Aspects of the Biology, Ecology and Management of the Green Mirid, *Creontiades dilutus* (Stål), in Australian Cotton

By

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Declaration

I hereby declare that the work presented in this thesis is original and has not been submitted, either in whole or in part, for a degree at this or any other university. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is provided.

(Moazzem H Khan)

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Abstract

The green mirid, *Creontiades dilutus* (Stål) (GM) is one of the important early season sucking pests in cotton in Australia. They prefer to feed on the growing parts of the cotton plant, particularly terminals and squares, causing shedding of squares and partial to complete wilting of terminals depending on the severity of feeding. GM feeding early in the season causes delay in maturity. Currently they are controlled mainly by the broad spectrum insecticides applied to control *Helicoverpa* spp., but this disrupts biological control of other major cotton pests. To increase our understanding of the pest, research on biology, behaviour, damage etiology and ecology of the GM was undertaken during the period 1994-97 in cotton growing areas of northern New South Wales.

C. dilutus was studied extensively on cotton plants under glasshouse and field conditions and its life cycle and biology was documented. Effects of temperature on female fecundity and egg and nymphal development were also studied. Both fecundity and development rate were found to be maximum at around 30°C. Threshold temperatures for development of eggs, individual stages of nymphs and the total nymphal period were calculated.

The oviposition pattern of GM on the cotton plants and the distribution of GM eggs in relation to the position of the main stem nodes were studied. GM preferred to deposit their eggs dorsally on the petiole end of 4 to 8 main stem node. Two factors, hardness and hairiness influence GM to discriminate preferred oviposition sites.

Damage etiology of GM on cotton was studied in detail under glasshouse and field conditions. Both nymphs and adults caused similar damage to the cotton plant. The damage caused by GM was cumulative and very apparent as nymphs entered the 4^{th} instar. GM confined their attack to young growing parts of cotton plants, especially squares and terminals, causing squares to shed and terminals to wilt. The shedding of squares depended on feeding site, frequency of feeding and the size or age of the square. The amount of damage increased with the increase of insect number. Their

feeding extended to the vascular bundle region and during feeding the insect released pectinase that destroyed surrounding cells of the feeding point. GM feeding in the field did not cause any significant yield loss in irrigated cotton. However, their feeding at early stages of plant growth caused significant delay in maturity.

The economic injury level for GM for the first time was calculated using maturity delay from field experiments by manipulating field population. An economic threshold (1 GM/m of row) was proposed which is higher than the currently recommended economic threshold and therefore will assist in the successful implementation of IPM programmes.

Extensive studies were made on GM ecology during 1994-97 in two locations. GM was more abundant in early to maximum squaring stage in all seasons in both locations. Analysis of GM population suggested that populations were regulated by contest competition during early cotton season (October - December) and scramble competition during middle and late cotton season (January - March). Egg populations may have been regulated by delayed density dependent factors, possibly predators. Insecticides targeted for *Helicoverpa*, temperature extremities, heavy rainfall along with strong wind, predators, alternative hosts, intraspecific competition and emigration may also have influenced GM populations in the field. Other than these factors, overwintering hosts allowed the build up of populations toward the end of spring, contributing to GM invasion of cotton. Study on the overwintering hosts also suggested that in northern NSW cotton growing regions, GM populations continued throughout the winter, though population growth was reduced.

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INTRODUCTION

Green mirid (GM), *Creontiades dilutus* (Stål) is one of the important sucking pests of cotton in Australia and is widely distributed from NE coastal to SE coastal and from SW coastal to NW coastal regions (Cassis and Gross 1995). Apart from cotton, GM is known to be a pest of lucerne, potatoes, stone fruits, grapes and sunflower (Woodward *et al.* 1970, Hori and Miles 1993, Malipatil and Cassis 1997).

In cotton it is considered to be an early to mid season pest and was first identified as a pest in the early 70s. In Queensland GM are considered to be a primary pest. In NSW, they are abundant in most years but less frequently reach the economic level (Room and Wardhaugh 1977, Adams and Pyke 1982, Miles 1995).

Currently, GM populations in the commercial cotton are suppressed by synthetic insecticides sprayed to control *Helicoverpa* spp. However, since commercialization of transgenic (Bt) cotton (INGARD®) (Monsanto Pty Ltd) in the 1996-97 season, use of synthetic insecticides against *Helicoverpa* has been reduced by 50-60% in INGARD® fields (Fitt 1998). With the general increase of growers adoption of integrated pest management (IPM) strategies in cotton (R. Mensah pers. comm. 1997), use of synthetic insecticide will be reduced further. As a result, GM will be a great threat to both conventional and INGARD® cottons under favourable conditions. The increasing pest status of GM will require a nondisruptive management strategy for the insect to enhance implementation of the IPM programmes within the cotton industry. The development of such strategies will need good understanding of the insect's biology, behaviour and ecology including population dynamics, sources, hosts and movement.

The information to date on the insect is very limited. Since its first identification as a pest, little work has been done. Recently, however, Miles (1995) has done more comprehensive work than previous researchers. Her work concentrated mainly on species identification, consultant perception about the pest (through questionnaire interview) and sampling methods. This means detailed knowledge of

GM biology, damage etiology and ecology is still lacking. Therefore, the objectives of this study were:

1. to develop a suitable technique for laboratory mass rearing of the insect
2. to conduct detailed studies of the insect's life cycle and behaviour
3. to study the damage etiology of the insect in detail on host plant
4. to study ecology of the insect including population dynamics and overwintering hosts of the insect and
5. to identify the factors that influence the insect behaviour and population change.

Thesis Structure

A literature review on the insect is presented in chapter 1 where the pest status of *Creontiades dilutus* (Stål) from the world as well as Australian perspective is described from available literature. Due to the paucity of literature on the insect, the review includes material on related species like *Lygus hesperus* and *Lygus lineolaris*. Chapter 2 deals with general materials and methods used in the experimental work presented in subsequent chapters.

In chapter 3, the biology of the insect including its lifecycle using a rearing method specially developed for the insect (see appendix 1) is covered. The oviposition behaviour of the insect and the factors influencing oviposition are also presented in this chapter, which also addresses the issue of whether dietary requirements, particularly sugar influence lifecycle parameters. Chapter 4 deals with the effect of temperatures on GM fecundity, egg and nymphal development. It also deals with temperature dependent development models of the insect and threshold temperatures.

In chapter 5 the investigation shifts to more applied aspects of GM, such as damage etiology of GM in cotton. This chapter looks at damage caused by different stages of the insect to the different parts of the cotton plants. Preferred feeding sites of the insect and consequences of feeding on the cotton plants are also presented in this chapter, which also deals with the insect damage-density relationship both in the field

and in the glasshouse. Feeding mechanisms and damage symptoms in the context of light microscopy are also described in this chapter.

In chapter 6 the insect's ecology is investigated. Here population dynamics of the insect and underlying factors influencing population changes are addressed. The chapter also deals with the overwintering hosts and the contribution of these hosts to GM invasion of cotton.

The information and insight gained from the previous chapters are discussed in general in chapter 7. The implications of the findings of each study for management and for future research are also discussed. Finally, suggestions are made for further research to increase our understanding of GM as a pest of cotton and to develop a management strategies for the pest compatible with existing IPM programs within the cotton industry.

CHAPTER 1

REVIEW OF THE LITERATURE

The literature on green mirid (GM), *Creontiades dilutus* (Stål), particularly as a pest of cotton, is very limited. Where appropriate, reference will be made to related species, particularly *Lygus hesperus* (Knight), *Lygus lineolaris* (Palisot de Beauvois) and *Pseudatomoscelis seriatus* (Reuter).

1.1 The green mirid, *C. dilutus* Stål - A world and Australian perspective

The genus *Creontiades* Distant is distributed world-wide and includes 54 described species. The subject of this thesis, *C. dilutus*, is an endemic Australian species. The placement of some species within *Creontiades* is questionable, because the limits of the genus and the closely related genus *Megacoelum* Fieber, are not clear cut (Malipatil and Cassis 1997). Earlier workers described the green mirid as *M. modestum* (Room and Wardhaugh 1977, Bishop 1980). Even when I began working on GM there was confusion among the cotton researchers as to whether the New South Wales GM species was *C. dilutus* or *M. modestum.* Later, with the help of Malipatil (pers. comm. 1994) all specimens collected from my study areas were identified as *C. dilutus* even if they came from different hosts including cotton, lucerne, sunflower or native weeds. Also Malipatil and Cassis (1997) in their recent work synonymised *M. modestum* with *C. dilutus*. In this thesis, any reference to *M. modestum* will be mentioned as *C. dilutus* (= *M. modestum*).

The species *C. dilutus,* as a pest of cotton, is only reported from Australia. However, a related species, the shedder bug, *Creontiades pallidus* (Rambur), was reported as a serious pest of cotton in the Tokar Delta and in Zeidab of Sudan. In 1950/51 the shedder bug was responsible for about 20-30 percent of the shedding of buds and young bolls (Ripper and George 1965). *C. pallidus* was also reported as a pest on cotton along the Euphrates river in Syria. At a mean population level of seven specimens per 50 sweeps, from early July till mid August, the shedder bug caused

50.4% yield loss (Stam 1987). Ewing (1929) found *C. debilis* as a pest of cotton in North America, but this is the only report so far been published about that species. Apart from *Creontiades* other mirids like *Lygus hesperus* in the San Joaquin Valley of California, the tarnished plant bug, *L. lineolaris* in the Delta areas of Mississippi and Arkansas and the cotton fleahopper, *P. seriatus* in central and southern Texas are considered serious pests of cotton (Falcon *et al.* 1971, Leigh *et al.* 1988, Scales and Furr 1968, Hanny *et al.* 1977, Lindell *et al.* 1986). On the other hand *L. vosseleri* was reported as a pest of cotton in East Africa (McKinlay and Geering 1957). Extensive work has been done by several workers on *Lygus* in North America.

In Australia *C. dilutus* is widely distributed in all states and territories. It has also been recorded from the Torres Strait Islands (Cassis and Gross 1995). The GM is known to be a pest of cotton, lucerne, potatoes, stone fruit, grapes, sunflower, safflower and beans (Woodward *et al.* 1970, Hori and Miles 1993, Malipatil and Cassis 1997).

In Australian cotton GM was identified as a pest in the early 1970's, soon after the establishment of large scale cotton growing (Room and Wardhaugh 1977, Room 1979 and Bishop 1980). Bishop (1980) first experimentally showed that GM damage to the cotton reduced the number of squares. However, little work has been done on GM, partly because of the difficulty of working with the insect and partly because populations in cotton are usually suppressed by broad-spectrum insecticides applied against other pests. Earlier workers like Room and Wardhaugh (1977), Bishop (1980), Adams and Pyke (1982), Foley and Pyke (1985), Chinajariywong *et al.*(1988) and Miles (1995) have discussed the GM problem in the Australian cotton industry.

1.2 Developmental biology of mirids

1.2.1 Life cycle features of mirids

The GM usually passes through five nymphal stages to become an adult (Chapter 3). However, Foley and Pyke (1985) found that out of 170 first instar

nymphs of GM, approximately 8% took 4 and 3% took 6 nymphal instars to complete development. The North American cotton mirids, *L. hesperus, L lineolaris* and *P. seriatus* pass through an egg and five nymphal stages before becoming an adult (Leigh 1963, Ridgway and Gyrisco 1960, Gaylor and Sterling 1975). Other mirids like *Eurystylus immaculatus* (Odhiambo), *Creontiades pallidus* (Rambur), *Calocoris angustatus* (Lethierry) and *Helopeltis corbisieri* (Schmitz) also take five nymphal instars to complete their development (Ratnadass *et al.* 1994, Sharma and Lopez 1990, Hiremath and Viraktamath 1992, Kumar and Ansari 1974). By the fifth instar female nymphs of GM can be distinguished from male nymphs by the presence of median cleft along the mid ventral line on the last abdominal segment (Chapter 3).

The reproductive biology of GM has not been studied, but that of *L. hesperus* has been studied in detail by Strong *et al.* (1970). They noticed that neither males nor females normally mated until they are about 5 days old. This phenomenon is correlated with maturation of the reproductive systems. Usually the sexually mature male has a pair of large testes, seminal vesicle and semen producing accessory glands. The 5th instar males possess fully developed testes, sperm and numerous sperm packets. However, they lack accessory glands and seminal vesicles. The newly moulted females contain completely undeveloped ovaries. Ovaries of one day old females are not fully developed and lack oocytes. Perhaps due to this phenomenon the pre-oviposition period for mirids is quite long. The pre-oviposition period for *C. pallidus* is 2-5 days (Sharma and Lopez 1990). Leigh (1963) found that pre-oviposition period of *L. hesperus* is 6-12 days under laboratory conditions. On the other hand *L. lineolaris* take 7-8 days to start laying after emergence (Ridgway and Gyrisco 1960a). The pre-oviposition period also varies with temperature. Gaylor and Sterling (1975) studied the effect of temperature on cotton fleahopper, *P. seriatus* and found that the pre-oviposition period decreases with the increase of temperature.

Green mirids are polymorphic and exhibit considerable variation in colour markings even within a population from the same habitat; this variation is marked for the hemelytra and hind femur (Miles 1995 and Malipatil and Cassis1997). Hemelytra may be evenly pale green to dark green and often have brown fascia on the calvus. Hind femur may be almost uniformly green with the distal half slightly darker to dark

green (Miles 1995). This sort of variation is also found in *C. pacificus* and in apple dimpling bug, *Campylomma liebknechti* (Malipatil 1992).

The GM is found throughout the year except in temperate regions of Australia where it is found only during summer months (September- May) (G. Fitt, P. Gregg, and R. Mensah pers. comm. 1997). However, it is not confirmed yet whether they undergo diapause during winter months. Miles (1995) found reproductively active females throughout winter. According to Beards and Strong (1966) in the Davis area of California, *L. hesperus* passes early winter (November) as diapausing adults, midwinter (December-February) as ovipositing adults, and late winter (February-March) as eggs. However *C. verbasci* overwinter as eggs (Womack and Schuster 1987, Smith and Borden 1991).

1.2.2 Biology of immature stages

Egg

Southwood (1956), Sanford (1964) and Hartley (1965) studied heteropteran eggs in detail. Southwood described the structure of the eggs of Pentatomorpha and Cimicomorpha. Although the eggs are similar, the shape, size, colour of the operculum, position on the branch are different and these characters may be used to distinguish one mirid from another (Sanford 1964). The eggs of GM are described by Miles (1995). They are banana shaped, slightly curved and water coloured, tapering towards the posterior end and narrowing to a neck below the operculum. The operculum is elliptical and slightly depressed on the centre and creamy white in colour (Miles 1995). One end of the operculum of a GM egg is slightly raised and forms the respiratory horn, through which gaseous exchange occurs (Southwood 1956, Hinton 1981). The respiratory horn is formed by a grouping of aeropyles, which open terminally and create a pattern of holes (Hinton 1981). The chorion, the part of the egg lying outside the oocyte cell membrane (Southwood 1956), of GM is smooth, opaque and dull white (Miles 1995).

Insect eggs change in volume and weight during development, and such changes are generally associated with the absorption of water (Banks 1949 and Constant *et al.* 1994). The egg of GM is usually embedded in plant tissue so that only the operculum and end of the rim are exposed to the outer environment. For such an egg, according to Hartley (1965), there are two possible sources of water, either free atmospheric water in contact with the exposed tip of the egg or water within the tissues of the plant.

Nymph

The rate or manner in which, an insect develops or grows may depend upon a number of factors, including temperature, the type and amount of food and the amount of moisture. Mirid development is temperature dependent. Several studies have examined this (Ridgway and Gyrisco 1960b, Beards and Strong 1966, Champlain and Butler 1967, Butler and Wardecker 1971, Dunbar and Bacon,1972, Gaylor and Sterling 1975, Khattat and Stewart 1977, Sevacherian *et al.* 1977, Alvarado-Rodriquez *et al.* 1987, Fleischer and Gaylor 1988). Development of *L. lineolaris* from hatched first instar (<24h old) to adult is a linear function of temperature between 18° and 30°C and most of the nymphal mortality occurs in the first two instars (Fleischer and Gaylor 1988). However, for *L. hesperus*, irrespective of instars, mortality occurs frequently during moulting (Alvarado-Rodriquez *et al.* 1987). Like *L. lineolaris*, development of nymphal stages of *L. hesperus* also showed linear relationship with temperature, the optimal within range (Champlain and Butler 1967). Temperature also has a pronounced influence on cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter). Survival is high during the first few days of nymphal life in the optimal temperature range. However, early nymphal mortality increases greatly as the temperature deviates from the optimal temperature range (Gaylor and Sterling 1975). Dunbar and Bacon (1972) studied the development of certain predaceous heteroptera. They found that most nymphs did not complete their development at temperatures above an upper threshold temperature and if some individuals did complete their development in such temperatures they required a considerably longer time.

Suitability of food is an important factor for insect development. Khattat and Stewart (1977) examined the development of *L. lineolaris* in relation to different plant foods. They found that nymphal survival varied with the suitability of food and first instars suffered greater mortality than any of the later instars, regardless of food source. They also found cannibalism among the test insects in the cages containing unsuitable food. However, the cause of cannibalism has been questioned by other workers. Solomon (1949) pointed out that cannibalism is density dependent. Beards and Leigh (1960) explained that cannibalism is a result of injury to individuals during handling making them more susceptible.

Sugars are important in the nutrition of many herbivorous insects, although they are not known to be essential nutrients (House 1965, Harvey 1974, van der Meijden *et al.* 1989). If an insect obtains adequate energy yielding substrates (such as sugar) from its food, it will direct most dietary protein into growth (Shaw *et al.* 1978). On the other hand, if energy substrates are deficient, and compensation is not possible, the insect will have to metabolise proteins for its energy content and will grow less. This theory implies that (1) higher levels of sugar should promote insect growth, survival and fecundity, and (2) positive responses to increased sugar should be more pronounced for food with low levels of protein (Clancy 1992). Butler (1968) studied the effect of sugar for the survival of *L. hesperus* on alfalfa. Alfalfa flower is a common source of sugar for lygus bugs in the field. Other sources include honeydew producing insects like aphids. After the alfalfa plant flowers, lygus bugs use the nectar secreted by the flowers nectaries. Butler (1968) found that when nymphs were caged on flowering alfalfa plants, 72% survived and only 5% survived of those caged on parts of the same plants without flowers. Hori and Miles (1993) also reported similar results for *C. dilutus*. They found that 65% of test insects completed their development on lucerne flower heads, none of the test insects completed development on foliage alone and only 25% completed development on lucerne green pods. Benedict *et al.* (1981) observed significantly lower survival of *Lygus* nymphs on nectariless cotton than on its nectaried counterpart.

Mellanby (1932), Gunn (1942), Broadbent and Hollings (1951) and Farnworth (1972) examined the effects of heat and humidity in combination on the vital limits of

insects. Mellanby (1932), after investigating four insects (*Xenopsylla cheopis* Roths., *Pediculus humanus corporis* de G., *Lucilia sericata* Mg. and *Tenebrio molitor* L.) concluded that above the critical temperature, the insects die from the effects of heat. Below this temperature they live several days at least, in moist air, and death occurs from starvation. In dry air, desiccation kills the susceptible insects at temperatures below the critical temperature. Fye (1982) evaluated the role of moisture during the winter and fall host plants on the winter survival of *L. hesperus* and *L. elisus* in eastern Washington. He found that moisture was more critical during exceptionally dry winters than during normal winters, and no lygus bugs survived the winter when no moisture was added to the overwintering site material. Survival doubled with each increment as the moisture in hibernation site material was increased. However, excessive moisture in winters with sustained precipitation had negative effects on survival.

1.2.3 Mating and oviposition behaviour

Literature on mating behaviour of mirids is very scarce. One reason for this may be the perception of the insects as minor pests, which usually get less attention from the cotton industry as well as from researchers. Another reason may be the difficulty of studying mating in this insect. In three years of working with GM I have never seen them mating either in the glasshouse or in the field. They may be nocturnal in mating, or it may be very brief, or the presence of the observer may inhibit it. However, a study on mating behaviour of *Lygus hesperus* was done by Strong *et al.* (1970). They reported that when males and females of the appropriate age (5-6 days) were placed together they were very active for 10-20 seconds, moving about haphazardly. Soon, however, they became quiet and remained motionless for a few seconds. Each partner then initiated a slow walk, and if the male touched the female with both antennae, an aggressive behavioural pattern immediately followed. This was characterised by a vertical jerking and quivering motion of his abdomen. If the male was on the female's right side he would move closer, bend his abdomen under the right side of the female's and attempt copulation. The male always directed the tip of his abdomen toward the right hand side of the base of the female's

ovipositor. If the female was receptive, copulation occurred. If not, she displayed an evasive behaviour and eluded him. The male would then often repeat his aggressive courtship and occasionally the female would accept him. They found that after an average 2 m 43 s, the test pair underwent copulation, which lasted an average of 2 m 23 s. The males would mate on consecutive days and could frequently be induced to mate three to four times. Females, however, would never mate on consecutive days.

Malipatil (1979) observed the mating behaviour of some lygaeid bugs. The male appears to recognize the female by vibrating and tapping with the antennae. He then attempts to climb over her from the back, in the meantime twisting and reversing the pygophore so that the parameres clasp her ovipositor. If the female is not receptive, the male leaves her after a few such attempts. Once they go into the usual end to end copulation they remain stationary, gently vibrating their antennae and feeding normally. When disturbed, the copulating pair tries to run away for shelter, often in the direction of the female, which drags the male after her.

Another aspect of mating is mediation by sex pheromone. Smith *et al.* (1991) first successfully identified a sex pheromone in the family Miridae in mullein bug, *Campylomma verbasci* (Meyer). They reported that the female mullein bugs emit a sex pheromone composed of 94% butyl butyrate and 6% (E)-crotyl butyrate. Apart from *C. verbasci* sex pheromone secretion in other mirid species has not been identified. However, there are several reports that female mirids emit sex pheromones which can attract males for mating (Scales 1968, Biovin and Stewart 1982, Smith *et al.* 1991 and 1994, Millar *et al.* 1997).

Quite a large number of studies have been done on mirid oviposition behaviour (Curtis and McCoy 1964, Sanford 1964, Stewart 1968, Mueller and Stern 1973, Tingey *et al.* 1973, Benedict *et al.* 1981a, Alvarado-Rodriquez *et al.* 1986). This is because insect oviposition behaviour is central to investigations of population dynamics, life-history evolution, plant-insect and host-parasitoid interactions, and biological control of insect pests (Minkenberg *et al.* 1992).

Knowledge of the preferred oviposition site is essential in insect pest management programmes where sampling the egg population is necessary for establishing the damage potential of a pest. Ovipositional site preference is also important in determining the way a plant may be resistant to a particular insect (Benedict *et al.* 1981a). Several factors influence the oviposition sites of the insects and there are different categories of insects according to oviposition sites (Southwood 1956, Benedict *et al.* 1981b, Rausher 1983, Alvarado-Rodriquez *et al.* 1986, van der Meijden *et al.* 1989, Hassan *et al.* 1990, Minkenberg et al. 1992, Messina and Dickinson 1993). Southwood (1956) grouped heteropteran oviposition sites into four categories based on their relationship to the environment:

(1) Exposed, e.g. on leaves of plants, as in Pentatomidae, Coreidae, Piesmidae, Neididae, some Lygaeidae, some Reduviidae.

(2) Semi-exposed, e.g. in the soil, under rocks, amongst fallen leaves, in the axis of stems, as in some Cydnidae, most Lygaeidae, Pyrrhocoridae, most Reduviidae, some Miridae.

(3) Embedded in plant tissue that is either dead or becomes so before the eggs hatch, as in Nabidae and some Miridae.

(4) Embedded in or intimately associated with living plant tissue, as in Tingidae, Anthocoridae and some Miridae.

In studying the oviposition behaviour of *L. hesperus, L. elisus,* and *L. lineolaris,* previous researchers indicate that differences in behaviour vary with species and host plant. Benedict *et al.* (1981a) found that 64-84% of all eggs of *L. hesperus* were deposited on the leaf petioles of cotton, and particularly in the leaf pulvinus. They also found that the mirid preferred to oviposit in the top third of cotton plants. Mueller and Stern (1973) reported that on safflower plants with buds and flowers about 33% of the *L. hesperus* eggs and 21% of the *L. elisus* eggs were found in the fruiting area of the plants, but on prebud stage plants, the majority of eggs of both species were found on the lower half of the plant. These bugs laid 90% of their eggs in the pedicels on blooming carrots (Carlson 1956). However, Alvarado-Rodriquez *et al.* (1986) in working with three age classes of common beans indicated that *L. hesperus* oviposition preference was not significantly influenced by the plant age, though they found 60% more eggs on the 50-day old plants (peak flower) than on

plants of the other classes. Latson *et al.* (1977) reported oviposition by *L. lineolaris* into the floral bud apex and into floral filaments of horseweed (*Erigeron canadensis* L.). Greenhouse studies on squaring cotton showed *L. lineolaris* were most abundant in the enlarged area of the leaf petiole at the base of the 4th, 5th, and 6th nodes above the soil (Bariola 1969). Sanford (1964) examined the oviposition sites of thirteen predaceous mirids on apple trees. He found that although the eggs were similar, there were differences in the position on the branch, age of wood, type of bark and numbers of eggs in one position. Stewart (1968) reported that the European lygus, *Lygus rugulipennis* Poppius embedded their eggs in the main stem of oat plants near the ground.

Plant tissue condition (tenderness) and developmental stage of the plant have been mentioned as factors that may influence ovipositional behaviour of lygus bugs (Sorensen 1939, Elmore 1955, Carlson 1956). New plant growth (characterised by tender tissues) as well as plants in the bloom stage are preferentially selected over other developmental stages by lygus bugs for oviposition (Alvarado-Rodriquez *et al.* 1986). GM also follow a definite pattern of oviposition in cotton plants either in glasshouse or in field (Chapter 3).

The number of oocytes available for oviposition influences many aspects of oviposition behaviour (Iwasa *et al.* 1984, Parker and Courtney 1984, Mangel 1987). However, Prokopy (1981) reported that ovipositing females of many phytophagous insects recognize and avoid hosts already bearing conspecific eggs. Host plant density also influences oviposition behaviour. Rausher (1983) working with *Battus philenor* butterflies noticed that oviposition rate increases as plant density increases. However, the relationship between oviposition rate and plant density is not linear. Numerous allelochemicals, present in the foliage of most plants, and which are often absent from flowers and fruits, also influence oviposition behaviour of the insects (Fitt 1991)

1.3 Temperature and mirid development

The environments of different insects vary to an enormous extent, but there is a group of environmental factors from which there is no escape for any terrestrial

insect, though their impact can be modified by behavioural patterns, especially microhabitat selection. These are the temperature, humidity and other physical conditions of the habitat. Any studies on a living insect must begin with investigations of its climatic environment (Uvarov 1931). Heat input and temperature extremes play a dominant role in determining the rate of increase of insect development. Therefore, knowledge of the effects of temperature on the development of the eggs and nymphs is important to an understanding of the fluctuations in population size (Butler and Wardecker 1971). The body temperature of most insects varies more or less directly with environmental temperature. An increase in temperature, within a favourable range, will speed up metabolism and consequently increase rate of development. Each species and each stage in the life history may develop at its own rate in relation to temperature. Laudien (1973) identified four different types of temperature range in respect of insect growth and development:

(a) Lower limiting temperature: below which an insect may survive, but cannot grow or develop;

(b) Central temperature range: temperatures above the developmental null point increase the rate of development up to an optimum;

(c) Optimum temperature: the highest rates of development are found at temperatures that are relatively normal;

(d) Supraoptimal temperatures: the zone between the optimum and the upper limiting temperature in which a relatively gradual decrease in the rate of development can be observed.

Leather (1994) pointed out that as far as reproductive and developmental processes of insects in relation to temperature are concerned, there is a lower threshold below which reproduction/development does not occur and an upper threshold beyond which reproduction/development ceases. Growth and fecundity usually increases linearly between these two thresholds up to an optimal point. However, Andrewartha and Birch (1954) pointed out that some development may occur at temperatures considerably below developmental thresholds.

Literature on the effect of temperature on insect development runs into tens of thousands of papers (Gregg 1981). However, for GM, only one paper by Foley and

Pyke (1985) has so far been published on this aspect. They estimated 280 day-degrees for total development of GM from a linear regression equation. A linear regression equation provided a good approximation of a certain part of the development curve but it did not explain the whole of it. In particular, the number of day-degrees required for development was underestimated near the lower threshold and overestimated near the optimum temperature. This problem occurs in many other insects, and is often dealt with by fitting a non-linear model such as the logistic (Hagstrum and Milliken 1988, Allsopp *et al.* 1990). Both logistic and day-degree models to describe GM development are provided in Chapter 4 of this thesis.

On the other hand many observations have been made on temperature effect on North American mirids (Ridgway and Gyrisco 1960b, Champlain and Butler 1967, Butler and Wardecker 1971, Mueller and Stern 1973, Gaylor and Sterling 1975). Champlain and Butler (1967) reported that 10°C is below the developmental threshold for eggs of *L. hesperus* and that 40°C is above the limit at which hatching can occur. Duration of the nymphal stage was reduced with an increase in temperature. Eggs of *L. lineolaris* hatched more quickly at higher temperatures (Ridgway and Gyrisco 1960b). The survival of cotton fleahopper was high during the first few days of nymphal life in the medium temperature range, and mortality increased greatly as the temperatures deviated from this temperature range (Gaylor and Sterling 1975). Temperature had no significant effect on the pre-oviposition or oviposition period or the number of eggs laid by each female. Almost all the above published data show developmental times of mirids reared under different temperatures, but a regression equations has only been fitted for two of these studies (Champlain and Butler 1967 and Gaylor and Sterling 1975). Such regression equations, linear or nonlinear, can provide a simple means of calculating developmental times in insect population models, or can be used for quantitative comparisons of the effect of temperature on the developmental times of different stages and species of insects (Hagstrum and Milliken 1988).

Kehat and Wyndham (1972a) examined the effect of temperature on an Australian heteropteran, the Rutherglen bug, *Nysius vinitor*. They found that the threshold temperatures for eggs and nymphs were 14.5°C and 15°C respectively and

40°C was lethal to both. They calculated 225 day-degrees were required from nymph to complete development. From their findings they suggested that warm springs followed by hot summers would favour a quick build-up of *N. vinitor* populations. Both *Helicoverpa armigera* and *H. punctigera*, major pests of Australian cotton, responded to temperature in very similar ways with maximum development rates of all stages occurring close to 35°C (Twine 1978, Kay 1981).

1.4 Pest status and damage etiology

Conway (1976) defined a pest as a living organism which causes damage. However, Turnbull and Chant (1961) pointed out that the concept "pest" is applied to a great variety of situations that may differ from each other ecologically. Southwood (1977), from the ecological point of view, divided pests into two categories: r-strategists and K-strategists. r-strategists are opportunists, selected for obtaining maximum food intake by the exploitation of their ephemeral habitats. K-strategists have been selected for harvesting food efficiently in a crowded environment. Their populations remain near the carrying capacity of their habitats. r-strategists tend to be small, mobile and with a short generation time; K-strategists are larger, with less migratory tendency and a long generation time (Southwood 1976). The importance of any pest is related to the value of the damage it causes.

Damage may be quantifiable, though qualitative judgements are often necessary. Thus, lower grades of produce or simply a delay in harvesting can have a considerable impact on the price paid (Matthews 1984). Damage can be direct or indirect. Direct damage is where losses are incurred when pests completely destroy plants or damage those parts of plants which are harvested. Indirect damage is where damage occurs to parts of plants other than the product, that is, damage is caused to leaves and other parts which reduces the ability of the plant to photosynthesize and transfer the products to the fruit, tubers or other harvested part.

GM cause both direct and indirect damage (Chapter 5). However, GM as a pest has been recognized only recently. Wright and Nikitin (1964) and Wright (1970) did not include GM in their lists of insects damaging to cotton in New South Wales.

Sterling (1976) did not include GM as a pest in Queensland either, when he developed a sequential sampling plan for cotton pest management in southeast Queensland. In studying seasonal occurrence of insects other than *Heliothis* spp. feeding on cotton in the Namoi valley of New South Wales, Room and Wardhaugh (1977) reported the presence of mirids in cotton field, but noted that they were in extremely low density and attributed no damage to them. Wilson and Greenup (1977) observed necrotic areas in some mature bolls, however, they could not pinpoint the causal agents. They thought the necrosis was the result of stings by an array of Heteroptera. Bishop (1980) first assessed the ability of GM, identified as *Megacoelum modestum*, to damage cotton in Queensland. von Mengersen (1982) reported field infestations of GM in the Namoi valley which caused considerable early season damage. Bishop (1980), Adams and Pyke (1982) and Adams *et al.* (1984) have done some experimentation to establish the pest status of sap-sucking bugs, in particular of GM. They found that among the sapsuckers, GM is the most damaging. GM was included in the 'Cotton Pesticides Guide' for the first time in the 1983-1984 season (Colton and Cutting 1983) and also incorporated into the cotton pest management program, SIRATAC (Ives and Hearn 1987). Miles (1995) has done more comprehensive work on GM than previous workers, and through questionnaire interview with cotton consultants in Australia concluded GM was considered by consultants and growers as a primary pest of cotton in Queensland.

1.4.1 Effect of mirid feeding

A few studies on GM feeding on cotton plants have been done since its recognition as a pest of cotton in Australia. Bishop (1980) reported that *C. dilutus (= M. modestum)* significantly reduced numbers of squares of all sizes, in particular squares less than 10mm. In 1982, von Mengersen observed that GM feeding caused poor fruit set in some fields of northern New South Wales. He also observed that young squares and small bolls turned brown inside at the point of damage and that tiny dark brown spot marks occurred at the point of entry. Adams and Pyke (1982), Adams *et al.* (1984) and Chinajariywong *et al.* (1988) made some sporadic observations on sap-sucking bugs. Their observations revealed that:

- the apple dimpling bug and the green mirid can cause tip damage to pre-squaring plants;
- they also can cause squares to blacken and shed;
- feeding at the early squaring stage can cause delay in maturity;
- the period of susceptibility is from the four true leaf stage to early flowering.

Later, Hori and Miles (1993) made a comprehensive study on GM feeding, but in lucerne (*Medicago sativa*), rather than cotton. They found that the GM targets the flower heads and seed pods in lucerne. Individual GM, by feeding frequently and causing loss of many whole flowers and their developing ovules, have the potential to cause heavy losses in seed crops, although the effect may not be readily distinguishable from normal flower drop. Unlike *C. dilutus*, the shedder bug, *C. pallidus*, prefers older rather than younger buds in cotton as the developing anthers are the most favoured source of food. The damaged anthers become shrunken and yellow to dark brown in colour (Ripper and George 1965, Stam 1987).

In contrast, *Lygus* spp. have received much attention from North American researchers. Extensive research has been conducted on the feeding effect of *L. hesperus* and *L. lineolaris* on cotton plants, including effects on plant anatomy (Ewing 1929, Flemion 1956, Scales and Furr 1968, Falcon *et al.* 1971, Tingey et al. 1975, Pack and Tugwell 1976, Tugwell *et al.* 1976, Hanny *et al.* 1977, Gutierrez *et al.* 1977, Tingey and Pillemer 1977, Mauney and Henneberry 1979, Williams *et al.* 1987, Leigh *et al.* 1988). Five generalized types of plant damage have been reported from feeding by lygus bugs: localized wilting and tissue necrosis, abscission of fruiting forms, morphological deformation of fruit and seed, altered vegetative growth and tissue malformation. Although these symptoms appear to be of diverse origin, they can all be related directly to the specific feeding processes of lygus bugs, the plant response to feeding injury and the specific feeding site (Tingey and Pillemer 1977). Hanny *et al.* (1977) listed more effects due to *L. lineolaris* feeding including swollen nodes, shortened internodes, deformed leaves, excessive branching of the main stem and delayed fruiting. Pack and Tugwell (1976) studied the effect of Clouded Plant Bug, *Neurocolpus nubilus* (Say) and Tarnished Plant Bug, *L. lineolaris* on cotton bolls of different ages. They found that feeding by these mirids made a dull, dark spot about 1 to 2 mm in diameter on the immature boll. They often observed a pinhead size

glossy black spot near the center of the larger dull spot where the stylets were inserted during feeding. Bolls with internal damage became smaller in size and were malformed to various degrees and, characteristically lint, was stained a tan colour near the point of injury.

Ewing (1929) noticed that observed feeding did not always result in apparent external injury but in most instances where external evidence of injury was not apparent, it was found by microscopic examination of sectioned material in the vicinity of the puncture that internal injury had occurred. Pack and Tugwell (1976) and Mauney and Henneberry (1979) examined histologically the damage on the squares. They found that the anthers and, more specifically the pollen grains, were the principal feeding sites and there were large areas of necrosis on the staminal column of the damaged anthers, the cells comprising staminal column being broken up. Normally, the pollen sacs are filled with maturing pollen grains, but due to mirid feeding the grains lost their normal shape and were grouped in the center of the pollen sacs. If the flower subsequently bloomed and set a boll, the level of anther damage increased the chance of the boll being malformed and shed.

The length of time the mirids feed has very little effect upon the extent of the injury. Both nymphs and adults cause identical damage to the cotton plant (Ewing 1929, Elmore 1955, Butts and Lamb 1990).

1.4.2 Mirid feeding and abscission of fruiting bodies

Guinn (1982) listed thirteen causes of square and boll abscission in cotton. Broadly they can be divided into two groups: (1) internal, physiological stresses due to shortages or excesses of moisture, light, or nutrients and (2) external, due to attack by insects or microorganisms. Mauney and Henneberry (1979) distinguished these two types of abscission by observing the visual symptoms. Morgan (1984) suggested that the abscission of cotton fruiting bodies is stimulated by biotic and abiotic factors and mediated by the phytohormones ethylene and indole acetic acid (IAA). Ethylene accelerates abscission and auxin retards abscission (Morgan 1969, Lipe and Morgan

1973). Ethylene production can be enhanced by either increases or decreases in IAA concentration (Morgan and Hall 1964). Thus, factors that either increase ethylene production directly or alter levels of IAA will induce a phytohormone imbalance that promotes abscission (Burden *et al.* 1989). Cotton plants themselves contain IAA, but not in sufficient amounts to induce abscission (Burden *et al.* 1989). Degradation of plant tissues by pectinases released by mirids during feeding may cause induction of ethylene synthesis to sufficient levels to cause abscission (Martin *et al.* 1988, Burden *et al.* 1989).

Embryo, endosperm and ovaries produce auxins which are responsible for fruit set or more technically, the prevention of abscission (Audus 1963). From this observation Strong (1970) inferred that when *Lygus* feed on a developing bud or a recently pollinated flower, the stylets will penetrate sufficiently deeply to reach (and enzymatically destroy) the auxin producing portions of the organ. In the case of a bud, these areas are the ovules and the pollen grains on the anthers. In a young fruit it is the embryo and the ovary wall which produce the auxins. If a sufficient amount of auxin-producing tissue is destroyed, the levels of the plant hormones directly associated with the formation of the abscission layer rise, and the organ soon abscises.

However, the likelihood of abscission depends on the size of the bud. Leigh *et al.* (1988) working with *L. hesperus* concluded that abscission and damage decreased with square age. Small squares and newly formed bolls were most susceptible to abscission (Stewart and Sterling 1989b and Chapter 5). In cotton buds longer than 8mm, the stylets cannot penetrate to sufficient depth to destroy or even injure the ovules, and only those pollen grains in the outer periphery of the bud are destroyed. It would appear then that such buds still had sufficient auxin producing sites to prevent the induction of abscission (Strong 1970).

1.5 Ecology of mirids

1.5.1 Polyphagy and host plant selection

The green mirid, like *Lygus* spp., is polyphagous. They forage on a wide variety of host plants, crop and noncrop, and many hosts are ephemeral (Chapter 6).

Polyphagy can be defined as the condition when a herbivore feeds on more than one plant species (Aiyer 1949, Dethier 1954, Cates 1981), or feeds on a large number of plants from different families (Fitt 1989, Bernays and Chapman 1994). The greater the number of plants the herbivore feeds on, the more polyphagous they are considered to be. Defining polyphagy in respect of the number of plant hosts overlooks other aspects of the herbivores life process, because the plant species and individuals can differ in quality or suitability for insect growth, survival, and reproduction (Dolinger *et al.* 1973, Scriber and Feeny 1979). However using taxonomic relationships, as Fitt (1989) and Bernays and Chapman (1994) did, focuses on some behavioural aspects of the insect. An insect feeding only on 10 plants from the same family is almost certainly using some characteristic of the plants that they have in common in determining their acceptability, while an insect feeding on 10 plants from different families probably employs a number of different cues. Polyphagous species may consist of generalist individuals, each with the capacity to accept and utilize a range of hostplants, or a collection of more specialized individuals forming host races which utilize a narrower spectrum of hosts (Fitt 1991). Andow (1991) divided polyphagous herbivores into two types: sequential polyphages that alternate hosts between generations in a temporal sequence and simultaneous polyphages that alternate hosts within a generation, with individuals moving from host to host. Fitt (1989) outlined the importance of polyphagy to the population dynamics and pest status of *Heliothis* spp., drawing conclusions which are also true for GM. They are: populations may develop simultaneously on a number of hosts within a region, populations may develop continuously during suitable periods by exploiting a succession of different cultivated and uncultivated hosts through the season and populations can persist at low density in seemingly unsuitable areas.

1.5.2 Alternate hosts and mirid movement

Kitching and Zalucki (1983) and Zalucki *et al.* (1986) defined hosts as plants, crop or noncrop, where an insect can complete development and be able to produce fertile offspring. On the other hand alternate hosts can be defined as plants, crop or non crop, other than main host where an insect can continue their generations. For

example, in the case of GM, lucerne may be an alternate host when cotton is the main host. Alternate hosts can influence the pest management decision making process in two ways: they may hinder control efforts by providing a source of migrants into agricultural fields or by maintaining pest populations when the crop host is unavailable, or alternatively they can have a net beneficial effect by acting as 'sinks' instead of sources of pest populations or by providing additional habitats for natural enemies (Sevacherian and Stern 1974, Stinner *et al.* 1983, Andow 1991, Messina *et al.* 1993, Mensah and Khan 1997). Altieri (1988) reviewed the role of weeds in the dynamics of insect populations in crop. He mentioned that the presence of weeds within or around crop fields influences the dynamics of the crop and associated biotic communities. In 1965 van Emden cited 442 references relating to weeds as reservoirs of pests. The presence of noncrop hosts in crop borders may have local effects on the population genetics of a crop pest. They may contribute to the genetic diversity of pest populations, at least if migration is limited. Thus they may help preserve genetic diversity in the local pest population, and decrease the rate of selection for new biotypes that have the ability to overcome host plant resistance, or withstand the application of pesticides (Thresh 1981).

A considerable amount of work has been done on alternate hosts and movement for *Lygus* spp. (Lipsey 1970, Sevacherian and Stern 1974, 1975, Poston and Pedigo 1975, Tugwell *et al.* 1976, Cleveland 1982, Fye 1982, Snodgrass *et al.* 1984, Fleischer and Gaylor 1987, Fleischer *et al.* 1988). The host range of *L. lineolaris* exceeds 300 species (Young 1986). *Lygus* spp. migrate to cotton from weedy hosts which serve as the source of *L. lineolaris* population increase in the southeastern United States (Tugwell *et al.* 1976, Cleveland 1982, Snodgrass *et al.* 1984, Fleischer and Gaylor 1987). They also indicated that movement into cotton commonly follows maturation of the weed host or destruction resulting from mowing and influenced by the availability and condition of weed hosts. Cleveland (1982) reported that the factors that mostly effect the emergence of wild hosts are temperature and rainfall. During periods of sufficient moisture, the luxurious growth of hosts plants helps support high populations of the insect. During dry periods they migrate in great numbers to more succulent plants such as cotton which are maintained by irrigation. However, most of the wild hosts are preferred over cotton. Sevacherian and

Stern (1975) discuss *L. hesperus* and *L. elisus* movement among the hosts plants in San Joaquin Valley, California. By late fall and early winter most of the annual field crops are harvested and the plant residue ploughed under *Lygus* find refuge in alfalfa (*M. sativa*) fields or on roadside weeds and foothill vegetation that has germinated from the early winter rains. When these latter host plants begin to dry or are ploughed in April and May the *Lygus* bugs find their way to alfalfa and safflower. When alfalfa is harvested and safflower begin to dry in June-July they move to cotton.

1.5.3 Seasonal phenology

In many places, the seasonal, cyclical and other variations of weather exert a profound influence on the population changes of insects. In particular the number of generations of an insect in each year is directly influenced by temperature. Host sequence and host suitability also influences the number of generations and the seasonal abundance of an insect. Rainfall indirectly influences seasonal abundance by affecting the abundance and suitability of host plants (Fitt 1989).

Temperature and abundance of hosts, especially during winter, affect the seasonal phenology of GM. Very hot summer and cold winter conditions may slow GM development. In cold areas, the GM may undergo facultative diapause during winter (Miles 1995). *Lygus* spp. have a similar pattern of seasonal abundance in North America. Stitt (1940) found small numbers of *L. hesperus, L. elisus* and *L. lineolaris* on alfalfa and weeds during December- February in Arizona and California. Beards and Strong (1966) reported that some insects remained active during winter in California, though they entered a facultative reproductive diapause in mid-September and remained in it through December. Bishop (1980) studied the seasonal abundance of *C. dilutus* (=*M. modestum*) in cotton in Queensland for two years. He found that the mirid reached peak densities around mid January, that is during the maximum squaring period of cotton in both years.

1.6 Population studies

1.6.1 Sampling of mirid populations

Quantitative estimation of insect populations is the base of any ecological or pest management study. It is impossible to count all the individuals in a habitat because it is time consuming and labour intensive. To overcome this problem researchers or farm managers sample a habitat, that is, estimate numbers in a representative portion of the habitat. There is no universal sampling method, and the sampling of a particular insect population must be appropriate to the distribution and life cycle of the insect (Southwood 1966). Broadly two types of samples, absolute and relative, can be used to estimate mirid populations in cotton. Southwood (1966) and Metcalf and Luckmann (1994) defined an absolute method as the successive estimation of the number of insects per unit area and relative method as the estimate of the number of insects from unknown units which allow comparisons in space or time. The type of sample depends on the objective of the study being undertaken. According to Andrewartha and Birch (1954) if the chief purpose of an investigation is to explain the influence of environment on the animal's chance to survive and multiply, it may be sufficient to measure relative changes in the density of the population without troubling to ascertain the absolute numbers in the population. Southwood (1966) lists five factors that may simultaneously affect the catch of any relative method:

1. The actual density or population size
2. The number of insects in a particular phase
3. The level of activity
4. The efficiency of relative method being used
5. The responsiveness of the particular sex and species to the trap stimulus

Details of the development of sampling programmes for various insects are given by Morris (1955), LeRoux and Reimer (1959) Harcourt (1961), Lyons (1964), Southwood (1966) and for mirids by Sevacherian and Stern (1972), Mukerji (1973), Adams *et al.* (1984), Ellington *et al.* (1984), Fleischer *et al.* (1985), Miles *et al.* (1992 and 1994), and Snodgrass (1993a).

Various sampling methods are used to estimate the arthropods in cotton including clamshell-trap (Leigh *et al.* 1970, Ellington *et al.* 1984), whole-plant-bag samples (Byerly *et al.* 1978, Garcia *et al.* 1982, Fleischer *et al.* 1985), a modified berlese funnel (Garcia *et al.* 1982), a variety of visual search techniques (Wilson and Gutierrez 1980, Garcia *et al.* 1982, Wilson and Room 1982, Fleischer *et al.* 1985), drop cloth (Young and Tugwell 1975, Fleischer *et al.* 1985), suction sampling devices (Young and Tugwell 1975, Byerly *et al.* 1978, Miles *et al.* 1992, 1994), and terminal examination (Young and Tugwell 1975, Miles *et al.* 1992, 1994). Wilson and Room (1983) proposed an alternative presence or absence (binomial) sampling which saves time when organisms are abundant. However, each method has its merits and demerits. Sweep netting is the most widely used method of sampling cotton pests because it is convenient and inexpensive. However, this method has been criticized for its inefficiency by different authors. Race (1960) compared a mechanical blowing device to a 38cm sweepnet and concluded that sweep net did not provide a dependable population index of *Lygus* bugs and stink bugs on cotton plants during the later half of the growing season. Byerly *et al.* (1978) found that adults and immatures of arthropods on cotton plants are usually underestimated with sweep net collection and estimates frequently do not correlate on a seasonal basis with those from more effective methods. Wilson and Gutierrez (1980) suggested that the sweep net is useful for estimating relative numbers of insects in cotton, but samples only a relatively small percentage of the insects found on plants; efficiency is influenced by the plant's phenology. Garcia *et al.* (1982) found that no single technique provided the best estimate for all stages of all insects, however, they concluded that carefully conducted visual searches provided the most reliable baseline for comparing the effectiveness of other methods.

In Australia several sampling methods are used commercially to estimate GM populations in cotton. Visual inspection of whole plants or plant terminals (often the same plants sampled for *Helicoverpa* spp. and other pests), shake sampling of lengths of row of cotton plants onto ground sheets or the ground and sweep net sampling (Miles *et al.* 1992). Adams *et al.* (1984), Bodnaruk (1987) and Miles *et al.* (1994) compared the efficiency of different sampling methods used to sample GM

population. They found that visual sampling method is the most efficient sampling method for GM for all stages of the crop and insects, but is very time consuming. Time spent sampling is a more important consideration to a cotton grower than obtaining the best estimate of absolute numbers per unit area (Adams *et al.* 1984). Considering this drawback of visual counting they recommended shake cloths and sweep nets for sampling sap-sucking bugs in cotton. However, since GM is a highly mobile insect, particularly in the adult stage, the efficiency of the shaking method is questionable. On the other hand the sweep net cannot be used on small seedlings and squaring cotton plants because it damages them. Another method involving small vacuum samplers is used widely by researchers (R. Mensah, pers. comm. 1994). There has also been recent trend for cotton consultants to use this sampling method (Miles *et al.* 1994). Stanley (1997) discusses factors affecting the efficiency of vacuum sampling for GM and many other insects in Australian cotton. He compared three different type of suction machines, Elecvac, Macvac and Bigvac and considered noise as possible means of increase or decrease of catch efficiency for GM. Other than noise, cotton plant phenology, position in the canopy (top or bottom of the canopy) and diurnal patterns of behaviour also contributed to catch efficiency. Though the vacuum sampling method is less efficient than visual count, it is faster and easy to use. To minimise variations in samples taken with vacuum sampler, the same machine and the person should be used in throughout the sampling period (R. Mensah pers. comm. 1994) I have used the same sampling machine (Homelite HB- 180V UT08010-F, Homelite Textron Inc., NC, USA) throughout the sampling period to sample GM populations.

CHAPTER 2

GENERAL MATERIALS AND METHODS

In this chapter general materials and methods used in various laboratory and field experiments are outlined. However, detailed experimental procedures for particular experiments are discussed in the relevant chapters.

2.1 Study sites

Three sites for population studies of GM were selected in the cotton growing areas of north west New South Wales: the Integrated Pest Management (IPM) field in the Australian Cotton Research Institute (ACRI), Narrabri (30° 13'S, 149° 47'E); Norwood, Moree (29° 30'S, 149° 31'E) and Alcheringa, Boggabilla (28° 37'S, 150° 22'E). Field trials other than population studies were also conducted at ACRI (Chapters 3, 5 and 6). In Moree, studies on overwintering hosts were also carried out (Chapter 6). The locations of the sites and other Australian cotton growing areas is shown in Figure 2.1. All the cotton was irrigated.

Though cotton was the main crop in the study sites, in winter wheat and barley and in spring safflower were also grown. The topography of the sites is characterised by extensive, black soil plains with heavy, impervious, cracking clay soils (Wilson and Greenup 1977). The study sites have semi-arid climates and experience mild winters (temperature range of -5° to 25°C) and hot summers (temperature range of 18° to 45°C). The hottest month is January and the coldest is July.

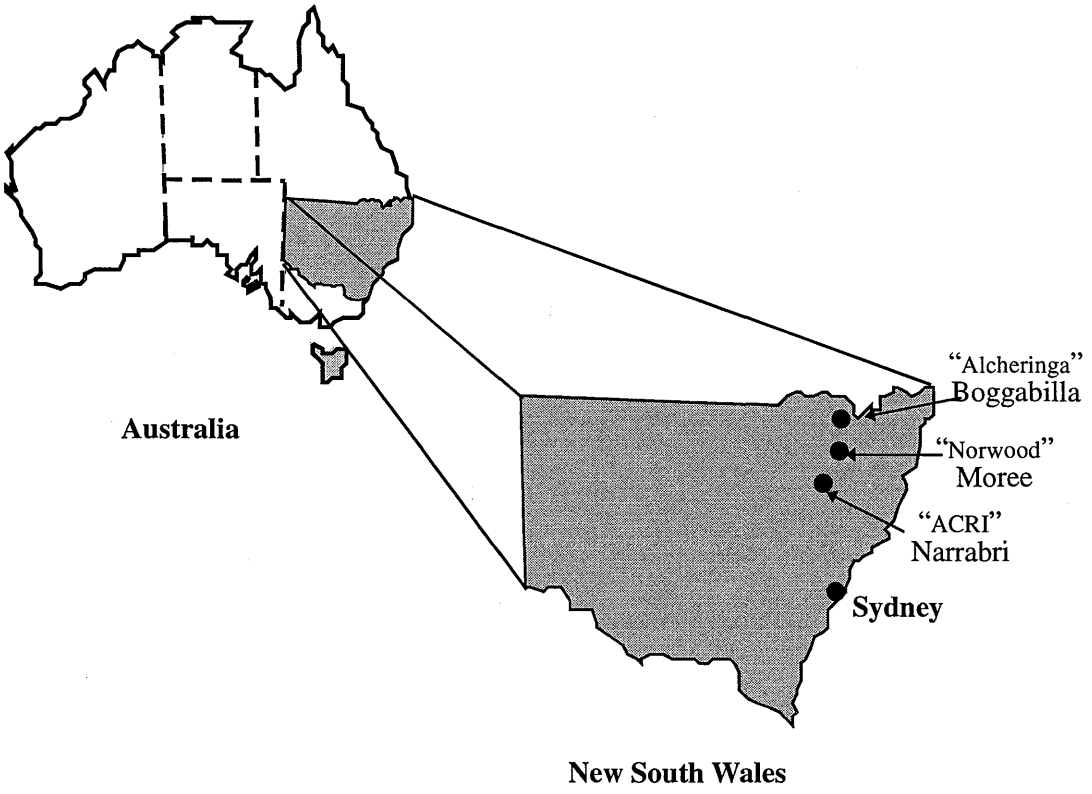


Figure 2.1. Location of study sites for population study of GM

2.2 Sampling of GM on cotton and on overwintering hosts

Sampling was done with a small portable vacuum machine, (Homelite HB-180V UT08010-F, Homelite Textron Inc., NC, USA) with a 120mm diameter cone and a nozzle speed of approximately 10m per second powered by a 2-cycle engine. A nylon cloth bag (25 cm deep) was inserted into the suction tube to collect insects sucked from the plants. The reasons for choosing the suction sampling method were firstly, suction sampling is faster and the machine is easy to move; and secondly, it is more efficient than sweep-netting in collecting GM throughout the season particularly for young insects which live mostly inside fruiting bodies. Also sweep-netting can cause severe damage to cotton plants, particularly to young seedlings. Since GM adults are highly mobile shaking methods are also impractical.

Suction sampling could not be used when plants were wet from rain or dew because that squashed the sample inside the collecting bag. In cotton, usually in the

later part of the season, and in lucerne at various times of the year the suction sampler collected many aphids which made insect counting tedious. Insects collected in the nylon cloth bag were transferred to a polyethylene bag with date, place and number of the sample on it. If there were any lepidopteran larvae in the sample, the heads of the larvae were crushed to prevent them making holes in the polyethylene bag, which created problems during washing as GM nymphs came out with water through the holes. I used an insulated container (Esky^{R} , designed by the Coleman Co Inc., made in Australia under licence by Nylex Corporation Ltd) with an ice box inside to keep the samples cool while transporting them to the laboratory. If the collection was for culturing insects, after reaching laboratory they were immediately transferred to the rearing cages using a mouth aspirator. Otherwise samples were kept in a refrigerator until counting and identification.

Although suction sampling was the main method used, to study the distribution pattern of GM, I also used a visual sampling method (whole plant check). This was done at ACRI. Insect counts were made by mainstem node number, with the first unfolded leaf on the terminal considered node 1. Plants were checked very carefully so that insects, particularly adults, were not disturbed. Checks were made for adults first, starting from top of the plant by very gentle touching of the plant. Once the whole plant was checked for adults then more rigorous checks were made for both adults and nymphs by uncovering all terminals, squares, flowers and bolls of the plant.

2.3 Sticky trap design

Sticky traps were placed around the study fields to catch both immigrating and emigrating GM. For the first year the traps consisted of 30 x 30 x 0.3cm iron sheet squares attached to a 1 m steel post. Iron sheets were used to avoid folding or any other damage from strong wind. Both sides of the square were painted first with a white primer undercoat and then with two coats of long-life gloss white enamel paint. Both sides of the traps were coated with Tangle-Trap, Tanglefoot® (The Tanglefoot Company, Grand Rapids, Michigan, USA), in either brushable or paste formulations. The brushable formulation was easy to put on the trap but dried up quickly. Therefore,

it was used only once, and thereafter the paste formulation was used. For the first year of field studies sticky traps were washed with kerosene weekly as dust and insects covered the traps, but this proved too tedious. After the first year sticky traps were made from .5mm Cadco® polystyrene sheet (Insulation Industries Pty Ltd, Broadmedow, NSW, Australia). The polystyrene sheets were cut to sticky trap size and clipped to both sides of the iron sheet of the traps. Paste type Tangle-trap was put on the polystyrene sheets. Both sheets and Tangle-trap were changed every week. The traps were painted white because GM is known not to respond to white colour (R. K. Mensah pers. comm. 1994). Any GM caught may be taken as moving out or coming into the study site depending on whether it was caught on the trap surface facing the site or away from the site.

2.4 Culturing GM in the laboratory for field experiments

A technique to culture GM in the laboratory was developed (see Appendix 1). Mirids cultured for a few generations in the laboratory may show changed viability and/or behaviour (R. Mensah and L. Wilson, pers. comm. 1994). For example GM from laboratory cultures suffered higher mortality in the field experiments than GM collected from the field. So, for field experiments 4th to 5th instar nymphs and adults were collected from lucerne with a suction sampler as described in section 2.2 of this chapter and reared in the laboratory on beans (*Phaseolus vulgaris* L.) (see Appendix 1). Adults and nymphs were reared separately in a plastic cage for 2 to 3 days before using them for experiments.

Laboratory conditions for culturing and life cycle study

All life cycle studies were conducted in a temperature-controlled environment room at ACRI. The temperature in the room was maintained at 25°C±1 using a thermostat controlled fan heater and an air conditioner. Light was provided in the control room by four 40w 'OSRAM' cool white one metre fluorescent tubes. The tubes were controlled by plate mounted 24hrs time clock (CLIPSAL) to maintain 14:10 hrs light-dark cycle.

2.5 Cages

Six different types of cages were used for experimenting and rearing GM. The design and construction of all the cages with names are given below, and the same names are used for cages throughout the thesis.

1. Field cage :

This cage was used to cage individual plants for field experiments. The frame of the cage was cylindrical and made of 5mm iron rods. Three straight 150cm high rods were welded with two 70cm diameter rod circles. The bottom circle was welded 40cm above the bottom end of the rods so that the frame could be pegged deep into the soil to avoid the risk of blowing over from strong winds. During experimentation the frame was covered with organdie material 160cm high and 90cm in diameter. Three slits were made on the bottom end of the organdie covering so that they could be pushed up to the base of the plant underneath the bottom circle of the frame to avoid any invaders from the soil. A zipper was provided on the middle of the organdie covering which facilitated plant checking and putting insects in and out of the cage.

2. Big plastic cage :

This cage was used for damage studies on 4 to 6-leaved plants in the glasshouse (Chapter 5). A transparent polypropylene sheet was folded and fixed with adhesive to make a cylindrical cage 45cm long and 12cm in diameter. Two windows, 12 x 9cm each located opposite to each other, were cut and covered with organdie cloth to facilitate ventilation.

3. Small plastic cage :

This cage was also used for damage studies in the glasshouse, however, only on plants up to 4-leaves. A rectangular cage 30cm high and 8cm in diameter was made from transparent polypropylene sheet. Two windows, each measuring 9 x 5cm,

located opposite each other were provided to allow air flow inside the cage. Windows were covered with organdie cloth.

4. Very small plastic cage :

This was used for caging individual squares and bolls. Two sizes of cages, one 9cm long x 5cm diameter and another 7cm long x 4cm diameter were made from polypropylene sheet. Two windows, each measuring 3.5 x 1.5 cm, covered with organdie cloth, were provided for proper ventilation.

5. Recycled soft drink bottle cage :

This cage was used for rearing experiments in the laboratory (Appendix 1). Round, transparent 125 ml empty soft drink bottles were cut from the top to make this cage. The size of the cage was 16cm high and 9cm diameter. The top end of the cage was covered by organdy cloth held in place by a rubber band during rearing to confine the insects and to facilitate air flow inside the cage.

6. Iron mesh cage :

This cage was also used for rearing experiments (Appendix 1). The frame of the cage measured 40 x 48 x 96cm, with a hinged door for convenient handling. All sides and the top were covered with fine iron mesh so that insects could not escape.

2.6 Multiple gradient temperature cabinet

A water-jacketed gradient temperature cabinet (Lindner and May, Brisbane, Australia), of the horizontal trough type with clear plexiglass removable box lids, was used to study the effect of temperature on GM development and fecundity. Constant temperatures were produced in different compartments of the cabinet which were separated by adjustable insulated partitions made from PVC sheet. Two thermostatic controls, one at each end, controlled the temperature gradient in the cabinet.

Temperature in each compartment was recorded by a maximum and minimum thermometer. Condensation was controlled by providing silica gel in a dessicator cartridge in each compartment. The silica gel was held in a container made from 32mm PVC pipe with two windows covered with fibre glass insect screen.

CHAPTER 3

THE BIOLOGY OF *Creontiades dilutus*

3.1 Introduction

Growth is an important component of an individual's ontogeny. Every insect passes through various growth phases from the egg, through immature development to the emergence of the adult. Embryonic development begins after fertilization as the zygote nucleus passes to the center of the yolk mass and begins to divide. The post embryonic development of insects is discontinuous, at least for the sclerotized cuticular part of the body. Size increase is by moulting. Morphological changes during ontogeny affect both external structures and internal organs, but the external changes are apparent at each moult.

The documentation of insect changes during different growth phases require studies of the basic biology. Broadly, basic biology is the foundation of ecological studies and thereby helps to develop a sound management strategy for the insect. Detailed information on biological characteristics of *C. dilutus* is lacking. Only one report (Foley and Pyke 1985) on the insect biology has so far been published, and it is not comprehensive. To obtain more comprehensive biological information, *C. dilutus* was studied extensively using the 'bean method'(see Appendix 1) in the laboratory, and on cotton plants in the glasshouse.

In this chapter the life cycle features of the insect including nymphal development, pre-oviposition, oviposition and egg incubation are described. Growth patterns of nymphs have been examined with respect of Dyar's hypothesis. Dyar (1890) stated that 'the widths of the head of an insect in its successive stages follow a regular geometrical progression' and this hypothesis can be expressed mathematically

Ln Y= a + bX, where Y= head capsule width and X= instar number.

The relationship between LnY and X should result in a straight line and a significant deviation from a straight line indicates a missing instar.

In addition adult longevity and female fecundity are also discussed. The sex ratio in the laboratory and in the field was determined. A description of the immature stages is also presented.

Oviposition is an important aspect of an insect's life history. The relationships between preference of ovipositing females for certain plant species and growth, survival and reproduction of offspring on those plants are central to insect-plant interactions (Thompson 1988). Therefore, the oviposition preference of GM was studied. The spatial distribution of GM eggs in relation to the node and petiole position on the cotton plants was determined. Later, the factors that influence the selection of oviposition sites by the insect were identified.

Green mirids prefer to feed on the growing parts of the cotton plant, particularly squares and small bolls (Bishop 1980, Adams and Pyke 1982, Adams *et al.* 1984). Perhaps sugar content of the feeding sites influences the insect to feed on sugar rich growing plant parts. Sugar is known to be important in the nutrition of many insect herbivores (House 1965, Harvey 1974). A laboratory experiment to investigate this phenomenon is also described in this chapter.

3.2 Life cycle features of *Creontiades dilutus* and the description of stages

3.2.1 Materials and methods

Unless otherwise stated, all the life cycle studies were done in the laboratory using the 'bean method'(see appendix 1) and all observations or measurements were taken by using a dissecting microscope (16x, Leica WILD M3B, Switzerland).

Egg incubation and nymphal development period

Insects used for the study were collected from the field on lucerne crops (*Medicago sativa* L). using a vacuum sampling machine (Chapter 2). Two pairs of GM were placed on four green beans, *P. vulgaris,* in a plastic bottle cage to feed and

oviposit for 5 days. Beans were checked daily and after oviposition, beans each containing 20 eggs (excess eggs were first crushed with forceps) were transferred into four different cages and placed on cotton wool kept moist with water until the eggs hatched. The incubation period was recorded. After eclosion, 60 newly hatched first instar nymphs were collected using a fine camel-hair brush and separated into 12 groups (i.e. 5 nymphs per group) to enable easier assessment and recording of the moulting periods. Each group was placed on beans in a different cage and observed for nymphal growth and development.

The nymphs in each cage were examined daily and records were taken of the number of insects that moulted and the duration of the stages until all insects emerged as adults. On each examination day, all dead nymphs and exuviae were removed to avoid nymphs becoming entangled in the exuviae and dying. When the adults emerged, they were transferred to a new cage, placed on a bean and sexed on the 3rd day. The presence of a black ovipositor in the lower abdominal segments which is visible through the plastic cages and found only in GM females was the method I used to sex the insects. This enabled the sex ratio of GM to be determined and recorded.

Determination of female fecundity, pre-oviposition and oviposition periods of GM adults

After the emergence of adults, a pair were placed in a cage and provided with two beans to oviposit on. In all fifteen pairs were used for the study. Beans were removed and replaced with fresh ones daily. After the beans have been removed, they were examined separately under a dissecting microscope and the numbers of eggs laid per female were recorded. The duration from emergence of adults to commencement of egg lay was determined and recorded as a pre-oviposition period. The experiment continued until the emerging adults died.

Description of developmental stages of GM

Eggs laid by the F1 generation were placed in cages until hatching. The newly hatched nymphs were followed through to adult. At each nymphal stage, 15 nymphs

were collected and measurements were taken of body length, width of head capsule and antennal length using a binocular microscope fitted with a micrometer eye piece. When adults emerged, weights, body lengths, head capsule widths and antennal lengths of the adults were also measured and recorded for both sexes.

3.2.2 Results and Observations

Egg and nymphal development period

The female of *C. dilutus* embedded their eggs into the plant tissue with their ovipositor. There was no apparent swelling or lumps in the plant tissue where the ovipositor was inserted to deposit the eggs. The egg is inserted so that the elliptically-shaped anterior part of the egg (known as the operculum) remained visible above the surface of the plant tissue (Plate 3.1). Eggs were laid singly. The egg is elongate, tapering towards the posterior end and slightly narrowing to the anterior end thus forming a neck just below the operculum. The average size of the egg was 1.56±0.01mm long with a range of 1.5 to 1.6mm (Table 3.2). Freshly laid eggs were light blue to hyaline in colour and turned to pale yellow before hatching. A pair of bright red spots which may develop to become the compound eye of the insect were

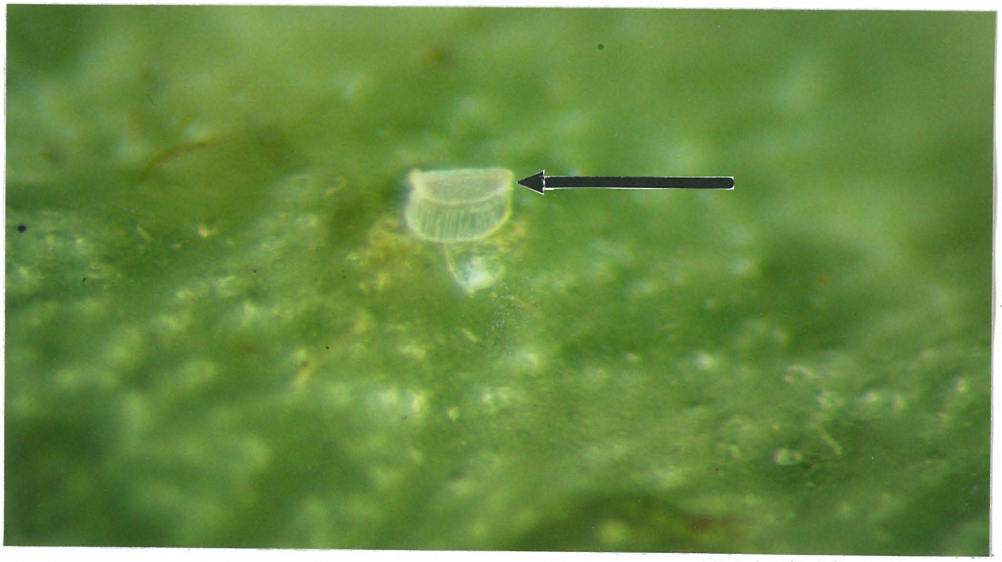


Plate 3.1 An egg of *Creontiades dilutus* inserted into the cotton plant tissue showing operculum.

visible prior to hatching. Eggs hatched from 6 to 9 days after oviposition (mean = 6.90±0.17 days) under laboratory conditions (Table 3.1).

Stage	Mean duration (x ±se)	Range
Egg	6.90±0.17	6-9
1st instar	2.97 ±0.06	2-4
2nd instar	2.95 ±0.11	2-4
3rd instar	3.10 ±0.07	2-4
4th instar	3.10 ±0.09	2-5
5th instar	3.58 ±0.14	2-5
Total	22.6 ±0.64	16-31
Preoviposition period	14.00 ±1.35	8-21
Oviposition period	9.40 ±1.04	3-15

Table 3.1 Time in days required for *C. dilutus* to develop on green beans under laboratory conditions (25°±1°C temperature and 40-60% relative humidity)

Nymphs emerged from the eggs by pushing themselves through the operculum. After emerging, the first instar nymph wandered around for about 5-10 minutes before settling down, usually at the tip of the beans. It then thrust its proboscis into the beans to commence feeding. GM has five nymphal instar stages. The duration of the 1st, 2nd, 3rd, 4th and 5th instar stages were 2.97, 2.95, 3.10, 3.10 and 3.58 days respectively (Table 3.1). The mean duration for total development from egg to adult in the laboratory was 22.6±0.64 days with a range of 16-31 days (Table 3.1)

Description of nymphal stages and adults

The measurements of body length, antennal length and head capsule width of nymphal stages are summarised in Tables 3.2 and 3.3.

The 1st instar nymph is pale green in colour with 6 bands of black shade dorsally on the thoracic region. Antennae have four segments, and translucent to light white. The scape is stouter and shorter than other segments, the 2nd and 3rd antennal segments are longer and are almost of equal size. The tip of the flagellum is light

brown. The entire abdomen is sparsely covered by fine setae, with a few setae also on the head and pedicel. The compound eyes are bright red and the tarsi are black.

The 2nd instar nymph is green in colour and devoid of black shade on the thoracic region. The eyes and tips of the antenna are reddish brown, and the antenna has a very light brownish tinge on the distal end of each segment. Abdominal segments are slightly wider than head and thorax. Setae are more prominent than in 1st instar nymph.

Stage	Average (x±se)	Minimum	Maximum
Egg	1.56±0.01	1.50	1.60
1st instar	2.07±0.09	1.40	2.60
2nd instar	3.01±0.08	2.50	3.50
3rd instar	4.40±0.11	3.90	5.20
4th instar	5.84±0.06	5.50	6.20
5th instar	7.46±0.15	6.50	8.50

Table 3.2 Length (mm) of the egg and each of the five nymphal stages of *C. dilutus* (Mean of 15 individuals)

The 3rd instar nymph is bright green in colour with dark setae throughout the body including the thoracic and leg regions. The important event of this stage is the development of wing pads. Mesothoracic wing pads extend half the length of the metanotum and metathoracic wing pads extend up to the first abdominal segment. Wing pads are light green in colour. Abdominal segments are wider than the head and thorax. Legs are pale green with a light brownish tinge on the distal end of the femur of hind legs. Claws are dark black.

In the 4^{th} instar nymphs, wing pads extend up to the third abdominal segment. The head and thorax are yellowish green. The abdominal segments are distinctly wider than the head and the thorax give them a flattened shape. The body of 4th instar nymphs is 5.84±0.06mm long and the head capsule is 1.22±0.02mm wide. Antennal length is 6.75±0.07mm. Eyes are brownish. Brown spots and coarse brown setae occur throughout the body dorsally, including the head and pronotum. Colour in the antenna

and legs is a discontinuous mixture of light green to light brown. The distal end of the femur of hind legs is light brown to reddish. Tarsi are light brown with black claws.

Average body length, antennal length and head capsule width of the 5th instar nymph is 7.46±0.15mm, 9.33±0.08mm and 1.50±0.02mm respectively. The head, thorax and abdomen are yellowish green. Wing pads extend up to the 5th abdominal segment. Outermost margins of the wing pad are brownish hyaline. The tips of the wing pads are blackened. Eyes are light brown with a light green tinge. The first segment of the antenna is short and stouter than the 2nd and 3rd segments, and the tip of the last segment is pointed and dark brown. Coarse black setae occur throughout the body, with a few setae also in the first antennal segment. Legs are light green to light brown in colour. Tibia of hind legs are pale green with coarse black setae. The distal end of tarsi and claws are black. Females can be distinguished from males under the microscope by the presence of a median cleft along the mid-ventral line on the last abdominal segment.

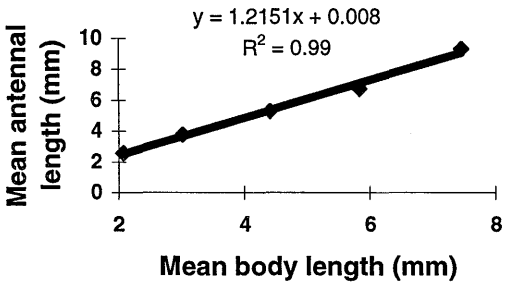
There was a significant positive correlation ($p < 0.001$) between body length of nymphal stage and (1) antennal length, (2) head capsule width (Figure 3.1A,B and Appendix 2.1). A significant positive correlation ($p < 0.001$) was also obtained between head capsule width and antennal length (Figure 3.1C and Appendix 2.1).

Stage	Antennal length (Mean±se)	Range	Head capsule width (Mean±se)	Range
1st instar	2.58±0.07	2.5-2.7	0.59±0.01	0.5-0.6
2nd instar	3.77±0.03	3.6-3.9	0.82±0.01	0.8-0.9
3rd instar	5.29±0.07	4.9-5.7	1.02±0.01	1.0-1.1
4th instar	6.75±0.07	6.2-7.2	1.22±0.02	1.1-1.3
5th instar	9.33±0.08	8.7-9.8	1.50±0.02	1.3-1.6

Table 3.3 Antennal length and head capsule width (mm) of nymphal stages of *C. dilutus* (Average of 15 individuals)

The frequency curve (Figure 3.2) of head capsule width showed 5 distinct peaks with modes at 0.6mm (peak 1), 0.8mm (peak 2), 1.0mm (peak 3), 1.2mm (peak 4) and 1.5mm (peak 5), confirming five nymphal instars.

A



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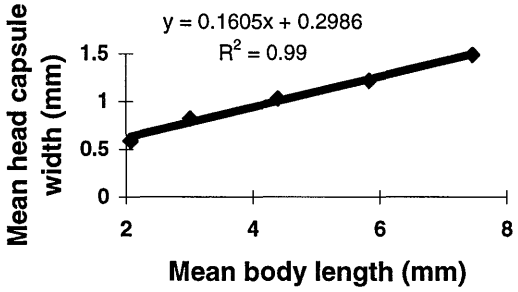

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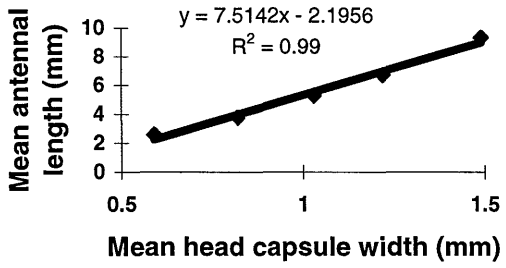


Figure 3.1 Relationship between body length of nymphal stage and (A) antennal length, (B) head capsule width and between head capsule width and (C) antennal length of *C. dilutus* in the laboratory

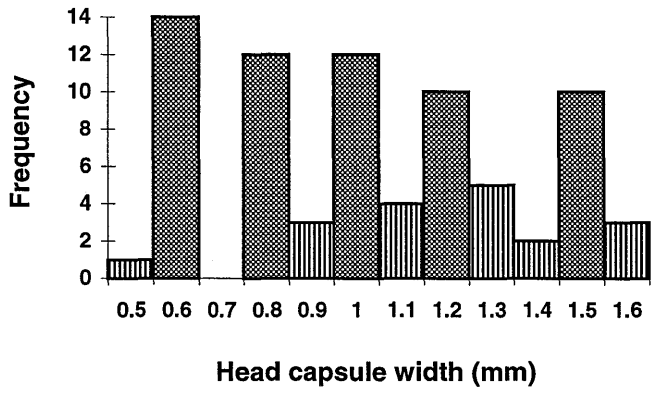


Figure 3.2 Frequency distribution of head capsule width of nymphs. The five peaks represent five instars

A regression line was then drawn of head capsule width against corresponding instar number (Figure 3.3). The near perfect straight line with equation LnY= 0.2252X - 0.6959; r^2= 0.99 indicated no missing instars. According to Dyar (1890) the increase in size from one instar to the next is geometric and the size ratio of one instar to the next is a constant. The ratio for *C. dilutus* was calculated from the straight line of Figure 3.3 and was 1.25.

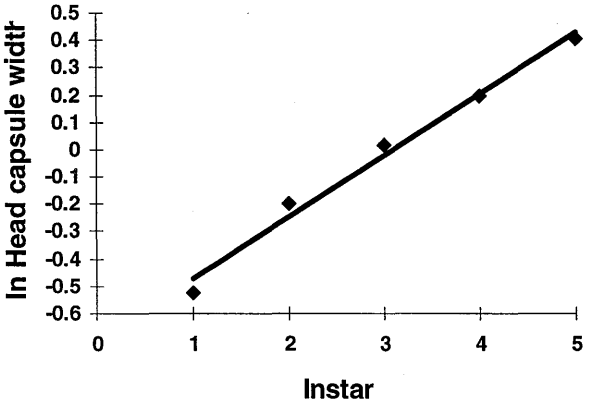


Figure 3.3 Regression of nymphal size (log head capsule width) against instar number showing geometric increase of nymphal size with corresponding instar number

Newly emerged adults are a uniform light green colour. Within a day the colour becomes yellowish green. The pronotum and scutellum are more yellowish than other parts. At old age the mirid becomes light brown in colour. The head including the eyes is wider than the base of the pronotum. Eyes are light brown with a

light yellowish tinge. Collar is light green with long brown setae. The pronotum is shiny with a brown line along the posterior margin. The scutellum is triangular, the tip being dark brown. The pronotum and scutellum are covered by sparse short setae. The base of the femur is greenish. The distal ends of femurs of hind legs are discontinuously bright brown, and the tibia are light brown with coarse dark setae. Wings are green with very light green to hyaline membrane. Wings taper posteriorly with round tips.

There was no significant difference between male and female adults in terms of weight, body and antennal length (Table 3.4 and Appendix 2.2). However, the width of the head capsule of the males were significantly different from the females ($P < 0.05$) (Table 3.4 and Appendix 2.2)

Sex	Live weight (Mean±se)	Body length (Mean±se)	Antennal length (Mean ±se)	Head capsule width (Mean±se)
Male	5.2±0.18 a (4.1-6.5)	8.9±0.18 a (7.8-9.9)	10.9±0.11 a (9.7-11.5)	1.8±0.03 a (1.6-1.9)
Female	5.5±0.36 a (3.4-8.4)	8.5±0.23 a (7.0-9.8)	10.9±0.15 a (9.6-11.7)	1.7±0.02 b (1.6-1.8)

Table 3.4 Weight (mg), length and head capsule width (mm) of adult *C. dilutus* under laboratory conditions. Figures in parenthesis indicate range. Means within a column followed by common letter are not significantly different (P>0.05), Fisher's least significant difference test (Average of 15 individuals).

Male and female longevity and sex ratio and female fecundity

Under laboratory conditions, the females live on average 2.4 days longer than males (Figure 3.4). Male longevity was 21.7±1.4 with a range of 16-36 days. Female longevity was 24.1±1.6 with a range of 16-37 days.

The male to female sex ratio is 1:1. Out of 218 adults that emerged in the life cycle experiments in the laboratory, 119 were males and 99 females, giving a sex ratio of 1.2:1. Under field conditions (Chapter 6), out of 995 adults sampled from the three study sites 486 were males and 509 females with a calculated sex ratio of 1.0:1.1 and

this was not significantly different ($P > 0.05$) from 1:1 ratio. A χ^2 test showed that there were no significant differences between the sex ratios under both laboratory and field conditions ($P > 0.05$) ($\chi^2 = 2.36$ with 1 degree of freedom).

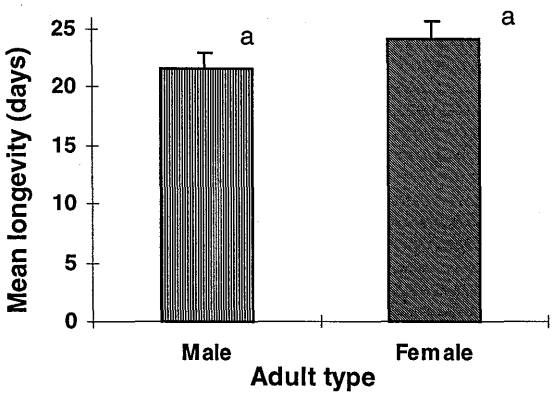


Figure 3.4 Adult longevity (days) of *C. dilutus* under laboratory conditions. Means in the bar with a common letter are not significantly different ($P > 0.05$), Fisher's least significant difference test. (Average of 20 individuals).

The female can lay an average of 39.25±4.21 eggs in her life time with a range of 14-82. The pre-oviposition period ranges from 8 to 21 days with a mean of 14.0±1.35 (Table 3.1). The oviposition period was 9.4±1.04 days (Table 3.1). The peak oviposition of *C. dilutus* females occurred 16-20 days after emergence (Table 3.5).

Days after emergence	No. of eggs/female (Mean±se)
1-5	0.00±0.00 a
6-10	5.25±1.38 c
11-15	11.75±1.92 d
16-20	13.50±1.46 d
21-25	5.60±1.40 c
26-30	2.10±0.81 abc
31-35	1.05±0.56 ab

Table 3.5 Number of eggs per female of *C. dilutus* laid on beans under laboratory conditions (Average of 20 females). Means followed by common letter are not significantly different ($P > 0.05$), Fisher's least significant difference test

3.2.3 Discussion

C. dilutus passes through an egg and five nymphal stages to become an adult which agrees with the findings of Adams *et al.* (1984) for *C. dilutus,* Ridgway and Gyrisco (1960a), Khattat and Stewart (1977) for *Lygus lineolaris,* Leigh (1963), Champlain and Butler (1967), Butler and Wardecker (1971) for *L. hesperus,* Mueller and Stern (1973) for *L. hesperus* and *L. elisus,* Gaylor and Sterling (1975) for *Pseudatomiscelis seriatus,* Sharma and Lopez (1990), and Ratnadass and Mallé (1994) for *C. pallidus*. Male and female development times of *C. dilutus* are similar (Foley and Pyke 1985), so in this study the development times of the sexes were not recorded separately.

Like *C. pallidus*, *C. dilutus* also inserted their eggs into the plant tissue leaving the egg cap outside (Sharma and Lopez 1990, Ratnadass and Mallé 1994). I did not find any swelling or lump around the egg on bean but when I studied oviposition preference using cotton plants (see Section 3.3, this chapter) I observed a brown ring around the egg on some occasions. Sharma and Lopez (1990) also reported a red-brown or black ring around the egg of *C. pallidus* on grain sorghum. This might be due to the cotton plant tissue being harder than green beans and during oviposition the female has to thrust the ovipositor more in harder tissue, causing more injury. Eggs of *C. dilutus* hatched in 6-9 days (Table 3.1) which was similar to *C. pallidus* (Sharma and Lopez 1990, Ratnadass and Mallé 1994), *L. lineolaris* (Khattat and Stewart 1977) and *L. hesperus* (Leigh 1963, Champlain and Butler 1967, Butler and Wardecker 1971, Mueller and Stern 1973). However the egg incubation period varies with temperature (Chapter 4). Lower temperatures result in a significantly longer time to hatch (Champlain and Butler 1967, Butler and Wardecker 1971, Mueller and Stern 1973, Khattat and Stewart 1977).

A few hours after eclosion nymphs settled down mostly on the tip of the beans, indicating their preference for tender tissue. Leigh (1963) also reported a similar type of behaviour for *L. hesperus*. Foley and Pyke (1985) reported 4-6 nymphal stages of *C. dilutus*. However, when I used Dyar's rule to corroborate my findings it clearly showed 5 distinct peaks in frequency curve (Figure 3.2) and nearly a perfect straight

line when regression was performed with log head capsule width and different instar (Figure 3.3). However, for *C. dilutus* the ratio of progression was 1.25, lower than Dyar's constant (which is 1.4). Enders (1976) explained Dyar's rule from an ecological point of view. According to him a large Dyar's constant implies low mobility and a small one implies high mobility. Obviously *C. dilutus* is a highly mobile insect. Ratnadass and Mallé (1994) calculated a similar growth ratio for West African sorghum head bug, *Eurystylus immaculatus* (Odhiambo).

Total developmental time, from egg to adult, for *C. dilutus* was 22.6 days, similar to the findings of Foley and Pyke (1985). They calculated egg to adult duration of 22.95 days at 25°C . However, they found 8.32 and 4.33 days for the egg stage and 5th instar respectively which is longer than my findings (Table 3.1). Egg incubation periods for *C. pallidus* reported by Sharma and Lopez (1990) and Ratnadass and Mallé (1994) were also similar to my findings, however, they reported lower nymphal duration. The findings of Ridgway and Gyrisco (1960b), Khattat and Stewart (1977) for *L. lineolaris* and Leigh (1963), Mueller and Stern (1973) for *L. hesperus* also agree with my findings. The greater longevity of *C. dilutus* females, compared with males (Figure 3.4), while not significant in my study, is consistent with findings in *L. hesperus* (Leigh 1963), *L. lineolaris* (Khattat and Stewart 1977) and in sorghum earhead bug, *Calocoris angustatus* (Hiremath and Viraktamath 1992). Fecundity was maximum in the early to mid stage of female lives (Table 4.6) which is similar to *L. lineolaris*, however, the numbers of eggs laid by *C. dilutus* were lower than in this species (Khattat and Stewart 1977). The pre-oviposition period of *C. dilutus* under laboratory conditions was longer than *C. pallidus* indicating that the insects take a long time to sexually mature. Strong *et al.* (1970) observed a similar phenomenon for *L. hesperus.*

The sex ratio of *C. dilutus* varied in the laboratory and in field. In the laboratory males outnumbered females and in field it was *vice versa.* Though this difference was not statistically significant, Ridgway and Gyrisco (1960a) also found two types of sex ratio for *L. lineolaris*; a male biased sex ratio when insects were caught with a sweep net and a female biased sex ratio when insects were caught with Tanglefoot^{R} traps. Field populations were collected from three different locations and

at different times of the year, which may have affected the sex ratio. Larger sample sizes may have increased the ability to detect small differences in sex ratios, but it is clear from my studies that there were no major deviations from a 1:1 ratio in either the field or the laboratory.

3.3 Oviposition preference of *Creontiades dilutus*

The following sections describe experiments conducted to assess the interaction between cotton plants and GM oviposition. Unless otherwise stated, the Sicala V1 cotton variety planted in 25cm pots was used in all the oviposition preference experiments in the laboratory and mesh house.

3.3.1 Oviposition pattern in relation to plant age, node number and petiole position under mesh house conditions

Materials and methods

The experiment was conducted in a Sarlon mesh house (4m x 10m x 1.8m) at the Australian Cotton Research Institute (ACRI), Narrabri during summer in the 1995-96 season. The cotton plants used for the study were planted in the glasshouse. Cotton plants at four different growth stages were used viz; (1) plants with 8 main stem nodal leaves (seedling stage), (2) plants with 14 main stem nodal leaves (squaring stage), (3) plants with 18 main stem nodal leaves (peak squaring and boll formation stage), and (4) mature plants with large bolls containing 20 or more main stem nodal leaves (boll maturing stage). Prior to the commencement of the study, all the cotton plants to be used for the study were transferred from the glasshouse to the mesh house 3 days before the trials to adjust to the environmental conditions. The holes in the sarlon mesh were larger than the size of the insects and there was the possibility that insects may escape when released on the plants so an organdie cage (130cm x 225cm x 140 cm) was used to cover the plants within the mesh house. The bottom of the cage was fixed firmly to the floor of the mesh house using an iron bar along the ends of the cage. This prevented insects escaping, and also spiders entering the cage to prey on the

mirids. The floor of the cage within the mesh house was covered by a polyethylene sheet (120cm x 200cm) to deny other soil inhabiting insects particularly earwigs and also spiders access to the cage.

Four plants from each growth stage representing four replicates of the treatment, were placed on the polyethylene sheet under the organdie cage. Thus sixteen plants (4 plants or replicates per treatment) were used. The plants were arranged randomly in a circle and thirty pairs of 7 to 8 day old first generation *C. dilutus* adults were released in the centre of the circle from a 'bean method' rearing cage (see Appendix 1). The behaviour of the insects were observed and recorded. The insects were allowed to feed and oviposit on the selected plants for 3 days. The number of *C. dilutus* females on each plant was recorded before the plants were cleared of insects by shaking and then taken to the laboratory where eggs on each treated plants were counted under a dissecting microscope (x16, Leica WILD M3B). Eggs were recorded as numbers per main stem nodal petiole since *C. dilutus* females oviposit mainly on the petioles of the cotton plants. Main stem nodal position was counted from the first unfolded leaf downwards. For each petiole position, a record of the number of eggs was deposited dorsally or ventrally (petiole aspect) was taken.

Data were analysed using one-way analysis of variance and means were separated by using Fisher's least significance difference test at the 5% level (MINITAB statistical package) (Ryan *et al.* 1992). In addition, a GLM (general linear model) was used to determine the interaction between plant age classes, petiole position and petiole aspect.

Results and observations

When the insects were released, they flew and remained at the top of the organdie cage for 2-3 hours before descending onto the test plants to oviposit. *C dilutus* laid significantly more eggs ($P < 0.001$) on plants with squares and also plants containing squares and small bolls than young and mature plants (Figure 3.5 and Appendix 2.3a). One way analysis of variance showed that the numbers of eggs laid

on plants with squares and plants with squares and bolls were not significantly different ($P > 0.05$) as also was the difference between eggs deposited on young and mature plants (Figure 3.5).

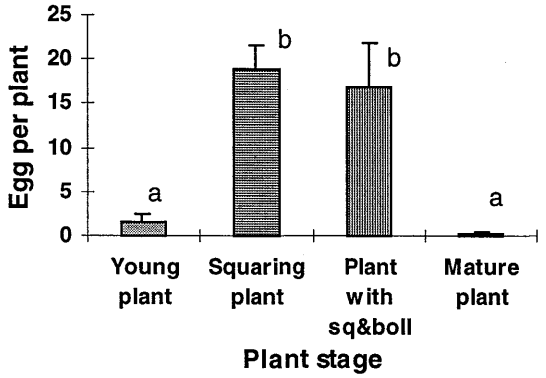


Figure 3.5 Oviposition of *C. dilutus* on different plant age classes under mesh house conditions. Means in the bar with common letter are not significantly different at 5% level, Fisher's least significant difference test.

Within each plant age group most eggs were laid on the 4^{th}, 5^{th}, 6^{th}, 7^{th} and 8th main stem nodal petiole (Figure 3.6). About 19.9, 19.2, 22.5, 16.6 and 11.9 percent of the total number of eggs were laid on the 4th, 5th, 6th, 7th and 8th main stem nodal petioles respectively. Other main stem nodal petioles contained negligible percentages of eggs (Table 3.6). One-way analysis of variance for all plant age class indicated a significant difference ($P < 0.001$) (see Appendix 2.3b) among the number of eggs deposited on the main stem nodes (Figure 3.6). Fisher's least significant difference test also revealed that the number of eggs deposited on the 4th to 8th main stem nodal petioles were significantly different ($P < 0.05$ and Appendix 2.3b) from the 2nd, 3rd, 9th and 10th main stem nodal petioles indicating that the 4^{th} to 8^{th} main stem nodal petioles were the preferred oviposition sites The analysis also indicated that there was no significant difference ($P > 0.05$) between the number of eggs deposited on the 7th, 8th and 9th nodal petioles (Figure 3.6 and Appendix 2.3b). When one-way analysis of variance was performed separately for each plant age class, the level of significance was different (Table 3.7) with the 4th to 8th main stem nodal petiole still the preferred sites for oviposition (Table 3.8).

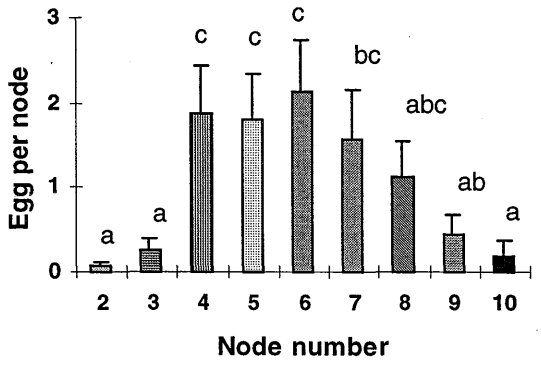


Figure 3.6 Number of eggs deposited by *C. dilutus* on different main stem nodal petioles under mesh house conditions (total of four plant age class). Means in the bar followed by the same letter are not significantly different at 5% level, Fisher's least significant difference test.

Node number	% Eggs deposited
2	0.7
3	2.7
4	19.9
5	19.2
6	22.5
7	16.6
8	11.9
9	4.6
10	1.9
Total	100

Table 3.6 Percentage of eggs oviposited on different main stem nodal petiole of cotton plants under mesh house condition.

Plant age class	Statistical parameter F value	P value	Level of significance
Young plant	1.35	0.262	NS
Squaring plant	3.40	0.008	***
Plant with sq. & boll	5.64	< 0.001	***
Mature plant	1.00	0.459	NS

Table 3.7 Level of significance for each plant age class analysed separately. NS= not significant; number of * indicates level of significance.

Node number	Young plant	Eggs per node Squaring plant	Plant with square and boll	Mature plant
2	0.00 a	0.25 a	0.00 a	0.00 a
3	0.00 a	0.75 ab	0.00 a	0.25 a
4	0.25 a	2.75 bcd	4.50 c	0.00 a
5	0.25 a	4.00 cd	3.00 bc	0.00 a
6	0.75 a	4.25 d	3.50 c	0.00 a
7	0.00 a	2.00 abcd	4.25 c	0.00 a
8	0.25 a	3.25 cd	1.00 ab	0.00 a
9	0.00 a	1.75 abc	0.00 a	0.00 a
10	0.00 a	0.00 a	0.75 ab	0.00 a

Table 3.8 Number of eggs of *C. dilutus* deposited on different main stem nodal petioles for each plant age class under mesh house conditions. Means within the column followed by the same letter are not significantly different ($P > 0.05$), Fisher's least significant difference test.

Highest numbers of eggs were recorded in the tip of the petiole, followed by the base with the lowest recorded in the middle of the petiole (Figure 3.7). The percentage of the total number of eggs deposited at the tip, base and middle of the petiole were 53.0%, 29.1% and 17.9% respectively. However, one-way analysis of variance for petiole positions in each age class showed that these differences were not statistically significant ($P > 0.05$) (Figure 3.7 and Appendix 2.4 a-d). When all the data were pooled and analysed, the analysis indicated that the difference between the number of eggs deposited in the tip of the petiole and the middle were significantly different ($P < 0.05$). However, the difference between eggs deposited in the base and middle of the petiole was not statistically significant ($P > 0.05$) so also was the difference between base and tip of the petiole (Figure 3.8 and Appendix 2.4e). *C. dilutus* deposited more eggs (69.5%) in the dorsal part of the petiole than the ventral part (30.5%) and this was statistically significant (Table 3.9 and Figure 3.9a and Appendix 2.4f). However, when analysis was done for each petiole position separately, a significant difference ($P < 0.05$) was found between dorsal and ventral aspects only for the petiole end (Figure 3.9b and Appendix 2.4g). Further analysis with GLM indicated significant interactions between plant age and petiole position ($P < 0.05$), plant age and petiole aspect ($P < 0.05$), petiole position and petiole aspect ($P < 0.01$) and among plant age, petiole position and petiole aspect ($P < 0.01$).

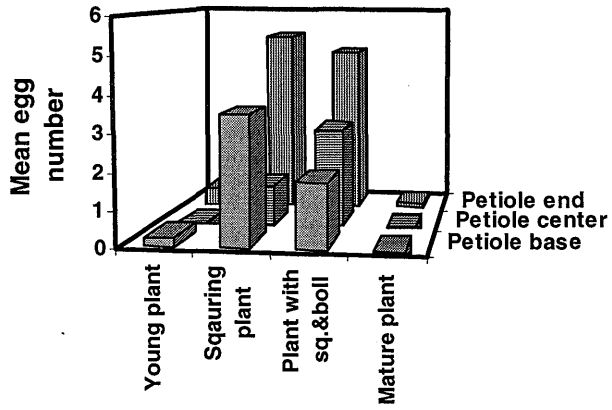


Figure 3.7 Number of eggs oviposited by *C. dilutus* on different petiole positions of each plant age class.

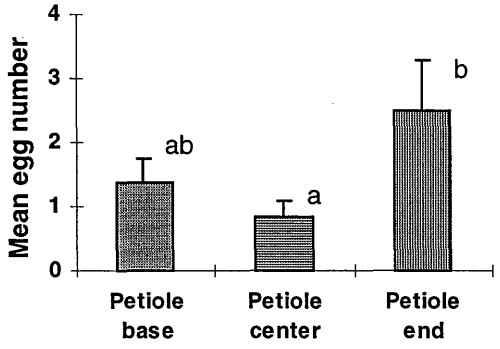


Figure 3.8 Pooled data on oviposition of *C. dilutus* on different petiole positions under mesh house conditions. Means in the bar followed by same letter are not significantly different at 5% level, Fisher's least significance difference test.

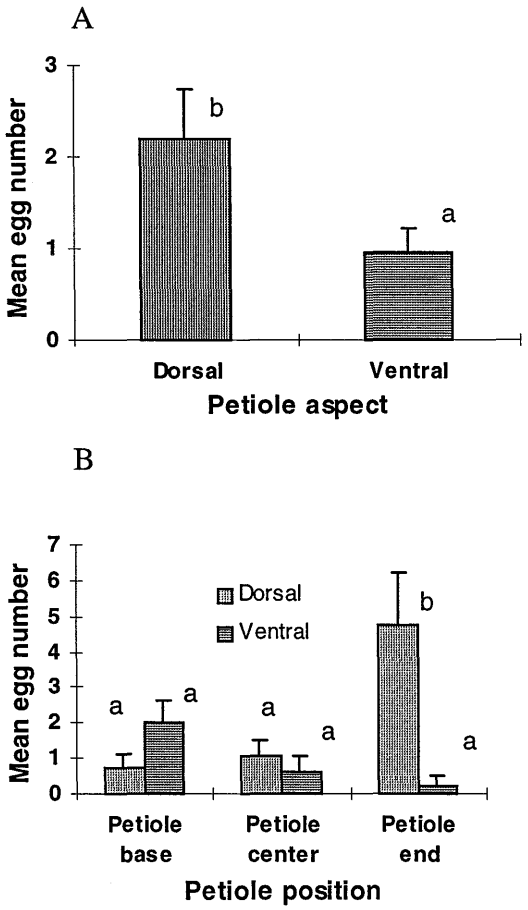


Figure 3.9 Effects of petiole position and aspect on oviposition of *C. dilutus* under mesh house conditions. (A) Pooled data showing a significant difference between dorsal and ventral aspect. (B) Oviposition for each petiole position with dorsal and ventral aspect. Means in the bar followed by common letters are not significantly different (P>0.05), Fisher's least significant difference test.

Petiole position	Percent egg laid		Total
	Dorsal	Ventral	
Petiole base	7.9	21.2	
Petiole center	11.3	6.6	
Petiole end	50.3	2.7	
Total	69.5	30.5	100

Table 3.9 Percentage of egg laid by *C. dilutus* in the dorsal and ventral aspects of each petiole position under mesh house.

3.3.2 Oviposition of *C. dilutus* in relation to hardness and hairiness of the petiole

Materials and methods

The experiment was conducted in the glasshouse in ACRI at Narrabri. Four squaring cotton plants of Sicala V 2 variety were used. Each plant was placed in a separate iron mesh cage and two pairs of seven day-old first generation *C. dilutus* adults were released in each cage for five days to oviposit on the plants. In total, eight pairs of insects were released in four cages. Each cage was considered as a replicate. After five days, the insects were removed and the number of eggs laid on the 3rd to 11th main stem nodal petiole of each plant were counted under a dissecting microscope (x16, Leica WILD 3MB). After counting, the hardness of each petiole was measured using a needle type penetrometer similar to that used by Mensah and Madden (1991). The penetrometer consisted of a metal tube, a needle holder and a small handle connected to the needle holder by an elastic spring to raise and lower the needle. The needle holder was fixed to the tube by a screw so that height could be adjusted by sliding up and down. A 0.2mm pin was poised above a hole in a square metal block placed on an electronic balance (Mettler PC 4400), which measured the weight that was exerted during penetration of the needle into the petiole. In operation, the petiole was placed on the squared metal block on the balance and the needle was lowered slowly with the aid of the handle. Hardness was measured as grams of penetration force required for each of nine penetrations for each petiole.

Hairiness of the petiole was measured using a 1 mm^2 grid. The grid was made by placing four pieces of coloured threads crosswise on the top of a plastic petridish and gluing them with epoxy gum. In operation, the petridish was placed on the petiole under a binocular microscope and the number of hairs in the grid was recorded. Three such samples were taken for each petiole.

The data were analysed by one-way analysis of variance in MINITAB. The relationships between the number of eggs per main stem nodal petiole and the absolute hardness and hairiness of the petiole were obtained by regression.

Results and observations

Data on hardness and hairiness of petioles in relation to eggs deposition by *C. dilutus* of different node numbers are given in Table 3.10. Hardness of the petiole increased significantly ($P < 0.001$) with increase node number whereas the hairiness of the petioles decreased significantly ($P < 0.001$) with increase node number (Appendix 2.5a and 2.5b). Figure 3.10 shows that the hardness and hairiness curves intersect each other at node number 5. The petioles surrounding node 5 are therefore probably the best compromise between the two characters. Egg deposition increased from node 5 and reached peak at node 7 then started to decrease from node 8.

Regression analysis showed no significant relationship between the number of eggs per node and the absolute hardness and hairiness of the 3rd to the 11th nodal petiole. However there was a significant and positive relationship between the number of eggs per node and the absolute hardness of the 3-7 nodal petiole (Figure 3.11 A) There was also a significant and positive relationship between the number of eggs and the hairiness of the 7-11 nodal petiole (Figure 3.11B). A significant and negative relationship ($P < 0.001$) was also found between hardness and hairiness of the petiole (Figure 3.12).

Node number	Hardness (g) (Mean±se)	Hairiness per sq mm (Mean±se	Egg per node (Mean±se)
3	6.80±0.37 a	17.81±0.81 d	0.0±0.0 a
4	7.62±0.48 ab	8.42±0.91 c	0.50±0.29 a
5	8.52±0.53 bc	8.25±1.61 c	1.25±4.48 abc
6	9.20±0.60 bcd	5.23±1.06 b	4.25±1.25 c
7	10.13±0.17 cde	5.08±1.14 b	8.0±2.65 d
8	10.56±0.33 de	2.94±1.08 ab	3.75±1.03 bc
9	10.96±0.58 e	2.04±0.48 a	1.0±0.41 ab
10	10.32±1.12 de	1.17±0.43 a	0.75±0.75 ab
11	10.91±0.37 e	1.21±0.36 a	0.0±0.0 a

Table 3.10 Hardness and hairiness of the petioles of different node number and numbers of eggs deposited by *C. dilutus* at different nodal petioles under glasshouse conditions. Means within the column followed by same letter are not significantly different at 5% level, Fisher's least significant difference test. LSD = 1.64, 2.77 and 3.16 for hardness, hairiness and eggs respectively.

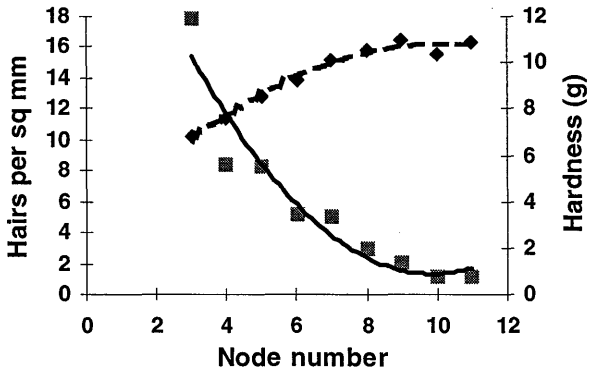


Figure 3.10 Hardness and hairiness of the main stem nodal petioles of cotton plants under glasshouse conditions. —■— = Hairiness and ---◆--- = Hardness.

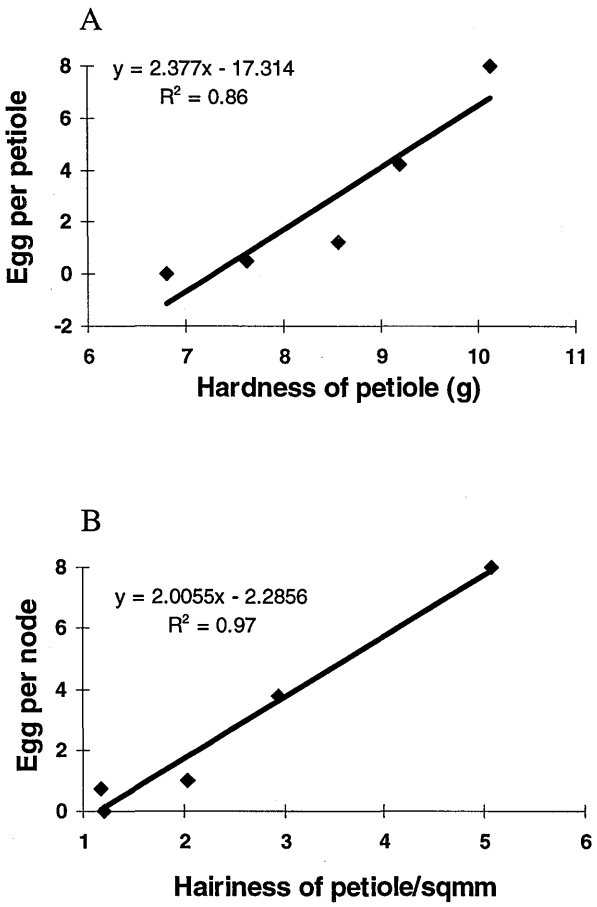


Figure 3.11 Relationship between egg number and (A) hardness for the 3-7 nodal petioles, (B) hairiness for the 11-7 nodal petioles under glasshouse conditions.

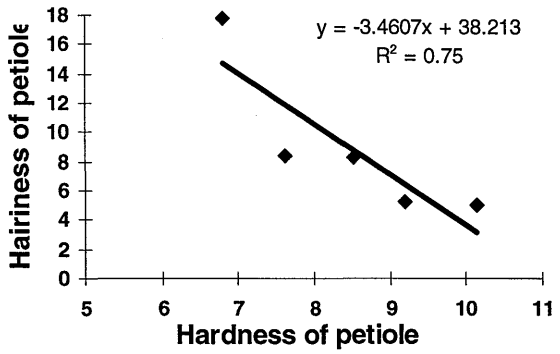


Figure 3.12 Relationship between hardness and hairiness of the petiole of cotton plants under glasshouse conditions.

3.3.3 Oviposition on plants which had been previously oviposited on

Materials and methods

Free choice and no-choice tests were conducted in the mesh house to determine whether *C. dilutus* will lay the same number of eggs on a previously oviposited and uninfested or fresh plants of similar age. In the no-choice tests, 3 uninfested or fresh cotton plants were each placed in an iron mesh cage (Chapter 2) named cages 1, 2,and 3. Four pairs of 8 day-old *C. dilutus* adults were released into the cages to oviposit as such-3 days prior to second oviposition in cage 1, 10 days in cage 2 and 15 days in cage 3. Each treatment was replicated 3 times. After oviposition the insects were removed, eggs and nymphs were counted without damaging the plants. The plants from each treatment were classified as previously oviposited plants and used in free choice tests. In the free choice tests, 3 uninfested or fresh plants of similar age and size as the previously oviposited plants were placed in an organdie cage. In all, four treatments viz; (1) a plant previously oviposited on by *C. dilutus* 3 days ago, (2) plants oviposited on 10 days ago (3) plants oviposited on 15 days ago and (4) uninfested or fresh plants (control). Each treatment consisted of 3 plants. Thirty pairs of *C. dilutus* adults were released in the cage to oviposit on the plants for three days. The insects were removed after 3 days and the number of eggs laid on the plants were counted.

The data were analysed using one-way analysis of variance and means were separated by Fisher's least significant difference test.

Results and observations

The mean numbers of eggs oviposited on plants previously oviposited upon are presented in Table 3.11. *C. dilutus* oviposited indiscriminately on both fresh and previously used plants under mesh house conditions. Table 3.11 showed that the relative attractiveness of the 6 or 7 nodal petioles as oviposition sites during second oviposition remained more or less consistent for all plant categories including the

control plants, even though most of the eggs were deposited on those sites during the first oviposition. One-way analysis of variance revealed no significant differences ($P > 0.05$) between the control plants and the previously used plants as oviposition sites during the second oviposition (Figure 3.13).

Treatment (days after first oviposition on plants)	Node number	Egg number (Mean±se) 1st oviposition	2nd oviposition
3 days after first oviposition	3	0.67±0.67	0.67±0.67
	4	1.67±1.20	1.33±0.88
	5	0.67±0.67	0.67±0.33
	6	3.67±1.86	6.00±3.06
	7	4.33±2.96	3.33±0.33
	8	0.33±0.33	4.00±2.31
	9	2.00±1.00	3.33±3.33
	10	2.00±2.00	1.33±1.33
10 days after first oviposition	3	0.33±0.33	0.00±0.00
	4	1.33±1.33	1.00±1.00
	5	1.33±1.33	2.67±1.45
	6	4.67±2.19	2.67±2.19
	7	2.33±1.45	1.00±1.00
	8	0.33±0.33	0.67±0.33
	9	2.33±2.33	2.00±2.00
	10	1.00±0.58	0.33±0.33
15 days after first oviposition	3	0.00±0.00	0.33±0.33
	4	0.67±0.67	0.00±0.00
	5	0.67±0.33	0.67±0.67
	6	3.00±1.73	2.67±2.67
	7	4.00±2.31	2.00±1.00
	8	3.33±1.33	2.33±1.86
	9	2.67±2.19	1.00±1.00
	10	0.00±0.00	0.67±0.33
Control (no prior oviposition)	3		0.33±0.33
	4		0.67±0.33
	5		2.33±2.33
	6		1.00±1.00
	7		4.33±1.86
	8		2.00±1.00
	9		0.67±0.67
	10		1.33±1.33

Table 3.11. Mean number of eggs deposited by *C. dilutus* on plants previously oviposited upon under mesh house conditions

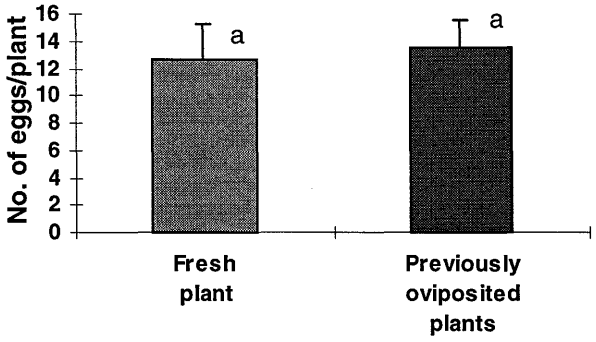


Figure 3.13 Number of eggs deposited on fresh (control) and previously oviposited plants under mesh house conditions. Means followed by the same letters are not significantly different at 5% level, Fisher's least significant difference test.

3.3.4 Oviposition pattern of *C. dilutus* in the field

Materials and methods

The experiment was conducted in a 2 ha irrigated field at ACRI, Narrabri during the 1995-96 and 1996-97 seasons to see the oviposition pattern of *C. dilutus* laid by wild insects. Cotton crops (Sicala VII variety), planted in 100m rows were used for the study. The field was divided into three subplots (Chapter 6). From each subplot ten whole plants up to the 10 leaf stage and later 10 tips containing at least 10-12 main stem nodal leaves were sampled by walking across the field. Plants were cut with secateurs and brought to the laboratory for observation. The sample was checked thoroughly under the microscope (x16) and the eggs were counted for each petiole position. Data were expressed as eggs per metre since this is the conventional unit of habitat used by consultants in the cotton industry. On average in the field one metre contained 10 plants.

Since *C. dilutus* did not deposit eggs on the petiole base and 1, 2, 11 and 12 nodal petioles, I excluded these sites for analysis. Means and standard errors of means were calculated using MINITAB. The data were further analysed using one-way analysis of variance.

Results and observations

C. dilutus deposited the same number of eggs in the 1995-96 and 1996-97 seasons (Figure 3.14 and Appendix 2.6a). However, the insects laid most of their eggs on the 4 to 8 main stem nodal petioles in both seasons (Table 3.12).

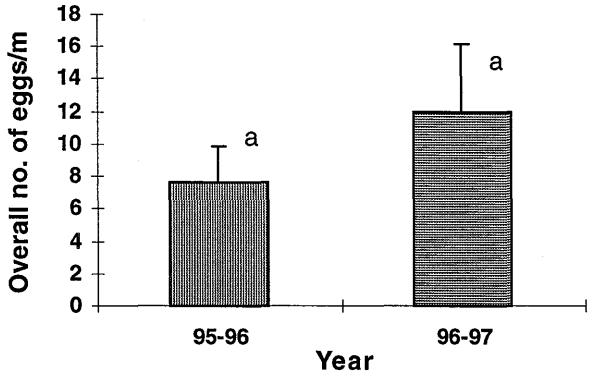


Figure 3.14 Number of eggs oviposited by *C. dilutus* in the field at ACRI, Narrabri during the 1995-96 and 1996-97 cotton seasons. Means in the bars followed by the same letter are not significantly different at 5% level, Fisher's least significant difference test.

Node number	Mean no. of eggs /nodal petiole	
	1995-96 season	1996-97 season
3	0.00a	0.67a
4	1.67ab	1.00a
5	2.00b	2.67a
6	2.00b	2.33a
7	1.67ab	2.67a
8	0.33ab	2.00a
9	0.00a	0.33a
10	0.00a	0.33a

Table 3.12 Mean number of eggs deposited by *C. dilutus* on different nodal petioles of cotton in the field at ACRI, Narrabri during the 1995-96 and 1996-97 cotton seasons. Means within the column with the same letter are not significantly different at 5% level, Fisher's least significant difference test. LSD= 1.94 and 2.74 for the 1995-96 and 1996-97 seasons respectively.

One-way analysis of variance indicated that the insects deposited eggs during the 1995-96 season varied significantly with node number. Fisher's least significant difference test revealed that the insects laid significantly more ($P < 0.05$) eggs on 5 and 6 nodal petioles (Table 3.12 and Appendix 2.6b). However, during the 1996-97

season the insects deposited eggs on 3 to 10 nodal petioles without any significant variation (P=0.33). When analysis was performed with two years' data pooled together a significant difference (P < 0.01) was found among the nodal petioles as oviposition sites (Figure 3.15 and Appendix 2.6c). The insects laid significantly more eggs on 5 to 7 nodal petioles.

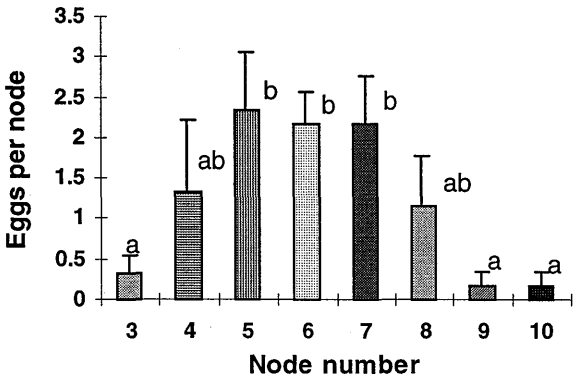


Figure 3.15 Number of eggs deposited by *C. dilutus* on different nodal petioles in the field at ACRI, Narrabri for both the 1995-96 and 1996-97 cotton seasons. Means in the bars followed by the same letter are not significantly different (P > 0.05), Fisher's least significant difference test. LSD=1.52.

C. dilutus oviposited more eggs on the petiole end than petiole base for both seasons (Figure 3.16). During the 1995-96 season 78.3% of eggs were deposited on the petiole end and 21.7% on the petiole base. However in the 1996-97 season the number of eggs deposited were 63.9% on the petiole end and 36.1% on the petiole base. A one-way analysis showed no significant difference (P > 0.05) between these petiole positions as oviposition sites for both years (Appendix 2.6d and 2.6e). On the petiole end the insect deposited more eggs dorsally and on the petiole base more eggs were deposited ventrally for both seasons. However, one-way analysis of variance revealed that the difference between dorsal and ventral aspect was also not significant (P=0.24 & 0.07 and 0.19 & 0.15 for petiole base and end during 1995-96 and 1996-97 seasons respectively) (Figure 3.17 A,B).

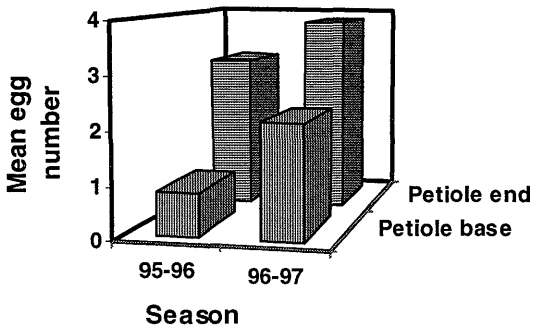


Figure 3.16 Number of eggs deposited by *C. dilutus* in the field at ACRI, Narrabri on petiole positions during the 1995-96 and 1996-97 cotton seasons. Means in the bars followed by the same letter are not significantly different at 5% level, Fisher's least significant difference test. LSD=2.96 and 3.72 for the 1995-96 and 1996-97 seasons respectively.

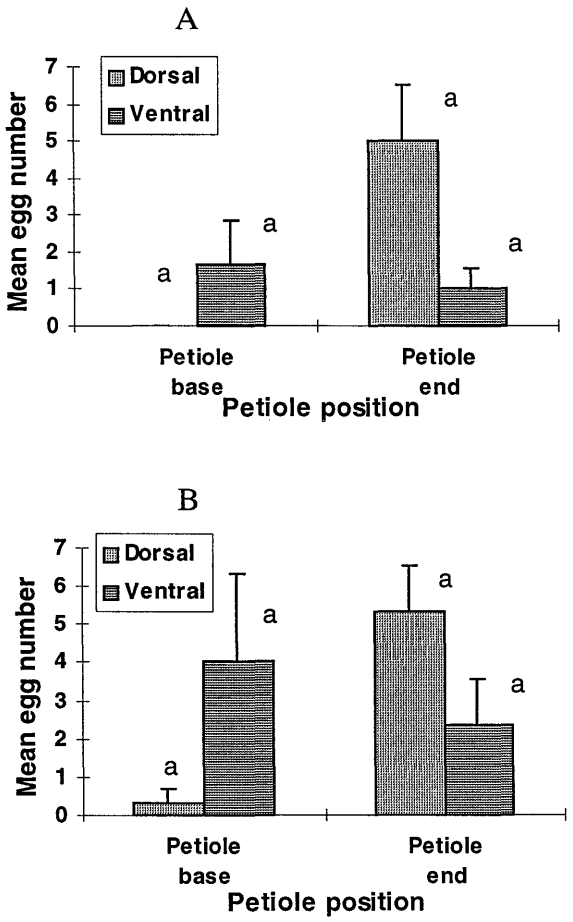


Figure 3.17 Number of eggs deposited by *C. dilutus* dorsally and ventrally on the petioles in the field, ACRI, Narrabri during (A) 1995-96 and (B) 1996-97 seasons. Means in the bars followed by the same letter are not significantly different at 5% level, Fisher's least significant difference test. LSD=3.34 & 4.53 and 6.48 & 4.72 for petiole base and end during the 1995-96 and 1996-97 seasons respectively.

3.3.5 Discussion

The study showed that *C. dilutus* preferentially oviposited on early to late squaring cotton plants (Figure 3.5). These plant organs provided both the adults and nymphs with food, shelter and oviposition sites better than the other cotton plant organs (Bishop 1980, Adams and Pyke 1982, Adams *et al.*1984). The selection of the squares as oviposition sites by *C. dilutus* adults is to ensure the survival of the offspring. This is because in many insects development of immature stages is confined to the plant parts upon which the adults deposited their eggs, indicating that maternal oviposition preference profoundly influences offspring performance (Chew 1975, Fox *et al.* 1994). The fruiting parts, squares and small bolls may provide carbohydrates in the form of sugars to mirids. Benedict *et al.* (1981b) and Alvarado-Rodriquez *et al.* (1986) also reported similar a oviposition pattern by *L. hesperus* on nectaried cotton and beans respectively.

Like other mirids, *C. dilutus* females embedded their eggs into the tissue of cotton plants leaving the operculum outside (Plate 3.1). In young and non fruiting cotton plants, the mirids deposited their eggs on leaf petioles. This finding agreed with those of Bariola (1969) for *L. lineolaris* and Benedict *et al.* (1981a) for *L. hesperus* and not that of Miles (1995) who indicated that *C. dilutus* eggs are usually inserted in the stem below terminals and around the base of squares. In damage-density relationship trials reported in this thesis (Chapter 5) in the glasshouse using 4-10 leafed cotton plants, I observed eggs in the stem of young cotton plants on a few occasions, but this may be due to the high population forcing the insects to deposit eggs in a non-preferred site. Apart from food, shelter, hardness, hairiness etc, other biotic and abiotic factors which may influence the mirid's oviposition preference included sun and shade effects (Rausher 1979, Moore *et al.* 1988) and leaf age and phenology (Thomas 1987, Auerbach and Alberts 1992, Kouki 1993).

Usually the females deposited eggs without producing any swellings or lumps surrounding the egg cap. However, on a few occasions a brown ring was detected surrounding the operculum, especially if the egg was deposited in relatively hard tissue like stem of the cotton plant. In trials in both mesh house and field GM

preferentially oviposited more eggs on the petiole end and base with few eggs deposited in the middle of the petiole (Figures 3.7 and 3.9B). In the field the insects did not deposit eggs on the middle of petiole (Figures 3.16 and 3.17B). This variation is due perhaps to the low mirid population in the field during the sampling time, which may have enabled them to discriminate between more favourable and less favourable sites. *L. hesperus* also preferred to lay eggs on the petiole end, particularly on the pulvinus area at the base of leaf blade (Benedict *et al.* 1981a). *C. dilutus* deposited eggs on the petiole either dorsally or ventrally (Figures 3.9 A,B and 3.19 A,B and Table 3.9). In the petiole end they put significantly more eggs dorsally and in the petiole base they put more eggs ventrally. However, for the petiole center they did not follow any definite pattern. The tenderness of the tissues of upper side of petiole end and lower side of petiole base may influence oviposition in those places.

Both in the field and in the mesh house, 4 to 8 nodal petioles were preferred oviposition sites (Figures 3.6 and 3.15 and Tables 3.7 and 3.13). Benedict *et al.* (1981a) reported that lygus bugs showed a significant oviposition preference for the upper one-third of glanded cotton plants and for the upper two-thirds of glandless plants. Mueller and Stern (1973) predicted that *L. hesperus* and *L. elisus* would oviposit near the top of the safflower plants when the field plants are in bloom and lower leaves had died. It seems that the softness of particular plant tissues contributes to selection of oviposition sites. Another plant morphological character, hairiness of the plant parts may also be involved. *C. dilutus* deposited fewer eggs on the top 3 nodal petioles. These top nodal petioles are softer than lower nodal petioles, but they contain more hairs. (Figure 3.10). It seems that *C. dilutus* exploited these two characters very efficiently and chose cotton plant parts as oviposition sites that are comparatively soft and less hairy. Dense hairs on the top petioles perhaps provide a mechanical barrier, but on the other hand the insect may find it difficult to penetrate hard plant tissues with its ovipositor. Tenderness of plant tissues was considered to be the determining factor for selection of lygus oviposition sites by Sorensen (1939), Elmore (1955), Carlson (1956) and Alvarado-Rodriquez *et al.* (1986). Hairiness has not previously been implicated by any worker as a factor in selection of oviposition sites for mirids. However, there is evidence of hairiness affecting feeding and oviposition behaviour in jassids, *Empoasca* spp., sap sucking insects in cotton in India

and Africa (Verma and Afzal 1940 and Parnell *et al.* 1949). Hairiness is also considered to be a determining factor for selection of oviposition sites by the insects in other crops. Schillinger and Gallun (1968) reported that leaf pubescence of wheat acted as a deterrent factor for selection of oviposition sites by cereal leaf beetle, *Oulema melanopus.*

Evidently *C. dilutus* followed a definite pattern of oviposition in cotton plants either in the mesh house or in the field. It is likely that two physical factors, tenderness and hairiness of the oviposition sites, shaped this pattern. Inclusion of this knowledge in sampling protocols for mirid management should give some degree of accuracy for determining population density in field. Existing sampling procedures fail to give accurate assessments of population density and only 50% of the actual population in a sampling unit can be predicted (Miles 1995). On the contrary egg counts will give more accurate estimate of population in a sampling unit. In that case, however, cotton growers and consultants have to have a dissecting microscope or strong magnifying glass and further research is necessary to determine egg threshold numbers for the insect.

3.4 Effect of sucrose on mirid development and survival under laboratory conditions

3.4.1 Materials and methods

These experiments were conducted in a controlled environment room using the bean culture method (Chapter 2 and Appendix 1).

On eclosion 40 first generation nymphs (nymphs emerged from eggs laid by field collected females), were placed in rearing cages with a bean, 20 for the sugar solution and 20 for the water treatment. Cotton wool soaked with water and 5% sucrose solution (white sugar procured from a grocery shop) were put on the base of the beans separately as an additional food source. The cotton wool was replaced every day so that the wool containing sugar solution did not became sticky and entangle

insects. Beans were changed every third day. The insects were checked every day and the dates of moults recorded until all insects became adults. At the end of the experiment 15 nymphs for each food source emerged as adults. Means and standard errors of means were calculated using MINITAB and analysis for total nymphal development time and development time of each nymphal instar were done by one-way analysis of variance.

For the survival study 10 first instar nymphs were confined in a rearing cage with two beans. Cotton wool soaked with 5% sugar solution and water was put on the base of the beans. Replacing cotton wool and beans was done as above. The number of insects out of ten which emerged as adults was counted as survival. The whole process was replicated four times for each food source. The data obtained were converted into proportions and then transformed using the arcsine transformation. Transformed data were analysed using one-way analysis of variance in MINITAB.

3.4.2 Results and observations

The data on development time for each food are summarised in Table 3.13. Total mean development time with and without sugar was 14.4±0.29 and 15.1±0.39 days respectively.

Stage	Development time (Mean±se)	
	Sugar	Without sugar
1st instar	2.80±0.14	2.93±0.15
2nd instar	2.53±0.17	2.60±0.19
3rd instar	2.47±0.13	2.53±0.13
4th instar	2.60±0.16	3.00±0.14
5th instar	4.00±0.10	4.07±0.12

Table 3.13 Development time in days required for *C. dilutus* on green beans with and without 5% sugar solution under laboratory conditions (25°±1°C temperature and 40-60% relative humidity) (Mean of 15 individuals)

Analysis of variance revealed that development time for each instar did not vary significantly ($P > 0.05$) with or without sugar solution. The times required for each instar were slightly lower with sugar solution (Table 3.14), but the analysis

showed that the total development times for each treatment were not significantly different (P=0.14) (Figure 3.18)

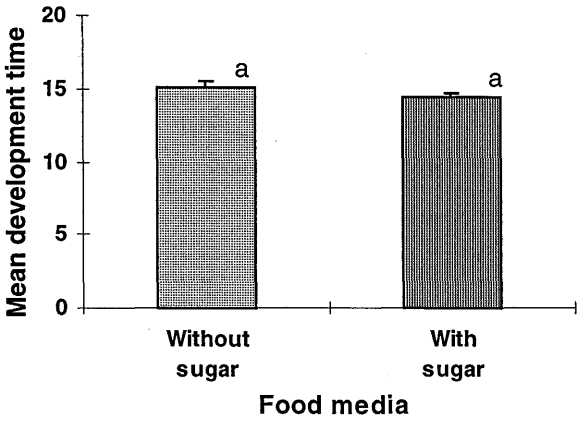


Figure 3.18 Total development time in days for *C. dilutus* on green beans with and without 5% sugar solution under laboratory conditions (25°±1°C temperature and 40-60% relative humidity). Means in the bars followed by the same letter are not significantly different at 5% level, Fisher's least significant difference test

Survival of *C. dilutus* in green beans with sugar solution was higher than without sugar solution. The percent survival for green beans with sugar solution and without sugar solution was 82.5% and 60.0% respectively (Figure 3.19).

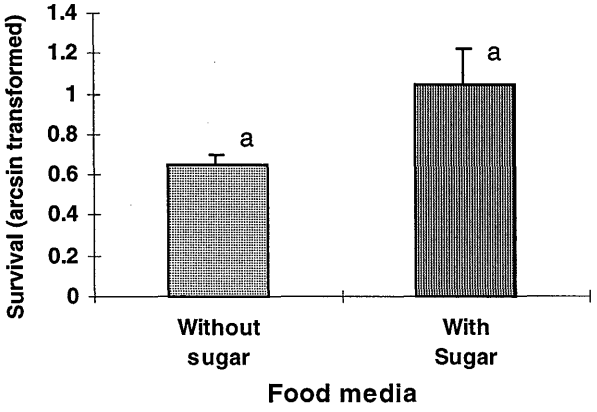


Figure 3.19. Survival of *C. dilutus* on green beans with and without 5% sugar solution under laboratory conditions (25°±1°C temperature and 40-60% relative humidity). Data were transformed using arcsine for analysis. Means in the bars followed by same letter are not significantly different ($p > 0.05$), Fisher's least significant difference test.

3.4.3 Discussion

The data suggest that sucrose has some positive impact on mirid survival. There was also a tendency for increased rate of development (lower development time) on green beans with sugar solution. The insects seemed more robust and active in cages containing sugar solutions. Butler (1968) reported 10% sucrose in diet increased survival by 80% in *L. hesperus* compared to insects grown only on water. The failure to obtain significant differences in my study might be due to the lower concentration (5%) of sugar solution used. Also, perhaps the parameters I used for measuring the effects of sucrose on mirid development failed to pick up other significant differences. It might be worth including body weights and lengths in future studies.

If an insect obtains adequate energy-yielding substrates (such as sugars) from its food, it will direct most of its dietary protein into growth (Shaw *et al.*1978). Otherwise it will have to metabolize proteins for their energy content and will grow less (Clancy 1992). Harvey (1974) reported that the adult weights and rates of larval development of eastern spruce budworm increase with increasing dietary level of sugars.

C. dilutus preferentially feed on squares, anthers and small bolls (Chapter 5, this thesis), which suggests their inclination towards sugar influences diet. Hori and Miles (1993) found higher survival of *C. dilutus* on flower heads than other parts of lucerne. One of the reasons may be that flower heads contain more sugar than other parts. Benedict *et al.* (1981b) reported that nectariless cotton increased mortality of *L. hesperus* adults and nymphs compared to other nectar sources which were available for 7 or more days. At the pre-squaring stage of cotton, the requirement of sugar in the diet of *C. dilutus* may be supplemented by mites (L. Wilson, pers. comm. 1995). Butler (1968) reported that alfalfa stem containing mealybug, pea aphids, *Acyrthsiphon pisum* (Harris) and spotted alfalfa aphids, *Therioaphis maculata* (Buckton) increased survival of *L. hesperus* many fold. However, he did not mention that the *Lygus* predate on those insects. He thought the honeydew the insects produce may supplement the sugar requirements of the diet.

CHAPTER 4

EFFECT OF TEMPERATURE ON FECUNDITY AND DEVELOPMENT OF *Creontiades dilutus*

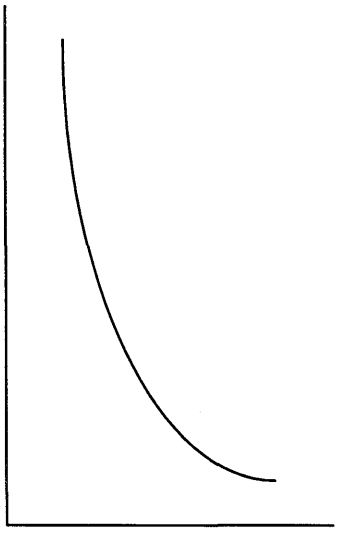
4.1 Introduction

As poikilotherms, all insects have certain temperature limits which Andrewartha and Birch (1954) termed 'temperature preferendum'. Above and below temperature preferendum the insect's development is retarded or, at extremes, death ensues. Thus measurement of the amount of heat required over time for an insect to complete development or a stage of development is more meaningful as a measure of development time than age in calendar time.

The principle of using temperature and time to describe poikilotherm and plant development has been recognized for more than 250 years (Réamur 1735, cited by Belehrádek 1935) and various mathematical models have been used by the entomologists to express the relationship between temperature and rate of development in insects. Temperature driven developmental rate models can be used to predict accurately the duration of developmental stages even when temperatures fluctuate (Eubank *et al.* 1973, Taylor 1982), to predict occurrence of damaging stages (Braman *et al.* 1992), to forecast the growth rate of pest populations (Siddiqui and Barlow 1973) or their cyclical peaks (Toscano *et al.* 1979), and to determine the timing for pest population census and control efforts so as to permit the survival of natural enemies (Johnson *et al.* 1979, Hawthorne *et al.* 1988). Developmental rate models may also be used as components in complex systems models (Gregg 1981). For example Butler and Scott (1976) developed a model for development of the corn earworm by utilizing a thermodynamic model to elucidate the detailed timing of events through the season, to produce daily life tables and to compare the effects of different treatments.

When the developmental periods for a stage or for the whole life cycle of any insect at a series of constant temperatures are plotted against these temperatures, a

backward "J"-shaped curve can be drawn through the points (Figure 4.1). If the reciprocals of development time (development rate) are plotted against temperature a shallow sigmoid ("S"-shaped) curve results (Figure 4.2). At the lower thermal limit, the development rate curve asymptotically approaches zero because insects often survive for long periods at cold temperatures with little or no development. As temperatures increase from the lower limit, development rates become proportional to temperature, and a linear response curve results in the mid region. Development begins to slow as the optimal temperature (i.e. the temperature associated with the fastest development rate) is reached; thereafter, development falls off sharply. More and more individuals die as temperatures increase beyond the optimum (Howe 1967, Wagner *et al.* 1984a,1985, Allsopp *et al.* 1990). Several equations have been used to describe this relationship. Broadly they can be divided into day-degree or linear models and more complex curvilinear (theoretical or empirical) models.



Temperature

Figure 4.1 Typical relationship between development time (t) and temperature at constant temperature.

The day-degree concept is based on the thermal summation principle that every stage in the life cycle requires a definite amount of heat for its development. The theory is based on the work of various authors, notably Sanderson and Pears, Krogh, Blunck and Bodenheimer (Uvarov 1931). The day-degree concept assumes that the rate of development is proportional to temperature:

$$1/y = a + bT$$

where 1/y is the rate of development and the threshold temperature can be calculated from the equation as (-a/b) degrees. This method with various modifications is widely used because of the simplicity of the model. It requires minimal data to formulate and is easy to calculate (Eckenrode and Chapman 1972, AliNiazee 1976, Butts and McEwen 1981, Gregg 1981, Wagner *et al.* 1984a). A curve similar to that shown in Figure 4.2 can be obtained by plotting 1/y against temperature. Though this method, which assumes a linear relationship between development rate and temperature (region A, Figure 4.2), is often accurate for intermediate temperatures, it yields considerable error when temperature conditions tend toward the extremes under variable conditions (region B and C, Figure 4.2). Problems with the day-degree model include:(a) the developmental zero, "-a/b", is not the true threshold for development (Sharpe and DeMichele 1977) and (b) gross errors in predicted development rates occur at the temperature extremes (Stinner *et al.* 1974).

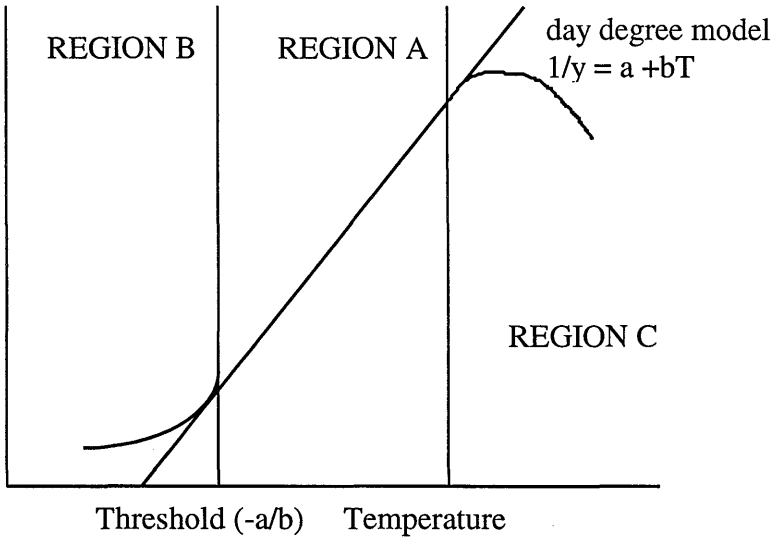


Figure 4.2 Typical relationship between temperature and developmental rate (r).

These shortcomings led many workers to develop curvilinear models of development. However, there is argument about whether this type of model should be theoretically or empirically based. Janisch (1932) argued that the model should have a theoretical basis and must correspond with the data observed. Other workers believe the model should rather be judged on empirical criteria such as goodness of fit and ease of manipulation.

Janisch (1932), Pradhan (1945, 1946), Logan *et al.* (1976), Sharpe and DeMichele (1977), and Lactin *et al.* (1995) derived several useful theoretical models. However, there is little to choose between different alternative models (Howe 1967). Very small errors in temperature control can result in highly significant deviations from model to model (Browning 1952) and that does not necessarily demonstrate the inadequacy of the model.

Sharpe and DeMichele (1977) formulated a complex biophysical model based on biological process rates incorporating both high and low temperature inactivation of enzymes. This model describes the entire response curve over a full range of temperatures, or only part of the curve over a restricted temperature range (Barfield *et al.* 1978, Palmer *et al.* 1981, Schoolfield *et al.* 1981, Wagner *et al.* 1984b). The model identifies six thermodynamic constants that appear to characterise many enzymatic responses. This advantage needs to be balanced against the complexity of fitting the model and the number of degrees of freedom lost in doing so (Gregg 1981).

The most widely used empirical model is that of Davidson (1942, 1944) that describes development rates as a function of temperature:

$$\frac{1}{y} = K / (1 + e^{a-bT})$$

where 1/y = reciprocal of the time required to complete development at a given temperature T

K = maximum development rate; a, b = constants

This equation is used by various workers including Birch (1944, 1945), Birch and Snowball (1945), Guppy and Mukerji (1974), Berkette *et al.* (1976), Thomas (1980), Lamb and Loschiavo (1981) although it is not very descriptive at one or both ends of the response curve (Browning 1952, Watt 1968). Stinner *et al.* (1974) described the effects of temperature on development rates with a modified sigmoid equation that eliminates the limitation of Davidson's model. Their model is:

$$\frac{1}{y} = C / (1 + e^{k_1 + k_2 T^1})$$

where C = (maximum developmental rate) x $\left(e^{k_1 + k_2 T_{opt}}\right)$, i.e. the asymptote

k_1, k_2 = empirical constants

T^1 = temperature T where $T \bullet T_{opt}$

$2.T_{opt} - T$ where $T > T_{opt}$

T_{opt} = temperature at which the maximum development rate occurs

This model was used by Barnes (1976), Butler and Hamilton (1976), Allsopp (1981) and Gregg (1981). However, it is symmetric around the optimum, and for this reason, it too has drawbacks at higher temperatures (Logan *et al.* 1976). Siddique and Barlow (1973) found it unsuitable for describing the effects of temperature on population growth in aphids.

Evidently, there is a need for quantitative data on the effect of temperature in *C. dilutus* development for using a complex logistic model as well as a day-degree model. Although Foley and Pyke (1985) studied the effect of temperature on *C. dilutus* development they used only a linear relationship to explain the data. The temperatures they used were very few. An approximate day-degree relationship can be obtained from few data but to derive a nonlinear relationship more temperatures are needed (Allsopp *et al.*1990).

In this chapter the effects of temperature on egg and nymphal development, survival and fecundity of *C. dilutus* are discussed. The data obtained from these experiments are described using logistic and day-degree models and threshold temperatures for total nymph and individual instars and egg development are determined.

4.2 Materials and methods

Unless otherwise stated all the cages used in this experiments were similar to the cages used for the 'bean method' (Chapter 2 and Appendix 1).

Temperature

The experiment was conducted in a controlled temperature cubicle at UNE, using a water-jacketed multiple gradient temperature cabinet (Chapter 2). In the cubicle light was provided by a skylight over the top and two 40w 'OSRAM' cool white fluorescent tubes. The tubes were controlled by a time clock (CLIPSAL^{R}) to maintain 14:10 hrs. light-dark cycle. Temperature in the temperature cabinet was maintained at 25°C±1 using a thermostat controlled fan heater and an air conditioner.

Since 8 temperatures could be produced at a time in the multiple temperature cabinet the experiment was conducted in 1995 and 1996 winter (Table 4.1) to get enough temperatures to fit a logistic model (Allsopp *et al.* 1990). Eight compartments were made by placing adjustable insulated partitions in such a way that 4 'recycled soft drink bottles' (Chapter 2), a maximum-minimum thermometer, and a desiccator cartridge with silica gel could fit in each compartment. Temperatures in the compartments were produced by adjusting the thermostatic control at each side of the water-jacketed temperature cabinet. Temperatures were monitored every day and the silica gel in the desiccator cartridge replaced every 3rd day.

Compartment number	Temperatures (°C) 1995	1996
1	12	11
2	17	15
3	21	20
4	24	23
5	27	26
6	30	30
7	33	32
8	38	38

Table 4.1 Temperatures (°C) produced in water jacketed multiple gradient temperature cabinet in 1995 and 1996, UNE, Armidale.

Stock cultures

Stock cultures were maintained in the controlled cubicle using the 'bean method'(Appendix 1 and Chapter 3). However, in this case 4-6 pairs of adults were confined in a cage with 4 beans. Beans were not replaced until they started drying. Beans containing eggs were kept in a separate cage and checked everyday for hatching.

GM adults were collected for this purpose from ACRI, Narrabri for both years. However, in 1995 beans containing eggs laid in the laboratory in ACRI by the field collected adults were also used. Insects were caught with a suction machine (Chapter 2) from lucerne on the day of transport to Armidale. Collected insects were aspirated out carefully from the collecting bag so they were not damaged, and confined in cages with lucerne tips.

Experimental design and recording data

***Fecundity*:**

Eight temperature regimes (11, 15, 20, 23, 26, 30, 32 and 38°C) were used to study the effects of temperature on fecundity. On emergence, a female and a male of first laboratory generation adults were placed in a rearing cage (Chapter 2) with a pair of fresh beans in each temperature regime for oviposition. Twenty to 25 pairs were used for each temperature regime. Every day beans were replaced with a pair of fresh beans and used beans were checked under the microscope for eggs until the female died. Thus the daily number of eggs for each female were recorded. If the male died before the female, it was replaced by another of the same age from stock culture. However, if the female died before laying that cage was discarded.

***Egg development*:**

First generation laboratory females were allowed to lay eggs on beans in the controlled cubicle. Six to ten females with the same number of males were confined in

a cage provided with six beans as oviposition sites. The next day the beans were replaced and eggs were counted under the microscope. Twenty eggs were placed in a cage in a temperature cabinet, thus four cages, considered as replications, were used for each temperature regime. Eight temperatures 11, 15, 20, 23, 26, 30, 32 and 38°C were used. Eggs were checked every day and any nymphs hatched were recorded and removed from the cage until all the eggs hatched. If in any temperature eggs did not hatch near the expected time they were allowed 15 days to hatch and after that unhatched eggs were checked under the microscope.

***Nymphal development*:**

On eclosion, 10 nymphs were confined in a cage with two fresh beans. Four cages, considered as replications, were placed in each compartment randomly. Care was taken so that cages were not misplaced during handling. Other operations like changing beans, keeping beans fresh with water soaked cotton wool, cleaning exuviae from the cage, etc. were the same as life cycle study methods (Chapter 3). Although in low temperature compartments beans remained fresh for 6 to 7 days, they were changed on the same day for all cages. Insects were checked every day and dates of moulting and death recorded. Nymphal instars were identified at moult. In low temperatures, particularly 11-15°C insects sometimes appeared moribund. If such insects did not move when prodded with the sharp end of a needle they were counted as dead.

Analysis

Since *C. dilutus* took more time as they progressed through the instars in lower temperatures the development time was calculated as median days because it is less sensitive to the influence of outliers than is the mean. The advantages of a median approach over mean are outlined by Messenger and Flitters (1958), Gregg (1981) and Wagner *et al.* (1984b). The median time was calculated by using a small BASIC program, TIME50.BAS, written and used by Gregg (1981). The program calculated 50% times (≈ median times) for the completion of developmental instars and

cumulative mortalities at those times. It was estimated by linear interpolation using the last available time when less than 50% had completed the moult, and the first available time when more than 50% had done so.

The effect of temperature on fecundity, oviposition rate and pre-oviposition period was determined by analysis of variance. Means were compared by Fisher's Least Significance different test at the 5% level.

Means of the median values were calculated for eggs and nymphal development and regression equations were derived for day-degree model using MINITAB. A BASIC program, WGTEXP, written by Dr. M. Adena (Gregg 1981) was used for fitting a logistic model.

4.3 Results and observations

Fecundity

Temperatures 11° and 38°C were not included in the analysis since no eggs were produced in those temperatures. At 11°C two females survived for 70 days but died without laying any eggs. At 38°C all insects died within 3 days. At 15°C only one female and at 20°C two females produced eggs. In other temperatures not all females laid eggs even though they lived for long periods. Mean fecundity varied from 5 eggs per female at 20°C to 62.6 eggs per female at 30°C (Figure 4.3). The highest number of eggs laid by one female (88) were laid at 30°C followed by 26°C where 80 eggs were laid by a female. Analysis of variance on oviposition per female revealed that the oviposition at 30°C was significantly higher ($P < 0.05$) than other temperatures (Figure 4.3 and Appendix 3.1). A consistent pattern was found between oviposition rate (number of eggs per female per day) and temperature. The oviposition rate increased with the increase of temperature. When regression was performed between oviposition rate and temperature, a significant ($P < 0.001$) positive relationship was found (Figure 4.4). By and large, daily oviposition rate was highest in the first third of the oviposition period (Figure 4.5 A & B). Females survived significantly longer ($P <$

0.05) in lower temperatures than higher temperatures (Figure 4.6). However, female longevity did not influence total fecundity significantly ($P > 0.05$).

An inverse relationship between temperature and pre-oviposition period was found. The pre-oviposition period decreased significantly ($P < 0.001$) with the increase of temperature (Figure 4.7). The relationship between oviposition period and temperature was not significant ($P > 0.05$). At 15°C oviposition period was fairly long, but it dropped at 20 and 23°C and rose again at 26°C (Figure 4.7).

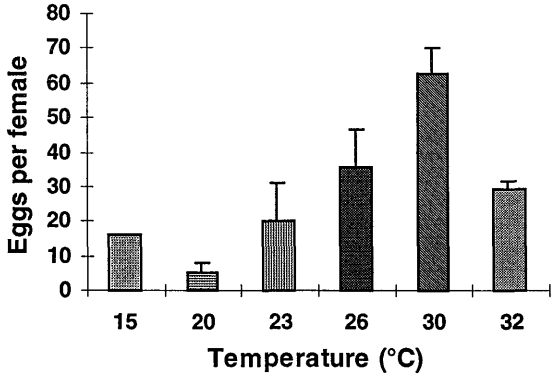


Figure 4.3 Oviposition of *C. dilutus* at constant temperatures. ($P < 0.05$, LSD = 25.7). Error bars indicate standard error of means.

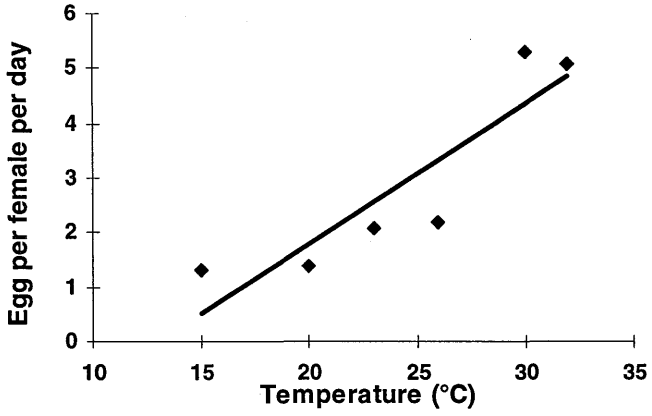


Figure 4.4 Relationship between oviposition rate of *C. dilutus* and temperature under laboratory conditions.

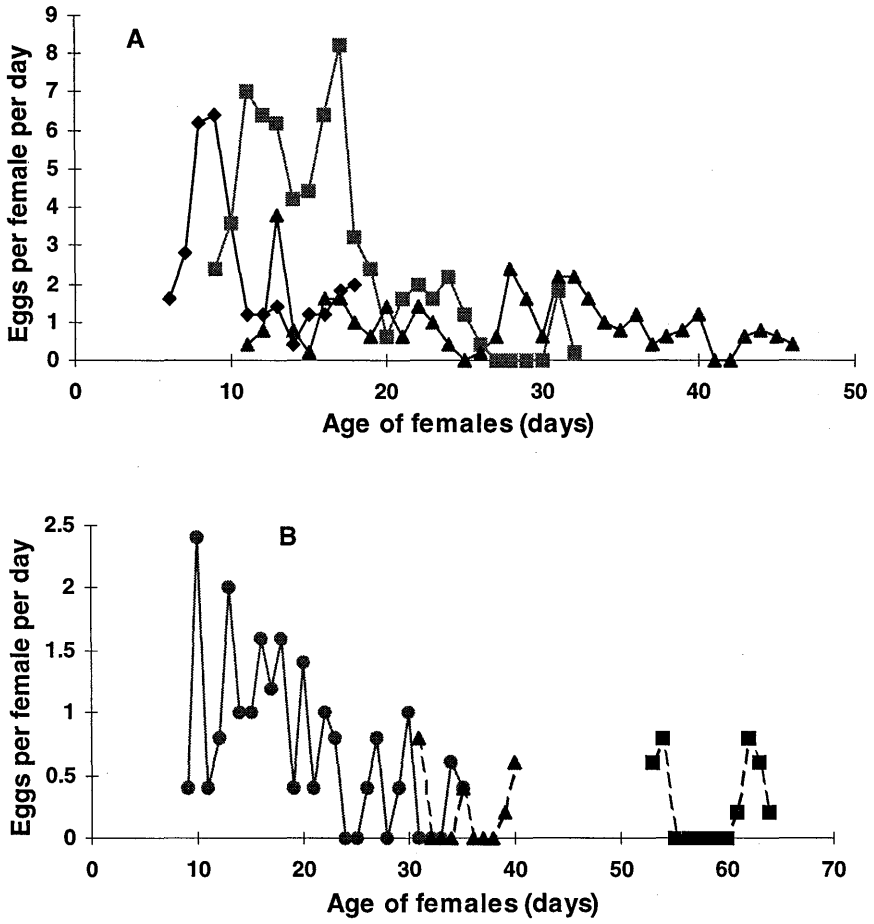


Figure 4.5 Age-specific fecundity curves for *C. dilutus* at constant temperature under laboratory condition. (A) for temperature 32°C —◆—, 30°C —■— and 26°C—▲— ; (B) for temperature 23°C—●—, 20°C ---▲---- and 15°C ---■----.

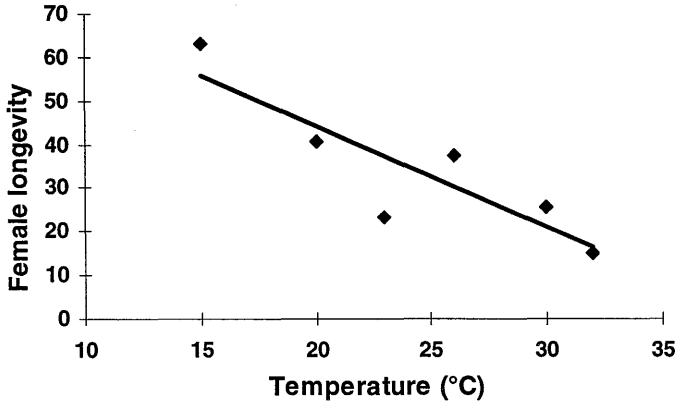


Figure 4.6 Female longevity of *C. dilutus* at constant temperature.

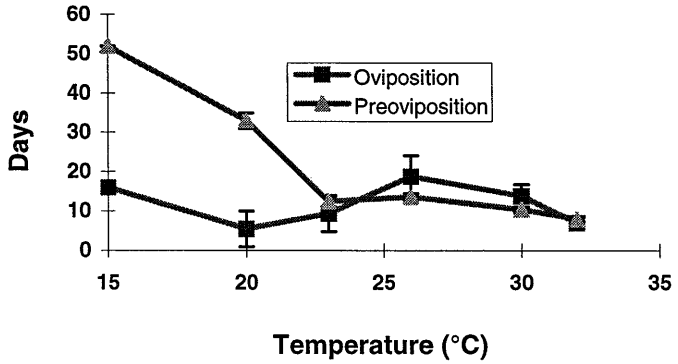


Figure 4.7 Oviposition and pre-oviposition periods of *C. dilutus* at constant temperature under laboratory conditions. Error bars indicate standard error of means.

Egg development and survival

Median egg development time and survival percentage are presented in Table 4.2. Temperatures 11, 15 and 38°C did not produce any nymphs. Development time decreased significantly ($P < 0.05$) with the temperature until 30°C, thereafter it increased again, but not significantly ($P > 0.05$) (Table 4.2).

Highest survival was observed at 26°C and analysis of variance and Fisher's least significance different test revealed that the difference from other temperatures was significant ($P < 0.05$) (Table 4.2 and Appendix 3.1b and 3.1c). Eggs at 11 and 15°C when checked under microscope showed no sign of development (bright red spots through the operculum, Chapter 3). At 38°C temperature the beans dried out which squeezed eggs to death.

Reciprocals of development time were taken to convert to development rate and when plotted against temperatures, the developmental rates showed a roughly sigmoid curve (Figure 4.8). Figure 4.8 and Table 4.2 reveal that the relationship between the development rate of GM eggs and temperature is linear up to certain levels. After that it is nonlinear and, therefore, to describe the curve a linear (day-degree) model as well as a logistic model was fitted.

Temperature °C	Median development time (days)	Survival percentage
11	*	0.00
15	*	0.00
20	15.84	75.26
23	9.84	91.34
26	6.68	95.55
30	4.92	92.60
32	5.14	89.40
38	*	0.00

Table 4.2 Median egg development time and percent survival of *C. dilutus* at constant temperatures. LSD = 1.28 for development time and LSD = 9.91 for survival. * not included for analysis since they did not hatch.

The day-degree model was derived from the regression equation of developmental rate on temperature. Since development rate slowed down at higher temperature (Table 4.2 and Figure 4.8) an assumption was made that the development rate above optimum temperature was the same as a corresponding value at the linear part of the curve when calculated from the formula-

$$2.\ T_{opt} - T$$

where, T_{opt}= optimum temperature for development;

T= temperature above optimum temperature.

Optimum temperature was determined from the regression analysis using different estimates of T_{opt} until the best correlation was found. Thus the day-degree model was described as-

$$1/y = a + bT \text{ for } T \leq T_{opt}$$

$$= a + bT^1 \text{ where } T^1 = 2.\ T_{opt} - T \text{ for } T > T_{opt}$$

where 1/y = development rate

Degree day (DD) requirement for development (the reciprocal of the slope b of the straight line) and threshold temperature (a/b) were calculated from the regression equation. For egg development of GM the regression equation was obtained from the data set where 30.5°C was found to be optimum. The equation from which linear model parameter was calculated was

$$1/y = -0.227 + 0.0144T \text{ for } (T \leq 30.5)$$

$$= -0.227 + 0.0144T^1 \text{ for } (T > 30.5)$$

where $T^1 = [(2 \times T_{opt}) - T]$

The r^2 value was 0.99. The lower development threshold temperature was calculated as 15.8°C and 69.4 DD above the lower threshold temperature would be required for complete egg development.

A modified logistic model was also fitted to the temperature-development rate data using an algorithm developed by Stinner *et al.* (1974):

$$\frac{1}{y} = C / (1 + e^{k1+k2T^1})$$

where C = (maximum developmental rate) x $\left(e^{k1+k2T_{opt}}\right)$, i.e. the asymptote

k_1, k_2 = empirical constants

T^1 = temperature T where $T \leq T_{opt}$

$= 2.T_{opt} - T$ where $T > T_{opt}$

T_{opt} = temperature at which the maximum development rate occurs

The model was fitted using the technique involving repeated regression analysis using different estimates of C and T_{opt} until the best correlation is found (Stinner *et al.* 1974, Gregg 1981). The fitted curve along with its inverse is depicted in Figure 4.8. The inverse equation is $y = a + be^{-cT^1}$. The r^2 value fitted with this model was 0.96. The parameter estimates from the logistic model with its inverse are given in Table 4.3.

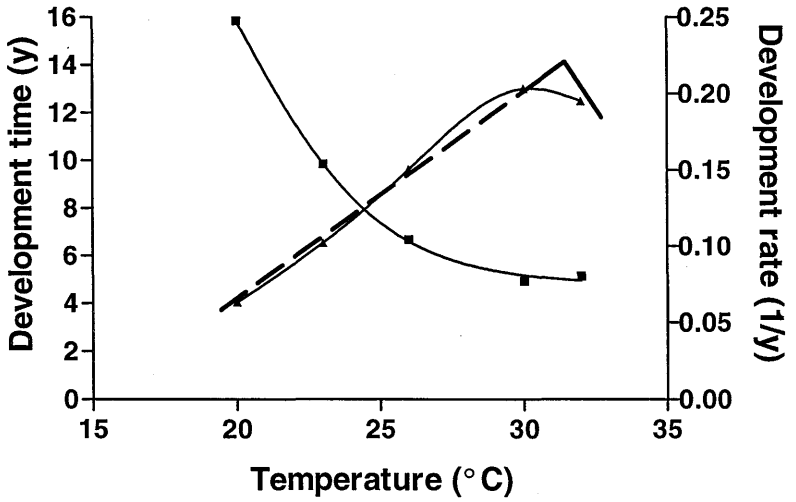


Figure 4.8 Egg developmental times of *C. dilutus* at constant temperatures showing linear (day-degree) and logistic models. ▲-----▲ linear (day-degree) model; ▲——▲ logistic model for development

Parameter				T_{opt}	r^2
Logistic[a]	C	k_1	k_2	30.5	0.96
	0.2807	5.7533	0.2259		
Logistic[b] Inverse	a	b	c		
	3.56	1122.9	0.2259		

Table 4.3 Nonlinear model parameters for egg development rate of green mirid at constant temperature. See previous page for the model equation.

No model was fitted for survival percentage data, however, an eye fitted curve is presented in Figure 4.9. Like development, survival also increased with the increase of temperature up to a certain level but thereafter it declined.

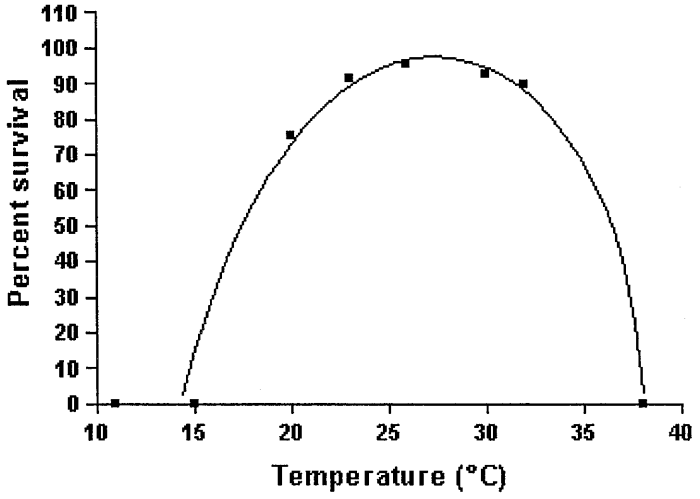


Figure 4.9 Survival percentage of green mirids egg at constant temperatures

Total nymphal development

Data of both sexes were pooled for analysis since male and female development times were similar (Foley and Pyke 1985).

Complete nymphal (from eclosion to adult) median development time and survival percentages are summarised in Table 4.4. Temperatures 11°C, 12°C, 15°C and 38°C (1995) were not included for the analysis of development time as not one nymph reached the adult stage in those temperatures.

At 17°C (1995), out of 40 insects, 8 insects from two replications (2 insects in one replication and 6 insects in another) completed their development and at 38°C temperature regime (1996) only one insect completed development. As expected, development time decreased significantly ($P < 0.01$) with the increase of temperature until the optimum temperature (Table 4.4). However, at temperature regimes in both extremes development slowed substantially (Table 4.4). Percent survival was also significantly lower at extreme high and low temperatures. Development time was shortest at 30°-32°C. Survival percentage was highest at 26°-32°C (Table 4.4). Analysis of variance indicated that percent survival was significantly higher ($P < 0.01$)

at 20°-33°C temperatures than at the extremes. However, there were no significant differences (P>0.05) between 20°-33°C (Table 4.4 and Appendix 3.1d).

When development rate (reciprocals of development time) was plotted against temperature, a roughly sigmoid curve was obtained (Figure 4.10). Since the relationship between complete nymphal development and temperature was nonlinear, as with egg, both linear (day-degree) and nonlinear models were fitted to describe the curve.

Temperature °C	Median development time (days)	Survival Percentage	Year of observation
11	*	0.00	1996
12	*	0.00	1995
15	*	0.00	1996
17	53.25	20.00	1995
20	33.78	64.38	1996
21	26.60	42.95	1995
23	19.68	56.78	1996
24	18.78	69.70	1995
26	13.05	80.00	1996
27	16.20	52.08	1995
30	10.73	80.00	1996
30	13.10	51.75	1995
32	10.68	77.50	1996
33	12.08	59.28	1995
38	15.50	2.50	1996
38	*	0.00	1995

Table 4.4 Complete nymphal median development time and percent survival of *C. dilutus* at constant temperatures. LSD = 20.9 for percent survival. * not included for analysis.

The day-degree model was derived from the regression equation of developmental rate on temperature. For complete nymphal development of *C. dilutus* the regression equation was obtained from the data set where 31.5°C was found to be optimum. The equation was

$$1/y = -0.0745 + 0.00538T \text{ for } (T \leq 31.5) \qquad (R^2 = 0.92).$$

$$= -0.0745 + 0.00538T^1 \text{ for } (T > 31.5)$$

$$\text{where } T^1 = [(2 \times T_{opt}) - T]$$

The lower threshold temperature and DD above the threshold temperature were calculated from the equation as 13.9°C and 185.9 respectively. A modified logistic model was also fitted to the temperature-development rate data. The fitted curve along with its inverse is illustrated in Figure 4.10. The inverse equation was

$$y = 9.36 + 2326.1e^{-0.2325T^1}$$

T^1= temperature T where $T \leq T_{opt}$
$= 2.T_{opt} - T$ where $T > T_{opt}$

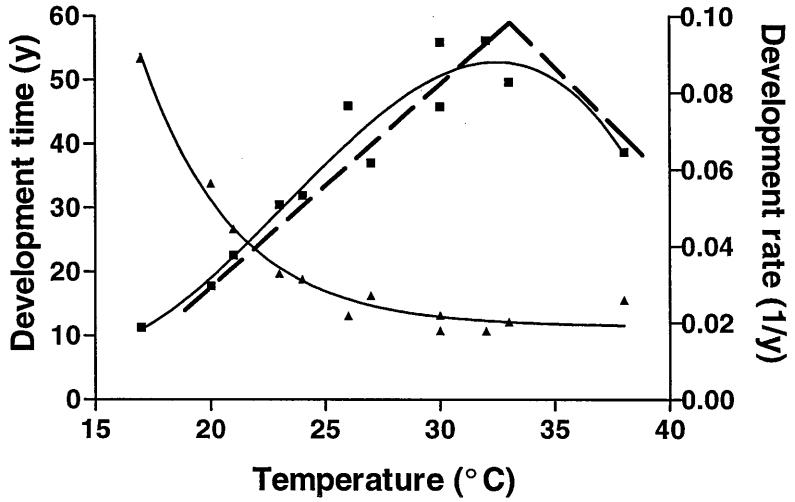


Figure 4.10 Complete nymphal developmental times of *C. dilutus* at constant temperatures showing linear (day-degree) and logistic models. ■-----■ linear (day-degree) model; ■——■ logistic model for development

The r^2 value of this model was 0.97. The parameter estimates from the logistic model with its inverse are given in Table 4.5.

No model was fitted for survival percentage data, however, an eye fitted curve is presented in Figure 4.11. Within the 20-33°C mortality was consistently around 50% and the curve became flat, indicating a fairly large temperature zone where GM nymphs could survive reasonably well. However, at temperature below 20°C and above 33°C GM survival fell steeply.

Parameter				T_{opt}	r^2
Logistic[a]	C	k_1	k_2	31.5	0.97
	0.1068	5.5151	0.2325		
Logistic[b] Inverse	a	b	c		
	9.36	2326.1	0.2325		

Table 4.5 Nonlinear model parameters for complete nymphal development rate of green mirid at constant temperature. See page 14 for the equation.

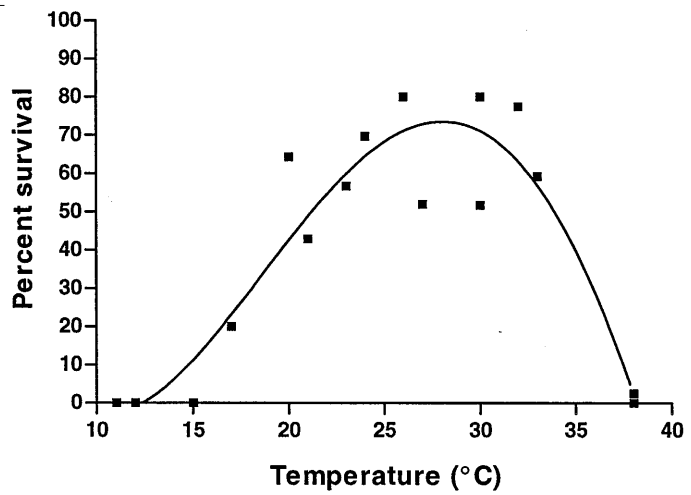


Figure 4.11 Survival percentage of nymphs of GM at constant temperature.

Development of individual instars

Median development time and survival percent for individual instars of GM are presented in Table 4.6.

First instars.

Development time for first instar nymphs of *C. dilutus* decreased steadily with temperature until 32°C, thereafter it increased again though the increase was not significant ($P > 0.05$) (Table 4.6). The lowest development time was observed in the 30°-32°C temperature range.

Survival percentage at 20°-33°C temperature range was quite high, but was lower above and below this zone and the difference was significant ($P < 0.05$). The optimum zone for first instar nymph survival was wide (Table 4.6). At a lower and

higher temperatures both development and survival were inconsistent compared to the optimum zone.

When development rate was plotted against temperatures a sigmoid curve resulted. The fitted curves and eye fitted survival curve are presented in Figure 4.12. The equation derived from the model was

$$1/y = 0.5359/1+e^{5.5906+.2536T}$$

and the inverse equation was $y = 1.8660 + 499.9\ e^{-.2536T}$

where if $T>31.5$ $T=2.T_{opt} - T$, 31.5°C is the optimum temperature.

Like total nymphal development, a day-degree model was also derived for first instar nymphs from the regression equation of developmental rate on temperature and the lower development threshold and DD requirement were also calculated. The lower development threshold temperature for first instar nymphs was 13.1°C and DD requirement was 32.2.

All the parameters calculated from modified logistic and its inverse and day-degree model for all individual nymphal instars are given in Table 4.7 at the end of this section.

A

Temperature (°C)	Median development time (day)					Year
	1st instar	2nd instar	3rd instar	4th instar	5th instar	
11	30.25	*	*	*	*	1996
12	29.65	8.60	*	*	*	1995
15	10.90	10.00	18.73	12.25	*	1996
17	9.73	10.18	12.40	9.28	11.25	1995
20	5.08	5.28	5.80	6.20	11.43	1996
21	4.65	4.98	4.93	4.58	7.48	1995
23	3.68	3.25	3.73	3.23	5.80	1996
24	3.13	3.13	3.48	3.70	5.35	1995
26	2.40	2.18	2.35	3.10	3.03	1996
27	2.55	2.75	3.53	3.35	4.03	1995
30	1.63	2.10	1.73	2.00	3.28	1996
30	1.90	2.53	2.55	3.63	2.50	1995
32	1.68	1.8	1.83	2.38	3.00	1996
33	2.60	2.13	1.85	2.60	2.90	1995
38	2.68	3.05	3.40	2.70	4.20	1996
38	3.03	2.85	2.25	3.00	*	1995

B

Temperature (°C)	Survival percentage					Year
	1st instar	2nd instar	3rd instar	4th instar	5th instar	
11	12.50	0.00	*	*	*	1996
12	36.82	75.65	0.00	*	*	1995
15	79.30	73.20	55.80	57.27	0.00	1996
17	61.28	86.85	92.15	90.35	48.75	1995
20	94.72	87.78	97.50	92.50	91.88	1996
21	100	95.00	90.00	81.45	76.50	1995
23	97.35	90.57	90.20	94.38	84.28	1996
24	95.00	86.28	96.22	99.57	92.62	1995
26	95.00	97.50	99.50	90.50	97.50	1996
27	96.88	86.80	83.32	87.78	97.30	1995
30	97.50	100	97.90	92.10	92.50	1996
30	93.22	87.62	83.78	93.13	94.00	1995
32	100	92.43	97.57	92.50	95.00	1996
33	93.93	81.40	91.28	93.40	99.03	1995
38	61.00	48.67	83.75	86.25	30.00	1996
38	50.40	33.75	0.00	*	*	1995

Table 4.6 Median development time (A) and survival percentage (B) for individual instars of *C. dilutus* at different constant temperatures. * indicate no insect passed through to that instar (A) and no insect survived (B).

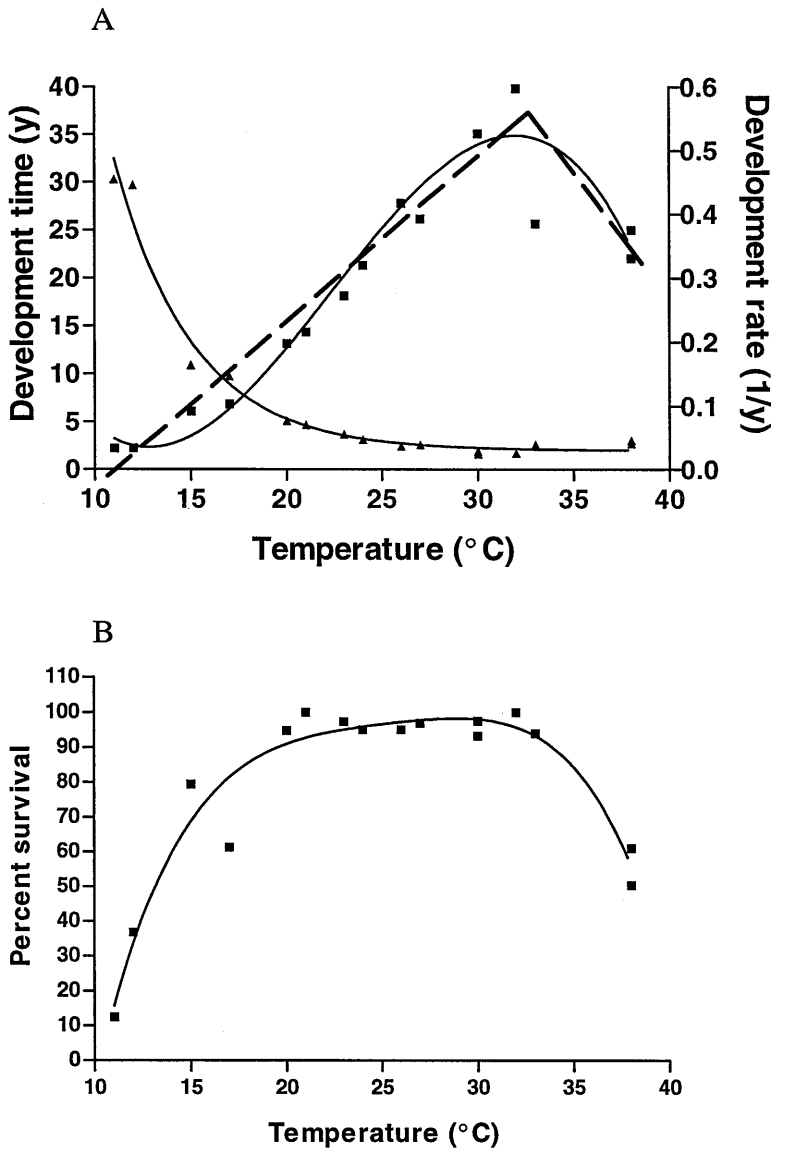


Figure 4.12 Development time (A) and survival percentage (B) of first instar nymphs at constant temperatures showing linear (day-degree) and logistic models.
■-----■ linear (day-degree) model; ■——■ logistic model for development

Second instars.

Development time decreased with temperature (Table 4.6). Development was fastest at 32°C, and after that started to slow. At 11°C not a single insect passed through to second instar. Mortality was higher at low and high temperatures (Table 4.6). At 17°-33°C mortality was fairly low.

Since development of second instar nymphs slowed down at high temperatures a modified logistic model was fitted. A day-degree model was also derived for linear development which occurred at 12°-32°C temperature range. The fitted curves and eye fitted survival curve are depicted in Figure 4.13. Unlike the first instars the sigmoid curve was shallow. The lower development threshold temperature and DD above the threshold temperature were calculated as 15.4°C and 25.8 respectively.

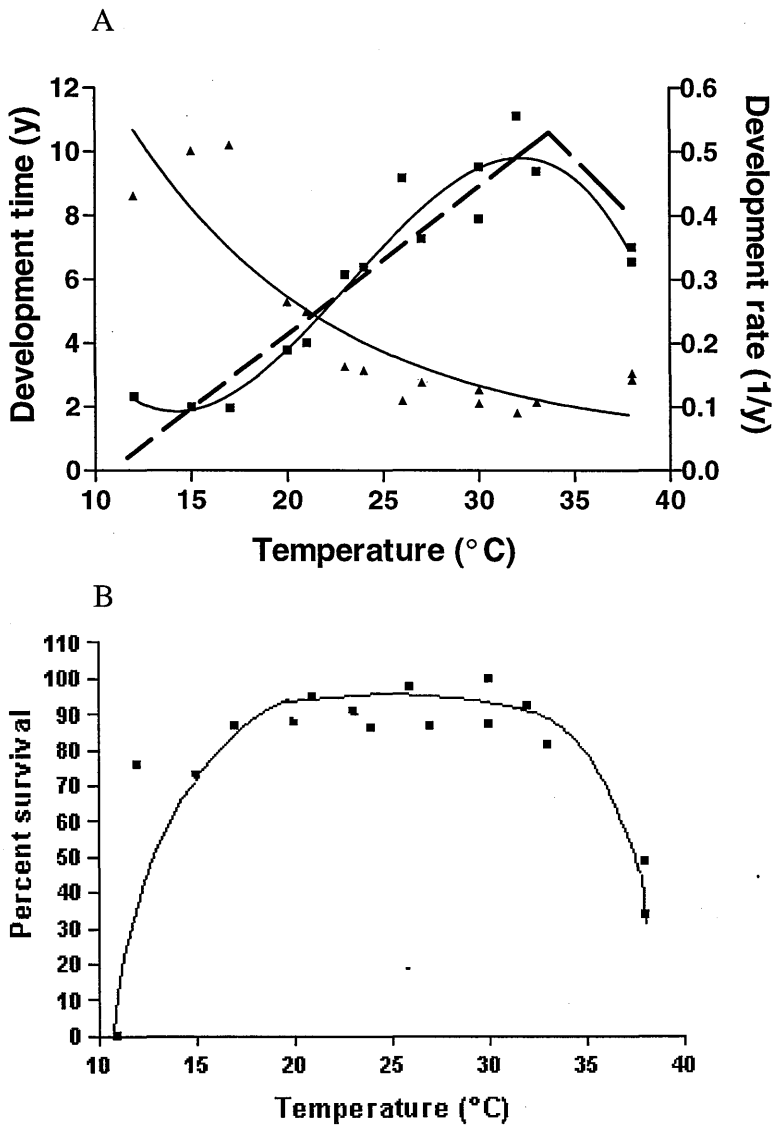


Figure 4.13 Development time (A) and survival percentage (B) of second instar nymphs at constant temperatures showing day-degree and logistic models.
■------■ linear (day-degree) model; ■————■ logistic model for development

Third instars.

Development in third instars was similar to that for the first and second instars (Table 4.6). Fastest development occurred at 26°-33°C. At 12°C and 38°C (1995) all nymphs died. Unlike first and second instars survival at high temperatures was reasonably high (Table 4.6). Analysis of variance indicated that there was no significant difference ($P > 0.05$) among the means at 15°-38°C temperature range.

The fitted curves for the modified logistic model are similar to that of first instars but sharper than second instars (Figure 4.14). Lower threshold temperature and degree day requirement above the threshold temperature were calculated from day-degree model as 14.3°C and 32.5 respectively. An eye fitted survival curve (Figure 4.14) illustrated a wide temperature zone ranging from 17°C to 38°C where mortality was low.

Fourth instars.

Significantly slower growth ($P < 0.05$) occurred at 15°C and 17°C and like previous instars fastest growth occurred at 30°-33°C. At high temperature development slowed down, though not significantly ($P > 0.05$). Except at 15°C survival percentage was quite high (Table 4.6) and there was no significant difference among the means ($P > 0.05$) (Table 4.6).

Figure 4.15 illustrates the development rate, the models fitted to describe development in fourth instars and the survival percentages. The lower threshold temperature calculated from the linear model was 10.4°C and the DD requirement above the threshold temperature was 50.8.

Fifth instars.

The development rate showed similar trends to those for fourth instar nymphs. With the increase of temperature development rate also increased, however, after

33°C development slowed down (Table 4.6). Fastest development occurred at 30°C. All nymphs died at 15°C. Unlike fourth instars, mortality at 17°C and 38°C was fairly high (Table 4.6).

Modified logistic and day-degree models were fitted to describe development. The fitted curves along with an eye fitted survival curve are presented in Figure 4.16.

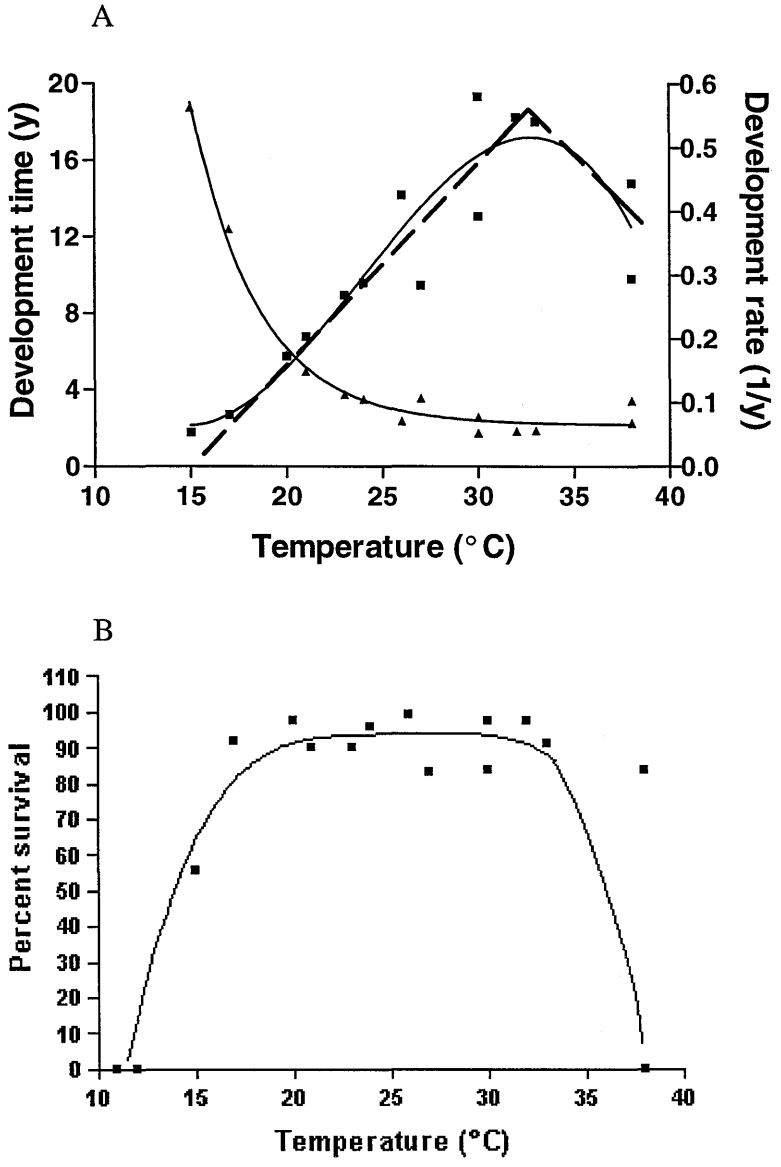


Figure 4.14 Development time (A) and survival percentage (B) during 3rd instar at constant temperatures showing linear (day-degree) and logistic models.
■------■ linear (day-degree) model; ■————■ logistic model for development

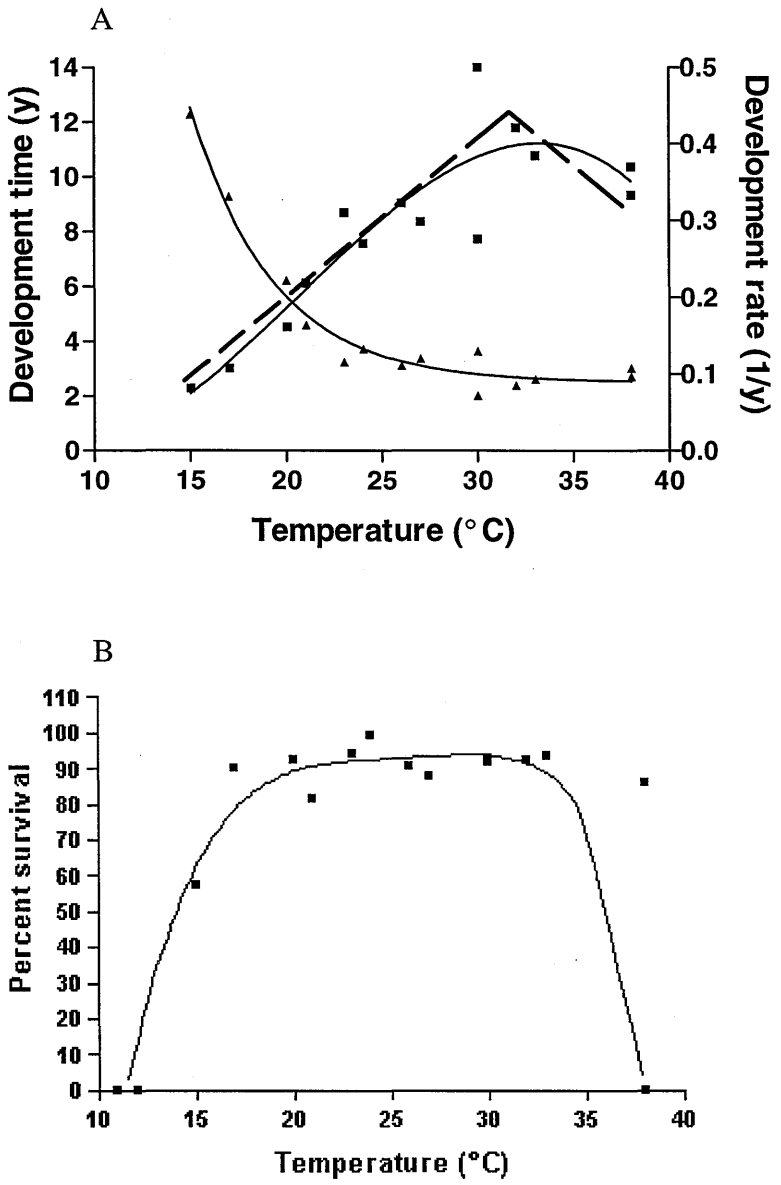


Figure 4.15 Development time (A) and survival percentage (B) during 4th instars at constant temperatures showing linear (day-degree) and logistic models.
■------■ linear (day-degree) model; ■————■ logistic model for development

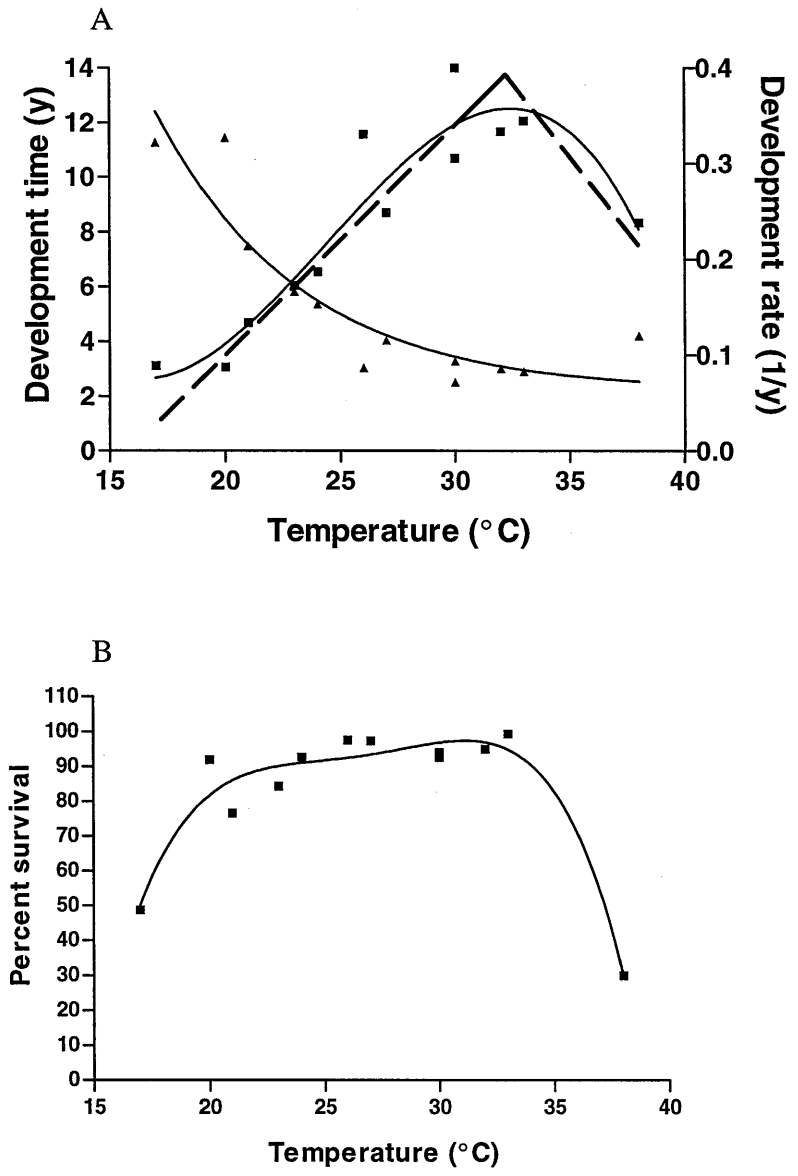


Figure 4.16 Development time (A) and percent survival (B) of fifth instar nymphs at constant temperatures showing linear (day-degree) and logistic models.
■------■ linear (day-degree) model; ■————■ logistic model for development

Parameter	1st instar	2nd instar	3rd instar	4th instar	5th instar
Logistic[(a)]					
T_{opt}	31.5	31.5	31.5	31.5	31.5
C	0.5359	2.4254	0.5566	0.4626	7.6687
k_1	5.5906	4.287	6.4816	4.7501	6.6343
k_2	0.2536	0.0989	0.2796	0.2135	0.1210
Logistic[(b)] **Inverse**					
a	1.8660	0.4123	1.7965	2.1618	0.1304
b	499.9	30.1	1173.1	249.9	99.2
c	0.2536	0.0989	0.2796	0.2135	0.1210
R^2	0.94	0.75	0.71	0.52	0.71
Linear[(c)]					
T_{opt}	30.9	32	32.4	31.9	30.9
a	-0.405	-0.278	-0.430	-0.210	-0.381
b	0.0310	0.0261	0.0304	0.02	0.0246
R^2	0.91	0.90	0.89	0.77	0.84
Day-degrees	32.26	40.49	32.89	50	40.65
Threshold	13.06	11.26	14.14	10.5	15.49

Table 4.8 Day-degree and logistic model parameters for individual instars of *C. dilutus* at constant temperature.

[a] $\frac{1}{y} = C / (1 + e^{k1 + k2T^1})$

[b] $y = a + be^{-cT^1}$

[c] $1/y = a + bT$

T^1 = temperature T where $T \leq T_{opt}$

= 2 x T_{opt} - T where $T > T_{opt}$

4.4 General Discussion

Temperature significantly influenced oviposition rate of *C. dilutus*. The average number of eggs laid per female per day was reduced as the temperature decreased. Khattat and Stewart (1977) also reported temperature effects for *L. lineolaris*. Female age is one important factor which influenced oviposition rate of *C. dilutus*. Early in their oviposition period the females laid more eggs per day. Old females thus contribute fewer offspring because their oviposition rate is low. This result agrees with those of Strong *et al.* (1970) for *L. hesperus* and of Kehat and Wyndham (1972b) for *Nysius vinitor*.

The number of eggs laid by a female of *C. dilutus* in her lifetime in relation to temperature was somewhat erratic and in almost all temperature regimens some females failed to oviposit though they lived for long enough. My findings agreed with those of Ridgway and Gyrisco (1960b) for *L. lineolaris*. They found that at 20°C some females oviposited and some failed to oviposit after a period of 28 days. It may be the females did not mate with the supplied male. This type of discriminatory behaviour of the female may arise from females being very selective in choice of mates or the inability of the males to attract females to mate (Alcock and Gwynne 1991).

I could not detect any significant relationship between temperature and the oviposition period of GM though temperature has significant influence on female longevity. However, unlike Jackson's (1987) work for *L. hesperus* where he found that the number of eggs deposited was related to longevity, in this work GM female longevity was not related to egg number. At lower temperature GM female lived longer but laid fewer eggs (Figures 4.3 and 4.6). Even in the higher temperatures where females deposited more eggs there was no positive relationship between fecundity and oviposition period or female longevity. This discrepancy may be due to the small sample size particularly when outliers dictated the overall result. For example at 30°C among the five females two females laid significantly more eggs than other three though they had significantly shorter oviposition period and longevity.

Temperature has a profound effect on egg and nymphal development of *C. dilutus*. Both egg and nymphal development increased with temperature until an optimum was reached, above which further increases in temperature resulted in retarded growth and in some cases increased mortality. For *C. dilutus* eggs and nymphs 38°C was found to be lethal which is comparable to results of Champlain and Butler (1967) for *L. hesperus* and Kehat and Wyndham (1972b) for *N. vinitor*. They found that 40°C is lethal to eggs and nymphs of both insects. Gaylor and Sterling (1975) also observed that high temperature is lethal to cotton fleahopper, *Pseudatomoscelis seriatus* nymphs. Development in an organism depends on rates of various biochemical reactions (Higley *et al.* 1986). Higher temperature within a certain range produces higher growth rates as reactions proceed more rapidly.

However, as temperature further increases diffusion of the reaction occurs (Metzler 1977, Higley and Pedigo 1984). Hunter-Jones (1970) and Sharpe and DeMichele (1977) suggested that low development rate at high and low temperature is a consequence of inactivation of a control enzyme or enzymes. In the case of *C. dilutus*, however, two physical factors seem to be responsible for mortality. At 33°C condensation contributed to mortality to some extent and at 38°C insects died due to desiccation. Whatever the reason, the symptom appearing during death was identical in all temperature regimens except 38°C. Prior to death the colour of the insects changed from green to dark green and the abdomen became flattened with pointed end and bent towards the head. At 38°C the colour of the insect changed from green to yellowish green. At 17°C the insects developing to adults were smaller in size and sometimes with deformed wings. These types of abnormalities due to high and low temperature are not uncommon. Jackson (1987) reported deformed *L. hesperus* emerging from continuous exposure to low temperature.

The lower development threshold temperature and day-degree requirement for *C. dilutus* egg was calculated to be 15.8°C and 69.4 DD respectively which is higher than Foley and Pyke (1985) but agrees with the findings of Kehat and Wyndham (1972b) for *N. vinitor*. The lower developmental threshold and DD requirements for individual instars and total nymphal development have been estimated from the linear models. Both threshold temperature and DD differed among developmental stages (see Table 4.6). The threshold value I got for complete nymphal development was 13.9°C which is comparable to that of Jackson (1987) for *L. hesperus*. The lower development threshold temperature for individual instars and complete nymphal development was 10-15°C indicated characteristics of insects adapted to mild to warm regions (Appendix 5.2). The study has also shown that the development rates for all individual instars were maximal at around 30°C. Early autumn, late spring and mild summer conditions therefore would be expected to favour development of *C. dilutus* populations. The study of population dynamics of the insect revealed similar population patterns in the field (Chapter 6), though developmental time data collected at constant temperatures in the laboratory can only be expected to provide rough estimates of developmental times in the field at fluctuating temperatures (Hagstrum

and Milliken 1991). Moreover, the development time I obtained from rearing on green beans may vary from development on cotton because some insects show significant effects of food on their development, for example *L. lineolaris* (Khattat and Stewart 1977).

Considering the value of coefficient of determination, R^2 for complete nymphal development nonlinear model (R^2=.97) fitted better than linear model (R^2=.92). However, for individual instars, except first instar, the coefficient of determination was lower in the nonlinear model. This may be due to lack of enough higher temperatures particularly above optimum. However, measuring the best fit only on R^2 value can be very misleading (Costanza 1989) because it can not detect where the lack of fit occurs (Kramer *et al.* 1991).

Though Foley and Pyke (1985) cautioned about the extrapolation of laboratory data to field situations, from this study it is evident that *C. dilutus* are likely to develop very slowly during winter in alternate hosts (Chapter 6). At the end of September, when temperatures start to rise, rates of development increase and produce large numbers of adults which may ultimately invade cotton during October onward and cause damage.

CHAPTER 5

THE DAMAGE ETIOLOGY AND PEST STATUS OF *Creontiades dilutus*

5.1 Introduction

The potential of the green mirid to damage cotton plants is well known in the cotton industry and most research effort has concentrated on the control of the pest (Bishop 1980, von Mengersen 1982, Adams and Pyke 1982, Adams *et al.* 1984, Chinajariywong *et al.* 1988) to the neglect of the basic biology and ecology of the pest. As a result, scientific knowledge of the basic biology, behaviour, ecology and even the pest status of this insect is lacking. Cotton growers and consultants are confused as to the nature or symptoms of green mirid damage as well as the insect's feeding behaviour. Miles (1995) attempted to establish an economic threshold for the green mirid, but her studies failed to give a definite threshold for the insect because of the cotton plant's ability to compensate for green mirid damage early in the season. She also failed to describe in detail the nature of the pest's damage and its feeding behaviour. The correct description of green mirid damage is important because it will enable cotton growers and consultants to differentiate correctly between green mirid damage and damage to cotton plants by other sucking pests such as apple dimpling bug (*Campylomma liebknechti*) and thrips (*Thrips tabaci, T. imaginis and Frankliniella schultzei*).

In this chapter, I investigated the preference of GM for cotton plants at different growth stages. I also investigate GM feeding symptoms on young plants, squares and bolls and determine the type of damage associated with each feeding symptom and on each plant part. In addition, I describe the damage-density relationship of GM for young plants, squares and bolls.

Unless otherwise stated all damage etiology experiments were conducted at the Australian Cotton Research Institute (ACRI), Narrabri. The plants used were Sicala V I and Sicala V II and GM reared in the laboratory were used.

5.2 Preference of green mirids for different stages of the cotton plant

Preference of GM for different stages of the cotton plants was measured by releasing GM adults on the cotton plants and recording the numbers of insects and eggs laid on each plant stage.

5.2.1 Materials and methods

Insects

GM adults were collected with a suction machine (Chapter 2) from lucerne, *Medicago sativa,* from an interplanted lucerne/cotton IPM field (Mensah and Khan 1997) at ACRI, 24 hours before the experiment was set up. The insects were collected from the suction machine bag using an aspirator, sexed and then each sex was placed in separate rearing cages. The insects were kept without food for 24 hours before being used for experiments.

Plants

The cotton plants were grown in a glasshouse in pots (25cm) at different dates so that plants of different sizes and of different growth stages (such as young plants, plants with squares only, plants with squares and bolls and plants with bolls) could be established. Plants in each pot were thinned to one per pot. The experiments were conducted in an open mesh house. Five days before the experiments were conducted the potted cotton plants were transferred from the glasshouse to the mesh house to enable plants to acclimatize with the outer environment.

Experiment set up

A randomized complete block design consisting of five treatments and four replicates was established in the mesh house. The treatments were:

1. Young plants with 6 true leaves
2. Plants with all squares removed but with tips present
3. Plants with squares, no bolls and tips removed
4. Plants with squares, bolls and tips
5. .Plants with bolls but no tips and squares

The plant parts were removed using scissors to establish the treatments two days before the insects were released. This was to make sure the wounds were completely healed and avoid fluids oozing out which may interfere with the behaviour of the green mirids (Mensah, pers. comm 1995). Pots from each treatment were placed in a nylon mesh cage (300 x 250 x 150 cm) and sixty pairs of GM adults were released into the cage to oviposit on the plants for seven days. After seven days the plants were removed to the laboratory and the numbers of eggs per plant were counted under the microscope (X 16) and recorded.

Data were analyzed using MINITAB (Ryan *et al.* 1992) and the means were separated by Fisher's Least Significant Difference test at 5% levels.

5.2.2 Results and observations

It was observed that when the insects were released in the cage they flew and landed on the top and sides of the cage. However, within 24 hours the insects had moved and colonised the plants. The highest numbers of mirids were recorded on plants with squares, bolls and tips followed by plants with bolls but no tips and squares (Figure 5.1A). Young plants with 6 true leaves had the lowest numbers of mirids (Figure 5.1A).

The highest numbers of eggs per plant was recorded in plants with squares, bolls and tips indicating they were the most preferred plant type. This was followed by plants with bolls but no tips and squares (Figure 5.1B). The least numbers of eggs were recorded on plants with 6 true leaves (Figure 5.1B).

A significant and positive relationship ($P < 0.05$) was established between green mirid numbers and the numbers of eggs laid on each of the treated plants (Figure 5.2).

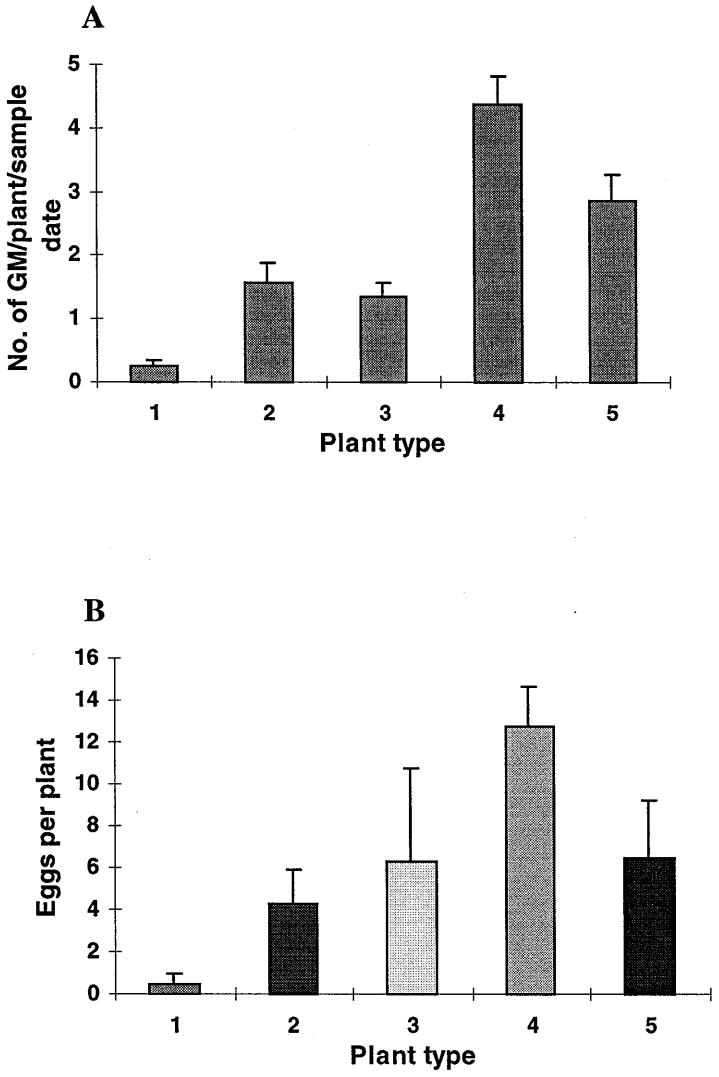


Figure 5.1 Preference of green mirid for different plant types in a free choice experiment in the mesh house. A. GM adult numbers, B. GM egg numbers. Plant types: 1= young plants with 6 true leaves; 2= plant with all squares removed but with tips; 3= plant with squares, no bolls and tips removed; 4= plant with squares, bolls and tips; 5= plant with bolls but no squares and tips.

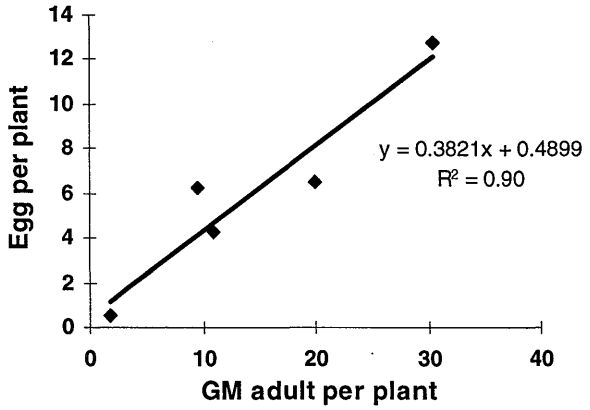


Figure 5.2 Relationship between green mirid adults per plant and eggs per plant in a free choice experiment in the mesh house.

5.2.3 Discussion

Green mirid adults prefer to feed and oviposit on plants with squares, bolls and tips. Whatever plant stage the green mirid selected, the insects preferred to stay inside the bract of squares, bolls and tip of the plants. The choice of the plants with squares as preferred feeding sites may be influenced by the nutritional status of the squares which may be rich in sugar. In the squares GM mainly feed on anthers, in particular pollen grains (see section 5.6, in this chapter), which are known to be rich in sugar. The importance of sugar in the development of mirids has been reported by several researchers in the USA for *Lygus hesperus* (Curtis and McCoy 1964, Landes and Strong 1965, Butler, Jr. 1968 and Benedict *et al.* 1981).

Host selection by GM may depend on several behavioural modifying cues. The chemical stimuli from the different parts of the host plant may play a role in the selection of a particular host plant. The role of the chemical stimuli in host selection of the adult females may be very important in determining the preferred host for oviposition and survival of the offspring (Jones 1991). Apart from the chemical cues, colour and other morphological factors such as the hardness and hairiness of the host plant, particularly at the oviposition sites (Chapter 3), may also play a significant role in host selection. The hardness and hairiness of the cotton plant can affect the choice of a plant as an oviposition or feeding site. The green mirid adults usually deposit their eggs in the leaf pulvinus and into the base of the petioles. These two oviposition sites

are relatively soft and less hairy in young cotton plants with squares and growing tips. As the plants grow and mature, the oviposition sites become relatively hard and hairy and less attractive to GM for feeding and oviposition. Walker *et al.* (1974) and Ring *et al.* (1993) found that the severity of yield loss from cotton fleahopper, *Pseudatomoscelis seriatus* was influenced by trichome density. The higher the trichome density, the lower the damage and hence yield loss.

This study shows that GM introduced in the cage moved from plant to plant before settling on preferred plants. This could indicate that green mirids after migrating into a cotton farm may take 1 or 2 days to select the most preferred plant to feed and oviposit on. It may therefore be possible that crop damage may not result from GM migration until after the period when host or plant selection is complete. The control of GM following migration should therefore not be solely based on insect numbers per plant but in conjunction with fruit retention.

5.3 Investigation of feeding mechanism of *Creontiades dilutus* using a light microscopy

Green mirids are known to feed on the host plant tissues by inserting their stylet into the phloem and xylem vessels of the plant. The insertion of the stylet into these vessels may destroy some of the plant tissues in the process. A study was therefore conducted in the laboratory to investigate the process of penetration of GM stylets into the plant tissues and determine the nature of damage caused to the plant tissues during penetration of the stylet. The experiment was conducted in the Department of Agronomy and Soil Science at the University of New England (UNE), Armidale in 1995 and 1996.

5.3.1 Materials and methods

Investigation of feeding mechanisms using a camera mounted dissecting microscope

The insects used for this study were 4th instar nymphs. The nymphs were hatched from eggs laid by the insects reared on green beans in a controlled environment cubicle (Chapter 4). The insects used in the study were starved for 24 hours before being introduced onto a feeding arena.

The feeding arena was made using a transparent 9 cm glass petridish (Pyrex, USA) so that feeding could be readily observed through the petridish covering. A cotton terminal 5 cm long was excised from a 6-true leaf Sicala VII potted cotton plant. The petridish was lined with a water soaked filter paper to keep the excised cotton terminal fresh for a longer period. A cotton leaf disc was placed on the filter paper along with a terminal on top of the leaf. The leaf disc was provided to make the insect recognize the feeding arena and the excised terminal as a cotton plant.

The movement of the stylets through the tissues of the excised cotton terminal was observed and photographs taken under a dissecting microscope (Zeiss SR, West Germany) with a mounted Olympus OM2 SP photographic camera.

Investigation of feeding mechanisms using light microscopy technique

In this study, GM adults were introduced onto a young cotton plant with 6 true leaves and squares to feed. The insects used for this observation were from the same culture mentioned above, and were confined to the young plants in a similar manner to the method described above. However, the insects were confined to the squares using a small plastic cage similar to the one described elsewhere (Chapter 2 and section 5.5.1). The different parts of the plants (terminals and squares) were examined for damage symptoms. When damage was observed in any of the parts, they were removed, cut into a small piece, about ½ cm and, along with a piece of undamaged plant part not exposed to the insects, immediately fixed with FPA solution for 48

hours. The specimens were then dehydrated through a series of 50, 70, 85, 95 and 100% of standard tertiary butyl alcohol, infiltrated in paraplast (Oxford Labware, USA) using Tissue Tek II (Tissue embedding center, AMES Co. Ltd., Australia). This was sectioned at 15 µm on a rotary microtome (Spencer AO 820, A. S. Aloe Co., St. Louis, MO, USA), mounted on glass slides, dewaxed, stained with 1% aqueous safranin, counterstained with 0.5% ethanolic fast green, coverslipped, dried up and then observed under a compound microscope (Zeiss Standard 20, West Germany). Photographs were taken using a photographic camera mounted on the microscope. A total of 100 stem paraffin sections and 50 square sections (damaged and undamaged) were examined in this study.

5.3.2 Results and observations

Feeding mechanisms of GM using the camera mounted dissecting microscope

The GM has piercing-sucking mouth parts. The mouth parts consist of maxillary and mandibullary stylets fused together into a tube. The tip of the mandibullary stylets is serrated and is the main organ used to pierce plant tissues. The stylets, when at rest, lie in a groove in the labium which is held below the prothorax. The mirids were observed to groom their stylets with the aid of forelegs before and in between feeding. They held the smooth part of the stylets with their forelegs and moved the stylets up and down. During feeding, the insects frequently probed several sites before forcing the stylets into the vascular tissues of the plant where feeding occurs. Feeding was observed on one occasion to last for 5 minutes. Once the insect has forced the stylets into the plant tissues, the flexible part of the stylet is moved rapidly in different directions in combination with plunging and withdrawing actions. During these processes, the insect secretes a saliva into the vascular tissues. It was observed under the microscope that the repeated feeding and watery secretions lysed the feeding cells immediately after feeding.

Feeding mechanisms of GM using the light microscopy technique

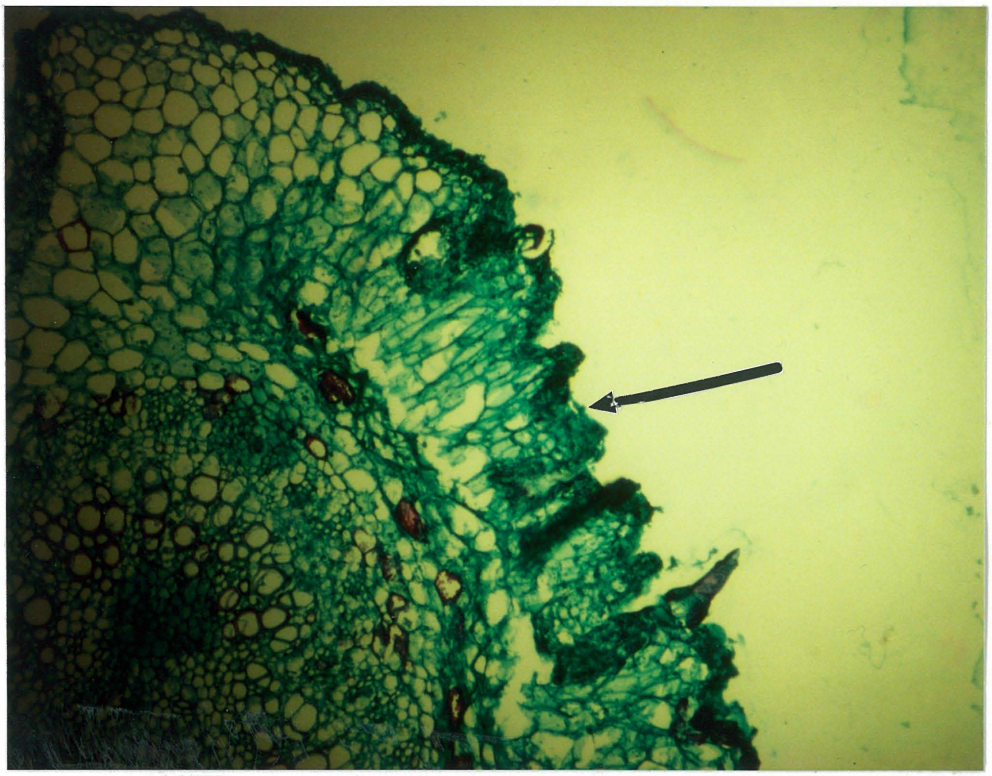
Light microscopy studies of the damaged cotton plant tips showed that the stylets of GM as they penetrate the plant tissues formed a tubular track which passed through several cells of the bundle sheath into the vascular bundle. The plant parts fed upon showed various degrees of internal cellular damage. The degree of damage depended on the magnitude of feeding. Both epidermal cells (Plate 5.1A) and vascular bundles (Plate 5.1B) were destroyed during GM feeding. This may result in either partial (Plate 5.4A) or complete damage to the tips (Plate 5.4C).

Light microscopy of the damaged cotton squares revealed that the anthers, particularly the pollen grains, were the main feeding sites. The pollen sacs of cotton flowers contain pollen grains. However, when these sacs were fed upon by green mirids, the pollen grains lost their turgidity and coalesced in the middle of the pollen sacs. This may have led to flattened pollen grains (Plate 5.2A). GM feeding also caused the break down of the cells of staminal columns (Plate 5.2B).

5.3.3 Discussion

The feeding mechanism shown in this study is similar to that of other heteropterans, particularly *Lygus* spp., which have been reported by several researchers such as Muir and Kershaw (1911), Flemion *et al.* (1954), Elmore (1955), Flemion (1956), Miles (1968), Pollard (1969), Strong (1970), Hori (1971a, 1971b) and Miles (1972). GM probed several times in several sites on the plant with the stylets before finally penetrating the plant tissues with the stylets and commencing feeding. This may be to test the host plant for suitable feeding sites or to determine the suitability of the plant for feeding, and if acceptable the stylets are inserted. GM insert stylets into the plant tissue by bending the labium as stylets do not elongate. Thus the greater the bend in the labium the deeper the penetration. Flemion *et al.* (1954) and Miles (1968) also reported similar patterns of stylet action for *Lygus* spp. Miles (1958) classified this stylet movement into three actions as 'advancing', when the stylets are

A.



B

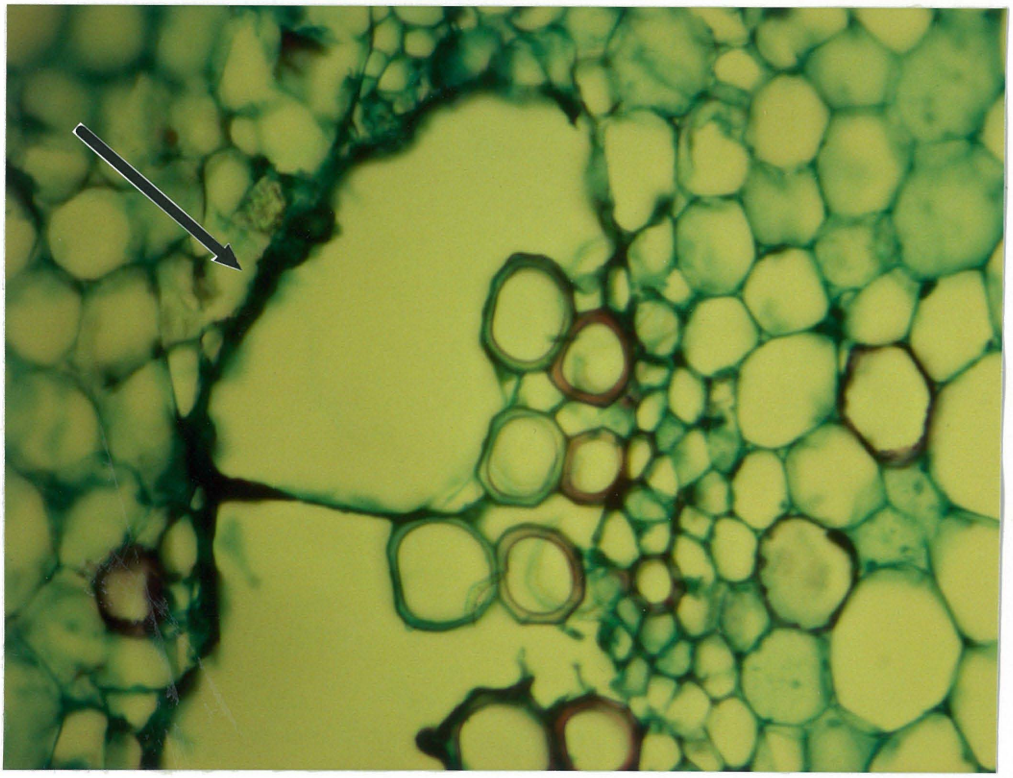


Plate 5.1 GM feeding damage showing destruction of epidermal cells (A) and vascular bundles (B)

moving into a region not pierced before; 'pumping', when the stylets are moving along tracks already made in the tissue; and 'retracting', when they are being withdrawn over some distance either prior to final withdrawal or to begin a new advance in some other direction. The watery saliva secreted during feeding in the plunging and withdrawal process may mainly consist of pectinase (Hori and Miles 1993; Tingey and Pillemer 1977) and this pectinase may be responsible for cell damage during GM feeding. The pectinase secreted by GM in the course of feeding dissolves the middle lamella, thus aiding the penetration and maceration of plant tissue. The mechanical damage identified in this study as a result of the penetration of the stylet is similar to that reported by Smith in 1926 and Flemion *et al.* in 1954 for *Lygus* spp.

It appears that a great deal of injury can occur during feeding processes. Histological study on damaged plant parts (stem apex and leaf petiole) showed that GM feeding caused destruction of epidermal cells and extended into the vascular bundle region. In severe cases cavitation might occur on feeding sites (Plate 5.1). Addicott and Romney (1950) and Flemion *et al.* (1954) also found similar types of damage for *L. hesperus* in guayule and *L. lineolaris* in bean crops. Depending on the degree of feeding and the severity of cell destruction, cotton plants, particularly young plants, can show different types of symptoms from localized to complete wilting. When cavitation occurs in the vascular bundle regions of the stem apex (base of the terminals) as a result of GM stylet penetration, it may hinder or prevent the conduction of plant nutrients from the roots to the terminals and leaves. This may result in the wilting of the terminals shown in plate 5.4C. If the cavitation occurs in the vascular bundle regions of the leaf petiole, this may result in the wilting of that particular leaf. However, if epidermal cells only are destroyed during feeding, the particular plant part may not show any wilting symptoms at all. Therefore, the wilting damage to the plant part may depend also on the type of cell destroyed during the course of feeding.

GM feeding on squares caused partial to complete damage to the anthers, particularly to pollen grains. Pollen grains lose their turgidity and coalesce in the middle of the pollen sac. Destruction of pollen grains may trigger abscission of squares. Ethylene is responsible for abscission and auxin retards abscission (Morgan

A

B

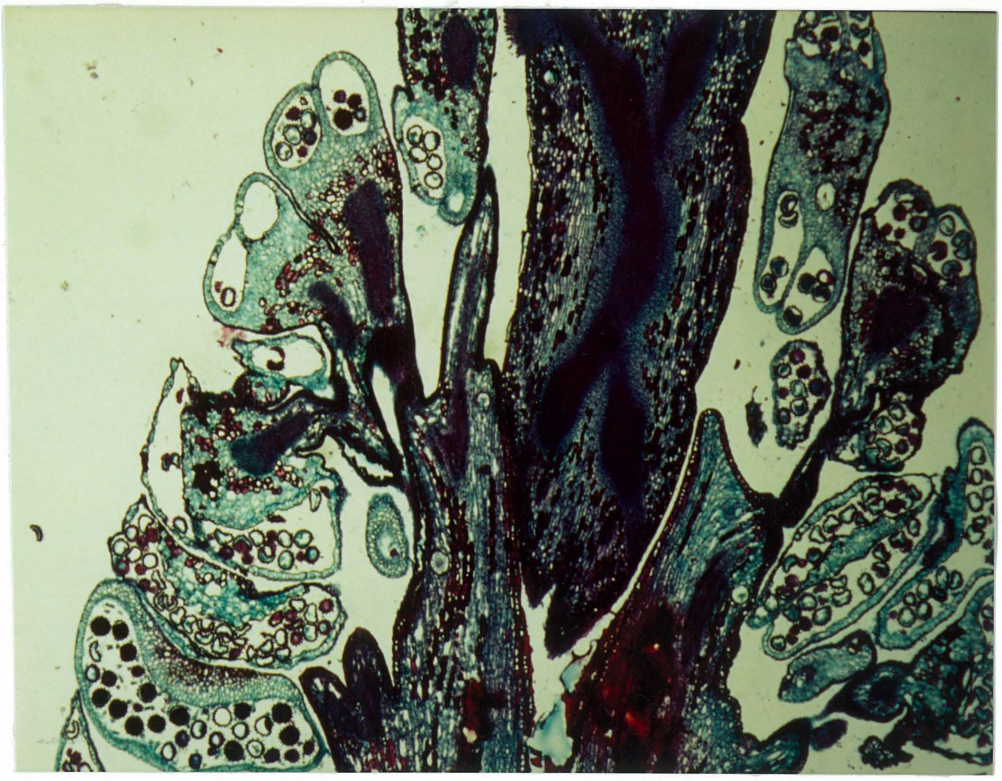


Plate 5.2 Damaged pollen grains (A) and staminal columns (B) due to GM feeding on squares

1969). Pollen grains in cotton contain auxin (Strong 1970). Due to GM feeding on squares, auxin producing pollen grains are destroyed, which may cause imbalance of hormones associated with abscission and as a result squares abscise. Pectinase may also have a major role in abscission. Pectinase may cause induction of ethylene synthesis to levels sufficient to cause abscission. Grisham *et al.* (1987) showed that cotton fleahopper feeding induced ethylene production at peak level within 72 hours. Sometimes necrosis developed on the staminal column and depending on the severity of the necrosis the staminal column may be completely destroyed. This type of damage has also been reported by Pack and Tugwell (1976) and Mauney and Henneberry (1979) for *L. lineolaris* and *L. hesperus* respectively.

5.4 Damage potential of different stages of green mirid in cotton plants

Damage caused by the GM is cumulative and is apparent from the 1st nymphal stage. In this experiment, therefore, I studied the cumulative effect of the different stages of GM and determined the extent to which the GM damage affects the growth of the cotton plants.

5.4.1 Materials and methods

Insects

Adult mirids were collected from lucerne *(M. sativa)* and reared in cages in the laboratory on beans as described elsewhere in the thesis (Chapter 2 and Appendix 1).

Plants

Cotton plants (variety Sicala V II) were used for the study. Plants were grown in pots (12cm) in the glasshouse and thinned to one plant per pot. The temperature in the glasshouse was not controlled and ranged from 35°-40°C with a relative humidity of 50-70 percent. The plants at 4 true leaf-stage were transferred to the laboratory

which was maintained at 25°C and a relative humidity of 40-60 percent (Chapter 3) for experimentation.

Experiment design

Forty plants at 4 true leaf stage (one plant per pot) were each infested with one GM first instar nymph. The first instar nymph was transferred to the plants using a small camel hair brush. The plants with the nymphs were enclosed in a plastic cage (Plate 5.3). Twenty four uninfested plants were also enclosed in similar plastic cages and used as controls. The treated and the control plants were checked daily and when the nymphs moulted to the next stage, four plants were randomly selected and the insects were removed. These four plants represented first nymphal stage damage. This procedure was done for second, third, fourth and fifth stage nymphs respectively. The four treated plants representing damage for each GM nymphal stage were given a damage score after 2 days by comparing them to the control plants. When the insects on the remainder of the forty treated plants turned adult, the insects were allowed to feed for three days after emergence. The three days allowed for the adult feeding corresponds to the feeding time available at each of the nymphal stages before moulting to the next stage. After three days of adult feeding, four plants were randomly selected (as was done for the nymphal stages) and a damage score was assigned to them. Immediately after assigning damage scores to each treated plant, leaf, stem and root, and the green leaf area for each treated and control plants were measured using an area meter (LI-COR L1-3100, USA). The fresh weight of the plant parts were also taken for each treated and control plants. Thereafter the leaves, stems and roots were put in paper bag separately and kept in a Forced Air Heater (Hurricane Forced Air Heater, Wessberg & Tulander Pty Ltd, Australia) at 70°C for 72 hours to dry, and the dry weights were recorded.

Damage score

GM feeding on young plants can cause localized damage such as browning around the feeding sites. When feeding occurs on the leaf, tips of leaves, terminals or

branch primordia, the feeding sites turn brownish to blackish and later dry off. The extent to which the leaf will dry off depends on the severity of GM feeding. My observations show that the damage symptoms resulting from GM feeding vary depending on the degree or duration of feeding. I quantified GM feeding damage by scoring damaged plants based on damage symptoms. Damage scores assigned were as follows;

Damage criteria	Score
No damage	0
Tips of folded leaves or tips of 20% branch primordia brownish	1
Tips of unfolded and folded leaves and 21-40% branch primordia partially damaged	3
Terminal and 41-60% branch primordia partially damaged	5
Terminal and >60% branch primordia damaged and dried off	7

Damage percentage can be calculated from the following formula:

$$\text{Damage percentage} = \frac{\sum damage\ scores\ for\ plant}{7\,(number\ of\ plant\ checked)} \times 100$$

Unless otherwise stated damage scores as calculated above were used to differentiate the extent of feeding damage.

Plate 5.3 Plastic cages used to study GM damage

5.4.2 Results and observations

Damage symptoms

Green mirids feed mainly on meristematic tissues, particularly on growing tips, branch primordia and young leaves on the seedlings. Depending on the feeding site and the severity of feeding, plants developed different damage symptoms which were usually visible within one or two days after feeding. When GM fed on the leaf margin or top of the unfolded leaves, the area around the feeding site turned brown, then black and eventually dried off (Plate 5.4A). However, if the insect fed on the base of the leaf petiole or tip of the leaf, the whole leaf or tip of the leaf became less turgid, droopy (Plate 5.4B) and then dried off. If the insect fed on the base of every leaf and tips of an infested plant, the whole plant dried off and died (Plate 5.4C). Apart from plant tips and leaves, GM can also feed on young stems and petioles. At all feeding sites, feeding lesions were characterised by brown areas with a black spot in the middle, indicating where the stylets penetrated the plant tissue.

Green mirid stages that are damaging to the young cotton plants

Both nymphs and adults caused similar types of damage to the cotton plants. Damage caused by the different nymphal and adult stages of green mirid are shown in Figure 5.3. The study showed a significant difference ($P < 0.001$) between cumulative damage caused by the different nymphal stages of GM (Appendix 4.2a). The lowest damage was caused by the 1st stage nymph followed by 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and adult stages respectively. However, the difference between the damages caused by the 3^{rd} to 5^{th} and adult stages were not significantly different ($P > 0.05$). In general, the damage caused by GM was cumulative and was maximal when the insect reached the 4^{th} and 5^{th} nymphal stage in its life cycle.

Plate 5.4 GM damage showing (A) partially damaged, (B) terminal drooping and (C) wilting of young cotton plants

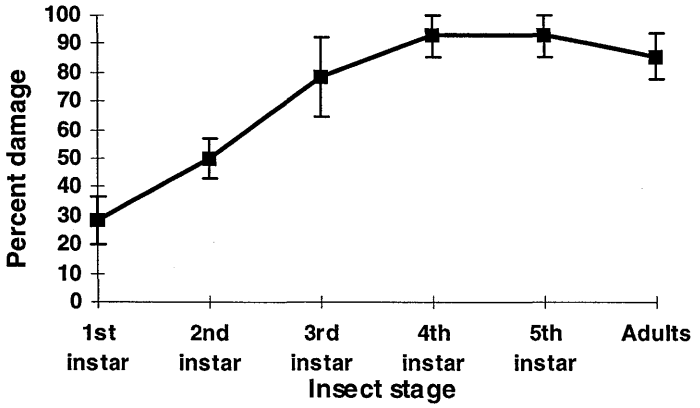


Figure 5.3 Percentages of damage caused by the different stages of *C. dilutus* on young cotton plants in the glasshouse at ACRI, 1994-95. (Error bars indicate standard error of mean).

Green mirid feeding and plant growth

Table 5.1 shows that the feeding of different stages of GM can affect the leaf area, and leaf and root weights of cotton plants and this directly affects the growth of the plants. The growth effect on the plant was cumulative and reached its peak when the insects were in their 4^{th} and 5^{th} stages. Analysis of variance on means indicated that there was a significant difference ($P < 0.05$) between the leaf area, and leaf and root weights of plants fed upon by the different stages of GM (Appendix 4.2b-4.2d). The reduction of stem weight was not significantly different ($P > 0.05$) (Table 5.1 and Appendix 4.2e). However, stem weight decreased when the insects reached the adult stage. The leaf area and leaf weights of plants fed upon by 3^{rd} to 5^{th} stage nymphs and adult GM were significantly different ($P < 0.05$) from the control plants (Table 5.1) However, the leaf area and weight of cotton plants infested with the 1^{st} and 2^{nd} stage nymphs were not significantly different to the control plants (Table 5.1 and Appendix 4.2b & 4.2c).

Insect stage	Leaf area (cm^2)	Leaf weight (g)	Stem weight (g)	Root weight (g)
1st instar	47.50ab	0.18a	0.08a	0.03ab
2nd instar	42.49ab	0.16a	0.08a	0.04ab
3rd instar	30.66bc	0.11b	0.08a	0.03ab
4th instar	10.37d	0.04c	0.07a	0.03ab
5th instar	5.97d	0.02c	0.06a	0.03ab
Adult	17.96cd	0.06bc	0.05a	0.02b
Control	52.25a	0.18a	0.05a	0.05a

Table 5.1 Effect of feeding by different stages of *C. dilutus* on the leaf area, leaf and root weights of potted cotton plants in the glasshouse at ACRI, 1994-95. Means within a column followed by the same letters are not significantly different (P > 0.05) (Fisher's least significant difference test).

As would be expected, a significant negative relationship was established between severity of damage per plant and leaf area and leaf weight of the plant (Figure 5.4).

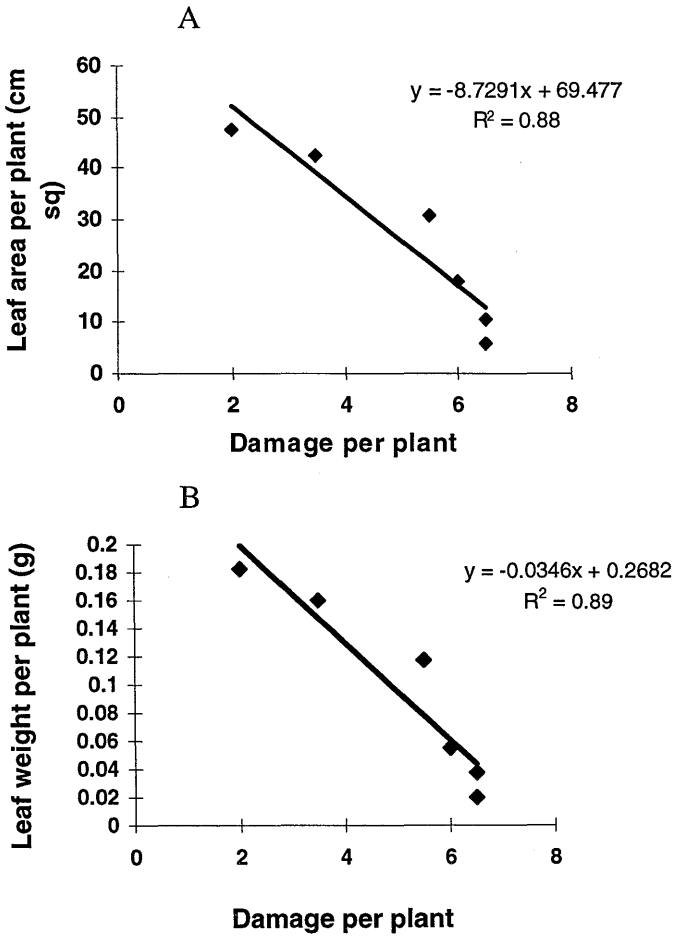


Figure 5.4 Relationship between severity of damage per plant and (A) leaf area and (B) leaf weight of cotton plants in the glasshouse at ACRI, 1994-95.

5.5 Densities of green mirids and damage and growth of cotton plants

All the studies so far have shown that GM feeding can cause damage to cotton plants which can affect the plant's growth. The damage caused by GM and its effect on the growth of the cotton plant is known to be cumulative and most evident during the 4^{th}, 5^{th} and adult stages of the insect. What is not known is whether the damage is economically important and if so, the density of GM required to cause economic damage (the economic threshold) in the field. In addition, the question as to whether cotton plants damaged by GM in the early stage compensate for it, or suffer yield loss during maturity, is also unknown. Studies were therefore conducted to answer these questions.

5.5.1 Materials and methods

Experiments were conducted in the mesh house under field conditions. Fourth instar green mirid nymphs reared from field collected adults (Section 5.2.1) were used. The plants used were Sicala V1 variety developed by CSIRO's Plant Breeding Division at ACRI in Narrabri. All the cotton plants used for this study were potted plants at the 4 true-leaf stage. Nine plants were each placed under a plastic cage and GM nymphal densities of 1, 2, 3, 4, 5, 6, 7 and 8 per plant were established. An uninfested plant of the same size was placed in a cage and used as the control. These treatments were replicated three times in a completely randomized design. The insects caged on each plant were allowed to feed for 72 hours. Insects were observed daily and if any died they were replaced with insects from the same culture and of the same age. After three days, all the insects were removed from each plant and the plant was kept for observation until boll maturity. Data were recorded for GM damage per plant (by scoring just after insects were removed from the plants), numbers of squares per plant (early and late stage), number of bolls at maturity, and days taken by each treated plant to commence and complete squaring, flowering and maturity (60% of bolls open).

Data were analysed using analysis of variance by MINITAB (Ryan *et al.* 1992). In addition, a regression analysis was performed on GM density and damage to find the relationship between density and damage.

5.5.2 Results and observations

Green mirid density and damage on young cotton plants

The damage scores caused by different densities of *C. dilutus* are given in Table 5.2 and Appendix 4.3a. The results showed that the damage caused to cotton plants tended to increase with increased density. However, the damage was not significantly different ($P > 0.05$) from the control for up to 3 insects/plant, indicating the ability of the cotton plants to recover from growth damage at up to 3 GM nymphs/plant. As densities increased from 4 to 8 per plant, the damage caused to the plants was significantly different ($P < 0.05$) from the control indicating that the cotton plants could not recover from growth damage at these high densities. Though analysis of variance and Fisher's least significant difference test showed that there was no significant ($P > 0.05$) difference between the damage caused by 4 - 8 insects per plant, the damage caused by lower densities (1-3 per plant) did not persist for long compared to those caused at higher densities (4 - 8 per plant) indicating that the plant's recovery

GM/plant	Damage score/plant
0	0.0d
1	1.3d
2	2.0cd
3	3.7bc
4	4.3ab
5	5.0ab
6	5.0ab
7	5.7ab
8	6.3a

Table 5.2 GM nymphal densities and damage caused to cotton plants under field conditions in the mesh house at ACRI, 1994-95. Mean within a column followed by the same letters are not significantly different ($P > 0.05$) (Fisher's least significant difference test).

was affected by the severity of damage. Regression analysis of GM density and percentage of plant damage indicated that the relationship between the mean percent damage and the mean number of green mirids per plant was highly significant ($P<0.001$, $R^2 = 0.94$) (Figure 5.5).

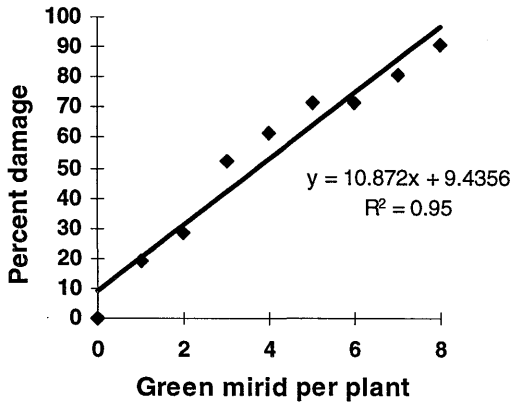


Figure 5.5 Relationship between green mirid nymphal densities and damage to cotton plants.

GM damage to young cotton plants and subsequent effect on yield

Plants which suffered severely (damage score 7) at a young stage showed abnormal growth in later stages. Secondary shoots emerged underneath the damaged tips and branch primordia, which made the plants bushy and broom-shaped (Plate 5.5). Also, those plants started squaring later than normal plants, although they recovered at a later stage.

Table 5.3 shows the numbers of squares at early and late stages, and the number of bolls at maturity. The results showed that plants infested with higher densities of insects (5 to 8 per plant) had the lowest number of squares during early squaring stage of the plant compared with the control. However, at completion of squaring (late squaring stage), the number of squares and bolls of plants infested with higher density of GM were not significantly different ($P > 0.05$) from the control (Appendix 4.3b-4.3d), indicating the cotton plant's ability to compensate for yield.

Green mirids per plant	Square number at early stage	Square number at late stage	Boll number at maturity
0	6.0a	13.7a	5.7a
1	5.3ab	21.0a	7.3a
2	3.3abcd	21.0a	6.7a
3	3.0abcd	20.0a	5.7a
4	3.7abcd	19.7a	6.7a
5	2.0bcd	19.3a	6.7a
6	4.0abc	15.7a	8.0a
7	1.0cd	18.3a	7.3a
8	0.3d	18.7a	8.3a

Table 5.3 Mean number of squares and bolls of plants infested with different GM densities at 4-leaf stage under field conditions in the mesh house at ACRI, 1994-95. Means within the columns followed by same letter are not significantly different (P>0.05), Fisher's least significant difference test.

Plate 5.5 Broom-shaped cotton plants due to GM feeding at young stage

A significant ($P < 0.001$) relationship was established between GM nymphal densities and the number of squares per plant at the early squaring stage indicating a decrease in number of squares per plant as GM densities increased (Figure 5.6).

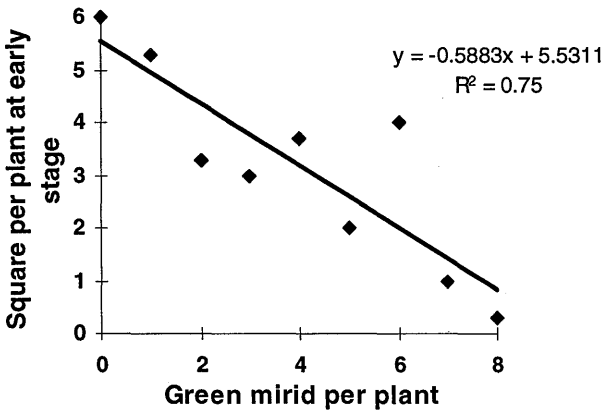


Figure 5.6 Relationship between number of squares per plant at early stage and green mirid nymphal density.

The cotton plants infested with different GM nymphal densities varied significantly in the number of days required to commence and complete squaring, flowering ($P < 0.001$) and maturity (60% of bolls open) ($P < 0.01$) (Table 5.4). In comparison with uninfested (control) plants, the infested plants had a delay ranging from 1 - 22; 2 to 25 and 3 to 18 days between commencing and completing squaring, flowering and maturity respectively (Table 5.4). In general uninfested plants took significantly fewer days to commence and complete squaring, flowering and maturity than plants infested with higher GM nymphal densities (Table 5.4).

Green mirid per plant	Days to square	Days to flower	Days to mature
0	31.3e	51.3d	104.3d
1	32.3e	53.7d	107.3cd
2	34.3de	56.0d	111.7bcd
3	37.3cde	61.0cd	111.3bcd
4	43.3bcd	67.3bc	113.3bc
5	47.0ab	74.3ab	113.3bc
6	46.7abc	68.3abc	116.7ab
7	51.3ab	76.0ab	117.3ab
8	53.3a	79.3a	122.7a

Table 5.4 Days required to square, flower and mature for plants infested with different densities of green mirids at 4-leaf stage in the mesh house. Means within the columns followed by the same letters are not significantly different ($P > 0.05$) (Fisher's least significant difference test).

A significant and positive relationship ($P < 0.05$) was established between (A) the densities of GM nymphs per plant and the number of days required by the plants to commence flowering and complete maturity and (B) the percentage of damage incurred by the plants at 4-leaf stage and the number of days used by the damaged plants to commence flowering and complete maturity (Figure 5.7).

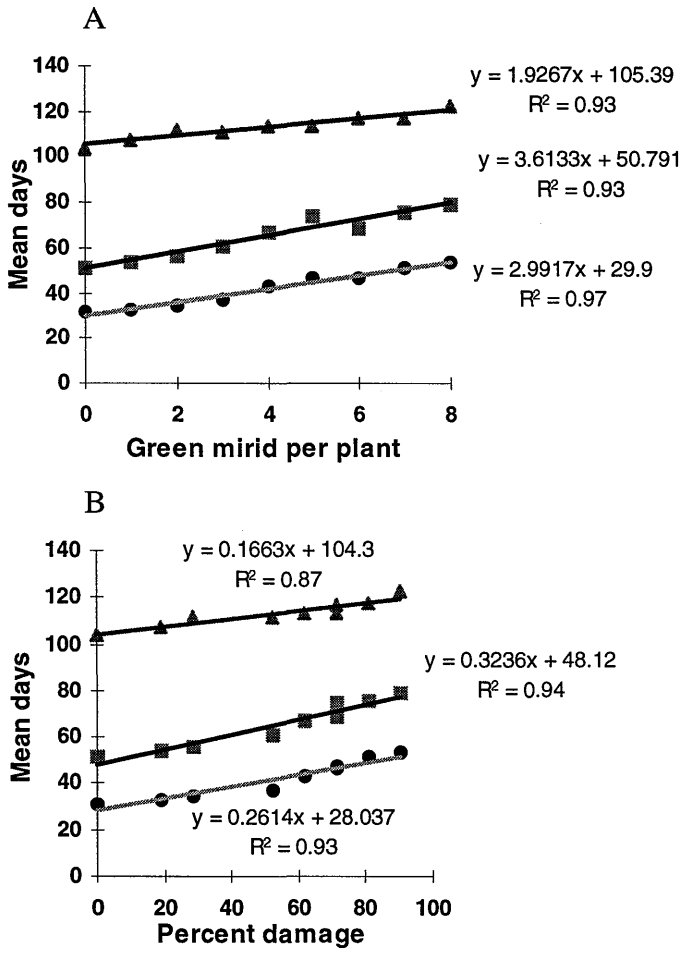


Figure 5.7 Relationship between (A) GM nymphal densities and the number of days required to square (●), flower (■) and mature (▲) and (B) percentage of damage incurred by cotton plants at 4-true leaf stage and days required by the plants to flower and mature.

5.6 Effect of green mirid feeding on squares under glasshouse conditions

It is known from the previous studies that GM feeding can cause square loss through shedding. The shedding of a square as a result of mirid feeding will depend on the size of the square. Squares that are too large to shed usually blacken. There is the

need to quantify the size of square that is vulnerable to shedding or blackening; and the relationship between GM density, square size and shedding or blackening of the fruits.

The objective of the following study was to determine the size of squares likely to shed as a result of GM feeding and the relationship between square sizes, GM density and shedding or blackening. In addition, I investigated the effect of GM feeding on large squares.

5.6.1 Materials and methods

Effect of densities of GM on cotton squares

This study was conducted in the glasshouse maintained at 30° ± 2°C using Sicala VI variety and GM 4th and 5th stage nymphs reared from field collected adults were used. Three sizes of cotton squares: <3 cm, 3-5 cm and > 5 cm, were used. The size of the squares was measured from the base to the tip of the bracts using a centimetre scale. Each square, still attached to the parent plant, was covered with a very small plastic cage (Chapter 2). In all, eight squares for each GM density of 1 to 8 nymphs/square were established. This was replicated three times. As a control (uninfested) squares of similar sizes were established in the same way as the infested squares. In all treatments, the insects were starved for 24 hours prior to infestation, then allowed to feed on the squares and bolls for 3 days before being removed. Each treatment was assessed daily and records were made for the numbers of squares shed.

Data for each square size and GM density were transformed using arcsine and analysis of covariance was performed using General Linear Modelling (GLM). In addition a regression analysis was performed on GM density and number of shed squares using MINITAB (Ryan *et al.* 1992).

Effect of GM feeding on large squares

Two experiments were conducted in this study. In the first experiment, a square (> 5cm) was infested with four 5th instar GM nymphs in a plastic cage and another square of the same size was placed in a plastic cage but uninfested with GM. In all, 15 infested and uninfested squares of similar sizes were set up for treated and control respectively. All the insect nymphs used for the study were starved for 24 hours prior to the experiment. The insects were allowed to feed for 3 days. Thereafter, each individual square was removed and damage assessed under the microscope.

In a second experiment, 30 large squares (> 5cm) were placed in a plastic cage as before and infested with two 4th instar nymphs. This was repeated three times. The insects were starved for 24 hours prior to the release and allowed to feed on each treated square for 3 days. After 3 days the nymphs were removed from the squares which were checked every day for bloom during anthesis (in the morning). The squares were tagged according to percentage of anther damaged and were observed daily for 2 weeks.

Analysis of variance was performed on the data (percent damaged anthers and subsequent effects) using MINITAB (Ryan *et al.* 1992).

5.6.2 Results and observations

Effect of densities of GM on cotton squares

Almost all insects died at densities 7 and 8 and results from these treatments are not included in the analysis. Figure 5.8 shows the number of squares of different sizes shed due to feeding by different densities of green mirids. Squares less than 3cm in size were more vulnerable to shedding than larger squares, irrespective of GM densities.

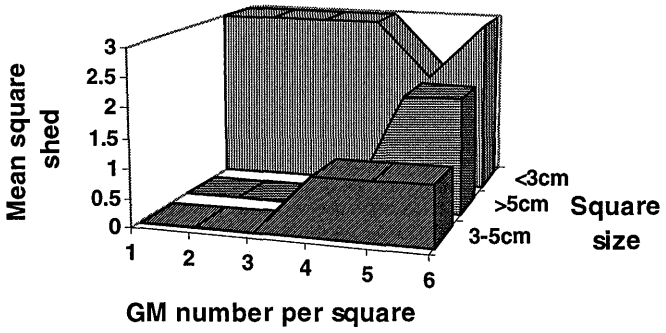


Figure 5.8 Effect of different densities of green mirids on cotton squares of different sizes under glasshouse conditions.

Cotton squares less than 3cm in size were shed consistently, irrespective of density (Figure 5.8). However, in squares between 3cm and 5cm shedding increased with increased mirid density. Covariate analysis showed that there was a significant difference between the proportion of squares shed and different sizes of squares ($P < 0.001$). The interaction between square sizes and GM densities was also significant ($P < 0.05$). However, the numbers of GM per square required to cause squares to shed were not significantly different ($P > 0.05$) (Appendix 4.4a).

A significant positive ($P < 0.05$) relationship was established between GM densities and the number of shed squares of sizes between 3-5 cm. However, an insignificant negative relationship ($P > 0.05$) was established between shed cotton squares of size less than 3 cm and GM density.

Effect of GM feeding on large squares

No visible GM feeding damage on larger cotton squares of size greater than 5 cm was evident on the square itself. However, when the calyx and corolla of the squares were removed to expose the anthers it was found that the anthers were damaged and their colour had turned brownish (Plate 5.6). The pollen sacs within the anthers were also shrunken and had lost their turgidity compared with those from uninfested squares.

The results show that 19% of squares showed no anther damage, 21% showed less than 30% anther damage, 18% showed 50% and 42% showed 70% or more anther damage after 3 days of GM feeding.

Plate 5.6 Damaged anthers due to GM feeding on the square

Damage to the anthers could result in flower or boll being shed or a boll being deformed. A deformed boll is characterised by a small, slender and parrot beak shaped head as shown in Plate 5.7. The degree of anther damage determined the percentage of flowers and bolls shed and also the percentage of bolls deformed (Table 5.5). Thirty per cent or less anther damage will result in no flowers or bolls shed or deformed bolls, indicating a threshold of 30% anther damage. This threshold information when

taken in addition to GM thresholds and fruit retention could be used to make a decision on GM control on cotton particularly during the peak squaring and boll forming stage of the cotton crop. Fifty per cent anther damage did not result in shedding of flowers but did result in the shedding of small bolls and also caused bolls to deform. Seventy per cent and over anther damage can cause both flowers and bolls to shed as well as bolls to deform (Table 5.5).

Plate 5.7 A deformed boll due to GM feeding on large squares along with a normal boll.

% Anther damage	% Flower shed	% Boll shed	% deformed boll
0	0.0a	0.0b	0.0b
30	0.0a	0.0b	0.0b
50	0.0a	5.6b	13.9b
70	5.6a	21.3a	20.4ab
>70	10.0a	27.3a	42.5a

Table 5.5 Effect of anther damage of large cotton squares infested with GM on deformed bolls, flower and boll shed under glasshouse conditions at ACRI in Narrabri, 1995-96. Means within a column followed by common letters are not significantly different (P>0.05) (Fisher's least significant difference test).

Overall, percentage boll deformity, boll and flower shed increased with anther damage. Analysis of variance showed that the effect of mirid numbers on boll shed and deformity as a result of anther damage was significant ($P < 0.05$) but the effect on numbers of flowers shed was not significant ($P > 0.05$). (Table 5.5 and Appendix 4.4b-4.4d).

5.7 Effect of GM feeding on cotton bolls of different ages

It has been established in previous experiments (sections 5.5 and 5.6) that GM feeding can cause considerable damage to young cotton plants and squares. GM feeding can also cause small bolls to shed. The effect GM feeding will have on bolls and yield would depend on the degree of damage and the age of the boll. Miles (1995) reported that GM can cause damage to the bolls by causing feeding spots on the bolls. She did not show that such damage can affect the harvestable yield of the cotton crop. Such information is crucial but lacking in decision making processes for management of GM in cotton fields. The objective of this study was to determine the effect of GM feeding on bolls shed, lint yield and delay in boll maturity at different ages.

5.7.1 Materials and methods

The experiment was conducted in the glasshouse on Sicala V II cotton plants with bolls that were at 2, 3, 4, 5, 6, 7, 10, 15 and 20 days after bloom. Each age group was replicated 5 times on different plants. Two GM 4^{th} instar nymphs starved for 24 hours were confined to each boll as described for the squares in the previous experiment and allowed to feed for 3 days (section 5.2.1). Bolls of the same age group were established on the same plant variety but uninfested with insects and used as controls. Insects were assessed daily and any dead insect was removed and replaced with another insect of the same age from the stock culture. After 3 days, GM nymphs which were now in their 5^{th} instar stage were removed. The number of feeding spots on each treated boll was recorded including bolls that were shed. The number of bolls shed was also recorded. Shed bolls were cut into two through the feeding spot using a knife and the lint examined under the microscope for damage. The bolls which did not

shed were kept until maturity and boll opening. After boll opening, records were taken of the number of bolls opened and the date the boll opened to enable calculation of maturity delay.

Data for shed and non shed bolls were transformed into arcsine before analysis. Analysis of variance was carried out for boll age and days to boll opening and also feeding spots. Regression analysis was also done for boll age, maturity, number of feeding spots and bolls shed.

5.7.2 Results and observations

GM feeding on bolls resulted in characteristic dark spots with a pin-head size shiny black spot in the middle (Plate 5.8A). The size of the spot ranged between 0.5 to 3 mm in diameter and this depended on the severity of feeding. The shiny spot in the middle was the point where the stylet penetrated the boll tissue. With mature bolls, external feeding spots on the carpel did not cause internal damage to the lint in the boll. However, with the younger bolls, larger feeding spots as a result of prolonged feeding can damage the lint in the boll. The lint characteristically changed from white to brown near the point of injury.

GM feeding can cause cotton bolls to either shed or crack depending on the age of the boll. Figure 5.9 shows that younger bolls from 1 to 7 days old were more likely to be shed than bolls 8 or more days old. Regression analysis revealed that there was a significant inverse relationship ($P < 0.01$, $R^2=0.70$) between boll age and proportion of bolls shed, and between boll age and the number of feeding spots. This indicates that GM feeding decreased as the bolls matured (Figures 5.9 and 5.10). Again, a significant positive relationship ($P < 0.05$, $R^2=0.53$) was also found between the number of feeding spots per boll and the proportion of bolls shed (Figure 5.9).

Significantly higher ($P < 0.01$) numbers of feeding spots were found on younger bolls (Figure 5.10). Cotton bolls 4, 5 and 6 days old took significantly more days ($P < 0.05$) to open after GM feeding than 7, 10, 15 and 20 day old bolls. Again 7

and 10 day old bolls took significantly more days to open ($P < 0.05$) than 15 and 20 day old bolls (Table 5.6). Days required for boll opening also depended on the number of feeding spots particularly in younger bolls. Regression analysis on days to boll opening and the number of feeding spots per boll revealed a significant positive relationship ($P=0.04$, $R^2 = 0.58$) (Figure 5.11).

Plate 5.8 Characteristics dark spots on the boll due to GM feeding.

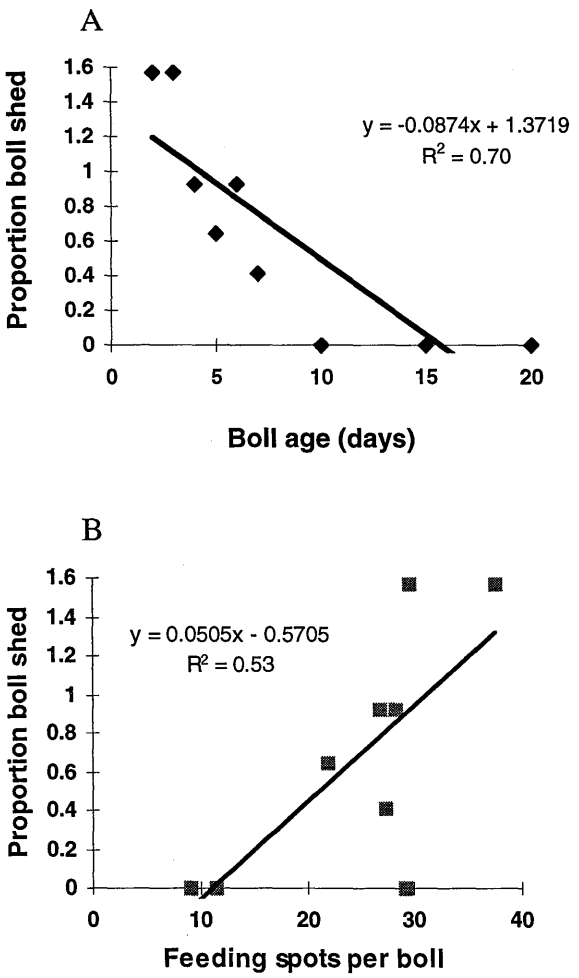


Figure 5.9 Relationship between (A) the age of cotton bolls and boll shedding and (B) the number of feeding spots per boll and boll shedding.

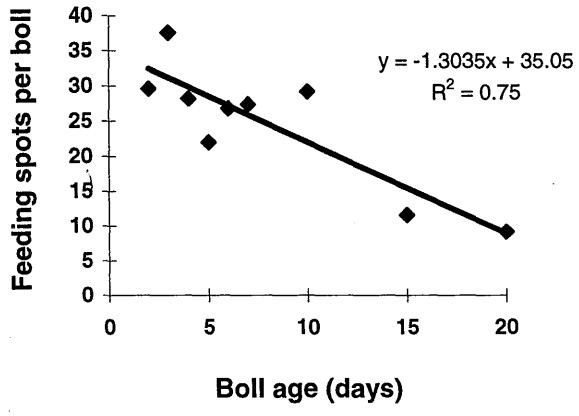


Figure 5.10 Relationship between boll maturity and the number of feeding spots per boll as a result of GM feeding under glasshouse conditions at ACRI, 1995-96.

Age of bolls (days)	Duration to boll opening (days) of infested bolls	Duration to boll opening (days) of uninfested bolls
2	*	104.6
3	*	105.0
4	115.0	103.8
5	113.0	104.6
6	114.0	104.6
7	108.3	104.0
10	109.0	104.0
15	104.2	102.6
20	103.4	103.0

Table 5.6 Days required for boll opening for different aged bolls fed upon by green mirid under glasshouse conditions. * boll age classes not included for analysis as they had already shed.

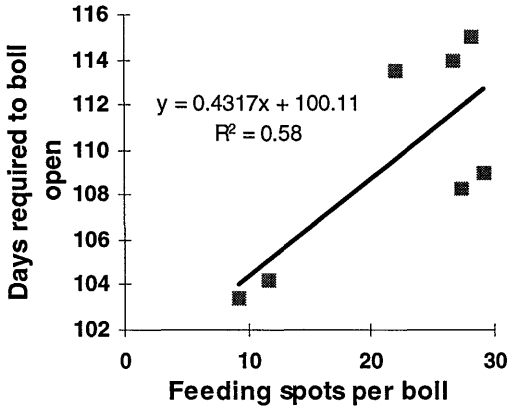


Figure 5.11 Relationship between feeding spots per boll and days required for boll opening under glasshouse conditions.

5.8 Field trials: Effect of GM feeding and damage to cotton plants

Two experiments were conducted in the irrigated cotton field at ACRI during the 1996-97 season to confirm previous data on GM feeding and damage to cotton plants under field conditions.

5.8.1 Materials and methods

Experiment 1

Transgenic (Bt) cotton crops (Ingard®) of the variety Sicala V II interplanted with 4 rows of lucerne strips located 100 metres apart were used for the study. The transgenic (Bt) cotton crops provided partial control of *Helicoverpa* spp. to avoid the need for synthetic insecticide sprays against these insects. The lucerne strips provided a source of GM for the studies. Six treatments consisting of GM densities of 1, 2, 3, 4 and 5 adults per plant and a control (no insects) were established by caging the insects in field cages (Chapter 2) on the cotton plants. These treatments were replicated three times in a randomized complete block design. GM adults were confined to the plants to feed for 3 days. GM adults used in this study were obtained from the stock collected from the lucerne strips within the study site. The insects were starved for 24 hours prior to release. After 3 days of feeding, cages were removed from the plants and records were taken of numbers of pinhead, small, medium and large squares. In addition records were taken of the number of aborted and shed squares in each treatment.

Analysis of variances and Fisher's least significant difference test were conducted to compare the means. Regression analysis was also done for GM density and squares shed.

Experiment 2

This experiment was also conducted in an irrigated transgenic cotton field at ACRI during the 1996-97 season. The transgenic (Bt) cotton crops were interplanted in 4 rows of lucerne strips located 100 metres apart. Three treatments: (1) untreated plot, (2) Dimethoate treated plot (Dimethoate 400g/l EC), @ 375 ml/ha) to control GM and (3) Endosulfan treated plot (Endosulfan 350 EC, @ 2.1 l/ha) to suppress *Helicoverpa* spp. were established. Each treatment was replicated four times in a randomized complete block design. Each replicated treatment consisted of cotton

crops 8 rows (metres) wide and 100 metres long and separated by a buffer of 5 rows of cotton plants. The lucerne strips which were established before the cotton was planted served as trap crops for the GM and as a source of GM for the trials when the lucerne was slashed after the experiment was set up.

Insecticide treatments were applied using a knapsack sprayer (Solo, Germany) at 10 day intervals from October 25, 1996 until January 15, 1997. The study field was infested by two spotted spider mites, *Tetranychus urticae* and both treated and control (untreated) plots were sprayed with Comite® (propargite) at 2.5 l/ha to control the mites.

Insect populations were sampled weekly from October 30, 1996 to January 20, 1997. Green mirids were sampled using a suction machine on 10m sections of row per plot (Chapter 2) and the data expressed as numbers per metre. *Helicoverpa* spp. eggs and larvae were counted visually from a 1m row per plot and the data was also expressed as numbers per metre. Cotton squares from 1m sections of row in each plot were counted every week from December 12, 1996 until January 15, 1997. Square count data were separated into early and mid season. The number of bolls per metre per plot were also recorded from mid season until maturity. Days of first flower and boll maturity (50% boll open) were also recorded. The cotton from each treatment was harvested and the lint yield per hectare was estimated from the harvest.

Analysis of variance was used to analysed the data. Data on green mirid and square numbers were analysed as early season (planting to December 20) and mid season (December 21 to January 20) and for bolls, data were analysed as mid season and at maturity (60% boll open). Green mirid numbers were also analysed for the whole season.

5.8.2 Results and observations

Experiment 1

Square size and squares shed

GM feeding caused pinhead, small and medium squares to shed but not large squares. Out of total squares shed, 47.2% were pinhead, 33.4 % were small and 19.4% were medium. The losses of pinhead and small sized squares were not significantly different ($P > 0.05$) nor were the losses of small sized and medium squares (Figure 5.12 and Appendix 4.5a). However, Fisher's least significant difference test revealed that pinhead squares shed significantly more ($P < 0.05$) than medium sized squares.

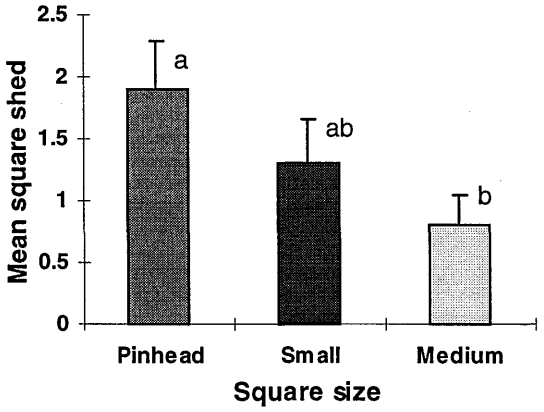


Figure 5.12 Numbers of squares shed due to green mirid feeding on caged plants in field trials at ACRI, 1996-97. Means in the bars followed by similar letters are not significantly different ($P > 0.05$) (Fisher's least significant difference test). Error bars indicate standard error of means.

GM density and square abscission

The effects of different densities of GM on the shedding of cotton squares are given in Table 5.7 and Appendix 4.5b-4.5e. Significantly higher ($P < 0.001$) numbers of squares per plant were shed with increased GM densities per plant. Fisher's least significant difference test revealed that 5 GM per plant caused significantly more

abscission of squares ($P < 0.05$) than all other densities tested. This was followed by 4 and 3 GM per plant with the least squares shed at 1 and 2 GM per plant. The number of squares shed at 1 and 2 GM per plant were not significantly different ($P > 0.05$) from the control (Table 5.7 and Appendix 4.5e).

GM/ plant	Mean square shed			
	Pinhead	Small	Medium	Total
0	0.33c	0.00b	0.00b	0.33d
1	1.00bc	0.33b	0.33ab	1.67cd
2	1.33bc	1.33ab	0.33ab	3.00cd
3	1.67bc	1.00ab	1.00ab	3.67bc
4	3.00ab	2.00ab	1.00ab	6.00b
5	4.00a	3.33a	2.00a	9.33a

Table 5.7 Number of squares shed due to feeding by different densities of green mirid on caged cotton plants in field at ACRI, 1996-97. Means within a column followed by common letters are not significantly different ($P > 0.05$) (Fisher's least significant difference test).

When regression analysis was performed between GM density and squares shed, a significant positive relationship ($P < 0.001$, $R^2 = 0.94$) was established between GM density and total squares, pinhead squares and small squares (Figure 5.13). Regression analysis showed that one green mirid adult per plant, feeding for three days on squaring cotton, can cause a total shedding of 1.7 squares.

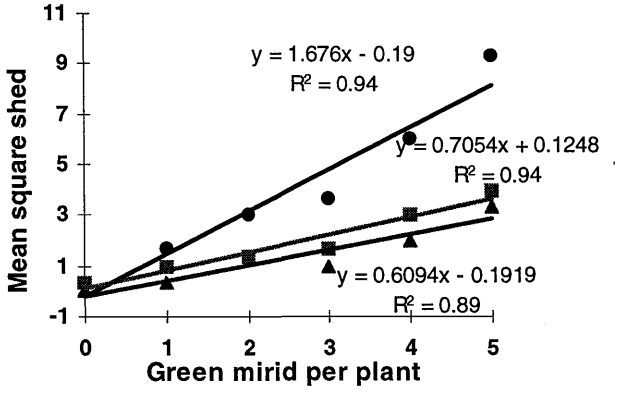


Figure 5.13 Relationship between green mirid density and squares shed.
-●- total square; -■- pinhead square; -▲- small square

Experiment 2

Insect populations

Green mirids, *Helicoverpa* spp. and two spotted spider mites (*T. urticae)* were the only pest groups found on the study plants apart from GM (Table 5.8). Green mirids were abundant early and mid season. Ingard® cotton gave good protection against *Helicoverpa* until first week of January. When the expression of Bt toxin by the transgenic crops was low, endosulfan was used to control *Helicoverpa* spp.

Numbers of GM were higher at mid than early season. Analysis of variances showed that green mirid populations for whole season and at mid season were significantly lower ($P < 0.05$) in the plots sprayed with dimethoate compared with endosulfan sprayed plots. The numbers of GM were not significantly different in all treated plots during the early season. (Table 5.8 and Appendix 4.6a-4.6c).

Treatments	No. of insects per metre			
	GM at early season	GM at mid season	Total GM	Helicoverpa spp.
Unsprayed plot (Control)	0.04a	0.10a	0.14a	0.70a
Plot sprayed with dimethoate to control GM	0.01a	0.03b	0.04b	0.95a
Plot sprayed with endosulfan to kill *Helicoverpa* spp. but not GM	0.04a	0.11a	0.15a	0.50a

Table 5.8 Green mirid and *Helicoverpa* spp. larval populations per metre in fields manipulated by insecticides, ACRI, Narrabri. Means within a column followed by common letter are not significantly different ($P > 0.05$) (Fisher's least significant difference test).

Number of squares and bolls

Table 5.9 shows the numbers of squares at early and mid season and also bolls at maturity in the different treated plots. Significantly higher ($P < 0.05$) numbers of squares were recorded mid season in the dimethoate GM controlled plots than the endosulfan and the unsprayed plots where GM were not controlled (Table 5.9 and Appendix 4.6d). Differences in average number of squares per metre early in the season were not significant ($P > 0.05$) for different treatments (Table 5.9 and Appendix 4.6e). Similarly the number of bolls per metre at maturity were not significantly different ($P > 0.05$) for different treatments (Table 5.9 and Appendix 4.6f) indicating that the cotton plants might have compensated for the square loss during mid season.

Treatments	Squares/m at early season	Squares/m at mid season	Bolls/m at maturity
Unsprayed plot (Control)	44.0a	158.8b	96.3a
Plot sprayed with dimethoate to control GM	60.3a	223.3a	112.5a
Plot sprayed with endosulfan to control *Helicoverpa* spp. but not GM	38.8a	165.8b	110.0a

Table 5.9 Average number of squares per metre at early and mid season and number of bolls per metre at maturity in irrigated cotton fields with and without GM control at ACRI, 1996-97. Means in the column followed by common letters are not significantly different ($P > 0.05$) (Fisher's least significant difference test).

Flowering, maturity and lint yield

Table 5.10 shows that the number of days the cotton crops took to flower (first flower) and to mature (60% bolls open) varied significantly ($P < 0.001$) between treatments. The number of days taken to first flower and to achieve 60% boll opening in plots where GM was controlled was significantly shorter than in plots where GM and other pests were not controlled. The GM controlled plot took 4-5 days less to first flower and 7-10 days less to mature compared to plots where GM and other pests were

not controlled. Analysis of variance showed that the differences between the treatments were highly significant ($P < 0.001$) (Table 5.10). However, the differences between days to flower and maturity between the unsprayed controls, and plots treated with endosulfan to control pests other than GM, were not significantly different ($P > 0.05$) (Table 5.10 and Appendix 4.6g and 4.6h).

Lint yields (bales/ha) were 7 bales/ha from GM controlled plots, 6.8 bales/ha from GM infested plots and from plots that were not controlled against cotton pests 6.1 bales/ha (Table 5.10). Analysis of variance showed that the differences between these yields were not significant ($P > 0.05$) (Table 5.10) indicating that significant GM population in mid season (Table 5.9) did not have any significant effect on lint yield. This is possibly due to the cotton plant's ability to compensate for early square loss.

Treatments	Days to 1st flower	Days to mature	Lint yield (bales/ha)
Unsprayed plot (Control)	76.3b	144.5b	6.1a
Plot sprayed with dimethoate to control GM	72.0a	136.0a	7.0a
Plot sprayed with endosulfan to kill *Helicoverpa* spp. but not GM	76.5b	143.8b	6.8a

Table 5.10 Effect of GM infestations on the number of days to first flower and maturity (60% boll open) and lint yields (bales/ha) in trials conducted in cotton fields at ACRI, Narrabri in the 1996-97 season. Means within a column followed by similar letters are not significantly different ($P>0.05$) (Fisher's least significant difference test).

Relationship between GM numbers, square and boll numbers and flowering or maturity dates

The relationship between square loss in early and mid season, number of bolls at maturity and numbers of GM (Tables 5.8 and 5.9) was not significant ($P > 0.05$). However, the relationship between GM numbers at early, mid or early plus mid

season, and the number of days required to first flower was significant and positive ($P < 0.05$). The number of days required to first flower increased with GM numbers (Figure 5.14). A significant positive relationship ($P < 0.05$) was also found between delay in maturity and GM numbers at early, mid and also early plus mid seasons (Figure 5.15) indicating the potential for GM to cause maturity delay in infested cotton plants.

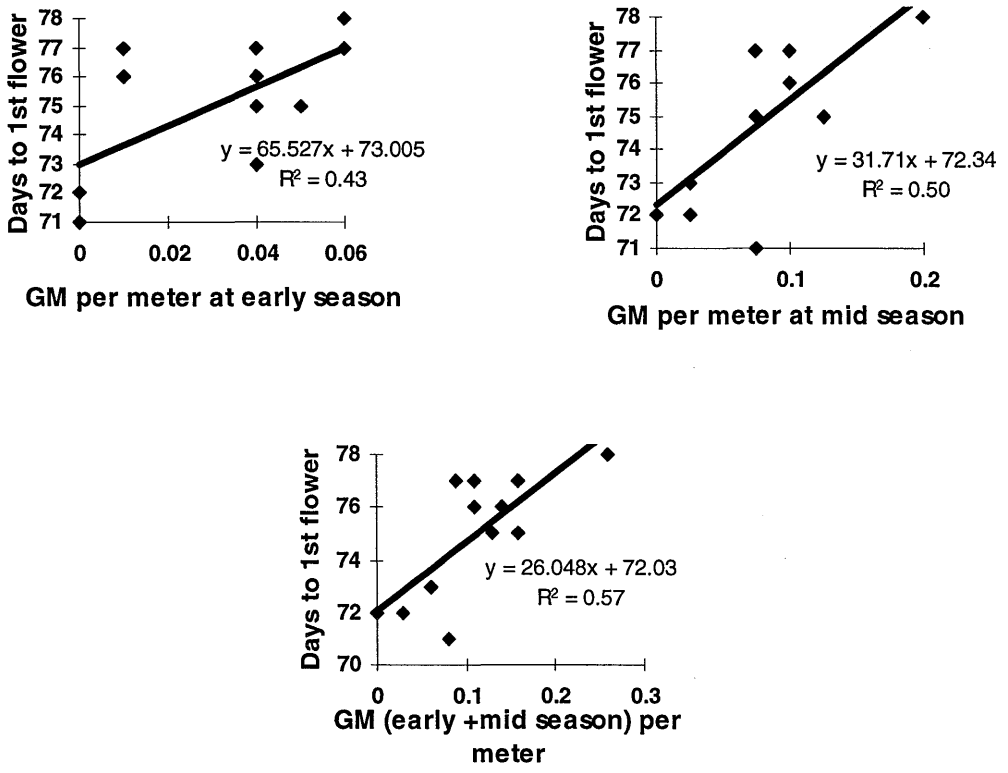


Figure 5.14 Relationship between numbers of GM and days required to first flower in the field, ACRI, Narrabri, 1996.

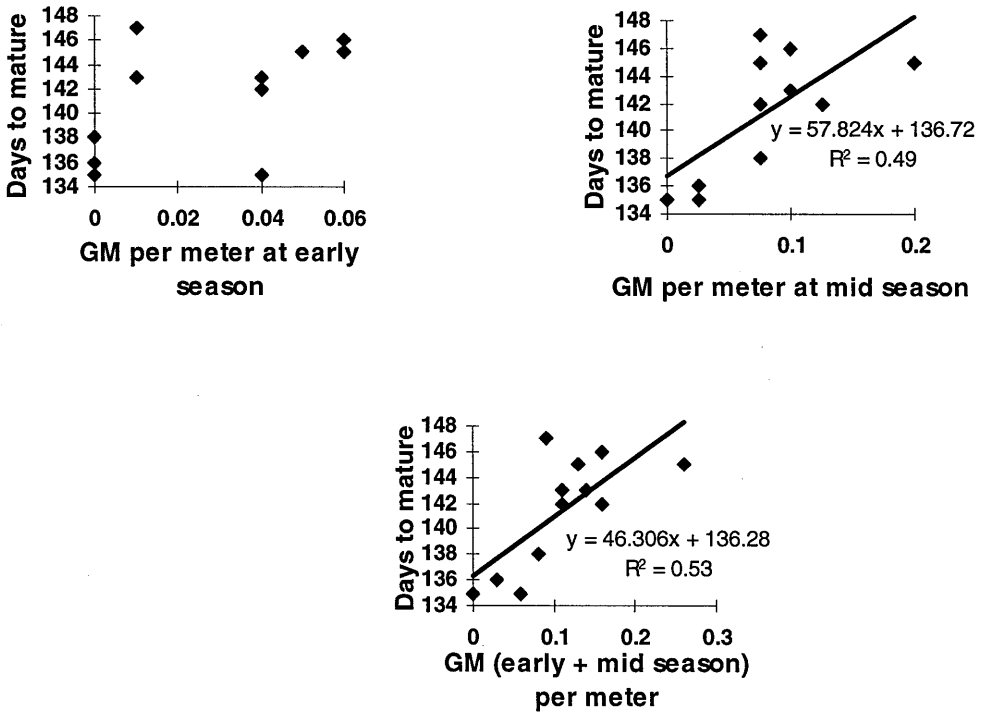


Figure 5.15 Relationship between numbers of GM and days required for bolls to reach maturity in the field, ACRI, Narrabri, 1996.

5.9 Discussion

GM feeding and damage to cotton plants

The symptoms associated with GM feeding on young plants are partial or complete blackening of folded terminal leaves, blackening of leaf margins and terminals of unfolded leaves, drooping of terminal leaves and tips and lesions of stems and petioles. Similar symptoms have been reported for *Lygus* bugs for different crops (Scales and Furr, 1968; Pack and Tugwell, 1976; Tugwell *et al.*, 1976; Hanny *et al.*, 1977; Mauney and Henneberry, 1979; Butts and Lamb, 1990). The degree of feeding, and growth stage of the crop when feeding occurred, will determine the type of damage the plant will incur and the subsequent effect on the plant's yield. If GM fed on young plants and the plant had a damage score below 5 (section 5.4, in this chapter), the effect on the plants was minimal during the later stages of the growth. However, if the damage rating of the plant was 5 or above, the plant became deformed, terminals dried off and lost the capacity to use all of the available resources

for growth and development. This may trigger the development of secondary shoots underneath the damage point making the plants become bushy.

Sadras (1996), reported that the light-use efficiency of damaged plants increased as a result of morphological changes induced by damage and this may enhance the production of resources and thus encourage plants to grow profusely. The problem with bushy cotton plants are that they are attractive to, and can be used as hiding places by other cotton pests (*Helicoverpa* spp., *Campylomma* spp, thrips etc.) to avoid insecticide sprays. The sprays, particularly ground rig sprays, never reach their target on the bushy plants. Damaged plants and their associated secondary shoots set more squares than undamaged plants. Most of the squares cannot be retained in the later stages of the plant growth (Table 5.3) due to competition between secondary shoots for available limited resources, especially photosynthates (Sadras, 1996). Apart from competition for available resources, several other physiological and environmental factors (Guinn 1982) can induce plants to shed squares. According to Mason (1922) and Guinn (1982), maturing fruits have a very high demand for carbohydrates and nitrogen. The demand for these resources varies with the size of the fruit thus when resources are in short supply, smaller fruits fail to accumulate enough of these resources to survive. These fruits are naturally shed by the plant. The shedding of squares as a result of GM feeding depended on size of the squares (Figure 5.8 and Table 5.7). Pinhead and small squares are most vulnerable to shedding. Similar findings were reported for *Lygus* bug by Strong (1970), Pack and Tugwell (1976), Mauney and Henneberry (1979), Leigh *et al.* (1988). As mentioned earlier, the level of ethylene and auxin in the fruiting parts, particularly the ovule and pollen grains, determines the subsequent fate of the square after GM feeding. Any reduction of auxin concentration in the fruiting parts encourages abscission. In small squares less than 3cm long GM may penetrate their stylets sufficiently deeply to destroy the auxin producing ovule and pollen grains, causing the square to abscise. In large squares of size greater than 3cm long, GM stylets may not reach the ovule to destroy the auxins. However, if a large number of pollen grains are damaged as a result of GM feeding, the squares may be shed as a flower or a small boll (Table 5.5). It is therefore difficult to differentiate between square shedding associated with GM feeding and natural shedding by cotton plants. Some methods to confirm shedding due to GM

feeding are (1) to remove the calyx and corolla of the fruit to expose the GM damage (2) to observe closely the anthers to reveal brown necrosis and shrivelled anthers during bloom and (3) to cut across the square longitudinally and expose the necrosis of the staminal column. Necrosis on anthers and staminal column and deformed bolls has also been reported by Pack and Tugwell (1976) and Mauney and Henneberry (1979) for *Lygus* spp. On the other hand if GM feed on the base of the squares, the light brown zone with a black spot in the middle at the point of insertion, is visible under a microscope.

GM injury to cotton bolls identified in this study is also similar to that observed by Pack and Tugwell (1976) and Tugwell *et al.* (1976) for *L. lineolaris* and *Neurocolpus nubilus*. The injury to the bolls can be easily distinguished from any other spots. The characteristic appearance of a spot caused by GM damage is a relatively shiny black spot with an even darker and glossier pinhead sized centre (Plate 5.8A). It is unusual for a large boll (more than 7 days old) to be shed as a result of GM feeding unless high infestations (5-6 insects per boll) occur in the field and concentrate their feeding on the fruit stalk. Such a situation is very rare under normal circumstances. However, a considerable number of younger bolls (2-7 days old) can be shed as a result of GM feeding (Figure 5.9). The results of this study also showed that GM feeding spots were significantly higher ($P < 0.05$) in younger bolls (2-7 day-old) suggesting that GM preferred younger to older bolls. Stam (1987) also reported that small or younger bolls are shed more than older bolls as a result of *C. pallidus* feeding on cotton plants in Syria.

The damage caused by GM to cotton plants was cumulative and evident during the 4^{th} and 5^{th} stages. All the nymphal stages of GM caused similar types of damage. The early stage nymphs (1^{st} and 2^{nd} instars/stages) caused the least amount of damage compared to the 3 -5^{th} stages and the adults (Figure 5.3). The degree of damage however depends on the GM infestation levels, site and duration of feeding, as well as size and age of bolls.

Relationship between GM density and damage

The relationship between GM density and percentage of damage in young plants was linear (Figure 5.5) indicating increased GM density resulted in increased damage. At the early squaring stage of cotton plants, the number of squares per plant decreases with increased GM density. However, such a reduction in square numbers is compensated for by the plant in the late stage of the crop thus averting yield loss (Table 5.3). Plants attacked by GM in the early stage of the plant's growth set 0.6 squares less for every GM per plant when compared to uninfested plants (Figure 5.6A). Plants attacked when young (4-leaf stage) gradually recover as the plant grows and by maturity may have more bolls per plant than undamaged plants (Table 5.3). This could be due to the fact that plants suffering GM damage at a young stage develop more vegetative growth (Plate 5.5) and thereby produce more photosynthates which ultimately induces them to set more fruit than undamaged plants. However a severe damage at the 4-leaf stage could lead to smaller fruits unable to accumulate carbohydrates and nitrogen, hence they are shed resulting in a lower fruit set. The capacity of cotton plants to compensate for yield after early vegetative bud damage is well documented (Evenson 1969, Bishop *et al.* 1977, Brook *et al.* 1992, Sadras 1996b). In this study, compensation occurred due to the development of extra secondary shoots and the formation of fruit buds through the activation of dormant buds .

The relationship between GM density and square loss when the insects were confined on a single square was somewhat erratic. Fewer squares were shed at 5 or more GM per square, in comparison to other densities. Tugwell *et al.* (1976) also found no differences in damage at different densities of *L. lineolaris* on cotton. The explanation for this could be intraspecific competition for space and feeding sites which possibly forced the insects into fighting each other instead of feeding. Most of the insects were found dead on the square at high densities per square. In younger or small squares less than 3 cm long, 1-4 GM per square caused 100 percent of squares to shed. This could mean that the shedding of a square may not depend solely on the number of insects per square but also on the site the insect fed. Nevertheless having more than one mirid per square in the field is a very unlikely situation. If an insect

feeds on the square stalk, there is the tendency for the square to be shed. This was quite different when the insects were caged on the whole plant. In this scenario the number of squares shed increased with increased GM density for small squares but the relationship was not significant with medium sized squares (Table 5.7-5.9 and Figure 5.13). This finding is in agreement with the findings of Stam (1987) for *C. pallidus*. Mauney and Henneberry (1979) proposed an abscission rate of about 1.7 squares per insect per day and Leigh *et al.* calculated a rate of 1.84 per insect per day for *Lygus* which is higher than 0.6 squares abscised per GM per day calculated in this study. GM is a very mobile insect. The decision to control these insects should therefore not to be based only on numbers of insect per plant or square but on damage and fruit retention as well.

GM density and yield

The results of this study have shown that infestation of GM early in the season or when the cotton plants are young may not significantly affect yield since plants do compensate. However, infestation during the middle of the cotton season during the peak squaring period of the cotton plants could affect lint yield in irrigated crops (Table 5.8). This agrees with the findings of Miles (1995) for irrigated cotton. Tugwell *et al.* (1976) and Parker *et al.* (1986) also failed to detect any significant yield loss for *Lygus* on cotton. In my case the GM densities at early and mid season was well below the existing threshold level which is up to 4 per metre (Shaw and Harris 1997). The highest population recorded was 0.15 GM per metre at mid season. The low density population caused very little damage to the cotton and thus did not cause any significant yield loss. Brook *et al.* (1992a) report similar results for medium and light early fruit damage. This small population could have impacted on yield had they been able to feed for a long time. However heavy rain and drifting of insecticide sprays (see chapter 6, in this thesis) limited their activities and may have greatly reduced the GM population. Timing, site and duration of feeding may be crucial for yield loss. Tugwell *et al.* (1976) reported that removal of squares for three weeks during the 4^{th} to 6^{th} weeks of squaring caused 45 percent yield loss but removal of squares during 1st to 3rd and 7th to 9th weeks of squaring did not reduce yield.

GM damage and maturity delay in cotton plants

Green mirids feeding on cotton at the young, square or boll stages caused significant delays in maturity of the cotton plant. The earlier the damage, the greater the delay. Delay also depended on GM density (which determined the severity of damage) (Tables 5.4 and 5.10) and in the case of bolls the boll age (Table 5.6). There was a maturity delay of 18 days with an increase of GM density from 1 to 8 per plant. Younger bolls with more feeding spots took more time to open than older bolls. Chinajariyawong (1988) and Miles (1995) also reported delay in maturity due to GM feeding in seedling and squaring stage. Delay in maturity as a result of mirids feeding is a common phenomenon in cotton crops and has been reported by several workers *Lygus* spp. (Hanny *et al.* 1977, Wilson 1982, Stewart and Sterling 1989b, Tonhasca and Luttrell 1991). The mechanism of delay is complementary to the compensation phenomenon. After cessation of growth due to GM damage (for example wilting of tips in young cotton) the cotton plants direct their available resources to the production of secondary tips and more vegetative growth, and produce more fruit to replace what has been lost. There is a time lag between cessation of growth and starting of new growth and this time lag is eventually reflected in maturity delays.

To the cotton grower, any delay is unacceptable (Miles 1995). Their perception of maturity delay is evident in the statement that *'earliness is next to godliness'* (Cox *et al.* 1991). The maturity delay may cost cotton growers in two ways: (1) an increase in management costs and (2) decrease in fibre quality. The cost of insecticide sprays to manage late season pests such as *Helicoverpa* spp. and *Aphis gossypii*, defoliants and irrigation water are some of the added or extra expenses incurred by the grower when his cotton crop is delayed. In addition, there are the interest charges on borrowed capital (Cox and Thomson 1989) to be taken into account. Also, delay in maturity may expose cotton to the risk of rain damage which may reduce lint quality. Reduction of fibre quality may also occur due to low night temperatures particularly in short season areas and this may reduce the micronaire value of lint, resulting in imposed penalties such as downgrading of lint and a lower sale price. (Gipson and Joham 1968, Constable 1988). If GM's early damage causes cotton maturity to be delayed by 2-3 weeks, there is every possibility that cotton in

NSW will experience low night temperatures during fibre development and will thereby incur price penalties. Growers usually aim for earliness of the crop, hence the importance of GM in the Australian cotton industry and it is necessary to derive an ETL for GM in respect of delay in boll maturity.

Calculation of an economic threshold (ET) using maturity delay

Economic thresholds are among the most crucial components of IPM. Stern *et al.* (1959) defined the economic threshold as 'the density at which control measures should be applied to prevent an increasing pest population from reaching the economic injury level (EIL)'. The EIL is 'the lowest population density that will cause economic damage' and economic damage is 'the amount of injury which will justify the cost of artificial control measures". Commonly EIL is calculated from the following model (Pedigo *et al.* 1986):

$$EIL = C/VID$$

where C = cost of the management activity per unit of production ($/ha), V = market value per unit of the produce ($/kg, or $/bale), I = injury unit per insect (insects/m) and D = damage per unit injury (kg reduction/ha).

'Although the EIL represent the critical level of damage relative to current biological and economic circumstances, the operable decision criterion is the ETL and the ETL is a direct function of the EIL' (Pedigo *et al.* 1986).

The yield loss I found for GM from field experiments was not statistically significant. However, the delay in boll maturity due to GM damage at early to mid season was significant and was calculated to be 46.31 days (Figure 5.16) if a single GM per metre fed for 83 days (from October 30, 1996 to January 20, 1997, see section 5.8.1 in this chapter) which means if 1 GM per metre feeds for 1 day, it will cause 0.56 days delay in maturity.

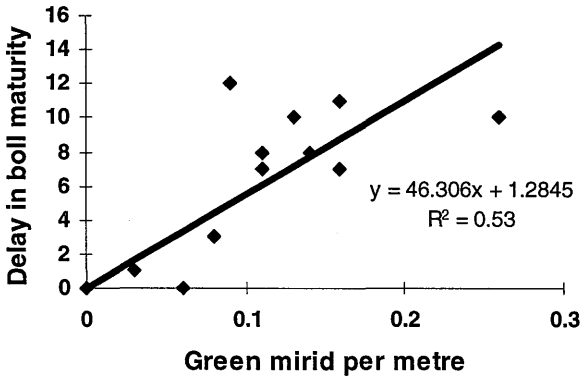


Figure 5.16 Relationship between GM per metre and delay in boll maturity in the field, ACRI, Narrabri, 1996-97.

In Fig. 5.16, the regression line was forced to run through the origin assuming that zero insects cause zero damage.

Hence if one days delay cost growers $X, one GM per metre per day gives Y days delay and since the relationship between GM number and delay is linear (Figure 5.16), then an EIL for GM is when

XY = Cost of spray

Therefore EIL = Cost of spray/XY

Where X = $ AUS 20/ha/day (Cox *et al.* 1991)

Y = 0.56 days

Cost of spray: chemical (imidacloprid or fipronil) AUS $16/ha + application (ground rig) $ AUS 4/ha, total = $ AUS 20/ha

Therefore the EIL = 1.78

For ease of communication to growers, this figure could be approximated to 2 GM/m. This is four times higher than the currently recommended threshold (0.5 GM/m), which suggests that insecticides are presently often used unnecessarily against GM. However, it should be recognized that this estimate depends on the accuracy of the delay costs estimated by Cox *et al.* (1991), who admit that some of their figures are only approximations. Moreover, it should be recognized that the figure of 2 GM/m is

an EIL, not an ETL. The ETL is commonly set lower than the EIL, both to give farmers time to respond to an increasing pest population and to accommodate the risk - averse nature of many farmers (Stern *et al.* 1959). Taking all these factors into account, I suggest that the current ETL for GM should be doubled, to one per metre of row.

CHAPTER 6

POPULATION DYNAMICS OF GREEN MIRIDS IN COTTON AND LUCERNE

6.1 Introduction

Understanding of the population dynamics of an insect pest in relation to particular hosts is a prerequisite for developing an effective management system for that insect pest. Effective pest management depends on knowledge of population changes over the season and their underlying causal factors (Tauber and Tauber 1976). For every species, populations may be regulated by several factors, either density-dependent or density-independent. Identifying those factors and how they act on populations will help to develop models to predict changes in population density and thereby influence management decisions.

As an initial step to understand the population dynamics of GM, in this chapter I examine the distribution pattern of the insect in the field as well as within the plant itself. The examination of these distribution patterns will give an idea of those aspects of GM's habitat that influence the growth and development of the insect. At the same time this finding will help to develop a sampling protocol for population studies. In this chapter I also examine the seasonal changes in the insect population in cotton and lucerne. These are then related to the following factors: GM's natural enemies, environmental factors such as temperature and rainfall and alternative hosts abundant during the cotton season. Furthermore, I examine the role of overwintering hosts grown around cotton growing areas as a population source for GM found in cotton.

6.2 Distribution pattern of GM in the field and on cotton plants

Miles (1995) from D-vac sampling counts determined the distribution pattern of GM. However, she did not distinguish between adults and nymphs. Since GM adults are mobile and nymphs are more or less sedentary the distribution pattern of the

adult may be different from that of the nymph. Therefore in this experiment I assess distribution patterns of GM in the field for adults and nymphs separately. I also examine the distribution of the insect within the cotton plant.

6.2.1 Materials and methods

The experiment was conducted in a 2ha field of 110 rows of Sicala VI at ACRI, Narrabri during the 1995-96 season. Each row measured 100m x 1m. The cotton was planted on 10th October, 1995 and no insecticides were used in the field during the study. Visual observation was made weekly from 2-leaf stage to maximum squaring stage. For each observation, one row in every 10 was selected from which 5 plants were sampled, selected randomly. Thus altogether 50 plants from 10 rows were investigated thoroughly on each occasion. Investigations were made for 8 occasions. Every time separate sets of plants were sampled. The data recorded included GM adults and nymphs. Data for within plant distributions were recorded according to plant parts (terminal, square and leaf) along with main stem node number. The first unfolded main stem leaf in the terminal counted as position 1. Information was recorded for dorsal and ventral sides of each leaf, and the inside and outside of terminals and squares.

To determine the distribution pattern in the field Taylor's power law (Taylor 1961) was used: $s^2 = am^b$ or, in logarithms, $\log s^2 = \log a + b \log m$ (where s^2 is the variance and m the mean). The parameters of the equation are dependent on the interaction between the species' behaviour and environment (Taylor 1984). The b slope is an index of distribution with values of $b<1$ indicating uniform, $b=1$ indicating random and $b>1$ indicating aggregated distributions. For within plant distribution, χ^2 tests were used to compare the numbers of adults and nymphs found in or on terminals and squares, dorsally or ventrally on leaves and the numbers of adults and nymphs found on upper (1-8 main stem node) and lower (9 and more main stem node) halves of the cotton plant.

6.2.2 Results and observations

Distribution pattern in the field

Overall insect numbers were very low. Out of a total population of 50, 26.8% were adult and 73.2% were nymphs. Only in 18.8% of cases were adult and nymphs found on the same plant. In 15.4% of cases two adults were found on the same plant whereas in 38.5% of cases more than two nymphs were found on the same plant. The spatial distribution in the field for adults and nymphs is described by Taylor's power law and estimated parameters are given in Table 6.1. The parameters indicated that the spatial distribution of adult GM was random and that of nymphs was aggregated.

Insect stage	b	r^2
Adult	1.00	0.98
Nymph	1.25	0.66

Table 6.1 Estimated parameters of Taylor's power law for green mirids in the field.

Distribution within the plant

Green mirid distribution within the plant is summarized in Figures 6.1 and 6.2 and in Table 6.2. Most GM adults were found on leaves (50%) followed by squares (33.3%) and terminals (16.7%). The percentages for nymphs were 54.1, 37.8 and 8.1% located respectively on squares, terminals and leaves (Figure 6.1).

Chi-square tests indicated a significant relationship between insect stage and distribution on squares and leaves ($\chi^2 = 9.27$, df =1, P=0.002) and on terminals and leaves ($\chi^2 = 9.54$, df=1, P=0.002). However, there was no significant relationship between insect stage and distribution on terminals and squares ($\chi^2 = 0.183$, df=1, P=0.669).

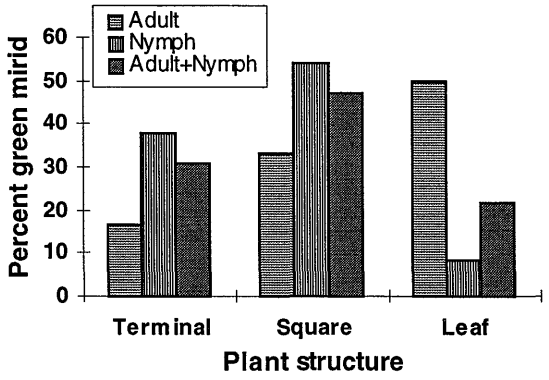


Figure 6.1 Distribution of green mirids within the cotton plant in the field at ACRI, Narrabri, summed for all dates. Total N = 50

With respect to plant parts most of the adults were found on the terminal and square whereas most of the nymphs were found inside terminals and squares. Both adult and nymph GM were predominantly abundant on top of the leaf surface.

Insect stage	Terminal		Square		Leaf	
	Outside	Inside	Outside	Inside	Dorsal	Ventral
Adult	66.7	33.3	66.7	33.3	100	0
Nymph	21.4	78.6	30.0	70.0	66.7	33.3

Table 6.2 Percentage of green mirids found in different aspects of terminals, squares and leaves of cotton plants in the field, ACRI, Narrabri, summed for all dates. Total N = 50

The relationship between adult or nymph distribution and node position (upper halves or lower halves) was not statistically significant (Figure 6.2; χ^2 =0.348, df=1, P=0.55).

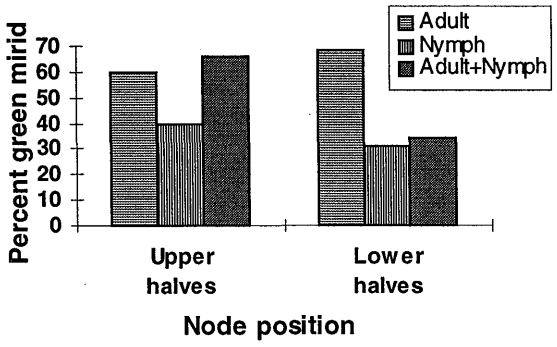


Figure 6.2 Distribution of green mirids on different main stem node positions of the cotton plant in the field, ACRI, Narrabri, summed up for all dates.

6.3 Population studies of GM on cotton and lucerne

Data were collected for three seasons (1994 to 1997) in two locations, ACRI in Narrabri and Norwood in Moree and for two seasons (1994 to 1996) in one location, Alcheringa in Boggabilla of northwest New South Wales (chapter 3, in this thesis) in an attempt to understand factors influencing the population changes of GM. Factors taken into consideration are competition, emigration, predation, weather and spraying of insecticides. In this section, however, I am presenting data for only two locations since in the third location (Alcheringa in Boggabilla), during the 1994-95 season, only two insects could be detected from 12 sampling occasions and in the 1995-96 season the field was inaccessible for most of the time due to heavy rain and flood. Sampling was possible on 4 occasions only. For both the ACRI and Norwood, populations estimates were obtained from October to March by weekly sampling on fields where lucerne was interplanted with cotton. Lucerne is used as a refuge for beneficial insects (Mensah 1998). Half of the lucerne strips are slashed on alternate occasions and at regular intervals to keep the lucerne fresh. I sampled both cotton and lucerne. I also studied GM adult movement in the field using sticky traps. All studies were conducted on irrigated cotton fields.

6.3.1 Materials and Methods

Description of fields

ACRI, Narrabri

The study was conducted in a 2.1ha Envirofeast® IPM field. Envirofeast® is a non-toxic attractant for beneficial insects (Mensah 1997). One hundred and seventy seven (177) rows of cotton were grown each 100m long 1m apart divided into three blocks containing 48, 121 and 8 rows of cotton. In between cotton blocks 4 rows of lucerne strips were grown (Figure 6.3). Lucerne strips developed well before cotton and were irrigated as for cotton. Sowing dates for the cotton crop were close to the recommended date (mid October) (Table 6.3) and plant densities ranged from 9 to 15 plants per metre with an average density of 10 plants per metre. Variety Sicala V II was used in all seasons. Standard agronomic practices were adopted for crop

management. Other than Envirofeast®, the miticide (Comite) was sprayed once in January 19, 1996 to control mites.

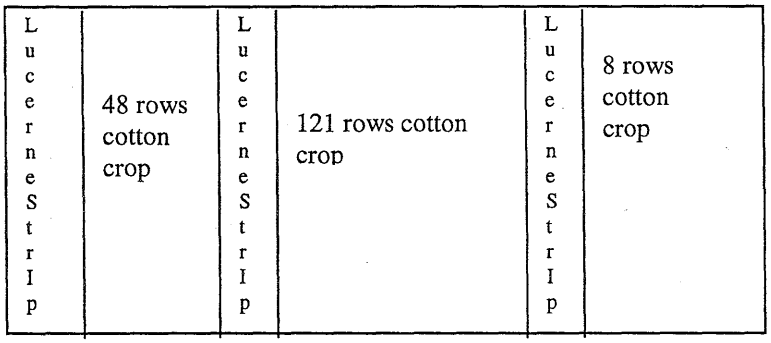


Figure 6.3 Field layout. ACRI, Narrabri.

Norwood, Moree

In this site the population estimates were obtained from a 48ha commercial field. Like ACRI, lucerne crop was interplanted with cotton (Figure 6.4) and the lucerne was established before the cotton was planted. The sowing date and cultivars used for the study are given in Table 6.3. Plant densities were on average 10 plants per metre. Normal agronomic practices were adopted to manage the crop. The field was initially managed using IPM strategies such as Envirofeast®, biological pesticides, beneficial insects, etc but due to insecticide drift from neighbouring farms which disrupted beneficial insect activity in the IPM plot the growers converted it to conventional pest management when *Helicoverpa spp.* pressure was high. The types and dates of insecticide spraying for three seasons are given in Appendix 5.1.

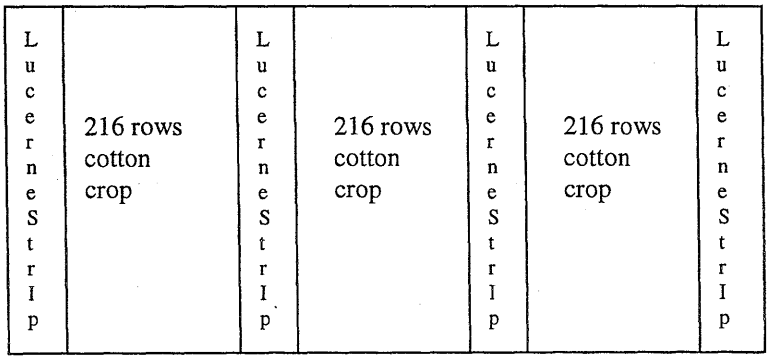


Figure 6.4 Field lay out. Norwood, Moree

Season	Site	Sowing date	Variety
1994-95	ACRI	12 Oct	Sicala V II
	Norwood	12 Oct	Sicala V15
1995-96	ACRI	06 Oct	Sicala V II
	Norwood	30 Sept	Sicala V15
1996-97	ACRI	10 Oct	Sicala V II
	Norwood	02 Oct	Sicala V II

Table 6.3 Seasons, sites, sowing dates and cultivars of population study fields.

Methods for sampling the population

Population estimates for GM adults, nymphs and eggs and other insect fauna including predators were obtained weekly from both sites (Table 6.4). GM adults and nymphs and other predatory insects were sampled using a small portable suction machine (Chapter 2) whereas eggs were counted by cutting shoots brought to the laboratory on each sampling date and examined under a binocular microscope.

Sampling adults and nymphs in cotton and lucerne

The suction tube of the vacuum sampler was passed over the tops of cotton plants or drawn along the sides of the plants and was also inserted inside the canopy when it grew thick, particularly toward the end of stage II (i.e. January 20) of the cotton season. In a single pass a 20m section of vacuum sampling constituted a sample. On each sampling date, 5 samples were taken from cotton rows and two samples from lucerne in ACRI for all three seasons. At Norwood, 8 samples from cotton and 4 samples from lucerne were taken during both the 1994-95 and 1995-96 seasons. During the 1996-97 season, 5 samples from cotton and 3 samples from lucerne were taken. Samples were taken randomly covering whole plots. GM adults, male and female and all five stages of nymph were counted on each sampling date.

Season	Site	Starting date	Ending date
1994-95	ACRI	17 Nov	22 Feb
	Norwood	15 Nov	15 Mar
1995-96	ACRI	31 Oct	11 Mar
	Norwood	25 Oct	19 Mar
1996-97	ACRI	30 Oct	5 Mar
	Norwood	24 Oct	28 Feb

Table 6.4 Starting and ending dates of population estimate in ACRI, Narrabri and in Norwood, Moree for different seasons. Samples were taken weekly between these dates.

Sampling adults and nymphs in alternative hosts

It was observed that some alternative hosts which were thought to be preferred overwintering hosts of GM were abundant near the base of a water reservoir in Norwood. To assess the role of these alternative hosts in the dynamics of GM in the cotton field I sampled them in the 1995-96 season. Due to their ephemeral nature hosts could not be sampled consistently on all sampling occasions. As for cotton, insects were sampled by passing the suction tube over the tops of the host plants. Because these hosts did not grow over large enough areas, replicated sampling was not possible. Ten to 20m sampling was made on each occasion, depending on availability of the host.

Sampling eggs

GM eggs are inconspicuous, concealed inside plant tissues in the upper halves of the plant (Chapter 3 and 5). To count eggs, whole plants early in the cotton season (stage I, October to December 10), and upper halves of the plant in mid cotton season, (stage II, December 11 to January 20) were taken to the laboratory to check under the microscope. Whole plants were uprooted and upper halves of the plant were cut on the main nodal stem using secateurs. Since on an average 10 plants grew in a metre, 10 whole plants or terminals constituted a sample and the estimates were expressed as egg number per metre. On each sampling date 3 samples were taken from each site. Plants were selected randomly covering whole blocks. In ACRI one sample from a 48 row cotton block and two samples from a 121 row cotton block (Figure 6.3) were

taken. In Norwood only one sample from each cotton block (Figure 6.4) was taken. Egg counts were made at Norwood during the 1995-96 and the1996-97 seasons.

Assessment of adult movement

To assess adult GM movement in the study site unattractive white sticky traps (Chapter 2) were used. The study was conducted during the 1994-95 and 1995-96 seasons at Norwood, Moree and during the 1995-96 and 1996-97 seasons at ACRI, Narrabri. At Norwood eight sticky traps, two on each side, and at ACRI 4 traps, one on each side, were placed at a height of 75cm (Mensah 1996) from the ground to the base of the panel. The insects caught on the side of the trap facing the study site is classified as emigrants and those caught on the opposite side *i.e.* facing the surrounding bushland as immigrants. The numbers of GM adults caught on each side of the sticky trap were counted and recorded separately every week. These were removed with a fine forceps and classified on the spot according to sex. Following counting the traps were washed with kerosene or new polystyrene sheets were fitted (Chapter 2) and recoated with the trapping adhesive. To understand the movement of GM adults, the number of adults caught was compared with the number of adults per metre found by suction sampling in the field. Comparisons were made between trap catches and GM egg and nymphal populations in the field to determine any relationship which might indicate factors controlling flight activity.

Analysis of the sampling data

Population estimates for eggs, nymphs and adults were expressed as numbers per metre and as crop stagewise (stage I indicated early season *i.e.*, seedling to squaring; stage II indicated mid season *i.e.*, peak squaring to boll setting and stage III indicated late season *i.e.*, boll maturation to harvest) unless otherwise stated.

Mortality

Mortality was estimated using a method developed by Richard in 1940. In this method it is assumed that the number of each stage found in a series of systematic samples corresponds with the time spent in that stage, any deviation from this representing the magnitude of mortality of that stage. The duration of each stage used for this method was calculated from the results of a laboratory study at 25°C±1 (Chapter 3). I used the same duration for all seasons because the average temperature during the sampling time was around 25°C (Appendix 5.2). No attempt was made to calculate mortality for lucerne since egg numbers were not recorded.

Population estimates

GM populations were very low in all seasons in both locations which made it difficult to apply conventional methods of analysis to sampling data to explain population dynamics of GM. Therefore the population data was analysed using Population Analysis System (PAS 4.0), a single species time series analysis (Berryman and Millstein 1994). Most insect populations are analysed by constructing life tables and key factor analysis is used to determine the key factor responsible for population regulation (Varley and Gradwell 1968). However, according to Berryman (1994), the high cost of collecting life-table data makes its application to real-life pest management problematic. Furthermore traditional life-table analysis looks only for direct density dependence and fails to detect density-dependent regulation in populations that are characterized by lags and complex dynamic behaviour (Turchin 1990). On the contrary, 'single species time series analysis' methods can identify factors that regulate population abundance from regular sampling data and they can also be used to build forecasting models. Models underlying classical time series analysis (Box and Jenkins 1976) are generally inappropriate for modelling self-replicating biological systems, however the time series analysis used in the PAS program is specially adapted to biological populations similar to GM.

In the time series analysis using PAS, the per-capita rate of change was estimated from the equation-

$$R = \log_e N(t) - \log_e N(t-1)$$

where R is the per-capita rate of change, N(t) is the density of population at time t and N (t-1) is its initial density. Since per-capita rate of change is influenced by both density dependent and density independent factors, these concepts were included into the following equation by allowing the per-capita rate of change to be a function of population density plus random environmental influences:

$$R = f\,[N(t-d)] + V$$

where f is a density dependent regulation function, or R-function, d is the average delay in response of the density dependent forces, and V is a random variable. Density independent forcing variables are assumed to act on the parameters of the model.

Before analysis, sampling data were transformed into $\sqrt{x+1}$ and then rescaled by multiplying by 100. As a first step of analysis, the stationarity and trends of the data were checked by using diagnostic statistics which included mean return time (MRT) and variance of the return time (VRT). The MRT and VRT were computed from the following formulae-

$$\text{MRT} = \sum_{i=1}^{I} RT(i) / I\,,$$

where RT is the return time, the time it takes for a trajectory to return to its mean value after disturbance and I is the number of times the trajectory crosses its mean.

$$\text{VRT} = \sum_{i=1}^{I} [RT(i) - MRT]^2 / (\text{I} - 1)$$

The mean and variance of the return time provide useful clues to the length of the time lag, and indicate whether the data show trends or discontinuities:

MRT < 2 implies a time lag of one, or d = 1,

MRT > 2 suggests that d > 1 and

VRT >> MRT implies a trend or discontinuity in the data, (*i.e.* a nonstationarity).

Nonstationary data were changed into stationary by sequencing and trend data were detrended before further analysis. Once the discontinuities were removed from the data set, delay in the feedback response was estimated by using partial rate correlation function (PRCF) and phase portraits. The PRCF provided partial correlation between the per-capita rate of change R and lagged population density n(t - d). The phase portrait is a plot of the per-capita rate of increase R on population density, N_{t-d} at lag d. Dominant lag was detected from phase plotting by changing d until the best correlation was obtained.

Once the lag structure of the dominant feedback mechanism acting on a population was detected, a linear or nonlinear logistic model was fitted to the data. Linear models were fitted by using a standard linear regression routine. The parameters in the model are:

A = the y-intercept or maximum per-capita rate of change

C = the slope of the regression line or the coefficient of density dependence

K = the equilibrium density (K=A/C)

s = the standard deviation, this is an estimate of density independent environmental variability,

r^2 = the coefficient of determination or percentage of the variation in the data explained by the regression line.

The nonlinear logistic model fitted to the data was:

$$R = A \{1 - [N(t - d)/K]^Q\}$$

where Q is the coefficient of curvature. If Q>1, the R-function bends downwards and has a convex form while if Q<1, it bends upwards and has concave shape. The shape of R-function describes the behaviour of the population.

I have used PAS analysis to analyse data for the 1996-97 season at ACRI as an example to see whether the factors regulating the GM populations could identify.

6.3.2 Results and observations

The population changes in GM in the cotton system

The trends of GM populations were similar both at ACRI and Norwood. However, significantly higher ($P < 0.05$) GM populations were recorded at ACRI than Norwood (Figure 6.5). In both study sites GM populations were highest in the 1995-96 and 1996-97 seasons. The lowest GM populations were recorded in the 1994-95 season at both study sites. The numbers of GM recorded in the 1994-95 season were 4 times less than numbers recorded during the 1995-96 and 1996-97 seasons. The lower GM numbers in 1994-95 may be due to the long period of drought experienced during that season resulting in the 'drying off' of alternative hosts plants of GM in spring thus suppressing the build up of GM population in cotton.

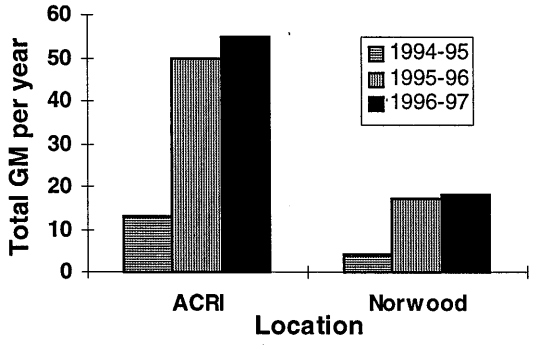


Figure 6.5 Green mirid populations per year in the cotton at ACRI and Norwood, Moree as determined by suction sampling.

GM on cotton occurred mainly at stage I and II (seedling to boll setting stage) in both locations in all seasons (Figures 6.6 and 6.7). However, in the 1994-95 and 1995-96 seasons, small numbers of adult populations were recorded at stage III (boll maturing to harvesting stage) (Figures 6.6A and 6.6B). This may be due to regrowths on the top of the mature cotton crops following 3 days of rainfall prior to crop defoliation. These regrowths serve as food for GM adults and nymphs because of their sugar content.

In ACRI, during the 1994-95 season highest numbers of adults (0.02/m) were recorded on the 15th and 22nd December 1994. These adults laid a considerable

number of eggs with the highest number of eggs (3.33) per metre recorded on the 12th January 1995. Thereafter numbers of adults declined to zero on the 3rd January 1995. The decline in the adult numbers coincided with the dates when synthetic insecticide (pyrethroid) was used by a neighbour on his farm to control *Helicoverpa* spp. (Figure 6.6A). On the 23rd January 1995, high numbers of adults and nymphs (0.01/m) were recorded. Both adults and nymphs declined to zero on the 7th February 1995 but the adult numbers recovered thereafter reaching a peak of 0.02 per metre on 22nd February 1995, 7 days prior to defoliation. The recovery of the adult population during this period coincided with development of new regrowths following rainfall. Also growers have ceased using insecticides waiting for the grounds to dry to defoliate the crop. In contrast to ACRI, no GM nymphs were recorded at Norwood during the 1994-95 season. The lower GM population at Norwood may also be attributed to drought and insecticide drift from neighbouring farms.

During 1995-96 season at ACRI, GM populations were found both in stage I and stage II (Figure 6.6B). The highest number of adults (0.07/m) were recorded on the 2nd January 1996. These adults laid significant amount of eggs with the peak number of eggs (3.33/m) recorded on the 8th January 1996. Despite the higher number of eggs laid on the plant only 4% nymphs were recorded indicating a high GM egg and nymphal mortalities. During the 1995-96 season at Norwood, GM populations were recorded in stage I only (Figure 6.7B). The highest numbers of adults (0.05/m) were found on the 24th November 1995. Following that the highest numbers of eggs (0.67/m) were recorded on the 29th November 1995. Despite the high numbers of eggs recorded no nymphs were found at this site indicating high egg and nymphal mortalities.

During 1996-97 season at both ACRI and Norwood GM populations were significantly higher ($P < 0.05$) in stage I and stage II than stage III (Figures 6.6C and 6.7C). At ACRI GM adults were first recorded in November 14, 1996. The adults commenced egg lay on the 5th December 1996 reaching its peak 3.0/m on the 19th December 1996 before declining to zero on the 17th January 1997. The decline of the egg numbers followed similar trends as the adults. The nymphal populations showed two peaks one on the November 14, 1996 and the other on the December 26, 1996.

The peak on the 14th November 1996 might have been laid by adults before sampling commenced. Like the adults and eggs the nymphal population collapsed on the 22nd January 1996, a period of high pyrethroid sprays by neighbours to control insecticide resistance *Helicoverpa* spp. At Norwood in the 1996-97 season, GM adults were recorded on the 7th November 1996 reaching its peak on the 5th December 1996 before declining to zero on the December 27, 1996. These adults laid two peaks of eggs on the 20th December 1996 and 2nd January 1997. Despite the high number of eggs (2.0/m) laid on the 20th December 1996 only 0.5/m nymphs were recorded indicating high egg and nymphal mortalities.

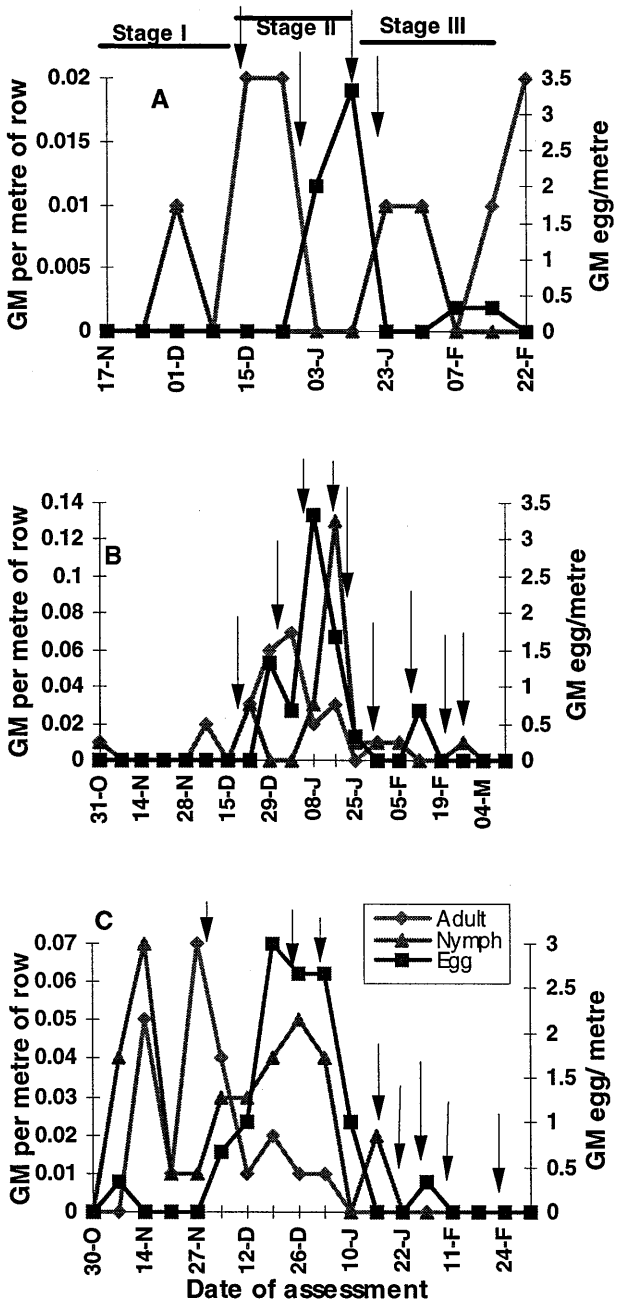


Figure 6.6 Seasonal density of green mirid population in the cotton at ACRI, Narrabri during (A) 1994-95, (B) 1995-96 and (C) 1996-97 seasons as determined by suction sampling. Arrows indicate insecticide spraying dates in the vicinity of the field. Horizontal lines in figure A indicate crop season. Stages I, II and III indicate early season (seedling to squaring); mid season (peak squaring to boll setting) and late season (boll maturation to harvest).

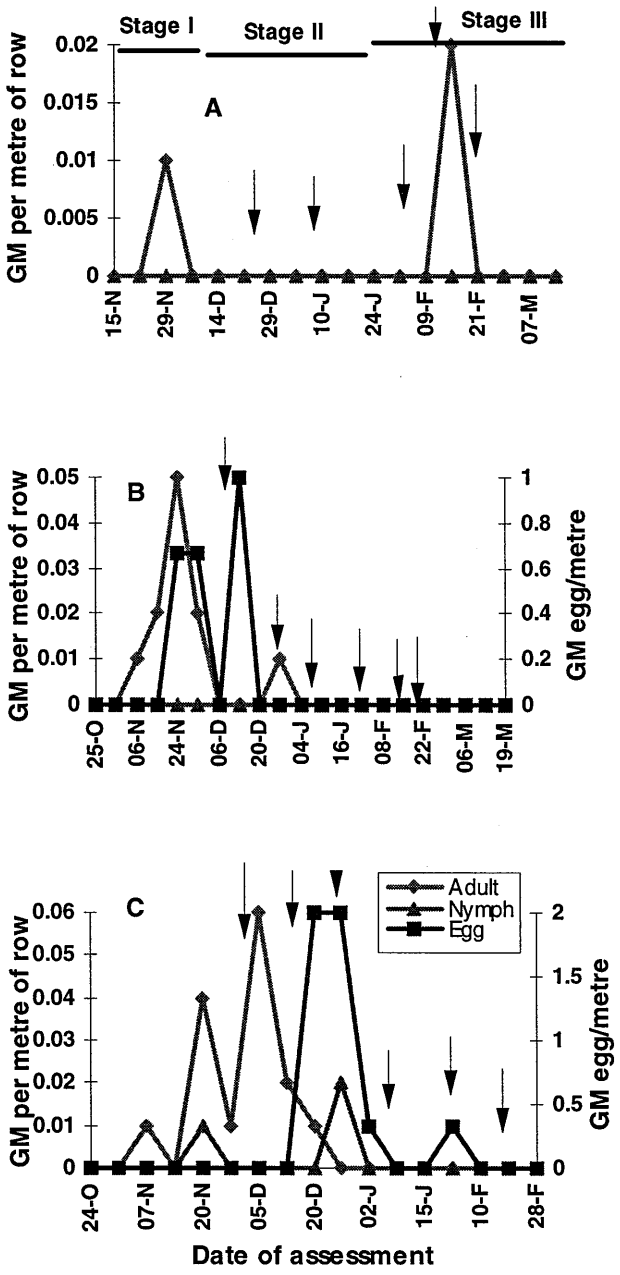


Figure 6.7 Seasonal density of green mirid population in the cotton in Norwood, Moree during (A) 1994-95, (B) 1995-96 and (C) 1996-97 seasons as determined by suction sampling. Arrows indicate insecticide spraying dates in the field. Horizontal lines in figure (A) indicate crop season. Stages I, II and III indicate early season (seedling to squaring); mid season (peak squaring to boll setting) and late season (boll maturation to harvest).

The population changes in GM in the lucerne system

GM populations were significantly higher ($P < 0.01$) in lucerne than in cotton in all seasons at both ACRI and Norwood. At ACRI, the total GM populations recorded in lucerne throughout the study was 17 times higher than in cotton and at Norwood they were 21 times higher. However, GM population recorded in lucerne at ACRI were significantly higher ($P < 0.01$) than those recorded in Norwood (Figure 6.8). At both study sites significantly higher numbers of GM ($P < 0.05$) were recorded in the 1995-96 season than any of the seasons (Figure 6.8). The lowest numbers of GM were recorded during the 1994-95 at ACRI and 1994-95 and 1996-97 at Norwood.

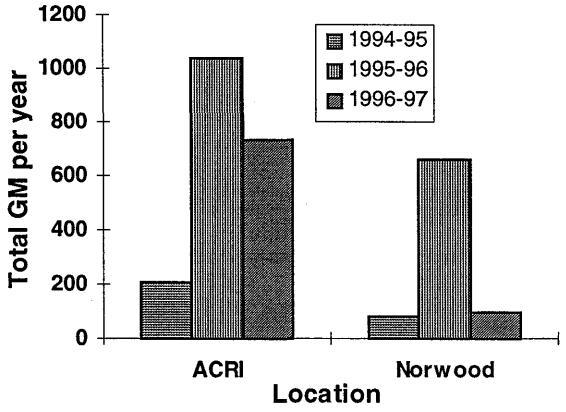


Figure 6.8 Numbers of green mirids in lucerne at ACRI and Norwood in different seasons as estimated by suction sampling.

GM eggs were difficult to find and count on lucerne terminal shoots even under the microscope due to the small size of the plants. As a result, GM eggs were not counted in the lucerne as in cotton in both study sites. GM populations in lucerne were more or less stable compared to cotton in both study sites with the exception of 1996-97 at ACRI, 1995-96 and 196-97 seasons at Norwood.

In the 1994-95 season at ACRI, both adult and nymphal populations were stable throughout the cotton season until the 16th February when numbers of both adults and nymphs started to increase reaching peak at 0.4/m for adults and 1.4/m for nymphs on the 22nd February 1995 (Figure 6.9A). In contrast the GM population at Norwood fluctuated at regular intervals (Figure 6.10A) with the highest adult

population (0.09/m) recorded on the 29th December 1994 and nymphal population (0.23/m) on the 15th March 1995.

GM populations during the 1995-96 season occurred earlier than 1994-95 seasons at all study sites indicating earlier infestation in cotton due to the good winter and spring conditions which enhanced the build up of GM population (Figures 6.9B and 6.10B). At ACRI, GM adult population peaked at 1.15/m on the 14th November 1995 and the nymphal population also peaked at 2.1/m on the 1st February 1996 (Figure 6.9B). At Norwood, the adult population peaked at 0.34/m on the 28th December 1995. In contrast the nymphal population peaked at 1.5/m on the 13th December 1995 (Figure 6.10B).

GM adults and nymphs were recorded at ACRI in November 1996. The nymphal population peaked at 5.32/m on the 8th November 1995 whereas the adult population peaked at 1.15/m on the 14th November 1995. Both adult and nymphal populations continue to increase until 22nd of January 1997, thereafter population declined to zero (Figure 6.9C). In contrast at Norwood, GM adults peaked at 0.2/m on the 20th November 1996 and nymphs also peaked at 0.58/m on the 27th December 1996 (Figure 6.10C). Both nymph and adult populations at Norwood crashed on the 9th of January (Figure 6.10C) as the lucerne crops were not irrigated and as such 'hay off'.

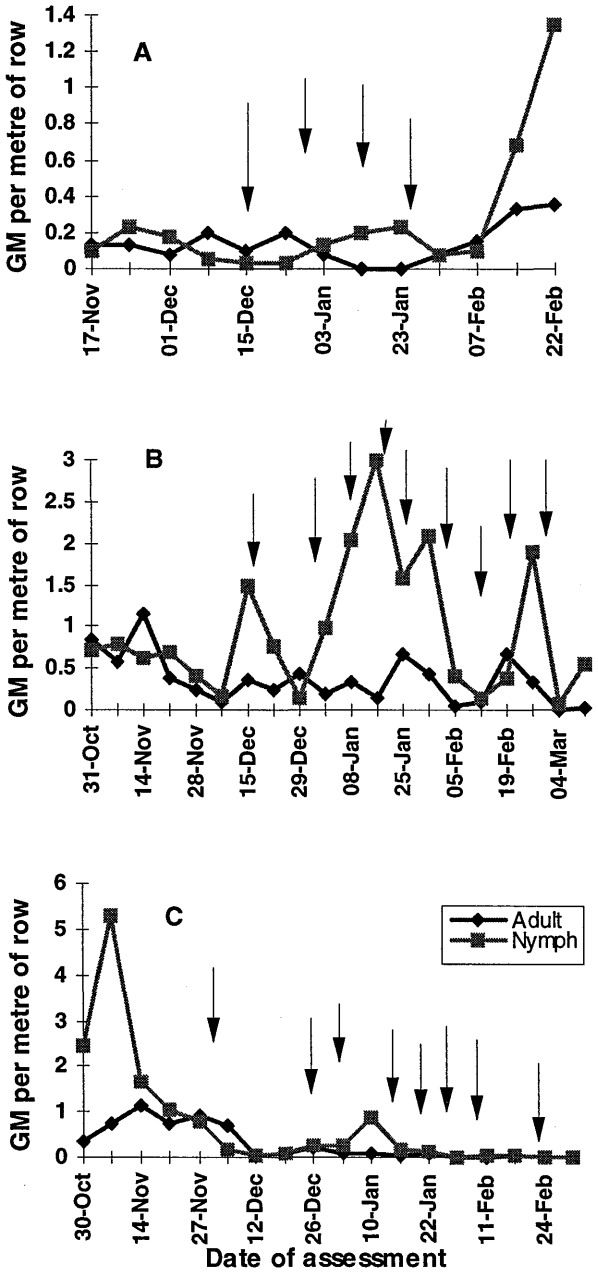


Figure 6.9 Green mirid population densities in lucerne at ACRI during the (A) 1994-95, (B) 1995-96 and (c) 1996-97 seasons as determined by suction sampling. Arrows indicate insecticide spraying dates in the vicinity of the field.

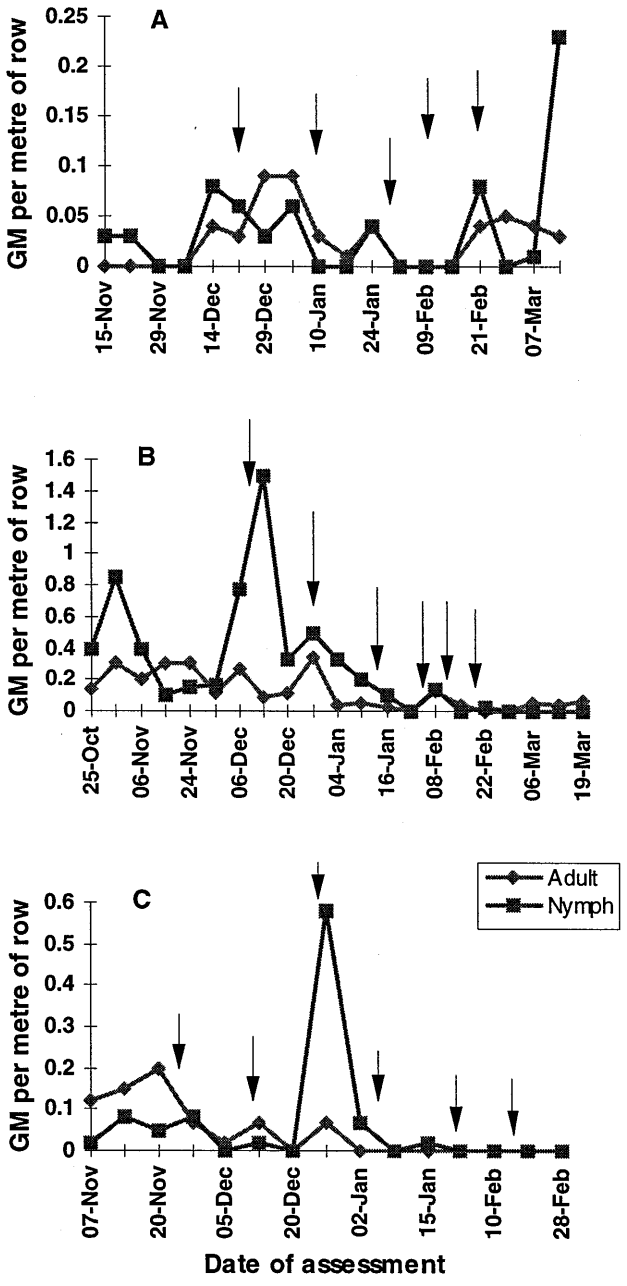


Figure 6.10 Green mirid population densities in lucerne at Norwood, Moree during the (A) 1994-95, (B) 1995-96 and (C) 1996-97 seasons as determined by suction sampling. Arrows indicate insecticide spraying dates in the field.

Mortality of nymphs in the cotton

The seasonal abundance data showed that the numbers of nymphs in cotton were very low in both ACRI and Norwood study sites. The high number of eggs recorded at these sites compared with low numbers of nymphs could indicate high nymphal mortalities. The percent nymphal mortalities (calculated using Richard 1940

method) for different cotton stages in the 1995-96 and the1996-97 seasons for ACRI are presented in Table 6.5. An example of Richards (1940) method used in the calculation of nymphal mortalities is given in Appendix 5.3. Mortality could not be calculated for the data from Norwood nor for the 1994-95 season at ACRI due to absence of nymphs.

Year	Crop stage	% mortality between successive stages			
		Instar 1-2	Instar 2-3	Instar 3-4	Instar 4-5
1995-96	Stage I	*	*	*	*
	Stage II	99	98.3	98.4	99.1
	Stage III	95.5	97.7	97.7	100
1996-97	Stage I	87.5	87.5	98	90
	Stage II	99.1	98.9	99.3	99.3
	Stage III	#	#	#	#

Table 6.5 Percent mortality of GM nymphs at different cotton stages in ACRI during the 1995-96 and the1996-97 seasons estimated using Richard's (1940) method. [a] stage I= planting to December 10 (seedling to pre-squaring); stage II= December 11 to January 20 (squaring to boll setting); stage III= January 21 (boll maturation) to harvesting. * indicates no eggs during the stage I in the 1995-96 season. # indicates no nymphs at stage III in the 1996-97 season.

The mortality of nymphs was very high in all stages and seasons (Table 6.5). Nymphal mortality was highest in stage II during the 1995-96 and 1996-97 seasons. The mortality was lowest in stage I in the 1996-97 season. Most mortalities (over 99%) occurred in the 4^{th} to 5^{th} instar nymphs. These are the stages which move out of their hiding places in the terminals and squares to seek new feeding sites. They may therefore be vulnerable to beneficial insects and other environmental factors.

Adult movement in the study area

Apart from nymphal mortality, depletion of populations may result from adult migration. Table 6.6 shows that there were more emigrating adults caught in sticky traps at Norwood than at ACRI. The ratio of numbers of adults in the field to numbers in sticky traps was highest in Norwood during 1994-95 season and lowest in ACRI during 1996-97 season. Overall, numbers of adults caught in the sticky traps corresponded with a decline of adults in the field when both cotton and lucerne

populations together were taken into consideration (Table 6.6). This suggests that very large numbers of adults might have been lost through emigration.

Location	Season	No. of adults in the field	No. of emigrating adults in the sticky traps	Ratio of no. of adults in the field to no. in sticky traps
ACRI	1996-97	225	18	1:0.08
	1995-96	317	48	1:0.15
Norwood	1996-97	56	14	1:0.25
	1995-96	219	46	1:0.21
	1994-95	40	33	1:0.82

Table 6.6 A comparison of the numbers of emigrating GM adults in the sticky traps with the numbers in the study field at ACRI and at Norwood.

In both locations and in all seasons immigration and emigration of the adult occurred simultaneously in the study fields. The t-test showed there was no significant difference ($P>0.05$) between immigration and emigration (Table 6.7).

Location	Season	Immigrated	Emigrated	t-statistic	P
ACRI	1996-97	1.06	1.13	0.15	0.88
	1995-96	3.19	3.0	0.17	0.86
Norwood	1996-97	0.94	0.88	0.14	0.89
	1995-96	2.81	2.19	0.69	0.50
	1994-95	1.61	1.83	0.28	0.78

Table 6.7 GM adults per sampling date caught in the sticky traps at ACRI and at Norwood in different seasons. Immigration = insects caught on the side of the trap facing away from the field, emigration = insects caught on the side facing the field.

In the study sites, the flight activity of the male and the female varied. T-statistics on male and female caught in the sticky traps indicated the male GM was caught significantly more often ($P<0.05$) in the sticky traps than females (Table 6.8) suggesting that the males may be more mobile than the females.

The relationship between females caught in the sticky traps and eggs per metre in the field was significant ($P<0.05$) except in the 1995-96 season at Norwood where numbers of eggs were detected on three occasions only (Figure 6.11).

Location	Season	Male	Female	t-statistic	P
ACRI	1996-97	1.56	0.63	2.37	0.026
	1995-96	5.37	0.87	3.61	0.002
Norwood	1996-97	1.38	0.56	1.82	0.083
	1995-96	4.14	0.90	3.31	0.003
	19994-95	2.61	0.83	2.24	0.035

Table 6.8 A comparison of male and female GM caught in the sticky traps at ACRI and Norwood at different seasons.

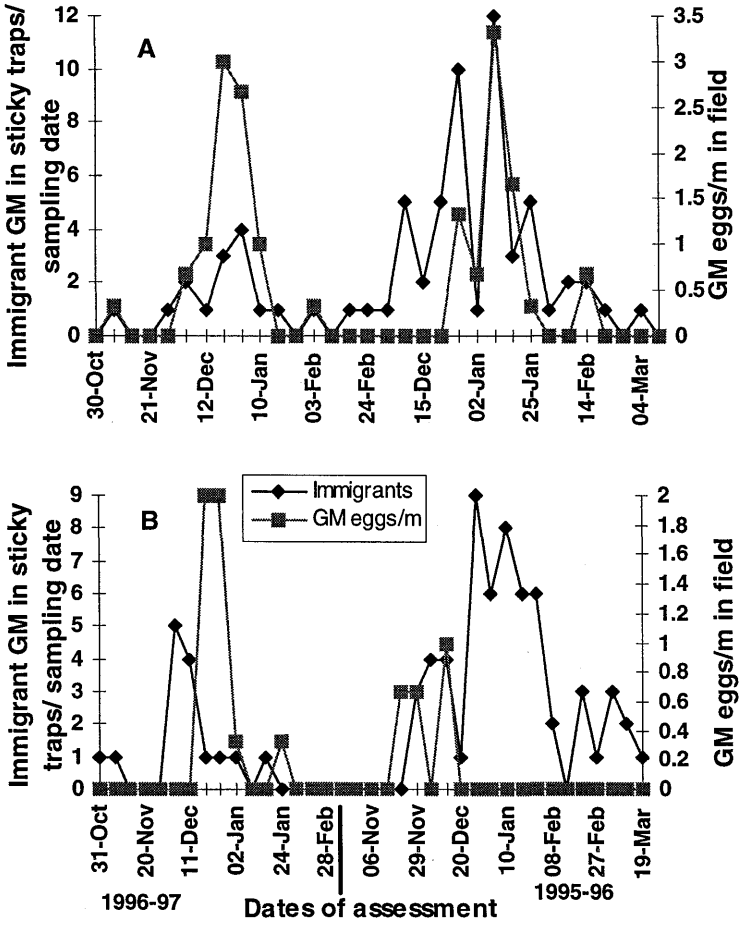


Figure 6.11 Relationship between immigrating GM adults caught in the sticky traps and GM eggs/m in the study fields in the 1996-97 and the 1995-96 seasons at ACRI (A) and at Norwood (B).

The causes of GM population regulation in cotton

Adults and nymphs

Data were split into two sequences (1 and 2) and each sequence was analysed separately. The first sequence was from October 30 to December 19 and second sequence was from December 26 to March 5. The time series plots for both sequences along with test statistics are given in Figure 6.12. It was revealed from the test statistics that Mean Return Time (MRT) was less than 2 and Return Time Variance (VRT) was less than MRT indicating a smaller time lag and that the population is being regulated by fast acting negative feedback. The dominant time lag was determined by the Partial Rate Correlation Function (PRCF) and phase portraits for both sequences (Figures 6.13 and 6.14). In all cases PRCF indicated dominant-feedback with lag 1 and regression on phase portraits also showed the largest regression coefficient at lag 1. When non-linear R-function was fitted to the data a convex shaped curve with larger coefficient of curvatures ($Q>1$) in sequence 1 and a concave shaped curve with smaller coefficient of curvatures ($Q<1$) in sequence 2 were found (Figure 6.15). This suggests that in the earlier case population was regulated due to contest competition and in the latter case the population was regulated due to scramble competition.

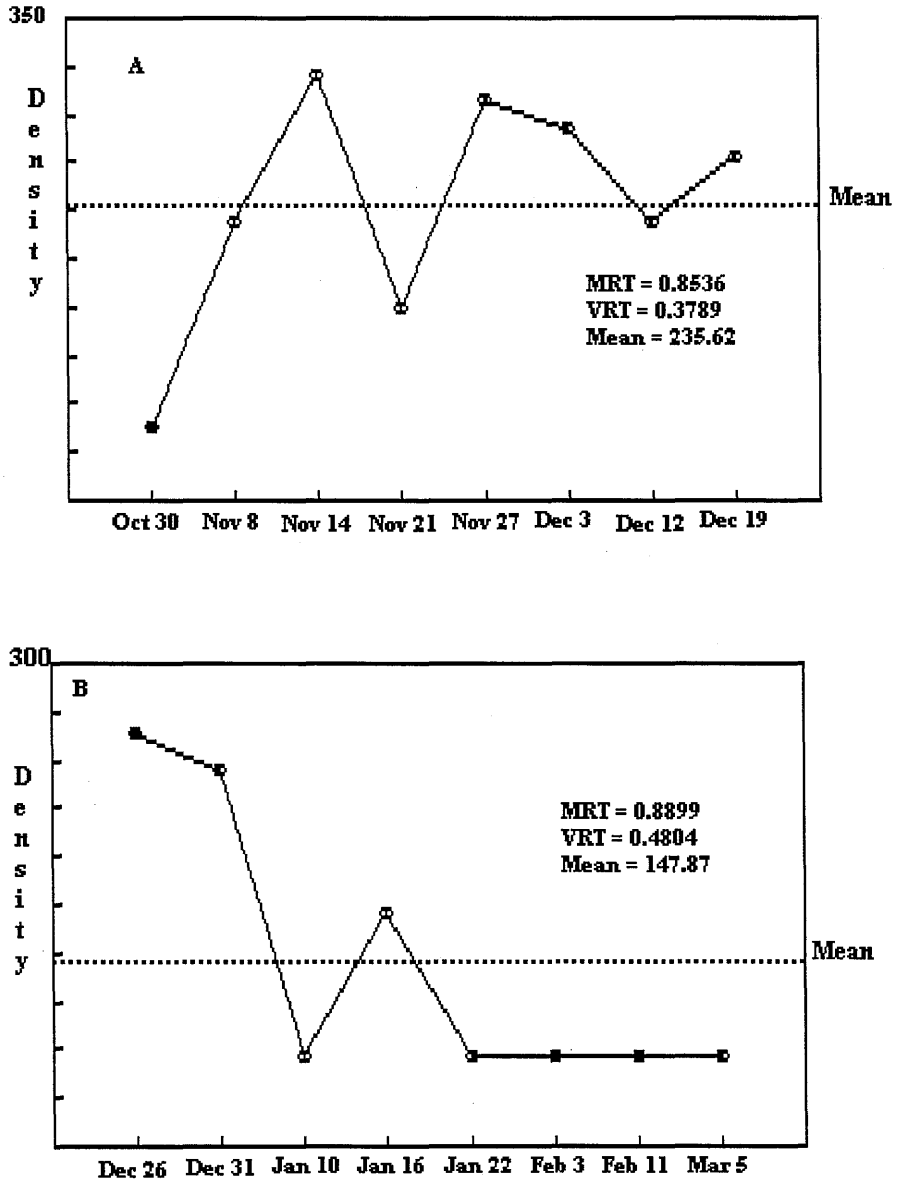


Figure 6.12 Time series of GM in cotton at ACRI in the 1996-97 season along with diagnostic statistics. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994).

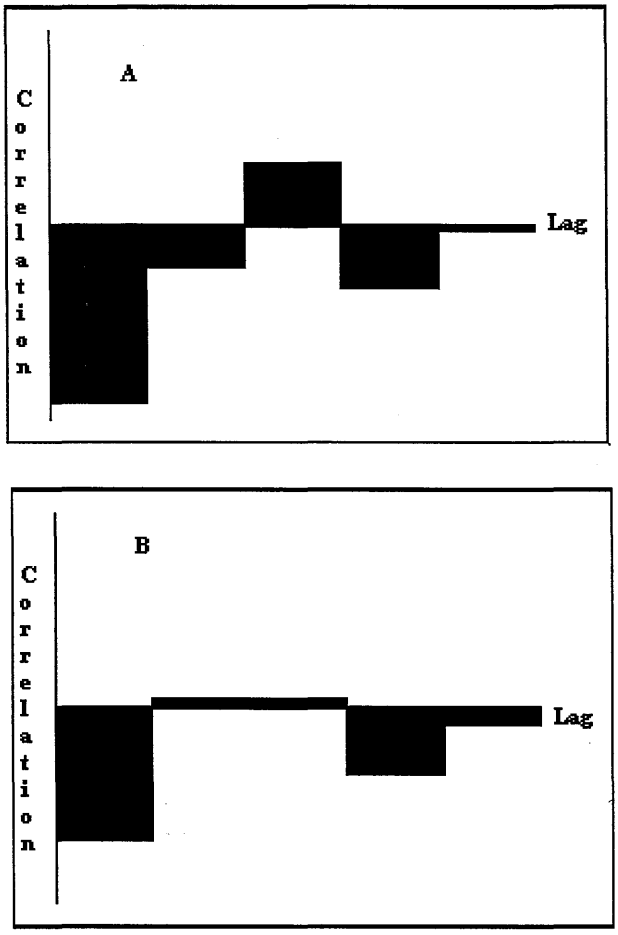


Figure 6.13 Partial rate correlation function (PRCF) for GM in cotton at ACRI during the 1996-97 season showing dominant time lag. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994).

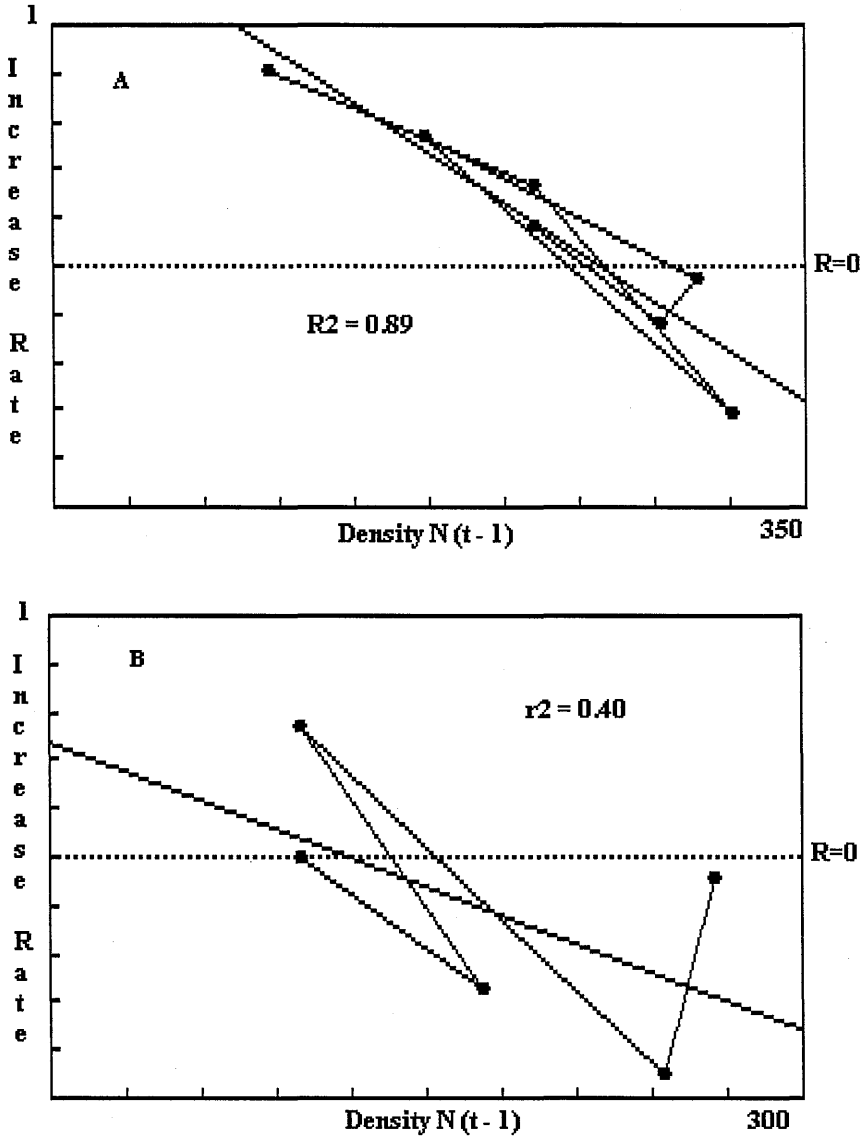


Figure 6.14 Phase portraits along with regression lines for the GM time series in cotton at ACRI during the 1996-97 season. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994).

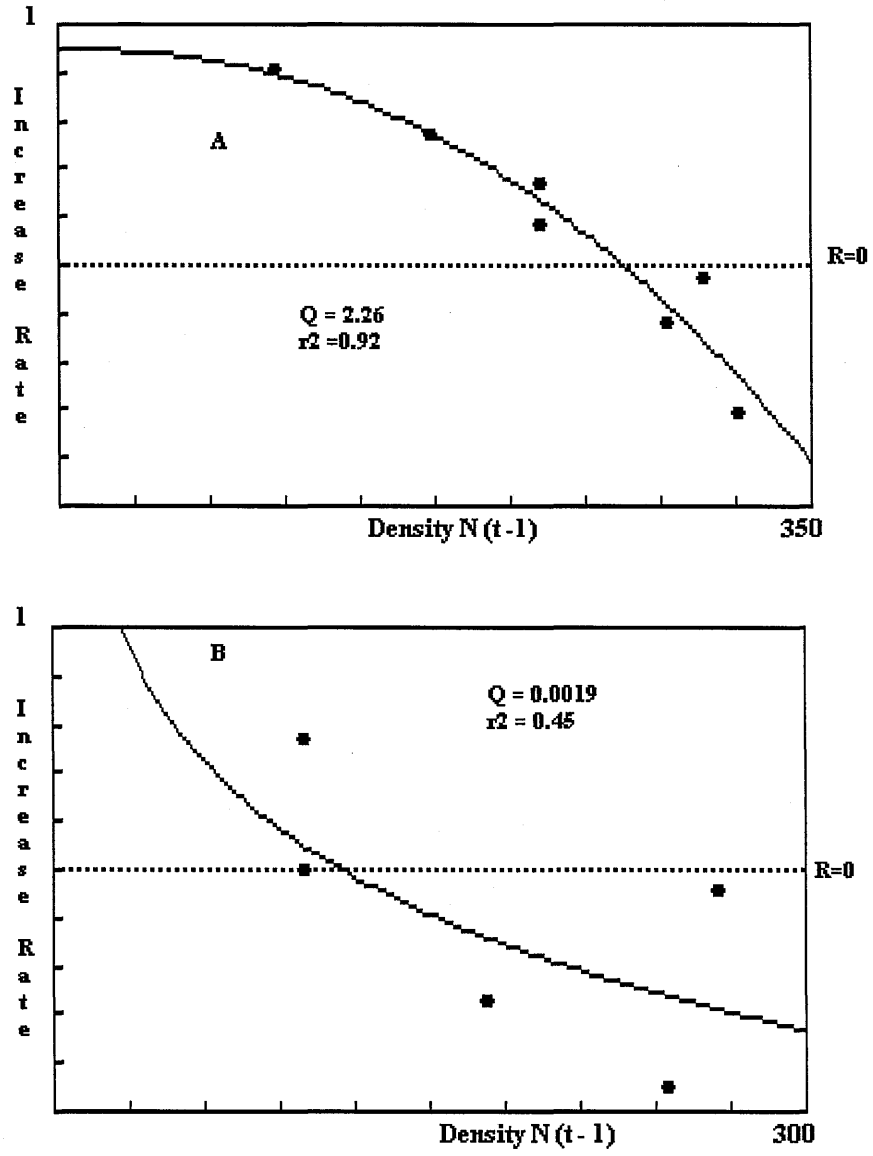


Figure 6.15 Non-linear R-functions with a time delay of 1 fitted to the GM time series data in cotton at ACRI during the 1996-97 season. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994).

Eggs

The de-trended time series data for GM eggs in cotton for both sequences are presented in Figure 6.16 along with test statistics. The MRT for both sequences was > 2, indicating the time lag was greater than 1. PRCF and regression on phase plot (Figures 6.17 and 6.18) showed dominant time lag at 3 for sequence 1 and at 2 for sequence 2. In sequence 2, however, dominant lag was not clear cut in PRCF (Figure 6.17B). When the regression line was drawn through the phase plot of different lag, the largest regression coefficient was found at lag 2, suggesting a delayed density

dependence mechanism acting on the GM egg population in the field for both sequences. Non-linear R-functions, when fitted to the de-trended time series of both sequences, formed concave shaped curves with smaller coefficients of curvature (Q<1) (Figure 6.19).

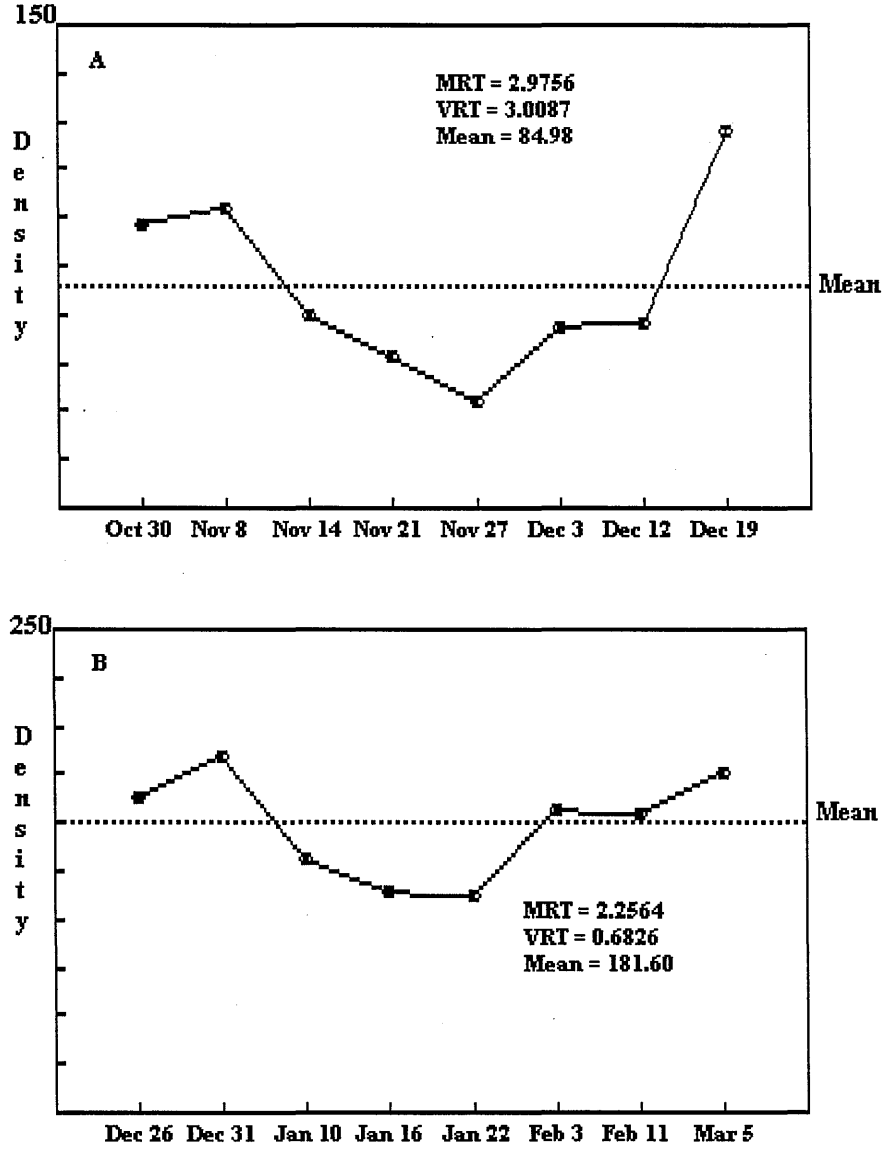


Figure 6.16 Detrended time series of GM egg in cotton at ACRI during the 1996-97 season along with diagnostic statistics. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994

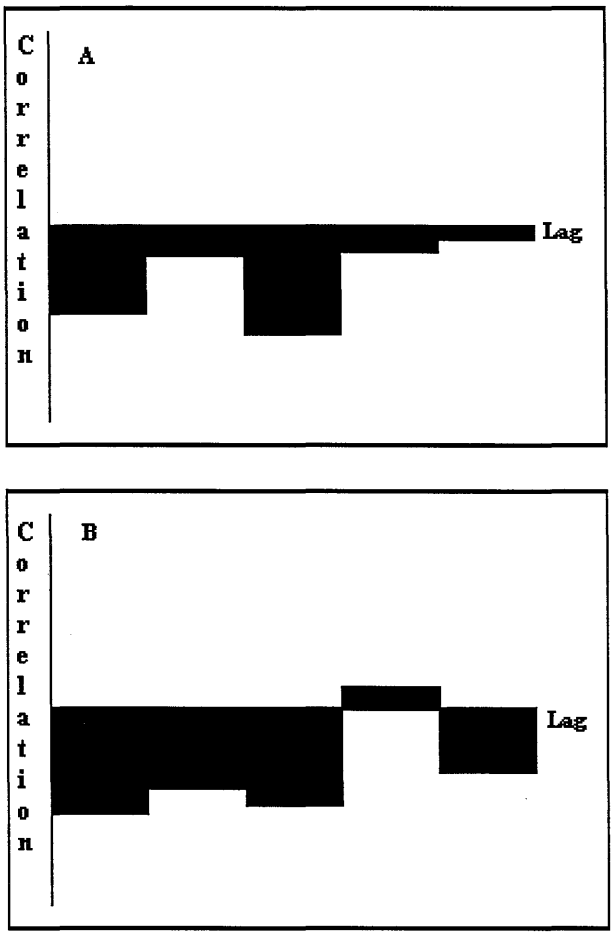


Figure 6.17 Partial rate correlation function (PRCF) for the de-trended GM eggs time series in cotton at ACRI during the 1996-97 season showing dominant time lag. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994

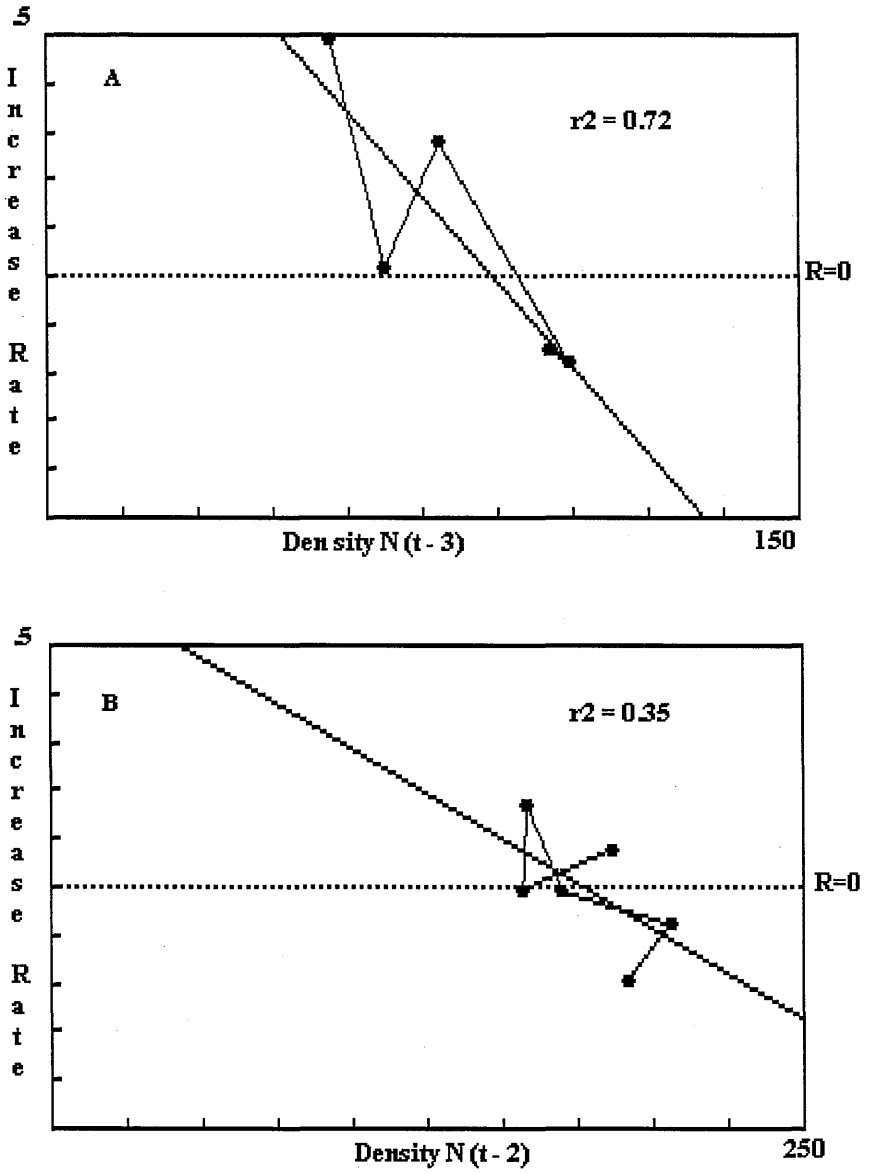


Figure 6.18 Regression line on phase plot for the de-trended GM eggs time series in cotton at ACRI during the 1996-97 season. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994

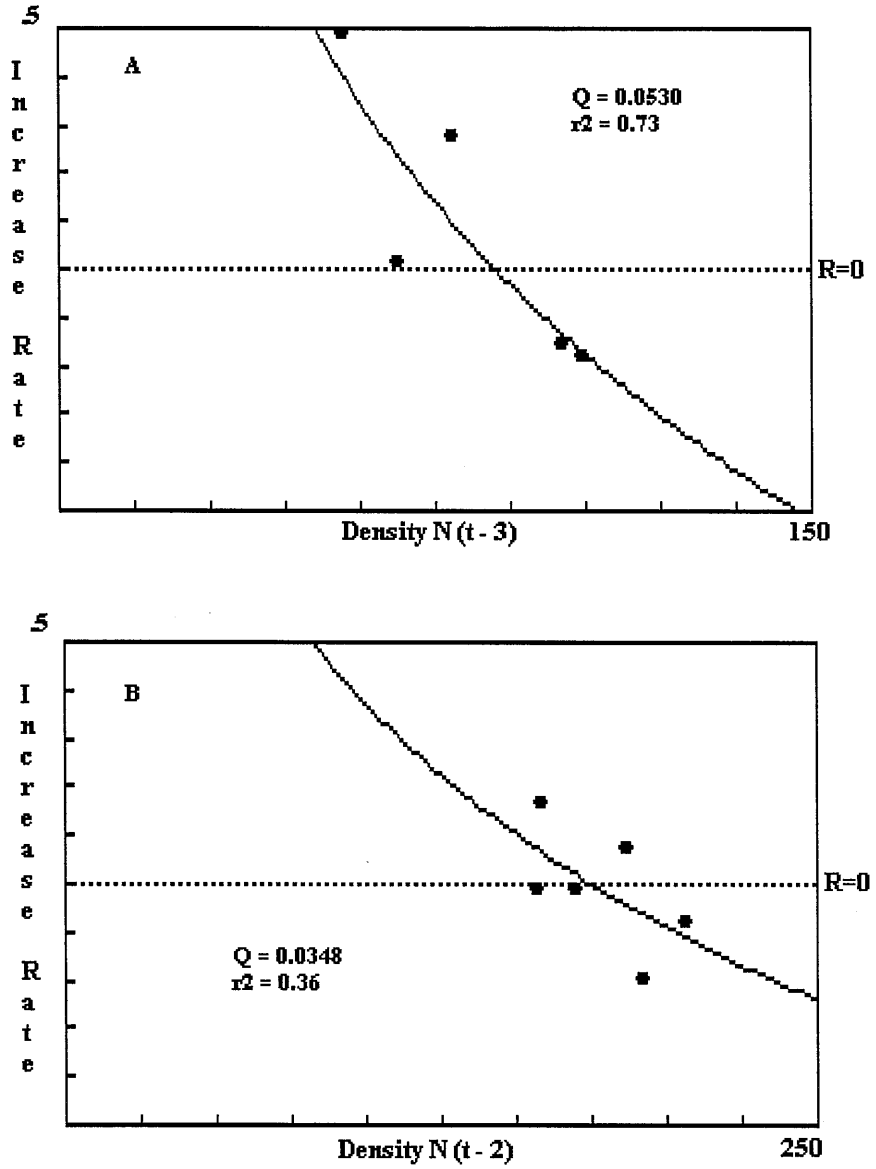


Figure 6.19 Non-linear R-functions fitted to the detrended GM egg time series in cotton at ACRI during the 1996-97 season. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994

The causes of GM population regulation in lucerne

De-trended time series plots of GM in lucerne during the 1996-97 season for sequence 1 and 2 are depicted in Figure 6.20. The MRT and VRT for sequence 1and 2 respectively were 0.8034, 0.2094 and 0.8115, 0.1915 which suggests small time lags. In lucerne, as for the cotton system, it was revealed from PRCF and phase portrait that dominant feedback acted on the population for time delays of one for both sequences

(Figures 6.21 and 6.22). Non-linear R-functions in lucerne showed concave shaped curves with small coefficients of curvature (Q<1) for both sequences (Figure 6.23).

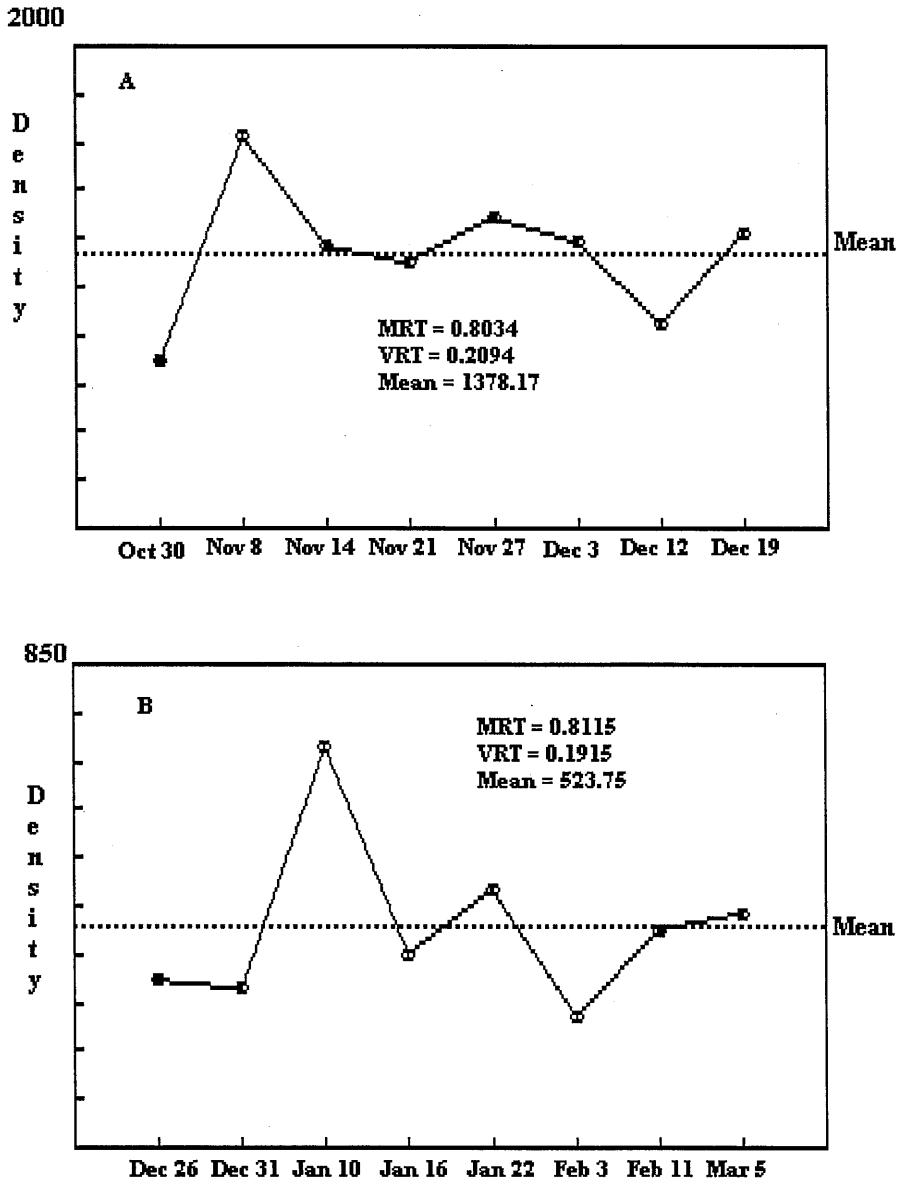


Figure 6.20 Time series of GM in lucerne at ACRI during the 1996-97 season along with diagnostic statistics. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994

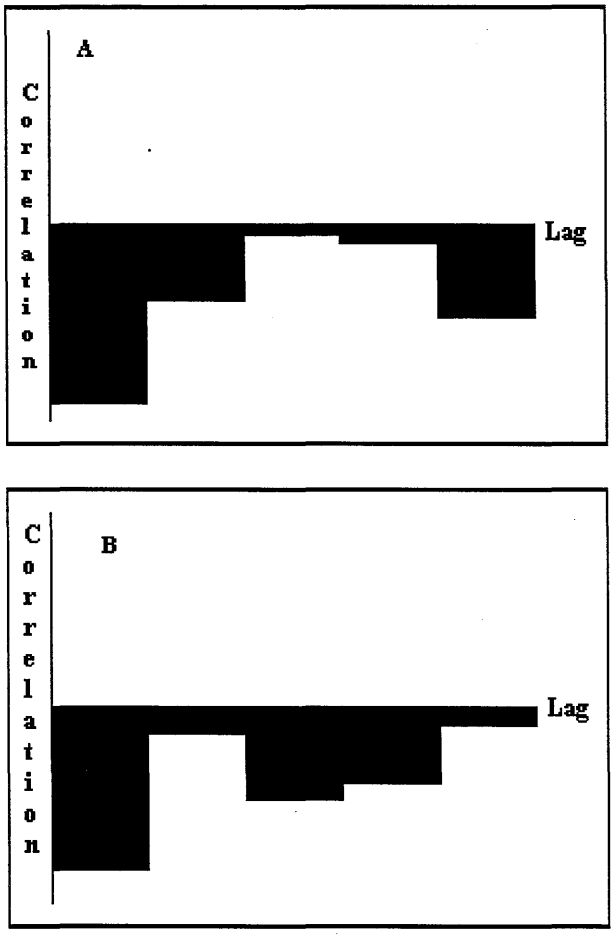


Figure 6.21 Partial rate correlation function (PRCF) for the detrended GM time series in lucerne at ACRI during the 1996-97 season showing dominant time lag. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994

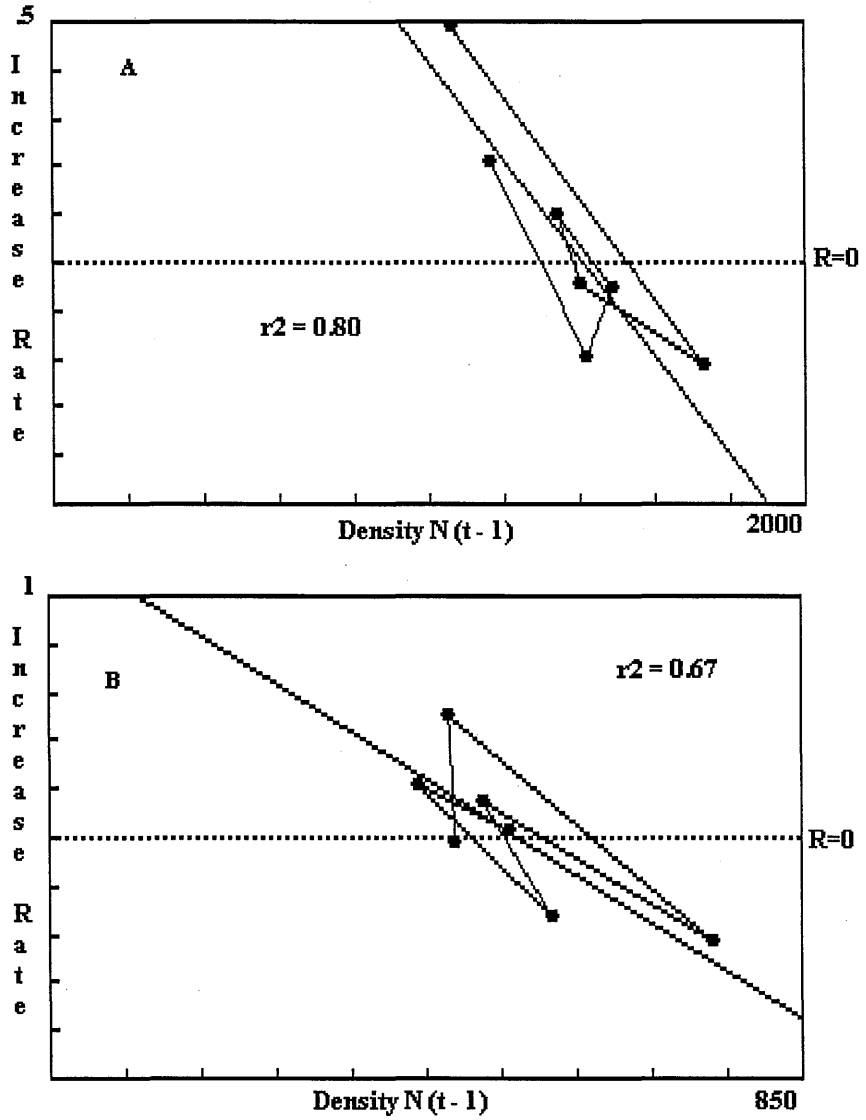


Figure 6.22 Phase plots along with regression lines for the detrended GM time series in lucerne at ACRI during the 1996-97 season. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994

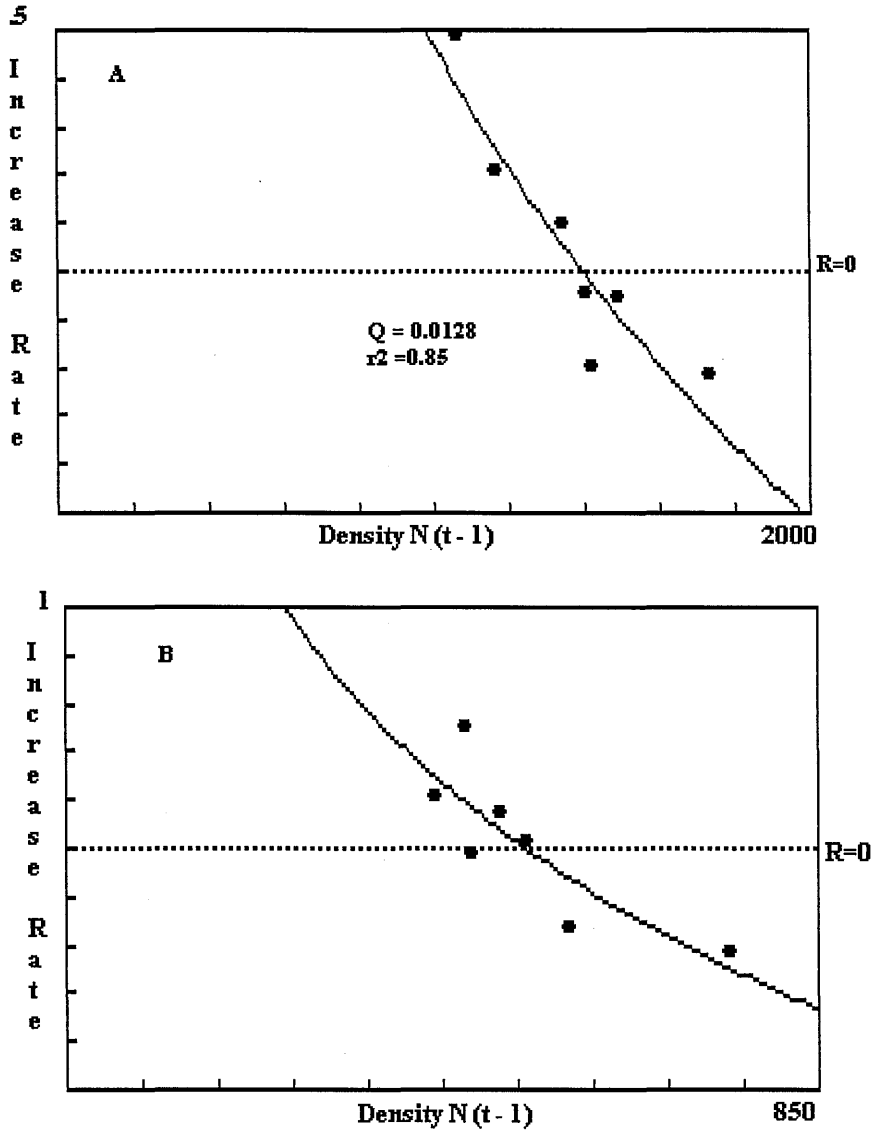


Figure 6.23 Non-linear R-functions with a time delay of 1 fitted to the detrended GM time series in lucerne at ACRI during the 1996-97 season. A = sequence 1 and B = sequence 2. Data were analysed using PAS (Berryman and Millstein 1994)

Factors affecting GM populations in cotton

Predators and GM population

Many predatory arthropods (Room 1979) were present with the green mirid populations in the cotton system. Most of them are generalist predators, preying on different insects. Amongst these predators only spiders were observed preying on

green mirid nymphs in the field. It was suggested by the PAS analyses in the previous section that green mirid egg populations were regulated by delayed density dependent factors, of which predators can be one. However, the eggs of green mirids are hidden inside the plant tissue, so only those predators which have sucking mouth parts, the heteropterans, could possibly feed on GM egg. However, no attempt has been made in this thesis to directly study predation of GM eggs in the field, since it is difficult to duplicate experimentally such a study because GM eggs are placed inside the plant tissues. Nor has an attempt been made to find a relationship between the predators and GM adults since adults are highly mobile and most likely to escape predation. The results presented here are abundance data on predators and GM populations in the field at ACRI.

Heteroptera

Campylomma liebknechti and *Nabis kinbergii* were the two most abundant heteroptera in the field. Occurrence of heteroptera more or less coincided with the occurrence of GM eggs and nymphs (Figure 6.24). The maximum number of heteroptera occurred at stage 2, that is at peak squaring stage, when GM egg and nymph populations were also maximum except in the 1994-95 season (Appendix 5.4a). This could mean that there is a primary predator-prey relationship between the heteroptera and GM, but it could also mean that the heteroptera may feed on other prey which also reach maximum numbers at peak squaring. Significant positive relationships were found between heteroptera and GM nymph ($P < 0.01$), heteroptera and GM egg ($P < 0.001$).

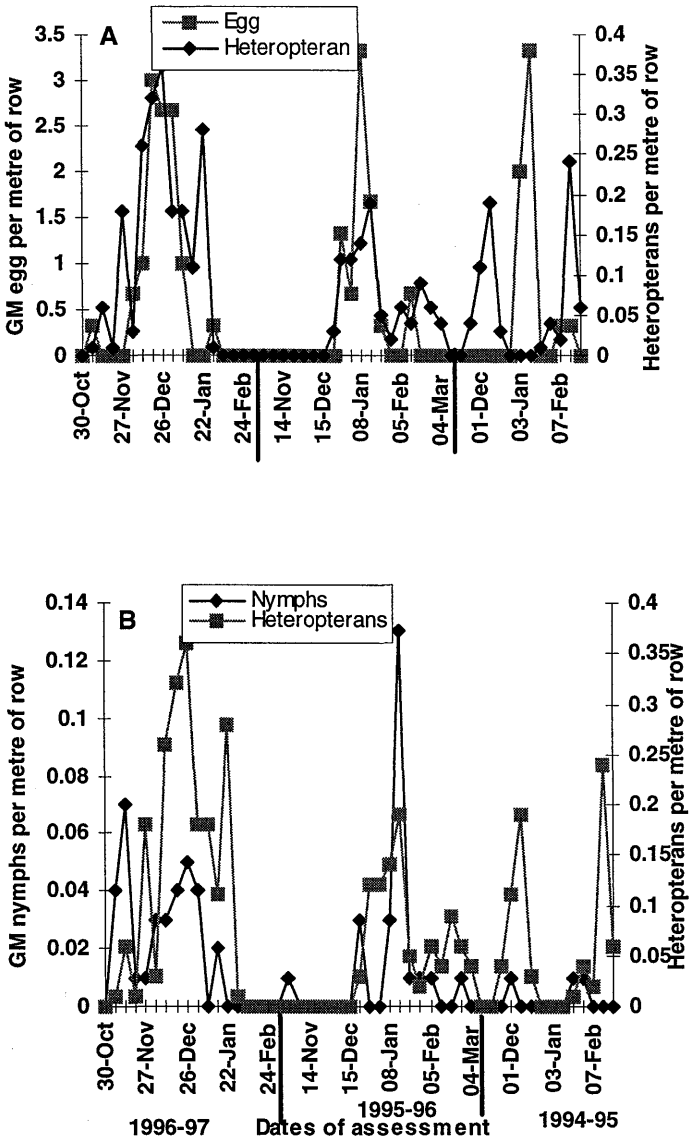


Figure 6.24 Comparison of the time of occurrence of other heteroptera (*Campylomma liebknechti* and *Nabis kinbergii*) with (A) GM egg, (B) GM nymphs in cotton at ACRI,1994-97.

Spiders

The most predominant spiders were lynx spiders (Oxyopidae) and jumping spiders (Salticidae). Others were Lycosidae and *Chiracanthium* Spp. (Clubionidae). The occurrence of spiders did overlap to some extent with GM nymphs (Figure 6.25) and significant positive relationships ($P<0.05$) were also found between them. In every season spiders were abundant at stage 1 and 2 as were GM nymphs (Appendix 5.4b).

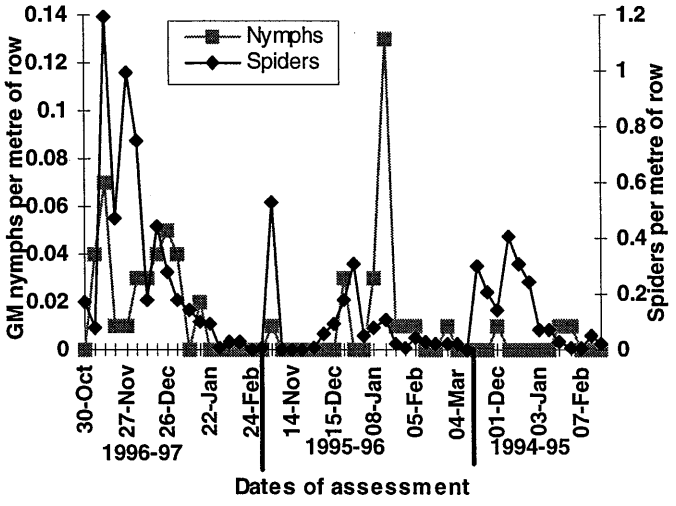


Figure 6.25 Comparison of the time of occurrence of spiders with GM nymphs in cotton at ACRI, 1994-97 .

Weather conditions and GM population

No detailed investigations were made of the effect of weather conditions on GM populations in the field. However, investigations were made to study the effect of temperature on GM development and fecundity in the laboratory (Chapter 4). It was revealed from these experiments that temperature extremities affect GM development profoundly. It was also noticed during sampling in the field that after heavy rain GM populations were reduced. Therefore I used both GM sampling data and weather data to look at how temperature extremities and heavy rain influenced GM populations in the field.

Effect of temperature on GM population in the field

Weekly average maximum and minimum temperatures during sampling periods (October 16 to March 21) for the 1994-97 seasons at ACRI and Norwood are given in Appendix 5.2. No significant relationship was found between average weekly temperature and GM population in the field through the season at all study sites. Laboratory experiments showed that GM development slowed down drastically or even resulted in death if the insect experienced temperatures greater than 35°C or less

than 10°C. The temperatures during sampling periods in the field were extremely high (>35°C) on several occasions each season. Table 6.10 showed total GM number per metre before and after heat waves averaging over 35°C at ACRI and Norwood. At ACRI heat waves were recorded on 3 occasions in the 1996-97, 4 occasions in the 1995-96 and on one occasion in the 1994-95 season during GM sampling periods. The 1994-95 heat wave lasted for 21 consecutive days and overall GM population was very low. Except on one occasion during the 1995-96 season, GM populations were reduced after heat shock by 1.2 to 4.8 times (Table 6.10). At Norwood heat waves were recorded for 3 occasions in 1996-97, 6 occasions in 1995-96 and 2 occasions in the 1994-95 season. Once again overall GM populations were very low at Norwood during heat wave periods which lasted for 10 and 7 consecutive days in the 1994-95 season. Except for the 1994-95 season, GM populations almost always decreased by 1.3 to 9.6 times after heat shock (Table 6.10).

Location	Season	No. of days /heat shock (>35°C)	GM/m before heat shock	GM/m after heat shock
ACRI	1996-97	4 (Nov 14-Nov 17)	6.14	1.79
		4 (Nov 27-Nov 30)	1.75	0.92
		2 (Feb 11 & 12)	0.03	0.00
	1995-96	2 (Nov 10 & 11)	1.37	1.77
		3 (Nov 27-Nov 29)	1.08	0.47
		3 (Dec 17-Dec 19)	1.83	0.86
		2 (Jan 31- Feb 1)	2.26	0.47
	1994-95	21 (Dec 12-Jan 1)	0.3	0.2
Norwood	1996-97	4 (Nov 14- Nov 17)	0.23	0.30
		4 (Dec 1- Dec 4)	0.16	0.08
		2 (Dec 30 & 31)	0.67	0.07
	1995-96	2 (Nov 2 & 3)	1.15	0.59
		2 (Nov 9 & 10)	0.60	0.42
		3 (Nov 26-Nov 28)	0.51	0.30
		3 (Dec 16-Dec 18)	1.59	0.44
		2 (Jan 30 & 31)	0.13	0.00
		2 (Feb 7 & 8)	0.26	0.04
	1994-95	10 (Dec 14-Dec 23)	0.11	0.11
		7 (Dec 27-Jan 2)	0.09	0.15

Table 6.10 Comparison of green mirid numbers per metre and heat waves (>35°C) at ACRI and Norwood for 1994-97 seasons. GM numbers for both lucerne and cotton were pooled together.

Effect of rainfall on GM population in the field

No significant relationship was found between weekly average rainfall and GM numbers per metre sampled every week at all study sites. However, occasionally, 1st and 2nd instar nymphs and adults were found dead, stuck on the leaves or squares after heavy rain in the field. Table 6.11 shows comparisons of GM numbers and rainfall where rainfall was more than 20mm cumulatively on any occasion. GM data for below 20mm rain on any occasion is not included. With some exceptions insect density was reduced after moderate to heavy rainfall in both locations and in all seasons (Table 6.11). On 2 occasions at ACRI and on 4 occasions at Norwood GM numbers increased after rain.

Location	Season	Dates of rainfall	Amount of rainfall (mm)	GM/m before rain	GM/m after rain
ACRI	1996-97	Dec 7 & 8	147	0.92	0.09
		Jan 30 & 31	105	0.23	0.00
	1995-96	Nov 15-22	98	1.78	0.65
		Jan 4-6	65	1.25	2.39
		Jan 21-24	103	3.31	2.26
		Mar 1 & 2	71	2.24	0.07
	1994-95	Jan 3	39	0.25	0.20
		Jan 18-21	159	0.22	0.23
Norwood	1996-97	Nov 5 & 6	26	0.00	0.14
		Dec 7 & 8	47	0.08	0.10
		Dec 18&19	23	0.10	0.01
	1995-96	Nov 17-22	215	0.42	0.51
		Jan 3-6	46	0.84	0.25
		Jan 23&24	46	0.13	0.00
	1994-95	Nov 17	32	0.03	0.03
		Jan 19-21	137	0.01	0.08
		Jan 29	46	0.08	0.00

Table 6.11 Comparison of green mirid numbers per metre and rainfall (>20mm on any occasion) at ACRI and Norwood, 1994-97. GM numbers for both lucerne and cotton were pooled together.

Alternative hosts and GM population in the cotton field

Green mirids used a variety of crops and weeds as alternative hosts to overwinter and also build up populations in spring (see next section). Some of these appeared to be preferred hosts for GM over cotton. Table 6.12 shows how one crop host, safflower, and three weed hosts available during the cotton season influenced GM numbers in cotton at Norwood during the 1995-96 season. The safflower was harvested in December and the two weed hosts, verbena (*Verbena supina*, Verbenaceae) and black berry nightshade (*Solanum nigrum*, Solanaceae) dried off in December and February respectively. Both glauca (*Haloragis glauca*, Haloragaceae) and *S. nigrum* dried off for a week in November, December and January and came up again after rain. During October, when cotton was just emerging, good numbers of GM were harboured in safflower and the weed hosts (Table 6.12). In November, safflower started to mature and weed hosts started drying up. As a result green mirid numbers increased in cotton and decreased in alternative hosts in this period. After summer rain at the end of December when the weed hosts, glauca and black berry nightshade, became fresh and succulent for a couple of weeks when cotton was sprayed with insecticides (see Figure 6.7, in this chapter). Consequently GM population dwindled in cotton and increased in weed hosts, continuing in them until the hosts died (Table 6.12).

Month	Green mirid per metre[1]				
	Cotton	Safflower	Verbena	*S. nigra*	Glauca
October	0.00	0.90	8.15	5.85	24.65
November	0.10	0.20 (mature)	3.65 (commencing drying off)	3.95 (drying off)	8.95 (start drying)
December	0.01	crop	host died	0.55 (rains)	0.15
January	0.00	harvested		0.75	8.20 (attractive)
February	0.00			host died	3.55

Table 6.12 Alternative hosts and GM numbers in cotton during the 1995-96 season at Norwood. 1= both nymph and adult together

6.4 Overwintering hosts and their subsequent effect on GM population in the cotton field

Overwintering hosts may play an important role in subsequent development of GM in the summer. It is obvious that the first GM in a given cotton field during the growing season originate from outside of that field, probably from overwintering hosts. Therefore, this section considers a number of winter crops or weeds acting as hosts of GM, and whether predictions of future GM populations in cotton can be made by knowing the numbers of GM on these overwintering hosts.

6.4.1 Materials and methods

GM populations on the overwintering hosts were sampled weekly with a portable suction machine as described in section 6.3.1 in this chapter. Sampling was done in the Narrabri and Moree areas of Northern New South Wales from April 1, 1994 to October 15, 1996. In 1994, from April to September all available green vegetation (herbs and shrubs) in the cotton farms (at ACRI, Narrabri and Norwood, Moree) and on the roadsides were sampled to identify the possible hosts of GM. Quantitative data were taken from selected plants identified as alternative hosts (if both adults and nymphs were found) at ACRI and Norwood. Quantitative data were also taken in 1995 and in 1996 from May to October and from April to October respectively. At ACRI only lucerne was an available host throughout the three years. Apart from the lucerne, wild turnip was sampled in 1994 at ACRI. At Norwood wild hosts were sampled from the inside of a water reservoir (the reservoir was empty during the winter) and the outside base of the water reservoir. Due to their ephemeral nature and because wild hosts do not grow in large areas, sampling was not possible throughout the sampling period or in replication. However, 10 to 20m sections of host plants were sampled weekly depending on the quantity of the host available. The insects were sorted as described in Chapter 2 in this thesis.

The host plants were identified by Mr. Graham Charles (Weed Agronomist) of NSW Agriculture, ACRI, and Dr Brian Sindel (Weed Agronomist) of the Department of Agronomy and Soil Science of University of New England, Armidale.

6.4.2 Results and observations

Plant species sampled during the winter are given in Table 6.13 along with their host status. Of the 37 species (covering a wide range of families) which were sampled, 17 plant species were classified as non-hosts. Non-hosts are species where not a single GM was detected for any sampling location.

Seven plant species were classified as incidental hosts, where only adult GM were found. In noogoora burr (*Xanthium occidentale*, Asteraceae), however, nymphs were found on a few occasions though in very small numbers, particularly when the primary hosts were absent.

Thirteen plant species covering 8 families were classified as primary hosts, where both adults and all stages of nymphs were found consistently. Of the 13 primary hosts 5 were crops and the rest were wild plants. Not all primary wild hosts were available in every year or at the same time of the year (Table 6.14). The three most important hosts supporting higher number GM population were wild turnip (*Rapistrum rugosum*, Brassicaceae) > hairy carpet weed (*Glinus lotoides,* Aizoaceae) > common joyweed (*Alternanthera nodiflora*, Amaranthaceae*)* in 1994, glauca *Haloragis glauca,* Haloragaceae) > black berry nightshade (*Solanum nigrum,* Solanaceae) > lucerne in 1995 and lucerne (*M. sativa,* Fabaceae)> verbena (*Verbena supina,*Verbenaceae)> black berry nightshade in 1996. Table 6.14 also shows that the GM reached its peak in most cases either before or after the winter. In a few cases GM reached peaks in the winter, however in low densities, except on wild turnip in 1994 where the winter peak was the dominant one (Table 6.15). During the winter both adults and nymphs were present in every year, though in low numbers, suggesting insects may not diapause in the true sense but respond to environmental changes that consequently lower insect numbers.

Family	Common name	Scientific name	Host status[1]
Asteraceae	sunflower	*Helianthus annuus*	PH
	safflower	*Carthamus tinctorius*	PH
	variegated thistle	*Silybum marianum*	PH
	noogoora burr	*Xanthium occidentale*	IH
	wild sunflower	*Verbesina encelioides*	IH
Amaranthaceae	common joyweed	*Alternanthera nodiflora*	PH
Aizoaceae	hairy carpet weed	*Glinus lotoides*	PH
	new zealand spinach	*Tetragonia tetragonoides*	IH
	black pigweed	*Trianthema portulacastrum*	NH
Boraginaceae	Paterson's curse	*Echium plantagineum*	NH
Brassicaceae	wild turnip	*Rapistrum rugosum*	PH
Chenopodiaceae	mealy saltbush	*Atriplex pseudocampanulata*	NH
Cucurbitaceae	desert cucumber	*Zehneria micrantha*	NH
	pumpkin	*Cucurbita pepo*	NH
Cyperaceae	nutgrass	*Cyperus alterniflorus*	NH
Fabaceae	burr medic	*Medicago polymorpha*	NH
	sesbania	*Sesbania cannabina*	IH
	lucerne	*Medicago sativa*	PH
	mungbean	*Vigna radiata*	PH
	soybean	*Glycene max*	NH
	cowpea	*Vigna sinensis*	NH
	bean	*Phaseolus vulgaris*	PH
	lupines	*Lupinus spp*	PH
Haloragaceae	glauca	*Haloragis glauca*	PH
Malvaceae	marshmallow	*Malva parviflora*	IH
Myoporaceae	emubush	*Eremophila longifolia*	NH
Myrtaceae	bottlebrush	*Callistemon branchyandrus*	NH
Poaceae	Rhode's grass	*Chloris gayana*	NH
	sorghum	*Sorghum bicolor*	NH
	Johnson grass	*Sorghum halepense*	NH
	wheat	*Triticum aestivum*	NH
Polygonaceae	wireweed	*Polygonum aviculare*	NH
Portulacaceae	pigweed	*Portulaca spp*	NH
Solanaceae	black-berry nightshade	*Solanum nigrum*	PH
	thornapple	*Datura inoxia*	IH
Umbellifereae	coriander	*Coriandrum sativum*	IH
Verbenaceae	trailing verbena	*Verbena supina*	PH

Table 6.13 List of plant species (along with their host status) sampled during 1994-96 in the Narrabri and Moree areas. 1 PH= primary host; IH= incidental host and NH= non-host.

The results shown in Tables 6.14 and 6.15 suggest that in almost all primary hosts large recruitment of GM populations occurred during the spring and these populations might have moved to the cotton when the overwintering hosts dried up or were cultivated at the beginning of the cotton season. Table 6.16 shows the positive relationship between GM population in the cotton field during the cotton season and GM populations during the spring in wild hosts in every season in both locations (r = 0.79 for ACRI and r = 0.99 for Norwood).

Year	Hosts	Location	Months (week)
			April 1 2 3 4 / May 1 2 3 4 / June 1 2 3 4 / July 1 2 3 4 / August 1 2 3 4 / September 1 2 3 4 / October 1 2 3
1994	Lucerne	ACRI	----------*----- ----------*-
		Norwood	-----*----------
	Wild turnip	ACRI	------*----------*----------*----------
		Norwood	----------*----------*----------*----------
	Common joy weed	Norwood	-----*----------*----------
	Hairy carpet weed	Norwood	--------*----------*----------
1995	Lucerne	ACRI	----------*----------*-
		Norwood	----*----------*--------- --------*--------
	Hairy carpet weed	Norwood	-------*----------
	Wild turnip	Norwood	----*----------*----------*----------
	Blackberry nightshade	Norwood	---*----------*-
	Glauca	Norwood	----------*----------*-
	Verbena	Norwood	----------*---
	Lupines	Norwood	-----*----------*----------
1996	Lucerne	ACRI	----------*----------*-
		Norwood	--------*----------*-
	Hairy carpet weed	Norwood	------*----------
	Blackberry nightshade	Norwood	---*---------- ----------*
	Wild turnip	Norwood	----------*
	Verbena	Norwood	------*----------*----------*

Table 6.14 Seasonal sequence of overwintering host plants of green mirid at ACRI and Norwood along with population peak in 1994-96. * indicates peak time.

Year	Host	GM/m at peak before winter	GM/m at peak in winter	GM/m at peak after winter
1994	Wild turnip	1.65	4.00	1.80
	Common joyweed	2.15	0.60	Host was not available
	Hairy carpet weed	4.50	3.3	Host was not available
1995	Wild turnip	3.10	0.35	2.40
	Glauca	Host was not available	1.20	12.65
	Lupines	Host was not available	0.10	3.20
	Hairy carpet weed	Host was not available	0.20	Host was not available
1996	Verbena	0.20	0.15	1.15

Table 6.15 Number of green mirids per metre at peak before and after winter and in winter on those different primary wild hosts that had GM peaks during winter in 1994-96 at Norwood.

Location	Year	Total GM/m in all overwintering hosts during spring	Total GM/m in cotton including lucerne strips in summer
ACRI	1994	87	219
	1995	411	1100
	1996	101	791
Norwood	1994	136	82
	1995	2448	676
	1996	242	114

Table 6.16 Comparison of GM numbers in all overwintering hosts during spring and in the cotton field (including lucerne strips) during summer, 1994-96 at ACRI and Norwood.

6.5 Discussion

The distributions of adults and nymphs in the field were different (Table 6.1). GM adults are highly mobile, move from one plant to another. Females repeatedly deposit their eggs in the plant's tissue. After hatching the nymphs live on the same

plant, feeding or wandering around on different plant parts, until they become adults. These differential behaviours of adults and nymphs might lead to different distribution patterns in the field. The plant parts GM use for their nutritional requirement are the growing parts of the plant, mainly squares and terminals. This might be the reason more GM were detected on those plant parts (Figure 6.1). The abundance of adults on leaves, however, could be attributed to the GM female's inclination to use leaf petioles as oviposition sites (see chapter 3, in this thesis). Similar types of distribution patterns within the cotton plant are also reported for *Lygus hesperus* (Wilson *et al.* 1984) and for *Lygus lineolaris* (Snodgrass 1993). It is likely that during the early stage of squaring this type of behaviour of GM sets them in competition amongst themselves for preferred feeding sites.

During the period of study, 1994-97, green mirids did not reach pest densities in any season in any location. In all seasons the GM population was higher at ACRI than Norwood. This was perhaps due to different crop management practice in the two locations. Study sites in both locations were IPM experimental fields but because the Norwood field is a commercial farm surrounded by many cotton farms which used insecticides early in the season, beneficial insects were disrupted and insecticides were used to manage the field by the end of December against *Helicoverpa* spp. GM was most abundant in the seedling to squaring stage i.e. in stage I and II. Thereafter the GM population collapsed in all seasons and locations (Figures 6.6 and 6.7). Mortality in the nymphal stages was very high in all seasons in both locations (Table 6.6) and may be attributable to (1) environmental factors, including temperature extremes and rainfall; (2) insecticide spray or drift; (3) competition and emigration; and (4) natural enemies, particularly predators.

Temperatures in the study sites were above 35°C on several occasions during the study period (Table 6.10). On almost all occasions, GM population dropped after heat shock. The driest season in the study period was 1994-95 when temperatures above 35°C were experienced over 21 consecutive days at ACRI and 10 consecutive days at Norwood. The total GM population was lowest in that season. Extreme high temperature might cause desiccation or inactivation of control enzymes in the insect and may cause death of the insect. Temperatures below 15°C were experienced on a

few occasions during summer in the study areas (however only at night and early in the season) but did not seem to have any effect on GM population. It is likely that the lowest threshold temperature was more critical to GM development in winter than summer because in summer average minimum temperature was almost always above the lower threshold temperature for development (Chapter 4). High temperature was also reported as detrimental to sorghum mirid head bugs, *Calocoris angustatus, Creontiades pallidus* and *Eurstylus bellevoyei* in ICRISAT, India (Sharma and Lopez 1990).

Heavy rainfall along with strong winds also appears to have had some effect on GM populations (Table 6.11). Rainfall did not seem to act on GM's internal system. Adult and nymphs were found stuck to the plants or drowned after heavy rain. During culturing of insects in the laboratory it was observed that if an adult's wing stuck to the wall of the rearing cage due to water condensation on the wall, insects could not detach themselves and ultimately died. The same thing happened to nymphs if they fell upside down on water at the base of the rearing cage (used to keep beans fresh; see chapter 3 and appendix 1, in this thesis).

Insecticide sprays, targeted mainly at *Helicoverpa* spp., played an important role in GM dynamics in the cotton system (Figures 6.6 - 6.9). In Australia, growers start to use broad spectrum insecticides from the beginning of stage II coinciding with the emergence of second generation GM nymphs and resulting in the reduction of GM populations in cotton. Survivors escape to the nearest alternative hosts and continue to build up until the host dies. In Norwood in every season insecticides were applied several times in the fields (Figure 6.7) whereas at ACRI the effect was from insecticide drift (Figure 6.6).

Analysis of GM population using Population Analysis Systems (PAS) (Berryman and Millstein 1994) has raised some interesting possibilities about the regulation of GM populations, and these possibilities require further study. It was detected in PAS analysis that GM population mortality was caused by density dependent factors such as competition for food and space on the host plant and natural enemies such as predators (e.g. heteropterans). These factors acted as negative

feedback mechanism in conjunction with other factors such as insecticides, heat shock, heavy rainfall and the availability of overwintering hosts to regulate the population. The PAS results suggest that GM population in cotton at ACRI in the 1996-97 season was regulated by competition at time lag 1 (see Figures 6.12 - 6.15). Studies have shown that cotton is not a preferred host plant for GM (Mensah and Khan 1998) because of the hairiness, hardness etc of the plant. GM adults and nymphs infesting cotton normally concentrate their feeding on the relatively soft and less hairy terminals and squares. During the seedling stage, feeding is concentrated on the terminals and in the squaring stage, on the squares. During both the seedling and early squaring stages of the cotton plant, the preferred feeding site for GM is therefore limited in supply due to the size of the plant. GM adults and nymphs therefore have to compete among each other and also with other insect pests such as apple dimpling bugs (*Campylomma liedknechti*), thrips (*Thrips tabacai*), aphids (*Aphis gossypii*) and jassids (*Amrasca terraereginae*) etc for food and space on the cotton plant which feed in the same plant part. Host plant deterioration and resulting competition for food and space reduced populations and increased emigration of adults from host plants. The effect of competition on GM nymphs could therefore be higher than the adults because of the higher adult mobility. Intra and interspecific competition in addition to predation may have acted as a negative feedback mechanism with a lag of at least 1 or 2 weeks. Apart from competition, insecticide drift from neighbouring farms, heat shock and heavy rainfall also suppressed GM population by either directly causing mortalities or forcing the GM adults to emigrate (see Tables 6.7 and 6.8).

Within the lucerne system, PAS analysis revealed that GM population mortality was primarily caused by scramble competition (see figures 6.20-6.23). In the study field, half of the lucerne was slashed alternatively every 4 weeks to keep the lucerne fresh and attractive. This practice also may have resulted in the emigration of some adults from the field to cotton or surrounding bush and also from the slashed to unslashed lucerne. Slashing of lucerne also may have forced nymphs to crawl from slashed to unslashed lucerne. However, since lucerne is preferred over cotton by GM, increasing numbers of GM adults and nymphs usually moved from slashed to the unslashed lucerne strip resulting in intraspecific scramble competition for food and space. As for the cotton system, host plant deterioration and resulting competition for

food and space may have reduced populations and increased emigration of adults. The competition thus acted as a negative feedback on the population with a time lag of 1 week. Apart from competition, depletion of GM population may also be due to insecticide drift from neighbouring farms, heat shock and heavy rainfall.

PAS analysis of GM egg populations also suggests that predators (mostly heteropterans) acted in a delayed density dependent manner with a negative feedback delay of 3 weeks (i.e. lag 3) to regulate egg populations on cotton plants during the early and mid cotton seasons (stages I and II) (see Figures 6.16 - 6.19). As mentioned earlier, GM deposit their eggs into plant tissues means that only insects with sucking mouth parts would be able to prey on it. The heteropterans were the only predators whose numbers coincided positively with GM egg population. In the 1996-97 season at ACRI two heteroptera *C. liebknechti* and *N. kinbergii* were dominant in stages I and II and synchronized well with GM egg occurrence (see Figure 6.24 and Appendix 5.4a). Apart from egg predation, these heteropterans along with spiders may also predate on GM nymphs. It was observed in the field data from ACRI that occurrence of both the heteropterans and spiders coincided with the occurrence of GM nymphs (see Figure 6.24 and 6.25).

GM populations in cotton during summer are also affected by the availability of suitable overwintering hosts. Several overwintering hosts of GM have been detected growing around the cotton fields (see Table 6.13). Some of them are preferred hosts over cotton and grow throughout the year. During the cotton season, there is usually GM adult movement in and out of cotton onto the alternate host plants particularly in the mid cotton season (stage II) when growers continuously apply insecticides to control *Helicoverpa* spp on cotton (see table 6.12). GM adult movement from cotton to the alternate host plants occurs throughout the season until the alternate hosts dry up in summer. After harvesting cotton, the autumn rains enable regrowth of host plants and the movement and build up of GM populations on these plants. The onset of winter reduces the availability of host plants thus reducing GM population to lower levels through winter until August when temperatures start to rise and availability of host plants also increases, resulting in GM population reaching their peak in almost every available hosts during September-October. In October,

cotton is planted and overwintering hosts start to dry up again or are cultivated or grazed. This may result in GM movement to seedling cotton where damage is caused to the young plants. Habitat diversity in cotton growing areas may therefore be important in keeping GM out of cotton and also in building up of GM population in outside the cotton system. A combination of these practices in conjunction with other mortality factors in cotton could be used to manage GM population in cotton and this should be taken into consideration for GM management.

CHAPTER 7

GENERAL DISCUSSION

This is the most comprehensive investigation of green mirid biology and ecology in Australia to date. My main objective was to acquire knowledge to increase the understanding of GM as a pest in cotton and identify the underlying factors influencing GM dynamics in the cotton system. In the preceding chapters the factors relating to the specific subject matter were discussed separately. In this chapter the information gathered and the insights gained into the biology, behaviour, damage etiology and ecology of GM are brought together. The implications of my findings for the management of the insect and for use in further research are also outlined. Finally from my understanding of GM biology and ecology I recommend what further investigation will be required to develop sound management systems for the pest.

Development of a rearing method

Since green mirid was first considered as a pest in cotton in the early seventies (Room and Wardhaugh 1977) no attempt has been made to develop a suitable rearing method for the insect, a prerequisite to any study on insect biology. Therefore a reliable rearing technique had to be developed to study the basic biology of the insect. The technique that was developed to consistently supply the required number of insects of similar bionomic background for the study involved the culturing of GM on green beans in transparent plastic bottle cages. The advantages of the technique are manifold. The method is cheap, simple, easy to handle and less time consuming than any method using artificial diet. The diet (beans) used in the method is readily available or the beans can be grown with minimum effort.

Implications

The mass rearing technique may not directly impact on the grower's decision process in GM management but it will create opportunities for the researcher to develop pest management systems against GM. A reliable mass rearing technique will

help to promote further study of the biology of the insect. This technique will also help in laboratory assays of control agents used against GM particularly when a consistent supply of insects of the same age and bionomic background are required.

Biology of *Creontiades dilutus*

C. dilutus passes through an egg and five nymphal stages before becoming adult. The description of egg and different nymphal stages were presented in chapter 4. The ratio for GM's increase in size from one instar to the next was calculated to be 1.25. The duration of total developmental period for the insect from egg to adult was 22.6 days at 25°C ± 1 which agrees with the findings of Foley and Pyke (1985). Females lived slightly longer than males (Figure 4.4) which agrees with the findings of Leigh (1963) for *L. hesperus* and Khattat and Stewart (1977) for *L. lineolaris*. The female in her life time laid an average 39.25 ± 4.21 eggs with a range of 14-82. The females deposited most of their eggs between 16 and 20 days after emergence. The pre-oviposition period for GM was relatively long for mirids, extending from 8 to 21 days [which is however similar to *L. hesperus* (Strong *et al.* 1970)].

Temperature has a profound effects on green mirid development. Laboratory experiments showed that the development rate increased with an increase in temperature until an optimum was reached, above which further increases in temperature resulted in retarded growth and in some cases increased mortality. The lower threshold temperature varied for different stages of the insect ranging from 10 to 15°C (Table 5.8). The lower threshold temperatures for egg and total nymphal development were calculated as 15.8 and 13.9°C respectively. Development still occurred at below threshold temperatures although very slowly. The development rate for all individual nymphal instar and eggs was maximal at around 30°C. Female fecundity also increased with an increase in temperature and at around 30°C, the highest numbers of eggs were laid. Pre-oviposition period decreased with increase in temperature. In cotton growing areas of north-west New South Wales, early autumn, late spring and mid summer therefore could be expected to favour development of GM populations.

Other than temperature, dietary content of the insect's food also influences insect survival and development. If an insect obtains adequate energy-yielding substrates (such as sugars) from its food, it will direct most of its dietary protein into growth otherwise it will metabolize proteins for their energy content and will grow less (Shaw *et al.* 1978 and Clancy 1992). My experiment on using sugar solution in GM diet showed that GM survival increased when sugar solution was added to the diet (Figure 4.21).

Implications

My investigations into green mirid biology clearly defined different stages of the insect thus eliminating any confusion about the insect's identity and the duration of their life cycle. Growers and consultants had difficulties in distinguishing between nymphs of *Campylomma liebknechti, Taylorilygus pallidulus* and GM during the study period. Knowledge of the insect's life cycle parameters will ensure that growers adopt management decisions in time to target the appropriate stage of insect. For example GM's pre-oviposition period is long, so in the early stage of cotton (when GM first move to cotton from surrounding alternative hosts) if growers could combat invading adults within a week using less disruptive insecticides (before deposition of eggs) they could avoid the 2nd generation nymphs which cause injury to early squares. However, this strategy will not work at the middle of the cotton season (stage II) when temperatures start to rise because at high temperature the pre-oviposition period will decrease. Temperature above 35°C slows down GM development and sometimes is lethal to GM, so, growers could avoid spraying when high temperatures persist for at least three consecutive days.

Oviposition behaviour of *Creontiades dilutus*

GM females lay eggs singly, inserting the eggs into plant tissues but leaving the egg cap outside for respiration purposes. Usually the females deposited eggs without producing any swellings or lumps surrounding the egg caps. Preferentially GM oviposited on early to late squaring plants (see Figure 4.5) since this plant type

category provide both adult and nymphs with food, shelter and oviposition sites better than any other cotton plant category (Bishop 1980, Adams and Pyke 1982, Adams *et al.*1984).

GM preferentially laid on leaf petioles and laid more eggs on the distal end than the petiole base (see Figures 4.7 and 4.9B). Benedict *et al.* (1981a) also found petiole end, particularly the pulvinus area at the base of leaf blade, to be the preferred oviposition sites for *L. hesperus*. The insect followed a definitive pattern in embedding eggs on the petiole. In the case of the petiole end, usually green mirid deposited eggs dorsally but for the petiole base they laid eggs ventrally (see Figure 4.9).

The plant's morphological character is exploited efficiently by the insect during oviposition. Experiments showed that GM preferred to put eggs on 4 to 8 main stem nodal petioles (see Figures 4.6 and 4.7). Two morphological characteristics, hardness and hairiness of the petiole, were identified as the major factors that determine discrimination between nodal position for oviposition (see Figure 4.10). GM deposited more eggs on comparatively soft and less hairy petioles. The top 3 nodal petioles were soft but had more hairs whereas petioles below the 8 main stem node had less hair but were hard. Dense hairs on the top petioles perhaps provided a mechanical barrier, but on the other hand the insect might find it difficult to penetrate hard plant tissues with its ovipositor. Sorensen (1939), Elmore (1955), Carlson (1956) and Alvarado-Rodriquez *et al.* (1986) also considered tenderness of plant tissues as the determining factor for selection of lygus oviposition sites. Cotton plants having more hair have been found more resistant to jassids (*Empoasca* spp.) feeding (Parnel *et al.* 1949). *Lygus* bugs also showed significant oviposition preference for the upper one-third of cotton plants (Benedict *et al.* 1981a).

Implications

The oviposition behaviour of green mirid can be exploited in two ways. First, successful cotton breeding work can be carried out using petiole hardness and hairiness as a guide. This can be done either by selecting those existing cotton

varieties that have the above mentioned morphological traits or by developing new varieties with these characteristics. For a quick outcome the former approach is preferable.

Secondly, effective sampling protocols can be developed by using the knowledge of oviposition patterns of the insect. In the existing sampling methods which utilise nymph and adult counts, only 50% of the actual population can be predicted (Miles 1995) leading to possible faulty management decisions. Moreover adults are highly mobile and move between cotton and the nearest alternative hosts frequently. It is therefore possible for insects to be out of the field at sampling time and for growers or consultants to gather the wrong information thereby undermining their decision making process. On the other hand management decisions based on egg counts will be more accurate. In this regard, however, further research is necessary to determine sample size and threshold density. Training consultants to accurately count the small and cryptic eggs of GM would also be necessary.

Damage etiology of green mirid in cotton

Both adults and nymphs of green mirid caused similar types of damage. The damage caused by the insect is cumulative and maximum damage occurred when the insect reached 4^{th} to 5^{th} nymphal stages.

Green mirid preferentially feed on the growing parts of plants, particularly terminals and squares (see chapter 6). Their feeding at seedling stage causes partial to complete damage to leaves and terminals. Severe feeding damage can result in complete tip out or wilting of terminals or whole plant. The study showed that GM feeding causes destruction of epidermal cells and feeding extending up to the vascular bundle region destroys phloem. In severe cases cavitation occurs on the feeding sites.

GM's feeding on a square causes the squares to shed. Experiments showed that the shedding of squares depends on the square size (see Figure 6.9). Squares less than 3cm are the most vulnerable to shedding due to GM feeding. The insect preferentially

fed on anthers, particularly pollen grains which consequently lost turgidity and coalesced in the middle of the pollen sac. GM also feed on the stalk of small squares. Destruction of both pollen grain and stalk may trigger abscission of squares. On the other hand feeding on large squares may not cause squares to shed although they may develop abnormal bolls (see Plate 6.15). The degree of abnormality will depend on the percentage of anthers damaged.

Though bolls are not a preferred feeding sites for GM, the mirid can still cause damage to the bolls particularly to the younger ones. Their feeding on bolls results in water soaked black spots on the carpel. In severe feeding, small bolls could shed and feeding spots could extend up to the lint resulting in characteristic brown coloured lint near the point of injury.

The mechanisms of terminal wilting and square shedding revealed by my study of different aspects of GM damage are as follows.

During the feeding process GM releases a chemical, pectinase (Hori and Mile 1993). For young plants, this pectinase enables the insect's stylet to penetrate the plant's tissue by dissolving middle lamella then lysing surrounding tissues of the feeding point, resulting in hindrance of conduction of nutrient material from bottom to top. Wilting of the plant parts above terminal or leaf feeding sites results.

There are similar consequences when GM feed on the stalk of the squares or small bolls. However, the mechanism is somewhat different. Feeding on the anthers of the squares may have resulted in an imbalance of hormones. This could occur in either of two ways. Ethylene causes abscission and auxin retards abscission (Morgan 1969). Pollen grains in cotton contain auxin (Strong 1970). The pectinase that was released during feeding on squares may have induced the production of ethylene and thus created an imbalance between ethylene and auxin causing squares to shed. Secondly, due to feeding on squares, auxin producing pollen grains were destroyed thus perhaps reducing the ratio of auxin to ethylene and causing shedding of squares.

GM feeding on young plants or squares did not translate into yield loss. It did however cause a delay in maturity of up to 3 weeks. GM damage at an early stages did not cause yield loss because of the ability of the cotton plant to compensate for the damage by developing more fruits. This may cause delay in maturity of the crop. Severe damage on tips caused cessation of terminal growth which encouraged the plant to redirect its available resources to producing more vegetative growth and fruits. However, there was a time lag between cessation of growth and starting of new growth and this time lag eventually reflected on delay in maturity. Delay in maturity may affect growers by increasing management costs or decreasing fibre quality. The additional costs of sprays, irrigation and interest charges on borrowed capital may increase management costs. Fibre quality may also decrease due to the longer exposure of the crop to the risk of rain damage and low night temperatures. Damage resulting in reduction in micronaire incurs high price penalties for the growers.

Implications

The nature of damage due to GM feeding on different parts of cotton and its related mechanisms have been clearly defined. This information will allow growers and consultants to distinguish between GM damage symptoms and symptoms of other sucking pests, particularly the thrips, *Helicoverpa* spp. and tipworms and should facilitate pest management decisions. Grower insight into the consequences of mirid damage (a delay in maturity) will allow them to avoid extra management costs or reduction of fibre quality. The relationship between GM number and delay in maturity has created an opportunity to calculate economic threshold if cost of delay per day is known.

Economic thresholds

The economic threshold (1 GM/metre of row) was proposed based on the calculation of EIL using maturity delay from field experiments. The value of delay cost ($ AUS 20/day/ha) used in the calculation was based on the additional costs and risks associated with a delay crop estimated by Cox *et al.* (1991). Thus the accuracy of

the EIL estimation depend on the accuracy of the delay costs estimation. Moreover, the estimation of the cost of delay for certain factors associated with delay is not straightforward. For example estimation of the cost of harvest delay in the coming season due to rainfall will depend on how closely historical weather patterns will repeat themselves.

The maturity delay that I have used for EIL calculation was from irrigated cotton which may differ from raingrown cotton. There is possibility that the compensatory ability of raingrown cotton to GM damage at early stage is less, which would result in more delay in boll maturity. Therefore the ET I have calculated will need to be validated for raingrown cotton.

Implications

The ET I have estimated is higher than the current recommended thresholds for GM which will assist in reducing insecticide spray at early stages of crop growth. Consequently this will help to make IPM programmes more successful in the cotton industry.

Population dynamics of green mirid

The green mirids first detected in cotton early in the season certainly moved in from the nearest alternative hosts. There is some belief that GM might invade eastern Australian cotton *en masse* through long distance migration from western Queensland (Gregg 1995, Miles 1995). However, Miles (1995) failed to confirm conclusively that mass migration of GM occurred from western Queensland to central Queensland. In my three year study period I did not perceive any influx of GM into cotton at any time in the study sites located in NSW. My studies showed that local migration from overwintering hosts grown around the cotton field were the main source of GM invading cotton in the study sites in NSW. Other than cotton and lucerne I have identified 12 primary hosts which could be a source of GM in cotton in summer. Five of these hosts were crop hosts and the remainder were wild hosts. Insects overwintering on these wild hosts grow slowly. At the onset of rising temperatures

during spring insects started to build up to large numbers, reaching peak population at the end of spring which coincides with the start of the cotton season in northern NSW. As temperatures rise and dry weather sets in due to low rainfall the wild hosts start to dry up triggering GM to move to newly emerged cotton. In the existing cotton management system they continue to increase until the beginning of squaring and boll setting stage of the plant (stage II). Thereafter populations start to decline- mainly due to the use of broad spectrum insecticides targeted for *Helicoverpa* spp. As use of insecticides continues, GM populations in cotton totally collapse. Some of them however escape to the nearest available wild hosts and continue to build up until spring, by switching from one available wild host to another as these wild hosts are ephemeral. Observations showed that lucerne and some of these wild hosts were preferred by GM over cotton (see Figures 6.8 and 6.9 and Table 6.12). Preference of GM for lucerne over cotton is well studied and lucerne is recommended for use as a trap crop for GM in IPM programmes in Australian cotton production (Mensah and Khan 1997). Further research is necessary to find out ways to use the remaining preferred hosts in managing the pest.

Apart from alternative hosts and insecticides, other factors influencing GM population are temperature, rainfall, predators, intraspecific competition and emigration.

Both extremely high and low temperatures had detrimental effects on GM populations. Observation showed that high temperatures, more than 35°C for few consecutive days, reduced GM numbers (see Table 6.10). During the study periods, the driest season was 1994-95 and GM numbers were also low during this season. Temperatures below threshold temperature during the winter slowed GM development (see Chapter 4 and Table 6.14). Heavy rainfall along with strong winds reduced insect numbers in the field. The intensity of rainfall at a particular time is probably more important than total rainfall in an area. That is why no relationship was found between average rainfall and GM number.

PAS analysis suggested that GM egg populations were regulated by a delayed density dependence factor operating at a time lag of 3 weeks. It is possible that the

heteropteran predators, *Campylomma liebknechti, Geocoris lubra* and *Nabis kinbergii*, are the only predators identified in the Australian cotton system during my study with sucking mouth parts and egg laying behaviours similar to GM could be the predator of GM eggs. Their life cycle was also well synchronized with GM eggs in the field. Life cycles of these heteropterans and also spiders were synchronized with GM nymphs also. As these are generalist predators in the cotton field GM may not be their main or only food source. It is therefore possible that heteropteran predators acted in concert with other density dependent factors not yet identified in this study to regulate GM egg population. The combined factors regulating GM population in the field are therefore not clear cut and further study are necessary to identify the key factors involved.

My study has also suggested that GM nymphal and adult populations were regulated by intraspecific competition in the seedling to squaring stage of the cotton plant (early season; stage I). The competition may be due to the insect's preference for feeding on particular plant parts (squares). At the beginning of stage I, cotton plants set fewer numbers of squares and competition for food may result. This competition however may only last for about one week. Thereafter when plants set more squares contest competition diminishes. At stage II insecticide spray targeted at *Helicoverpa* spp. killed GM or forced insects to emigrate thereby reducing competition.

Implications

Any control measure taken for a particular pest should be based on the ecology of that pest. At present the only control options we have for GM, other than use of lucerne strips as trap crops, are broad spectrum insecticides. Since GM is an early season pest, insecticide spraying against GM early in the season also disrupts the activities of beneficial insects which creating congenial environments for major pests, particularly mites and *Helicoverpa* spp. to build up. To manage GM in the cotton field we should follow a 'prevention is better than cure' philosophy. This can be achieved by managing preferred wild hosts along with the lucerne strips. Growers have two options in this respect, either destroy all the wild hosts including lucerne at least one week before the emergence of cotton or leave them growing throughout the summer.

Lucernes should be grown and become well established before cotton emergence so that they can serve as a sink for GM moving onto cotton from wild hosts as they dry off.

Future research priorities

Assessment of the role of generalist predators in managing GM population

Analysis of GM population dynamics suggested that GM eggs may be regulated by predators and, as mentioned earlier, it is possible that GM eggs and nymphs were being preyed on by those insects with sucking mouth parts and by the spiders. Quite a few generalist predators were always present in the cotton field. Some of them have been recorded as predators of *Helicoverpa* spp. eggs and larvae (Room 1970). Therefore assessment of the role of these predators in managing GM population is vital for developing a successful IPM programme for the pest.

Assessment of the role of preferred alternative hosts in managing GM populations

Lucerne is being used as a trap crop for GM as well as a refuge or source for beneficial insects. Other than lucerne there are a number of preferred wild hosts. Some of them grow in summer around the cotton field. The use of these alternate hosts as trap crops for GM should be studied in detail so that this knowledge can be integrated into the IPM system.

Study of sex pheromones of GM

Miles (1995) reported that the females released long distance pheromones to attract males. However, she could not identify the sex pheromones. Further research is necessary to identify sex pheromones and to develop a pheromone based management strategy for the insect such as attract and kill method.

Finding new chemistry for insecticides against GM which is less disruptive

Without doubt, insecticides are still some of the most powerful tools used in pest management systems in Australian cotton. Currently, the insecticides recommended for controlling GM are highly disruptive and destructive to beneficial insects. Evaluation of insecticides against GM that are less disruptive is highly desirable. Further research is also necessary to evaluate other alternative pest control agents such as petroleum spray oil, pathogens etc to be incorporated into IPM programs in cotton.

Concluding remarks

The work presented in this thesis is the most comprehensive so far on GM biology, behaviour, feeding mechanisms, damage etiology and ecology. The way I addressed the problem of understanding the pest answered some but not all of the fundamental questions. Some of the findings may have direct implications for managing the pest, others may have indirect effects. But the information or insight gained from this work will aid the pursuit of applied research to resolve management problems associated with this insect pest in cotton crops in Australia and other parts of the world.

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APPENDIX 1

MASS REARING TECHNIQUE FOR *C. dilutus*

1.1 Introduction

A reliable and satisfactory mass rearing technique for an insect is a prerequisite for basic biological and behavioural studies, as well as for experiments with chemical and biological agents to develop control methods. This is especially so when large numbers of insects of uniform age and physiological state are required. Knipling (1966) listed several aspects of entomology that are based on a successful rearing method under controlled conditions. A successful rearing method for *C. dilutus* is long overdue (Foley and Pyke 1985). Such a method should help research on the development, fecundity, longevity and behaviour of *C. dilutus*.

This Appendix presents studies of different rearing techniques tested to develop a reliable and easy mass rearing technique for *C. dilutus* which would supply the required number of insects needed to study the basic biology of the insect in this thesis. The capacity of *C. dilutus* to survive is compared between different methods.

1.2 Materials and Methods

The trials were conducted in a controlled temperature room (5m x 2m) provided with two 40w fluorescent tubes, a fan heater and an air conditioner to maintain temperature at 25±1°C and humidity at 40-60%, as measured by a 7-day recording thermohygrograph. The fluorescent tubes were controlled by a time clock to maintain a 14:10 L:D. light-dark cycle. However, one technique was tested in a glasshouse where temperature was maintained at 30-32°C by a thermostatically controlled fan heater and an air conditioner and the trial was conducted with natural light-dark cycle.

C. dilutus adults were collected from lucerne, *Medicago sativa* L., in September 1994 at Norwood, Moree using a suction sampler (see Chapter 3). The

collected mirids were sexed morphologically (Chapter 4) and placed in pairs of male and female in the rearing cages.

Four different methods of rearing *C. dilutus*, the 'cotton seedling', 'bean', 'potato', and 'lettuce' methods, were tested.

1.2.1 Cotton seedling method

This technique was tested in the glasshouse. In this method four iron mesh cages (see Chapter 2) were used. Twelve cotton plants, variety Sicala V1, at the 8 leaf stage, with three plants arranged in each 25cm pot, were provided for feeding and oviposition. The base of the plant and floor of the cage were covered with white paper so that dead insects could be easily located, and the paper coverings were changed when they got dirty. One pot containing three plants were placed in each cage. Before putting them into the cages, plants were thoroughly checked to avoid spiders, mites or aphids; plants with mites or aphids were not used because mirids were assumed to predate on mites and aphids (L. Wilson pers. comm. 1994). Spiders were killed if there were any on the plant since they predate on mirids. Four pairs of adults, male and female, were confined in each cage and their subsequent development was observed.

1.2.2 Bean method

In this method recycled soft drink bottle cages (see Chapter 2) were used in the controlled temperature room. Two whole fresh, soft and succulent green beans, *Phaseolus vulgaris* L., variety stringless dwarf snapbean, were put in each cage as feeding and oviposition sites. Injured or spotted beans were avoided. The bases of the beans were placed in one of five depressions made on the bottom of the cage and were covered with water soaked cotton wool to keep the bean fresh, longer and moist inside the cage. Cotton wool was soaked with water every day with a plastic dropper and fresh cotton wool was provided every 4-5 days. The cotton wool was always soaked with water before placing it in the cages because mirid nymphs, particularly 1st instars, became entangled in dry cotton wool and died. Over-watering was avoided because if the insects fell into free water they get drowned and died. Beans were changed when they started drying. The changed beans were placed in a separate cage

for hatching if any eggs were laid on them. During changing of the beans a small camel brush was used to dislodge adult mirids. For nymphs, insects were gently tapped off the beans which were then taken out from the cage with small forceps. Cages were washed first with bleaching agents and then thoroughly with hot water and air dried every 2 weeks.

Fresh green beans were procured from a grocery store. Beans were thoroughly washed with warm water in a sink to avoid any contamination (for example, with insecticides) and sorted according to size and tenderness. Sorted beans were stored in a plastic bag and kept in a refrigerator for further use. Depending on the freshness of the beans, they could be used for 4-5 days. Very young and mature beans were not used.

Care was taken during handling so that insects, particularly adults, could not escape. During changing beans and soaking cotton wool with water I placed a hand over the organdie covering before opening the cage and handled the cage very gently so that insects were disturbed as little as possible.

Altogether 10 cages with two pairs of mirids, male and female, in each cage were used for this method.

1.2.3 Potato method

In this method, bottle cages as used in the bean method, with small modifications were used. Two opposite windows of 9 x 6cm covered with organdie were provided for more ventilation so that the potatoes did not rot quickly due to condensation.

Potatoes, *Solanum tuberosum*, uncleaned (because growing buds of washed potatoes might be damaged), were procured from a grocery store. They were kept on sand in a dark room until they sprouted. Sprouted potatoes were washed and dried carefully to avoid any damage to the sprout. A single potato was placed on the bottom of each cage to serve as a feeding and oviposition site. Two pairs of adult mirids, male and female, were confined in each cage. In this way 10 cages were used.

1.2.4 Lettuce method

The same bottle cages as in the potato method were used. Lettuce were purchased from a grocery store and one quarter of a lettuce was placed inside a cage. Two pairs of adult mirids, male and female, were confined in each cage and subsequent development was observed. Altogether 10 cages were used.

1.2.5 Survival of *C. dilutus* in different rearing methods

The survivorship of mirids was tested using the above four rearing methods. Ten 2nd instar nymphs collected from lucerne (Chapter 2) were placed in each cage of each rearing method using a mouth aspirator. This was replicated four times for each method, thus 160 nymphs were used. Insects were tapped gently from the vials of the aspirator into the cages. First instar nymphs were not used because they are very small and delicate to handle and vulnerable to injury during collection with the suction samplers. Survivorship was counted as the number of nymphs from ten which emerged as adults.

1.2.6 Hatchability of *C. dilutus* egg laid on different media

Ten pairs of field collected mirid adults, male and female, were allowed to feed and oviposit on cotton seedlings and on green beans for 3 days. The number of eggs were counted under a microscope very carefully so that the operculum (see Chapter 3) remained undamaged. All but 20 eggs were destroyed with a needle, and those 20 were placed into the 'cotton seedling method' and 'bean method' cages and allowed to develop for 12 days. They were checked every day and the hatched nymphs were recorded and aspirated out from the cage. The whole experiment was replicated four times.

Data for this and the survival experiments were transformed using arcsine transformation, and analysed using the one-way analysis of variance. Means of the transformed data were compared using Fisher's least significance difference test.

1.3 Results

1.3.1 Cotton seedling method

This rearing method was only partially successful. All the released insects died within 10 days. Though they laid a few eggs and 20 nymphs hatched out, it was difficult to locate newly hatched nymphs. Only 5 nymphs were located at 3rd instar stage and none of them were recovered as adults. However, some adults may have escaped during checking, and some spiders were found on a few occasions

1.3.2 Bean method

This method was successful. Adults were alive for 22-39 days. Females laid a considerable number of eggs during that period and 266 nymphs hatched out. Fifty percent of them were recovered as adults. The first batch of adults emerged 25 days after the parent insects were released into the cage.

1.3.3 Potato method

In this method all the adults died within 5days. No eggs were laid. Most of the time the insects were on the organdie covering. The potatoes may not have been suitable as food, for example, perhaps the potato sprout was not long enough to attract the insects.

1.3.4 Lettuce method

This method was also unsuccessful. As in the potato method, all adults died within 5 days and no eggs were laid. Almost all the time insects were on the organdie covering. The lettuce started going rotten within three days of setting up the experiment.

1.3.5 Survival of *C. dilutus* in different rearing method

The results on survival of *C. dilutus* in the different rearing methods are depicted in Figure.1.1

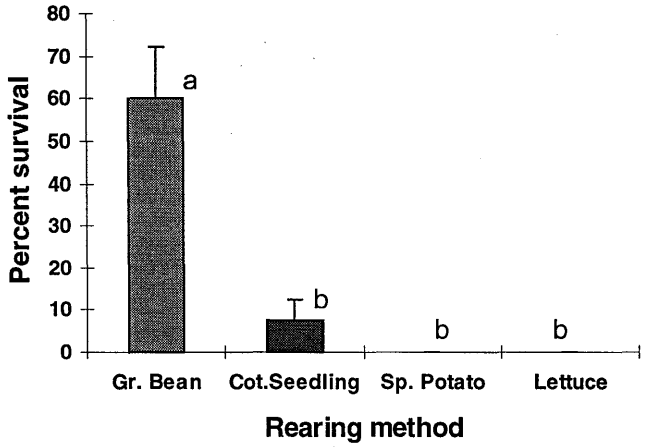

Figure 1.1. Percent survival of *C. dilutus* nymph to adult, in different rearing methods. (Gr. Bean= Green Bean; Cot. Seedling = Cotton seedling; Sp. Potato = Sprouted potato). Means followed by the same letter are not significantly different ($p<0.001$) (Fisher's least significance difference).

Survival was highest in the bean method followed by the cotton seedling method. No mirid survived in the sprouted potato and lettuce methods. The mean survival was 60 percent with the range of 30-90 percent for the bean method and for the cotton seedling method it was 7.5 percent with the range of 0-20 percent. A one way analysis of variance showed that survival was significantly higher in the bean method than other methods (Figure 1.1).

1.3.6 Hatchability of *C. dilutus* eggs laid on different media

A one way analysis of variance revealed that there was no significant difference between the treatments (Figure 1.2). The mean hatches for beans and cotton seedlings were 76.2 and 83.8 percent respectively with a range of 50-95 and 70-100 percent respectively.

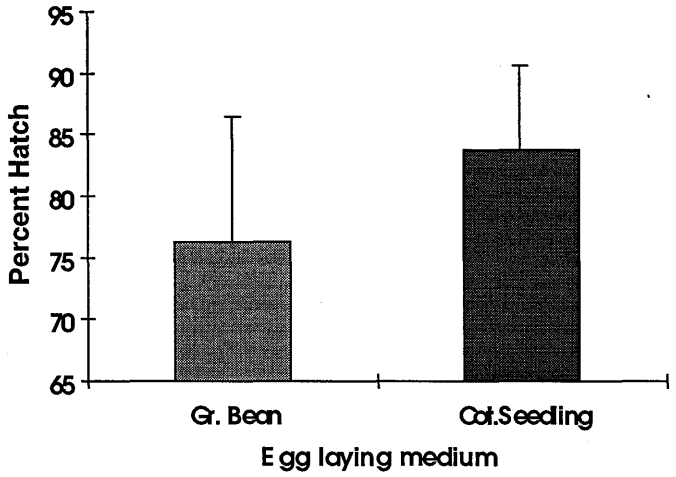

Figure 1.2. Percent hatch of *C. dilutus* eggs laid on green beans and cotton seedlings.

1.4 Discussion

A successful, simple and less time consuming rearing method for *C. dilutus* was developed. This is the first detailed report on *C. dilutus* rearing. Though Foley and Pyke (1985) used beans for studying mirid development in relation to temperature, they did not report the rearing method in detail. They put lucerne clippings inside the cage which could make observation difficult as insects may hide in the lucerne clippings.

Among the four rearing methods tested, the 'bean method' was clearly the best. Rearing mirids, other than *C. dilutus*, using beans is not uncommon. Several reports have been published on rearing North American mirids using green beans (Beards and Leigh 1960, Ridgway and Gyrisco 1960a, Leigh 1963, Strong *et al.* 1970, Wilson 1973, Parrott *et al.* 1975, Bryan *et al.* 1976, Khattat and Stewart 1977, Bailey 1986, Snodgrass and McWilliams 1992). The advantages of using the 'bean method' for rearing *C. dilutus* are many fold: beans are cheap, easy to handle and readily available throughout most of the year and can be grown with little effort; it is not necessary to change beans every day; cages are readily available and easy to observe inside through the transparent wall so the method is less time consuming; the size of the cage is convenient for cleaning (dead insects or exuviae) with simple forceps or a mouth aspirator.

However, overflowing of water during soaking of the cotton wool could be lethal particularly to the early instar nymphs, though water is vital for their development. The importance of water during rearing is reported by several authors for different hemipterans (Kehat and Wyndham 1972b, Harris and Todd 1981, Chinajariyawong *et al.* 1989, Jackai 1989).

Some beans exuded an adhesive substance from the feeding punctures which trapped the proboscis of the nymphs and caused their death, particularly when large numbers of mirid fed on small amounts of bean. This could be avoided by putting an optimum density of insects for the amount of food. Later I used two beans per 10 nymphs or per 2 pairs of adults for 2-3 days in a cage and avoided this problem. Khattat and Stewart (1977) also reported a similar problem with broccoli for *Lygus lineolaris.*

Cannibalism was reported by Beard and Leigh (1960) for *Lygus hesperus*, Khattat and Stewart (1977) for *L. lineolaris* and Harris and Todd (1981) for *Nezara viridula.* I did not observe cannibalism for *C. dilutus,* though sometimes insects were observed sitting on dead mates. Khattat and Stewart (1977) found that cannibalism was prevalent in cages containing unsuitable food and Beard and Leigh (1960) explained that cannibalism is due to injury to individuals during handling. Solomon (1949) and Harris and Todd (1981) mentioned that cannibalism is density dependent and can be reduced by lowering densities and changing food and water regularly.

One problem experienced occasionally with the 'bean method' was pesticide contamination of commercially purchased beans. However, this problem can be avoided by growing the beans without pesticides.

In the 'cotton seedling' method survival was very low. Leigh (1963) also failed to rear *L. hesperus* on cotton. Nymphs could not survive to adult in the 'potato' or 'lettuce' methods, indicating that *C. dilutus* might have a different host range than *L. lineolaris,* which was successfully reared on potato shoots (Slaymaker and Tugwell 1982) and on lettuce (Stevenson and Roberts 1973). In the experiments reported here, the size of the cage (which may have been too long to force the insects into contact

with the food) might also have affected their food finding ability. This behaviour of *C. dilutus* was also observed in plant type preference experiments in a mesh house (Chapter 5).

In the bean method, an average 60 percent of the nymphs survived to become adult, which is similar to the results of Foley and Pyke (1985), who had 59% survival from 1st instar to adult. The survival percentage for North American mirids reared on beans is almost the same as my results (Strong *et al.* 1970, Khattat and Stewart 1977, Snodgrass and McWilliams 1992).

The hatchability of mirid eggs on beans and cotton seedlings did not differ significantly, indicating that beans were a good oviposition media, as good as cotton plants. The eggs are mostly laid in the portion of the beans uppermost in the cage, possibly because they preferred the tenderest tissue to oviposit. The egg laying behaviour of the mirid on cotton, including its relationship to plant hardness, is discussed in Chapter 3 of this thesis.

Seventy six percent hatchability of green mirid eggs in beans is similar to the 73 percent *L. lineolaris* egg hatchability obtained by Khattat and Stewart (1977) in beans. Even though the beans I used for the study have moderately dried, it did not significantly affect the egg hatchability. This supports the observation of Khattat and Stewart that moderate drying of the plant material containing eggs does not influence hatchability. Leigh (1963) mentioned that small failures could happened in portions of the host tissue if the beans dried excessively, which apparently constricted the plant tissue around the egg.

APPENDIX 2

RESULTS AND STATISTICAL ANALYSES FOR CHAPTER 3

Appendix 2.1

(a) Regression analysis on the relationship between mean body length and mean antennal length of five nymphal stages

The regression equation is
Antennal length = 0.008 + 1.22 Body length

Predictor	Coef	Stdev	t-ratio	p
Constant	0.0080	0.3030	0.03	0.981
BodyL	1.21510	0.06122	19.85	0.000

s = 0.2646 R-sq = 99.2% R-sq(adj) = 99.0%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	27.575	27.575	393.97	0.000
Error	3	0.210	0.070		
Total	4	27.785			

(b) Regression analysis on the relationship between mean body length and mean head capsule width of five nymphal stages

The regression equation is
Head capsule width = 0.299 + 0.161 Body length

Predictor	Coef	Stdev	t-ratio	p
Constant	0.29859	0.04211	7.09	0.006
BodyL	0.160538	0.008510	18.87	0.000

s = 0.03678 R-sq = 99.2% R-sq(adj) = 98.9%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	0.48134	0.48134	355.89	0.000
Error	3	0.00406	0.00135		
Total	4	0.48540			

(c) Regression analysis on the relationship between mean headcapsule width and mean antennal length of five nymphal stages

The regression equation is
Antennal length = - 2.20 + 7.51 Head capsule width

Predictor	Coef	Stdev	t-ratio	p
Constant	-2.1956	0.5481	-4.01	0.028
H.capW	7.5142	0.5093	14.75	0.001

s = 0.3549 R-sq = 98.6% R-sq(adj) = 98.2%

Analysis of Variance

SOURCE	DF	SS	MS	F	p
Regression	1	27.407	27.407	217.65	0.001
Error	3	0.378	0.126		
Total	4	27.785			

Appendix 2.2

(a) Analysis of variance on body weight (mg) of male and female GM

SOURCE	DF	SS	MS	F	p
Sex	1	0.41	0.41	0.34	0.563
Error	28	33.35	1.19		
Total	29	33.75			

(b) Analysis of variance on body length (mm) of male and female GM

SOURCE	DF	SS	MS	F	p
Sex	1	1.200	1.200	1.83	0.187
Error	28	18.392	0.657		
Total	29	19.592			

(c) Analysis of variance on antennal length (mm) of male and female GM

SOURCE	DF	SS	MS	F	p
Sex	1	0.005	0.005	0.02	0.885
Error	28	6.973	0.249		
Total	29	6.979			

(d) Analysis of variance on head capsule width (mm) of male and female GM

SOURCE	DF	SS	MS	F	p
Sex	1	0.04800	0.04800	5.20	0.030
Error	28	0.25867	0.00924		
Total	29	0.30667			

Appendix 2.3

(a) Analysis of variance on GM oviposition in relation to different plant age class

SOURCE	DF	SS	MS	F	p
Plant age	3	1184.2	394.7	12.28	0.001
Error	12	385.7	32.1		
Total	15	1569.9			

LSD = 8.73

(b) Analysis of variance on GM oviposition in relation to node number

SOURCE	DF	SS	MS	F	p
Node no.	8	86.72	10.84	3.70	0.001
Error	135	395.94	2.93		
Total	143	482.66			

LSD = 1.19

Appendix 2.4

(a) Analysis of variance on GM oviposition in relation to petiole position for young plants

SOURCE	DF	SS	MS	F	p
Petiole position	2	1.000	0.500	0.91	0.417
Error	21	11.500	0.548		
Total	23	12.500			

Petiole position	Mean egg no	Standard deviation
Base	0.2500	0.7071
Centre	0.0000	0.0000
End	0.5000	1.0690

LSD = 0.77

(b) Analysis of variance on GM oviposition in relation to petiole position for squaring plants

SOURCE	DF	SS	MS	F	p
Petiole position	2	57.6	28.8	1.94	0.169
Error	21	311.7	14.8		
Total	23	369.3			

Petiole position	Mean egg no	Standard deviation
Base	3.500	2.673
Centre	1.125	1.458
End	4.875	5.939

LSD = 4.0

(c) Analysis of variance on GM oviposition in relation to petiole position for the plants with squares and bolls

SOURCE	DF	SS	MS	F	p
Petiole position	2	34.3	17.2	1.41	0.265
Error	21	255.0	12.1		
Total	23	289.3			

Petiole position	Mean egg no	Standard deviation
Base	1.750	1.909
Centre	2.250	1.832
End	4.500	5.425

LSD = 3.62

(d) Analysis of variance on GM oviposition in relation to petiole position for mature plants

SOURCE	DF	SS	MS	F	p
Petiole position	2	0.0833	0.0417	1.00	0.385
Error	21	0.8750	0.0417		
Total	23	0.9583			

Petiole position	Mean egg no	Standard deviation
Base	0.0000	0.0000
Centre	0.0000	0.0000
End	0.1250	0.3536

LSD = 0.21

(e) Analysis of variance on GM oviposition in relation to petiole position for all the plant types together

SOURCE	DF	SS	MS	F	p
Petiole position	2	45.77	22.89	2.58	0.081
Error	93	823.72	8.86		
Total	95	869.49			

Petiole position	Mean egg no	Standard deviation
Base	1.375	2.136
Centre	0.844	1.462
End	2.500	4.458

LSD = 1.48

(f) Analysis of variance on GM oviposition in relation to petiole aspect for all the plant types and petiole position together

SOURCE	DF	SS	MS	F	p
Petiole aspect	1	36.26	36.26	4.09	0.046
Error	94	833.23	8.86		
Total	95	869.49			

(g) Analysis of variance on GM oviposition in relation to petiole aspect for petiole position of all plant types together

Petiole base

SOURCE	DF	SS	MS	F	p
Petiole aspect	1	12.50	12.50	2.91	0.099
Error	30	129.00	4.30		
Total	31	141.50			

Petiole centre

SOURCE	DF	SS	MS	F	p
Petiole aspect	1	1.53	1.53	0.71	0.406
Error	30	64.69	2.16		
Total	31	66.22			

Petiole end

SOURCE	DF	SS	MS	F	p
Petiole aspect	1	162.0	162.0	10.70	0.003
Error	30	454.0	15.1		
Total	31	616.0			

Appendix 2.5

(a) Analysis of variance on hardness of the petiole of different node number

SOURCE	DF	SS	MS	F	p
Node no.	8	72.80	9.10	7.11	0.000
Error	27	34.54	1.28		
Total	35	107.35			

Node no.	Mean hardness	Standard deviation
3	6.795	0.734
4	7.615	0.965
5	8.515	1.064
6	9.200	1.204
7	10.125	0.335
8	10.555	0.650
9	10.962	1.161
10	10.318	2.243
11	10.910	0.741

LSD = 1.64

(b) Analysis of variance on hairiness of the petiole of different node number

SOURCE	DF	SS	MS	F	p
Node no.	8	891.32	111.41	30.50	0.000
Error	27	98.64	3.65		
Total	35	989.96			

Node no.	Mean hairiness	Standard deviation
3	17.810	1.632
4	8.417	1.823
5	8.250	3.219
6	5.227	2.124
7	5.075	2.271
8	2.938	2.160
9	2.040	0.959
10	1.168	0.867
11	1.207	0.726

LSD = 2.77

Appendix 2.6

(a) Analysis of variance on GM oviposition in the field for two years

SOURCE	DF	SS	MS	F	p
Time	1	28.2	28.2	0.85	0.409
Error	4	132.7	33.2		
Total	5	160.8			

Time	Mean egg no.	Standard deviation
1995-96	7.667	3.786
1996-97	12.000	7.211

LSD = 13.06

(b) Analysis of variance on GM oviposition in the field in relation to node number in the 1995-96 season

SOURCE	DF	SS	MS	F	p
Node no.	7	18.96	2.71	2.17	0.095
Error	16	20.00	1.25		
Total	23	38.96			

Node no.	Mean egg no.	Standard deviation
3	0.000	0.000
4	1.667	2.887
5	2.000	1.000
6	2.000	0.000
7	1.667	0.577
8	0.333	0.577
9	0.000	0.000
10	0.000	0.000

LSD = 1.94

(c) Analysis of variance on GM oviposition in the field in relation to node number (data for two years were pooled)

SOURCE	DF	SS	MS	F	p
Node no.	7	36.31	5.19	3.04	0.012
Error	40	68.17	1.70		
Total	47	104.48			

Node no.	Mean egg no.	Standard deviation
3	0.333	0.516
4	1.333	2.160
5	2.333	1.751
6	2.167	0.983
7	2.167	1.472
8	1.167	1.472
9	0.167	0.408
10	0.167	0.408

LSD = 1.52

(d) Analysis of variance on GM oviposition in the field in relation to petiole position in the 1995-96 season

SOURCE	DF	SS	MS	F	p
Petiole position	1	14.08	14.08	2.67	0.134
Error	10	52.83	5.28		
Total	11	66.92			

Petiole position	Mean egg no.	Standard deviation
Base	0.833	1.602
End	3.000	2.828

LSD = 2.96

(e) Analysis of variance on GM oviposition in the field in relation to petiole position in the 1996-97 season

SOURCE	DF	SS	MS	F	p
Petiole position	1	8.33	8.33	1.00	0.342
Error	10	83.67	8.37		
Total	11	92.00			

Petiole position	Mean egg no.	Standard deviation
Base	2.167	3.251
End	3.833	2.483

LSD = 3.72

APPENDIX 3

RESULTS AND STATISTICAL ANALYSES FOR CHAPTER 4

Appendix 3.1

(a) Analysis of variance on GM fecundity at constant temperatures under laboratory conditions

Source	DF	SS	MS	F	P
Temperature	5	7180	1436	3.87	0.016
Error	17	6314	371		
Total	22	13494			

Temperature	Mean egg no.	Standard deviation
15	16.00	0.00
20	5.00	4.24
23	20.00	24.34
26	35.60	25.35
30	62.60	17.53
32	29.40	5.59

LSD = 25.7

(b) Analysis of variance on GM egg development at constant temperatures under laboratory conditions

Source	DF	SS	MS	F	P
Temperature	4	332.53	83.13	115.55	0.000
Error	15	10.79			
Total	19	343.32			

Temperature	Development time	Standard deviation
20	15.84	0.595
23	9.84	1.190
26	6.68	0.881
30	4.92	0.730
32	5.14	0.721

LSD = 1.28

(c) Analysis of variance on GM egg survival at constant temperatures under laboratory conditions

Source	DF	SS	MS	F	P
Temperature	7	60183.5	8597.6	186.40	0.000
Error	24	1107.0			
Total	31	61290.5			

Temperature	Percent survival	Standard deviation
11	0.00	0.00
15	0.00	0.00
20	75.26	11.54
23	91.34	8.15
26	95.55	5.26
30	92.60	8.89
32	89.40	7.92
38	0.00	0.00

LSD = 9.91

(d) Analysis of variance on GM nymph survival at constant temperatures under laboratory conditions

Source	DF	SS	MS	F	P
Temperature	15	60866	4058	18.75	0.000
Error	48	10389			
Total	63	71254			

Temperature	Percent survival	Standard deviation
11	0.00	0.00
12	0.00	0.00
15	0.00	0.00
17	20.00	28.28
20	64.34	25.03
21	42.95	9.10
23	56.78	21.47
24	69.70	21.61
26	80.00	18.26
27	52.08	12.72
30	80.00	14.14
30	51.75	11.21
32	77.50	9.57
33	59.28	9.39
38	2.50	5.00
38	0.00	0.00

LSD = 20.9

APPENDIX 4

RESULTS AND STATISTICAL ANALYSES FOR CHAPTER 5

Appendix 4.1

(a) Analysis of variance on GM preference (GM no.) for different plant types

SOURCE	DF	SS	MS	F	p
Plant types	4	277.74	69.44	24.64	0.000
Error	135	380.39	2.82		
Total	139	658.14			

Plant types	GM no./plant type/day	Standard deviation
1	0.250	0.441
2	1.571	1.597
3	1.357	1.096
4	4.357	2.392
5	2.857	2.103

LSD = 0.89

Plant types-1= young plants with 6 true leaves; 2= plant with all squares removed but with tips; 3= plant with squares, no bolls and tips removed; 4= plant with squares, bolls and tips; 5= plant with bolls but no tips and squares.

(b) Analysis of variance on GM preference (eggs/plant type) for different plant types

SOURCE	DF	SS	MS	F	p
Plant types	4	316.7	79.2	2.88	0.059
Error	15	412.3	27.5		
Total	19	729.0			

Plant type	Egg no./plant type	Standard deviation
1	0.500	1.000
2	4.250	3.403
3	6.250	8.958
4	12.750	3.862
5	6.500	5.447

LSD = 7.9

Plant types-1= young plants with 6 true leaves; 2= plant with all squares removed but with tips; 3= plant with squares, no bolls and tips removed; 4= plant with squares, bolls and tips; 5= plant with bolls but no tips and squares.

Appendix 4.2

(a) Analysis of Variance on percentage of damage to the young cotton plants caused by different stages of GM

Source	DF	SS	MS	F	p
GM stage	5	13888	2778	8.74	0.000
Error	18	5720	318		
Total	23	19608			

GM stage	Mean damage percent	Standard deviation
1st inst	28.55	16.45
2nd inst	49.95	14.30
3rd inst	78.55	27.38
4th inst	92.85	14.30
5th inst	92.85	14.30
Adults	85.70	16.51

LSD = 26.49

(b) Analysis variance on leaf area (sq cm)of young cotton plants fed upon by different stages of GM

Source	DF	SS	MS	F	P
GM stage	6	8259	1377	7.63	0.000
Error	21	3790	180		
Total	27	12049			

GM Stage	Mean leaf area (sq cm)	Standard deviation
1st inst	47.50	5.72
2nd inst	42.49	10.53
3rd inst	30.66	19.17
4th inst	10.37	12.48
5th inst	5.97	11.93
Adults	17.96	21.22
Control	52.25	1.94

LSD = 19.73

(c) Analysis variance on leaf weight (gm) of young cotton plants fed upon by different stages of GM

Source	DF	SS	MS	F	P
GM stage	6	0.11767	0.01961	9.53	0.000
Error	21	0.04320	0.00206		
Total	27	0.16087			

GM Stage	Mean leaf area (sq cm)	Standard deviation
1st inst	0.18250	0.02062
2nd inst	0.16000	0.05033
3rd inst	0.11750	0.06021
4th inst	0.03750	0.04349
5th inst	0.02000	0.04000
Adults	0.05500	0.06557
Control	0.18250	0.00500

LSD = 0.07

(d) Analysis variance on root weight (gm) of young cotton plants fed upon by different stages of GM

Source	DF	SS	MS	F	P
GM stage	6	0.003000	0.000500	3.85	0.010
Error	21	0.002725	0.000130		
Total	27	0.005725			

GM Stage	Mean root weight (gm)	Standard deviation
1st inst	0.03250	0.00500
2nd inst	0.03500	0.00577
3rd inst	0.03250	0.01258
4th inst	0.02750	0.01500
5th inst	0.02500	0.00577
Adults	0.02000	0.02000
Control	0.05500	0.00577

LSD = 0.02

(e) Analysis variance on stem weight (gm) of young cotton plants fed upon by different stages of GM

Source	DF	SS	MS	F	P
GM stage	6	0.004543	0.000757	1.71	0.169
Error	21	0.002725	0.000130		
Total	27	0.005725			

GM Stage	Mean stem weight (gm)	Standard deviation
1st inst	0.08250	0.01258
2nd inst	0.08250	0.02986
3rd inst	0.08250	0.00957
4th inst	0.06750	0.02217
5th inst	0.06250	0.00957
Adults	0.05250	0.03594
Control	0.05250	0.00957

LSD = 0.03

Appendix 4.3

(a) Analysis of variance on damage to the young cotton plants caused by different densities of GM in the mesh house.

SOURCE	DF	SS	MS	F	p
GM/plant	8	110.30	13.79	8.46	0.000
Error	18	29.33	1.63		
Total	26	139.63			

GM/plant	Mean damage score	Standard deviation
0	0.000	0.000
1	1.333	1.528
2	2.000	1.732
3	3.667	1.155
4	4.333	1.155
5	5.000	0.000
6	5.000	2.000
7	5.667	1.155
8	6.333	1.155

LSD = 2.19

(b) Analysis of variance on number of squares at early stage infested with different GM densities at 4-leaf stage in the mesh house.

SOURCE	DF	SS	MS	F	p
GM/plant	8	83.41	10.43	2.45	0.055
Error	18	76.67	4.26		
Total	26	160.07			

GM/plant	Mean square no. at early stage	Standard deviation
0	6.000	0.000
1	5.333	2.082
2	3.333	0.577
3	3.000	2.000
4	3.667	3.512
5	2.000	2.000
6	4.000	3.606
7	1.000	0.000
8	0.333	0.577

LSD = 3.54

(c) Analysis of variance on number of squares at late stage infested with different GM densities at 4-leaf stage in the mesh house.

SOURCE	DF	SS	MS	F	p
GM/plant	8	144.5	18.1	0.85	0.572
Error	18	382.0	21.2		
Total	26	526.5			

GM/plant	Mean square no. at early stage	Standard deviation
0	13.667	2.517
1	21.000	5.000
2	21.000	3.606
3	20.000	5.196
4	19.667	8.083
5	19.333	5.132
6	15.667	4.163
7	18.333	2.082
8	18.667	2.517

LSD = 7.90

(d) Analysis of variance on number of bolls at mature stage infested with different GM densities at 4-leaf stage in the mesh house.

SOURCE	DF	SS	MS	F	p
GM/plant	8	20.52	2.56	1.47	0.235
Error	18	31.33	1.74		
Total	26	51.85			

GM/plant	Mean boll no. at mature stage	Standard deviation
0	5.667	1.155
1	7.333	0.577
2	6.667	1.155
3	5.667	0.577
4	6.667	1.155
5	6.667	1.155
6	8.000	2.646
7	7.333	0.577
8	8.333	1.528

LSD = 2.26

(e) Analysis of variance on days required to appearance of first square for plants infested with different GM densities at 4-leaf stage in the mesh house.

SOURCE	DF	SS	MS	F	p
GM/plant	8	1655.3	206.9	6.83	0.000
Error	18	545.3	30.3		
Total	26	2200.7			

GM/plant	Mean days required to 1st square	Standard deviation
0	31.333	0.577
1	32.333	1.528
2	34.333	2.517
3	37.333	4.163
4	43.333	4.509
5	47.000	2.646
6	46.667	11.504
7	51.333	5.859
8	53.333	7.234

LSD = 9.44

(f) Analysis of variance on days required to flower for plants infested with different GM densities at 4-leaf stage in the mesh house.

SOURCE	DF	SS	MS	F	p
GM/plant	8	2525.2	315.6	7.40	0.000
Error	18	768.0	42.7		
Total	26	3293.2			

GM/plant	Mean days required to flower	Standard deviation
0	51.333	0.577
1	53.667	2.517
2	56.000	2.646
3	61.000	2.646
4	67.333	9.815
5	74.333	3.512
6	68.333	14.572
7	76.000	4.583
8	79.333	4.619

LSD = 11.21

(g) Analysis of variance on days required to boll maturity for plants infested with different GM densities at 4-leaf stage in the mesh house.

SOURCE	DF	SS	MS	F	p
GM/plant	8	712.7	89.1	4.26	0.005
Error	18	376.0	20.9		
Total	26	1088.7			

GM/plant	Mean days required to boll mature	Standard deviation
0	104.33	2.52
1	107.33	4.73
2	111.67	5.13
3	111.33	4.62
4	113.33	6.35
5	113.33	2.08
6	116.67	7.37
7	117.33	0.58
8	122.67	3.51

LSD = 7.84

Appendix 4.4

(a) Analysis of covariance for arcsine transformed data on squares shed versus GM density and square size.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Sizeclass	2	5.4445	4.1019	2.0509	22.34	0.000
mirids	1	0.0348	0.0348	0.0348	0.38	0.546
Sizeclass*mirids	2	1.1890	1.1890	0.5945	6.48	0.008
Error	18	1.6523	1.6523	0.0918		
Total	23	8.3206				

(b) Analysis of variance on percent flower shed due to GM feeding on anthers of large squares in the glass house.

SOURCE	DF	SS	MS	F	p
Damage %	4	262.9	65.7	1.70	0.225
Error	10	386.1	38.6		
Total	14	649.0			

Anthers damage %	Percent flower shed	Standard deviation
0	0.00	0.00
=30	0.00	0.00
=50	0.00	0.00
=70	5.56	9.62
>70	10.37	10.02

LSD = 11.30

(c) Analysis of variance on percent boll shed due to GM feeding on anthers of large squares in the glass house.

SOURCE	DF	SS	MS	F	p
Damage %	4	1932.9	483.2	10.42	0.001
Error	10	463.1	46.4		
Total	14	2396.8			

Anthers damage %	Percent boll shed	Standard deviation
0	0.00	0.00
=30	0.00	0.00
=50	5.56	9.62
=70	21.30	4.24
>70	27.33	11.02

LSD = 12.39

(d) Analysis of variance on percent deformed bolls due to GM feeding on anthers of large squares in the glass house.

SOURCE	DF	SS	MS	F	p
Damage %	4	3707	927	5.18	0.016
Error	10	1790	179		
Total	14	5497			

Anthers damage %	Percent deformed boll	Standard deviation
0	0.00	0.00
=30	0.00	0.00
=50	13.89	12.73
=70	20.37	26.25
>70	42.50	6.61

LSD = 24.34

Appendix 4.5

(a) Analysis of variance on numbers of squares shed due to GM feeding on caged plants in field trials at ACRI, 1996-97.

Source	DF	SS	MS	F	p
Square size	2	11.11	5.56	2.70	0.077
Error	51	104.89	2.06		
Total	53	116.00			

Square size	Mean square shed	Standard deviation
Pinhead	1.889	1.641
Small	1.333	1.534
Medium	0.778	1.060

LSD = 0.96

(b) Analysis of variance on pinhead squares shed due to feeding by different densities of GM on caged plants in field trials at ACRI, 1996-97.

SOURCE	DF	SS	MS	F	p
GM density	5	27.78	5.56	3.70	0.029
Error	12	18.00	1.50		
Total	17	45.78			

GM/plant	Mean square shed	Standard deviation
0	0.333	0.577
1	1.000	1.000
2	1.333	0.577
3	1.667	1.528
4	3.000	1.000
5	4.000	2.000

LSD = 2.18

(c) Analysis of variance on small squares shed due to feeding by different densities of GM on caged plants in field trials at ACRI, 1996-97.

SOURCE	DF	SS	MS	F	p
GM density	5	22.00	4.40	2.93	0.059
Error	12	18.00	1.50		
Total	17	40.00			

GM/plant	Mean square shed	Standard deviation
0	0.000	0.000
1	0.333	0.577
2	1.333	1.155
3	1.000	1.000
4	2.000	1.000
5	3.333	2.309

LSD = 2.18

(d) Analysis of variance on medium squares shed due to feeding by different densities of GM on caged plants in field trials at ACRI, 1996-97.

SOURCE	DF	SS	MS	F	p
GM density	5	7.778	1.556	1.65	0.222
Error	12	11.333	0.944		
Total	17	19.111			

GM/plant	Mean square shed	Standard deviation
0	0.0000	0.0000
1	0.3333	0.5774
2	0.3333	0.5774
3	1.0000	1.7321
4	1.0000	1.0000
5	2.0000	1.0000

LSD = 1.73

(e) Analysis of variance on numbers of squares shed due to feeding by different densities of GM on caged plants in field trials at ACRI, 1996-97.

SOURCE	DF	SS	MS	F	p
GM density	5	157.33	31.47	13.17	0.000
Error	12	28.67	2.39		
Total	17	186.00			

GM/plant	Mean square shed	Standard deviation
0	0.333	0.577
1	1.667	1.155
2	3.000	2.000
3	3.667	1.528
4	6.000	2.000
5	9.333	1.528

LSD = 2.75

Appendix 4.6

(a) Analysis of Variance on GM/m at early season in field.

Source	DF	SS	MS	F	p
Treatments	2	0.002370	0.001185	2.90	0.106
Error	9	0.003672	0.000408		
Total	11	0.006042			

Treatments	GM/m at early season	Standard deviation
Plots infested with GM & other pest	0.03750	0.02041
GM controlled plot	0.00938	0.01875
GM infested plot	0.04063	0.02135

LSD = 0.03

(b) Analysis of Variance on GM/m at late season in field.

Source	DF	SS	MS	F	p
Treatments	2	0.01531	0.00766	4.64	0.041
Error	9	0.01484	0.00165		
Total	11	0.03016			

Treatments	GM/m at late season	Standard deviation
Plots infested with GM & other pests	0.10000	0.02041
GM controlled plots	0.03125	0.03146
GM infested plots	0.11250	0.05951

LSD = 0.06

(c) Analysis of Variance on GM/m for whole season in field.

Source	DF	SS	MS	F	p
Treatments	2	0.02971	0.01486	5.72	0.025
Error	9	0.02336	0.00260		
Total	11	0.05307			

Treatments	GM/m for whole season	Standard deviation
Plots infested with GM & other pests	0.13750	0.03536
GM controlled plots	0.04063	0.03442
GM infested plots	0.15313	0.07315

LSD = 0.08

(d) Analysis of Variance on Square/m during mid season in fields with and without GM infestation.

Source	DF	SS	MS	F	p
Treatments	2	10021	5010	4.42	0.046
Error	9	10192	1132		
Total	11	20213			

Treatments	Squares/m at mid season	Standard deviation
Plots infested with GM & other pests	158.75	44.44
GM controlled plots	223.25	30.35
GM infested plots	165.75	22.40

LSD = 53.82

(e) Analysis of Variance on Squares/m at early season in fields with and without GM infestation.

Source	DF	SS	MS	F	p
Treatments	2	1005	503	2.38	0.148
Error	9	1897	211		
Total	11	2903			

Treatments	Squares/m at early season	Standard deviation
Plots infested with GM & other pests	44.00	13.29
GM controlled plots	60.25	16.13
GM infested plots	38.75	13.99

LSD = 23.23

(f) Analysis of Variance on Bolls/m at mature stage in fields with and without GM infestation.

Source	DF	SS	MS	F	p
Treatments	2	612	306	1.42	0.292
Error	9	1946	216		
Total	11	2558			

Treatments	Bolls/m at mature stage	Standard deviation
Plots infested with GM and other pests	96.25	11.93
GM controlled plots	112.50	13.96
GM infested plots	110.00	17.64

LSD = 23.51

(g) Analysis of Variance on days required to first flower in fields with and without GM infestation.

Source	DF	SS	MS	F	p
Treatments	2	51.17	25.58	23.62	0.000
Error	9	9.75	1.08		
Total	11	60.92			

Treatments	Days to first flower	Standard deviation
Plots infested with GM and other pests	76.250	0.957
GM controlled plots	72.000	0.816
GM infested plots	76.500	1.291

LSD = 1.66

(h) Analysis of Variance on days required to mature (60% boll open) in field with and without GM infestation.

Source	DF	SS	MS	F	p
Treatments	2	177.17	88.58	26.80	0.000
Error	9	29.75	3.31		
Total	11	206.92			

Treatments	Days to mature	Standard deviation
Plots infested with GM and other pests	144.50	2.38
GM controlled plots	136.00	1.41
GM infested plots	143.75	1.50

LSD = 2.91

APPENDIX 5

RESULTS AND STTISTICAL ANALYSIS FOR CHAPTER 6

Appendix 5.1

Dates and types of insecticides spraying during study periods in Norwood, Moree study site.

Season	Spraying date	Trade name	Active ingredient
1994-95	December 24, 1994	Endosulfan 350 EC	350 g/L EC
	January 8, 1995	Endosulfan 350 EC	350 g/L EC
	February 2, 1995	Larvin 375	375 g/L SC
	February 10, 1995	Talstar	100 g/L EC
	February 21, 1995	Comite 600®	600 g/L
1995-96	December 14, 1995	Endosulfan 350 EC &	350 g/L EC
	December 28, 1995	Dipel ES	-
	January 13, 1996	Endosulfan 350 EC &	350 g/L EC
	January 30, 1996	Dipel ES	-
	February 12, 1996	Endosulfan 350 EC &	350 g/L EC
	February 2, 1996	Dipel ES	-
		Endosulfan 350 EC &	600 g/L
		Dipel ES	-
		Talstar	100 g/L EC
		Comite 600® &	600 g/L
		Rogor 400	400 g/L EC
1996-97	November 27, 1996	Tracer	480 g/L
	December 19, 1996	Endosulfan 350 EC	350 g/L EC
	December 31, 1996	Tracer	480 g/L
	January 8, 1997	Endosulfan	350 g/L EC
	February 5, 1997	Talstar & Parathion	480 g/L & 500 g/L
	February 21, 1997	500 M	EC
		Curacron 250 ULV &	250 g/L ULV &
		Bulldock 8 UL	8 g/L ULV

Appendix 5.2

Weekly temperature (°C) during sampling periods at study sites

Study site	Week	1994-95			1995-96			1996-97		
		Max	Min	Avg	Max	Min	Avg	Max	Min	Avg
ACRI	October-04	*	*	*	30.9	16.5	23.7	28.9	12.7	20.8
	November-01	*	*	*	31.5	14.7	23.1	26.3	11.2	18.8
	November-02	*	*	*	26.8	17.0	21.9	33.9	15.4	24.7
	November-03	27.4	16.4	21.9	27.2	15.4	21.3	26.1	11.2	18.6
	November-04	32.9	19.3	26.1	31.3	16.6	24.0	36.8	17.3	27.1
	December-01	30.6	16.6	23.6	31.0	17.5	24.3	29.9	17.4	23.6
	December-02	32.0	17.7	24.9	29.5	15.8	22.7	30.6	17.4	24.0
	December-03	37.3	22.1	29.7	29.6	13.9	21.7	28.3	17.0	22.6
	December-04	35.1	20.7	27.9	32.7	19.5	26.1	32.8	19.4	26.1
	January-01	35.8	19.9	27.9	28.9	20.0	24.5	29.3	16.4	22.8
	January-02	29.6	19.4	24.5	30.4	19.4	24.9	29.2	15.7	22.4
	January-03	31.9	19.2	25.6	32.8	20.1	26.5	31.8	19.3	25.6
	January-04	25.6	17.5	21.5	29.3	16.6	23.0	*	*	*
	February-01	32.4	19.9	26.2	34.3	17.9	26.1	26.7	20.0	23.4
	February-02	29.0	18.3	23.7	29.6	16.4	23.0	31.3	18.8	25.0
	February-03	29.2	18.7	24.0	27.3	14.6	20.9	32.5	19.3	25.9
	February-04	30.2	16.1	23.1	31.1	17.3	24.2	28.1	18.0	23.1
Norwood	October-03	*	*	*	29.5	14.5	22.0	31.2	13.5	22.3
	October-04	*	*	*	31.4	16.1	23.7	28.4	13.2	20.8
	November-01	*	*	*	33.6	16.3	24.9	27.4	11.3	19.4
	November-02	27.9	18.5	23.2	25.2	18.2	21.7	35.6	16.2	25.9
	November-03	31.6	17.8	24.7	31.7	16.7	24.2	28.9	10.7	19.8
	November-04	30.1	17.3	23.7	31.0	18.6	24.8	38.7	18.7	28.7
	December-01	31.6	17.9	24.7	29.1	16.6	22.8	31.7	16.8	24.2
	December-02	36.7	21.5	29.1	32.7	16.7	24.7	32.8	18.7	25.7
	December-03	35.3	21.9	28.6	28.5	14.3	21.4	31.6	17.1	24.4
	Decmebr-04	37.1	20.8	28.9	32.2	20.4	26.3	35.3	18.8	27.1
	January-01	32.3	20.4	26.3	27.7	19.4	23.5	32.8	15.7	24.3
	January-02	33.8	19.8	26.8	31.7	19.4	25.6	31.5	17.1	24.3
	January-03	26.7	19.5	23.1	29.7	20.2	24.9	33.2	18.5	25.9
	January-04	30.4	19.4	24.9	33.6	18.2	25.9	33.2	20.3	26.7
	February-01	32.0	20.4	26.2	34.1	18.4	26.3	33.1	22.9	28.0
	February-02	28.4	17.7	23.0	28.8	15.4	22.1	32.4	20.0	26.2
	February-03	28.4	18.6	23.5	30.3	15.4	22.9	35.3	22.5	28.9
	February-04	29.3	17.6	23.4	30.9	16.0	23.4	*	*	*
	March-01	30.1	17.9	24.0	29.4	17.2	23.3	*	*	*
	March-02	32.4	17.6	25.0	31.3	14.7	23.0	*	*	*
	March-03	31.0	15.4	23.2	31.8	15.9	23.9	*	*	*

* indicate data unavailable

Appendix 5.3

Calculation of nymphal mortalities for stage II in the 1996-97 season at ACRI using the method of Richards (1940).

Rows	Sampling date	Number of eggs and nymphs per metre					
		Eggs	1st inst	2nd inst	3rd inst	4th inst	5th inst
1	12th Dec. 1996	1	0.03	0.00	0.00	0.00	0.00
2	19th Dec. 1996	3	0.00	0.01	0.00	0.00	0.00
3	26th Dec. 1996	2.67	0.00	0.02	0.01	0.02	0.00
4	31st Dec. 1996	2.67	0.01	0.01	0.02	0.00	0.00
5	10th Jan. 1997	1	0.00	0.00	0.00	0.00	0.00
6	16th Jan. 1997	0	0.00	0.01	0.00	0.01	0.00
7	Total	10.34	0.04	0.05	0.03	0.03	0.00
8	Duration of stage (days)	6.09	3	3	3.1	3.1	3.6
9	Expected proportions	3.2	1.4	1.4	1.4	1.4	1.7
10	Proportions expected with egg	10.34	4.5	4.5	4.5	4.5	5.5
11	Loss 7-10	0	-4.46	-4.45	-4.47	-4.47	-5.5

Row 7 in the table gives the total of each stage found at stage II in ACRI. In general, the numbers of each stage found should correspond to the time spent in that stage.

The duration of each stage given in row 8 was calculated from the results of a laboratory study at 25°±1 since average temperature in study site during the sampling time was around 25°C (see appendix 5.2).

Row 9 in the table shows the total number of eggs and nymphs divided up into numbers proportional to the duration of each stage. The number of eggs actually found was far more and nymphs far less than would have been expected.

Row 10 shows the numbers of each stage which might have been expected in a random collection of 10.34/m eggs.

The discrepancy between the numbers of first-, second-, third-, fourth- and fifth instar nymphs in rows seven and ten gives a measure of the mortality.

Appendix 5.4

(a) Occurrence of heteropterans according to cotton stage for GM egg and GM nymph in the field at ACRI,1994-97.

Season	Stage	Heteroptera/m	GM egg/m	GM nymph/m
1996-97	1	0.55	2.00	0.19
	2	1.43	9.34	0.15
	3	0.01	0.33	0.00
1995-96	1	0.00	0.00	0.01
	2	0.60	7.00	0.19
	3	0.36	1.00	0.04
1994-95	1	0.37	0.00	0.01
	2	0.01	5.33	0.01
	3	0.36	0.66	0.01

(b) Occurrence of spiders and GM nymphs in cotton at ACRI during the 1994-97 seasons according to cotton stage

Season	Cotton stage	Spiders/m	GM nymphs/m
1996-97	1	3.83	0.19
	2	1.23	0.15
	3	0.08	0.00
1995-96	1	0.69	0.01
	2	0.73	0.19
	3	0.16	0.04
1994-95	1	1.37	0.01
	2	0.41	0.01
	3	0.09	0.01