THE BIOLOGY AND ECOLOGY OF THE PINK-SPOTTED BOLLWORM, *PECTINOPHORA SCUTIGERA* (HOLDAWAY) (LEPIDOPTERA: GELECHIIDAE)

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A thesis submitted in accordance with the requirements for the degree of Doctor of Philosophy in the University of Queensland.

DECLARATION

The material presented in this thesis is, to the best of my knowledge and belief, original, except as acknowledged in the text, and has not been submitted, either in whole or in part, for a degree at this or any other institution.

Paul W. Walker

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ABSTRACT

Despite it's status as a pest of cotton (*Gossypium hirsutum* L.) in Central Queensland, the biology and ecology of the pink-spotted bollworm, *Pectinophora scutigera* (Holdaway), is poorly understood. This study was undertaken to gain a better understanding of the biology and ecology of *P. scutigera* in order to suggest ways of improving current control strategies. The approach I adopted was that advocated by Clark et al. (1967), whereby the life system of a pest is studied in an attempt to recognize key factors which control its abundance.

The effect of temperature on *P. scutigera* development and reproduction was investigated at four constant (18, 21, 25 and 30^{o} C) and one fluctuating (25/15^{o} C) temperature regimes. A novel technique for rearing larvae on artificial diet is described. The relationship between temperature and development was linear for eggs, first and second instar larvae and pupae but curvilinear for third and fourth instar larvae. Although overall larval and moth development was curvelinear, it was adequately described by a simple linear regression model. The fourth larval instar was very variable in duration and constituted over 50% of the larval stage. Development of all life stages was accelerated, but not significantly, at the fluctuating temperature regime when compared to the constant temperature equivalent. Realized fecundity and capacity for increase was highest at 25^{o} C and lowest at 18^{o} C.

Larval diet significantly affected the rate of development and reproduction. The effect of diet on larval development was much more pronounced than on pupal development. Larvae reared on excised flowers of *Hibiscus tiliaceus* L. and *H. rosa-sinensis* L. developed fastest, while those reared on excised cotton squares developed slowest. Larvae reared on excised cotton bolls had intermediate developmental rates which were very similar to larvae reared on artificial diet. Larval development on excised flowers of *Brachychiton australis* (Schott *et* Endl) was slower than all other diets except cotton squares. Pupal development was significantly slower when larvae were reared on cotton squares, but was identical for all other diets. Realized fecundity (not measured for *P. scutigera* reared on cotton squares) was highest

when reared on artificial diet, closely followed by cotton bolls, and lowest on *B. australis* flowers. Excluding artificial diet, capacity for increase was highest on *H. rosa-sinensis* flowers and lowest on *B. australis* flowers.

The seasonal incidence of *P. scutigera* infesting unsprayed and sprayed cotton fields over three consecutive seasons (1984-1987) is described. In confirmation with previous reports, *P. scutigera* is a late season pest, causing economic damage to cotton bolls. Although larvae infest squares in the field, levels of infestation ~~are~~ in this 3-yr study were never high enough to warrant control with insecticides. Low infestation of squares was attributed to the ineffective carry-over of *P. scutigera* populations from one cotton season to the next, and the unsuitability of squares as a larval food source. In glasshouse experiments, neonate larvae avoided infesting squares when released onto cotton plants bearing bolls.

The behaviour of larvae infesting bolls is described. The first three larval instars are spent feeding within the tissue of the boll wall. On moulting to the fourth instar, larvae attack the developing lint and seed. At this stage most economic damage to the boll occurs. The relationship between boll age and larval infestation was unclear. First instar larvae were found infesting bolls of all ages, although there was evidence that survival of young larvae was lower in bolls less than ~~ca.~~ about 2 weeks and more than 7 weeks old due to unfavourable moisture content. Accurate detection of larvae in bolls was very difficult and time consuming. During the first year of investigation, significantly more larvae were found in bolls incubated in plastic boxes for 10-14 days than when inspected for larvae immediately after collection. This discrepancy was attributed to inexperience in locating neonate larvae. The optimum number of samples required to determine larval infestation at the 10% level of precision was unacceptably high. The use of a lower level of precision (20%) reduced the number of samples needed to more acceptable levels, but only when larval populations were at or above 5 larvae/100 bolls. Consequently, a sequential sampling plan for larvae is proposed.

The site of oviposition of *P. scutigera* varied according to the age and physiological condition of the cotton plant. Contrary to previous reports, eggs are very rarely laid on

squares or bolls, but mainly on leaves or leaf buds. Bolls became attractive oviposition sites only when fully open at the end of the cotton season. Wherever laid, the eggs were always well concealed and difficult to locate with the naked eye. Most eggs laid on leaves were deposited inside the abandoned larval mines of *Bucculatrix gossypii* Turner. Such concealed location, together with their small size and variability in location on the plant, made it impractical to develop an egg sampling plan for estimating *P. scutigera* populations.

Trichogrammatoidea bactrae Nagaraja was recorded parasitizing *P. scutigera* eggs for the first time but due to problems in sampling *P. scutigera* eggs, the importance of this parasitoid in regulating moth populations could not be assessed. An unidentified species of *Apanteles* parasitized *P. scutigera* larvae. Levels of parasitism were very low in all three cotton seasons, even in the unsprayed field. Similarly, levels of prepupal/pupal parasitism by a *Brachymeria* sp. and *Lissopimpla excelsa* (Costa) were very low at the end of the cotton season despite very high *P. scutigera* populations in unsprayed cotton.

Contrary to previous reports, the main site of pupation in cotton was not inside bolls but at the base of plants at the leaf litter/soil interface. Pupation inside cotton bolls only occurred at the end of the season in dry, partially opened bolls that had been previously damaged by *P. scutigera* larvae.

During summer, the nocturnal activity of adult moths could be divided into two distinct phases; a dispersal and oviposition phase between dusk and midnight, and a sexual phase between midnight and dawn. Temperature affected the duration of each phase; on cool nights the sexual phase was advanced while on warm nights it was delayed. Males only responded to traps baited with synthetic pheromone during the sexual phase of activity.

It is recommended that synthetic pheromone baits for monitoring male *P. scutigera* should contain a 9:1 ratio of Z,Z and Z,E isomers of 7, 11-hexadecadienyl acetate and not the Z,Z isomer alone. However, pheromone traps should not be used for timing insecticide sprays against *P. scutigera* due to the inconsistent relationship between trap catch and larval infestation. Factors responsible for this poor correlation are discussed. Of four pheromone

trap designs compared, the Delta trap was the most suitable for monitoring male *P. scutigera*. Traps placed immediately outside the cotton crop caught significantly fewer males than those placed within the crop. Synthetic pheromone baits remained suitable for monitoring males for at least eight weeks.

Four new alternative host plants of *P. scutigera* were discovered; *Brachychiton australis*, *B. populneus* (Scott *et* Endl), *Gossypium sturtianum* J. H. Willis, and *Prunus persicae* L. Also, *H. rosa-sinensis* and *H. cannabinus* L. were confirmed as alternative hosts of *P. scutigera* in Central Queensland. Only one host plant, *B. australis*, is considered to be important in assisting *P. scutigera* populations to carry-over from one cotton season to the next. Mass flowering of ornamental *B. australis* trees coincided with peak emergence of moths from cotton crop residues in September and October, thus providing *P. scutigera* an important larval resource at a time when cotton is unavailable. The significance of *B. australis* in the population dynamics of *P. scutigera* in inland Central Queensland is discussed.

The major factor controlling the development of overwintering *P. scutigera* larvae was the moisture content of dry, open cotton bolls. Larvae were unable to develop in bolls containing ≤38% moisture. In response to low moisture, third and fourth larvae entered a period of quiescence in which they could survive for long periods (>63 days). Quiescence was terminated immediately on absorption of adequate moisture by the boll through rainfall or irrigation. This adaptation could be related to the life history of *P. scutigera* on native host plants whereby larvae infest reproductive forms which undergo similar changes in moisture content when detached from the plant.

Burial of infested bolls was a beneficial cultural control practice as the overlying layer of soil increased boll moisture. This advanced the development of larvae thus reducing the number of moths emerging during the following cotton season. More importantly, burial severely reduced the overall number of moths emerging due to the formation of a crust impenetrable to pupating larvae. In dry soil, mature larvae tunnelled out of buried bolls and burrowed to the soil surface to pupate. Movement of larvae to the surface was restricted when

a soil crust formed after rain or irrigation. Unless cracks in the soil were located, larvae were forced to pupate underneath the crust thus trapping the emerging moths.

The survival and rate of emergence of *P. scutigera* from buried bolls was significantly affected by soil type; moth survival was lowest and development slowest in bolls buried underneath sandy soil compared to clay and alluvial soils. Both factors were related to differences in the water retention properties of each soil type, and the degree of soil crusting.

The carry-over of *P. scutigera* populations from one cotton season to the next may be aided by the long-lived adult stage. Larval diet significantly affected the longevity of *P. scutigera*; moths reared on *H. rosa-sinensis* had significantly shorter life-spans than those reared on dry cotton bolls or *B. australis* flowers. Longevity of *P. scutigera* reared on *B. australis* flowers at 25^{o} ranged up to 126 days in males and 68 days in females. Oviposition of moths emerging from *B. australis* flowers and cotton crop residues during September and October, extended until late December when moths were placed in an insectary under ambient temperature conditions.

Delaying mating for 10, 20 and 30 days reduced the number of eggs laid by 39, 55 and 65%, respectively, when compared to moths paired immediately after eclosion. Delayed mating had no significant affect on the preoviposition period, egg fertility and 'effective longevity' of females (number days from eclosion to the last fertile egg laid). The duration of oviposition declined linearly with increasing delay in mating.

In the general discussion the results from previous chapters are collated to give an overview of the life system of *P. scutigera* in cotton crops. The major factor thought to influence the abundance of *P. scutigera* is a relative shortage of larval resources. This shortage is brought about by the scarcity of alternative host plants in the inland areas of Central Queensland, and by the unsuitability of pre-squaring and squaring cotton crops for development, survival and reproduction. It is proposed that high quantities of the terpenoid aldehyde, gossypol, may be responsible for the unsuitability of cotton squares for larval infestation.

The shortage of larval resources is offset by two important factors that favour population increase: the ability of larvae to utilize dry cotton bolls as a larval resource, and the comparatively long life-span of adult moths. Both of these characteristics of *P. scutigera* can be related to the adaptations this species has evolved for living on the reproductive forms of native host plants.

Ways of improving current control strategies are suggested. It is recommended that the cotton season is shortened to prevent the build-up of *P. scutigera* populations at the end of the season. This population build-up ensures the carrying-over of moth populations into the next cotton season through the slow development of larvae infesting dry, open cotton bolls.

The limited pest status of *P. scutigera* in Australia is discussed and compared with the closely related species, *Pectinophora gossypiella* (Saunders). Suggestions for further research are also made.

ACKNOWLEDGEMENTS

I am sincerely grateful to Dr Vivian Harris for initiating this project and for her enthusiastic supervision during the first year of study. I thank Professor Hugh Paterson for taking over as supervisor and for his guidance throughout the remainder of the study. I am indebted to Dr Myron Zalucki for his prompt and critical reviewing of draft chapters of the thesis. I thank all other members of staff and postgraduate students in the Department of Entomology, for their constant encouragement and assistance.

I am also sincerely grateful to past and present members of staff at the Queensland D.P.I. Research Station, Biloela, who gave unstinted support while I was based in Central Queensland. Special thank go to: Mr Alan McTaggart for providing excellent facilities; Dr Peter Lawrence and Dr John Standley for getting me started; Mr Frank Page, Mr Mark Modini and Mr Angus McColl for invaluable entomological advice; Mr Peter Walsh for assisting with cultural control trials; and Mr Bob Noble for his interest in developing a technique for determining the age of adult moths.

Thanks also go to: Dr Roger Lewis, Department of Physics, University of Wollongong, for developing a technique to determine the elemental composition of adult moths while he was at the Capricornia Institute of Advance Education, Rockhampton; Mr Bruce Pyke and Mr Mike Stone, Integrated Pest Management Systems, Biloela, for their extensive knowledge of *P. scutigera*; the Cotton Marketing Board in Biloela for providing facilities at the start of the project, Mr Bruce Sabine, Queensland D.P.I., Brisbane, for supplying *P. scutigera* host plant records, and Dr G. H. L. Rothschild, C.S.I.R.O., Canberra, and Dr H. Flint, U.S.D.A., Phoenix, Arizona, for supplying synthetic pheromone baits.

I thank the many farmers in Central Queensland who tolerated my use and abuse of their cotton fields, especially Messrs. Allister and Ivan Shepherdson, Ian Wilkie and Bob Breeze.

Finally, I thank the Australian Cotton Research Council for generous financial support.

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CHAPTER 1

General Introduction

The pink-spotted bollworm, *Pectinophora scutigera* (Holdaway), is a serious pest of cotton in Central Queensland. Larvae cause severe damage to cotton bolls, usually late in the season (Page 1981, Passlow 1964, Sloan 1946). It is a notoriously difficult pest to control as moths are inconspicuous, eggs are too small to detect readily, and the neonate larvae bore directly into cotton bolls leaving little external evidence of their presence (Passlow and Sabine 1963, Page *et al.* 1984a). Farmers are frequently unaware of an infestation until populations are well established, at which time control with insecticides is difficult due to the protected location of larvae deep inside the cotton boll (Sabine 1969a).

Despite its pest status and potential ability to spread to other cotton growing districts (Rothschild 1983), the biology and ecology of *P. scutigera* is poorly understood. Current knowledge is largely based on casual observations that have not been verified empirically. Excluding notes that have appeared in agricultural journals, which make statements about the pests biology but give no experimental data, there are only 12 publications which deal with aspects of *P. scutigera* biology. Six of these papers concern trapping with artificial sex pheromones and give little biological or ecological information about the species.

The following literature review is a comprehensive summary of all publications on *P. scutigera*, and emphasizes the need for further studies to improve current pest control.

1.1. Literature review

1.1.1. Discovery and nomenclature

When larvae of *P. scutigera* were first discovered infesting cotton in Central Queensland they were misidentified as those of the pink bollworm, *Pectinophora gossypiella* Saunders (Holdaway 1926). After the discovery of *P. gossypiella* in Western Australia and the Northern

Territory it was feared that this notorious pest of cotton may invade the newly established Queensland cotton industry. Crops were constantly monitored for its possible presence and in 1923, knowing that *P. gossypiella* infested native Malvaceae in other parts of the world, an inspection of wild *Hibiscus* species growing around the Brisbane area was conducted (Tryon 1926). Larvae very similar in appearance to *P. gossypiella* were commonly found infesting seed capsules of *Hibiscus heterophyllus* Vent., *H. diversifolius* Jacq. and *H. tiliaceus* L. although none were found infesting near-by cotton crops (Anon. 1923, Tryon 1926). Adult specimens were sent to J. A. Turner, an amateur specialist on Australian microlepidoptera, for identification while preserved larvae were examined by a Queensland government entomologist, F. G. Holdaway (Tryon 1926). Turner was unable to verify that specimens were *P. gossypiella* but suggested, on the basis of host plant records and lack of infestation of near-by cotton, that it may be a distinct species. Holdaway reached a similar conclusion as larvae differed from those of *P. gossypiella* described by Busck (1917) (Tryon 1926).

In 1924 a number of cotton crops growing in coastal Central Queensland became heavily infested with *P. gossypiella*-like larvae (Holdaway 1926, Tryon 1926, Wells 1925). Adult specimens were sent to Turner for identification who was again unable to distinguish them from *P. gossypiella* (Holdaway 1926). Fearing the pest would spread to other cotton growing districts, government entomologists released an announcement to the press proclaiming the discovery of *P. gossypiella* in Queensland cotton. This announcement received considerable media attention and was published in the leading state newspaper, the Brisbane Courier, on April 14, 1924.

Holdaway, who was assigned to conduct a study of the so-called 'Queensland pink bollworm' (Anon. 1924), disagreed with the identification of specimens as *P. gossypiella*. He found that larvae infesting cotton in Central Queensland were similar to ones previously collected from *Hibiscus* spp. in the Brisbane area but distinct from *P. gossypiella* larvae from Western Australia, the Northern Territory and Egypt. On the basis of detailed comparisons of larval and pupal characters, and host plant records, Holdaway (1926) put forward the hypothesis that the Queensland pink bollworm was a distinct species, indigenous to Australia,

and proposed the name *Platyedra scutigera*. Later, Holdaway (1929a) followed the opinion that Busck's genus of *Pectinophora* should be used rather than Meyrick's *Platyedra*.

Holdaway's hypothesis was rejected by Ballard (1925), who considered the Queensland pink bollworm to be a geographic race of *P. gossypiella* and not a distinct species. Furthermore, adult moths collected from Central Queensland cotton were also sent to 2 world authorities on microlepidoptera; E. Meyrick of the Imperial Bureau of Entomology in England, and A. Busck of the U.S. National Museum in Washington, who both agreed with the identification of specimens as *P. gossypiella* (Holdaway 1926, Zimmerman 1978). Ballard (1925) confidently wrote in the Journal of Economic Entomology:

> " all doubts as to whether or not we were dealing with *Platyedra gossypiella* Saunders, or an allied species, have been removed, Mr E. Meyrick whose opinion was taken, having given it as his considered judgment that the Queensland species is *gossypiella* and no other."

In this paper Ballard also referred to 2 races of *gossypiella* in Queensland -a "cotton" race and a "Hibiscus" race whose larvae were bright red in colouration and was never found in cotton. The cotton race was in fact *P. scutigera* while it seems most likely that the Hibiscus race was *Pectinophora endema* Common.

Ballard (1927a) further criticized Holdaway for having described the species using only larval and pupal characters and considered evidence that the Queensland pink bollworm was a distinct species to be "powerful but not conclusive". Ballard continued to use the name *gossypiella* in subsequent publications (Ballard 1927b, 1927c). In response Holdaway (1929b) presented a paper at the 1928 International Congress of Entomology in an attempt to clarify the matter and to justify the use of larval and pupal characters for describing species. In this paper Holdaway also included a disclaimer to point out that while in a joint publication (Ballard and Holdaway 1926) reference was made to *P. gossypiella* in Queensland, he denied ever having subscribed to the opinion that this species occurred in the state. According to Holdaway (1929b) the incorrect statement appeared due to: "my departure from Queensland necessitated the final form of the paper being left to the co-author".

The controversy was finally settled when Holdaway (1929a) confirmed the species status of *P. scutigera* on the basis of detailed studies of the genitalia. These findings were later confirmed by Common (1958) in a revision of the genus *Pectinophora* in Australia, and by Zimmerman (1978) who compiled a detailed comparison of all life stages of *P. scutigera* and *P. gossypiella.*

To avoid further confusion with *P. gossypiella*, Passlow and Sabine (1963) proposed changing the common name of *P. scutigera* from Queensland pink bollworm to pink-spotted bollworm. This new name was derived from the pattern of pink colouration in *P. scutigera* larvae which is the major character that readily distinguishes it from *P. gossypiella.* Common (1958) and Zimmerman (1978) presented comprehensive keys for distinguishing *P. scutigera* from *P. gossypiella* and *P. endema.*

1.1.2. Distribution and alternative host plants

P. scutigera occurs in coastal and sub-coastal Australia, Papua New Guinea, Micronesia and Hawaii (Common 1958, Zimmerman 1978) and may be widely distributed throughout the western Pacific (Rothschild 1975). All known host plants of *P. scutigera* belong to the family Malvaceae and are listed in Table 1.1.

Zimmerman (1978) considered *P. scutigera* to be an introduced species in Hawaii where it infested cotton, *H. tiliaceus* and *Thespesia populnea* (L.) Soland. ex Corr. on the island of Oahu. As in Australia, *P. scutigera* was initially confused with *P. gossypiella*, which also occurs in Hawaii, until publication of Holdaways' work (Zimmerman 1978). Details on the distribution and host plants of *P. scutigera* in Micronesia were not given by Common (1958).

In Australia, Holdaway (1926) considered *P. scutigera* to be a coastal species with its distribution closely associated with the two primary native hosts, *H. tiliaceus* and *T. populnea.* The former is restricted to the coast and estuaries of rivers along the entire coast of Queensland, northern New South Wales and the Northern Territory (Holdaway 1926, Sabine 1969b, Sands and Hill 1982). *T. populnea* is distributed along the coast of northern Western Australia, the Northern Territory and Queensland (Fryxell 1965). The most southerly record of *P. scutigera* in Australia is from *H. tiliaceus* growing on the Clarence River in New South Wales (Wilson 1972) while the most northerly record comes from Hicks' Island, off Cape

Table 1.1. Host plants of *Pectinophora scutigera*.

Host Plant	Locality	Reference
Gossypium hirsutum (Cotton)	Queensland	Holdaway (1926)
	Papua New Guinea	Ballard (1927a)
	Hawaii	Zimmerman (1978)
Gossypium sp.	Queensland	Holdaway (1926)
Hibiscus tiliaceus (Cottonwood tree)	Queensland & N.S.W.	Holdaway (1926)
	"	Q.D.P.I.[1]
	"	Tryon (1926)
	Northern Territory	Common (1957)
	" "	Sands & Hill (1982)
	Hawaii	Zimmerman (1978)
	Papua New Guinea	Ballard (1927a)
Hibiscus divaricatus	Queensland	Holdaway (1926)
Hibiscus diversifolius	Queensland	Tryon (1926)
Hibiscus heterophyllus[2]	Queensland	Holdaway (1926)
		Tryon (1926)
Hibiscus rosa-sinensis (Ornamental Hibiscus)	Queensland	Q.D.P.I.
Hibiscus cannibinus (Kenaf)	Queensland	Q.D.P.I.
Thespesia populnea (Milo)	Queensland	Holdaway (1926)
	Hawaii	Zimmerman (1978)

1. Q.D.P.I. = Queensland Department of Primary Industry records (kindly provided by Mr B. Sabine). See Appendix A for detailed listing of Q.D.P.I. distribution records for *P. scutigera* infesting cotton and alternative host plants.

2. Sabine (1969b) was unable to confirm *H. heterophyllus* as an alternative host plant of *P. scutigera* in Central Queensland.

York Peninsula, Queensland, where it heavily infested cotton during the 1920's (Holdaway 1926). A single female was collected from *H. tiliaceus* at Darwin during the 1920's (Common 1958) and its presence in the Northern Territory was confirmed by Sands and Hill (1982).

Sabine (1969b) searched for alternative host plants of *P. scutigera* in Central Queensland during the 1960's but found no species, other than *H. tiliaceus*, supporting larvae. Larvae could be reared on the fruit of *Abutilon asciatum* G. Don. in the laboratory but *P. scutigera* was never found infesting plants in the field (Sabine 1969b). Although Holdaway (1926) and Tyron (1926) reported *P. scutigera* attacking seed capsules of *H. heterophyllus*, Sabine (1969b) could only find larvae of the closely related *P. endema* infesting this plant. Fig. 1.1 shows the known distribution of *P. scutigera* in Queensland, as recorded by the Queensland Department of Primary Industries (B. Sabine pers. comm.). Appendix A lists the localities and grid reference for each record.

Recent pheromone trap surveys by Murray and Page (1982) and Page *et al.* (1982, 1984a) revealed that *P. scutigera* is not restricted to coastal or sub-coastal areas but is widely distributed throughout Central and S.E. Queensland (Fig. 1.1). Males were caught in traps as far inland as Emerald and up to 15 km away from the nearest commercial cotton (Murray and Page 1982, Page *et al.* 1982). *P. scutigera* was also recorded for the first time between Taroom and Wandoan, and near Macalister on the Darling Downs (Page *et al.* 1984a).

This widespread distribution either indicates that moths have the ability to disperse over long distances, or that it is established on alternative host plants throughout the region. In 1985 *P. scutigera* larvae were found infesting the flowers of *H. rosa-sinensis* in S.E. Queensland (B. Sabine pers. comm., Appendix A). Both *H. rosa-sinensis* and *H. tiliaceus* are popular ornamental plants and today they can be found in parks and gardens throughout Queensland. Therefore, both of these host plants may have helped *P. scutigera* to extend its range into inland areas of Queensland. Ornamental *H. tiliaceus* trees planted in Emerald and Biloela are known to support *P. scutigera* larvae (D. Murray unpublished D.P.I. report, F.D. Page pers. comm.).

1.1.3. History of pest status

Fig. 1.2. shows the distribution of cotton in Australia up to 1925, as given by

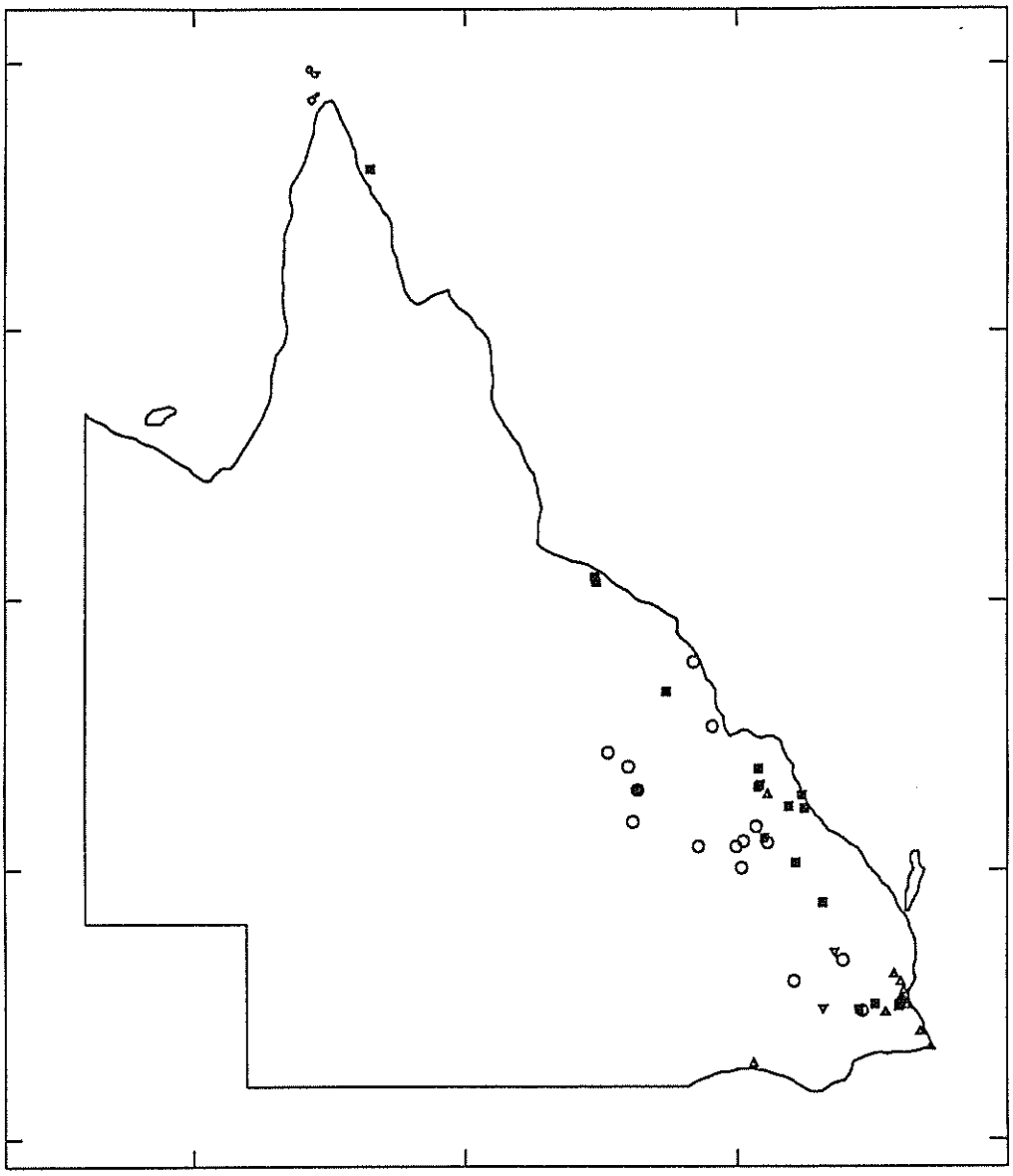

Fig. 1.1. Known distribution of *P. scutigera* in Queensland as recorded by the Queensland Department of Primary Industries. (see Appendix A for localities and grid references).

Legend:

- ○ Pheromone trap catch
- ■ Cotton
- ▲ *Hibiscus tiliaceus*
- ▼ *Hibiscus rosa-sinensis*

Holdaway (1926). At this time cotton was mainly grown in coastal areas, less than 180 km inland. *P. scutigera* commonly infested cotton growing between Bundaberg and Marlborough but the heaviest infestations occurred in coastal areas of Central Queensland around Sand Hills, Yeppoon, The Caves and Mount Larcom (Holdaway 1926, Wells 1926). *P. scutigera* was noticeably absent from cotton grown in inland areas. Holdaway (1926) explained its absence in these areas due to cotton being grown too far away from native hosts such as *H. tiliaceus*.

Cotton was first grown in the Callide Valley, around Biloela, in 1923 and by the end of 1924-25 cotton season *P. scutigera* was noticed infesting bolls (Holdaway 1926). Ballard (1927a) traced this infestation to a large field of standover cotton that was planted during the cotton growing boom and later abandoned. Levels of boll infestation were much lower at 1%, compared to over 70% in annual cotton at Gladstone and 90% in standover cotton at Sand Hills. Infestations in the Callide Valley remained low throughout the 1920's while cotton grown in coastal areas continued to suffer heavy damage, particularly between 1926-29 (Wells 1925, 1926, 1927, 1928, 1929, 1930).

However, in the 1932-33 cotton season very high infestations of *P. scutigera* were reported in sections of the Callide Valley (Wells 1934). Wells (1934) attributed this sudden increase in *P. scutigera* numbers to the presence of large areas of standover cotton. During this time the early settlers of the Callide Valley grew cotton as a pioneer crop in areas of newly burned scrub. Before harvesting, Rhodes grass (*Chloris gayana* Kunth) was sown amongst the cotton plants to establish improved pastures for dairy cattle. Under favourable climatic conditions, establishment of grass was good and when burnt off in the following spring, to stimulate fresh growth, all old cotton plants were meticulously destroyed. Unfortunately, in dry winters establishment of grass was poor and burning was not sufficient to destroy old cotton plants. This led to large areas of stand-over cotton in which considerable numbers of *P. scutigera* bred when new shoots were produced. Moths then spread from this standover cotton into adjacent fields of annual cotton.

P. scutigera infestations were again high in the Callide Valley during the 1933-34 season when it was recorded for the first time infesting cotton at Gayndah (Wells 1935). In the 1941-

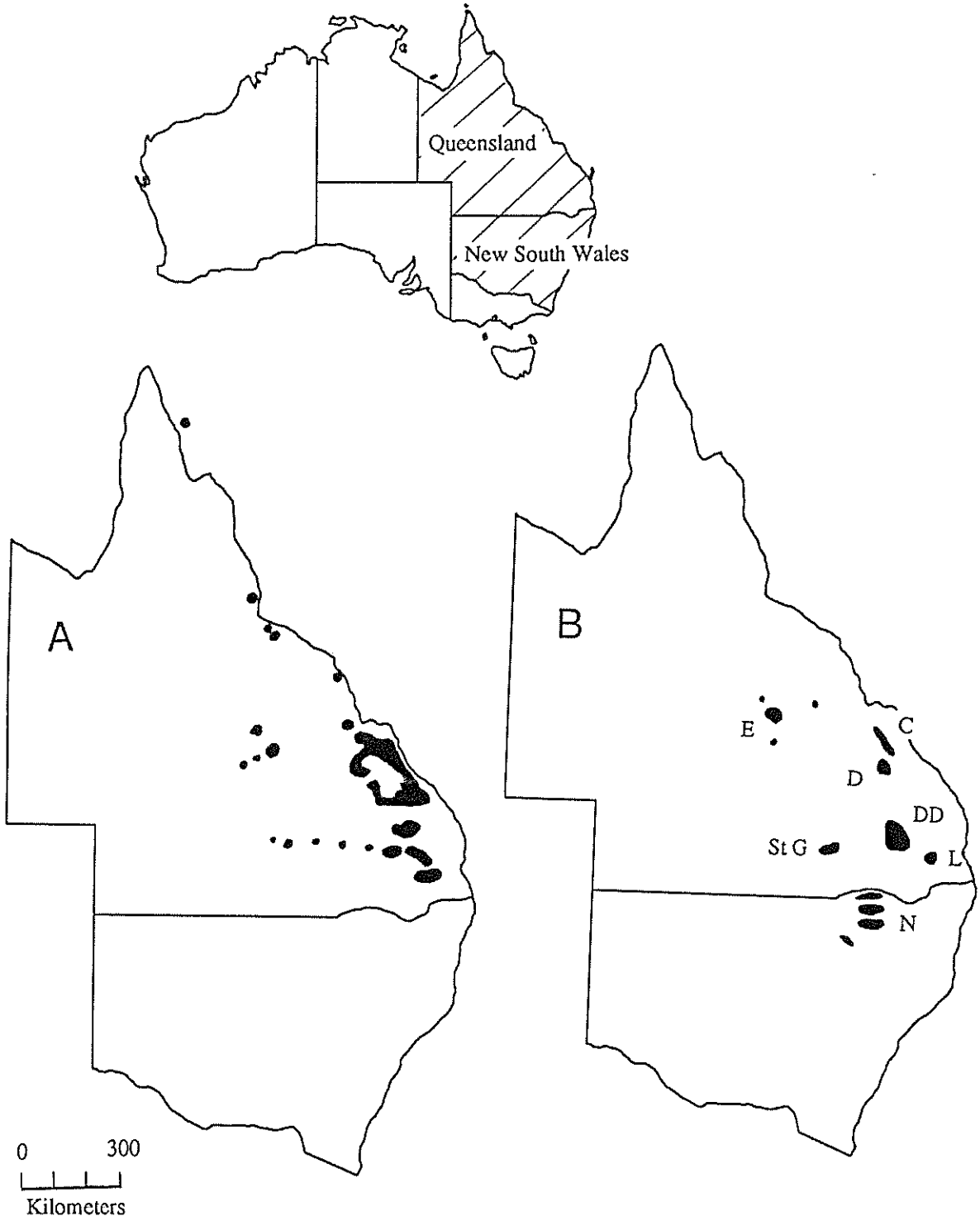

Fig. 1.2. Major cotton growing areas in Australia: (A) Distribution during the 1920's (after Holdaway (1926)). (B) Present distribution (after Lawrence *et al.* (1984) and Page *et al.* (1984)). C = Callide Valley, D = Dawson Valley, E = Emerald Irrigation Area, DD = Darling Downs, L = Lockyer Valley, N = Namoi/Gwydir/Macintyre/Macquarie, St G = St. George.

42 season *P. scutigera* was also recorded for the first time infesting cultivated cotton in south-east Queensland district of Lowood in the Lockyer Valley (Wells 1943).

During the 1940's the Australian cotton industry underwent a serious decline after official encouragement to grow cotton was discontinued (Lloyd 1982). No further reports on the pest status of *P. scutigera* appeared until the late 1950's, when the cotton industry recovered. This recovery saw great changes in the areas of cotton production (Fig. 1.2). Areas of production moved inland and cotton was no longer grown in coastal areas of Central Queensland where *P. scutigera* infestations were most severe. Large areas of cotton were planted on the Darling Downs, Lockyer Valley and St. George districts of south-east Queensland and in northern New South Wales. During the 1970's large-scale cotton production began in the Central Highlands after completion of the Emerald Irrigation Scheme (Lloyd 1982). In the early 1980's, there was a further increase in the cultivation of dryland or raingrown cotton in all areas, particularly around Emerald, the Darling Downs, and areas west of the Callide Valley around Bauhinia.

With the rapid expansion of the cotton industry it was feared that *P. scutigera* would become a pest in other areas (Page *et al.* 1982, Rothschild 1983). Despite the widespread distribution of the moth throughout Queensland, as indicated by pheromone trap surveys (Murray and Page 1982, Page *et al.* 1982, 1984a), this has not occurred. Intensive searches for *P. scutigera* during the 1960's failed to find any larvae infesting cotton on the Darling Downs, Lockyer Valley and St. George areas of southern Queensland (Sabine 1969a). Wright and Nikitin (1964) did not mention *P. scutigera* in their extensive survey of cotton insects occurring in New South Wales.

P. scutigera was rarely mentioned in reports on cotton pests published during the 1950's and 1960's. Changes in agronomic practices, such as the introduction of broad spectrum insecticides and improved cultural control, may have helped to reduce the impact of this pest (Sabine 1969a) (see section 1.1.8). Passlow (1958), in a review on the control of cotton insects, considered populations of *P. scutigera* to be rarely sufficiently large to cause concern. However, in the 1959-60 cotton season severe infestations of *P. scutigera* were reported in some areas of Callide and Dawson Valleys.

Today *P. scutigera* remains a pest of cotton only in the Callide and Dawson Valleys of Central Queensland and around Bauhinia, *ca* 146 km west of Biloela (Fig. 3 1.). Mysteriously, it has failed to become established as a pest of cotton in the Emerald district, situated *ca* 300 km west of Biloela, where it is known to infest *H. tiliaceus* and is regularly caught in pheromone traps throughout the year (Page *et al.* 1982, D. Murray unpublished report). Only on one occasion has *P. scutigera* been reported infesting cotton in Emerald; in February 1982 larvae were found infesting a crop that was grown adjacent to a field of unploughed cotton (Murray and Page 1982, D. Murray pers. comm.). The infestation was successfully controlled by a single application of a pyrethroid insecticide and no further problems were encountered.

Very recently, *P. scutigera* was recorded infesting cotton in the Lockyer Valley, south-east Queensland, for the first time since 1942. In 1986 larvae were found infesting a small plot of unsprayed cotton at the Q.D.P.I. research station near Gatton and infestations persisted in the following 2 cotton seasons (B. Sabine pers. comm., Appendix A). Whether this marks the establishment of *P. scutigera* in south-east Queensland cotton remains to be seen.

1.1.4. Biology

Vickers (1982a, 1982b) records are the only detailed studies on *P. scutigera* biology. Nearly all of his experiments were laboratory based, rearing larvae on artificial diet under constant temperatures and controlled photoperiods. Total development was curvilinear between 17-31^{o} C but linear between 17-25^{o} C. Development from egg hatch to moth emergence at 25^{o} C took an average of 39.5 days. Day-degree calculations, using an estimated lower developmental threshold of 13.5^{o} C for the combined stages, suggested that *P. scutigera* could complete four generations in one cotton season under Central Queensland temperatures. On the basis of head capsule width Vickers (1982a), in agreement with Holdaway (1926), estimated that there were four larval instars.

At 25^{o} C (14:10 light/dark photoperiod) females laid on average 326 eggs after a preoviposition period of 4.1 days (Vickers 1982b). Under the same conditions, mean longevity of males was 56 days (ranging up to 113 days) and females 42 days (ranging up to 65 days). Adult moths were entirely nocturnal with activity beginning immediately after the

start of the scotophase. Ninety-seven per cent of eggs were laid within the first 4 hours of darkness. Mating started eight hours after the onset of the scotophase and lasted around 54 minutes. Both sexes were capable of multiple mating and size of the first spermataphore in males increased linearly with age (Vickers 1982b).

Adult females produce a sex pheromone which is highly attractive to males. Rothschild (1975) was able to attract male *P. scutigera* with the major components of the synthetic pheromone used to monitor *P. gossypiella*; Z,Z and Z,E isomers of -7,11-hexadecadienyl acetates. In *P. gossypiella* a 1:1 ratio of the 2 isomers is most attractive but Z,Z alone was highly attractive to male *P. scutigera* (Rothschild 1975). Catches of males increased, though not significantly, when small quantities of the Z,E isomer were added to Z,Z but were reduced when the level of Z,E exceeded 50%. Rothschild (1975) concluded that the Z,Z isomer alone was adequate for monitoring purposes and this was used in his subsequent studies (Rothschild 1983) and those by Page *et al.* (1982, 1984a, 1984b). Monitoring studies conducted by Flint and Stone (1985) found that a 9:1 ratio of Z,Z and Z,E was significantly more attractive to male moths than Z,Z alone. They stated that further monitoring tests were required before a standard pheromone bait for *P. scutigera* could be recommended.

Vicker s (1982b) only field study involved determining the time of capture of males in pheromone traps placed in a cotton field over one night during March. Ninety-nine percent of males were caught between 7.5 and 9.5 hours after darkness.

LaChance and Ruud (1979) conducted interspecific crosses between *P. scutigera* and *P. gossypiella* to examine the feasibility of using hybrid sterility and cytoplasmic incompatibility for the control of the latter species in the U.S.A. Under laboratory conditions *P. scutigera* was able to mate with *P. gossypiella* originating from the U.S.A. and Australia, but crosses were characterized by reduced mating, eupyrene sperm transfer, low fecundity and very low egg hatch. Both male and female F1 progeny, obtained by crossing *P. scutigera* females with *P. gossypiella* males, were sterile when backcrossed to *P. gossypiella* but fully fertile when backcrossed to *P. scutigera.*

No intensive field studies on *P. scutigera* attacking cotton have been published. The following description is mainly based on short reports in which no experimental data were

presented to support the statements made. Some statements, especially those made by Ballard (1925), must be considered with caution as they may be based on the biology of *P. gossypiella* rather than *P. scutigera*.

According to Passlow and Sabine (1963) eggs are laid on young leaves, stems, and on the bases of the squares and bolls. Veitch (1938) claimed that bolls are the favoured site for egg-laying. Eggs laid on bolls were usually found in the crack along which mature bolls split or at the base of bracts (Ballard 1925).

All parts of the cotton plant are liable to attack; squares, flowers, bolls in all stages, terminal shoots, boll pedicels, and even fairly wooden stems (Holdaway 1926). However, larvae have a distinct preference for bolls (Veitch 1938) and only very rarely infest squares or any other plant part (Passlow and Sabine 1963, Sabine 1969a). Bolls of all ages may be infested but usually not until they are at least half-grown (Veitch 1938). Young bolls are generally shed when attacked while older bolls remain on the plant (Veitch 1938). Mature bolls are preferred to younger ones and severe infestations usually commence only when large firm bolls with well developed lint and seed are present (F. D. Page unpublished report). This agrees with observations that *P. scutigera* is usually a pest only towards the end of the crop's growing period during March and April (Passlow 1958, 1961, Sabine 1969b).

Immediately after egg hatch, neonate larvae search for a boll to infest, although some may feed on green tissue before entering a boll (Holdaway 1926). Bolls are infested from any point; under or near the edge of the calyx, on the exposed boll wall or through the apex (Holdaway 1926), but most appear to enter through the base (Page *et al.* 1984b). On entry larvae quickly bore into the carpel wall and tunnel though the tissue just below the outer surface. Damage is only visible on the inner surface of the carpel wall (Passlow 1964). Larvae eventually move from the carpel wall to feed on developing lint and seed. Feeding causes lint staining and damage to seeds which interferes with lint production (Passlow and Sabine 1963). Larval damage may be restricted to only one or two locules but fungal rots often enter feeding areas and completely destroy the boll (Passlow and Sabine 1963).

Larval development is usually completed inside a single fruit and pupae are found within

the boll (F. D. Page unpublished report, Passlow 1964, Passlow and Sabine 1963, Veitch 1938). Holdaway (1926) commonly found pupae within ripening bolls or on the outside between the involucre and boll wall but suspected that some larvae pupated elsewhere, away from the site of infestation, due to the presence of large holes in boll walls presumably made by mature *P. scutigera* larvae. When pupation takes place within the boll it can occur at the base of a locule, in the cracks between drying carpels, in damaged seed or in the lint (Holdaway 1926).

1.1.5. Parasitoids

Holdaway (1926) bred an unidentified species of microbracon parasitoid from *P. scutigera* larvae infesting cotton. Sabine (1969a) reported a species of *Apanteles* emerging from cotton bolls highly infested with overwintering *P. scutigera* larvae. During surveys for parasitoids of *P. gossypiella* in the Northern Territory, Sands and Hill (1982) found three species of Braconidae parasitizing *P. scutigera* larvae infesting *H. tiliaceus*. These were: *Apanteles oenone* Nixon, *Chelonus* sp. and *Phanerotoma* sp.

1.1.6. Carry-over of populations from one cotton season to the next

Alternative host plants are thought to play a minor role in the carry-over *P. scutigera* populations from one cotton season to the next (Sabine 1969a). However, during the 1920's when cotton was grown in coastal areas of Central Queensland, *H. tiliaceus* was considered an important source of *P. scutigera* (Holdaway 1926, Sloan 1946, Veitch 1938). *H. tiliaceus* was very common in coastal and sub-coastal vegetation and when trees grew directly adjacent to cotton crops their destruction was recommended (Anon 1924b, Sloan 1946). Since cotton production in Central Queensland moved away from coastal areas, into the Callide and Dawson Valleys, the importance of *H. tiliaceus* as a source of *P. scutigera* infestation in cotton is considered to have diminished. *H. tiliaceus* does not occur naturally in these valleys, although a small number of trees have been planted as ornamentals in some parks and gardens (Page *et al.* 1984b, F. D. Page pers. comm.).

Sabine (1969a) considered the carry-over of *P. scutigera* from one cotton season to the next, was through the survival of overwintering larvae in crop residues remaining at the end of the season. Overwintering larvae do not appear to enter diapause, unlike *P. gossypiella*, but

remain in a slow feeding stage (Holdaway 1926). Sloan (1942) was unable to find double cotton seeds which are characteristic of diapausing *P. gossypiella* larvae. The uninterrupted emergence of moths from residual cotton bolls throughout winter and spring months further suggested to Sabine (1969a) that *P. scutigera* does not enter a true diapause or any other form of quiescence.

Neither Sloan (1942) or Sabine (1969a) were able to explain why or how, in the absence of a diapause or quiescent stage, larval development was so prolonged. Sloan (1942) observed that larvae collected in late autumn and early winter, held with dry linted seed and cotton trash, did not pupate for periods of up to 16 weeks. Ballard (1927b) claimed to have received live 'pink bollworm larvae' (presumably *P. scutigera*) from a sample of cotton stored in an office for nearly two years. Also, Sabine (1969a) found that moths continued to emerge from infested dry cotton bolls left on the soil surface for a period of approximately nine months. Moth emergence appeared to be stimulated by rainfall (above 12.5 mm) or irrigation, with peaks of emergence occurring three to four weeks later. Burial of infested boll material hastened moth emergence, significantly reducing the period of emergence and numbers of moths emerging (Sabine 1969a).

1.1.7. Cultural control

The recommended control procedures for *P. scutigera* largely relies on prevention rather than treatment due to problems in detecting larval infestations (Passlow 1964). Before the widespread availability of insecticides to treat pests, control relied solely on cultural methods, including: 1) destruction of all cotton plants by the end of July, or early August at the latest, to ensure a close season of cotton until the planting of annual crops in November; 2) cultivation of cleared areas to help destroy pupae in soil and crop residues; 3) destruction of all ratoon and standover cotton; 4) heat treatment of all cotton seed to be used for planting (Anon. 1924a, Ballard 1927a, Currie 1928, Sloan 1946, Veitch 1938).

Holdaway (1926) believed that the main method of dispersal in *P. scutigera* was through the distribution of cotton seed infested with larvae, as in the case of *P. gossypiella* (Sloan 1946). To prevent the spread of larvae into other cotton growing areas it was decided to heat

treat, at 140° C, all cotton seed used for sowing in a specially built Simon's Heater (Veitch 1938, Sloan 1946). Ballard (1927b) claimed that heat treatment of seeds was effective as no new areas of cotton had been infested by *P. scutigera* since this control measure was adopted. However, Sloan (1946) found levels of infestation of cotton seed by *P. scutigera* to be so negligible that heat treatment was not warranted. Of 98,413 seeds examined only two dead *P. scutigera* larvae were found. Sloan (1946) considered the absence of larvae in seeds was largely due to the selection of only high grade cottons which are unlikely to contain many infested seeds.

The most important and effective control measure, the strict eradication of all ratoon and standover cotton, was poorly supported by farmers during the 1920's (Anon. 1924b, Ballard 1927b). Special anti-ratoon legislation was introduced by the state government in 1924 to ensure the immediate destruction of such cotton (Anon. 1924c). Unfortunately, the law was difficult to enforce (Ballard 1927a, Holdaway 1926) and many farmers continued to grow ratoon and standover cotton, suffering heavy infestations of *P. scutigera* as a consequence (Ballard 1927b). Since the decline of the cotton industry in the 1940's, the practice of growing ratoon or standover cotton has largely disappeared.

Sabine (1969a) clearly demonstrated the importance of disposing of cotton crop residues ('cotton trash') by burial. Burial significantly reduced the number of adult moths emerging from cotton trash and also hastened moth emergence. Timing of burial was critical; early burial, no later than the end of August, caused moths to emerge before squaring of new cotton crops (usually mid-November). Such emergence, in the absence of alternative host plants, was considered 'ineffective' by Sabine (1969a) as no new generation of moths could be established. If cotton trash was left unburied on the soil surface or buried after August, moth emergence extended well into spring and summer months, when new cotton crops were susceptible to larval infestation.

1.1.8. Chemical control

Successful chemical control of *P. scutigera* relies on monitoring crops frequently to detect the presence of neonate larvae. The standard method of monitoring is to check 50-100 large green bolls sampled at random per 20 ha of cotton; insecticides are usually applied when 3-

5% of bolls are infested (F. D. Page unpublished report). This method of scouting is slow and tedious as each individual boll must be split open to check for larval infestation (Page *et al.* 1984a). Newly emerged larvae are very difficult to detect due to their small size and hidden location within the boll tissue (Passlow 1964). The distribution of *P. scutigera* in the field is often patchy and many larvae may be missed (Page *et al.* 1984b). Crop losses due to larval feeding can often exceed 20%, and when infestations become established before control is attempted, losses of 40% may occur (F. D. Page unpublished report).

The widespread use of DDT to control other cotton pests (mainly *Heliothis* = *Helicoverpa* spp.) and the early disposal of cotton trash was thought to be responsible for the excellent control of *P. scutigera* during the late 1950's and 60's (Page 1981, Page *et al.* 1984, Sabine 1969a). When DDT was phased out in Australia, *P. scutigera* caused considerable losses in many crops in Central Queensland as Endosulfan, which largely replaced DDT, failed to give satisfactory control (Page 1981). The introduction of synthetic pyrethroids in the early 1980's gave viable alternatives, with fenvalerate and permethrin being more effective than DDT (Page 1981). The use of synthetic pyrethroids in cotton is presently restricted by an insecticide management strategy to combat pyrethroid resistance in *Helicoverpa armigera* (Hübner) (Page *et al.* 1984a) (see section 1.1.10).

1.1.9. Monitoring populations with synthetic pheromones

Because of the problems in accurately detecting *P. scutigera* larval infestations there was considerable interest in using synthetic pheromone baited traps to monitor male moths (Page *et al.* 1984a, 1984b). Rothschild (1983) conducted trials to determine whether pheromone trap capture could be used to predict crop damage and time insecticide sprays. While males were captured throughout the cropping period, Rothschild (1983) found no consistent relationship between pheromone trap catch and level of boll damage in the crop. Flint and Stone (1985) reached a similar conclusion stating that much more work was needed to determine if pheromone traps can be used to time insecticide sprays. In contrast, Page *et al.* (1984a, 1984b) in their monitoring trials found that pheromone traps were a useful indicator of the likely occurrence of larval infestations, and hence the need to apply insecticide treatments.

They claimed that when a mean catch of 15 or more moths/night was recorded on 3 consecutive nights, economically damaging larval infestations invariably developed.

Today, despite conflicting evidence in the literature, pheromone traps are used by some commercial cotton scouts to monitor *P. scutigera* populations and time insecticide applications. Sprays are usually applied within 24 hours of a peak in moth activity (Page *et al.* 1984a).

Murray and Page (1982) used pheromone traps to indicate the seasonal activity of *P. scutigera* in three cotton growing areas, Biloela, Theodore and Emerald, on a monthly basis between July 1981 and June 1982. Two distinct periods of activity were evident in all three areas; the first occurred between August and November and the second between March and June. Very few moths were caught in December, January and February. The trap catch in Biloela was greater in the first peak than in the second, whereas in Theodore the opposite was true. Surprisingly, in Emerald, where *P. scutigera* is not a pest of cotton, trap catch followed a very similar pattern although far fewer moths were trapped than in the other two sites.

Flint and Stone (1985) also demonstrated the potential of synthetic pheromones to control *P. scutigera* populations by mating disruption. When a 9:1 ratio of Z,Z and Z,E isomers or Z,Z isomer alone was released at a rate of 7.2 g a.i./ha, pheromone trap catch was disrupted by 95-100%. Due to the small size of experimental plots (0.1 ha) boll infestation and the immigration of mated females into the area was not monitored.

1.1.10. Current pest management practices in Australian cotton

Cotton pest management in Australia is presently in a transitional phase due to the unexpected and rapid development of resistance to synthetic pyrethroids in a key pest, *H. armigera* (Sawicki and Denholm 1987).

In the early 1970's, when *H. armigera* developed significant resistance to organochlorine and some organophosphate insecticides in New South Wales (Goodyer *et al.* 1975), it was recognized that the Australian cotton industry needed to reduce its dependence on insecticides (Room 1979). Integrated pest management (IPM) programmes were initiated with the development of an 'on-line' pest management system aimed at minimizing further selection for insecticide resistance and exploiting natural mortality factors (Hearn and Room

1979). These programmes were successful in reducing chemical insecticide usage to 50% of that used commercially while maintaining commercial yields (Room 1979).

Unfortunately, IPM was unsuccessful in forestalling the development of resistance to synthetic pyrethroids (SPs) which had, when first introduced in the 1970's, restored total control over *H. armigera*. Facing the possible loss of SPs to control key pests a relatively new pest control concept, Insecticide Resistance Management (IRM), was adopted. IRM differs from IPM in that the basic premise is that insecticides are too valuable to be wasted through resistance, so the emphasis is on the rational use of insecticides and on the restriction of treatments to prevent selection for resistance, thus prolonging the viability of these products (Sawicki and Denholm 1987).

An IRM approach was adopted with the dramatic failure of SPs to control *H. armigera* on cotton and other summer crops at Emerald in January 1983 (Forrester and Twine 1988). The Australian Field Crops Insecticide Strategy, was developed to prevent further selection for resistance to SPs over other areas of Eastern Australia. The cotton season was divided into 3 stages: Stage 1 (from September to January 10) corresponded to the early growth of cotton, Stage 2 (January 10 to February 20) to peak squaring, and Stage 3 covered the remainder of the season. The application of SPs was restricted to Stage 2 only, a 42 day period that corresponded to one complete life-cycle of *H. armigera*. Thus selection by SPs was believed to be restricted to only one of 4-5 generations of this bollworm produced annually (Forrester 1989). After Stage 2, any SP resistant survivors should be controlled by alternative insecticides. This program, although voluntary, was widely supported by farmers and has been partially successful in containing pyrethroid resistance in *H. armigera* (Forrester 1989).

1.1.11. Implications of the IRM strategy in the control of *P. scutigera*

The implications of the IRM strategy in the control of *P. scutigera* in Central Queensland are two fold. Firstly, the strategy has limited the use of SPs to control *P. scutigera* to a specified 'window'. Pyrethroids (maximum of three sprays) cannot be used after February 20, when *P. scutigera* damage is usually most severe (Passlow 1958). Outside this period farmers

are forced to rely on the only other insecticides presently registered for control of *P. scutigera*, the organophosphate Chlorpyrifos, and the highly toxic insecticides such as Methyl Parathion. Other insecticides registered for use in cotton, e.g. Endosulfan, Methomyl and Monocrotophos, are generally ineffective at controlling *P. scutigera* (Page 1981).

Secondly, the IRM strategy has developed an impetus to minimize the number of SPs sprayed on cotton to as few as possible. Additional sprays of SPs to control other cotton pests, such as *P. scutigera*, must be minimized as much as possible in order to prevent further selection for resistance in *H. armigera*.

1.2. Approach of the thesis

As can be seen from the above literature review our understanding of *P. scutigera* is very incomplete. My aims in this thesis are to improve our knowledge of the biology and ecology of *P. scutigera*. Without such knowledge an effective pest management programme cannot be designed or implemented.

1.2.1. An ecological approach to pest management

It is my contention, developed in this thesis, that a more ecological approach to pest management in Australian cotton must be adopted in order to improve current control strategies.

Approaching a pest situation ecologically has proven useful in discovering or improving control tactics (Rabb *et al.* 1984). For those pests which have no or little susceptibility to natural control, detailed ecological research makes possible greatly improved timing, and more effective and minimal use of chemical control agents (Croft *et al.* 1984).

Clark's (1970) life systems approach to pest situations forms a basis to this thesis. By understanding the life system of *P. scutigera* in cotton a more efficacious control programme can be designed.

Clark *et al.* (1967) defined the life system as that part of an ecosystem which determines the abundance and evolution of a particular species population. To understand and solve pest problems Clark (1970) recommended that three closely-linked phases of investigation should be made:

Phase I - deals with causation of injury and identification and description of the major biological and physical influences involved.

Phase II - investigation of economics of injury and functioning of the relevant life systems.

Phase III - modification of life systems to achieve satisfactory management.

The first phase is an intensive and systematic study of life histories, the object of which is to identify and describe relevant elements of the pest situation. The second phase is concerned with the actual operation of injurious interactions and the ecological processes entailed. The third phase completes the analysis of the pest situation by combining the findings of phases I and II. Decisions are then made as to how the functioning of life systems could be usefully modified or circumvented and courses of action or strategies are adopted (Clark 1970).

1.2.2. Outline of the thesis

In Chapter 2 the effect of temperature and larval diet on *P. scutigera* development and reproduction is investigated, to extend the work of Vickers (1982a, 1982b). This information is used in subsequent chapters to interpret the population performance of *P. scutigera* in the field.

Chapter 3 investigates the biology of *P. scutigera* attacking cotton to verify sites of oviposition, behaviour of larvae, age of bolls attacked, the behaviour of adult moths and species of parasitoids attacking all life stages. The seasonal incidence of larval infestations in sprayed and unsprayed cotton fields in the Callide Valley is determined over three consecutive seasons.

In Chapter 4 the potential of synthetic pheromones for monitoring *P. scutigera* populations and timing insecticide sprays is further evaluated. The best bait formulation to use for monitoring is investigated to settle conflicting data from previous publications, as well as the best trap type and trap location. The correlation between pheromone trap catch, light trap catch and larval infestations is compared to assess the reliability of trap catch in indicating the need to spray.

Chapter 5 readdresses the possible role of alternative host plants in the carry-over of *P. scutigera* populations from one cotton season to the next. Intensive searches for host plants

are conducted, recording their numbers and location in the Callide and Dawson Valleys, rate of infestation and relative importance in supporting moth populations.

Chapter 6 assesses the role of cotton crop residues in the carry-over of moth populations from one cotton season to the next. Factors controlling the development of larvae in dry cotton bolls are determined. The effect of soil type on larval survival and adult moth emergence is investigated. Methods of burying cotton crop residues are compared to determine which method maximizes moth mortality and minimizes the chance of moths emerging during the next cotton season.

Chapter 7 investigates the possible role of adult longevity in the carry-over of populations from one cotton season to the next. The longevity, fecundity and oviposition rates of *P. scutigera* emerging from cotton trash and a major alternative host during spring months is followed in an insectary.

The General Discussion, Chapter 8, consolidates the findings of all chapters to give an overview of the life strategy of *P. scutigera* in cotton. Hypotheses are generated to explain the major factors limiting the abundance of *P. scutigera* in Central Queensland. The pest status of *P. scutigera* is discussed in relation to its adaptations to native host plants. Ways of reducing the impact of *P. scutigera* on cotton and improving methods of control are given. The limited pest status of *P. scutigera* in Australia is also discussed and suggestions for further research are given.

CHAPTER 2

Effects of temperature and larval diet on development and reproduction in *P. scutigera*

2.1. Introduction

Population performance of insects, expressed as reproductive potential or effort, is complicated by the temperature-mediated 'time-scale' experienced by insects as heterotherms (Kitching 1977). Ambient temperatures dictate the appearance and dynamics of insect populations (Ratte 1985). Consequently, a knowledge of the relationship between temperature and rate of insect development is essential for ecological studies and basic to the formulation of pest management strategies (Lysyk and Nealis 1988, Taylor 1981, Uvarov 1931, Wagner *et al.* 1984b).

An extensive literature exists on the effects of temperature on insect growth and development. Growth is defined as a change in size of an individual (or any part of it) while development is a change in form; both are complex, variable, interrelated and hard to quantify (Gordan 1984). The temperature range in which insects can survive is much wider than the range for normal activity and non-arrested development (Ratte 1985). When developmental time of an insect is measured over a wide range of temperatures, the resulting plot typically gives a backward "J" shaped curve. If these times are converted to developmental rates (1/time) the curve becomes a shallow sigmoid or "S" shape (Wagner *et al.* 1984b). At the lower thermal limit of the insect, the developmental curve slowly approaches the point of zero development. As temperatures increase the rate of development becomes proportional to temperature giving a linear response in the mid-region of the curve. Development slows as the optimum temperature is reached and thereafter falls sharply.

Many empirical and biophysical models have been devised to describe the relationship between developmental rate and temperature to enable predictions of insect development in

the field. Historically, the most widely known and used approach is the degree-day or thermal summation model (Curry *et al.* 1978a, Ratte 1985, Taylor 1981, Wagner *et al.* 1984b). This model only uses the linear portion of the rate versus temperature curve, fitting simple linear regression to the observed values. The regression line is extrapolated to the temperature axis to give a theoretical developmental threshold (DT) or zero (DZ), below which no development is assumed to occur. The duration of development can then be predicted by temperature summing: adding up the number of thermal units (e.g. degree-days) accumulated over time until development is completed.

Thermal summing has received much criticism as it is only valid for temperatures within the favourable range for development i.e. where development increases linearly with increasing temperature (Ratte 1985). Extrapolation of the regression line to the temperature axis to determine the developmental zero is often inaccurate due to the sigmoid shape of the entire developmental curve (Wagner *et al.* 1984b). Despite its limitations, thermal summing has been widely used because it requires minimal data for formulation, is easy to calculate and often yields approximately correct values. Simple linear regression has provided a good fit to development data over a wide range of temperatures for a number of insects (Allsopp *et al.* 1983, Bari and Lange 1980, Campbell *et al.* 1974, David *et al.* 1989, Gangavalli and Aliniazee 1985, Hanula *et al.* 1987, Hutchison *et al.* 1986, Lysyk and Nealis 1988, Zalucki 1982) and degree-day calculations have been used to predict insect outbreaks (e.g. Ackers and Nielsen 1984, Burden and Hart 1989, Butts and McEwen 1981, Eckenrode and Chapman 1972, Ives 1973, Knutson *et al.* 1989, Levine 1989, Neal *et al.* 1987, Sanderson *et al.* 1989, Sevacherian *et al.* 1977a).

When rates of insect development are not strictly linear functions of temperature, more complex non-linear models are required for accurate prediction (Stinner *et al.* 1974). A large number of empirical non-linear models have been developed. The most widely used are those of Pradhan (1946) and Stinner *et al.* (1974). Pradhan (1946) used an exponential equation similar to the normal distribution while Stinner *et al.* (1974) used a modified sigmoid equation. Both models have been criticized as they incorrectly assume that developmental

rates on either side of the optimum temperature are symmetric (Wagner *et al.* 1984a). Even so, they have often provided more accurate predictions of insect development at constant and fluctuating temperatures than thermal summation (e.g. Allsopp 1981, Butler 1976, Butler and Hamilton 1976, Hawthorne *et al.* 1988, Smith 1984, Siddique *et al.* 1973).

An alternative non-linear method, polynomial regression, has rarely been used but was found to give excellent empirical descriptions of developmental rates in mites and insects by Tanigoshi *et al.* (1975, 1980) and Tingle and Copland (1988). The latter authors also successfully used polynomial regression, for the first time, to predict insect developmental periods under fluctuating temperatures. These predictions were more accurate than those given by the thermal summation method.

The above developmental models are concerned with predicting mean emergence times of an insect population or cohort. However, insect development is a stochastic process with cohort stadium transitions occurring over extended time periods (Curry *et al.* 1978a). Knowledge of the variability in development provides valuable information on the day of first, peak and last emergence of an insect population which can then be utilized to improve sampling programmes or the timing of insecticide treatments (Sharpe *et al.* 1981). Models incorporating stochastic aspects of insect development have been developed by Curry *et al.* (1978b), Reinchenbach and Stairs (1984), Sharpe *et al.* (1981), Stinner *et al.* (1975) and Wagner *et al.* (1984a). These stochastic models have been successfully integrated into studies on insect development by Allsopp (1981), Casagrande *et al.* (1987), Hagstrum and Millikin (1988) and Narajo and Sawyer (1987).

The resemblance of developmental rate curves to those of chemical reaction rates has led to suggestions that development may be determined by one "master reaction" or one rate-determining enzymatic process (Sharpe *et al.* 1977). Such ideas have been extended, giving rise to complex models of insect development that are based on biophysical laws. Sharpe and DeMichele (1977) consolidated earlier work to formulate a 6 parameter biophysical model that described the entire response curve of insect development over a full range of temperatures. Their model was subsequently modified by Schoolfield *et al.* (1981). Wagner *et al.* (1984b) were aware of no other poikilotherm developmental rate model with greater

flexibility, goodness-of-fit capability, or stronger theoretical foundation. Unfortunately, the model is complex and requires the user to have experience in non-linear parameter estimation.

Non-linear models are undoubtedly more accurate in describing the entire developmental rate versus temperature curve than simple linear models (Kitching 1977). However, the amount of discrepancy between developmental rates in the field with those predicted by linear (i.e. thermal summation) and non-linear models will depend on how often the insect experiences temperatures outside the linear portion of the developmental rate-temperature curve. Gilbert *et al.* (1976) and Kitching (1977) argue that most insects will rarely experience field temperatures that are outside the linear range of development to warrant the use of more complex models. Both authors compared the thermal summing approach with other more complex non-linear models and showed that they gave no appreciable improvement in their predictive value.

Temperature can also profoundly affect insect size and weight, activity, longevity, fecundity and fertility (Engelmann 1984, Gordan 1984, Kitching 1977, Ratte 1985). The effects of temperature on reproduction can be indirect, through a change in insect size or weight, or direct through reduced oviposition rates and egg fertility (Andrewartha and Birch 1954, Danthanarayana 1975, Graham *et al.* 1967, Mack and Backman 1984, Nadgauda and Pitre 1983, Smith 1986).

Other factors, besides temperature, must be considered when attempting to predict insect development in the field and explain population performance. Larval diet can significantly affect the developmental rate of many insects, according to the host plant species utilized (Allsopp *et al.* 1983, Baker and Miller 1974, Cave and Gutierrez 1983, Nadgauda and Pitre 1983, Solomon 1988) or nutritional quality of the diet (House 1966, Lukefahr and Griffin 1962, Taylor 1984). Similarly, larval diet quality and quantity can also influence reproduction by significantly affecting levels of fecundity (Adkisson 1961a, Beckwith 1970, Cave and Gutierrez 1983, Page 1983, Smith 1986, Solomon 1988, Taylor and Sands 1986). In some insects, e.g. *H. armigera*, the length of the photoperiod also affects larval developmental rates

and fecundity (Dimetry *et al.* 1987).

The only empirical studies on developmental rates and aspects of reproduction in *P. scutigera* are those of Vickers (1982a, 1982b). Results from these studies were summarized in Chapter 1 and they will be discussed in more detail in section 4 of this chapter. Vickers did not investigate the effects of larval diet on development and fecundity; in his studies all larvae were reared on artificial diet, and aspects of reproduction were investigated at only one constant temperature (25^{o} C). Also, the number of larval instars in *P. scutigera* has only been determined indirectly by measuring head capsule widths (Holdaway 1926, Vickers 1982a). While both authors estimated that there were four larval instars, this method has been shown to be unreliable, especially when the number of larval instars is variable (Kishi 1971, Schimdt *et al.* 1977).

The aim of this chapter is to report in more detail the effects of temperature and larval diet on the development and reproduction of *P. scutigera*. This information will be used in subsequent chapters to interpret the population performance of *P. scutigera* in the field. The fit of three developmental rate models; thermal summation, Pradhan (1946) and polynomial regression, will be compared to determine which model is best for predicting development of *P. scutigera*.

2.2. Materials and methods

2.2.1. General rearing methods

A novel rearing method was used to culture *P. scutigera* in the laboratory on artificial diet. The diet of Shorey and Hale (1965) was used throughout this study. Vickers (1982a) added ground cotton seed meal to the diet when rearing *P. scutigera* but preliminary experiments showed that this was unnecessary as the development and reproduction of moths was not affected by its omission. Larvae were reared in sections of plastic drinking straws (Woolworths brand, 207 mm long, 5 mm diameter), filled with diet and sealed at both ends by a cotton wool plug. Straws were cut into three equal lengths and repeatedly pressed into a block of diet, ca 55 mm deep, until about three-quarters full. Any diet or moisture on the outside of the straw was removed by wiping with tissue-paper. A tight cotton wool plug was

inserted into the end of the straw filled with diet. This first plug was used to push the diet until it was located centrally within the straw. Once the diet was in the correct position, the plug was immediately pulled away to leave a 5 mm gap, to prevent absorption of moisture from the diet. A second cotton wool plug was then inserted into the other end of the straw, again leaving a 5 mm gap between the cotton wool and the diet.

The amount of diet contained in a single straw was adequate for the development of two larvae, placed in either end of the straw. It was necessary to store straws in sealed plastic containers as all larvae, when ready to pupate, chewed through the plastic straws or cotton wool plugs. One litre plastic food containers (Decor ^{R}), with a ventilation hole made in the lid and covered by wire insect mesh, were used to store straws (up to 100 straws per container). The containers were lined with tissue paper to absorb excess moisture.

For mass rearing purposes two neonate larvae were placed into each straw (one larva at either end). Usually cannibalism or larval interaction was not a problem with a mean survival rate to pupation of 93.3% (N = 186 straws) for 2 larvae/straw compared to 92.7% (N = 126 straws) for 1 larva/straw. Cannibalism or larval injury was higher if the diet used to fill the straws was of poor quality and shrank away from the sides allowing free interaction between the larvae. For temperature development studies, only one larva was placed into each straw to eliminate possible larval interaction and injury from affecting development or survival rates.

Larvae pupated in the tissue paper lining or on the outside of the cotton wool plugs. Prepupae or pupae were carefully removed with the aid of forceps and placed into plastic petri dishes. Pupae were sexed according to the position of the genital pore on the final abdominal segments.

Pupae or newly emerged adults were placed in either 400 ml glass beakers or 100 x 65 mm clear plastic containers and provided with 5% sugar solution for food. The top of each container was covered with a piece of nylon insect screen plus a layer of two-ply facial tissue paper as an oviposition substrate, securing each layer with separate rubber bands. Moths readily laid their eggs through the insect screen and onto the tissue paper. This system allowed tissue papers (=egg sheets) to be changed while keeping the insect screen in place,

thus preventing moth escape and eliminating the need to immobilize moths (e.g. with carbon dioxide) which may affect their longevity and/or fecundity.

It was found that females only readily laid eggs when provided with two-ply facial tissue paper. Eggs were either laid on the inner, exposed surface of the tissue paper or more commonly in between the two layers of paper. Single-ply paper towelling was not a suitable substrate for oviposition.

Egg sheets were placed in 15 cm diam petri dishes sealed with Parafilm^{R} to prevent larval escape. Neonate larvae were transferred onto diet using a fine paint brush moistened with distilled water.

2.2.2. Effects of temperature on development and reproduction

The rate of egg, larval and pupal development was determined at four constant temperatures, 18, 21, 25 and 30^{o} C, and one fluctuating temperature 25/15^{o} C (mean=20.8^{o} C) (all cabinets $\pm$ 1^{o} C, 14:10 light/dark photoperiod). The time of egg hatch was also determined at all these temperature regimes, except 18^{o} C. The fluctuating temperature cabinet was set so that the warm period coincided with the photophase and the cool period with the scotophase.

Eggs laid on tissue paper over a single night were obtained from females (ten or more) held in oviposition cages at each respective temperature regime except in the case of the fluctuating temperature where eggs less than four hours old were obtained from moths held at 21^{o} C. As most eggs are laid within the first four hours of the scotophase (Vickers 1982a), a mean oviposition time of two hours after the start of the scotophase was used as a starting point to calculate the number of days to 50% egg hatch. All moths used in these experiments originated from cotton collected larvae at different times of the year and reared on artificial diet in the laboratory for at least one generation.

To determine the time of egg hatch, sections of egg sheets, containing less than 80 eggs, were placed in 6 cm or 15 cm diam petri dishes for observation. Eggs were observed at hourly intervals at the start of the photoperiod, counting the number of unhatched eggs remaining. The day of egg hatch could be predicted as it was observed to occur on the day following the blackhead stage (i.e. when the head capsule of the developing larva darkens) at all

temperature regimes. Egg hatch at 18° C was checked four times daily, at ca 2, 4, 6 and 10 hours after the start of the photoperiod.

Egg development was recorded for three separate egg batches at each temperature regime. The number of eggs observed varied according to their availability (see Table 2.3).

The duration of each larval instar was determined by checking larvae daily (usually during the eighth hour of the photoperiod) for ecdysis, based on the shedding of the head capsule.

Pupae were sexed and held separately in 15 cm diam petri dishes or 2 x 6 cm glass vials and checked daily as above until all moths emerged. Samples of pupae resulting from larvae reared at 18, 21 and 25° C were weighed soon after hardening of the cuticle.

Realized fecundity of females (defined as the total number of eggs laid during the lifespan of a female) was compared at 18, 21, 25 and 30° C, using moths reared on artificial diet at the same temperature. Females, held singly, were paired on the day of emergence with two males (1-2 days old) and provided with 5% sugar solution. Two-ply tissue paper was provided as an oviposition substrate and changed every 1-5 days until all females died. The preoviposition period and duration of oviposition was also recorded at each temperature regime.

2.2.3. Effects of larval diet on development and reproduction

The effect of larval diet on development was determined at various constant temperatures and compared with larvae reared on artificial diet. The larval diets used were:

i) Cotton squares

ii) Green cotton bolls

iii) *Hibiscus rosa-sinensis* flowers

iv) *Hibiscus tiliaceus* flowers

v) *Brachychiton australis* flowers

Squares (SQ) and green bolls (GC) were collected from unsprayed cotton at the D.P.I. Research Station, Biloela. All squares and bolls were carefully checked for *P. scutigera* infestation and only those with no visible evidence of larval damage were used. Squares were infested with a single neonate larva (less than 12 hours old) while bolls were infested with two or four larvae. Squares were placed in glass jars (3.5 cm diam, 6 cm ht) lined with tissue

paper. Fresh squares were provided as necessary. Bolls were stored in 15 cm diam. plastic cups (Lily Plastics Pty. Ltd., P125-77) lined with tissue paper. Ventilation was provided by inserting pieces of cotton wool through two small holes in the lid. Excess condensation was removed and tissue paper linings were replaced every 2-3 days. Bolls were not replaced throughout the development period of larvae. Development on squares was determined at constant temperatures of 21, 25 and 30° C, and on bolls at 23, 25, 28 and 30° C.

Flowers of *H. rosa-sinensis* (OH), *Hibiscus tiliaceus* (HT) and *B. australis* (BT) were collected in Biloela (see Chapter 5 for details on each host species) and carefully checked for larval infestations. Flowers were placed in tissue lined plastic cups as above or in 2 x 8 cm glass vials, plugged with cotton wool, and infested with either 2 (OH and HT) or 4 (BT) neonate larvae, less than 12 hours old. Fresh flowers were added when necessary (usually every 3-4 days). Development rates were determined at constant temperatures of 25, 28 and 30° C and also at 21° C for OH flowers only.

All host plant material was checked daily for pupae, usually during the eighth hour of the photoperiod. Pupae were removed, sexed and held in petri dishes at each temperature until adult emergence. Samples of pupae from larvae reared at 25° C on all host plants (except SQ and BT) were weighed and compared with those reared on artificial diet.

Aspects of reproduction of moths reared on the above larval diets, except SQ, were measured at 25° C (± 1° C, 14:10 L:D photoperiod) and compared with moths reared on artificial diet (AD). Also included in these comparisons were moths resulting from overwintering larvae feeding in dry cotton bolls (DC), collected from an unsprayed cotton field at the D.P.I. research station, Biloela (see Chapter 6 for further details). Single females were paired with two males on the day of emergence. The preoviposition period, number of eggs laid per female and duration of oviposition was recorded. The number of females used depended on the availability of moths (see Table 2.2A). Most of the moths used in the fecundity experiments were derived from the above larval development studies conducted at 25° C, while others were obtained from infested host plant material, incubated in ventilated plastic boxes at the same temperature.

2.2.4.Data analysis

Three models were used to describe the relationship between temperature and rate of development:

i) simple linear regression $Y = A + BX$

ii) Pradhans' (1946) model: $Y = A \exp [B(C - X)]$

iii) polynomial regression $Y = Bo + B_1T + B_2T^2$ etc.

where Y = developmental rate (1/days), X = temperature, A, B, C and T are empirical constants.

Predicted values of developmental rates were converted back into developmental times (days) and compared with the observed results.

The threshold temperature for development (DZ) for eggs, each larval instar, pupae, and total development was estimated by extrapolating the linear regression line to the X-axis. The predicted number of degree-days (DD) required to complete development at each stage was calculated as the reciprocal of the slope of the linear regression line (B). Standard errors for DZ and DD were calculated using the methods of Campbell *et al.* (1974).

The observed number of degree-days to complete development at each temperature was calculated by the formula:

$DD = P (T-DZ)$

where P is the developmental period (days) and T is the temperature at which the moths were reared (^{o} C).

Capacity for increase (rc) was calculated using the equation of Laughlin (1965):

$rc = (\ln Ro) / Tc$

where Ro is the net reproductive capacity: $Ro = lx.mx$, lx is the proportion of moths surviving to adult emergence multiplied by the proportion of mated females laying fertile eggs, and mx is the number of female eggs laid per female (50% of total fecundity, assuming a 1:1 sex ratio). Tc is the mean generation time i.e. the time from oviposition of a female egg to the day she lays 50% of her egg compliment.

Students t-test and one-way analysis of variance (Proc TTEST and Proc GLM, SAS

Institute 1985) were used to test for significant differences in development periods, pupal weights, preoviposition periods and fecundity for each temperature regime or larval diet. Means were separated, if the F ratio was significant, using the GT2 test for unequal comparisons. Data were transformed where necessary to meet the assumptions of these parametric tests but only untransformed data is presented in the tables and figures. If data could not be adequately transformed, the non-parametric Kruskal-Wallis test and Wilcoxon's 2-sample test were used (Proc NPAR1WAY, SAS Institute 1985).

2.3 Results

2.3.1. Temperature and egg development

The rate of egg development increased linearly with increasing temperature and was adequately described by the simple linear regression model (r^2=0.9998, p<0.0001) (Table 2.1, Fig. 2.1). Predicted values for egg development, calculated from the regression equation, were similar to observed values except at 18° C where development was underestimated by 1.4 days (Table 2.1). The developmental zero was estimated as 12.5° C (± 0.9 S.E.) by extrapolation of the regression line to the X-axis. The predicted number of degree-days (DD) required to reach 50% egg hatch was estimated from the regression line as 74.3 DD (± 6.4 S.E.). The observed number of DD required to reach 50% egg hatch ranged from a mean of 80.0 DD at 18° C to 70.0 DD at 25° C. Egg survival was over 90% at all temperature regimes (Table 2.1).

The timing of egg hatch was gated to the start of the photoperiod on the day after the black head capsule was visible (Fig. 2.2). Egg hatch followed a sigmoid pattern at all temperature regimes. At 21° C the first eggs emerged 3-4 hours after the start of the photophase on the tenth day after oviposition (=203-204 developmental hours). Most eggs hatched between the hours 204-211 and at lights off on day 10 a mean of 80.5% eggs had hatched. The remaining eggs hatched at much slower rates; observations were not resumed until 2 hours after the start of the photophase on day 11 when 6.5% of the eggs still remained unhatched, suggesting that very slow rates of emergence occurred over the scotophase. All remaining

Table 2.1. Mean number of days and degree-days (± S.D.) *P. scutigera* required to reach 50% egg hatch at three constant and one fluctuating temperature regimes. Three replicate egg batches were observed at each temperature regime. R = residuals (observed - predicted values).

Temp. (° C)	No. eggs	% egg survival	No. of days to 50% hatch			Observed[2] number of degree-days
			Observed	Predicted[1]	R	
18	391	92.9	14.8 (0.06)	13.4	1.4	80.0 (0.8)
21	901	95.4	8.7 (0.03)	8.7	0	73.8 (0.2)
25	613	98.7	5.6 (0.05)	5.9	-0.3	70.0 (0.7)
30	492	93.3	4.4 (0.01)	4.2	0.2	76.4 (0.1)
25/15	338	93.7	8.5 (0.04)	8.9	-0.4	71.2 (0.7)

1. Calculated from the simple linear regression equation of developmental rates against temperature (see Appendix B for parameters).

2. Calculated using a developmental zero of 12.5° C.

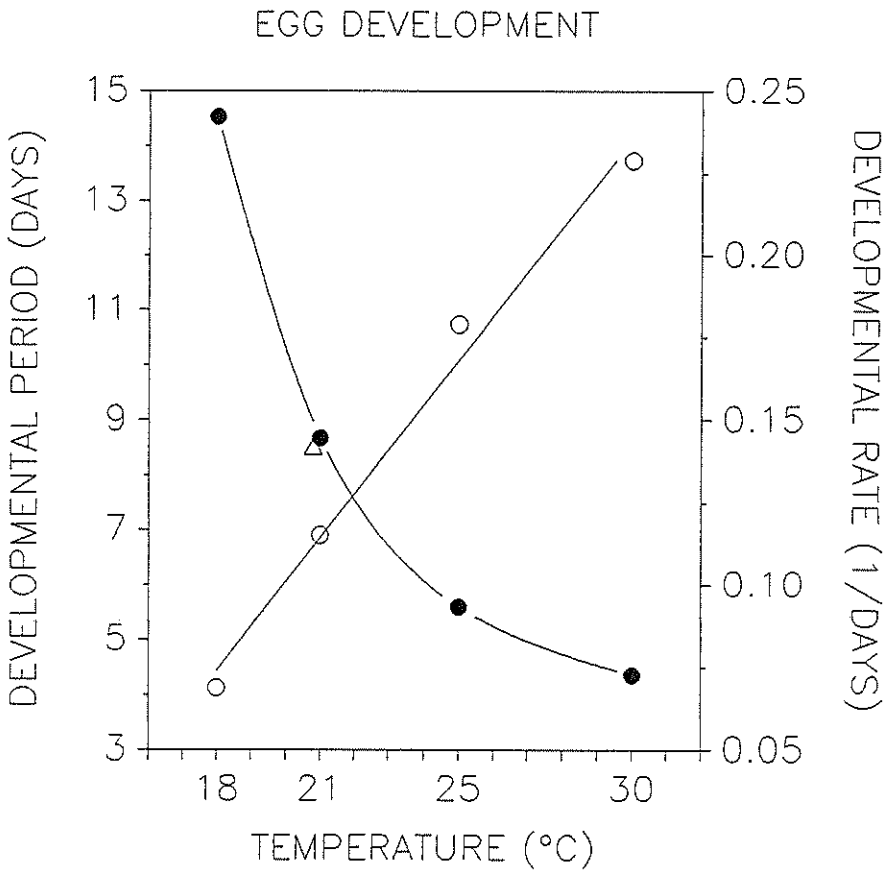

Fig. 2.1. Developmental periods (days) and rates (1/days) for *P. scutigera* eggs incubated at four constant temperatures. Linear regression fitted to rates data (see Appendix B for regression parameters). Triangle symbol represents period of development under the fluctuating temperature of 25/15° C.

eggs hatched within 8 hours of the start of the photophase on day 11 (231 developmental hours).

The rate and pattern of egg hatch at 25° C was very similar to 21° C, although there was greater variation between replicates (Fig 2.2). Egg hatch began 3-4 hours after the start of the photophase on the sixth day after oviposition (developmental hour 130-131). There was a steep rise in rates of egg hatch between 131-139 hours, but levelled-off with emergence extending into day 7. At lights off on day 6 a mean of 19.3% eggs had not hatched, only a further 10.3% emerged during the scotophase (a rate of 1.3% eggs per hour), the remaining eggs hatching within 3 hours after the start of the photophase on day 7.

At 30° C egg hatch started immediately at the onset of the photophase on the fifth day after oviposition (developmental hour 103) and proceeded at a very fast rate so that within 2 hours a mean of 71.8% of eggs had hatched (Fig. 2.2). Egg hatch continued at a very fast rate until developmental hour 107 when the rate levelled off with a mean of 96.3% of eggs hatched. All remaining eggs hatched by developmental hour 114, 11 hours after the start of the photophase on day 5.

Egg hatch under the fluctuating temperature of 25/15° C was much more synchronized than at constant temperatures of 21 and 25° C, being similar to egg hatch at 30° C (Fig. 2.2). Egg hatch began immediately at the onset of the photophase on day 7 (developmental hour 202) and increased sharply with a mean of 41.6% of all eggs emerging by developmental hour 204. In 2 of the replicate experiments all eggs hatched by day 7, by hour 211 or 212, but in the third replicate 1.3% (N=3) of the eggs remained unhatched at lights off. These eggs had hatched by development hour 227 on the eight day after oviposition.

2.3.2. Temperature and larval development

The mean duration of each larval instar at 4 constant and 1 fluctuating (25/15, mean=20.8° C) temperatures is shown in Table 2.2. All larvae passed through 4 instars at all temperature regimes. The fourth instar was considerably longer than the first 3 instars, taking 52-62% of the total developmental time (Table 2.3). The first 3 instars were of similar duration although the second instar was consistently shorter at all temperatures.

At all temperature regimes the frequency distribution of moulting in the first 3 instars was

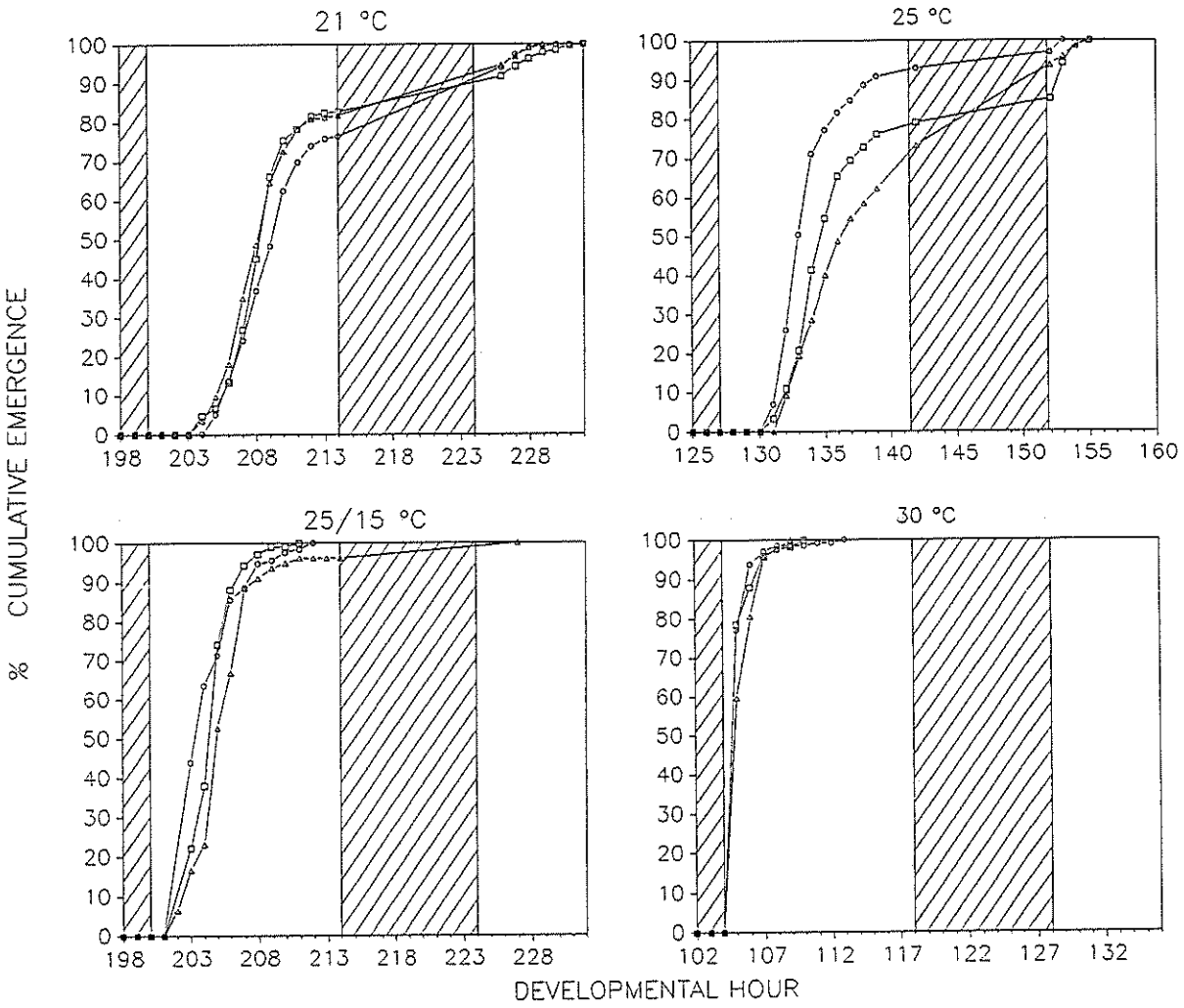

Fig. 2.2. Time of *P. scutigera* egg hatch at three constant and one fluctuating temperature regimes (14L:10D photoperiod). Number of egg batches observed at each temperature regime = 3. Period of scotophase is indicated by hatched bars.

unimodal, occurring over a relatively short period of time (Fig. 2.3). In contrast, the length of the fourth instar was much more variable and not unimodal, particularly at lower temperatures. Female larvae developed slightly faster than males at all temperatures, except the fluctuating temperature regime, but the difference was only significant at 25° C ($p<0.05$).

Developmental rates increased linearly with increasing temperature (Fig. 2.4 and 2.5). Simple linear regression (see Appendix B for regression parameters) gave a good fit to the data for the first, second and fourth larval instars ($p<0.01$, $p<0.01$ and $p<0.05$, respectively) but not for the third instar ($p>0.05$) due to the effects of the extreme temperatures, 18 and 30° C, on development. For this instar Pradhan's (1946) equation and second order polynomial regression gave better predictions of larval development than the linear method, particularly at 18 and 30° C (Table 2.3 and Appendix C). All methods overestimated the number of days to complete development under the fluctuating temperature of 25/15° C.

Linear regression of total larval development against temperature was significant when both sexes were considered together ($r^2=0.954$, $p<0.05$) or when males ($r^2= 0.953$, $p<0.05$) and females ($r^2=0.955$, $p<0.05$) were considered separately (Table 2.4 and Appendix B).

The developmental threshold (DZ) for each larval instar varied from 4.1° C in third instar larvae to 14.1° C in first instar larvae (Table 2.5). However, the DZ estimate for third instar larvae must be considered unreliable because of the highly non-linear relationship between developmental rate and temperature. The overall DZ for all larval instars was estimated to be 11.1° C or 11.0 and 11.1 when male and female larvae were considered separately.

The observed number of DD required to complete each larval instar at each temperature regime was calculated using the above DZ's (Table 2.5). The first larval instar took between 40.3 to 46.2 DD, depending on the temperature regime, with a predicted estimate of 42.3 DD. The second larval instar was of similar duration requiring between 37.6 and 43.5 DD (predicted estimate = 40.7 DD). The number of DD required for the third larval instar was much higher and more variable between temperatures, ranging from 72.7 DD to 107.0 (predicted estimate = 87.3 DD). Similarly, the fourth larval instar was very variable between temperatures ranging from 154.8 DD at 25/15° C to 261.8 DD at 18° C, with a predicted estimate of 205.3 DD.

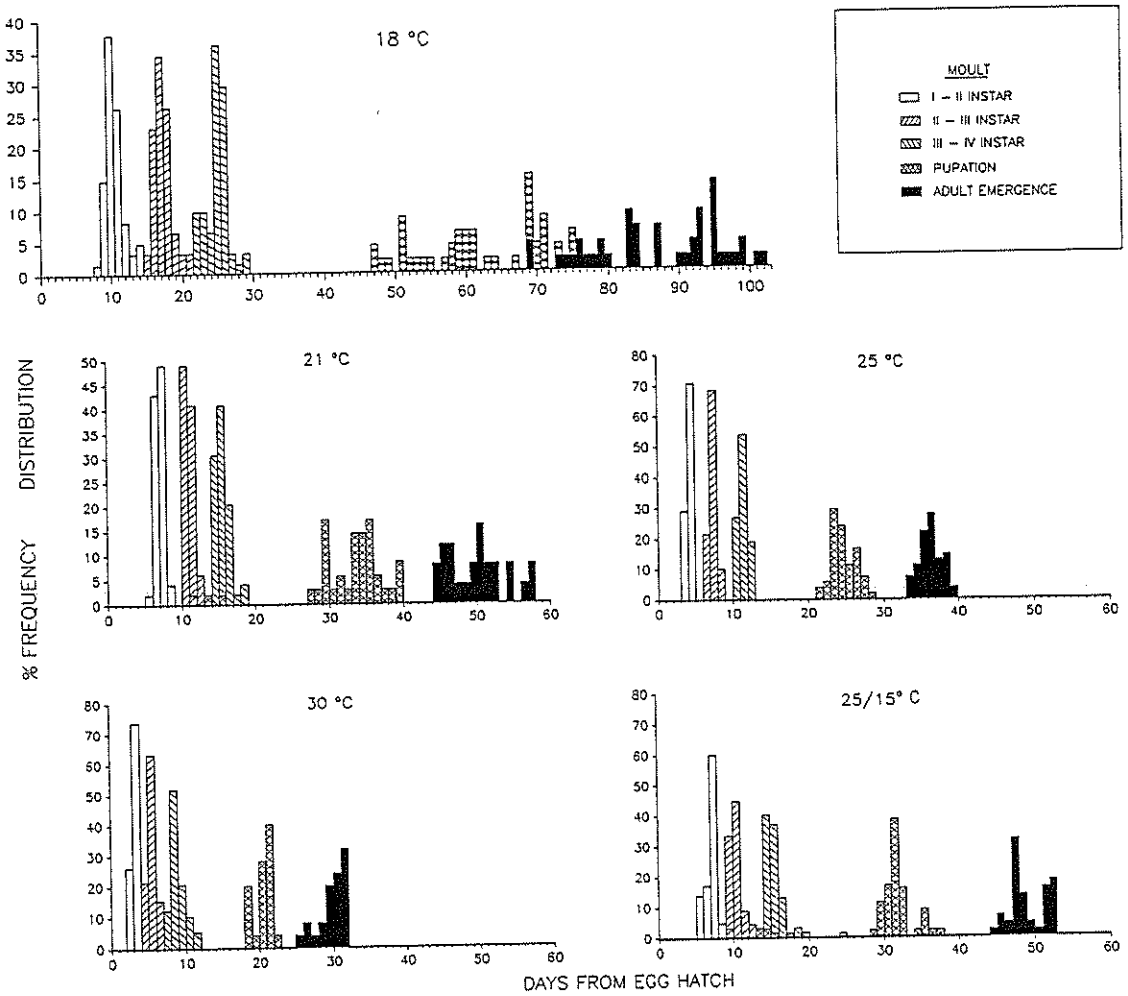

Fig. 2.3. Frequency distribution of *P. scutigera* development, from egg hatch to moth emergence, when reared on artificial diet at four constant and one fluctuating temperature regimes.

The number of degree days DD required for total larval development ranged from 313.0 at 21° C to 404.38 at 18° C, while the value predicted from the regression equation was 362.3 DD (Table 2.10). Female larvae consistently required fewer DD to complete development than male larvae, although the difference was only significant at 25° C ($p<0.05$). Values predicted from the regression equations indicated that females required 357.1 DD, compared to 367.5 DD for males, to complete larval development.

2.3.3. Temperature and pupal development

The relationship between pupal developmental rates and temperature was highly linear ($r^2=0.9998$, $p<0.001$) (Fig. 2.8, Table 2.6), therefore it was unnecessary to apply non-linear models. Female pupae developed faster than male pupae at all temperature regimes, with emergence starting 1 day earlier. However, development was only significantly different at 18 and 21° C ($p<0.01$ and $p<0.0001$, respectively). There was no difference in pupal development at the fluctuating temperature of 25/15° C and the constant temperature equivalent of 21° C. Extrapolation of the regression lines indicated a DZ of 11.6° C for male pupae, 12.3° C for female pupae, or 11.9° C when data for the sexes was combined.

Emergence of female pupae occurred over a four day period at all temperatures except 21° C when females emerged within three days. The period of male emergence was more variable taking 7, 5, 3 and 4 days at 18, 21, 25 and 30° C, respectively. Unlike total larval development, the frequency distribution of pupal development was unimodal at all temperature regimes (Fig. 2.7).

Table 2.10 gives the estimated number of DD required to complete pupal development. While developmental periods (days) between the sexes differed significantly only at 25 and 30° C, when the number of DD required to complete the pupal stage was considered, there was a highly significant difference between the sexes at all temperature regimes. This was because two different developmental thresholds were used to calculate the number of DD required. Predicted values for completion of the pupal stage, obtained from the regression equations, were 162.3 and 139.9 DD, for male and female pupae, respectively (Table 2.10).

The effect of temperature on pupal weight was only measured at 18, 21 and 25° C (Table 2.7). Both male and female pupal weight increased with decreasing temperature. Pupae

resulting from larvae reared at 18 and 21° C were significantly heavier than those reared at 25° C (GT2 test, p=0.05). Female pupae were always significantly heavier than males.

2.3.4. Temperature and total development

Total developmental periods, from egg hatch to moth emergence, for each temperature regime are given in Table 2.8. Variability in the length of the fourth larval instar caused moth emergence to be spread out over a number of days, particularly at lower temperatures, although some of the variability was dampened out by synchronized pupal development (Fig. 2.8). Females developed faster than males at all constant temperatures but the difference was only significant at 25° C ($p<0.01$). At the fluctuating temperature treatment development of the sexes was very similar. The combined development times at 25° C, from egg hatch to moth emergence was 35.2 days. Development at the fluctuating temperature of 25/15 was slightly (but not significantly) faster than at the constant temperature equivalent of 21° C (48.6 days and 49.5 days, respectively, $p<0.05$).

All models adequately predicted total development, although polynomial regression was more accurate due to the effects of the extreme temperatures, 18 and 30° C on development (Table 2.9). All models over-estimated total development time at the fluctuating temperature by 5-10%.

The DZ for larvae to adult development was estimated as 11.3° C with both sexes having an identical DZ of 11.4° C when analysed separately (Table 2.10). The number of DD required for total development ranged from 480.3 at 21° C to 584.1 at 18° C, while 461.6 DD were required at the fluctuating temperature of 25/15° C (Table 2.10). The predicted value from the regression line was 511.7 DD. Males required significantly more DD for development at 25 ($p<0.01$) and 30° C ($p<0.05$) but not at 18, 21 or 25/15° C ($p>0.05$). Predicted values for each sex were 523.7 and 499.1 DD for males and females, respectively.

Combining this data with egg development data gives an overall developmental period, from oviposition to moth emergence, of 91.7, 58.4, 40.8, 32.7 and 57.1 days at 18, 21, 25, 30 and 25/15 ° C, respectively. Simple linear regression of this data estimated the overall DZ at 11.5° C (± 1.3 S.E.), and predicted that 583.8 DD (± 64.0 S.E.) would be required to

Table 2.2. Comparison of observed and predicted (linear and Pradhan (1946) equations) developmental periods for each *P. scutigera* larval instar at four constant and one fluctuating temperature regimes. N = number of larvae observed. R = residuals (observed-predicted development periods).

Instar/ temp. (°C)	N	Developmental period (days) Observed	Predicted Linear	R	Pradhan	R	Polynomial	R
L1								
18	64	10.5	11.0	0.5	-[1]	-	-	-
21	49	6.7	6.2	0.5	-	-	-	-
25	79	3.7	3.9	-0.2	-	-	-	-
30	65	2.7	2.7	0.0	-	-	-	-
25/15	65	6.8	6.4	0.4	-	-	-	-
L2								
18	61	6.8	6.4	0.4	-	-	-	-
21	49	4.0	4.4	-0.4	-	-	-	-
25	79	3.2	3.0	0.2	-	-	-	-
30	65	2.2	2.2	0.0	-	-	-	-
25/15	65	3.6	4.4	-1.2	-	-	-	-
L3								
18	61	7.7	6.2	1.5	6.8	0.9	7.1	0.6
21	49	4.3	5.1	-0.8	4.9	-0.6	4.7	-0.4
25	70	4.0	4.1	-0.1	3.8	0.2	3.7	0.3
30	65	3.5	3.3	0.2	3.6	-0.1	3.6	-0.1
25/15	64	5.3	5.2	0.1	5.0	0.3	4.8	0.5
L4								
18	61	37.4	29.1	8.3	33.4	4.0	36.5	0.9
21	49	18.2	20.5	-2.3	19.8	-1.6	18.7	-0.5
25	63	13.3	14.7	-1.4	12.9	0.4	13.1	0.2
30	58	11.5	10.8	0.7	11.6	-0.1	11.5	0.0
25/15	63	15.8	20.9	-5.1	20.4	-4.6	19.3	-3.5

1. Pradhans' equation and polynomial regression not calculated due to the highly linear relationship between temperature and development in first and second instar larvae (see text for further details).

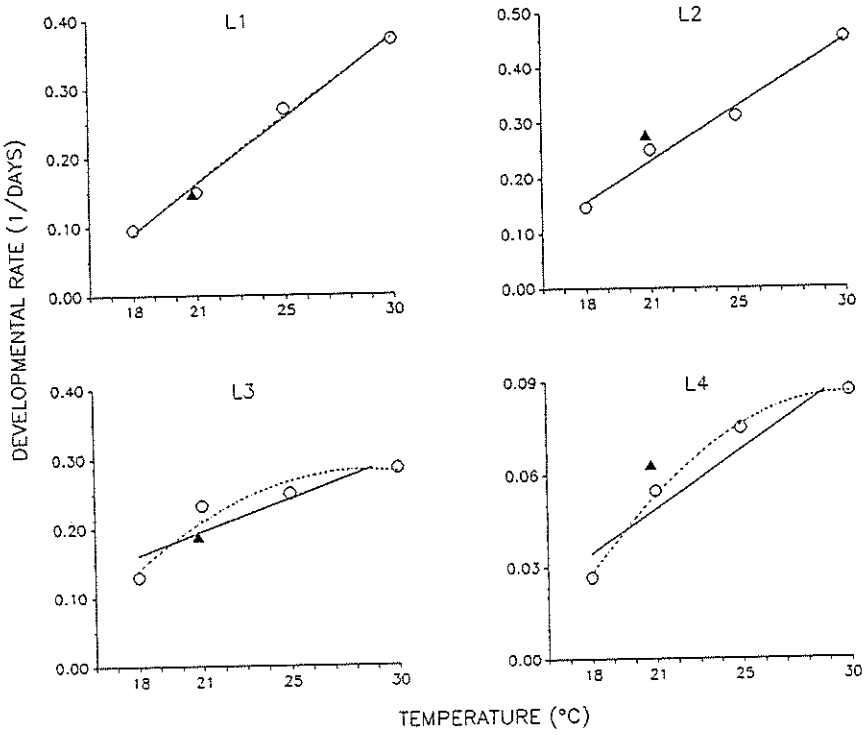

Fig. 2.4. Developmental rates (1/days) for each *P. scutigera* larval instar when reared on artificial diet at four constant temperature regimes. Linear regression (solid line) and polynomial regression (dashed line) was fitted to the data (see Appendix B and C for regression parameters). Triangle represents period of development under the fluctuating temperature of 25/15^{o} C.

Table 2.3. The percentage of *P. scutigera* larval development spent in each instar at each temperature regime.

Temp. (° C)	Percentage of total larval development/instar			
	L1	L2	L3	L4
18	16.8	10.9	12.3	59.9
21	20.2	12.0	13.0	54.8
25	15.3	13.2	16.5	55.0
30	13.6	11.1	17.6	57.8
25/15	21.6	11.4	16.8	50.2
Mean	17.4	11.8	15.3	56.0

Table 2.4. Duration of the entire larval stage of *P. scutigera*, reared on artificial diet at four constant and one fluctuating temperature regimes, for each sex separately and combined. N = number of individuals observed.

Temp. (° C)	Mean developmental time in days (± S.D.)						
	Combined	N	Males	N	Females	N	t-test[1]
18	62.4 (8.7)	46	63.6 (8.8)	21	61.3 (8.6)	25	0.860
21	33.2 (3.3)	35	33.1 (3.4)	15	33.3 (3.3)	20	0.087
25	24.2 (1.6)	58	24.8 (1.8)	25	23.8 (1.3)	33	2.231*
30	20.0 (1.3)	29	20.2 (1.2)	15	19.8 (1.4)	14	0.713
25/15	31.5 (2.0)	47	31.3 (1.9)	28	31.8 (2.0)	19	0.540

1. T-test comparisons between sexes, * denotes a significant difference in developmental periods at $p<0.05$.

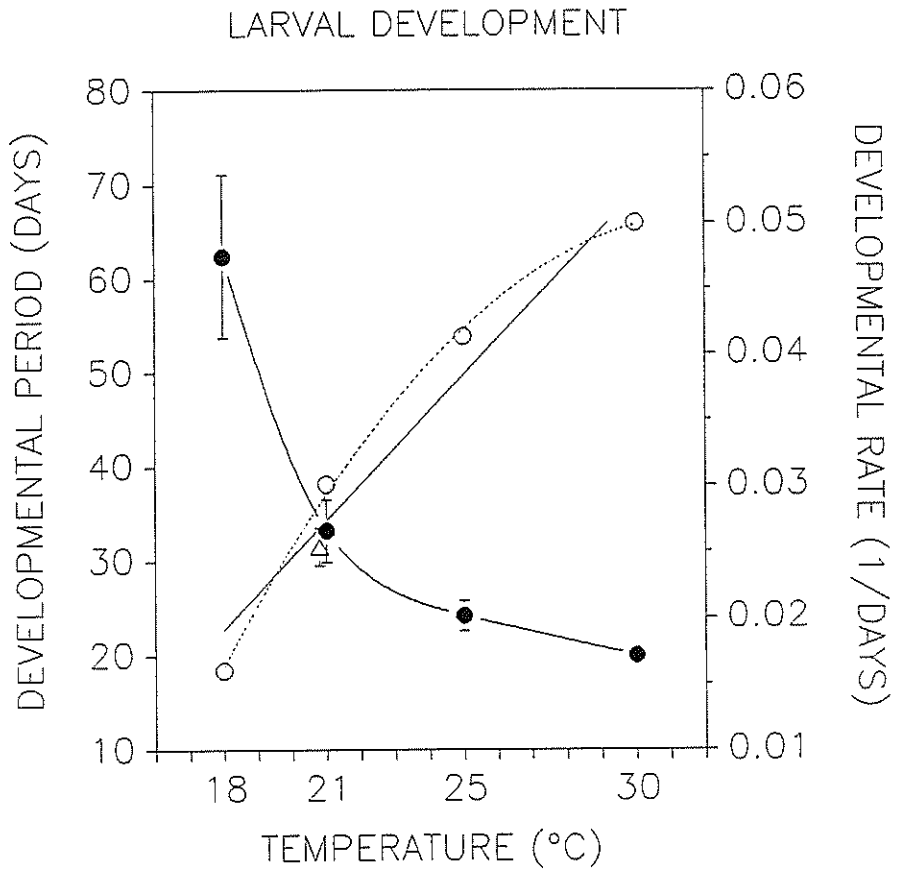

Fig. 2.5. Developmental periods (days ± S.D.) and rates (1/days) for *P. scutigera* larvae (L1-L4) when reared on artificial diet at four constant temperature regimes. Lines and symbols as in Fig. 2.4.

Table 2.5. Estimated and predicted number of degree days required for *P. scutigera* to complete each larval instar at each temperature regime. DZ = developmental zero (± S.E.).

Instar	DZ	Observed DD/temperature regime (° C) 18	21	25	30	25/15	Predicted DD (± S.E.)[1]
L1	14.1 (0.7)	41.0	45.9	40.4	43.5	45.5	42.3 (2.8)
L2	11.6 (1.1)	43.5	37.6	42.9	40.5	33.1	40.7 (3.6)
L3	4.1 (6.9)	107.0	72.7	83.6	90.6	88.5	86.5 (29.9)
L4	11.9 (2.8)	261.8	182.0	186.0	218.5	154.8	205.5 (43.6)

1. Predicted values obtained from linear regression equations.

Table 2.6. Duration of the pupal period of *P. scutigera*, reared on artificial diet at four constant and one fluctuating temperature regimes, for each sex separately and combined. N = number of individuals observed.

Temp. (° C)	Mean developmental time in days (± S.D.) Combined	N	Males	N	Females	N	t-test[1]
18	24.6 (1.5)	43	25.4 (1.5)	18	24.0 (1.1)	25	3.337*
21	16.8 (1.2)	25	17.6 (1.2)	11	16.1 (0.7)	14	4.604***
25	11.7 (1.0)	54	11.8 (0.7)	23	11.5 (0.9)	31	1.763
30	8.4 (1.0)	25	8.9 (0.8)	13	7.8 (0.9)	12	1.891
25/15	17.1 (1.6)	44	17.2 (1.8)	26	16.9 (1.2)	18	0.334

1. T-test comparisons between sexes, * and *** denotes a significant difference in developmental periods at $p<0.05$ and $p<0.0001$, respectively.

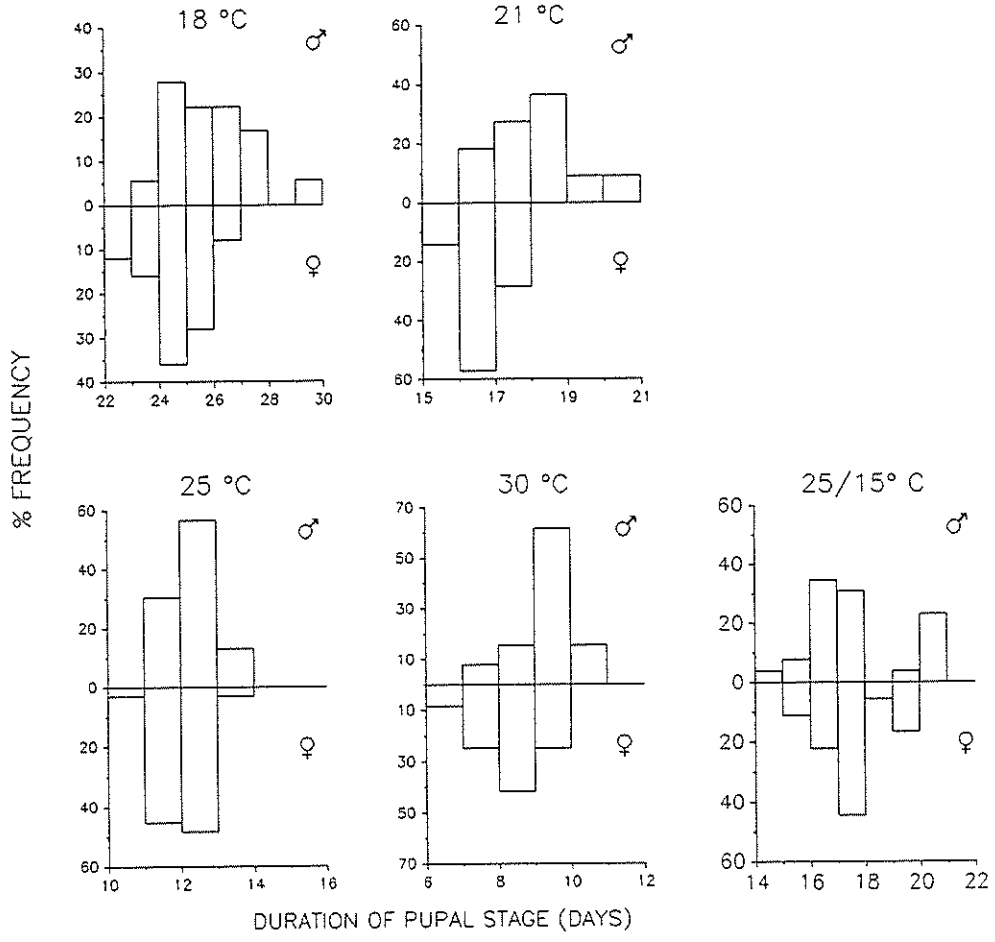

Fig. 2.6. Frequency distribution of *P. scutigera* pupal development for male (upper bars) and female (lower bars) when reared on artificial diet at four constant on fluctuating temperature regimes.

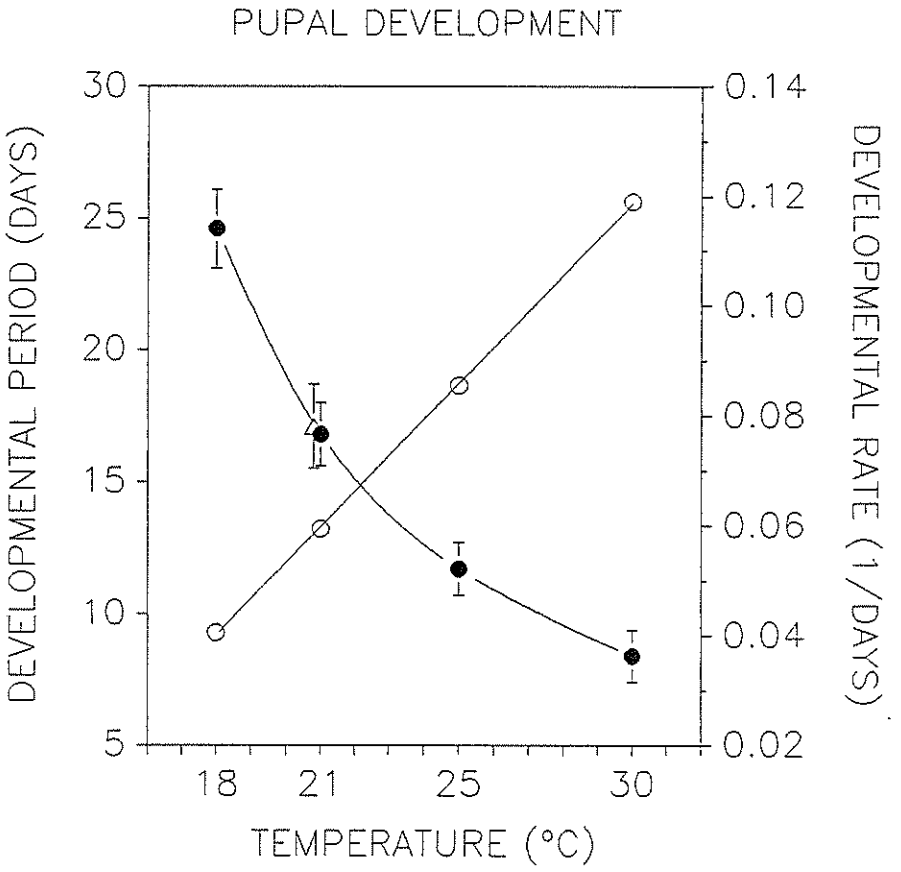

Fig. 2.7. Developmental periods (days + S.D.) and rates (1/days) for *P. scutigera* pupae when reared on artificial diet at four constant temperature regimes. Lines and symbols as in Fig. 2.4.

Table 2.7. Weight of *P. scutigera* pupae when reared at three constant temperature regimes on artificial diet. N = number of pupae weighed.

Temperature (° C)	Weight (mg ± S.D.)[1] Males	N	Females	N	t-test[2]
18	249.7a (21.1)	10	315.3a (24.8)	6	5.48***
21	230.6a (21.9)	10	305.9a (18.4)	10	8.14***
25	189.0b (17.1)	10	229.5b (31.0)	10	3.78**

1. Means within columns followed by the same letter are not significantly different (GT2 test, p=0.05). ANOVA F (MALES, $\log_{10}$ transformed) =25.78, df=2/27, p<0.0001
F (FEMALES, $\log_{10}$ transformed) =30.21, df=2/23, p<0.0001
2. T-test comparisons between sexes, ** and *** denotes a significant difference in pupal weight at p<0.001 and p<0.0001, respectively.

Table 2.8. Total developmental periods (egg hatch to moth emergence) of *P. scutigera*, reared on artificial diet at four constant and one fluctuating temperature regimes, for both sexes separately and combined. N = number of individuals observed.

Temp. (° C)	Mean developmental time in days (± S.D.) Combined	N	Males	N	Females	N	t-test[1]
18	87.2 (9.1)	43	89.7 (9.1)	18	85.4 (8.8)	25	1.543
21	49.5 (4.0)	25	50.5 (3.8)	11	48.8 (4.1)	14	1.096
25	35.2 (1.8)	54	36.6 (1.5)	23	35.2 (1.2)	31	3.238**
30	28.3 (1.8)	25	29.0 (1.5)	13	27.6 (1.8)	12	1.480
25/15	48.6 (2.4)	44	48.5 (2.6)	26	48.7 (2.1)	18	0.364

1. T-test comparisons between sexes, ** denotes a significant difference in developmental periods at p<0.01.

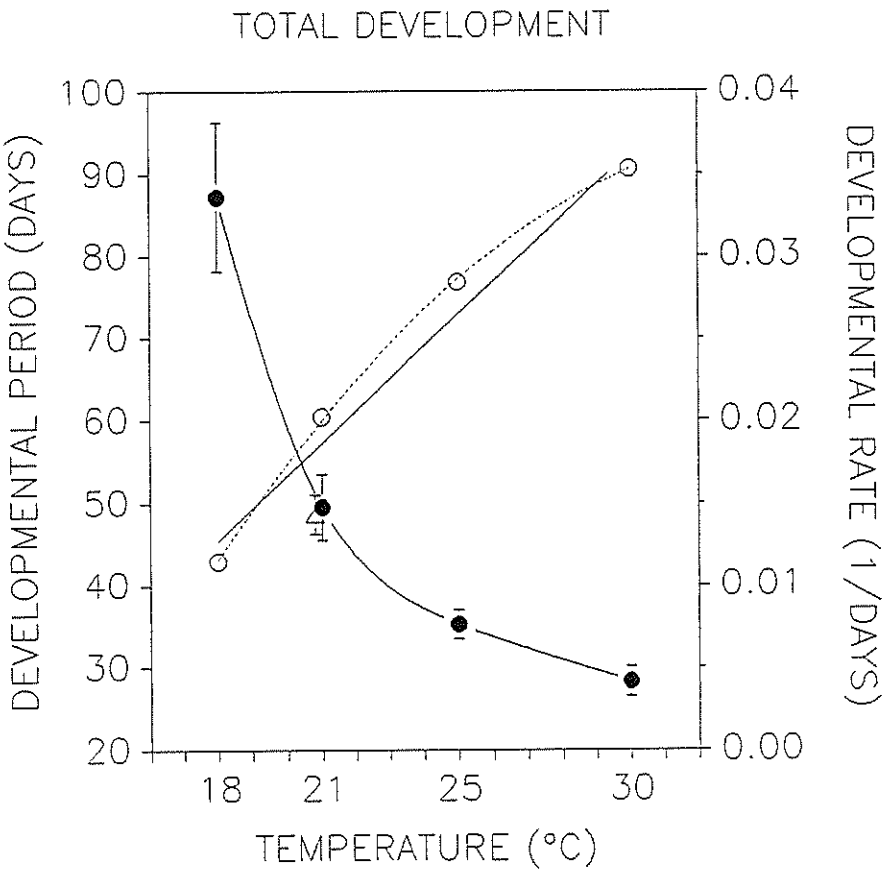

Fig. 2.8. Developmental periods (days + S.D.) and rates (1/days) for *P. scutigera* total development (from egg hatch to moth emergence) when reared on artificial diet at four constant temperature regimes. Lines and symbols as in Fig. 2.4.

Table 2.9. Observed and predicted *P. scutigera* larval, pupal and total (egg hatch to moth emergence) developmental periods at four constant and one fluctuating temperature regimes. R = residuals (observed-predicted development periods).

Stage/ temp. (°C)	Developmental period (days)						
		Predicted					
	Observed	Linear	R	Pradhan	R	Polynomial	R
Larvae (L1-L4)							
18	62.4	52.1	10.3	57.3	5.1	61.2	1.2
21	33.2	36.5	-3.3	35.6	-2.4	34.1	0.3
25	24.2	26.0	-1.8	23.6	0.6	23.9	0.3
30	20.0	19.1	0.9	20.0	0.0	20.1	-0.1
25/15	31.5	37.2	-5.7	36.6	-5.1	35.4	-3.9
Pupae							
18	24.6	24.9	-0.3	-[1]	-	-	-
21	16.8	16.7	0.1	-	-	-	-
25	11.7	11.6	0.1	-	-	-	-
30	8.4	8.4	0.0	-	-	-	-
25/15	17.1	17.1	0.0	-	-	-	-
Total							
18	87.2	76.3	10.9	82.3	4.9	86.2	1.0
21	49.5	52.6	-3.1	52.1	-2.6	50.1	-0.6
25	35.2	37.3	-2.1	34.6	0.6	35.0	0.2
30	28.3	27.3	1.0	28.4	-0.1	28.4	-0.1
25/15	48.6	53.8	-5.2	53.5	-4.9	51.4	-2.8

1. Pradhans' equation and polynomial regression were not calculated for pupae because of the linear relationship between development and temperature.

Table 2.10. Observed and predicted number of degree-days (DD) required for both sexes combined (M+F), males (M) and females (F) to complete larval, pupal and total (egg hatch to moth emergence) development at four constant and one fluctuating temperature regimes. DZ = developmental zero (DZ).

Stage	DZ (± S.E.)	Observed DD (± S.D.)/temperature regime (°C) 18	21	25	30	25/15	Predcted DD (± S.E.)[1]
Larvae							
M+F	11.1 (2.1)	430.2 (60.0)	328.7 (32.6)	336.7 (22.4)	378.8 (23.3)	305.8 (19.3)	362.1 (56.6)
M	11.0 (2.0)	445.0 (61.7)	331.3 (33.6)	347.0 (25.3)	382.9 (23.1)	305.4 (15.9)	367.6 (58.0)
F	11.1 (2.1)	423.1 (59.6)	329.2 (33.0)	330.9 (18.5) *[2]	373.1 (25.5)	307.7 (20.2)	357.5 (54.7)
Pupae							
M+F	11.9 (0.2)	150.1 (8.8)	152.9 (10.8)	152.6 (8.5)	151.3 (18.0)	151.9 (14.0)	152.9 (1.4)
M	11.6 (0.4)	162.5 (9.6)	165.8 (11.3)	158.5 (8.7)	162.8 (14.7)	156.7 (15.9)	162.3 (5.4)
F	12.3 (0.5)	137.0 (6.5) ***	140.4 (5.8) ***	146.2 (7.9) ***	138.6 (16.5) ***	144.0 (10.3) **	140.0 (6.5)
Total							
M+F	11.3 (1.5)	584.1 (60.8)	480.3 (38.6)	491.4 (21.3)	529.6 (33.1)	461.6 (22.7)	511.7 (59.2)
M	11.4 (1.5)	592.2 (60.1)	484.4 (36.8)	497.9 (20.4)	539.4 (28.4)	456.3 (24.4)	523.8 (57.8)
F	11.4 (1.3)	563.4 (58.0)	468.3 (39.2)	479.8 (18.7) **	513.0 (33.1) *	457.8 (20.7)	499.1 (49.4)

1. Predicted values obtained from linear regression equations.

2. * , ** and *** indicates a significant difference in the number of DD required for development between the sexes at $p<0.05$, <0.01 and <0.001, respectively (Student's t test, values not shown).

Table 2.11. Survival of *P. scutigera*, from egg hatch to moth emergence, on artificial diet at four constant and one fluctuating temperature regimes. Number (N) and percentage (%) survival to the next developmental stage is shown.

Temp. (°C)	Initial number of larvae	Larval survival		Pupal survival		Percent total survival
		N	%	N	%	
18	64	46	71.9	43	93.5	67.2
21	49	35	71.4	25	71.4	51.0
25	79	58	73.4	54	93.1	68.4
30	65	29	44.6	25	86.2	38.5
25/15	65	47	72.3	44	93.6	67.7

complete development (see Appendix B for regression parameters).

2.3.5. Temperature and survival rates

Larval survival ranged between 71.4 to 73.4% at all temperature regimes except 30° C where only 44.6% of larvae survived to pupation (Table 2.11). Most larval mortality occurred in the fourth instar during pupation (see Appendix E for a more detailed life table). Pupal survival was above 90% at 18, 25 and 25/15° C but was only 71.4 and 86.2% at 21 and 30° C, respectively. Overall survival rates, from egg hatch to moth emergence, were highest at 25° C (68.4% survival) and lowest at 30° C (38.5% survival).

2.3.6. Temperature and reproduction

Temperature significantly affected the preoviposition period ($p<0.01$) but there was considerable variation within temperatures, particularly at 21° C where it ranged from 4 to 38 days with a mean of 10.9 (Table 2.12). The preoviposition period was shortest and least variable at 25° C with a mean of 4.7 days, ranging from 2-8 days. Pairwise comparisons of means, using Wilcoxon's 2-sample test, revealed that the preoviposition period at 18 and 21 was significantly longer than at 25 and 30° C.

The percentage of females laying fertile eggs was highest at 25° C (100%) and lowest at 18° C (47%) while at 21 and 30° C all but 1 female failed to lay any eggs (93 and 92% of females paired, respectively).

Significantly fewer eggs were laid at 18° C than at all other temperatures ($p<0.001$, Table 2.12). Levels of fecundity varied greatly within each temperature regime but was highest at 25° C with a mean of 494.7 eggs per female. The highest number of eggs laid by an individual female was 678 at 30° C.

Temperature also significantly affected the mean duration of oviposition ($p<0.001$) being longest at 21° C (50.9 days) and shortest at 30° C (24.8 days). At 18 and 25° C the duration was similar (39.3 and 45.7 days, respectively). Oviposition lasted significantly fewer days at 30° C than at 21 or 25° C (Table 2.12, GT2 test, $p=0.05$). At 25 and 21° C 50% of the females were still laying eggs 55 and 63 days after eclosion, respectively (Fig. 2.10).

The rate of oviposition was very low at 18 and 21° C with no marked peak in the number of eggs laid, while at 25 and 30° C most eggs were laid within the first 2 weeks after eclosion

Table 2.12. Effect of temperature on aspects of *P. scutigera* reproduction. Consecutive rows within each aspect of reproduction represent the mean, followed by the standard deviation of the mean in brackets, and the observed range of values.

	Temperature (^{o} C)			
	18	21	25	30
No. of females paired	15	15	10	12
% of females laying eggs	46.7	93.3	100	91.7
Preoviposition period (days)	9.9a (4.1) 6-14	10.9a (9.2) 4-38	4.7b (1.7) 2-8	6.6b (6.5) 2-23
No. of eggs /female	99.0a (145.6) 30-406	395.5b (192.0) 85-642	494.7b (95.0) 352-644	372.9b (202.4) 28-678
Duration of oviposition (days)	39.3ab (24.1) 3-67	50.9a (12.2) 31-68	45.7a (13.6) 21-60	24.8b (9.0) 8-39
Time to 50% oviposition (days)	19.6ab (8.9) 7-32	26.1b (9.7) 16-50	14.7c (3.1) 10-19	14.5c (8.6) 7-36

1. Means within rows followed by the same letter are not significantly different (Wilcoxons' two-sample rank test or GT2 test, $p<0.05$).

Kruskall-Wallis test: Preoviposition period Chisq=12.333, df=3, $p<0.01$
Fecundity Chisq=23.124, df=3, $p<0.0001$

ANOVA: Duration (log_{10} transformed data) F=7.09, df=3/39, $p<0.001$
50% eggs (log_{10} transformed data) F=6.76, df=3/39, $p<0.001$

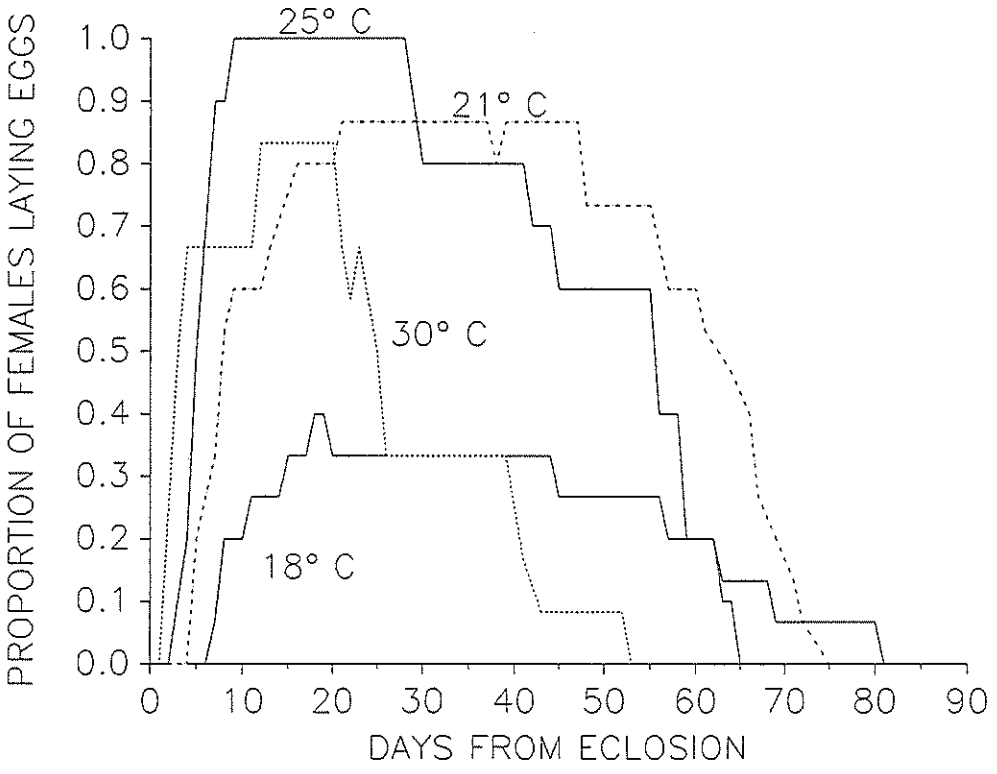

Fig. 2.9. Proportion of *P. scutigera* females laying eggs over time when reared on artificial diet at four constant temperature regimes.

Table 2.13. Correlation between preoviposition period (PRE), realized fecundity (FEC) and duration of oviposition (DUR) for female *P. scutigera* reared at four constant temperature regimes.

Temp. (° C)	Correlation coefficient (r)		
	PRE v. FEC	PRE v. DUR	FEC v. DUR
18	0.219	0.217	0.898**[1]
21	0.152	0.075	0.174
30	0.134	0.021	0.601**

1. ** indicates a significant correlation at $p<0.01$

Table 2.14. Capacity for increase for *P. scutigera* reared on artificial diet at four constant temperature regimes.

Temp. (° C)	Net reproductive capacity (Ro)	Cohort generation time (To)	Capacity for increase (rc)
18	32.1	96.9	0.0358
21	95.8	68.9	0.0662
25	166.7	45.3	0.1129
30	67.1	39.1	0.1076

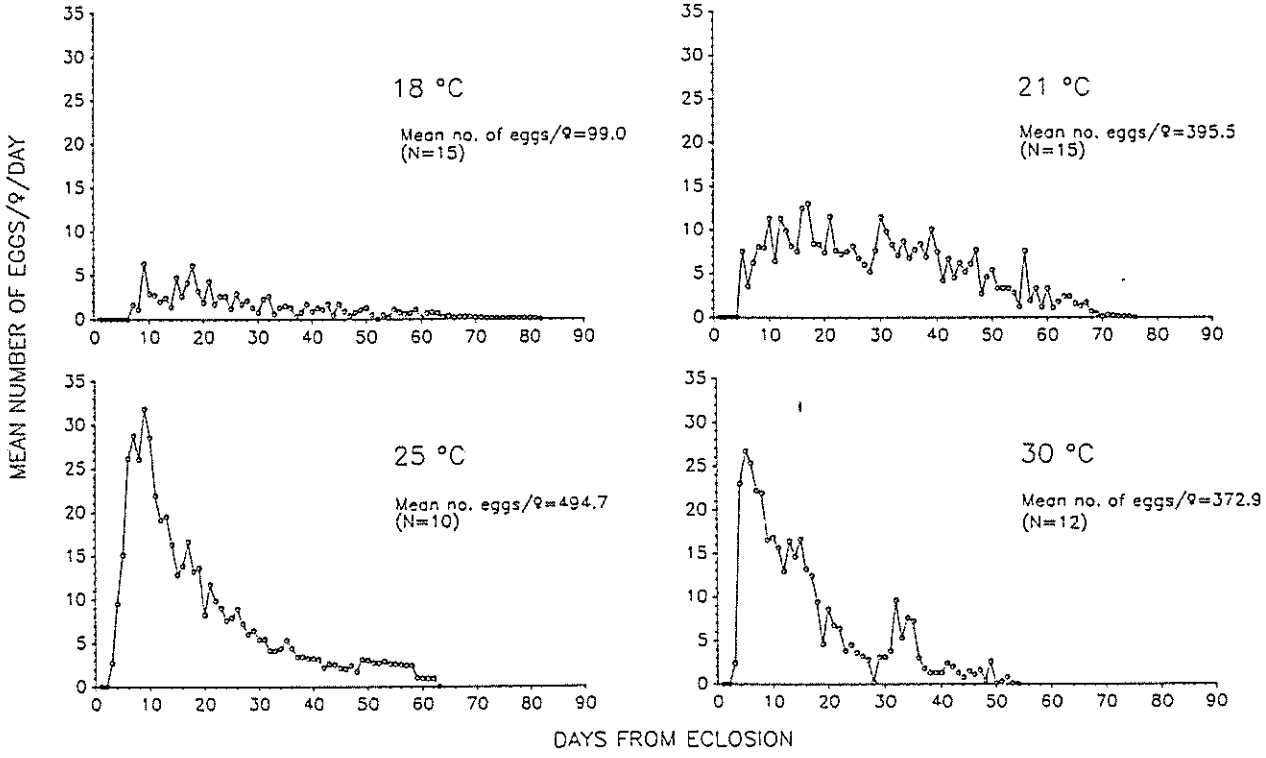

Fig. 2.10. Age specific oviposition curves for female *P. scutigera* reared on artificial diet at four constant temperature regimes. N indicates the number of females paired at each temperature.

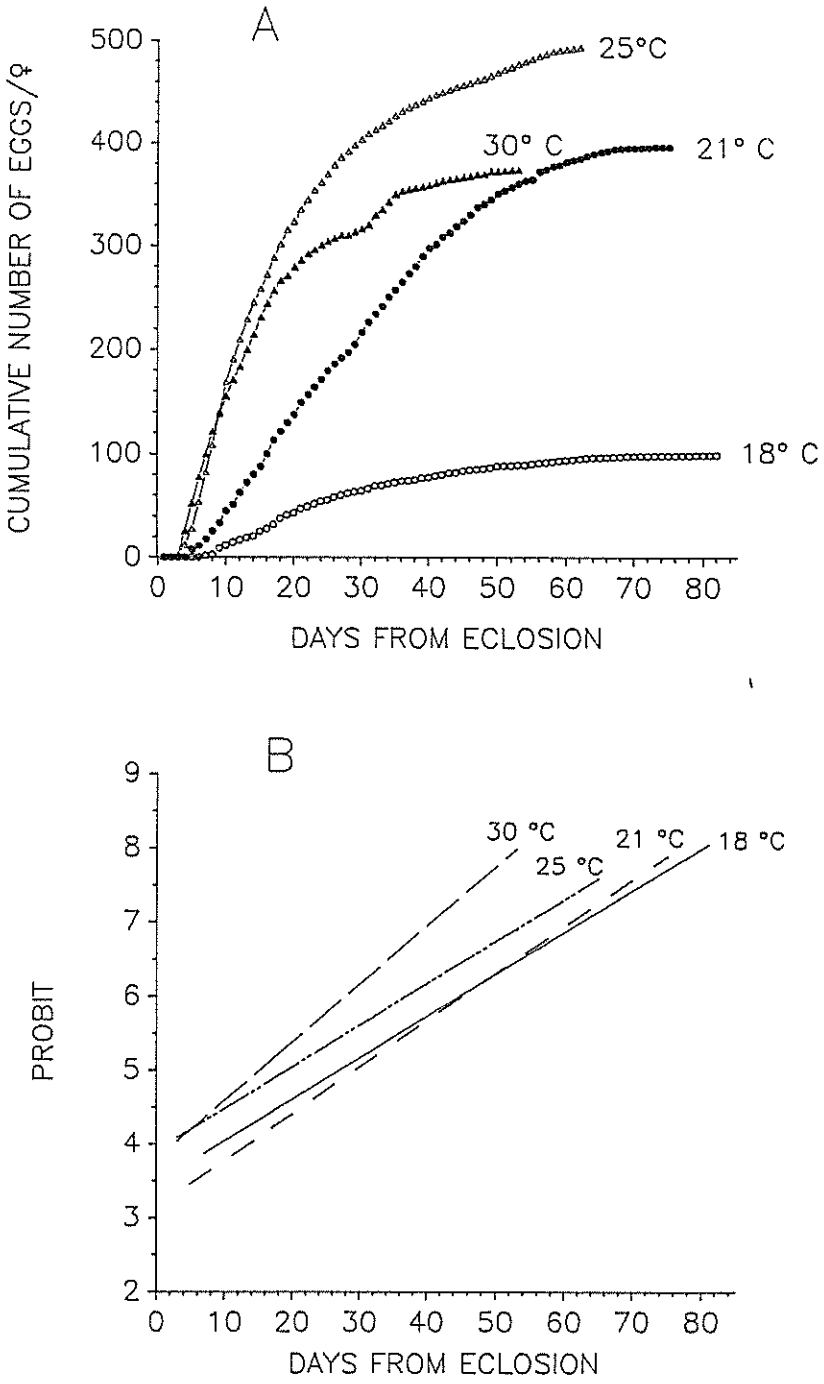

Fig. 2.11. (A) Cumulative number of eggs laid by *P. scutigera* reared on artificial diet at four constant temperature regimes from the day of moth eclosion. (B) Ditto plotted as probits against time and linear regression fitted to the lines.

(Fig. 2.11). The number of days to reach 50% of eggs laid was significantly longer at 21° C (26.1 days) than at 25 and 30° C (14.7 and 14.5 days) but not 18° C (19.6 days) (Table 2.12, GT2 test, p=0.05).

The preoviposition period was not correlated with either realized fecundity or the duration of oviposition at all temperature regimes (Table 2.13). However, there was a significant correlation ($p<0.01$) between realized fecundity and the duration of oviposition at the 2 temperature extremes, 18 and 30° C, but not at 21 or 25° C.

Cumulative percentage oviposition curves for each temperature were plotted as probits against time and linear relationships were fitted (Fig. 2.12). From the slope of the fitted regression lines (b) the rate of egg-laying in relation to temperature was compared using t-tests (Steel and Torrie 1960). Rate of oviposition at 30° C was significantly faster than at 18 or 21° C ($p<0.05$) but not 25° C ($p>0.05$).

The capacity for increase (rc) was highest at 25° C and lowest at 18° C (Table 2.14). The cohort generation time (To) decreased with increasing temperature, as would be expected. The net reproductive capacity (Ro) was lowest at 18° C, due to the low proportion of moths laying fertile eggs, peaked at 25° C, and decreased sharply at 30° C mainly due to poor survival rates at this temperature. This resulted in the capacity for increase at 25° C being 3.2, 1.7 and 1.1 times higher than at 18, 21 and 30° C, respectively.

2.3.7. Diet and larval development

Larval development on SQ was significantly slower than on all other diets, taking a mean of 57.1 days compared to 24.0 days for larvae reared on GC at 25° C (Table 2.15). Development was fastest on OH and HT (17.6 and 17.1 days at 25° C, respectively), but of intermediate duration on BT (21.5 days at 25° C). Development on GC was very similar to that on AD (compare Table 2.4 with Table 2.15). Survival rates on BT were very low; at 25° C only 5 of the initial 30 larvae infested onto flowers survived (16.7%), On other diets survival rates at 25° C ranged from 37.5% (GC) to 65.0% (OH).

At 25° C larvae reared on SQ did not start pupating until day 52 compared to day 13 for OH and HT and day 19 for GC. All larvae reared on SQ reached pupation on day 62 while those reared on OH, HT and GC had completed the larval stage by day 22, 21 and 31,

respectively.

Differences in developmental rates between the sexes were not significant when reared on SQ, HT and BT but male larvae reared on OH, at 28 and 30° C, developed significantly faster than female larvae ($p<0.05$). GC female larvae developed significantly faster than male larvae at 25° C ($p<0.05$) but not at any other temperature regime (Table 2.15).

The number of DD required to complete larval development at 25° C on each diet was calculated using a common DZ of 11.1° C derived from artificial diet data (Table 2.10). Significantly more DD were required to complete larval development on SQ than any other diet; at 25° C larvae required a mean of 793.5 DD when reared on SQ compared to 334.6 when reared on GC. Significantly fewer DD were required to complete larvae development when reared on OH and HT than when reared on AD, GC, SQ or BT (GT2 test, $\log_{10}$ transformed data, $p=0.05$, Table 2.19).

2.3.8. Larval diet and pupal development

Pupal development was much less affected by larval diet than larval development. While *P. scutigera* reared on SQ had significantly longer pupal periods than all other diets (GT2 test, $p=0.05$, Table 2.16) the difference in development was much smaller than that for larvae. There was no significant difference in pupal development between any other diet. At 25° C, SQ pupae took a mean of 13 days to develop, compared to 11.4, 11.0, 10.9 and 9.8 for GC, OH, HT and BT pupae, respectively. There was no significant difference in pupal periods between the sexes for any diet or temperature regime (t-test, $p=0.05$, Table 2.16).

The number of degree days required to complete pupal development at 25° C was calculated for each diet using a common DZ of 11.9° C derived from artificial diet data (Table 2.10). The mean number of DD required for pupal development ranged from 128.4 DD for BT pupae up to 170.3 DD for SQ pupae (Table 2.19). SQ pupae required significantly more DD to complete development than all other diets (GT2 test, $\log_{10}$ transformed data, $p=0.05$). BT pupae required significantly fewer DD for development than all diets except OH pupae.

Male and female GC pupae were significantly heavier than AD, SQ, OH or HT pupae

Table 2.15. Effect of larval diet on *P. scutigera* larval development at various constant temperature regimes, for each sex separately and combined. Larval diets were: green cotton bolls (GC), cotton squares (SQ), *Hibiscus rosa-sinensis* flowers (OH), *Hibiscus tiliaceus* flowers (HT), and *Brachychiton australis* flowers (BT). N = number of individuals observed.

Diet/ temp. (° C)	Mean number of days (± S.D.)						
	Combined	N	Males	N	Females	N	t-test[1]
GC 23	28.0 (3.3)	23	28.8 (3.3)	16	26.1 (2.7)	7	1.88
25	24.0 (3.4)	15	25.6 (3.4)	9	21.7 (1.5)	6	2.59*
28	20.9 (2.2)	17	21.1 (1.9)	11	20.5 (2.9)	6	0.52
30	19.3 (4.3)	17	20.3 (3.6)	7	18.6 (4.8)	10	0.78
SQ 21	62.3 (5.7)	20	63.0 (6.4)	11	61.4 (5.0)	9	0.61
25	57.1 (3.7)	12	55.7 (5.5)	3	57.6 (3.2)	9	0.56
30	48.0	18	47.8	12	48.9	6	0.46
OH 21	25.1 (2.2)	14	25.6 (2.5)	5	24.8 (2.1)	17	0.66
25	17.6 (2.2)	22	18.0 (1.9)	8	17.3 (2.4)	14	0.71
28	15.4 (2.5)	19	14.1 (1.0)	10	16.8 (3.0)	9	2.72*
30	15.0 (1.7)	22	14.0 (1.8)	11	16.0 (2.0)	11	2.60*
HT 25	17.1 (2.6)	17	17.7 (2.4)	9	16.4 (2.9)	8	1.09
28	15.1 (2.4)	15	14.1 (2.0)	8	16.3 (2.3)	7	1.94
30	13.1 (1.5)	21	12.8 (1.5)	13	13.5 (1.5)	8	1.09
BT 25	21.6 (0.6)	5	22.0 (0.0)	2	21.3 (0.6)	3	0.28
28	20.0 (2.3)	11	20.3 (2.1)	4	19.9 (2.5)	7	0.31
30	18.2 (1.1)	5	18.0 (1.4)	2	18.3 (1.1)	3	0.28

1. T-test comparisons between sexes, * indicates a significant difference in larval developmental times at $p<0.05$.

Table 2.16. Effect of larval diet on *P. scutigera* pupal development at various constant temperature regimes, for each sex separately and combined. Larval diet abbreviations as in Table 2.15.

Diet/ temp. (° C)	Mean number of days (± S.D.)						
	Combined	N	Males	N	Females	N	t-test[1]
GC23	13.7 (0.8)	23	13.6 (0.7)	16	13.9 (0.9)	7	0.66
25	11.4 (1.2)	15	11.1 (1.3)	9	11.8 (1.2)	6	1.11
28	10.8 (1.8)	17	10.7 (1.9)	11	10.8 (1.7)	6	0.03
30	7.4 (1.2)	17	8.1 (1.9)	7	6.9 (1.2)	10	1.68
SQ 21	16.8 (1.5)	20	17.2 (1.2)	11	16.3 (1.9)	9	1.19
25	13.0 (1.1)	12	13.7 (0.6)	3	12.8 (1.2)	9	1.71
30	7.3 (0.9)	18	7.3 (1.1)	12	7.3 (0.5)	6	-
OH21	18.0 (0.7)	14	18.6 (0.6)	5	17.7 (0.5)	9	1.99
25	11.0 (1.2)	22	11.6 (1.5)	8	10.6 (0.9)	14	1.92
28	9.1 (0.5)	19	9.2 (0.6)	10	8.9 (0.3)	9	1.31
30	7.2 (1.3)	22	7.1 (1.0)	11	7.3 (1.5)	11	0.32
HT 25	10.9 (0.7)	17	10.8 (0.7)	9	11.1 (0.6)	8	1.10
28	9.3 (0.8)	15	9.6 (2.3)	8	9.0 (0.5)	7	1.56
30	8.4 (0.8)	21	8.1 (2.3)	13	8.0 (0.5)	8	2.04
BT 25	9.8 (0.5)	5	10.0 (0.0)	2	9.7 (0.6)	3	1.26
28	10.1 (1.2)	11	10.7 (1.5)	4	9.9 (1.1)	7	0.82
30	7.6 (1.3)	5	8.0 (1.4)	2	7.3 (1.5)	3	0.50

1. T-test comparisons between the sexes, * indicates a significant difference in pupal developmental times at $p<0.05$.

Table 2.17. Effect of larval diet on *P. scutigera* pupal weight when reared at 25° C.

Abbreviations as in Table 2.15. Data for artificial diet (AD) is also included for comparison.

Larval diet	Mean pupal weight (mg ± S.D.)[1] Males	N	Range	Females	N	Range	t-test[2]
GC	220.9a (31.5)	20	135-264	305.5a (23.3)	20	261-344	8.213***
SQ	175.7bc (36.7)	3	137-210	229.6b (46.5)	8	178-315	1.861
OH	173.4b (23.2)	22	131-230	228.1b (37.5)	30	121-296	6.035***
HT	143.0c (25.7)	39	86-202	162.8c (35.7)	45	99-250	2.695*
AD	185.4b	29	141-235	264.9b	43	138-331	8.754***

1. Means within columns followed by the same letter are not significantly different (GT2 test, p=0.05).
ANOVA F (MALES, $\log_{10}$ transformation) =27.98, df=4/108, p<0.0001
ANOVA F (FEMALES, $\log_{10}$ transformation) =57.03, df=4/141, p<0.0001

2. T-test comparisons between sexes, * and *** indicates a significant difference in pupal weights at p<0.05 and p<0.001, respectively.

Table 2.18. Effect of larval diet on *P. scutigera* total development (egg hatch to moth emergence) at various constant temperature regimes. Larval diet abbreviations as in Table 2.15.

Diet/ temp. (°C)	Mean number of days (± S.D.) Combined	N	Males	N	Females	N	t-test[1]
GC23	43.7 (3.5)	23	42.4 (3.6)	16	40.0 (2.7)	7	1.59
25	35.4 (2.4)	15	36.7 (2.3)	9	33.5 (0.8)	6	3.21**
28	31.7 (3.7)	17	31.8 (3.5)	11	31.3 (4.3)	6	0.26
30	26.7 (4.8)	17	28.4 (5.4)	7	25.5 (4.3)	10	1.25
SQ 21	79.5 (5.9)	20	80.2 (6.5)	11	78.6 (5.3)	9	0.62
25	70.1 (3.6)	12	69.3 (4.9)	3	70.4 (3.4)	9	0.36
30	55.3 (3.0)	18	55.1 (2.3)	12	55.7 (4.4)	6	0.31
OH21	43.1 (2.2)	14	44.2 (2.3)	5	42.4 (2.0)	9	1.50
25	28.6 (2.4)	22	29.6 (2.3)	8	28.0 (2.3)	14	1.41
28	24.4 (2.3)	19	23.3 (0.8)	10	25.7 (2.8)	9	1.80
30	22.2 (2.2)	22	21.1 (1.6)	11	23.3 (2.2)	11	1.96*
HT 25	28.0 (2.8)	17	28.4 (2.2)	9	27.5 (3.4)	8	0.68
28	24.5 (1.2)	15	23.8 (1.2)	8	25.3 (1.2)	7	1.27
30	21.5 (1.2)	21	21.5 (1.2)	13	25.5 (1.2)	8	0.07
BT 25	31.4 (1.1)	5	31.7 (0.6)	2	31.0 (1.0)	3	0.87
28	30.0 (1.6)	11	30.3 (1.5)	4	29.9 (1.7)	7	0.43
30	24.4 (2.7)	5	24.0 (2.8)	2	24.7 (3.2)	3	0.24

1. T-test comparisons between sexes, * and ** indicates at significant difference in total development at $p<0.05$ and $p<0.01$, respectively.

Table 2.19. Estimated number of degree days *P. scutigera* required to complete larval, pupal and total (egg hatch to moth emergence) development at 25° C on six larval diets. Larval diet abbreviations as in Table 2.15. Data for artificial diet (AD) is also included for comparison.

Larval diet	Mean number of degree-days (± S.D.)[1]		
	Larvae	Pupae	Total
GC	334.6a (48.6)	149.3a (16.3)	485.0a (33.1)
SQ	793.5b (51.8)	170.3b (14.8)	961.3b (49.8)
OH	243.9c (30.8)	144.1ac (16.2)	381.7c (30.0)
HT	237.1c (36.6)	143.3a (8.6)	383.6c (38.1)
BT	300.2a (7.6)	128.4c (5.9)	430.2d (12.3)
AD	336.7a (22.4)	152.6a (8.5)	491.4a (21.3)

1. Means within columns followed by the same letter are not significantly different, GT2 test, $p<0.05$.

ANOVA F: (LARVAE, $\log_{10}$ transformation) =246.17, df=5/112, $p<0.0001$
(PUPAE, $\log_{10}$ transformation) =8.42, df=5/99, $p<0.0001$
(TOTAL, $\log_{10}$ transformation) =405.78, df=5/134, $p<0.0001$

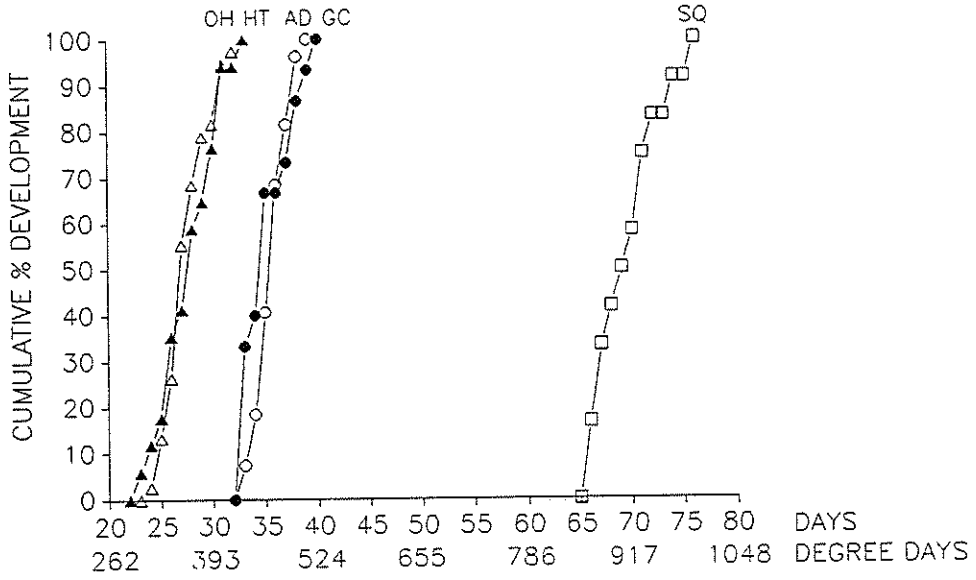

Fig. 2.12. Cumulative developmental rates of *P. scutigera* reared on four larval diets at 25° C, expressed in days and degree-days (above 11.3° C). Larval diets were green cotton bolls (GC), cotton squares (SQ), *H. rosa-sinensis* flowers (OH) and *H. tiliaceus* flowers (HT). Data for artificial diet (AD) is also included for comparison.

(GT2 test, p=0.05, Table 2.17). HT pupae were significantly lighter than pupae from all diets except for male SQ pupae. Within diets, female pupae were always heavier than male pupae with the exception of SQ pupae where the number weighed was very low (3 male and 8 female pupae). The difference between the sexes was greater in GC and AD pupae than in OH, HT or SQ pupae.

2.3.8. Larval diet and total development

The effects of larval diet on larval development strongly influenced total development (Fig. 2.15A). At 25° C *P. scutigera* reared on SQ took a mean of 70.1 days to reach the adult stage, compared to 35.4, 35.2, 27.8, 28.0 and 31.4 days for GC, AD, OH, HT and BT diets, respectively (Table 2.18).

Developmental rates were similar in both sexes, except females reared on GC developed significantly faster than males at 25° C ($p<0.01$), and at 30° C OH reared moths males developed significantly faster than females ($p<0.05$).

The number of DD required to complete total development at 25° C on each diet (calculated using a common DZ of 11.3° C, derived from artificial diet data) ranged from 381.7 DD for OH to 961.3 DD for SQ (Table 2.19). When the number of DD required for total development on each diet was compared, including moths reared on artificial diet (AD), the only comparisons that were not significantly different were between AD and GC, and OH and HT (GT2 test, $\log_{10}$ transformed data, $p<0.05$).

2.3.10. Larval diet and reproduction

The preoviposition period was significantly affected by larval diet ($p<0.05$) but varied greatly, being shortest for moths reared on GC at 4.9 days (range 1-11 days) and longest and most variable when reared on DC at 12.9 days (range 5-28 days), followed by those reared on BT at 12.9 days (range 3-27 days). Moths reared on OH and HT had similar preoviposition periods of 7.1 and 8.4 days, respectively. Pairwise comparison of means (Wilcoxon's 2-sample test) revealed a significant difference in the preoviposition period between moths reared on: GC and BT, GC and DC, AD and DC (Table 2.20, Appendix F).

Realized fecundity was also significantly affected by larval diet ($p<0.001$) but again varied greatly within diet groups (Table 2.20, Fig. 2.18). Highest levels of fecundity were achieved

Table 2.20. Effect of larval diet on aspects of *P. scutigera* reproduction at 25° C. Larval diet abbreviations as in Table 2.15, including moths reared on dry cotton bolls (DC) and artificial diet (AD) for comparison. Consecutive rows within each aspect of reproduction represent the mean, followed by standard deviations of the mean in brackets and the range of values observed.

	Larval diet					
	GC	OH	HT	BT	DC	AD
No. females paired	20	30	20	15	9	10
% females laying eggs	85.0	83.3	70.0	60.0	77.8	100
Preoviposition period (days)	4.9a (2.8) 1-11	7.1ab (3.8) 3-16	8.4b (4.6) 3-22	10.9b (8.0) 3-27	12.9b (11.5) 5-28	4.7a[1] (1.7) 2-8
Number of eggs/female	373.4ab (266.8) 26-720	238.6bc (194.7) 13-683	208.9c (194.7) 27-820	143.7c (161.0) 27-455	163.7c (165.2) 6-474	494.7a (95.0) 352-644
Duration of oviposition (days)	44.9a (15.8) 5-63	17.8b (9.9) 2-33	20.4b (7.3) 1-27	34.0a (15.9) 3-51	21.0b (14.0) 3-31	45.7a (13.6) 21-60
Time to 50% oviposition (days)	13.0a (6.2) 3-28	12.6a (5.6) 5-23	15.1a (6.1) 7-26	23.0a (14.1) 4-48	20.3a (10.9) 10-42	14.7a (3.1) 10-19

1. Means within rows followed by the same letter are not significantly different (Wilcoxons' two-sample test, ($p<0.05$, see Appendix F for pairwise comparisons).

Kruskall-Wallis test:

Preoviposition period Chisq=15.39, df=4, $p<0.01$
Fecundity Chisq=19.041, df=4, $p<0.001$
Duration Chisq=35.0, df=4, $p<0.001$
50% oviposition Chisq=7.43, df=4, $p>0.05$

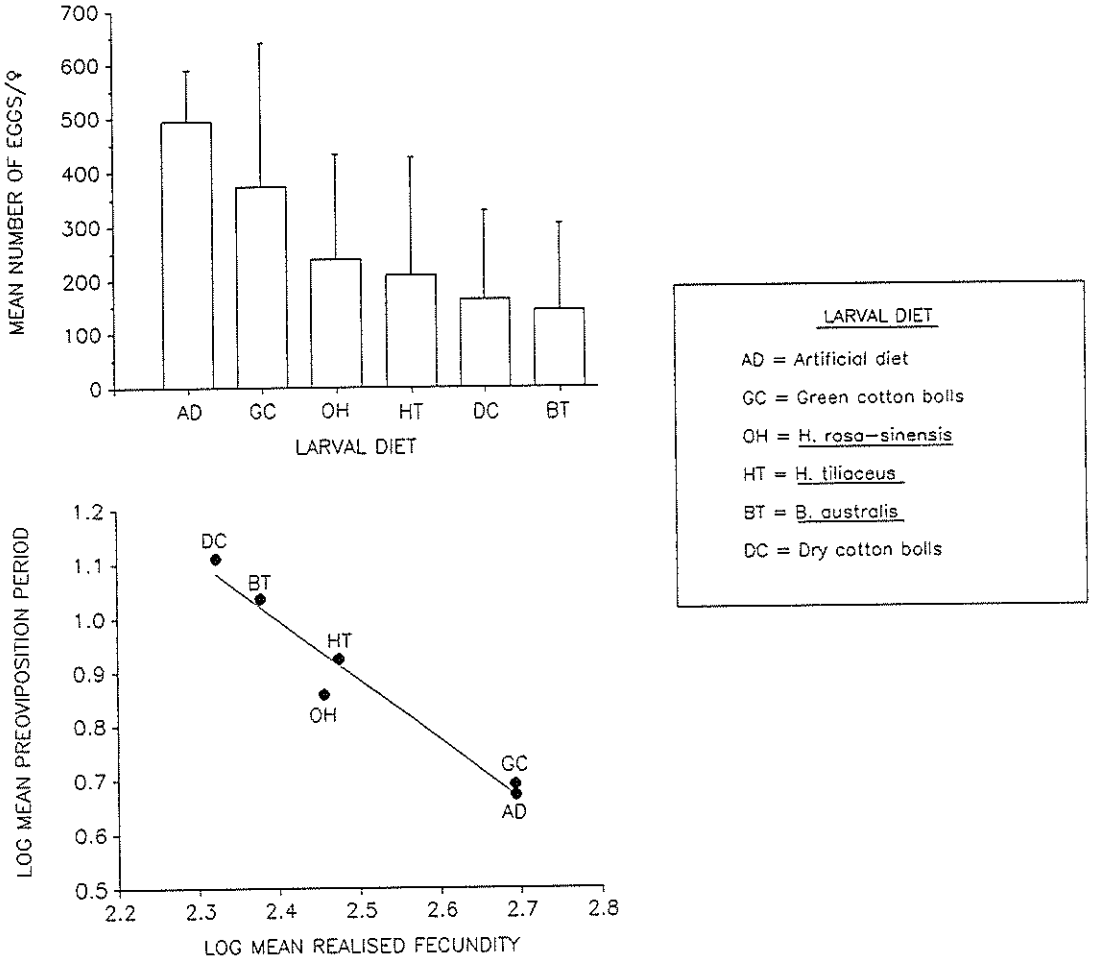

Fig. 2.13. Effect of six larval diets on realized fecundity of *P. scutigera* (top graph) and the relationship between $\log_{10}$ mean preoviposition period and $\log_{10}$ mean realized fecundity of *P. scutigera* reared on each larval diets (bottom graph). Linear regression equation: $\log(y) = 2.864 - 0.829 \log(x)$ ($r^2 = 0.956$).

Table 2.21. Correlation between preoviposition period (PRE), realized fecundity (FEC), pupal weight (PUPW) and duration of oviposition (DUR) for female *P. scutigera* reared on five larval diets at 25° C. Larval diet abbreviations as in Table 2.20.

Larval diet	Correlation coefficient (r)			
	PRE v. FEC	PRE v. DUR	FEC v. DUR	PUPW v. FEC
GC	0.0004	0.268	0.610***[1]	0.011
OH	0.044	0.002	0.407***	0.493
HT	0.004	0.038	0.694***	0.025
BT	0.046	0.239	0.259	-[2]
DC	0.042	0.012	0.516	-

1. *** indicates a significant correlation at $p<0.001$
2. Pupal weight not determined for BT and DC reared moths.

Table 2.22. Capacity for increase of *P. scutigera* reared on four larval diets at 25° C. Larval diet abbreviations as in Table 2.20.

Host plant	Net reproductive capacity (Ro)	Cohort generation time (Tc)	Capacity for increase (rc)
GC	59.7	54.8	0.0746
OH	64.4	47.6	0.0875
HT	27.2	49.5	0.0667
BT	27.2	60.7	0.0412

by moths reared on GC at a mean of 493.3 eggs per egg laying female which was very similar to that of moths reared on AD (494.7 eggs per female). However, 3 moths (15%) failed to lay any eggs giving an overall fecundity rate of 373.4 eggs per female.

Moths reared on all other diets laid significantly fewer eggs than GC or AD moths (Wilcoxon's rank test, $p=0.05$, see Appendix F). The number of eggs (per egg laying female) was similar for OH and BT moths at 286.2 and 298.4, respectively, while BT and DC moths laid 239.6 and 210.4 eggs, respectively. Only 60% of BT moths laid fertile eggs compared to 83, 70 and 78% for OH, HT and DC moths. The highest number of eggs laid by a single female was 820 for a moth reared on HT.

The mean duration of oviposition was significantly affected by larval diet ($p<0.0001$) but the mean number of days to 50% egg lay was not ($p>0.05$). The majority of eggs were laid within the first 2 weeks after eclosion regardless of larval diet. Most moths took 12-15 days to lay 50% of their egg complement, except those reared on BT and DC which took a mean of 23 and 20.3 days, respectively (Table 2.20). The duration of oviposition ranged from 1 (HT) to 63 (GC) days being more prolonged in moths reared on AD, GC and to a lesser extent BT than those reared on OH, HT or DC (Table 2.20, see Appendix F for pairwise comparison of means by Wilcoxon's 2-sample test).

There was no significant correlation between the preoviposition period and realized fecundity or the duration of oviposition for any diet ($p>0.05$, Table 2.21). However, there was a strong correlation ($p<0.001$) between realized fecundity and the duration of oviposition when moths were reared on GC, OH and HT but not when reared on BT or DC. Pupal weight was not correlated with realized fecundity for moths were reared on GC, OH or HT ($p>0.05$, Table 2.21). Capacity for increase was highest for OH moths (0.0875), closely followed by GC moths (0.0746), and was lowest for HT (0.0667) and BT moths (0.0412) (Table 2.22).

2.4 Discussion

Synchronization of egg hatch in *P. scutigera* to the early part of the photoperiod may be an adaptation to favour the survival of neonate larvae. *P. scutigera* eggs are rarely laid on the site of larval infestation (Chapters 3 and 5), therefore, newly emerged larvae must actively search over the plant to locate feeding sites. Local weather conditions would strongly favour egg hatch to occur in the early morning when it is cooler. Mid-day temperatures in Central Queensland can exceed 35^{o} C during summer and most heavy rain storms occur late in the afternoon or at night (Chapter 3). Chapman *et al.* (1983) observed a similar pattern of egg in *Chilo partellus* Swinhoe and suggested that levels of parasitism and predation may also be lower in the early morning.

The mechanism controlling egg hatch in *P. scutigera* cannot be deduced from the experiments conducted in this study. In the closely related *P. gossypiella* egg hatch is controlled by a circadian rhythm which can be initiated by a single light or temperature pulse, but only if these signals are administered after the midpoint in embryogenesis (Minis and Pittendrigh 1968, Pittendrigh and Minis 1964). In *C. partellus* egg hatch cannot be entrained by a circadian rhythm but is controlled by the "lights-on" stimulus when the eggs have reached the blackheading stage (Chapman *et al.* 1983).

Hatching was more synchronized when *P. scutigera* eggs were incubated under the fluctuating temperature of 25/15^{o} C than at constant temperatures of 21 and 25^{o} C. It is possible that the combination of a temperature and a light pulse may help to synchronize egg hatch in *P. scutigera* more effectively than a light pulse alone.

Developmental rates of insects are often accelerated under fluctuating temperatures when compared to rates at the constant temperature equivalents (Ratte 1985). The possible reasons for this will be discussed later.

Variation in egg hatch times between replicates was highest at 25^{o} C. This was the only temperature regime where egg batches for each replicate experiment were obtained from different cultures of *P. scutigera*. These cultures had been reared under laboratory conditions

for an unknown but probably unequal number of generations. Changes in the behaviour of laboratory reared insects have been documented in many species, including *P. scutigera*. Vickers (1982a) observed that females from an eleventh generation culture mated more times than females from the first generation in culture.

P. scutigera invariably underwent four larval instars at all temperature regimes thus confirming previous estimates of the number of larval instars based on head capsule widths by Holdaway (1926) and Vickers (1982b). Many Lepidopterous larvae undergo a variable number of larval instars, and this has been related to larval nutrition or temperature (Beck 1949, Smith 1984, Taylor 1984, Uvarov 1931). The number of instars in *P. scutigera* was only determined for larvae fed on artificial diet. Those reared on less suitable diets, such as cotton squares where development was greatly prolonged, may have a different number of instars.

The first larval instar was of slightly longer duration than the second instar at all temperature regimes. This may suggest that there is an initial period in which no growth takes place (Richards 1949). The fourth larval instar was longest, representing 50-60% of the total larval stage, and was extremely variable in duration, particularly at 18^{o} C. The possible adaptive significance of a variable fourth larval instar is discussed in Chapter 8.

The thermal summation model gave adequate predictions of developmental rates (except for third instar larvae), but due to the effects of the extreme temperatures (18^{o} and 30^{o} C) the non-linear model of Pradhan (1946) and, more particularly, polynomial regression gave a better fit to the data (Table 2.2 and 2.9). The thermal summation model is more useful as it is readily applied and allows the calculation of degree-days (DD) for predicting development under fluctuating temperatures experienced in the field (Kitching 1977).

The use of linear models for predicting insect development may be justified if the insect experiences temperatures within the linear range of the developmental rate-temperature curve. Mean temperatures in the Biloela district during the hottest and coolest months generally fall within this range (Chapter 3). Daily maximum temperatures may exceed 35^{o} C during summer months but only for a few hours. Constant exposure to such extreme temperatures would undoubtedly be detrimental to *P. scutigera* development and survival. However, it is

unlikely that short exposures would have any adverse effects. Casagrande *et al.* (1987) found that short exposures of *Lymantria dispar* (L.) larvae to supraoptimal temperatures did not slow developmental rates as indicated by constant temperature studies.

The DZ for all growth stages (except third instar larvae) ranged from 11.0 to 14.1° C. Differences in the DZ within insect life stages have been observed by numerous authors (e.g. Bari and Lange 1980, David *et al.* 1989, Hutchison *et al.* 1986, Rodriguez *et al.* 1989). Johnson and Smith (1980) suggested the possible existence of different rate-limiting enzymes for each life-stage rather than a single control enzyme for total development as proposed by Sharpe and DeMichele (1977). The DZ for third instar larvae was very low at 4.1° C. While this value must be rejected, due to the very high standard error (6.9), it is evident from the shape of the developmental rate-temperature curve (Fig. 2.4) that the true DZ value for third instar larvae would be much lower than that for all other life stages.

The decision to include data for development at 30° C in the linear regression calculations is debatable. Inclusion of 30° C data gave a DZ estimate for larval and total development approximately 2° lower than when the data was excluded (Table 2.24). This influenced the estimate of the number of DD required to complete larval and total development by 81.2 or 94.1 DD, respectively. Vickers (1982b) excluded data for *P. scutigera* development at 31° C because of the departure from linearity. Hence, his DZ estimates are approximately 2-3° C lower than those obtained in this study. In past literature the decision to include extreme temperature regimes appears to have been made somewhat arbitrarily according to the extent developmental rates were retarded. For example, Lysyk and Nealis (1988) omitted values from the regression analysis if there was a 'strong' temperature effect on development. Usually, the inclusion of extreme temperature regimes is warranted if they only have a marginal effect on developmental rates, as seen in this study for *P. scutigera* development at 30° C.

In order to evaluate which DZ estimates are the most suitable for predicting *P. scutigera* development it would be necessary to follow development in the field. However, as a rough guide for predicting developmental rates, a difference of 2° C in the DZ estimate may not be

Table 2.23. Comparison of the developmental zero (DZ) and the estimated number of degree-days (DD) required for *P. scutigera* development when data from this study and Vickers (1982b) is used alone or combined together, including or excluding values for 30+^{o} C.

Stage of development	DD/DZ	This study		Vickers study		Both studies	
		Inc 30+	Exc 30+	Inc 30+	Exc 30+	Inc 30+	Exc 30+
Egg	DZ	12.5	13.7	13.8	14.1	13.3	14.0
	DD	74.3	63.0	61.2	57.3	66.8	59.8
Larva	DZ	11.1	13.2	11.3	13.0	11.2	13.1
	DD	362.1	280.9	359.0	285.7	360.2	283.3
Pupa	DZ	11.9	11.7	13.2	14.0	12.7	13.2
	DD	152.9	155.8	133.3	116.6	140.9	130.8
Larva-adult	DZ	11.3	13.0	12.0	13.3	11.7	13.2
	DD	511.7	417.6	489.3	402.6	498.2	408.4
Egg-adult	DZ	11.5	13.1	12.3	13.5	11.6	13.2
	DD	583.8	479.6	548.7	457.5	574.2	470.0

all that critical.

Criticism can be directed at the small number of temperature regimes used in this study to calculate the DZ values by regression analysis. If, however, these results are combined with those of Vickers (1982b), so that three extra temperature regimes (17, 20 and 31° C) and an extra data set for 25° C are included in the regression analysis, the outcome of the estimates are very similar (Table 2.23, Fig. 2.14). When all temperature regimes are used in the analysis (i.e. including 30 and 31° C) the DZ for larvae and total development are 11.2 and 11.7° C, respectively, compared to 11.1 and 11.3° C when data from this study alone is used. The overall DZ estimate for pupae is higher at 12.7° C when both data sets are combined, compared to 11.9° C when data from this study only is used. This higher value is more similar to that estimated for females separately (12.4° C) and was mainly due to the influence of Vickers value for pupal development at 25° C on the slope of the regression line (Fig. 2.14). It is possible that the sex ratio of Vickers moths at 25° C may have been heavily biassed towards females giving a faster developmental rate than expected under a 1:1 sex ratio.

The rate of development at the lower temperature of a fluctuating temperature regime can be estimated if the rate is known at the higher temperature (Fielding and Ruesink 1988). Hence, by subtracting the observed rate of development at 25° C from the fluctuating temperature of 25/15° C, development of *P. scutigera* at 15° C can estimated as 16.3, 104.2, 37.0 and 128.2 days for egg, larval, pupal and total development, respectively. All of these values fall close to the extrapolated regression line when developmental rates are plotted against temperature (Fig. 2.14).

Developmental rates under the fluctuating temperature of 25/15° were slightly, but not significantly, faster than that at the constant temperature equivalent of 21° C. Such an acceleration of developmental rates under fluctuating temperature regimes has been observed in many other insects (e.g. Fielding and Ruesink 1988, Welbers 1975, Zalucki 1982). Fielding and Ruesink (1988) hypothesized that if insect development is controlled by rate-determining enzymatic processes, as proposed by Sharpe and de Michele (1977), then an apparent acceleration in developmental rates may occur under fluctuating temperatures if there is a

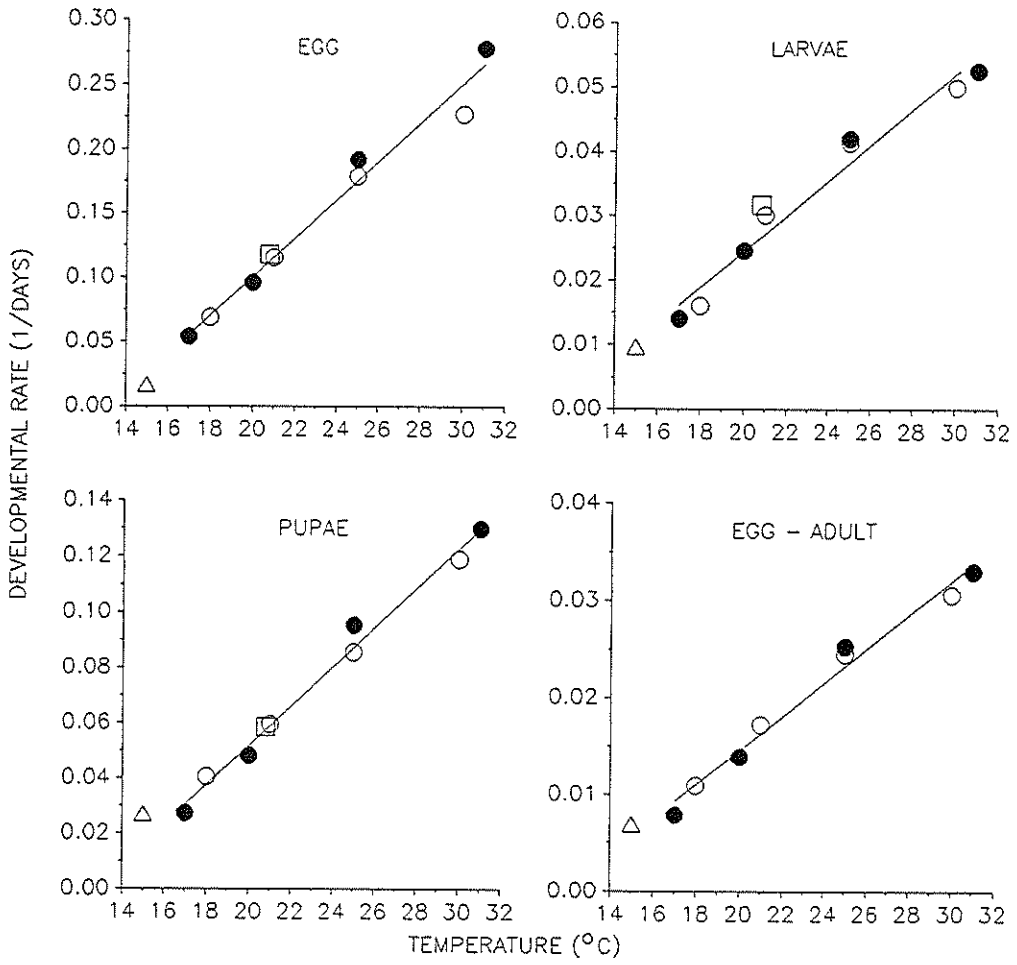

Fig. 2.14. Developmental rates (1/days) for *P. scutigera* eggs, larvae, pupae and total development using the combined data from Vickers (1982b) (solid circles) and this study (open circles). Linear regression model fitted to the combined data. Square symbol represents developmental rate at fluctuating temperature of 25/15^{o} C. Triangle symbol represents estimated developmental rate at 15^{o} C (see text for details).

time lag between the switch from one-rate controlling enzyme to another. This hypothesis presumes that the rate-controlling enzymes are part of the same metabolic pathway. Ratte (1985) gave an alternative explanation based on his concept of a dual temperature action on insect development and reproduction. This concept is based on evidence from the past literature and purports to show that temperature does not affect the growth process alone, but also the control of metamorphosis and reproduction. The effects are mediated by the neuro-endocrine system and are recognized by the temperature-dependant sizes of adults and eggs. Ratte (1985) proposed that the acceleration or retardation of developmental rates shown by some insects under different temperature regimes is achieved not by temperature compensation of the growth rate, but by modification of the critical size. This implies that developmental rate is not a true rate in a chemical sense as it is in the case of growth rate. Developmental rate and growth rate will only be closely related if environmental factors, such as thermoperiods, do not considerably affect adult size. However, if a thermoperiod leads to considerably smaller or larger adults than arise under a constant temperature, then developmental rates will not be the same. Ratte (1985) was unable to test his hypothesis because previous studies on insect development rarely measured the size of individuals. The weight of *P. scutigera* pupae significantly changed with temperature (Table 2.7). Pupae resulting from larvae reared at 18 and 21^{o} C were significantly heavier than those reared at 25^{o} C. If Ratte's (1985) theory is correct, it may not be possible to predict accurately *P. scutigera* development under variable temperature regimes by the thermal summation technique.

The frequency distribution of developmental times of first, second and third instar larvae and pupae was unimodal at all constant temperature regimes (Fig. 2.3). In contrast, the distribution of developmental times of fourth instar larvae appeared to be trimodal at 18^{o} C, bimodal at 21 and 30^{o} C, and unimodal only at 25^{o} C. There was also bimodality in the developmental times of fourth instar larvae under the fluctuating temperature regime. This may simply be an artifact of sample size or indicate the presence of several thermal biotypes (Reichenbach and Stairs 1984). The very variable fourth larval instar influenced the

distribution of developmental times at moth emergence. Some of this variation was dampened out by the pupal stage which had very uniform distributions of developmental times over all constant temperatures (Fig. 2.6). There was evidence of bimodality, however, in pupal developmental times under the fluctuating temperature regime.

Stochastic models of insect development assume that the frequency distribution of emergence times is uniform at each temperature regime, i.e. the same-shape property (Wagner *et al.* 1984a). However, when the frequency distributions for total development of *P. scutigera* were normalized to a mean of 1 and compared using the Kolmogorov-Smirnov test (Sokal and Rohlf 1981), moths reared at 18^{o} C had significantly different frequency curves from those reared at all other temperature regimes (Fig. 2.15). Wagner *et al.* (1984a) listed several reasons why distributions of normalized developmental time may not have identical shapes at all temperature regimes. One source of error, involves the sample interval at which insects are observed. If the time interval between observations are too long, then the shape of the frequency distribution will be affected adversely. This is more likely to be a problem at high temperature regimes, where the duration of development is very short, rather than at low temperature regimes. Different sample sizes can also affect the shape of measured distributions. Sample size is difficult to control because of differential mortality of insects between temperature regimes. Another problem, related to sample size, involves the selective mortality of insects held at the extreme temperature regimes. This selection reduces the variability in distributions by eliminating individuals that would complete development in longer times. Such mortality may be responsible for the very truncated shape of the frequency distribution of total developmental times for *P. scutigera* at 30^{o} C (Fig. 2.3).

Changes in food quality and quantity or changes in rearing conditions between replicates may also affect the frequency distribution of developmental times (Wagner *et al.* 1984a). While rearing conditions were kept constant in this study, changes in food quality may have occurred at the two extreme temperature regimes. The artificial diet used to rear larvae was not replenished throughout the developmental period. Because of the very prolonged larval period at 18^{o} C and high temperatures at 30^{o} C, some changes in the quality of the diet, such as loss of moisture, may have occurred and induced variability in developmental times.

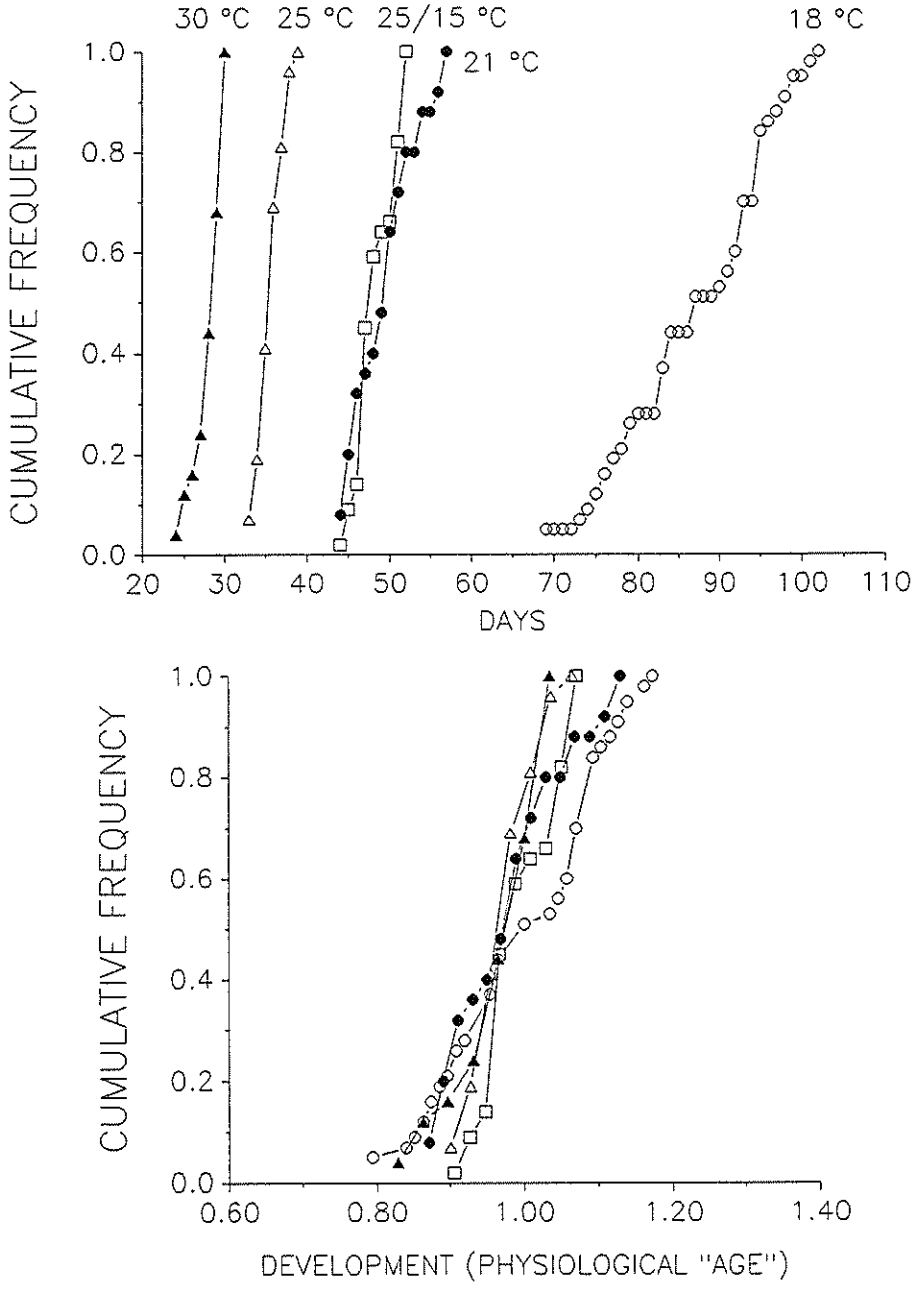

Fig. 2.15. (A) Cumulative frequency development of *P. scutigera* at four constant and one fluctuating temperature regimes. (B) Normalized cumulative frequency developmental times for *P. scutigera* at each temperature regime.

Larval diet had a very pronounced effect on *P. scutigera* development (Tables 2.15-18). Larvae reared on OH and HT developed approximately 1.4 times faster than those reared on GC or AD. Larvae reared on BT had intermediate developmental rates although survival rates on this diet were very poor. The most dramatic effect on development was when larvae were reared on SQ. At 25^{o} C, SQ larvae took 2.4 times longer to develop than those fed on AD or GC and 3.2 or 3.3 times longer than OH or HT fed larvae, respectively. Pupal development was much less affected; SQ pupae only took 1.1-1.2 times longer to develop than other diets. Hence, total development was prolonged by a factor of 2.0 (AD and GC) or 2.5 (OH and HT) times.

It is now recognized that food intake, utilization and allocation are active, dynamic processes which can be altered by an insect in an adaptive manner (Slansky and Wheeler 1989). Insects are known to increase the rate of food consumption in response to diets containing low levels of nutrients, allowing some species to maintain growth rates at or near levels occurring on a more nutritious diet (Karowe and Martin 1989, Slanksy and Wheeler 1989). In other cases, growth rates may decline but the decrease may be lessened compared with what would have occurred had the insect not increased it's food consumption. The rate of food consumption was not measured in this study but it is possible that differences in developmental rates between diets may have partly resulted from differences in the nutritional value of the diet. Nitrogen is one the key elements controlling the growth and development of insects. White (1978, 1984) maintains that the single most important factor limiting the abundance of insects, if not most animals, is the shortage of nitrogenous food for the very young. This hypothesis has been supported to some extent by studies on the quantity and accessibility of nitrogen in host plants of insects. For example, Taylor (1984) found that the development and fecundity of *Samea multiplicalis* (Guenée) was dependent on early larval intake of nitrogen. Larvae developed more rapidly when their food plant, *Salvinia molesta*, was richer in nitrogen. Development on plants containing low nitrogen was slower and larvae underwent a supernumery moult. In this case total nitrogen content alone was sufficient to explain the observed effect on development, suggesting that compounds which reduce the availability of nitrogen in the food were not important. However, secondary plant compounds

such as tannins (Feeny 1970) or low water content of the diet (Hough and Pimental 1978, Martin and Van't Hof 1988, Scriber 1977) may indirectly affect development of the insect by reducing the utilization or assimilation of nitrogen.

The effects of diet on the growth and development of insects may be further complicated by temperature and the balance of nutrients. House (1966) fed larvae of the fly *Pseudosarcophaga affinis* Auct. on four dietary levels of glucose in all combinations with three levels of thiamine at three constant temperatures. Relatively high levels of glucose in the diet had a less deleterious effect on larval development at low temperatures than at high. House (1966) found that this effect of temperature was dependent on the balance of nutrients in the diet. Temperature increments increased the rate of growth and development of larvae on diets containing a more optimum balance of nutrients but decreased rates on suboptimally balanced diets. Thus the metabolism of insect larvae already strained by a suboptimally balanced diet may be further impaired by another stress, temperature. The increase in developmental times of *P. scutigera* larvae reared on SQ at 25 and 30^{o} C was almost identical. Larvae took 2.4-2.5 times longer to develop on SQ than on AD or GC when reared at either 25 or 30^{o} C. Similarly, SQ larvae took 3.2 and 3.3 times longer to develop than OH or HT fed larvae at 25^{o} C compared to 3.2 and 3.7 times longer on the same diets, respectively, at 30^{o} C. This may suggest that the effects of SQ on *P. scutigera* larval development is not through an imbalance on nutrients but due to other factors.

A variety of plant allelochemicals reduce the growth of insects (Bowers and Puttick 1989). Cotton plants contain a number of allelochemicals that are known to have adverse effects on the development of Lepidopterous pests, such as condensed tannins (Zummo *et al.* 1984) and terpenoid aldehydes e.g. gossypol (Shaver and Lukefahr 1969). The effects of secondary plant compounds on insect growth may vary according to the quality of the protein content of the diet. In *Spodoptera exigua* (Hübner) protein quality significantly altered the toxicity of a soybean protein inhibitor to the larvae; i.e. the more nutritious the protein, the less toxic the inhibitor (Broadway and Duffey 1988). The possible influence of tannins and terpenoid aldehydes on the population performance of *P. scutigera* on cotton will be discussed in

Chapter 8.

Gordon (1984) warns that studies on the adequacy of food sources for growth of insects can be misleading if their duration is restricted to a few instars or to a single generation. He cited the case of a polyphagous species (*Hyphatina cunea* Drury) which could not be maintained for several generations on many host plants that allowed good growth and reproduction of a single generation. Furthermore, care must also be taken in the interpretation of results when using laboratory strains of insects. Bowers and Puttick (1989) found that larvae of a laboratory strain of *L. dispar* more readily accepted diets containing increasing concentrations of a feeding deterrent, irridoid glycosides, than did wild strains. This resulted in the laboratory strain achieving significantly higher rates of growth.

The timing of metamorphosis in insects appears to be dependent on attainment of a critical size (Ratte 1985). This is supported by the fact that *P. scutigera* larvae reared on SQ underwent very prolonged developmental periods yet attained pupal weights that were equal to those reared on OH and AD (Table 2.17). Faster development rates on OH and HT may have been at the expense of a reduction in pupal weight compared to moths reared on GC. Size and/or weight has often been considered as a measure of potential fecundity, hence a reduction in pupal weight implies a reduction in fecundity (Gilbert 1984). Leather (1988) argues that this relationship may only hold true in insects where no ovulation occurs after the adult moult. If ovulation does occur after adult moult, as for most Lepidoptera, then size and/or weight will only give a measure of initial rates of reproduction. This appears to be true for *P. scutigera* as Vickers (1982a) found a highly significant relationship between female pupal weight and the number of large oocytes at emergence but not between pupal weight and realized fecundity. Similarly, in this study there was no relationship between pupal weight and realized fecundity for moths reared on OH, HT and GC. However, when mean pupal weights for each diet group were plotted against mean realized fecundity, the relationship was significant; i.e. the mean pupal weight of a sample of *P. scutigera* reared on the same host plant may give a rough measure of potential fecundity. The fecundity of *P. scutigera* reared on SQ was not measured, but as mean pupal weights were very similar to OH moths the number of eggs laid may also be similar.

Reproduction in *P. scutigera* was significantly affected by temperature. Very few eggs were laid at 18° C and only 46.7% of the females paired laid fertile eggs (Table 2.12). Although there was no significant difference in the mean number of eggs laid at 21, 25 and 30° C egg numbers were highest at 25° C. Such effects of temperature on fecundity have been observed in many other insect species (see section 2.1. for citations).

In this study, mean realized fecundity of moths reared on artificial diet at 25° C was 494.7 eggs/female which is much higher than that reported by Vickers (1982a) at 326 eggs/female. The reason for this is unknown, but may have been partly due to the fact that Vickers reared his moths at 27° C, rather than 25° C.

The preoviposition period may represent a period of ovarian maturation that is needed before a female starts to oviposit. Like all other developmental processes, this preoviposition period is affected by temperature (Norris 1967). Vickers (1984a) measured the mean number of large oocytes at emergence in *P. scutigera* and found that it was less (253) than the mean number of eggs (326) laid by females. In this study temperature significantly affected the length of the preoviposition period, being longer at 18 and 21° C than at 25 or 30° C. However, egg-laying females were not given access to host plants, therefore, the observed preoviposition period may be unrepresentative of what actually happens in the field. In *Panolis flammea* (Denis and Schiffermüller) the preoviposition period changes according to the provenance of the *Pinus contorta* presented to egg-laying moths (Leather *et al.* 1985).

Alternatively, the preoviposition period may represent a dispersal phase in the biology of *P. scutigera*. Females may not oviposit and/or mate until they have flown a predetermined distance. Vickers (1982a) observed that while some females mated one day after emergence, the majority mated on the fourth or fifth day.

Within larval diets or temperature regimes there was no correlation between the length of the preoviposition period and realized fecundity. However, when the group means of each larval diet were considered, there was a significant negative correlation between the preoviposition period and fecundity i.e. larval diets that were poorer for reproduction resulted in a longer mean preoviposition period (Fig. 2.13). The reason for this is difficult to answer

without knowing what the preoviposition period actually represents. If it represents a period of ovarian maturation, the onset of oviposition or mating may depend on the attainment of a critical number of mature oocytes in the ovaries. Inadequate larval diets may give rise to females with smaller or less developed ovaries, hence lengthening the time taken to reach this critical state of maturation.

The duration of oviposition can be considered as a measure of 'effective' longevity of females moths i.e. the time over which offspring are produced. The longevity of females after all their eggs have been laid is irrelevant to studies on population performance unless they continue to attract males and hence affect reproduction of virgin females by reducing or delaying mating. The effects on reproduction of delaying mating in *P. scutigera* are considered in Chapter 7. However, it was observed that most females died within 1-2 days after oviposition had ceased. At 30° C the duration of oviposition was approximately half of that at 21 and 25° C. Similar effects of temperature on the reproduction of other insects have been published. In *P. gossypiella* high temperatures (32° C or higher) have a detrimental effect on reproduction by reducing moth longevity, mating frequency and the success of sperm transfer to the spermatophore of the female. Low temperatures may have a more profound effect on reproduction in insects which undergo postmetabolous gonadal development. In such insects the favourable range of temperatures for reproduction is often much narrower than that for development (Ratte 1985).

Larval diet significantly affected the fecundity of *P. scutigera*, with moths reared on AD and GC laying the most number of eggs (Table 2.21). Similar effects of larval diet on insect fecundity have been observed in many other species e.g. *Epiphyas postvittana* (Walker) (Danthanarayana 1975), *H. virescens* (Nagauda and Pitre 1983), *P. gossypiella* (Adkisson 1961). The effects of adult diet on longevity and fecundity was not investigated. In most Lepidoptera the availability of carbohydrate (e.g. sugar or honey solution) significantly increases longevity and fecundity (Leather 1984).

If the capacity for increase, rc, is a measure of suitability of the environment, then maximum rc will reveal conditions most appropriate for population increase (Allsopp 1981). For *P. scutigera* reared on artificial diet, the most optimal temperature for population increase

was 25° C. The most suitable larval diet (excluding artificial diet) for population increase was OH, due to the short cohort generation time on this diet, closely followed by GC which had the highest net reproductive capacity (Ro) for any diet. Moths reared on BT had the lowest rc because of the relatively long cohort generation time and very low reproductive capacity. Unfortunately, it was not possible to calculate the capacity for increase of *P. scutigera* reared on SQ or DC, but values would probably be very low due to the adverse effects of these diets on development and reproduction, respectively.

It is fortuitous that development and reproduction on artificial diet was very similar to that on green cotton bolls. This means that laboratory studies, rearing larvae on artificial diet, may be used to predict the development and population performance of *P. scutigera* attacking cotton bolls.

CHAPTER 3

Biology of *P. scutigera* infesting cotton

3.1. Introduction

The biology of *P. scutigera* infesting cotton is poorly documented. If effective pest management programs for the control of *P. scutigera* are to be designed then more detailed information is needed.

In this Chapter aspects of the biology of *P. scutigera* in unsprayed and sprayed (commercial) cotton fields are reported over three consecutive cotton seasons, identifying sites of oviposition and pupation, the rate and incidence of larval infestations, egg, larval and pupal parasitoids, and the nocturnal activity of adult moths. The spatial distribution of larvae in unsprayed cotton is described and optimal sample sizes required for detecting larval infestations at fixed levels of precision are given. A sequential sampling plan is proposed for detecting larval infestations in bolls.

3.2. Materials and methods

3.2.1. Weather data and degree-day calculations

Local weather records, for the period July 1984 to May 1987, were obtained from the D.P.I. Research Station, Biloela. Daily maximum and minimum screen temperatures, the number of days when the terrestrial minimum was below -0.9^{o} C (frost), and daily rainfall figures were extracted from the records. Average monthly rainfall figures for each year of observation were compared with a 62 year mean (1924-1985) compiled by the D.P.I. Research Station, Biloela (J. Page unpublished data).

A BASIC computer program (DEGDAY) devised by Higley *et al.* (1986) was used to calculate degree-day development of cotton under field temperatures, choosing the sine wave method of calculation. Degree-day requirements for cotton as given by Hearn (1989) were used to approximate the date of seedling emergence, first square, first flower and first open

boll.

3.2.2. Oviposition sites

Oviposition sites of *P. scutigera* were determined by confining moths onto pot-grown plants and by sampling cotton plants from an unsprayed field when *P. scutigera* populations were high.

Cotton seedlings (variety DPL61) were planted in 26 cm diameter plastic pots containing alluvial soil on 25 October, 1984. The plants were placed in an unheated shadehouse at the D.P.I. Research Station, Biloela, and were regularly watered and fed with a nutrient solution. When plants started flowering, cardboard tags bearing the date were tied onto open yellow flowers each day so that the age of all bolls was known.

Plants were used when 57 and 80 days old, selecting five plants of equal height, growth form and fruit production. The 57 day old plants had on average 8.8 squares and 9.2 bolls per plant. The bolls ranged from one to 18 days old. In the 80 day old plants growth had prematurely terminated due to water stress. No squares were present on plants but there was an average of 9.6 bolls per plant, ranging from 10 to 39 days old.

Each plant was covered by a cylindrical cage (60 cm diam., 120 cm ht) constructed from steel wire and nylon insect screen. The cage was large enough to allow the free movement of moths around and above the plant. Cages were suspended from the frame of the shadehouse with string so that the base was flush with the concrete floor. The cage base was sealed to the floor with masking tape to prevent escape of moths. Access to the cotton plants was possible through a zip fastener in the side of each cage. Five pairs of laboratory reared *P. scutigera* (ex artificial diet), that were allowed to mate for three days prior to release, were introduced into each cage for a period of five nights. After the fifth night plants were removed from the cages and all parts were immediately searched for *P. scutigera* eggs under a binocular microscope. Leaf size was arbitrarily classified as small (<5 cm wide), medium (5-10 cm wide) or large (>10 cm wide).

A further experiment was conducted on cotton plants (variety DPL90) grown in the grounds of the D.P.I. Research Station, Biloela, on 28 October, 1986. Growth of these plants had terminated prematurely due to water stress and heavy insect damage. An average of 6 squares, 6.7 green bolls and 4.2 open bolls were present on plants. Three cylindrical cages were placed over groups of three plants one week before moths were released into the cages. On 31 March, 1987, when plants were 93 days old, five pairs of *P. scutigera* (as above) were introduced into each cage for a period of five nights. After the fifth night all plant parts were searched immediately for *P. scutigera* eggs under a binocular microscope.

The site of oviposition on field collected cotton plants was determined on four occasions during 1985 and 1986. Three large branches, bearing an average of 2.4 squares and 3.5 bolls per branch, were sampled on 24 April, 1985 (181 days after planting). Further samples of plants were collected after defoliation and picking of the cotton to determine whether old plants remained attractive to ovipositing females. Six plants were collected on 28 May 1985 (215 days after planting) when plants had begun to produce fresh leaves, squares and flowers alongside old damaged, dry bolls left on the plants after picking. An average of 5.5 squares and 1.7 damaged open bolls were present on the sampled plants. Three entire plants, bearing an average of 5 squares, 0.3 green bolls and 6 damaged open bolls, were collected on 6 June 1985 (224 days after planting). Three frost damaged plants were collected on 3 July 1985 when plants bore on average of 5 frost damaged green bolls and 6.5 open damaged bolls. In the following cotton season, three entire plants were collected on 12 April, 1986, 167 days after planting. These plants had on average, 12.7 squares and 3.7 green bolls.

All plants sampled were carefully pulled out of the ground so that the entire stem, including that just below the soil surface, could be searched for eggs. Soil surrounding the base of plants was not searched for eggs.

3.2.3. Egg parasitoids

All *P. scutigera* eggs found on cotton plants sampled in section 3.2.2. were incubated in the laboratory to determine levels of parasitism. The presence of egg parasitoids in cotton was also determined by exposing *P. scutigera* eggs obtained from laboratory colonies in the field for three days. Tissue papers bearing 1-3 day old eggs (see Chapter 2 for details) were cut into

ca 3 x 5 cm sections. Eggs were placed in the field by pinning the piece of tissue paper onto the underside of cotton leaves situated at the top of the plant. After three days exposure in the field the eggs were retrieved and incubated at 25° C until the emergence of a larva or parasitoid. The number of eggs exposed varied according to their availability (Table 3.14). Eggs were exposed in unsprayed cotton on four occasions, between April 1985 and February 1987, and in commercial cotton (Site 1) on 26 April, 1985.

3.2.4. Larval infestation of cotton

The incidence and rate of *P. scutigera* larval infestations in cotton was closely monitored in one unsprayed field (D.P.I. Research Station, Biloela) and two or three commercial fields for three consecutive seasons between 1984-1987. All fields were located near Biloela in the Callide Valley, although commercial fields in the Dawson Valley and around Bauhinia Downs were occasionally inspected for larval infestations. Fig. 3.1 shows the area of study and approximate locations of each sampling site.

Larval infestation was monitored by taking random samples of 70 - 200 squares or bolls (2-4 weeks old) from each field at approximately weekly intervals. Squares were usually sampled only at the beginning of the season until enough bolls (over 2 weeks old) were present on the plants. Boll age was estimated using the technique of Van Steenwyck *et al.* (1976). Descriptions of each field and sampling methods are given in the Results section of this Chapter. Records of insecticides sprays applied in each field were obtained from farmers when available. Sticky traps baited with synthetic sex pheromone were placed in all fields to simultaneously monitor male moth activity alongside larval infestation (see Chapter 4).

In the 1984/85 and 1986/87 cotton seasons the number of *P. scutigera* found in bolls that were dissected immediately on collection was compared with the number of larvae found in bolls incubated at 25° C for 10-14 days, according to the method of Fye (1976). Bolls were incubated in plastic boxes (Lily Plastics Pty Ltd, product T-1000), 18 x 12 x 6.5 cm, lined with tissue paper to absorb condensation. A large hole (12 x 6 cm), covered with nylon insect screen, was cut into the lid of each box to provide ventilation.

In the 1984/85 season, the 2.7 ha unsprayed cotton field was divided into 12 areas of equal

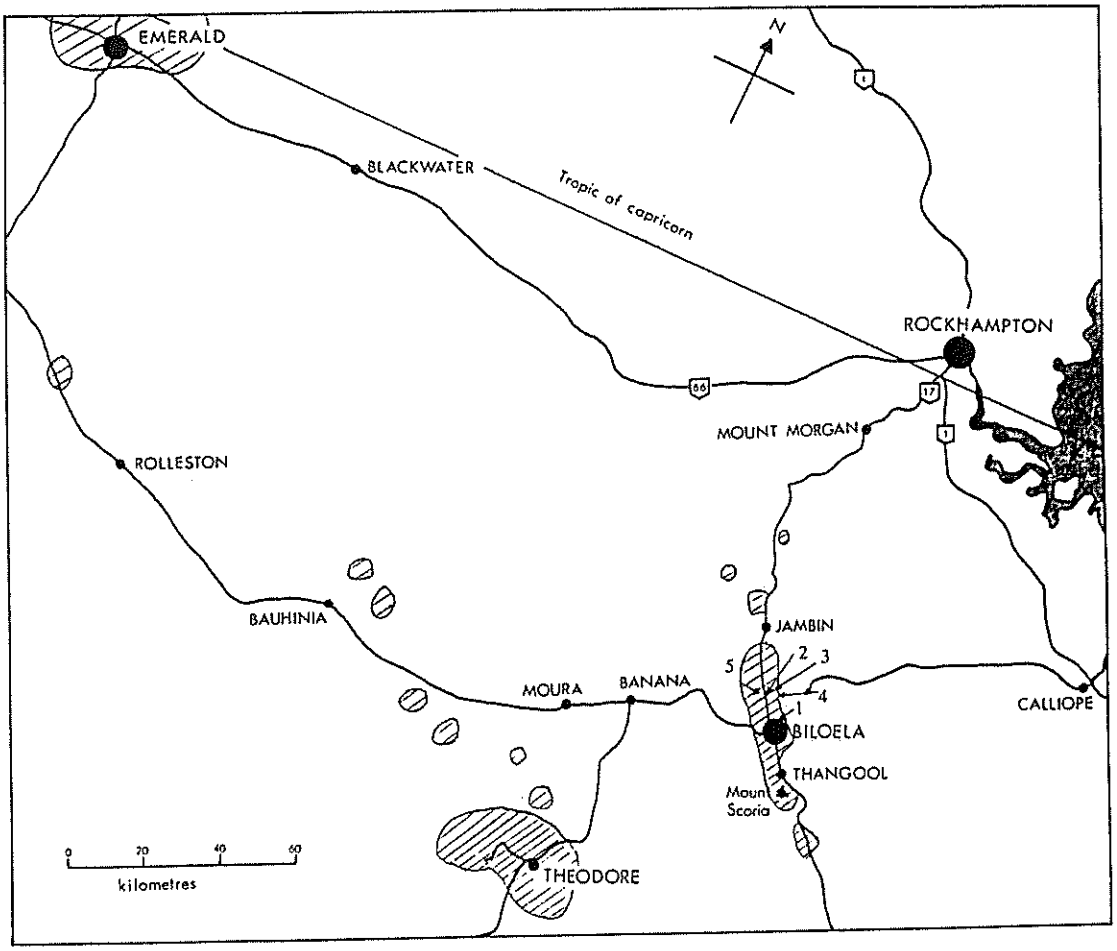

Fig. 3.1. Area of study in Central Queensland, showing the distribution of cotton (hatched areas, after Page *et al.* 1982) and localities mentioned in the text.

Location of field sites:

1 = D.P.I. Research Station
2 = Commercial cotton Site 1
3 = " " Site 2 1984/85 and 1985/86 seasons
4 = " " Site 2 1986/87 season
5 = " " Site 3 1984/85

size. Twenty bolls were randomly sampled from each area (i.e. 240 bolls) at *ca* weekly intervals between 3 February and 11 April, 1985. Half of the bolls sampled from each area were inspected immediately for *P. scutigera* infestation, while the other half were incubated at 25^{o} C in ventilated plastic boxes for 10-14 days.

In the 1985/86 season, all collections of squares and bolls made from unsprayed and commercial fields were incubated in ventilated plastic boxes before searching for larvae. In other seasons, bolls collected from commercial fields were inspected for *P. scutigera* infestation immediately after collection. The growth stage of all larvae found was estimated by comparing the size of their head capsules with those of known instars obtained from laboratory cultures (see Chapter 2).

The behaviour of larvae infesting squares and bolls was recorded while conducting field experiments, observing the point of larval entry, areas of feeding and damage caused. The sex ratio of *P. scutigera* pupae, resulting from larvae infesting bolls collected from unsprayed cotton, was determined during the 1985/86 season. Chi-square tests were performed for each sampling date to test whether the sex ratio departed from an expected ratio of 1 male to 1 female.

The rate and incidence of larval parasitism for each field was recorded when bolls were incubated. Parasitoids were identified by taxonomists at the Queensland Department of Primary Industries (Q.D.P.I.), Brisbane, and representative specimens have been lodged at the University of Queensland insect collection, Brisbane.

3.2.5. Spatial distribution of larvae in unsprayed cotton, optimal sample sizes, and a sequential sampling plan

The spatial distribution of *P. scutigera* larvae in unsprayed cotton was determined using data from incubated bolls sampled during the 1984/85 season. Means and variances, based on 12 sampling units (comprising of 10 bolls/unit) for each of the ten sampling occasions, were calculated to test if the distribution of larvae conformed to the Poisson (random) distribution. Dispersion indices were calculated using Morisita's index (Morisita 1964), Iwao's patchiness regression (Iwao 1968) and Taylor's power law (Taylor 1961).

If a population follows the Poisson distribution, the mean density ($\bar{x}$) will equal the

variance (s^2), so that the departure of $s^2/\bar{x}$ from unity will be a measure of the departure from the Poisson distribution (Southwood 1978). This was tested by calculating the index of dispersion (I_D):

$$I_D = s^2(N - 1)/\bar{x}$$

where N is the number of units comprising a sample. The significance of I_D was tested as a Chi-square with N - 1 degrees of freedom. If the Chi-square value falls between the Chi-square table values at the 0.975 and 0.025 probability levels (P>0.05), agreement with a Poisson distribution is accepted. Values for Chi-square less than the 0.975 probability level suggest a regular pattern, whereas Chi-square values greater than the 0.025 probability level suggest a clumped or aggregated pattern (Southwood 1978).

Morisita's index is given by the formula:

$$Io = N\ \Sigma x^2 - \Sigma x/(\Sigma x)^2 - \Sigma x$$

where N is the total number of samples and Σx is the sum of the number of individuals found in all samples. This index has the advantage of being relatively independent of the type of distribution, the number of samples and the size of the mean (Southwood 1978). When the distribution is Poisson (random) the index will give a value of unity, when the distribution is aggregated the index will be greater than one, and when the distribution is regular less than one.

The significance of the departure from a random distribution shown by Morisita's index may be tested by comparing F_o calculated:

$$F_o = I\sigma(\Sigma x-1) + N - \Sigma x/N - 1$$

with the value of F (variance ratio) in tables, the appropriate values being that for F where $N_1 = N - 1$ and N_2 = infinity (Southwood 1978).

Iwao's patchiness regression utilizes Lloyd's (1967) mean crowding index ($\overset{*}{m}$) by regressing $\overset{*}{m}$ on the mean ($\bar{x}$):

$$\overset{*}{m} = \alpha + \beta\,\bar{x}$$

where α and β are regression constants. The intercept (α) of the regression line, the index of basic contagion, is 0 for distributions where a single animal is the basic unit, but positive

where populations exist as groups of individuals (Iwao 1968). The slope of the regression line (β) is the density-contagiousness coefficient and describes how individuals distribute themselves in the habitat. When individuals are arranged in uniform, random or aggregated patterns then B is less than 1, equal to 1 or greater than 1, respectively. Lloyd's (1967) mean crowding index ($\overset{*}{m}$) was calculated as:

$$\overset{*}{m} = \bar{x} + [(s^2/\bar{x}) - 1]$$

where $\bar{x}$ is the density of a species.

Taylor's power law relates sample variance to mean density through:

$$\ln s^2 = \ln a + b \ln \bar{x}$$

where a, the exponential of the intercept, is a sampling factor, and the slope b is an index of aggregation, which is constant for a species and/or stage. A value of $b<1$ implies a uniform distribution, $b=1$ a random distribution, and $b>1$ an aggregated distribution (Southwood 1978).

The description of the distribution of *P. scutigera* larvae infesting bolls given by Iwao's regression method and Taylor's power law was used to determine the optimum sample size required for estimating mean larval density (Smith and McDonald 1989). Karandinos (1976) defined the optimum sample size (n) as:

$$n = s^2/(C^2.\bar{x}^2)$$

where C is a measure of precision expressed as a proportion of the mean ($\bar{x}$). s^2 was substituted with $s^2 = (\alpha + 1)\bar{x} + (\beta - 1)x^2$, derived from Iwao's regression method to give:

$$n = [(\alpha + 1)/\bar{x} + (\beta - 1)]/C^2$$

and in Taylor's power law to give:

$$n = a\bar{x}^{(b-2)}/C^2$$

Precision levels of 10 and 20% (i.e. C equal to 0.10 and 0.20, respectively) were used in both formulae. For estimates of cost in man hours, the optimum number of samples were multiplied by the time taken to pick and process one sample unit of ten bolls, adding on to this estimate the time taken to walk between samples.

A sequential sampling plan was formulated using Iwao's (1975) method which allows classification of populations relative to a critical density, such as the economic threshold. The

upper and lower limits of the plan were calculated from:

$$q_n = nm_o \pm t \sqrt{n(am_o^b)}$$

where q_n is the upper or lower limit of the confidence interval for the cumulative number of larvae collected; n = the number of samples required; m_o = the critical density (economic threshold); a and b are coefficients from Taylor's power law. A nominal critical density of five *P. scutigera* larvae/100 bolls (0.5 larvae/sample unit) was used, based on the upper economic threshold currently recommended for making spray decisions (Q.D.P.I. unpublished report).

3.2.6. Relationship of cotton boll age to larval infestation

The relationship of cotton boll age to larval infestation was determined in unsprayed cotton at the D.P.I. Research Station, Biloela, during the 1984/85 and 1986/87 seasons. In 1985, aluminum plant tags, bearing the date, were attached to 100-200 open flowers every 3-4 days from 6 January until 18 March. The resulting bolls from all age categories were collected on 28 February (N=241 bolls) and 19 March (N=73 bolls). All boll samples were incubated in ventilated plastic boxes at 25^{o} C for 11 and 18 days, respectively, before determining total *P. scutigera* infestation. In 1987, plant tags were attached to 200 open flowers every 3-4 days from 13 January to 7 March. Bolls of all age categories were collected on 6 March (N=201) and 9 March (N=306). Bolls were searched immediately for *P. scutigera* larvae, rather than incubated in ventilated boxes, so that the relationship of boll age to larval attack could be determined for each larval instar. The length and width of all unopened bolls was recorded in both cotton seasons. Data from the two boll collections in each cotton season were combined for analysis.

3.2.7. Recovery of larvae artificially infested onto cotton squares and bolls

Neonate larvae were placed onto squares and bolls of potted cotton plants grown in a shadehouse at the D.P.I. Research Station, Biloela. Cotton plants (variety DPL61) as described in section 3.2.2. were used for infestation. During peak squaring (57 days after planting) 51 squares on a total of eight plants were infested with four neonate larvae (less than six hours old). Squares were collected nine days later and searched for larvae. Fifty bolls on a

total of five plants were infested with four neonate larvae when the plants were 112 days old. The bolls, ranging from 21 to 50 days old, were collected seven days after infestation and searched immediately for larvae.

3.2.8. Dispersal and survival of larvae emerging from eggs placed on cotton plants

The dispersal and survival of neonate larvae after emerging from eggs was investigated in a shadehouse using potted cotton plants, planted in March 1987. Twenty eggs, obtained from laboratory cultures and laid on tissue paper, were attached to the underside of large leaves at the base of eight cotton plants (variety DP90), 87 days old. Three and five plants were searched for larvae 24 and 48 hours after egg hatch, respectively, noting the number and position of larvae found.

3.2.9. Sites of pupation and pupal parasitism

The site of pupation of *P. scutigera* in unsprayed cotton was determined when larval populations in bolls was high. All entire plants sampled to determine sites of oviposition (see section 3.2.2.) were also searched for any *P. scutigera* prepupae or pupae.

Further entire cotton plants were searched for *P. scutigera* prepupae and pupae on 3 April, 1985. Plants were selected by randomly throwing a 0.5 m^2 quadrat in three locations. The quadrats covered a total of ten cotton plants which were inspected *in situ* for prepupae and pupae. All leaf litter (a mixture of shed leaves, flowers, squares and bolls) and the top 10cm of soil situated underneath each quadrat was transferred to the laboratory and carefully searched for prepupae and pupae by hand.

The presence of prepupae or pupae inside green bolls, collected while monitoring larval infestations in unsprayed and commercial cotton (section 3.2.4.), was noted. Also, the presence of pupae in open damaged cotton bolls, collected for experiments described in Chapter 6, was recorded.

The number of *P. scutigera* prepupae and pupae in the 'leaf litter' situated at the base of cotton plants was determined in unsprayed cotton on 12 and 20 June, 1986. Approximately 1000 decaying fallen forms (shed cotton flowers, squares and bolls) that comprised the leaf litter were sampled. All *P. scutigera* larvae, prepupae and pupae found were incubated in the laboratory at 25^{o} C to determine levels of parasitism. Parasitoids were identified by Q.D.P.I.

taxonomists, and representative specimens have been lodged at the University of Queensland insect collection, Brisbane.

3.2.10. Nocturnal activity of adult moths

The nocturnal activity of *P. scutigera* was monitored in unsprayed cotton at the D.P.I. Research Station, Biloela, from dusk to 5 am (Australian Eastern Standard Time) for a total of 12 nights between 14-18th March 1987, 4-6th April 1985, 24-26th June 1987 and 24-26th November 1985.

The number of males caught in pheromone traps was recorded hourly throughout each night of observation, using the Delta trap design (see Chapter 4). In April, 1985, traps contained 1 mg baits of (Z,Z)-7, 11-hexadecadienyl acetate but during all other observations traps contained 1 mg baits of a 9:1 ratio of (Z,Z) and (Z,E) -7, 11-hexadecadienyl acetate (see Chapter 4 for further details). Traps were placed at plant canopy height, and within the crop at 2 m from the edge. The number of traps observed each night varied from one in April 1985, to two in March 1987 and November 1985, and three in June 1987.

The onset of nocturnal activity of laboratory reared moths, held in oviposition chambers (see Chapter 2) placed in the cotton field, was recorded each night. The number of moths per container varied according to availability. Moths were held in an outdoor insectary, under ambient temperature and photoperiod conditions, for at least one night prior to moving into the cotton field and provided with 5% sugar solution for food. Activity was recorded in detail on 7 April 1985, observing the time of feeding, walking, flying and mating. In March 1987 the time of oviposition was recorded by changing egg sheets at hourly intervals starting from 18.00 h.

Moths were observed with the aid of a battery powered headlamp covered by a red Kodak filter. Air temperature was recorded throughout the night and weather conditions noted. Wind speed was recorded hourly using an anemometer.

In March 1987 the number of *P. scutigera* caught in an ultraviolet light trap was recorded hourly over the three night period. The light trap was situated 2 m from the edge of the cotton field and approximately 200 m from the pheromone traps. A block of plaster of paris soaked

with Dichlorvos insecticide was placed inside the collection bucket to kill trapped insects. The trap was switched on before dusk and emptied every hour from 19.00 h. The catch was sorted the next day, recording the number of male and female *P. scutigera* caught.

3.3. Results

3.3.1. Weather data

Daily maximum and minimum screen temperatures fluctuated between 40.6° C and -2.7° C during the period of study (Fig. 3.2). Weather data is summarized in Table 3.1, giving monthly averages for each year of observation. The hottest months were December, January and February with mean monthly maximum temperatures of 33-35° C and minimum temperatures of 19-20° C. The coolest months were June, July and August with mean monthly maximum temperatures of 21-24° C and minimum temperatures of 6-10° C. Frosts (defined as days when the terrestrial minimum was less than -0.9° C) were most frequent during July and August but also occurred in June, September and October (Table 3.1).

Rainfall is extremely variable in the Callide and Dawson Valleys. Most rain results from localized storm activity which usually falls in the late afternoon during hot weather. Occasionally, very heavy periods of rain may occur through the random influence of cyclones from coastal areas. Based on mean rainfall over a 62 year period (1924-1985); December, January and February are the wettest months (Table 3.1). During the period of study, October and December received over 100 mm of rain in all three years but rainfall in January and February was extremely variable. The driest months are usually August and September although in 1984 68 mm of rain was recorded in September. Unusually heavy rain was also experienced in June 1985, totaling 142 mm. All of this rain fell over a two day period (3rd and 4th June) giving the highest recorded rainfall for a single day of 101 mm (Fig. 3.2).

3.3.2. Oviposition sites on cotton

Moths confined onto 57, 80 and 93 day old plants laid a mean of 59.8, 33.0 and 48.3 eggs per plant, respectively (Table 3.2). The distribution of eggs on 57 and 80 day old plants was similar with the majority being laid on leaves (55.5 and 44.2%, respectively) or leaf buds (40.2

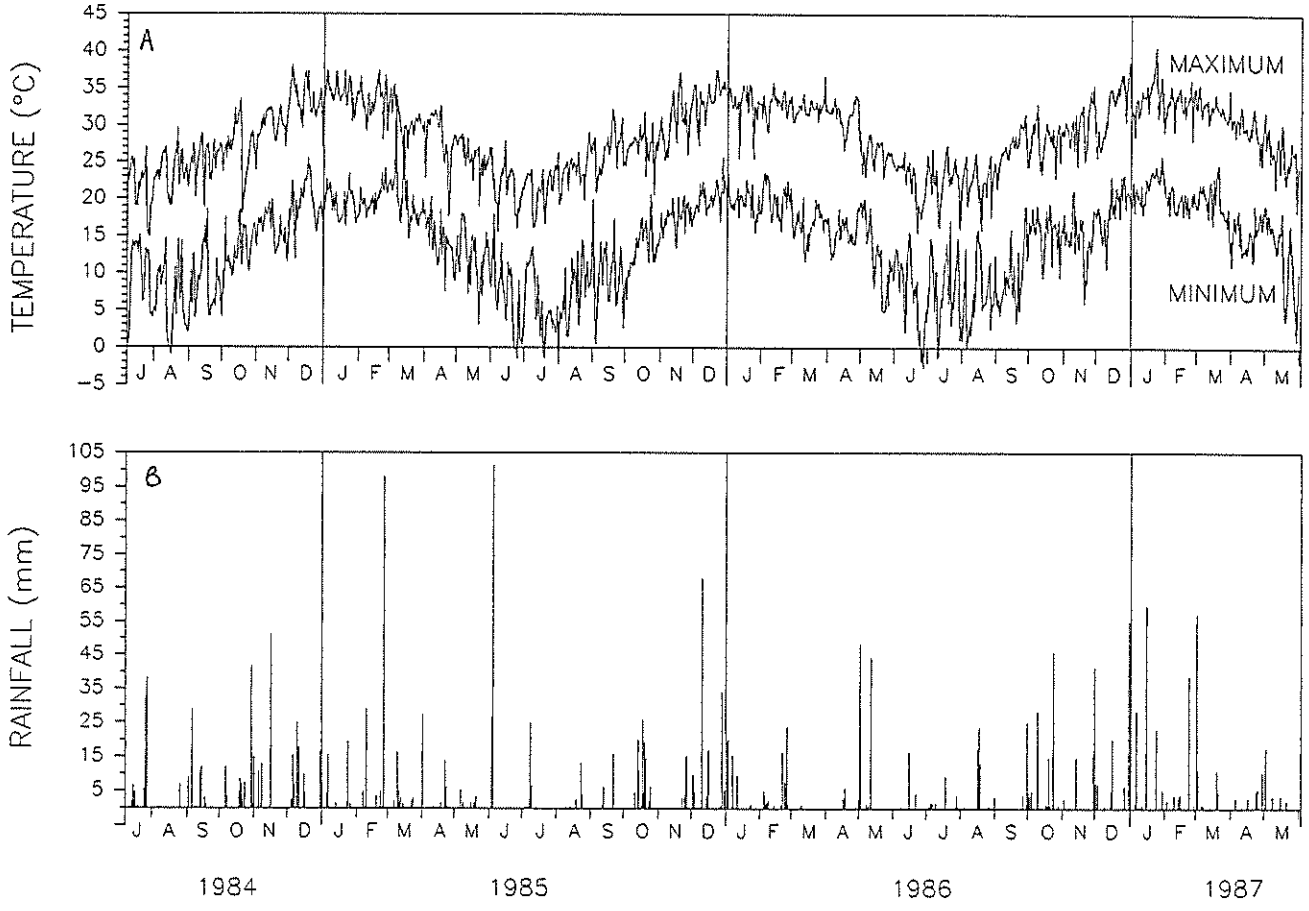

Fig. 3.2. Temperature and rainfall data recorded at the D.P.I. Research Station, Biloela, weather station between July 1984 and May 1987; (A) daily maximum and minimum screen temperatures, (B) daily rainfall.

Table 3.1. Summary of weather data recorded at the D.P.I. Research Station, Biloela, weather station between July 1984 and May 1987.

	Month											
Year	J	A	S	O	N	D	J	F	M	A	M	J
Mean maximum temperature (° C)												
1984-85	22	24	25	27	30	34	34	33	31	28	26	21
1985-86	22	24	27	31	34	34	33	33	32	31	26	22
1986-87	23	23	27	28	29	33	35	33	32	29	26	-
Mean minimum temperature (° C)												
1984-85	10	6	9	13	16	20	19	20	20	14	12	6
1985-86	6	7	10	14	18	20	20	20	17	16	12	7
1986-87	8	7	9	16	16	19	20	19	18	17	12	-
Number of days when terrestrial minimum <-0.9° C (frost)												
1984-85	9	6	5	2	0	0	0	0	0	0	0	1
1985-86	7	3	4	0	0	0	0	0	0	0	0	0
1986-87	5	16	0	1	0	0	0	0	0	0	0	-
Rainfall (mm)												
1984-85	96	8	68	104	91	106	50	142	36	49	14	128
1985-86	34	20	23	110	23	146	65	66	1	12	123	21
1986-87	19	59	39	127	74	101	119	52	85	16	37	-
62 year mean												
1924-85	32	21	24	54	80	99	107	112	65	41	40	38

and 54.0%, respectively).

Eggs were laid on all leaf sizes, both on the topside and underside (Table 3.2), and were always well concealed between or against main veins at the top of the petiole. Most eggs laid on leaf buds were located within axillary buds situated in the angle between the main-stem leaf and the stem (57.0 and 27.5%), or in buds growing on main branches or sub-branches (42.1 and 70.3%). Very few eggs (0.8 and 2.2%) were laid in terminal buds at the top of the main-stem. The proximity of bolls did not appear to influence which buds were chosen as an oviposition site. Eggs laid in leaf buds were again well concealed either within the unfolding leaves or on the underside of the strap-shaped leaf of the axillary bud.

Squares and green bolls were not attractive sites for oviposition (Table 3.2). In the 57 day old plants 0.7% (N=2) and 2.7% (N=8) of the eggs found were laid on squares and bolls, respectively. In the 80 day old plants only a single egg (0.6% of all eggs laid) was laid on a boll. Eggs laid on bolls were usually located on the outside of a bract rather than on the calyx. No eggs were found on flowers that were fully open during the period of exposure to ovipositing moths.

The distribution of eggs on 93 day old plants was markedly different from that on 57 and 80 day old plants. While a large percentage (37.3%) were found on leaves, the majority of eggs (60.1%) were laid in open bolls (Table 3.2). Two eggs were laid on the dry carpel wall of an open boll but the remainder (86 eggs) were laid within the cotton lint. Up to 22 eggs were found in the lint of a single open boll. No eggs were laid on or near green bolls but one egg was laid on a square. Only 1.4% of all eggs found were laid in leaf buds. Very few buds were present on plants because of their stressed condition.

A significant change also occurred in the site of oviposition on leaves of 93 day old plants. Leaves of these plants were heavily damaged by the cotton leaf perforator, *Bucculatrix gossypii* Turner. Larvae of this moth initially mine between the upper and lower epidermis of leaves but in later instars they feed externally making characteristic leaf perforations (Room 1979a). Seventy-two percent of *P. scutigera* eggs laid on leaves were located inside abandoned *B. gossypii* leaf mines. Other eggs were found within perforations made by later instars of *B. gossypii* larvae, and against or within empty *B. gossypii* larval cocoons, usually located on the

Table 3.2. Distribution of *P. scutigera* eggs on cotton plants 57, 80 and 93 days old when moths were confined onto plants for five days. Figures represent the total number of eggs found on five replicate plants for each age category.

Site of oviposition	No. (%) of eggs laid/plant part Plant age (days) 54	80	93
Small leaves (<5 cm wide)			
Topside	28 (9.4)	13 (7.9)	1 (0.7)
Underside	28 (9.4)	8 (4.8)	4 (2.8)
Perforator mines	0	0	10 (6.9)
Medium leaves (5-10 cm)			
Topside	27 (9.0)	15 (9.1)	2 (1.4)
Underside	30 (10.0)	28 (17.0)	2 (1.4)
Perforator mines	0	0	16 (11.0)
Large leaves (>10 cm)			
Topside	7 (2.3)	1 (0.6)	0
Underside	40 (13.4)	6 (3.6)	5 (3.4)
Perforator mines	0	0	13 (9.0)
Leaf petiole	6 (2.0)	2 (1.2)	1 (0.7)
Leaves total	166 (55.5)	73 (44.4)	54 (37.2)
Leaf bud - stem	69 (23.1)	25 (15.2)	2 (1.4)
" " - branches	51 (17.1)	64 (38.8)	0
Terminal bud	1 (0.3)	2 (1.2)	0
Leaf buds total	121 (40.5)	91 (55.2)	2 (1.4)
Open flower	0	-*	0
Square (>0.5 cm)	0	-	0
" bract	1 (0.3)	-	0
" stalk	1 (0.3)	-	1 (0.7)
Square total	2 (0.7)	0	1 (0.7)
Green boll calyx	2 (0.7)	0	0
" " bract	4 (1.3)	1 (0.6)	0
" " stalk	2 (0.7)	0	0
Green boll total	8 (2.7)	1 (0.6)	0
Open boll	-	-	88 (60.7)
Stem or branch	2 (0.7)	0	0
Mean no. eggs/plant	59.8	33.0	48.3

* - indicates not present on plants.

top-side of leaves.

Table 3.3 summarizes the distribution of eggs found on field collected plants. A mean of 6.0 eggs were found on three large branches collected in April, 1985. The distribution of eggs was similar to that on artificially infested cotton plants 57 and 80 days old. Most eggs were either laid on leaves (44.4%) or in leaf buds (38.9%) with very few laid on fruiting forms. One egg was found on the peduncle of a green boll, but none was laid directly on squares or bolls. Eggs were laid on the upper or lower side of leaves, usually between veins. Little leaf perforator damage was present on these plants and no eggs were found in the old larval mines.

A mean of 54.3 eggs were found on three entire plants sampled in April 1986, with 97.5% laid on leaves. The remainder were found in leaf buds with no eggs on any fruiting structures despite the presence of 12.7 squares and 3.7 bolls per plant. Leaves on these plants were heavily damaged by the cotton leaf perforator and 79.8% of all eggs found were laid in old larval mines of *B. gossypii*. No open bolls were present on the plants.

Plants that had been defoliated and picked were still attractive to ovipositing females. A mean of 23.3, 36.3 and 11.0 eggs per plant were found in May, June and July, 1985, respectively. The distribution of eggs was markedly different from that on younger plants. Fewer leaves and leaf buds were available as sites for oviposition. Only between 3.0-10.1% eggs were found on leaves. Plants sampled in May had 23.6% eggs laid in leaf buds, whereas in June and July only 4.6 and 6.1% of the eggs were laid in leaf buds. The majority of eggs were laid in areas of branches and stems that had been damaged by the cotton picking machines. These damaged areas allowed moths to lay eggs underneath loose bark or within the exposed fibrous, woody tissue. For the three sampling dates, 57.9, 56.0 and 72.7% of the eggs were laid in damaged branches or stems. Another 5.7, 29.4 and 6.1% of the eggs laid on plants in May, June and July, respectively, were found in the lint of open bolls. No eggs were found on any fresh squares or green bolls.

The frequency distribution of egg clumping was similar for both artificially infested and field collected plants (Fig. 3.3). Most eggs laid on leaves or within leaf buds were laid singly or in pairs. Eggs laid within larval mines of *B. gossypii*, open cotton bolls, or in damaged stems were often clumped together in groups of up to 19 eggs.

Table 3.3. Distribution of *P. scutigera* eggs on field collected cotton plants. Figures represent the total number of eggs found on three to six entire plants sampled from unsprayed cotton, except on 24/4/85 when three large branches were sampled.

Site of oviposition	No. (%) of eggs/plant part Date plants collected 12/4/86	24/4/85	28/5/85	6/6/85	8/7/85
Small leaves: (<5 cm wide)					
Topside	2 (1.2)	1 (5.6)	0	0	0
Underside	0	0	0	0	0
Perforator mine	0	0	5 (3.6)	6 (5.5)	0
Medium leaves: (5-10 cm)					
Topside	0	4 (22.2)	5 (3.6)	1 (0.9)	0
Underside	1 (0.6)	3 (16.7)	1 (0.7)	0	0
Perforator mine	30 (18.4)	0	0	4 (3.7)	0
Large leaves (>10 cm)					
Topside	15 (9.2)	0	1 (0.7)	0	0
Underside	11 (6.7)	0	1 (0.7)	0	0
Perforator mine	100 (61.3)	0	0	0	0
Leaf petiole	0	0	0	0	0
Leaves total	159 (97.5)	8 (44.4)	13 (9.3)	11 (10.1)	1 (3.0)
Leaf bud - stem	1 (0.6)	0	13 (9.3)	0	2 (6.1)
" " - branches	3 (1.8)	7 (38.9)	20 (14.3)	5 (4.6)	0
Terminal bud	0	0	0	0	0
Leaf buds total	4 (2.5)	7 (38.9)	33 (23.6)	5 (4.6)	2 (6.1)
Open flower	0	0	0	0	0
Square (>0.5 cm)	0	0	0	0	0
" bract	0	0	0	0	0
" stalk	0	0	0	0	0
Square total	0	0	0	0	0
Green boll calyx	0	0	0	0	0
" " bract	0	0	0	0	0
" " stalk	0	1 (5.6)	0	0	0
Green boll total	0	1 (5.6)	0	0	0
Open boll	-*	-	8 (5.7)	32 (29.4)	2 (6.1)
Stem or branch	0	2 (11.1)	5 (3.6)	0	4 (12.1)
Damaged stem	-	-	81 (57.9)	61 (56.0)	24 (72.7)
Mean no. eggs/plant	54.3	6.0	23.3	36.6	11.0

* - indicates not present on plants

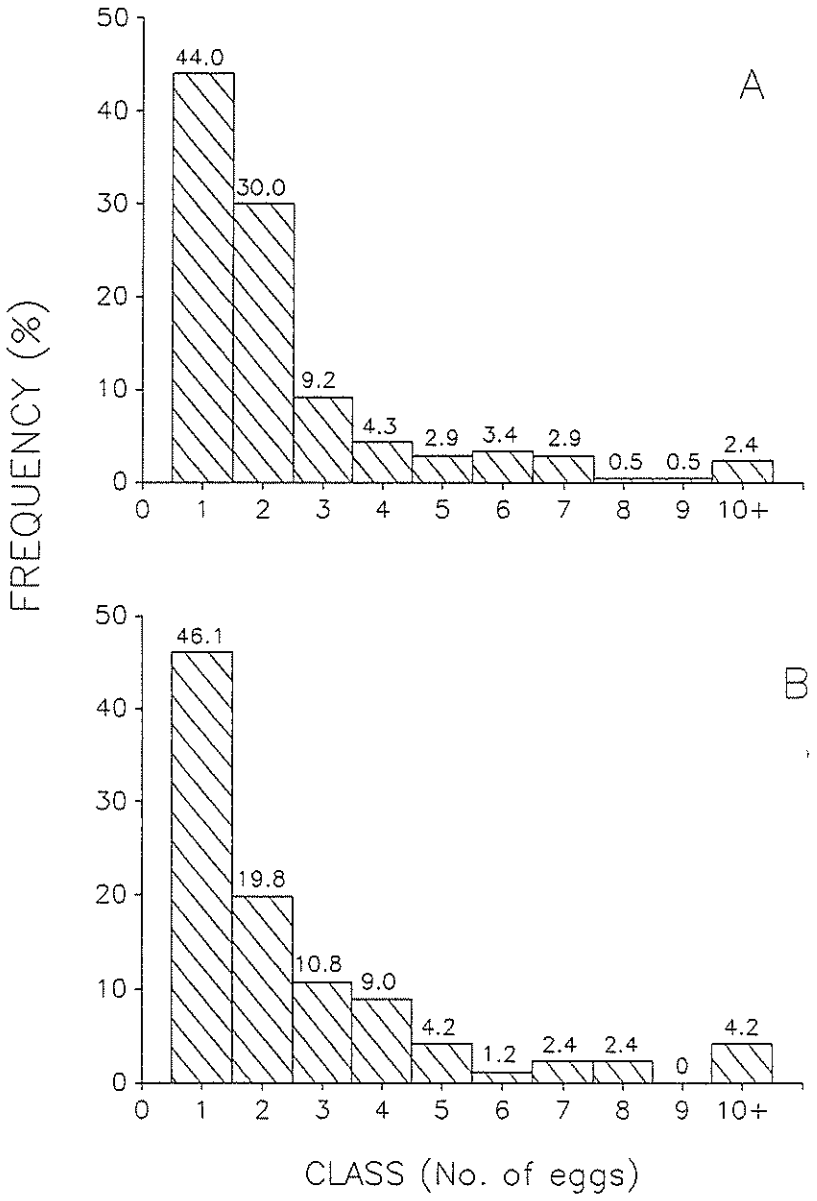

Fig. 3.3. Frequency distribution of clumping of *P. scutigera* eggs laid on cotton; (A) artificially infested plants, (B) field collected plants. Numbers above bars indicate the percentage of eggs in each class.

3.3.3. Larval infestation of unsprayed cotton

A 2.7 ha field of irrigated cotton was grown at the D.P.I. Research Station, Biloela, under conventional agronomic practices. The field was located at the same site over the three cotton seasons and did not receive any insecticide sprays during the period of sampling.

In the 1984/85 season, cotton (variety DPL61) was planted on 25 October. Random samples of 100 squares were taken between 13 December and 13 January and searched immediately after collection but no *P. scutigera* larvae were detected. Initially, bolls were sampled in the same way, starting on 21 January. In this first sample three larvae were found and on 27 January one larva was found in another random sample of 100 bolls (Fig. 3.4).

From 3 February the field was divided into 12 areas of equal size and the number of larvae found in incubated and non-incubated bolls was compared. Significantly more larvae were found consistently in incubated than non-incubated bolls (Table 3.4). On 3 February, no larvae were detected in the bolls searched immediately after collection, but in the sample of bolls incubated for 10 days 7.5 larvae/100 bolls were found (Table 3.4). The number of larvae found in incubated bolls was 21.7 larvae/100 bolls by the end of February and 53.3 larvae/100 bolls by 11 April, compared to only 1.7 and 44.2 larvae/100 bolls in non-incubated bolls.

In the 1985/86 season, cotton (variety DPL90) was planted on 31 October. All samples of squares and bolls were made by randomly selecting 100 forms and incubating in ventilated plastic boxes before searching for larvae. One larva was detected in the first sample of 100 squares taken on 27 December. Infestation increased to 2 larvae/100 squares on 2 January, but thereafter no larvae were found in samples of squares until 23 February when 3 larvae/100 squares were detected (Fig. 3.5). In contrast boll infestation was very high; 12 larvae/100 bolls were found in the first sample taken on 18 January and by 23 February boll infestation had reached 62.5 larvae/100 bolls (Fig. 3.5).

Between 9 February and 17 March boll infestation along two crop edges was compared with infestation at 100 m away from both crop edges. The level of boll infestation was not significantly different at any sample site (t-test, $p>0.05$), therefore the data was pooled and random sampling of bolls was resumed on 25 March. Boll infestation was very high, particularly towards the end of the growing season, reaching 221 larvae/100 bolls on 25 March

Table 3.3. Rate of larval infestation of unsprayed cotton during the 1984/85 season: comparison of the number of *P. scutigera* found in cotton bolls searched immediately (non-incubated) or 10-14 days after collection (incubated). Means of 120 bolls (12 samples of 10 bolls) for each sampling date.

Date bolls sampled	Mean no. larvae/100 bolls (± S.D.)		t-test[1]
	Not incubated	Incubated	
3/2	0	7.5 (1.48)	-
11/2	0.8 (0.29)	10.8 (1.51)	2.261*
20/2	6.7 (0.65)	16.7 (0.78)	4.311***
27/2	1.7 (0.39)	23.3 (1.56)	4.677***
6/3	8.0 (0.83)	46.7 (2.67)	4.740***
14/3	10.0 (1.13)	51.7 (2.41)	5.432***
20/3	23.3 (1.50)	45.0 (2.75)	2.399*
27/3	17.5 (1.36)	75.8 (2.94)	6.245***
3/4	29.2 (1.73)	86.7 (3.60)	4.986***
11/4	44.2 (2.15)	129.2 (2.87)	8.200***

1. * and *** indicates significant difference between means at $p<0.05$ and $p<0.001$, respectively, df=11,11.

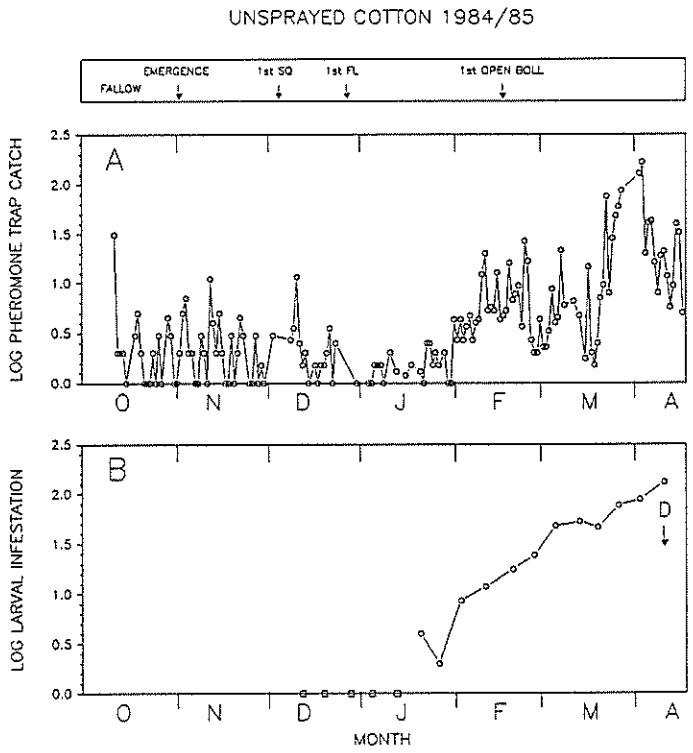

Fig. 3.4. Seasonal incidence of *P. scutigera* in unsprayed cotton during the 1984/85 season; (A) Pheromone trap catch expressed as $\log_{10}$ (x + 1) number of males/trap/night, (B) Larval infestation expressed as $\log_{10}$ (x + 1) number of larvae/100 forms, for squares (open squares) and bolls (open circles). Crop phenology is shown in the box above (A), indicating the approximate date of seedling emergence, first square, first flower and first open boll. Arrow marked D indicates the date of crop defoliation.

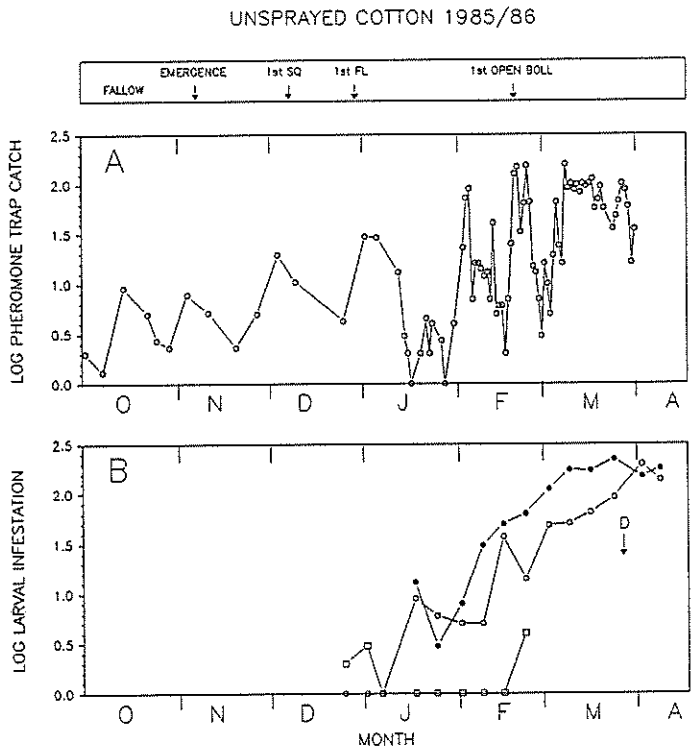

Fig. 3.5. Seasonal incidence of *P. scutigera* in unsprayed cotton during the 1985/86 season: (A) Pheromone trap catch expressed as $\log_{10}$ (x + 1) number of males/trap/night, (B) Larval infestation expressed as $\log_{10}$ (x + 1) number of larvae/100 forms, for squares (open squares), fallen forms (open circles) and bolls (closed circles). Crop phenology is shown in the box above (A), indicating the approximate date of seedling emergence, first square, first flower and first open boll. Arrow marked D indicates the date of crop defoliation.

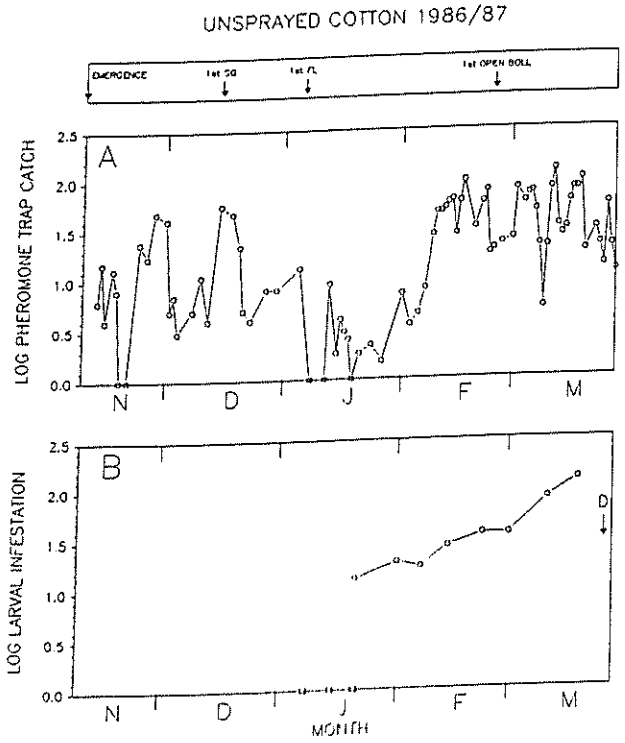

Fig. 3.6. Seasonal incidence of *P. scutigera* in unsprayed cotton during the 1986/87 season; (A) Pheromone trap catch expressed as $\log_{10}$ (x + 1) number of males/trap/night, (B) Larval infestation expressed as $\log_{10}$ (x + 1) number of larvae/100 forms, for squares (open squares) and bolls (open circles). Crop phenology is shown in the box above (A), indicating the approximate date of seedling emergence, first square, first flower and first open boll. Arrow marked D indicates the date of crop defoliation.

(Fig. 3.5). Bolls produced after defoliation and picking of cotton were also heavily infested reaching 280 larvae/100 bolls on 28 May.

Freshly fallen forms (newly shed squares and bolls) were first sampled on 18 January and were highly infested with *P. scutigera* larvae. Infestation increased from 8 larvae/100 forms on 18 January to 197 larvae/100 forms on 3 April (Fig. 3.5).

In the 1986/87 season, cotton was planted on 1 November. One half of the field was planted with cotton variety DPL90 while the other half was planted with a mixture of varieties as part of a *Helicoverpa* spp. host plant resistance trial. Random samples of 100-200 squares and bolls were taken from DPL90 cotton only. Half of each sample was searched immediately for larvae while the other half was incubated in plastic boxes for 10-14 days. No larvae were found in any samples of squares taken in January (Fig. 3.6). When boll sampling was initiated on 21 January, larval infestation was high at 12 larvae/100 bolls (Fig. 3.6). As in the 1984/85 season, more larvae were consistently found in incubated bolls than in non-incubated bolls. However, the difference was much smaller and only significant in bolls sampled on 11 March (t-test: $t=3.642$, $df=3,3$, $p<0.05$) due to greater experience in detecting first instar larvae (Table 3.5).

Table 3.6 shows the number and percentage of each larval instar found in non-incubated bolls. In the first boll sample on 21 January only fourth instar larvae were found (N=2) while on 1 February only first (N=5) and second (N=2) instar larvae were present. In all subsequent boll samples first instar larvae were constantly present and numbers increased sharply from a mean of 9.5/100 bolls on 2 March to 31.3/100 bolls on 11 March (constituting 58.4% of all larvae found). The number of first instars remained high in the following 2 weeks at 44.5 and 38.0/100 bolls. Other instars tended to increase in numbers through the season as expected with the progression of larval development between sampling dates (see Chapter 4).

3.3.4. Larval infestation of commercial cotton

The commercial cotton fields sampled in all three seasons were located in the Callide valley, approximately 10 km north of Biloela. All fields were irrigated, except where indicated, and ranged between 10 and 50 ha in size.

Table 3.5. Rate of larval infestation in unsprayed cotton during the 1986/87 season: comparison of the number of larvae found in bolls searched immediately (non-incubated) or 10-14 days after collection (incubated).

Date bolls sampled	No. of bolls sampled	Mean no. of larvae/100 bolls (± S.D.)		
		Non-incubated	Incubated	t-test[1]
21/1	100	2.0 (2.0)	12.0 (12.7)	1.555
1/2	100	7.0 (1.7)	18.0 (16.8)	1.299
7/2	100	9.0 (0.5)	16.0 (8.6)	1.028
14/2	100	22.0 (6.9)	26.0 (17.4)	0.426
27/2	200	32.0 (10.1)	34.5 (11.8)	1.034
2/3	200	31.0 (11.1)	34.0 (12.2)	1.162
11/3	400	53.7 (17.3)	78.0 (18.2)	3.642*
20/3	200	128.5 (4.1)	121.0 (16.7)	0.872

1. * indicates a significant difference between means at $p<0.05$, df=3,3.

Table 3.6. Number and percentage of each *P. scutigera* larval instar infesting unsprayed cotton during the 1986/87 season. All samples of bolls were searched for larvae immediately after collection.

Date bolls sampled	No. of bolls sampled	No./percentage of each larval instar			
		I	II	III	IV
21/1	100	0/0	0/0	0/0	2.0/100
1/2	100	5.0/71.4	2.0/28.6	0/0	0/0
7/2	100	3.0/33.3	4.0/44.4	2.0/22.2	0/0
14/2	100	6.0/27.3	8.0/36.4	5.0/22.7	3.0/13.6
23/2	200	9.5/29.7	10.5/31.1	7.0/21.9	5.5/17.2
2/3	200	9.5/28.8	8.5/25.8	6.5/22.7	6.5/22.7
11/3	400	31.3/58.4	13.0/24.3	7.5/14.0	1.8/3.4
20/3	200	44.5/34.6	38.5/30.0	32.0/24.9	13.5/10.5
27/3	200	38.0/26.4	40.0/27.8	38.0/26.4	28.0/19.4

1984/85 season:

i) Site 1 (A. Shepherdson's property) was planted on 13 and 14 November (varieties Sicot I and DPL61) and received a total of 10 insecticide sprays, mainly early in the season to control *Helicoverpa* spp. and sap-sucking bugs. Random samples of 100 squares were taken from throughout the field, starting on 25 January, but no *P. scutigera* were found. Bolls were sampled in the same way from 26 February when one larva was detected. Infestation increased to 3 larvae/100 bolls on 14 March but declined to 2 larvae/100 bolls on 21 March (Fig. 3.7) with the application of Profenofos on 15 March.

During the course of boll sampling, it was noticed that larval infestation was substantially higher along one crop edge. This crop edge bordered a creek lined with tall trees. It was suspected that the trees impeded the flight of aerial sprayers, causing, along with an adverse wind direction, poor spray coverage of cotton growing near the creek. Subsequently, samples of 100 bolls were taken along this crop edge and also at 50 and 100 m parallel to this edge. On 28 March *P. scutigera* infestation at the crop edge was 14 larvae/100 bolls compared to 4 larvae/100 bolls at 50 and 100 m away from the crop edge (Fig. 3.7). Infestation of bolls along the crop edge increased to 34 larvae/100 bolls on 4 April, despite the application of Methyl Parathion plus Methomyl on 3 April. Infestation at 50 and 100 m from the crop edge remained comparatively low at 4 and 2 larvae/100 bolls, respectively. Infestation at the crop edge fell to 22 larvae/100 bolls on 11 April but increased to 8 larvae/100 bolls at both 50 and 100 m away from the edge. A final application of Methyl Parathion was made on 15 April in an attempt to contain *P. scutigera* infestation.

ii) Site 2 (B. Breeze's property) was planted on 10 October with variety DPL61. This field was sprayed much less than the other two commercial fields monitored, with only four applications made for the control of *Helicoverpa* spp. and sap-sucking bugs. Fruiting of cotton plants was severely delayed due to extensive hail damage on 1 January. No *P. scutigera* larvae were found in random samples of 100 squares collected between 21 December (before hail damage) and 11 January. When the first boll samples were taken on 22 January, infestation was comparatively high at 6.7 larvae/100 bolls but no larvae were found in bolls sampled on 4 February. On 26 February boll infestation increased to 9 larvae/100 bolls but decreased again to

1 larvae/100 bolls on 6 March. Thereafter, boll infestation increased steadily from 4 to 8 larvae/100 bolls between 14 March and 11 April (Fig. 3.8). The last insecticide application was made on 22 February but the crop was not defoliated until 15 April.

iii) Site 3 (I. Wilkies' property) was planted on 15 October (varieties DPL61 and DPL55) and sprayed 12 times with insecticides, mainly to control *Helicoverpa* spp. and sap-sucking bugs. No *P. scutigera* larvae were found in random samples of 100 squares collected between 21 December and 4 January. Random samples of 100 bolls were first taken on 11 January but no *P. scutigera* larvae were detected until 26 February (Fig. 3.9). Two insecticide sprays (Fenvalerate and Chlordimeform) were applied directly for the control of *P. scutigera* on 26 January and 12 February. These insecticides were applied as part of a D.P.I. insecticide trial to compare their effectiveness in preventing *P. scutigera* larval infestations from developing. They were applied on the basis of large increases in pheromone trap catches of *P. scutigera* males (see Chapter 4), rather than on the presence of larvae infesting bolls. No further insecticide sprays were applied after 12 February. On 26 February, boll infestation increased to 3 *P. scutigera* larvae/100 bolls but decreased to 1 larva/100 bolls on 6 March (Fig. 3.9).

The crop was defoliated on 4 and 12 March but due to incomplete crop defoliation plants continued to produce fresh flowers and bolls. Defoliation was particularly poor along one crop edge which bordered power lines. Random samples of bolls along this edge revealed an infestation of 9 larvae/100 bolls on 14 March. Harvesting of the cotton commenced on 18 March but due to wet weather slashing of plants and burial of crop residues was not completed until late May. As a result plants produced many more fresh squares, flowers and bolls. The number of *P. scutigera* larvae in bolls increased from 53 larvae/100 bolls on 11 April to 170 larvae/100 bolls on 19 May, shortly after which the crop was destroyed.

1985/86 season:

Throughout the 1985/86 season, in all three commercial cotton fields sampled, infestation of squares and bolls (N = 100) along a crop edge was compared with the mid-crop region (100 + m from the crop edge).

i) Site 1 (A. Shepherdson) was planted on 28 November (variety DPL90) and was heavily

sprayed, receiving ten insecticide applications mainly against *Helicoverpa* spp. (Fig. 3.10). On 16 January, when squares were first sampled, 3.3 larvae/100 squares were found at the crop edge, but no larvae were present in squares sampled from mid-crop. No larvae were found in subsequent samples of squares made on 24 and 29 January. Infestation of bolls was comparatively low in February but rose very sharply in bolls sampled from the crop edge during March despite the application of insecticides (Fig. 3.10). Boll infestation along the crop edge increased from 1.4 to 14.3 larvae/100 bolls on 28 February and 7 March, respectively. On the same sampling dates infestation at mid-crop was 0 and 1.4 larvae/100 bolls. Infestation at the crop edge stayed at 17.1 larvae/100 bolls during the next 3 sampling periods compared to 2.9 larvae/100 bolls at mid-crop. The last insecticide spray was applied on 28 March but the crop was not defoliated until 28 April and 6 May. Boll infestation continued to remain high along the crop edge while staying comparatively low at mid-crop. On 8 and 17 April infestation of bolls at the crop edge was 21.4 and 14.3 larvae/100 bolls compared to 2.9 and 4.3 larvae/100 bolls at mid-crop. Bolls were also sampled from the crop edge on 29 April and 18 May when there were 69 and 121 larvae/100 bolls, respectively.

ii) Site 2 (B. Breeze) was planted on 26 October (variety DPL90). The number of insecticide sprays applied to this crop was not recorded but was very few (3-4 sprays), particularly during boll formation. Infestation of squares was very low and larvae were only found in samples taken on 3 and 24 January at 1.4 larvae/100 squares (Fig. 3.11B). Boll infestation was severe; while more larvae were usually found infesting bolls at the crop edge than those at mid-crop, the difference was much less than in the other two commercial crops monitored (Fig. 3.11B). Larvae were found in the first samples of bolls taken on 14 January at 2.9 and 1.4 larvae/100 bolls at the crop edge and mid-crop, respectively. Infestation increased to very high levels and by 16 March there were 90 and 77.1 larvae/100 bolls at the crop edge and mid-crop, respectively.

iii) Site 3 (I. Wilkie) was planted on 29 October (variety DPL90) and received ten insecticide sprays, mainly for the control of *Helicoverpa* spp. Squares were first sampled on 27 December when 0.3 larvae/100 squares were detected in the mid-crop sample. Further samples of squares on 3 and 8 January did not contain any *P. scutigera* larvae.

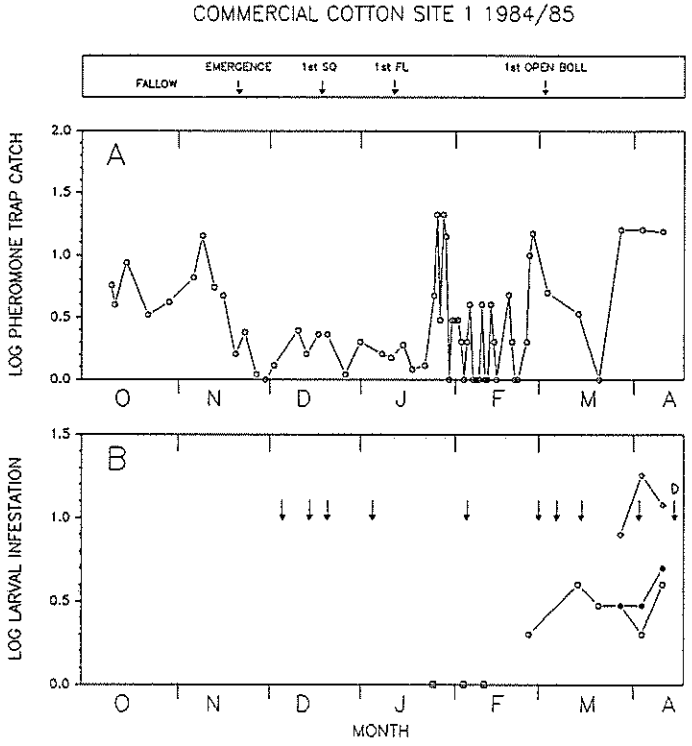

Fig. 3.7. Seasonal incidence of *P. scutigera* in commercial cotton at Site 1 during the 1984/85 season; (A) Pheromone trap catch expressed as $\log_{10}$ (x + 1) number of males/trap/night, (B) Larval infestation expressed as $\log_{10}$ (x + 1) number of larvae/100 forms, for squares (open squares) and bolls sampled at the crop edge (open diamonds), 50 m from the edge (closed circles) or 100 m from the edge (open circles). Crop phenology is shown in the box above (A), indicating the approximate date of seedling emergence, first square, first flower and first open boll. Arrows represent applications of insecticide sprays except for the arrow marked D which indicates the date of crop defoliation.

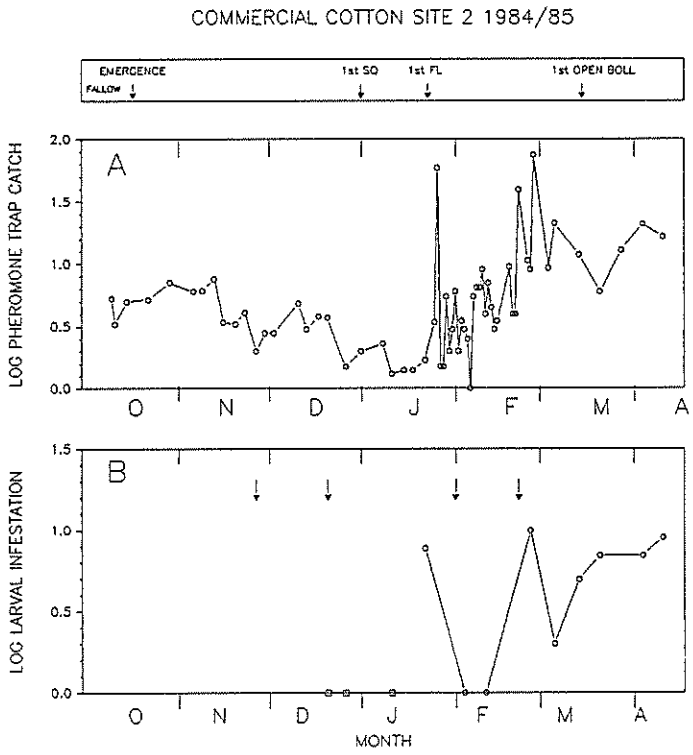

Fig. 3.8. Seasonal incidence of *P. scutigera* in commercial cotton at Site 2 during the 1984/85 season; (A) Pheromone trap catch expressed as $\log_{10}$ (x + 1) number of males/trap/night, (B) Larval infestation expressed as $\log_{10}$ (x + 1) number of larvae/100 forms, for squares (open squares) and bolls (open circles). Crop phenology is shown in the box above (A), indicating the approximate date of seedling emergence, first square, first flower and first open boll. Arrows represent applications of insecticide sprays.

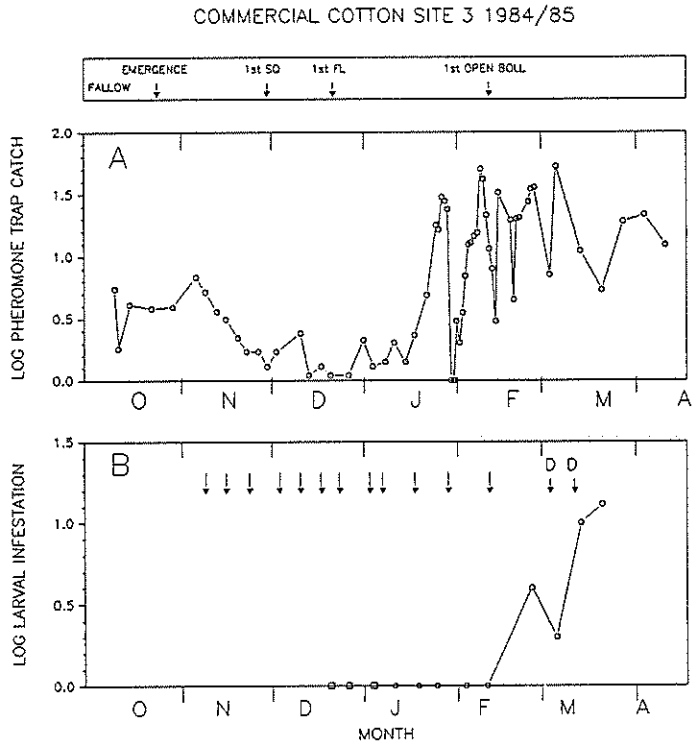

Fig. 3.9. Seasonal incidence of *P. scutigera* in commercial cotton at Site 3 during the 1984/85 season; (A) Pheromone trap catch expressed as $\log_{10}$ (X + 1) number of males/trap/night, (B) Larval infestation expressed as $\log_{10}$ (X + 1) number of larvae/100 forms, for squares (open squares) and bolls (open circles). Crop phenology is shown in the box above (A), indicating the approximate date of seedling emergence, first square, first flower and first open boll. Arrows represent applications of insecticide sprays except for the arrows marked D which indicate the date of crop defoliation.

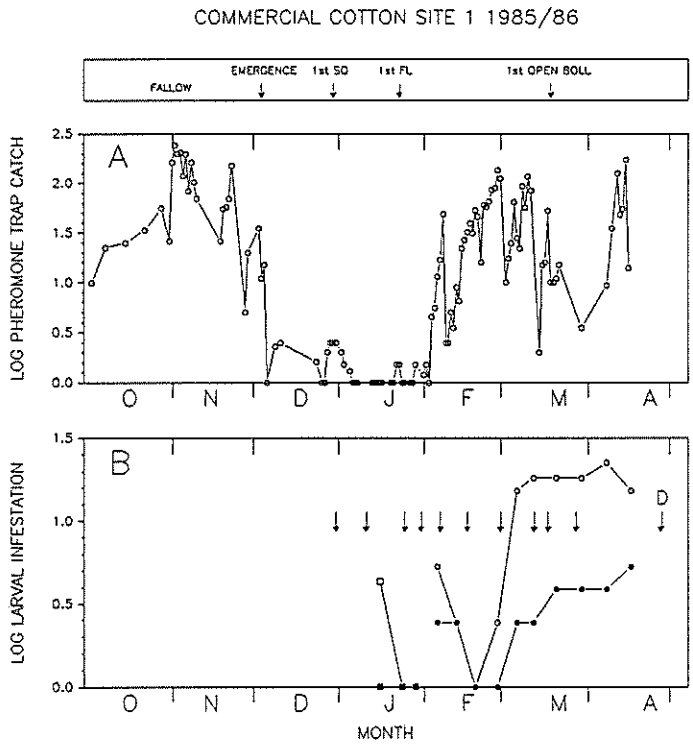

Fig. 3.10. Seasonal incidence of *P. scutigera* in commercial cotton at Site 1 during the 1985/86 season; (A) Pheromone trap catch expressed as $\log_{10}$ (x + 1) number of males/trap/night, (B) Larval infestation expressed as $\log_{10}$ (x + 1) number of larvae/100 forms, for squares sampled at the crop edge (open squares) or 100 m from the crop edge (closed squares), and bolls sampled at the crop edge (open circles) or 100 m from the crop edge (closed circles). Crop phenology is shown in the box above (A), indicating the approximate date of seedling emergence, first square, first flower and first open boll. Arrows represent applications of insecticide sprays except for the arrow marked D which indicates the date of crop defoliation.

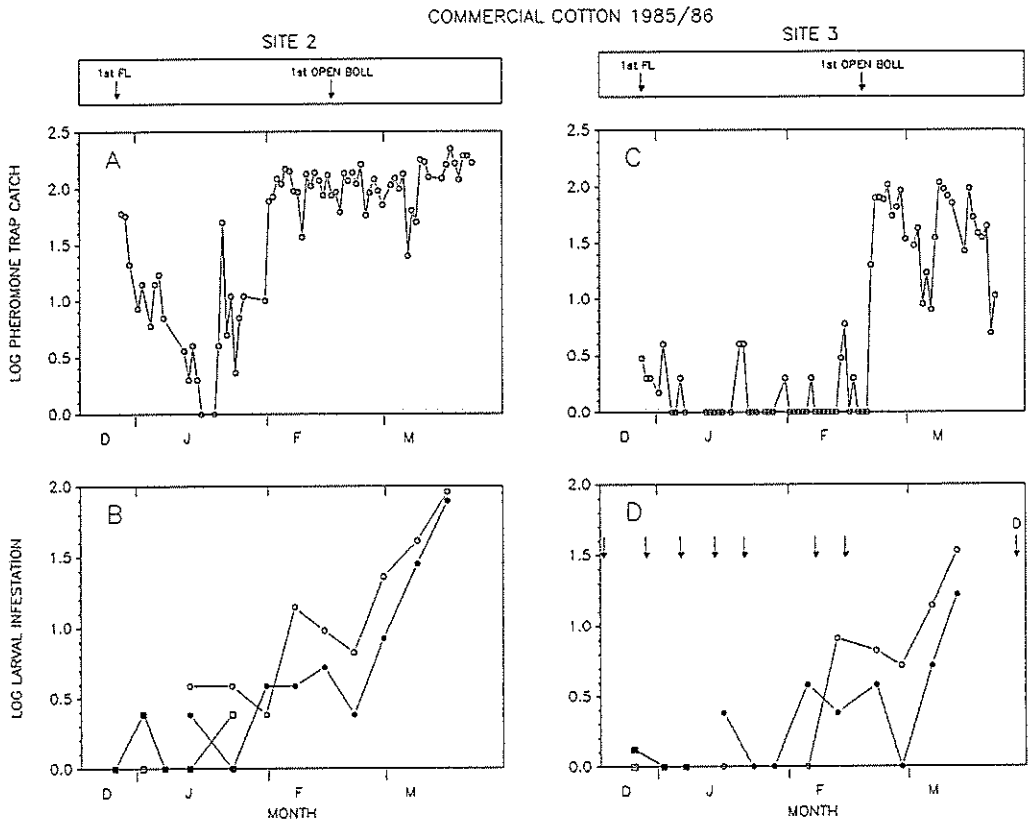

Fig. 3.11. Seasonal incidence of *P. scutigera* in commercial cotton at Site 2 and 3 during the 1985/86 season; (A and C) Pheromone trap catch expressed as $\log_{10}$ (x + 1) number of males/trap/night, (B and D) Larval infestation expressed as $\log_{10}$ (x + 1) number of larvae/100 forms, for squares sampled at the crop edge (open squares) or at 100 m from the crop edge (closed squares), and bolls sampled at the crop edge (open circles) or 100 m from the crop edge (closed circles). Crop phenology is shown in the box above (A and C), indicating the approximate date of first flower and first open boll. Arrows represent applications of insecticide sprays except for the arrow marked D which indicates the date of crop defoliation.

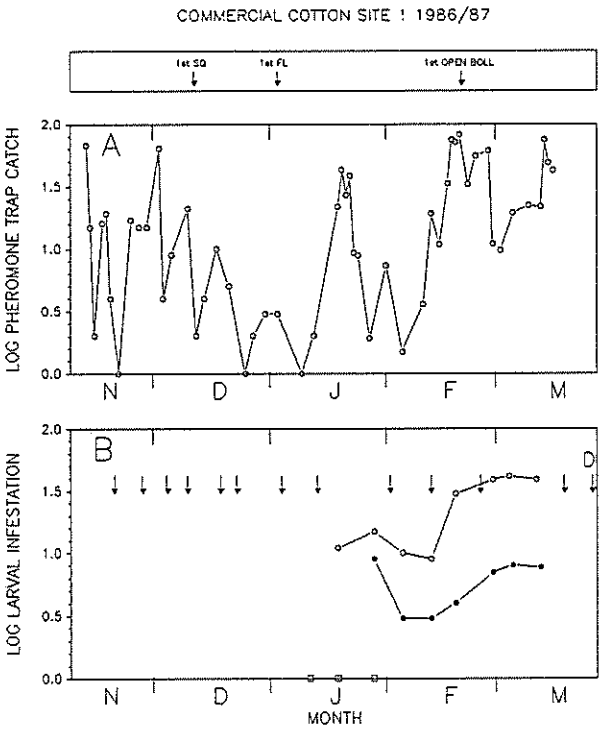

Fig. 3.12. Seasonal incidence of *P. scutigera* in commercial cotton at Site 1 during the 1986/87 season; (A) Pheromone trap catch expressed as $\log_{10}$ (x + 1) number of males/trap/night, (B) Larval infestation expressed as $\log_{10}$ (x + 1) number of larvae/100 forms, for squares (open squares) and bolls sampled at the crop edge (open circles) or 100 m from the crop edge (closed circles). Crop phenology is shown in the box above (A), indicating the approximate date of first square, first flower and first open boll. Arrows represent applications of insecticide sprays except for the arrow marked D which indicates the date of crop defoliation.

Bolls were first sampled on 17 January when 1.4 larvae/100 bolls were detected in the mid-crop sample only. No larvae were found in any samples of bolls collected in the following two weeks. Larvae were first detected in the crop edge on 13 February at 7.1 larvae/100 bolls, compared to 1.4 larvae/100 bolls in the mid-crop sample (Fig. 3.11D). The last insecticide application was made on 15 February and while infestation at the crop edge decreased on the following two sampling dates, it remained higher than at mid-crop. Large increases in boll infestation occurred on 7 and 13 March with 12.9 and 32.9 larvae/100 bolls at the crop edge and 4.3 and 15.7 larvae/100 bolls at mid-crop, respectively. The crop was defoliated on 27 March and 5 April, more than one month after the last insecticide application.

1986/87 season:

i. Site 1 (A. Shepherdson) was planted on 29 October (variety DPL90) and received 12 insecticide sprays, mainly early in the season to control *Helicoverpa* spp. (Fig. 3.12). Four sprays were applied for the control of *P. scutigera* but levels of boll infestation remained high, compared to the two previous seasons, particularly at the crop edge. Table 3.7 compares the number of each larval instar found in bolls sampled from the crop edge and mid-crop.

On 14 January a random sample of bolls made at the crop edge revealed a mean of 10 larvae/100 bolls; 80% of larvae were first instars while 1 second and 1 fourth instar were also found. Only 1 first instar larva was found in a sample of 100 squares on the same date. On 23 January boll infestation at the crop edge was similar at 11 larvae/100 bolls but fewer (18.2%) first instar larvae were found. In comparison, infestation of mid-crop bolls was 4 larvae/100 bolls and no first or second instar larvae were found. Infestation of squares remained at 1 first instar larva/100 squares and no further samples were taken.

Infestation at the crop edge remained at 11 larvae/100 bolls on 30 January. No first instar larvae were detected in this sample. Infestation decreased to two larvae/100 bolls in mid-crop bolls after the aerial application of Endosulfan plus Methyl parathion on 27 January.

Deltamethrin was applied on 6 February when infestation at the crop edge was 8 larvae/100 bolls (3 first instar larvae found). Despite spraying, infestation at the crop edge increased further to 40 larvae/100 bolls on 12 February. This was mainly due to a large influx of first instar larvae, which constituted 50% of all larvae found. Such an increase in the

Table 3.7. Comparison of the number of *P. scutigera* larvae infesting bolls sampled from a crop edge (E) and 100 + m away from the crop edge (M) at Site 1 during the 1986/87 cotton season. N = total number of bolls sampled.

Date sampled	N	Boll sample	No. larvae/instar/100 bolls I	II	III	IV	Total	t-test[1]
14/1	100	E	8.0	1.0	0	1.0	10.0	-
23/1	100	E	0.5	2.0	4.5	4.0	11.0	2.05
	100	M	0	0	2.0	2.0	4.0	
30/1	100	E	0	3.0	7.0	1.0	11.0	2.27
	100	M	1.0	1.0	0	0	2.0	
6/2	100	E	3.0	1.0	0	4.0	8.0	3.00*
	100	M	0	0	2.0	0	2.0	
12/2	100	E	20.0	9.0	5.0	6.0	40.0	5.98**
	100	M	2.0	0	1.0	0	3.0	
21/2	200	E	16.0	18.5	20.0	5.0	59.5	5.21**
	200	M	1.0	1.5	2.5	1.0	6.0	
25-26/2	300	E	9.3	20.3	16.3	10.0	55.9	6.87***
	300	M	2.0	1.0	3.7	0.7	7.4	
4-5/3	300	E	2.3	9.0	23.7	14.7	49.7	6.29***
	300	M	1.0	2.3	3.3	0.3	7.0	

1. *, **, and *** indicate a significant difference at p = 0.05, 0.01 and 0.001, respectively, between infestation in edge and mid-crop bolls (d.f. = 6, except for last 2 sampling dates when d.f. = 22)

Table 3.8. Infestation of cotton bolls by *P. scutigera* larvae at Site 2 during 1987. Number and percentage of each larval instar/100 bolls randomly sampled from the crop.

Date bolls sampled	No. bolls sampled	No. larvae/instar /100 bolls					Percentage of larvae/instar			
		I	II	III	IV	Total	I	II	III	IV
5/1	100	0	0	2.0	2.0	4.0	0	0	50.0	50.0
20/1	200	3.5	2.0	1.5	0.5	7.5	46.7	26.7	20.0	13.3
3/2	200	0.5	0	1.0	0	1.5	33.3	0	0	66.7
10/2	200	0.5	0.5	0.5	0	1.5	33.3	33.3	33.3	0
13/2	100	0	2.0	2.0	3.0	7.0	0	28.6	28.6	42.9
21/2	200	0	0.5	1.0	0	1.5	0	33.3	66.7	0

numbers of first instars did not occur in bolls sampled from mid-crop. Infestation of mid-crop bolls remained static at 2 larvae/100 bolls on 6 February and only two first instars were found on 12 February when boll infestation was 3 larvae/100 bolls (Table 3.7).

In the next three boll samples larval infestation rates at the crop edge remained at very high levels of 59.5, 55.9 and 50.7 larvae/100 bolls compared to 6, 7.4 and 7/100 bolls at mid-crop on 21 and 25 February and 4 March, respectively. Numbers of first instar larvae at the crop edge remained high but decreased from 16/100 bolls on 21 February (26.9% of all larvae found) to 2.3/100 bolls on 4 March (4.5%), while numbers of other larval instars changed as would be expected with the progression in larval development over time. The numbers of first instar larvae in mid-crop bolls remained low at 1 or 2/100 bolls.

A late application of Methyl Parathion was made on 11 March in an attempt to contain *P. scutigera* populations, and the field was defoliated on 18 and 28 March.

ii) Site 2 (T. Jenson) was a field of dryland cotton (variety DPL90) planted in skip-rows in late October. The number of insecticide sprays applied was not recorded but was few due to relatively low insect pressure. The number of *P. scutigera* larvae/100 bolls was 5 on 5 January, and increased to 7.5 on 20 January. Infestation decreased to 1.5 larvae/100 bolls on 3 February after an application of Lambda-cyhalothrin on 1 February (Table 3.8). Infestation remained at 1.5 larvae/100 bolls on 10 February, increased to 7 larvae/100 bolls on 13 February, but decreased again to 1.5 larvae/100 bolls on the final date of sampling on 21 February.

3.3.5. Spatial distribution of larvae in unsprayed cotton

Except on the first two sampling occasions, the variance-mean relationship (I_D) was not significantly greater than one (Chi-square test, d.f. = 11, $p<0.05$), suggesting agreement with a Poisson (random) distribution (Table 3.9). Larvae sampled on 3 and 11 February had a I_D that was significantly greater than one ($p<0.05$), implying an aggregated pattern of distribution. Larvae sampled on 20 February had an I_D of 0.36 which gave a calculated Chi-square outside the 0.05 probability limits, implying a regular pattern of distribution.

Calculation of Morisita's index of aggregation gave a similar result; larvae sampled on the first two occasions had a value significantly greater than 1 (ANOVA, d.f. = 11 and infinity, $p<0.01$ and 0.05), implying an aggregated distribution, while in the remainder of the samples

the index was not significantly greater than one ($p>0.05$), suggesting a random pattern of distribution.

Iwao's regression of mean crowding index (*m) on the mean (x) gave the following equation:

$Y = 0.82 + 0.916*X$ ($r^2 = 0.967$)

which was a better description of the data than Taylor's power law of log variance (S^2) on log mean (x):

$Y = 0.20 + 0.793*X$ ($r^2 = 0.637$)

As the slope (b) is close to 1 in Iwao's equation, a random pattern of distribution is suggested. The smaller value of b in Taylor's power law suggests a more regular than random pattern of distribution.

3.3.6. Optimum sample sizes and sequential sampling

It was estimated that it would take an experienced operator about eight minutes to pick and process one sample unit of ten bolls. This estimate includes one minute to pick the bolls, six minutes to thoroughly check bolls for larval infestation (see Discussion of this Chapter), and an additional minute to walk to the next sampling point.

The optimum number of samples required to detect a larval infestation and costs involved were much lower under Taylor's power law than Iwao's regression method (Table 3.10). If larval infestation is at 5 larvae/100 bolls, the optimum sample size required at a 10% level of precision, would be 46 samples of 10 bolls under Taylor's power law or 356 samples under Iawo's regression method (Table 3.10). The costs involved in obtaining such a large number of samples would be unacceptable at 6 or 47 man hours, respectively. If a 20% level of precision was used the optimum sample size for Taylor's power law was reduced to 11 (cost = 1.5 man hours) but remained high for Iwao's regression method at 89 (cost = 12 man hours).

Fig. 3.13 gives a sequential sampling for *P. scutigera* infesting bolls, based on an economic threshold of 5 larvae/100 bolls. (F. D. Page unpublished report) Use of this sampling plan is discussed in section 3.4 of this Chapter.

reference

Table 3.9. Indices of dispersion for *P. scutigera* larvae infesting unsprayed cotton during the 1984/85 season. Means and variances based on a sample unit of 10 bolls collected from 12 sites on each sampling occasion.

Date bolls sampled (day/month)	Sample mean	Sample variance	Dispersion index[1]	
			ID	Morisita
3/2	0.75	2.19	2.92*	3.67**
11/2	1.08	2.28	2.11*	2.00*
20/2	1.67	0.61	0.36	0.63
27/2	2.33	2.43	1.04	0.86
6/3	4.67	7.13	1.53	1.11
14/3	5.17	5.81	1.12	1.02
20/3	4.50	7.56	1.68	1.14
27/3	7.58	8.64	1.14	1.02
3/4	8.67	13.00	1.50	1.05
11/4	12.92	8.24	0.64	0.97

1. For ID * indicates value significantly greater than 1 at $p<0.05$ (Chi-square test, df = 11), while for the Morisita index * and ** indicates value significantly greater than 1 at $p<0.05$ and $p<0.01$, respectively (ANOVA, df = 11, ∞).

Table 3.10. Optimum sample sizes and costs (in ~~man~~ person hours) for different population densities of *P. scutigera* larvae infesting bolls, at 10% and 20% levels of precision, given by Taylor's power law and Iwao's regression method (see text for equations). One sample unit = 10 bolls.

Mean density of larvae/ sample unit	Optimum sample size			
	Taylor's power law		Iwao's regression method	
	10% (cost)	20% (cost)	10% (cost)	20% (cost)
0.1	322 (43)	80 (11)	1812 (242)	453 (60)
0.3	85 (11)	21 (3)	598 (80)	150 (20)
0.5	46 (6)	11 (1.5)	356 (47)	89 (12)
1.0	20 (3)	5 (0.7)	174 (23)	43 (16)
5.0	3 (0.4)	1 (0.1)	28 (4)	7 (1)

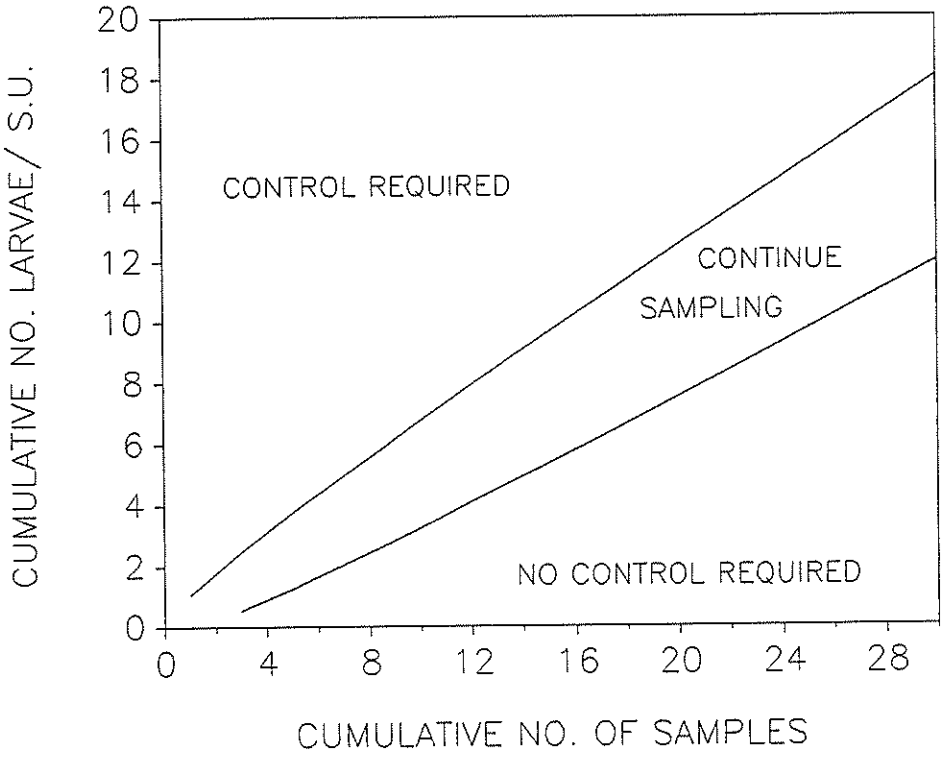

Fig. 3.13. Sequential sampling decision plan for *P. scutigera* larvae infesting bolls with a confidence probability level of 90%. Sample unit (S.U.) = 10 bolls.

3.3.7. Description of larval infestation of squares and bolls

Because of the low rate of infestation, few observations on the behaviour of larvae attacking squares were made. When neonate larvae were placed onto squares in glasshouse experiments, minor larval damage was often observed but no *P. scutigera* larva could be found. In the squares which were successfully infested, larvae entered the flower bud through any point; through the base, sides or apex of the corolla. Large larvae (third and fourth instars) caused extensive damage to the ovary and other reproductive parts, resulting in the square being shed from the plant. One fourth instar was observed to web squares onto the plant which prevented them from falling to the ground.

Larvae also entered bolls from any point; through the boll base (underneath the inner bracts), or through the boll wall (calyx). Entrance through the boll wall was usually in a protected area; either underneath the large outer bracts (boll sides) or at the very apex of the boll underneath the old flower. In bolls collected from unsprayed cotton during the 1986/87 season (N=800 bolls), 66% of larvae entered bolls through the base, while 25% entered through the sides and 8% entered through the apex (Table 3.11A). In sprayed cotton (Site 1) during the 1986/87 season, the point of boll entry differed (Table 3.11B). Of 137 larvae found in bolls sampled from the crop edge (N=300), 47% entered through the sides of bolls, 28% through the base and 25% through the apex. Only 20 larvae were found in 300 bolls sampled from mid-crop; 55% entered through the boll base, 6% through the apex and 39% through the boll sides (Table 3.11B).

Larval behaviour on infesting a boll varied according to the age of boll at infestation. The following description is applicable for bolls aged approximately 1 to 6 weeks. Some larvae made several holes before entering the boll, but often only a single entrance hole was visible. Newly made entrance holes were extremely difficult to detect. Entrance holes became more visible as the larvae fed, with the accumulation of frass and/or discolouration of the surrounding tissue.

Larval development was completed within the same boll, even when bolls were removed from the plant. Larvae were sometimes observed on the outside of bolls, in protected locations, and the presence of head capsules suggested that this was the site of ecdysis. Generally, the

Table 3.11. Point of *P. scutigera* larval entry in bolls collected from (A) unsprayed cotton on three occasions during March, 1987, and (B) sprayed cotton (Site 1) on 4 and 5 April, 1987. In sprayed cotton, bolls were collected from the crop edge or 200 m away from the crop edge (mid-crop bolls).

(A) Unsprayed cotton

Date sampled	No. bolls sampled	Total no. larvae	Point of larval entry (no. / % of larvae) Apex	Side	Base
11 March	400	212	22 10.4	34 16.0	156 73.6
20 March	200	256	13 5.7	73 28.5	170 66.4
27 March	200	277	27 9.7	83 30.0	167 60.3
Total	800	745	62 8.3	190 25.5	493 66.2
(S.D.)			(2.9)	(7.7)	(6.7)

(B) Sprayed cotton

Position of bolls sampled	No. of bolls sampled	Total no. of larvae	Point of larval entry (no. / % of larvae) Apex	Side	Base
Crop edge	300	137	34 24.8 (1.2)	66 47.2 (3.8)	37 27.9 (3.5)
Mid-crop	300	20	2 5.6 (9.6)	10 55.6 (17.3)	8 38.9 (12.7)

entire larval stage was spent feeding internally within the boll. When the point of entry was through the base or sides, the larva initially tunneled and fed within tissues of the boll wall, just below the surface. When larvae entered through the apex, initial damage was confined to the very top of the wall or down the central axis. Larvae remained feeding within the boll wall during the first three instars. These larvae often penetrated through the inner wall allowing the entry of boll rots (*Rhizopus* spp.) into the developing lint and seed. On moulting to the fourth instar, the larvae attacked the lint and seeds of the boll. At this stage most economic damage was done. On completion of feeding, the fourth instar larva cut out of the boll leaving a large characteristic hole.

Bolls previously damaged by *P. scutigera* larvae were often reinfested, particularly at the end of the cotton season. When this occurred, all larval instars could be found within the damaged areas of lint and seed.

3.3.8. Relationship of cotton boll age to larval attack

In the 1984/85 cotton season a total of 566 tagged bolls were examined and 128 larvae found (0.23 larvae/boll) (see Appendix G for the size and number of bolls collected in each age category). Larvae were found in bolls of all ages: from one day old up to 63 days old when bolls were mature and fully open (Fig. 3.14A). No larvae were found in bolls 55, 61, 67 and 71 days old. Levels of infestation varied greatly with no discernible pattern in relation to boll age. The highest number of larvae were found in bolls between 21 and 53 days old, with numbers peaking in bolls 47 days old at a mean of 0.84 larvae/boll (Fig. 3.14A, Appendix G).

In the 1986/87 cotton season 501 tagged bolls were examined and 136 larvae found (0.27 larvae/boll) (Appendix H). A relationship between boll age and peak larval infestation was evident only when each larval instar was considered separately (Fig. 3.14B-F). While all larval instars were found in a wide range of boll ages, generally the age of boll in which peak infestation occurred increased with increasing larval instar. First instar larvae were found in bolls of all ages, from two to 46 days old, numbers were highest in bolls five and 18 days old (0.33 and 0.11 larvae/boll, respectively) and few were found in bolls older than 31 days old.

Second instar larvae infested bolls of all ages, from two to 46 days old, except those 18-27 days old. Most were found infesting 30 and 37 day old bolls at levels of 0.14 and 0.12

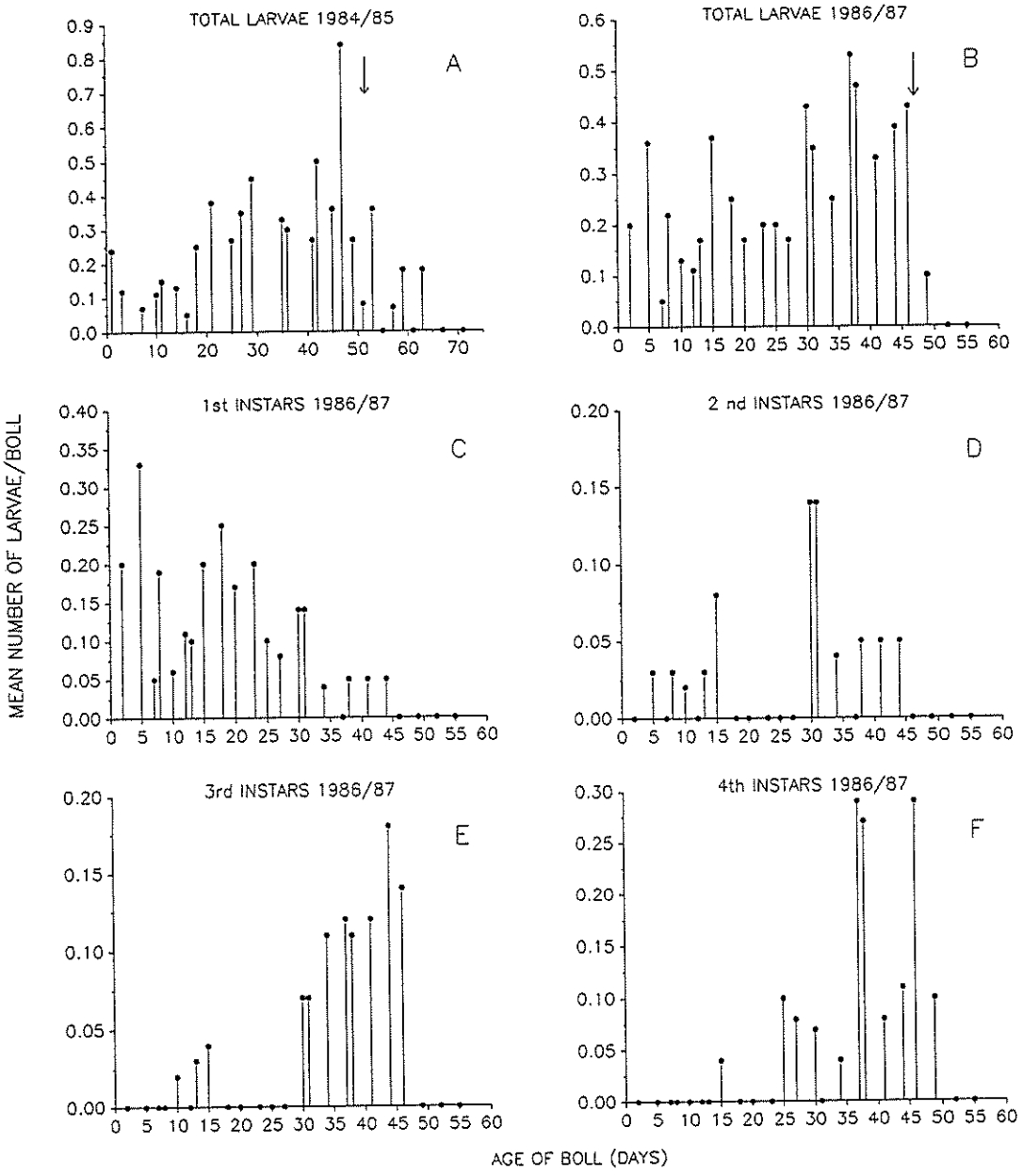

Fig. 3.14. Relationship between cotton boll age and *P. scutigera* larval infestation for; (A) all larval instars during the 1984/85 cotton season, (B) all larval instars during the 1986/87 cotton season, (C) - (F) for each larval instar separately during the 1986/87 cotton season. Arrow in graphs (A) and (B) indicate the age at which bolls split open.

larvae/boll, respectively.

Third instar larvae were absent in bolls less than ten days old and, as for second instars, no larvae were found in 18-27 day old bolls. Infestation by third instars was highest in bolls 34-46 days old, peaking in 44 day old bolls at 0.18 larvae/boll.

Very few fourth instar larvae were found in bolls younger than 25 days old with most being found in bolls between 37-49 days old at 0.29 larvae/boll.

As in the 1984/85 season, when total larval numbers were considered there was no discernible pattern of infestation in relation to boll age (Fig. 3.14). Total infestation was highest in bolls 36-46 days old and in bolls five and 15 days old due to the presence of more first instar larvae. Infestation peaked in bolls 37 days old at 0.53 larvae/boll rather than in bolls 47 days old as observed in the 1984/85 cotton season.

Yield loss was not measured but when a single *P. scutigera* larva infested bolls less than six weeks old, total loss of yield invariably resulted. Very young bolls (less than one week old) were shed from the plant when damaged. Older bolls damaged by larvae remained on plants and continued to develop. While larval damaged was usually confined to a single locule, total yield loss often resulted due to poor lint development in the remaining locules. Also, damaged bolls only partially opened and could not be effectively harvested by mechanical cotton pickers. If the bolls were infested when the lint and seed was almost mature (i.e. when over six weeks old), little economic damage resulted. Usually, only the very basal seeds of these bolls were damaged by larvae.

3.3.9. Recovery of larvae artificially infested onto cotton squares and bolls

Recovery of neonate larvae placed on bolls was much higher than when larvae were placed on squares (Table 3.12). Of 204 larvae placed on 51 squares, 15 were recovered nine days after infestation, a mean recovery of 6.8% (± 4.7 S.D., range = 0-12.5%). Of 200 larvae placed on 50 bolls, 48 were recovered seven days after infestation, a mean recovery of 23.2% (± 4.5 S.D., range = 19.4-28.9%).

3.1.10. Dispersal and survival of larvae emerging from eggs

Of the 200 eggs placed on eight cotton plants, 141 larvae emerged (70.5% egg hatch). A total of 56 larvae were recovered from all eight plants, 24 and 48 hours after eggs hatched.

Percent recovery ranged from 31.3 to 57.9%, giving a mean rate of recovery of 40.0% (± 9.0 S.D.) per plant. In plants sampled 24 hours after egg hatch, 21 out of 35 (60%) larvae recovered were found in bolls, 13 larvae were in small leaf buds (37.1%) and one larva was found in an old dry flower attached to a boll. No larvae were found in squares or on leaves. In plants sampled 48 hours after egg hatch, 17 out of the 21 (81%) larvae found were located in bolls, three larvae were found in old dry flowers attached to bolls (14.3%), one larva was located in a large square (4.8%), and no larvae were found in leaf buds or any other plant part (Table 3.13).

3.3.11. Site of pupaton

Prepupae and pupae were found in high numbers at the base of cotton plants in the decaying leaf litter, close to or on the soil surface. In a sample of 1000 fallen forms, collected from unsprayed cotton on 12 June 1986, 479 larvae and 176 pupae (26.9%) were found (the number of prepupae was not recorded). In a further sample of 1000 fallen forms, collected on 20 June 1986, 354 larvae, 68 prepupae and 230 pupae (35.3%) were found. Prepupae and pupae were also found in samples of leaf litter and soil collected from the base of cotton plants on 3 April, 1986. Two prepupae (one in the leaf litter and one on the soil surface) and seven pupae (three in leaf litter and four on or near the soil surface) were found. All prepupae and pupae were located inside a loose cocoon made of leaf litter and/or soil particles.

No prepupae or pupae were ever encountered inside the 4200 and 5400 green bolls that were inspected immediately after collection during the 1984/84 and 1986/87 cotton seasons, respectively. This was despite the presence of 3566 and 1705 *P. scutigera* larvae inside these bolls. Similarly, no prepupae or pupae were encountered on any part of cotton plants (N = 15 plants) sampled to determine oviposition sites (see section 3.2.2.).

Low numbers of pupae were found inside dry, open cotton bolls, previously damaged by *P. scutigera* larvae, at the end of the cotton season. In 300 dry bolls collected on 29 May, 1986, a total of 268 larvae and 20 pupae (7.5%) were found. In another 300 dry bolls, sampled on 25 June, 1987, 243 larvae and three pupae (1.2%) were found. The number of larvae in the prepupal stage was not recorded but was observed to be very low. Of those pupae found in open bolls most were located inside damaged locules while a few pupae were found on the

Table 3.12. Recovery of neonate *P. scutigera* larvae placed on squares or bolls of cotton plants grown in pots in a shadehouse. Four newly emerged larvae were infested onto each square or boll and inspected nine and seven days later, respectively.

Plant	No. of squares/ bolls infested per plant	No. of larvae introduced	No. of larvae recovered	% larvae recovered
Squares				
1	8	32	4	12.5
2	4	16	1	6.3
3	6	24	3	12.5
4	7	28	1	3.6
5	5	20	1	5.0
6	9	36	4	11.1
7	7	28	1	3.6
8	5	20	0	0
Total	51	204	15	-
Mean	6.4	25.5	1.9	6.8
(S.D.)	(1.7)	(6.7)	(1.6)	(4.7)
Bolls				
1	9	36	7	19.4
2	12	48	13	27.1
3	13	52	15	28.9
4	5	20	4	20.0
5	11	44	9	20.5
Total	50	200	48	-
Mean	10.0	40.0	9.6	23.2
(S.D.)	(3.2)	(12.6)	(4.4)	(4.5)

Table 3.13. Recovery of neonate *P. scutigera* larvae on glasshouse cotton plants artificially infested with 20 eggs. Eggs, laid on tissue paper, were pinned onto the underside of large leaves at the base of each plant.

Plant[1]	No. eggs emerged	No. larvae recovered	% larvae recovered	Location of larvae			
				Squares	Flowers	Bolls	Buds
1	16	5	31.3	0	0	2	3
2	19	11	57.9	0	1	3	7
3	12	6	50.0	0	0	6	0
4	19	7	36.8	0	0	4	3
5	16	6	37.5	0	0	6	0
6	20	7	35.0	1	2	4	0
7	20	7	35.0	0	0	7	0
8	19	7	36.8	0	1	6	0
Total	141	56	-	1	4	38	13
Mean	17.6	7.0	40.0	0.1	0.5	4.8	1.6
(S.D.)	(2.8)	(1.8)	(0.9)	(0.4)	(0.8)	(1.8)	(2.6)

1. Plants 1-5 were searched for larvae 24 hours after egg hatch.
Plants 6-8 were searched for larvae 48 hours after egg hatch.

outside of bolls between the dry carpel wall and bract.

3.3.12. Egg parasitism

Placement of *P. scutigera* eggs in unsprayed cotton on 15 April, 1985, led to the discovery of an egg parasitoid, later identified by Dr H. Nagaraja (Agricultural Consultancy Services Inc., Davao del Sur, Philippines) as *Trichogrammatoidea bactrae* Nagaraja. Of the original 1639 eggs exposed in the field for three days, 113 or 7.0% were parasitized by *T. bactrae*, although many of the eggs disappeared presumably through predation and/or dislodgement by wind or rain. Percentage recovery of eggs exposed in unsprayed cotton for three days on 8 January and 14 February, 1987, was recorded. Only 24.0 and 58.3% of the original number of eggs exposed were recovered, respectively. Of those eggs recovered, 0 and 27.4% were parasitized by *T. bactrae* (Table 3.14A).

Many of the eggs placed in a commercial cotton field during 1985 (Site 1, A. Shepherdson's property) at the end of the season also disappeared after three days exposure. The percentage of eggs recovered decreased from 66.7% at the crop edge to 30.5% at 50 m away from the crop edge, and 13.3% at 100 m from the crop edge (Table 3.14B). Levels of parasitism of the eggs remaining was higher at the crop edge than within the crop; 83.4% at the crop edge, 14.8% at 50 m and 6.4% at 100 m from the crop edge. Expressed as the percentage parasitism of the original number of eggs exposed, this gives 35.3, 6.6 and 4.8% parasitism at the crop edge, 50 m and 100 m from the crop edge, respectively.

Levels of parasitism of eggs collected during studies on oviposition sites in unsprayed cotton ranged from 6.1% in regrowth cotton during June 1986, to 50.0% during April 1985. Parasitized eggs were also found in commercial cotton fields at the end of the growing season. At Site 3 (I. Wilkie's property) 25.0 and 43.8% of eggs collected from entire cotton plants on 30 April and 19 May, 1985, respectively, were parasitized. In another cotton field near Biloela (T. Jensen's property) the level of egg parasitism was 21.4% on 10 April 1986.

A laboratory colony of *T. bactrae* was readily established on *P. scutigera* eggs. Development of *T. bactrae*, from parasitism of eggs to adult emergence, took 12.0 days at 25^{o} C. Most parasitized eggs (45.8% of 384 eggs examined) contained a single female *T. bactrae*, although up to five parasitoids could develop in a single egg. Multiple parasitized eggs

Table 3.14. Parasitism of *P. scutigera* eggs by *Trichogrammatoidea bactrae* when exposed in cotton fields for three days: (A) unsprayed cotton; and (B) commercial cotton (Site 1). Eggs exposed in the commercial cotton field were placed in three locations on 26 April, 1985.

(A) Unsprayed cotton:

Date eggs exposed	Number of eggs exposed	Number of eggs parasitized	Percent of eggs recovered	Percent of eggs recovered parasitized	Percent of eggs exposed parasitized
15/4/85	1639	113	NR[1]	-	7.0
8/1/87	649	0	24.0	0	0
21/1/87	1236	38	NR	-	3.1
14/2/87	357	57	58.3	27.4	16.0

1. NR = not recorded

(B) Commercial cotton:

Position of eggs in crop	Number of eggs exposed	Number of eggs parasitized	Percent of eggs recovered	Percent of eggs recovered parasitized	Percent of eggs exposed parasitized
Crop edge	490	218	66.7	83.4	35.3
50 m from crop edge	379	25	30.5	14.8	6.6
100 m from crop edge	454	12	13.3	6.4	4.8

Table 3.15. Parasitism of *P. scutigera* larvae by *Apanteles* sp. in unsprayed cotton over three consecutive seasons. All larvae were collected from bolls. BI = number of *P. scutigera* larvae/100 bolls, %P = percentage of larvae parasitized by *Apanteles* sp.

Cotton season 1984/85			1985/86			1986/87		
Date	BI	%P	Date	BI	%P	Date	BI	%P
21/1	3	0	18/1	12	0	21/1	12	0
27/1	3	0	25/1	2	0	1/2	18	0
3/2	7	0	2/2	7	0	7/2	16	0
11/2	11	0	9/2	30	0	14/2	26	0
20/2	17	0	16/2	50	0	23/2	34	0
27/2	23	0	23/2	62	1.6	2/3	34	0
6/3	47	0	8/3	111	1.8	12/3	78	3.2
14/3	52	0	10/3	176	2.6	20/3	121	3.3
20/3	45	0	17/3	170	1.2			
27/3	76	8.2	25/3	221	2.7			
3/4	87	12.7	3/4	150	6.0			
11/4	129	6.9	9/4	180	8.9			
17/4	90	11.1	21/4	231	19.5			
3/5	125	6.4	6/5	178	8.5			
28/5	136	16.2	16/5	257	13.2			
6/6	172	18.6	28/5	280	16.1			
12/6	187	17.6						

contained all possible combinations of males and females or one sex only. In the absence of males, female *T. bactrae* readily parasitised *P. scutigera* eggs and produced all male progeny.

Longevity of *T. bactrae* varied according to sex and the number of individuals emerging from the host egg. Females that emerged singly from parasitized eggs were the longest lived; at 25° C longevity was 3.1 days (+ 1.3 S.D., range = 0.9 - 5.9 days) when provided with a 5% sugar solution for food. Under the same conditions, mean longevity of females emerging from multiple parasitized eggs was 1.99 days (± 1.04 S.D.), while males emerging from multiple and single parasitized eggs lived for 1.03 (± 0.55 S.D.) and 1.28 (± 0.85 S.D.) days, respectively. Females (N = 10), emerging from eggs containing one parasitoid, parasitized a mean of 30.8 eggs during their lifetime at 25° C, when confined to 15 cm diam. petri dishes containing 60 *P. scutigera* eggs. Seventy-one percent of those eggs were laid on the first day of emergence.

3.3.13. Larval parasitism

Parasitized *P. scutigera* larvae were first detected in collections of bolls from unsprayed cotton at the D.P.I. Research Station, Biloela, in late March 1985. Specimens of adult parasitoids were identified as *Apanteles* sp. (Braconidae) by Dr I. Galloway, Entomology Branch, Q.D.P.I., Brisbane. Levels of parasitism were low despite very high *P. scutigera* populations (Table 3.15). On 27 March, 1985, 8.2% of *P. scutigera* larvae were parasitized when there were 76 larvae/100 bolls. Parasitism increased to 12.8% in the following week but decreased to 6.9% on 11 April. On 28 May parasitism levels increased to 16.2% but remained at a similar level during the following two weeks despite an increase in *P. scutigera* infestation from 136 to 187 larvae/100 bolls.

Similarly, in the 1985/86 season larval parasitism was very low in unsprayed cotton despite very high *P. scutigera* populations (Table 3.15). *Apanteles* sp. were first detected on 23 February when there were 62.5 *P. scutigera* larvae/100 bolls, of which 1.6% were parasitized. Levels of parasitism increased slowly while *P. scutigera* numbers increased rapidly. At the end of May when boll infestation was 280 larvae/100 bolls only 16.1% of larvae were parasitized.

In the 1986/87 cotton season parasitized larvae were not detected in unsprayed cotton until 12 March at a level of 3.2% when *P. scutigera* boll infestation had reached 78 larvae/100 bolls.

Although boll infestation increased to 121 larvae/100 bolls on 20 March, parasitism remained at a similar level of 3.3% (Table 3.15).

Parasitized larvae were rarely found in commercial cotton fields. In the 1984/85 cotton season parasitized larvae were only detected at the very end of the season when *P. scutigera* populations increased in bolls produced after defoliation of the crop. At Site 1, on 29 April 12.2% of larvae were parasitized when boll infestation reached 134 *P. scutigera* larvae/100 bolls. Parasitism remained at this level on 12 and 19 May despite increases in boll infestation to 160 and 170 larvae/100 bolls, respectively. At Sites 2 and 3 parasitoids were only detected in bolls sampled on 7 May at 10.3 and 23.5% level of parasitism when larval populations had increased to 145 and 51 larvae/100 bolls, respectively.

Although boll infestation was very high at Site 1 in 1986, particularly along one crop edge, parasitoids were only detected in bolls at the very end of the season on 17 April and 18 May (10.0 and 2.5% parasitism, respectively). In April, 1986, parasitized larvae were also found in other commercial cotton crops near Biloela and Theodore where substantial increases in *P. scutigera* had occurred in fresh regrowth after crop defoliation. Levels of parasitism ranged from 6.3 to 10.7% in fields where boll infestation ranged from 97.5 to 206 *P. scutigera* larvae/100 bolls.

Apanteles sp. only parasitized first and second instar *P. scutigera* larvae when confined to petri dishes containing each larval instar. A single parasitoid larva emerged from a third or fourth instar larva. Before emergence of the parasitoid larva, the host larva underwent behaviour normally associated with ecdysis or pupation. Parasitized larva exited from the cotton boll and usually built a loose silk cocoon. Soon after completion of the cocoon the parasitoid larva emerged from the host.

On emergence from the host larva, the *Apanteles* sp. larva usually fed on any remaining internal fluids before constructing a cocoon. Some parasitoid larvae did not feed at all and the degree of feeding also varied; some only partially removed the internal fluids of the host larva while others removed all fluids including cuticular pigments, causing the parasitoid larva to change in colour from creamy-white to pink. The parasitoid larva constructed a white silk cocoon, *ca* 4 x 1.75 mm, in which pupation occurred. The remains of a parasitized larva (head

capsule and cuticle) remained attached to the outside of the cocoon. These cocoons were often observed on the outside of the cotton boll in which the *P. scutigera* larva infested, towards the end of the season.

Development of *Apanteles* sp., from parasitism of a host larva to formation of a pupal cocoon, took approximately ten days at 25^{o} C, while emergence of the adult occurred on day 13 or 14 after parasitism.

Two species of hyperparasite were found attacking *Apanteles* sp. in unsprayed and commercial cotton: larvae were parasitized by a species of *Perilampus* (possibly *P. queenslandensis* Girault, Dr I. Galloway pers. comm.) while cocoons were multiple parasitized by an unidentified species of micro-Hymenoptera. The degree of hyperparasitism was not assessed but appeared to be very low.

3.3.14. Prepupal and pupal parasitism

Prepupal and pupal parasitism was very low; no parasites emerged from the pupae resulting from 374 fourth instar larvae recovered from fallen forms sampled on the 12 June 1986. From 176 pupae found at the time of sampling, five parasitoids emerged (2.8% parasitism). Two of the parasitoids were identified by Q.D.P.I. taxonomists as *Lissopimpla excelsa* (Costa) (Ichneumonidae) and the remainder were an unidentified species of *Brachymeria* (Chalcididae).

None of the fourth instar larvae (N = 280) or prepupae (N = 68) found in fallen forms sampled on 20 June 1986, were parasitized. However, four parasitoids emerged from 230 pupae found in the same sample (1.7% parasitism). Three of the parasitoids were identified as *L. excelsa* and one as *Brachymeria* sp.

3.3.15. Sex ratio

The sex ratio of 2351 pupae, resulting from larvae infesting green bolls between 23 February and 20 June, 1986, did not significantly depart from an expected 1:1 ratio of males to females (Chi-square test, P = 0.05, Table 3.16).

Table 3.16. Sex ratio of *P. scutigera* pupae reared from larvae infesting bolls in unsprayed cotton during the 1985/86 season.

Date bolls collected	Number of pupae		Sex ratio (M : F)	Chi-square
	Male	Female		
23/2	22	19	1.2:1	0.11 NS[1]
3/3	68	64	1.1:1	0.06 NS
10/3	87	97	0.9:1	0.27 NS
17/3	71	68	1.0:1	2.16 NS
25/3	168	132	1.3:1	2.16 NS
3/4	122	108	1.1:1	0.43 NS
9/4	154	121	1.3:1	1.98 NS
21/4	115	109	1.1:1	0.08 NS
6/5	38	43	0.9:1	0.15 NS
16/5	48	33	1.5:1	1.39 NS
28/5	16	23	0.7:1	0.63 NS
8/6	93	87	1.1:1	0.10 NS
12/6	109	126	0.9:1	0.61 NS
20/6	104	106	1.0:1	0.01 NS
Total	1215	1136	1.1:1	1.32 NS

1. NS = Not significant from expected sex ratio of 1 male : 1 female (P = 0.05).

3.3.16. Nocturnal activity of adult moths

Figs 3.15-17 show the time of capture of *P. scutigera* males in pheromone baited traps. Local air temperatures are also shown but data on wind speed has been omitted as no detectable wind was present during periods of pheromone trap capture on all nights of observation.

In November, March and April the time of sunset was similar at ca. 18.58, 18.55 and 18.52 h, respectively, while in June sunset occurred at 17.37 h. On all nights of observation moths become active at or soon after twilight before total darkness. On the night of April 7, 1985, when moth behaviour was closely observed, activity began at twilight (18.17 h) and steadily increased over the next few minutes. Feeding was first observed at 18.28 h and all moths seen were active by 18.32 h. Females started laying eggs at 18.32 h but oviposition was sporadic with frequent bouts of feeding and resting taking place. Moths remained very active until around 21.30 h when there was a noticeable decrease in the number of moths walking or flying. By 22.57 h all activity had ceased and did not resume until 00.45 h when three females were observed calling. Calling females were recognized by their characteristic stance with wings held over the body and the pheromone gland extruded. Males immediately responded by walking and wing fanning towards the calling female with their anal claspers extended. Activity was much lower and more sporadic than during the earlier part of the night probably due to much cooler temperatures. Mating pairs were observed at 00.45, 00.55, 01.00, 01.10, 01.19 and 01.30 h. At 02.25 h all walking and flying ceased but five pairs remained mating. By 04.30 h no pairs remained mating and no further activity was observed.

A total of 97, 306 and 318 males were caught in pheromone traps over the three nights of observations made in April 1985, November 1985 and March 1987, respectively. In June 1987 males were only caught on the first night of observations (N=53) as temperatures were too low for activity on the two other nights.

On all occasions, except June 1987, the majority of males were caught between midnight and 04.00 h but one male was caught between 22.00-23.00 h on the first night of observations in March 1987. The hour in which the majority of moths were caught appeared to be associated with local air temperature. On the coolest nights of observation between November and March, most males were caught between 02.00-03.00 h whereas on warmer nights most males were

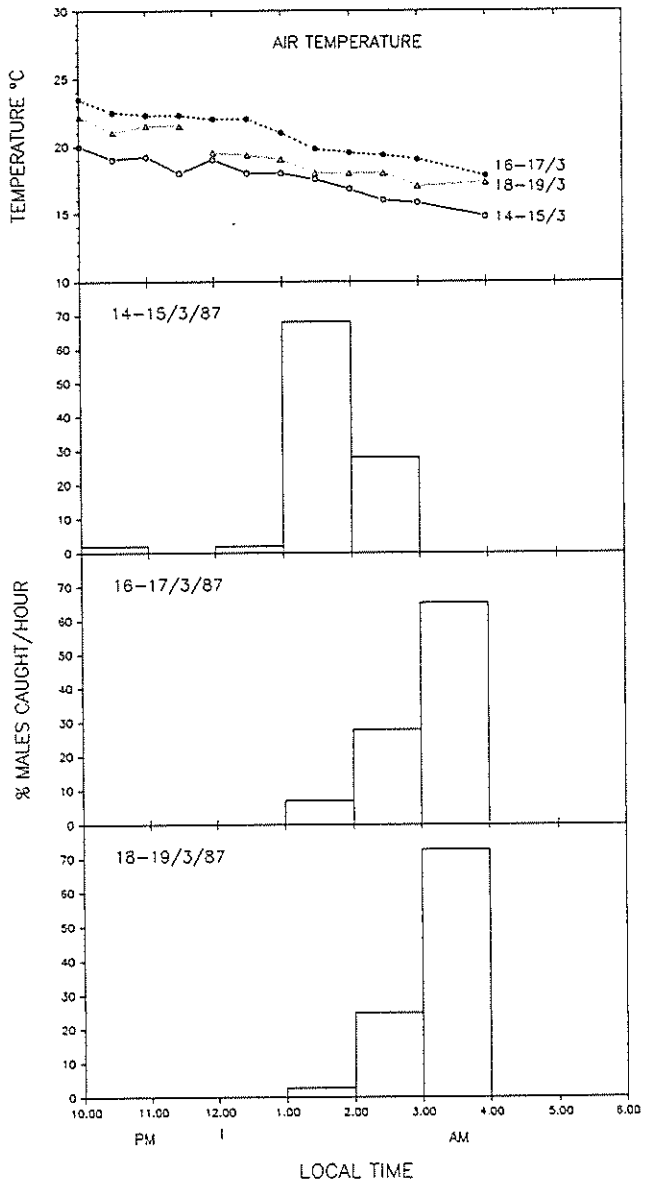

Fig. 3.15. Hour of capture of male *P. scutigera* in pheromone traps placed in unsprayed cotton for three nights during March 1987. Air temperatures recorded throughout each night are shown in the top graph.

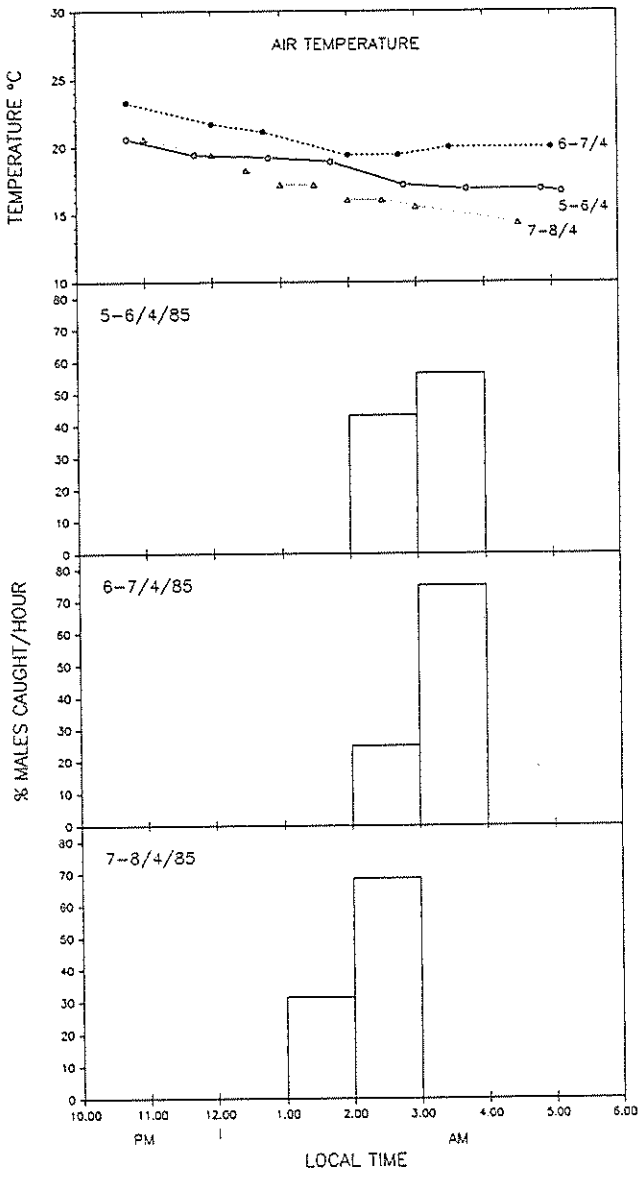

Fig. 3.16. Hour of capture of male *P. scutigera* in pheromone traps placed in unsprayed cotton for three nights during April 1985. Air temperatures recorded throughout each night are shown in the top graph.

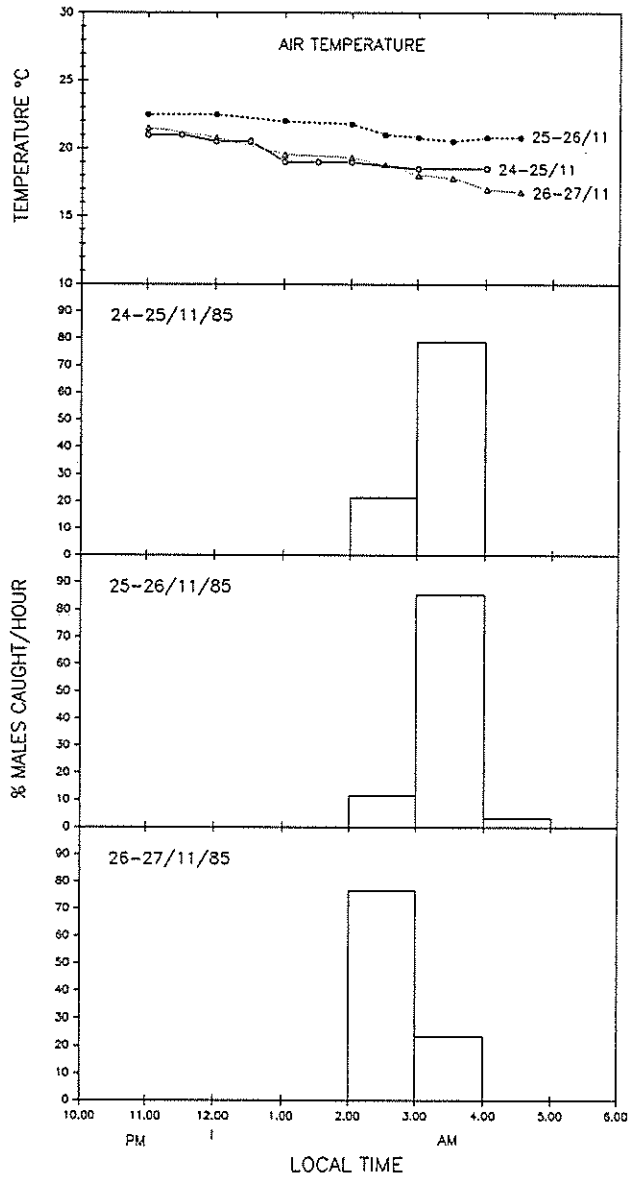

Fig. 3.17. Hour of capture of male *P. scutigera* in pheromone traps placed in unsprayed cotton for three nights during November 1985. Air temperatures recorded throughout each night are shown in the top graph.

caught between 03.00-04.00 h.

The effect of temperature on pheromone trap catch was clearly evident in June 1987. On the first night (24/6) pheromone traps were checked at twilight (17.15 h) when the air temperature was 21° C. Traps were not checked again until 18.45 h when the temperature had dropped sharply to 11.1° C. Unexpectedly, by this time the three pheromone traps had caught a total of 53 males (mean=17.7/trap). No further moths were caught during that night as temperatures remained very low. On the two following nights in June no males were caught in the pheromone traps as temperatures were even cooler at around 16.7° C at 17.30 h, dropping to 11.1° C by 18.30 h.

A total of 333, 690 and 676 eggs were laid on the three nights of observation in March, 1987, respectively. Temperature appeared to influence the period of egg lay and the time of peak oviposition (Fig. 3.18). On the warmest night (night 2) oviposition extended from the first hour of darkness until 02.00 h. There was no distinct peak in oviposition but most eggs (over 80%) were laid between 07.00-09.00 h. On the coolest night (night 1) oviposition started in the first hour of darkness but only extended up until midnight, with very few eggs (0.3%) laid between 11.00-12.00 h. There was a distinct peak in oviposition between 08.00-09.00 h when over 50% of the eggs were laid. On the third night temperatures were slightly warmer than night 1 and oviposition occurred over a similar period of time but more eggs were laid between 11.00-12.00 h (3.1%). Peak oviposition occurred one hour earlier than on night 1 at between 07.00-08.00 h. While less than half the number of eggs were laid on the coolest night than on the two warmer nights, it is difficult to ascertain temperature as the cause of this as the reproductive status of the females may have changed (see Chapter 2).

Generally, the hour of capture of *P. scutigera* in a light trap over three nights in March, 1987, reflected the pattern of activity observed during oviposition and pheromone trap capture (Fig. 3.19). On night 1, male and female capture was bimodal occurring between 19.00-21.00 h and 01.00-03.00 h in the males and between 19.00-22.00 h and 01.00-02.00 h in the females. The former peak coincided with the period of feeding, oviposition and perhaps dispersal while the latter peak coincided with the period of reproductive activity and male capture in

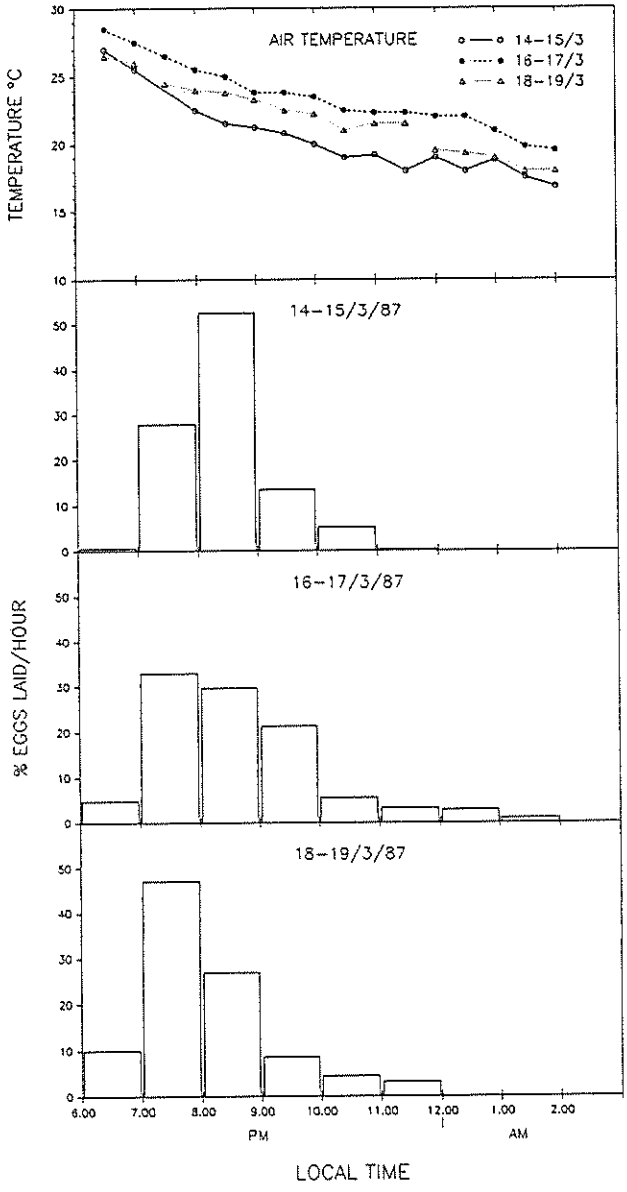

Fig. 3.18. Hour of oviposition of *P. scutigera* confined to oviposition chambers and placed in unsprayed cotton for three nights during March 1987. Air temperatures recorded throughout each night are shown in the top graph.

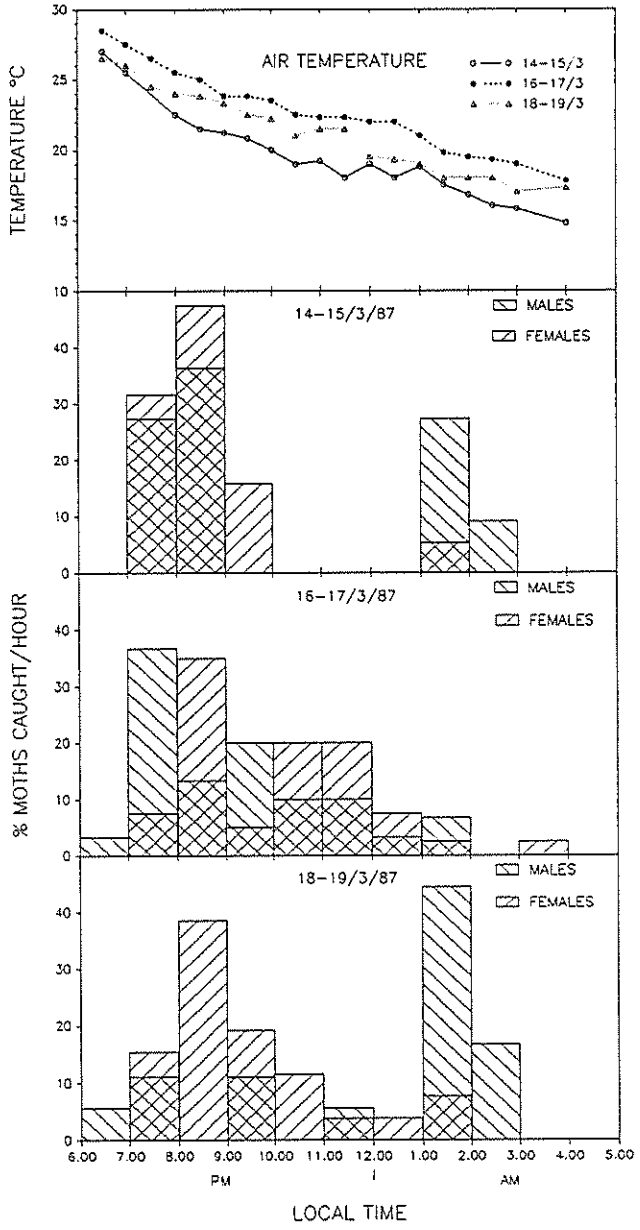

Fig. 3.19. Hour of capture of male and female *P. scutigera* in an ultraviolet light trap placed in a cotton field for three nights during March 1987. Air temperatures recorded throughout each night are shown in the top graph.

pheromone traps. On the second night, males were caught continuously from the first hour of darkness until 02.00 h while females were first captured at 19.00 h and continued to be caught up to 04.00 h except for a break in capture between 02.00-03.00 h. Most males and females were caught during the time of peak oviposition while a smaller number were caught during the estimated period of calling and mating. On the third night few males were caught before midnight with the majority being trapped between 01.00-03.00 h, coinciding with the time of peak pheromone trap capture. Females were caught continuously from 19.00-02.00 h with peak capture coinciding with the time of peak oviposition of caged moths. More females than males were caught in the light trap at a ratio of 1 female to 0.6, 0.8 and 0.7 males for the three nights of trapping.

3.4. Discussion

The seasonal incidence of *P. scutigera* infesting cotton observed in this study confirms previous reports that this species is largely a late season pest and of economic importance only when bolls are present on plants (Passlow 1961, Page *et al.* 1984b, Sabine 1969b). Rates of larval infestation of squares was always very low, even in unsprayed cotton, and never warranted control with insecticides.

The low rate of infestation of squares early in the cotton season may be related to the ineffective carry-over of moth populations from one cotton season to the next. This aspect of *P. scutigera* biology is discussed in Chapters 6 and 8. However, infestation of squares remained low throughout the cotton season even when boll infestation increased to very high levels, as seen in unsprayed cotton during the 1985/86 season. This suggests that squares are either unattractive to larvae and/or that survival is poor.

The unattractiveness of squares was evident in the glasshouse experiment where the distribution of larvae, hatching from eggs placed onto cotton plants, was determined. Only one larva hatching from these eggs was found inside a square when plants were searched 24 and 48 hours after egg hatch, compared to 42 larvae found in bolls or dry flowers attached to bolls (Table 3.13). In Chapter 2 it was shown that although larvae confined onto

squares successfully developed to the adult stage, development took twice as long as larvae reared on bolls. Such slow development suggests an allelochemical effect and is further discussed in Chapter 8.

The incidence of *P. scutigera* larvae infesting bolls was similar in both unsprayed and sprayed cotton. Infestations were generally low until the end of January or early February, after which populations increased sharply. No discrete generations of *P. scutigera* larvae could be identified in any field or season. First instar larvae were present in bolls sampled throughout the 1987 season, in both unsprayed and sprayed cotton.

The relationship of boll age and susceptibility to larval attack was unclear. In both years of observation, bolls aged between 30 to 48 days old contained the highest number of larvae. This result may merely be an accumulative effect whereby older bolls have greater chance of becoming infested with a larva as time progresses. Only when each larval instar is considered separately can inferences about susceptibility to larval attack be made. First instars were found in bolls of all ages, except those over 46 days old. Bolls older than 46 days are probably unattractive because of their low moisture content. At this age the lint and seed is mature and the boll begins to split open. Very little moisture is present in the carpel walls where initial infestation by a *P. scutigera* larva takes place. The absence of second and third instar larvae in bolls between 16 and 29 days old may be a consequence of poor survival rates through antibiosis or allelochemical effects (see Chapter 8). Although first instar larvae were found in these bolls, mortality may not occur until the larva starts to feed and tunnel within the boll wall.

Few fourth instar larvae were found in bolls less than 15 days. This was expected as *P. scutigera* larvae tend to feed within the same boll until completion of development. Development to the fourth larval instar takes 10.9 and 15.0 days at 25 and 21^{o} C constant, respectively (Chapter 2). Mean monthly temperatures during February and March range between 20-26.5^{o} C (Table 3.1), therefore, for bolls to contain a fourth instar larva they would have to be older than *ca* 10 to 15 days.

Larvae entered bolls through any point in the calyx, as observed by Holdaway (1926). Infestation of bolls through the apex may have been associated with the retention of flower

corollas. Farrer and Bradley (1985) found that retention of flower corollas by developing cotton bolls increased the likelihood of infestation by *Heliothis zea* (Boddie) larvae. In unsprayed cotton the major point of entry was through the boll base whereas in sprayed cotton it was the boll sides (Table 3.14). This difference may have reflected greater levels of predation in unsprayed cotton. Larvae entering through the boll base were probably better protected from predators than those entering through more exposed regions of the boll sides. In varieties of cotton other than DPL90, the site of boll entry may differ, especially those with frego bracts such as the Siokra variety.

The within-field distribution of *P. scutigera* larvae infesting bolls in unsprayed cotton was initially aggregated but became random when populations reached more than 16.7 larvae/100 bolls. On the final sampling occasion (11 April, 1985) populations approached a regular distribution when the number of larvae was extremely high at 129.2 larvae/100 bolls. Apparent or real changes in the distribution of insects with increasing density have often been observed. More commonly, the insect changes from a random distribution when at low population density to an aggregated distribution at higher population densities, although many forest insects and periodic cicadas show a tendency to become more randomly distributed at higher densities (Southwood 1978).

Random patterns in a population of organisms imply environmental homogeneity and/or nonselective behavioural patterns (Ludwig and Reynolds 1988). Clumping suggests that individuals are aggregated in more favourable parts of the habitat. While a monoculture of cotton may appear homogeneous, the fruiting pattern of individual plants can vary greatly according to a number of agronomic factors and previous insect damage. Early in the season, boll production may be uneven across the field causing an aggregated distribution of *P. scutigera* larvae through selective oviposition by females and/or increased survival of larvae in areas with a higher number of bolls per plant. As the season progresses and the plants approach the peak fruiting stage, the density and distribution of infestable bolls per plant will become more homogeneous resulting in a random distribution of *P. scutigera* larvae. However, many other factors (abiotic and biotic) may influence the pattern of distribution of

an organism.

The pattern of distribution of *P. scutigera* larvae in sprayed cotton fields may also differ according to the efficiency of insecticide penetration and coverage. Poor spray coverage was thought to be responsible for the observed 'edge effect' at Site 1 (A. Shepherdson's property) over three consecutive cotton seasons. At this site cotton was repeatedly grown adjacent to a creek bordered by tall trees which impeded the flight of crop sprayers. Spray coverage along the crop edge was further affected by an adverse wind direction.

An 'edge effect' has been frequently described in studies of butterfly oviposition, where individual host plants at the edge of a clump receive more eggs than plants in the centre. Courtney and Courtney (1982), studying *Anthocharis cardamines* (L.), suggested that the effect was due to two or more aspects of searching behaviour of ovipositing females. Females tended to fly towards the nearest host plant on a flight path and lay an egg before dispersing. Also, females appear to be more responsive to host plants after flying long distances, resulting in plants at the edge of clumps receiving more attention.

Such behavioural patterns may also occur in female *P. scutigera* when searching for a host plant to infest. However, the persistence of larval infestations in bolls along the crop edge at Site 1, despite the application of insecticides, suggests that poor spray coverage was at least partly responsible. Furthermore, an 'edge effect' was not apparent in unsprayed cotton during the 1985/86 cotton season. Poor spray coverage became obvious when crops were chemically defoliated at the end of the season. Areas inaccessible to aerial sprayers remained green in contrast with the rest of the defoliated field. This problem is further discussed in Chapter 8.

The detection of first instar larvae in bolls is a difficult and time consuming task. In the 1984/85 season, significantly more *P. scutigera* larvae were found in bolls incubated for 10-14 days than in bolls inspected immediately after collection. Undoubtedly, this discrepancy arose through poor detection of first instar larvae. Incubation of bolls for 10-14 days allowed small larvae to develop into third or fourth instars which were easy to detect. In the 1986/87 season the discrepancy in larval counts between incubated and non-incubated bolls was not as apparent due to greater experience in locating first instar larvae. Based on this experience, the following method for detecting larvae in bolls is recommended:

(1) Pick bolls off plants complete with the boll stalk.

(2) Fold back the bracts and inspect both the boll calyx and the inside surface of each bract for larvae.

(3) Pull away the boll stalk so that the inner bract becomes detached to expose the base of the boll. Inspect the inner bracts as well as the boll base for larvae. As first instar larvae found in this location are often difficult to see unless they move, it is recommended that the inner bracts and boll base are carefully observed for a 3-5 seconds before proceeding to the next step.

(4) Split open the boll by inserting a knife into a suture, twisting the knife blade side-ways. The knife should be inserted about mid-way between the boll base and apex to prevent damage to any larvae infesting the apex.

(5) After splitting, pull the boll open and inspect the apex and central axis for larvae. Again, first instar larvae are difficult to see unless they move, therefore, observe the apex and central axis for 3-5 seconds before proceeding to the next step.

(6) Split apart all locules by inserting the knife between each suture. Pull away the lint to expose the inner wall of each locule section.

(7) Inspect the inner walls for larval mines; if a mine is suspected peel away the covering skin to expose the damaged area and verify the presence of a larva. This is necessary as previous insecticide sprays, predation, natural antibiosis or other mortality factors may have killed the larva. Also, damage caused by sap-sucking bugs can often be mistaken for *P. scutigera* larval damage.

Once experience was gained, it took approximately six minutes to inspect a sample of ten bolls in this way.

The optimal sample size needed to estimate the density of larvae within a 10% level of precision was unacceptably high (Table 3.10). The use of a lower level of precision (20%) gave more acceptable sample sizes but only when larval populations were more than or equal to 5 larvae/100 bolls. Consequently, a sequential sampling plan would be more useful and is given in Fig. 3.13. Under this plan, the grower or pest adviser would repeatedly inspect

samples of ten bolls, collected from different points in the field, until the cumulative number of *P. scutigera* larvae exceeds the upper limit (initiate control procedures) or is less than the lower limit (no control required). Sampling should continue if the cumulative number of larvae remains between the upper and lower limits but stop when a 'maximum' number of samples have been taken. This maximum number can be set according to the constraints of sampling costs (Smith and McDonald 1989) but it is recommended that at least ten samples of ten bolls are taken to achieve acceptable levels of precision.

Such a sampling plan needs to be tested in commercial cotton fields but it could be easily incorporated into existing plans devised for other cotton insects e.g. that given by the SIRATAC computer programme (Hearn *et al.* 1981). Bolls could be collected during normal SIRATAC insect sampling procedures, moving the recommended distance of five paces (*ca* 5 m) along the row and seven rows across between each sampling point.

The SIRATAC plan recommends leaving a buffer zone of 50 m around the edge of the cotton field (except when sampling for aphids). Such a buffer zone cannot be recommended for *P. scutigera*, especially if poor aerial spray coverage along crop edges is suspected. Separate sampling plans for field edges may be required from the main body of the field.

The very low infestation of squares by *P. scutigera* larvae makes it unnecessary to sample cotton crops for this pest until bolls are present on plants. Initiation of boll sampling for *P. scutigera* larvae may be decided by using pheromone traps to monitor male activity (see Chapter 4) or by using SIRATAC's recommendation for roughbollworm (*Earias huegeli* Rogenhofer) as a guideline. The SIRATAC plan recommends initiating sampling bolls for *E. huegeli* when more than 5 bolls/m over two weeks old are present on plants. The SIRATAC plan also recommends terminating sampling bolls for *E. huegeli* when there are less than 5 bolls/m less than two weeks old, and again this may be a useful guide for sampling *P. scutigera*. Sampling should be conducted twice weekly or every three days to ensure *P. scutigera* infestations are detected while larvae are in the first or second instar.

The presence of high numbers of *P. scutigera* larvae in freshly fallen forms can be explained by the behaviour of larvae to remain in squares, flowers and bolls when shed by the cotton plant. Once shed, larvae continue to feed on the fallen form and complete development

on the ground. This behaviour was also seen when larvae infested the flowers of alternative host plants (Chapter 5). The presence of a large larval population in fallen forms is significant. These larvae would be largely unaffected by any insecticide applications, due to protection by the plant canopy. Also, development of larvae to the adult moth would be extremely variable according to the moisture content of the fallen form (see Chapters 5, 6 and 8 for further discussion). Consequently, moth emergence would be spread over a long period resulting in a constant source of moths infesting the cotton crop.

The small size of *P. scutigera* eggs (0.56 x 0.32 mm), together with their concealed placement and variability in location on the cotton plant, makes it impractical to develop an egg sampling plan for monitoring moth populations. Hutchison *et al.* (1986, 1988) were able to devise an egg sampling plan for *P. gossypiella*, which has similar sized eggs, as a consistent proportion were laid on bolls where they were readily visible to the naked eye.

In contradiction with previous reports on oviposition sites of *P. scutigera* (Ballard 1927b, Passlow 1964, Passlow and Sabine 1963), eggs are very rarely laid on squares or green bolls, but mainly on leaves and leaf buds. The proportion of eggs laid on leaves and leaf buds changed according to the physiological condition of the plant. At the end of the cotton season, open bolls became attractive sites for oviposition.

The concealed placement of eggs suggests that ovipositing females actively search over cotton plants for sites that afford some protection to the developing embryo. Inducement to lay an egg may occur only if tactile receptors on the female ovipositor are sufficiently stimulated. The absence of eggs on squares and bolls may be simply due to the lack of adequately protected surfaces. While the concealed position of eggs may afford some protection from predators, parasitoids and adverse weather conditions, the resulting neonate larva must be very vulnerable to these mortality factors. As few eggs are laid on or close to the site of larval infestation, the newly hatched larvae need to actively search over the cotton plant to locate a suitable feeding site.

The influence of *T. bactrae* on the population dynamics of *P. scutigera* infesting cotton could not be assessed due to the difficulties in sampling moth eggs. Rates of larval parasitism

were very low in all cotton fields sampled. *Apanteles* sp. was not detected in unsprayed and sprayed cotton until the very end of the season when *P. scutigera* larval populations were very high. Sands and Hill (1982) identified *Apanteles oenone* Nixon parasitizing *P. scutigera* infesting *H. tiliaceus* in the Darwin region. Whether *A. oenone* was the species of *Apanteles* found parasitizing *P. scutigera* in this study is unknown.

The absence of *Apanteles* sp. early in the cotton season may be partly due to the inability of this species to effectively carry-over from one season to the next when host larvae are rare or absent (Chapter 7). However, the slow increase in *Apanteles* sp. populations during the remainder of the season may indicate that *P. scutigera* is not a suitable host species. Similarly, very low levels of prepupal/pupal parasitism in unsprayed cotton at the end of the season may be due to the unsuitability of *P. scutigera* as a host to *L. excelsa* and *Brachymeria* sp. There are many factors which affect the suitability of a host for parasitization, such as size, nutritional quality, host defenses and kairomones (Vinson 1976, Vinson and Iwantoch 1980). Parasitism of *P. scutigera* by these species may only occur in the absence of normal hosts. Both *L. excelsa* and the genus *Brachymeria* are known to parasitize *Helicoverpa* spp. in cotton. The former was recorded in Queensland and New South Wales crops, while the latter was recorded from the Ord River irrigation area (Bishop and Blood 1977, Evenson and Basinski 1973, Room 1979, Wright and Nitikin 1964, Zalucki *et al.* 1986).

Contrary to previous reports (Passlow 1964, Passlow and Sabine 1963), the site of pupation in *P. scutigera* during the growing season is not inside bolls but at the base of cotton plants at the leaf litter and soil surface interface. No prepupae or pupae were found on any part of the cotton plant until the very end of the season when some pupae were found in dry, partially opened bolls damaged by *P. scutigera* larvae.

Observations on the nocturnal activity of *P.scutigera* reported in this study closely agree with those made by Vickers (1982a). Two distinct phases in the activity of moths can be recognized; a dispersal and oviposition phase, and a sexual phase. The dispersal and oviposition phase is marked by a high level of moth activity and feeding. It starts at dusk and usually ends around midnight. The sexual phase is marked by lower moth activity and usually occurs between midnight and dawn. Males respond to pheromone traps during this phase,

coinciding with the period of calling in females. Differences in the level of activity of moths during each phase may be partly related to temperature, but are probably also behavioural. Females were stationary during calling, while only those males responding to calling females were active.

The onset and termination of each phase was affected by temperature. On cool nights the period of sexual activity was advanced while on warm nights it was delayed. On very cool nights, such as that observed in June, there may only be a sexual phase. Similar effects of temperature on the nocturnal activity of moths have been observed in many other species e.g. *P. gossypiella* (Lingren *et al.* 1989) and various Arctiid species (Webber and Conner 1986).

Threshold temperatures for *P. scutigera* oviposition and male capture in pheromone traps can be approximated from the data. As no eggs were laid after midnight on the two coolest nights of observation, a threshold temperature for oviposition can be estimated to lie between 17 and 19^{o} C (Fig. 3.18). Based on the time of termination of pheromone trap capture on the coolest nights of observation, the threshold temperature for male flight activity can be estimated to lie around 15 or 16^{o} C (Figs. 3.15-17).

Interestingly, the nocturnal activity of *P. scutigera* is very similar to that of *P. gossypiella*, with peak pheromone trap capture, oviposition, calling and feeding occurring at approximately the same time of night (cf. Leppa 1972, Lingren *et al.* 1989, Lukefahr and Griffen 1957, Van Steenwyk *et al.* 1978).

CHAPTER 4

Monitoring *P. scutigera* populations with synthetic sex attractants

4.1. Introduction

Traps baited with synthetic sex attractants (herein called pheromone traps) are probably the most specific, economical and convenient biological monitoring tool available today. They are ideally suited for monitoring pests where an accurate assessment of the timing of emergence and/or size of the adult population is needed (Riedl *et al.* 1976). Insecticides or other control methods can then be coordinated more precisely with the life cycle of the insect to achieve control.

The basic premise for monitoring insect populations with pheromone traps is simple but problems are often encountered in the development of accurate and predictive sampling methods (Birch and Haynes 1982). Interpretation of trap catch is complicated by the effects of local environmental variables (such as temperature, wind speed and direction, moonlight and cloud cover) on the number of insects caught over a single night (Dent and Pawar 1988). One of the least understood variables that may affect pheromone trap catch is the degree of competition imposed by the presence of virgin females (Croft *et al.* 1986).

It is often assumed that pheromone trap catch is well correlated with adult emergence or other phenological events e.g. oviposition, egg hatch, damage (Wall 1989). Such a relationship has been verified in relatively few studies (e.g. Allen *et al.* 1986, Carroll and Rummel 1985, Johnson 1983, Ramos *et al.* 1989). Often, when investigated, the correlation between pheromone trap catch and various phenological events has been found to be poor (Batiste *et al.* 1973, Legg and Chiang 1984, Oloumi-Sadeghi *et al.* 1975, Shepherd *et al.* 1985, Trimble 1986). In some insects, such as *Cydia pomonella* (L.) and *Ostrinia nubilalis* (Hubner), pheromone traps are only useful for monitoring the first flight or generation but not the second (Riedl *et al.* 1976, Roelof *et al.* 1970).

Similarly, for *P. gossypiella* in cotton, pheromone traps are useful for timing the first insecticide spray but thereafter trap catch is poorly related to larval infestation (Henneberry and Beasley 1985, Henneberry and Clayton 1982).

The impetus to develop a synthetic pheromone trapping system to monitor *P. scutigera* arose from the difficulties in detecting larval populations in the field (Chapters 1 and 3). Despite contradictory results on their reliability for monitoring *P. scutigera* populations (cf. Flint and Stone 1985, Page *et al.* 1984a, Rothschild 1983), pheromone traps are presently used by some pest advisors for timing insecticide sprays in Central Queensland (M. Stone pers. comm.).

In this Chapter, experiments are presented to resolve questions on the usefulness of pheromone traps for monitoring populations of *P. scutigera*. As outlined in Chapter 1, Rothschild (1975) reported that pheromone traps baited with the Z,Z- isomer of 7, 11-hexadecadienyl acetate were adequate for monitoring *P. scutigera*. He found that the addition of small quantities of the Z,E- isomer to Z,Z- baits increased trap catch but not significantly. However, Flint and Stone (1985) caught significantly more *P. scutigera* in traps baited with a 9:1 ratio of Z,Z- to Z,E- isomers than in traps containing the Z,Z- isomer alone. Baits were obtained from both authors and tested in the field simultaneously to determine which was most suitable for monitoring *P. scutigera*. The effects of pheromone trap design, trap placement and bait age on the number of males caught is also reported. The correlation between pheromone trap catch, light trap catch and subsequent larval infestation in cotton is determined, using data given in Chapter 3.

4.2. Materials and methods

4.2.1. Comparison of baits

The number of males caught in pheromone traps baited with the Z,Z- isomer only and a 9:1 ratio of Z,Z- to Z,E- isomers of 7, 11-hexadecadienyl acetate was compared using baits obtained from Dr G. H. L. Rothschild, C.S.I.R.O., Canberra, A.C.T., Australia, and Dr H. M. Flint, U.S.D.A., Phoenix, Arizona, U.S.A. Baits from each source are abbreviated to:

RZZ - Rothschild's Z,Z- isomer only

R9:1 - " 9:1 ratio of Z,Z- to Z,E- isomers

FZZ - Flint's Z,Z- isomer only

F9:1 - " 9:1 ratio of Z,Z- to Z,E- isomers.

Attractants obtained from Dr Flint originated from Farchan Chemical Co., Willoughby, Ohio, U.S.A. The Z,Z- isomer was declared to be 98.8% pure with 1.2% Z,E- contaminant. The Z,E- isomer was 96.9% pure, with 2.2% Z,Z- and 0.9% unknown contaminant.

The Z,Z- isomer obtained from Dr Rothschild originated from Chemical Samples Division, Albany International, U.S.A., and was declared to be 95% pure with 5% unknown contaminant. The Z,E- isomer originated from Bayer AG, LeverKüsen, West Germany, and was stated to be 96% pure with 4% unknown contaminant.

F9:1 and FZZ baits consisted of 1-F red rubber sleeve stoppers (The West Company, Phoenixville, Pennsylvania, U.S.A.), impregnated with 1 mg active ingredient of attractant diluted in methylene chloride. The desired ratios of each isomer were prepared as described by Flint and Stone (1985). Initially, the R9:1 and RZZ baits consisted of 1.5 cm lengths of red rubber tubing (internal diam. 5 mm), impregnated with 1 mg active ingredient of attractant diluted in toluene. However, for the 1986 and 1987 trials Dr Rothschild's baits were standardized with Dr Flint's by using the same solvent and dispensers.

Trials were conducted in experimental (D.P.I. Research Station, Biloela) and commercial cotton fields over three consecutive cotton seasons (1984-87). Details of trials conducted in each field site over the three cotton seasons are listed in Table 4.1. In the 1984/85 season, the commercial cotton fields used were the same as those where larval infestation was monitored throughout the season, as described in Chapter 3. Only three of the four synthetic pheromone baits were tested in these trials (F9:1, FZZ and RZZ) due to the unavailability of R9:1 baits. On the D.P.I. Research Station during the 1984/85 season, field 1 (V2) was not sprayed with insecticide during the period of observation and was described in Chapter 3. Fields 2 (M1) and 3 (K1) were part of a cotton variety trial consisting of a large number of varieties with DPL61 planted around the borders. Control traps, i.e. traps containing no synthetic baits, were

also included in these trials. In field K1, one entire row of traps was placed on a fence 2 m from the crop border while the other two rows were placed within the crop. In the 1985/86 season, the number of males caught in traps arranged in three 3 x 4 grids or four single isolated lines was compared (Table 4.1). Trials were conducted in the same commercial cotton fields (Sites 1-3) plus an additional site (Site 4, I. Shepherdson's property), located 8 km NE of Biloela, where a single isolated line of traps was placed. In the 1986/87 season, commercial cotton Site 1 was the same site as in previous seasons, but Site 2 was a large field of dryland cotton (T. Jensen's property) as described in Chapter 3. The distance between rows of traps and traps within rows was maximised according to the size of each field and are given in Table 4.1.

In all trials the Delta trap design was used (see section 4.2.2.). Each row of traps contained one representative of each treatment being compared. The initial order of treatments within a row was assigned randomly, using random number tables. On counting the number of moths caught, each bait type was rotated to the next position within the row to overcome any positional bias in trap capture. Traps were suspended from metal posts and maintained at plant canopy height as recommended by Rothschild (1983). On checking traps, moths were counted and removed, replacing the trap when necessary. Fresh baits were used in all trials except in the 1986/87 season when baits that had been exposed in the field for 14 days during the 1985/86 trials were re-used. Used baits were wrapped in aluminum foil, and stored in a freezer until required in the 1986/87 season.

Two-way ANOVA was applied to compare the number of males caught by each bait type and trap row. Trap data collated from the three commercial cotton fields in the 1984/85 season were combined, as were data for single lines of traps in the 1985/86 season. In the 1984/85 season trap catch over the first 20 trapping days (1-20 February) was selected for analysis. In the 1986/87 season trap catch between 13 January and 16 March was used, while in the 1985/86 season all trapping data (9-22 March) was used for analysis. This was done to reduce the amount of time analysing trap data. In both seasons the dates selected for analysis were representative of the number of moths caught over the entire trapping season.

Table 4.1. Details of trials conducted over three cotton seasons to compare the trapping efficiency of four synthetic pheromone baits (F9:1, FZZ, R9:1 and RZZ). The size of each field, dates over which trap catch was monitored, trap arrangement (number of rows x treatments) and the distance between traps is listed for (A) D.P.I. Research Station fields and (B) commercial cotton fields.

A) D.P.I. Research Station, Biloela.

Season/field		Field size (ha)	Dates traps monitored	Trap arrangement (rows x trt)	Distance between traps (m)	
					Rows	Within rows
1984/85@	M1	4.3	1/2-2/3	4 x 5 grid	50	80
	K1	5.6	"	3 x 5 grid	80	50
	V2	2.7	"	3 x 5 grid	70	50
1985/86	K1	5.6	9-22/3	1 x 4 line	-	50
	V2	2.7	19/3-1/4	3 x 4 grid	70	50
1986/87	V2	2.7	13/1-31/3	2 x 4 grid	80	50

@ 1984/85 trials included control traps containing no synthetic bait.

B) Commercial cotton

Season/field		Field size (ha)	Dates traps monitored	Trap arrangement (rows x trt)	Distance between traps (m)	
					Rows	Within rows
1984/85#	1	30	1/2-2/3	1 x 3 line	-	100
	2	25	"	2 x 3 grid	200	100
	3	25	"	2 x 3 grid	200	100
1985/86	1	30	9-22/3	2 x 4 grid	700	50
	2	25	"	3 x 4 grid	80	50
	3	10	"	3 x 4 grid	80	100
	4	70	"	1 x 4 line	-	100
1986/87	1	30	13/1-8/3	4 x 4 grid	100	100
	2	100	14/1-21/2	3 x 4 grid	100	100

In the 1984/85 trials only F9:1, FZZ and RZZ baits were compared due to the unavailability of R9:1 baits.

4.2.2. Comparison of pheromone trap design

Four pheromone trap designs were compared to determine the most efficient and convenient design to use for monitoring male *P. scutigera*:

a) Delta trap (Fig. 4.1.A) - a prism-shaped trap measuring 18 cm long, 10 cm wide at the base, and 12 cm high when assembled. Traps were obtained from commercial suppliers (Sandia Die and Cartridge, Albuquerque, New Mexico, U.S.A.) and consisted of waxed cardboard, red on the outside with a white internal sticky surface. Originally designed by the U.S.D.A. for monitoring gypsy moths (Foster *et al.* 1977), Delta traps are the most widely used trap design for monitoring *P. gossypiella* in the U.S.A. (Flint and Merkle 1983). They are also currently used in Australia by some pest advisors to monitor *P. scutigera.*

b) Pherocon 1C trap (Fig. 4.1.B) - manufactured by Zoecon Corporation (Palo Alto, California, U.S.A.) for monitoring Tortricid moths. Traps consisted of an upper and lower section (26 x 22 cm), made of white waxed cardboard, held together by a wire frame (9 cm high when assembled). The removable lower section was coated with a sticky paste.

c) Cup trap (Fig. 4.1.C) - an open ended cup-shaped trap used by Page *et al.* (1984a, 1984b) for monitoring *P. scutigera.* A similar trap design was used by Rothschild (1983) in some of his *P. scutigera* monitoring studies. Traps were constructed from white plastic cups (Hygienic Lily 30SP), 11 cm long, 12.6 (top) and 9.3 (bottom) cm diam., with the base removed and coated with Tanglefoot ^{R}.

d) Funnel live trap (Fig. 4.1.D) - designed by Lingren *et al.* (1980) for trapping male *P. gossypiella* without the use of a sticky surface. The funnel live trap (FLT) was constructed from a large yellow plastic funnel (21 cm top diam., 24 cm long, 1.5 cm diam. spout) with a clear plastic vial (5 x 10 cm) attached to the bottom spout. A roof made of white waxed cardboard was glued onto the top of the funnel. Four equidistant entrance holes (5 cm diam.) were drilled into the side of the funnel at 2 cm from the top rim.

All traps were baited with RZZ baits, pinned onto the top inside surface. Traps were suspended from metal posts, using wire, at plant canopy height. The trial was conducted in field V2 on the D.P.I. Research Station, Biloela, over a 12 night period between 5-16 April 1985. Three rows of traps, 80 m apart, were arranged in a randomized block design so that

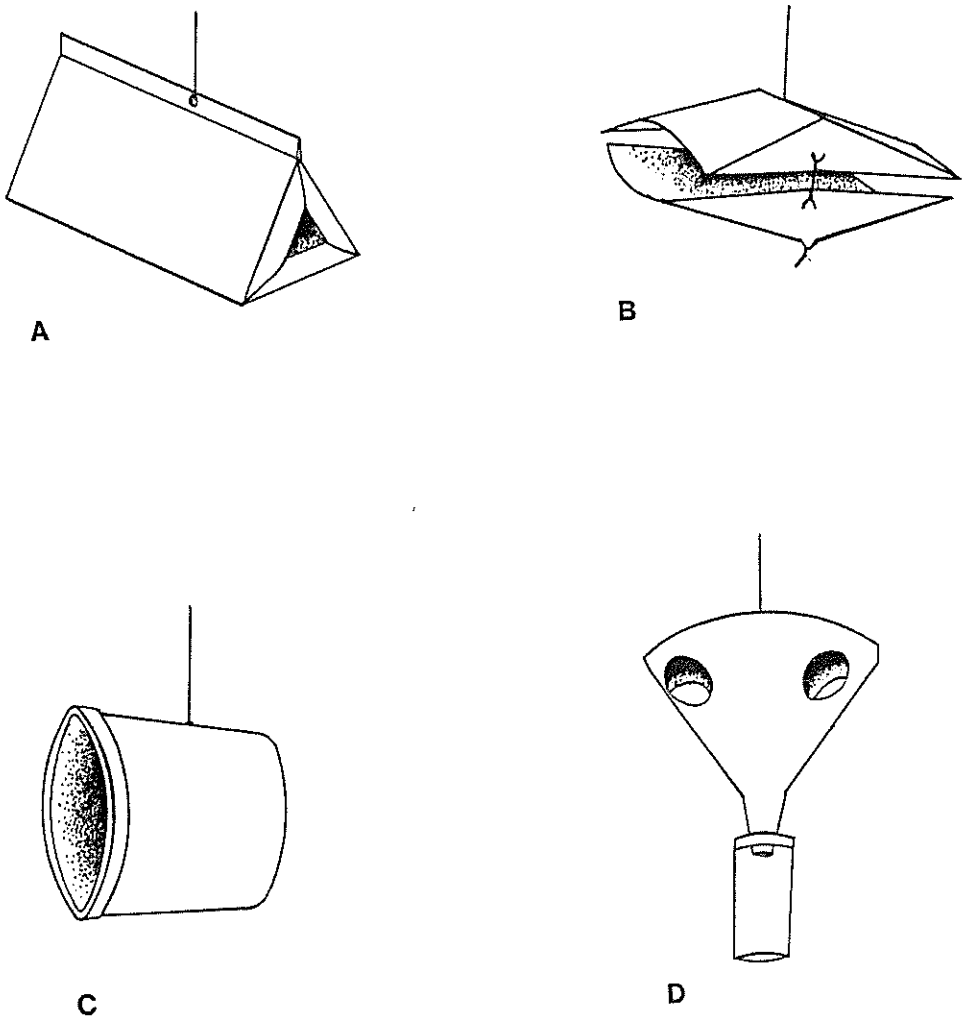

Fig. 4.1. Four pheromone trap designs compared for monitoring male *P. scutigera*: A) Delta trap, B) Pherocon trap, C) Cup trap, D) Funnel live trap.

each row contained one of each trap design. Traps within rows were spaced 50 m apart. Trap catch was checked daily, rotating each trap design to the next position within the row to overcome any positional bias in moth capture. The mean number of males caught/night in the four trap designs was compared using a one-way ANOVA.

The capture efficiency of each trap design was determined by observing the number of males caught over a 10 minute period during peak pheromone response (Chapter 3). Males approaching within 1 m of the trap were counted at the start of the 10 minute period and compared with the actual number caught at the end of the period. This was done on two consecutive nights for Pherocon, cup and FLT traps and on three consecutive nights for Delta traps. Observations were made using a 6 V battery operated head-lamp covered by red Kodak filter.

4.2.3. Effect of bait age on trap catch

F9:1 baits were aged inside Delta traps suspended from a metal fence on the grounds of the D.P.I. Research Station, Biloela, for 2, 4, 6 and 8 weeks, beginning in February 1986. When all baits had reached the required age they were placed in fresh Delta traps and transported to the field for testing. Three replicates of each bait age were compared with unexposed baits (0 weeks), arranging traps in three randomized rows; one row was placed at Site 1, while two rows (spaced 100m apart) were placed in field V2 at the D.P.I. Research Station. Traps were spaced 50-80 m apart within rows and the number of moths caught was recorded over five consecutive nights between 12-16 April 1986. Traps were rotated each day to overcome any positional bias in moth capture. Data from all three rows of traps were combined for analysis, to give the mean number of males caught/trap/night for each bait age. A one-way ANOVA was applied to test for significant differences in attractancy of the aged baits.

4.2.4 Effect of trap position

While conducting comparisons of attractants at Site 2 during the 1984/85 season, the effect of trap placement on moth catch was also investigated. One row of traps (baited as described in section 4.2.1.) was placed 2 m away from the crop edge and another row was placed in the centre of the cotton field. Total trap catch in each row was compared for 16 days. On day 17,

traps placed 2 m away from the crop edge were moved into the first row of cotton and further comparisons in trap catch were made with traps left in the centre of the field over another 18 days.

The effect of row position on trap catch in all other cotton fields was also determined (see section 4.2.1.). Data from Site 1 during the 1986/87 season was further analyzed to determine whether traps situated along the crop edge caught significantly more moths than traps placed at increasing distances away from this edge. As reported in Chapter 3, larval infestation of bolls in this field was consistently higher along the crop edge than in the mid-crop region. The total number of moths caught in four traps, positioned in four parallel rows at 1, 100, 200 and 300 m from the crop edge, was compared over six trapping periods. Trapping periods were based on one full rotation of traps, so that each type of pheromone bait (F9:1, FZZ, R9:1 and RZZ) was present once in each row position. This period varied according to the frequency traps were checked. The dates of each period were: 1) 13-17 January, 2) 18-26 January, 3) 27 January-8 February, 4) 9-13 February, 5) 14-21 February, and 6) 22 February-5 March. For statistical comparison, using a two-way ANOVA, trap catch in each period was expressed as the mean number of moths caught/trap/night.

4.2.5. Seasonal incidence of male *P. scutigera*

The seasonal incidence of male *P. scutigera*, caught in F9:1 baited pheromone traps was monitored in all cotton fields described in Chapter 3. The trapping period varied in each site and season (see Chapter 3 and Figs. 3.4-3.12), but was usually initiated in the spring and terminated on defoliation or harvesting of the cotton crop. Single traps (Delta design) were placed on metal posts in each field and maintained at crop height. When traps were placed in the field before the emergence of cotton seedlings, trap height was standardized to ca 1.5 m above the ground. Trap catch was monitored every 1-5 days, replacing traps when necessary. Baits were replaced at approximately monthly intervals or until trials to compare different baits were conducted (see section 4.2.1.).

The time interval between major peaks in pheromone trap catch, at least 20 days apart, was measured in day-degrees (DD) above 11.5^{o} C and compared with the expected generation time of 1031 DD and 555 DD for *P. scutigera* reared on squares and bolls, respectively

(Chapter 2). Degree-day accumulations were based on weather data presented in Chapter 3. The DEGDAY program of Higley *et al.* (1983) was used, choosing the sine-wave method for calculating degree-days.

4.2.6. Correlation between pheromone and light trap catch

During the 1986/87 cotton season a battery-powered ultraviolet light trap was operated alongside pheromone traps placed in unsprayed cotton at the D.P.I. Research Station. The light trap was situated on the edge of the cotton field, ca 200 m away from the pheromone traps. A block of plaster of paris soaked with Dichlorvos insecticide was placed inside the collection bucket to kill trapped insects. The light trap was operated between dusk and dawn for 29 nights between 17 January and 31 March, 1987. All *P. scutigera* caught in the light trap were removed and sexed. Light trap catch was correlated with the number of males caught in pheromone traps baited with F9:1, FZZ, R9:1 and RZZ baits, during the same night.

4.2.7. Correlation between pheromone trap catch and larval infestation of cotton

The number of male *P. scutigera* caught in pheromone traps, placed in cotton fields where larval infestation was simultaneously monitored, was correlated with the number of larvae detected in cotton squares and bolls. Details of larval sampling procedures were given in Chapter 3.

Two methods of correlating trap catch with boll infestation were attempted. In Method 1, simple correlations between accumulative trap catch and larval infestations were calculated for each field and cotton season. Pheromone trap data from F9:1 baited traps was used throughout, except for unsprayed cotton during the 1984/85 season when R9:1 trap catch data was used. This was necessary due to the abnormally low number of moths caught by F9:1 baited traps in this field (see section 4.3.1.).

The cumulative number of males caught in traps for -3, -5, -7, -9 and -11 days before bolls were sampled, was correlated with the number of first, first and second or all larval instars found in bolls during the 1984/85 and 1986/7 cotton seasons. In the 1985/86 season, because cotton bolls were incubated in ventilated plastic boxes before searching for larvae (Chapter 3), the number of young larvae (second and third instar larvae) found in the

incubated bolls was used to calculate correlations with trap catch and larval infestation.

The second method (Method 2) of correlating trap catch with larval infestation took into account a delay from the time moths were detected in traps to the time when egg laying and egg hatch occurs. In Chapter 2 it was estimated that 74.3 degree-days (DD) were required for *P. scutigera* eggs to complete development with an average preoviposition period of 42.3 DD. The number of males caught in traps over -1, -3, -5 and -7 days was calculated at either 74.3 DD (egg hatch alone) or 116.6 DD (preoviposition period plus egg hatch) before the date of boll sampling. Calculations were made using data from F9:1 baited traps during the 1986/87 season only.

4.2.8. Statistical analysis

All data was transformed to $\log_{10}$ (adding 1 to overcome problems with zero catches) before statistical analysis to normalize the means. Only untransformed data is presented in the tables and figures unless stated. Whenever ANOVA was applied to trap data, the 5% L.S.D. test was used to separate means if the F value was significant.

Pearsons product mean correlation (Ryan *et al.* 1985) was applied to determine the correlation between pheromone and light trap catch, and between pheromone trap catch and larval infestation. The significance of the correlation coefficient (r) was determined by comparing with critical values of r, as tabulated by Sokal and Rohlfs (1973).

4.3. Results

4.3.1. Comparison of baits

The results of all trials are summarized in Tables 4.2-4.5, giving the mean number of males caught for each bait type and trap row.

1985/85 commercial cotton trials - 2,828 males were caught in all traps over the 38 day test period (Fig 4.2). Comparison of the first 20 days of trapping with a two-way ANOVA, revealed that significantly more males were caught at Site 3 than at Site 1 and 2 ($p<0.01$) (Table 4.2). F9:1 baited traps caught significantly more males than both FZZ and RZZ baited traps, while RZZ baited traps caught significantly more males than FZZ baited traps ($p<0.05$) (Table 4.2). The overall percentage trap catch was 66.7, 7.2 and 26.2% for F9:1, FZZ and

RZZ baited traps, respectively (Table 4.5).

1984/85 D.P.I. trials - 3,304 males were caught in all three D.P.I. fields over the 29 day trapping period. The results contrast markedly from the above commercial field trials. Traps baited with F9:1 caught very few males and trap catch was not significantly different from FZZ baited traps ($p>0.05$) (Table 4.2). R9:1 and RZZ baited traps caught similar numbers of males ($p>0.05$), but significantly more than either F9:1 or FZZ baited traps ($p<0.05$). Fig. 4.3 shows the number of males caught in field V2 which is representative of trap catch in fields M1 and K1. Mean percent trap catch over all three fields was 10.7, 9.0, 36.2 and 44.2% for F9:1, FZZ, R9:1 and RZZ baited traps, respectively (Table 4.5).

1985/86 trials - 37,718 males were caught over all fields over the 14 day trapping periods. Single lines of traps placed in field K1 and Site 4 caught significantly more males than the two lines placed at Site 1 ($p<0.05$) (Table 4.3). F9:1 baited traps caught significantly more males than all other bait types ($p<0.05$) at all sites except Site 2 where there was no significant difference between F9:1 and R9:1 baited traps (Table 4.3). Although R9:1 baited traps caught more moths than RZZ baited traps at most sites, the difference was never significant ($p>0.05$). Both of these baits caught significantly more moths than FZZ baited traps ($p<0.05$). Fig. 4.4 shows trap catch for each bait type in V2 which is representative of all other field sites. Overall, the mean percent trap catch was; F9:1 39.6%, FZZ 10.8%, R9:1 25.8% and RZZ 23.8% (Table 4.5).

1986/87 trials -23,748 moths were trapped in all fields over the entire trapping period. For the period of trapping compared with a two-way ANOVA, F9:1 baited traps caught significantly more moths than all other baited traps in all fields ($p<0.05$) (Table 4.4). Both R9:1 and RZZ baited traps caught significantly more moths than FZZ traps ($p<0.05$) except at Site 2, where very high numbers of moths were caught throughout the trapping period (Table 4.4). As seen in the 1985/86 season, R9:1 baited traps consistently caught more moths than RZZ baited traps but the difference was never significant ($p>0.05$). Fig. 4.5 shows the number of males caught by all bait types placed in V2 over the entire trapping period, and again is representative of the other two field sites. The overall mean percent trap catch was; F9:1

Table 4.2. Comparison of synthetic baits for monitoring male *P. scutigera*. Mean trap catch for all sites during the 1984/85 cotton season.

(A) Commercial cotton Sites 1-3

Site	Mean no. males/bait/night F9:1	FZZ	RZZ	Site mean[1]
1	3.6	0.5	1.6	1.9a
2	5.8	0.1	1.0	2.3a
3	14.4	0.6	5.4	6.8b
Mean	7.9a	0.4b	2.7c	

ANOVA:

Source of variation	df	F ratio	p
Site	2	14.59	<0.01
Bait	2	42.22	<0.01
Interaction	4	3.48	<0.05
Error	171		

(B) V2 D.P.I. Research Station (unsprayed cotton)

Row	Mean no. males/bait/night F9:1	FZZ	R9:1	RZZ	Row mean
1	1.2	0.7	4.4	6.6	3.2a
2	1.8	1.2	8.6	8.8	5.1a
3	1.4	1.0	3.8	5.9	3.0a
Mean	1.5a	0.9a	5.6b	7.1b	

ANOVA:

Source of variation	df	F ratio	p
Row	2	1.08	>0.05
Bait	3	24.83	<0.01
Interaction	6	0.13	>0.05
Error	228		

(C) K1 D.P.I Research Station

Row	Mean no. males/bait/night F9:1	FZZ	R9:1	RZZ	Row mean
1	0	0.4	1.3	1.2	0.7a
2	2.9	2.2	12.3	7.5	6.2b
3	3.3	2.7	4.6	6.2	4.2a
Mean	2.1a	1.8a	6.0b	5.0b	

ANOVA:

Source of variation	df	F ratio	p
Row	2	14.26	<0.01
Bait	3	5.43	<0.01
Interaction	6	0.63	>0.05
Error	228		

(D) M1 D.P.I. Research Station

Row	Mean no. males/bait/night F9:1	FZZ	R9:1	RZZ	Row mean
1	0.5	0.5	1.9	3.4	1.6a
2	0.6	0.4	2.0	5.2	2.0a
3	1.0	1.0	2.3	2.9	1.8a
4	0.1	0.2	0.7	0.7	0.4b
Mean	2.1a	1.8a	6.0b	5.0b	

ANOVA:

Source of variation	df	F ratio	p
Row	3	9.72	<0.01
Bait	3	0.73	<0.01
Interaction	9	0.73	>0.05
Error	304		

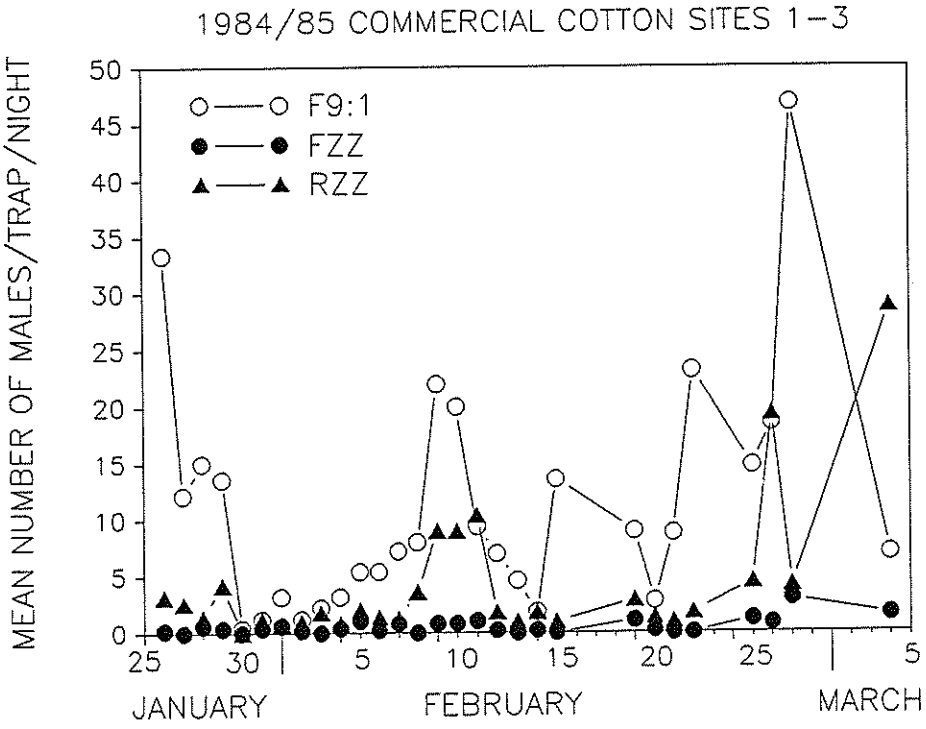

Fig. 4.2. Comparison of the number of male *P. scutigera* caught in pheromone traps baited with F9:1, FZZ and RZZ placed in three commercial cotton fields during the 1984/85 season.

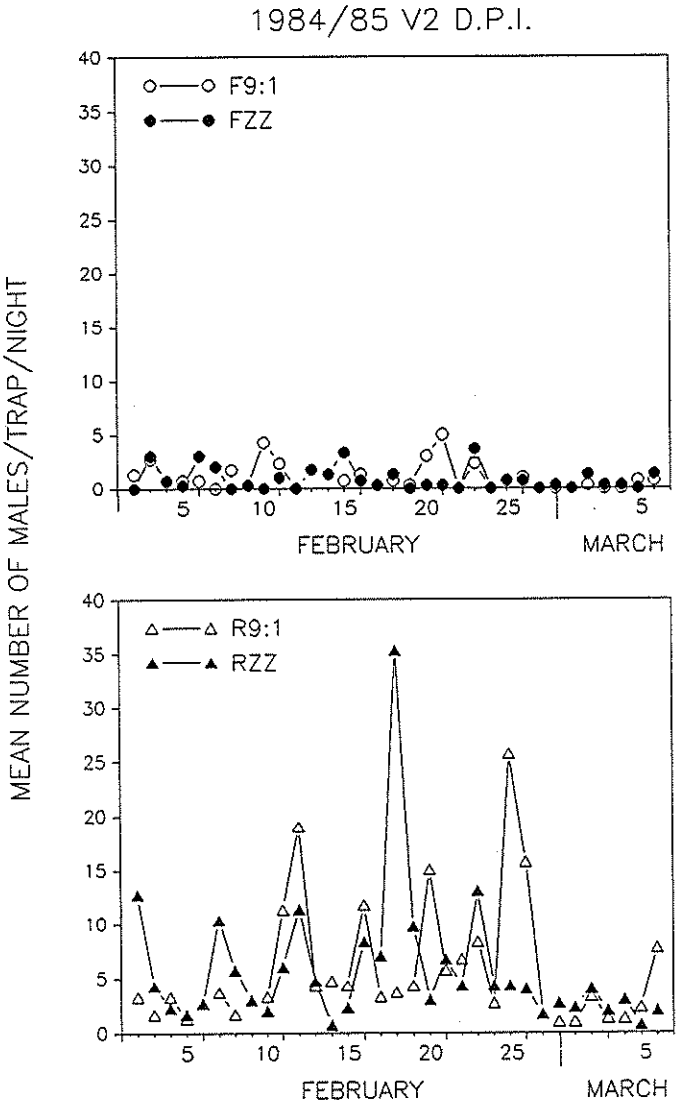

Fig. 4.3. Comparison of the number of male *P. scutigera* caught in pheromone traps baited with F9:1, FZZ, R9:1 and RZZ in field V2 (unsprayed cotton) at the D.P.I. Research Station, Biloela, during the 1984/85 season. Data for the F9:1 and FZZ baits have been plotted separately from the R9:1 and RZZ baits for clarity.

Table 4.3. Comparison of synthetic baits for monitoring male *P. scutigera*. Mean trap catch for all sites during the 1985/86 cotton season.

(A) Single trap lines

	Mean no. males/bait/night				
Row	F9:1	FZZ	R9:1	RZZ	Row mean
1A	39.2	13.7	24.2	29.0	26.5a
1B	73.2	20.7	58.0	49.3	50.3b
K1	141.3	47.3	108.5	80.3	94.3c
4	119.8	69.5	112.1	116.6	104.5c
$\bar{x}$	93.4a	37.8b	75.7c	68.8c	

ANOVA:

Source of variation	df	F ratio	p
Site	3	33.72	<0.01
Bait	3	8.26	<0.01
Interaction	9	0.58	>0.05
Error	160		

(B) Site 2

	Mean no. males/bait/night				
Row	F9:1	FZZ	R9:1	RZZ	Row mean
1	174.6	57.8	137.7	83.9	113.5a
2	141.6	53.9	133.9	128.8	114.5a
3	174.6	36.3	131.1	103.4	111.4a
$\bar{x}$	163.5ac	49.3b	134.2cd	105.4d	

ANOVA:

Source of variation	df	F ratio	p
Row	2	0.83	>0.05
Bait	3	29.63	<0.05
Interaction	6	1.82	>0.05
Error	132		

(C) Site 3

	Mean no. males/bait/night				
Row	F9:1	FZZ	R9:1	RZZ	Row mean
1	50.8	5.0	18.2	21.4	23.9a
2	58.3	10.3	15.5	22.3	26.6a
3	55.3	6.2	19.0	32.8	28.3a
$\bar{x}$	54.8a	7.2b	17.6c	25.5c	

ANOVA:

Source of variation	df	F ratio	p
Row	2	0.10	>0.05
Bait	3	21.04	<0.01
Interaction	6	0.83	>0.05
Error	132		

(D) V2 D.P.I. Research Station (unsprayed cotton)

	Mean no. males/bait/night				
Row	F9:1	FZZ	R9:1	RZZ	Row mean
1	87.7	5.9	47.3	29.1	42.5a
2	59.7	6.3	35.2	23.4	31.2a
3	33.4	9.3	16.7	14.2	18.4b
$\bar{x}$	60.3a	7.2b	33.1c	22.2c	

ANOVA:

Source of variation	df	F ratio	p
Row	2	8.05	<0.01
Bait	3	32.82	<0.01
Interaction	6	2.71	>0.05
Error	132		

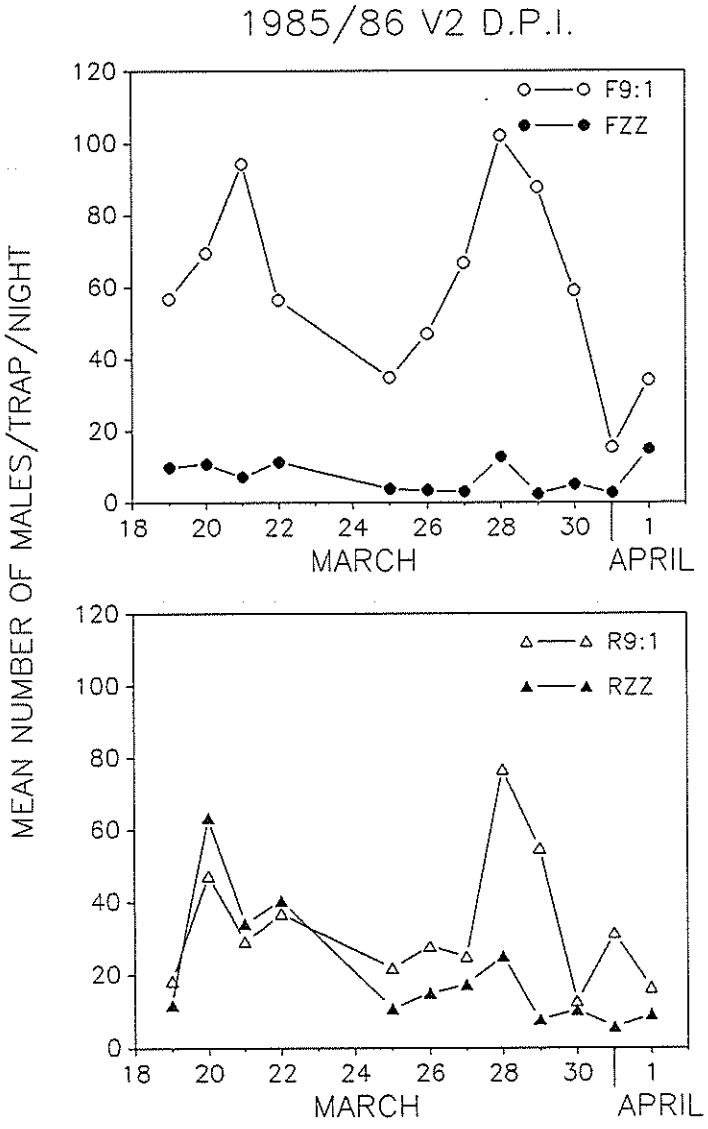

Fig. 4.4. Comparison of the number of male *P. scutigera* caught in pheromone traps baited with F9:1, FZZ, R9:1 and RZZ in field V2 (unsprayed cotton) at the D.P.I. Research Station, Biloela, during the 1985/86 season. Data for the F9:1 and FZZ baits have been plotted separately from the R9:1 and RZZ baits for clarity.

Table 4.4. Comparison of synthetic baits for monitoring male *P. scutigera*. Mean trap catch for all sites during the 1986/87 cotton season.

(A) Site 1

	Mean no. males/bait/night[1]				
Row	F9:1	FZZ	R9:1	RZZ	Row mean
1	42.1	3.9	22.2	21.6	22.4a
2	31.4	9.0	28.5	22.2	22.8a
3	29.5	5.5	23.5	15.3	18.4a
4	30.3	4.6	27.0	23.2	21.3a
$\bar{x}$	33.3a	5.7b	25.3c	20.6c	

ANOVA:

Source of variation	df	F ratio	p
Row	3	0.21	>0.05
Bait	3	21.66	<0.01
Interaction	9	0.29	>0.05
Error	256		

(B) Site 2

	Mean no. males/bait/night				
Row	F9:1	FZZ	R9:1	RZZ	Row mean
1	10.3	3.7	16.0	5.7	8.9a
2	24.3	2.2	5.3	5.8	9.4a
3	41.6	4.0	15.5	9.9	17.8a
$\bar{x}$	25.4a	3.3b	12.3c	7.1b	

ANOVA:

Source of variation	df	F ratio	p
Row	2	2.35	>0.05
Bait	3	14.14	<0.01
Interaction	6	1.14	>0.05
Error	132		

(C) V2 D.P.I. Research Station (unsprayed cotton)

	Mean no. males/bait/night				
Row	F9:1	FZZ	R9:1	RZZ	Row mean
1	22.7	0.8	9.7	13.1	11.6a
2	18.9	5.5	14.8	3.0	10.6a
$\bar{x}$	20.8a	3.2b	12.3c	8.1	

ANOVA:

Source of variation	df	F ratio	p
Row	1	0.16	>0.05
Bait	3	12.42	<0.01
Error	132		

1. Means within columns and rows followed by the same letter are not significantly different (L.S.D. test, p=0.05).

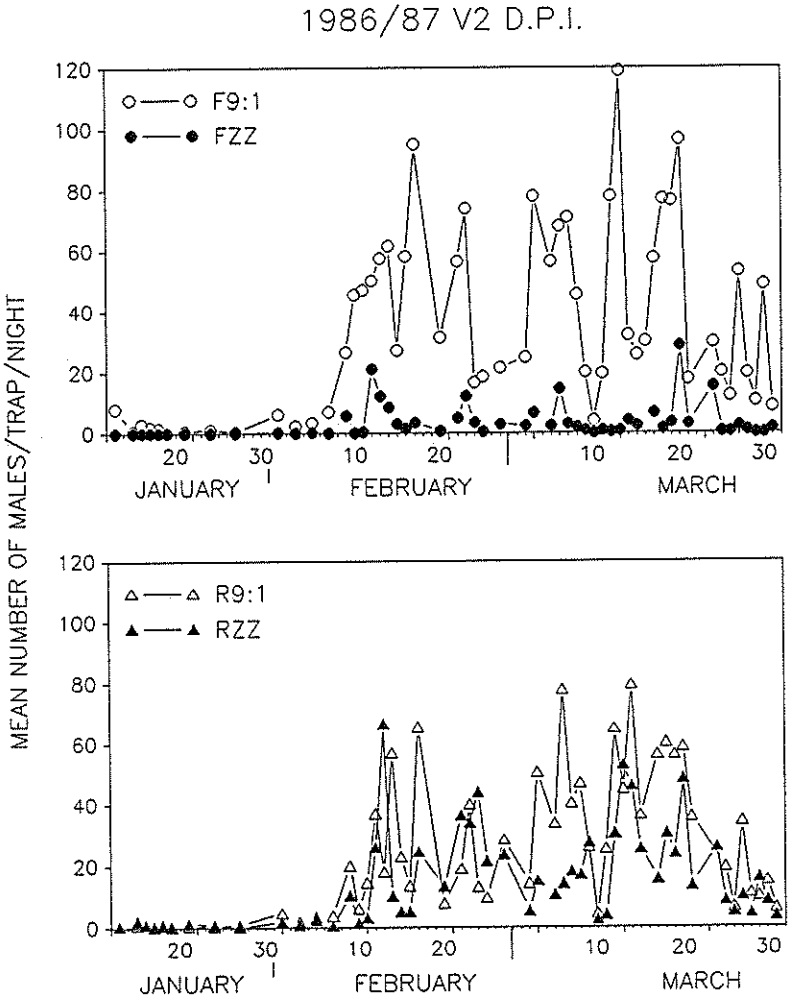

Fig. 4.5. Comparison of the number of male *P. scutigera* caught in pheromone traps baited with F9:1, FZZ, R9:1 and RZZ in field V2 (unsprayed cotton) at the D.P.I. Research Station, Biloela, during the 1986/87 season. Data for the F9:1 and FZZ baits have been plotted separately from the R9:1 and RZZ baits for clarity.

Table 4.5. Summary of bait comparison trials: the percentage trap catch for each bait type, field site and cotton season.

(A) Commercial cotton 1984/85

	% trap catch/bait			
Site	F9:1	FZZ	R9:1	RZZ
1	71.2	3.5	-	25.3
2	81.1	2.6	-	16.3
3	47.7	15.4	-	36.7
Mean	66.7	7.2	-	26.2
S.D.	17.2	7.1		10.3

(B) D.P.I. 1984/85

	% trap catch/bait			
Site	F9:1	FZZ	R9:1	RZZ
V2	8.0	6.2	42.6	43.3
K1	12.7	10.6	40.4	36.3
M1	11.3	10.3	25.5	52.9
Mean	10.7	9.0	36.2	44.2
S.D.	2.4	2.5	9.3	8.3

(C) 1985/86

	% trap catch/bait			
Site	F9:1	FZZ	R9:1	RZZ
1A	36.9	13.0	22.8	27.3
1B	36.4	10.3	28.8	27.9
2	36.1	10.9	29.7	23.3
3	52.3	6.7	16.8	24.3
4	28.7	16.6	26.8	24.5
V2	49.1	5.9	27.0	18.1
K1	37.4	12.5	28.7	21.3
Mean	39.6	10.8	25.8	23.8
S.D.	8.2	3.7	4.6	3.4

(D) 1986/87

	% trap catch/bait			
Site	F9:1	FZZ	R9:1	RZZ
1	34.2	10.9	29.3	25.6
2	53.7	6.4	25.5	14.5
V2	44.9	4.2	31.5	19.4
Mean	10.7	9.0	36.2	44.2
S.D.	9.8	3.4	3.0	5.6

44.3%, FZZ 7.2%, R9:1 28.8% and RZZ 19.8% (Table 4.5).

4.3.2. Comparison of pheromone trap design

The number of male *P. scutigera* caught in four pheromone trap designs was highly variable, but over the entire period the Delta trap caught significantly more males than all other trap designs ($p<0.05$) (Table 4.6). There was no significant difference in trap catch between the Pherocon, cup and FLT designs ($p>0.05$). The overall mean number of males/trap/night was 21.6 for Delta traps, compared to 8.9, 6.5 and 4.7 for the cup, Pherocon and FLT traps, respectively.

Trap capture efficiency was highest in Pherocon traps, which caught 18.2% of all *P. scutigera* males attracted to within 1 m of the trap, compared to 15.3%, 10.4% and 8.5% for Delta, cup and FLT traps, respectively. The mean number of moths attracted to within 1 m of Pherocon traps was low at 6.6 moths/10 minute observation compared to 23.6 and 25.8 moths/10 minute observation for Delta and FLT traps, respectively. Only 9.6 moths/10 minute observation were attracted to within 1 m of cup traps.

4.3.3. Effect of bait age

There was no significant difference in the number of males caught in traps containing baits aged between 0 (unexposed) and 8 weeks (ANOVA $F=0.52$, $df=4/20$, $p>0.05$). A total of 8,589 males were caught in all traps over the 5 night trapping period. The mean number of males/trap/night was 127.5, 104.8, 130.0, 106.9, and 103.4, for baits 0, 2, 4, 6 and 8 weeks old, respectively.

4.3.4. Effect of trap position

At Site 3 during the 1984/85 cotton season, pheromone traps placed 2 m outside the cotton crop caught significantly fewer males than traps placed within the centre of the crop (paired t-test, $t=5.383$, $df=15$, $p<0.001$). Centre traps caught a mean of 11.8 males/trap/night compared to 2.6 males/trap/night in traps situated outside the crop, over the 16 day test period. When traps placed 2 m outside the crop were moved into the first row of cotton, there was no significant difference in trap catch compared to centre traps over the following 18 nights (6.9 and 10.1 males/trap/night, respectively, $t=0.776$, $df=12$, $p>0.05$).

Table 4.6. Comparison of four pheromone trap designs for monitoring male *P. scutigera* over a 12 night period between 3-16 April, 1985. The number of replicates per trap design = 3.

	Mean no. males/trap design (± S.D.)			
Night	Delta	Pherocon	FLT	Cup
1	19.0 (10.6)	6.3 (1.2)	8.3 (0.6)	4.7 (4.0)
2	40.3 (22.3)	12.7 (11.0)	17.7 (4.9)	4.3 (2.9)
3	42.3 (41.9)	9.0 (3.5)	11.0 (14.9)	8.0 (4.4)
4	15.3 (17.0)	6.0 (2.6)	1.7 (2.1)	5.7 (3.5)
5	7.0 (6.9)	2.3 (0.6)	3.3 (0.6)	0.3 (0.6)
6	17.7 (17.8)	6.0 (4.3)	0.3 (0.6)	7.3 (4.7)
7	19.7 (19.1)	0.7 (1.1)	2.0 (1.7)	5.3 (3.2)
8	10.7 (15.1)	1.7 (2.1)	0.3 (0.6)	2.7 (2.3)
9	4.7 (0.6)	1.7 (1.5)	0.7 (1.1)	4.3 (6.7)
10	9.3 (4.2)	1.0 (1.0)	0	3.0 (2.6)
11	40.0 (7.0)	4.3 (2.5)	5.0 (7.8)	30.7 (10.2)
12	32.7 (17.2)	26.3 (27.5)	6.0 (7.8)	30.3 (9.9)
Mean[1]	21.6a	8.9b	6.5b	4.7b
± S.D.	(20.1)	(10.9)	(10.1)	(7.0)
% catch	51.8	21.4	15.6	11.3

1. Means followed by the same letter are not significantly different (L.S.D. test, P=0.05).

ANOVA: ($\log_{10}$ catch + 1 transformed data) F=7.91, df=3/44, p<0.001.

Table 4.7. Effect of pheromone trap position on the number of male *P. scutigera* caught at Site 1 over six trapping periods between 13 January and 5 March, 1987. Number of traps per row = 4.

Trap row	Distance from crop edge (m)	Mean no. of males/trap/period[1]						
		1	2	3	4	5	6	Mean (± S.D.)
1	1	9.4	1.6	1.3	39.2	25.7	17.0	15.7
2	100	15.0	1.4	1.9	37.9	22.1	17.5	15.9
3	200	14.7	1.7	1.0	39.5	20.6	16.0	15.6
4	300	10.9	1.1	1.4	43.3	20.9	17.2	15.8
Mean (± S.D.)		12.5	1.5	1.4	39.9	22.3	16.9	15.8

1. ANOVA: ($\log_{10}$ transformed data)
Row position F=0.495, df=3/15, p>0.05
Trapping period F=261.826, df=5/15, p<0.001

A similar effect on trap catch was seen in field K1 during the 1984/85 season. Significantly fewer males were caught in the row of traps placed on a fence 2 m outside the crop (Row 3), than in two rows placed within the crop ($p<0.05$) (Table 4.2). There was no significant difference in trap catch between the three rows (all placed within the cotton crop) in V2 during the same season, but row 4 in M1 caught significantly fewer males than the three other rows (Table 4.2).

In the other two cotton seasons, all rows of traps placed within the same field caught similar numbers of males, except field V2 during the 1985/86 season when row 3 caught significantly fewer males than rows 1 and 2 ($p<0.05$) (Table 4.2-4.4). This row of traps was situated in cotton planted approximately six weeks later than the main body of the field. No significant interaction between rows of traps placed within the same cotton field and bait types was evident in any field or season (Table 4.2-4.4).

At Site 1 during the 1986/87 cotton season, there was no significant difference in the number of males caught in traps situated along the crop edge (1 m within the crop) and traps placed 100, 200 and 300 m away from this crop edge ($p>0.05$) (Table 4.7). The number of males caught over the six trapping periods was significantly different ($p<0.01$) (Table 4.7).

4.3.5. Seasonal incidence of male *P. scutigera*

The seasonal incidence of male *P. scutigera*, caught in F9:1 baited traps, varied greatly between cotton fields and seasons (compare Figs. 3.4-3.12 presented in Chapter 3). Trap catch was lowest during the 1984/85 season and highest during the 1985/86 season.

The general pattern of trap catch was similar in both unsprayed and commercial cotton fields. Large numbers of males were caught on placing traps in the field during the spring. Trap catch was particularly high in late September, October and early November, before the availability of squaring cotton. Usually, trap catch declined in late November/early December, and remained at very low levels until late January/early February. At this time a sharp, well defined peak in trap catch occurred, after which trap catch stayed at high levels for the remainder of the trapping period.

The number of males caught in unsprayed cotton during spring was much lower than in

commercial cotton, particularly at Site 1 during the 1985/86 season (compare Figs. 3.5 and 3.10). During the 1985/86 season, two broad peaks in trap catch occurred in unsprayed cotton in early December and January which were not observed at Site 1. A sharp increase in trap catch was observed in both fields in early February, after which trap catch remained at very high levels. In the 1984/85 season, a very sharp increase in trap catch occurred simultaneously in all three commercial cotton fields at the end of January (Figs. 3.7-3.9). The peak was greatest at Site 2, where trap catch increased from 1 male/trap/night on 22 January to 58 males/trap/night on 26 January. A corresponding increase in trap catch was not observed in unsprayed cotton until early February (Fig. 3.4).

In February and March there was considerable daily variation in trap catch at all sites. Peaks in trap catch during these months were too closely spaced to represent successive generations of moths and could not be related to general changes in air temperature or rainfall. The distance between major peaks in pheromone trap catch, that were spaced at least 20 days apart, varied greatly from 191 DD to 743 DD (Table 4.8). The majority of peaks ranged between 350 and 500 DD apart. The longest distance between peaks occurred during the low period of moth activity in December and January when between 580 to 743 DD accumulated. The choice of pheromone peaks measured late in the cotton season was made entirely subjective by the large number of closely spaced peaks.

The application of insecticides usually affected trap catch for only a few days or not at all. For example at Site 1 during the 1985/86 season, an aerial application of pyrethroids (Deltamethrin plus Lambdacyhalothrin) was made on 7 February. Trap catch decreased from 48 to 2 males/trap/night after spraying but immediately increased again over the following days (Fig. 3.8). Another aerial application of the same insecticides on 17 February did not result in any decrease in pheromone trap catch.

4.3.6. Correlation between pheromone and light trap catch

The number of *P. scutigera* caught in a light trap, situated in unsprayed cotton during the 1986/87 season, ranged from 0 (17 January) to 103 (25 March) moths/night. Male and female catches generally increased together and were significantly correlated ($r=0.7$, $df=27$, $p<0.01$).

The correlation between the number of *P. scutigera* caught in pheromone and light traps

Table 4.8. Distance between major peaks in *P. scutigera* pheromone trap catch (>20 days apart), expressed in degree-days (DD) above 11.5°.

Site/season	Dates of pheromone peaks compared	Distance between peaks	
		Days	DD
V2 1984/85	3/11-11/12	38	487
	11/12-6/1	26	324
	6/1-11/2	36	537
	11/2-8/3	25	404
Site 1 1984/85	9/11-10/12	32	384
	10/12-26/1	46	714
	26/1-27/2	32	485
	27/2-28/3	29	404
Site 2 1984/85	6/11-11/12	35	452
	11/12-26/1	45	743
	26/1-22/2	27	404
	26/1-27/2	32	485
Site 3 1984/85	7/11-11/12	34	439
	11/12-27/1	48	728
	27/1-27/2	31	359
	9/2-6/3	25	403
	6/3-28/3	22	283
V2 1985/86	4/11-4/12	30	393
	4/12-3/1	30	493
	3/1-5/2	33	489
	5/2-9/3	31	457
Site 1 1985/86	4/11-3/12	29	380
	3/12-31/12	28	443
	31/12-8/2	39	580
	8/2-11/3	31	439
	11/3-12/4	31	409
Site 2 1985/86	6/2-10/3	32	457
	22/1-24/2	33	481
Site 3 1985/86	22/1-23/2	32	466
	23/2-16/3	21	273

Table 4.8. continued...

Site/season	Dates of pheromone peaks compared	Distance between peaks	
		Days	DD
V2 1986/87	25/10-28/11	34	381
	14/11-15/12	31	367
	15/12-13/1	29	450
	13/1-14/2	32	507
	14/2-13/3	27	390
Site 1 1986/87	6/10-14/11	39	410
	3/11-2/12	29	327
	2/12-15/1	44	643
	8/12-15/1	38	580
	15/12-15/1	31	482
	15/1-11/2	29	429
	11/2-6/3	23	336
Site 2 1986/87	19/11-12/12	23	270
	28/11-8/1	40	585
	4/12-8/1	35	513
	12/12-17/1	36	421

Table 4.9. Correlation between *P. scutigera* pheromone and light trap catch in unsprayed cotton over 29 nights during the 1986/87 season.

	Correlation coefficient (r)			
	Synthetic bait			
Light trap catch	F9:1	FZZ	R9:1	RZZ
Males	0.18	0.17	0.36	0.29
Females	0.02	-0.001	0.13	0.001

on the same night was very poor regardless of which synthetic attractant was used, particularly for female light trap catch (Table 4.9). Pheromone traps baited with R9:1 attractant gave the highest correlation coefficients of 0.36 and 0.13 for male and female light trap catch, respectively, but these r values were not significant ($p>0.05$) (Table 4.8).

4.3.7. Correlation between pheromone trap catch and larval infestation

The correlation between trap catch and larval infestation in commercial cotton fields using Method 1 was inconsistent between fields and seasons. At Site 1 significant correlations were obtained in the 1984/85 and 1986/87 season but not in the 1985/86 season. Within seasons, correlations were inconsistent between fields. For example in the 1984/85 season, significant correlations were obtained at Site 1, but at Site 2 and 3 the correlation between trap catch and larval infestation was very poor (Table 4.11). In the 1985/86 season, the converse was true: Site 2 and 3 gave significant correlations but Site 1 did not. During this season, correlation was improved in all fields when larval infestation at the crop edge was used in the calculations, rather than the mean rate of infestation for the entire field, or infestation at the mid-crop region (see Chapter 3 for further details on larval sampling procedures).

In the 1986/87 season very high correlation coefficients were obtained at Site 1. Values of r increased when larval infestation at the crop edge only was considered, and when only numbers of first instar larvae were used. At Site 2, correlation coefficients were very low and often negative (Table 4.11).

In unsprayed cotton, significant correlations were obtained in all three seasons (Table 4.10). In the 1984/85 season, correlations were only significant when total larval infestation was correlated with pheromone trap catch. In the 1986/87 season correlations were improved when the numbers of first instar larvae or first plus second instars was used. Similarly, in the 1985/86 season correlations were improved when the number of young larvae found in incubated cotton bolls was used, as opposed to total larval numbers (Table 4.10).

Generally, the longer the period over which pheromone trap catch was accumulated prior to boll sampling, the higher the correlation with larval infestation.

Method 2 of correlating trap catch and larval infestation, where a delay of 74.3 and 116.6

Table 4.10. Correlation between *P. scutigera* pheromone trap catch and larval infestation in unsprayed cotton using Method 1 (see section 4.2.7 for details). N = number of data sets correlated.

		Correlation coefficient[1]				
		No. days trap catch accumulated				
Larval infestation	N	-3	-5	-7	-9	-11
1984/84						
All larvae	12	**0.67**	**0.80**	**0.84**	**0.86**	**0.88**
1st instars only		-0.48	-0.30	-0.19	-0.16	0.05
1st + 2nd instars		0.01	0.22	0.32	0.28	0.56
1985/86						
All larvae	13	0.52	**0.57**	**0.68**	**0.75**	**0.77**
Young larvae only		0.52	0.55	**0.64**	**0.70**	**0.73**
1986/87						
All larvae	9	**0.70**	**0.73**	**0.74**	**0.80**	**0.80**
1st instars only		**0.82**	**0.83**	**0.82**	**0.85**	**0.85**
1st + 2nd instars		**0.87**	**0.87**	**0.86**	**0.88**	**0.89**

1. Bold type indicates a significant correlation coefficient ($p<0.05$, df=N-2).

Table 4.11. Correlation between *P. scutigera* pheromone trap catch and larval infestation in commercial cotton using Method 1 (see section 4.2.7 for details). N = number of data sets correlated.

			Correlation coefficient[1]				
			No. days trap catch accumulated				
Site	Larval infestation	N	-3	-5	-7	-9	-11
1984/85							
Site 1	Mean infestation	9	**0.68**	0.65	0.52	0.66	**0.71**
Site 2	" "	9	-0.27	-0.18	-0.09	0.16	0.19
Site 3	" "	11	0.15	0.20	0.27	0.29	0.17
1985/86							
Site 1	Mean infestation	12	0.10	0.36	0.36	0.42	0.48
	Crop edge all larvae		0.20	0.43	0.43	0.47	0.52
	" young larvae		0.16	0.39	0.39	0.46	0.49
	Mid-crop all larvae		0.02	0.21	0.18	0.23	0.30
	" young larvae		0.07	0.28	0.28	0.29	0.25
Site 2	Mean infestation	11	**0.64**	**0.65**	**0.69**	**0.65**	**0.65**
	Crop edge all larvae		**0.73**	**0.74**	**0.76**	**0.73**	**0.74**
	" young larvae		0.56	0.59	0.60	0.59	**0.61**
	Mid-crop all larvae		0.41	0.42	0.44	0.40	0.40
	" young larvae		0.40	0.40	0.40	0.36	0.36
Site 3	Mean infestation	11	0.58	**0.62**	**0.65**	**0.67**	**0.64**
	Crop edge all larvae		**0.73**	**0.73**	**0.74**	**0.73**	**0.71**
	" young larvae		0.35	0.40	0.43	0.43	0.44
	Mid-crop all larvae		0.50	0.57	**0.61**	**0.63**	**0.62**
	" young larvae		0.42	0.46	0.51	0.56	0.53
1986/87							
Site 1	Mean infestation	8	0.70	**0.78**	**0.89**	**0.88**	**0.87**
	Crop edge all larvae		0.64	**0.77**	**0.90**	**0.89**	**0.87**
	" 1sts only		**0.91**	**0.88**	**0.77**	0.63	0.59
	" 1sts + 2nds		0.82	**0.87**	**0.88**	**0.80**	**0.77**
	Mid-crop all instars		0.50	0.49	0.70	**0.79**	**0.82**
	" 1sts only		0.39	0.48	0.54	0.49	0.46
	" 1sts + 2nds		0.32	0.45	0.50	0.46	0.44
Site 2	All instars	6	-0.19	0.11	0.09	-0.29	-0.42
	1st instars only		-0.63	-0.30	-0.26	**-0.98**	**-0.96**
	1st + 2nd instars		-0.37	-0.06	-0.07	-0.70	-0.79

1. Bold type indicates a significant correlation coefficient ($p<0.05$, df=N-2).

Table 4.12. Correlation between *P. scutigera* pheromone trap catch and larval infestation during the 1986/87 season using Method 2 (see section 4.2.7 for details). N = number of data sets correlated.

Site	Delay in DD	N	Correlation coefficient (r)[1] No. days trap catch accumulated -1	-3	-5	-7
V2 D.P.I.	74.3	9	**0.82**	**0.80**	**0.76**	**0.73**
	116.6		**0.82**	**0.76**	**0.79**	**0.81**
Site 1 Crop edge	74.3	8	0.60	0.61	0.46	0.42
	116.6		0.59	0.56	0.39	0.11
Mid-crop	74.3	8	0.67	0.72	0.53	0.47
	116.6		0.52	0.42	0.29	0.44
Site 3	74.3	6	0.09	-0.02	-0.19	-0.29
	116.6		-0.64	-0.40	-0.43	-0.35

1. Bold type indicates a significant correlation coefficient ($p<0.05$, df=N-2).

DD was included to allow for a preoviposition period and/or egg development, did not improve correlation coefficients in either unsprayed or commercial cotton fields (Table 4.12

4.4. Discussion

The seasonal incidence of male *P. scutigera* observed in this study agrees closely with that reported by Murray *et al.* (1982), Page *et al.* (1984a, 1984b) and Rothschild (1983), and is discussed further in Chapter 8. In support of Rothschild (1983), there was no consistent correlation between pheromone trap catch and larval infestation. In commercial cotton fields, high pheromone trap catch was not always associated with a subsequent increase in larval infestation. This was also true in unsprayed cotton early in the season before plants produced bolls.

Many factors may have been responsible for the inconsistent relationship between pheromone trap catch and larval infestation. Pheromone traps are probably much more efficient at detecting the presence of low density *P. scutigera* populations in a crop than current larval sampling techniques (Chapter 3). Early in the cotton season, larval survival is probably very poor due to the unsuitability of seedling and squaring cotton plants to support larvae. Hence, while a high pheromone trap catch may indicate the presence of egg-laying females in the crop, the poor survival of larvae does not lead to a detectable infestation. Larvae survival will be further limited in commercial cotton fields due to intense spraying of insecticides during this stage of cotton, mainly against *Helicoverpa* spp. and sap-sucking bugs (Chapter 3).

The application of insecticide sprays often had little or no impact on the number of male *P. scutigera* caught in pheromone traps; a phenomenon also noted by Flint and Stone (1985). This may indicate that males caught in pheromone traps originated from areas outside the crop and/or emerged from locations within the crop inadequately covered by insecticide. The site of pupation in *P. scutigera* is mainly at the base of cotton plants (Chapter 3) where insecticide penetration is poor (Uk and Courshee 1982). The presence of high larval populations along the crop edge of some commercial cotton fields, as described in Chapter 3,

was thought to have be due to poor insecticide coverage, and may have been another source of males caught in pheromone traps. This will be discussed further in Chapter 8.

The continuous capture of male *P. scutigera* in pheromone traps immediately following the application of insecticides, but the absence of a corresponding increase in larval populations, may indicate that spray residues were effective at reducing subsequent population events such as reproduction, oviposition, egg hatch and/or larval infestation. The sublethal effects of insecticides on *P. scutigera* mating and behaviour are unknown. Although Floyd and Crowder (1981) reported no effect of sub-lethal doses of permethrin on the mating ability of male *P. gossypiella* when confined to small cages with females, Haynes and Baker (1985) found that similar concentrations of permethrin did affect the mate-locating behaviours of males and the rate of pheromone release in females.

Female *P. scutigera* may be more susceptible to insecticide residues remaining on cotton plants than males through increased exposure during oviposition. As reported in Chapter 3, female *P. scutigera* appear to actively search over cotton plants for suitable oviposition sites. Perhaps more importantly, or in conjunction with this effect on females, eggs are very rarely laid on the site of larval infestation (Chapter 3), forcing the neonate larva to search over the cotton plant for food and increasing its' exposure to insecticide residues. The period over which insecticides remain effective varies according to the species of insect, type of chemical used, environmental conditions and the growth rate of the crop (Wilson *et al.* 1983). Wilson (1984) found that cotton leaves sprayed with insecticides, such as pyrethroids, remained 90% effective at killing small *Helicoverpa* spp. larvae for up to 10 days at 25^{o} C.

The significance of the very poor correlation between pheromone and light trap catch is unknown. More data is needed to compare the two trapping methods and to verify whether light traps are any better at indicating actual moth populations in the field than pheromone traps. A comparison of pheromone and light trap catch is complicated by the differential effect of environmental variables, such as moonlight, cloud cover, wind speed and temperature, on the trapping efficiency of both methods (cf. Dent and Pawar 1982, Kaae and Shorey 1973, McGeachie 1989).

Within fields, trap catch varied considerably between replicate traps and between nights.

Rothschild (1983) also noted considerable daily fluctuations in *P. scutigera* pheromone trap catch which could not be related to air temperature during the main nocturnal flight period of males, or the lunar cycle. This variability, together with the inconsistent relationship with larval infestation make it impossible to use a threshold trap catch for timing insecticide sprays against *P. scutigera* as suggested by Page *et al.* (1984a). The measurement of the distance between major peaks in pheromone trap catch gave variable results. The majority of peaks selected measured between 350 and 500 DD (above 11.5^{o} C) apart which is shorter than the expected generation time of 1031 and 555 DD for *P. scutigera* feeding on cotton squares and bolls, respectively (Chapter 2). Selection of peaks was subjective due to the large number of closely spaced peaks in some fields, particularly at the end of the cotton season.

Again many factors may have caused variability in pheromone trap catch e.g. interaction between closely spaced traps, competition with feral females, and trap location. The effect of trap interaction was not analyzed in this study but some authors have found that upwind traps often suppress the number of males caught in downwind traps (Elkington and Cardé 1988, Perry and Wall 1984, Wall and Perry 1978, 1980). Several studies have shown that the efficiency of pheromone traps declines with increasing insect density and this has been attributed to competition with feral females (Croft *et al.* 1986, Howell 1974, Knight and Croft 1987, Minks and DeJong 1975, Riedl *et al.* 1976).

The effect of trap location within rows was minimized by rotating traps each time the number of males caught was checked. Row position did not significantly affect trap catch except when traps were placed outside the crop or in younger cotton. Rothschild (1983) found no significant difference between total catches of *P. scutigera* in trap lines set 'within or along the edge' of a block of cotton. Unfortunately, it is unclear from his paper whether the edge traps were placed within the last row of cotton or actually outside the crop. Page *et al.* (1984a) interpreted the paper as meaning traps were placed outside the crop, and subsequently placed their traps 1 m from the edge of the cotton. The results from this study imply the opposite interpretation.

The conflicting results obtained by Rothschild (1975, 1983) and Flint and Stone (1985),

concerning the attractancy of baits to male *P. scutigera*, were confirmed in this study. When attractants obtained from Dr Rothschild were used, there was no significant difference in the number of males caught in pheromone traps baited with the Z,Z- isomer alone, or with a 9:1 mix of the Z,Z- and Z,E- isomers. In contrast, excluding the anomalous results from the 1984/85 D.P.I. trials, the Z,Z- isomer alone obtained from Dr Flint caught significantly fewer males than a 9:1 mix of Z,Z- and Z,E isomers.

The most plausible explanation for these results is the difference between the purity of attractants obtained by each author. The Z,Z- isomer used by Dr Flint was comparatively pure (98.8%) and was known to contain 1.2% of the Z,E- isomer as contaminant. Dr Rothschild's source of Z,Z- isomer was less pure (95%). The amount of Z,E- present as a contaminant was not known but may have been as high as 5%. If high levels of the Z,E- isomer as contaminant were present in RZZ then this would explain it's greater attractiveness to male *P. scutigera* than the more pure FZZ baits.

As F9:1 baited traps consistently caught significantly more males than R9:1 baited traps (except in the 1984/85 D.P.I. trials), the optimum ratio of the Z,Z- and Z,E- isomers for attracting *P. scutigera* may be more critical than previously thought. Rothschild (1975, 1983) found no significant difference in trap catch when 1, 10 or 100 ug of Z,E- isomer was added to 1000 ug of Z,Z- isomer but these tests need to be repeated using more pure formulations of the Z,Z- isomer.

The results obtained in the 1984/85 D.P.I. trials, where F9:1 baited traps did not catch significantly more males than FZZ baited traps, are considered unrepresentative of normal F9:1 trap catch and due to experimental error. Unfortunately, the cause of this error is unknown. Baits used in D.P.I. trials originated from the same batch as those used in the 1984/85 commercial cotton trials, where F9:1 baited traps caught significantly more males than FZZ and RZZ baited traps. The density of traps placed in the D.P.I. fields was too low to have caused disruption of sexual communication in *P. scutigera*. Placement of fresh baits in the same fields, at similar trap densities, during the next two cotton seasons did not give the same anomalous results. The possibility that F9:1 baits were mistaken for FZZ baits can be discounted as not enough FZZ baits were available during this season to allow twice the

intended number of traps to be baited. The only possible explanation for the unusual result is that the F9:1 baits used in the D.P.I. trials were inadvertently contaminated with an unknown substance, thus reducing their attractiveness to male *P. scutigera.*

During the course of this study the sex pheromone of *P. scutigera* was identified by Dr C. Whittle, Division of Entomology, C.S.I.R.O., Canberra. Both the Z,Z- and Z,E- isomers of 7,11 hexadecadienyl acetate were found. Most individuals contained 13-14% of the Z,E-isomer together with the main Z,Z- component, with only a 1-2% deviation between females originating from different host plants (G. H. L. Rothschild pers. comm.). Minor trace components, of undisclosed identity, were also detected in the female pheromone. In preliminary trapping trials, conducted at the end of the 1986/87 cotton season, the addition of the trace components did not significantly increase trap capture (unpublished results).

With the identification of the female pheromone there is a need to repeat trials conducted by Rothschild (1975) where the attractiveness of Z,Z- baits with different amounts of Z,E-isomer added was compared. Until such work is completed it is recommended that baits used for male monitoring *P. scutigera* are standardized to contain both the Z,Z- and Z,E- isomers in a 9:1 ratio.

The release rate of synthetic pheromone from baits used in this study was not measured. Baits containing a 9:1 mix of Z,Z- and Z,E- isomers obtained from Dr Flint remained suitable for monitoring *P. scutigera* for at least eight weeks in the field. Flint *et al.* (1978) reported that baits for monitoring *P. gossypiella*, containing 1:1 mix of the same isomers at the same concentration, produced a 1st-order release curve and had an average half-life of 120 days in the field.

Of the four pheromone trap designs compared, the Delta trap was the most suitable for monitoring male *P. scutigera* populations. The Delta trap was the most efficient design at attracting and capturing male moths. Although the funnel live-trap (FLT) attracted a similar number of males to within 1 m of the trap, the Delta trap had a much higher capture efficiency. Moths approaching FLTs rarely entered the traps and those that did readily escaped. In Delta traps, more approaching males entered traps, usually by landing on the

folded ends of the trap opening and walking inside. Very few of the moths observed entering Delta traps escaped.

Lingren *et al.* (1980) also found that both trap types attracted a similar number of *P. gossypiella* males but their FLTs had a higher capture efficiency (38%) than Delta traps (23%). While the FLT design reported in their paper was closely followed it is possible that slight differences in the materials used to construct traps and/or trap dimensions, may have affected the capture efficiency of FLTs used in this study. Alternatively, differences in the behaviour of the two moth species may have contributed to the low catches of *P. scutigera* in FLT traps.

The capture efficiency of Delta traps (15.3%) is lower than that reported in other studies using the same or a similar trap design. As already stated, Lingren *et al.* (1980) found that Delta traps were 23% efficient at catching male *P. gossypiella.* Lewis and Macaulay (1976) reported that triangular traps, similar to the Delta trap design, caught 21-29% of pea moths, *Cydia nigricana* (Steph), attracted to within 2 m of a trap. The low capture efficiency of Delta traps in this study may reflect that an inferior synthetic attractant (RZZ) was used.

Lewis and Macaulay (1976) related the efficiency of a pheromone trap design to attract *C. nigricana*, on the shape of the pheromone plume emitted. They found that the most efficient trap design, the triangular trap, emitted a long thin plume which persisted even in partial cross winds. A covered funnel trap, similar to the FLT used in this study, produced a much broader plume that retained a directional element what ever the wind direction. In contrast, the plume from a 'Pherocon' 1C trap was erratic, being elongated when the wind blew directly lengthwise but diffuse in partial cross winds.

The colour of each pheromone trap design was not standardized and this may have influenced the number of males approaching or landing on a trap. The effect of trap colour has rarely been investigated (Cardé and Elkington 1984, Wall 1989), although some studies have shown that colour can significantly affect the number of insects trapped (McLaughlin *et al.* 1975, Mitchell *et al.* 1989).

For monitoring purposes, where the foremost concern is sensitivity of the pheromone trap to detect the presence *P. scutigera*, the Delta trap was the most suitable design tested. The

plastic cup trap design, previously used in some studies (e.g. Page *et al.* 1982), is not as sensitive as the Delta trap in detecting the presence of *P. scutigera* and its use should be discontinued.

The Delta trap, however, is not a suitable design for studying the population dynamics of *P. scutigera* due to rapid trap saturation under high moth densities. When more than approximately 80 moths were caught within in a single night the wing and body scales of trapped moths covered the layer of sticky glue, thus preventing the retention of moths subsequently entering the trap. Trap saturation may have caused the difference in the attractancy of F9:1 and FZZ baited traps to have been underestimated. The difference in trap catch varied considerably from 1.7 to 30.9 more moths in F9:1 baited traps than FZZ baited traps. It can be envisaged that under high moth populations F9:1 baited traps would quickly reach the point of saturation where no or very few of the moths subsequently attracted to the traps are retained. Over the same period, the less attractive FZZ baited traps would continue to accumulate moths, albeit at a slower rate. Consequently, the difference in the number of moths caught by each bait would decrease the longer the period over which F9:1 traps remained saturated. In order to make more accurate comparisons of the attractancy of pheromone baits, it may be necessary to omit data whenever trap saturation occurs or to use an alternative trap design.

A more suitable pheromone trap design for studying *P. scutigera* population dynamics may be the Huber oil trap (Huber and Hoffman 1979) which employs a liquid rather than a sticky surface to retain moths. Huber and Hoffman (1979) found that the oil trap caught on average six times more male *P. gossypiella* than the Delta trap.

CHAPTER 5

Alternative host plants of *P. scutigera* in central Queensland

5.1. Introduction

Alternative host plants are considered to play a minor role, if any, in the carry-over of *P. scutigera* populations from one cotton season to the next, or as a direct source of moths infesting cotton (Chapter 1). *H. tiliaceus* is the only known alternative host plant of *P. scutigera* in the cotton growing regions of Central Queensland, where it is not native but has been planted as a shade tree in some parks and gardens. As so few trees exist it is unlikely to act as a source of moths infesting cotton. Furthermore, *P. scutigera* infests *H. tiliaceus* in Emerald where it is not a pest of cotton (D. Murray unpublished report).

There is indirect evidence, however, to suggest that other host plants of *P. scutigera* may exist in Central Queensland. Firstly, peak emergence of *P. scutigera* from overwintering larvae in cotton bolls occurs during September and October when there is no suitable cotton to infest (Chapter 7, Sabine 1969a). Sabine (1969a) termed this emergence as 'ineffective', in the apparent absence of any alternative host plants, because no new generation of *P. scutigera* could be established. This closely parallels the so-called 'suicidal' emergence of *P. gossypiella* in the U.S.A., which also emerges from overwintering larvae before the availability of squaring cotton plants (Fife 1961, Bariola 1983). It is possible that the emergence of *P. scutigera* from overwintering larvae may in fact coincide with the fruiting period of an unidentified host plant on which moths can complete a new generation and then infest squaring cotton crops.

Secondly, pheromone trap surveys by Page *et al.* (1982, 1984a) revealed that *P. scutigera* is widely distributed throughout Central and S.E. Queensland, in areas where it is not known to infest cotton (Fig. 1). Unless moths are capable of long distance migration, populations must be established on alternative host plants in these areas. The identity of such hosts is

unknown although *H. rosa-sinensis*, which has only recently been discovered as a host of *P. scutigera* (Sabine pers. comm.), and *H. tiliaceus* are now widely distributed throughout the state. The role of *H. rosa-sinensis* in the population dynamics of *P. scutigera* in Central Queensland has not been addressed.

Past surveys for alternative hosts of *P. scutigera* have concentrated on plants belonging to the same family as cotton; the Malvaceae (Holdaway 1926, Sabine 1969b, Tryon 1926). There is no evidence to suggest that *P. scutigera* is restricted to this plant family. The closely related *P. gossypiella*, although mainly confined to Malvaceous plants, has a very wide host range infesting 70 species of plants belonging to 7 families (Shiller *et al.* 1962).

The aim of this chapter is to report on surveys conducted to search for alternative hosts plants of *P. scutigera* in Central Queensland and to assess their role in the population dynamics of moths infesting cotton.

5.2. Materials and methods

Searches for alternative host plants of *P. scutigera* were conducted around the Callide and Dawson Valleys and Bauhinia Downs (see Fig. 3.1 for map of area), mainly during the period of peak emergence of moths from overwintering larvae and before the availability of cotton plants bearing bolls (September-December). Initially, searches were concentrated near cotton fields where severe *P. scutigera* infestations were known to occur each season. Fruits and seed capsules of any common species growing on the borders of or within cotton fields were sampled. After it was discovered that *P. scutigera* readily infested the fallen flowers of *H. rosa-sinensis* and *H. tiliaceus*, searches were broadened to include any common native or ornamental plants which flowered prolifically between September and December.

Samples of flower buds, flowers, fruits or seed capsules were collected from the field and searched immediately for larvae. If no larvae were found further samples were collected and incubated in ventilated plastic boxes for at least 1 week to ensure no first instar larvae were missed.

The number and location of *H. tiliaceus*, *H. rosa-sinensis*, and other *P. scutigera* host plants discovered during the course of surveys, was determined in each area. Samples of fallen flowers and/or seed capsules from these species were made at irregular intervals throughout the period of study in Central Queensland, between August or September and June, 1984-1987.

Parasitoids bred from *P. scutigera* larvae infesting each host plant were identified by Dr B. Cantrell, Entomology Branch, Queensland Department of Primary Industries, Brisbane, and his assistance is gratefully acknowledged. Representative specimens of parasitoids have been deposited at the University of Queensland Insect Collection, Brisbane.

5.3. Results

Four new alternative host plants of *P. scutigera* were discovered during this study, only one of which, *Gossypium sturtianum* J. H. Willis, belongs to the family Malvaceae. Two species, *Brachychiton australis* (Schott et Endl) and *B. populneus* (Schott et Endl), belong to the Sterculiceae, while the other, *Prunus persicae* L. (Peach), is a member of the Rosaceae. *P. scutigera* were also recorded infesting *H. rosa-sinensis* and *Hibiscus cannabinus* L. (Kenaf) in Central Queensland for the first time.

Another previously unrecorded host plant of *P. scutigera*, *Hibiscus sabdariffa* L. (Roselle), was noticed in the insect collection of the D.P.I. Research Station, Biloela. Mr F. D. Page bred 2 moths from larvae infesting the fruit of cultivated *H. sabdariffa* growing in Biloela, on 18 and 21 June, 1981. No *H. sabdariffa* plants were encountered in the Biloela area during this study. Appendix I lists all the species of plants examined in which *P. scutigera* were not found, giving the time of year, location and plant part of each species sampled.

The vegetation of Central Queensland forms part of the brigalow belt; areas of open-forest and woodland communities dominated by brigalow, *Acacia harpophylla* F. Muell. ex Benth. (Johnson 1984). The most speciose genera in the brigalow belt are *Acacia* (*ca* 120 spp.) and *Eucalyptus* (*ca* 90 spp.), which determine the character of most vegetation types of the region.

Generally, the only areas of natural vegetation surviving in the vicinity of irrigated cotton fields in the Callide and Dawson Valleys were along creeks or amongst areas left to cattle

grazing. Plant diversity in these areas was very low being highly disturbed by logging, fire, grazing and the invasion of exotic weeds. Areas were dominated by various *Eucalyptus*, *Acacia* and grass species, while *Argemone ochrolemea* Sweet, *Ricinus communis* L., and *Ficus* spp. were abundant in some areas. Other relatively abundant weeds were: *Datura stramonium* L., *Sesbania cannabina* (Retz.) Poiret and *Kalanchoe tubiflora* (Harvey) Hamet. None was ever found to be infested by *P. scutigera* larvae.

The only host plant of *P. scutigera* that occurred near cotton fields was *B. populneus*, although ornamental *B. australis* trees were often planted on the properties of cotton farmers, particularly at Gibber Gunyah near Theodore. The only Malvaceous plants encountered in or around cotton fields were *Malva parviflora* L., *Sida cordifolia* L. and *Hibiscus trionum* L.; all were scarce and never infested by *P. scutigera.*

The only native species of *Hibiscus* encountered (other than *H. tiliaceus*) was *H. divaricartus* R. Grah.. This shrub was infrequently found on the border of forests in ranges surrounding the Callide valley, but never near cotton fields. Although it was listed as a host plant of *P. scutigera* by Holdaway (1926) only larvae of the closely related *P. endema* were found.

When searches for host plants were broadened to include any common flowering plants, the flowers of ornamental *Bauhinia* sp., *Brachychiton rupestris* Benth., *Jacaranda mimosifolia* D. Don, *Rosa* sp. and *Spathodea campanulata* P. Beaux growing in parks and gardens around Biloela were sampled but no *P. scutigera* larvae were found. Species of plants infested by *P. scutigera* are detailed separately below.

5.3.1. *Hibiscus tiliaceus*

Thirty-nine *H. tiliaceus* trees were located in Biloela, of which 32 trees grew in an abandoned caravan park. Only 3 trees could be found in Theodore, and another 2 trees were located on a property near a cotton farm (10 km north of Biloela) and in the township of Jambin.

While *H. tiliaceus* trees can grow up to 10 m in height (Chin 1986), nearly all the trees located in Biloela were small at less than 3 m tall and flowered very sporadically. Flowering

occurred throughout all months of observation, except September and early October. Initially, only the seed capsules were sampled but after it was discovered that *P. scutigera* readily infested the flowers of *H. rosa-sinensis*, fallen flowers of *H. tiliaceus* were sampled from May, 1985.

The seed capsules and flowers of all trees sampled in Biloela and Theodore yielded *P. scutigera* on all sampling occasions (N = 11 trees). Two large trees growing in private gardens were sampled most frequently as they consistently produced more fruiting structures than other smaller trees. Table 5.1 gives the rate of infestation of a selected number of *H. tiliaceus* trees in Biloela and Theodore sampled at irregular intervals between 1984 and 1987.

The seed capsules of *H. tiliaceus* measure about 3 cm in length and have thick, dry walls, densely covered by short bristly hairs. The yellow flowers measure 6 cm in length and remain open for 1-2 days before turning orange-red and then falling to the ground. Fallen flowers were always more highly infested with *P. scutigera* larvae than seed capsules, ranging from 20 to 119 larvae/100 flowers compared to 0 to 42.1 larvae/100 seed capsules (Table 5.1).

Larvae were found inside new and old fallen flowers regardless of their condition; whether they were completely dry or saturated with water after rain. All larval instars were encountered inside fallen flowers but only 1 pupa was ever found. Up to 6 larvae were found in a single fallen flower. Larvae appeared to feed only on the petals and not on any part of the staminal column, pollen, anthers or ovaries. Feeding and development was arrested if the flowers dried out. Larvae confined to dry flowers in the laboratory became visibly dehydrated but third and fourth instars were capable of surviving in this condition for long periods (at least 6 weeks). Feeding and development was resumed if the flowers were sufficiently moistened with water or if the dehydrated larvae were transferred onto fresh flowers or artificial diet. (Chapter 2 gives the rate of development for larvae reared on fresh *H. tiliaceus* flowers incubated under constant temperature and photoperiod conditions).

P. scutigera larvae infesting fallen flowers were often parasitized at levels ranging up to 48%, but parasitoids were absent in larvae collected from seed capsules (Table 5.1). All parasitoids were identified as *Apanteles* sp. (Braconidae) except for a single specimen of a *Eriborus* sp. (Ichneumonidae) collected from Theodore on 19 December, 1986. The species of *Apanteles*

Table 5.1. Infestation of *H. tiliaceus* seed capsules and fallen flowers by *P. scutigera* larvae in Biloela (two trees sampled) and Theodore (three trees sampled) between 1984 and 1987.

SEED CAPSULES

Locality	Date sampled	No. seed capsules sampled	No. larvae /100 seed capsules	% larval parasitism
Biloela	22/10/84	20	0	NR[1]
	9/1/85	25	20.0	0
	16/1/85	19	42.1	0
	22/2/85	22	13.6	0
	4/6/85	96	11.5	0
	22/11/85	22	13.6	0
	18/3/86	68	0	0
Theodore	31/12/86	150	4.0	0
	16/1/87	100	6.0	0
	31/3/87	50	12.0	0

FLOWERS

Locality	Date sampled	No. flowers sampled	No. larvae /100 flowers	% larval parasitism
Biloela	16/5/85	92	108.7	NR
	4/6/85	150	25.0	48.0
	30/10/85	67	103.7	10.7
	11/11/85	54	55.6	3.3
	22/11/85	100	49.0	10.2
	27/11/85	100	20.0	0
	8/12/85	100	25.0	8.0
	24/12/85	70	20.0	0
	31/12/85	80	30.0	0
	17/1/86	90	40.0	0
	24/1/86	80	45.0	0
	5/2/86	29	44.8	3.5
	14/2/86	48	35.4	5.9
	18/3/86	77	61.0	1.3
Theodore	19/12/86	350	74.2	6.2
	31/12/86	270	49.2	4.7
	9/1/87	155	51.8	3.4
	16/1/87	100	17.0	11.8
	27/2/87	80	22.5	4.5
	31/3/87	100	48.0	0
	24/4/87	100	119.0	3.3
	5/5/87	64	76.0	6.3

1. NR = not recorded.

looked similar to the one found parasitizing *P. scutigera* in cotton (Chapter 3).

Branches (20 cm long), bearing flower buds, open flowers and seed capsules, were randomly sampled from trees in Biloela and all parts searched for eggs and larvae. No eggs were found on ten branches sampled in December, 1984, but six first instar larvae were found infesting seed capsules and flower buds. Two eggs were found in two samples of ten branches collected from different trees in January, 1985. One egg was laid on rough bark near a small leaf bud while the other egg was laid directly inside a leaf bud, in between the unfolding leaves. Six first instar larvae were found infesting seed capsules and one larva was located inside a flower bud. No eggs were found in a sub-sample of fallen flowers (N = 50) collected on 16 May, 1985, although they were highly infested with larvae.

5.3.2. *Hibiscus rosa-sinensis*

Sixteen *H. rosa-sinensis* bushes were found along street paths and parks in the township of Biloela although more probably exist in private gardens. Another 22 small bushes were found in the grounds of the D.P.I. Research Station, Biloela. Only three *H. rosa-sinensis* bushes could be found in the township of Theodore.

H. rosa-sinensis is thought to have originated from China or Vietnam and was probably introduced into Australia after World War II (Chin 1986). It is an evergreen shrub, varying from two to six m in height. Many different varieties exist; the bushes located in Biloela were mainly of the open-branched upright varieties bearing single, reflexed-shaped flowers. A few double-flowered varieties were present, as well as the 'Chinese-Lantern' variety with small tunnel-shaped flowers. All bushes flowered very sporadically during all months of observation.

None of the bushes located set seeds which is typical of *H. rosa-sinensis* growing in tropical regions (Chin 1986). *P. scutigera* larvae were found infesting fallen flowers of all *H. rosa-sinensis* bushes sampled (N = 13), except the variety 'Chinese Lantern'. A single *H. rosa-sinensis* bush growing in the grounds of the D.P.I. Biloela Research Station was most frequently sampled as it was the largest and most prolifically flowering bush located. Larvae were found infesting flowers on every sampling occasion except 6 February, 1986, when the

Table 5.2. Infestation of *H. rosa-sinensis* fallen flowers by *P. scutigera* larvae at the D.P.I. Research Station, Biloela, between 1985 and 1987.

Date sampled	No. flowers sampled	No. larvae /100 flowers	% larval parasitism
9/9/85	50	28.0	0
15/9/85	50	46.0	0
25/9/85	80	57.5	0
4/10/85	50	72.0	0
9/10/85	70	52.9	0
16/10/85	50	58.0	0
23/10/85	80	55.0	0
29/10/85	100	53.0	0
4/11/85	100	59.0	0
19/11/85	80	73.8	0
27/11/85	50	12.0	0
9/12/85	100	45.0	0
24/12/85	100	58.0	0
30/12/85	80	31.3	0
6/1/86	100	18.0	0
14/1/86	80	12.5	0
22/1/86	50	28.0	0
28/1/86	70	22.9	0
6/2/86	40	0	-
11/2/86	30	6.7	0
21/2/86	25	4.0	0
9/4/86	20	40.0	0
14/6/86	18	5.6	0
22/6/86	23	56.5	0
26/6/86	40	65.0	0
25/8/86	20	37.5	6.7
1/9/86	50	36.0	5.6
22/9/86	50	22.0	0
30/9/86	40	22.5	0
6/10/86	70	61.4	0
15/10/86	115	60.0	0
28/10/86	40	85.0	0
26/11/86	70	54.3	0
3/12/86	70	24.3	0
12/12/86	75	38.7	0
18/12/86	90	22.2	0
22/12/86	100	17.0	0
2/4/87	35	42.9	0
22/4/87	25	80.0	0
27/4/87	45	55.6	0

number of flowers was very low (Table 5.2). Infestation ranged from 4 to 72 larvae per 100 flowers. Peak infestation occurred in late September and October in both years of sampling but remained high into November in 1986. Only on two sampling occasions were parasitized larvae found (Table 5.2). All parasitoids appeared to be the same species of *Apanteles* found attacking *P. scutigera* larvae in *H. tiliaceus* and cotton.

Flowering branches (15-20 cm long) were randomly sampled from the *H. rosa-sinensis* bush located at the D.P.I. Research Station, Biloela, and searched for eggs and larvae. Only 1 egg was found on five branches sampled on 16 October, 1985, laid on the bark in a crevice positioned away from leaves or flowers. Twenty-five eggs were found on another five flowering branches sampled on 1 June, 1987. Ten eggs (40%) were laid on fully expanded leaves; two on the topside and eight on the underside. All were laid in hollows on the leaf laminar caused by insect feeding damage. The other 15 eggs were laid on the bark of branches, usually situated in crevices close to a leaf bud. No eggs were found on flowers buds or open flowers.

As for *H. tiliaceus*, *P. scutigera* larvae were found in fallen *H. rosa-sinensis* flowers regardless of their age or condition. Generally, *H. rosa-sinensis* flowers only open for one day before wilting and falling to the ground, although a few varieties retain their flowers for 2-3 days (Chin 1986). *P. scutigera* could survive in dry *H. rosa-sinensis* flowers for long periods of time until the flowers were replaced or moistened with water. Larval development on fallen *H. rosa-sinensis* flowers, held under constant temperatures and photoperiods, was very similar to that on *H. tiliaceus* flowers (Chapter 2).

5.3.3. *Brachychiton australis*

On 10 September, 1985, *P. scutigera* larvae were noticed infesting the flowers of *B. australis* trees growing in the grounds of the D.P.I. Research Station, Biloela. Twenty-eight *P. scutigera* larvae were found in a random sample of 100 fallen flowers from three adjacent trees.

Subsequently, the number and location of all *B. australis* trees in the Biloela and Theodore areas was determined. Twenty-three large ornamental trees were located around Biloela, ten

of which flowered heavily during 1985. In Theodore, 14 large ornamental trees were found nine of which flowered heavily. Ornamental *B. australis* trees were also present in Thangool, Banana and Gibber Guynah near Theodore where trees were commonly planted on the properties of cotton farmers (see Fig. 3.1 for locations).

B. australis is a medium-sized tree with a thick columnar trunk. The flowers are white, 2 cm long, heavily scented, with thick petals and staminal columns. Open flowers remain on trees for only one day before falling to the ground. The fruit are thick-walled, hairless, boat-shaped follicles, up to 15 cm long, that contain several hard, dry yellow seeds set amongst bristly hairs.

All ornamental *B. australis* trees sampled in the Callide and Dawson Valleys (N = 22 trees) were found to be infested with *P. scutigera* larvae. Table 5.3 and 5.4 give the rate of infestation of fallen flowers for a selected number of ornamental trees sampled during 1985 and 1986. Peak flowering occurred between late September and early October during both years. In 1985 trees ceased flowering by mid-November (with the exception of one tree which continued to flower until the end of November), while in 1986 trees ceased flowering by mid-October. Rates of infestation in 1985 ranged from 27 to 109 larvae/100 fallen flowers, while in 1986 infestation was lower, ranging from 6 to 39 larvae/100 fallen flowers.

Close inspection of ornamental trees at the D.P.I. Research Station, Biloela, revealed that *P. scutigera* laid their eggs underneath loose bark on flowering branches but not on flower buds or open flowers. First instar larvae appeared to infest open flowers while attached to the tree. Flower buds were never infested. Larvae remained inside flowers when detached from the tree but due to their small size and rapid dehydration, larvae had to move into freshly fallen or undamaged flowers to complete development. A few large larvae (mainly fourth instars) were occasionally noticed infesting open flowers attached to the tree. These larvae had webbed a number of flowers together on a flowering panicle which prevented them falling onto the ground. It is unknown whether these larvae had remained on the flowering branches throughout their development or crawled up the tree from fallen flowers.

All larval instars, predominately first instars, were found inside fallen flowers but no eggs

Table 5.3. Infestation of *B. australis* fallen flowers by *P. scutigera* larvae in Central Queensland during 1985. Flowers (N=100) were collected from the base of single trees and searched immediately for larvae.

Locality /tree	Date sampled	No. larvae/100 flowers/instar				
		I	II	III	IV	Total
Theodore						
Tree A	27/9/85	8	8	6	5	27
"	9/10/85	20	8	5	7	40
Tree B	27/9/85	33	12	6	0	51
"	9/10/85	25	12	3	3	43
Tree C	27/9/85	16	8	3	2	29
"	9/10/85	20	10	3	3	36
Tree D	9/10/85	6	16	4	6	31
Tree E	2/10/85	31	7	6	4	48
Banana	27/9/85	21	9	7	0	37
	9/10/85	37	10	4	2	53
Thangool	24/9/85	7	15	17	4	43
Biloela						
Tree A	12/9/85	63	7	2	0	72
"	25/9/85	34	6	7	1	48
"	2/10/85	18	4	11	10	43
"	10/10/85	21	3	1	0	25
"	22/10/85	24	15	22	43	104
Tree B	22/10/85	32	13	19	33	97
"	28/10/85	43	30	16	20	109
Tree C	13/9/85	12	5	3	0	20
"	25/9/85	35	4	0	0	39

Table 5.4. Infestation of *B. australis* fallen flowers by *P. scutigera* larvae in Central Queensland during 1986. Flowers were collected from the base of single trees and searched immediately for larvae. N = number of flowers sampled.

Locality /tree	Date sampled	N	No. larvae/100 flowers/instar				
			I	II	III	IV	Total
Theodore							
Tree A	16/9/86	350	0	3.4	6.6	2.3	12.0
"	26/9/86	200	18.0	4.0	1.0	1.0	24.0
"	3/10/86	200	16.0	3.0	3.5	5.0	27.5
"	10/10/86	200	1.0	7.5	10.0	10.0	28.5
Tree B	16/9/86	278	0	2.9	15.8	10.8	29.5
"	26/9/86	200	8.5	2.5	2.5	0	14.0
"	3/10/86	200	16.0	2.5	2.0	2.0	22.5
"	10/10/86	200	4.0	6.0	4.5	20.0	34.5
Tree C	16/9/86	200	0	5.5	12.5	7.5	26.5
"	26/9/86	200	11.0	7.5	3.5	1.0	23.0
"	3/10/86	200	8.0	0	1.0	1.0	10.0
"	10/10/86	200	7.5	2.5	3.0	7.5	19.0
Banana	27/8/86	200	1.0	5.0	5.0	0.5	11.5
"	16/9/86	200	0.5	4.0	2.5	0.5	7.5
"	26/9/86	200	3.0	0.5	2.0	2.5	7.0
"	3/10/86	200	2.0	0.5	2.0	2.5	7.0
"	10/10/86	200	1.0	2.5	0.5	1.5	5.5
Thangool	27/8/86	81	9.9	9.9	4.9	0	24.7
"	18/9/86	100	0	2.0	1.0	4.0	7.0
"	2/10/86	200	21.5	9.5	1.0	1.0	33.0
"	8/10/86	200	16.0	11.5	6.0	5.0	39.0
Emerald							
Tree A	12/9/86	85	1.2	3.5	4.7	3.5	12.9
B	"	64	0	4.7	0	0	4.7
C	"	163	0	3.1	1.2	0.6	3.7
D	"	230	2.2	5.2	5.7	0.9	13.9
E	"	263	1.1	2.3	8.4	0.4	12.2
F	"	215	0	0	0.9	0.9	1.9
Biloela							
Tree A	17/9/86	200	1.5	20.0	16.5	4.5	42.5
"	25/9/86	200	44.0	10.0	2.5	0	56.5
"	6/10/86	200	37.5	7.5	6.0	10.0	62.4

or pupae were ever encountered (Table 5.5). Larvae attacked all parts of both male and female flowers. First, second and small third instars were mainly found feeding at the base of flowers or tunnelling through the centre of the staminal or pistil column. Very few larvae fed on the globular mass of anthers or lobed stigmas. First instars were also found tunnelling through the thick fleshy petals. Third and fourth instars rapidly consumed the entire stamen or pistil leaving the petals intact. Petals were consumed only if fresh entire flowers were not available. Fourth instars were often found inside groups of 3-10 fallen flowers webbed together. It was suspected that pupation occurred on or near the soil surface where the flowers lay. Developmental rates of *P. scutigera* reared on fallen *B. australis* flowers are given in Chapter 2.

In 1985, a De-vac^{R} suction sampler was used to collect fallen flowers beneath 3 adjacent trees at the D.P.I. Research Station. When *P. scutigera* larvae were first noticed infesting flowers in early September, two trees were flowering very heavily and reached their peak around 30 September, while the third smaller tree peaked in early October. Flowering in all trees declined rapidly after mid-October and ceased completely by the end of that month.

Flowers were sampled on four occasions between 16 September and 8 October, 1985, and immediately placed under a pyramid emergence cage (see Chapter 6 for description) after estimating the number collected by weighing and counting sub-samples. Four random samples of 50 flowers were taken to determine levels of larval infestation. Approximately 36,828 flowers were collected which contained an estimated 6,984 *P. scutigera* larvae. Moth emergence was recorded every 1-3 days alongside the number of males caught in a pheromone trap placed in the lower canopy of the central *B. australis* tree.

A total of 652 moths and nine parasitoids emerged from the caged flowers. This represents a 9.5% survival rate of the original number of *P. scutigera* estimated to be present in the flowers. Moths started to emerge on 11 October, 25 days after the first flowers were placed inside the cage. Rate of emergence was low throughout October but increased sharply in early November, peaking on 10 November at 71 moths/day. Emergence sharply declined after 14 November but low numbers of moths continued to emerge until 12 December (Fig. 5.1).

Table 5.5. Infestation of *B. australis* fallen flowers by *P. scutigera* larvae at the D.P.I. Research Station, Biloela, during 1985 and 1986. Flowers were collected from the base of adjacent three trees and searched immediately for larvae, unless indicated. N = number of flowers sampled.

Date sampled	N	No. larvae/100 flowers/instar				
		I	II	III	IV	Total
10/9/85	100	15.0	10.0	2.0	1.0	28.0
12/9/85	100	28.0	5.0	1.0	1.0	35.0
16/9/85	400	19.0	15.8	3.2	1.2	39.0
23/9/85	180	21.1	4.4	4.4	0.6	30.6
30/9/85	400	1.6	4.2	1.8	3.0	10.6
8/10/85	400	2.6	3.8	3.2	1.8	11.4
23/10/85	100	38.0	8.0	8.0	1.0	39.0
25/8/86	86	16.3	5.8	0	2.3	24.4
30/8/86*	100	2.0	13.0	5.0	2.0	22.0
3/9/86	134	29.9	7.5	2.2	0	39.6
11/9/86	200	22.5	9.0	0.5	0	27.0
14/9/86*	200	0	2.0	7.5	4.0	12.5
21/9/86	200	16.5	9.0	3.0	1.5	30.0
23/9/86	200	14.0	8.0	14.5	1.5	37.5
29/9/86	200	5.0	4.0	2.0	0.5	11.5
3/10/86	200	5.5	3.0	3.0	0	11.5
9/10/86	200	2.5	0.5	1.5	2.5	3.5

* Flowers collected on 30/8/86 and 14/9/86 were incubated in the laboratory for 5 and 8 days, respectively, before searching for larvae.

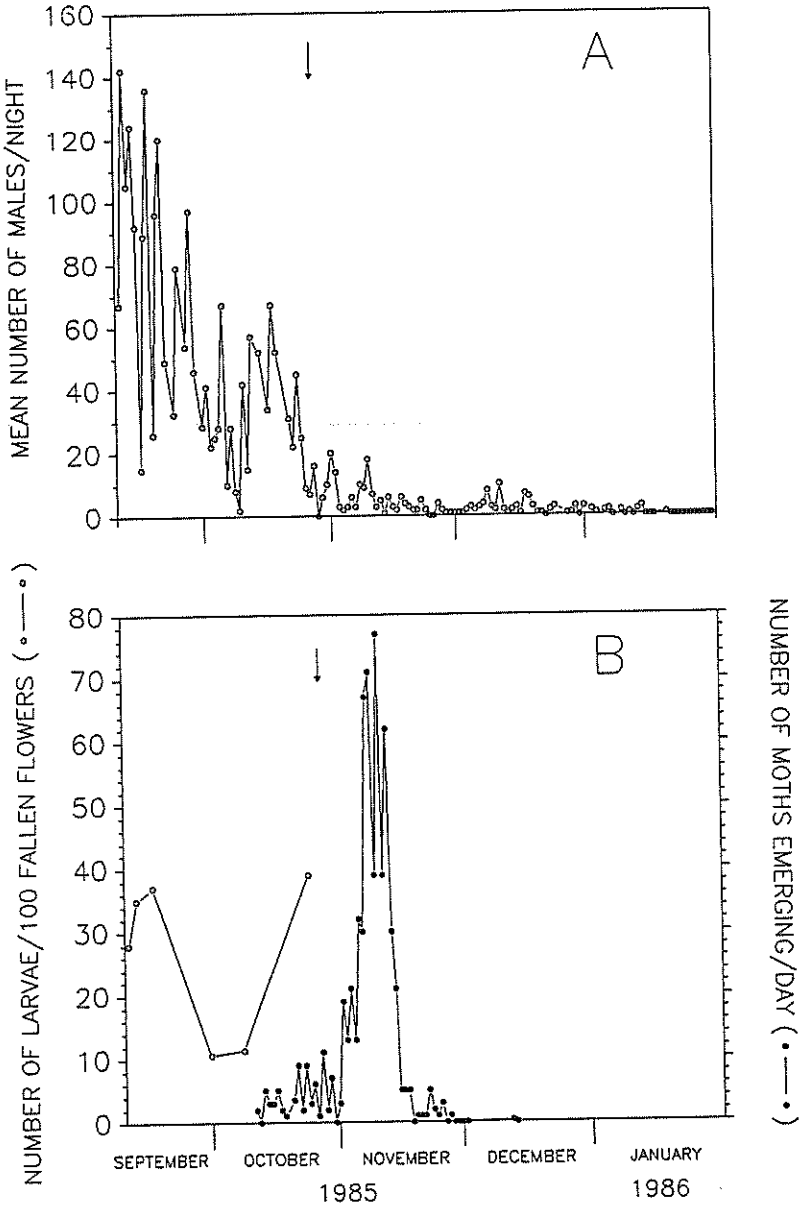

Fig. 5.1. (A) Number of male *P. scutigera* caught in a pheromone trap placed in the canopy of a *B. australis* tree at the D.P.I. Research Station, Biloela, during 1985/86. (B) Emergence of *P. scutigera* from *B. australis* fallen flowers at the same locality. Flowers were placed underneath a single pyramid emergence cage between 16 September and 8 October, 1985. Arrow indicates the approximate end of the mass flowering period.

Six of the nine parasitoids that emerged were identified as a species of *Apanteles*, different from that found parasitizing *P. scutigera* in cotton and *Hibiscus* spp. The other parasitoids were identified as 2 specimens of a *Eriborus* sp. (probably the same species found parasitizing a *P. scutigera* larva infesting *H. tiliaceus* flowers at Theodore) and a specimen of *Chelonus* sp. (Braconidae).

Pheromone trap catch was high during the flowering period of the trees, peaking at 142 moths per night in early September. As flowering decreased pheromone trap catch declined rapidly, although a small peak in trap catch occurred in mid-October. No corresponding increase in pheromone trap catch occurred during the main period of emergence of moths from caged flowers (Fig. 5.1).

In 1986 flowers beneath the same three trees at the D.P.I. Research Station were counted every 2-3 days using a 0.75 m^2 quadrat. The quadrat was randomly placed underneath the canopy of each tree, counting only freshly fallen flowers; five and ten replicate counts were taken under the one smaller and two large trees, respectively.

Production of masses of flowering panicles in 1986 began during August with the first flowers opening towards the end of that month. On 3 September there was an overall mean of 1.8 fallen flowers/0.75 m^2 underneath the trees. Flowering peaked on 27 September at a mean of 40.3 flowers/0.75 m^2, but rapidly declined after 9 October, and ceased entirely on 15 October, 1986 (Fig. 5.2).

The density of *P. scutigera* larvae in fallen flowers beneath each tree averaged 0.7 larvae/0.75 m^2 on 3 September and peaked at 9.0 larvae/0.75 m^2 on 23 September, declining to 0.2 larvae/0.75 m^2 on 9 October (Fig. 5.2). Expressed as the number of larvae/100 flowers, infestations ranged from 22 larvae on 30 August to 39.6 larvae on 3 September, and declined to seven larvae/100 flowers on 9 October. Random collections of 200-1000 freshly fallen flowers were made by hand every 2-3 days until trees stopped flowering. All flowers were immediately placed under a single pyramid emergence cage located near the trees. A total of 11,900 flowers, infested by an estimated 2,162 larvae, were placed underneath the cage between 11 September and 11 October, 1986.

Moth emergence from caged flowers began on 7 October, 1986, 27 days after the first

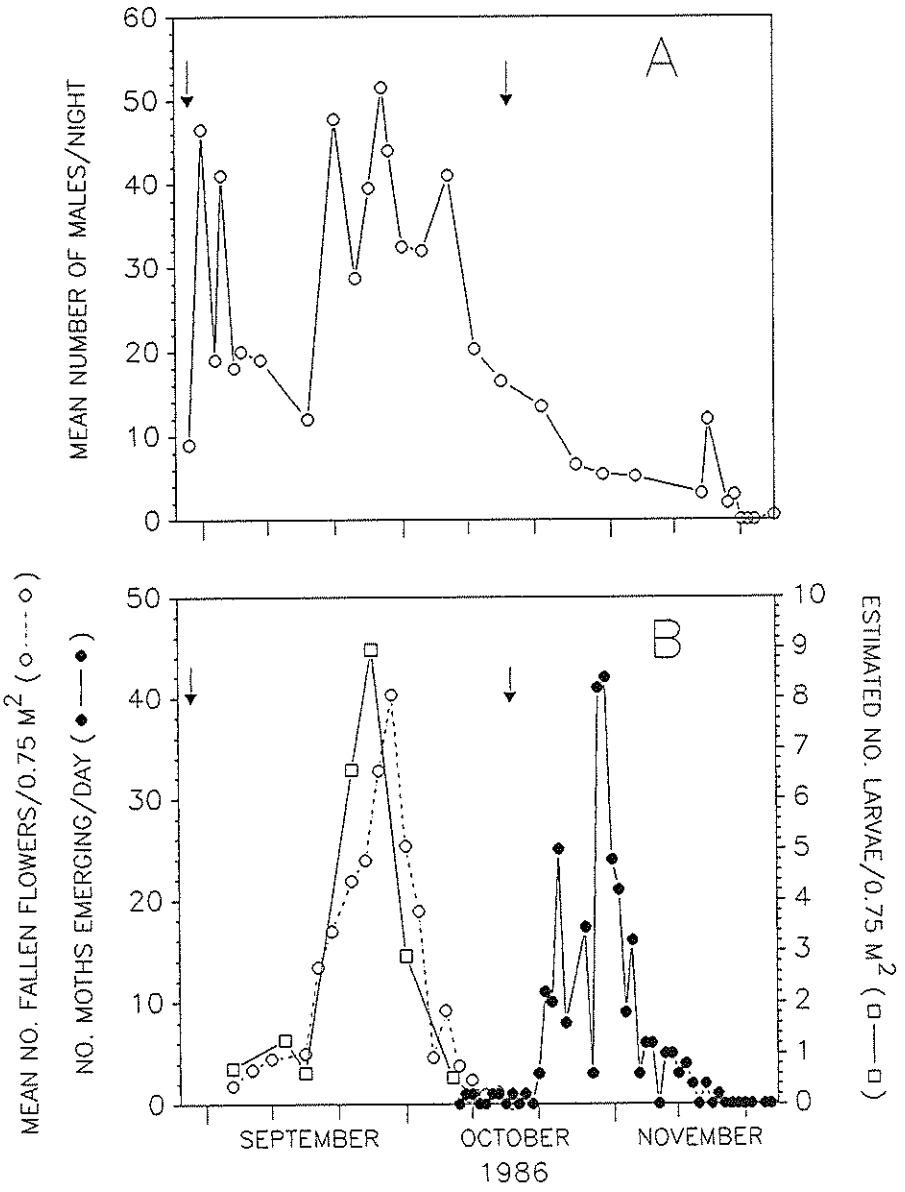

Fig. 5.2. (A) Number of male *P. scutigera* caught in a pheromone trap placed in the canopy of a *B. australis* tree at the D.P.I. Research Station, Biloela, during 1986. (B) Mean density of *B. australis* fallen flowers (o) and *P. scutigera* larvae (□) underneath the canopy of three adjacent trees at the same locality during 1986; emergence of adult moths from *B. australis* fallen flowers (●) placed underneath a single cage between 11 September and 11 October, 1986. Arrows indicate the approximate start and finish of the mass flowering period.

flowers were collected. A total of 309 moths, but no parasitoids, emerged which represented 14.3% of the original number of *P. scutigera* estimated to be in the flowers. Emergence peaked on 30 October at 42 moths per day (Fig. 5.2) and the last moth emerged on 23 November, 1986.

The number of males caught in a pheromone trap, placed in the lower canopy of the central *B. australis* tree, varied greatly but was highest between 30 August and 20 October, 1986, during the flowering period of the trees. Trap catch peaked on 26 September at 51.5 moths/night (Fig. 5.2). Males were caught continuously in low numbers until removal of the trap on 18 December.

The number of degree-days (DD) required for emergence of *P. scutigera* from caged *B. australis* flowers in 1985 and 1986 was calculated using screen temperatures recorded at the D.P.I. Research Station, Biloela, and the DEGDAY programme of Higley *et al.* (1986). A developmental zero of 11.3^{o} C was used, derived from Chapter 2 data for total *P. scutigera* development on artificial diet. The modified sine wave method of Allen (1976) for calculating DD was chosen in the DEGDAY programme. The number of DD accumulated from an arbitrary date, 1 September, to first, peak and last moth emergence in 1985 and 1986 is given in Table 5.6. Also compared are the number of DD accumulated from first, mid or last flowers placed underneath cages to first, peak or last moth emergence.

The extended emergence of moths in 1985 resulted in a greater number of DD accumulating until last moth emergence, compared to 1986. In 1985, 1074 DD were accumulated from 1 September to last moth emergence compared to 810 DD in 1986. However, the number of DD from first flowers placed underneath cages to first moth emergence was similar at 214 and 255 DD in 1985 and 1986, respectively. The number of DD from 1 September to peak emergence was 625 in 1985 and 550 in 1986.

Green seed pods were collected from *B. australis* trees in 1985 and 1986 but few were sampled because of the problems in reaching the upper canopy of large trees. Also, despite the mass production of flowers, very few fruits were set. In 62 seed pods sampled from 5 adjacent trees near Biloela, on 22 October, 1985, 16 *P. scutigera* larvae were found (25.8

Table 5.6. Number of degree-days (DD) above 11.3° C required for emergence of *P. scutigera* from caged *B. australis* fallen flowers in 1985 and 1986. Flowers were placed underneath a single pyramid emergence cage between 16 September and 8 October, 1985, and between 11 September and 11 October, 1986. Accumulated number of DD calculated between the first, mid and last flowers placed in the cages to first, peak and last moth emergence. The number of DD accumulated from an arbitrary starting date, 1 September, to first, peak and last emergence is also presented for both years.

	No. DD accumulated	
Period of DD accumulation	1985	1986
First flower - first moth emergence	214	255
Mid flower - peak moth emergence	431	371
Last flower - first moth emergence	798	462
1 September - first moth emergence	313	315
1 September - peak moth moth emergence	625	550
1 September - last moth emergence	1074	810

Table 5.7. Infestation *G. sturtianum* reproductive forms by *P. scutigera* larvae at Bauhinia Downs, Central Queensland. N = number of each form sampled.

	No. larvae/100 forms							
Date sampled	Flower buds	N	Open flowers	N	Fallen flowers	N	Seed capsules	N
2/1/85	-[1]	-	-	-	-	-	1.6	250
30/9/85	0	100	11.3	200	0	200	-	-
23/10/85	-	-	-	-	1	100	4.5	200
21/11/85	-	-	-	-	-	-	5.2	154
27/1/86	-	-	-	-	-	-	4.0	100
1/10/86	4.4	90	-	-	-	-	18.8	32
16/10/86	4.5	110	15.0[2]	80	-	-	4.4	90

1. Dash indicates form not present on plants
2. Includes one larva parasitized by *Apanteles* sp.

larvae/100 seed pods). In 80 fruits sampled from 3 trees at the D.P.I. Research Station, Biloela, on 3 December, 1985, 11 *P. scutigera* larvae were found (13.8 larvae/100 seed pods). No larvae were found in a further ten seed pods sampled from the same trees on 21 January, 1986.

In both years of observation, many ornamental *B. australis* trees started flowering again in April or May. The number of flowers produced was very variable and much lower than during spring months. *P. scutigera* larvae were found infesting the fallen flowers of all trees inspected in all areas, ranging from 9 larvae/100 flowers in Theodore to 26 larvae/100 flowers in trees near Biloela. Trees continued to flower at low levels throughout winter months until August/September when masses of flowering panicles were produced.

B. australis is endemic to Central Queensland although very few wild trees were found in the Callide and Dawson Valley. The largest stand of trees located near Biloela was at Thangool where about eight large trees grew on the slopes of Mt. Scoria (Fig. 3.1). These trees produced very few flowers when inspected between September and November, and none were infested by *P. scutigera* larvae. Other isolated *B. australis* trees were also noticed on the banks of some creeks around Thangool but none were flowering when inspected in September and October. The only other large stand of wild trees found was near Bauhinia where *B. australis* grew in remnant patches of softwood scrub on the tops of hills. In one patch, located on the Oombabeer Road about 10.7 km N.E. of Bauhinia, more than 20 large trees were found. These trees produced very few flowers during the spring but flowered prolifically in April and May, 1986. Twelve *P. scutigera* larvae were found on 4 May in a sample of 600 fallen flowers from eight trees but no larvae were found in a further sample of 400 flowers collected from seven trees on 7 May.

Other *B. australis* trees encountered while travelling in Central and S.E. Queensland were inspected when possible. An isolated tree located about 10 km south of Calliope on Tableland Road, (102 km east of Biloela) was inspected on 29 August, 1986, but no larva was found in a sample of 43 fallen flowers. No larva was found infesting 25 fallen flowers collected from an ornamental tree at Mt Morgan (77 km north-east of Biloela) on 31 August, 1986. Similarly,

no larva was found in a sample of 300 fallen flowers collected from trees at the Queensland Agricultural College, Gatton, in S.E. Queensland, on 18 September 1986. *P. scutigera* larvae were found infesting the fallen flowers of ornamental *B. australis* growing in Emerald. Flowers, randomly collected from 6 ornamental trees on 12 September, 1986, were infested at rates from 2 to 14 larvae/100 flowers. Larvae were parasitized by *Eriborus* sp. although rates of parasitism were not determined.

5.3.4. *Brachychiton populneus*

B. populneus is a small tree, growing up to 15 m in height, with a dense rounded crown. Trees were scarce in the Biloela and Theodore districts, but were found in vegetation bordering creeks, often in the vicinity of cotton fields. A stand of ornamental trees (*ca* 15 trees) was also located on the Biloela D.P.I. Research Station farm.

The flowers of *B. populneus* are very different from those of *B. australis*; they are of similar size but have very thin petals and small staminal columns. The fruits of the 2 species are similar in appearance but smaller in *B. populneus*. Trees were observed flowering between December and February. The intensity and timing of flowering varied considerably between trees and years. Few trees flowered prolifically; two out of 14 trees located near cotton farms, 10 km north of Biloela, flowered heavily in 1985/86.

Two *P. scutigera* larvae were found in 200 fallen flowers sampled from the base of a heavily flowering tree on 24 December, 1985. The tree was growing on the border of a field that was planted with cotton in the previous season. No larva was found in a further sample of 200 fallen flowers collected on 30 December, 1985, but 11 larvae were found in 500 flowers (2.2 larvae/100 flowers) collected from the same tree on 2 January, 1986. In another heavily flowering *B. populneus* tree, located about 500 m away, 4 *P. scutigera* larvae were found in a sample of approximately 1,500 flowers (0.3 larvae/100 flowers) on 3 January, 1986. Eighteen seed pods collected from this tree on 6 January, 1986, did not contain *P. scutigera*.

Infestation of *B. populneus* flowers was lower in 1987. One large tree, located close to the first tree sampled in 1985/86, flowered prolifically during February. Approximately 2,500 fallen flowers were collected on 7 February but no *P. scutigera* larvae were found. Similarly, no larva was found in 600 fallen flowers and 8 seed pods collected from the first tree sampled

in 1985/86 on 21 January, 1987. A further sample of 250 fallen flowers were also uninfested but 1 *P. scutigera* larva was found in a sample of 57 seed pods (1.7 larvae/100 pods).

Nineteen other *B. populneus* trees, located around Biloela and on the D.P.I. Research Station farm, produced very few flowers during 1986/87. Fallen flowers and seed pods were occasionally sampled between December and January but no *P. scutigera* larva was ever found.

5.3.5. *Gossypium sturtianum*

In December, 1984, Mr M. Stone of Biloela, noticed *P. scutigera*-like larvae infesting the seed capsules of *G. sturtianum*. Plants were located on the edge of a small patch of softwood scrub, 10.7 km N.E. of Bauhinia, along the Oombabeer Road (where *P. scutigera* was later found infesting the flowers of *B. australis*). Four *P. scutigera*-like larvae and one other Gelechiid larva were found in 250 green seed capsules sampled on 2 January, 1985. Larvae were reared to adults on artificial diet and subsequently identified as *P. scutigera* and a *Pexicophora* sp. by Q.D.P.I. taxonomists.

G. sturtianum produced few flowers or fruits, remaining dormant for most of the year. Flowering appeared to be stimulated by heavy rainfall. Infestation of seed capsules was low, ranging from 4 to 5 larvae/100 capsules (Table 5.7). Open flowers and flower buds were sampled if enough were available. Infestation of flower buds was similar to seed capsules but open flowers, picked off plants, contained 11 and 19 larvae/100 flowers when sampled on 30 September, 1985 and 1 October 1986, respectively (Table 5.7).

Despite extensive searches, no *G. sturtianum* plants were found in any other locality. Areas of softwood scrub around the Biloela dam, Mt Scoria near Thangool, Theodore airport and along the Theodore-Nipan-Moura road were searched during February, 1985. Other areas of softwood scrub located near Thangool airport, Biloela, and in the Expedition Range were also searched in March 1985.

5.3.7. *Prunus persica*

In November, 1985, a single *P. scutigera* larva was found infesting a Peach fruit (*P. persica*) growing on a cotton farm 10 km north of Biloela (B. Breeze's property). Cotton

grown on this farm suffered heavy *P. scutigera* infestations each season. No larva was found in further samples of fruit from this tree but 87 fruit collected from a tree on the outskirts of Biloela (F.D. Page's property) on 8 and 9 December, 1985, contained two larvae (3% infestation). Thirty-nine nectarine fruit collected from R. Brengman's property, 10 km north of Biloela, on 4 December, 1985, were not infested with *P. scutigera* larvae.

5.3.7. *Hibiscus cannabinus*

A small plot of *H. cannabinus* was grown on the D.P.I. Research Station farm, Biloela, in 1987. A sample of 32 fallen flowers, collected on 24 April, contained 1 *P. scutigera* larva (3.1% infestation).

5.4. Discussion

B. australis is probably the most important alternative host of *P. scutigera* in the Callide and Dawson Valleys. The main period of flowering of ornamental trees coincides with peak emergence of moths from overwintering larvae in cotton trash (Chapter 6), thus providing a very important larval food source at a time when no suitable cotton is available for infestation. Hence, the emergence of *P. scutigera* from cotton trash during September and October can no longer be considered as 'ineffective' (Sabine 1969a). Infestation of fallen flowers allowed moths to produce a new generation which emerged mainly in November and extended into December, enabling *P. scutigera* to carry-over directly into squaring cotton crops. *B. australis* trees may also provide adult moths emerging from cotton trash with an important nectar resource, thus increasing their survival and longevity until the availability of squaring cotton (see Chapter 7).

However, it is still difficult to assess the actual importance of *B. australis* in assisting the carry-over of *P. scutigera* populations from one cotton season to the next. While flowering trees were heavily infested with *P. scutigera* larvae at all locations, survival of larvae, as indicated by the number of moths emerging from caged fallen flowers, was very low (9.5% in 1985 and 14.3% in 1986). Survival of larvae would undoubtedly be much lower in flowers left beneath the trees. Rapid dehydration of flowers and their small size forced larvae to actively search for fresh or undamaged flowers in order to complete development. This would

result in high levels of larval mortality and cannibalism, particularly towards the end of the trees' flowering period as the number of fallen flowers decreased. Large larvae voraciously fed on fallen flowers even if they were infested by neonate *P. scutigera* larvae. Ants, which were always present amongst fallen flowers, were probably very important predators. Another important mortality factor was the mowing of grass underneath ornamental trees where fallen flowers accumulated. Mowing was observed to completely shred all fallen flowers lying on the surface of the grass.

Furthermore, the results of laboratory experiments in Chapter 2, where larvae were continuously provided with fresh flowers, indicated that *B. australis* is a poor host. Survival rates and levels of fecundity were lowest when reared on *B. australis* than for any other larval diet. The capacity for increase of *P. scutigera* reared on *B. australis* flowers was estimated at only 0.0412, compared to 0.0746, 0.0875 and 0.0667 for moths reared on green cotton bolls, *H. rosa-sinensis* and *H. tiliaceus*, respectively (Table 2.22).

The difference in the number of DD required for the emergence of *P. scutigera* from caged *B. australis* flowers in 1985 and 1986 (Table 5.6) may be attributed to differences in temperature, rainfall and rates of larval infestation between the years. Peak emergence from flowers occurred when 625 and 550 DD had accumulated from 1 September in 1985 and 1986, respectively. In Chapter 2 it was estimated that *P. scutigera* reared on *B. australis* flowers would require 430.2 DD for total development (from egg hatch to moth emergence). However, this estimate was based on the development of larvae that were continuously supplied with fresh *B. australis* flowers.

Survival or development of *P. scutigera* larvae infesting seed pods of *B. australis* was not recorded but may be higher than those infesting fallen flowers. Few seed pods were sampled because of the difficulties in reaching the canopy of large trees.

Wild *B. australis* trees supported at most low populations of *P. scutigera* in the Callide and Dawson Valleys. Very few wild trees remain in the heavily farmed valleys, being mainly found in remnant patches of softwood scrub (semi-evergreen vine thickets) on elevated parts of the landscape.

Although wild trees appear to have the potential to produce masses of flowering panicles, as seen at Bauhinia Downs in April and May, 1985, they rarely do so. The intensity and timing of flowering in *B. australis* may be highly dependent on rainfall and/or soil moisture. Wild *B. australis* trees occurred on well drained soils, usually derived from basalts. Very heavy rainfall may be required to stimulate mass flowering in these trees. Mass flowering of *B. australis* trees at Bauhinina Downs was noted to occur after very heavy, localized rainfall. The ability of ornamental trees consistently to produce masses of flowers during the spring may be related to the more fertile and thicker alluvial soils on which they grow. These soils, which are often irrigated, may retain a critical level of moisture during spring rains which stimulates flowering. Alternatively, ornamental trees may represent a strain of *B. australis* specially selected for its' flowering characteristics. The origin and history of cultivation of ornamental *B. australis* is unknown.

B. populneus is unlikely to be of importance in the carry-over of *P. scutigera* populations from one cotton season to the next. *B. populneus* flowered between December and February, when most cotton crops would bear squares, flowers and small bolls. The trees may act as a direct source of moths infesting cotton crops although their importance is dubious. Only 17 larvae were found in more than 5,500 *B. populneus* fallen flowers sampled (0.003% infestation). It is suspected that survival of larvae in *B. populneus* flowers is very low due to the thinness of the petals and staminal or pistil columns which rapidly dry out once detached from the tree. Larvae were only found in fallen flowers where large numbers accumulated in shady areas beneath the tree canopy. Flowers falling in sunny areas or that were thinly scattered did not contain *P. scutigera* larvae.

In order to utilize fallen flowers as a food source larvae must be tolerant to extreme changes in the moisture content of their food. Under the normally hot and dry climatic conditions of Central Queensland, flowers of all *P. scutigera* host plants, particularly *B. australis* and *B. populneus*, rapidly desiccated within 3-4 days of falling from the tree or bush. Some moisture was regained if a heavy dew occurred but the flowers soon dehydrated again during the heat of the day. After rainfall, flowers often became completely saturated with water, but if dry weather prevailed immediately afterwards, the flowers would quickly lose

their moisture. Third and fourth instar larvae confined to dry flowers became visibly dehydrated in appearance but were able to survive in this condition for long periods of time (up to 6 weeks). Development was completely arrested on dry flowers but was resumed when the flowers were sufficiently moistened with water or if fresh flowers were supplied.

The ability of *P. scutigera* larvae to survive and develop on fallen flowers, which constantly undergo extreme changes in moisture content, may help to explain how populations of this pest successfully carry-over from one cotton season to the next in dry cotton bolls. This will be discussed further in Chapters 6 and 8.

Although *H. tiliaceus* and *H. rosa-sinensis* were always infested by *P. scutigera*, both plants are relatively scarce in the cotton growing areas of Central Queensland. Flowering was sporadic and at low intensities throughout the year. Hence, they are unlikely to play a significant role in the carry-over of *P. scutigera* populations from one cotton season to the next. Similarly, the low levels of infestation and rarity of *G. sturtianum* and *P. persicae* in the Callide and Dawson Valleys excludes these hosts from being important.

The infestation of *H. cannabinus* and *H. sabdariffa* by *P. scutigera* suggests that large-scale cultivation of these crops may be unwise in cotton growing districts. Commercial production of *H. cannabinus* has been suggested in a number of cropping regions in Queensland as a source of bast fibre. *H. sabdariffa* was introduced into Australia from Africa in the 19th century and grown for its fleshy calyces, which were processed and marketed as 'rosella jam' (Wilson 1974). It has now become established in Australia by escaping from cultivation, although no wild plants were encountered in Central Queensland during this study.

The Callide and Dawson Valleys do not appear to contain a more diverse or unique range of *P. scutigera* host plants than other cotton growing districts. Ornamental *B. australis*, *H. tiliaceus* and *H. rosa-sinensis* occur in other cotton growing districts such as Gatton, Dalby and Emerald, where *P. scutigera* is not a pest of cotton. *P. scutigera* has been recorded infesting *H. rosa-sinensis* at Gatton in the Lockyer valley and Toowoomba near the Darling Downs (Q.D.P.I. unpublished records, see Appendix A). In Emerald *P. scutigera* infests *H.*

tiliaceus (F. D. Page pers. comm.) and *B. australis* (Table 5.4), and has been caught in pheromone traps placed in *H. tiliaceus* trees throughout the year (Murray and Page 1982). *B. populneus* is distributed throughout Queensland, as far north as Townsville, extending into New South Wales and eastern Victoria (Boland *et al.* 1984).

Alternative host plants alone cannot explain the limited pest status of *P. scutigera* in Australia. However, they may act in combination with other factors operating in Central Queensland that favour the carry-over of *P. scutigera* populations from one cotton season to the next. This will be discussed further in Chapter 8.

CHAPTER 6

Carry-over of *P. scutigera* populations from one cotton season to the next - the role of overwintering larvae in cotton crop residues

6.1. Introduction

The early disposal of cotton crop residues by burial is believed to be essential to prevent the direct carry-over of *P. scutigera* populations from one cotton season to the next (Chapter 1). If crop residues are left on the soil surface, emergence of adult *P. scutigera* extends well into the next cotton season. Burial of crop residues not only causes high larval mortality but also stimulates moth emergence so that most moths emerge before the availability of new squaring cotton crops (Sabine 1969a, 1969b).

It is not understood why burial of crop residues is so effective at reducing larval survival and stimulating moth emergence. *P. scutigera* overwinters as a larva inside dry, open cotton bolls. According to Sabine (1969a) and Sloan (1946) larvae do not enter any form of diapause or quiescence. However, evidence to reject the presence of a larval diapause or quiescence in *P. scutigera* is weak. Sabine (1969a) considered that the uninterrupted emergence of *P. scutigera* moths from dry cotton bolls proved that this species did not enter a period of dormancy, but did not explain why or how moth emergence extended over periods of up to 10 months. Sloan's (1942) criteria for rejecting the presence of larval diapause in *P. scutigera* was based on the absence of a hibernaculum constructed from two cotton seeds webbed together which are characteristic of diapausing *P. gossypiella* larvae.

The pattern of *P. scutigera* emergence from dry cotton bolls is similar to that of *P. gossypiella* which undergoes a faculative diapause at the end of the fourth larval instar (Adkisson *et al.* 1963, Fife 1961). Induction of larval diapause in *P. gossypiella* is primarily controlled by photoperiod although other factors, such as larval diet and temperature, may exert an influence on the proportion of larvae entering diapause (Adkisson 1961b, Adkisson *et*

al. 1963, Bull and Adkisson 1960, Raina and Bell 1974).

In this Chapter, experiments are presented to determine factors controlling the emergence of *P. scutigera* from dry, open cotton bolls, buried under soil or left on the soil surface. The effect of 7 different tillage practices, artificial irrigation and soil type on the moth emergence is investigated in field and pot experiments. The rate of emergence of *P. scutigera* infesting green, unopened bolls, buried under soil or left on the soil surface, is also examined. The effect of a short-day photoperiod and larval diet containing high concentrations of cotton seed oil on the development of *P. scutigera* is compared in the laboratory at 25° C.

6.2. Material and Methods

6.2.1. Comparison of tillage practices

Seven tillage systems were compared to determine which method was most effective in reducing the survival and emergence of *P. scutigera* infesting dry cotton bolls. The tillage systems chosen were based on those commonly used by cotton farmers in the Callide valley. This experiment was conducted in collaboration with Peter Walsh, Agricultural Engineer at the D.P.I. Research Station, Biloela, whose interest and participation is gratefully acknowledged.

Dry, open bolls, infested with *P. scutigera* larvae, were collected from unsprayed cotton at the D.P.I. Research Station, Biloela, at the end of May 1986. Bolls were stored in the laboratory at room temperature until needed. Three random samples of 100 bolls were taken to determine *P. scutigera* infestation prior to placing the bolls in the field. Mean infestation was 113.7 *P. scutigera* per 100 bolls - 9.3, 22.4 and 65.6% were second, third and fourth instar larvae respectively, and 2.6% were pupae.

The trial was conducted in part of a 1.4 ha fallow field (previously planted with sorghum) on the D.P.I. Research Station, Biloela. A cotton field was not used because of the complicating presence of an unknown *P. scutigera* population in the cotton trash and soil. The field was cultivated to remove weeds *ca* one week prior to use, hilling-up the soil into 1 m furrows to simulate an irrigated cotton field. Twenty-four 5 x 10 m plots were marked out in a

3 x 8 grid. In the centre of each plot 200 dry, open cotton bolls were evenly spread along the bottom of a furrow over a distance of 60 cm. About 1.8 kg of cotton trash (shredded cotton plants with bolls removed) was evenly spread over and around the bolls. This quantity of trash approximated the density found in slashed cotton fields. The trash was added to ensure that the behaviour of soil on boll burial would be similar to that in a slashed cotton field.

The tillage treatments were as follows:

i) Offset discs, ploughed once.

ii) Offset discs, ploughed once and soil hilled-up immediately after burial.

iii) Offset discs, ploughed twice.

iv) Spadovator plough.

v) Spadovator plough and soil hilled-up after burial.

vi) Rotary-hoe.

vii) Ratoon method- this burial method was proposed by a farmer who wanted to leave old cotton root stocks left standing in the soil (so that a ratoon crop of cotton could be grown in the following season) while burying cotton trash lying in between furrows. This was achieved by using triple-disc hillers which turned over soil on the outside of furrows, burying the cotton trash, while leaving the soil in the centre of the hill undisturbed.

viii) Unburied bolls -control treatment where bolls were left undisturbed on the soil surface.

Each treatment was replicated three times and arranged in a 3 x 8 randomized block design. All bolls were placed in the field and buried on 18 June 1986. Ploughing was initiated 5 m before the position of the bolls to ensure that the plough was operating effectively before reaching the bolls. Where appropriate, hilling-up of the soil was done immediately after boll burial. Four extra plots, containing 200 bolls, were buried using methods i, iii, iv and vi to determine the depth of boll burial after ploughing.

Pyramid-shaped emergence cages were placed over the site of boll burial on the 18 or 19 June 1986. Cages were constructed from a sheet of plastic or fibreglass insect screen suspended from a 1 m steel fence post, buried into the soil at an angle of 45^{o}. The screen was folded to form a pyramid shape so that the basal area of the cage covered 1 m^2 of ground. The sides of the cage were buried into the soil to prevent escape of moths. At the apex of each

cage an inverted plastic funnel (15 cm basal diam.) was inserted through a hole in the screen. A 5 x 10 cm clear plastic vial, with a hole cut into the screw-on lid, was attached to the funnel's spout. The sloping sides of the pyramid shaped cage ensured that emerging moths crawled or flew upwards, entered the funnel and became trapped inside the plastic vial.

In some treatments the plough dragged a proportion of the bolls and trash over the ground. This was such a large problem in the ratoon method that it was necessary to place two emergence cages over each plot to ensure adequate coverage of buried bolls. However, in most treatments the buried bolls stayed within a 1 m area. After placing cages over the bolls all surrounding soil was sifted to determine the number of bolls not covered by the cages.

Moth emergence was recorded every 1-7 days from 1 July 1986 until termination of the experiment on 5 January 1987. A single spray of Roundup^R herbicide was applied to each cage in October to control weed growth. Before statistical analysis, the results of moth emergence were corrected for missing bolls by expressing emergence as a percentage of the estimated number of *P. scutigera* originally in the bolls. One-way ANOVA was performed on the data, after a $\log_{10}(x + 1)$ transformation, to test for significant differences between the overall number of moths emerging in each treatment. ANOVA was also used to compare the percentage of moths emerging from each treatment for each month. All data was transformed to arcsines, adding 0.05 to overcome problems with 0 values, before analysis. The least significant test (L.S.D.) was used to separate means if the ANOVA was significant.

Cumulative moth emergence on each sampling date was calculated for each burial treatment as a proportion of total moth emergence over the entire period. Cumulative proportions were transformed to a probit scale, omitting the values 1.0 and 0.0 as these are undefined for a probit transformation (Gray and Tollefson 1988). Linear regression, $y = a + bx$, was used to express cumulative moth emergence (y) on Julian date (x) for each tillage practice. T-tests were performed to test if the slopes (b) of the regression lines were significantly different.

6.2.2. Effect of artificial irrigation of buried and unburied bolls on *P. scutigera* development

Dry, open cotton bolls, infested with *P. scutigera* larvae, were collected from unsprayed

cotton at the D.P.I. Research Station, Biloela, in April 1986. On 16 April, five bolls were placed in plastic plots (11 cm ht x 12.5 cm top diam.), part-filled with alluvial soil, and either buried by covering with 5 cm of soil or placed on the soil surface. The tops of each pot were covered with nylon insect screen and sealed with glue. One-hundred and twenty pots containing buried and unburied bolls were set up. Immediately after bolls were placed in the pots, half of each treatment were irrigated once with distilled water to simulate 2.5 cm of rainfall, while all other pots were kept dry. In another 30 pots of buried and unburied bolls watering of half of the pots (as above) was delayed until the 49th day after bolls were placed in pots. All pots were stored in an unheated shadehouse until examined for larvae.

Ten random samples of 10 bolls, taken on the day experiments were set up, revealed the presence of 100 *P. scutigera* larvae/100 bolls; 3, 18, 25 and 54% were first, second, third and fourth instars, respectively. No prepupae or pupae were present.

Ten pots from each treatment (a total of 50 bolls) were examined 7, 14, 21 and 35 days after the start of the experiment. A further ten pots that received delayed watering were sampled on day 63 and compared with bolls which had been kept dry.

On each sampling occasion, all soil and bolls were thoroughly searched for larval, pupal and adult *P. scutigera*. The position of each life form within the pot and the physiological condition of larvae was recorded. The moisture content of 9 bolls, randomly selected from each treatment, was determined by weighing the plant material before and after drying in an oven at 100^{o} C for 48 hours.

6.2.3. Effect of soil type on the emergence of *P. scutigera* from buried bolls

The effect of soil type on the emergence and survival of *P. scutigera* from buried bolls was investigated in 1986. Three contrasting soil types were chosen and are broadly classified as:

i) alluvial (collected from the D.P.I. Research Station, Biloela).

ii) heavy clay (I. Shepherdson's property, Biloela).

iv) sandy loam (T. Jensen's property, Biloela).

Alluvial soil represented the typical soil type found in the Callide Valley while soils ii) and iii) were rarer, extreme types. All soils were collected from cotton fields on 1 June 1986 and

sifted through a 2 cm sieve to remove large stones and organic matter. The soil was stored dry in a shadehouse until needed.

Open bolls were collected from unsprayed cotton on the D.P.I. Research Station, Biloela on 29 May 1986. Two samples of 100 bolls each were taken prior to the start of the experiment to determine *P. scutigera* infestation. A mean of 50 *P. scutigera*/100 bolls were found - 11, 16 and 64% were 2nd, 3rd and 4th instar larvae respectively and 9% were pupae.

On 8 June 1986, large plastic plant pots (26 cm top diam., 18 cm bottom diam., 23.5 cm height) were part-filled with each soil type and 25 dry bolls placed on top. Bolls were carefully spread in the centre of the pots away from the sides, and then covered with a 10 cm layer of dry soil. Thirty replicate pots of each soil type were set up plus another 10 pots containing unburied bolls (placed on top of alluvial soil) as a control. All pots were moved from the shadehouse into the grounds of the D.P.I. research station on 10 June 1986 so that ambient temperatures and natural rainfall were experienced.

Each pot was covered by a conical top (26 cm high) made from fibreglass insect mesh. An inverted plastic cup (6 cm diam.) was attached to an opening at the top of the cone to collect emerging moths. The conical tops were sealed onto the rims of pots with glue to prevent moths from escaping. The efficiency of these conical tops to trap emerging moths was tested in a preliminary experiment by releasing a known number of moths into the pots and recording date of capture. Of ten moths released into two pots, 80 and 90% were trapped in the collecting cup. In one pot moths were trapped 1-4 days after release but in the other pot two moths were not caught until day five and one moth until day nine. The untrapped moths presumably died inside the pots.

Pots were checked for emerging moths every 1-7 days until 20 December 1986, when the experiment was terminated due to no further emergence. ANOVA was used to compare the overall mean number of moths emerging from each treatment, after a $\log_{10}(x + 1)$ transformation. Linear regression was used to express cumulative moth emergence (probit scale) on Julian date for each treatment and the slopes of regression lines compared as described in section 6.2.2.

6.2.4. Emergence of *P. scutigera* infesting green, unopened cotton bolls

Green, unopened cotton bolls were collected from unsprayed cotton between 4-6 June, 1986. A random sample of 200 bolls revealed a mean of 203 *P. scutigera* larvae/100 bolls. Of these larvae 15.3% were parasitized by *Apanteles* sp. (see Chapter 3). On 7 June, 1986, the green bolls were either placed on the soil surface or buried under 10 cm of soil, in the grounds of the D.P.I. Research Station, Biloela. Four replicates, consisting of 200 bolls, were conducted for each treatment. Each replicate was covered by a separate pyramid emergence cage . The design of emergence cages was based on that described by Sabine (1969a). The cages measured 0.75 m^2 at the base and in height, and consisted of a wooden frame covered with nylon insect screen. A funnel (6 cm basal diam.) was placed at the top of each cage, covered by a glass jar, to trap the emerging moths. The base of cages was buried into the soil to prevent the escape of larvae and adult moths. Moth and parasitoid emergence was checked every 1-7 days, according to the rate of emergence. Linear regression was used to express cumulative moth emergence (probit scale) from buried and unburied green bolls on Julian date, comparing the slopes of regression lines as described in section 6.2.2.

6.2.5. Effects of photoperiod and cotton seed oil on the development of *P. scutigera*

The development of *P. scutigera* reared on artificial diet containing no, low or high levels of cotton seed oil, under two photoperiod regimes, was compared at 25 $\pm$ 1^{o} C. Refined cotton seed oil (CSO) was thoroughly blended into artificial diet (Shorey and Hale 1965), to form 1% and 5% of the total volume, before adding the agar. The diet was dispensed into sections of plastic drinking straws as described in Chapter 2. One neonate larva was transferred to a straw containing each diet. The number of larvae used in each treatment varied according to their availability (see Table 6.7).

Development to pupation and moth emergence on each diet was compared under a short-day (10:14 light/dark) photoperiod and a long-day (14:10 light/dark) photoperiod. Pupae reared under the long-day photoperiod were sexed and weighed within 1-2 days after hardening of the cuticle. The main criterion used by Raina and Bell (1974) for recognising diapause in *P. gossypiella* was applied to *P. scutigera*; i.e. the failure of fully fed larvae to advance to the pupal stage within a reasonable period (2 weeks at 25^{o} C).

ANOVA was performed on the data to test for significant differences between treatment means. The data was $\log_{10}$ transformed if necessary, to meet the assumptions of this parametric test. When the ANOVA was significant, pairwise comparisons of means was performed using a GT2 test for unequal replicates (PROC GLM, SAS Institute, 1985). Male and female development was compared using Student's t-tests.

6.3. Results

6.3.1. Comparison of tillage practices

When offset discs were used once, burial of bolls was very poor; over 50% of the bolls remained on the soil surface and nearly 20% were located just below the soil surface (0-3cm) (Fig. 6.1). Few bolls were buried over 3 cm deep and none was buried over 15 cm deep. Burial was improved when the offset discs were used twice, reducing the proportion of bolls lying on the soil surface or 0-3 cm deep to about 20 and 9%, respectively. The majority of bolls were buried between 9-12 cm deep with some located 18-21 cm deep. In comparison, the rotary hoe and spadovator ploughs were much more efficient at burying bolls, with most lying 6-9 cm deep and only a few on or near the soil surface (Fig. 6.1).

A total of 226 moths emerged from unburied bolls between 1 July to 29 December, 1986. The total number of moths emerging from buried bolls ranged from 71 (spadovator treatment) to 127 (offset discs + soil hilled-up treatment) moths, over the period 1 July to 22 December, 1986. Fig. 6.2 compares the pattern of moth emergence for unburied bolls and all buried boll treatments combined. Peak moth emergence occurred in September, when between 41.4 and 69.0% of all moths emerged from each treatment (Fig. 6.2, Table 6.1). In some treatments emergence during October remained high, while in other treatments the proportion of moths emerging was significantly lower (Table 6.1, $p<0.001$). Emergence during November and December declined to very low levels, but remained highest in the unburied bolls and offset discs + soil hilled-up treatments.

Variation in the number of moths emerging from each replicate cage was high in some treatments e.g. ratoon and spadovator (Table 6.2). Overall emergence from the ratoon

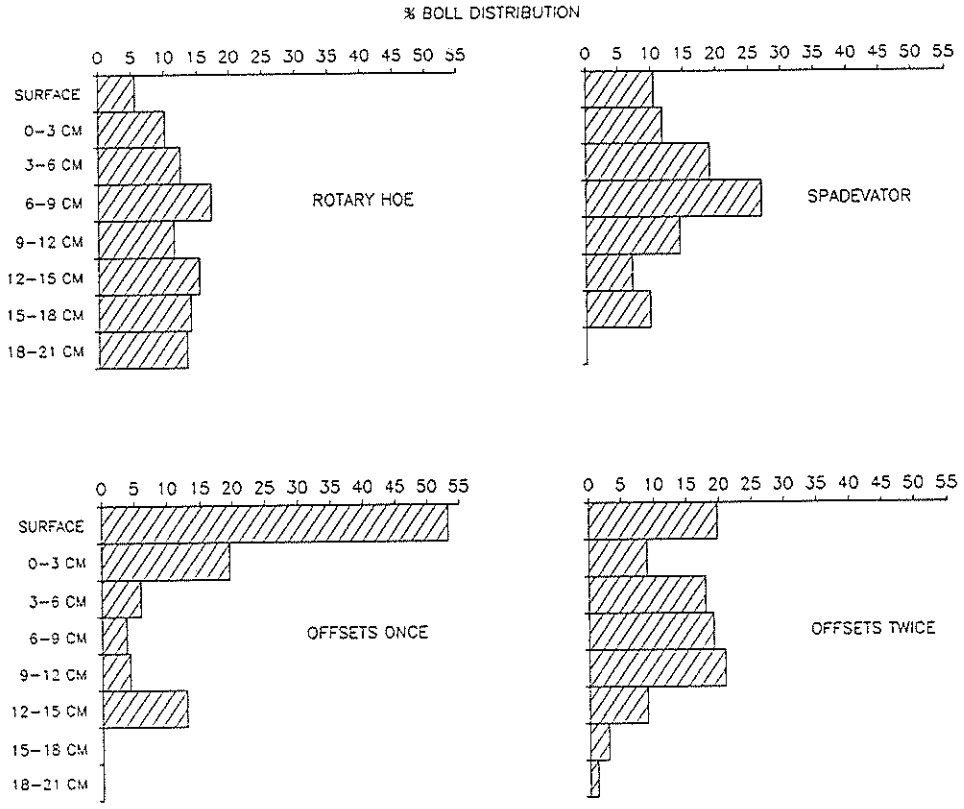

Fig. 6.1. Vertical distribution of open cotton bolls buried under soil using four tillage systems; offset discs once, offset discs twice, rotary hoe and spadovator plough. N = 200 bolls per treatment.

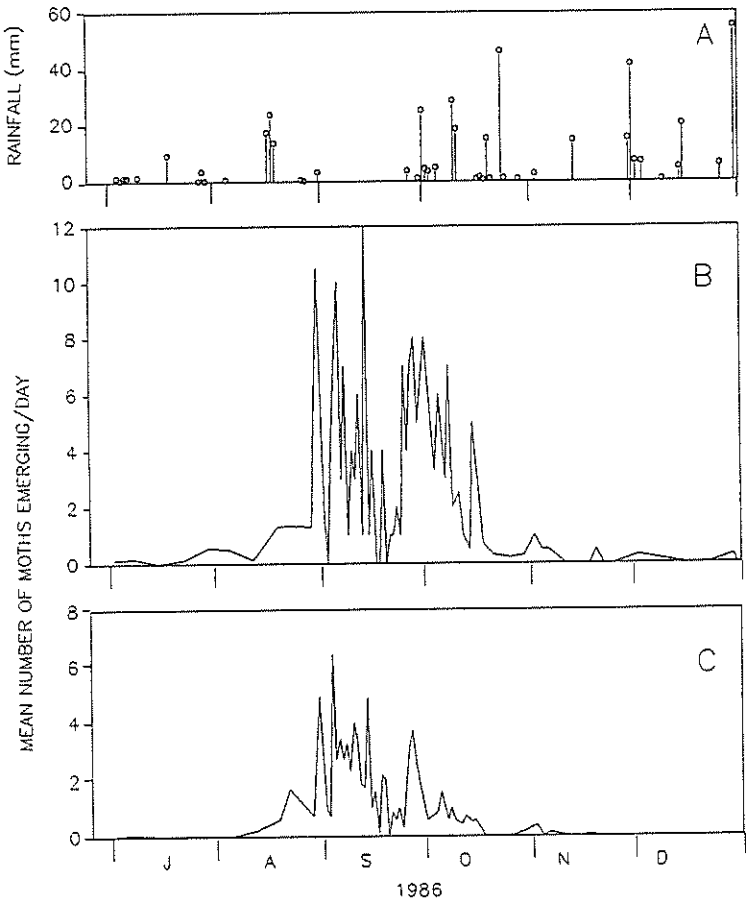

Fig. 6.2. Mean number of *P. scutigera* emerging/cage/day from unburied bolls (B) and buried bolls (C) at the D.P.I. Research Station, Biloela, during 1986. Emergence from buried bolls represents the mean of 7 tillage treatments (see text for details). All bolls were placed in the field on 18 June 1986. Graph (A) shows daily rainfall records during the period of moth emergence.

Table 6.1. Monthly emergence of *P. scutigera* (%) from dry cotton bolls, unburied or buried under soil using seven tillage practices, during 1986. The percentage of moths emerging after October 15 (>15/10) and November 26 (>26/11) is also shown. N = three replicate cages per tillage treatment.

Tillage treatment	Mean % moth emergence/cage/date (± S.D.)[1]							
	J	A	S	O	N	D	>15/10	>26/11
Unburied	1.9a (0.1)	21.8a (4.0)	50.3a (2.5)	22.1ad (2.1)	2.8a (0.2)	1.1a (1.7)	6.8a (2.0)	1.8a (1.6)
Offsets twice	0.7a (1.1)	30.1a (4.0)	64.2a (5.7)	4.5bce (1.5)	0.5a (0.9)	0a -	2.1a (3.6)	0a -
Offsets once	1.0a (1.2)	17.7a (4.6)	67.7a (3.2)	12.9ace (8.4)	0.7a (1.2)	0.1a (0.2)	1.5a (1.3)	0.8a (1.4)
Offsets + hilled-up	1.5a (2.0)	18.2a (6.5)	56.5a (13.7)	17.4ad (8.1)	5.8a (5.2)	0.5a (0.4)	8.3a (5.5)	3.5a (3.1)
Spadovator	0.8a (1.5)	48.2a (32.2)	46.7a (27.4)	4.3bce (3.9)	0a -	0a -	1.7a (3.0)	0a -
Spadovator + hilled-up	2.2a (2.4)	24.1a (10.3)	61.2a (3.2)	9.7ae (2.2)	2.0a (3.5)	0.3a (0.6)	2.7a (4.6)	0.7a (1.1)
Rotary-hoe	0a -	29.8a (1.9)	69.0a (1.8)	0.6bc (1.0)	0.6a (1.0)	0a -	1.2a (2.1)	0a -
Ratoon	1.5a (1.4)	19.8a (6.3)	41.4a (14.9)	34.3d (20.1)	3.0a (3.1)	0a -	10.7a (12.9)	0a -

1. Means within columns followed by the same letter are not significantly different (L.S.D. test, p = 0.05).

ANOVA (all data transformed to arcsin (x + 0.05)):
July F=0.91, df=7/16, p>0.05
August F=1.92, df=7/16, p>0.05
September F=1.95, df=7/16, p>0.05
October F=7.72, df=7/16, p<0.001
November F=1.99, df=7/16, p>0.05
December F=1.38, df=7/16, p>0.05
>15/10 F=1.37, df=7/16, p>0.05
>26/11 F=2.05, df=7/16, p>0.05

Table 6.2. Effect of boll burial, using seven tillage treatments, on the emergence of *P. scutigera* moths from dry cotton bolls. N = three replicate cages per treatment.

Tillage treatment	Mean (± S.D.) No. of bolls/cage	Estimated no. larvae/cage	No. of moths emerged/cage	Percent[1] emergence
Unburied	200.0 (0)	227.4 (0)	75.3 (10.7)	33.1a (4.7)
Offsets twice	180.0 (6.6)	204.6 (7.5)	39.0 (10.4)	19.1b (5.3)
Offsets once	197.7 (2.5)	224.7 (2.9)	42.3 (2.5)	18.8b (1.4)
Offsets + hilled-up	188.7 (1.1)	214.5 (1.3)	40.0 (4.6)	18.6b (2.0)
Spadovator	180.7 (18.5)	205.4 (21.0)	23.7 (13.6)	11.5b (6.5)
Spadovator + hilled-up	170.3 (27.1)	201.3 (36.6)	32.3 (15.5)	15.9b (6.2)
Rotary-hoe	196.3 (2.1)	223.2 (2.3)	32.7 (10.8)	14.7b (5.0)
Ratoon	165.7 (24.5)	188.3 (27.9)	33.3 (34.0)	16.7b (14.6)

1. Means within column followed by the same letter are not significantly different (L.S.D. test, p=0.05).

ANOVA: ($\log_{10}$ transformed data) F=2.67, df=7/16, p<0.05

Table 6.3. Linear regression parameters for the relationship between cumulative proportion moth emergence (plotted as probits) and Julian date for seven tillage treatments and a control of unburied bolls.

Tillage treatment	Regression parameter		
	a (+ S.E.)	b (+ S.E.)	r^2
Unburied	-4.0278 (0.2608)	0.0349a[1] (0.0010)	0.9543***[2]
Offsets twice	-8.5533 (0.4333)	0.0542bcf (0.0017)	0.9543***
Offsets once	-6.2971 (0.4854)	0.0489b (0.0018)	0.9102***
Offsets + hilled-up	-4.0379 (0.3545)	0.0349ae (0.0013)	0.9218***
Spadovator	-7.3420 (0.2979)	0.0503c (0.0016)	0.9748***
Spadovator + hilled-up	-5.0638 (0.4772)	0.0392de (0.0017)	0.8950***
Rotary-hoe	-9.7757 (0.7615)	0.0600f (0.0029)	0.9069***
Ratoon method	-5.1278 (0.2419)	0.0396d (0.0009)	0.9721***

1. Values of b followed by the same letter are not significantly different ($p>0.05$).

2. *** indicates regression is significant at $p<0.0001$.

treatment was lower than expected as a large proportion of cotton trash and bolls remained on the soil surface after ploughing. This may have been caused by a lower capture efficiency of emerging moths through the use of two cages (as opposed to one cage in all other treatments) to cover the widely spread bolls. Also, a significant proportion of bolls were not covered by the cages due to the triple-disc hiller dragging cotton trash and bolls over a long distance. In the replicates with the lowest moth emergence 48 and 49 of the original 200 bolls buried (24 and 25%) were not covered by the emergence cages. Similarly, in the first spadovator treatment replicate 60 (30%) bolls were recovered outside the cage. In other treatments between 0-26 bolls were missed by the emergence cages.

Because of the unequal number of bolls in each treatment, overall moth emergence was expressed as a percentage of the estimated number of *P. scutigera* present in the 200 cotton bolls before burial, adjusting the results for missing bolls (Table 6.2). ANOVA on the adjusted results, detected a significant difference ($p<0.05$) in the overall percentage emergence of moths between treatments (Table 6.2). However, comparison of means using a L.S.D. test, revealed that only unburied bolls were significantly different from the tillage treatments ($p<0.05$). No tillage treatment was statistically better than any other (Table 6.2).

Hilling-up of the soil immediately after boll burial did not affect the overall percentage emergence of moths in the offset discs treatment. When the spadovator plough was used, hilling-up of the soil increased the percentage of moths emerging from 11.5 to 15.9% (Table 6.2).

The rate of moth emergence from unburied bolls, as indicated by the slope of the regression line (b), was significantly lower ($p<0.05$) than for all other treatments, except for the offset discs once + soil hilled-up treatment which had an identical slope value (b=0.0349) (Table 6.3). The fastest rate of moth emergence occurred in the rotary-hoe treatment (b=0.06), closely followed by the offset discs twice (b=0.0542) and the spadovator (b=0.0503) treatments.

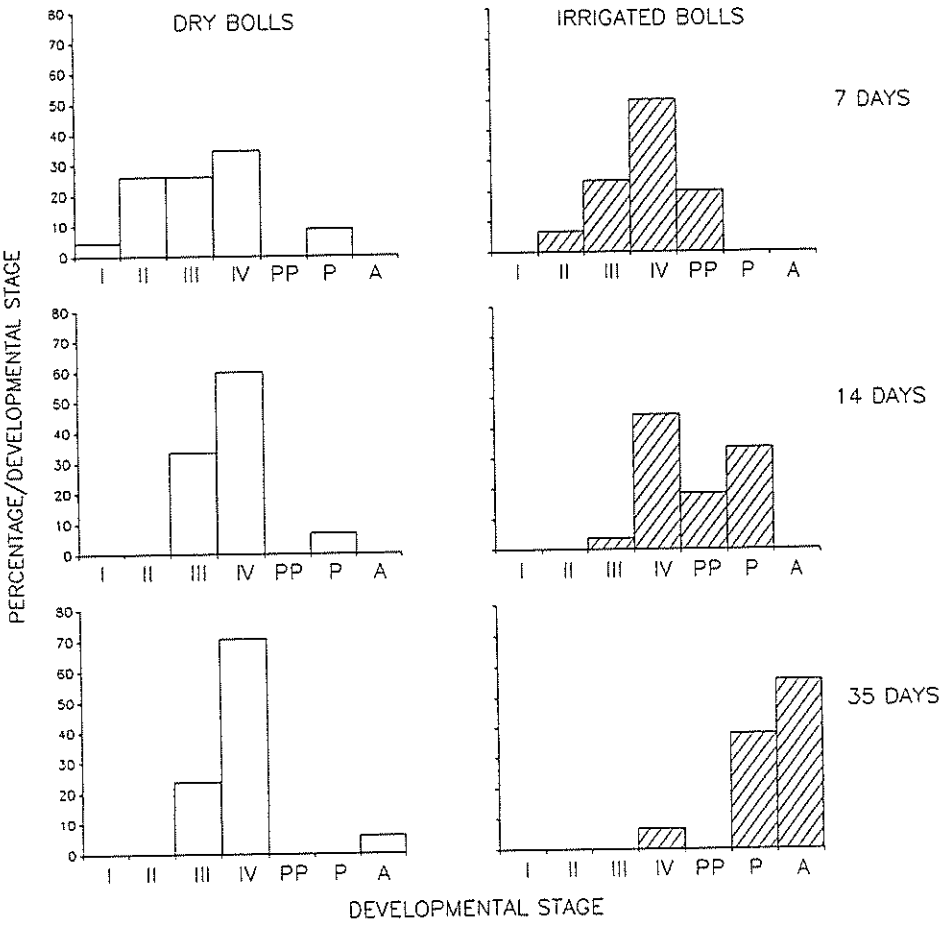

Fig. 6.3. Development of *P. scutigera* infesting open cotton bolls buried under 5 cm of soil and kept dry (open bars) or irrigated (hatched bars) with 2.5 cm of water on day 1. Bolls were sampled on day 7, 14, and 35 (N = 10 pots/treatment). The percentage of each larval instar (I-IV), prepupae (PP), pupae (P) and adult moths (A) found in each treatment is shown.

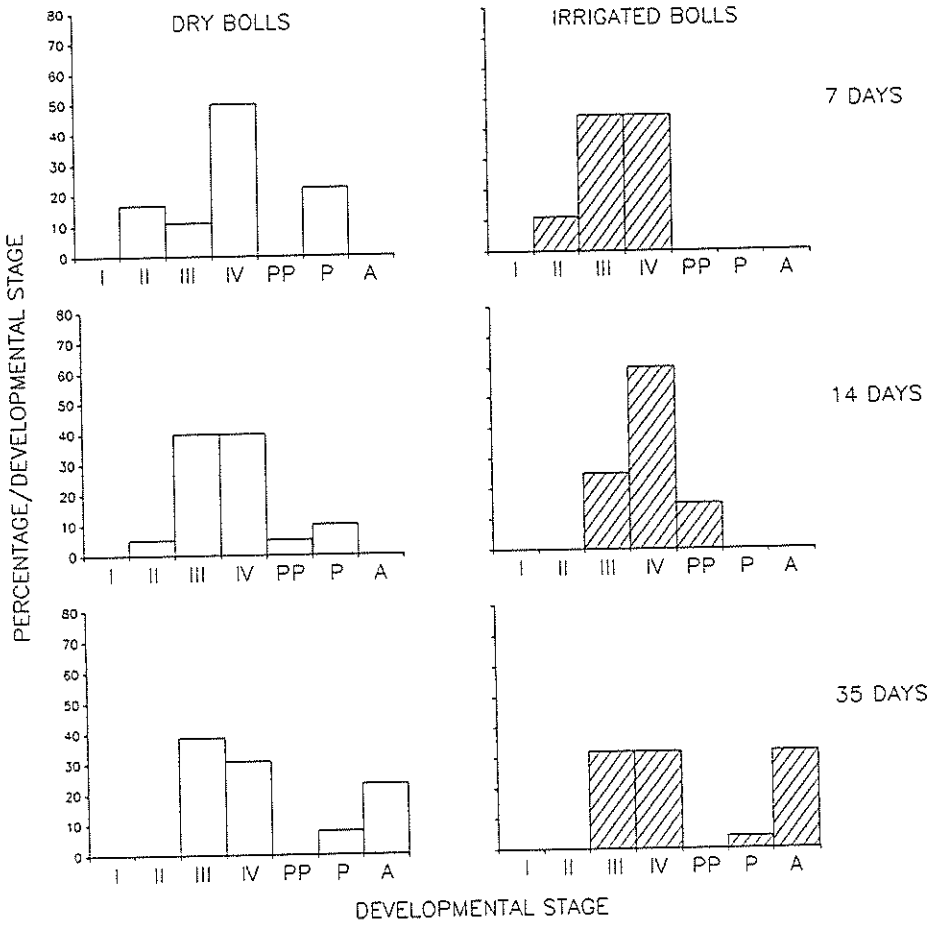

Fig. 6.4. Development of *P. scutigera* infesting open cotton bolls placed on the soil surface and kept dry (open bars) or irrigated (hatched bars) with 2.5 cm of water on day 1. Bolls were sampled on day 7, 14, and 35 (N = 10 pots/treatment). The percentage of each larval instar (I-IV), prepupae (PP), pupae (P) and adult moths (A) found in each treatment is shown.

6.3.2. Effect of artificial irrigation of buried and unburied bolls on *P. scutigera* development

Development of *P. scutigera* was much faster in dry cotton bolls that were buried and irrigated with water than in all other treatments (Fig. 6.3 and 6.4). Seven days after experiments were set up, 70% of the larvae found in buried, irrigated bolls were in the fourth instar. Seventy-one percent of these fourth instars were in the prepupal stage, being found within loose silken cocoons. On the same sampling date, only 34.8% of all *P. scutigera* found in buried, unirrigated bolls were in the fourth instar. None of these larvae were in the prepupal stage, although 8.7% of all *P. scutigera* found were pupae. Similarly, in both unburied boll treatments, the number of larvae in the fourth instar was lower and none were in the prepupal stage. Pupae (22.2% of all *P. scutigera* found) were only present in the unirrigated treatment.

Fourteen and 35 days after treatment, the advancement in the development of *P. scutigera* in buried, irrigated bolls was even more marked compared to moths in all other treatments (Fig. 6.4). There was also a marked difference in the physiological condition of larvae found inside bolls. In buried, irrigated bolls larvae remained in a normal, healthy state while in all other treatments larvae were smaller and had a dehydrated appearance. These smaller, dehydrated larvae were always found within bolls, usually inside damaged cotton seeds. Larvae were active when removed from the boll and if placed on artificial diet they immediately started feeding.

The physiological condition of larvae was clearly related to the moisture content of bolls. The moisture content of irrigated, buried bolls was significantly greater than for bolls in all other treatments at 7 and 14 days after irrigation (Table 6.4, $p<0.05$). Seeds of these bolls were soft which enabled larvae to actively fed on the tissue. In contrast, bolls left on the soil surface absorbed very little water on irrigation. The moisture content of these bolls was not significantly greater than unirrigated bolls (Table 6.4, $p>0.05$). Seeds within these bolls remained hard and dry. Larvae appeared to be unable to feed on the dry material, leading to suppressed development.

After 35 days, the moisture content of bolls in all treatments was very similar. Immature larvae remaining in the irrigated, buried bolls (6.7% of all *P. scutigera* found), started to

Table 6.4. Change in the moisture content of buried and unburied dry, open cotton bolls over time when artificially irrigated with 2.5 cm of water or left dry. In all treatments bolls were irrigated on day 0, except in the delayed irrigation treatment when water was applied on day 49. N = 8-9 bolls/treatment/date.

Boll/irrigation treatment	% boll moisture (± S.D.) on experimental day[1]			
	7	14	35	63
Buried bolls irrigated	39.9a (7.5)	42.6a (4.9)	35.0a (8.4)	-
Buried bolls unirrigated	24.5b (5.8)	27.7b (5.0)	31.9a (6.2)	37.8a (6.4)
Buried bolls delayed irrigation	-	-	-	48.8b (6.4)
Surface bolls irrigated	27.3b (3.6)	32.4b (5.6)	34.2a (8.8)	-
Surface bolls unirrigated	23.4b (4.8)	27.4b (4.5)	32.7a (8.2)	37.1a (3.4)
Surface bolls delayed irrigation	-	-	-	39.3a (4.8)

1. Means within columns followed by the same letter are not significantly different (L.S.D. test, p=0.05).

ANOVA:

Day 7 $F=16.6$, $df=3/32$, $p<0.05$.
Day 14 $F=18.4$, $df=3/31$, $p<0.05$.
Day 35 $F=0.3$, $df=3/31$, $p>0.05$.
Day 63 $F=8.8$, $df=3/32$, $p<0.05$.

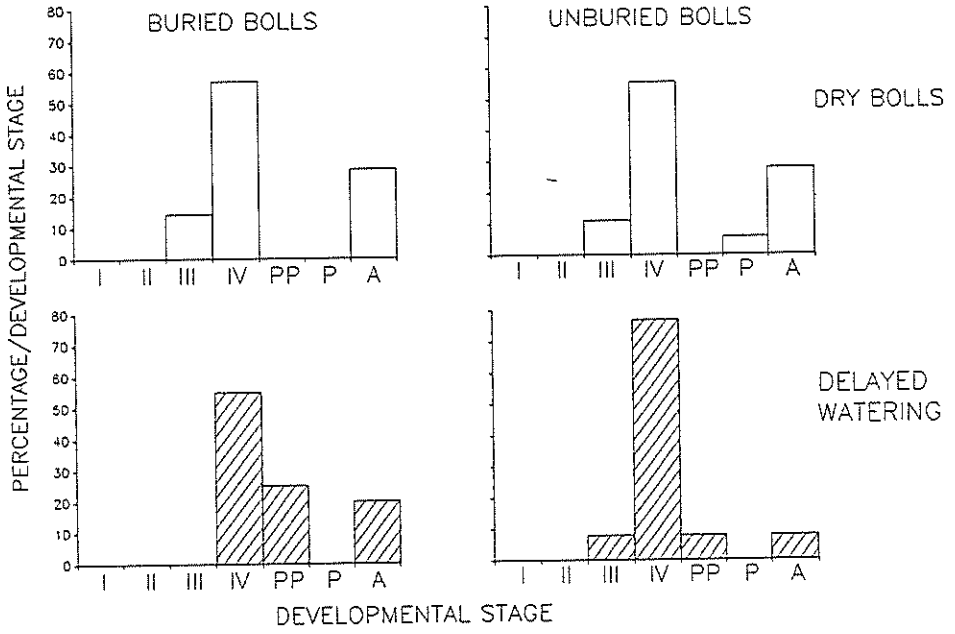

Fig. 6.5. Development of *P. scutigera* infesting open cotton bolls placed on the soil surface or buried under 5 cm of soil, kept dry (open bars) or irrigated (hatched bars) with 2.5 cm of water on day 49, and sampled on day 63. The percentage of each larval instar (I-IV), prepupae (PP), pupae (P) and adult moths (A) found in each treatment is shown.

become dehydrated in appearance as the boll material dried out.

Delayed irrigation of bolls on day 49, also had a dramatic effect on the development of larvae in buried bolls (Fig. 6.5). After irrigation the percentage of fourth instar larvae found within buried bolls increased to 80% by day 63. Twenty-five percent of these larvae were in the prepupal stage. In unirrigated bolls 14.3% of *P. scutigera* found were still in the third larval instar, and of the 57.1% fourth instar larvae found none were in the prepupal stage. Both buried treatments contained a similar proportion of emerged pupae. Again, there was a marked difference in the physiological condition of larvae in the delayed irrigated and unirrigated buried boll treatments. Larvae in the irrigated buried bolls were of healthy appearance and were observed actively feeding inside the soft cotton seeds. The moisture content of irrigated, buried bolls increased to 48.8% compared to 37.9-39.3% in all other treatments. Larvae in unirrigated treatments were very small and many dead larvae were encountered within the bolls.

The location of prepupae and pupae within pots differed in the two burial treatments. No prepupae/pupae in either burial treatment were found within buried bolls. In the unirrigated, buried boll treatment, the majority of prepupae/pupae (85.7%) were found within the top 1 cm of soil, usually on the soil surface. In contrast, very few prepupae/pupae in the irrigated treatment were found on or near the soil surface. Most prepupae/pupae (89.8%) were located directly underneath the 2-3 cm hard soil crust that formed after watering. This crust appeared to form a barrier to pupating larvae, preventing them from reaching the soil surface. Dead adult moths were also found underneath the soil crust, as they were unable to reach the surface on emergence from trapped pupae. Only when large cracks were present in the crust were prepupae/pupae found on the soil surface in the irrigated treatment. In both unburied boll treatments, all prepupae/pupae were found on the soil surface, usually directly underneath the bolls.

6.3.3. Effect of soil type on moth emergence and survival

Soil type significantly affected the number of *P. scutigera* emerging from buried bolls; the mean number of moths emerging/pot was 26 for unburied bolls and 7.7, 3.2 and 1.1 moths

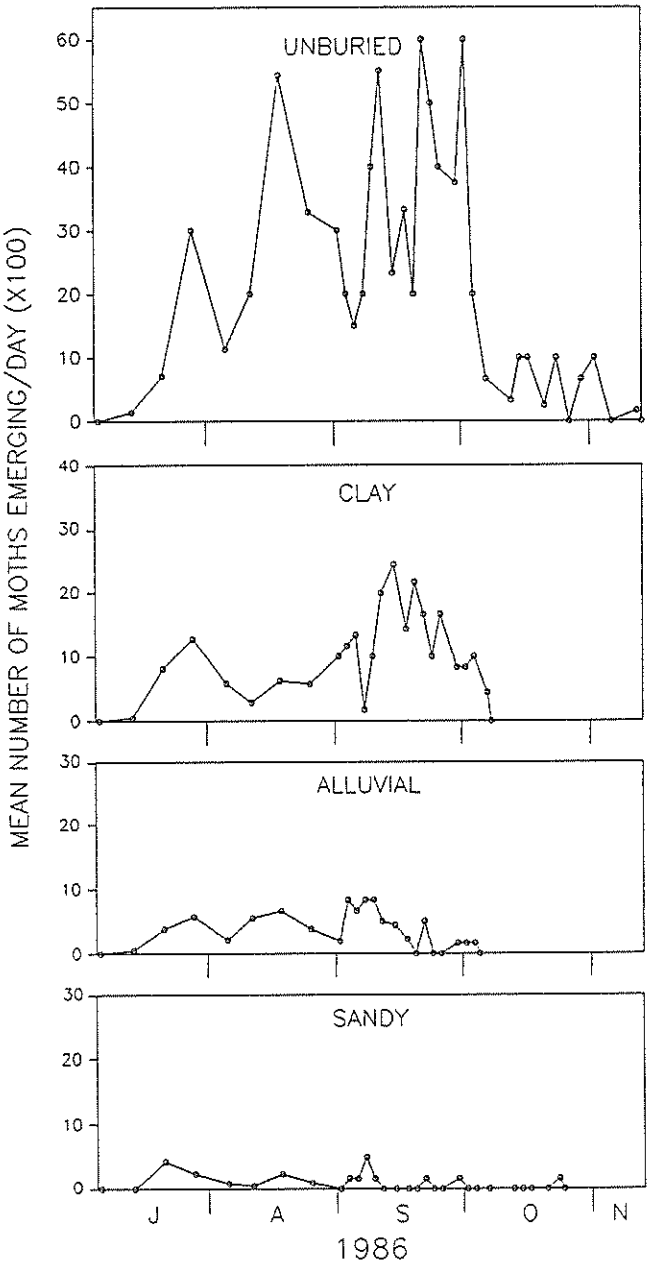

Fig. 6.6. Mean number of *P. scutigera* emerging/pot/day (x 100) from unburied bolls and bolls buried under 10 cm of three contrasting soil types; clay, alluvial and sandy.

Table 6.5. Effect of three soil types on the emergence of *P. scutigera* from buried dry, open cotton bolls, compared with a control of unburied bolls. Number of bolls/pot = 25.

Treatment	No. pots	Mean no. (± S.D.)[1] moths emerging/pot	Range
Unburied	10	26.0a (6.0)	16-32
Clay	30	7.9b (5.1)	1-24
Alluvial	30	3.1c (2.4)	0-8
Sandy	30	1.1d (1.4)	0-6

1. Means within columns followed by the same letter are not significantly different (GT2 test, p=0.05).

ANOVA ($\log_{10} x + 1$ transformed data): F=72.58, df=3/96, p<0.0001.

Table 6.6. Effect of soil type on the emergence of *P. scutigera* from buried and unburied bolls; linear regression parameters for cumulative moth emergence plotted as probits against Julian date.

	Regression parameter		
Treatment	a (+ S.E.)	b (+ S.E.)	r^2
Unburied	-5.3425 (0.2466)	0.0420a[1] (0.0009)	0.985***[2]
Alluvial	-6.6872 (0.4176)	0.0504b (0.0018)	0.976***
Clay	-5.7535 (0.7261)	0.0445a (0.0029)	0.919***
Sandy	-1.3478 (0.3660)	0.0293c (0.0014)	0.946***

1. Values of b followed by the same letter are not significantly different (p>0.05).

2. *** indicates regression is significant at p<0.001.

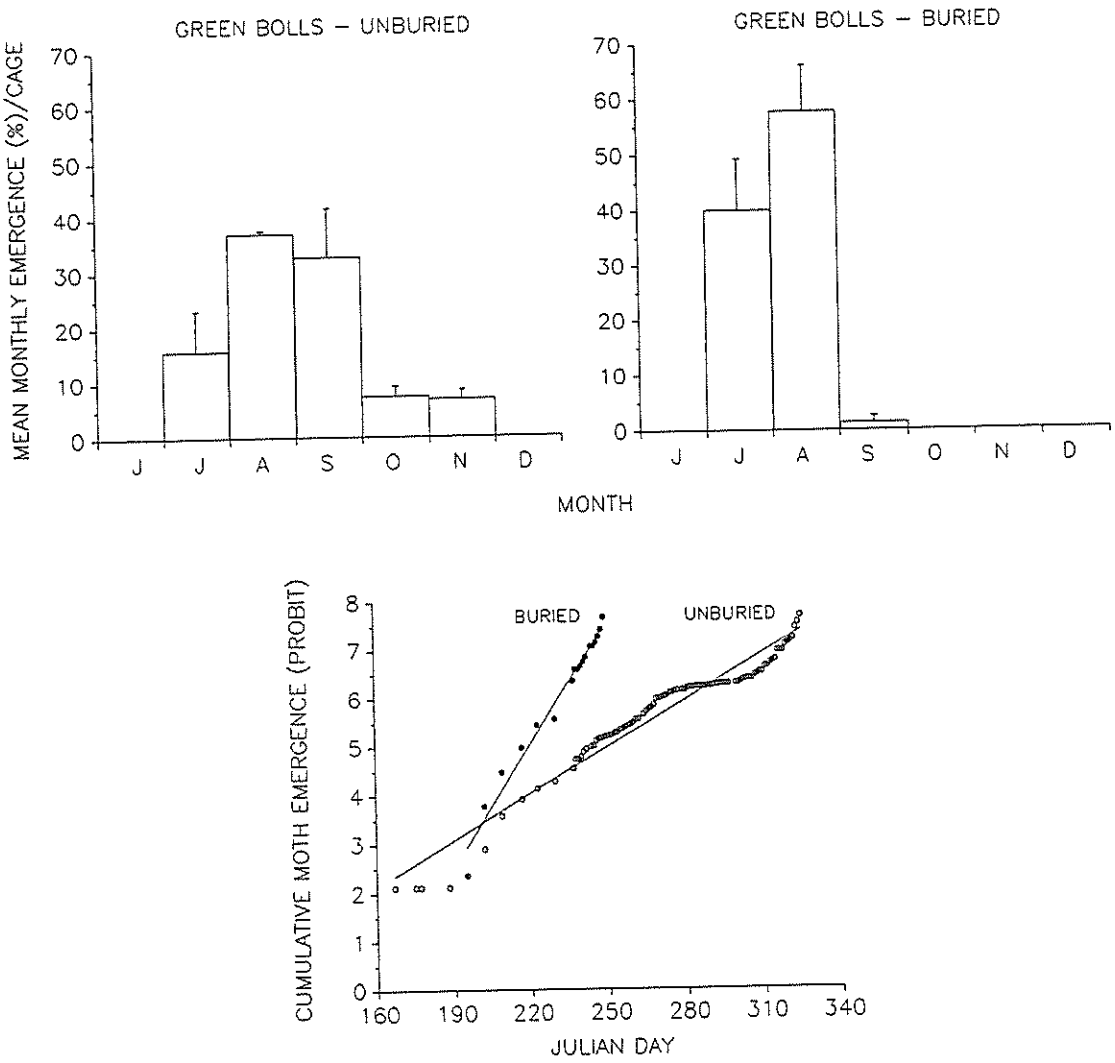

Fig. 6.7. Emergence of *P. scutigera* infesting green, unopened bolls when placed on the soil surface (unburied) or buried under 10 cm of soil. Top graphs show the percentage monthly rate of moth emergence (mean/cage ± S.E.) for each treatment, and the bottom graph shows the cumulative rate of moth emergence plotted as probits against Julian date. Linear regression equations for each treatment are:

Unburied bolls: $y = -3.027 + 0.03205x$ ($r^2 = 0.947$)

Buried bolls: $y = -13.490 + 0.08415x$ ($r^2 = 0.979$)

Regression slopes were significantly different ($t = 13.103$, $df = 4$, $p<0.001$)

for bolls buried under clay, alluvial and sandy soil, respectively (Table 6.5). Moths emerged from unburied bolls over the period 7 July to 11 November 1986 (Fig. 6.6). The last moth to emerge from bolls buried under alluvial soil was on 6 October and 10 October 1986, from clay soil on , but 23 October for bolls buried under sandy soil. Three main peaks in moth emergence from unburied bolls occurred; a minor peak in late July, followed by two major peaks in late August and in late September/early October. The pattern of moth emergence from buried bolls differed; peaks in emergence were advanced by *ca*1 week and the peak in late August was much smaller (Fig. 6.6).

The rate of moth emergence, as indicated by the slope of the regression line (b) of cumulative moth emergence (probits) against Julian date, was significantly faster when bolls were buried under alluvial and clay soil, than for bolls unburied or buried under sandy soil (Table 6.6).

6.3.4. Emergence of *P. scutigera* from green, unopened bolls

A total of 502 *P. scutigera* emerged from green, unopened cotton bolls left on the soil surface, compared to 248 moths emerging from buried green bolls. This represents a survival rate of 30.9 and 15.0% for *P. scutigera* infesting unburied and unburied bolls, respectively. In addition to these moths a total of five and six *Apantales* sp. emerged from cages containing the buried and unburied bolls, respectively.

The rate of moth emergence differed greatly between buried and unburied bolls (Fig. 6.7). The majority of moths emerged from buried bolls in July (40.0%) and August (57.8%). Very few moths emerged from buried bolls in September (1.3%) and the last moth emerged on 8 September. When the bolls were placed on the soil surface they rapidly dried out and had fully opened within a period of *ca* two weeks. Emergence of moths from unburied green bolls extended until 24 November, 1986, with the majority of moths emerging during August (37.1%) and September (32.8%). When the last moth emerged from buried bolls (on 8 September) only 58.3% of moths had emerged from unburied bolls. Comparison of linear regression lines for cumulative moth emergence, plotted as probits against Julian date, revealed a significant difference between regression slopes (Fig. 6.7).

Table 6.7. Effect of photoperiod and larval diet containing cotton seed oil (CSO) on the development of *P. scutigera* at 25° C. Mean number of days (± S.D.) to reach pupation and moth emergence when reared on artificial diet containing, no, low (1%) or high (5%) levels of CSO, under a long (14L:10D) or short (10L:14D) day photoperiod. N = number of moths observed.

Photo -period	Larval diet	N	Days to pupation[1]	Days to moth emergence
Long day	No CSO	74	24.6a (2.8)	35.1a (2.9)
	1% CSO	75	29.3b (3.6)	39.9b (3.8)
	5% CSO	76	31.0b (3.5)	41.7b (3.5)
Short day	No CSO	56	33.2c (3.6)	47.3c (3.6)
	1% CSO	51	36.5d (5.4)	50.7d (5.9)
	5% CSO	33	42.8e (7.7)	56.4e (7.9)

1. Means within columns followed by the same letter are not significantly different (GT2 test, p=0.05).

ANOVA:
Pupation (log_{10} transformed data) F=114.7, df=5/359, p<0.0001.
Emergence F=156.0, df=5/336, p<0.0001.

6.3.5. Effects of photoperiod and cotton seed oil on the development of *P. scutigera*

Both a short-day photoperiod and the addition of cotton seed oil (CSO) significantly lengthened larval development but the increase was too short to constitute a form of diapause (Table 6.7). Under the long-day photoperiod, the mean number of days to pupation increased from 24.6 days, when no CSO was added to the diet, to 31.0 days when the diet contained 5% CSO. Under the short day photoperiod, larvae took 33.2 days to reach pupation on no CSO diet and 42.8 days on 5% CSO diet. The increase in the number of days to reach moth emergence was similar (Table 6.7).

6.4. Discussion

The low moisture content of dry cotton bolls was the major factor limiting the development of overwintering *P. scutigera* larvae. Development was suppressed in dry, open bolls kept dry (<38% moisture content) as larvae were unable to feed on the lint and seed. In response to low moisture content, larvae became inactive. Third and fourth instars were capable of surviving in this condition for long periods of time (>63 days). Larval development proceeded immediately when boll moisture increased after irrigation or rain. Development was completed if boll moisture remained favourable, but became suppressed again if moisture decreased through evapouration.

In disagreement with Sabine (1969a), the ability of *P. scutigera* larvae to survive for long periods of time in dry, open bolls suggests that they enter a form of dormancy. However, unlike *P. gossypiella*, dormancy in *P. scutigera* appears to be a period of quiescence and not a true form of diapause. Quiescence is defined as a condition of growth retardation which is induced directly by the environment in response to a limiting factor (Mansingh 1971). It is the simplest type of insect dormancy and the reaction of individuals is spontaneous, resulting in growth retardation only (Tauber *et al.* 1984). As seen in *P. scutigera* larvae, termination of quiescence is relatively immediate, depending on the speed of re-acclimation of individuals to improved conditions. In contrast, diapause is a more profound interruption of development that is not controlled simply by the direct action of environmental factors (Danks 1987,

Mansingh 1971). Diapause is induced well before the adversity occurs, while termination is complex and can be maintained for some time regardless of environmental conditions (Behrens 1984).

A short-day photoperiod, in combination with a larval diet containing high levels of cotton seed oil, did not induce diapause in *P. scutigera*, as seen in *P. gossypiella* (Bull and Adkisson 1960). Both factors did significantly lengthen developmental rates, when compared to larvae reared on a long-day photoperiod and on diet containing no cotton seed oil. The effect of cotton seed oil on development may have been due to the presence of gossypol, which is known to reduce larval growth rates in other species of Lepidoptera (Chan *et al.* 1978, Shaver and Lukefahr 1969). The independent effect of photoperiod on *P. scutigera* larval development is difficult to explain. Photoperiod has been reported to affect the growth rate of many other insects but usually in association with a diapause response (Saunders 1982).

The effect of exposure to low temperatures on *P. scutigera* development was not investigated as a possible diapause inducing factor. Temperature as a primary diapause inducing factor, has been demonstrated in relatively few species (Tauber *et al.* 1984). In *P. gossypiella*, the effect of low temperatures on the proportion of larvae entering diapause is not readily apparent except under diapause inducing photoperiods (Adkisson *et al.* 1963).

The ability of *P. scutigera* larvae to survive in dry, open cotton bolls can be related to it s life history on alternative host plants, where larvae feed on the fallen forms which undergo similar extreme changes in moisture content (Chapter 5). The significance of this adaptation, in terms of the pest status of *P. scutigera*, is discussed in Chapter 8.

The low moisture content of dry cotton bolls, as a major factor limiting the development of *P. scutigera* larvae, explains why burial and irrigation treatments are beneficial in reducing the carry-over of moth populations from one cotton season to the next, as reported by Sabine (1969a, 1969b). Burial of cotton bolls advanced the development of larvae, compared to bolls left on the soil surface, due to higher moisture levels. The surrounding layer of soil acted as a reservoir of water from which the dry bolls absorbed moisture. Also, the overlying soil layer considerably reduced the loss of boll moisture through evapouration. Bolls left on the soil

surface absorbed significantly less water than buried bolls (Table 6.4), and in the field, moisture would be quickly lost due to direct exposure to the sun and wind.

Moth emergence from open bolls was very variable and spread over a long period of time (Fig. 6.2). Developmental rates of *P. scutigera*, reared on artificial diet and at constant temperatures, are inherently variable, particularly during the fourth larval instar and at low temperatures (Chapter 2). Further variation in larval development rates would be induced according to the position of the cotton boll within the soil, the rate of water absorption and evapouration, and quality of the food for larval development.

Further support for the hypothesis that moisture is the key factor controlling the development and emergence of *P. scutigera* from dry cotton bolls was apparent from the rate of emergence of moths from green, unopened bolls. Moth emergence from green bolls buried under 10 cm soil was very fast compared to unburied green bolls and dry bolls (compare Figs. 6.2, 6.5 and 6.6). This would be expected due to the much higher moisture content of green bolls, allowing larval development to proceed at a much faster rate than larvae infesting dry bolls. When green bolls were left on the soil surface they rapidly desiccated, hence causing larval development to be prolonged. This not only further illustrates the importance of burying cotton bolls as a cultural control method, but also demonstrates the potential of larvae infesting green unopened bolls to carry-over moth populations into the next season.

The rate and period of moth emergence from dry bolls would vary each year according to the incidence and pattern of rainfall. As indicated in Chapter 3, rainfall in the Callide and Dawson Valleys is extremely variable between years (Table 3.1). The pattern of moth emergence observed by Sabine (1969a), from bolls placed in the field in early June, differed greatly from that observed in this study. Accordingly, rainfall figures during Sabine's trials in 1961-62 and 1962-63 differed greatly from 1986, particularly in September 1961 and October 1962 when rainfall was very low. However, differences in rainfall cannot entirely explain why emergence of moths from unburied bolls in Sabine's (1969a) experiments was so prolonged compared to that observed in this study. While rainfall was comparatively low between July and October 1961, very heavy rainfall occurred in November 1961 (135 mm

compared to 74 mm in 1986) yet moth emergence extended until February, 1962. Such extended emergence may indicate that a small proportion of *P. scutigera* do in fact enter a true form of diapause.

Alternatively, the extended period of emergence in Sabine's (1969a) trial may have been due to the reinfestation of unburied boll material by moths emerging within cages, giving rise to a new generation of *P. scutigera*. Preliminary experiments during this study showed that only 80-90% of moths released inside emergence cages were recovered. Furthermore, recovery of moths was spread over a period of nine days, within which mating and oviposition could have taken place. In Chapter 3, it was demonstrated that open cotton bolls are an attractive oviposition substrate to egg-laying *P. scutigera*.

Further advancement in larval development may occur in buried bolls due to the insulating effect of the overlying soil. Temperature would only influence the rate of larval development when boll moisture was high enough for feeding to occur. If temperature was the only factor controlling the rate of development of overwintering *P. scutigera*, then we would expect all moths to have emerged from unburied bolls placed in the field on 18 June 1986, within approximately 515 degree-days (DD), based on developmental rate data presented in Chapter 2. When the number of DD accumulated over the emergence period of moths was calculated, using the DEGDAY programme of Higley *et al.* (1986) and weather data presented in Chapter 3, a total of 1652 DD accumulated from 18 June, 1986, to the emergence of the last moth on 29 December, 1986. A total of 515 DD was accumulated by 25 September 1986, when only 74 and 86% of moths had emerged from unburied and buried bolls, respectively.

The reduction in the number of moths emerging from buried bolls, as observed by Sabine (1969a) and in this study, was probably a consequence of soil crusting rather than the physical action of burial. All larvae infesting dry bolls were well protected, being located within damaged seeds. On burial, larvae appeared to remain within damaged seeds until ready for pupation. Most mortality occurred through the formation of a hard soil crust overlying the buried bolls after rain or irrigation. In pot experiments, the hard crust formed a layer impenetrable to pupating larvae attempting to reach the soil surface. This forced pupation to occur directly underneath the soil crust, resulting in the emerging moth becoming trapped and

dying. Only if larvae located cracks in the soil crust did pupation take place on the soil surface.

The degree of crusting varied according to the physical properties of the soil. Sandy soil formed a much harder crust than alluvial and clay soils, and this factor was probably responsible for the significantly lower number of moths emerging from bolls buried underneath sandy soil. The emergence of *P. gossypiella* from bolls buried under sandy soil is also significantly lower than for other soil types (Chapman *et al.* 1960, Richard and Clark (1965).

The rate of microbial breakdown (bacterial and fungal) of boll material may also affect the survival of *P. scutigera* larvae in buried bolls. The more moist conditions experienced in buried bolls would ensure a more rapid rate of decay than in drier bolls on the soil surface. This may reduce the survival, hence emergence, of *P. scutigera* if microbial breakdown of boll material occurs before all larvae complete development.

Soil type significantly affected the rate of moth emergence, as indicated by the slope of the regression line of accumulative moth emergence plotted as probits against Julian date (Table 6.6). The rate of moth emergence was lower when bolls were buried under sandy soil than both alluvial or clay soil, while emergence from bolls under alluvial soil was significantly slower than under clay soil. This may be related to differences in the drainage characteristics of each soil type. The well-draining sandy soil had a low water retention capacity compared to the sticky clay soil, while the alluvial soil was of intermediate type. Consequently, the absorption and loss of moisture by cotton bolls buried under each soil type differ, affecting the speed of larval development.

Of the seven tillage practices compared in this study, there was no significant difference in the total number of *P. scutigera* moths emerging. However, there were significant differences in the rate of moth emergence. Generally, the most efficient tillage treatments (spadovator plough and rotary-hoe), in terms of the proportion of bolls covered by soil (Fig. 6.1), had the fastest rates of moth emergence.

The slower the rate of moth emergence, then the greater the chance that moths may carry-

over directly into the next cotton season. Table 6.1 shows the percentage of moths emerging from each tillage treatment after October 15 and November 26, the usual date of planting and squaring of cotton in the Callide and Dawson valleys (Sabine 1969a). In all treatments, including unburied bolls, very few moths emerged after both dates. The worst tillage treatment was offset discs once + soil hilled-up, in which 8.3 and 3.3% of moths emerged after October 15 and November 26, respectively. In four of the tillage treatments no moths emerged after November 26.

Regardless of the tillage treatment, peak moth emergence from dry bolls coincided with the mass flowering period of ornamental *B. australis* trees (Chapter 5). Between 51 and 80.5% of moths emerged from buried bolls during September and October (Table 6.1, Fig. 6.2) at a time when no squaring cotton was available for infestation. The importance of *B. australis* trees in the carry-over of *P. scutigera* populations from one cotton season to the next is discussed further in Chapters 5 and 8.

CHAPTER 7

Longevity of adult *P. scutigera* in relation to the carry-over of populations from one cotton season to the next

7.1. Introduction

When considering how *P. scutigera* populations carry-over from one cotton season to the next, the role of the adult moth must not be overlooked (Holdaway 1926). Vickers (1982a) found that male and female *P. scutigera* lived for up to 113 and 65 days, respectively, at 25^{o} C, with a mean longevity of 56 days for males and 42 days for females. If similar life-spans can be attained in the field, *P. scutigera* emerging from cotton crop residues as early as September may be capable of surviving until new cotton crops begin squaring in November. More importantly, in terms of larval performance (Chapter 2), moths emerging from cotton trash and *B. australis* flowers (Chapter 5) in October or November may be able to survive until cotton starts to produce bolls in late December.

The longevity and rate of oviposition of *P. scutigera* under ambient temperatures during spring months are unknown. Some species of Lepidoptera undergo a reproductive diapause if emergence occurs during periods unsuitable for population increase (Harcourt and Cass 1966, Jacobson 1960). Conversely, rates of development and oviposition may be higher under fluctuating temperatures than constant temperatures (Ratte 1984, Welbers 1975). Larval diet may also affect longevity and rate of oviposition. Vickers (1982a) only measured the longevity of moths reared on artificial diet. Less suitable larval diets may result in shorter life-spans of adult moths or lower rates of oviposition (Chapter 2).

In this Chapter the longevity and rate of oviposition of *P. scutigera* at 25^{o} C and under ambient temperature conditions is compared, using moths originating from experiments in Chapters 2, 5 and 6. Also in this Chapter the effects of delayed mating on reproduction of *P. scutigera* is reported. A delay in mating has been shown to have a negative effect on the

reproductive capacity of some Lepidoptera by altering the females readiness to mate, or by lowering the production or the proportion hatching of eggs (van der Kraan and van der Straten 1988). Delayed mating may occur in *P. scutigera* when population densities are low during emergence from overwintering sites or from *B. australis* flowers. A delay in mating would be further prolonged if the development of the sexes is asynchronous. Such asynchrony may occur due to the extremely variable development rates of larvae infesting dry cotton bolls and fallen flowers of alternative host plants (Chapters 5 and 6).

7.2. Materials and Methods

7.2.1. Longevity and rate of oviposition of *P. scutigera* at 25° C

The longevity and rate of oviposition of moths reared on *B. australis* flowers, dry cotton bolls and *H. rosa-sinensis* flowers was determined at 25° C (± 1° C, 14 hour photoperiod). All moths originated from experiments reported in Chapter 2 and were held in oviposition chambers (two males and one female per container) with a 5% sugar solution for food. Tissue paper was provided as an oviposition substrate. Mortality of moths and the number of eggs laid was checked daily until all individuals had died. The number of males and females observed respectively, was 30 and 14 for *B. australis*, 16 and 9 for dry cotton, and 20 and 10 for *H. rosa-sinensis*.

7.2.2. Longevity and rate of oviposition of *P. scutigera* emerging from *B. australis* flowers in an insectary

The longevity and rate of oviposition of moths emerging from caged *B. australis* flowers (see Chapter 5 for details) was determined in an insectary at the D.P.I. Research Station, Biloela, during 1986. Moths emerging each day were removed from cages and placed in oviposition chambers as above. The number of male and female moths placed in each chamber varied according to emergence rates. A total of 45 males and 38 females that emerged from *B. australis* flowers between 26 October and 9 November, 1986, were observed.

7.2.3. Rate of oviposition of overwintered *P. scutigera* emerging from cotton trash in an insectary

The rate of oviposition of moths emerging from cotton crop residues (cotton trash) was monitored in an insectary as above. Longevity of moths was not recorded. Moths were removed from emergence cages daily and placed in oviposition chambers between 1 September and 9 November. A total of 75 males and 53 females were placed in the insectary and the number of eggs laid was recorded every 1-4 days until all females died.

7.2.4. Effects of delayed mating on reproduction

The effects of delayed mating on reproduction of *P. scutigera* was determined by pairing moths 10, 20 and 30 days after eclosion. The results were compared with those reported in Chapter 2 where moths were paired immediately after eclosion (<1 day). All moths were reared on artificial diet at 25^{o} C ($\pm$ 1^{o} C, 14 hour photoperiod). Pupae were sexed and held in petri dishes until emergence. Newly emerged adults were held in oviposition chambers (see Chapter 2), provided with 5% sugar solution for food and incubated at 25^{o} ($\pm$ 1) C. A total of 15 females were paired with a single, unmated male of the same age 10, 20 or 30 days after eclosion. Each pair of moths was held in separate oviposition chambers and egg sheets were checked daily for eggs until death of the female. The effective longevity (number of days from eclosion to last egg laid), preoviposition period, number of eggs laid, duration of oviposition and percentage egg hatch was recorded. The number of progeny (first instar larvae) produced by each female was calculated by multiplying the total number of eggs laid by the proportion of fertile eggs (Lingren *et al.* 1988).

7.2.5. Data analysis

The number of degree-days above 16^{o} C, accumulated during the period of observation of moths in the insectary, was calculated using the DEGDAY program of Higley *et al.* (1986). Maximum and minimum air temperatures reported in Chapter 3 were used in the programme, choosing the sine wave option for calculating degree-days. A lower temperature threshold of 16^{o} C was used as this is thought to approximate the flight activity threshold of adult moths, based on field observations reported in Chapter 3.

Cumulative rates of oviposition and mortality were converted to percentages and plotted as

probits against the cumulative number of degree-days for each diet and temperature combination. Moths from *B. australis* flowers were considered as a single cohort and analysed together. Moths emerging from cotton trash within the same seven day period, starting from 1 September, were grouped together to give six separate plots. Linear regression lines were fitted to the data and the slope of each line (b) was compared to determine if the rate of oviposition or mortality was statistically different.

7.3. Results

7.3.1. Longevity and rate of oviposition of *P. scutigera* at 25° C

At 25° C *P. scutigera* reared on *B. australis* flowers (BT) and dry cotton bolls (DC) had similar life-spans while those reared on *H. rosa-sinensis* flowers (OH) lived for significantly shorter periods of time ($p<0.05$, Table 7.1, Fig. 7.1). Males lived significantly longer than females ($p<0.05$); mean longevity of males was 59.2, 55.3 and 39.3 days for BT, DC and OH moths, respectively, and female longevity was 39.1, 34.3 and 22.5 days for each diet, respectively. Expressed as degree-days (DD) above 16° C, mean longevity ranged from 353.7 to 504 DD for males, and 202.5 to 351.6 DD for females (Table 7.1). The longest lived male and female both originated from BT, attaining life-spans of 126 days (1134 DD) and 68 days (612 DD), respectively.

The rate of oviposition differed in each diet group (Fig. 7.2) and reflected the longevity of females. Although OH moths laid the greatest number of eggs per female (mean = 225.0), oviposition ceased 32 days (288 DD) after moth emergence. BT moths laid a mean of 143.7 eggs per female but oviposition continued up to 60 days (540 DD) after emergence. DC moths laid a mean of 163.7 eggs per female and oviposition ceased on the 44th day after emergence (396 DD). Further details on aspects of reproduction for each diet group were given in Chapter 2.

When percentage cumulative oviposition was plotted as probits against cumulative DD, the relationship was highly linear (Table 7.2). Comparison of regression slopes (b) revealed a significant difference between all diet groups ($p<0.05$) with OH moths having a much steeper

Table 7.1. Longevity of adult *P. scutigera* reared on 3 larval diets at 25° C, expressed in (A) days and (B) degrees days accumulated above 16° C. Larval diets were: *B. australis* flowers (BT), dry cotton bolls (DC) and *H. rosa-sinensis* flowers (OH). In table (B) the longevity of moths emerging from *B. australis* flowers and placed in an insectary (BT INS) is also shown for comparison (N = 45 males and 38 females).

(A)

	Longevity (days)					
	Males			Females		
Larval diet	N	Mean (+ S.D.)	Range	N	Mean (+ S.D.)	Range
BT	30	59.2 (40.9)	6-126	14	39.1 (19.8)	12-68
DC	16	55.3 (30.6)	15-103	9	34.3 (15.7)	8-31
OH	20	39.3 (31.1)	10-112	10	22.5 (8.1)	9-33

(B)

	Longevity (degree-days)[1]						
	Males			Females			
Larval diet	Mean	(± S.D.)	Range	Mean	(± S.D.)	Range	t-test[2]
BT	504	(366)a	54-1134	352	(178)a	108-612	2.20*
DC	498	(276)ab	117-927	309	(142)ab	72-450	2.26*
OH	354	(278)bc	90-1008	202	(72)bc	81-297	2.28*
BT INS	244	(108)c	116-500	222	(104)c	52-539	1.01

1. Means within columns followed by the same letter are not significantly different (L.S.D. test, p=0.05).

ANOVA Males F=9.46, df=3/107, p<0.005
ANOVA Females F=5.57, df=3/67, p<0.05

2. T-test between sexes, * indicates a significant difference in longevity between males and females within rows at p<0.05.

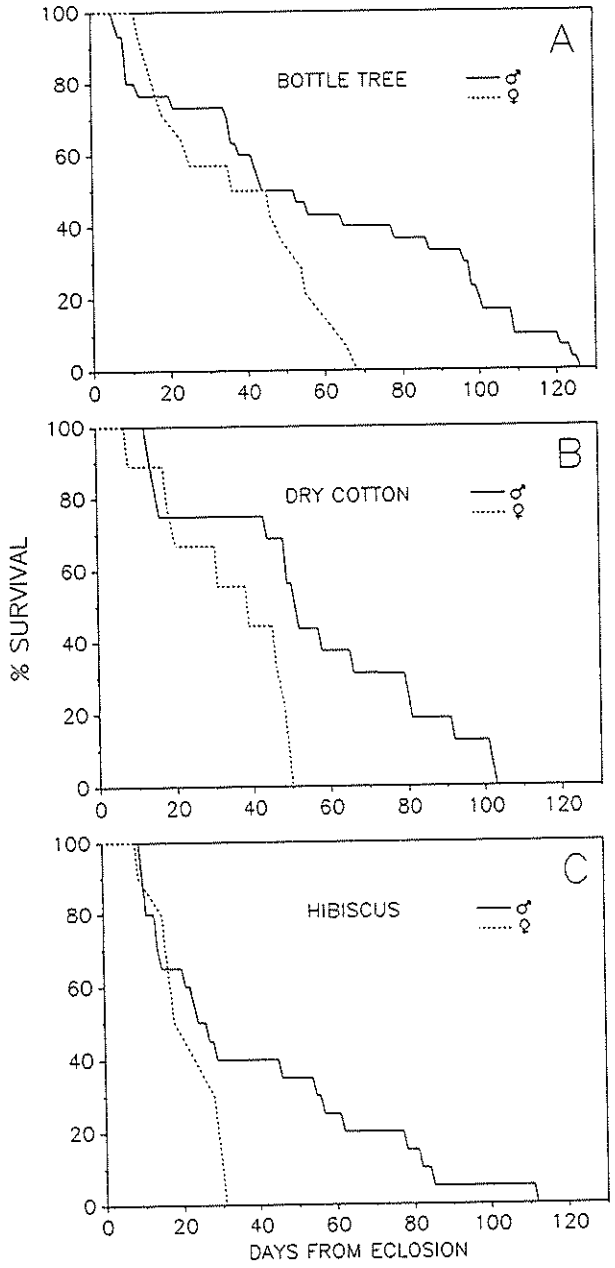

Fig. 7.1. Survival of adult *P. scutigera* when reared on three larval diets at 25° C: (A) Bottle tree (*B. australis*) flowers; (B) dry cotton bolls; and (C) ornamental hibiscus (*H. rosa-sinensis*) flowers.

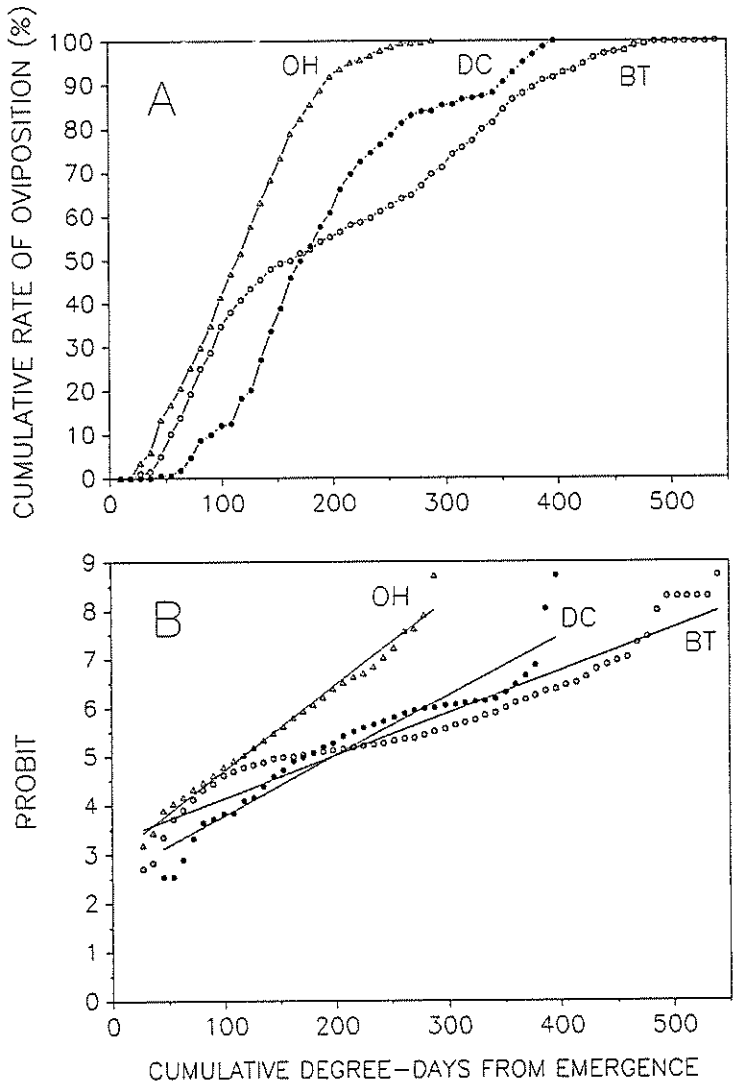

Fig. 7.2. Rates of oviposition of *P. scutigera* at 25° C for moths reared on three larval diets; *B. australis* flowers (BT), overwintered dry cotton bolls (DC), and *H. rosa-sinensis* flowers (OH): (A) percentage cumulative rate of oviposition plotted against cumulative degree-days above 16° C; (B) probit cumulative oviposition plotted against cumulative degree-days and linear regression lines fitted (see Table 7.2 for regression parameters).

regression line than BT or DC moths (Fig. 7.2).

7.3.2. Longevity and rate of oviposition of *P. scutigera* emerging from *B. australis* flowers in an insectary

Survivorship curves for the cohort of 45 males and 38 females emerging from *B. australis* flowers and kept in an insectary are given in Fig. 7.3C. There was no significant difference in male and female longevity ($p>0.05$) at a mean of 33.0 and 31.2 days, respectively (= 244 and 221.8 degree-days above 16 ^{o} C) (Table 7.1). Longevity of males ranged between nine and 62 days (68-500 DD) with the last male dying on 27 December. Longevity of females ranged between three and 65 days (52-539 DD) and the last female died on 30 December.

A total of 8,833 eggs were laid by the 38 females (mean of 233.6 eggs per female). Most eggs were laid during the first three weeks of November, however, oviposition extended until 25 December (Fig. 7.3B). When percentage cumulative oviposition was plotted as probits against cumulative DD, the relationship was linear (Table 7.2). The slope of the regression line (b) was identical to BT moths kept at 25^{o} C but was significantly lower than that for DC and OH moths ($p<0.05$, Table 7.2).

7.3.1. Rate of oviposition of *P. scutigera* emerging from cotton trash in an insectary

Moths emerging from cotton trash between 4 and 26 September, 1986, laid a mean of 211.4 eggs per female (N=53 females), ranging from 35 to 444.5 eggs per female. Females emerging during week 1 (1-7 September) started laying eggs on 4 September and ceased on 1 December. In moths emerging during week 4 (22-28 September) oviposition extended until 18 December, while moths emerging in the final week (3-9 November) ceased laying eggs on 7 December.

When accumulative rate of oviposition was plotted as probits against accumulative DD, the relationship was highly linear (Table 7.2). Comparison of regression slopes (b) for each week of emergence revealed significant differences in the rate of oviposition (Table 7.2). Moths emerging in weeks 1, 2, 3 and 5 had similar regression slopes ranging between 0.0094 and 0.0129. Moths that emerged in week 4 had the lowest regression slope (b = 0.0071) while moths emerging in week 6 had the highest at b = 0.0225.

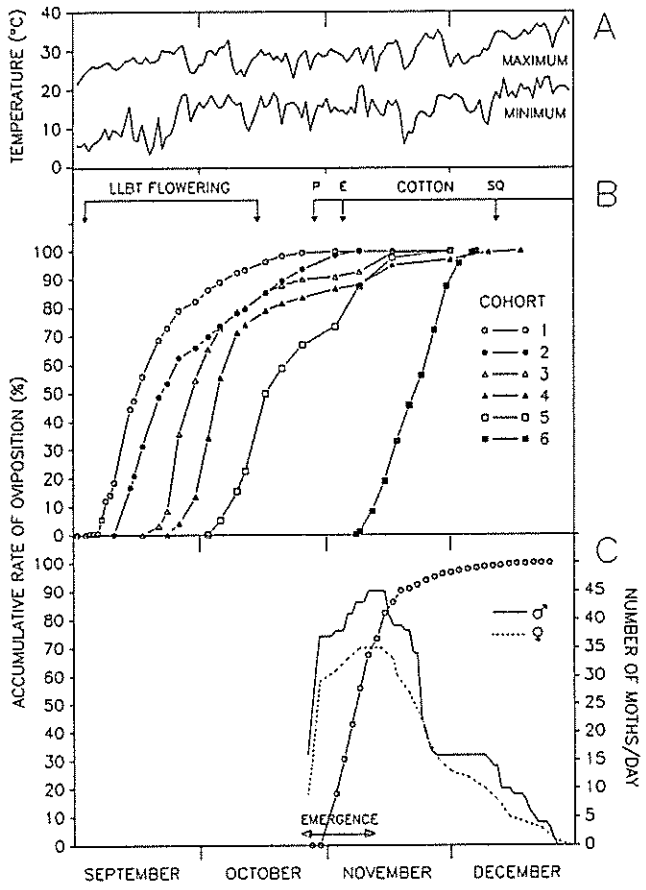

Fig. 7.3. Rates of oviposition of *P. scutigera* kept in an insectary at the D.P.I. Research Station, Biloela, during 1986: (A) Daily maximum and minimum air temperatures; (B) Accumulative rate of oviposition (%) of moths emerging from cotton crop residues over six weekly periods between 1 September and 9 November, 1986 (see Table 7.2. for date of emergence). Arrows indicate the mass flowering period of *B. australis* trees and cotton crop phenology for a commercial field (Site 1) during the 1986/87 cotton season. P indicates the date of sowing, E the date of seedling emergence, and S the date of first square production. (C) Percent accumulative oviposition (open circles) and survivorship curves of moths emerging from *B. australis* flowers between 25 October and 11 November, 1986.

Table 7.2. Linear regression parameters for cumulative rates of oviposition of *P. scutigera* plotted as probits against cumulative degree-days for: a) moths reared from *B. australis* flowers (BT25), dry cotton bolls (DC25) and *H. rosa-sinensis* flowers (OH25) and incubated 25° C constant; b) moths emerging from *B. australis* flowers and placed in an insectary (INSBT); and c) moths emerging from dry cotton bolls during weekly periods between 1 September and 9 November and placed in an insectary (INSDC1-6).

Origins of moths	Period of emergence	Regression parameter		
		a (± S.E.)	b (± S.E.)[1]	r^2
BT25	-	3.275 (0.103)	0.0087a (0.0003)	0.929
DC25	-	2.564 (0.149)	0.0123b (0.0006)	0.915
OH25	-	2.969 (0.072)	0.0175c (0.0004)	0.985
INSBT	26/10-9/11	4.850 (0.082)	0.0087a (0.0003)	0.938
INSDC1	1-7/9	3.862 (0.281)	0.0113b (0.0011)	0.826
INSDC2	8-14/9	4.123 (0.156)	0.0104b (0.0009)	0.912
INSDC3	15-21/9	4.041 (0.226)	0.0094ab (0.0010)	0.871
INSDC4	22-28/9	4.180 (0.219)	0.0071a (0.0007)	0.874
INSDC5	1-7/10	3.324 (0.170)	0.0129b (0.0008)	0.967
INSDC6	3-9/10	2.815 (0.117)	0.0225c (0.0010)	0.953

1. Values of b followed by the same letter are not significantly different (p=0.05).

7.3.3. Effects of delayed mating on reproduction of *P. scutigera*

All females laid fertile eggs when paired immediately after eclosion. Delaying mating for 10, 20 or 30 days decreased the percentage of females laying fertile eggs to 86.7, 66.7 and 66.7%, respectively (Table 7.3). There was no significant difference in the preoviposition period between treatments ($p>0.05$), although it was highest at a mean of 5.4 days after a 30 day delay in mating compared to 4.7, 4.6 and 4.3 days when mating was delayed for <1, 10 and 20 days, respectively (Table 7.3).

. fecundity decreased as the delay in mating increased, but there was considerable variation within treatments (Table 7.3). Significantly fewer eggs were laid when mating was delayed for 10, 20 or 30 days than when there was no delay in mating ($p<0.05$, <0.001 and <0.001, respectively). There was no significant difference in fecundity between 10 and 20 day delay moths or between 20 and 30 day delay moths ($p>0.05$). Females in which mating was delayed for 30 days laid a mean of 174.5 eggs (range 0-514 eggs) compared to 494.7 eggs (range 352-644 eggs) when there was no delay in mating.

The duration of oviposition decreased linearly with an increasing delay in mating ($r^2 = 0.9996$, Fig. 7.4). Moths mated immediately and 10 days after eclosion had a significantly longer period of oviposition compared to moths that were mated 20 or 30 days after eclosion ($p<0.05$, Table 7.3). The duration of oviposition lasted up to 45.7 days when there was no delay in mating, compared to 33.7, 25.2 and 18.7 days when mating was delayed 10, 20 and 30 days, respectively.

The 'effective' longevity of egg laying females (the number of days from eclosion to the last egg laid) was not significantly different between treatments. Delaying mating also had no effect on egg viability, which remained high at over 95% regardless of when females were paired. The mean number of first instars produced per female was estimated as 490, 293, 217 and 167 larvae when mating was delayed for <1, 10, 20 and 30 days, respectively.

Table 7.3. Effect of delayed mating on aspects of *P. scutigera* reproduction at 25° C.

	Moth age at pairing (days)			
	<1	10	20	30
No. of females paired	10	15	15	15
No./% of females laying fertile eggs	10 /100	13 /86.7	10 /66.7	10 /66.7
Effective longevity of females (days ± S.D.)	50.1a[1]	47.1a	49.4a	52.6a
	(13.1)	(8.9)	(11.3)	(8.3)
Range	29-65	36-71	32-75	41-68
Preoviposition period (days ± S.D.)	4.7a	4.5a	4.3a	5.4a
	(1.7)	(2.5)	(2.2)	(3.8)
Range	2-8	3-12	2-10	2-11
No. of eggs per female (± S.D.)	494.7a	302.9b	227.1bc	174.5c
	(95.0)	(201.8)	(176.1)	(175.8)
Range	352-644	0-603	0-434	0-514
Duration of oviposition (days ± S.D.)	45.9a	33.7a	25.2b	18.7b
	(13.6)	(8.0)	(9.3)	(9.4)
Range	21-60	24-55	10-45	2-33
Percentage egg hatch (± S.D.)	97.4a	97.3a	95.6a	95.2a
	(3.6)	(3.9)	(4.9)	(6.8)
Range	95.8-99.8	84.8-99.7	82.4-100	77.5-100
Mean no. of progeny per female (± S.D.)	481.8a	292.2b	218.1bc	169.0c
	(92.5)	(199.6)	(171.0)	(170.5)
Range	343-591	0-591	0-424	0-496

1. Means within rows followed by the same letter are not significantly different (least significant difference test, p=0.05).

ANOVA:

Effective longevity F=0.53, df=3/39, p>0.05
Preoviposition period F=0.34, df=3/39, p>0.05
Eggs/female F=7.59, df=3/51, p<0.001
Duration of oviposition F=13.32, df=3/39, p>0.05
Progeny/female F=7.60, df3/51, p<0.001

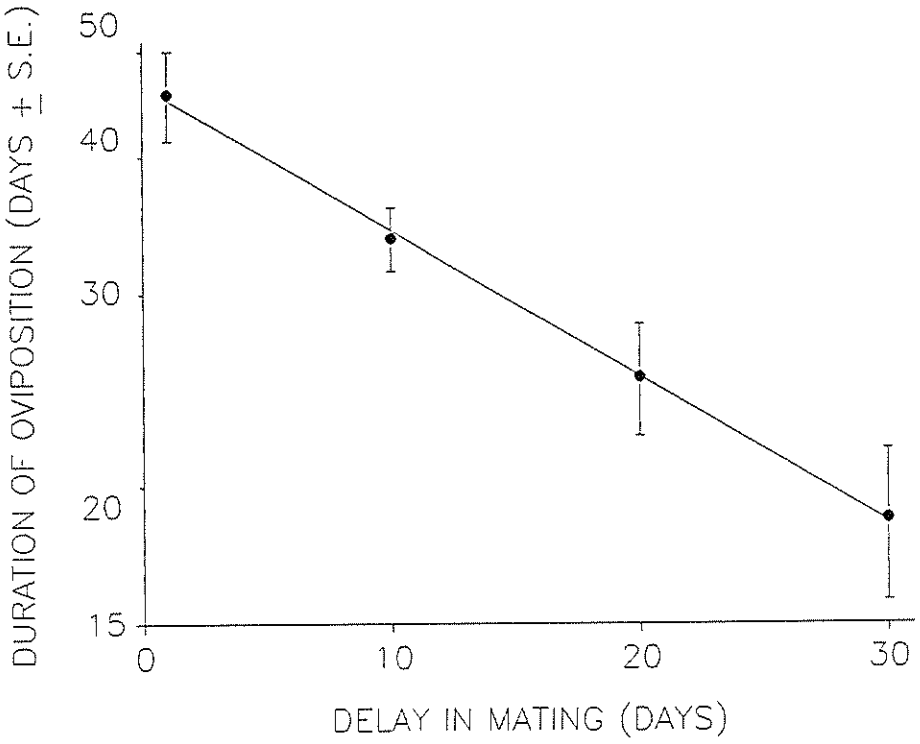

Fig. 7.4. Duration of oviposition ($\log_{10}$ scale) for *P. scutigera* when mating was delayed for <1, 10, 20 and 30 days at 25°. Linear regression line fitted to the data gave: log (y) = 1.663 - 0.013x (r^2 = 0.9996).

7.4. Discussion

The life-spans attained by adult *P. scutigera* kept in an insectary confirm that moths emerging from cotton trash as early as September have the potential to survive until squaring cotton is available. Cotton in the Callide and Dawson Valleys is usually planted at the end of October and starts squaring in mid-November (Sabine 1969a). Moths emerging from cotton trash between early September and early November continued to lay eggs until 18 December, while a cohort of moths emerging from *B. australis* flowers in October and November did not cease laying eggs until 25 December.

Obvious caution must be taken when extrapolating these results to the field situation. Moths in this study were provided with a continuous source of carbohydrate (5% sugar solution) and complete protection from predators. In pre-squaring cotton, sources of carbohydrate would be rare. The requirement to search for food would decrease longevity through increased activity and increased exposure to predators. Furthermore, female *P. scutigera* were not given access to host plant material. This may have artificially prolonged the oviposition period through the absence of any sensory cues to stimulate oviposition.

Predictions of adult moth longevity under ambient temperature conditions are not possible using data from constant temperature studies. Longevity of BT moths kept in an insectary was much shorter than moths kept at 25^{o} C constant, when expressed as the number of degree-days accumulated above 16^{o} C (Table 7.1). This discrepancy may have been due to the lethal effects of high day time temperatures experienced in the insectary. Maximum air temperature exceeded 30^{o} C on many days (Fig. 7.3). Female moths appeared to be more tolerant to extreme temperatures than males, as under the insectary conditions there was no significant difference in the longevity of the sexes, while at 25^{o} C mean male longevity was always significantly longer than mean female longevity.

Despite differences in longevity, rates of oviposition of BT moths were the same when kept in the insectary or at 25^{o} C constant. However, the rate of oviposition of DC moths changed according to the week of moth emergence (Table 7.2). Moths emerging from cotton trash during early September and incubated in the insectary had similar rates of oviposition compared to those kept at 25^{o} C constant. Moths emerging from cotton trash in early

November had a significantly faster rate of oviposition, similar to BT moths emerging over the same period. Moths emerging from cotton trash during the last week of September may have experienced optimum temperature conditions for oviposition as these moths had a significantly lower rate of oviposition than all other moths (including those incubated at 25^{o} C constant) and continued to lay eggs for the longest period of time. Average air temperatures during this week of emergence were around 21.2^{o} C compared to 15.4 - 18.6^{o} C in early September and 22.4^{o} C in early November.

P. scutigera could successfully reproduce even after long delays in mating. When mating was delayed for 10, 20 and 30 days the number of fertile eggs laid was reduced by 39, 55 and 65%, respectively, compared to moths paired immediately after eclosion. In other species of Lepidoptera much shorter delays in mating have been reported to have a more profound effect on reproduction. This may be partly related to the life-span of the adult moth. For example, in the shorter lived species *Ephestia cautella* (Walker) and *H. virescens*, mating delays of four and eight days reduced the number of progeny produced by 44.4% and 100%, respectively, when compared to individuals that were paired immediately after eclosion (Barrer 1976, Proshold *et al.* 1982). However, in *P. gossypiella*, which has a similar life-span to that of *P. scutigera* (cf. Butler and Foster 1979), a mating delay of 15 days reduced the number of first instar larvae by 97% (Lingren *et al.* 1988). Here the major difference in the effect of delayed mating from that observed in *P. scutigera* was the reduction in egg viability. In *P. gossypiella* egg viability decreased from 80% when mating was allowed to occur immediately after eclosion, to only 15% when mating was delayed for 15 days. Delayed mating had no effect on *P. scutigera* egg viability, which remained over 95% regardless of when mating was allowed to occur. Other species of Lepidoptera have also shown no reduction in egg viability through delayed mating e.g. *H. virescens* (Proshold *et al.* 1982), *Adoxophyes orana* F. v. R. (van der Kraan and van der Straten 1988).

The reduction in the total number of eggs laid by *P. scutigera* when mating was delayed can largely be explained by the shortening of the number of days over which oviposition occurred. The duration of oviposition declined linearly with increasing delay in mating (Fig.

7.4). A delay of 30 days reduced the duration of oviposition to 18.7 days compared to 45.7 days when females were paired immediately after eclosion.

It is unknown whether females that failed to lay fertile eggs had actually mated or not. The effects of delayed mating on males was not investigated. In many species of Lepidoptera the stimulus for oviposition appears to be the transfer of normal eupyrene sperm, perhaps in conjunction with other seminal products (Proshold *et al.* 1975). *P. scutigera* females oviposit readily after pairing but virgin females normally retain their eggs. Of 10 virgin females observed by Vickers (1982a) only one laid eggs (N = 16) 42 days after eclosion. Similarly, of the 11 females that did not produce any fertile eggs when mating was delayed for 10, 20 or 30 days, only three laid infertile eggs. Two females (10 and 30 day delay) laid a single infertile egg, while one female (10 day delay) laid 23 infertile eggs starting on the 59th day after eclosion.

The retainment of eggs by virgin or unmated females may be an adaptation to conserve essential nutrients, through oosorption, which would otherwise be lost if infertile eggs were laid (Lum 1983). Oosorption or oocyte degeneration is a common reproductive strategy in insects when normal reproductive events are disrupted (Bell and Bohm 1975). When normal reproduction can occur, oosorption may cease, and the ovary again becomes productive.

Often virgin females, or females in which mating is delayed, live longer than mated females (Barrer 1976, Ellis and Steele 1982, Lingren *et al.* 1988). This may be a manifestation of oosorption where essential nutrients obtained from degenerated oocytes are used to maintain longevity (Barrer 1976). In *P. scutigera* longevity of females did not increase significantly with increasing delay in mating, supporting Vickers (1982a) observation that virgin female *P. scutigera* do not live significantly longer than mated females. This does not necessarily indicate that oosorption is absent in *P. scutigera*. Oosorption is known to occur in virgin females of *Plodia interpunctella* Hübner and *Cadra cautella* (Walker) but it does not significantly increase their longevity (Lum 1983).

CHAPTER 8

General Discussion

8.1. Introduction

The aim of this thesis, as stated in Chapter 1, is to improve our knowledge of the basic biology and ecology of *P. scutigera* in Central Queensland. It was argued that such information is essential for the design of effective pest management programmes. The approach I adopted was that advocated by Clark *et al.* (1967) whereby the life system of a pest is studied in an attempt to recognize key factors which control its abundance. As defined in Chapter 1, the life system is that part of the ecosystem which determines the existence, abundance, and evolution of a particular population (Clark *et al.* 1967, Clark 1970). Clark *et al.* (1967) considered that the persistence and abundance of a population are the outcome of interactions between the inherited properties of individuals and the intrinsic attributes of the effective environment. In other words, the intrinsic qualities of the subject species and those of its environment are the co-determinants of abundance.

Within the time frame of this thesis it has only been possible to conduct a partial analysis of the life system of *P. scutigera.* I believe this study has achieved an important insight into the functioning of this species life system. In the following discussion the results of previous chapters are collated to give an overview of the life system of *P. scutigera* in Central Queensland. The discussion considers the inherited properties of this species, and the effective environment of the cotton ecosystem. Hypotheses are proposed to explain the pest status of *P. scutigera* in Central Queensland. Ways in which the life system may be modified to achieve satisfactory control are discussed. Also, the limited pest status of *P. scutigera* in Australia is addressed and suggestions for further research are given.

8.2. Life system of *P. scutigera* in Central Queensland

The major factor influencing the abundance of *P. scutigera* in Central Queensland is the relative shortage of larval resources in time and space. This shortage is brought about by the scarcity of alternative host plants in the inland areas of Central Queensland, and by the unsuitability of pre-squaring and squaring cotton crops for development, survival and reproduction.

The relative shortage of larval resources is offset by two important factors that favour population increase: the ability of larvae to utilize dry cotton bolls as a larval resource and the comparatively long life-span of adult moths.

Each factor affecting population increase in *P. scutigera* is discussed in detail below.

8.3. Factors favouring population increase

I believe that the success of *P. scutigera* as a pest of cotton in Central Queensland can largely be related to the adaptations this species has evolved for living on the reproductive forms (i.e. flowers and fruiting structures) of native host plants. These preadaptations constitute the inherited properties of the life system that enable *P. scutigera* populations to persist in the effective environment.

The most significant adaptation is a larval stage which is tolerant to extreme and repeated changes in the moisture content of its food. It is hypothesized that this adaptation arose to enable larvae to continue feeding on the reproductive forms of native hosts even after they detach from the plant and begin to desiccate (Chapter 5).

Subsequently, this adaptation has allowed *P. scutigera* to survive and develop in dry cotton bolls. The development of larvae in dry bolls is prolonged due to the limiting moisture content (Chapter 6). This enables moth populations to overwinter at a time when larval resources are scarce and ensures that a proportion of the population is carried-over into the next cotton season.

Another characteristic of the *P. scutigera* life-cycle is a relatively long adult life-span. Mean longevity of males and females at 25^{o} C was 59.2 and 39.1 days, respectively, ranging up to 126 days for males and 68 days for females when reared on *B. australis* flowers

(Chapter 7). This contrasts markedly with other Australian pests of cotton, e.g. longevity of female *H. armigera* at a similar temperature is *ca* 14 days (Fitt 1984) and female cotton tipworm (*Crocidosema plebejana* Zeller) live on average 13.3 days at 25° C (Hamilton 1987).

In Chapter 6, I proposed that the long life-span of adult moths may further assist populations to carry-over from one cotton season to the next. A long life-span is interpreted as another preadaptation associated with the biology of *P. scutigera* on native host plants that has evolved in response to:

a) an unpredictable larval resource;

b) a very variable rate of development during the larval stage.

The availability of larval resources is dependent on the right conditions for flowering and fruiting of host plants. Above all, rainfall is probably the most important variable affecting the timing and intensity of flowering of native host plants. This was apparent in wild *B. australis* trees and *G. sturtianum* at Bauhinia Downs which flowered only after heavy localized rainfall (Chapter 5). In frequently irrigated gardens, *H. rosa-sinensis* and *H. tiliaceus* were able to flower almost all year round, but when unirrigated, rainfall clearly affected the intensity of flowering of both species.

Due to the highly variable and localized pattern of rainfall in Central Queensland (Chapter 3), it follows that the availability of larval resources would also vary in an unpredictable manner. Accordingly, a long adult life-span may help to overcome the adverse effects of an unpredictable larval resource by spreading oviposition over a long period of time (Chapter 2 and 7).

The rate of larval development in dry fallen forms of native hosts would also depend on the pattern and intensity of rainfall. Development of individual larvae would vary according to: a) the type of form infested and its suitability for larval development (nutritional value, size, and allelochemical constituents); b) the microclimate in which the form is located (e.g. in sun or shade); c) the initial moisture content of the form, and d) the rate of loss/absorption of moisture of that form. In combination, these factors result in an unsynchronized moth emergence over a long period of time.

Again, a long adult life-span would help to overcome problems associated with such

unsynchronized emergence. As seen in Chapter 7, *P. scutigera* can successfully reproduce even when mating is delayed for up to 30 days. Although delaying mating significantly reduced the number of eggs laid it had no affect on egg fertility.

It could be argued that if both larval development and flowering of host plants are dependent on rainfall, as suggested, then there may be a degree of synchrony in the emergence of adult moths and the availability of flowers. However, it is suspected that the amount of rainfall needed to allow larval development to proceed in dry fallen forms would be much less than that required to stimulate flowering in a large host plant such as *B. australis*.

8.4. Factors limiting population increase

A) Unsuitability of pre-squaring and squaring cotton crops for larval development

The most notable feature about the seasonal incidence of *P. scutigera* in cotton was the marked absence of larvae in pre-squaring and squaring crops (Chapter 3). During this study the number of *P. scutigera* larvae found infesting cotton squares rarely exceeded 2 larvae/100 squares, even in unsprayed cotton fields. The low incidence of larvae may have been due to:

1) Sampling error - larval populations existed within the fields but were missed due to sampling the wrong part of the cotton plant.

2) Low levels of oviposition due to the comparative rarity of adult moths early in the cotton season.

3) Pre-squaring and squaring cotton is unattractive to ovipositing females.

4) Pre-squaring and squaring cotton is unsuitable for larval growth and development.

5) Poor survival of eggs and larvae early in the season due climatic factors, high levels of parasitism and/or predation.

Point (1) is highly unlikely as pest control advisors thoroughly check all plant parts when scouting for other insects early in the season. Although *P. scutigera* larvae are known to infest terminal buds and stems of young cotton plants, causing damage similar to the cotton tipworm (*C. plebejana*), the incidence of such infestations is very rare (M. Stone pers. comm.). Similarly, there is no apparent reason why the survival of *P. scutigera* eggs and larvae should

be poorer earlier in the season unless differences in the microclimate of young and old cotton crops are important. In sprayed cotton, insecticide penetration may be greater when plants are smaller, but this does not explain the absence or rarity of larvae in squares of unsprayed cotton.

The most likely explanation for the low incidence of *P. scutigera* larvae in young cotton crops is that squares are an unsuitable larval food source and are unattractive to neonate larvae. This interpretation is supported by: a) the slow developmental rates of larvae confined to squares (Chapter 2); b) poor recovery of larvae placed onto squares of glasshouse plants; c) distribution of larvae hatching from eggs placed on plants bearing squares and bolls; and d) very low infestation of squares late in the cotton season even when boll infestation was very high (Chapter 3).

Cotton plants are known to contain many allelochemicals which have been implicated in resistance to diseases and insects. The two most important group of compounds are the condensed tannins and the terpenoid aldehydes, principally gossypol (Zummo *et al.* 1984). The effectiveness of these compounds varies with pest species. For example, high levels of condensed tannins are thought to confer resistance to *C. plebjana* (Hamilton 1987) and spider mites (*Tetranychus urticae* Koch) but not *Helicoverpa* spp. (McColl and Noble 1990). Tannins are also unlikely to confer significant resistance to *P. scutigera* as levels generally increase with increasing cotton plant age and stage of growth (Zummo *et al.* 1984). Levels of tannins remain high throughout the fruiting stage of growth, when *P. scutigera* damage is also high.

In contrast, gossypol decreases in concentration with plant age: levels are very high in squares (0.5% of dry weight), intermediate in small bolls (0.29%), but very low in medium bolls (0.04%) (Hedin *et al.* 1983). Therefore, a high gossypol content may explain the poor performance of *P. scutigera* larvae on cotton squares. The reduction in gossypol content with increasing boll size or age needs to be defined more closely. Unfortunately, Hedin *et al.* (1983) did not specify the age of the 'small' and 'medium' bolls they analyzed. The low numbers of second and third instar larvae found in young bolls, particularly those aged between 15 and 30 days old (Chapter 3), may be due to changes in the gossypol content.

The low rate of moth emergence from dry cotton bolls during the pre-squaring and squaring stage of cotton may also contribute to the low levels of larval infestation. During 1986 only between 0 and 3.5% of moths emerged from dry cotton bolls during the time cotton crops were squaring (Chapter 6). This poor carry-over may be offset by the long life-span of adult moths and alternative host plants, primarily *B. australis* (Chapter 5, also see section 8.4).

The attractiveness of pre-squaring and squaring cotton to ovipositing female *P. scutigera* is unknown. Host finding behaviour and the selection of oviposition sites in *P. scutigera* is not understood. Female *P. scutigera* may avoid ovipositing eggs on plants that are poor for larval development or survival. Such discrimination seems unlikely given the occurrence of larvae on alternative host plants that are poor for larval survival and development, such as *B. populneus* and *B. australis* (see next section). Current understanding of the relationship between oviposition preference and offspring performance in insects is poor and has been hampered by lack of data on how the two are be related genetically (Thompson 1988). The presence or absence of chemical cues to stimulate oviposition is likely to be of more importance. Such cues have been frequently demonstrated as important factors in the acceptance of a host for oviposition, after initial contact with the plant has been made (e.g. Fenemore 1988).

B) Alternative host plants

In the Callide and Dawson Valleys there appears to be a scarcity of *P. scutigera* host plants. This is compounded by the fact that host plants are only suitable for larval development when flowering or fruiting. Therefore, the timing of the life-cycle of *P. scutigera* is critical with respect to the suitability of the host plant (Chapter 5 and section 8.2.). The scarcity of alternative host plants supports the hypothesis that *P. scutigera* is not native to this region but was introduced by man with the spread of cotton cultivation (Holdaway 1926).

The most important alternative host plant discovered was *B. australis*. The mass flowering period of ornamental trees coincided with peak moth emergence from cotton crop residues

(Chapter 5). Population increase of *P. scutigera* on *B. australis* flowers was limited by: a) the scarcity of trees; b) short mass flowering period; and c) poor survival of larvae infesting fallen flowers. Moths emerged from *B. australis* flowers mainly during early to mid-November at a time when no other alternative host plants were available (except for low numbers of *H. rosa-sinensis* and *H. tiliaceus* trees in towns) and when cotton was still in the squaring stage of growth. Therefore, flowering *B. australis* trees are viewed as an important sink for *P. scutigera* populations emerging from fallow cotton fields during September and October, but not as a limited source of moths invading cotton crops. The role of *B. australis* in the population dynamics of *P. scutigera* warrants further investigation (section 8.11).

Other alternative host plants discovered were either too scarce or very rarely infested to be considered as important in the population dynamics of *P. scutigera* in the region (Chapter 5). The listing of Peach (*P. persicae*) and *B. populneus* as alternative hosts of *P. scutigera* should be treated with caution. Kitching and Zalucki (1983) considered that defining a food plant as a species on which a larvae is seen to feed is inadequate. They proposed that a more satisfactory definition would be "a plant on which the species can complete its immature feeding period and then successfully complete pupal/adult metamorphosis". This should be extended to determine whether the offspring developing on that host can then produce a new generation.

In conclusion to this section, it is postulated that the scarcity of alternative host plants in time and space severely restricts the successful carry-over of *P. scutigera* populations from one cotton season to the next. Population increase in pre-squaring and squaring cotton is further constrained by the unsuitability of plants for larval development. A diagrammatic representation of the life-cycle of *P. scutigera* in Central Queensland cotton crops is given in Fig. 8.1.

8.5. Natural control of insect populations

The above interpretation of the life system of *P. scutigera* should be considered together with the idea that one of the most important factors controlling insect populations is the carrying capacity of the habitat.

The most commonly held theory is that animal populations exist in a dynamic equilibrium with their environment, at densities that are well below the level at which resources are fully

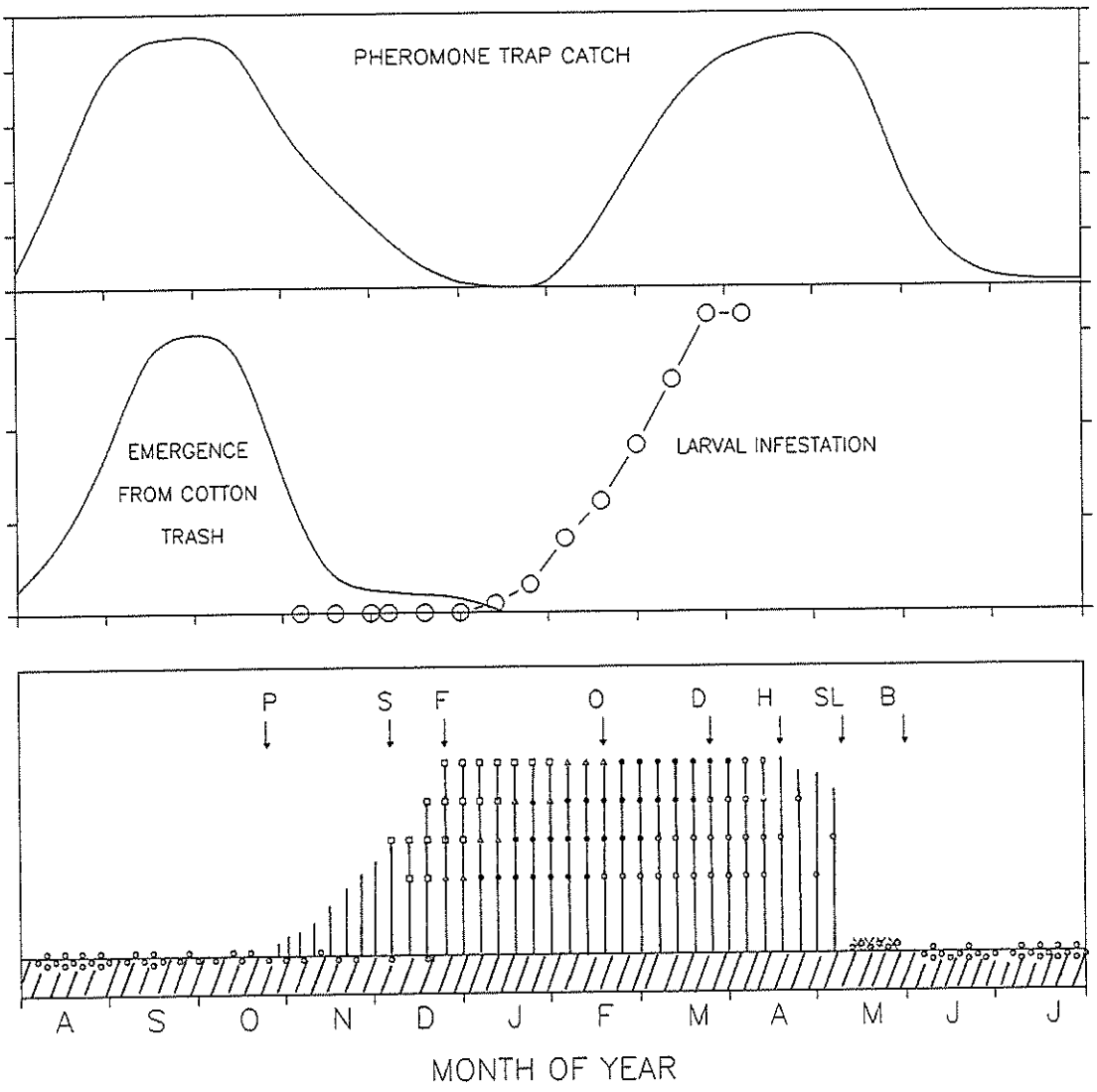

Fig. 8.1. Diagrammatic representation of the population dynamics of *P. scutigera* in relation to cotton phenology in Central Queensland. The bottom graph illustrates the crop phenology; square symbols represent cotton squares, triangles are cotton flowers, filled circles are closed cotton bolls, and open circles are open cotton bolls. The usual date of crop events are indicated by the letters: P = date cotton planted, S = first square, F = first flower, O = first open boll, D = defoliation, H = harvest, SL = slashing and shredding of crop residues, and B = burial of crop residues.

utilized (Dempster 1983). This 'equilibrium model' stems from the concept of 'the balance of nature' and the realization that birth-rates and death-rates may be affected by population size, i.e. through the action of density-dependent processes. The equilibrium theory has been readily accepted by insect ecologists and forms the basis of many population studies. The most important density-dependent mechanisms invoked in regulating insect populations vary according to the species involved, but are usually either intraspecific competition (e.g. Nicholson 1954) or natural enemies (e.g. Hassell 1985, Varley *et al.* 1973).

The main alternative theory of population control, considered today, is the 'ceiling' or resource limitation model which stems from the ideas of Milne (1957). In this model a population faces an upper limit to its size, set largely by the carrying capacity of the habitat. This upper limit, or 'ceiling', varies in time and from one locality to another. A population also faces a 'floor'; a minimum size below which extinction is likely to occur unless re-established by immigration from other areas. Between these two extremes the population will fluctuate in response to a complex of interacting factors, e.g. weather and natural enemies (Dempster 1983).

Dempster and Pollard (1981) strongly favour of the resource limitation model. They severely criticize the extensive application of equilibrium theory to insect population studies as it has concentrated attention on 'spurious' regulatory processes, at the expense of considering the impact of variations in the carrying capacity of the habitat on the population. Dempster and Pollard (1981) maintain that variations in the carrying capacity of the habitat are often the most important factors in the determining yearly fluctuations in the population size of insects.

Both models of natural population control involve density-dependent mechanisms, but the form of the density-dependent response differs (Dempster 1983). In the equilibrium model any disturbance of the population away from the equilibrium will be countered by density-dependent compensation. In the ceiling model, the limitation of resources may be density dependent, when it involves intraspecific competition, but the density response will only occur at relatively high densities. At densities below the carrying capacity, no density

dependence should be identifiable. Furthermore, a shortage of resources (in time or space) may not necessarily result in competition. Population growth may be limited simply as a result of the insect failing to find adequate resources in the time available (Andrewartha and Birch 1954, Dempster 1983, Kitching 1978). In these circumstances no density dependence will be found.

According to Dempster and Pollard (1981), acceptance of the resource limitation model by insect ecologists has been slow for two reasons. Firstly, it is often difficult to believe that food supply is limiting, especially in the case of phytophagous insects where there is an apparent abundance of food. Secondly, the general acceptance of the equilibrium theory of population control has led to the view that resources are rarely limiting.

There is increasing evidence in the literature to suggest that the abundance of many herbivores is limited by the availability of food of the right quality. The importance of food quality and the ability of animals to utilize their food efficiently, has been underestimated in many ecological studies (White 1978). During the last 15 to 20 years ideas about the nature of plant/insect interactions have gradually moved away from the classic theory of passive plant defenses against herbivores as expounded by e.g. Fraenkel (1959). It is now recognized that a variety of factors may combine to modify plant chemistry and thus change the nutritional quality of the plants for phytophagous insects (Jermy 1984). These factors include environmental stress, mechanical damage, and temporal, seasonal and genetic variability (Parry *et al.* 1990). One of the most important aspects of the plant/insect relationship frequently implicated is the quality and quantity of nitrogen in plant tissues (Mattson 1980, McNeill and Southwood 1978, Taylor 1984, White 1984).

In many species of Lepidoptera the timing of the life-cycle to the growing season of their larval host plants can have an enormous effect on the quality and quantity of food available to them (Dempster and Lakhani 1979, Varley *et al.* 1973). This is most apparent in species that attack deciduous trees in which survival is dependent on the synchronization of larval hatch and bud-burst of trees. If larval hatch is too early then mortality is high through starvation (Varley *et al.* 1973); if too late then survival and development may be adversely affected by changes in the quality of nitrogen in leaves (Feeny 1970).

In summary the resource limitation model of population control is applicable to the life system of *P. scutigera* infesting cotton in Central Queensland. The population dynamics of *P. scutigera* is severely limited by the shortage of larval resources of the right quality throughout the winter and spring.

Hamilton (1987) proposed that the population dynamics of another pest of cotton in Australia, *C. plebejana*, is also primarily determined by the availability and quality of host plants. In this species the suitability of cotton for larval development and survival declines after the late squaring stage of growth. Hamilton and Gage (1986) hypothesized that weather was the ultimate driving force behind the population dynamics of *C. plebejana* through its effects on the abundance and phenology of the main alternative host plant, *Malva parviflora* L.

Further research is needed to determine the importance of other factors in regulating *P. scutigera* populations, particularly natural enemies. Although the role of larval parasitoids did not appear to be important in regulating *P. scutigera* numbers infesting cotton or alternative host plants (Chapters 3 and 5), the importance of egg parasitism and predation (particularly by ants) was not defined.

Also the influence of migration on the population dynamics of *P. scutigera* needs to be examined. It is possible that *P. scutigera* may migrate from coastal areas, where alternative host plants such as *H. tiliaceus* are abundant, into the Callide and Dawson Valleys during the summer and invade cotton crops. In southern California, long distance movement of *P. gossypiella*, which is of similar size to *P. scutigera* (cf. Zimmerman 1978), appears to be frequent and is probably aided by local weather systems (Toscano *et al.* 1979). Adult *P. gossypiella* have been caught in traps attached to an airplane at altitudes of over 300 m (Glick 1955).

8.6. Pest status of *P. scutigera* in cotton

Given the above constraints on population increase, how is *P. scutigera* able to persist as a pest of cotton in Central Queensland?.

The ineffective carry-over of moths into the next cotton season and the unsuitability of

squaring cotton plants for population increase means that the initial population of *P. scutigera* colonizing cotton is small. However, as already discussed (section 8.1), the long life-span of adult moths may allow females to continue laying eggs or survive over a long period of time until cotton crops becomes more suitable for larval development. Perhaps more importantly, the period of fruiting of cotton in Central Queensland is long, usually extending from mid-December until late March. This long period allows time for *P. scutigera* populations to increase considerably in numbers before the destruction of crop residues. Furthermore, the control of *P. scutigera* larval infestations is difficult, mainly due to the problems in accurately monitoring larval populations and because of their protected location from insecticides deep inside the cotton boll (Chapters 1 and 3).

The build-up of *P. scutigera* populations is also favoured by the following factors:

1) Few insecticide sprays are applied towards the end of the season when *P. scutigera* numbers are high, due to low pressure from *Helicoverpa* spp. and sap-sucking bugs which are normally sprayed.

2) Cotton remains attractive to ovipositing *P. scutigera* even after defoliation and harvest (Chapter 3). Therefore, females continue to lay eggs on plants and the resulting neonate larvae reinfest dry, partially-open cotton bolls previously damaged by *P. scutigera* larvae.

3) A long period between the last insecticide spray and the destruction of crop residues. This period is often extended by rainfall which disrupts normal farming practices, and results in cotton plants entering a second fruiting cycle. Any new bolls produced after defoliation were heavily infested by *P. scutigera.*

8.7. Management of *P. scutigera* in cotton

In the above discussion it was suggested that the persistence of *P. scutigera* in cotton crops of Central Queensland is dependent on overwintering larvae infesting dry cotton bolls to carry-over populations into the next cotton season. Consequently, this dependence makes *P. scutigera*, perhaps more than any other pest of cotton in Australia, an ideal candidate for control by cultural methods. This has been realized since *P. scutigera* first became a pest of cotton in Queensland during the 1920's (Holdaway 1926).

In the past, emphasis has been placed on the importance of early burial of crop residues to

limit the effective emergence of *P. scutigera* in the following cotton season (Sabine 1969a, 1969b). While agreeing with the importance of this cultural control method, I make a further recommendation, namely that the interval between the last insecticide spray and the destruction of crop residues be shortened as much as possible.

The importance of this recommendation cannot be overemphasized as it is essential to limit the end of season build-up in *P. scutigera* populations. The larvae that are most likely to carry-over into the next cotton season are those which reinfest previously *P. scutigera* damaged bolls that have partially opened and dried out (Chapter 6). It follows then, that the level of boll infestation in a crop during the growing season will largely determine the size of the overwintering population. The longer *P. scutigera* populations are allowed to increase at the end of the cotton season, the more larvae there will be to reinfest damaged bolls and carry-over into the next cotton season.

In this context, together with other observations made in Chapter 3, the following recommendations for limiting the impact of *P. scutigera* in cotton crops are made:

1) Avoid planting cotton late (e.g. after mid November). Late-planted crops often develop heavy larval infestations at the end of the season probably due to considerable local movement of *P. scutigera* from early-planted crops that have been defoliated, harvested and slashed.

2) Avoid planting cotton in areas that are inaccessible to aerial sprayers e.g. close to power lines or trees. This is not only relevant to the insecticidal control of all insect pests but also to the chemical defoliation of crops to prevent plants from entering a second fruiting cycle.

3) Defoliate, harvest, slash and shred cotton crops as soon as possible to reduce the interval between the last insecticide spray and the destruction of crop residues.

4) Bury crop residues as soon as possible, using tillage methods which are efficient at covering a high proportion of bolls with soil.

In accordance with the above recommendations, ratoon or standover cotton should never be grown as this would allow *P. scutigera* populations to continue to increase throughout winter and spring at a time when larval resources are normally very scarce.

Effective control of *P. scutigera* will only be achieved if strict adherence to the above cultural practices is implemented throughout the entire cropping area, and not just on individual farms. *P. scutigera* appears to be highly mobile and may undergo considerable local movement when larval resources are scarce. Present legislation to enforce the destruction of cotton crops at the end of the season is ineffective due to the absence of a date by which cotton crops must be destroyed. If area-wide control of *P. scutigera* is to be achieved, it may be necessary to amend the law.

8.8. Comparison of the pest status of *P. scutigera* and *P. gossypiella*

Given the similarity between the two species, it is of interest to ask why isn't *P. scutigera* as serious pest of cotton as *P. gossypiella*?. For example, in southern California, cotton growers apply an average of 13 insecticide treatments annually for the control of *P. gossypiella*, costing an average of US $400-600/ha (Hutchison *et al.* 1990). In Central Queensland, it is rare for more than two insecticide sprays to be applied directly for the control of *P. scutigera* (Chapter 3).

Many environmental and agronomic differences between the cotton growing regions may be responsible for difference in pest status of the two species. However, there are two major biological differences between *P. scutigera* and *P. gossypiella*: larval diapause and the level of infestation of cotton squares.

The presence of a larval diapause in *P. gossypiella* may help this species to carry-over from one cotton season to the next more effectively than *P. scutigera*. In the U.S.A., the percentage of *P. gossypiella* emerging at the time of cotton squaring varies from 20 to 40% according to the season and locality (Bariola 1978). This is much higher than in *P. scutigera* which does not diapause (Chapter 7). As already mentioned, during 1986 between 0 and 3.5% of *P. scutigera* emerged from buried cotton bolls when cotton crops were squaring.

P. gossypiella infests cotton squares much more readily than *P. scutigera*. In field cages, Henneberry and Clayton (1989) reported up to 51.6% infestation of squares by *P. gossypiella* larvae even when large numbers of bolls were present on plants. Furthermore, unlike *P. scutigera*, the rate of development of *P. gossypiella* larvae confined to squares is not prolonged and is very similar in duration to that of larvae reared on bolls (Noble and

Robertson 1964). Together, these factors would enable *P. gossypiella* to establish much larger populations in squaring cotton fields than *P. scutigera*, leading to increased levels of boll infestation later in the season.

Another important difference in the biology of the two species is the site of oviposition: *P. scutigera* very rarely lays eggs on bolls (Chapter 3) while *P. gossypiella* often does so (Hutchison *et al.* 1988). Undoubtedly, larvae hatching from eggs laid on bolls would have a greater chance of surviving than those emerging from eggs laid on vegetative parts, particularly if insecticide residues were present on plants. The number of *P. gossypiella* eggs laid on bolls is significantly correlated with the percentage of bolls infested (Henneberry and Clayton 1989).

8.9. Restricted pest status of *P. scutigera* in Australia

Why is *P. scutigera* only a pest of cotton in Central Queensland and not in other cotton growing regions where it may be present on alternative host plants? (Chapters 1 and 7). Possible reasons are:

1) More diverse and/or abundant alternative host plants in Central Queensland. This idea was rejected in Chapter 5. Furthermore, the importance of alternative host plants in the population dynamics of *P. scutigera* infesting cotton in Central Queensland is dubious (see section 8.4.). Unless there are undiscovered host plants of *P. scutigera* in Central Queensland, other factors must be responsible.

2) Climatic differences between cotton growing regions. Climate may affect the population dynamics of *P. scutigera* in two ways:

1) through its influence on the rate of development and survival of overwintering larvae infesting dry cotton bolls;

2) by affecting the availability of larval resources in time. Primarily this may occur through the effects of climate on the phenology of cotton crops i.e. the time available for *P. scutigera* populations to increase during the fruiting stage of growth.

A convenient way of summarizing the climate of a region is to plot average monthly rainfall against average monthly temperature to form a climograph (Andrewartha and Birch

1954). Fig. 8.2 compares the climograph for Biloela with that for six other cotton growing regions. Each climograph is based on mean monthly rainfall and screen temperatures over the same 10 year period (1966-1975), as supplied by the Australian Bureau of Meteorology. For the locality of each cotton growing area refer to Fig. 1.1.

Not surprisingly, the climographs for the principal towns of the Callide and Dawson Valleys (namely Biloela and Theodore) are very similar. In contrast, Emerald (Central Highlands) has a much hotter and drier climate than Biloela, particularly during spring and early summer. St. George has a hotter summer, cooler winter and is much drier in spring and early summer. Narrabri in the Namoi Valley of New South Wales, has the most extreme climate that is much cooler in the winter with hotter and drier summers. The most similar climographs to Biloela, after Theodore, are those for Dalby on the Darling Downs and Gatton in the Lockyer Valley. Dalby experiences slightly cooler winters while Gatton receives more rain during the summer.

Ashburner *et al.* (1984) calculated the start and end of boll set for eight different cotton growing regions in Queensland and New South Wales. The calculations were based on the day-degree requirements of cotton, long term weather data, and using a sowing date of 1 October for all regions. From this analysis, it was evident that Biloela and the Lockyer Valley had the longest fruiting period of *ca* 13 December to 23 March. Other regions, had shorter fruiting periods, ranging between 7 January to 19 February (Macquarie Valley) and 25 December to 6 March (Darling Downs). The only exception was Emerald where the fruiting period given was 3 December to 6 April.

In summary, climatic differences between cotton growing regions exist and may be responsible (at least in part) for the restricted pest status of *P. scutigera*. These climatic differences may affect the carry-over of *P. scutigera* populations from one season to the next by influencing crop phenology, and the rate of development and survival of overwintering larvae in dry cotton bolls. The above analysis suggests that *P. scutigera* should occur in the Lockyer Valley, and possibly the Darling Downs. This is supported by the recent discovery of *P. scutigera* infesting cotton at Gatton in the Lockyer Valley (Chapter 1, Appendix A). However, further research is needed to define more exactly the ideal climatic conditions for *P.*

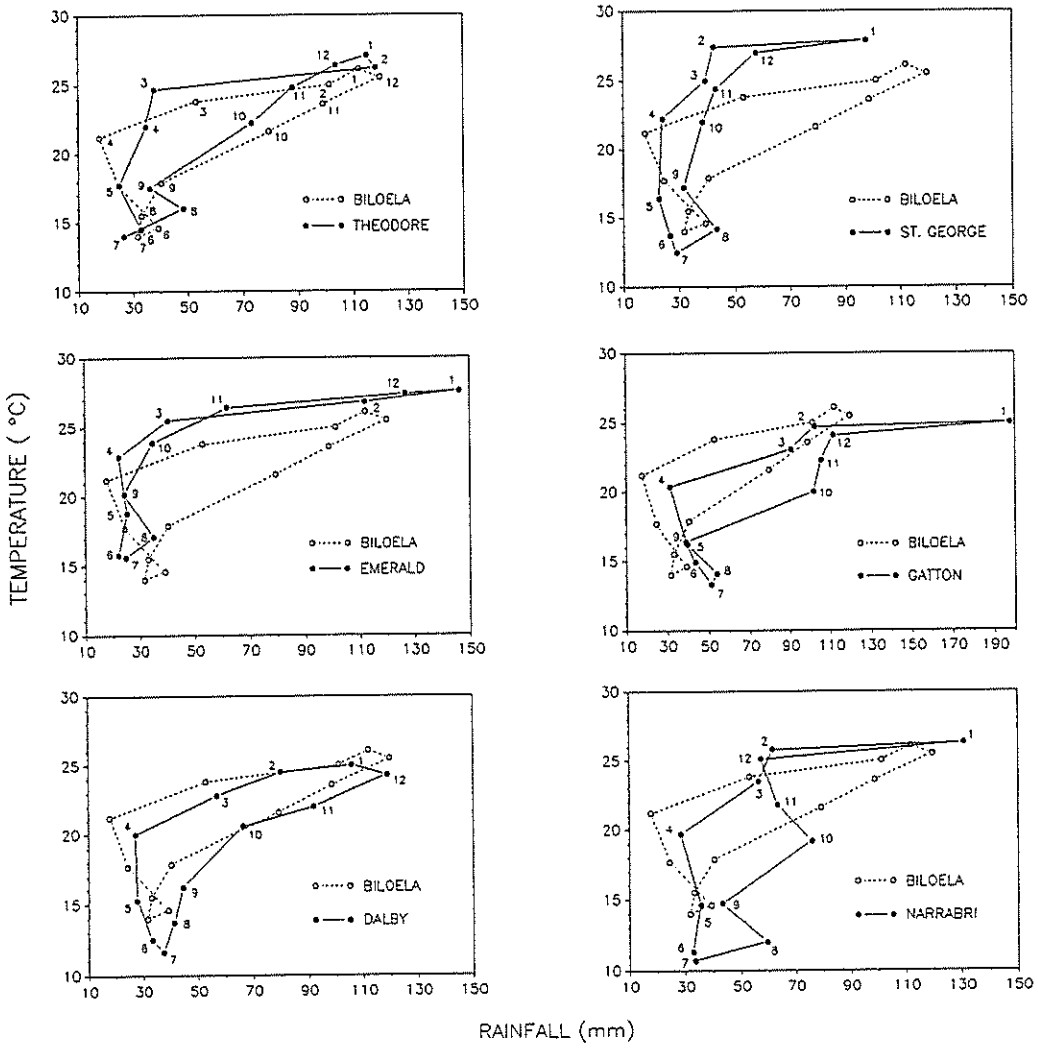

Fig. 8.2. Climographs for seven cotton growing regions of Australia (for location of regions see Fig. 1.2). In each graph the climograph for Biloela is compared with the climograph for another region. Numbers represent each month of the year.

scutigera population increase.

3) *P. scutigera* is a complex of cryptic or sibling species i.e. *P. scutigera* which infests cotton in Central Queensland is a separate species to that found in other areas where it infests alternative host plants but not cotton crops. This possibility needs urgent investigation. During this study, preliminary crossing experiments were conducted to determine whether populations of *P. scutigera* collected from various localities and alternative host plants were able to mate successfully and lay fertile eggs (Appendix J). Populations of *P. scutigera* were collected from larvae infesting *B. australis* at Emerald and Biloela, *H. rosa-sinensis* at Brisbane and Biloela, *H. tiliaceus* at Biloela and *G. sturtianum* at Bauhinia Downs. Each population was established on artificial diet and maintained at 25^{o} C. Males and females of each population were crossed with males and females collected from cotton in Biloela. The F_1 generation of each cross was tested to determine if they were also fully fertile. The results of these crosses gave no evidence of incompatibility between the populations investigated.

Such crossing experiments are not conclusive. In the conifer-feeding *Choristoneura* species complex, all crosses between species produce viable offspring that survive to produce adults (Volney 1989). More detailed experiments are needed to examine whether any assortative mating takes place when males and females are given a choice of mates from the same and a different population under near natural conditions.

Electrophoretic studies of *P. scutigera* collected from different geographic localities and host plants have also shown no evidence for a species complex (Hughes *et al.* 1989). A copy of this paper is included in Appendix K. Again this study was only preliminary and further work is required to determine conclusively the taxonomic status of the various populations.

4) Differences in host plant utilization between *P. scutigera* populations. Many studies have revealed significant genetic variability in the relative performance of herbivores on various host plants (Via 1990). For example intra-population variation in the host plants used for oviposition have been documented in some species of insect (e.g. Tabashnik *et al.* 1981). It is possible that populations of *P. scutigera* from different geographical areas are be genetically different in their choice of host plants i.e. populations in Central Queensland

readily use cotton as a host plant while populations elsewhere do not. Such a possibility requires investigation.

8.10. Further research

The following suggestions for future research on *P. scutigera* are made:

1) Cryptic species: as explained in the above section the possibility that *P. scutigera* is a complex of species needs urgent investigation. More detailed crossing experiments of *P. scutigera* collected from host plants in different localities should be conducted, alongside further electrophoretic examination of the material.

2) Larval sampling methods: the sequential sampling plan presented in Chapter 3 needs to be tested in commercial cotton fields over a number of seasons. The cause of higher *P. scutigera* larval infestations along the crop edge in some commercial fields needs to be verified to determine whether separate sampling plans for these edges should be devised.

3) Development of an egg trap: Valencia (1981) successfully developed an egg trap for monitoring populations of the potato tuber moth, *Phthorimaea operculella* (Zeller). It may be possible to develop an egg trap for monitoring *P. scutigera* populations given the preference of females to lay eggs in concealed locations on the cotton plant (Chapter 3). If an attractive oviposition substrate could be found, then by attaching it around the stems or branches of cotton plants, it may be possible to monitor egg numbers as an alternative to sampling for larvae.

4) Economic thresholds or injury levels: at present spraying decisions are based on a working threshold of 3-5% larval infestation of bolls. The validity of this threshold needs to be tested by conducting studies to determine the relationship between larval damage and yield loss.

5) Alternative host plants and migration: further studies are required to determine the importance of alternative host plants and migration on the population dynamics of *P. scutigera*. Ideally a method for determining the host plant origin of field collected moths is needed. The development of such a technique was attempted during the course of this study by determining the elemental composition of adult moths reared on known larval diets. Insects may contain a unique combination and quantity of chemical elements (chemoprint) that is characteristic of the environment in which they live (Bowden *et al.* 1979). Many

studies have been conducted to investigate whether chemoprints can be used to identify the host plant or geographic origin of an insect with varying degrees of success (e.g. Bowden *et al.* 1984, 1985, Dempster *et al.* 1986, Sherlock *et al.* 1985).

In collaboration with Dr R. Lewis, Department of Physics, Capricornia Institute of Advance Education, Rockhampton, a method for determining the elemental composition of adult *P. scutigera* was developed. The results of preliminary studies were published in a joint paper (Lewis and Walker 1987) is included as Appendix L. of this thesis. The elemental composition of adult moths reared on three larval diets (cotton, *H. tiliaceus* flowers and artificial diet) was examined using a scanning electron microscope based on energy dispersive spectroscopy of X-rays. The initial results looked promising in that classification of moths by larval diet was possible on the basis of the quantity of 13 elements, using discriminant and principal component analysis. Further work is needed to fully evaluate the potential of this method to determine the host plant origin of field collected *P. scutigera*.

6) Longevity of adult moths under field conditions: studies similar to the ones conducted for *P. gossypiella* by Henneberry and Clayton (1989) should be conducted to determine the longevity of adult *P. scutigera* in pre-squaring and squaring cotton crops and their ability to establish an F_1 generation.

A technique for determining the age of field collected adult moths would greatly assist our understanding of the population dynamics of this species. Such a technique was investigated during the course of this study in collaboration with Mr B. Noble, Agricultural Chemistry Branch, Queensland Department of Primary Industries, Biloela. Mail *et al.* (1983) found that the concentration of fluorescent pteridine pigments in the head capsules of *Stomoxys calcitrans* (L.) increased linearly with age. By measuring total fluorescence, a prediction of fly age could be made. This technique was successfully extended to predict the age of field collected flies and other species of Diptera (Langley *et al.* 1988, Lehane and Hargrove 1988). When applied to *P. scutigera* this technique looked promising for determining the age of adult moths. However, due to differences in the rate of change of pteridine pigments in the heads of adult moths, it was not possible to measure total fluorescence. The level of an unidentified

pteridine, termed 'modified xanthopterin', significantly decreased with moth age in male and female heads. The amounts of xanthopterin, pterin and 6-biopterin also decreased with age but at slower rates than modified xanthopterin. In contrast, the concentration of isoxanthopterin decreased in males with increasing age, but increased in females with increasing age. In summary, this preliminary research demonstrated that the measurement of the concentration of pteridine pigments in the heads of *P. scutigera* may be a useful technique for determining the age of moths. This work needs to be extended to determine the relationship between pteridine concentration and temperature.

7) Biological control: the egg parasitoid, *T. bactrae*, may be an ideal candidate for the biological control of *P. scutigera*. This species has the additional attraction in that it also parasitizes the eggs of *Helicoverpa* spp. in cotton (Scholz 1988). The potential of *T. bactrae* to control *P. gossypiella* is presently being investigated in the U.S.A. (Hutchison *et al.* 1990).

8) Pheromone trapping: in Chapter 4 it was concluded that pheromone traps are not reliable indicators of potential larval damage in cotton and their use for timing insecticide sprays could not be recommended. With the recent identification of the *P. scutigera* sex pheromone (see Chapter 4), further monitoring trials using a closer synthetic copy than that used in this study may give different results.

The relationship between trap catch and subsequent population events, such as the timing of mating and oviposition, needs to be defined, as well as the influence of natural female populations on trap catch.

9) Control with synthetic pheromones: over recent years synthetic pheromones have been used with considerable success to control *P. gossypiella* by mating disruption (Campion *et al.* 1990). Control was achieved by the widespread application of synthetic pheromone over cotton fields using slow-release formulations. This success, together with the similarity in the biology and pheromone of *P. gossypiella* and *P. scutigera*, led to a project to evaluate the potential of pheromones for controlling *P. scutigera* in Central Queensland. The project was run after field work for this study had been completed, over two consecutive cotton seasons (1988/89 and 1989/90), and the results were reported by Walker *et al.* (1989). Synthetic pheromone (a 95:5 ratio of Z,Z- and Z,E- 7,11-hexadecadienyl acetate) was formulated in

polyethylene tube dispensers and applied over cotton fields by hand at a rate of 1,000 dispensers/ha (*ca* 78 g a.i./ha). One application of dispensers was successful in disrupting mating over the entire cotton season (see Walker *et al.* 1989 for further details). However, in most fields treated with pheromone larval infestations invariably developed. It was suggested that this was due to the movement of mated females from untreated areas into the pheromone treated area where they laid fertile eggs. In summary, this work demonstrated that control of *P. scutigera* with synthetic pheromone may be possible but large areas of cotton need to be treated. Further research, using a synthetic pheromone formulation that can be sprayed over large areas of cotton, is warranted.

9) Host plant resistance: the screening of high gossypol lines of cotton for resistance to *P. scutigera* should be conducted. Varieties of cotton developed specifically for resistance to *P. gossypiella* (cf. Wilson 1989) should also be screened.

Other varieties worth investigating are: A) Okra-leaf varieties (e.g. Siokra), which have more open plant canopies, may lower the survival of neonate *P. scutigera* larvae by allowing greater penetration of insecticides. B) Frego bract varieties in which the bracts of boll are free and do not touch the boll wall. This may be beneficial in lowering larval survival by providing fewer suitable points of entry into bolls (Chapter 3) and/or by making larvae more exposed to insecticide sprays, predators and parasitoids. C) early maturing varieties to shorten the time available for *P. scutigera* population increase.

10) Improvement of cultural control methods: ways of reducing the survival of *P. scutigera* larvae in cotton crop residues needs further investigation e.g.: a) feasibility of artificially irrigating crop residues to stimulate moth emergence; b) spraying of infested cotton bolls with insecticide; c) further comparison of boll burial methods to determine the most efficient technique or plough; d) more efficient ways of shredding crop residues to lower survival of overwintering larvae.

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Appendix A. Queensland Department of Primary Industries distribution records for *P. scutigera* in Australia. All localities in Queensland unless indicated.

Host/locality	Date	Collector	Grid reference Latitude (S)	Longitude (E)
Cotton (*Gossypium hirsutum*)				
Rockhampton	11/7/23	NR[1]	23 31	148 10
Mt Larcom	30/4/24	"	23 49	150 59
Sand Hill	16/4/24	"	23 37	151 14
Gladstone	16/4/24	"	23 51	151 16
Rockhampton	7/5/24	"	23 31	148 10
Gayndah	?/5/41	"	25 37	151 37
Lowood	14/6/42	"	27 28	152 35
Home Hill	15/8/49	W. A. Smith	19 40	147 25
Biloela	?/7/53	T. Passlow	24 24	150 31
Ayr	19/3/54	W. A. Smith	19 34	147 25
Rockhampton	?/4-5/55	T. Passlow	23 31	148 10
Biloela	?/4-5/55	"	24 24	150 31
Milman	22/7/55	"	23 08	150 24
Gracemere	25/2/59	"	23 26	150 27
Monto	11/2/59	"	24 52	151 07
Biloela	23/4/63	B. Sabine	24 24	150 31
Nebo	?/?/63	"	21 42	148 42
Kabra	1965-1970	"	23 28	150 24
Brisbane[2]	?/3/83	"	27 28	153 01
Brisbane	8/7/83	"	27 28	153 01
Gatton	4/4/86	"	27 34	152 17
"	18/4/86	"	"	"
"	20/3/87	"	"	"
"	6/5/87	"	"	"
Ornamental Hibiscus (*Hibiscus rosa-sinensis*)				
Annerley	29/1/70	NR	27 28	153 01
Brisbane	?/1-5/85	N. Gough	27 28	153 01
Toowoomba	10/4/85	B. Sabine	27 34	151 57
Kingaroy	?/4/85	H. Brier	26 32	151 50
Brisbane	?/6/85	N. Gough	27 28	153 01
"	?/8/85	"	"	"
"	?/7/85	"	"	"
Native Hibiscus (*Hibiscus* sp.)				
Kingaroy	?/4/85	H. Brier	26 32	151 50

1. NR = not recorded
2. Ornamental cotton in suburban garden

Appendix A. continued....

Host/locality	Date	Collector	Grid reference Latitude (N)	Longitude (E)
Kenaf (*Hibiscus cannibinus*)				
Brandon	17/3/86	I. R. Kay	19 33	147 21
Cottonwood tree (*Hibiscus tiliaceus*)				
Southport	23/4/23	NR[1]	27 58	153 25
Sand Hill	3/3/24	"	23 37	151 14
Archer	27/10/65	B. Sabine	23 35	150 35
Shorncliffe	8/12/83	"		
Nudgee Beach	8/12/83	"	27 21	153 06
Ipswich	15/12/83	"	27 37	152 47
Nudgee Beach	1/1/84	"	27 21	153 06
Nudgee Beach	31/1/84	"	"	"
Manly	20/4/84	"	27 28	153 11
Nudgee Beach	27/4/84	"	27 21	153 06
Yeronga	27/4/84	"	27 31	153 01
Goondiwindi	?/6/85	"	28 33	150 18
Kingscliffe (NSW)	2/7/85	"	28 15	153 36
Sandgate	1/5/86	"	27 20	153 04
Glasshouse Mountains	17/7/86	"	26 54	152 57
Toorbul	17/7/86	"	27 02	153 03
Sandgate	?/6/87	"	27 20	153 04
Scarborough	4/9/87	"	27 12	153 07
"	9/10/87	"	"	"

1. NR = not recorded.

Appendix B. Parameters for the linear regression model to describe the relationship between *P. scutigera* developmental rates on artificial diet and temperature, for eggs, each larval instar, all larval instars combined, pupal development and total development.

add a column with sample size ✓

Stage/ instar	n	Regression parameter (± S.E.) a	b	r^2
Eggs		-0.1685 (0.0113)	0.0135 (0.0005)	0.988***[1]
Larvae				
L1		-0.3346 (0.0369)	0.0237 (0.0005)	0.992**
L2		-0.2860 (0.0957)	0.0246 (0.0021)	0.985**
L3		-0.0470 (0.0096)	0.0116 (0.0040)	0.807
L4		-0.0534 (0.0247)	0.0049 (0.0010)	0.917*
L1-L4				
Males		-0.0300 (0.0103)	0.0027 (0.0004)	0.953*
Females		-0.0310 (0.0102)	0.0028 (0.0004)	0.955*
Combined		-0.0311 (0.0103)	0.0028 (0.0004)	0.953*
Pupae				
Males		-0.0714 (0.0049)	0.0062 (0.0002)	0.998**
Females		-0.0883 (0.0079)	0.0072 (0.0003)	0.996**
Combined		-0.0775 (0.0015)	0.0065 (0.0001)	0.999***
Larvae-adult				
Males		-0.0217 (0.0050)	0.0019 (0.0002)	0.976*
Females		-0.0229 (0.0047)	0.0020 (0.0002)	0.981**
Combined		-0.0221 (0.0054)	0.0019 (0.0002)	0.974*
Egg-adult				
Males		-0.0190 (0.0043)	0.0017 (0.0002)	0.977*
Females		-0.0202 (0.0040)	0.0017 (0.0002)	0.982**
Combined		-0.0197 (0.0049)	0.0017 (0.0002)	0.977*

1. *, **, *** indicate regression significant at $p<0.05$, 0.01 and 0.001, respectively.

Appendix C. Parameters for the Pradhan (1946) model to describe the relationship between *P. scutigera* developmental rate on artificial diet and temperature, for third and fourth larval instars, all larval instars and total development (from egg hatch to moth emergence).

Stage of development	Parameter (± S.E.) A	B	C	r^2
L3	0.2865 (0.0291)	-0.00621 (0.00510)	28.3732 (3.3688)	0.897
L4	0.0880 (0.00493)	-0.00948 (0.00332)	28.6626 (1.36617)	0.983
L1-L4	0.0498 (0.0024)	-0.00788 (0.0027)	29.5406 (1.5990)	0.989
Total	0.0352 (0.0015)	-0.00704 (0.00191)	30.2987 (1.4662)	0.994

Appendix D. Parameters for the polynomial regression model to describe the relationship between *P. scutigera* developmental rates on artificial diet and temperature, for each larval instar, all larval instars combined and total development (from egg hatch to moth emergence).

Life stage	Parameter (± S.E.) Bo	B_1	B_2	r^2
L1	-0.40579 (0.34597)	0.02978 (0.02953)	-0.00013 (0.00061)	0.992
L2	-0.29296 (0.49067)	0.02515 (0.04188)	-0.00001 (0.00087)	0.985
L3	-0.78734 (0.58972)	0.07543 (0.05033)	-0.00133 (0.00104)	0.920
L4	-0.28432 (0.03796)	0.02474 (0.00324)	-0.00041 (0.00007)	0.998
L1-L4	-0.12662 (0.01845)	0.01103 (0.00157)	-0.00017 (0.00003)	0.998
Total	-0.07297 (0.00564)	0.00633 (0.00048)	-0.00009 (0.00001)	0.999

Appendix E. Life table for *P. scutigera*, from egg hatch to moth emergence, reared on artificial diet at four constant and one fluctuating temperature regimes.

Life stage /moult	Number entering stage	Number dying	Proportion of total dying	Probability of dying[1]	Cumulative survival (%)
			18° C		
L1	64	-	-	-	100.0
L2	61	3	0.047	0.047	95.3
L3	61	0	0	0	95.3
L4	61	0	0	0	95.3
Pupation	46	15	0.234	0.246	71.9
Eclosion	43	3	0.047	0.065	67.2
			21° C		
L1	49	-	-	-	100.0
L2	49	0	0	0	100.0
L3	49	0	0	0	100.0
L4	49	0	0	0	100.0
Pupation	35	14	0.286	0.286	71.4
Eclosion	25	10	0.204	0.286	51.0
			25° C		
L1	79	-	-	-	100.0
L2	79	0	0	0	100.0
L3	70	9	0.114	0.114	88.6
L4	63	7	0.089	0.100	79.7
Pupation	58	5	0.063	0.079	73.4
Eclosion	54	4	0.051	0.069	68.4
			30° C		
L1	65	-	-	-	100.0
L2	65	0	0	0	100.0
L3	65	0	0	0	100.0
L4	58	7	0.108	0.108	89.2
Pupation	29	29	0.446	0.500	44.6
Eclosion	25	4	0.062	0.138	38.5
			25/15° C		
L1	65	-	-	-	100.0
L2	65	0	0	0	100.0
L3	64	1	0.015	0.015	98.5
L4	63	1	0.015	0.016	96.9
Pupation	47	16	0.246	0.254	72.3
Eclosion	44	3	0.046	0.064	67.7

1. Probability of dying = proportion of individuals which enter a stage and die during that stage.

Appendix F. Effect of larval diet on aspects of *P. scutigera* reproduction at 25° C. Pairwise comparisons of means using Wilcoxons' two-sample test. Values of Z and their level of significance (df=1) as calculated by PROC NPAR1WAY (SAS Institute, 1985).

Diet[2] comparison	Value of Z[1]				
	PRE	FEC1	FEC2	50%	DUR[3]
AD-GC	0.148	0.770	0.126	1.326	0.440
AD-OH	-1.787	3.500***	3.285***	0.337	3.818***
AD-HT	-2.593*	3.379***	2.723**	-0.352	3.520***
AD-BT	1.853	3.881***	-3.225**	1.106	-1.557
AD-DC	2.751**	-3.308***	-2.977**	1.176	-2.339*
GC-OH	-1.911	1.755	2.347*	-0.682	4.242***
GC-HT	2.498*	2.081*	-1.846	-1.430	3.658**
GC-BT	2.068*	-2.572**	-2.102*	1.706	-1.573
GC-DC	2.570**	-1.924*	-2.159*	1.646	-2.769*
OH-HT	0.780	-1.014	-0.028	1.165	-0.099
OH-BT	0.962	-1.755	-0.472	1.721	2.480*
OH-DC	1.377	-1.102	-0.925	1.656	0.509
HT-BT	0.253	-0.817	-0.567	1.043	2.289*
HT-DC	0.862	-0.262	-0.923	0.375	1.282
BT-DC	0.425	0.486	-0.318	-0.478	-1.624

1. Values of Z followed by *, ** and *** indicate a significant difference between diets compared at p <0.05, 0.01 and 0.001, respectively.

2. Larval diet abbreviations: AD = artificial diet, GC = green cotton bolls, OH = ornamental *Hibiscus* flowers, HT = *Hibiscus tiliaceus* flowers, BT = *B. australis* flowers, DC = dry cotton bolls.

3. PRE = preoviposition period, FEC1 = fecundity (all females), FEC2 = fecundity (egg laying females only), 50% = days to reach 50% oviposition, DUR = duration of oviposition.

Appendix G. Relationship between cotton boll age and size to *P. scutigera* larval infestation in unsprayed cotton during the 1984/85 season.

Boll age (days)	No. of bolls	Mean boll size (± S.D.)		Mean no. of larvae/boll (± S.D.)
		Length	Width	
1	41	10.2 (0.4)	4.6 (0.5)	0.24 (0.43)
3	42	15.0 (0.9)	8.5 (0.5)	0.12 (0.33)
7	29	16.5 (3.7)	11.2 (2.7)	0.07 (0.26)
10	45	20.4 (2.4)	12.7 (2.7)	0.11 (0.32)
11	27	25.8 (3.4)	16.9 (2.8)	0.15 (0.36)
14	40	22.8 (2.0)	13.6 (2.6)	0.13 (0.33)
16	20	31.8 (4.4)	22.2 (3.2)	0.05 (0.22)
18	32	29.9 (3.5)	28.9 (1.7)	0.25 (0.44)
21	29	39.8 (6.5)	28.3 (3.8)	0.38 (0.49)
25	15	34.2 (3.5)	24.4 (3.1)	0.27 (0.46)
27	23	39.7 (3.6)	27.0 (3.1)	0.35 (0.49)
29	11	34.9 (5.1)	21.4 (2.9)	0.45 (0.52)
36	27	44.9 (4.4)	31.7 (1.9)	0.30 (0.47)
41	11	40.6 (4.7)	33.0 (2.1)	0.27 (0.47)
42	30	48.0 (3.2)	30.1 (1.5)	0.50 (0.51)
45	11	38.8 (4.5)	30.4 (4.0)	0.36 (0.50)
49	11	44.0 (4.9)	30.8 (3.5)	0.27 (0.47)
51	12	Open		0.08 (0.29)
53	11	"		0.36 (0.50)
55	15	"		0
57	15	"		0.07 (0.26)
59	11	"		0.18 (0.40)
61	6	"		0
63	11	"		0.18 (0.40)
67	12	"		0
71	7	"		0

Appendix H. Relationship of cotton boll age and size to *P. scutigera* larval infestation in unsprayed cotton during the 1986/87 season.

Boll age (days)	No. of bolls	Mean boll size (MM) (± S.D.) Length	Width	Mean no. of larvae/boll (± S.D.)
2	5	11.5 (0.7)	9.5 (0.7)	0.20 (0.45)
5	33	14.7 (1.7)	12.0 (1.0)	0.36 (0.60)
7	19	16.1 (3.2)	12.9 (2.1)	0.05 (0.23)
8	36	18.7 (2.6)	14.6 (1.7)	0.22 (0.54)
10	47	19.7 (3.3)	15.8 (2.5)	0.13 (0.34)
12	18	26.0 (5.2)	20.6 (3.6)	0.11 (0.32)
13	29	25.8 (6.1)	19.2 (4.1)	0.17 (0.38)
15	49	32.8 (5.2)	24.7 (3.5)	0.37 (0.53)
18	8	31.9 (5.6)	24.3 (4.8)	0.25 (0.46)
20	6	30.8 (6.1)	22.3 (4.5)	0.17 (0.41)
23	10	30.8 (4.7)	22.5 (3.9)	0.20 (0.63)
25	10	35.2 (4.0)	26.4 (2.1)	0.20 (0.42)
27	12	36.3 (2.0)	27.1 (1.5)	0.17 (0.39)
30	14	35.6 (2.5)	27.3 (1.9)	0.43 (0.65)
31	14	35.6 (2.5)	27.3 (1.9)	0.43 (0.65)
34	28	36.8 (3.1)	27.1 (2.7)	0.25 (0.44)
37	17	33.7 (3.9)	25.8 (3.2)	0.53 (0.62)
38	19	39.2 (4.8)	29.1 (1.6)	0.47 (0.51)
41	40	40.6 (6.0)	28.4 (3.3)	0.39 (0.47)
44	44	43.2 (4.5)	30.9 (3.5)	0.39 (0.58)
46	7	Open		0.48 (0.53)
49	21	"		0.10 (0.30)
52	12	"		0
55	3	"		0

Appendix I. Species of plants sampled during host plant surveys in which *P. scutigera* were not found infesting. Plant parts sampled were: flower buds (FB), flowers (FL), fruits (FR) and seed capsules (SC). Localities were: Biloela (B), Bauhinia Downs (BD) and Theodore (T).

Plant family/ species	Plant part sampled	Month sampled	Locality sampled
SOLANACEAE:			
Datura stranionium	SC	N, D	B
PAPAVERACEAE:			
Argemone ochroleuca	FB, SC	S, O	B
LEGUMINOSAE:			
Acacia spp.	FL	S, O	B
Sesbania cannabia	SC	JAN, F	B, T
STERCULIACEAE:			
Brachychiton rupestris	FL, SC	O,N,D	B, BD
MALVACEAE:			
Abutilon sp.	SC	F	B
Hibiscus divaricartus	SC	O, N	B
Hibiscus rosa-sinensis var. Chinese Lantern	FL	O, N, D	B
Hibiscus trionum	SC	D, JAN	B
Malva parviflora	SC	D, JAN	B
Sida cordifolia	SC	O, N, D	B
EUPHORBIACEAE:			
Ricinus communis	SC	S, O	B
ROSACEAE:			
Prunus persica var. Nectarine	FR	N, D	B
Rosa sp.	FL	D	B
CRASSULACEAE:			
Kalanchoe tubilflora	FL	F	B
MYRTACEAE:			
Eucalyptus spp.	FL	MAR	B
CAESALPINIACEAE:			
Bauhinia sp.	FL	N, D	B
BIGONIACEAE:			
Jacarandra mimosifolia	FL	N	B
Spathodea campanulata	FL	F	B

Appendix J. Summary of crosses of *P. scutigera* obtained from larvae infesting cotton at Biloela with moths obtained from three alternative host plants and four localities. All moth populations were reared for one generation on artificial diet at 25° C before conducting crosses. Larval hosts and localities were: BTB = *B. australis* ex Biloela; BTE *B. australis* ex Emerald; OHB = *H. rosa-sinensis* ex Brisbane; GSD = *G. sturtianum* ex Bauhinia Downs; C = cotton ex Biloela. F1 represents the fertility of the progeny of each inter-population cross.

Cross ♀ X ♂	No. replicates	No. moths paired ♂	♀	Mean no. eggs /females (± S.D.)	% egg hatch (± S.D.)
BTB X C	5	20	10	362.6 (78.0)	95.3 (1.4)
C X BTB	5	20	9	384.6 (163.7)	95.0 (2.1)
BTB X BTB	5	14	13	315.4 (156.5)	95.0 (2.5)
F1 (BTB X C)	5	15	15	464.8 (26.3)	94.5 (2.1)
F1 (C X BTB)	5	15	15	418.3 (32.1)	95.4 (1.7)
BTE X C	7	28	14	282.9 (180.0)	96.8 (1.2)
C X BTE	3	12	6	380.7 (147.7)	95.8 (0.5)
BTE X BTE	6	18	15	331.2 (142.3)	91.6 (7.2)
F1 (BTE X C)	5	20	10	499.2 (37.1)	NR
F1 (C X BTE)	4	12	12	373.9 (59.7)	92.3 (4.9)
OHB X C	8	30	15	401.7 (109.6)	NR
C X OHB	8	30	15	372.6 (134.6)	NR
OHB X OHB	5	20	10	423.0 (96.6)	NR
F1 (OHB X C)	5	20	10	288.8 (81.4)	NR
F1 (C X OHB)	5	20	10	365.1 (147.0)	NR
GSD X C	7	28	13	252.0 (87.0)	97.3 (0.8)
C X GSD	5	20	10	360.7 (131.7)	96.8 (1.0)
GSD X GSD	2	5	5	137.8 (92.3)	91.3 (0.4)

NR = % egg hatch not recorded but was observed to be normal.

Appendix K.

J. Aust. ent. Soc., 1989, 28: 197–200

ELECTROPHORETIC STUDIES OF THREE POPULATIONS OF *PECTINOPHORA SCUTIGERA* (HOLDAWAY) (LEPIDOPTERA: GELECHIIDAE) IN QUEENSLAND

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Abstract

Samples of pinkspotted bollworm *Pectinophora scutigera* (Holdaway) were collected from a range of different host plants at 3 locations in Queensland. Cellulose acetate electrophoresis was used to determine if there was evidence that more than one species was present. Of 12 genetic loci examined, only 2 were polymorphic and allele frequencies differed very little among samples taken from different host plants or between locations. While the evidence is not conclusive, it suggests that all 3 populations are conspecific.

Introduction

The pinkspotted bollworm, *Pectinophora scutigera* (Holdaway), has been a serious pest of cultivated cotton *(Gossypium hirsutum)* in the Callide and Dawson Valleys of central Queensland since it was first grown there in the 1920's (Holdaway 1926; Sabine 1969; Walker and Harris 1985). However, despite its widespread distribution throughout central and southeast Queensland, *P. scutigera* has not become a pest of cotton in other areas of Queensland, or in New South Wales. It also utilises a number of native and ornamental plants throughout Queensland, the most important being *Hibiscus tiliaceus, Hibiscus rosa-sinensis* and *Brachychiton australis* (Queensland Department of Primary Industries unpubl. records; Walker and Harris 1985). Page *et al.* (1982, 1984) conducted pheromone trap surveys and caught male *P. scutigera* throughout central and southeast Queensland. At Emerald, *ca* 300 km northwest of the Callide Valley, adult moths have been caught in pheromone traps throughout the year and larvae have been found infesting *H. tiliaceus* and *B. australis* flowers (F.D. Page pers. comm.; P.W. Walker unpubl. data). Yet cotton in Emerald remains free of *P. scutigera,* which has been recorded as a pest there on only one occasion (D.A.H. Murray pers. comm.). Similarly, while *P. scutigera* infests ornamental cultivars of *H. rosa-sinensis* throughout southeast Queensland, commercial cotton on the Darling Downs and Lockyer Valley does not have problems with this pest. However, a small plot of experimental cotton at Gatton in the Lockyer Valley was infested by *P. scutigera* during the 1985/86, 86/87 and 87/88 seasons, but infestations did not apparently spread to surrounding commercial fields (B. Sabine pers. comm.; P.W. Walker pers.obs.).

This limited pest status of *P. scutigera* is difficult to explain. Variation in host plant utilisation may be responsible, with moths in central Queensland more readily utilising cotton as a host than those in other areas of Queensland. Alternatively, there may be more than one species of moth each with a different host plant range. We investigated this possibility by using allozyme electrophoresis to compare genotypes of *P. scutigera* collected from various host plants in central and southeast Queensland.

Materials and methods

Collections

P. scutigera larvae were collected from cultivated cotton, *H. tiliaceus, H. rosa-sinensis* and *B. australis* in Biloela, central Queensland. Adult moths reared from these larvae were directly compared with collections of *P. scutigera* made in southeast Queensland at Brisbane *(H. tiliaceus* and *H. rosa-sinensis)* or at Gatton in the Lockyer Valley (cultivated cotton and *H. rosa-sinensis), ca* 92 km west of Brisbane.

Infested flowers or fruiting structures were sampled from each host plant and either incubated in ventilated containers until pupation of *P. scutigera* larvae, or the larvae were immediately removed and reared on artificial diet. All collections were kept at 28°C (± 2°), 75% relative humidity and 14L:10D photoperiod. Table 1 shows the number of *P. scutigera* analysed and the collection dates for each locality. The identity of all larvae and pupae was checked using the characters given by Holdaway (1926). Pupae were sexed and kept in separate containers. Emerging moths were provided with a 5% sugar solution.

Table 1. Details of *P. scutigera* collections

Locality/ sampling date	Number of moths analysed from each host plant			
	Gossypium hirsutum	*Hibiscus tiliaceus*	*Hibiscus rosa-sinensis*	*Brachychiton australis*
CENTRAL QUEENSLAND				
Biloela				
1- 4.ix.1985	28	-	34	23
7- 8. v.1986	30	11	-	-
4-18.vi.1987	81	-	87	-
11. v.1988	28	-	28	-
Total	167	11	149	23
SOUTHEAST QUEENSLAND				
Brisbane				
15.xi. 1985	-	23	37	-
7. v. 1986	-	-	26	-
10.iii. 1987	-	39	20	-
Gatton				
10.v. 1988	17	-	27	-
Total	17	62	110	-

Electrophoresis

Moths were killed with CO_2 on the day they were to be analysed. The thorax and abdomen were ground separately with 0.2 ml of buffer (0.1M Tris EDTA, 2 mg/L NADP, adjusted to pH 8.2 using conc. HCl). Samples were spun at 10.000 rpm for 20 minutes below 4°C in a Sorvall RC5B centrifuge. The supernatant was decanted into vials and analysed immediately.

Electrophoresis was performed on cellulose acetate plates (Titan III, Helena Laboratories). For the enzymes glucose phosphate isomerase, phosphoglucomutase, alcohol dehydrogenase, isocitrate dehydrogenase, hydroxybutyrate dehydrogenase, malic enzyme and malate dehydrogenase, 0.1 M tris citrate PH 7.0 was used as electrode buffer and for esterase and aspartate amino transferase 0.75M tris glycine pH 8.5 was used.

For each enzyme, moths collected concurrently from each locality were run on the same plate to ensure that fixed differences between them could be identified. For the polymorphic loci, samples from a particular host and location were pooled so that sufficient data could be generated to analyse differences in allele frequency between host plants and locations. Wright's F_{ST} statistic was calculated for those alleles which had frequencies of greater than 0.10 in at least one sample (Wright 1943).

Results and discussion

Twelve genetic loci were represented in the 9 enzymes examined. Only 2 loci, Pgi and Pgm in Table 2 were polymorphic (Table 2). There was no evidence of fixed differences between samples from different hosts within locations or between different locations. Five alleles were identified for each of the polymorphic loci and the frequencies of these for each host plant and location are shown in Table 3. In all samples, for both Pgi and Pgm, the same allele was very common (ranging from 0.775-0.983 in Pgm and from O.858-1.00 in Pgi) with the other 4 in very low frequencies. When F_{ST}'s were calculated with all 7 samples included, only 1 allele, Pgm^2, showed significant differentiation between samples ($p < 0.05$, Table 4). Because of the small samples for *B. australis* and Biloela *H. tiliaceus*, the analysis was repeated with only *H. rosa-sinensis* and cotton samples included. Again only Pgm^2 showed significant differentation among samples. Yet, tests of proportions (Freund 1979) showed no significant differences in allele frequencies between any 2 samples, which suggests that the differentiation is very slight.

Although some samples, for example Biloela cotton, appear to have a greater number of rare alleles present, this is very probably an artifact caused by the

Table 2. Enzymes examined, the number of loci and whether they were plymorphic (most common allele < 0.95)

Enzyme	I.E.C.C.	No. of loci	Polymorphic
Alcohol dehydrogenase (Adh)	1.1.1.1	1	monomorphic
Esterase (Est)	3.1.1.1	1	"
Aspartate amino transferase (Got)	2.6.1.1.	2	"
Hydroxybutyrate dehydrogenase (Hbdh)	1.1.1.30	1	"
Isocitrate dehydrogenase (Idh)	1.1.1.42	2	"
Malate dehydrogenase (Mdh)	1.1.1.37	2	"
Malic enzyme (Me)	1.1.1.40	1	"
Phosphoglucomutase (Pgm)	2.7.5.1	1	polymorphic
Glucose-phosphate isomerase (Pgi)	5.3.1.9	1	"

Table 3. Gene frequencies at the PGM and PGI loci for *P. scutigera*

		Locality/host plant						
		SOUTHEAST QUEENSLAND			CENTRAL QUEENSLAND			
Loci/ alleles		G.h.	H.t.	H.r.	G.h.	H.t.	H.r.	B.a.
Pgm	1	–	0.025	0.023	0.012	–	0.007	0.025
	2	0.031	0.051	0.099	0.036	0.100	0.062	0.150
	3	0.938	0.907	0.854	0.899	0.900	0.886	0.776
	4	–	–	–	0.009	–	–	–
N		16	59	106	167	5	145	20
Pgi	1	–	0.025	0.005	–	–	0.003	–
	2	0.059	0.108	0.071	0.081	–	0.057	–
	3	–	–	–	0.011	–	–	–
	4	0.941	0.858	0.919	0.904	0.954	0.926	1.000
	5	–	0.008	0.005	0.003	0.045	0.003	–
N		17	60	105	167	11	149	19

G.h., *Gossypium hirsutum;* H.t., *Hibiscus tiliaceus;* H.r., *Hibiscus rosa-sinensis;* B.a., *Brachychiton australis*

Table 4. F_{ST} values for 2 loci with all 7 samples included. Only alleles with frequencies > 0.10 in at least one sample were included

Allele	F_{ST}	χ^2	p
Pgm^2	0.11375	15.672	$0.01 < p < 0.05$
Pgm^3	0.002237	8.115	n.s.
Pgi^2	0.001710	7.77	n.s.
Pgi^4	0.004938	10.856	n.s.

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variability is sample size. Certainly, the 3 samples with the lowest number of alleles, Gatton cotton, Biloela *H. tiliaceus* and Biloela *B. australis*, all contained less than 25 individuals.

These results suggest that we are dealing with a single species. Even the polymorphic loci have similar distributions of alleles among samples. Most studies of morphologically similar species have found at least some fixed differences between them e.g. Milton *et al.* 1983, Mather (in press). However, this is not always the case. For example, Drew and Lambert (1986) found no fixed differences between 2 species of fruit fly *(Dacus tryoni* (Froggatt) and *D. aquilonis* (May)), using cellulose acetate electrophoresis. However they did find large differences in frequency, with different alleles most common, in 3 out of a total of 30 loci examined and what appeared to be significantly different frequencies at 4 other loci.

Preliminary cross-mating experiments found no evidence of reproductive incompatibility between *P. scutigera* populations collected from different host plants and different localities. Such crosses have been extended to the F_2 (P.W. Walker, unpubl. data).

The results suggest that other factors may be responsible for the limited pest status of *P.scutigera* in cotton. Its establishment as a pest of cotton in any area will depend on the ability of populations to carry-over from one cotton season to the next. Successful carryover of *P. scutigera* populations in the Callide and Dawson Valleys of central Queensland may occur due to a more favourable local climate, more abundant and diverse alternative host plants and/or differences in agronomic practices.

Further work is required to determine conclusively the taxonomic status of the various populations. Genitalia of adult samples from each host should be examined using the key of Common (1958) and host plant preferences of adults reared on different host plants should be investigated. As well, further investigation is needed of the mating system (such as differences in sex pheromones) and any evidence of positive assortative mating among individuals from different host plants and/or localities needs to be examined (see Lambert and Paterson 1982).

Acknowledgments

We thank Mr Tony Clark and Dr Viv Harris who assisted with collections of samples around Brisbane, Chris Fitzgerald who helped grind animals and Professor Hugh Paterson for comments on an earlier draft of the manuscript. Queensland Department of Primary Industries provided some funds for cellulose acetate plates, as did Griffith University and the University of Queensland. We also thank Mr B. Sabine, Queensland Department of Primary Industries, Brisbane, for providing host plant records of *P.scutigera*.

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Appendix L.

Scanning Microscopy, Vol. 1, No. 2, 1987 (Pages 871-878)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/87$3.00+.00

DISTINGUISHING ADULT PECTINOPHORA SCUTIGERA (HOLDAWAY) ACCORDING TO LARVAL DIET BY X-RAY MICROANALYSIS

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(Received for publication June 23, 1986, and in revised form February 26, 1987)

Abstract

In an exploratory study using scanning electron microscope (SEM) based energy dispersive spectroscopy (EDS) of X-rays, differences were detected in the elemental compositions of adult Pectinophora scutigera raised on contrasting diets - cotton, Hibiscus tiliaceus and laboratory medium.

X-ray spectra were collected from the thorax and abdomen of 13 male and 13 female moths. X-ray yields are optimised by ashing the insects for 1 h at 400-600^{0}C, and by operating the SEM at 25 kV.

Spectrum matching, element matching, discriminant analysis and principal components analysis were used to classify the specimens on the basis of diet with 54-100% success. Spectra were considered as a whole or processed using either a digital filter to remove background or a ZAF program to compute semiquantitative elemental concentrations. Background corrected peaks is the favoured data format, having high discriminating power and being simple to obtain and interpret.

Better classification is apparent when subsets of the same sex, body part or both are employed; and when the data for each element is normalised to a mean of 0 and a standard deviation of 1. The chief discriminating elements appear to be F, Na, Mg, P, Cl, K and Ca with Si, S, Mn, Fe and Zn being of little assistance.

KEY WORDS: Insects, pink-spotted bollworm, X-ray microanalysis, bulk biological specimens, energy dispersive spectroscopy.

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Introduction

X-ray spectrometric methods have been applied to diverse biological species. For example, Calaprice (1971) studied sockeye salmon by X-ray fluorescence (XRF) energy dispersive spectroscopy (EDS). Using discriminant analysis of the spectral data he was successful in separating both adult and fry salmon on the basis of their geographical origins. Kelsall and Burton (1977), with some success, applied the same experimental and statistical techniques to feathers from lesser snow geese.

A number of X-ray spectrometric investigations have been directed towards insects.

McLean and Bennett (1978), using XRF/EDS and discriminant analysis, found that ambrosia beetles could be readily separated on the basis of geography, sex or the presence of adhesive from sticky traps. McLean (1980) reported on a practical extension, identifying the origins of beetles found in the Chemainus Sawmill. McLean et al. (1979) separated western spruce budworms on the basis of their source stands. Later, McLean et al. (1983) identified a partitioning of elements in the thoraces and abdomens of these insects, and discovered that elemental variations in adult insects were much smaller than those in the original food sources.

Another substantial contribution to the entomological exploitation of X-ray analysis is a series of papers by Bowden and co-workers. The first in the series (Bowden et al. 1984) concerned the moth Noctua pronuba, the subject of a previous study (Bowden et al. 1979). XRF/wavelength dispersive spectroscopy (WDS) was employed in both cases. Principal components analysis (PCA) gave no discrimination until subsets were considered. When this was done, not only could insects from different host plants be distinguished, but also insects from the same host plant growing on different soils. The second article (Bowden et al. 1985a) examined two species of aphid, using SEM/EDS. No discrimination was discernible for any aphid species, element, plant or soil subset. The third paper (Bowden et al. 1985b) again examined the same two aphid species, but collected from different host plants and with a refined PCA, and reported successful discrimination. Another moth, Agrotis segetum, was the subject of a fourth paper (Sherlock et al. 1985), in which robust means PCA was applied to XRF/WDS data. Provided host plant subsets were considered separately, the moths could be differentiated on the basis of soil type. A fifth paper (Sherlock et al. 1986) examined two aphid morphs collected at various localities mainly from the same host plant, using SEM/EDS. While the morphs were well differentiated, there was no clear separation, in either morph, on the basis of host plant locality.

Preliminary reports on another X-ray study of moths, Heliothis species, have been given by Fitt (1985, 1986). Dempster et al. (1986) have conducted XRF/WDS studies of the Brimstone butterfly. Compositional differences were observed between insects of different sexes, sites and seasons, but the host plant differences diminished as the adults fed and aged.

The broad conclusion that may be drawn from this literature survey is that X-ray methods for geographically discriminating insects have met with mixed success. In some instances, at least, for identifiable reasons or unknown, there is a lack of any measurable compositional variation in insects from different sources.

The subject of the present study is the pink-spotted bollworm, Pectinophora scutigera (Holdaway) (Lepidoptera:Gelechiidae), a serious pest of cotton in Central Queensland. Larvae tunnel into developing cotton bolls damaging lint and seed. Of particular interest is the question as to how populations carry over from one season to the next. Sabine (1969) stressed the importance of post-harvest cotton trash, containing infested bolls, and considered alternative host plants to be of little significance. However, the recent discovery of additional host plants (Walker and Harris 1985) suggests that alternative host plants may play a significant role in the carry over of moth populations. It is toward investigating this possibility that the method described here will be directed.

The purpose of this paper is to demonstrate that the larval diet of adult bollworms can be deduced with a good chance of success, and to suggest some ways to improve the success rate. The method set forth here is not claimed to be the best possible means of discriminating P. scutigera. A method meriting such a claim might be expected to include a complete quantitative elemental analysis of each moth as well as to take into account other variables - colouration, body weight, moisture content, age and so on. The proposed method does not require an accurate analysis of the detected elements. It is generally recognised that quantitative analysis of bulk biological specimens by EDS is difficult and prone to substantial error (Roomans 1980, Heinrich 1982, Boekestein et al. 1980). Detection of all the elements present in the specimens has not been attempted. Most EDS systems have a lower atomic number limit of Z=9, although windowless detectors may be pressed to Z=4 (Marshall 1984). Hydrocarbons are therefore difficult and hydrogen impossible to detect. An alternative spectroscopy, such as secondary ion mass spectrometry (SIMS), is required to probe for light elements.

Materials and Methods

Specimens

Adult P. scutigera were reared from larvae fed on 3 contrasting diets: artificial medium, cotton bolls and Hibiscus tiliaceus flowers.

A laboratory culture of P. scutigera, originally established from larvae already infesting cotton bolls, was maintained on artificial medium as described by Vickers (1982). The medium consisted mainly of soya beans (66.2% by weight of dry ingredients) with a small amount (2.3%) of ground cotton seed added to act as a larval feeding stimulant. The culture had been maintained on this medium for approximately 3 generations.

P. scutigera infested cotton bolls were collected from cotton (Deltapine 61) near Biloela, Central Queensland, in May 1985. Fallen H. tiliaceus flowers were collected from the base of a single tree in Biloela during March and April 1985. Cotton bolls and H. tiliaceus flowers were held in ventilated plastic boxes at 25°C until the larvae had pupated. All pupae were sexed and incubated at 25°C. On emergence adult moths were not fed, but immediately killed by freezing.

Specimens were deep frozen until required. To reduce the contribution of the hydrocarbon matrix to the X-ray spectrum, the specimens were ashed using a Townson and Mercer furnace. To establish the optimum ashing temperature, 5 entire air-dried moths (all males collected from cotton) were ashed for 1 h at each of the following temperatures: 200, 300, 400, 500, 600 and 700°C.

Ashed moths were mounted on Al stubs using double-sided adhesive tape. From 1 to 3 moths were mounted on each stub. The moths were systematically aligned on the stubs to facilitate identification of body parts.

The main data base comprised X-ray spectra from 26 moths. Of the 26 moths, 5 of each sex (M, F) came from cotton (C), 5 of each sex from laboratory medium (L) and 3 of each sex from H. tiliaceus (H). The thorax (T) and abdomen (A) of each moth were examined separately, yielding a total of 52 spectra. The abbreviations we employ to refer to data subsets having the same sex and body part are MT, MA, FT and FA.

SEM/EDS system

The prepared specimens were placed in an ISI-60A SEM. For all the measurements, the working distance was fixed at 53 mm and the working magnification was 1000x. The specimens were irradiated with 30 keV electrons, the highest energy available, to ensure any elements with high atomic number were excited. During the acquisition of the data base, no elements with Z>29 were detected.

Later, to ascertain the optimum beam voltage, a series of X-ray spectra were obtained at electron energies of 15, 20, 25 and 30 keV. A single set of 5 specimens (male moths from cotton) was used in this investigation.

X-ray collection and sorting was via a PGT4 system. The X-ray detector was located 27 mm above the specimen and 21 mm from the beam axis; the take-off angle was 52°. X-ray spectra were collected for 200 s. The beam current was measured using a Faraday cup before and after each spectrum. Each X-ray spectrum was transferred through an RS232 interface to an IBM Personal Computer (IBM PC) for further processing.

Data format

The X-ray spectral data was presented for statistical analysis in three forms. These will be described in order of increasing spectrum processing.

First, the raw channel-by-channel data was used. The energy calibration was 20 eV per channel. The lower threshold on the main amplifier rejected signals corresponding to less than 500 eV. No peaks were observed above 10 keV. So the data in channels 25 to 500 was used.

Second, background-corrected peak intensities were computed. The background was removed using a "top-hat" digital filter as described by Statham (1977). Given the detector resolution (152 eV at 5894 eV), the energy range over which peaks were observed (676 to 8630 eV) and the variation of resolution with energy (Goldstein et al. 1981), an optimal filter was chosen according to the prescription of Statham (1977). The filter had a central lobe 7 channels wide and side lobes 3.5 channels wide. Regions of interest were set up to record counts in peaks which consistently occurred, corresponding to the elements F, Na, Mg, Si, P, S, Cl, K, Ca, Mn, Fe, Cu and Zn. (To avoid interference from the K K-beta peak in measuring the Ca K-alpha peak, the Ca K-beta peak was used.)

Finally, semiquantitative elemental concentrations were calculated. An algorithm based on the FRAME C program (Myklebust et al. 1979) was used to determine the concentration of the elements Na, Mg, Al, P, S, Cl, K, Ca and Cu. The code uses the ZAF technique and background modelling. As all the assumptions of the program were not met (flat surface, at most 1 element unknown), the results are appropriately described as semiquantitative.

The data was subjected to various statistical treatments, which will be detailed in the Results and Discussion section. The simpler analyses were performed on the IBM PC. The filtered peak and semiquantitative concentration information was further analysed using the SPSS package (Nie et al. 1975) running on an HP3000 computer.

Results and Discussion

Ashing temperature

The effect of ashing temperature on X-ray yield is shown in Figure 1. The net-peak to background ratio of the K K-alpha line is

shown. A monotonic improvement in yield with temperature is observed, with a large step between 300 and 400°C.

As temperature increases, progressively more of the specimen is volatilised and oxidised, with it disappearing completely at 700°C. That ashing may cause irreproducible loss of elements from biological specimens has previously been noted (Goldstein et al. 1981, Turner and Bowden 1983). Our data lends some support to this suggestion, as larger absolute errors are associated with higher temperatures. However, the relative errors from 400-600°C ashing are smaller than those at lower temperatures.

A suitable ashing temperature will balance the gain in sensitivity due to matrix removal against the loss in precision due to the removal of analytic elements. The range 400-600°C fits these criteria: ashing at 400°C was employed in obtaining all the other data reported here.

Electron energy

Figure 2 shows the dependence of X-ray yield on electron energy over the range 15 to 30 keV. Of the four energies tried, 25 keV gave the greatest yield, in terms of the net-peak to background ratio for the K K-alpha line. This result is not explicable simply in terms of the over-voltage ratio increase, which would lead to an increase in yield with accelerating voltage. The decrease in detectability may be attributed to high energy backscattered electrons depositing energy in the Si(Li) X-ray detector. The further data reported in this paper had previously been obtained using 30 keV electrons.

Spectrum matching

Initial examination of the data base was by spectrum matching. The general principles of this technique are described by Russ (1984). In essence, the differences between a "test" spectrum and several "standard" spectra are computed - the smaller the difference, the better the match.

The test and standard spectra were generated as follows. Counting error spectra were generated for each of the original spectra by taking the square root of the counts in each channel. Each original spectrum and its associated error spectrum was then normalised with respect to the electron beam current flowing during collection. This produced the 52 test spectra and the 52 related error spectra. Standard spectra for cotton, hibiscus and laboratory medium were obtained by averaging the relevant test spectra. S(i) and T(i) denote the beam current corrected counts in channel i of the standard and test spectra respectively; s(i) and t(i) are the respective errors.

A number of statistics were used to sum, channel-by-channel, the differences between each of the 52 test spectra and each of the 3 standard diet spectra. The spectrum being tested was assigned to the diet from whose standard spectrum it differed least. The number of successful classifications was then tallied.

As expected, a squared difference statistic, $(S(i)-T(i))^2$, is more efficacious than either a difference, (S(i)-T(i)), or an absolute difference statistic, |S(i)-T(i)|, the classification success rates for these being respectively 67, 38 and 58%. These success rates may be compared with that expected on the basis of random guessing, 33%. The statistic favoured formally, $(S(i)-T(i))^2/(s(i)^2+t(i)^2)$, equivalent to the Mahalanobis D^2, gave a success rate of 71%.

The percentages of the previous paragraph are biased estimates of the success of matching. Each test spectrum contributes a small proportion to one of the standard diet spectra against which it is being matched. To measure this bias a "jackknife" (or "leave-one-out") procedure was followed. The last statistic was computed again with the contribution of the test spectrum under consideration being removed from the relevant standard spectrum. Proceeding this way gave the same success rate as before, 71%. This suggests that no great bias was introduced in using the same spectra to generate and evaluate the standard spectra.

Next, subsets of the data were considered separately. For instance, standard spectra for each diet were generated from the male spectra alone and the male spectra alone were matched with these standards. Classification success for each of the subsets is

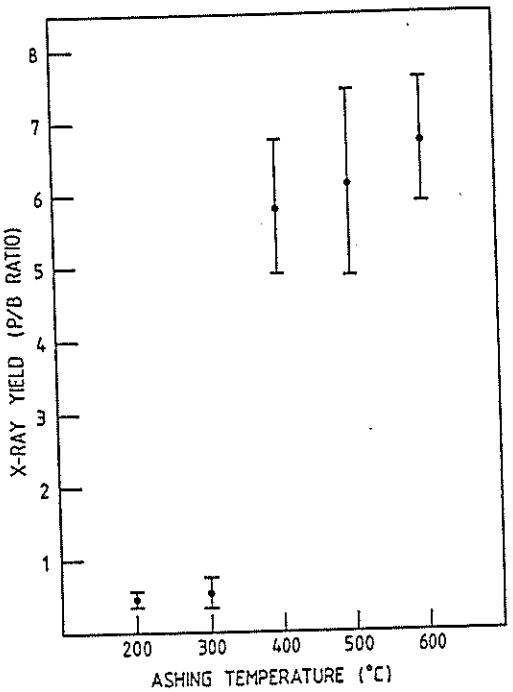

Figure 1. X-ray yield as a function of ashing temperature. The net-peak to background ratio for the K K-alpha line is shown. A bar representing the standard error of the mean is shown on either side of each data point.

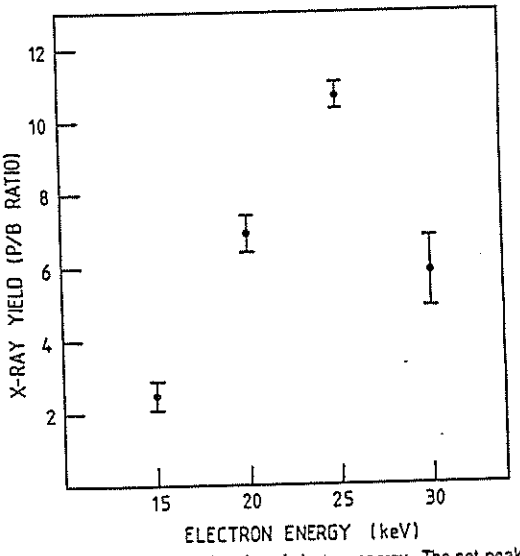

Figure 2. X-ray yield as a function of electron energy. The net-peak to background ratio for the K K-alpha line is shown. A bar representing the standard error of the mean is shown on either side of each data point.

given in Table 1. Several observations may be made concerning this table. Generally, the more homogeneous the subset, the higher the classification success rate. The spectra taken together were classified with 71% success: grouped according to sex and body part the success rate was 65-77%: grouped according to both sex and body part the success rate was 69-100%. The advantage in using subsets has previously been recognised with other moth species (Bowden et al. 1984, Sherlock et al. 1985). The same calculations were performed using a jackknife procedure and exactly the same success rates were found.

It is suggested that specimens of the same sex, or body part, or, preferably, of the same sex and same body part, be used whenever practical to improve the classification of P. scutigera. Although the data is not conclusive on this point (given the small sample sizes of the various sex-body part subsets), it appears that the male-thorax subset is the most homogeneous.

While not the main purpose of this study, it is of some interest to see how well the sex of adult P. scutigera may be distinguished and also how well the body parts (abdomen or thorax) may be distinguished.

This has been attempted using spectrum matching. No subsets were considered: the standard abdomen spectrum, for example, was the average of all abdominal spectra, regardless of sex or diet. A success rate of 50% would follow on the basis of random classification; spectrum matching did not yield much better. Using the statistic $(S(i)-T(i))^2/s(i)^2$, the correct sex of the moths was given in 52% of the cases, and the correct body part in 62%. These figures concern the data set as a whole and using more homogeneous groups (for example, of the same diet) may well increase the chance of correct classification, as it did previously. Moreover, other methods described below are shown to be superior to spectrum matching in classifying diets, and may similarly enjoy a greater success than spectrum matching in classifying moth sex and body part. However that may be, distinguishing sex and body part is not of practical entomological importance as these may be determined directly by inspection.

Element matching

Rather than match spectra channel-by-channel, the faster and more meaningful strategy of matching a small number of regions of interest, or elemental concentrations, may be employed (Russ 1984). Table 2 shows the results of such an approach using both regions of interest from digitally filtered spectra and semiquantitative elemental concentrations. The elements matched were Na, Mg, P, S, Cl, K, Ca and Cu. The statistic $(S(i)-T(i))^2/s(i)^2$ was used.

In one respect the results of Table 2 differ from those discussed under spectrum matching: in Table 2, the jackknife comparisons are not as successful as those that include the test specimen in a standard. This is expected; the earlier results are unusual in showing no difference with jackknifing.

In several respects the results shown in Tables 1 and 2 concur. Breaking the data base down into subsets increases the classification success rate. The success rates using all data formats - original spectra, digitally filtered spectra and semiquantitative concentrations - are similar, with not one of these clearly superior.

Other researchers have favoured our first and third data formats. Calaprice (1971) used a selection of channel-by-channel data as did Kelsall and Burton (1977). These workers used 40 and 80 channels respectively as variables in multivariate analyses. On the other hand, Turner and Bowden (1983), for example, examined a smaller number (10) of elemental concentrations. The results given in Tables 1 and 2 do not especially commend one or other of the data formats on the basis of classification success. The second data format - peaks from digitally filtered spectra - combines simplicity with interpretability. A small number of variables, identifiable with particular chemical elements, is readily derived from the original spectrum. This data format will be the main one used in the rest of the analyses reported here.

Character of the data base

A closer examination of the data base will precede the discussion of further classification strategies. The data base is here taken to comprise 52 cases containing backgound subtracted peaks for 13 elements.

A basic question is whether the data comes from a multivariate normal distribution. If it does, it is unlikely that moths from the 3 diets will be separable. Calculation of the skew and kurtosis for each of the elements across the 52 spectra suggested the data was not normally distributed. A more formal examination was afforded by performing on each element the Kolmogorov-Smirnov test (Hull and Nie 1979); only Fe and Cu appeared to be normally distributed. This leaves open the possibility of three distinct dietary subpopulations, with the elements Fe and Cu expected to be of little help in distinguishing them.

The same tests were run on each of the dietary subpopulations. For the purpose of distinguishing moths on the basis of their diets, it would be most convenient to find each diet subpopulation was multivariate normal, with the means widely separated in multivariate space. This was not the case. While some of the elements appeared to be normally distributed within each diet subpopulation (F, Na, K, Fe and Cu in cotton; Cl, K and Cu in hibiscus; Fe and Cu in laboratory) most of the elements show non-normality. To reduce non-normality due to skew, a logarithmic transformation may be applied (Srivastava and Carter 1983). A logarithmic transform also gives equal weighting to relative, rather than absolute, changes in the elemental data. The data within each diet was transformed according to $\ln(x+\bar{x}/10^4)$, where $\bar{x}$ is the mean of x, the concentration of the element under consideration. The Kolmogorov-Smirnov test was run on the transformed data for each element across each diet. As with the original data, only a few elements appeared to be normal in each diet (F, Na, Si and P in cotton; F, Mg, P and K in hibiscus; K in laboratory). The chief conclusion that can be drawn from these tests is that none of the three diets show marginal normality among most of the variables in it, let alone multivariate normality. A second conclusion is that transforming, by taking logarithms, the data within any diet changes which elements may be normal but does not improve the overall distribution.

Smaller groupings still (defined by sex or body part or both, as well as diet) were not tested for multivariate normality because of their small populations. Whether the elements in these prove to be normally distributed, or whether further factors still are needed to define normal populations, cannot be answered from our data.

The data for each element within each diet was also transformed to Z-scores (Nie et al. 1975). This is accomplished for each datum by subtracting the mean and dividing by the standard deviation. Thus the Z-score data for any element within any diet has a mean of 0 and a standard deviation of 1.

Discriminant analysis

Multivariate discriminant analysis aims to classify a multivariate observation into one of a number of distinct groups. As such, it seems well-suited to the present study and was used in similar circumstances by Calaprice (1971), Kelsall and Burton (1977) and McLean and Bennett (1978). However, certain problems accompany discriminant analysis in the current context. For a start, assumptions of multivariate normality underlie most discriminant programs (although Lachenbruch (1975) discusses methods not constrained by these assumptions). While in practice standard discriminant programs are generally robust to departures from normality (Jackson 1983), their suitability to the present data base must be regarded as tentative. More seriously, the discriminant method assumes that the distinct groups into which it classifies unknowns are comprehensive. However, it is gratuitous to assume that field-collected P. scutigera must have had a cotton or hibiscus larval diet. Field collected specimens may have come from an altogether different, perhaps as yet unknown, host plant. In spite of these reservations concerning its formal basis, discriminant

Table 1. Correct classification of moths according to larval diet using channel-by-channel data grouped in subsets.

Subset	Correct classifications (%)
ALL	71
M	77
F	65
T	77
A	77
MT	100
MA	69
FT	77
FA	92

Table 2. Correct classification (%) of moths according to larval diet using element-by-element matching.

Subset	Background corrected peaks		Semiquantitative concentrations	
		jackknife		jackknife
ALL	83	77	79	77
M	81	73	84	84
F	69	65	81	54
T	81	77	81	65
A	92	84	88	84
MT	84	69	92	77
MA	77	69	84	62
FT	77	62	77	54
FA	92	84	100	69

Table 3. Correct classification (%) of moths according to larval diet using discriminant analysis.

Subset	Background corrected peaks	Semiquantitative concentrations
ALL	67	67
M	81	81
F	88	85
T	92	89
A	92	85
MT	100	100
MA	100	100
FT	100	100
FA	100	100

analysis may be cautiously used as a descriptive tool with which to explore the data base.

Discriminant analysis was undertaken using both the background subtracted peaks and the semiquantitative elemental concentrations, with the aim of distinguishing moths on the basis of larval diet. The successful classifications ensuing are recorded in Table 3. It should be noted that the same data was used both to generate the discriminating functions and to evaluate them, so to this extent the success rates in Table 3 are biased. Table 3 is thus most directly comparable with Table 1 and columns 1 and 3 of Table 2. In comparing results across these tables, the discriminant analysis can be seen to be slightly more effective than the other methods. While discriminant analysis is not so powerful when all the data is considered together (67% success compared with 71-83%), it excels when subsets are considered separately (81-100% success

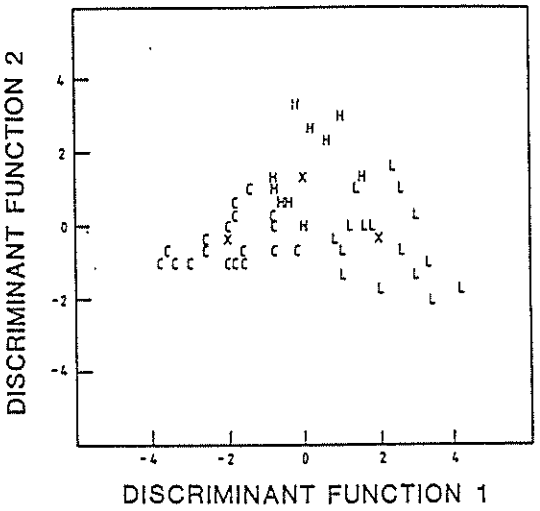

Figure 3. Discriminant analysis scatterplot for Z-score data. "C" stands for cotton, "H" for hibiscus, "L" for laboratory diet; "X" indicates group centroid.

compared with 65-100%). It is of interest to note that the background corrected peaks provide a slightly better discrimination than the semiquantitative concentrations; the extra spectral processing needed to obtain the concentrations again appears unwarranted.

Next, discriminant analysis was performed on the Z-score transformed data bases. Without grouping the data into subsets, greater differentiation was obtained than before: 94%. A plot of the Z transformed data in its chief discriminating plane is shown in Figure 3. Use of Z-scores, in which each element can contribute equally to the analysis, is thus seen to be preferable to using arbitrarily scaled or weight percent data.

Finally, stepwise discriminant analysis was carried out using the Z transformed data. In this approach, variables are added to the discriminating function one at a time, in order of discriminating power. Again, a final success rate of 94% was obtained. The variables used were, in order: P, Cu, K, Mg, Cl, F, Na and Ca. Thus as good results were obtained using 8 elements as 13. The nondiscriminating elements are Si, S, Mn, Zn and Fe. In the previous normality tests these appeared erratically across the specimens. They would seem to aid little in bollworm classification. As far as EDS analysis is concerned, the question of which are the best discriminating elements is of little importance as all elements are detected simultaneously.

Principal components analysis

The aim of PCA is to restate multivariate data in terms of new variables. In the common case in which much of the variance is accounted for by a few principal components, PCA allows the reduction of a many variable problem to a few-variable one. A difference between PCA and discriminant analysis is that the former does not presume the samples to be classified into distinct groups, whereas the latter does. Hence PCA gives a more natural representation of the data. However, PCA operates by minimising the difference between the data and the principal components and, as Jackson (1983) emphasises, this procedure may work against extracting the most important information from the data base.

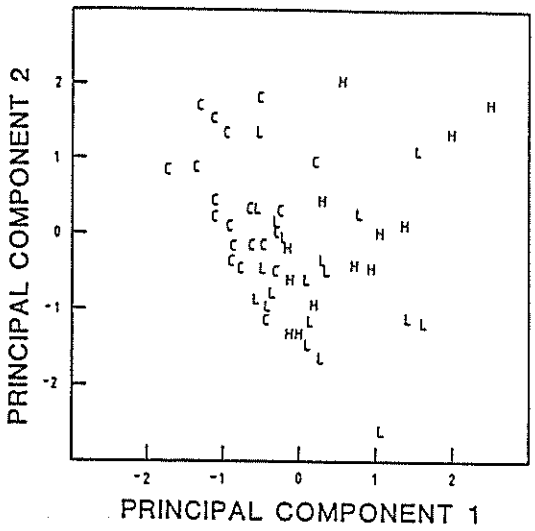

Figure 4. Principal components analysis scatterplot for Z-score data. "C" stands for cotton, "H" for hibiscus, "L" for laboratory diet.

The Z transformed data was subjected to PCA. The data is shown plotted against the first two principal components in Figure 4.

Extension

The results and discussion so far have been concerned with producing a data base and drawing descriptive information from it. The question now addressed is how and with what confidence this data base may be extended to classify field-collected specimens.

At the outset it is recognised that many factors, apart from the larval host plant, may affect elemental concentrations in P. scutigera. Just as the composition of a larva depends on the plant which nourished it so in turn the plant's composition depends on the soil on which it grew. In some cases, at least (Sherlock et al. 1985), the characteristics of the plant dominate differences in soil type, but the reverse may sometimes hold. We are presently investigating the elemental composition of insects reared on the same host plant grown on different soils. It is possible that the differences identified in this paper are merely differences between the soils on which the plants that made up the three diets grew.

Many other factors could conceivably affect elemental concentrations in moths. Bowden et al. (1984) list candidate contributors to the elemental composition of an insect, such as: soil - type, uniformity; host plant - species, variety, genetic makeup, stage of development, part consumed; insect - sex, physiological state, feeding in the adult stage, genetic makeup, disease, parasitism. Certainly the comprehensive data needed to determine the role played by all these factors is not yet at hand.

Conclusions

The major conclusion of this study is that adult P. scutigera may be distinguished on the basis of larval diet. This outcome is not trivial since similar studies have reported a range of results, from sharp distinction in some insects to no distinction in others.

The subsidiary conclusions drawn from this work concern the enhancement of the discrimination procedure. The first step in the procedure is data acquisition. Pretreating the specimen by ashing to remove the organic matrix improves X-ray yields. Too high a temperature leads to loss of analytic elements - ashing at 400-500°C for 1 h is proposed as a suitable compromise. An electron energy of around 25 keV results in greatest X-ray yields. The preferred format for the data is as background corrected peaks, obtained by digitally filtering each of the original spectra. Little spectrum processing is needed to derive this data set, which then comprises a small number of variables identifiable with particular chemical elements.

The second step in the procedure is data analysis. For the larval diets studied, the data is unlikely to be multivariate normal. The use of Z-scores, rather than the original data, and the use of specimens of the same sex and body part, assist in the discriminant analysis.

Acknowledgements

This work was financially supported by the Australian Department of Primary Industry Cotton Research Committee and the Capricornia Institute of Advanced Education Research Committee. Stephen Carter helped with some data reduction and Barry Cochrane assisted in the use of SPSS.

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Discussion with Reviewers

A. Kiss: Criticism can be directed at the study in that the data base was small and limited.
K. Kiss: Do the authors plan to expand this work to obtain a wider data base which uses simpler and more reliable statistics?
J. A. McLean: My major concern is the very low 'n' in each category.
G. M. Roomans: The main problem with the paper is that the database is too small.
Authors: The work reported here is of a preliminary nature, undertaken to discover if x-ray microanalysis can be used to classify field-collected adult P. scutigera. Even with this relatively small sample, the results are quite encouraging, showing a high classification success for laboratory reared moths. Enough data has been gathered to refine specimen preparation and analysis conditions. We are presently using the methods developed in this paper to broaden the data base.

Our sample size of 52 spectra falls within the range reported in other studies - from 22 spectra (Bowden et al. 1979) to 728 (Bowden et al. 1985a).

K. Kiss: Did the authors attempt to use the "two-voltage" technique for quantitative EDX analysis? This yields a more precise and more accurate analysis of specimens with rough surfaces than the commonly used ZAF method. (See Kiss, K: "Quantitative" Electron Probe Analysis of Low-Atomic Number Samples with Irregular Surfaces. Applied Spectroscopy 37, 1, 1983.)
G. M. Roomans: In the case of a sample with a rough surface, a FRAME P based program would have been better than a FRAME C program.
A. J. Morgan: Have you considered ashing your specimens in a low-temperature oxygen plasma, since this may provide for both element retention and high analytical sensitivity?
Authors: We have not attempted any of these suggestions, all of which may well improve classification success.

A. J. Morgan: How large were the moths? Apart from PIXE and SIMS, would it be possible to analyse such specimens by other multielement chemical techniques, or does size preclude this possibility?
Authors: For an adult, the average body length is 7 mm and the width of the thorax about 2.5 mm. Other chemical techniques could be utilised: while the microscopic capabilities of SEM/EDS were one consideration, the speed and the simultaneous acquisition of multielement data were also important.

J. A. McLean: Did females oviposit any eggs? Had all moths voided their meconium? (This is often rich in elements not assimilated into the adult tissue.)
Authors: The females were unmated. As the moths void their meconium immediately after emergence we assume that all had done so before they were frozen.

G. M. Roomans: Since F can be measured in Teflon and related substances, couldn't the tape be the source of the signal?
J. A. McLean: Was an analysis of the adhesive tape carried out? Depending on the brand, this can contain Ti and/or Ca and Fe.
Authors: Prior to this work, spectra had been collected from the tape and no spurious signals were detected.

G. M. Roomans: Are the authors certain that they measure a true bulk specimen, i.e. that they do not excite the substrate? The lower P/B value at 30 kV may be due to the fact that the excitation volume exceeds the sample depth.
Authors: The excitation volume may affect the P/B ratio as suggested. The areas of the specimens analysed were chosen to minimise the possibility of probing the tape.

J. A. McLean: What effort was made to correct for different counting rates in a spectrum?
Authors: In each specimen, a suitably oriented residue was found to ensure a counting rate of around 20%.