooked in their field studies. Development rates for the combined larval and pupal period at constant temperatures were satisfactorily described by the linear model with the threshold temperature and the thermal constant estimated at 10°C and 720 Degree-Days (DD), respectively. Due to their inherent differences, individual insects completed development at different times. The differences increased as temperature decreased. Distribution of development times were well fitted to the Weibull distribution (see Wagner et al., 1984). A sample-size- and stage-size-dependent phenology model (Dennis et al., 1986) was used to describe the stage-specific proportions of individuals at a given amount of DD.
9.1.4 Reproductive Behaviour

All moths emerged in the first 3.5 hours of the scotophase, with nearly half emerging in the half-hour interval of 1.0-1.5 h after dark. Calling by females commenced 3 hours after light-off and terminated at the end of the scotophase. Mating was enhanced by blowing wind through the mating cage. This finding effectively solved the long-standing problem of obtaining mating in indoor cages and paved the way for successful artificial rearing of the insect. Most females were available for mating during the second half of the scotophase. They were most receptive to males on the second and third night after their emergence, agreeing to the peak age of pheromone production reported for *H. grandella* (Holsten and Gara, 1977a). Males and females may mate more than once, as some females resumed calling after mating and some mated females showed courtship behaviour. Unlike female calling and mating, egg-laying activities showed no distinctive peaks and were observed at almost any time in the scotophase. However, observations of individual mated females showed a concentration of physical activities of mated females in the first 3 hours of the scotophase.

Gravid females, instead of virgin females, as suggested for *H. grandella* (Gara et al., 1973; Holsten and Gara, 1977b), may be responsible for host finding. Females were quite inactive in the first scotophase of their emergence. Then physical activities peaked in the third scotophase, when many of them had already mated. In fact, virgin females were, on average, less active than gravid females. Mating before dispersal increases the chance of reproduction in sparse populations, which is characteristic of *H. rubasta* in its natural habitat. Indeed, searching by gravid females is the norm, rather than the exception, among lepidopteran species (see Ramaswamy, 1988; Renwick and Chew, 1977).

Results of olfactory experiments suggest that host odour may not be involved in the orientation phase of host finding but may act as arresting cues at close range. Absence of directional response to host odour has been documented in many oligophagous lepidopteran species (Ramaswamy, 1988). In fact, typical odour-mediated anomotaxis with characteristic odour plume tracking has so far been reported for only a few moth species (Ramaswamy, 1988).
Long-range host searching by this insect may be random. *Hypsipyla* species appear to possess behavioural attributes that are specially adapted to the random searching strategy. It is reported that females of *H. grandella* laid only a few eggs on each tree (Grijpma, 1974). Such a behaviour increases the chance of searching females finding a host plant in a diverse plant community. In addition, once a host patch has been found, moths of later generations do not usually disperse out and continue to reproduce in existing host plants (Grijpma and Cara, 1970). This behaviour ensures the continuation of local populations despite the high risk of failing to find new host habitats by the random searching strategy.

The fact that females were able to discriminate against the foliage of some non-host plants paired with the host in cages suggests the involvement of contact chemical and/or mechanical cues in host acceptance. Leaves of the two rejected plants species either have a heavy layer of wax or are leathery, neither of these two characteristics are shown by the leaves of host and other accepted species.

### 9.2 Implications for the Management of *H. robusta*

The damage characteristics of *H. robusta* and the desire for long lengths of straight timber suggests that a very low economic threshold exists for the management of the insect. Attack by one larva on a host tree at an early age can destroy the tree form. Furthermore, the present study indicates that one larva can inflict multiple wounds to the host tree (Chapter 7) and terminal shoots would be among the first to be attacked (Chapter 3). Considering the seriousness of the problem, the best strategy appears to avoid or delay attack by the insect.

While total avoidance of attack appears unlikely to be attained, in the absence of resistant strains or varieties, some naturally-regenerated host trees did escape attack for a considerable period of time (Plate 5), probably due to their sporadic distribution. This suggests the advantage of planting host trees at low density in their natural habitat. A similar suggestion has been made with regard to the management of *H. grandella* (Grijpma, 1974). However, this planting scheme may be uneconomical to most growers. An alternative is to
plant the host trees in small groups inside an established forest. Results of this study have demonstrated that Red Cedars planted in this way suffered less frequent attack than those planted in the open (Chapter 3). If planting in the open is desired, attack may be delayed by keeping the planting sites at some distance from existing infestation loci or mature Red Cedar trees (Beeson, 1919). Once grown to a certain size, a tree could probably tolerate some degree of damage without losing its height growth and form (Chapter 3).

Delaying attack in open sites may also be achieved by planting rows of non-host trees around the host trees (Stanton, 1985). An ideal candidate for the non-host trees appears to be White Cedar. This tree was readily accepted for egg-laying (Chapter 8) and feeding (Chapter 6) but larvae invariably died (Chapter 6). With wide buffer zones consisting of this tree, a small invading population of the insect may vanish before reaching the host trees in the interior. Consequently, attack on some host trees, if not all, may be delayed. Considering that White Cedar grows naturally within the geographic range of Red Cedar and that it also produces good wood (Floyd, 1980), this planting scheme looks promising.

Finally, attack can be delayed by special protective measures, such as the application of insecticides, during the early stage of plantation establishment, to maximise first-branch height of trees and consequently increase the timber value. Maintaining young trees in good form may also reduce their risk of being attacked after the withdrawal of protective measures, since attack frequency was negatively associated with initial tree form (Chapter 6). Based on the same finding, branched trees should be pruned regularly during the first few years of their planting. Regular pruning has two additional advantages. First, it encourages terminal growth and hence the growth of the base of minor stems. Second, it reduces the number of sites available for attack and, in the long term, the size of the local populations of the insect.

Since females of the insect probably mate before emerging from groups of host finding (Chapter 7), control by mating disruption or mass trapping of aphid pheromones should be applied at the site of current infestations rather than healthy host stands surrounding the infestation site.
Observations on the feeding process of larvae (Chapter 6) indicate a re-emergence of larvae from previously established feeding sites during much of the 3rd and early 4th stadia. Such a period may be used in timing the application of insecticides. The actual time can be estimated from field phenology models of the insect, similar to those established in this study (Chapter 5). However, the practice will be of value only for the first post-winter generation of the insect, as later generations are likely to show a high degree of overlapping of individuals of different development stages (Chapter 5).

9.3 Recommended Areas for Future Research

Based on the above discussion, the following recommendations are made for future research on the insect. These recommendations are not intended as a comprehensive list of important research areas, but merely provide a place to start. The order in which each recommendation appears does not reflect its relative importance.

1. Examination of plants in areas surrounding mature Red Cedar trees on which attack by H. robusta has been recorded, to see if other plants are also attacked naturally and, if such plants are found, whether they are capable of supporting the complete life cycle of the insect.

2. Flight distance and height above ground of flying males and females.

3. Flight path of females within and without the host patch.

4. Comparative study of the dispersal pattern of adults emerged from host trees grown in the forest and open habitat.

5. Further experiments on the olfactory responses, directional or non-directional, of females to host volatiles.

6. Discriminate bioassays to assess the role of contact chemical and mechanical cues in host acceptance.

7. Life-table studies of the insect in Red Cedar plantations to determine the rate of population growth and key factors regulating population growth.

8. Development rate of the insect reared on host plants and field phenology models.
9. Controlled experiments to quantify the relationship between attack levels and tree growth and to estimate the economic threshold of the insect.

10. The efficiency of female sex pheromones in attracting males in the field.

11. Planting trials to see if mixed planting of Red Cedar and White Cedar has any effects in reducing attack levels on Red Cedar trees.

9.4 Conclusions

Attack by *H. rothschildi* is a serious problem to the cultivation of the valuable Red Cedar. Effective control of the insect requires good knowledge of its interactions with the hosts and other biological and physical components of its environment. To this end, the present study has provided some new information about the ecology and behaviour of the insect. Some findings have direct implications for the management of the insect, such as those on infestation patterns. Others may be useful in future research, such as those on artificial rearing and temperature-dependent development. All findings are new to Australia. Some of the investigations reported in this thesis represent pioneering work on the insect worldwide, such as those on the feeding, behaviour and reproductive behaviour. However, studies of the insect in Australia are just beginning. Our knowledge of the insect is still fragmentary. However, multi-national effort is now being sought to combat the insect (Proceedings of the 1996 International workshop on *Haplopappus rothschildi*). Hopefully the effort would bring us closer to a satisfactory solution to reduce the impact of the *H. rothschildi* problem.
Plate 3. Three naturally-grown Red Cedar (Toona australis (F. Muell.) Harms) trees (with no leaves) in a forest patch close to Kempsey, NSW. The three trees must have been free of attack by H. robusta for a period of time, as shown by the long and straight boles.
References


Beeson, C.F.C. (1941) *The ecology and control of the forest insects of India and neighbouring countries*. Vasant Press, Dehra Dun, India.


Hanson, F.E. (1983) The behavioral and neurophysiological basis of food plant selection by lepidopterous larvae. In: *Herbivorous Insects - Host-seeking*


Roberts, H. (1968) An outline of the biology of *Hypsipyla robusta* Moore, the shoot borer of the Meliaceae (mahoganies) of Nigeria, together with brief comments on two stem borers and one other lepidopteran fruit borer also found in Nigerian Meliaceae. *Commonwealth Forestry Review* 47, 225-232.


Appendix

Estimation of Larval Instars of *Hypsipyla robusta* Moore
(Lepidoptera: Pyralidae) By Larval Frass Widths.

Introduction

Red Cedar Tip Moth, *Hypsipyla robusta* Moore (Lepidoptera: Pyralidae), is a serious pest of a number of Meliaceae species, including Australian Red Cedar, *Toona australis* (F. Muell.) Harms (Beeson, 1919). The larvae feed inside various tissues of host plants, especially the growing shoots, and pupate inside larval tunnels (Beeson, 1919). Due to their cryptic nature, the development stages of larvae cannot be directly determined. As an indirect approach, this paper explores the possibility of using larval frass widths (FW) to estimate larval head capsule widths (HCW) and therefore larval instars. Such a technique can be used by foresters in fine-tuning the timing of control measures against this pest.

Frass of Red Cedar Tip Moth larvae is found as conspicuous clumps at the openings to the larval tunnels (Roberts, 1968). The inside of the tunnels, however, contains little frass (personal observation), suggesting that fresh frass is constantly being pushed out. It is therefore possible to relate the exterior frass with the current development stages of the larvae.

Methods

Larvae were obtained from a laboratory stock originated from mature larvae collected in a Red Cedar plantation in Macksville, NSW, and maintained on the artificial diet of Couilloud & Guiol (1980). To enhance feeding, a small amount of macerated fresh, young Red Cedar shoots was incorporated into the diet. Larvae from the original site were introduced into the stock at least twice a year.

One hundred newly-hatched larvae were reared separately in glass vials (50 x 12 mm) until pupation. The instar of a larva was determined from the number of head capsules it shed. 1st-3rd instar larvae were fed with the terminal parts of young shoots while the older larvae were supplied with cuttings from the stouter parts of young shoots, in accordance with their natural feeding habits (personal observation). Food was replaced every 1-3 days, depending on the consumption rate and freshness of the tissue. Frass was removed daily from the glass vials. Rearing was in a room with temperature at 26 ± 1°C and light period at 14L:10D. Humidity was not controlled but the room was maintained humid by a vaporiser (KAZ Model 76).

At least 20 larvae at each instar were measured for HCW and FW under a stereo microscope fitted with an eyepiece scale to the nearest 1/40 mm. Due to the frequent rupture of head capsules in the last moult, the HCW’s of the last instar larvae were replaced with the corresponding larval head widths just before pupation. For each larva measured for HCW, 20 air-dried frass pellets produced by that larva were measured and the mean FW calculated.

A separate set of data involving 30 larvae was collected in a Red Cedar plantation in Macksville, NSW, to test the effectiveness of FW in estimating larval instars in the field. The frass was first transferred from the infested shoots to glass vials and then the shoots were dissected for larvae. The frass and the associated larvae were taken back to the laboratory and the larvae were further reared to obtain their head capsules for HCW measurements.

Results

Larvae molted either 5 (32%) or 6 (68%) times before pupation as previously noted by Atuahene & Souto (1983). A recent study by the authors showed 5- and 6-instar forms in larvae of both sexes (82% and 75% of 6-instar forms in males and females respectively), hence the variation in the number of larval instars is not likely to be sex dependant.
Larvae of the 5- and 6-instar forms showed similar HCW ranges in the 1st-5th instars. Hence data were pooled and the joint mean and ranges are given in Table 1. Total separation was achieved by HCW for the first four instars, whereas the 5th and 6th instar larvae showed some overlapping in their HCW ranges. Further examination of the data showed that among the 25 5th instar larvae measured, only one had its HCW fall in the HCW range of the 6th instar larvae. Thus HCW can still be considered as a reliable predictor of larval ages. The inter-instar boundaries in HCW for any two non-overlapping instars were arbitrarily determined as the average of the maximum HCW of the former instar and the minimal HCW of the following instar, and that for the 5th and 6th instars as the minimum HCW of the 6th instar (Figure A1.1).

Overlapping in FW started in the 4th instar and the relative within-instar variations (expressed as SE/mean) were consistently higher than that in HCW (Table A1.1). However, FW showed apparent positive correlation with HCW (Figure A1.1) and the correlation was significant (t=51.37, df=133, p<0.001). The relationship was well fitted by linear regression (Figure A1.1). Assuming the regression equation correctly described the true relationship between HCW and FW, the inter-instar boundaries of FW obtained by supplanting the HCW boundaries into the equation (Figure A1.1) should perform equally well in delimiting larval instars. In effect, the percentages of correct estimations of larval instars by comparing individual FW’s with the FW boundaries were 100% for the first 3 instars, 95% for the 4th instar, 76% for the 5th instar, and 90% for the 6th instar. Overall, 93% of the measured larvae were assigned to their original instars by their FW’s. Most of the misclassifications occurred in the 5th and 6th instar, which is probably due to the overlapping of the HCW ranges of these two instars.

With field data, 26 larvae (87%) were assigned to the same instars by both HCW and FW. Two larvae that were assigned to the 5th instar by HCW were estimated as 6th instar by FW, and 2 larvae that were determined as 5th instar by HCW were estimated as 4th instar by FW.
Discussion

The above analysis demonstrates that FW is a useful predictor of larval instars of Red Cedar Tip Moth, especially for the first four instars. The degree of predictiveness is comparable to that of HCW. But, as the FW data are more easily accessed than the HCW data, the FW approach appears promising.

When applied to field situations, care should be taken to measure only those frass pellets of apparently larger sizes to minimise the possibility of accidently including frass pellets produced at earlier developmental stages. The number of frass pellets required varies with instars. Under the assumption of normal distribution of FW, a minimal number of 16 frass pellets is recommended to keep the relative sampling error below 10%.

Finally, the inter-instar boundaries of FW given here are based on larvae reared in an artificial environment. Although they were validated by one set of field data, further validation and possibly modification may be needed before widespread application of the method.
References


Table A1.1. Larval head capsule widths and frass widths (mm)

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<tr>
<th>larval instar</th>
<th>head capsule width</th>
<th>frass width</th>
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<tr>
<td></td>
<td>mean±SE(n)</td>
<td>range</td>
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<tr>
<td></td>
<td>(SE/mean)</td>
<td></td>
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<tr>
<td>1st</td>
<td>0.29±0.01(20)</td>
<td>0.28-0.30</td>
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<td></td>
<td>(0.03)</td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>0.49±0.04(20)</td>
<td>0.45-0.53</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>3rd</td>
<td>0.85±0.08(20)</td>
<td>0.70-0.95</td>
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<td></td>
<td>(0.09)</td>
<td></td>
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<tr>
<td>4th</td>
<td>1.24±0.13(20)</td>
<td>1.05-1.40</td>
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<td></td>
<td>(0.10)</td>
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<tr>
<td>5th</td>
<td>1.78±0.15(25)</td>
<td>1.53-2.20</td>
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<td></td>
<td>(0.08)</td>
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<tr>
<td>6th</td>
<td>2.25±0.15(30)</td>
<td>2.00-2.55</td>
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<td>(0.07)</td>
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Figure A1.1. The relationship between average frass width (FW) and larval head capsule width (HCW), with their inter-instar boundaries shown as dotted lines. Data from larvae reared on host plant material (see text for detail).