Confidential

DAQ 81C
Field trials of transgenic cotton (INGARD) in central Queensland

Final report
November 1999

Dr. Richard Sequeira, Entomologist
Queensland Department of Primary Industries
Emerald, Queensland.

Contents

0.0 Introduction

0.1 Issues and objectives

Transgenic cotton (INGARD®) expressing the Cry1A(c) δ-endotoxin gene from Bacillus thuringiensis subsp. Kurstaki, with efficacy against Helicoverpa species was released for limited commercial production for the first time in parts of Australia at the beginning of the 1996-97 season. The limited commercial release was confined to New South Wales and southern parts of Queensland.

The decision by the National Registration Authority (NRA) to exclude central Queensland (CQ) from the commercial release area was based on two main reasons. The first reason was the concern expressed by the Genetic Manipulation Advisory Committee (GMAC) that the likelihood of the gene escaping into native Gossypium species was higher in CQ than in the release areas because of the greater abundance of native Gossypium species in northerly latitudes.

The second reason for excluding CQ from the limited INGARD registration was the concern that the national INGARD resistance management strategy for Helicoverpa spp. (heliothis[1] hereafter) would be ineffective under CQ conditions. The strategy is based on the concept of gene dilution through the production of susceptible moths using 'refuge' crops, and post-harvest tillage of the soil to destroy diapausing pupal stages.

Under CQ conditions, destruction of diapausing pupae is thought to be ineffective as a means of eliminating end-of-season resistant individuals from the population for two reasons. First, the incidence of diapause in heliothis is thought to be low in any given year, generally somewhat less than 50% of the total population. Second, simulation modelling and somewhat limited field data reveal that cotton is picked in CQ well before the onset of diapause. Thus, INGARD-resistant moths would be likely to migrate out of cotton, searching for alternate hosts, before post-harvest tillage operations to destroy pupae under cotton could be undertaken.

In response to requests by local grower organisations in CQ, the Cotton Research & Development Corporation (CRDC) commissioned research project DAQ81C to address GMAC and NRA concerns regarding the effectiveness of the national INGARD resistance management strategy in CQ. The challenges facing the CQ cotton industry for securing INGARD registration were manifold. First, the field parameters of the product had to be quantified for the local environment. The second and more difficult challenge was to develop a novel mechanism for end-of-season resistance management specific to CQ. Before such a mechanism could be developed, the ecology and population dynamics of heliothis spp. as related to INGARD cotton needed to be studied and quantified.

The trials were designed to address the following main issues:

1. The effectiveness of INGARD cotton, i.e., changes in insecticidal efficacy over the season.

[1] For traditional reasons, the more familiar generic name *Heliothis* is retained here in preference over the correct name *Helicoverpa*.

2. Identification of suitable 'refuge' crops (crops that produce substantial numbers of heliothis pupae or moths) that could be planted with INGARD cotton as part of strategic resistance management.

3. The 'refuge value' of conventional cotton and non-cotton crops grown commercially within the irrigation area in terms of pupae or moths per hectare.

4. The extent of diapause in populations of heliothis across CQ.

In addition to addressing the above four issues, the research protocols and plan of work were designed to provide some information on the distribution of native Gossypium species in the region and the impact of INGARD cotton on local invertebrate fauna relative to conventional cotton.

A key objective of the trials was also to establish a framework of research activities that would facilitate the identification of novel resistance management options. The hope was that such options could be developed into a resistance management strategy specific to CQ.

The research protocols and plan of work for the trials are detailed in Appendix 1.

1.0. 1996-97: Trials of INGARD in CQ

1.1. Block layouts and descriptions

The 5 INGARD blocks in the Emerald Irrigation Area (E1 - E5; see Appendix 1) were planted between the first and last weeks of October, giving a 4-week planting window. Of the 4 blocks in the Dawson/Callide valley (DC1-DC4), DC4 was planted in mid-September whereas the others were planted in the second and third weeks of October. Block descriptions (plot sizes and treatments) are provided in Appendix 2. Because of discordant planting dates, a common time scale (days after 20 September) will be used hereafter in this section in conjunction with calendar dates for all descriptive purposes.

1.2. Seasonal above-ground dynamics of heliothis - Emerald Irrigation Area

Dynamics of egg pressure

Fig. 1.2.1 shows the dynamics of heliothis eggs (white + brown) on INGARD treatments within blocks E1-E5, from the commencement of crop checking to defoliation. The corresponding data for conventional cotton (= treatment CONVENTIONAL) are shown in Fig. 1.2.2. In both sets of corresponding treatments, egg pressure varied considerably between plots and over the season. Blocks E1, E4 and E5 sustained higher egg pressure throughout the season than E2 and E3. A two-way analysis of variance (ANOVA) on total eggs/metre/check using 'block' and 'treatment' as factors indicated significant differences in egg pressure between blocks ($\alpha = 0.05$, $P < 0.001$) but not between treatments, i.e., INGARD and CONVENTIONAL ($\alpha = 0.05$, $P > 0.10$). In both treatments, seasonal egg pressure was higher on blocks E4 and E5 compared to the others.

The dynamics of egg pressure appear to be synchronised right across the EIA. The synchrony in egg-laying is discernible in both treatments, but more clearly so in Fig. 1.2.2 (CONVENTIONAL) than in Fig. 1.2.1 (INGARD). In all five graphs in Fig. 1.2.2, egg pressure begins to increase rapidly beginning at day 50. The density peak is followed by two more peaks, before and after day 75, and a third, well defined peak, before day 100. The pattern of synchrony in egg-laying in INGARD treatments is less clear probably because of greater distortion resulting from the increased action of beneficial insects relative to that in CONVENTIONAL treatments. INGARD could be expected to harbour greater numbers of beneficial insects because of significantly reduced insecticide usage, thereby having a greater impact on heliothis egg and larval populations (see section 1.5 below).

Dynamics of larval survival and chemical control

Larval survivors on INGARD plants were observed in all plots by mid-December. Figs. 1.2.3 and 1.2.4 show the seasonal dynamics of neonates (very small) and higher instars (small + medium + large larvae) for INGARD and CONVENTIONAL, respectively. In blocks E1-E3, INGARD provided effective control for approximately one half of the crop duration. On blocks E4 and E5, survival of neonates through to the second and later instars was observed somewhat earlier than on the other blocks.

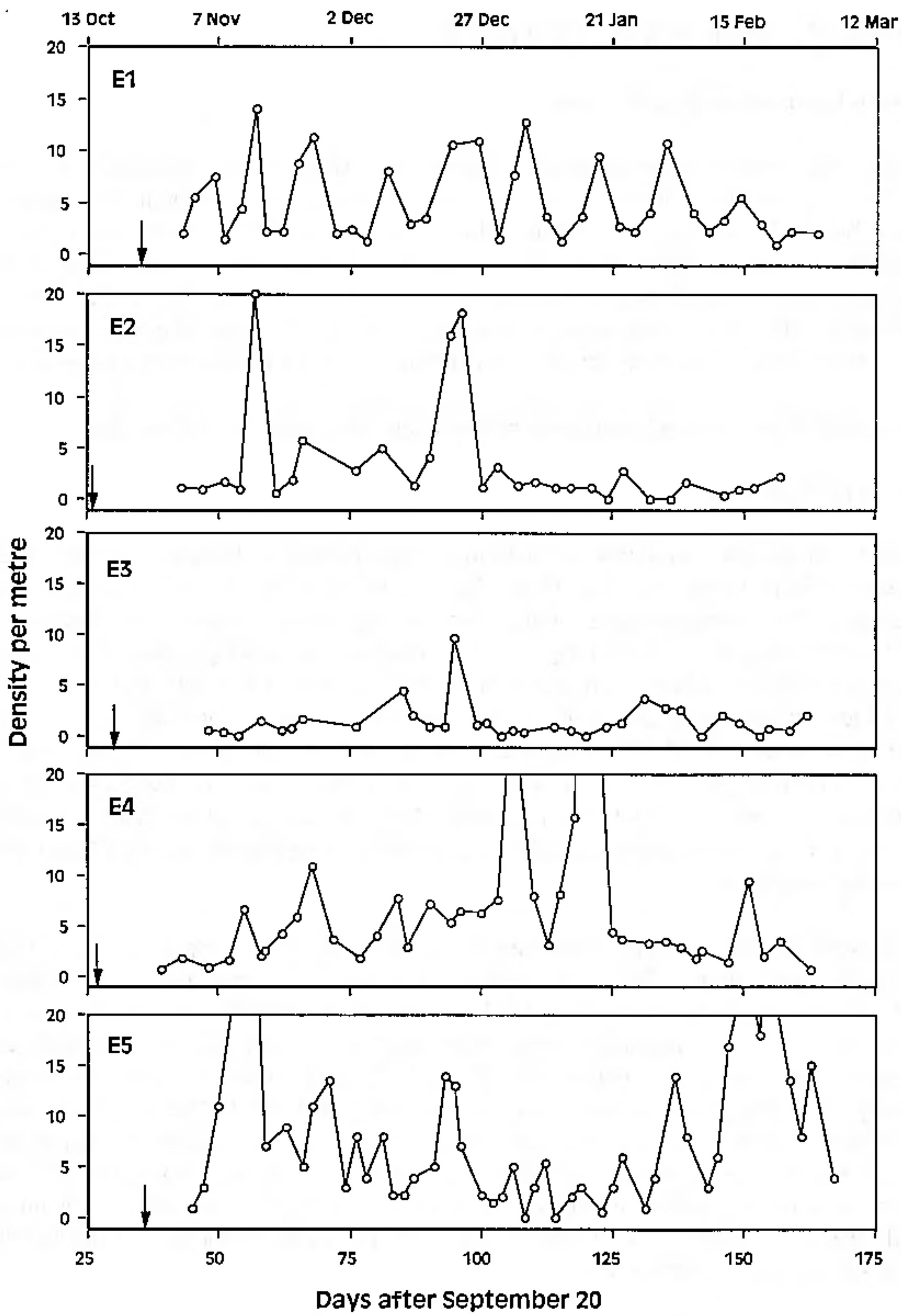


Fig. 1.2.1. Total number of eggs (o) per metre on INGARD in blocks E1-E5 over the 1996-97 season. Each point represents a check. Arrows indicate planting date.

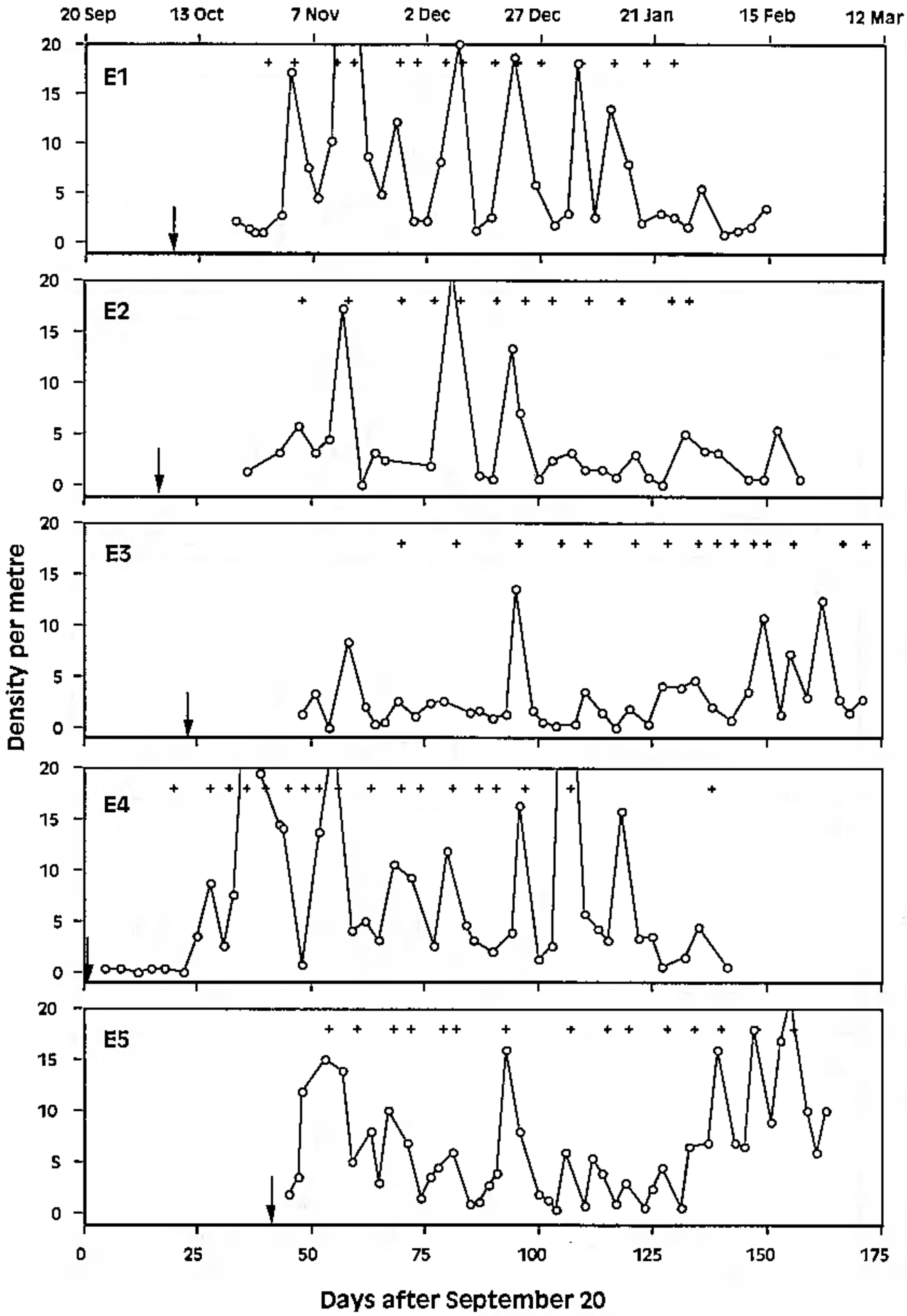


Fig. 1.2.2. Total number of eggs (o) per metre on CONVENTIONAL cotton in blocks E1-E5 over the 1996-97 season. Each point represents a check. Arrows indicate planting date. + indicates insecticide application.

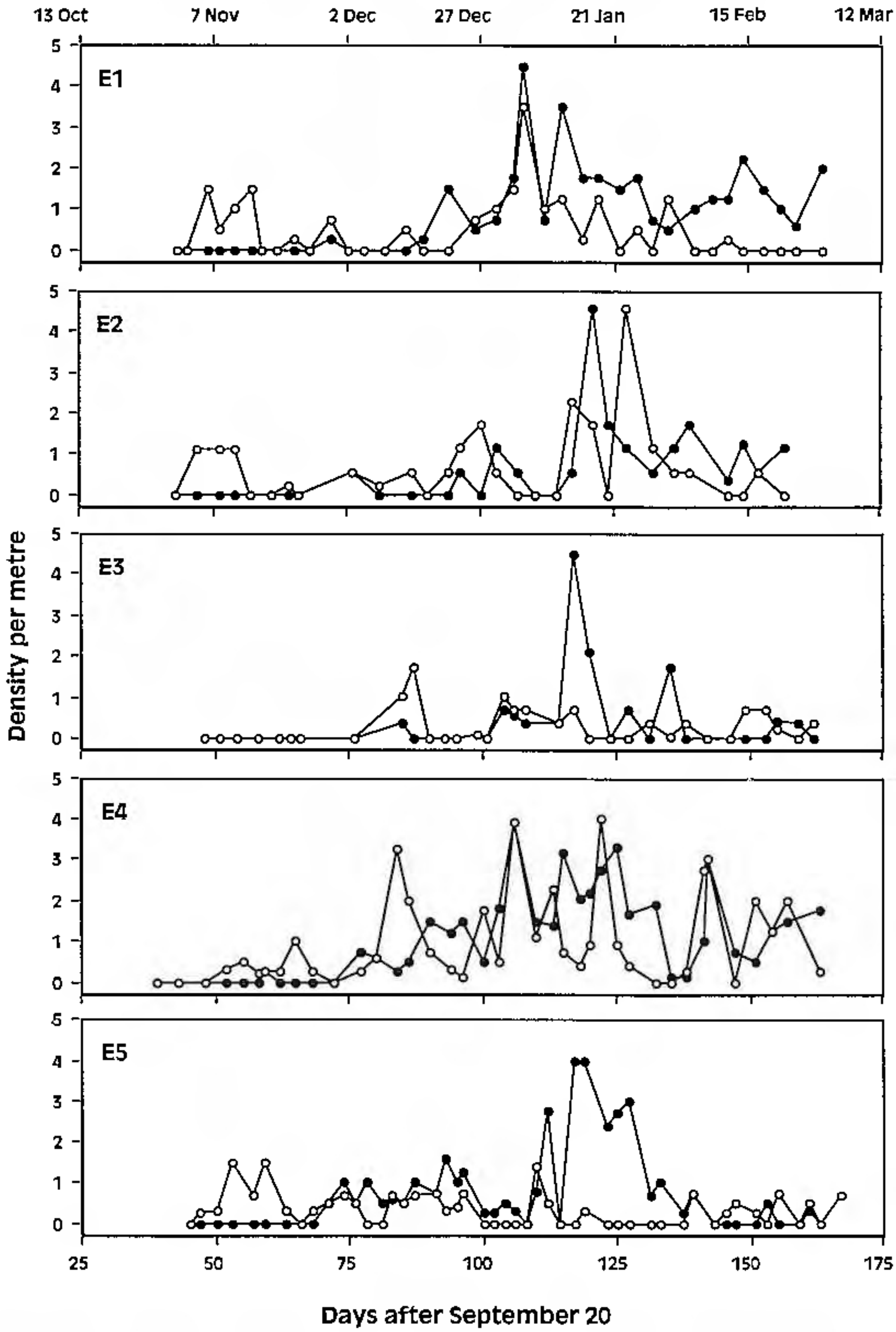


Fig. 1.2.3. Total number of neonate (o) and second- and higher-instar larvae (•) per metre on INGARD cotton in blocks E1-E5 over the 1996-97 season. Each point represents a check.

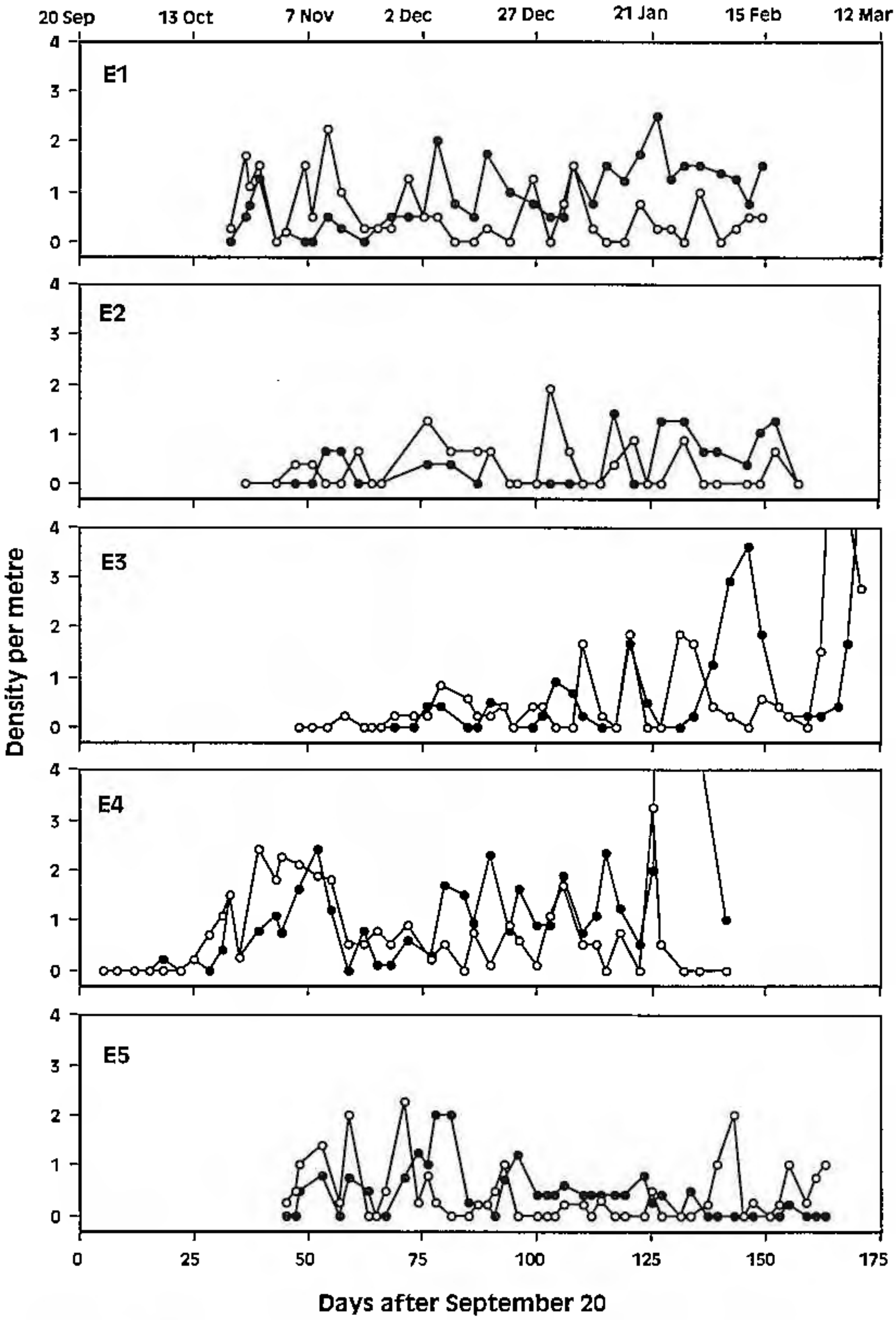


Fig. 1.2.4. Total number of neonate (o) and second- and higher-instar larvae (•) per metre on CONVENTIONAL cotton in blocks E1-E5 over the 1996-97 season. Each point represents a check.

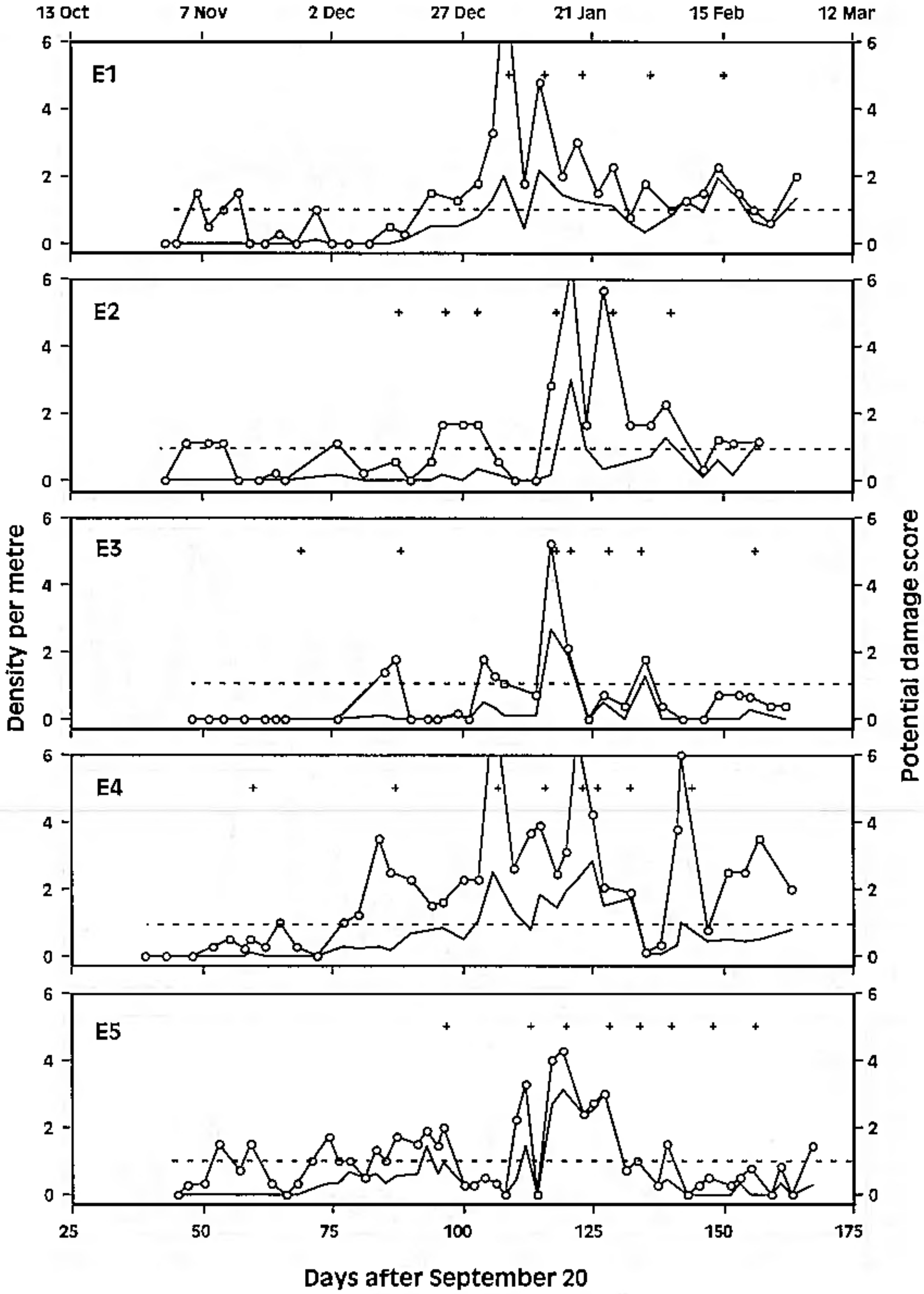


Fig. 1.2.5. Potential damage score (solid line) in relation to total number of larvae (all stages, o) on INGARD treatments on blocks E1-E5 over the 1996-97 season. The dashed horizontal line represents the TIMS recommended threshold for treatment (see text for explanation). Each point represents a check. + indicates insecticide application.

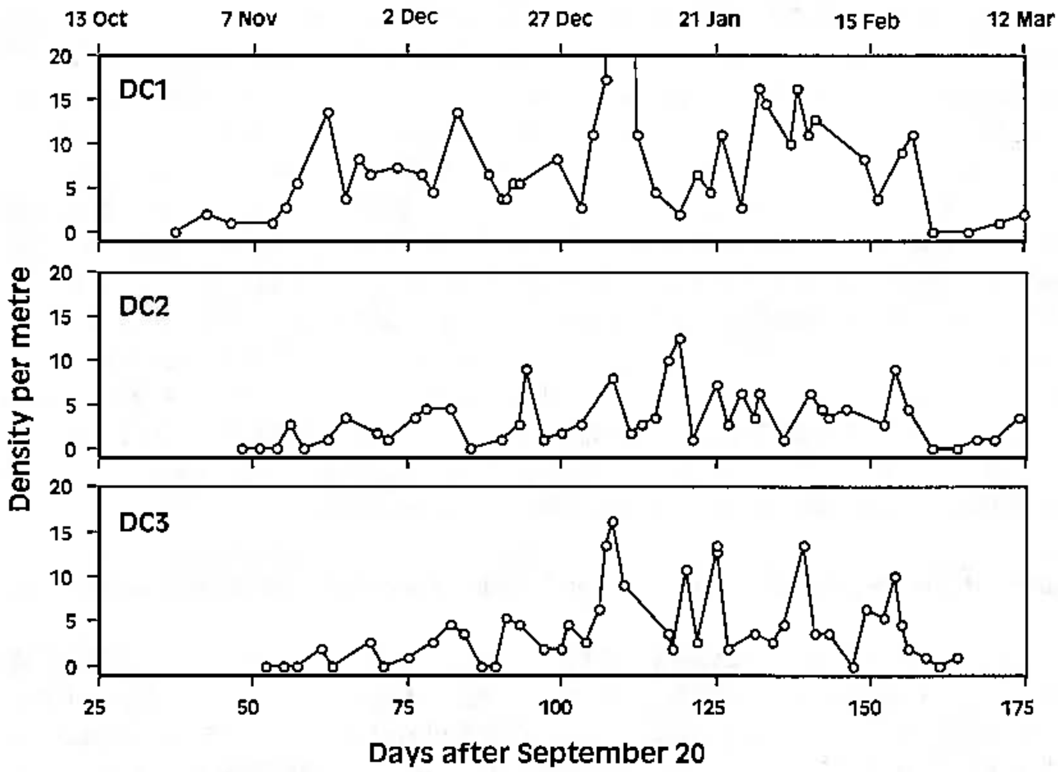


Fig. 1.3.1. Total number of eggs (o) per metre on INGARD cotton in blocks DCI-DC3 over the 1996-97 season. Each point represents a check.

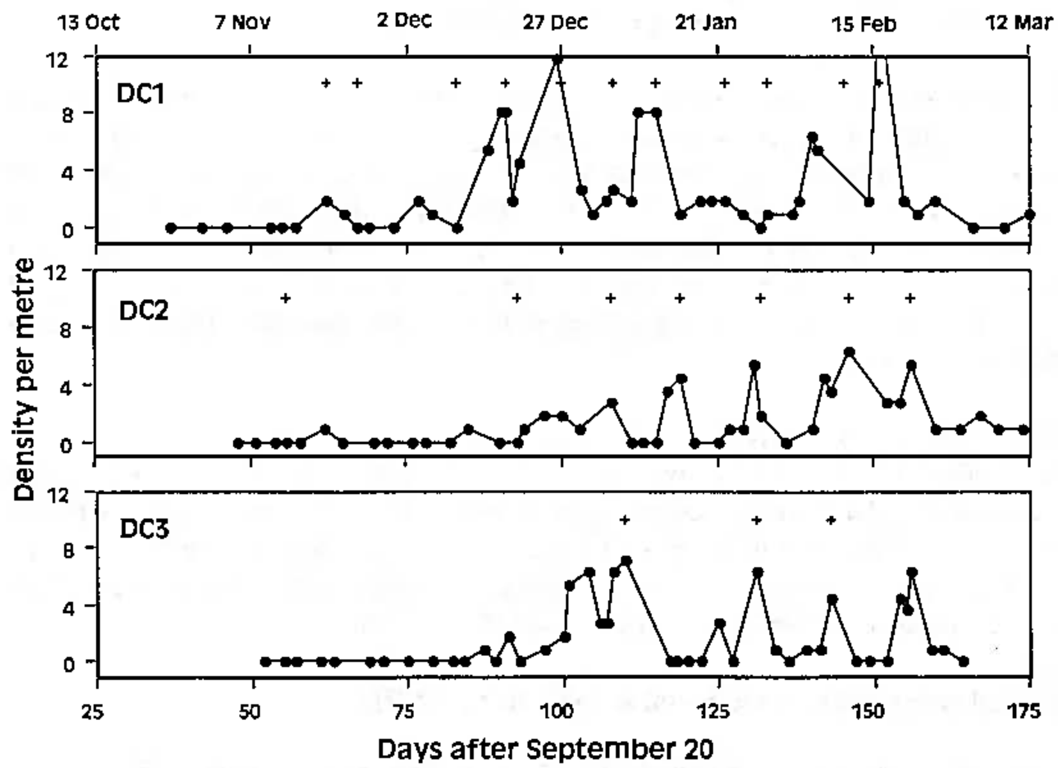


Fig. 1.3.2. Total number of larvae (all stages; •) per metre on INGARD cotton in blocks DC1-DC3 over the 1996-97 season. Each point represents a check.

Fig. 1.2.5 shows the seasonal dynamics of total larvae on INGARD in relation to the 'potential damage score.' The latter was computed as the sum of numerical values assigned to bug checking data as follows: 0 to non-feeding first-instar (very small), 0.33 to feeding first-instar and second-instar (small), 1 to medium and 1 to large larvae per metre of row. In accordance with the spray threshold recommended by the Transgenic and Insect Management Committee (TIMS) for INGARD, a potential damage score of 1 on two consecutive checks indicates that the larval population is above the threshold and spray action is warranted. The number and timing of all spray applications to INGARD are shown in Fig. 1.2.5. INGARD plots were sprayed between five (E1) and eight (E4, E5) times. Control of early-season mirid infestations was essential and sprays were targeted as follows: E1 - no sprays, E2 - 2 of 6 sprays, E3 - 2 of 7 sprays, E4 - 1 of 8 sprays, E5 - no sprays. CONVENTIONAL received significantly more insecticidal treatments than INGARD. The total number of sprays on CONVENTIONAL ranged from 12 (E2) to 18 (E4). In general, insecticidal treatment for control of heliothis on INGARD was not required for roughly the first half of the season.

1.3 Seasonal above-ground dynamics of heliothis - Dawson/Callide Irrigation Area

Figs. 1.3.1 and 1.3.2 show the dynamics of eggs (white + brown) and larvae (total of all stages), respectively, on INGARD blocks DC1 - DC3. Data for DC4 were not available. Variation in egg pressure was similar to that observed in EIA blocks. Egg pressure on INGARD plots in DC2 and DC3 was within the range of variation observed in the Emerald blocks. However, DC1 sustained the highest egg pressure in comparison to all other INGARD plots in the CQ trials, with an average of 9.15 eggs/metre/check over the season. The pattern of egg-laying on CONVENTIONAL was similar to that on INGARD. DC1 sustained higher egg pressure than DC2 and DC3.

Visual comparison of the dynamics of egg pressure between CONVENTIONAL blocks (Fig. 1.3.3) reveals some periods of synchronous egg laying. For example, a peak in egg density is evident after 2 December (DC1 and DC3), before 27 December (DC1 and DC2), early January (DC1 and DC3), end of January and 15 February. A dramatic increase in egg pressure in late December (see Fig. 1.3.1 - DC1) is clearly associated with large numbers of moths produced after mid-December by a field of unsprayed sunflower adjacent to and planted at the same time as the INGARD block. (see also section 8 on pupae data below).

Larval survivors on INGARD blocks DC2 and DC3 were observed by mid-December (Fig. 1.3.2). On DC1, larval survivors were observed somewhat earlier, toward the end of November. INGARD plots were sprayed between 3 (DC3) and 11 (DC1) times. Control of early-season mirid infestations were required as follows: DC1 - 2 of 11 sprays, DC2 - 1 of 7 sprays, DC3 - no sprays. By comparison, CONVENTIONAL treatments received 11-12 sprays over the season (Fig. 1.3.3).

1.4 Seasonal variation in insecticidal activity of INGARD

Leaf bioassays to assess the insecticidal activity of INGARD over the season were conducted at regular intervals, beginning approximately 4 weeks after planting (53 days on the standardised scale). Leaves for the bioassays were collected from E2 and DRYLAND (see Appendix 2). H. armigera larvae used in the bioassays were supplied

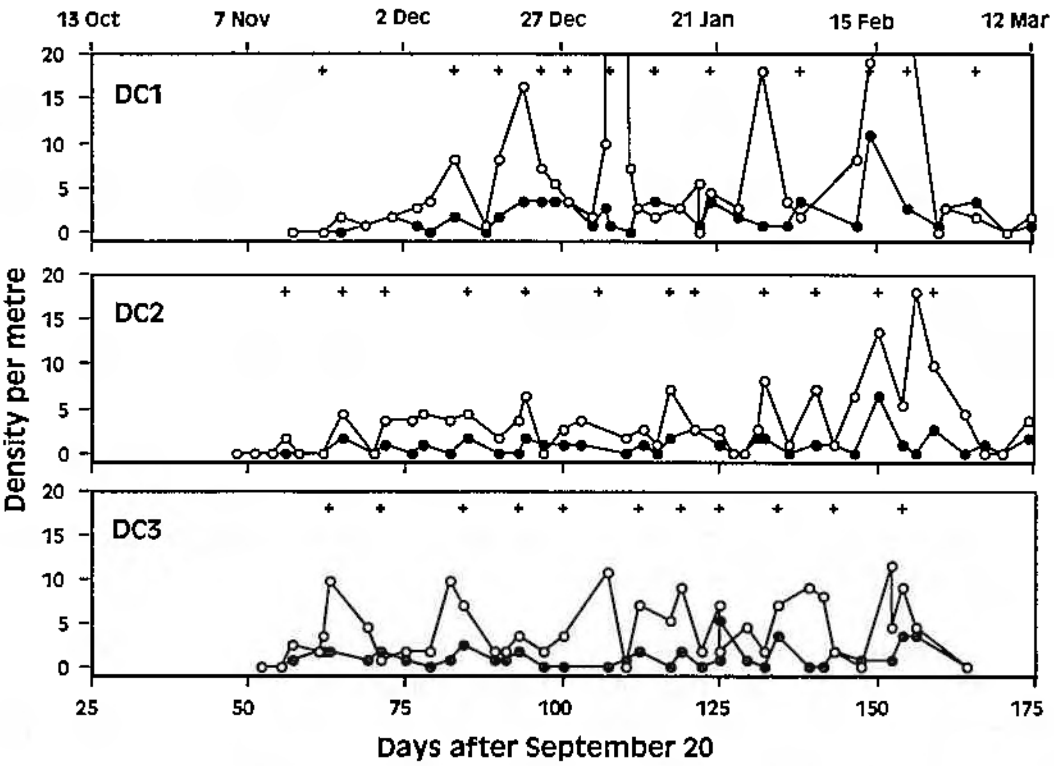


Fig. 1.3.3. Total number of eggs (white + brown; o) and larvae (all stages; •) per metre on CONVENTIONAL cotton in blocks DC1-DC3 over the 1996-97 season. Each point represents a check. + indicates insecticide application.

by Dr. David Murray of QDPI in Toowoomba. Fresh eggs were sent to Emerald by overnight express as and when needed, and neonate larvae were used in the bioassay soon after hatching.

The results of the bioassays are presented in Table 1.4.1. Survivorship in the control group declined rapidly after the first run. This decline is most likely the result of H. armigera cultures being infected with the stunt virus. Entomology staff in Dr. Murray's laboratory have identified the stunt virus as the cause of significant mortality in their cultures.

Table 1.4.1. Survivorship of test heliothis larvae after 5 days on INGARD and unsprayed conventional (= CONTROL) leaves.

		% survival (raw data)			% survival (adjusted)	
Date	Days	CS50i	CS50i (dry)	control	CS50i	CS50i (dry)
10/11/9	53	2	-	93	2	-
25/11/96	68	25	0	62	40	0
15/12/96	88	28	16	55	51	29
12/1/97	116	70	0	**90**	77	0
26/1/97	130	-	-	-	-	-
14/2/97	149	79.5	-	90	88.3	-
9/3/97	172	93		72	100	-

Date = date of bioassay setup; Days = days after 20 September; CS50i = INGARD.

After adjustment of the data to account for mortality in the CONTROL group, an increase in survivorship on INGARD leaves from 2 to 25% is evident by day 68 (Table 1.4.1). After remaining stable for approximately 5-6 weeks, insecticidal activity of INGARD dropped dramatically by day 116, permitting 70% of neonate larvae assayed to reach the third instar stage. The last scheduled bioassay done just prior to defoliation (day 149, Table 1.4.1) indicated very low insecticidal activity with 80% survival of neonates. The dramatic drop in insecticidal activity under laboratory conditions by day 116 coincides with a steep increase in the number of second- and larger instar larvae observed in the field at approximately the same time (see Fig. 1.2.3, block E2). This clearly indicates that the laboratory leaf bioassay results are a good reflection of INGARD effectiveness in a field situation.

An additional leaf bioassay was conducted on re-growth of defoliated INGARD following a significant rainfall event. The result of this bioassay (shaded row, bottom of Table 1.4.1) indicates virtually no residual insecticidal activity.

Leaf bioassays on dryland INGARD cotton could not be conducted beyond the middle of January 1997 because of drought-related stress on the plants. The results of the three bioassays that were conducted (Table 1.4.1) indicate significantly greater insecticidal activity relative to irrigated INGARD.

Larvae surviving on INGARD leaves appeared to grow at rates noticeably lower than those of their counterparts developing on CONTROL leaves. However, changes in size of larvae on the different treatments were not specifically estimated. The insecticidal activity of INGARD against heliothis, although marginal, appears to have been sufficient to provide effective early season control under field situations. Mortality of heliothis caused by beneficial insects is likely to have contributed significantly to the overall mortality, supplementing that caused by INGARD insecticidal action. Parasitised medium to large larvae were commonly observed over the course of the season (see also section 1.7, data on pupal parasitism). Presumably, even higher mortality of very small and small larvae could be attributed to the action of predators and parasitoids.

1.5 Relative impact of INGARD on local invertebrate fauna

Samples of the invertebrate fauna in INGARD, CONVENTIONAL and CONTROL treatments were collected at regular intervals throughout the season by suction sampling. Five 20-m lengths of row were sampled in each cotton treatment on blocks E1 and E2. The collections were preserved in 70% ethanol and sent to the Australian Cotton Research Institute (ACRI), Narrabri, NSW, for identification by Dr. Lewis Wilson. The invertebrate fauna in each suction sample were identified down to Family.

Fig. 1.5.1 shows the aggregate data (means) at the level of Order. The two blocks differed greatly in the level of overall invertebrate abundance but not in the relative composition of invertebrates (level of representation of each Order) within blocks. INGARD generally harboured higher mean densities of invertebrates than the corresponding CONVENTIONAL treatments. Dipterans (flies), hemipterans (bugs), hymenopterans (wasps, etc.) and lepidopterans (moths, primarily heliothis eggs and larvae) were more abundant than representatives of other Orders.

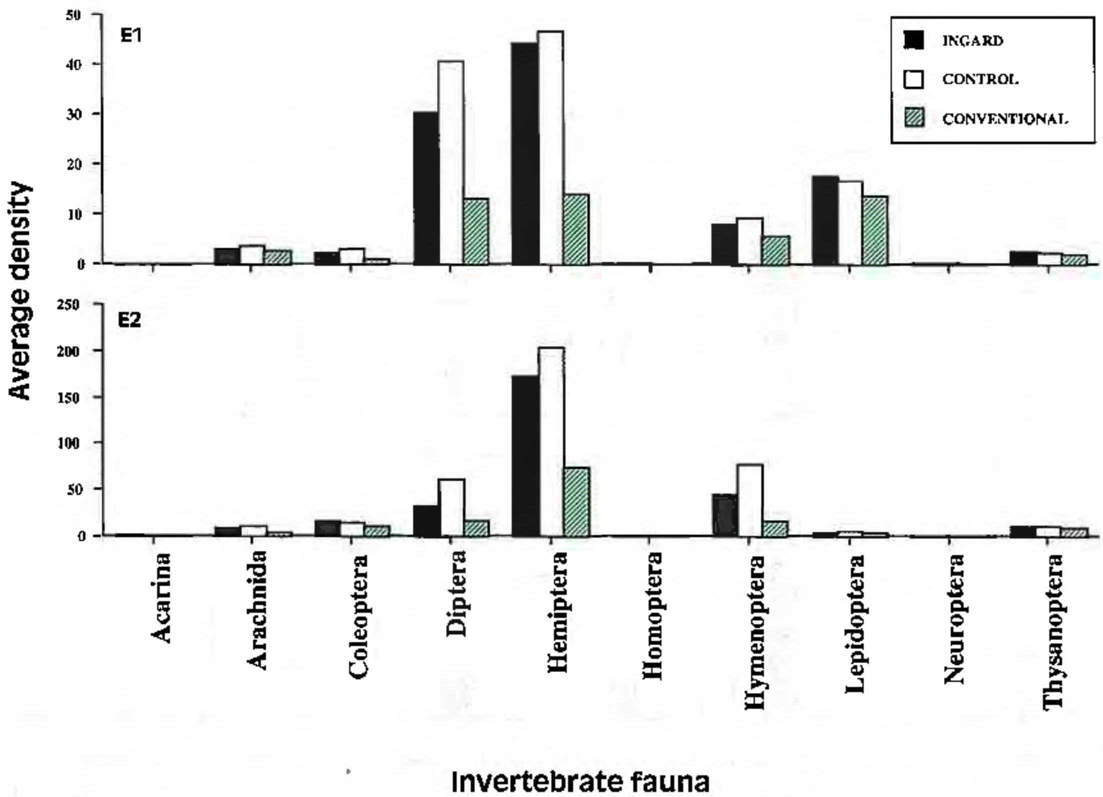


Fig. 1.5.1. Mean number of individuals belonging to different invertebrate Orders found in fortnightly suction samples on cotton treatments in blocks E1 and E2 from November to February.

1.6 Seasonal dynamics of fruit retention and plant stand characteristics

Estimates of fruit retention (percentage) in the first position over all fruiting branches (whole plant = WP) and on the top five branches (T5) for INGARD and CONVENTIONAL are shown in Figs. 1.6.1 and 1.6.2, respectively. The estimates were obtained by counting the number of first-position fruit per plant in three samples of 10 plants each.

Retention varied considerably over the season, between treatments and between blocks. Retention figures for INGARD on blocks E1 and E2 were estimated on the completely unsprayed strips within the INGARD plots (see Appendix 2). WP retention on INGARD in blocks E1 and E2 dropped below 60% nearing the end of the season. Block E1 suffered somewhat less early season damage by mirids than E2. The latter suffered severe fruit loss as a result of mirid infestation, requiring two spray applications prior to day 100. WP retention on both INGARD and CONVENTIONAL was considerably below 60% in block E4 as a result of sustained high heliothis pressure throughout the season.

A two-way ANOVA on arcsine-transformed data (α = 0.05) using 'block' and 'treatment' as factors indicates no significant difference between mean WP retention on INGARD and CONVENTIONAL, but significant differences between blocks. T5 retention is much less variable than its counterpart. A drop in T5 retention is evident only on the last check (Day 148, Fig. 1.6.2), when the plants were at cutout. A two-way ANOVA (as above) indicated no significant differences between blocks or treatments.

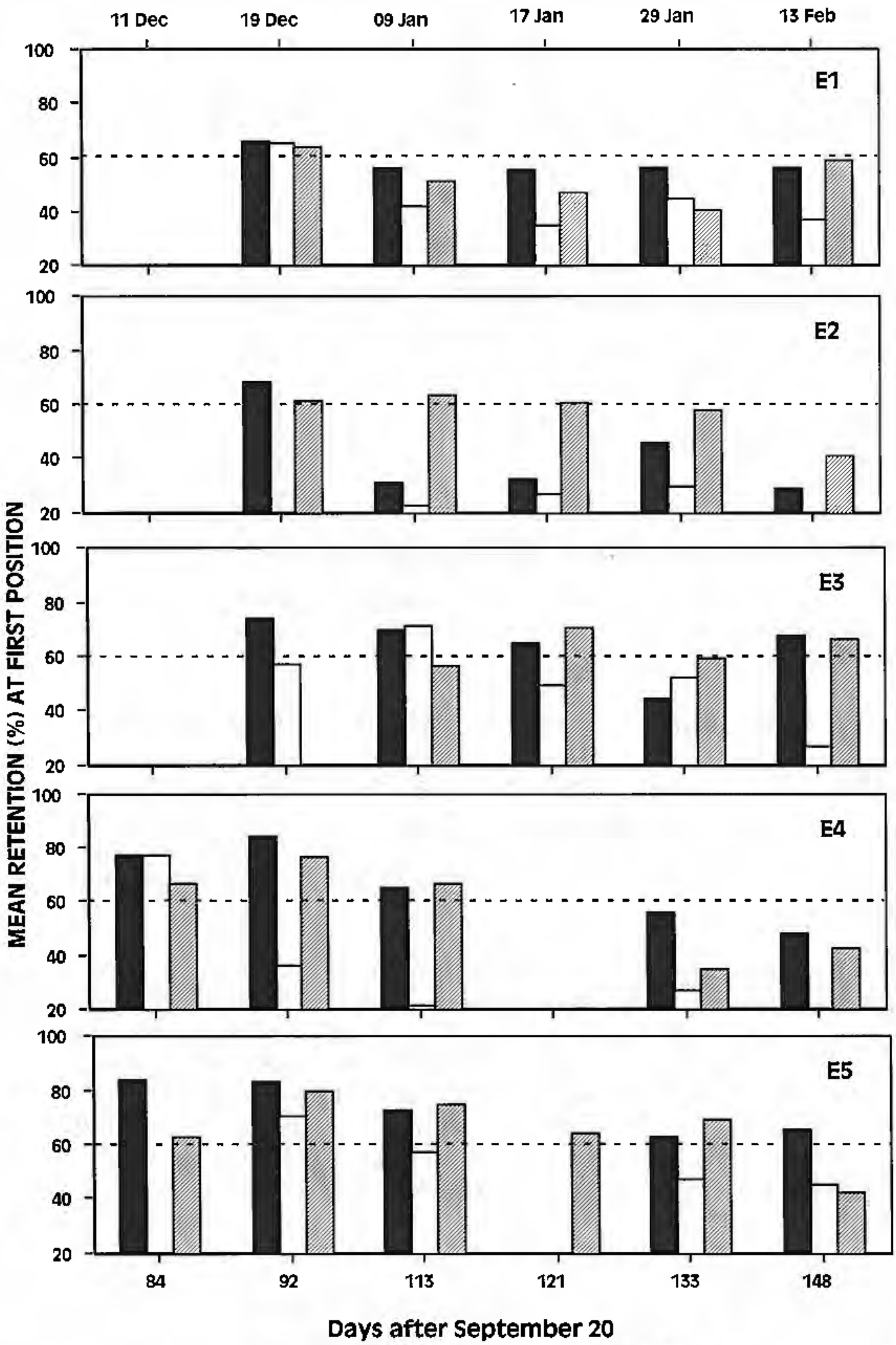


Fig. 1.6.1. Mean fruit retention in the first position on all fruiting branches on INGARD (solid bars), CONTROL (open bars) and CONVENTIONAL (shaded bars) plants on blocks E1-E5 over the 1996-97 season.

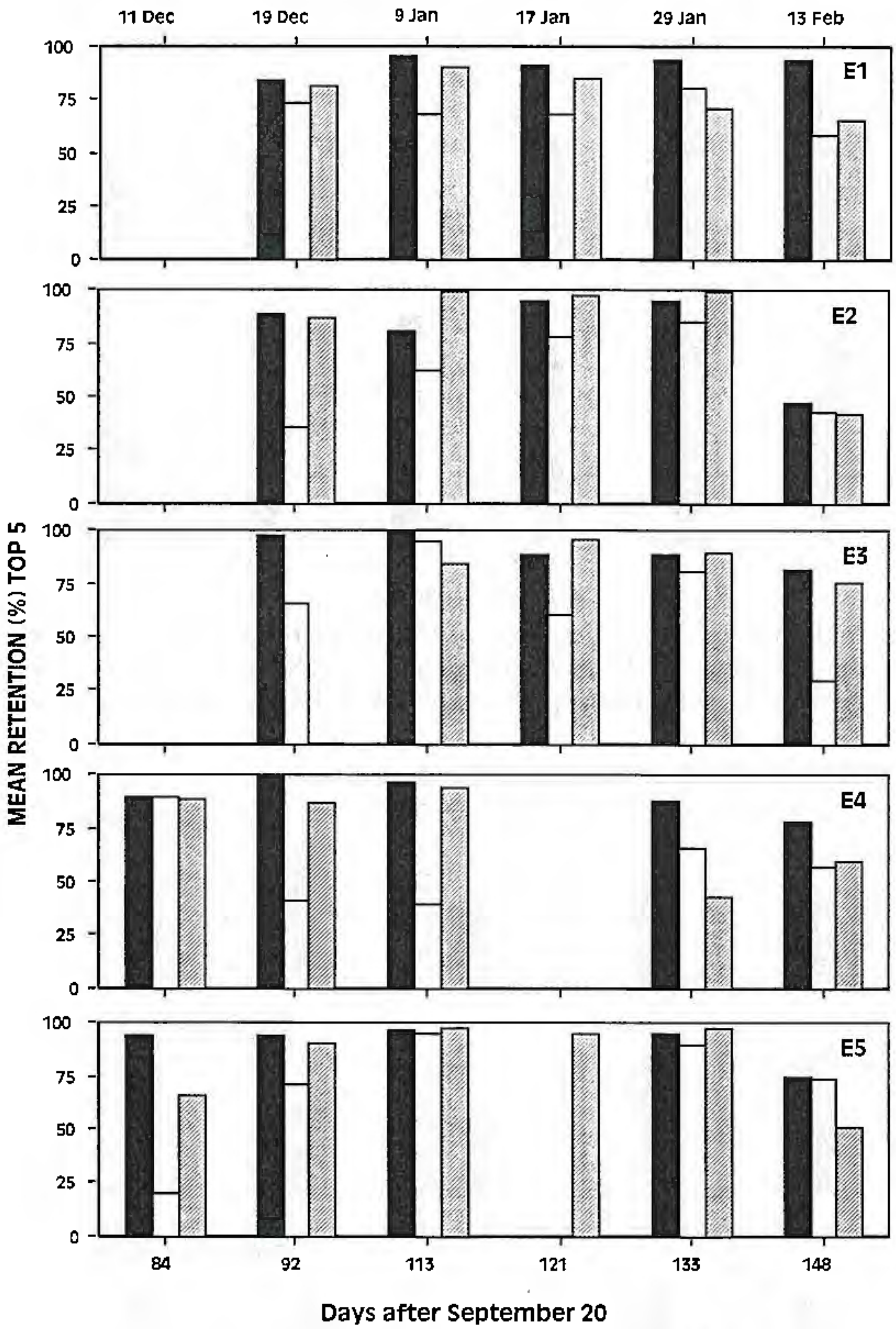


Fig. 1.6.2. Mean fruit retention in the first position on the top five fruiting branches on INGARD (solid bars), CONTROL (open bars) and CONVENTIONAL (shaded bars) plants on blocks E1-E5 over the 1996-97 season.

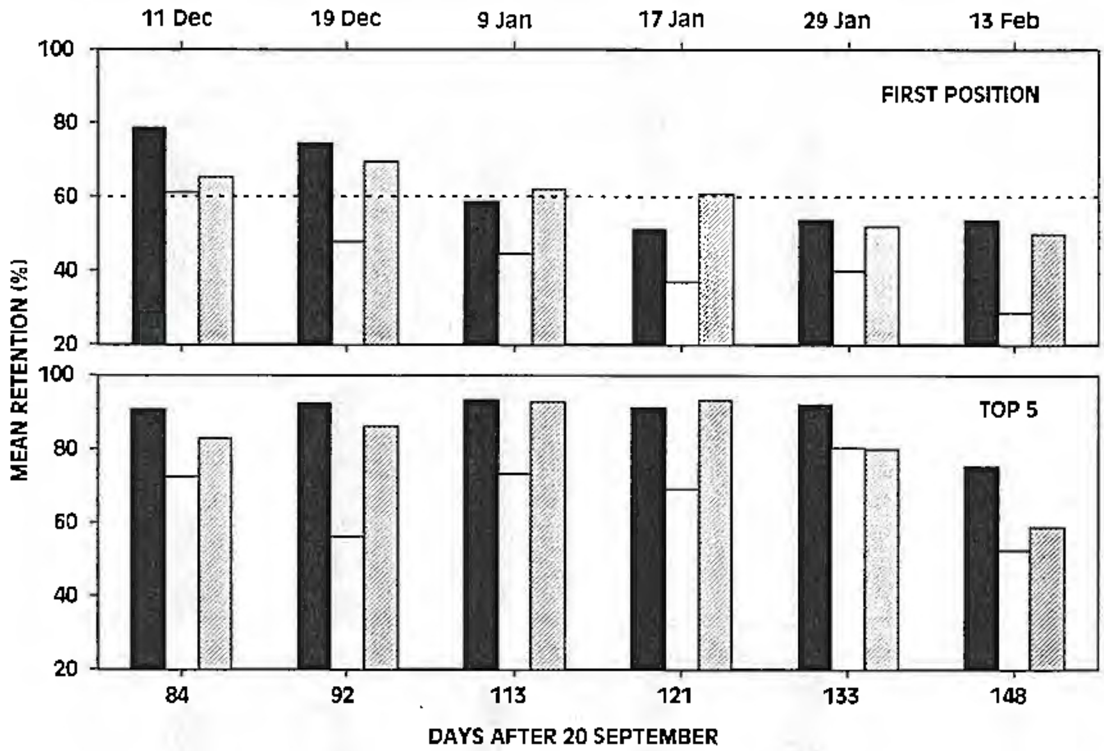


Fig. 1.6.3. Mean fruit retention in the first position on all fruiting branches and on the top five branches on INGARD (solid bars), CONTROL (open bars) and CONVENTIONAL (shaded bars) plants on blocks E1-E5 over the 1996-97 season.

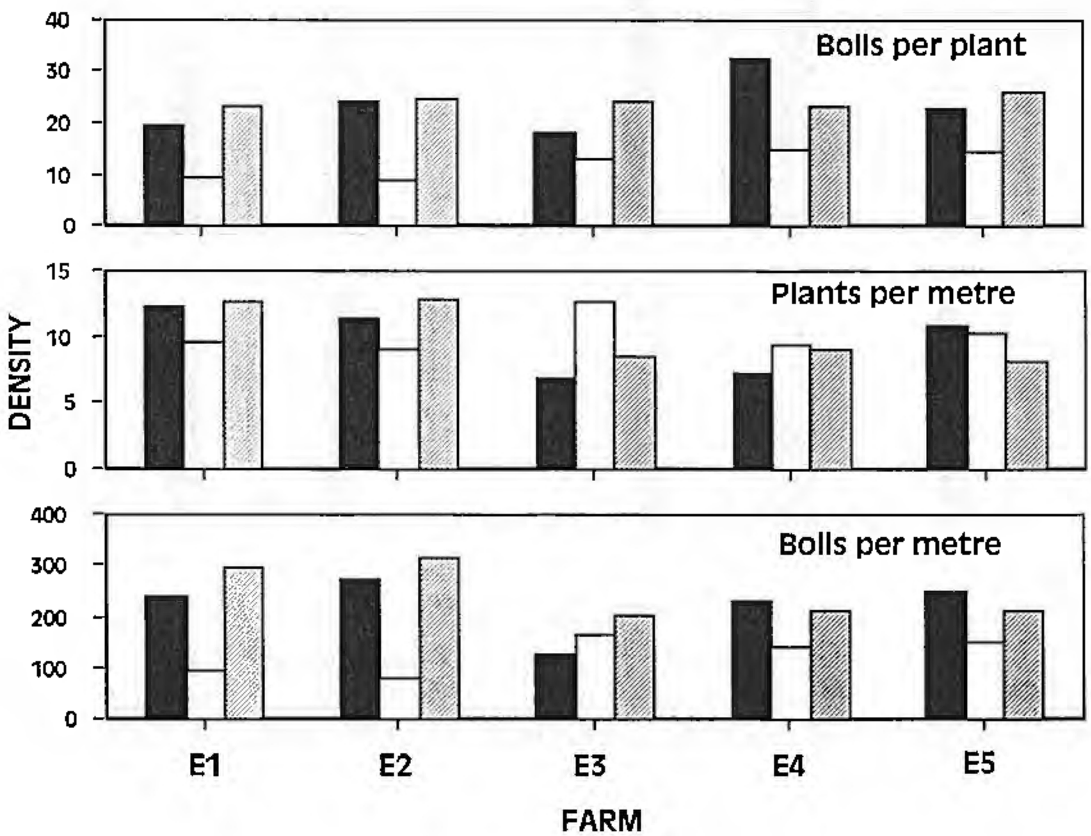


Fig. 1.6.4. Mean fruit load and plant stand on blocks E1-E5 over the 1996-97 season.

Fig. 1.6.3 shows aggregate retention estimates for all three treatments summarised across block. The CONTROL treatment recorded the lowest retention estimates as expected. T5 retention is generally uninformative whereas WP retention shows a clear declining trend over the season. This and other data (see sections 1.7 and 1.8 below) suggest that insecticidal control in the second half of the cotton season may be ineffective.

Dryland INGARD appears to be more effective in heliothis control than irrigated INGARD. Fruit retention, especially on the lower part of dryland INGARD plants, appeared to be distinctly superior to that on all irrigated INGARD blocks until the second week of January 1997 (at which point in time, extreme water stress resulted in a large-scale shedding of bolls and squares). Unfortunately, fruit retention on dryland INGARD could not be quantified before the large-scale loss of fruit after the second week of January. Dryland INGARD deserves greater attention in trials next season.

Fig. 1.6.4 shows mean estimates of bolls-per-plant at defoliation, plants-per-metre and the composite variable, bolls-per-metre. A two-way ANOVA (as above) indicates no significant difference in average bolls per plant between INGARD and CONVENTIONAL, but significant differences between blocks. The pattern of significance is the same for mean plants-per-metre. ANOVA results indicate that for CONVENTIONAL, estimates of bolls per plant are not significantly different between blocks, but E1 and E2 had more plants per metre than the other three blocks (Fig. 1.6.4).

1.7 Refuge value of cotton and other crops - Emerald Irrigation Area

Cotton and other crops on research blocks

Pupae were recovered from almost all treatments by mid-January (day 125) of the season (Fig. 1.7.1). CONVENTIONAL and INGARD treatments generally produced moths at the same time (Fig. 1.7.1); In block E1, CONVENTIONAL began producing moths well before INGARD. Assuming an area of 20 hectares of CONVENTIONAL on each of the five blocks, an estimated 3.5 million moths were produced prior to picking over all treatments and blocks over the season. INGARD blocks collectively produced 2.8 million moths over the season whereas a comparable area of CONVENTIONAL treatments in the same blocks produced approximately 1.3 million moths.

Table 1.7.1 provides a summary of the refuge value of cotton and non-cotton crops on the five research blocks (see Appendix 2 for details) in terms of heliothis pupae and moths per unit area. Of the five non-cotton refuge crops specifically evaluated, two plots of pigeon pea produced more pupae and moths per unit area over the season than the combined seasonal totals per unit area of INGARD and CONVENTIONAL (Table 1.7.1). Pupal parasitism accounted for a significant reduction in the number of moths produced (Table 1.7.1). Fig. 1.7.2 shows the relative timing and frequency of moth production by the five non-cotton refuge crops. Pupae were recovered from all five treatments by day 125, in line with the recovery of pupae from INGARD and CONVENTIONAL.

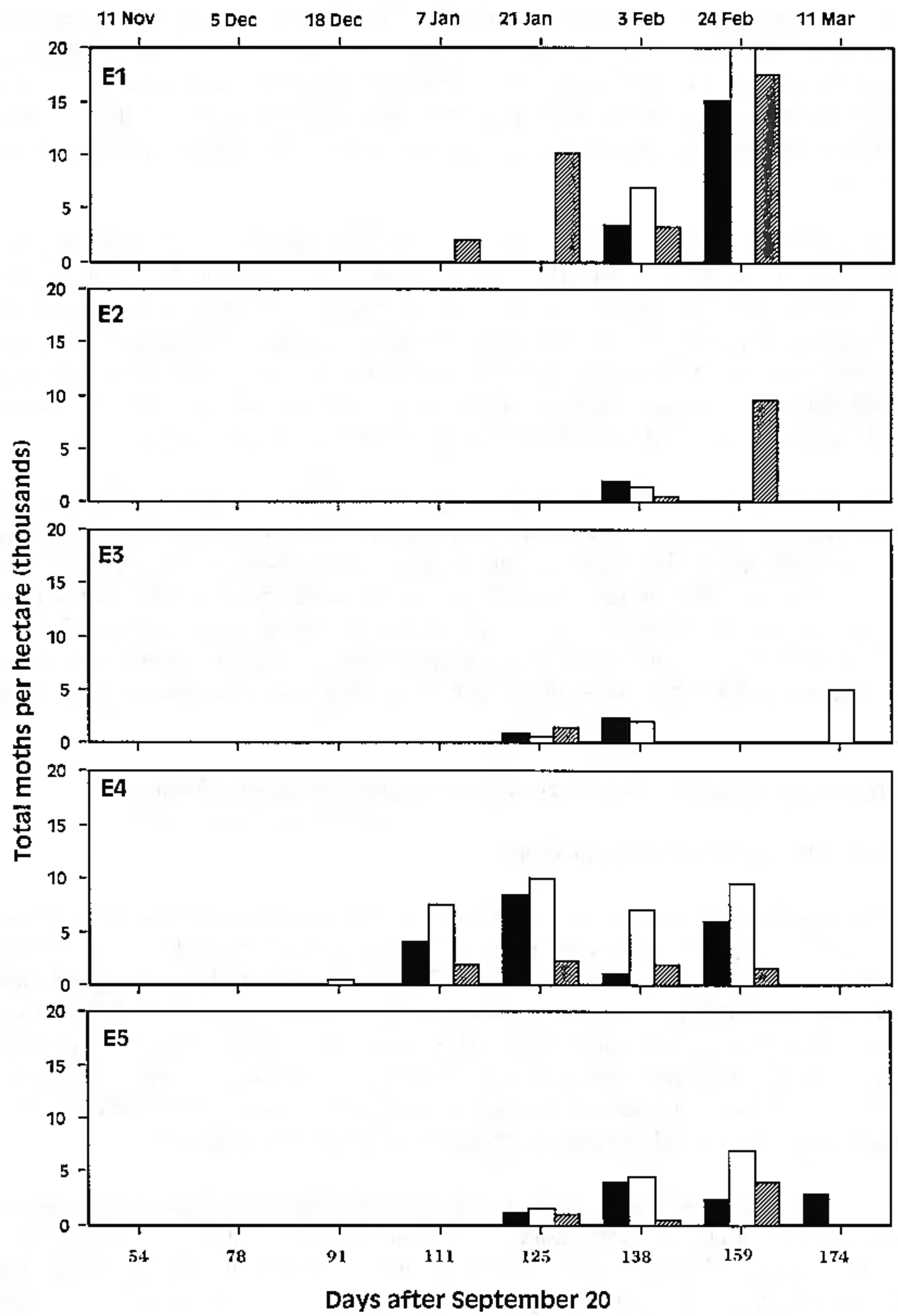


Fig. 1.7.1. Total number of moths generated under INGARD (solid bars), CONTROL (open bars) and CONVENTIONAL (shaded bars) cotton treatments, on blocks E1-E5 over the 1996-97 season.

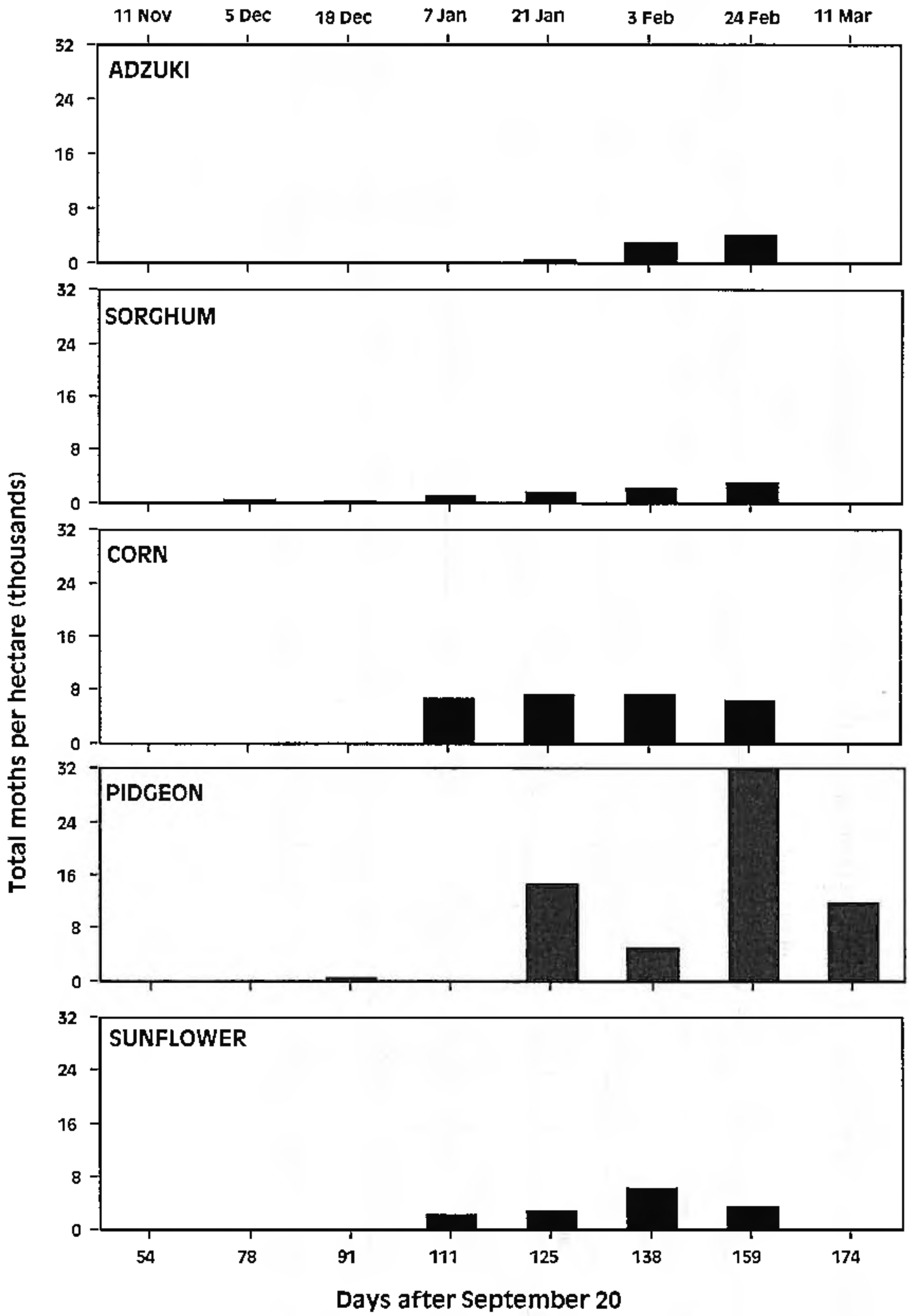


Fig. 1.7.2. Total number of moths generated under various refuge crops on blocks E1-E5 over the 1996-97 season.

Table 1.7.1. Seasonal totals of number of pupae, percentage parasitism and number of heliothis moths by farm and crop.

		Cotton crops			Other crops				
FARM	VARIABLE	Ingard	Control	Conventional	Adzuki	Sorghum	Corn	Pigeon Pea	Sunflower
E1	pupae in 20 m^2	72	104	75	45				
	% parasitism	49	33	24	63				
	moths per hectare	18360	34840	28500	8550				
E2	pupae in 20 m^2	15	21	20	10				
	% parasitism	40	83	0	20				
	moths per hectare	4500	1785	10000	4000				
E3	pupae in 20 m^2	8	20	3		55	83		
	% parasitism	20	50	0		79	34		
	moths per hectare	3200	5000	1500		5775	27390		
E4	pupae in 20 m^2	39	108	25		17	82	342	42
	% parasitism	0	37	67		17	37	67	45
	moths per hectare	19500	34020	4125		7055	25830	56430	11550
E5	pupae in 20 m^2	24	32	12				238	46
	% parasitism	20	22	50				41	25
	moths per hectare	9600	12480	3000				70210	17250

The principal agents of mortality of recovered pupae were the ichneumonid wasp Heteropelma scaposum (accounting for over 90% of all parasitism) and to a lesser extent species of tachinid fly. From the % parasitism estimates in Table 1.7.1, it is evident that natural biological control is a major regulatory component of heliothis population dynamics in CQ. It is noteworthy that significant levels of parasitism are evident in conventional cotton despite frequent insecticide applications over the season.

Table 1.7.2. Species composition of moths emerging from field collected pupae.

Date	Day	Number of moths	% H. armigera
07 Jan '97	113	19	89.5
22 Jan '97	125	124	77.4
03 Feb '97	138	174	87.4
24 Feb '97	159	61	88.5
11 Mar '97	174	78	88.5

Table 1.7.2 shows the species composition of moths that successfully emerged from pupae collected in the field. The percentage of H. armigera in the population remained remarkably stable throughout the season. Although some of the percentages in Table 1.7.2 are based on rather small sample sizes (a consequence of high rates of pupal parasitism), the results are in close agreement with results of large scale resistance testing data collected for Emerald over the season.

Refuge value of commercial cotton and other crops

Table 1.7.3 shows the number of pupae and estimated number of moths produced by crops outside the trial blocks but within the EIA. Large numbers of pupae and moths were recovered right through the season on various crops. Winter chickpea crops produced the largest number of moths early in the season whereas CONVENTIONAL produced significant numbers of moths after mid-January.

1.8 Refuge value of cotton and other crops - Dawson/Callide Irrigation Areas

A total of six pupal digs were conducted under cotton treatments in the DCIA. Because of greater spatial segregation of the four blocks relative to EIA blocks, pupal digs could not always be conducted on all four blocks at the same time. The data for the 6 digs are summarised in Fig. 1.8.1. Estimates of moths/ha were computed assuming 50% parasitism of all pupae produced. The estimates of moths produced in each treatment are likely to be conservative because not all pupae present in the sampling area are likely to be found.

Pupae were recovered from CONVENTIONAL and CONTROL (=unsprayed conventional) on DC4 as early as mid-December. This could be due to the fact that all treatments on DC4 were planted 4-5 weeks before the other three blocks. On DC1-DC3, pupae were recovered beginning early February, but could have been present by mid-January, as evidenced by the recovery of pupae under CONVENTIONAL on DC3 in early January. From Fig. 1.8.1 it is evident that CONVENTIONAL produced as many, and often more, pupae than INGARD.

Table 1.7.3. Total number of pupae, number of crops sampled (N), percentage parasitism[1] and mean number of heliothis moths[2] under various crops grown within the Emerald irrigation area (excluding INGARD trial farms) during the 1996-97 season.

		Sample date							
Crop	Variable	30 Oct	20 Nov	23 Dec	08 Jan	23 Jan	10 Feb	10 Mar	03 Apr
cotton	pupae in $20m^2$					1	133		
	N					4	4		
	% parasitised						26		
	moths per ha					125	12303		
sorghum	pupae in 20 m^2	0	97	29	19			165	
	N	1	1	2	1			3	
	% parasitised		57					80	
	moths per ha	0	21000	7250	9500			5500	
corn	pupae in 20 m^2								15
	N								1
	% parasitised								
	moths per ha								7500
sunflower	pupae in 20 m^2			36					
	N			1					
	% parasitised			32					
	moths per ha			5760					
soyabean	pupae in 20 m^2	0						29	11
	N	1						2	3
	% parasitised							41	
	moths per ha	0						3000	1833
mungbean	pupae in $20m^2$		2						
	N		1						
	% parasitised								
	moths per ha		1000						
chick pea	pupae in 20 m^2	188	170						
	N	1	2						
	% parasitised								
	moths per ha	94000	42500						
peanut	pupae in 20 m^2								0
	N								1
	% parasitised								
	moths per ha								0

[1] Empty cells represent missing or unavailable data.

[2] Where % parasitism estimates were not available, moths/ha was computed assuming 50% of pupae collected were parasitised

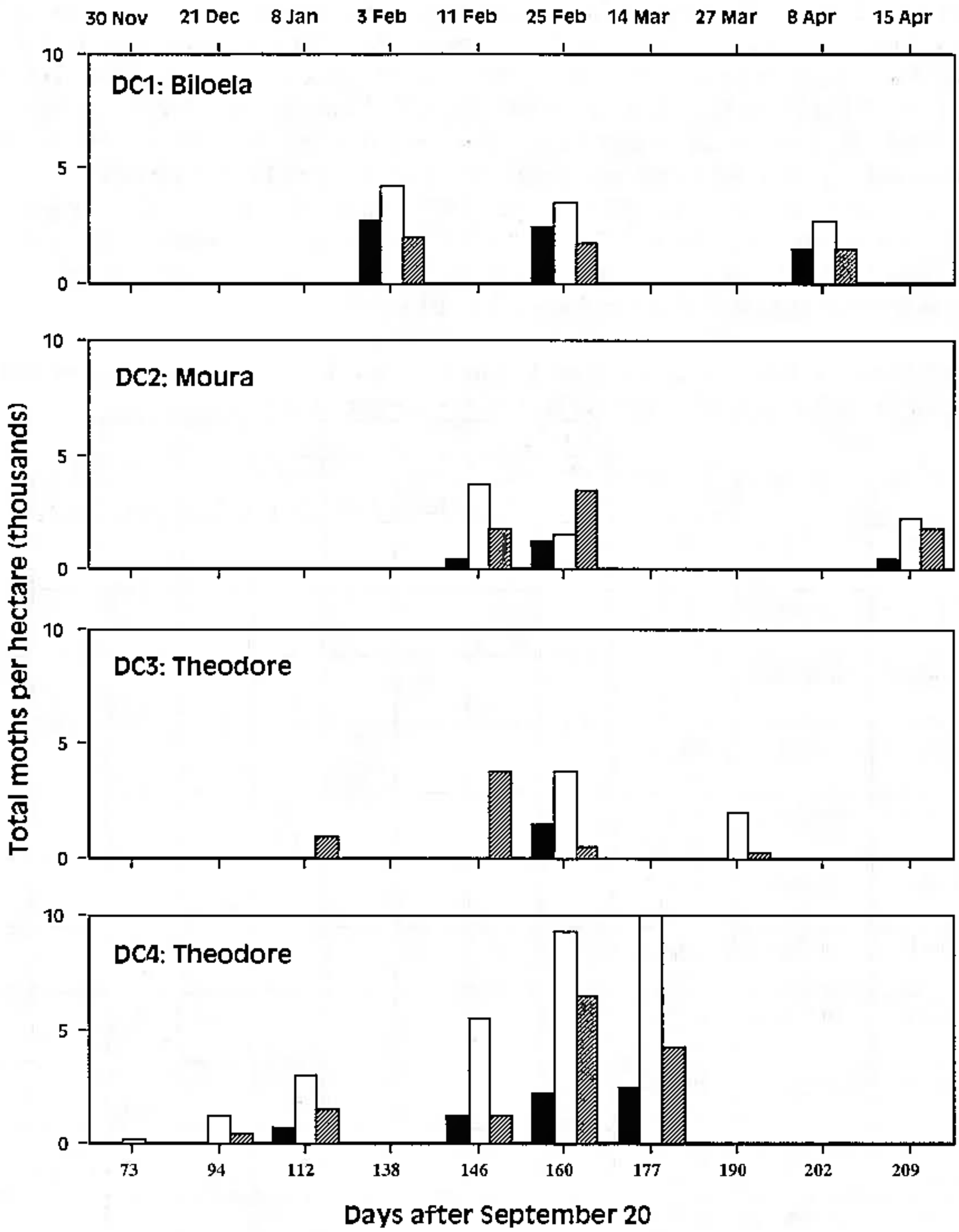


Fig. 1.8.1. Total number of moths generated under INGARD (solid bars), CONTROL (open bars) and CONVENTIONAL (shaded bars) cotton treatments, on blocks DC1-DC4 over the 1996-97 season.

Table 1.8.1 shows the refuge value of cotton and other crops in Biloela, Moura and Theodore. Commercial cotton, sunflower and sorghum crops produced substantial numbers of moths from January to April. The commercial sunflower crop sampled on 8 January (Table 1.8.1) was adjacent to the INGARD block on DC1. Of the 31 pupae recorded, 26 were empty pupal cases. This indicates that the bulk of the moth population pupating under the sunflower crop had emerged by late December. This would account for the large spike in egg-laying activity on cotton in DC1 in early January (see Figs.3.0.1 - DC1, 3.0.3 - DC1). The previous generation emerging by early December from the same sunflower crop could have contributed largely to the egg-laying spike in mid-December (see Figs. 1.3.1 - DC1, 1.3.3 - DC1).

Table 1.8.1. Number of pupae[1] and heliothis moths[2] under various crops grown within the Dawson/Callide irrigation areas during the 1996-97 season.

Locality	Crop	Variable	Date 08 Jan	16 Jan	20 Jan	06 Feb	07 Mar	07 Apr	17 Apr	18 Apr	21 Apr
Biloela	sunflower	pupae moths	31 7750								
Theodore	cotton	pupae moths		14 3500							
Theodore	dolichos	pupae moths			0						
Baralaba	cotton	pupae moths				3 750					
Theodore	sorghum	pupae moths					16 4000				
Moura	cotton	pupae moths						22 5500			
Biloela	cotton	pupae moths							28 7000		
Biloela	sorghum	pupae moths							21 5750		
Biloela	mungbean	pupae moths							7 1750		
Biloela	sorghum	pupae moths								22 5500	
Biloela	navybean	pupae moths									7 1750

[1] in 20 m^2

[2] moths/ha, computed assuming 50% of pupae collected were parasitised.

1.9 Outcrossing and volunteer cotton

Potential for gene escape to native Gossypium species

All aspects of commercial production of transgenic crops were discussed at a workshop entitled 'Commercialisation of transgenic crops: risk, benefit and trade considerations' held in Canberra from 11-13 March 1997. At the workshop, A. H. D. Brown et al. (Centre for Plant Biodiversity Research, CSIRO Division of Plant Industry, Canberra) presented an in-depth assessment of the risk of cotton transgene escape into native

Australian Gossypium species. In their published report, Brown et al. (1997) conclude that the barriers to transgene escape from cotton into indigenous Gossypium species are formidable. They consider it generous to allow an order-of-magnitude (one tenth) probability of occurrence of each of the ten barriers to gene escape identified in their analysis. In their opinion, no Australian diploid indigenous Gossypium species combines the traits of ecological flexibility, crossability and meiotic fertility required to constitute a conceivable risk of transgene escape.

Native Gossypium species and volunteer cotton in the Emerald irrigation area

A search for native Gossypium species was conducted in the immediate neighbourhood of INGARD blocks in September 1996. The area within 1 km of the border of each INGARD block in EIA and DCIA was scouted for the presence of G. sturtianum and G. australe. Specimens of the latter were collected from a location 11 km. west of Blackwater on the Capricorn Highway and distributed to the survey team to help in identification. The results of the survey were negative. Records maintained by the Queensland Herbarium of previous collections of Gossypium spp. from the EIA (Wills road) in 1991 were checked again but specimens were not recovered. Although CQ is clearly within the range of native Gossypium spp., to date, specimens have not been found anywhere in the EIA or DCIA.

An assessment of the number and distribution of volunteer cotton plants was conducted in March 1997, at the end of the cotton season. Volunteer cotton can be found growing along side the major roads in the EIA, within 3 m of the road shoulder. These plants are established from raw cotton blown off trucks carrying cotton modules, and survive largely because they get run-off water from the road surface. They are controlled on a regular basis by roadside slashing and herbicide treatment. Both the shire council and the Department of Main Roads have roadside weed control programs in place. Volunteer cotton was not evident along any of the side roads in the irrigation area or in the vicinity of the trial blocks.

1.10 Summary of 1996-97 results

The performance of INGARD cotton throughout the season, judged on the basis of laboratory as well as field data, can be best described as variable. Insecticidal activity, as indexed by leaf bioassays, declined sharply less than 8 weeks after planting. Field performance, as documented by commercial scouting data and spray records, appeared to support the conclusion of a decline in insecticidal activity roughly half way through the season. Such a decline in efficacy appears to be in line with observations on INGARD performance in other areas.

The data show clearly that conventional (sprayed) cotton and other crops grown commercially in CQ produce large numbers of heliothis moths throughout the growing season. In view of the data on moth production in CQ, the strategy aimed at dilution of resistance to the INGARD toxin should be more effective in CQ than in many other cotton growing regions of Australia. It should be noted that estimates of pupae presented in all the foregoing tables and graphs are probably conservative because it is unlikely that all pupae actually present were found.

Assuming an area of 20 hectares, the single chick pea crop sampled in late October produced in excess of 1.9 million moths over a 2 week period. This is more than half the total number of moths produced by 120 hectares of conventional cotton over the entire season. The data in Tables 1.7.1, 1.7.3 and 1.8.1 indicate that various crops able to generate large numbers of heliothis are readily available throughout the season within irrigation areas in CQ. This is undoubtedly the result of the opportunistic nature of crop production in the irrigation areas.

A further implication of the data in Tables 1.7.3 and 1.8.1 is that irrigation areas in CQ could be the nuclei from which heliothis infestations, particularly H. armigera, may be spreading to areas beyond. It is highly probable that the late chickpea crop in October (Table 1.7.3) was a source for the spread of infestations in other parts of the irrigation area.

The yield data (Appendix 3) show that yields from INGARD treatments were similar to the corresponding CONVENTIONAL, while requiring 1/3 to 2/3 fewer insecticidal applications. The suction sampling data indicate that INGARD cotton does not adversely affect invertebrate fauna relative to commercial cotton. On the contrary, the significant reductions in chemical pesticide usage facilitated by INGARD should have a positive impact on the environment.

1.11 INGARD resistance management strategy for CQ

It was clear from the outset that development of an INGARD resistance management tactic or strategy that would serve as an alternative to pupae busting in CQ was critical in determining the future of INGARD in the region. Accordingly, an important objective of the research program was the identification and development of novel tactics for heliothis control. These tactics could then be used in an INGARD resistance management strategy specific to CQ.

2.0 1997-98: First season of commercial INGARD production in CQ

At the end of the 1996-97 season, Monsanto Australia Ltd. filed an application with the NRA for commercial registration of INGARD cotton in CQ. Included in the application were data on the insecticidal efficacy and agronomic performance of INGARD during the 1996-97 season together with a proposed INGARD resistance management strategy for CQ (see Appendix 4 for details). Following discussions with the research team (Dr. Richard Sequeira, Entomologist - QDPI, and Mr. Michael McCosker, Cotton Development and Extension Officer - QDPI) on the feasibility of the proposed resistance management strategy for CQ, the NRA approved the application by Monsanto Australia Ltd in September 1997.

Resellers' records show that a total of 1645.3 hectares of commercial INGARD cotton were planted in the Emerald irrigation area between 15 September and 25 October 1997. The bulk of the INGARD area was planted to NuCotn37*i* and the remainder to CS50*i*. In addition, 35 ha of INGARD cotton was planted as a research trial to evaluate its insecticidal activity under fully irrigated and simulated rainfed conditions. The replicated trial was designed as follows: irrigated and rainfed NuCotn37*i* (10-15 ha and 5 ha, respectively), irrigated and rainfed L23*i* (10-15 ha and 5 ha, respectively). Irrigated and rainfed treatments were located on adjacent blocks within the same paddock. Commercial as well as trial INGARD crops were managed independently by cotton consultants. The crops were scouted for heliothis throughout the season using INGARD thresholds recommended by the Transgenic and Insecticide Resistance Management (TIMS) committee.

The research activities described in the following sections are in accordance with the protocols given in Appendix 1. Certain research activities, including leaf bioassays, environmental impact assessment and plant mapping, were restricted to cotton crops on four farms, F1 to F4. The treatments (crops) evaluated on each farm were as follows: **F1** – CS50*i*, CS50 Unsprayed (control), CS50 Sprayed; **F2** – CS50*i*, NuCotn37*i* Irrigated, NuCotn37*i* Dryland, L23*i* Irrigated, L23*i* Dryland, NuCotn37 Sprayed, NuCotn37 Unsprayed (control); **F3** – NuCotn37*i*, NuCotn37.

2.1 Seasonal variation in efficacy of INGARD cotton (Protocol 1)

Leaf bioassay results

Leaf bioassays to assess the insecticidal activity of INGARD were conducted at regular intervals throughout the season, beginning 6 weeks after planting. Leaves for the bioassays were collected from unsprayed conventional and INGARD cotton crops on farms F1 and F2. The experimental protocol followed in conducting the bioassays is given in Appendix 1. The results of the bioassays are presented in Table 2.1.1.

Larval survivorship after five days showed that insecticidal activity (efficacy) of CS50*i* appeared to decline abruptly soon after the onset of squaring, around 7 weeks after planting (Table 2.1.1). Subsequent leaf bioassays showed that efficacy of CS50*i* remained depressed throughout the season. A small increase in insecticidal activity was recorded on 20 December (Table 2.1.1). However, it is debatable whether this increase was the result of improved INGARD efficacy or higher larval mortality on the test leaves due to insecticide residue resulting from spray drift. The latter explanation cannot be ruled out because insecticide usage in the area increased dramatically after the middle of December.

Table 2.1.1. Survivorship and growth rating of test H. armigera larvae after 3 and 5 days on unsprayed conventional (control) and INGARD (*i*) cotton leaves from Farm F1.

Variety	Treatment	Assay	Date[1]	Crop[2]	Day 3		Day 5		
		No.		age	Alive	%	Alive	%	Growth*
CS50	irrigated	1	15-Nov	49	99/100	99	99/100	99	2.00
	(control)	2	21-Nov	55	100/100	100	148/150	99	2.06
		3	4-Dec	67	135/150	90	128/150	85	2.45
		4	12-Dec	75	92/100	92	14/100	86	2.41
		5	20-Dec	83	142/150	95	139/150	93	2.50
		6	7-Jan	101	116/145	80	111/145	77	1.95
		7	23-Jan	117			64/140	46	1.92
CS50*i*	irrigated	1	15-Nov	49	31/150	21	26/150	17	2.00
		2	21-Nov	55	110/150	73	99/150	66	20.60
		3	4-Dec	67	108/150	72	101/150	67	2.38
		4	12-Dec	75	107/150	71	85/150	57	2.28
		5	20-Dec	83	64/150	43	44/150	29	2.10
		6	7-Jan	101	120/150	80	112/150	75	1.90
		7	23-Jan	117			7/150	4	1.07

Notes: [1]Date of bioassay; [2] Days after planting; *Growth = larval growth index (see Appendix 1).

By comparison, efficacy of NuCotn37*i* and L23*i* was variable but larval survival was generally low, ranging from 0 to 13 % (Table 2.1.2). Efficacy appeared to dip sharply around the middle of December for a brief period before recovering. The sudden decline coincided with a prolonged period of overcast weather. The overall pattern of change in efficacy over the season was similar in NuCotn37*i* and L23*i* and, as well, in irrigated and dryland treatments for each variety. Within each variety, insecticidal activity of plants did not differ significantly between treatments (Paired sample *t*-test, Analysis of Variance, $P < 0.01$). However, the result of bioassay #5 (Table 2.1.2) provides the only indication of differential performance of INGARD under dryland and irrigated conditions.

Larval survival in bioassay #5 was markedly lower on dryland than on irrigated INGARD leaves of both varieties (Table 2.1.2). Because this leaf bioassay was conducted during a prolonged period of overcast weather, it is tempting to relate the anomaly in larval survivorship between treatments to differential response of INGARD plants to weather-related stress. However, the singularity of the anomaly precludes any definitive conclusions.

The larval growth index (see Appendix 1 for details) enables comparisons of INGARD efficacy between treatments. The larger the value of the index, the greater is the average size (and implicitly growth rate) of the surviving larvae. Thus, the average size of larvae feeding on leaves of the CS50*i* crop on farm F1 was only slightly less than that of larvae feeding on non-INGARD leaves throughout the season (Table 2.1.1). INGARD crops on farm F2 had a greater and more variable effect on larval growth. Greater reductions in size of larvae on INGARD leaves relative to non-INGARD leaves were observed earlier in the season when the efficacy of the plants was higher (Table 2.1.1).

Table 2.1.2. Survivorship and growth rating of test H. armigera larvae after 3 and 5 days on unsprayed conventional (control) and INGARD (*i*) cotton leaves from Farm F2.

Variety	Treatment	Assay	Date[1]	Crop[2]	Day 3		Day 5		
		No.		age	Alive	%	Alive	%	Growth*
NuCotn37	irrigated	1	15-Nov	26	99/100	99	99/100	99	2.00
	(control)	2	21-Nov	32	100/100	100	100/100	100	2.03
		3	4-Dec	44					
		4	12-Dec	52	95/100	95	94/100	94	2.50
		5	20-Dec	60	96/100	96	95/100	95	2.47
		6	7-Jan	78	76/100	76	74/100	74	1.89
		7	23-Jan	94			21/55	38	1.48
NuCotn37*i*	irrigated	1	15-Nov	26	48/100	48	13/100	13	1.04
		2	21-Nov	32	48/100	48	7/100	7	1.07
		3	4-Dec	44	0/100	0	0/100	0	
		4	12-Dec	52	12/100	12	7/100	7	2.07
		5	20-Dec	60	63/100	63	52/100	52	2.03
		6	7-Jan	78	5/100	5	3/100	3	1.50
		7	23-Jan	94	0/100	0	0/100	0	
NuCotn37*i*	rainfed	1	15-Nov	26	34/100	34	10/100	10	1.50
		2	21-Nov	32	21/100	21	7/100	7	1.00
		3	4-Dec	44	0/100	0	0/100	0	
		4	12-Dec	52	8/100	8	5/100	5	2.00
		5	20-Dec	60	27/100	27	22/100	22	2.02
		6	7-Jan	78	3/100	3	1/100	1	1.50
		7	23-Jan	94	0/100	0	0/100	0	
L23*i*	irrigated	1	15-Nov	26	28/100	28	12/100	12	1.42
		2	21-Nov	32	29/100	29	7/100	7	1.07
		3	4-Dec	44	17/100	17	0/100	0	
		4	12-Dec	52	17/100	17	4/100	4	1.75
		5	20-Dec	60	45/100	45	35/100	35	2.17
		6	7-Jan	78	4/100	4	2/100	2	1.75
		7	23-Jan	94			1/100	1	1.50
L23*i*	rainfed	1	15-Nov	26	36/100	36	7/100	7	1.07
		2	21-Nov	32	29/100	29	5/100	5	1.10
		3	4-Dec	44	11/100	11	0/100	0	
		4	12-Dec	52	6/100	6	3/100	3	2.00
		5	20-Dec	60	24/100	24	18/100	18	2.08
		6	7-Jan	78	6/100	6	4/100	4	1.75
		7	23-Jan	94			0	0	

Notes: [1]Date of bioassay; [2] Days after planting; *Growth = larval growth index (see Appendix 1).

The leaf bioassay results appeared to become less reliable indicators of efficacy after the end of December. The apparent marked decrease in larval survival on INGARD leaves in January (see Tables 2.1.1 and 2.1.2) could have been related to frequent insecticide spraying for heliothis control in late December and early January rather than increased insecticidal activity of the INGARD plants *per se*. Insecticide drift residue resulting from frequent spraying for heliothis control around the area after mid-December is the most likely cause of high larval mortality on INGARD leaves. This argument would appear to be supported by the significant mortality of larvae on control (non-INGARD) leaves after December (Tables 2.1.1 and 2.1.2). High mortality of larvae on INGARD leaves after three days may also be indicative of external mortality factors, including insecticide residue on the leaves.

The bioassay data in Tables 2.1.1 and 2.1.2 provide only a rough indication of the insecticidal activity of INGARD cotton under laboratory conditions. Because of the large number of factors that can influence larval heliothis mortality under field conditions, the data do not permit any direct inferences of INGARD efficacy under field conditions. However, some level of correlation between the lab bioassay results, heliothis larval dynamics on INGARD in the field, and the timing and frequency of insecticidal applications may be expected but only for the particular crops tested.

Soil water conditions and INGARD efficacy

It is a well-known fact that the efficacy of INGARD is adversely affected by stress, particularly waterlogged soil conditions. The seasonal soil moisture profile for the paddock housing the CS50*i* crop on Farm F1 (the subject of leaf bioassays in Table 2.1.1 above) was monitored and documented using an ENVIROSCAN® unit. The output is shown in Fig. 2.1.1. The rate of water uptake by (and implicitly growth of) plants is directly proportional to the rate of decline in the soil moisture profile. Flattening of the curve indicates low uptake of water and/or waterlogged conditions.

Fig. 2.1.1A shows the profile of average soil water content between depths of 10 and 70 cm. Fig. 2.1.1B shows the profile at a depth of 30 cm. The decline in soil water content between 21 October and 05 November in Fig. 2.1.1A suggests optimum growth conditions for seedling growth. However, during the remainder of the growing season, average soil water content appears to have been maintained at or close to the saturation point, resulting in long periods of wet or waterlogged conditions in the paddock. Waterlogging is particularly evident over a period of roughly 20 days, from the middle of December until the first week of January. From a total of 126 days required from planting to maturity, the crop experienced sub-optimal (exceedingly wet or waterlogged) conditions over roughly 52 days.

The profile of soil moisture at a depth of 30 cm reveals several plateaus over the course of the growing season (Fig. 2.1.1B). The profile at 70 cm is essentially flat, with few and minor dips. This indicates that soil moisture at or below a depth of 30 cm was poorly utilised.

The data from Figs. 2.1.1A, B suggest that the poor efficacy of the CS50*i* crop on Farm F1 (see also Fig. 2.1.2C below to compare performance in the field) may have resulted partly from sub-optimal soil moisture conditions caused by over-irrigation, untimely rainfall or a combination of both.

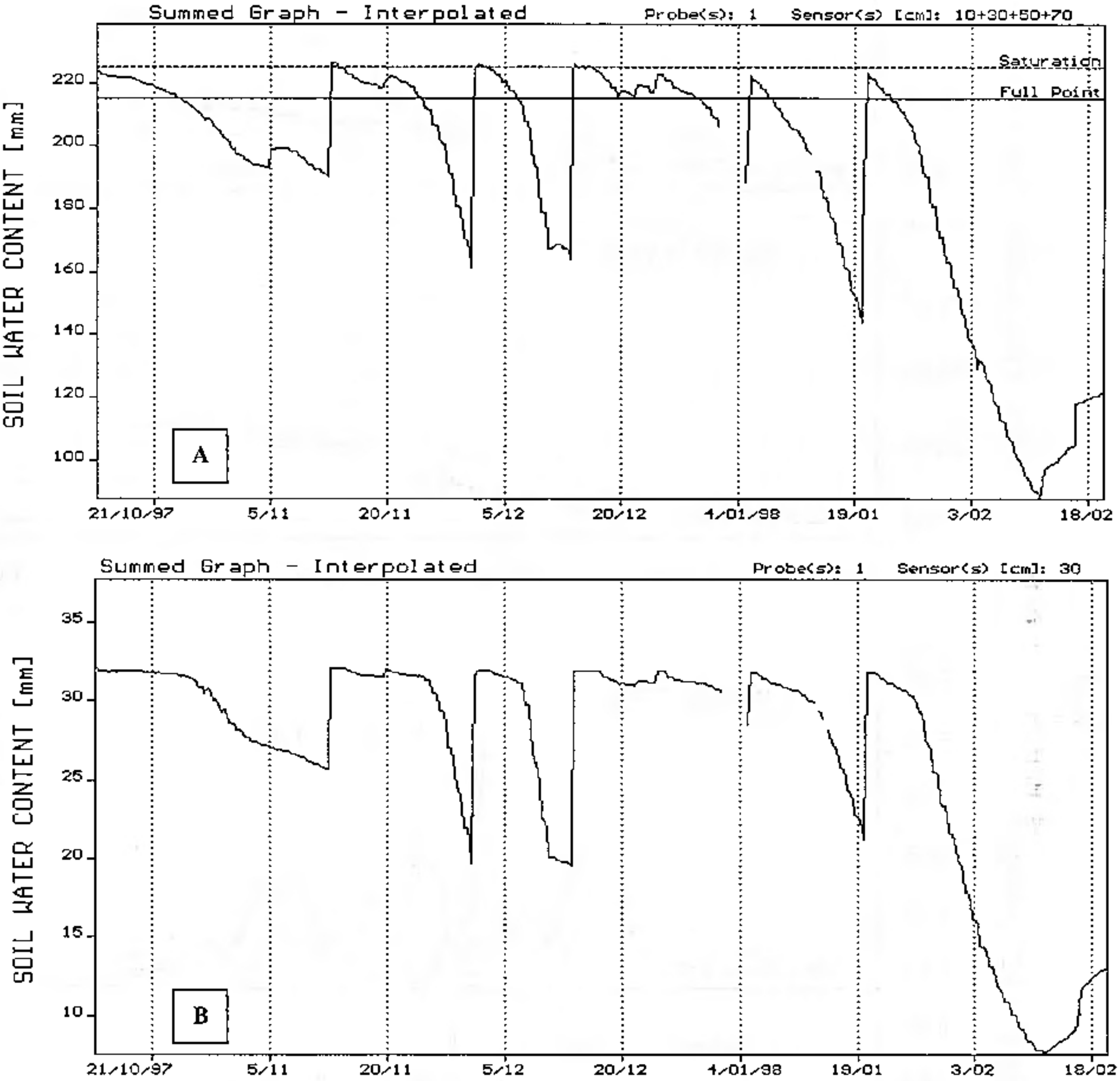


Fig. 2.1.1. Profile of seasonal changes in soil water content for the CS50*i* crop in Table 1A as measured by an ENVIROSCAN® unit.

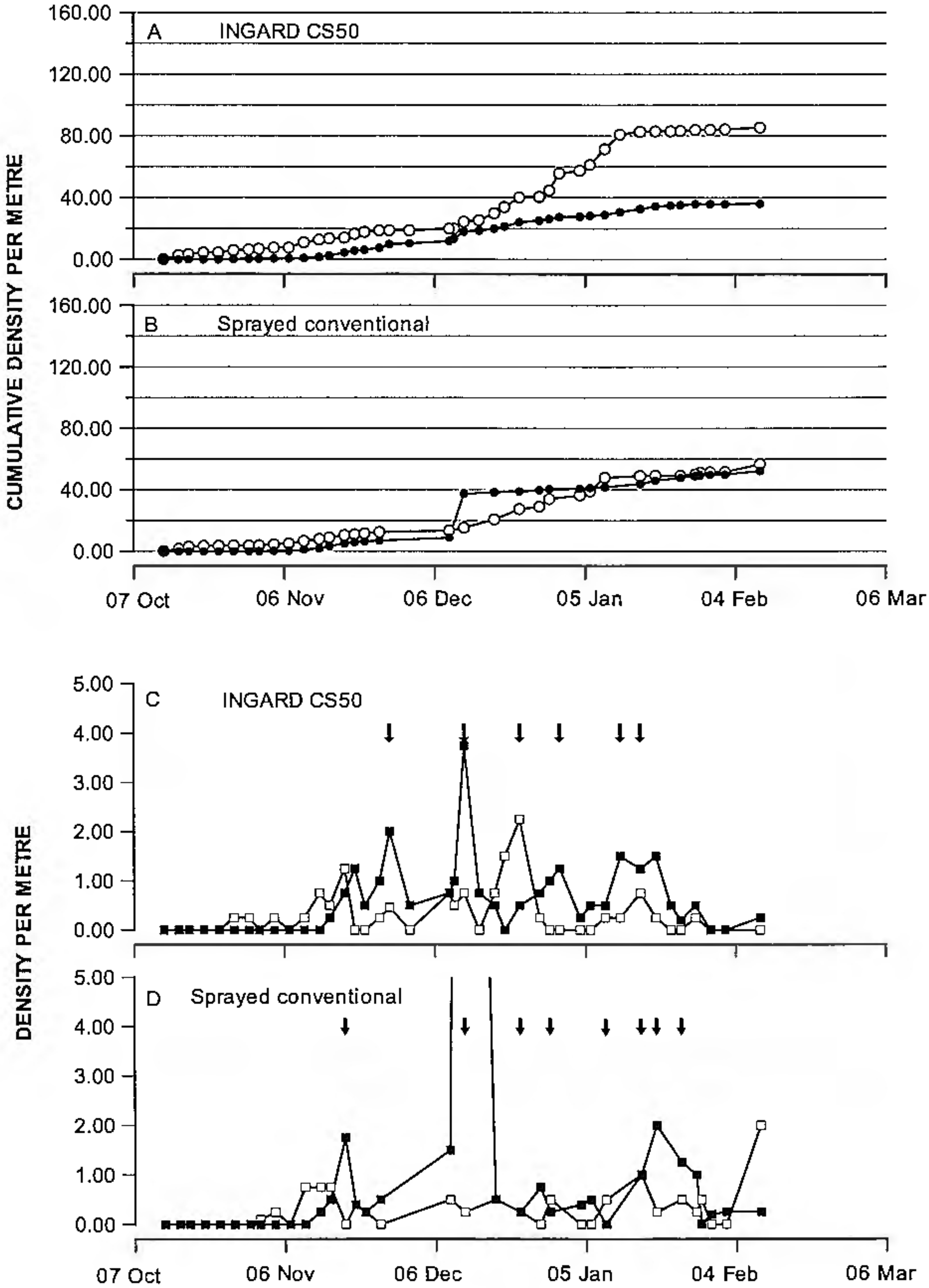


Fig. 2.1.2. Seasonal dynamics of heliothis on cotton crops on Farm F1: A) Cumulative density of eggs [O] and all larval stages [●]; B) As in A; C) Density of neonates/first instars [□] and higher instars [■]; D) As in C. Arrows indicate spray application.

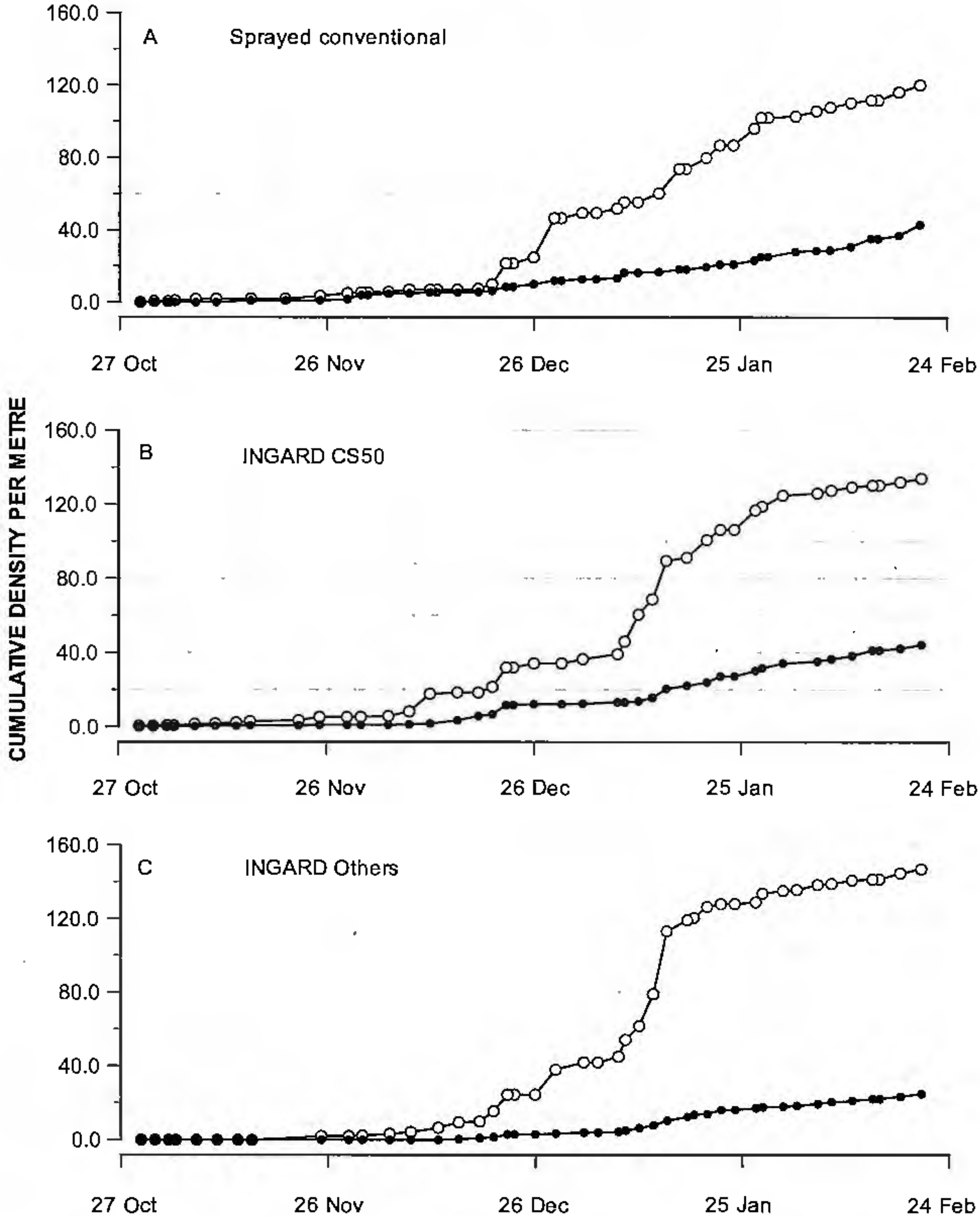


Fig. 2.1.3. Seasonal dynamics of heliothis on cotton crops on Farm F2: Cumulative density of eggs [O] and all larval stages [●]. INGARD varieties in 'C' are Nucotn37 and L23.

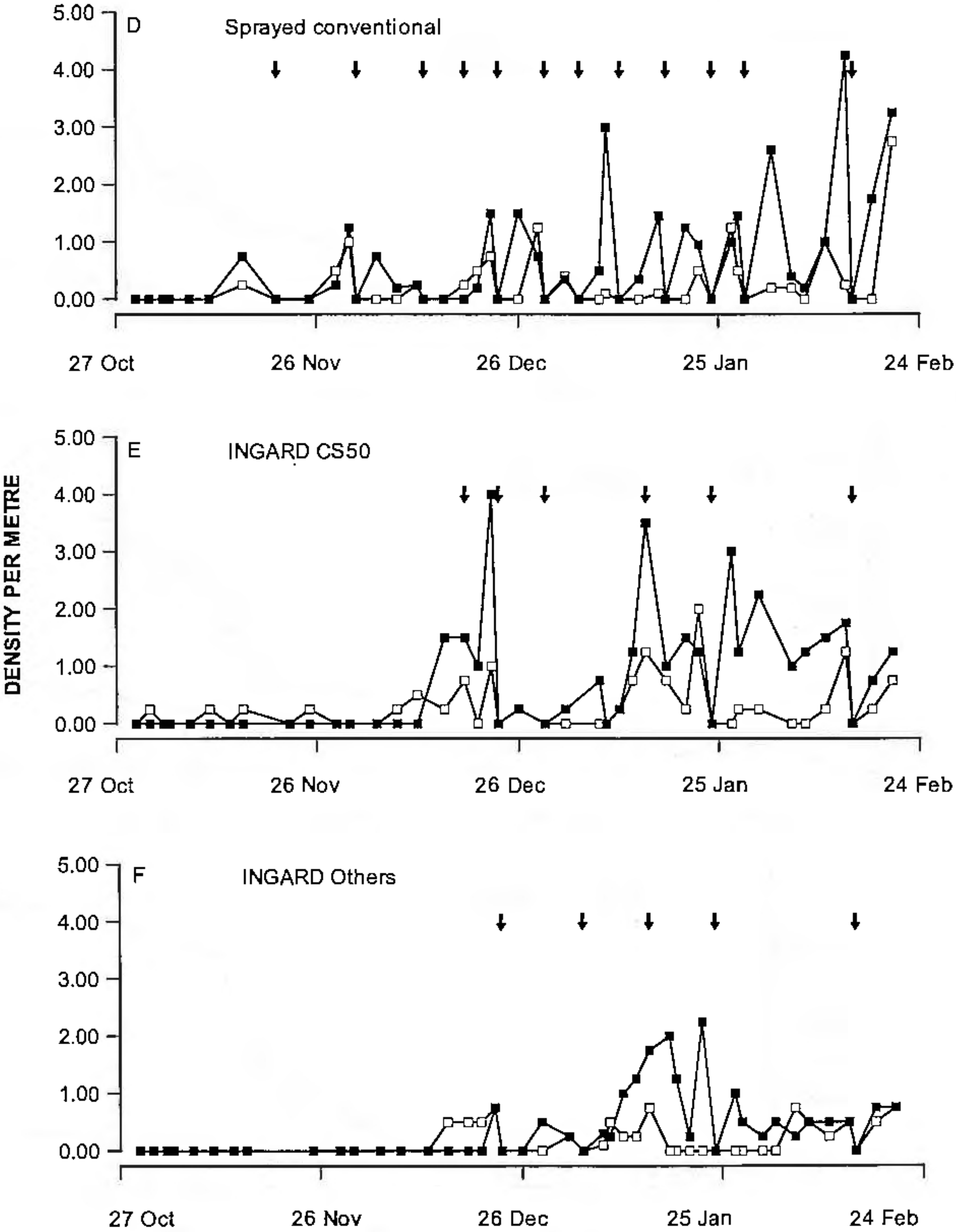


Fig. 2.1.4. Seasonal dynamics of heliothis on cotton crops on Farm F2: Density of neonate/first instars [□], and older instars [■]. INGARD varieties in 'F' are NuCotn37 and L23. Arrows indicate spray application.

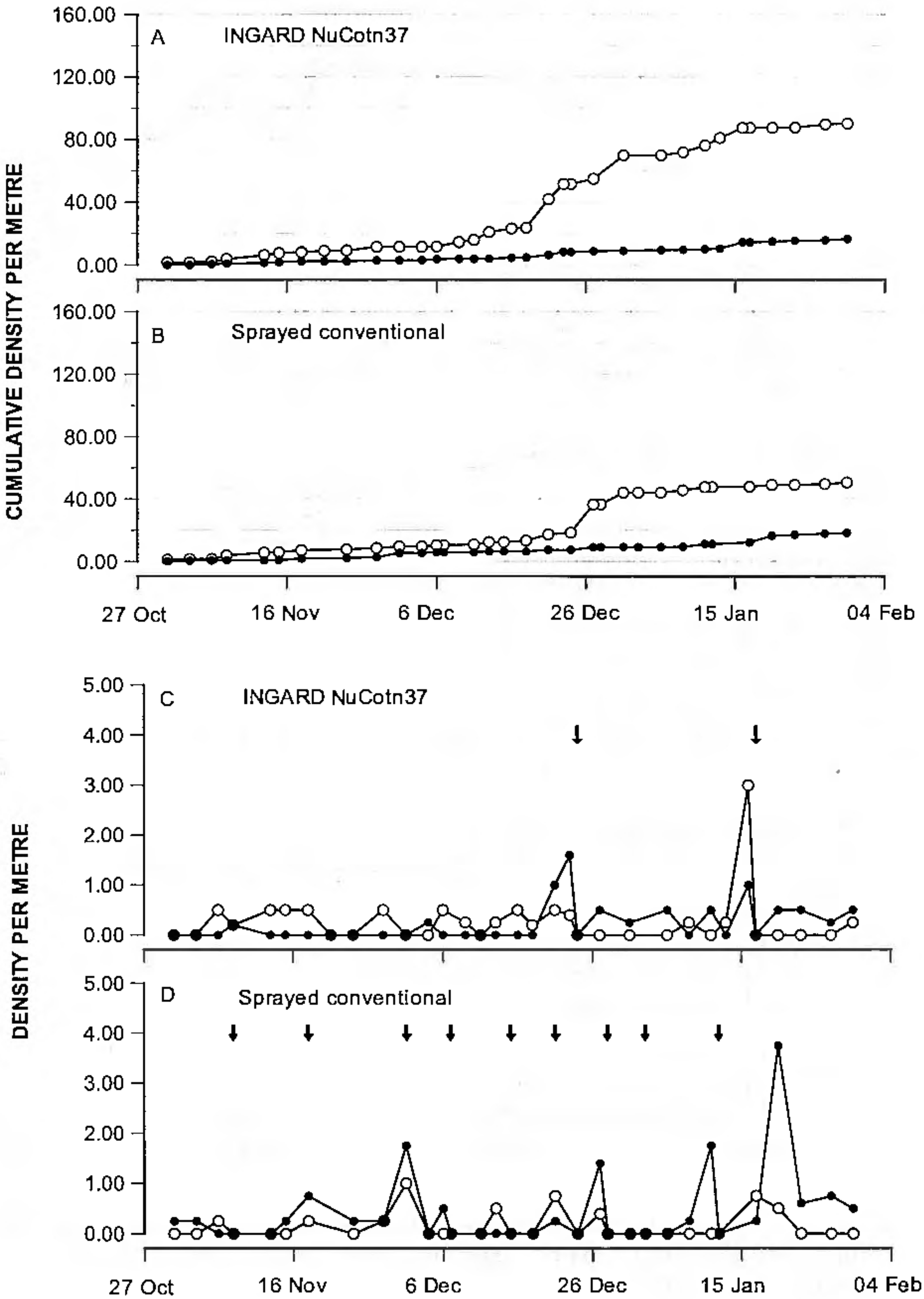


Fig. 2.1.5. Seasonal dynamics of heliothis on cotton crops on Farm F3: A) Cumulative density of eggs [O] and all larval stages [●]; B) As in A; C) Density of neonates/first instars [□] and higher instars [■]; D) As in C. Arrows indicate spray application.

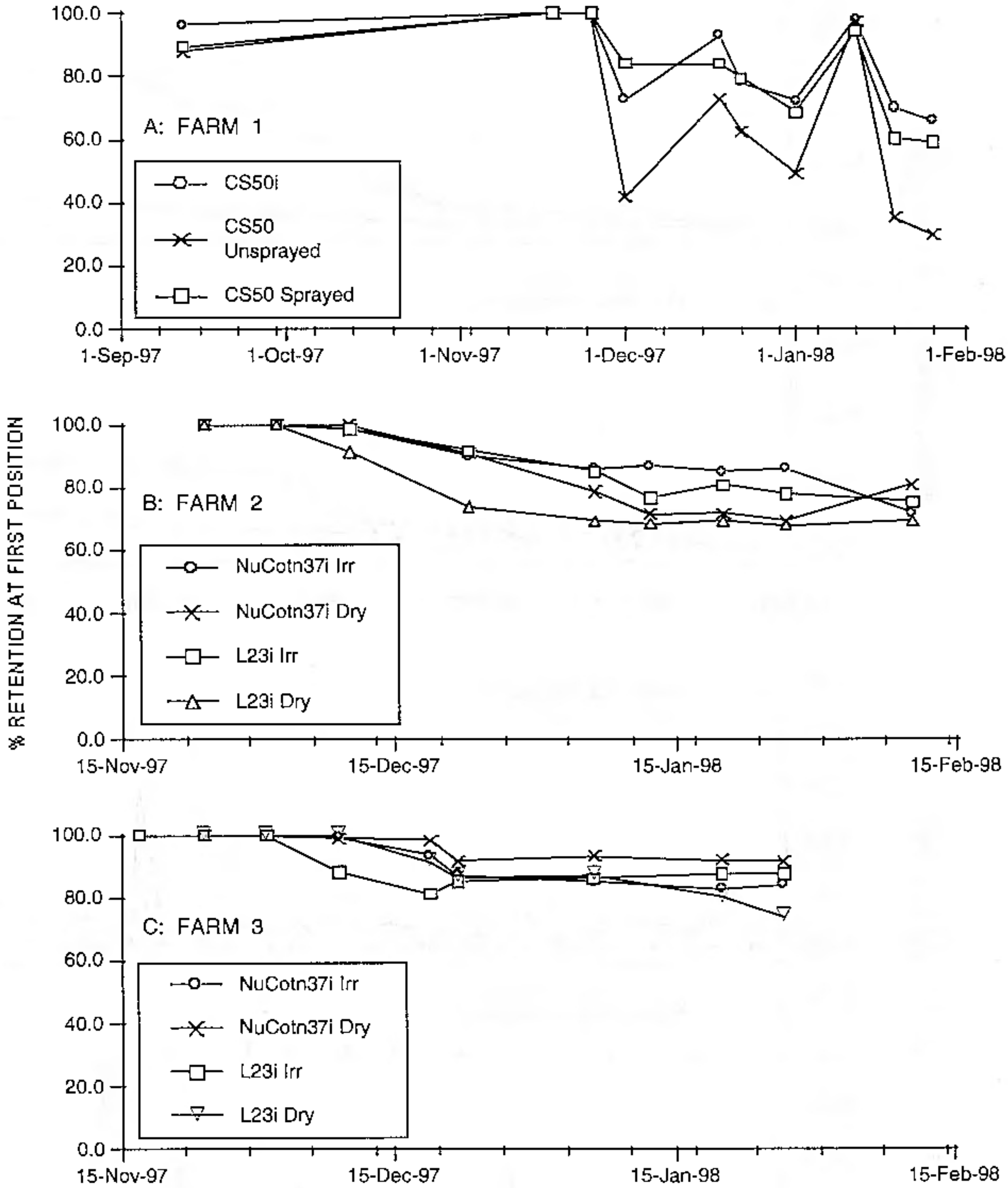


Fig. 2.1.6. Seasonal profile of average fruit retention in the first position on all fruiting branches on INGARD (*i*) and conventional cotton. Abbreviations: Irr = irrigated; Dry = dryland

A proportion of the survivors from the heliothis populations on grain crops in autumn initiate diapause and spend the winter months under the crop stubble. The proportion in diapause each year is determined by weather conditions. Over 75% of the pupae found in the Emerald area between 15 May and 15 July 1998 were in the state of diapause.

Few non-cotton crops were grown within the irrigation area between October 1997 and March1998. A single mung bean crop on the outskirts of the irrigation area appears to have contributed significantly to the initial heliothis pressure in December (Table 2.2.2).

The data in Tables 2.2.1 and 2.2.2 indicate that the CQ cropping area produces large numbers of heliothis moths on a wide range of field crops throughout the year. In the context of INGARD cotton, this implies that there is good potential for dilution of the INGARD resistance gene(s).

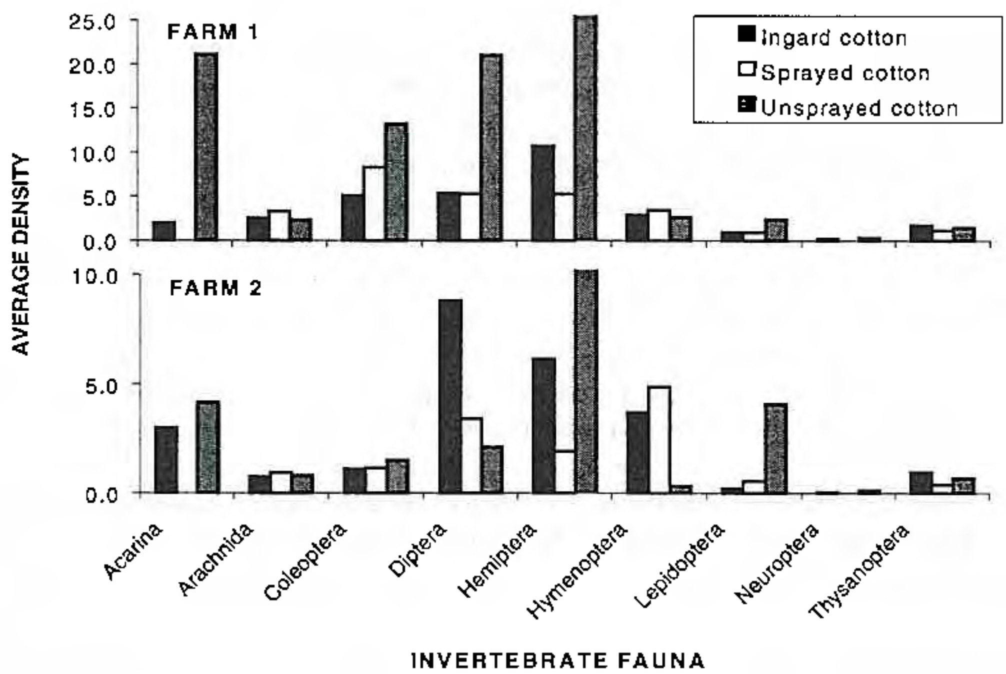


Fig. 2.3.1. Mean number of individuals belonging to different invertebrate Orders found in fortnightly suction samples on cotton crops on farms F1 and F2 from November to February.

2.3 Relative impact of INGARD on local invertebrate fauna

Samples of the invertebrate fauna on INGARD, conventional and unsprayed cotton crops were collected at regular intervals throughout the season by suction sampling. Five 20-m lengths of row were sampled in each cotton treatment on Farms F1 and F2. The collections were preserved in 70% ethanol and sent to the Australian Cotton Research Institute (ACRI), Narrabri, NSW, for identification by Dr. Lewis Wilson. The invertebrates in each suction sample were identified down to Family.

Fig. 2.3.1 shows the aggregate data (means) at the level of Order. The two farms differ with respect to the level of overall invertebrate abundance and representation between orders. As one would expect, unsprayed conventional cotton generally harboured more invertebrate fauna than the other crops. INGARD generally harboured a similar or higher mean density of invertebrates than conventional cotton. Dipterans (flies), hemipterans (bugs), hymenopterans (wasps, etc.), lepidopterans (moths, primarily heliothis eggs and larvae) and mites were more abundant than representatives of other orders. The data in Fig. 2.3.1 suggest that the impact of INGARD cotton on invertebrate fauna is likely to be similar or more positive (facilitating greater abundance of beneficial insects) than that of conventional sprayed cotton.

2.4 INGARD resistance management strategy for CQ

In accordance with the INGARD resistance management strategy (see Appendix 4 for details) pigeon pea crops (1% of farm area) were planted at the start of the 1997/98 season, along with cotton. Table 2.4.1 below shows the refuge value of different cotton crops in the irrigation area in relation to pigeon pea during the season.

Cotton started producing heliothis pupae (moths) by the middle of December and continued to do so until the end of February (Table 2.4.1). Unsprayed cotton blocks, planted as refuge for INGARD cotton, produced large numbers of moths, thereby contributing significantly to the heliothis build up in Stage III. Pigeon pea crops could be classified into two phenologically distinct groups. One group, consisting of the majority of crops in the area, was characterised by stunted crops that stopped growing and flowering by mid-December. These crops experienced hot and dry conditions during October and November. A few crops escaped early season stress as a result of frequent irrigation, which resulted in tall (in excess of 150 cm in height), continuously flowering crops. Both groups of pigeon pea crops produced large numbers of pupae, with the tall crops producing two to three times as many pupae as the other.

Table 2.4.1. Abundance and time of production of heliothis pupae on pigeon pea trap crops in relation to cotton in the Emerald area from April 1997 to March 1998.

CROP	Pupae per hectare (thousands)					
	Apr 97	May 97	Dec 97	Jan 98	Feb 98	Mar 98
Cotton						
Conventional	13	14	3	3	3	0
INGARD	0			4		
Unsprayed				56		
Rainfed					0	
Trap crops						
Pigeon pea (short)			41	27	51	22
Pigeon pea (tall)					174	675

The number of pupae produced by pigeon pea is evidence of the crop's ability to attract eggs that would otherwise have been deposited on cotton. The timing of pupae production by pigeon pea indicates clearly that moths emerging from cotton at the end of February are being successfully lured into pigeon pea. Furthermore, the data indicate that the placement of the trap crops (February and March) is strategically correct.

Field performance of INGARD

Larval survival.

Figs. 2.1.2-2.1.5 show seasonal dynamics of heliothis egg and larval pressure on cotton on farms F1 – F3 as recorded by the professional consultant managing the crops. The crops were managed using appropriate heliothis larval thresholds recommended by the TIMS Committee. The data formed the basis of spray recommendations by the consultant.

Changes in the profiles of cumulative egg and larval density show that heliothis pressure increased dramatically after mid-December on all three farms. The number of eggs recorded over the season was generally higher on INGARD than on conventional cotton crops (Figs. 2.1.2A, B; 2.1.3A-C; 2.1.5A, B).

Survival of heliothis larvae beyond the first-instar stage in the field was observed earlier on the two CS50*i* crops (Figs. 2.1.2C, 2.1.4B) than on the other INGARD crops (Figs. 2.1.3C, 2.1.5C). The INGARD crops represented in Figs. 2.1.2-2.1.5 varied in their effectiveness against heliothis as is evident from the frequency and timing of insecticide applications. This variation in efficacy is representative of the variability in performance of commercial INGARD crops observed in the Emerald area during the season. In general, INGARD crops required approximately half the number of insecticide applications in comparison to conventional cotton.

Beginning in mid-November, a sampling protocol was developed and initiated to determine season-long changes in the survivorship of eggs to first-instar larvae under field conditions. Under the protocol, three groups of 10 plants were selected in each of three treatments (INGARD, sprayed conventional cotton and pigeon pea crops) on each of two farms. The plants were monitored twice weekly for heliothis eggs and larvae with an interval of 2-3 days between consecutive observations. The data thus collected were not indicative of a correlation between the number of eggs observed at any given time and subsequent estimates of neonate and first-instar larvae.

Fruit retention.

Estimates of fruit retention (percentage) in the first position over all fruiting branches for INGARD and CONVENTIONAL on farms F1-F3 are shown in Figs. 2.1.6A-C, respectively. The estimates were obtained by counting the number of first-position fruit per plant in three samples of 10 plants each.

The profile of fruit retention by crops on farm 1 was erratic from December to the end of January (Fig. 2.1.6A). This is particularly so for the unsprayed conventional and INGARD crops. Retention declined steadily on all crops on F2 and F3 after the beginning of December (Figs. 2.1.6B, C). The crops on F3 showed higher retention than those on the other two farms. In general, average retention at the end of the season was above 60%. Analysis of variance on the data (arcsine transformed, $\alpha = 0.05$) indicate no significant differences between treatments (crops) within each farm. Average retention (across all crops) also did not differ significantly between farms.

Changes in fruit retention cannot easily be related to heliothis feeding damage because of the multitude of factors that can influence the plant's ability to hold on to fruit. The only outstanding feature of the fruit retention data is the erratic fruit retention profiles of crops, particularly unsprayed conventional and INGARD, on farm 1 (Fig. 2.1.6A). An erratic profile is to be expected for unsprayed cotton but in the case of INGARD cotton it could be linked to the poor insecticidal efficacy of the crop.

2.2 Refuge value of cotton and other crops (Protocol 2)

The refuge value (number of pupae/moths produced per unit area of crop) of conventional, INGARD and unsprayed conventional cottons was evaluated throughout the season. Table 2.2.1 shows a summary of the data. Detailed data, including estimates of parasitism and sampling details are presented in Appendix 5. Conventional cotton began producing heliothis moths as early as mid-December. By comparison, the first heliothis pupae under INGARD cotton were found in early January. In general cotton crops produced significant numbers of heliothis moths after mid-December.

Table 2.2.1. Abundance and time of production of heliothis pupae under cotton in the Emerald area from April 1997 to March 1998.

CROP	Pupae per hectare (thousands)											
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
<u>Cotton</u>												
Conventional	13	14							3	3	3	0
Ingard	0									4		
Unsprayed										56		
Rainfed											0	

Table 2.2.2. Abundance and time of production of heliothis pupae under non-cotton crops in the Emerald area from April 1997 to March 1998.

Crop	Pupae per hectare (thousands)											
	Apr 97	May 97	Jun 97	Jul 97	Aug 97	Sep 97	Oct 97	Nov 97	Dec 97	Jan 98	Feb 98	Mar 98
Navy bean	9	0										
Soy bean	5	17	4								0	
Sorghum	16	0	31									0
Sunflower	0	1						0				0
Mung bean	0							0	32		3	
Corn		20										
Chickpea			51	16								
Linseed						8						

Table 2.2.2 shows summary data on the refuge value of non-cotton crops in the Emerald area. Detailed data are presented in Appendix 5. The data in Table 2.2.2 are indicative of the dynamics and movements of heliothis in late summer and winter, leading up to the start of the cotton season in September/October. Heliothis moths emerging from cotton toward the end of the season (late February through to April) tend to migrate out of the irrigation area on to rainfed grain crops. These crops, including sorghum, beans and sunflower, sustain large heliothis populations from April to June (Table 2.2.2). However, parasitism is a major mortality factor for these populations (see Appendix 5). Winter chickpea crops form the final link in the heliothis cycle, enabling the survivors to emerge from pupation under chickpeas in time to re-infest early spring crops.

Table 2.4.2. Abundance and distribution of heliothis larvae on pigeon pea trap crops in Emerald: 1997/98.

Crop type*	Date	Area m^2	Number and instar of larvae collected				
			L_1 & L_2	L_3	L_4 & L_5	L_6	Total
Short	26/2/98	10 x 1	10	36	86	19	151
Tall	26/2/98	10 x 1	60	117	128	92	397
Short	2/3/98	10 x 1	0	20	45	11	76
Tall	2/3/98	10 x 1	0	19	94	31	144

* See text for explanation

The data in Table 2.4.2 lend further support to the foregoing discussion on the effectiveness of the pigeon pea trap crops. Large numbers of larvae of all instars can be found on the crops throughout February and early March. Note that very small (L_1) and small (L_2) larvae were not represented in the second sample collected in March (Table 2.4.2). This indicates that no new eggs were laid on pigeon pea after the end of February. By implication, the bulk of heliothis moths from that generation appear to have emerged from pupation and finished laying all their eggs prior to the end of February. Thus, the trap crops were able to soak up the bulk of the eggs laid by the final generation of moths from cotton.

Management of pigeon pea trap crops to destroy heliothis

Limited trials at the end of the 1996/97 season seemed to indicate that cultivation of standing pigeon pea crops was feasible and likely to be an effective means of destroying heliothis pupae. The rationale for planting pigeon pea along with cotton at the start of the 1997/98 season was that growers would be able to cultivate the trap crops from the onset of flowering to destroy pupae and thereby obtain insecticide-free control of heliothis. In reality, however, cultivation proved ineffective as the data in Table 2.4.3 below indicate. This was so partly because pupae tended to be found on the top of the ridge, beyond the reach of all cultivation equipment.

Table 2.4.3. Effectiveness of soil cultivation using two cultivation implements on heliothis pupae survival under pigeon pea.

Option & Indicator	Treatment	Date	Area (m^2)	Number of pupae/moths	
				Total	per m^2
Tyne cultivator					
Pupae collected	Uncultivated	6/2/98	10	196	19.6
Moths emerged	Uncultivated	16/2/98	27	181	6.7
Moths emerged	Cultivated	16/2/98	24	98	4.1
Lilliston cultivator					
Pupae collected	Uncultivated	20/2/98	10	128	12.8
Moths emerged	Uncultivated	7/3/98	30	39	1.3
Moths emerged	Cultivated	7/3/98	30	55	1.8

The data in Table 2.4.3 show that the tyne cultivator trial resulted in a 40% reduction in the number of moths emerging from the cultivated area relative to the uncultivated. In the Lilliston cultivator trial there was essentially no difference in the number of moths emerging from both treatments. Cultivation was also operationally difficult to manage because all growers could not do it at the same time, in the same way and with the same equipment across the management area.

2.5 Area-wide management strategy (AWMS) for heliothis - Winter trap crops

In compliance with the recommended AWMS for heliothis management in CQ (see Appendix 4), winter chickpea trap crops were grown on most cotton farms in the Emerald irrigation area. The crops were planted in June 1997 and ploughed out in the second half of August 1997.

Table 2.5.1. Abundance and distribution of heliothis larvae on chickpea trap crops in the Emerald area.

Variety	Date	Metres checked	Number and size of larvae collected				
			VS	S	M	L	Total
Amethyst	2/8/97	10 x 1	5	7	5	7	24
Amethyst	10/8/97	10 x 1	3	9	5	6	23
Macareena*	25/8/97	10 x 1	37	59	113	11	220
Amethyst*	26/8/97	10 x 1	12	42	67	12	133

* Data provided by Dr. David Murray, Queensland Department of Primary Industries, Toowoomba.

The trap crops began accumulating grubs in early August (Table 2.5.1). The density of grubs on the plants increased dramatically toward the end of August. The data in Table 2.5.1 and grower observations indicate that the Macareena variety of chickpea is much more attractive to heliothis than Amethyst. Over 85% of all larvae examined were found to be H. armigera.

2.6 Summary of 1997-98 results

The pattern of seasonal changes in INGARD efficacy is similar to the pattern of the 96-97 season. Insecticidal activity measured by means of leaf bioassays appears to decline roughly half way through the season. Insecticidal activity under field conditions, as indicated by commercial scouting data, indicates good protection against heliothis even when leaf bioassay results indicate declining efficacy under laboratory conditions. This discrepancy between field and laboratory assessments serves to highlight the danger of extrapolating field performance purely on the basis of laboratory assessments.

INGARD cotton required between 1/2 and 1/3 the number of insecticide applications for heliothis control as compared to conventional cotton, thereby providing significant savings to growers on the cost of insect control.

The data on the end-of-season trap crops lead to the conclusion that pigeon pea has the potential to serve as a highly effective population sink. However, appropriate management of the trap crops and the larval populations on them will be critical for the success of the strategy.

3.0 1998-99: Second season of commercial INGARD production in CQ

The start of the 1998-99 cotton season marked the beginning of the second season of commercial INGARD production in CQ. Resellers' records show that an estimated total of 8082 hectares of INGARD cotton were planted in the Emerald irrigation area between September 15 and October 25. Commercial INGARD varieties that were grown included NuCotn 37i and Sicot 189i. A number of experimental lines of INGARD cotton were also grown as part of variety trials and assessed for efficacy and agronomic performance in the area.

The research activities described in the following sections are in accordance with the protocols given in the original grant proposal (see Appendix 1).

3.1 Seasonal variation in efficacy of INGARD cotton (Protocol 1)

Leaf bioassay results

Leaf bioassays were conducted to assess the insecticidal activity of INGARD cotton against heliothis larvae at regular intervals throughout the season, beginning 6 weeks after planting. Leaves from plants with and without the INGARD gene were collected from trial sites and commercial crops in the area. There were three sites of which two were on Farm F1 and the third was on Farm F2. Two experimental protocols, A and B, were followed in conducting the bioassays. The protocols differed in the number of heliothis larvae tested per leaf. Protocol A was provided by Monsanto Australia Limited (S. Addison) whereas protocol B employed methodology used in the previous two seasons. Protocols A and B were used on leaves collected from Sites 1 (completely unsprayed) and 2 (commercial INGARD blocks with an area of unsprayed cotton to serve as a control), respectively, on Farm F1. Protocol B was used on leaves from Site 3 on Farm F2. INGARD varieties on Site 3 were bioassayed for larval performance at the specific request of Cotton Seed Distributors Ltd.

Estimates of larval survival obtained using protocol A were based on a single larva per leaf whereas estimates based on protocol B were based on 5 larvae per leaf. The two protocols were employed for two reasons. The first reason was to make bioassay results from CQ comparable to similar data on INGARD performance in southern Queensland and New South Wales. The second reason was to ensure continuity with bioassay data from the previous two seasons. The results of leaf bioassays on a range of INGARD cotton varieties are summarised in Tables 3.1.1-3.1.3.

Table 3.1.1 shows estimates of larval survival on INGARD and unsprayed control (non-INGARD) leaves based on protocol A. In all the control treatments (shaded rows), percent survival on control leaves was considerably below the expectation (100%) early in the season but increased after mid-November. The most likely explanation for this pattern of survival is the high frequency of pesticide application for heliothis control and the resulting insecticide drift onto unsprayed cotton in the first 10-12 weeks of the season.

With the exception of NuCotn37*i*, estimates of larval survival on INGARD varieties listed Table 3.1.1 are variable but generally below 25%. The survival estimate of 87% for NuCotn37*i* on 29 October is substantially higher than all other estimates and, as such, is difficult to explain.

Table 3.1.1. Survivorship and larval growth index* of Helicoverpa armigera larvae when reared for 5 days on INGARD and non-INGARD leaves using protocol A (see text for explanation). Leaves used in the bioassay procedures were obtained from Site 1 on Farm A.

		Date/Month					
Variety	Variable	29/10	6/11	12/11	20/11	27/11	4/12
Siokra V15 (Block 1)	Crop age (days)	31	39	45	53	60	67
	% Survival	**42**	**29**	**49**	**89**	**93**	**91**
	Growth index	1.85	1.80	1.27	2.27	2.10	2.80
Siokra V15i (Block 1)	Crop age (days)	31	39	45	53	60	67
	% Survival	**11**	**2**	**13**	**49**	**49**	**47**
	Growth index	1.30	1.50	1.70	2.07	1.60	2.03
Siokra V15ii (Block 1)	Crop age (days)	31	39	45	53	60	67
	% Survival	**16**	**9**	**27**	**9**	**53**	**56**
	Growth index	1.00	1.75	1.90	1.43	1.20	1.73
Siokra V15 (Block 2)	Crop age (days)	31	39	45	53	60	67
	% Survival	**60**	**36**	**69**	**93**	**100**	**87**
	Growth index	1.33	1.70	2.07	2.33	1.80	2.70
Siokra V15i (Block 2)	Crop age (days)	31	39	45	53	60	67
	% Survival	**27**	**2**	**13**	**67**	**38**	**56**
	Growth index	1.30	1.50	1.80	1.67	1.63	2.00
Siokra V15ii (Block 2)	Crop age (days)	31	39	45	53	60	67
	% Survival	**27**	**0**	**9**	**13**	**62**	**51**
	Growth index	1.40		1.17	1.10	1.27	1.70
Dp5690	Crop age (days)	31	39	45	53	60	67
	% Survival	**89**	**20**	**84**	**62**	**100**	**93**
	Growth index	1.97	1.47	1.93	2.40	2.03	2.90
Dp5690i RR	Crop age (days)	31	39	45	53	60	67
	% Survival	20	**2**	**2**	**73**	**89**	**89**
	Growth index	1.63	1.00	1.50	1.53	1.13	2.07
Dp5690i	Crop age (days)	31	39	45	53	60	67
	% Survival	**16**	**0**	**13**	**80**	**87**	**76**
	Growth index	1.17		1.10	1.77	1.17	2.17
Delta pearl	Crop age (days)	31	39	45	53	60	67
	% Survival	**69**	**42**	**87**	**82**	**98**	**98**
	Growth index	1.87	1.77	1.73	2.30	1.90	2.73
Nu pearl (Block 1)	Crop age (days)	31	39	45	53	60	67
	% Survival	**29**	**0**	**22**	**71**	**76**	**76**
	Growth index	1.40		1.10	1.83	1.10	2.07
Ingard nucot 37i	Crop age (days)	31	39	45	53	60	67
	% Survival	**87**	**2**	**13**	**58**	**91**	**87**
	Growth index	2.07	1.50	2.03	1.73	1.30	2.13
Nupearl (Block 2)	Crop age (days)	31	39	45	53	60	67
	% Survival	**27**	**0**	**21**	**39**	**70**	**72**
	Growth index	1.50		1.47	1.65	1.20	1.33

*See Appendix 1

The bioassay done on 20 November indicates a sharp increase in larval survival in all single-gene INGARD lines and continuing high survival over the remainder of the bioassay period (early December, Table 3.1.1). The increase in survival observed on single-gene INGARD varieties was manifested a week later in the double-gene variety (Siokra V15*ii*).

The results of bioassays on commercial INGARD on Farm F1 (site 2) are presented in Table 3.1.2. The bioassay on 20 November was not done because all commercial cotton on the farm had been sprayed the previous day. Larval survival on the two INGARD varieties was variable but low until 12 November (Table 3.1.2). The last two bioassays indicated markedly increased survival, indicative of sub-optimal expression of the toxin gene. The marked increase in larval survivorship is likely to have commenced at the same time as in site 1 because both sites were on the same farm, within 100m of each other.

Table 3.1.2. Survivorship and growth index* of Helicoverpa armigera larvae when reared for 5 days on INGARD and non-INGARD leaves using protocol B (see text for explanation). Leaves used in the bioassay procedures were obtained from Site 2 on Farm A.

		Date/Month					
Variety	Variable	29/10	6/11	12/11	11/20	27/11	4/12
Sicot 189i	Crop age (days)	31	39	45		60	67
	% Survival	**13**	**5**	**9**		**66**	**54**
	Growth index	1.42	2.90	1.44		1.14	2.22
Siokra V15	Crop age (days)	31	39	45		60	67
	% Survival	**85**	**47**	**56**		**98**	**88**
	Growth index	2.48	2.01	1.70		1.62	2.60
Nucot 37i	Crop age (days)	31	39	45		60	67
	% Survival	**7**	**0**	**23**		**88**	**70**
	Growth index	1.43		1.20		1.24	2.01

*See Appendix 1

Farm F2 did not have any unsprayed non-INGARD cotton. As a consequence, the bioassay procedure for Farm F2 did not include a control treatment. The absence of a control treatment makes the data in Table 3.1.3 difficult to interpret. However, the similarity in the pattern of larval survival on INGARD varieties in Tables 3.1.1-3.1.3 suggests that INGARD performance on Farm F2 was not substantially different from that on Farm F1.

The larval growth index (see Appendix 1 for details) enables comparisons of INGARD efficacy between treatments. Growth index scores varied between bioassay dates, varieties and sites (Tables 3.1.1-3.1.3). In general, growth index scores on INGARD leaves were lower than corresponding scores on non-INGARD leaves. This indicates that survivors on INGARD leaves grew at lower rates than their counterparts maintained on non-INGARD leaves even after the onset of a decline in efficacy as measured by markedly higher survival of larvae.

Table 3.1.3. Survivorship and growth index* of Helicoverpa armigera larvae when reared for 5 days on INGARD and non-INGARD leaves using protocol B (see text for explanation). Leaves used in the bioassay procedures were obtained from Site 3 on Farm B.

		Date/Month					
Variety	Variable	29/10	6/11	12/11	20/11	27/11	4/12
Sicot 50i	Crop age (days)	36	45	51		65	72
	% Survival	**4**	**0**	**15**		**42**	**62**
	Growth index	1.38	1.75	1.60		1.36	2.27
Sicala V2i	Crop age (days)	36	45	51		65	72
	% Survival	**0**	**0**	**5**		**22**	**26**
	Growth index					1.41	2.15
Exp 666	Crop age (days)	36	45	51		65	72
	% Survival	**7**	**0**	**0**		**8**	**16**
	Growth index	1.43				1.00	1.81
Sicot 189i	Crop age (days)	36	45	51		65	72
	% Survival	**4**	**0**	**17**		**52**	**36**
	Growth index	2.00		1.32		1.50	1.97

*See Appendix 1

Performance under field conditions

Actual estimates of larval survival under laboratory conditions (Tables 3.1.1-3.1.3) did not reflect survival or damage to INGARD crops under field conditions. Fig. 3.1.1 shows the number of times INGARD cotton was sprayed for heliothis control in relation to adjacent conventional cotton on five randomly selected farms. On all five farms insecticide application for heliothis control was recommended half the number or fewer times on INGARD than on adjacent conventional cotton (Fig. 3.1.1).

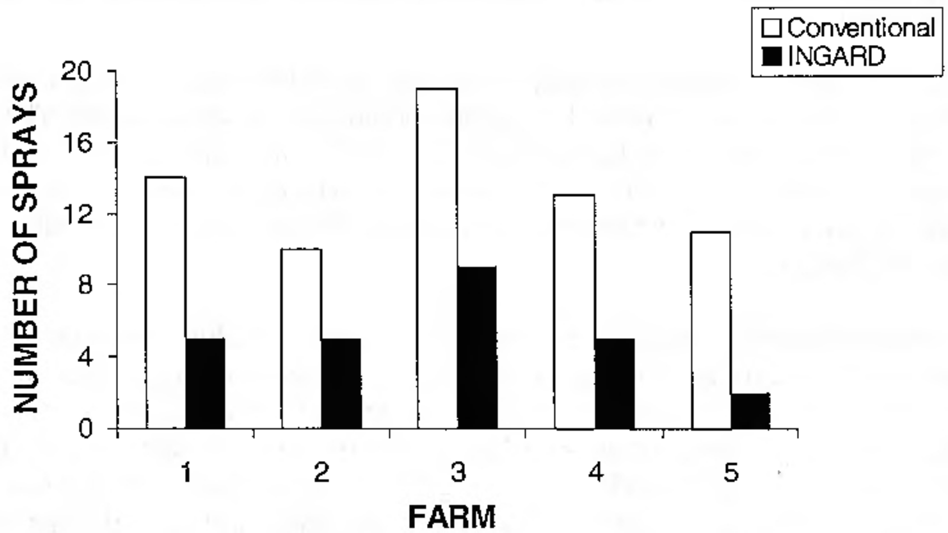


Fig. 3.1.1. Heliothis control on INGARD and conventional cotton on five randomly selected farms in the Emerald area during the 1998-99 season.

Most insecticide applications to INGARD occurred later in the season, beginning roughly at the time that the jump in larval survival was observed in the leaf-bioassay results. Feed back from growers and consultants indicated a high level of satisfaction with the general performance of INGARD cotton in terms of heliothis control.

3.2 Refuge value of cotton and other crops (Protocol 2)

The refuge value (number of pupae/moths produced per unit area of crop) of conventional, INGARD and unsprayed conventional cottons was evaluated throughout the season. Table 3.2.1 shows a summary of the data collected from May 1998 to June 1999. Detailed data, including estimates of parasitism and sampling details are presented in Appendix 5. Sources of heliothis populations were distributed in and around the irrigation area in the winter and spring of 1998. Substantial numbers of heliothis pupae were collected under commercial chickpea crops throughout October and November (Table 3.2.1).

With the exception of a small area of dolichos, there were few, if any, sources of moths within the irrigation area in the first part of the season 1998-99 cotton season. The intense pressure on early-season cotton within the irrigation area was most likely the result of moths emerging from dryland chickpea crops and migrating into the irrigation area. As in previous years, cotton produced substantial numbers of pupae in the second half of the season, primarily January and February (Table 3.2.1).

Table 3.2.1. Abundance and time of heliothis pupae production under cotton and other crops in the Emerald area from May 1998 to May 1999.

			Month and Year										
Crop	Zone[+]	Variable	May 98	Jun 98	Jul 98	Oct 98	Nov 98	Dec 98	Jan 99	Feb 99	Mar 99	Apr 99	May 99
Corn	1	pupae/ha	22000	17000									
Dolichos	1	pupae/ha		35500			500						
Mung bean	1	pupae/ha							0				
Cotton	1	pupae/ha							217	2100			
Cotton (Ingard)	1	pupae/ha								1375			
Cotton	2	pupae/ha		70000									
Chickpea	2	pupae/ha				231000	85250						
Mung bean	2	pupae/ha									0		
Sunflower	2	pupae/ha										0	
Corn	2	pupae/ha											17000

+ 1 = within Emerald Irrigation Area; 2 = outside Emerald Irrigation Area

The data in Table 3.2.1 suggest that a likely pattern of heliothis population movement was from mid-summer crops (corn, dolichos) in May/June 98 to commercial chickpea crops (August-November 98), and in turn from chickpeas to cotton. Moths leaving cotton at the end of the season are likely to have migrated out to dryland crops in late summer of 1999. Thus there is potential for dilution of INGARD and conventional insecticide resistance genes after the end of the cotton season.

The timing and extent of diapause initiation in the Emerald area was quantified by recording observations of pupal morphology from pupae collections made between June and October 1998. From a total of 1379 pupae, 765 or 55.47% were found to be in diapause.

3.3 *INGARD resistance management strategy for CQ*

In accordance with the resistance management strategy (Appendix 4) and licence requirement for growing INARD cotton, 1% of the cropping area of every farm planning to grow INGARD cotton was planted to a pigeon pea trap crop. In a variation of the previous season's protocol wherein pigeon pea trap crops were planted contemporaneously with cotton, at the start of the 1998-99 season growers were advised to plant pigeon pea later, in the second half of November. The reason for recommending this variation was the concern expressed by some growers that pigeon pea crops could have served as nurseries for heliothis at least for a short period of time during the previous season when the crops were carrying substantial numbers of larvae in late-December and January. Although there was no evidence to substantiate this concern, it was decided to trial a later planting of pigeon pea so as to minimise the risk of creating in-season nurseries.

Table 3.3.1. Totals of heliothis larvae on pigeon pea trap crops in the Emerald area during the 1998-99 season.

		Rep	Instars				
Farm	Date	(1 metre)	1st & 2nd	3rd	4th & 5th	6th	Total
F3	16-Feb-99	10 x 1	37	191	563	29	819
F4	18-Feb-99	10 x 1	9	134	221	3	367
F5	18-Feb-99	10 x 1	18	67	174	8	267

Poor seed quality and adverse weather conditions appear to have combined to produce poor stands, many of which were overtaken by weeds and subsequently abandoned. The overall result was that there were only a few pigeon pea patches that had reasonable stands (4-6 plants/m) and were able to serve as traps for end-of-season heliothis populations. Pupae digs under four such pigeon pea crops in February indicated an average of 4875 pupae per hectare, with a pupal parasitism rate of 28%. Drop-sheet samples of larvae on the pigeon pea plants indicate that the crops with reasonable stands were carrying substantial numbers of grubs, as shown in Table 3.3.1 above.

3.4 Area-wide management strategy (AWMS) for heliothis - Winter trap crops

In accordance with AWMP protocols, growers within the irrigation area also planted 1% of cultivated farm area to a chickpea trap crop in June/July. The majority of growers planted chickpea trap crops and got them fully established. The crops picked up large egg lays in early July, and by mid-August some were carrying in excess of 30 larvae/metre (Table 3.4.1).

The majority of crops in the area were ploughed out around the 15th of September. This turned out to be timely as some crops were still carrying in excess of 30 larvae/metre (Table 3.4.1). If these crops were carried any longer they would have lost attractiveness because of water stress.

Three of the most attractive looking crops in the area were given an extra irrigation in early September and sprayed with Gemstar®. The objective of the extra irrigation and spraying with Gemstar® was to determine whether or not the trapping potential of the trap crops could be extended by reducing the larval population on them and thereby minimising the potential for moth emergence (and risk to cotton) from these crops.

Gemstar® applications at 250 ml with milk powder in 100 litres of water gave very poor results. An application of 500 ml with milk powder in 100 litres of water on another crop resulted in around 70% mortality. Thus, in CQ a 'cotton' rate of Gemstar® (minimum 375ml) may be the lowest rate at which acceptable results can be obtained.

Table 3.4.1. Totals of heliothis larvae on chickpea trap crops in the Emerald area during the 1998-99 season.

Date	Location	Sample size (x 1m²)	Number of heliothis grubs							Plant Height
			W.E.	B.E	Vs	Sm	M	L	Total	
7/29/1998	A	10	13	6	52	33	3	0	**88**	14 cm
8/10/1998	A	10	6		14	66	49	3	**132**	20cm
8/20/1998	A	10			1	11	62	2	**76**	30cm
7/31/1998	B	10	1		27	52	20	0	**99**	36cm
8/11/1998	B	10			12	87	56	2	**157**	45cm
8/21/1998	B	10			1	14	85	13	**113**	45cm
7/30/1998	C	10		2	49	103	29	0	**181**	15 cm
8/10/1998	C	10			22	80	179	11	**292**	23cm
8/20/1998	C	10				24	122	9	**155**	33cm
7/31/1998	D	10			65	179	59	7	**310**	35cm
8/11/1998	D	10			3	55	59	8	**125**	40cm
8/21/1998	D	10			1	10	102	18	**131**	50cm
7/30/1998	E	10	3		64	56	35	6	**161**	25 cm
8/12/1998	E	10			20	124	108	18	**270**	43cm
8/19/1998	E	10			4	73	207	31	**315**	45cm
7/30/1998	F	10	2		42	69	19		**130**	25 cm
8/12/1998	F	10			25	111	99	11	**246**	31cm
8/19/1998	F	10			2	43	45	19	**109**	43cm

The above exercise to extend the trapping potential of chickpea trap crops was only partially successful in that Gemstar® at 375 ml was effective, but the crops cut out and dried off within 3 weeks of the extra irrigation. The most plausible explanation for this result is the susceptibility of chickpea to water logging.

3.5 Summary of 1998-99 results

The pattern of seasonal changes in INGARD efficacy both in the laboratory and field proved to be similar to that seen in previous years. The majority of growers in the area expressed satisfaction with the performance of the product.

After two seasons of experience with the pigeon pea trap-cropping technique, there is now a better understanding of the strengths and weaknesses of the tactic. On the positive side, the data show that appropriately managed crops have the potential to become effective heliothis sinks and thereby play an important role in INGARD resistance management. A potential weakness is that the trap cropping programs calls for highly coordinated action and synchronous implementation by growers across the area. This is not always easy to achieve especially when weather conditions are not favourable. During the current cotton season, rain-delayed planting and poor seed quality affected plant stand in many pigeon pea trap crops. These crops were planted in a window extending from November though to the end of December, effectively putting some crops out of sink with cotton.

4.0 Summary of project achievements

Cotton now ranks as the second most important export crop from Australia, generating export income of about $1 billion annually. Cotton growers in Australia spend about $100 million annually on insect control, most of it on spraying insecticides for the control of heliothis species. Cotton has been grown in central Queensland for almost 30 years, albeit at a cost. CQ cotton growers currently face mounting pressure from H. armigera and other, non-lepidopteran, pests because of increasing levels of insecticide resistance. INGARD cottons expressing the δ-endotoxin gene from the bacterium Bacillus thuringiensis (*Bt*) offer new hope for the management of heliothis_and other lepidopteran pests, and so pave the way for a greatly reduced dependence on insecticides.

Aside from generating data on the insecticidal efficacy of INGARD over a period of three years, the principal objective of project DAQ81C was to develop a resistance management strategy for INGARD that was specifically tailored to CQ conditions. This objective was achieved within 12 months of the project being commissioned. Success in achieving the objectives of the project is reflected in the decision by the NRA to approve use of the INGARD product in CQ. The NRA's decision was based on data from a single season of trials and the proposed AWMS for heliothis in the region.

Three seasons of leaf bioassay data on seasonal changes in INGARD efficacy indicate that the efficacy of the product appears to decline 8-10 weeks after planting under CQ conditions. The experiences of growers and crop consultants in southern cotton growing areas seem to indicate that this pattern of efficacy decline is not unique to CQ. Under field conditions, INGARD cotton growers can expect to save approximately 50% of chemical costs for heliothis control. This has been shown to be the case in other regions as well. Therefore, it is reasonable to conclude that INGARD cotton crops grown under CQ conditions perform similarly to their counterparts in other cotton growing areas of Australia.

Whilst the heliothis attracting qualities of pigeon pea appears to be ideally suited to strategic manipulation for INGARD resistance management, the results of the last two seasons of pigeon pea trap cropping do not as yet permit a definite conclusion. The first season, 1997-98, was apparently very successful, with pigeon pea crops attracting large numbers of grubs at the end of the season. The second was plagued by logistical problems, untimely rainfall and issues related to seed quality and plant stand. However, those crops that grew even under less than ideal conditions were successful in trapping substantial numbers of grubs at the end of the season.

The experiences of the last three seasons of growing pigeon pea suggest that the difficulties associated with the use of pigeon pea trap crops for INGARD resistance management are almost entirely related to agronomic management rather than strategic manipulation. Adequate testing of the INGARD resistance management plan for CQ has proven to be beyond the scope of this project, and warrants further evaluation.

A novel outcome of the project has been the development of an AWMS centred on trap cropping for heliothis in CQ. Currently in its third cycle, the trap-cropping program continues to show good promise as a tool for heliothis management.

5.0 Acknowledgments

The scouting data on heliothis eggs and larvae on INGARD and conventional cotton (Sections 1.0 and 2.0) were provided by Duane Evans, Rodger Lindeman, Amanda Noone and Simon Struss. Their assistance in providing the data and helpful discussions are gratefully acknowledged. Guidance and support from Drs. Gary Fitt, Neil Forrester, David Murray, Ian Titmarsh and Lewis Wilson are gratefully acknowledged. Financial support for this work was provided by the Cotton Research and Development Corporation.

6.0 Literature cited

Brown, A. H. D., C. L. Brubaker and M. J. Kilby, 1997. Assessing the risk of cotton transgene escape into wild Australian Gossypium species. Pages 83-94. **In**: Commercialisation of transgenic crops: Risk, benefit and trade considerations. Proceedings of a workshop held in Canberra, 11-13 march 1997. G. D. McLean, P. M. Waterhouse, G. Evans and m. J. Gibbs (eds.). Cooperative Research Centre for Plant Science and Bureau of Resource Sciences, Canberra.

Appendix 1

Research program and protocols for trial plots and commercial blocks of INGARD cotton.

- **Protocol 1: Effectiveness of INGARD against heliothis.** Laboratory leaf bioassays to be conducted fortnightly, throughout the cotton season, to quantify changes in insecticidal action over the season. Excised cotton leaves (node 3 from the top) to be brought in from the field and put into FALCON petridishes. 5 neonate H. armigera larvae to be put on each of 40 INGARD cotton leaves, 20 conventional, unsprayed cotton leaves (control), 10 dryland INGARD cotton leaves. Larval survival to be recorded after 3 and/or 5 days.

  Larval growth index. Larvae used in bioassays will be assigned scores after 5 days on test leaves as follows: Dead = 0; 1st instar, non-feeding = 1; Late 1st instar (feeding) = 1.5; Early 2nd instar = 2; Late 2nd instar = 2.5; 3rd instar = 3.

- **Protocol 2: Assessing the refuge value of various crops relative to INGARD cotton for the management of resistance to the Bt toxin.** The field research plan is based on five irrigated plots (E1-E5) at Emerald and four irrigated plots (DC1-DC4) at in the Dawson/Callide valley. Each plot at Emerald and Dawson valley will consist of three treatment options, viz., INGARD cotton (20 hectares), a refuge crop (6 hectares of a non-cotton crop) and a control (1-2 hectares of unsprayed non-Bt cotton). Plots E1 and E2 will include an additional treatment, viz., conventional cotton (10 hectares). The non-cotton refuge crops to be evaluated are Adzuki bean, Pigeon pea, Sorghum, Corn and Sunflower. Every non-cotton refuge option will be replicated twice. Of the six plots at the Emerald site, E1 will be located at the Emerald Agricultural College, whereas E2-E5 will be located on commercial cotton farms. The Dawson valley plots (DC1-DC4) also will be located on commercial cotton farms. An additional plot consisting of dryland-INGARD and control treatments (labelled DRYLAND) will be located at Emerald. Cotton is scheduled to be planted by the first week of October whereas the refuge crops will be staggered; the first planting by the third week of October, the second and third plantings 1-1.5 months apart.
  - *Aspect 1.* Specific refuge crops.

    Monthly monitoring of Helicoverpa pupae and moths, from September to April, by soil digging (plots E1, E2, DC1-DC4) and emergence cages (plots E1-E6). Methods and equipment adopted from protocol of Dr. Gary Fitt (ACRI) for similar research in NSW.
  - *Aspect 2.* Other summer crops in irrigation/dryland area.

    Monthly monitoring of Helicoverpa pupae, from September to April, by soil digging. Crops to be sampled include Sorghum, Mung bean, Dolichos and dryland Cotton.
  - *Aspect 3.* Quantification of diapause in pupal Helicoverpa.

    Digging for pupae once during winter at 16-20 sites in and around Emerald Irrigation Area (including dryland crops).
  - *Aspect 4.* Effect of cultivation on pupal survival.

    Digging for pupae once prior to cultivation in each INGARD plot (E1-E6) followed by post-cultivation erection of cages to trap emerging moths.
  - *Aspect 5.* Refuge value of winter crops.

Monthly monitoring of heliothis pupae, from May to August, by soil digging. Crops to be sampled for pupae include Chickpea (cv. Amethyst and Macareena) and Lucerne.

- **Protocol 3: Environmental impact assessment.**

Fortnightly monitoring, from October to March, of fauna present in INGARD cotton relative to conventional (sprayed) and unsprayed (control) cotton. Suction and pitfall-trap samples to be collected from plots El and E2 and sent to Dr Fitt, ACRI Narrabri, for identification by his team.

- **Protocol 4: Native Gossypium species.**

Prior to planting INGARD cotton, surveys will be conducted to examine the proximity of selected sites to stands of native Gossypium species. The density of native Gossypiums will be recorded within a 1 km radius of each site.

- **Protocol 5: Harvest and cleanup of trial sites.**

  INGARD cotton will be machine harvested, pressed into modules and ginned as a job lot through a gin at Emerald and Biloela. After ginning, lint will be baled and sold; seed will be isolated from conventional seed and be on-sold within Australia for crushing purposes only. The sites used for INGARD cotton crops in 1996/97 will not be used for cotton production the following season. Instead, the sites will be rotated into an alternative crop such as winter wheat or faba bean immediately following the transgenic cotton (sown in May 1997, harvested in November 1997) and followed by either a summer fallow or a summer legume such as Dolichos. Any volunteer cotton plants emerging in the rotation crops will be obvious and removed through cultivation, hand chipping, or use of an appropriate herbicide.

Appendix 2

Plot descriptions for INGARD trials of the 1996-97 season.

Farm	Locality	Treatment	Area (ha)	Plant date
E1	Emerald	Ingard (CS50i)	15.407	23 Oct
		Unsprayed Ingard (CS50i)	5.538	23 Oct
		control	2.496	23 Oct
		Adzuki bean	6	
		conventional	50	6 Oct
E2	Emerald	Ingard (CS50i)	15.401	14 Oct
		Unsprayed Ingard (CS50i)	5.5216	14 Oct
		control	1.1424	14 Oct
		Adzuki bean	6	
		conventional		5 Oct
E3	Emerald	Ingard (CS50i)	15.614	18 Oct
		Ingard (NuCotn37i)	5.2656	18 Oct
		control	1.073	20 Oct
		sorghum	3	
		corn	3	
		conventional		11 Oct
E4	Emerald	Ingard (CS50i)	37.377	15 Oct
		Ingard (NuCotn37i)	5.639	15 Oct
		control	2.154	15 Oct
		pigeon pea	3	
		sunflower	3	
		sorghum	3	
		corn	3	
		conventional	100	20 Sep
E5	Emerald	Ingard (CS50i)	23.204	24 Oct
		control	0.967	24 Oct
		pigeon pea	3	
		sunflower	3	
		conventional		29 Oct
DC1	Biloela	Ingard (CS50i)	20	14 Oct
		control	2	14 Oct
		conventional		27 Oct
DC2	Moura	Ingard (CS50i)	20.166	25 Oct
		control	1.44	25 Oct
		conventional		25 Oct
DC3	Theodore	Ingard (NuCotn37i)	20	28 Oct
		control	2	10 Oct
		conventional		12 Oct
DC4	Theodore	Ingard (CS50i)	25.2	18 Sep
		control	2	18 Sep
		conventional		17 Sep
DRYLAND	Emerald	Ingard	2.2	

Appendix 3

Yield data for 1996-97 INGARD trials.

Farm	Treatment	Raw Weight kg	Bales / ha
E1	CS50i	69060	7.50
E1	Unsprayed CS50i	14380	4.35
E1	Conventional	204794	6.73
E1	Control	2760	1.85
E2	CS50i	67880	7.38
E2	Unsprayed CS50i	19840	6.01
E2	Conventional	588250	9.20
E2	Control	3720	5.45
E3	CS50i	78900	8.46
E3	NuCotn 37i	28350	9.01
E3	Conventional	276550	9.02
E3	Control	4470	6.98
E4	CS50i	187200	8.38
E4	Nucotn 37i	26900	7.99
E4	Conventional	486500	7.95
E4	Control	5575	4.33
E5	CS50i	98460	7.49
E5	Conventional	107600	8.58
E5	Control	2180	3.77
DC1	CS50i	98270	8.08
DC1	Conventional	111162	9.14
DC1	Control	6543	5.38
DC2	CS50i	99449	8.04
DC2	Conventional	103555	8.04
DC2	Control	5596	6.33
DC3	NuCotn37i	93918	7.43
DC3	Conventional	198243	8.15
DC3	Control	9050	7.16
DC4	CS50i	150750	10.05
DC4	NuCotn37i		
DC4	Conventional	116666	9.8
DC4	Control	5704	4.69
D/L	CS50i		

Appendix 4

Area-wide management strategy (AWMS) for heliothis species in CQ

Richard Sequeira
Farming Systems Institute, Queensland Department of Primary Industries, Locked Mail Bag 6, Emerald, Queensland, Australia 4720.

Introduction

Helicoverpa armigera and H. punctigera, commonly called heliothis, continue to be the bane of field crops in central Queensland (CQ). The need to control heliothis translates into a continually increasing production cost for cotton. H. armigera is the problem species with high levels of resistance to most commonly used insecticides. The typical cost of insect control on cotton in CQ has risen from roughly $30/ha in 1966 to $800-1000/ha in 1999. The bulk of this cost arises from the need to control heliothis species throughout the season.

What is often described as high heliothis pressure in other cropping areas of eastern Australia may be considered normal in CQ. For example, during most of the 1997-98 season, cotton growers on the Darling Downs had to contend with heliothis pressure of around 15 eggs/metre, coupled with insecticide-resistant H. armigera comprising around 80% of the population as early as mid November. This was an unusual season for the Darling Downs not only because heliothis pressure was unusually heavy throughout, but also because H. armigera which usually appears much later in the season put in a strong showing very early in the season.

In comparison to the southern cotton growing areas, the Dawson and Callide irrigation areas of CQ experienced 100% H. armigera right from the start of the 1997-98 season, with heliothis pressure often in excess of 30 eggs/metre. Many crops sustained extensive damage and yield loss. In CQ, it is not unusual for the season to begin with almost 100% H. armigera. This is particularly true of the last few years. The traditional pattern of heliothis manifestation in which H. punctigera appears early in the season and is gradually displaced by H. armigera later in the season has been severely distorted in CQ. In general, the heliothis situation over the last few years has placed the CQ cotton industry in a 'serious, bordering on critical' condition.

The heliothis problem in CQ

The nature of the heliothis pest problem in any area must be examined within the context of heliothis pupae production under various crops during the whole calendar year. Table 1 shows the abundance and distribution of heliothis pupae under various crops in the Emerald irrigation area (EIA) and the surrounding rain-fed farming area over the period 1996-1999. Substantial numbers of pupae are produced under various crops within the EIA from between October and June. The pattern of pupae production within the irrigation area is complimented by pupae production in the rain-fed area between July and November (Table 1).

Given that the EIA is surrounded by rain-fed cropping areas, the pattern of pupae (and implicitly moth) production in the greater Emerald area (Table 1) is indicative of a cycle of migration to and from the irrigation area in spring and late summer,

Table 1: The pattern of Helicoverpa pupae production in relation to the time of year, crops grown and production system[1] in the Emerald area over the period 1996-1999.

1996-97		July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Cotton	Irrigated							1	4	4	4	4	
Chickpea	Irrigated				5	4							
Sorghum	Irrigated					4	2						
Sunflower	Irrigated						4						
Sorghum	Irrigated							3	3	4	4		
Soybean	Irrigated									3	3	3	4
Corn	Irrigated										3	4	
Navy Bean	Irrigated										3		
Sunflower	Dryland											3	
Sorghum	Dryland												4
Chickpea	Dryland												4
1997-98		**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**
Cotton	Irrigated						2	2	2				
Corn	Irrigated											4	4
Mungbean	Dryland						4						
Cotton	Dryland												5
Chickpea	Dryland	4											
1998-99		**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**
Cotton	Irrigated							1	2				
Mungbean	Dryland									2			
Chickpea	Dryland				5	5							
Corn	Dryland											4	

Legend	1	2	3	4	5
Pupae/ Ha	1-1,000	1,000-5,000	5,000- 10,000	10,000-50,000	>50,000
Pupae/m	0.0001- 0.1	0.1 – 0.5	0.5 - 1	1 - 5	>5

[1] Irrigated or rain fed crop production systems

respectively. The most likely pattern of moth movement involves large numbers of moths emerging from cotton in the second half of stage III (around mid-February) and infesting succeeding non-cotton summer crops in the EIA and surrounding rain-fed cropping areas. Re-infestation of spring crops and cotton in the EIA at the start of the following growing season is most likely facilitated by locally diapausing pupae and inward migration of moths from rain-fed winter crops (mainly chickpea) in spring (August-September).

The above pattern of heliothis population dynamics suggests that management of a heliothis pest problem on any crop in the EIA will be influenced by a number of factors. These factors include the presence of absence of other crops in the neighbourhood, locality and region, the heliothis history of the previous crops in the area and the time of year. The observed population dynamics in the Emerald area implies that management of heliothis should ideally be undertaken on an area-wide basis.

Development of the trap-cropping strategy

The principle of trap cropping for insect pest management has been prevalent in crop production for a very long time (Hokkanen 1991). Most studies on trap cropping have examined the potential of trap crops as in-season companion crops, growing adjacent to the main crop so as to divert insect pressure from the main to the trap crop throughout the life of the main crop (see Hokkanan (1991) for examples). However, in the published literature there are few examples of in-season trap crops that have provided a sufficiently high level of pest diversion to make the practice economically viable in commercial crop production.

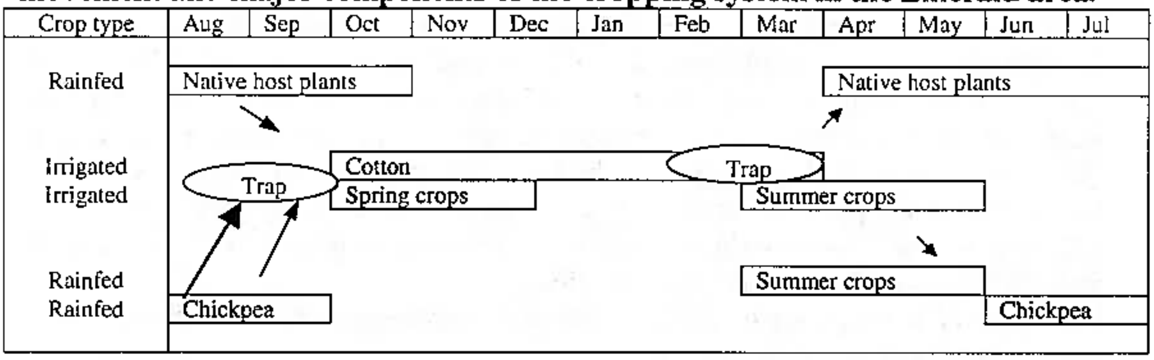
In contrast to the in-season model, the Emerald trap-cropping program is designed as a 'beginning-and-end-of-season' strategy (BEOS hereafter). The strategic objectives of the BEOS model of trap-cropping are as follows:

1. Trap and destroy final generation of heliothis emerging from cotton so that
    - The frequency of insecticide-resistant individuals (*H. armigera*) can be minimised,
    - carry-over of the cotton population to late-summer crops is minimised, and
    - migration of moths out of the irrigation area is minimised.
2. Trap and destroy first spring generation so that
    - early-season build-up of the pest is delayed,
    - early-season insecticide usage is minimised,
    - the frequency of insecticide resistance genes built up in the previous season could be minimised, and
    - an early-season build-up of beneficial insects is promoted

Fig. 1 shows the strategic positioning of the trap crops in relation to the components of the crop and moth production system in the greater Emerald area and the strategic objectives listed above. There are four distinct components of the production system that contribute to the heliothis problem in the EIA. These components are (1) uncultivated and cultivated volunteer host plants, (2) cotton and other spring/early summer crops such as corn, sorghum and mung bean, (3) winter crops, particularly chickpea, and (4) mid-summer crops such as sorghum, sunflower, grain and ley legumes, and horticultural crops.

In the absence of long-term scientific data on the population dynamics of the pest in the area, the development of the trap-cropping model is based on a largely conceptual framework and on grower/crop consultant experiences. Development and refinement of the trap-cropping program is expected to continue as more data on the population dynamics of the pest become available after implementation of each cycle of the program.

Fig. 1. Placement of trap crops in relation to perceived patterns of heliothis movement and major components of the cropping system in the Emerald area.



Trap cropping protocol

The current recommendation is that the program be implemented on a 'Farm' scale, that is, one patch of trap crop on every farm in the EIA. The action plan calls for patches comprising 1% of green acres to be planted to traps crops in June (chickpea) and October (pigeon pea). The rationale for the proposed patch size is a compromise between the willingness of growers to sacrifice potential cotton area and potential efficacy of the patch in attracting moths. The expectation is that if the patches are well distributed throughout the area, moths flying around the area are likely to find at least one of these patches and deposit their eggs.

Trap crops may be sprayed with nuclear polyhedrosis virus (NPV) to reduce larval load prior to their destruction. Pigeon pea crops should be left untreated for at least 3-4 weeks prior to destruction so as to ensure their attractiveness to the last generation of moths to emerge from cotton. It should be noted that chemical insecticides, especially the pyrethroid group, could potentially lower the attractiveness of the pigeon pea trap crops to heliothis moths for egg laying.

Chickpea trap crops planted in June should be destroyed by slashing and thorough soil disturbance. The crops can be destroyed either before the oldest larvae on the crops initiate pupation or 1-2 weeks before cotton crops are planted, depending on larval load on the crops and the perceived risk to young cotton.

Pigeon pea crops planted at the same time as cotton should be destroyed at the end of the cotton season. The timing of pigeon pea destruction will depend on recruitment of eggs and the development stage of the oldest larvae on pigeon pea relative to cotton. As a rough indication, pigeon pea destruction is likely to be within one week of cotton defoliation.

Strategy evaluation procedures

The effectiveness of an AWMS is difficult to assess because comparison with an untreated control is often not feasible. By definition, area-wide IPM strategies include all crops grown in the area that are susceptible to the pest. If the domain of an AWMS (the specific area included in the AWMS) is only a part of a larger cropping area, then the program is predestined for failure because insect pests do not recognise or abide by arbitrary boundaries. Thus, the definition of the target 'area' in an AWMS is critical in determining the outcome of the strategy.

In the absence of rigorous comparative measures of success or failure, the criteria by which the Emerald trap-cropping program could be evaluated include:

1. A historical perspective on average seasonal pest pressure and frequency of insecticide usage for control before and after the introduction of the AWMS.
2. The relative abundance of the two heliothis species before and after the implementation of specific components of the trap cropping program. For example, the predominance of H. armigera on chickpea trap crops prior to their destruction, followed by a sharp decline in H. armigera numbers relative to H. punctigera on the following cotton crops would indicate that the spring trap crops were effectively reducing levels of the 'troublesome' species.
3. The growers' perceptions on the benefits of the program and their willingness to continue participating in the project
4. Heightened awareness of IPM techniques and increased communication between groups of growers.

It is clear from Fig. 1 above that the trap-cropping program described above is restricted to component (2) of the system, namely, cotton and other summer crops within the irrigation area. The reason for this is ease of management and provision of research support in the early phases of program implementation and development. It is expected that after establishment of the program in component (2), it will be extended to broad-acre winter crops (component 3).

The trap-cropping program discussed above does not equate to an AWMS but rather constitutes one component of such a strategy. The development of an AWMS for the heliothis in the Emerald area will require the development of IPM options for other heliothis-producing components of the cropping system (see Fig. 1).

INGARD resistance management for CQ

The national INGARD resistance management strategy is based on i) the concept of gene dilution through the production of susceptible moths using 'refuge' crops, and ii) post-harvest tillage of the soil to destroy diapausing individuals (pupae) that may be carrying INGARD resistance genes. Under CQ conditions post-harvest soil cultivation is thought to be ineffective as a means of eliminating resistance genes that have been selected for during the season for two reasons. First, the incidence of diapause in Helicoverpa is thought to be low and unpredictable, often somewhat less than 50% of the total population. Second, simulation modelling and somewhat limited field data reveal that cotton is picked in CQ well before the onset of diapause. This implies that a vital component of the strategy used down south - post-harvest cultivation - will be ineffective in CQ.

The recovery of substantial numbers of Heliothis pupae from chick pea crops in October and pigeon pea after December (see Section 1.0) suggest the potential for development of an INGARD resistance management strategy that is likely to work in CQ. Accordingly, the strategy for CQ proposed to the NRA is as follows:

1. *Specific tactics for diluting the frequency of Bt-toxin resistance gene:*. INGARD refuge options approved for southern cotton producing areas should be approved for CQ. Specific requirements to be detailed by Monsanto Australia Ltd.

   Substantial numbers of moths are produced under various crops in the irrigation area (see Section 1.0) throughout the year, thereby indicating ample opportunity for dilution of INGARD resistance genes. Conventional cotton by itself produces large numbers of moths. Thus conventional cotton should be adequate as the refuge crop in the context of the dilution tactic. All non-cotton crops evaluated in the CQ trials produced substantial numbers of heliothis pupae/moths thereby indicating that sorghum and corn would be suitable refuges in the CQ environment.

2. *Specific tactics for managing end-of-season resistance build up:*
   - Post-harvest cultivation of INGARD fields. Specific requirements to be detailed by Monsanto Australia Ltd.
   - Every farm with INGARD cotton should be planted to a pigeon pea trap crop comprising not less than 1% (minimum 2 ha) of the cropping area of the farm.

   The pigeon pea trap crop is an additional component of the CQ INGARD resistance management strategy. The additional component is designed to compensate for the perceived ineffectiveness of post-harvest cultivation in CQ. The trap crop is to be planted and managed in line with the recommendations of the AWMS for heliothis discussed in the preceding sections.

Literature cited

Hokkanen, H. T. 1991. Trap cropping in pest management. Annual Review of Entomology 36: 119-38.

Appendix 5

Heliothis pupae collection data (1996-1999) categorised by date, crop[1] and location[2]

Year	Month	Crop	Location	Pupae/ha	%Parasitism
1996	10	Chickpea	1	85000	0.0
1996	10	Sorghum	1	0	0.0
1996	10	Soybean	1	0	0.0
1996	11	Chickpea	1	22500	0.0
1996	11	Chickpea	1	61500	0.0
1996	11	Mung bean	1	1000	0.0
1996	11	Sorghum	1	46500	59.8
1996	12	Sorghum	1	12000	0.0
1996	12	Sorghum	1	1000	0.0
1996	12	Sunflower	1	16500	36.8
1997	1	Cotton	1	0	0.0
1997	1	Cotton	1	0	0.0
1997	1	Cotton	1	500	0.0
1997	1	Cotton	1	0	0.0
1997	1	Sorghum	1	9500	0.0
1997	2	Cotton	1	39000	20.3
1997	2	Cotton	1	1500	50.0
1997	2	Cotton	1	22500	47.6
1997	2	Cotton	1	2000	0.0
1997	3	Sorghum	1	25000	79.2
1997	3	Sorghum	1	31000	91.1
1997	3	Sorghum	1	21000	93.8
1997	3	Soybean	1	14500	41.2
1997	3	Soybean	1	0	0.0
1997	4	Corn	1	7000	0.0
1997	4	Cotton	1	16000	100.0
1997	4	Cotton	1	5000	100.0
1997	4	Cotton	1	10000	100.0
1997	4	Cotton	1	2000	100.0
1997	4	Cotton	1	60000	0.0
1997	4	Cotton (dry)	2	0	0.0
1997	4	Cotton (dry)	2	0	0.0
1997	4	Cotton (Ingard)	1	0	0.0
1997	4	Mung bean	2	0	0.0
1997	4	Navy bean	1	9000	100.0
1997	4	Peanut	2	0	0.0
1997	4	Sorghum	1	16000	0.0
1997	4	Soybean	1	5500	0.0
1997	4	Soybean	2	0	0.0
1997	4	Soybean	2	0	0.0
1997	4	Soybean	1	5000	100.0
1997	4	Sunflower	2	0	0.0
1997	4	Sunflower	2	0	0.0
1997	4	Sunflower	2	0	0.0
1997	5	Corn	1	29600	60.9
1997	5	Corn	1	6000	75.0
1997	5	Cotton	1	14000	100.0
1997	5	Navy Bean	1	0	0.0
1997	5	Navy bean	1	0	0.0
1997	5	Navy bean	1	0	0.0
1997	5	Navy bean	1	0	0.0
1997	5	Navy bean	1	0	0.0
1997	5	Navy bean	1	0	0.0
1997	5	Pumpkin	1	0	0.0
1997	5	Sorghum	2	0	0.0
1997	5	soybean	1	0	0.0
1997	5	Sunflower	2	1000	100.0
1997	5	Sunflower	2	1000	100.0
1997	5	Sunflower	2	0	0.0
1997	5	Sunflower	1	0	0.0
1997	6	Chickpea	2	37143	51.4
1997	6	Sorghum	2	31000	52.4
1997	6	Soybean	1	19000	100.0
1997	7	Chickpea	2	30667	34.0

1997	9	Linseed	2	7500	66.7
1997	11	Mung bean	1	0	0.0
1997	11	Sunflower	1	0	0.0
1997	12	Cotton	1	3000	0.0
1997	12	Mung bean	2	31500	52.4
1997	12	Pigeon Pea (T)	1	101000	28.7
1997	12	Pigeon pea (S)	1	44000	6.3
1997	12	Pigeon pea (S)	1	3000	0.0
1997	12	Pigeon pea (S)	1	16000	6.3
1998	1	Cotton	1	7000	0.0
1998	1	Cotton	1	0	0.0
1998	1	Cotton	1	1000	0.0
1998	1	Cotton	1	5000	0.0
1998	1	Cotton (Ingard)	1	1000	0.0
1998	1	Cotton (Ingard)	1	2000	0.0
1998	1	Cotton (Ingard)	1	0	0.0
1998	1	Cotton (Ingard)	1	2000	0.0
1998	1	Cotton (Ingard)	1	2000	0.0
1998	1	Cotton (Ingard)	1	4000	0.0
1998	1	Cotton (unsp)	1	0	0.0
1998	1	Cotton (unsp)	1	20000	0.0
1998	1	Cotton (unsp)	1	39000	4.0
1998	1	Cotton (unsp)	1	1000	0.0
1998	1	Cotton (unsp)	1	193000	20.4
1998	1	Cotton (unsp)	1	83000	21.7
1998	1	Pigeon Pea (S)	1	22000	0.0
1998	1	Pigeon Pea (S)	1	27000	19.2
1998	1	Pigeon Pea (S)	1	55000	25.0
1998	1	Pigeon Pea (S)	1	3000	0.0
1998	2	Cotton	1	0	0.0
1998	2	Cotton	1	5000	0.0
1998	2	Pigeon pea (T)	1	226400	29.4
1998	2	Pigeon pea (T)	1	191000	29.4
1998	2	Pigeon pea (S)	1	80000	0.0
1998	2	Pigeon pea (T)	1	150000	36.8
1998	2	Pigeon pea (S)	1	42000	22.9
1998	2	Pigeon pea (S)	1	31000	38.7
1998	2	Pigeon pea (T)	1	127500	31.4
1998	3	Cotton	1	0	0.0
1998	3	Cotton	1	0	0.0
1998	3	Cotton	1	0	0.0
1998	3	Cotton	1	0	0.0
1998	3	Cotton (dry)	2	0	0.0
1998	3	Cotton (dry)	2	0	0.0
1998	3	Mung bean	2	3000	0.0
1998	3	Pigeon pea (S)	1	32000	84.4
1998	3	Pigeon pea (S)	1	12000	58.3
1998	3	Pigeon pea (T)	1	675000	57.6
1998	3	Sorghum	2	0	0.0
1998	3	Sorghum	1	0	0.0
1998	3	soybean	1	0	0.0
1998	3	Sunflower	2	0	0.0
1998	5	Corn	1	22000	25.0
1998	6	Corn	1	17000	17.4
1998	6	Cotton	2	70000	53.6
1998	6	Dolichos	1	35500	34.3
1998	10	Chickpea	2	231000	0.0
1998	11	Chickpea	2	22500	100.0
1998	11	Chickpea	2	148000	10.0
1998	11	Dolichos	1	500	0.0
1999	1	Cotton	1	435	0.0
1999	1	Cotton	1	0	0.0
1999	1	Mung bean	1	0	0.0
1999	2	Cotton	1	6500	0.0
1999	2	Cotton	1	1000	0.0
1999	2	Cotton	1	500	0.0
1999	2	Cotton	1	0	0.0
1999	2	Cotton	1	2500	0.0
1999	2	Cotton (Ingard)	1	2000	0.0

1999	2	Cotton (Ingard)	1	0	0.0
1999	2	Cotton (Ingard)	1	3500	0.0
1999	2	Cotton (Ingard)	1	0	0.0
1999	2	Pigeon pea (S)	1	2500	0.0
1999	2	Pigeon pea (S)	1	7500	60.0
1999	2	Pigeon pea (S)	1	6500	50.0
1999	2	Pigeon pea (S)	1	3000	0.0
1999	3	Mung bean	2	0	0.0
1999	4	Sunflower	2	0	0.0
1999	4	Sunflower	2	0	0.0
1999	5	Corn	2	17000	4.8

[1] dry=dryland; S=short phenotype, T=tall phenotype; unsp=unsprayed
[2] 1=Emerald irrigation area, 2=rainfed cropping area