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**Project CSE65C**

**A reappraisal of sampling relationships  
and *Helicoverpa* feeding behaviour  
in INGARD cotton.**

**CSIRO Entomology  
Australian Cotton CRC  
Narrabri**

**A final report to the  
Cotton R&D Corporation**

January 2001



## SUMMARY

### *Distribution of Life stages of Helicoverpa on Conventional versus INGARD cotton varieties.*

The within-plant distribution of life stages of *Helicoverpa* spp. was compared in field crops of two conventional and two INGARD cotton varieties over two growing seasons, 1997/8 and 1998/9. The placement of eggs and subsequent distribution of larvae was lower in the canopy on INGARD than conventional cotton varieties over the first two phases of crop growth. Generally, a lower proportion of *Helicoverpa* was found in the terminal region of the canopy than in previous studies (Dillon and Fitt, 1995).

### *Movements of 1<sup>st</sup> instar larvae on Conventional versus INGARD cotton.*

First instar larvae travelled an average of 8 cm in the first 40 minutes after release on INGARD and conventional cotton varieties in the glasshouse. There was no significant difference in the distance travelled on each type. Approximately 50% of neonates had reached young leaves in 40 minutes, 20% were on terminals, 10% old leaves, 5% squares and the remainder on stems or lost.

### *Comparison of Whole-plant and Presence-Absence sampling protocols for INGARD versus Conventional Cotton varieties.*

A close relationship between presence-absence and whole-plant sampling of *Helicoverpa* life stages was confirmed. This further supports the validity of presence-absence sampling as an efficient technique for cotton scouting. The relationship of whole-plant counts to whole-plant presence-absence sampling was very similar for conventional and INGARD varieties. With terminal presence-absence sampling there was a difference in the relationship to whole plant counts between INGARD and conventional cotton. The relationship derived from conventional cotton underestimated the density of very small and small larvae on INGARD cotton. The relationships obtained here differed from those established in previous sampling studies. We note however, that our analysis depends largely on one season when *Helicoverpa* was abundant. Overall, we conclude that INGARD varieties do not require a sampling strategy markedly different from that used on conventional varieties, although further recalibration of sampling relationships will be valuable in the future.

## Objectives

- To compare the distribution of *Helicoverpa* life stages on conventional and INGARD cotton varieties in relation to sampling protocols.
- To assess the suitability of sampling protocols developed for *Helicoverpa* on conventional cotton varieties, for use with INGARD cotton varieties.

## Staffing

The research was conducted by an Experimental Scientist in CSIRO Entomology, Ms. Kirsti Abbott from 2/12/97 to 22/1/99, supervised by Dr. G. P. Fitt. The timing of Ms. Abbott's commencement and departure affected the quantity of useful data collection during cotton growing periods. After Ms. Abbott's departure, the data analysis and preparation of this report was completed by Dr. John Stanley (CSIRO Entomology, Canberra).

## Background

The introduction of INGARD cotton to the Australian Cotton Industry represents a major change in the approach to the management of *Helicoverpa* spp. INGARD cotton varieties possess the Cry IAc gene from *Bacillus thuringiensis*. This gene allows tissues of the plant to produce a Bt toxin effective against Lepidopteran larvae such as *Helicoverpa* when ingested during feeding. The toxicity to larvae depends on the dose of toxin each receives which in turn depends on the extent to which the plant tissues express the Bt gene. There is considerable variation in the toxicity of various plant tissues; for example, leaf tissues contain relatively high concentrations whereas flowers have relatively little Bt. This within-plant variability has raised concern that *Helicoverpa* larvae might be distributed differently on INGARD cotton compared to the conventional varieties. One possibility is that the insects may oviposit or establish on plant parts which do not express the insecticidal Bt gene as strongly as others.

Sampling protocols rely heavily on a known distribution of the pest within the crop to greatly increase sampling efficiency (Ives and Hearn, 1987, Dillon and Fitt, 1995). Past research has established a strong relationship between the number of insects on cotton plants and the proportion of plants infested with insects. Furthermore, a significant proportion of *Helicoverpa* spp. eggs and young larvae are concentrated in the terminal (top 20 cm) region of the plant. Using relationships between numbers in the terminal and numbers on the whole plant it is possible for crop scouts to search a smaller area of the plant whilst maintaining an accurate estimation of pest density. These relationships were determined in the past for conventional cotton.

If *Helicoverpa* spp. are distributed differently on INGARD varieties, the suitability of currently practiced sampling protocols may need modification for INGARD. The current sampling protocols were developed during the 1970's (Ives and Hearn 1987) and revalidated for current varieties and farming practices in the mid 1990's (Dillon and Fitt 1995). They form the basis of sampling routines in the computer based decision support package, CottonLOGIC.

## **Methodology**

### **Section 1**

#### ***Distribution of Life stages of Helicoverpa on Conventional versus INGARD cotton varieties***

The positions of life stages of *Helicoverpa* spp. were recorded in field crops of two conventional and two INGARD cotton varieties (Conventional; Sicala V2 and Siokra V15/V16, and INGARD; Sicala V2 and Siokra V15) over two growing seasons, 1997/8 and 1998/9. Plant parts were characterised as: upper young leaf, lower young leaf, upper old leaf, lower old leaf, square, bract, boll, stem, flower bud (square immediately before unfurling into flower), flower or terminal. "Terminal" in this dataset was the small region of unexpanded growth at the end of each stem, as opposed to the "terminal region" used by CottonLOGIC, which is the top 12 to 20 cm region of each plant. All crops were managed according to current agronomic practices. This ensured that the results are relevant to *Helicoverpa* spp. behaviour under current agronomic and pest control practices. With enough records of oviposition sites and position of very small to large larvae, the pattern of *Helicoverpa* spp. distribution on conventional and INGARD cotton can be compared.

Glasshouse experiments were also conducted to observe the establishment behaviour of first instar larvae on INGARD and conventional varieties. Newly emerged larvae were released onto potted cotton plants and their position recorded at 5-minute intervals for a period of forty minutes.

Although this study is primarily concerned with sampling relationships, it is possible that *Helicoverpa* spp. behave quite differently on INGARD cotton without appreciably violating sampling protocols. By directly recording the distribution and behaviour in the approach above, a basis is established for answering future questions about *Helicoverpa* spp. on INGARD cotton.

### **Section 2**

#### ***Presence-absence sampling protocols for INGARD versus conventional cotton varieties***

Using the same crop scouting data used in the life-stage distribution study, presence-absence tables were constructed. These compare the estimates of *Helicoverpa* spp. density from whole plant searches with estimates derived from presence-absence per plant or presence-absence per terminal (top 12 to 20cm of plant canopy). Two questions are of interest in this approach: a) Is the relationship between whole-plant counts and presence-absence in INGARD cotton reliable for predicting *Helicoverpa* spp. density? If so, b) Is the same relationship appropriate for conventional and INGARD cotton varieties?

## **Likely Impact of results for Cotton Industry**

The continuing priority of the cotton industry to reduce dependence on insecticides and implement an effective integrated approach to pest management (IPM) can not be achieved without accurate and reliable standards of sampling and appropriate action thresholds. Confirming, or otherwise, that sampling protocols established for *Helicoverpa* crop scouting are suitable for INGARD cotton varieties is essential for maintaining confidence in industry standards and the general relevance of previous research on the behaviour of *Helicoverpa* as a pest of cotton.

## **Project Technology (Commercial significance)**

The results of this work will continue to underpin the sampling protocols used by consultants and supported by the computer software of CottonLOGIC.

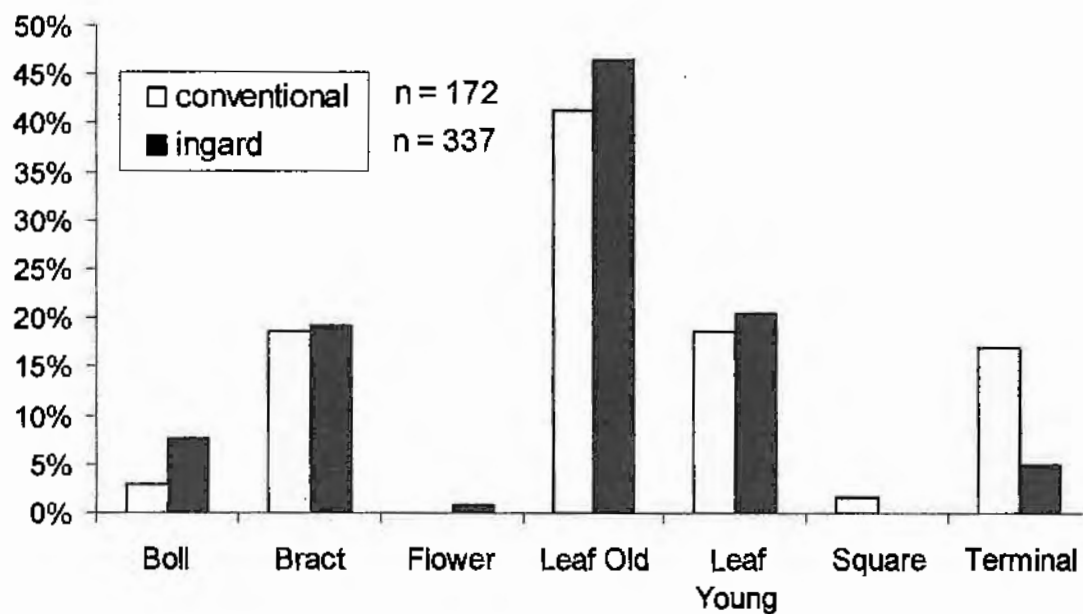
## **Section 1: Results and Discussion**

### ***Distribution of Life stages of Helicoverpa on Conventional versus INGARD cotton varieties***

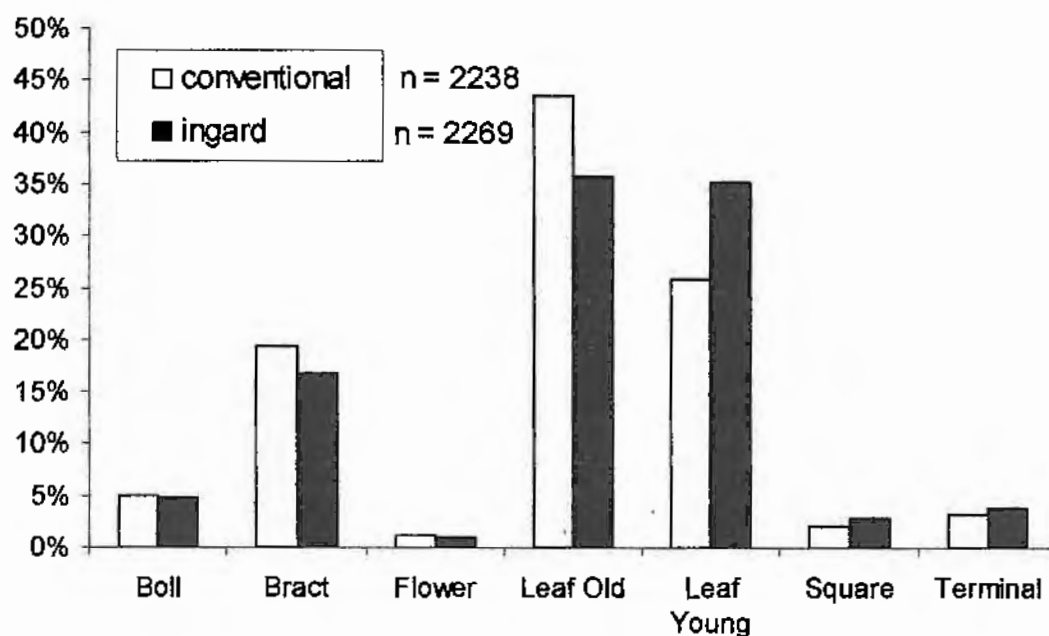
#### **Eggs**

The insecticidal properties of the INGARD protein do not affect eggs, therefore a comparison of the distribution of eggs is a direct indication of whether adult female *Helicoverpa* spp. prefer different sites for oviposition on INGARD or conventional cotton varieties. A consistent pattern of oviposition preference was displayed in each season, with older leaves receiving the most eggs, followed by young leaves and bracts, terminals, then squares (Fig. 1 and 2). Considerable variation in the proportion laid on each structure between seasons suggests that the displayed differences between INGARD and conventional cotton are not significant. Far fewer eggs were recorded in the 1997-98 season than in 1998-99. Our results should be more reliable in that year, where very little difference in egg distribution among plant structures was observed overall.

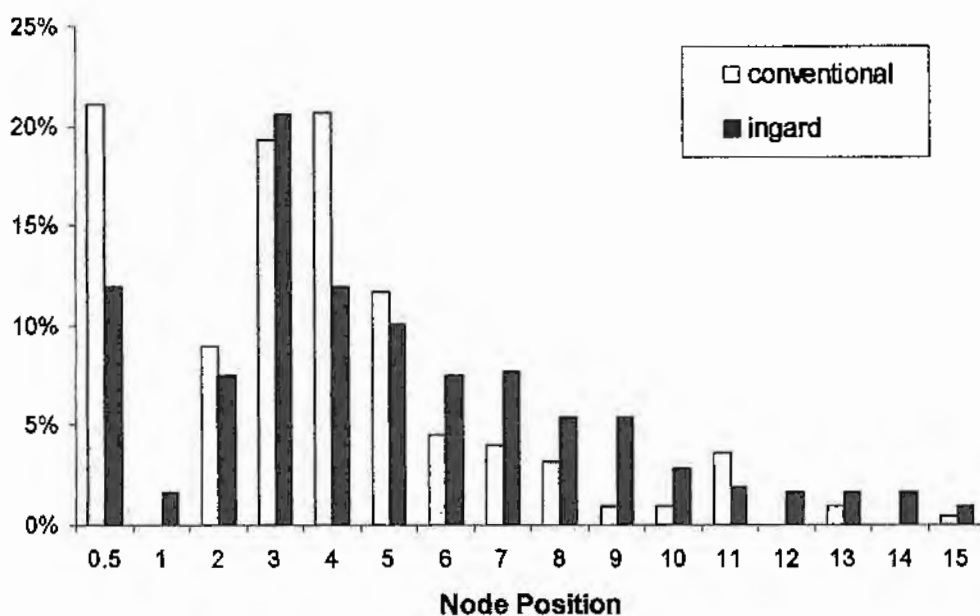
Figures 3 and 4 show the position of eggs by node position (counting down from the terminal) rather than plant structure. Again, the 1998-99 season (Figure 4) provides considerably more data and shows a very similar distribution for eggs on INGARD and conventional varieties overall.



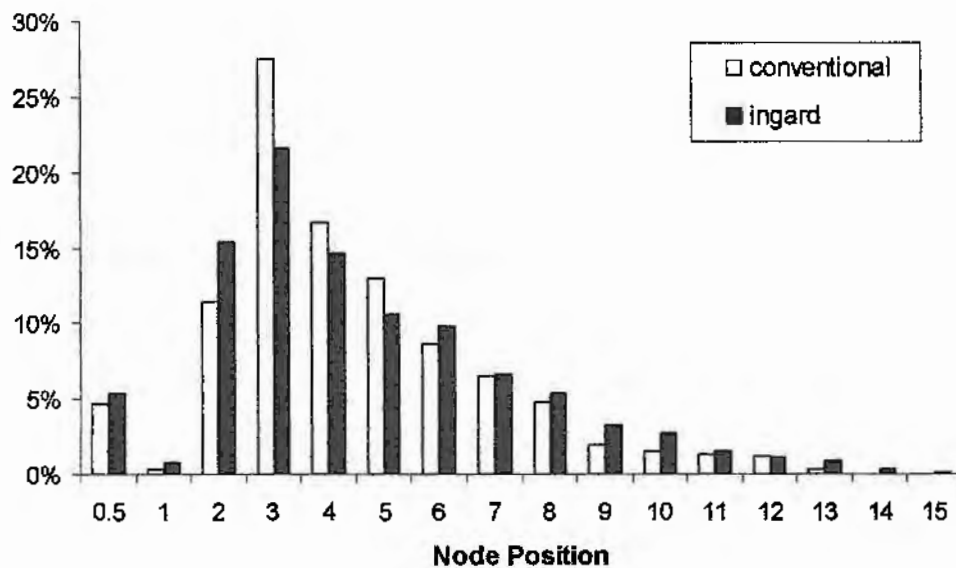
**Figure 1** The proportion (%) of White and Brown eggs on different plant structures of INGARD and conventional cotton in 1997-98.



**Figure 2** The proportion (%) of White and Brown eggs on different plant structures of INGARD and conventional cotton in 1998-99.



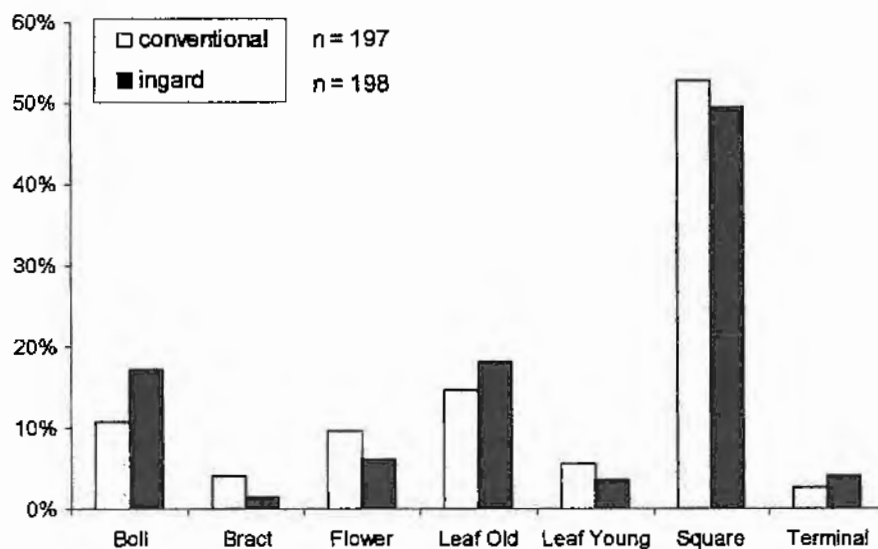
**Figure 3** The proportion (%) of White and Brown eggs on different nodes of INGARD and conventional cotton in 1997-98. Note: 0.5 node position is the region of terminal growth.



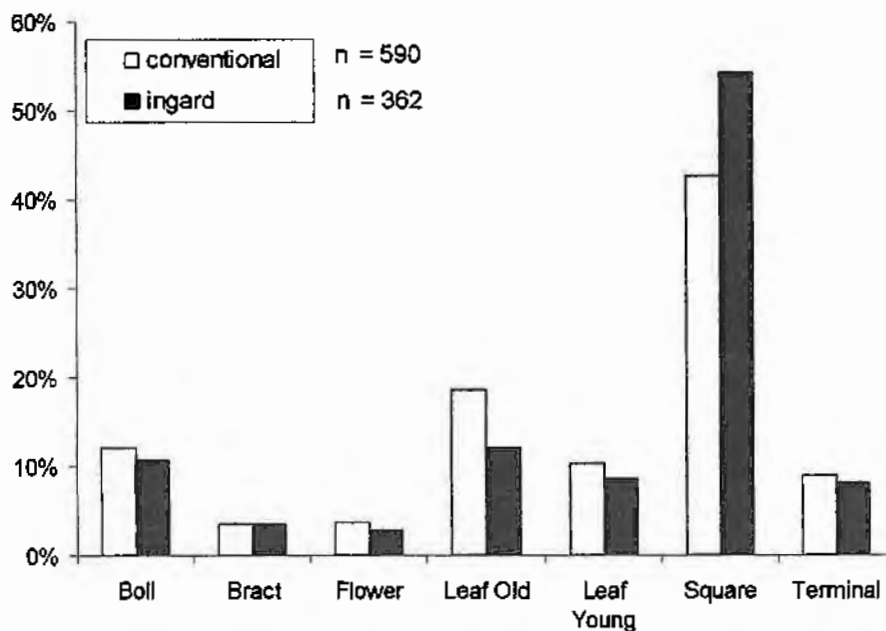
**Figure 4** The proportion (%) of White and Brown eggs on different nodes of INGARD and conventional cotton in 1998-99. Note: 0.5 node position is the region of terminal growth.

### Very Small and Small Larvae

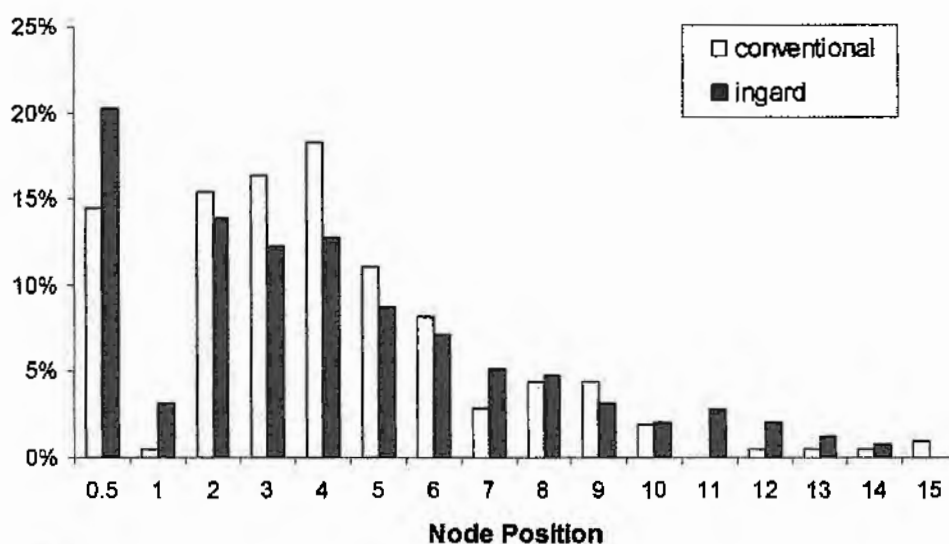
The preferred feeding sites of very small and small larvae were ranked similarly in the 1997-98 and 1998-99 seasons (Figs. 5 and 6). In 1997-98 the distribution is very similar on INGARD and conventional cotton but in 1998-99 a larger proportion of larvae on INGARD were apparently found in squares. There are also other differences displayed in 1998-99 (Fig. 6) but the lack of confirmation in the previous season questions that any of these differences are real. The distribution described by node position (Figs. 7 and 8) suggests that there is very little difference between the INGARD and Conventional varieties, at least for sampling purposes. However again during phases 1 and 2 a smaller proportion of very small and small larvae were present in the terminals of INGARD (Figs. 13 to 15).



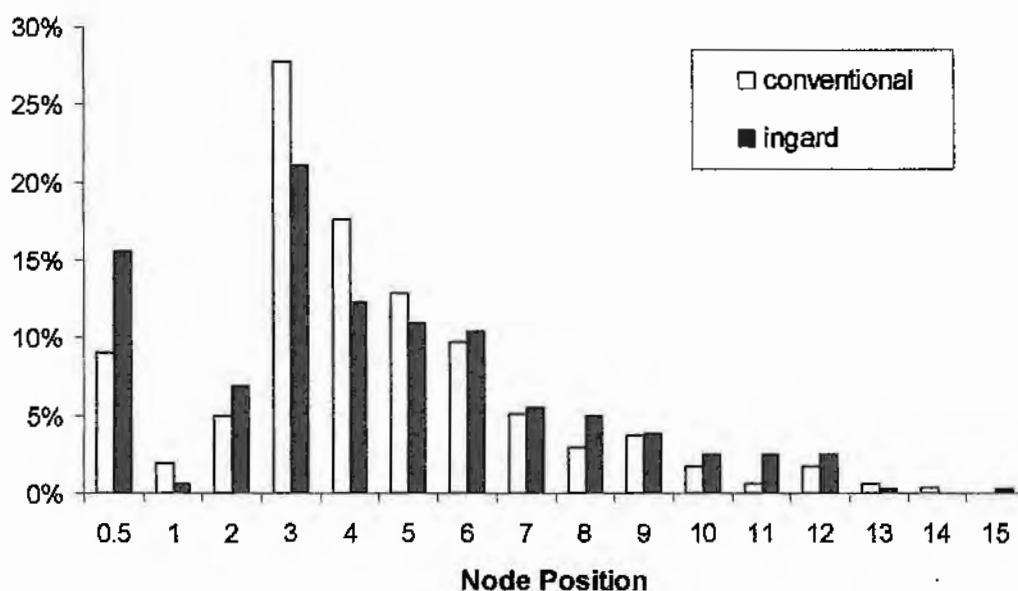
**Figure 5** The proportion (%) of Very Small and Small larvae on different plant structures of INGARD and conventional cotton in 1997-98.



**Figure 6** The proportion (%) of Very Small and Small larvae on different plant structures of INGARD and conventional cotton in 1998-99.



**Figure 7** The proportion (%) of Very Small and Small larvae at each node position of INGARD and conventional cotton in 1997-98. Note: 0.5 node position is the region of terminal growth.

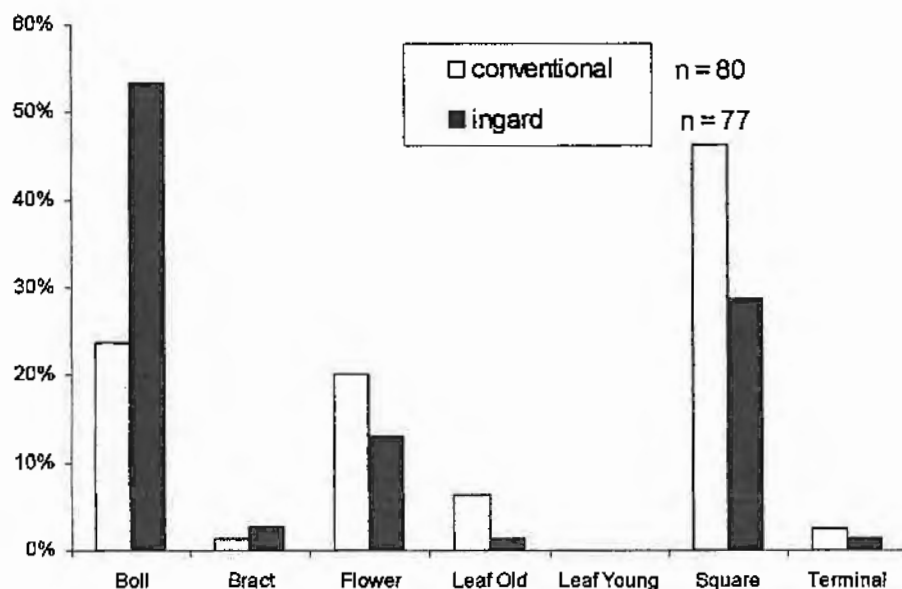


**Figure 8** The proportion (%) of Very Small and Small larvae at each node position of INGARD and conventional cotton in 1998-99. Note: 0.5 node position is the region of terminal growth.

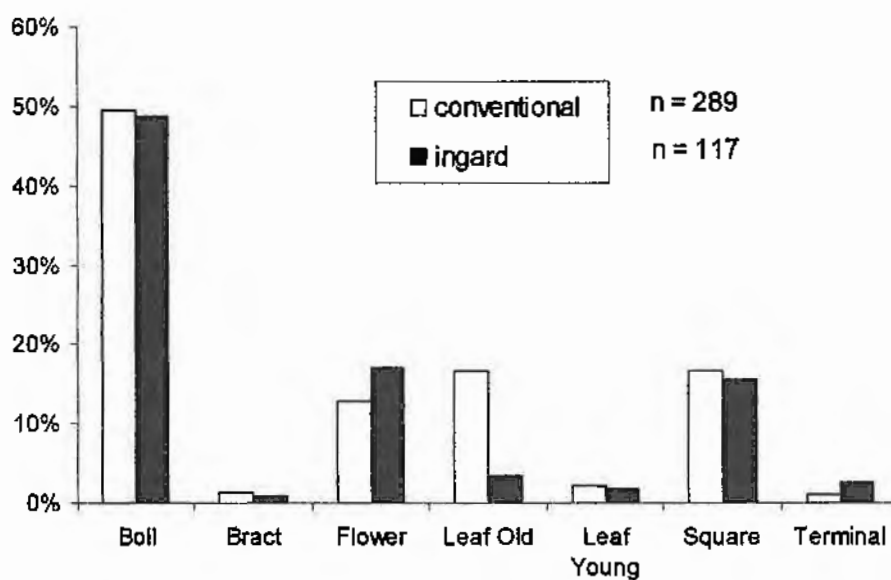
### Medium to Large Larvae

Relatively large differences are displayed between INGARD and conventional in the proportion of medium and large larvae on the various plant structures (Figs. 9 and 10), however, the large variation between seasons suggests that the differences are not significant. These differences between seasons would also reflect differences in the stage of crop development when the larvae were found. Most of the large larvae were

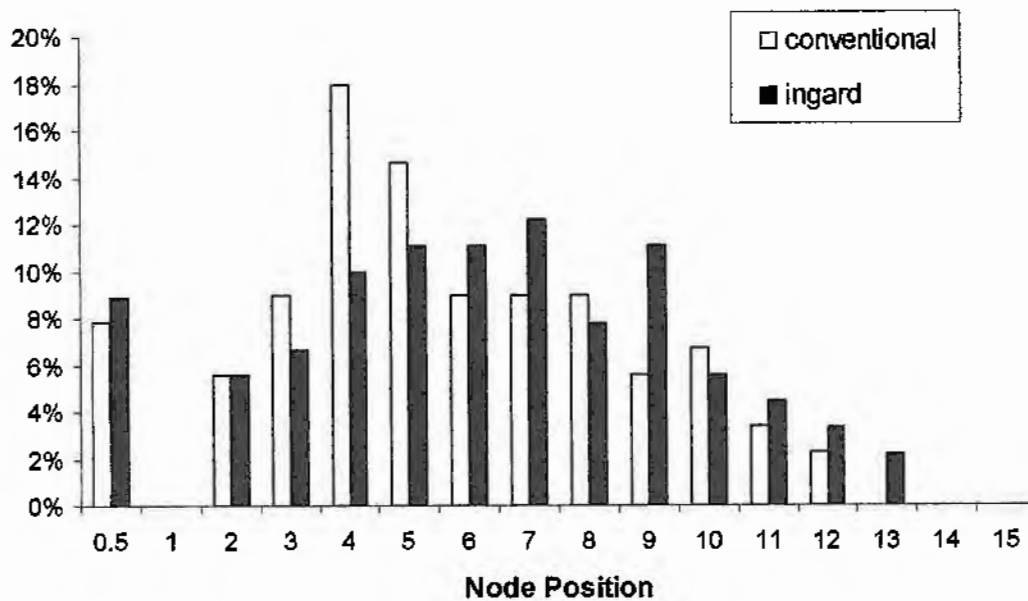
present late in the second season, when bolls were plentiful and other structures less acceptable. In the first year medium to large larvae were found throughout the season. The distribution by node position suggests very little difference in the distribution of larvae between INGARD and conventional varieties (Fig. 12). A lower proportion of medium and large larvae are present in the terminals of INGARD during crop phases 1 and 2 (Figs. 13 to 15).



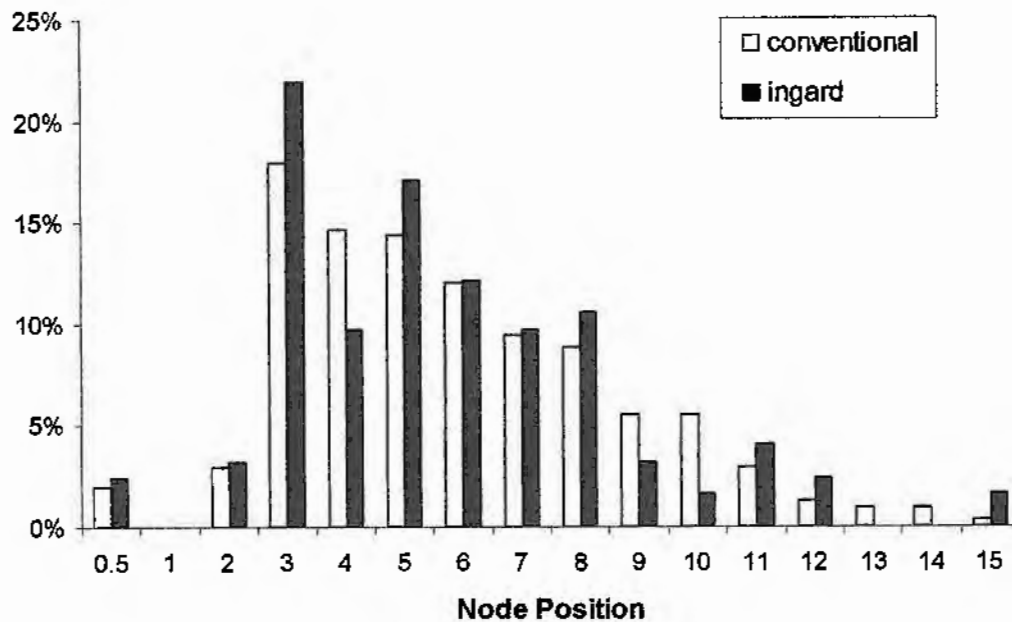
**Figure 9** The proportion (%) of Very Small and Small larvae on different plant structures of INGARD and conventional cotton in 1997-98.



**Figure 10** The proportion (%) of medium to large larvae on different plant structures of INGARD and conventional cotton in 1998-99.



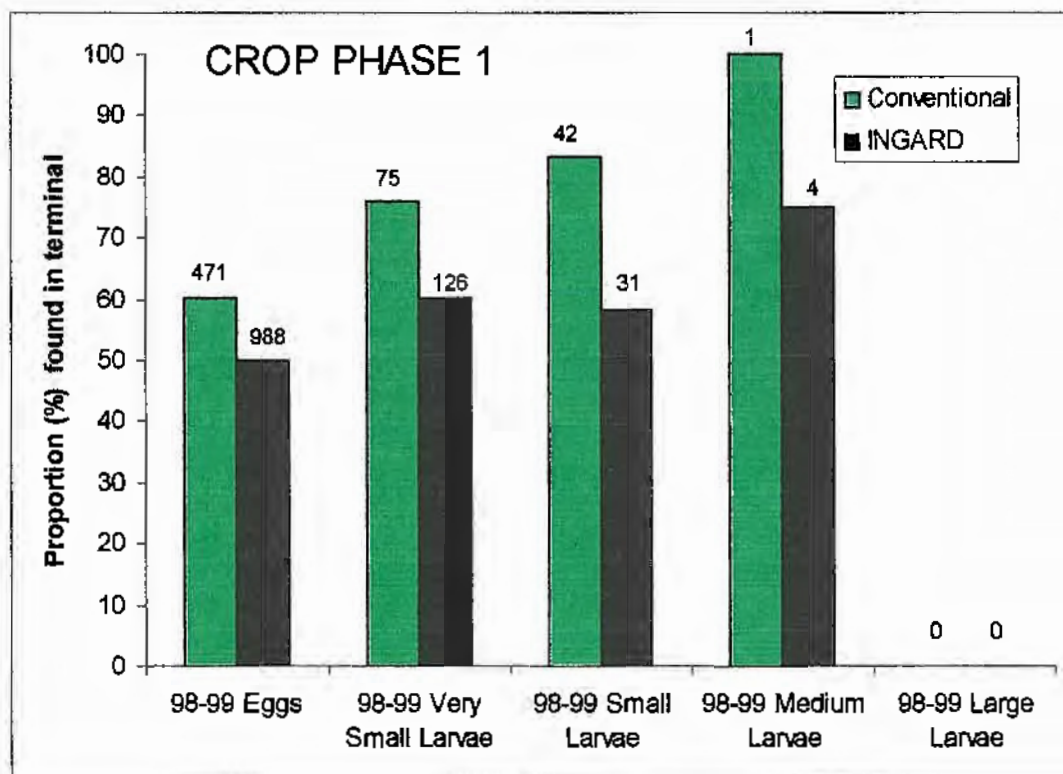
**Figure 11** The proportion (%) of medium to large larvae at each node position of INGARD and conventional cotton in 1997-98. Note: 0.5 node position is the region of terminal growth.



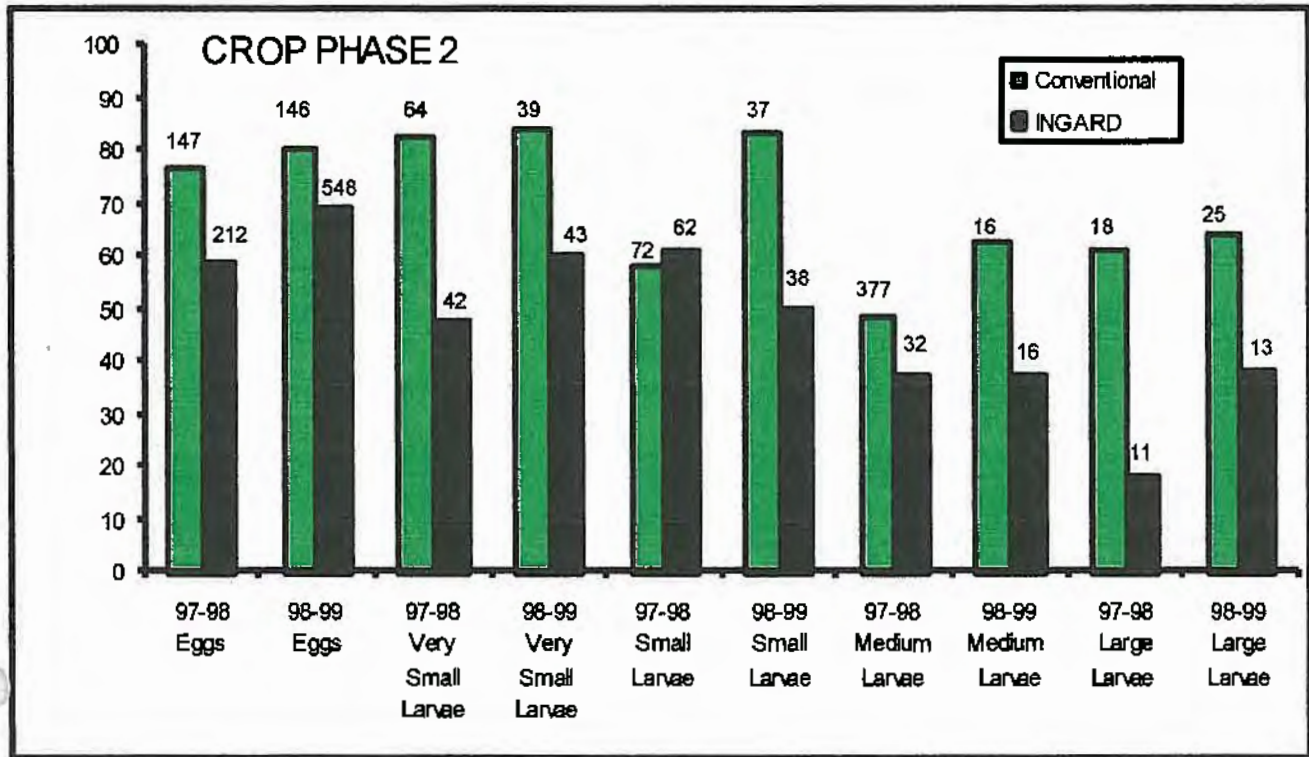
**Figure 12** The proportion (%) of medium to large larvae at each node position of INGARD and conventional cotton in 1998-99. Note: 0.5 node position is the region of terminal growth.

### Proportion of *Helicoverpa* on terminals in each Crop Phase.

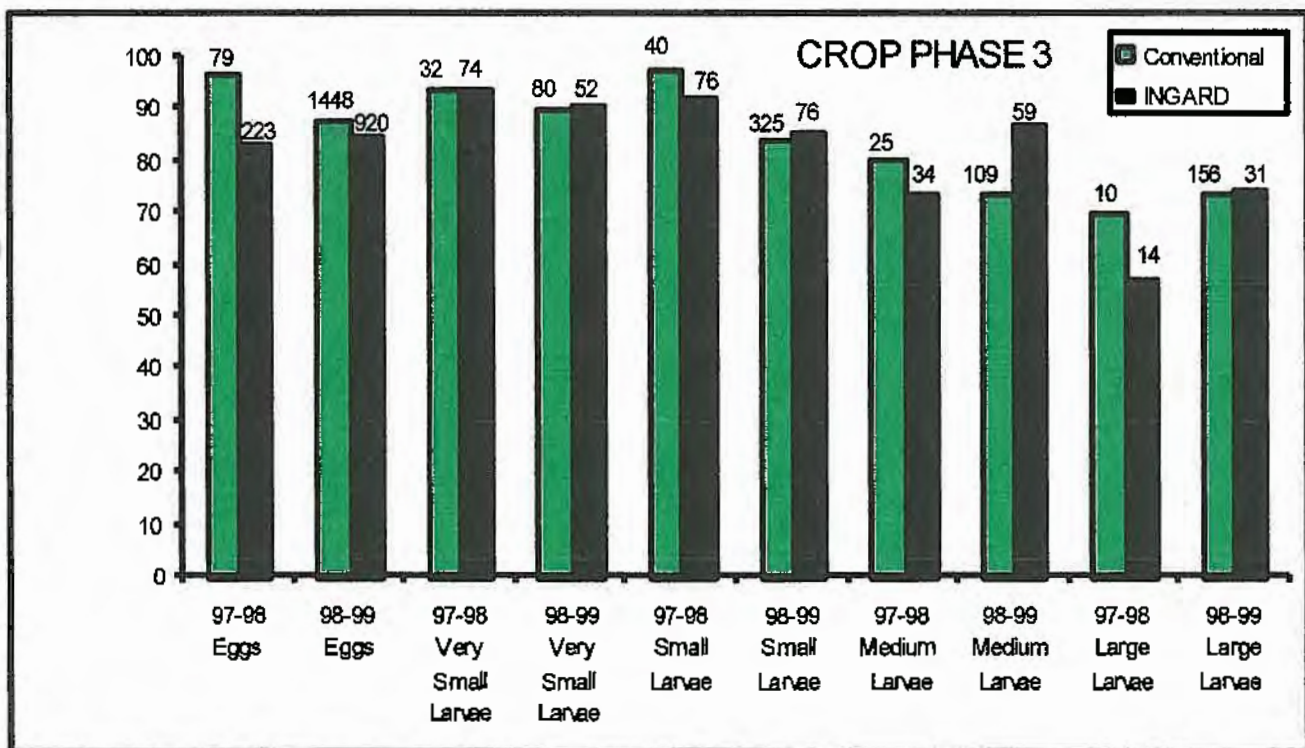
The following graphs (Fig. 13, 14 and 15) show *Helicoverpa* position for crop phase 1, 2 and 3 respectively. Note that for the first two crop phases a lower proportion of *Helicoverpa*, are present in the terminal region of the plants for all except one comparison. The difference disappears in Phase 3. This result is consistent with studies by Pietrantonio & Heinz (in press) in the USA who found a significantly smaller proportion of living *Helicoverpa* spp. larvae in the terminal region of the plants. They explained the differences in larval distribution by the differential expression of the Bt toxin throughout the plant. Of particular interest in our study is that eggs show a similar distribution to larvae. Since eggs are unaffected by Bt, this suggests that at least some of the difference is explained by a difference in the oviposition behaviour of the adult moths.



**Figure 13** The percentage of insects present in the terminal region of the plants for INGARD and conventional varieties of cotton during cropping phase 1. Numbers above each column denote 'n' observations. Note there was no data for the 1997-98 season for Phase 1.



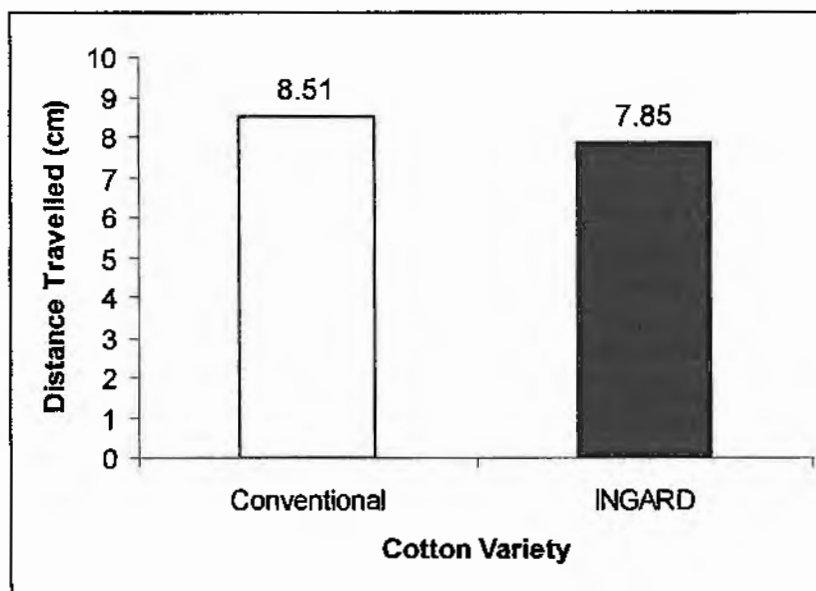
**Figure 14** The percentage of insects present in the terminal region of the plants for INGARD and conventional varieties of cotton during cropping phase 2. Numbers above each column denote 'n' observations.



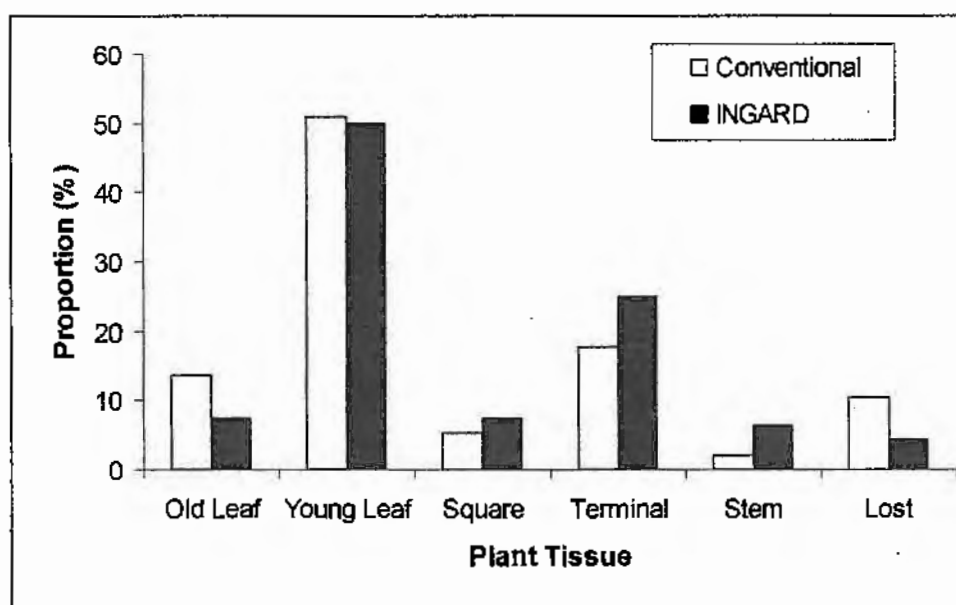
**Figure 15** The percentage of insects present in the terminal region of the plants for INGARD and conventional varieties of cotton during cropping phase 3. Numbers above each column denote 'n' observations.

### Observations on Movements of First Instar Larvae

Our glasshouse study showed that 1<sup>st</sup> instar larvae behaved similarly on INGARD and conventional cotton plants. The position of each neonate larvae was recorded at five-minute intervals for forty minutes following release onto the terminal and upper leaves (up to node position five) of potted Sicala V2 or Siokra V15 conventional and INGARD cotton plants. 12-24 new larvae were recorded each day on eight days of recording. The average distance travelled in 40 minutes from release was 8 cm on both conventional and INGARD varieties ( $P=0.53$ ,  $t = 1.99$ ) (Fig. 16). The plant tissue reached in forty minutes was also similar for each variety (Fig. 17).



**Figure 16** Total distance travelled by first instar larvae over the first forty minutes following release onto potted conventional or INGARD cotton plants in glasshouse trials. Columns are not significantly different ( $P = 0.53$ ,  $t = 1.99$ )



**Figure 17** The proportion of first instar larvae on each plant tissue forty minutes after release onto Conventional or INGARD cotton varieties in glasshouse trials.

## Section 2: Results and Discussion

### *Comparison of Whole-plant Counts and Whole Plant Presence-Absence Sampling for INGARD versus Conventional Cotton Varieties*

The following three figures (18, 19 and 20) present the relationship of **whole-plant sampling to whole-plant presence-absence sampling**. In each case; white and brown eggs, very small and small larvae, and medium and large larvae, the linear model fits very well ( $R^2$  values range from 88% to 98%). That is, for practical purposes, the estimate of pest density for each insect stage from presence-absence sampling provides a useful estimate for whole-plant counts. Furthermore, it is difficult to distinguish the difference between the relationship determined on INGARD cotton to that determined on conventional varieties, i.e. the regression lines appear virtually identical (none significantly different, refer to Table 1 for 'f' and 'P' values). From this we can conclude that the protocols for **presence-absence sampling on whole-plants** for conventional cotton are equally appropriate to INGARD cotton varieties.

The results of presence-absence of whole-plants show a greater level of clumping in this dataset for both INGARD and conventional varieties compared to the previous study. i.e. The same proportion of plants infested would correspond to a larger population of insects using the models from this study compared to the previously established model. The difference this makes is shown in Table 2 where, for a threshold of 3 very small to small larvae per metre, the previous model would have underestimated the pest density by 17%. However, the present study did not produce exactly the same relationship determined in Dillon and Fitt (1995).

The transformation used in the following figures for the presence absence data (x axis) is  $\ln(-\ln(1-P_{iw}))$  where  $P_{iw}$  is the proportion of whole plants infested. This follows the linear models used in CottonLOGIC (Dillon and Fitt 1995).

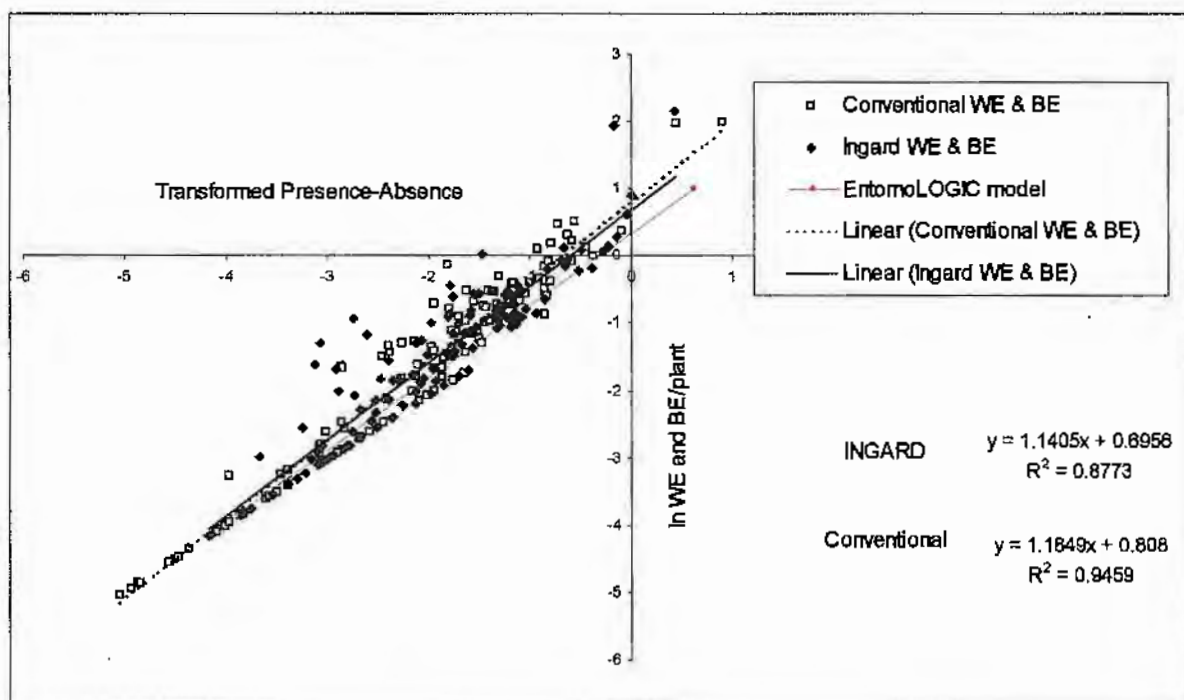
**Table 1** The statistical comparison (GLM) of the linear model for INGARD versus that for conventional cotton using **whole-plant presence-absence** sampling as a predictor of whole plant counts. None of the linear models had significantly different slopes. Values report statistical test for difference in intercept.

Life stage	f	P
White Eggs	1.06	0.30
Brown Eggs	0.08	0.78
White and brown Eggs	0.19	0.66
Very Small Larvae	0.20	0.66
Small Larvae	0.04	0.84
Very Small and Small Larvae	0.00	0.98
Medium Larvae	0.71	0.41
Large Larvae	0.30	0.59
Medium and Large Larvae	0.52	0.48

**Table 2** A comparison of **whole-plant presence-absence** predictions of *Helicoverpa* very small to small larval density per metre around the critical threshold level of 3 larvae/m (shaded).

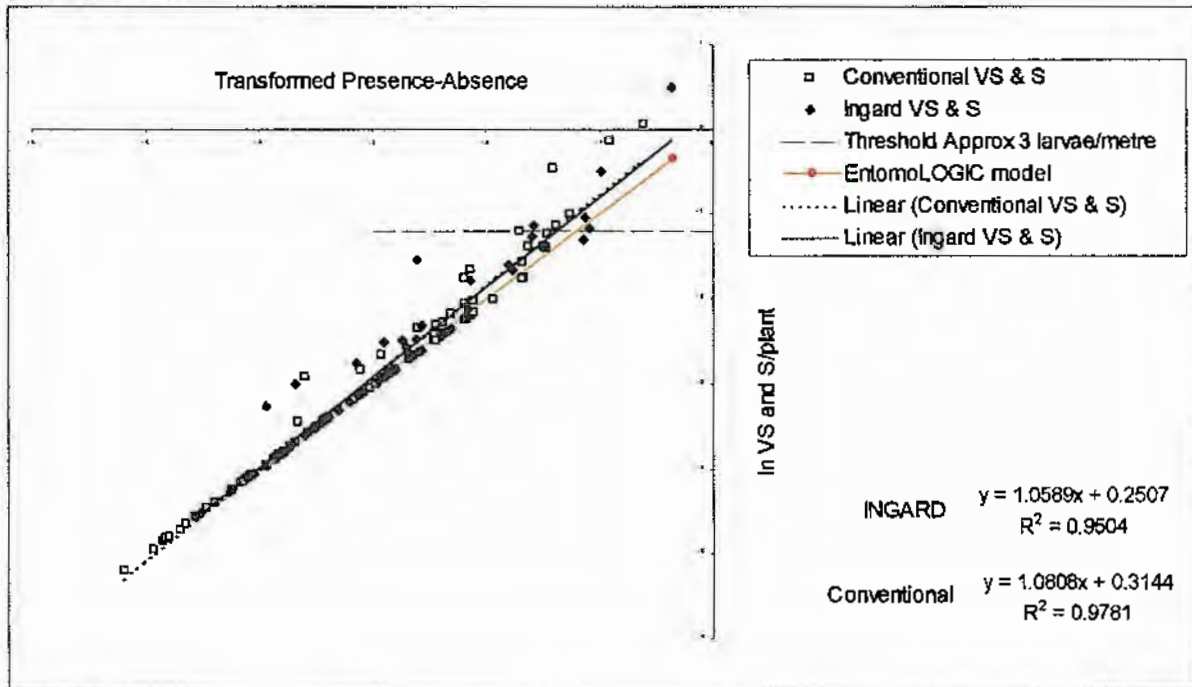
Transformed presence-absence	Proportion Infested	INGARD Model	Conventional Model	Conventional CottonLOGIC
X axis of graphs	(Proportion)	(VS & S/m)	(VS & S/m)	(VS & S/m)
-1.75	0.16	2.0	2.1	1.7
-1.62	0.18	2.3	2.4	2.0
-1.50	0.2	2.6	2.7	2.2
-1.39	0.22	2.9	3.0	2.5
-1.29	0.24	3.3	3.4	2.7
-1.20	0.26	3.6	3.7	3.0

### White and Brown Eggs



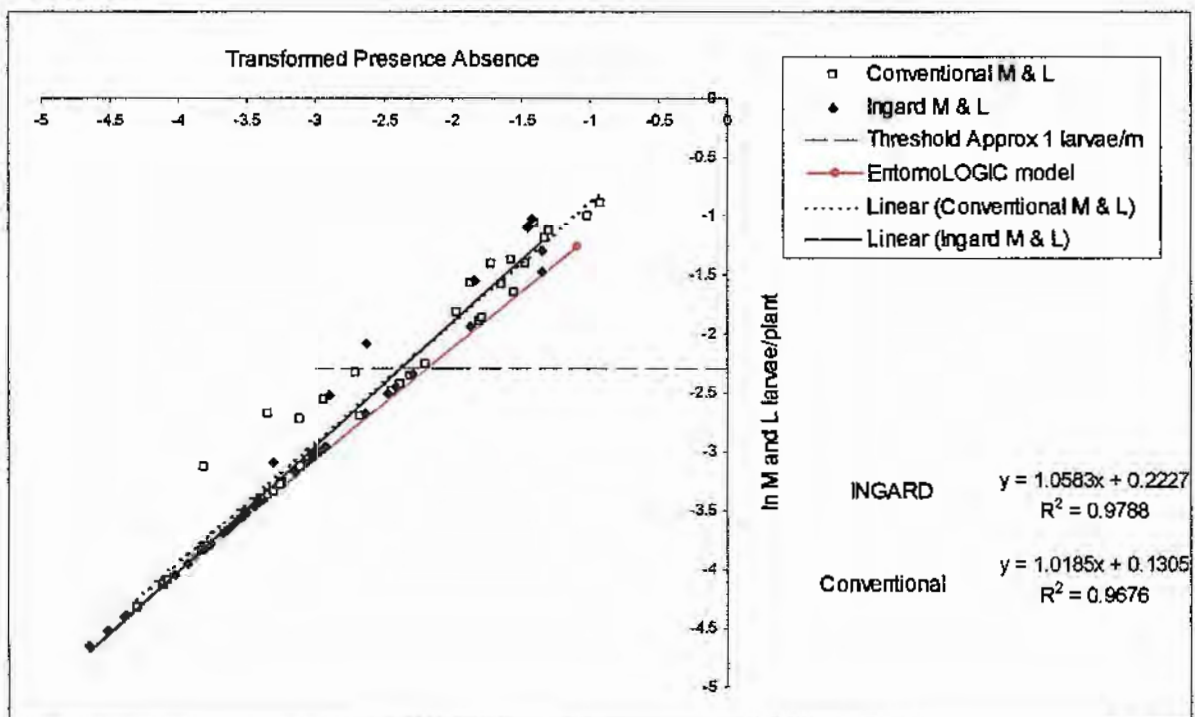
**Figure 18** White and Brown Eggs: Comparison of two regression lines of whole plant counts predicted by **whole-plant presence-absence** sampling for *Helicoverpa* in INGARD and conventional cotton.

### Very Small and Small larvae



**Figure 19** Very Small and Small larvae: Comparison of two regression lines of whole plant counts predicted by whole-plant presence-absence sampling for *Helicoverpa* in INGARD and conventional cotton.

### Medium and Large Larvae



**Figure 20** Medium and Large larvae: Comparison of two regression lines of whole plant counts predicted by whole-plant presence-absence sampling for *Helicoverpa* in INGARD and conventional cotton.

***Comparison of Whole-plant Counts and Terminal Presence-Absence Sampling for INGARD versus Conventional Cotton Varieties***

The following three figures (21, 22 and 23) present the relationship of **whole-plant sampling to terminal presence-absence sampling**. As before, the linear model fits very well for each life-stage grouping; white and brown eggs, very small and small larvae, and medium and large larvae ( $R^2$  values ranging from 80% to 97%). Therefore, presence-absence sampling of terminals would again provide a reasonable estimate of pest density for management purposes. However, unlike the relationships between whole-plant counts to whole-plant presence-absence, the relationships are not always the same for INGARD and conventional varieties (refer to Table 3 for 'f' and 'P' values).

For the white and brown eggs, and medium to large larvae groupings the relationships appear similar (but note significant difference in for brown eggs  $P=0.03$ ). However, the INGARD model for very small to small larvae predicts higher insect density than the conventional model for the same proportion infested. This would cause a conventional model to underestimate the number of very small to small larvae in INGARD crops, particularly since the currently used CottonLOGIC model (Dillon and Fitt, 1995) is even lower on the graph. The horizontal dashed line indicates the position of the three-larvae/metre threshold, showing that the discrepancy is applicable to the area of interest for decision making. At this point the conventional model would have initiated action based on 3 larvae per metre whereas the INGARD model would have suggested 4 larvae per metre were really present. An indication of the magnitude of the difference in predictions by each model for very small to small larvae is shown in Table 4.

That there was little difference in the models derived in this study for **whole-plant presence-absence** between INGARD and conventional cotton, suggests that these differences for terminal presence-absence are caused by a greater proportion of eggs and larvae on INGARD varieties being lower in the canopy than on conventional plants. This is consistent with the insect distribution data reported earlier for the first two crop phases.

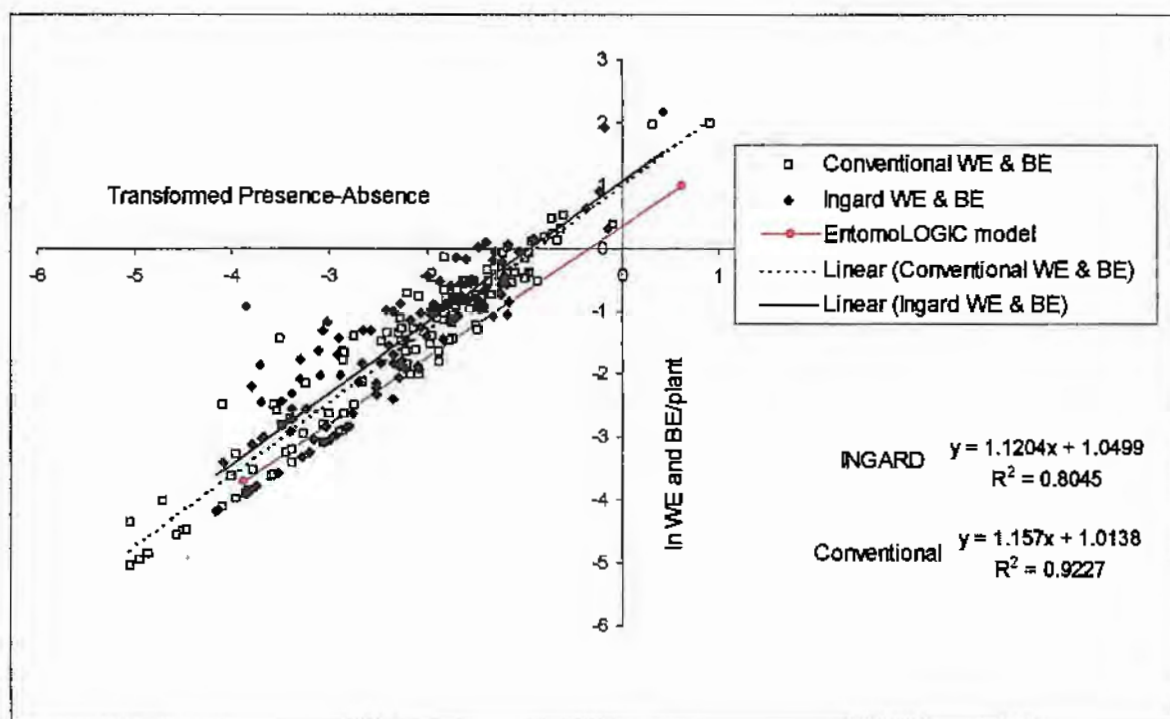
**Table 3** The statistical comparison (GLM) of the linear model for INGARD versus that for conventional cotton using **terminal presence-absence** sampling as a predictor of whole plant counts. \*, \*\* and \*\*\* refer to levels of statistical significance of 5%, 1% and 0.1% respectively. None of the linear models had significantly different slopes. Values report statistical test for difference in intercept.

<b>Life stage</b>	<b>f</b>	<b>P</b>
White Eggs	0.20	0.66
Brown Eggs	4.66	0.03 *
White and brown Eggs	3.70	0.06
Very Small Larvae	39.13	0.00 ***
Small Larvae	8.30	0.01 **
Very Small and Small Larvae	39.49	0.00 ***
Medium Larvae	2.64	0.11
Large Larvae	0.18	0.68
Medium and Large Larvae	2.23	0.18

**Table 4** A comparison of terminal presence-absence predictions of *Helicoverpa* very small to small larval density per metre around the critical threshold level of 3 larvae/m (shaded).

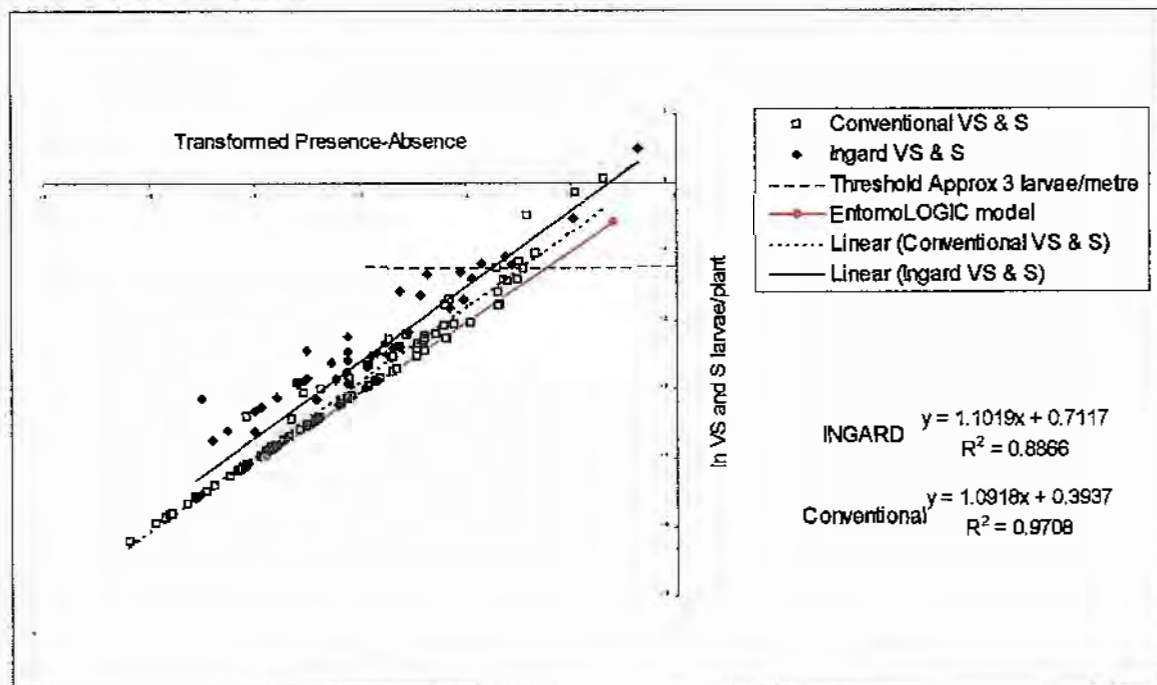
Transformed presence-absence	Proportion Infested	INGARD Model	Conventional Model	Conventional CottonLOGIC
X axis of graphs	(Proportion)	(VS & S/m)	(VS & S/m)	(VS & S/m)
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-1.62	0.18	3.4	2.5	2.0
-1.50	0.2	3.9	2.9	2.2
-1.39	0.22	4.4	3.2	2.5
-1.29	0.24	4.9	3.6	2.8
-1.20	0.26	5.4	4.0	3.1

### White and Brown Eggs



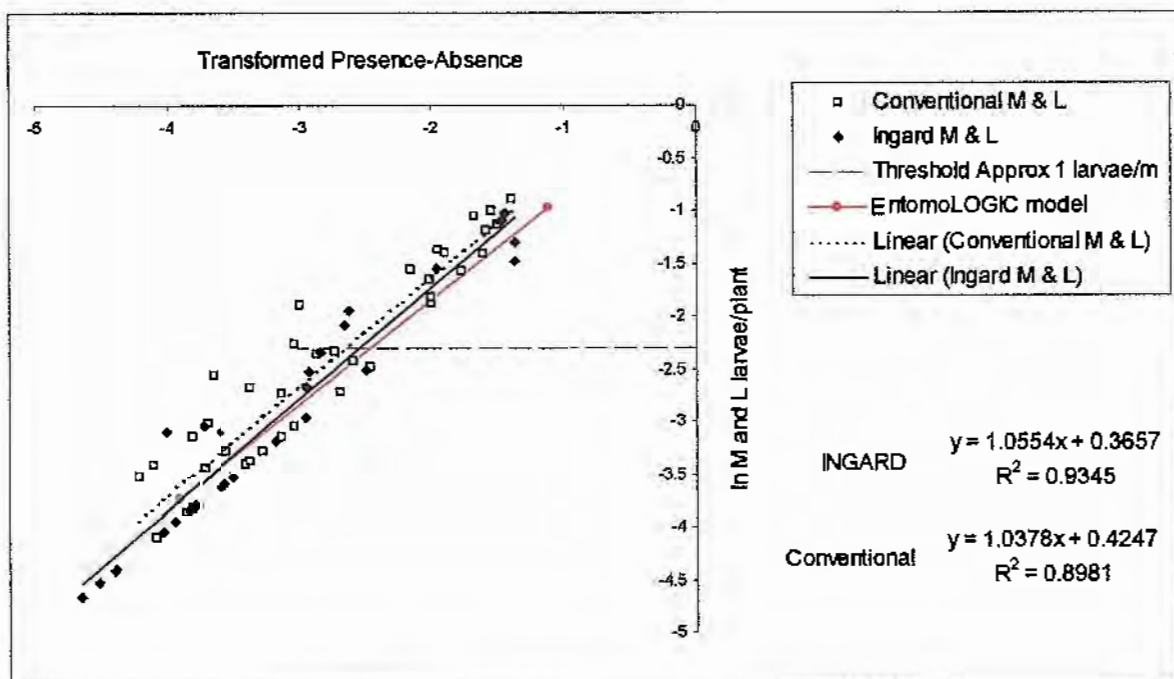
**Figure 21** White and brown Eggs: Comparison of two regression lines of whole plant counts predicted by terminal presence-absence sampling for *Helicoverpa* in INGARD and conventional cotton.

### Very Small and Small Larvae



**Figure 22** Very Small and Small larvae: Comparison of two regression lines of whole plant counts predicted by terminal presence-absence sampling for *Helicoverpa* in INGARD and conventional cotton.

### Medium and Large Larvae



**Figure 23** Medium and Large larvae: Comparison of two regression lines of whole plant counts predicted by terminal presence-absence sampling for *Helicoverpa* in INGARD and conventional cotton.

## Conclusions

Data from the position of *Helicoverpa* life-stages on various plant structures suggests that there are some differences in distribution on INGARD and conventional cotton varieties during crop phases 1 and 2. However a preliminary study of the movement of first instars on glasshouse plants failed to show a difference in insect behaviour on the two types of cotton.

The revalidation of terminal presence-absence sampling has identified a difference between INGARD and conventional varieties for eggs, very small and small larvae suggesting that during the seasons studied a larger proportion of the insects were present lower in the crop canopy of INGARD varieties. The distribution of medium and large larvae may also be affected but the number of observations was much lower for these. The magnitude of the difference in estimated larval densities which this change in distribution causes is important to management decisions around threshold levels. Furthermore, the models constructed in this current study are not consistent with earlier established models currently being used in CottonLOGIC.

The significance of these findings are somewhat tempered by the reliance, to a large extent, on one seasons data when large numbers of insects were scored.

## Recommendations from this Research

1. Presence-absence sampling remains an appropriate means of increasing sampling efficiency for cotton and is supported by CottonLOGIC
2. Terminal presence-absence models are likely to be different for INGARD and conventional cotton varieties.
3. Relationships developed in this study differ from established sampling relationships currently used in CottonLOGIC. Current relationships may underestimate the true density of *Helicoverpa* lifestages in INGARD crops.
4. More data is needed to improve our confidence in these conclusions because the relationships determined in this study differ from previously established models, but are based on limited datasets.

### **Publications arising from this project**

**Abbott, K.L. and Fitt, G.P. (1999)** Distribution of *Helicoverpa* (Lepidoptera: Noctuidae) eggs and larvae in INGARD and conventional cotton: Implications for sampling techniques. In: Proceedings of the 9th Australian Cotton Conference, Gold Coast, August 1998

### **Links with other CRDC and CRC projects**

Collaboration with:

Gary Fitt and Colin Tann, CSIRO Narrabri (INGARD refuge crops)

### **Acknowledgements**

Thanks to Richard Morton (CSIRO Mathematical and Information Sciences, Canberra) for guidance with the statistical analysis.

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