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NSW AGRICULTURE

Project title: **Ecology and management of apple dimpling bug in conventional and transgenic cotton**

Project code: **DAN 114C**

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A final report prepared for the Cotton Research and Development Corporation

Project DAN 114C **Ecology and management of apple dimpling bug in conventional and transgenic cotton**

SUMMARY

Development of a rearing technique for ADB

I developed a new rearing method to study the basic biology of ADB. Three methods were tested. The most effective rearing method was the culturing of ADB on cotton leaves and *Helicoverpa* spp. eggs in a small petridish. In this method over 60% of ADB's survived from nymphal stage to adult compared to the other methods where less than 40 per cent survived.

Species of apple dimpling bug found in cotton

Two species of apple dimpling bug, *Campylomma liebknechti* (Girault) and *C. seminigricaput* (Girault) were found in cotton growing areas of NSW during the study. Out of these two, only *Campylomma liebknechti* (Girault) was found to occur and cause damage in cotton.

Life cycle of ADB and description of different stages

ADB passes through an egg and five nymphal stages before becoming an adult. The females laid their eggs inside the plant tissues. The egg cap is left exposed for respiration purposes. Eggs took 5-11 days to hatch. The first instar nymphs were initially pale white in colour but gradually became pale to bright yellow as they mature. Wing pads developed at the third instar stage. The two distinguishing characters of ADB are dark spicules on the hind legs and dark brownish rings at the far end of second antennal segment. These characters were visible at the 5th instar stage. ADB males and females took 18.5 and 18.1 days respectively to develop from egg to adult stage. Females lived 4 days longer than males. ADB female can lay an average of 41.3±4.3 eggs in its life time. The peak oviposition period occurred between 6-10 days after emergence.

Temperature and ADB development

Temperamre has profound effect on ADB fecundity and development. Laboratory experiments showed that fecundity and development increased with temperature until an optimum was reached, thereafter, fecundity and development slowed down and in some case caused mortality. The optimum temperature for ADB fecundity and development was 32°C. The threshold temperatures for egg and nymphal development was 9.2° and 12.1°C respectively.

Pest and predator status of ADB

Both adult and nymphs of ADB caused similar types of damage. However, the degree of damage caused by different ADB stages differ. The late stage nymphs, 4th and 5th instar stages caused more damage than the early stage/younger nymphs (1-3rd instar stage). ADB prefer to feed on growing parts of plants, particularly terminals and squares. Feeding on terminals and leaves caused characteristic blackening and curling of leaf margin at the feeding point. Field and glasshouse experiments showed that seedling damage by ADB did not cause yield loss but caused 4 to 5 days delay in boll maturity. ADB prefer to feed on very small squares. Their feeding can cause necrosis of the anthers and cause squares to shed particularly pin-head squares. The shedding of squares as a result of ADB feeding was dependent on square size, feeding frequency, duration and point of feeding.

Experiments showed that ADB need *Helicoverpa* spp. eggs for their development. The presence of *Helicoverpa* spp. eggs did not significantly reduce ADB feeding on cotton plants.

The economic threshold for ADB based on visual counting was determined in this study as 10 per metre row of cotton. ADB was found mostly in the upper halves (1 to 8 nodes from first unfolded leaf) of the cotton plant than the lower halves.

Population dynamics of ADB

A wide range of crops and weeds were identified as overwintering hosts for ADB. Field study showed a linear relationship between densities of ADB adults on overwintering hosts during spring and infestations on cotton during summer. There was no difference in the densities of ADB population on both Ingard® and conventional cotton. On both Ingard® and conventional cotton crops, ADB occurred mainly from seedling through to squaring stage. High temperature (>38°C) for few consecutive days and the use of synthetic insecticides to control *Helicoverpa* were the two most limiting factors for ADB population. Lucerne was found to harbour more ADB than any other available hosts.

Recommendations for Best Management Practices

1. Growers should not use synthetic insecticides against ADB at seedling stage
2. An economic threshold of 10 ADB per metre row and 50% fruit retention should consider deciding on ADB control.
3. Lucerne can be used as a trap crop for ADB.

Project DAN 114C **Ecology and management of apple dimpling bug in conventional and transgenic cotton**

BACKGROUND OF THE PROJECT

The current pest management strategy in cotton in Australia relies heavily on the use of synthetic insecticides for the control of cotton pests particularly *Helicoverpa* spp. With the introduction of transgenic cotton and the continuous adoption of IPM by growers, the use of synthetic insecticides to control *Helicoverpa* spp. especially early in the cotton season is reducing. While the use of IPM strategies and transgenic cotton can manage *Helicoverpa* spp. with reduced insecticide sprays, sucking pests stand out as a major threat to the successful implementation of these technologies. One of the important sucking pests the green mirid, can be managed successfully in cotton by interplanting lucerne crop as strips in cotton fields. However, apple dimpling bug, another sucking pest, cannot be managed using lucerne strips and will require the use of synthetic insecticides especially during the early cotton season for control. The use of synthetic insecticides during this season will disrupt beneficial insects and affect IPM programmes. The apple dimpling bug is indigenous to Australia and occur in all cotton growing areas. Despite the insect's wide range of distribution, only little information is available on the insect's biology, behaviour and pest status on cotton. Lack of biological and ecological knowledge of this pest in the cotton industry has led to a series of misconceptions about the pest status of the apple dimpling bug on cotton. Some growers and consultants regard it as a pest while others regard it as a predator of *Helicoverpa* spp. eggs, mites and thrips.

A study of the basic biology, behaviour, pest status, population dynamics, sources and movements will provide the industry enough information to enable them to understand the ecology of apple dimpling bug and how to manage them in the context of IPM programmes.

The aim of the study is to undertake biological and ecological studies of the apple dimpling bug to increase the Australian cotton industry's knowledge of the biology, behaviour and ecology of the apple dimpling bug including their population dynamics, sources, hosts, movement etc. In addition to investigate the insects damage and develop economic thresholds for this pest for control in commercial cotton farms.

OBJECTIVES

- To confirm the identity of the species of the apple dimpling bug and develop a laboratory protocol for mass rearing of the insect.
- To conduct detailed studies of the insect's life cycle, behaviour and feeding mechanisms on conventional and transgenic cotton.
- To assess the pest status of apple dimpling bug, evaluate its damage to cotton plants and develop economic threshold for the management of the insect in cotton.
- To study the population dynamics of apple dimpling bug including its sources, principal host plants and movement.

METHODOLOGY

Development of a rearing technique for ADB

A reliable and satisfactory mass rearing technique for an insect is a pre-requisite for basic biological and behavioural studies, as well as for experiments to develop control options for the insect. This is especially so when large numbers of insects of similar bionic backgrounds are required for studies. In developing a suitable rearing method for ADB, the adults were collected from lucerne in Maules Creek near Narrabri.

Three different methods were used. They were 'cotton seedling', 'bean' and 'petridish' methods. The 'cotton seedling' and 'bean' methods used were similar to those used for rearing green mirids and is described by Khan 1999. In the 'petridish' method, ADB was reared on a diet of cotton leaf and *Helicoverpa* spp. eggs. In this method, a 50mm filter paper was placed in a 9 x 50 mm petridish. A fresh cotton leaf with soft and swollen leaf veins was cut into different sizes and placed on the filter paper in the petridish. The filter paper was moistened to keep the cotton leaf fresh for longer period of time. Special care was taken not to over wet the filter paper to avoid water condensing inside the petridish and drowning the insects.

Ten 3rd instar ADB nymphs collected from lucerne were placed on beans in the "bean method" and on cotton leaves in the other methods. The set up in each method was placed in cages and the survivals of the insects were assessed for each rearing method. This procedure was replicated four times for each method. Survivorship was determined from the total number, which completed development to an adult.

Confirmation of specie identity of apple dimpling bug

It is important in any entomological studies that one know the identity of the insects one is studying to enable appropriate literature of the insect in question or closely related species or genera to be searched. To confirm the identity of the species of apple dimpling bug in cotton crops and also on surrounding weeds, I did a survey and collected ADB's in cotton and other weeds in 4 cotton growing areas in NSW viz; Boggabilla, Moree, Narrabri and Warren. The collection was done during the early, mid and late cotton seasons in 1997-98. Most of the weeds surveyed were those which remained consistently fresh and green during the survey. All the ADB's collected were stored in 70% alcohol and sent to Dr. B. Malipatil (Agriclture, Victoria) for identification. .

Studies of the life cycle of ADB

The life cycle of ADB was studied in the laboratory maintained at a temperature of 25 ±1°C. The insects were cultured in the laboratory using 'petridish' method. Duration of development of the different stages of ADB was recorded. Female fecundity, pre-oviposition period and the longevity of male and female were also recorded. In addition, the developmental period of ADB under 8 different temperature regimes viz, 15, 20, 25, 30, 32, 35, 38 and 42°C was studied.

Pest status of ADB

A series of experiments were conducted both in the field and mesh house at ACRI using different densities of ADB to assess the damage caused by ADB adults and nymphs to seedling and squaring cotton plants. Insects were confined on the plants using either Perspex or Organdie cages.

For seedling cotton, ADB damage was assessed using a damage score method developed for green mirids (Khan, 1999). In addition, plant partitioning was also used to assess damage. The consequences of ADB seedling damage to the growth of the plant was also studied by allowing the damaged plants to grow until maturity and then damaged assessed by recording the number of squares and bolls per plant at weekly intervals. The dates of appearance of the first square, flower and boll maturity (60% boll open) were also assessed.

For squaring plants, the number of cotton squares on each treated plant was recorded prior to and after infestation.

Predator status of ADB

Studies were conducted in the mesh house and in the field to determine whether ADB can prey on *Helicoverpa* spp. eggs and also whether the presence of *Helicoverpa* spp. eggs on a cotton plant can influence the degree of damage ADB can cause to the plant. Two treatments were established on the cotton plants using perspex cage in the mesh house. The treatments were (1) cotton plants infested with ADB alone and (2) cotton plants infested with ADB and *Helicoverpa* eggs. The number of eggs consumed and the damage caused by ADB to each treated cotton plants were recorded. In the field, a 3 x 1 metre row cotton were checked visually on Ingard® and conventional cotton crops at ACRI and Norwood near Moree and the number of ADB and *Helicoverpa* eggs were recorded.

Economic Threshold trials

Previous experiments using ADB and green mirids showed that ADB's damage to cotton plants particularly early in the season irrespective of density did not translate to yield loss. It was therefore difficult to assess economic threshold for ADB on cotton crops. A new approach of determining the economic threshold for ADB on cotton was developed. This approach was to determine the number of ADB required causing the same economic damage as green mirids. This is because since ADB and green mirids (GM), do similar types of damage (Khan and Mensah 1998) and the economic threshold for green mirid is one per metre (see IPM Guidelines for Cotton 1999), instead of directly calculating ADB threshold from damage-density relationships. The equivalent number of ADB that can cause the same degree of damage as one green mirid was calculated and used as an economic threshold for ADB. This was calculated from regression models for ADB and GM in seedling and squaring cotton plants.

Distribution pattern in the field and on cotton plants

The experiment was conducted in a 20 ha irrigated cotton fields at Norwood during 1998-99 season. Visual observation was made weekly from 2-leaf stage to peak squaring stage. For each observation, one metre row of cotton was randomly selected and 5 randomly selected plants were sampled for ADB adults and nymphs. Data for within plant distributions were recorded according to plant parts (terminal, square and leaf) along with main stem node number.

To determine the distribution pattern in the field, Taylor's power law (Taylor, 1961) $S^2 = am^b$ or, in logarithms, $\log S^2 = \log a + b \log m$ (where S^2 is the variance and m the mean) was used. The slope b of the regression is an index of the distribution with values $b < 1$ indicating uniform, $b = 1$ indicating random and $b > 1$ indicating aggregated distributions. For within plant distribution, χ^2 test was used to compare the number of adults and nymphs found in the terminals, squares, leaves, upper (1-8 main stem node) and lower (9 and more main stem node) of the cotton plant.

Determination of the most efficient sampling method for ADB.

Four different sampling methods viz, D-vac, sweepnetting, visual counting and shaking were tested to determine the most efficient method to estimate the abundance of ADB on cotton crops. All four sampling methods tested are currently being used by growers and consultants in their day-to-day scouting of cotton plants. The study was conducted in transgenic cotton crops at Yarral near Narrabri during 1997-98 and 1998-99 seasons. Sampling was undertaken during the seedling, early and late squaring stages of the cotton crops.

Diurnal activities of ADB

An experiment was conducted in transgenic cotton at Yarral to determine the best times of the day to sample ADB in cotton fields. ADB populations were sampled at 0600, 0100, 1300, 1600 and 1900 hours during early and late squaring stages of the cotton plants. Three different sampling methods viz, visual, D-vac and sweepnetting were used.

Population studies of ADB on cotton and lucerne

The study was undertaken in commercial cotton crops at (1) the Australian Cotton Research Institute (ACRI) in Narrabri; (2) Yarral farm near Narrabri and (3) Norwood farm near Moree. At ACRI and Norwood study sites, sampling was done for 1997-98, 1998-99 and 1999-00 seasons and at Yarral for 1997-98 and 1998-99 seasons. At each site, population estimates were obtained from October to March by sampling once every week. The cotton fields in each study site were interplanted with lucerne to trap green mirids and conserve beneficial insects (Mensah and Khan 1997). ADB was sampled from both transgenic and conventional cotton using a portable suction machine (Homelite HB - 180V UT08010 - F, Homelite Textron Inc., NC USA). Samples were taken randomly every week in each plot. A single 20 metre suction sampling of canopy of cotton plants constitute a sample. Five samples each were collected from both Ingard® and conventional cotton at all study sites with the exception of ACRI during 1997-98 season where only 3 samples were taken from transgenic cotton crops because of the small size of the plots. ADB adults, male and female and all five stages of nymphs were counted on each sampling date. Sample commenced when the cotton crops were in the seedling (2-4 true leaf) stage. Insect samples were processed in the laboratory and counted under a dissecting microscope.

Overwintering hosts and their subsequent effect on ADB population on commercial cotton

ADB populations on overwintering hosts were sampled weekly with a portable suction machine as in cotton. Sampling was done in the Narrabri and Moree areas from May 1998 to October 1999. In May to October 1998, all green vegetation (weeds and shrubs) surrounding cotton farms (at ACRI, Yarral and Norwood) and also along access roadsides to these sites were sampled for possible hosts plants for ADB. Samples were taken once every week from selected plants and sent to the laboratory for identification and counting. In 1999, suspected overwintering hosts for ADB were sampled at ACRI from crop rotation experimental fields of Dr Ian Rochester in Narrabri. in 1999. At Norwood wild hosts surrounding a water reservoir used by the grower to irrigate his field were also sampled. Due to the ephemeral nature of the wild hosts and the fact that the wild hosts did not grow in large areas, sampling was not possible throughout the sampling period.

Statistical analysis

Unless otherwise stated all data were subjected to one-way analysis of variance and where necessary means were separated by using Fisher's least significance difference test at the 5% level (MINITAB statistical package, Ryan *et al.*1992). Regression analysis was also performed on various data set combinations as appropriate.

RESULTS

Development of a rearing technique for ADB

Out of the three methods tested, 'petridish' method came out as the most effective rearing method for ADB. This was followed by 'cotton seedling' method and the 'Bean' method was the least successful method with all adults dying within three days after infestation (Figure 1).

In the 'petridish' method, adults survived for 9 to 22 days. In this method, the ADB females laid about 200 eggs and 150 nymphs hatched from these eggs (i.e. 75 % hatchability). Out of the 150 nymphs 90 nymphs survived to adults (i.e. 60 % survivorship).

In the 'cotton seedling' method, ADB adults did not survived as long as adults caged on cotton leaves and *Helicoverpa* spp. eggs in the petridish method. In the seedling method, the ADB females laid about 50 eggs of which 30 nymphs hactched from these eggs (60 % hatchability). Out of the 30 nymphs only 12 could make it to adults stage (i.e. 40 % survivorship).

Species of apple dimpling bug found in cotton

Two species of apple dimpling bug, *Campylomma liebknechti* (Girault) and *C. seminigricaput* (Girault) were found in cotton growing areas of New South Wales. Despite the fact that all these two species occur on weeds surrounding cotton fields in all the sampling sites, only *C. liebknechti* occured on cotton (Table 1).

Table 1. Apple dimpling bug species found on cotton, other crops and weeds at Narrabri, Boggabilla, Moree and Warren in New South Wales, 1997-98.

Location	Early season		Mid season		Late season	
	C. liebknechti	*C. seminigricaput*	*C. liebknechti*	*C. seminigricaput*	*C. liebknechti*	*C. seminigricaput*
Narrabri						
Cotton	9	0	1	0	2	0
Lucerne	8	1	2	0	16	0
Saltbush	-	-	-	-	-	-
Pig weed	-	-	-	-	-	-
Boggabilla						
Cotton	3	0	4	0	0	0
Lucerne	4	0	0	0	1	0
Saltbush	1	1	0	0	0	1
Pig weed	-	-	-	-	0	0
Moree						
Cotton	18	0	5	0	0	0
Lucerne	3	0	1	1	0	0
Saltbush	-	-	-	-	-	-
Pig weed	-	-	-	-	-	-
Warren						
Cotton	17	0	4	0	2	0
Lucerne	13	0	2	0	0	0
Saltbush	4	58	12	2	0	0
Pig weed	-	-	8	0	0	0

Life cycle of ADB and description of different stages

ADB has the same egg laying behaviour as the green mirid. The female ADB embedded their eggs singly into the plant tissue leaving only the egg cap exposed for respiration. Eggs are kidney-shaped and when newly laid are transparent whites. The egg turned to pale yellow with two red spots on top within 3 days before hatching. The two red spots indicate the compound eyes of the developing nymph. Eggs hatched between 5 to 11 days after oviposition (mean = 7.3 ± 0.12 days). Nymphs emerged from the eggs by pushing themselves through the egg cap. After emerging, ADB passes through 5 nymphal stages to become an adult. The duration taken by ADB egg and nymphal stages for their development are given in Table 2. The development ADB males and females from egg to adult takes 18.5 and 18.1 days respectively. From the 1st instar nymph to adult, the males and females take 11.2 and 10.8 days to develop respectively.

The first instar nymph is initially pale white in colour and gradually changes to pale bright yellow as it matures. The antennae have four segments, each translucent with light white flagellum. The compound eyes are bright red and the tarsi are black. The second instar nymphs are yellow in colour. Tips of the antennae are yellowish. The third instar nymphs are pale yellow in colour with light black setae on the body. Wing pads are light brown in colour. Abdominal segments are slightly wider than the head and thorax. The compound eyes are light red in colour.

In the fourth instar nymphs, wing pads extend up to the third abdominal segment. The head and thorax are brownish yellow and the abdomen is greenish yellow.

Table 2. Time in days required for *Campylomma liebknechti* to develop in the laboratory (25±1°C temperature and 40-60% relative humidity).

Stage	Mean duration (days) ±se		Range (days)	
	Male	Female	Male	Female
Egg	7.3±0.12	7.3±0.12	5-11	5-11
1st instar	2.6±0.16	2.6±0.17	2-4	2-4
2nd instar	2.1±0.18	2.0±0.17	1-4	1-3
3rd instar	1.6±0.12	1.7±0.17	1-2	1-3
4th instar	2.2±0.15	1.8±0.13	1-3	1-3
5th instar	2.7±0.18	2.7±0.17	1-4	1-4
Total	18.5±0.91	18.1±0.93	11-28	11-28
Preoviposition	-	5.8±0.48	-	3-8
Oviposition	-	6.80±1.40	-	3-15

Wing pads extend up to the 8th abdominal segments in the fifth instar nymphs. The tips of the wing pads are blackened. A dark spicules on the hind legs and a dark brownish ring at the far end of the second antennal segment are also developed in the fifth instar stage. At this development stage, the 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 segments. The males and females also can be distinguished by colour and shape. Males are dirty yellow whereas females are light yellow in colour. The colour of the pronotum of males are dark yellow and shiny. Males are slender than females and the last abdominal segment of the males are pointed.

ADB female can lay an average of 41.3±4.3 eggs in her lifetime with a range of 34-49 at 25±1°C. Pre-oviposition and oviposition period ranged from 3 to 8 days and 3 to 15 days respectively. The peak oviposition period of ADB females occurred from 6-

10 days after emergence (Table 3). The females live longer than males, 15.4 and 11.4 days respectively.

Table 3. Number of eggs laid per female after emergence. Means followed by common letters are not significantly different (P > 0.05).

Days after emergence	No. of eggs/female (Mean± se)
1 - 5	2.9±1.42 a
6 - 10	32.1±5.56 c
11 - 15	17.20±6.35 b
16 - 20	3.0±2.19 a

Temperature and ADB development

ADB females laid significantly (P > 0.05) more eggs at 25° and 32°C (Figure 2). Oviposition increased with temperature up to 32°C, thereafter oviposition decrease with increase in temperature. ADB laid the highest number of eggs at 32°C and lowest at 40°C.

Table 4 shows ADB egg development time, nymphal development time and per cent nymphal survival. Development time for both egg and nymphs decreased significantly (P < 0.05) from 15- 32°C, thereafter egg and nymphal development time increased. At 15°C, ADB failed to complete development and at 42°C all insects died within a day.

Table 4. Mean egg, nymphal development time and nymphal survivorship at different temperature regimes.

Temperature °C	Mean egg development time	Mean nymphal development time	Nymphal survival percentage
15	20.00±0.91	*	*
20	11.50±0.65	18.25±0.78	37.5±7.22
25	7.50±0.29	11.08±0.47	62.5±7.22
30	-	8.15±0.15	87.5±7.22
32	4.75±0.25	7.83±0.18	87.5±7.22
35	5.00±0.00	8.43±0.18	56.3±15.7
38	-	12.75±0.25	25.0±0.00
42	-	*	*

- not included in the experiment and * failed to complete development

Reciprocals of development time were taken to convert to development rate and plotted against temperature (Figure 3). The relationship between temperature and ADB development was described by using a day-degree model:

$$1/y = a + bT$$

where 1/y is the rate of development. Since development rate slowed down at higher temperature (Table 4) an assumption was made that the development rate above optimum temperature was same as corresponding value at 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-

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

Day degree (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 and nymphal development the regression equation was obtained from the data set where 33.5°C and 31.5°C were found to be optimum. The equation from which linear model parameter for egg was calculated was

$$1/y = -0.0778 + 0.00845T \text{ for } (T = 33.5) \quad (R^2 = 96.4)$$
$$= -0.0778 + 0.00845\ T^1 \text{ for } (T > 33.5)$$
$$\text{where } T^1 = [(2 \times T_{opt}) - T]$$

The lower threshold temperature for egg development was calculated from day-degree model as 9.2°C and 118.3 DD above the lower threshold temperature would be required for complete egg development.

For nymphal development the regression equation was

$$1/y = -0.0835 + 0.00692T \text{ for } (T = 31.5) \quad (R^2 = 93.5)$$
$$= -0.0835 + 0.00692T^1 \text{ for } (T > 31.5)$$
$$\text{where } T^1 = [(2 \times T_{opt}) - T]$$

The lower threshold temperature and DD for nymphal development was calculated from day-degree model as 12.1°C and 144.5 respectively.

Pest status of ADB

Effect of ADB damage to seedling cotton

The types of damage caused by ADB to cotton plants are similar to green mirids but the degree of damage is less than that of the green mirid. Both adults and nymphs do similar types of damage. ADB prefer to feed on growing parts of seedling cotton, particularly terminals, tips of folded and unfolded leaves and branch primordia. Feeding on tips of leaves causes a characteristic blackening and curling of the leaf margin at the feeding points. An abrasive brown spot also occur at the feeding site on the plant. Experiments conducted in the mesh house and field showed that seedling cotton infested with 10 - 15 ADB per plant suffered significantly ($P < 0.05$) more damage than the control plants and the damage increased with insect density (Figure 4). When cotton plants are severely damaged at two-leaf stage, the plant growth rate is reduced. In an experiment where 10 ADB adults were placed on cotton plants for two weeks and the growth of the plant compared with uninfested plant of the same age, it was found that infested plants had 60 per cent less nodes than the uninfested plants (Figure 5A). The study also showed that ADB infested plants had reduced leaf area, leaf dry weight, stem and root dry weights than uninfested plants (Figure 5B and C).

Plants which were severely damaged (damage score 5-7) by ADB at seedling stage started squaring later than normal or undamaged plants. These damaged plants, however, recovered at a later stage. Table 5 gives the consequences of ADB damage to cotton plants at two-leaf stage. The results showed that plants infested with ADB had significantly ($P < 0.05$) lower number of squares during early squaring stage of the plant than uninfested control plants. However, at later stage, the number of squares and bolls of ADB infested plants were not significantly different ($P > 0.05$) from the control plants (Table 5), indicating the cotton plant's ability to compensate for ADB damage.

Cotton plants infested with 15 ADB took significantly ($P < 0.05$) more days to commence and complete squaring, flowering and boll maturity (60% of boll open) than nninfested (control) plants (Table 5). The plants infested with 15 ADB adults per plant suffered 5, 7 and 5 days delay at first square, flower and boll maturity compared to control plants.

Table 5. Consequences of ADB feeding on cotton plants at two leaf stage in the field. Means followed by common letters are not significantly different ($P > 0.05$).

Density of ADB per plant	Square no. at early squaring stage	Square no. at max. squaring stage	Boll no. at maturity	Delay in first squaring (days)	Delay in flowering (days)	Delay in boll maturity (days)
0	10.3 a	38.3 a	18.0 a	0.0 b	0.0 c	0.0 b
4	7.7 b	33.3 a	16.3 a	0.3 b	0.7 bc	0.3 b
6	6.0 bc	32.7 a	15.3 a	1.7 b	1.3 bc	0.7 b
10	6.3 bc	32.3 a	15.0 a	2.3 b	3.3 b	2.0 b
15	5.0 c	25.3 a	16.0 a	5.3 a	7.0 a	4.7 a

ADB damage to cotton squares

ADB adults and nymphs prefer to feed on very small squares. The symptoms of ADB damage to squares are necrosis in the internal organs, particularly anthers. Feeding on small squares caused squares to shed particularly the pinhead squares. Small and medium-sized squares are usually not shed by ADB feeding (Figure 6). The shedding of squares will depend in part on the frequency of ADB feeding and the point of feeding. Experiments conducted with ADB adults and nymphs showed that square shedding increased with ADB density. For both adults and nymphs, 10 per plant can significantly ($P < 0.05$) cause square shed (Figure 7).

Helicoverpa eggs and ADB damage to cotton plants

The presence of *Helicoverpa* spp. eggs on cotton plants can reduce ADB feeding damage (Figure 8). Field observations showed a trend of increased *Helicoverpa* spp. egg on cotton plants with increased densities of ADB during early squaring stage of the plants. This relationship, however, was not significant. The highest relationship ($r = 0.55$) was found on Ingard® cotton at Moree (Figure 9).

Economic threshold of ADB

The green mirid and ADB cause the same type of damage, however, green mirids cause more damage than ADB. The damage caused by both insects increases with density of the insects. The density-damage relationship between ADB and GM on seedling cotton and squares are shown in Figures 10 and 11. The regression models show that when seedling cotton was infested with either 1 ADB or 1 GM the cotton plant suffered 2.63 and 19.78 percent damage respectively. The density-damage relationship of ADB and GM for squaring cotton plants were $Y = 0.4701X + 1.7477$ and $Y = 1.9431X + 4.4643$ respectively (Figure 11). Based on these relationships, it was calculated that 11.3 and 9.8 ADB's could cause the same degree of damage as 1 GM to seedling and squaring cotton plants respectively. Since, the economic threshold for green mirids in commercial cotton is calculated as 1 per metre, the economic threshold for ADB is 11.3 and 9.8 respectively for seedling and squaring plants. This is the threshold of ADB that can cause equivalent damage of 1 green mirid.

Spatial distribution of ADB in the field

Taylor's power law describes the spatial distribution of ADB adults and nymphs and the estimated parameters are given in Table 6. The parameters indicated that the spatial distribution of ADB adults and nymphs were clumped.

Table 6. Spatial distribution of ADB in cotton fields using Taylor's Power Law

Insect stage	b	r^2
Adult	1.31	0.79
Nymph	2.21	0.78

Distribution of ADB in cotton plant

ADB adults and nymphs are found mostly on cotton terminals (including tips of branches and pinhead squares) followed by leaves (Table 7). Squares had least numbers of ADB. Chi-square tests showed that there was no significant relationship between insect stage and distribution on terminals and squares ($\chi^2 = 0.026$ df = 1, P = 0.87); and on squares and leaves ($\chi^2 = 3.214$, df =1, P = 0.07). The relationship between insect stage and distribution on terminals and leaves was however significantly different ($\chi^2 = 11.80$, df = 1, P = 0.001).

Table 7. Percent ADB found in different parts of cotton plants in the field, Moree, 1998-99.

Plant parts	Adult[1]	Nymph[1]	Total[1]
Terminal	23.24	62.94	86.18
Leaf	4.82	4.39	9.21
Square	1.32	3.29	4.61

[1]summed for all dates

Most ADBs' were found on upper halves (1 to 8 nodes) of the cotton plant (81.53%). The lower halves had 18.47 percent of ADB (Figure 12). Chi-square test indicated a significant relationship between insect stage and distribution on upper and lower halves ($\chi^2 = 17.94$, df = 1, P = 0.000).

Determination of efficient sampling method for ADB

The results showed that visual counting is the most effective method to sample ADB. This was followed by D-vac and sweepnetting. However, during the 1997-98 season, visual, D-vac and sweepnetting were found to be equally effective in sampling ADB during the seedling stage (Figure 13). In general, D-vac and sweepneting were found to be less effective against ADB nymphs throughout the cotton season (Figure 13). The shaking method was the least effective method.

Diurnal activities and sampling of ADB

The results of this study are summarised in Figure 14. Early morning (6 am to 10 am) or late afternoon (4 pm) were found to be the optimum times of the day to sample ADB effectively irrespective of the sampling method and crop stage.

Population changes of ADB on Ingard® and conventional cotton in commercial cotton fields

ADB population density changes were not significantly different throughout the season on both Ingard and normal cotton crops at all the study sites (Figure 15). ADB population density recorded at all study sites during the 1998-99 season were significantly higher ($P < 0.05$) than 1997-98 and 1999-00 seasons with the exception of Norwood study site where the population densities on conventional cotton, was not significantly different ($P > 0.05$) (Figure 15B). ADB population density in 1997-98 and 1999-00 were not significantly different at all the study sites.

ADB numbers recorded on both Ingard® and normal cotton plants were not significantly different ($P > 0.05$) throughout the season at all the study sites (Figure 16) with the exception of the 1998-99 season where, numbers were significantly higher on Ingard® than on conventional cotton at Yarral (Figure 16 B) and Norwood (Figure 16C). In contrast, ADB densities during 1998-99 season at ACRI were significantly higher on conventional cotton than Ingard® (Figure 16A).

In both cotton types ADB occurred mostly at seedling and early squaring stage (Figures 17-19). ADB adults were also recorded in boll maturing stage at ACRI during 1998-99 and 1999-00 seasons and at Norwood during 1999-00 season (Figure 17 & 18).

During 1999-00 season at ACRI, the highest number of ADB adults were recorded on 6 December on both Ingard® and conventional cotton crops. This coincided with the period when cotton crops have commenced to square. After this period, the number of insects continued to decline reaching their lowest levels at crop maturity stage. Nymphal populations were very low on both Ingard and conventional cotton crops on 6 December but peaked on 30 December. Thereafter, nymphal populations reduced to zero (Figure 17A). At Norwood, ADB adults reached its peak density on 2 and 16 December on Ingard cotton. In contrast, the ADB population density peaked 16 December on the conventional cotton crops. The population however, crashed after 30 December on both Ingard® and conventional cotton crops. The ADB adult population on conventional cotton crops recovered on 20 January through to 10 February, before collapsing on 17 February (Figure 18A). The nymphal populations, on the other hand, peaked on 9 December and then crashed on 16 December. Unlike the adult population, the nymphal population failed to recover after the crash (Figure 18A).

During 1998-99 season at the ACRI study site, the density of ADB adults was highest on 23 December on both Ingard® and conventional cotton crops. Thereafter, population continued to decrease reaching its lowest level (0.01/m) on 9 February (Figure 17B). The ADB nymphal population density was highest on 23 December and 12 January on both conventional and Ingard® cotton. The ADB nymphal population, however, collapsed on 12 January (Figure 17B). At Norwood and Yarral study sites, ADB adults were recorded earlier at these sites than the ACRI site (Figures 18 & 19). At the Norwood site, the highest adult numbers were recorded on 18 November on both Ingard® and conventional cotton crops. However, the peak densities of ADB nymphs were recorded on 2 and 15 December on Ingard® and conventional cotton respectively (Figure 18B). The populations of both adult and nymphs collapsed after 31 December at Norwood (Figure 18B). At Yarral, the population densities of both adults and nymphs peaked on 30 November (Figure 19A), thereafter, the adult populations continued to decline reaching its lowest level (0.02/m) on 25 January. The nymphal population collapsed after 17 December on both Ingard® and conventional cotton crops (Figure 19A).

During 1997-98 season, the highest number of ADB adults was recorded on 19 November at ACRI on both Ingard® and conventional cotton crops. Thereafter, populations continued to decline until the 3rd week of January (Figure 17C). The nymphal population peaked on 11 November. At the Norwood site, the population of both adults and nymphs reached its peak numbers on 18 November in both Ingard® and conventional cotton crops (Figure 18C). The population, however, crashed in the 1st week of January. At Yarral the highest adult populations were recorded on 27 November on both Ingard® and conventional cotton crops (Figure 19B) but no nymphs were recorded in any of the cotton crops during the 1997-98 season.

Overwintering hosts and their subsequent effect on ADB population in commercial cotton field

ADB has a very wide host range from field crops to weeds. Some of the host plant species which can support the development of ADB adults and nymphs are given in Table 8. In all, 19 crop and weed plants were found as alternative hosts of ADB. Table 8 shows that out of the 9 host crops and 10 weed hosts sampled 7 crops and 6 weeds respectively were found to support the development of both adults and nymphs. Population changes of ADB on alternative host plants at ACRI and Norwood are given in Figure 20. The alternative crop found to support higher densities of ADB was lucerne (*Medicago sativa*). This was followed by fababeans (*Vicia faba*) and vetch (*Vicia villosa*) at ACRI and wild turnip (*Brassica spp*) at Norwood. Generally, the population density of ADB was highest on the alternative host plants during October (Figure 20) indicating the possibility that the ADB found on cotton crops during the early cotton season might have migrated from weeds and other crop hosts surrounding cotton fields. Migration from these host plants may be enhanced when the host plants dried up, cultivated or grazed at the beginning of the cotton season.

Table 8. List of crop and weed hosts that support ADB population

Common Name	Scientific Name	Support ADB stage development
Crop Hosts		
Lucerne	*Medicago sativa*	Adult and Nymph
Sunflower	*Helianthus annuus*	Adult and Nymph
Safflower	Carthemus tinctorius	Adult
Sorghum	*Sorghum bicolor*	Adult
Mungbean	*Vigna radiata*	Adult and Nymph
Bean	*Phaseolus vulgaris*	Adult and Nymph
Fababean	*Vicia faba*	Adult and Nymph
Vetch	*Vicia villosa*	Adult and Nymph
Pigeonpea	*Cajanus cajan*	Adult and Nymph
Weed Hosts		
Burr medic	*Medicago polymorpha*	Adult
Cat head	*Tribulus terrestris*	Adult
Common joyweed	*Alternenthera nodiflora*	Adult
Gluaca	*Haloragis glauca*	Adult and Nymph
Hairy carpet weed	*Glinus lotoides*	Adult and Nymph
Noogoora burr	*Xanthium spp.*	Adult and Nymph
Paterson's curse	*Echium plntagineum*	Adult and Nymph
Salt bush	*Atriplex spp.*	Adult and Nymph
Variegated thistle	*Silybum marianum*	Adult
Wild turnip	*Brassica spp.*	Adult and Nymph

DISCUSSION

This study has been the most detail so far on apple dimpling bug on cotton in Australia to date. The study has increased our knowledge on the issue of species identity, sampling, thresholds, basic biology including lifecycle, pest and predator status, seasonal phenology, sources and movement of apple dimpling bug.

There are 5 species of apple dimpling bug distributed throughout Australia (Malipatil 1992). Out of the 5, two species *Campylomma liebknechti* (Girault) and *C. seminigricaput* (Girault) occur on alternative hosts such as lucerne. Only one species, *C. liebknechti* occur on cotton (see Table 1). *C. liebnknechti* can be distinguished from *C. semingiricaput* by head colour. *C. liebnknechti* has black head. *C. seminigricaput* has no black head and dark spicules on the dorsal surface of the hind femur.

ADB, like green mirid, passes through an egg and five nymphal stages before becoming an adult. The development rate of ADB is however faster than GM. From egg to adult, ADB and GM take 18 and 23 days respectively at 25°C ± 1. ADB females also lay more eggs than GM. It can lay an average of 41.3±4.3 in its life time. The peak oviposition period of ADB occurs at 6-10 days after emergence (see Table 3). Therefore targeting control measures to coincide with newly emerged adults during the pre-oviposition period may reduce ADB population in the next generation. Newly emerged ADB adult can easily be differentiated from the older ones by colour. Newly emerged ADB adult females (2-3 days old) are pale yellow in colour and as they grow old it becomes brownish yellow.

ADB development is temperature dependent. The study has shown that ADB fecundity and development rate increases with an increase in temperature until an optimum temperature is reached where fecundity and development level up. Above the optimum temperature fecundity and development reduce significantly to a level where any increase resulted in high mortality. The optimum temperature for ADB fecundity and development is 32°C, which is higher than GM (Khan 1999). Therefore unlike GM, ADB could survive well and damage cotton plants at higher temperatures especially in stage III where temperatures are often high. In stage III, ADB population may only be limited by insecticide sprays used by growers to manage *Helicoverpa* spp. The lower threshold temperatures for ADB egg and nymphal development were 9.2°C and 12.1°C respectively. These threshold temperatures are lower than that of GM (Khan 1999) indicating that ADB could continue development and survive better at lower temperatures than GM.

The study has shown that ADB can act as a pest or predator. ADB can survive and develop better if it has access to both plants (carbohydrates) and insects (proteins) duriug feeding. The study showed that ADB need *Helicopverpa* egg for their survival and development. The insect's abdomen was found or observed to be transparent when it has fed on only cotton plant tissue but white or brown if they have fed on *Helicoverpa* spp. white or brown eggs respectively. In the cotton field, the presence of *Helicopverpa* spp. eggs though did not significantly reduce ADB feeding on cotton plants, it however reduced the damage to these plants (see Figures 8 and 9) indicating that ADB may feeds on cotton plants and *Helicoverpa* spp. eggs simultaneously. The availability of prey on cotton plants in the course of ADB feeding will reduce the feeding damage (pest status) of ADB and increase its predatory ability. ADB should therefore be controlled only if it exceeded the economic threshold and reduces fruit retention. This will enable growers to utilise the insect as a predator particularly during the early cotton season.

A wide range of overwintering hosts for ADB have been identified in this study (see Table 7). Some of these hosts can support the development of ADB adults and nymphs. ADB overwintering on these hosts develop slowly but during spring (September – October) ADB population starts to build up as the temperature rises, reaching peak densities at the end of spring when growers commence to plant cotton. As the cotton plant grows and the surrounding host plants dry up due to dry weather, or grazing by cattle or cultivation by growers, there is a movement of ADB onto the cotton plants. ADB adult populations recorded on cotton early in the cotton season might have come from surrounding alternative host plants.

ADB adults and nymphs can cause similar types of damage. The older nymphs (4th and 5th instars) and adults are more damaging than younger nymphs (1-3 instars). Though both ADB and GM can cause similar type of damage the degree of damage caused by ADB to cotton are less than that of GM. ADB prefer to feed on growing parts of plants, eg, terminals, tips of folded and unfolded leaves, branch primordia and squares. ADB can also cause damage to cotton plants from seedling through to squaring stage. The damage caused by ADB to seedling cotton though does not translate into yield loss they can cause delay in boll maturity. ADB feeding on cotton squares can cause the squares to shed particularly pinhead squares. Shedding also depend on the frequency and duration of feeding and also the point of feeding. Unlike GM, it is not clear whether shedding is associated with pectinase (Hori and Miles 1993) or induction of ethylene (Morgan 1969). The economic threshold for ADB was calculated as the number of ADB which can cause the same degree of damage as one GM (economic threshold for GM per metre for cotton). This number was calculated as 11 and 10 ADB's for seedling and squaring cotton respectively and recommended in this project as the economic threshold for ADB on cotton. The most efficient way of assessing ADB population in the field is by visual counting. Since most ADB's were found mostly on the upper halves of the plant (1 to 8 nodes of the plant starting from first unfolded leaf) (see Figure 12), visual counting or assessment of ADB should be done on the upper halves of the plants during early morning or late afternoon.

The Australian Cotton industry is increasingly adopting IPM strategy and transgenic cotton technology to manage insect pests. The adoption of these technologies will reduce synthetic insecticide use and increase ADB survival. Any decision therefore to control ADB should be taken in the context of IPM strategy and should be based on the economic threshold. This study has shown that the damage caused by ADB at seedling stage is not significant or economically important since it does not cause any yield loss. The damage, though, according to this study, can cause a maturity delay of 4-5 days but this delay is not significant economically. Considering these findings, it is worthwhile not to use synthetic insecticide sprays at seedling stage against ADB. Feeding by ADB during the squaring stage of cotton plants can shed pin-head squares but cotton plants can compensate for this damage.

In the light of this findings, it is crucial that growers should avoid the use of broad spectrum and indiscriminate sprays against ADB and any decision to control ADB during the squaring stage of cotton plants should be based on the economic threshold and fruit retention. .

CONCLUSIONS AND RECOMMENDATIONS

Based on the findings of this project, I suggest the following recommendations in the IPM Guidelines and the Pesticide Management Guide:

1. ADB's feeding on seedling cotton does not result in yield loss. The delay in maturity of cotton plants caused by ADB feeding is economically negligible. Synthetic insecticides should not be used against ADB at seedling stage. During periods of high infestations above the threshold recommended in this project, selective rather than broad-spectrum insecticides should be used to control ADB.

2. At squaring stage, ADB's feeding can cause shedding of pinhead squares. Ten ADB per metre row of cotton along with 50% fruit retention is recommended as the economic threshold for ADB at squaring stage. Use visual counting to assess ADB population in the field. Early morning or late afternoon is the best time to sample ADB population.

3. Lucerne harbours more ADB than cotton or other alternative hosts. Lucerne is being used as trap crop to manage green mirid (Mensah and Khan 1997) in the cotton field. Lucerne can also be used as trap crop for ADB. The area of lucerne required and its management in cotton fields are similar to those given in the IPM Guidelines.

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ACKNOWLEDGEMENTS

I would like to thanks CRDC for funding this project. I also thank Robert Wenzel, Sandra Pearson, John Rehburg and Nicky Lee for technical assistance. Special thanks to Peter Glennie and Kylie May (Norwood, Moree), Chris Lehmann and Vic Melbourne (Yarral, Narrabri), John and Ross O'Brien (Berllevue, Warren) for their cooperation with the trial.

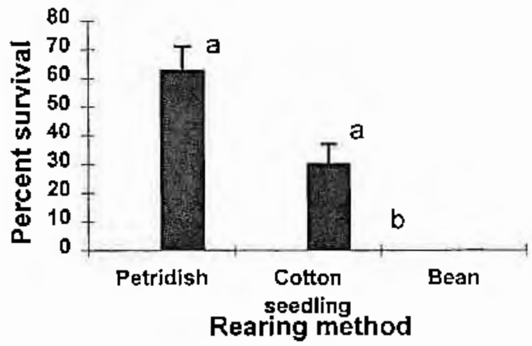


Figure 1. Percent survival of ADB nymph to adult, in different rearing methods. Means followed by same letter are not significantly different ($P > 0.05$). Error bars indicate standard error of means.

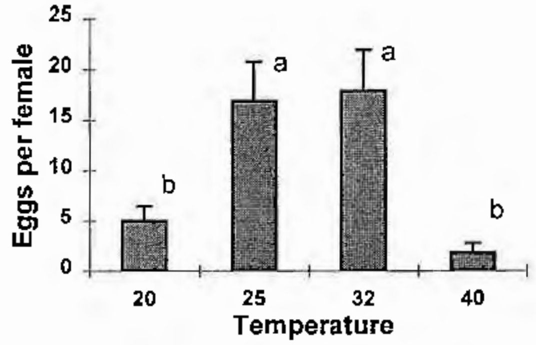


Figure 2. Oviposition of ADB at constant temperatures. Means followed by same letter are not significantly different ($P > 0.05$). Error bars indicate standard error of means

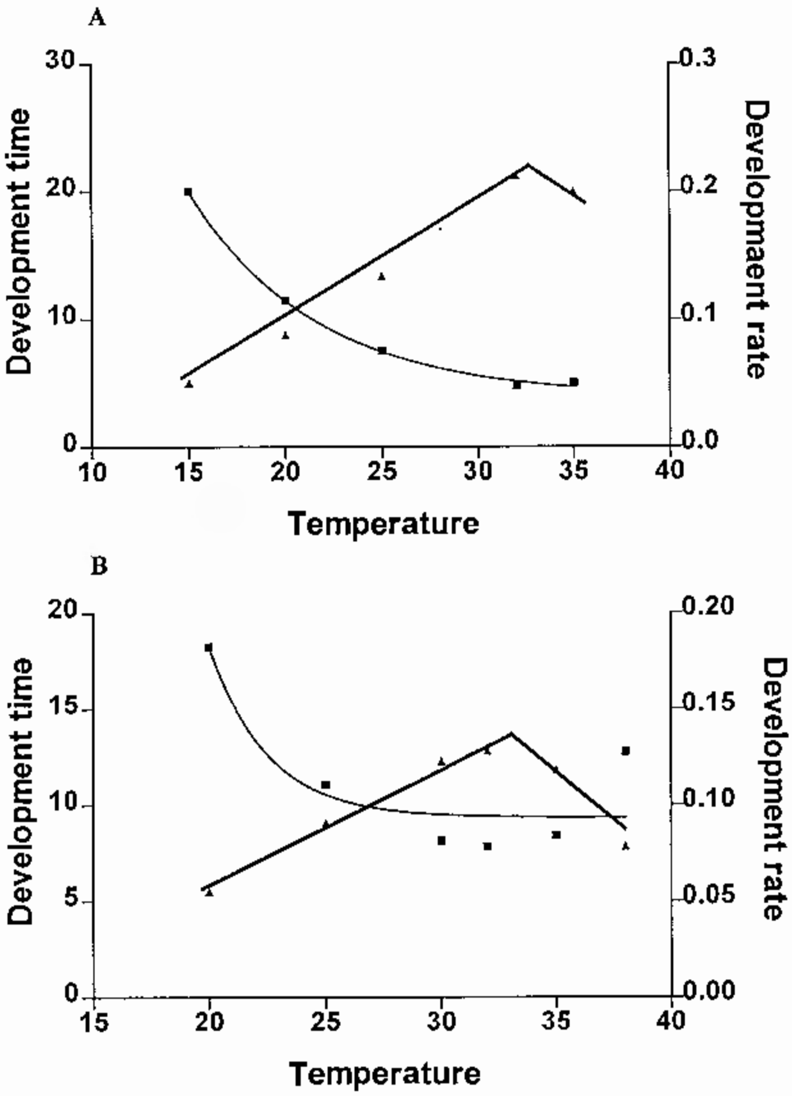


Figure 3. Egg (A) and nymphal (B) development times and rates of ADB at constant temperatures showing day-degree models.

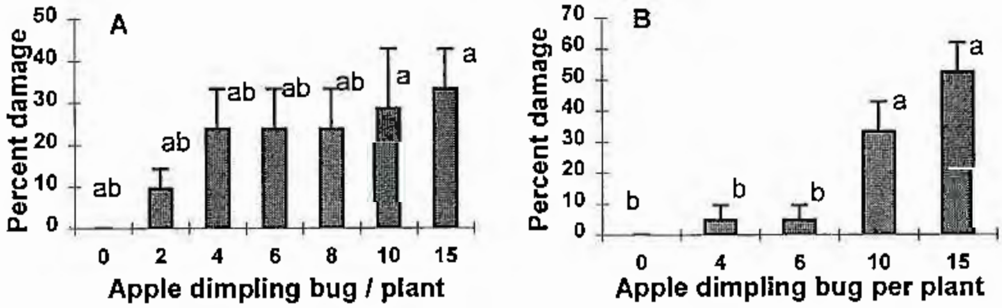


Figure 4. Damage caused by apple dimpling bug on seedling cotton in mesh house (A) and in field (B). Means followed by same letter are not significantly different (P > 0.05). Error bars indicate standard error of means.

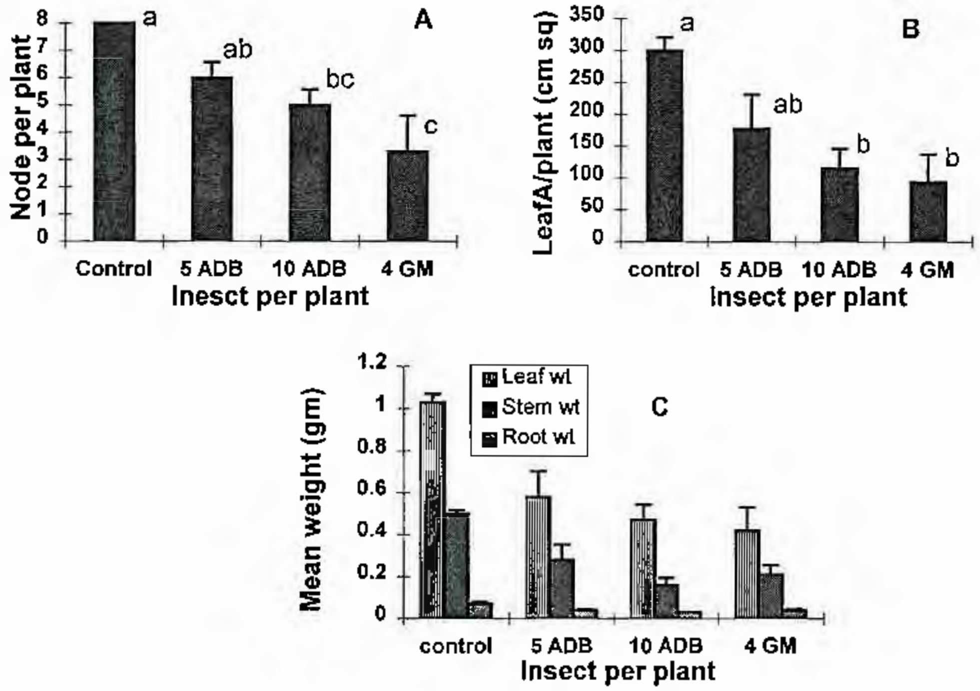


Figure 5. Feeding by apple dimpling bug on seedling cotton in the glass house reduces node production (A), leaf area (B) and dry weights of leaf, stem and root (C). Means followed by same letter are not significantly different (P > 0.05). Error bars indicate standard error of means.

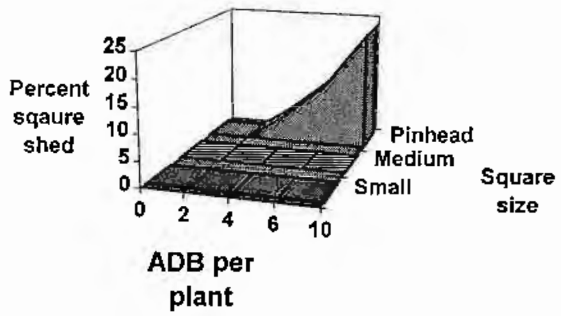


Figure 6. Effect of ADB feeding on different sizes of cotton squares in the field

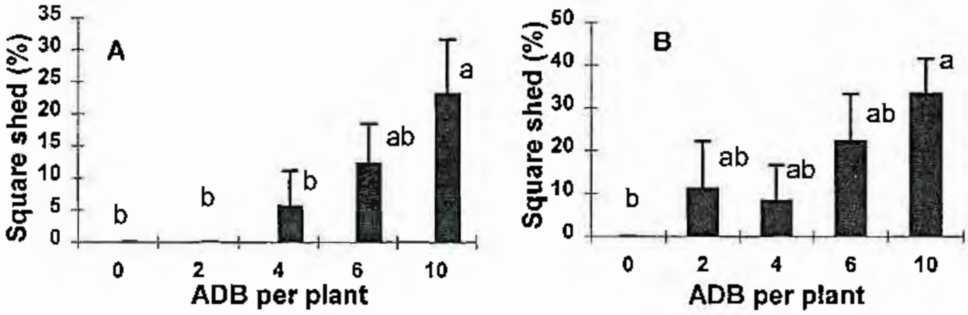


Figure 7. Percent square shed due to feeding of adults (A) and nymphs (B) of ADB in the field. Means followed by same letter are not significantly different (P > 0.05). Error bars indicate standard error of means.

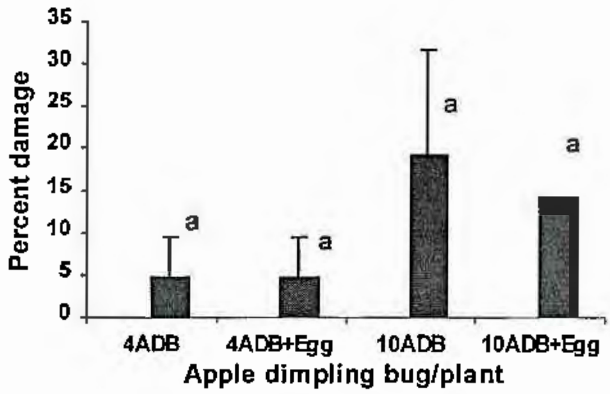


Figure 8. Provision of *Helicoverpa* spp. eggs on cotton plants and damage caused by ADB to plants in the mesh house.

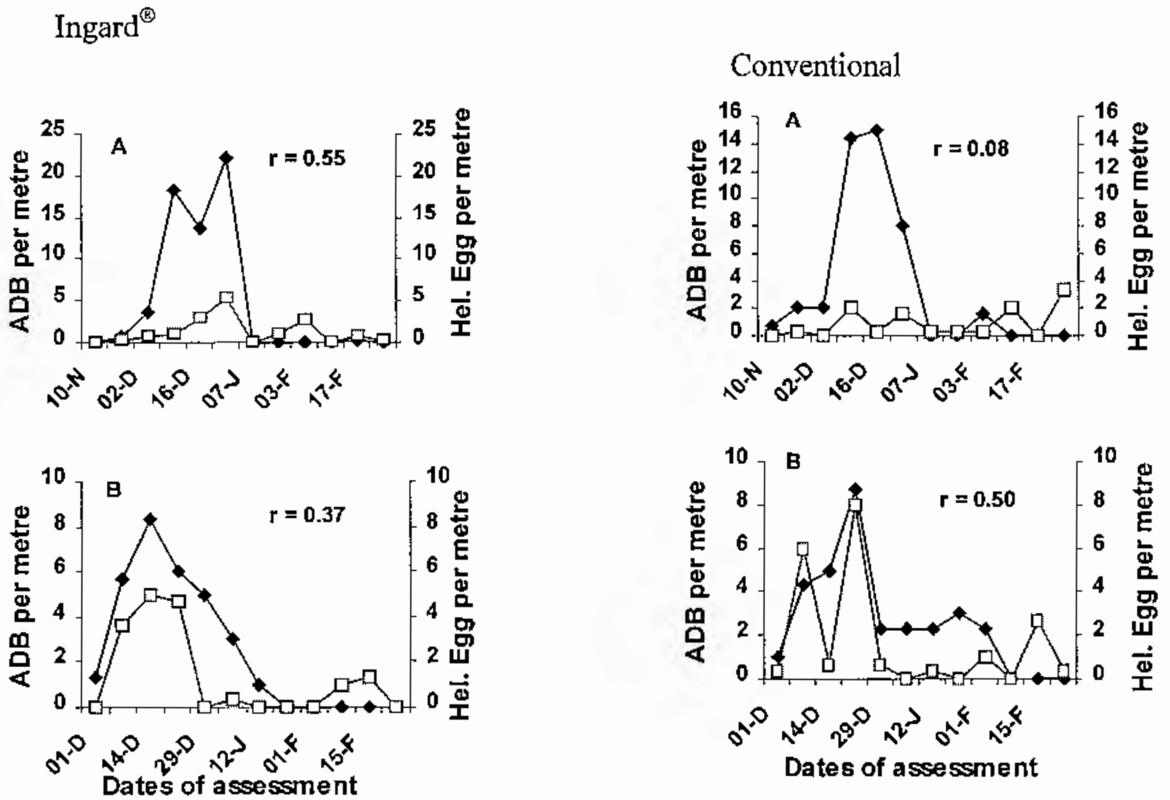


Figure 9. Relationship between presence of *Helicoverpa* spp. egg and incidence of apple dimpling bug in field in Ingard® and conventional cotton at Norwood, Moree (A) and at ACRI, Narrabri (B) during 1999-00 season

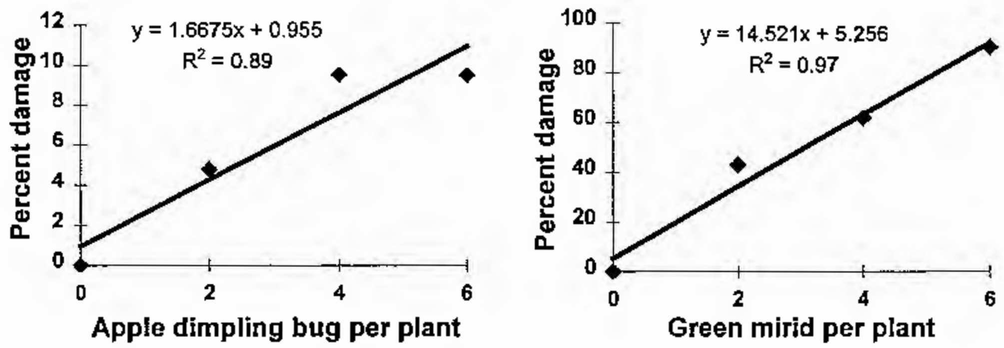


Figure 10. Density-damage relationship of ADB and GM on seedling cotton

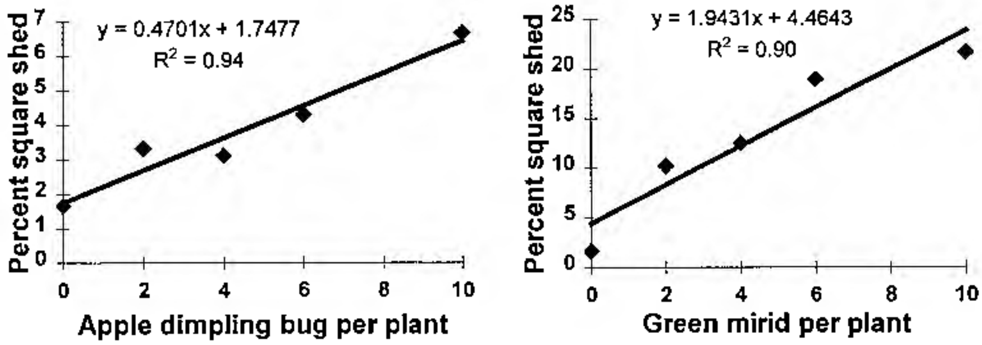


Figure 11. Relationship between damage and density of ADB and GM for squaring plants

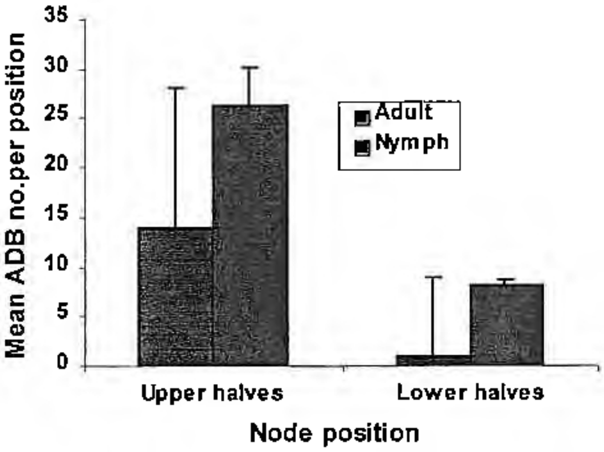


Figure 12. Distribution of ADB on different main stem node position of the cotton plant in the field, Norwood, Moree during 1998-99 season

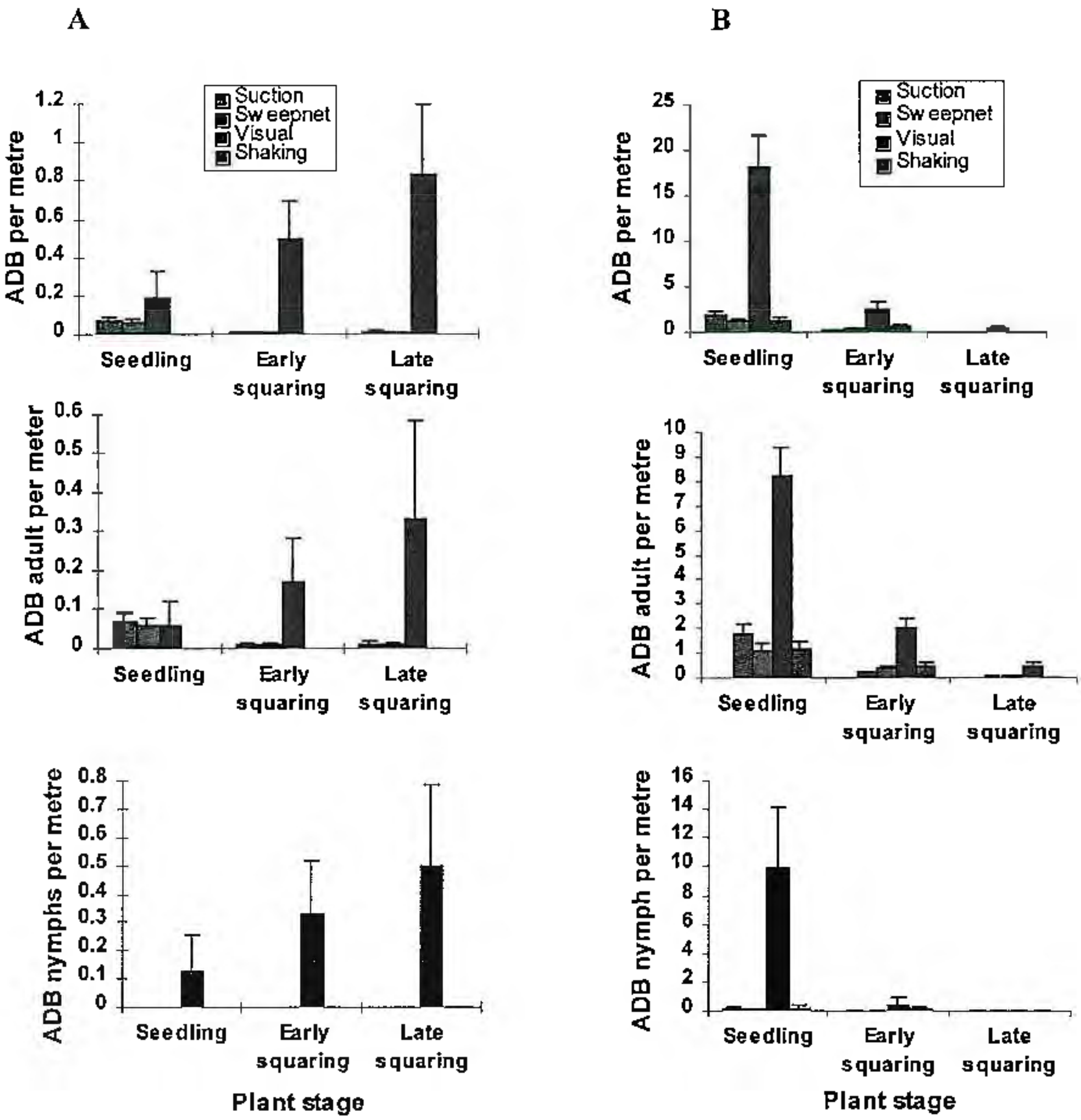


Figure 13. Comparisons of sampling methods for apple dimpling bug in cotton fields at Yarral, Narrabri during 1997-98 (A) and 1998-99 (B) seasons. Error bars indicate standard error of means

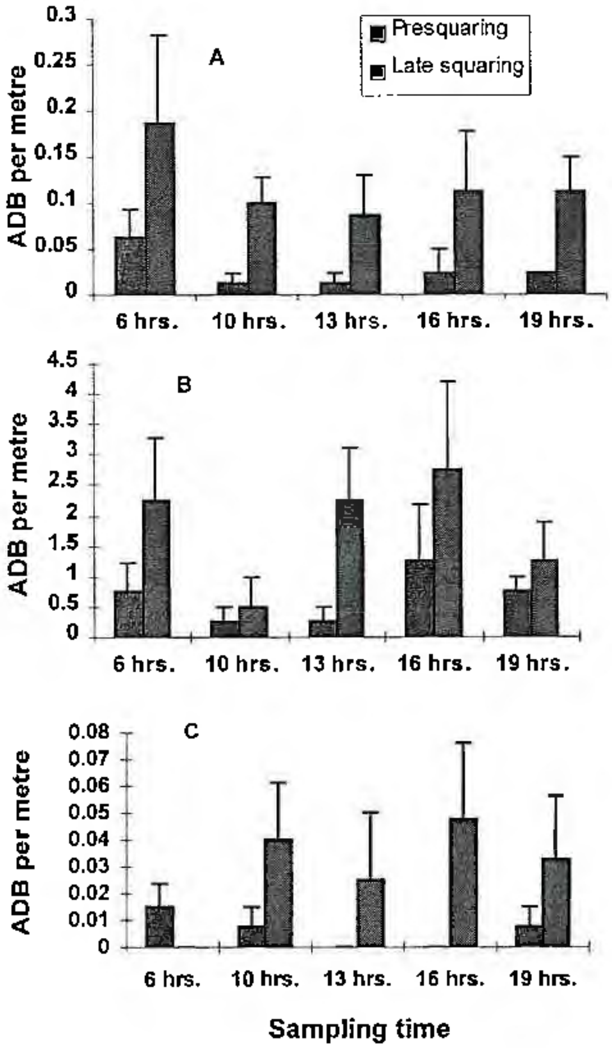


Figure 14. Number of apple dimpling bug caught using suction machine (A), visual counting (B) and sweepnetting (C) at different times of the day in cotton fields at Yarral, Narrabri, 1997-98

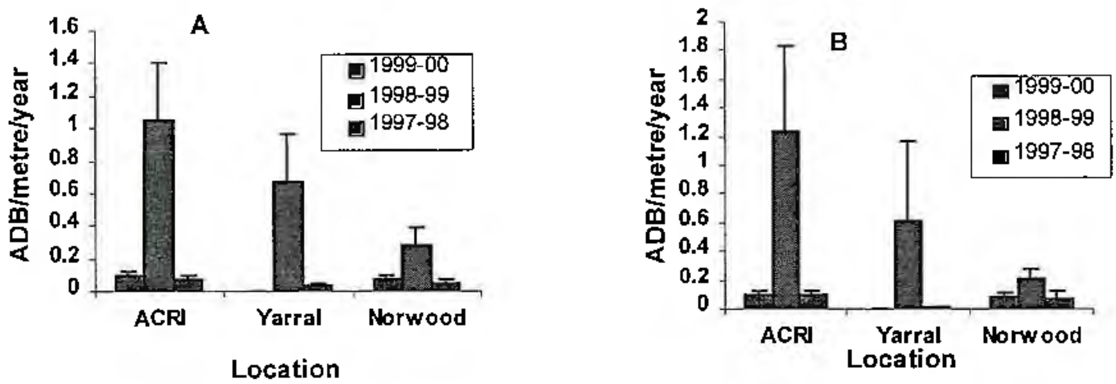


Figure 15. ADB populations sampled from Ingard (A) and conventional (B) cotton crops at ACRI, Yarral and Norwood during 1997-98, 1998-99 and 1999-00 seasons.

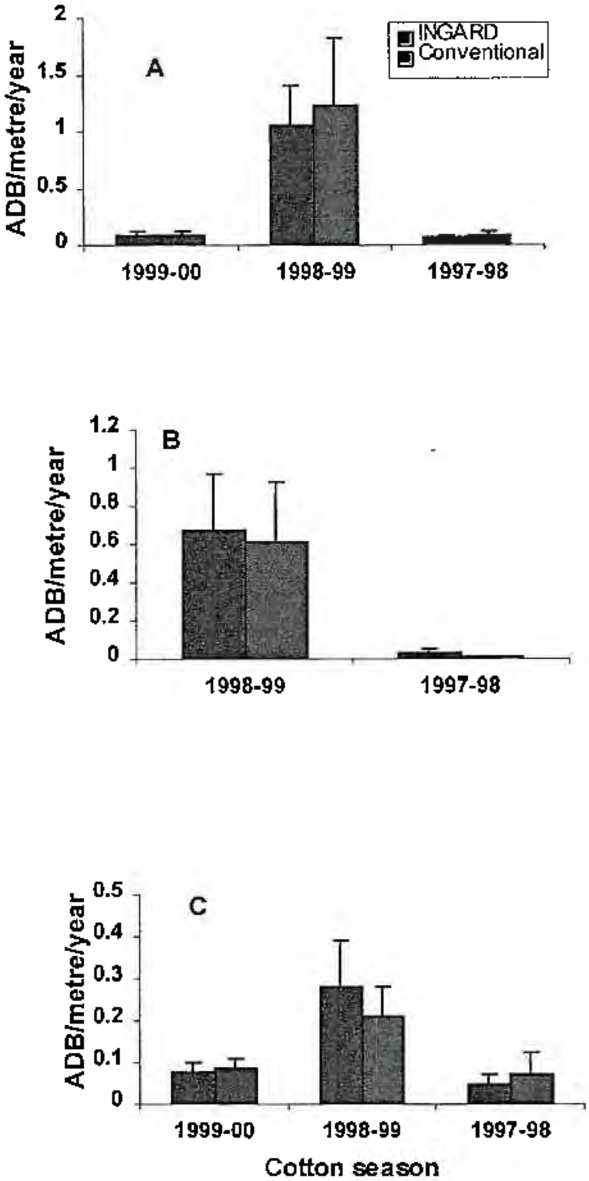
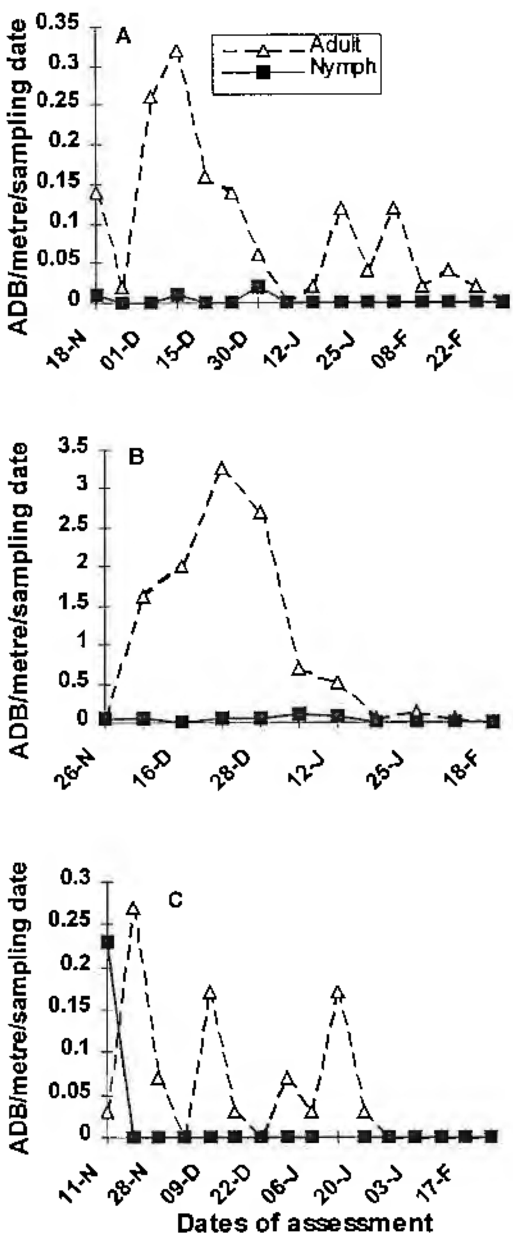


Figure 16. ADB populations on Ingard® and conventional cotton crops at ACRI (A), Yarral (B) and Norwood (C) during 1997-00 seasons

ACRI, Narrabri

Ingard®



Conventional

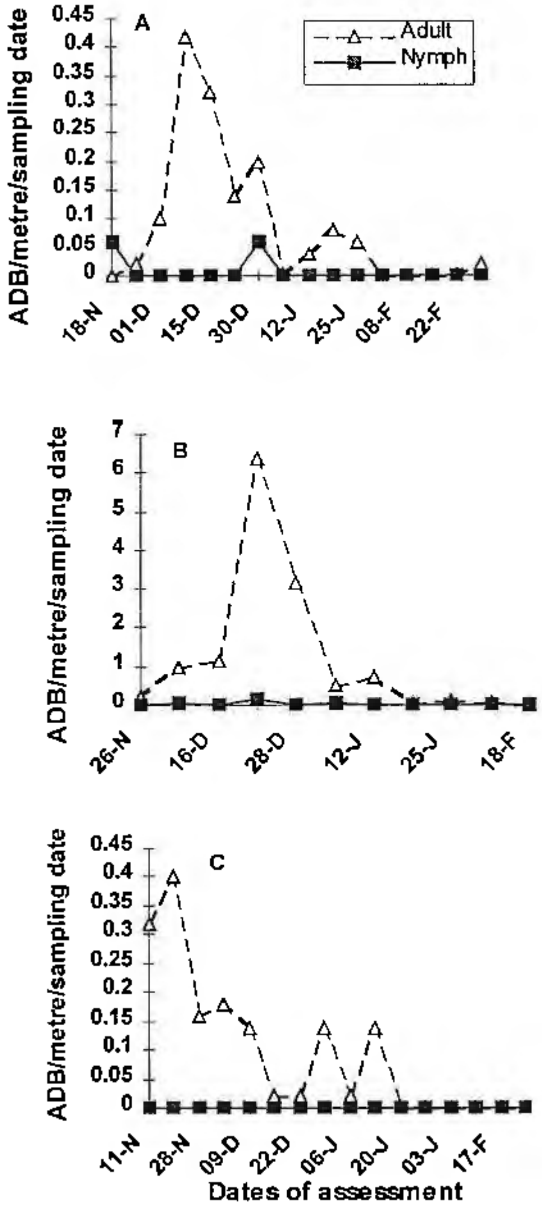


Figure 17. Seasonal abundance of ADB on Ingard® and conventional cotton crops at ACRI in Narrabri during 1999-00 (A), 1998-99 (B) and 1997-98 (C).

Norwood, Moree

Ingard® | Conventional

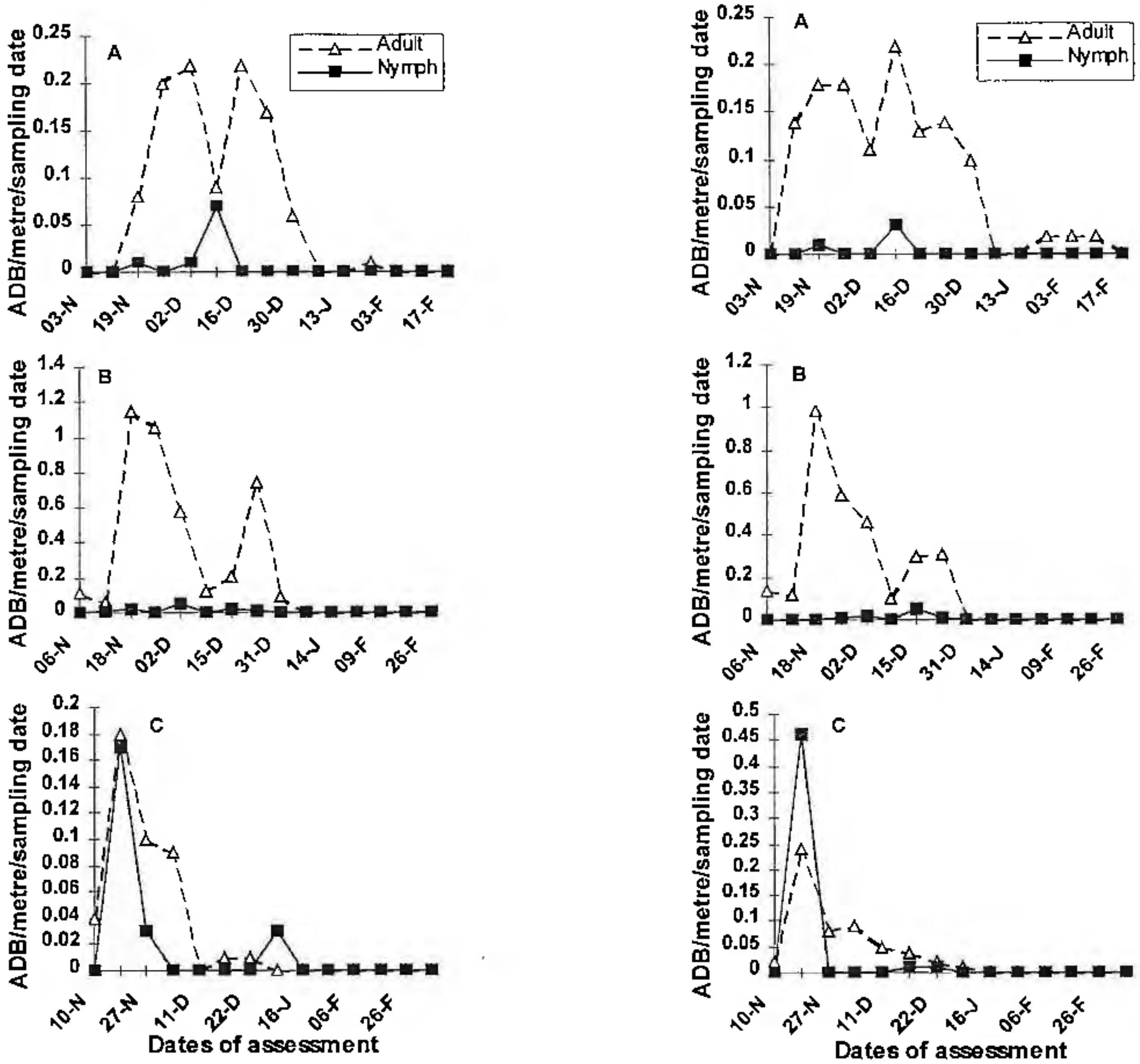


Figure 18. Seasonal abundance of ADB on Ingard® and conventional cotton crops at Norwood, Moree during 1999-00 (A), 1998-99 (B) and 1997-98 (C) seasons.

Yarral, Narrabri

Ingard®

Conventional

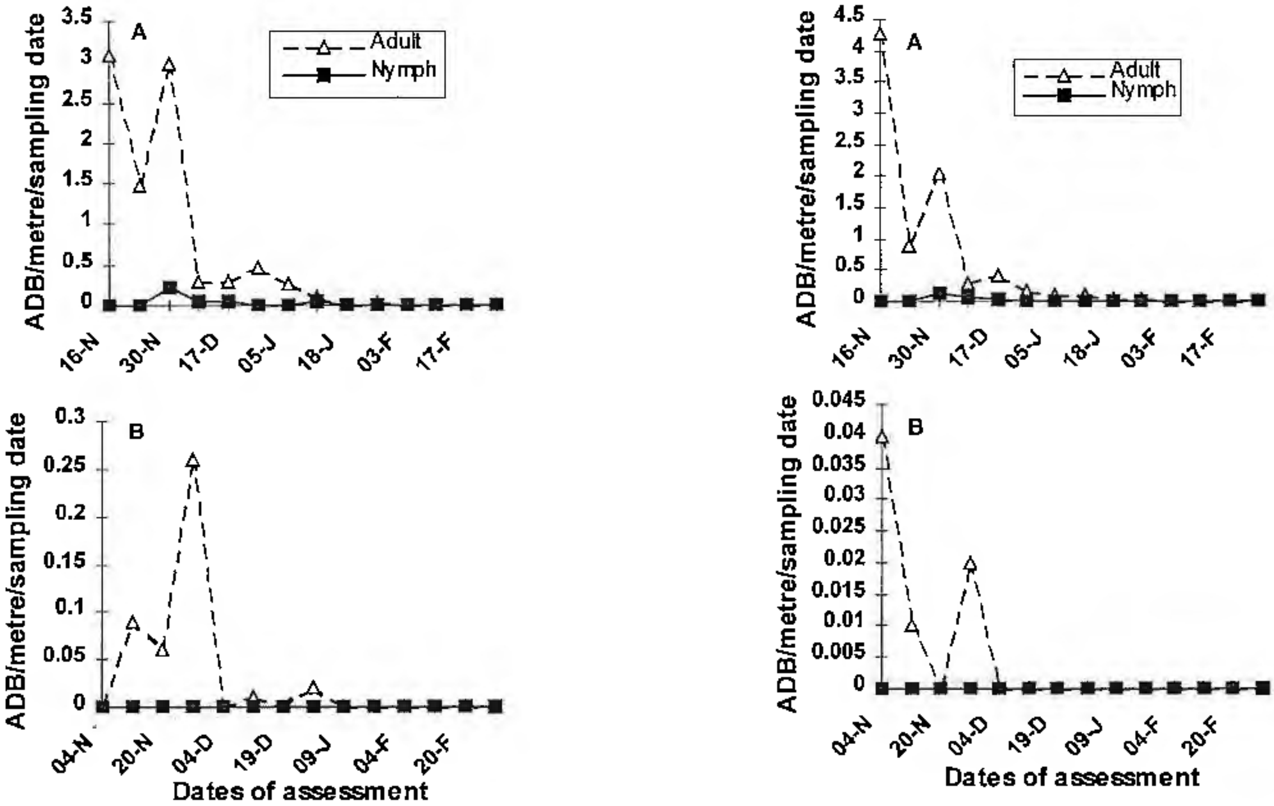


Figure 19. Seasonal abundance of ADB population in Ingard® and conventional cotton crops at Yarral, Narrabri during 1998-99 (A) and 1997-98 (B) seasons.

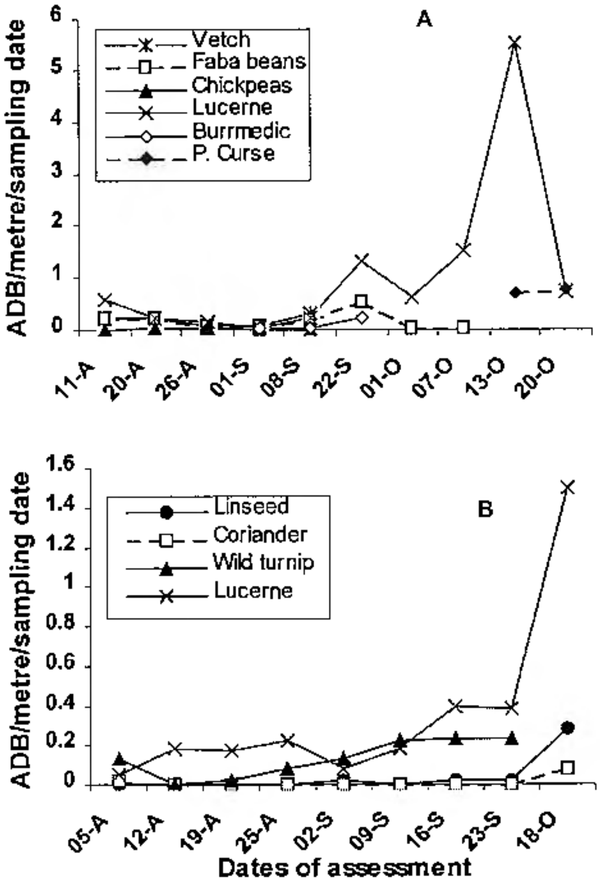


Figure 20. Population changes of ADB on alternative hosts at ACRI (A) and Norwood (B) during spring 1999.