



SUMMER SCHOLARSHIP Final Report

Part 1 - Summary Details

Cotton Catchment Communities CRC Project Number: 5.10.03.27

Project Title: Evaluating flush sampling for *Helicoverpa* moths

Project Commencement Date: 01/01/10

Project Completion Date: 26/02/10

Research Program: The Farm

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Part 3 – Scholarship Report

The points below are to be used as a guideline when completing your final report.

1. Background:

Sampling for management of *Helicoverpa* spp. in cotton has been based on counting eggs and/or larvae in the crop. This is because previous technologies have targeted these life stages. Attempts have been made to use pheromone and light trap catches of adult moths as a proxy for estimating egg numbers. However, these have not been adopted by industry because the correlation between trap catch numbers and egg/larvae numbers is often poor.

Consultants usually have to go and count the eggs and larvae anyway. The registration of Magnet® gives us a technology which directly targets the adult stage. We therefore need a method for estimating moth populations, both for research and for the operational use of Magnet®. In developing Magnet®, we devised a method of estimating adult populations by flush counting. This involves walking along a row of cotton and throwing dirt at the plants ahead, counting moths as they fly out. We used this only as a guide to applying Magnet® in trials, to ensure there were enough moths present to determine the impact of treatments. However it is simple, quick and cheap, and has potential to be used in management if it meets the criteria for good sampling methods such as efficiency, accuracy and repeatability. This project aimed to test those criteria, and will advance the strategic goals of the CRC by facilitating the adoption of Magnet® and by contributing to the adoption of IPM for insects.

2. Aims and Objectives:

To determine the value of flush counting moths for monitoring populations of *Helicoverpa* spp. Specifically, the objectives were:

1. to examine the repeatability and accuracy of flush counts
2. to determine the efficiency of flush counting
3. to examine the variability of flush counts between operators
4. to compare flush counts with other sampling methods (pheromone and light traps, egg counts, Magnet® moth kills)

3. Methodology:

1. Technique of flush counting

Flush counts were made at least 50m into the field from either the head ditch or tail ditch end. A section of row approximately 50 m long was paced out

and marked using PVC conduit poles. A plastic bucket containing approximately 2 litres was filled with dry, loose dirt from the field. The operator then moved 10 rows to the side, so as to sample in undisturbed cotton, and walked back along the 50 m section, throwing handfuls of dirt every 3-5 m (Fig. 1) Moths which flew from the cotton plants in response to the noise and vibration from the dirt were counted and classified as "*Helicoverpa*" or "Other" on the basis of whether they resembled *Helicoverpa* spp. in regard to size, colour and flight pattern.



Fig. 1. Method of flush counting

2. The site

The experiments were conducted in a 75 ha field of Bollgard II® cotton on the property "Oakville", approximately 3 km west of Narrabri (Fig. 2).

Six sampling points were established, three each at 100m in from the head ditch and tail ditch ends. Three pheromone traps (AgriSense design) for *H. punctigera*, and three for *H. armigera*, were established at these points, and designated A1 to A3, and P1 to P3 (Fig. 2). Three light traps (8 w fluorescent tubes, Australian Entomological Supplies design) were established at intermediate locations between the pheromone traps (L1 - L3 in Fig. 2). Light and pheromone traps were cleared daily during experiments.

Throughout the experiments, *Helicoverpa* numbers at the site were low, whether judged by egg counts or the various indicators of moth abundance.

Several attempts were made to find an alternative site in the lower Namoi valley which might have provided greater numbers, but no such site was found.

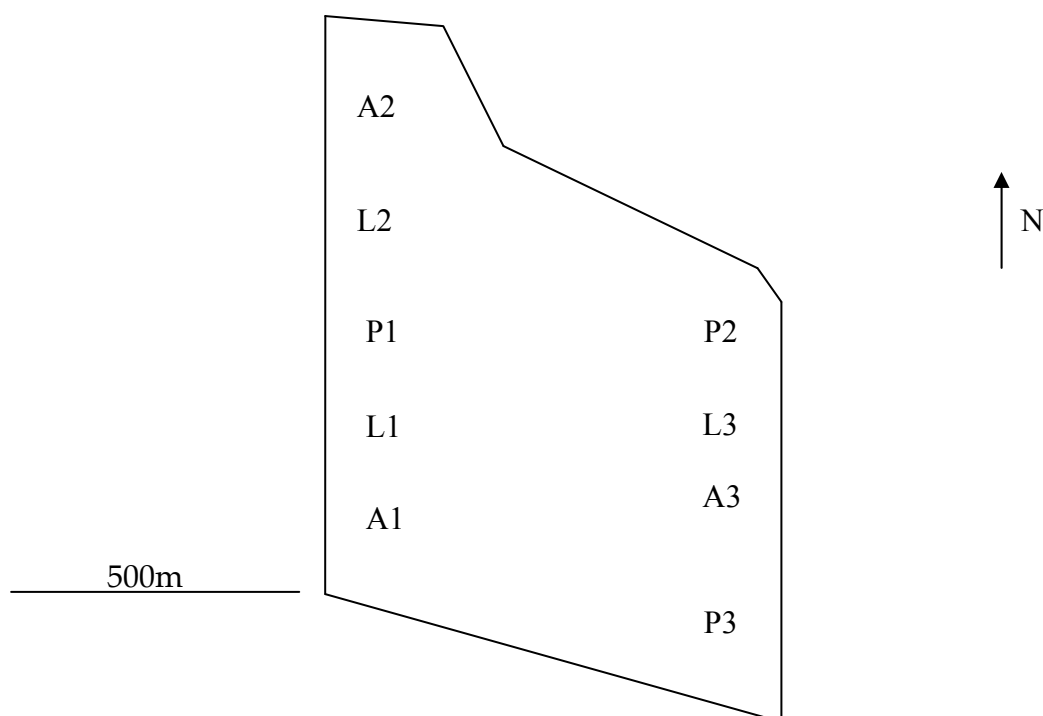


Fig.2. Layout of sampling sites and traps on Bollgard II® field, "Oakville", Narrabri. A=*H. armigera* pheromone traps, P=*H. punctigera* pheromone traps, L=light traps. Flush count and egg sampling sites were associated with each pheromone (but not light) trap, and were located 50-100m away from the trap.

Hourly meteorological data for each day for Narrabri airport (approximately 8 km to the east) were downloaded from the Weatherzone website (<http://www.weatherzone.com.au/index.jsp>)

3. Determination of flush count efficiency

The efficiency of the flush count method was determined on two occasions, 18/12/2009 and 11/02/2010. One operator ("thrower") performed flush counts as described above. A second operator ("beater") followed closely behind on the same row, shaking and beating individual plants with a stick to check for any moths left behind after the thrower. A third operator ("watcher"), standing several rows to the side of the sample row watched for any moths coming back into the sampled row between the passage of the thrower and the beater. These moths were discarded from the analysis. The efficiency of the technique was estimated by:

$$E = 100 * (\text{moths counted by thrower} / (\text{moths counted by thrower} + \text{beater}))$$

The roles of the three operators, the Summer Student Joel Eulenstein (JE) and researchers Peter Gregg (PG) and Alice Del Socorro (ADS) were rotated between thrower, beater and watcher so that each operator performed each role three times, on each day. These experiments were performed in the afternoon. Data were analysed using a GLM model with operator (thrower) and day as factors. Preliminary analyses indicated that the data were normally distributed so no transformations were made.

4. Effect of time of day and operator variation on flush counts

This experiment was conducted over two days, 27/01/2010 and 12/02/2010. On the first occasion, two operators were involved, JE and PG. On the second occasion, an additional operator (ADS) was also involved.

Flush counts were done by each operator at six locations in the field (Fig. 2), every two hours between 0730 and 1930 h (Eastern Daylight Saving Time). Data were analysed using two-way AoV in which operator and time of day were factors. Preliminary analysis indicated that flush counts were normally distributed so no transformations were applied to the data.

4. Correlations between flush counts, pheromone traps and egg counts

Four experiments (two pairs of consecutive days) were done on the Bollgard field at "Oakville" (Fig. 2) on 17/12/2009, 18/12/2009, 10/1/2010 and 11/1/2010. For each experiment, 3x *H. armigera* and 3x *H. punctigera* pheromone traps, and 3x light traps were set up. Traps were cleared daily, and catches of *Helicoverpa* and other noctuid moths recorded. Light trap catches were dissected to determine sex and mated status. Flush counts (6 x 50m) and egg counts (6 x 1m) were done by each of three operators (JE, PG, ADS) at locations associated with the pheromone traps, during each experiment.

Following the pair of experiments on 18/12/2009, the moth attractant Magnet® was applied on 6 x 50m strips of cotton at the rate of 500ml/50m + 0.5% methomyl. Dead moths were collected the following morning and dissected. This information, along with catches in light traps, was used to determine the relative proportions of *H. armigera* and *H. punctigera*, and to determine what other species of moths were present which might have been confused with *Helicoverpa* spp. during flush counting.

Data were analysed using regression analysis of egg and flush count means (across the three operators) and pheromone trap catches. Egg and flush counts were normally distributed and were not transformed. Pheromone trap catches required transformation ($\log_{10} x+1$) to normalise the data prior to analysis.

Numbers of *Helicoverpa* spp., both moths and eggs, were very low throughout these experiments. The opportunity was therefore taken to include in the analysis data from a previous experiment (Project 1.05.10; "Auscott" Narrabri, 2008/09 season) in which similar spatially related flush counts, egg counts and *H. armigera* pheromone trap catches were recorded, and *Helicoverpa* numbers were much higher.

4. Results and discussion:

1. Efficiency of flush counting

Preliminary analysis indicated that the efficiency data were not significantly different from normal, so they were analysed untransformed, using a GLM model in which the operator (thrower) and the date were factors. A GLM (as opposed to AoV) was necessary because there were several replicates in which no moths were seen, and these had to be deleted from the analysis since it was not possible to calculate percentage efficiency for them, which led to unequal numbers of replicates.

There were no significant effects of operator or date on the efficiency of the flush counting technique. Mean efficiency levels for the three operators, for both dates combined, are shown in Fig. 3.

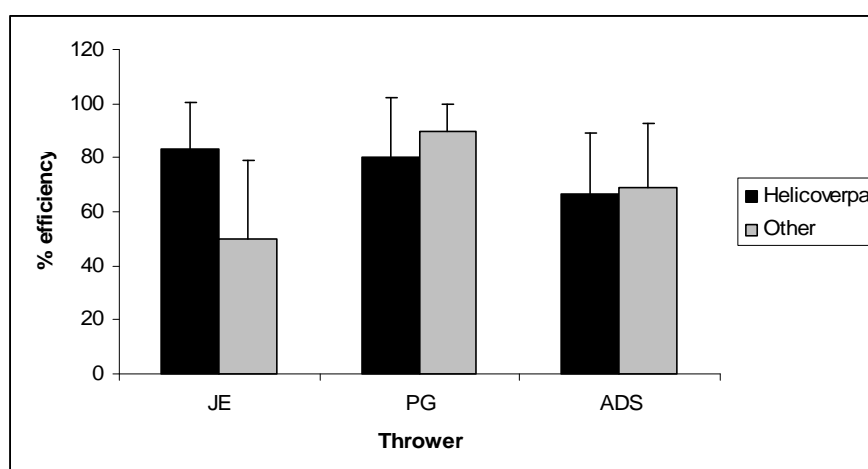


Fig. 3. Mean and s.e. of efficiency (moths counted by the thrower/(moths counted by thrower + beater, as %) for three operators (throwers), with two dates pooled.

The overall efficiency, across all operators, was 78.2% for "*Helicoverpa*", and 71.2% for "Other" moths.

Weather data (temperature and wind speed) for the two days are shown in Fig. 4. While the patterns throughout the two days were rather different, during the period when the experiments were conducted (1230 - 1430 h), both days were fairly similar, with moderate temperature (28 - 30°C) and low wind speed (10 - 15 km/h).

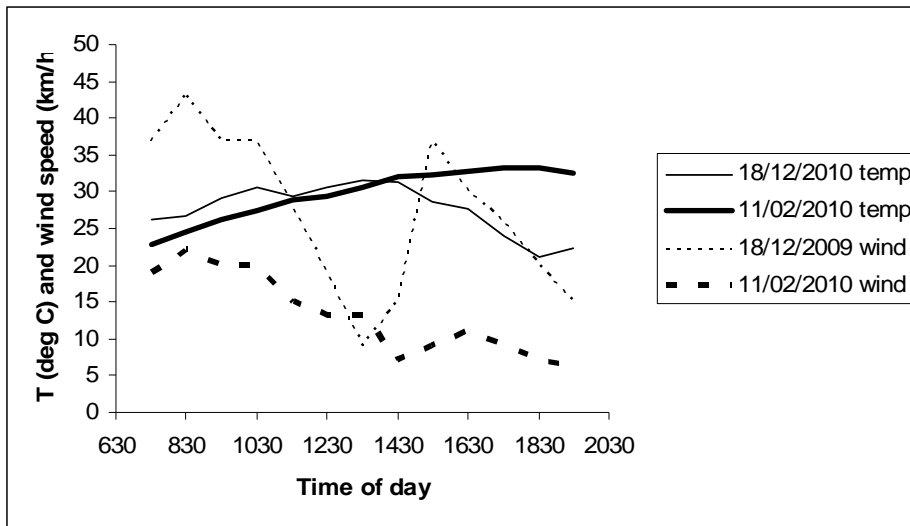


Fig. 4 Temperature and wind speed for the two days of the flush count efficiency experiments

In these conditions flush counting appeared to be quite efficient. Encouragingly, no significant differences were found between operators, although PG and ADS were much more experienced with the technique than JE. Furthermore, no significant differences were found between days even though on 18/12/2009 the cotton was in the squaring stage, only about 50 cm high whereas on 12/02/2010 it was in the flowering and boll setting stage, about 100cm high and much bushier. It might have been expected that flush counting would be less efficient in bigger cotton but this was not so.

However, previous experience has indicated that there are conditions in which flush counting might be less efficient than it was here. One is when wind speeds are high (> 30 km/h). It becomes difficult to accurately throw dirt at the row in such conditions. Also, moths seem to be less responsive, perhaps because the noise is masked by the rustling of leaves. Finally, in windy conditions moths are often carried quickly from the observer, leaving insufficient time to assess colour, size and flight patterns, which may lead to misidentification. Another condition in which flush counting may not be efficient is within 30 min of sunset. At this time moths often begin spontaneous flight, especially if the temperature is mild. It becomes difficult to determine whether moths observed in flight originated from the row being sampled.

A final condition in which flush counting may be inefficient is if there are large numbers of moths which might be confused with *Helicoverpa* spp. Identifying moths in flight from a range of several meters is an inexact procedure. This can be countered in two ways. One is to approach a separate sample of moths more closely. This has to be done separately from the 50 m quantitative sample, for example, when entering the field. Moths which are flushed usually only fly 10-15 m before alighting. Often the position where

they alight can be noted and the moths stealthily approached to a range of 1 -2 m, from where they can generally be identified as *Helicoverpa* or not, though it is usually still difficult to pick the two *Helicoverpa* species apart. This procedure was adopted on numerous occasions during the two experiments reported here, and almost all "*Helicoverpa*" identifications were confirmed.

The second way of minimising identification errors is to employ an independent technique which captures or kills moths, allowing close examination. This also allows separation of the two *Helicoverpa* species. However, the possibility that the independent technique may be differentially effective for *Helicoverpa* and other species must be borne in mind. In these experiments light traps and Magnet® were used for this. Results are shown in Table 1.

Date	Method	<i>H. armigera</i> .	<i>H punctigera</i>	Similar to <i>Helicoverpa</i>	Other moths
18/12/2009	Light	0.2 ± 0.2	16.2 ± 4.4	3.0 ± 0.9	0
18/12/2009	Magnet	0.5 ± 0.2	3.2 ± 1.6	0.5 ± 0.2	0.2 ± 0.2
12/2/2010	Light	11.3 ± 4.2	9.8 ± 3.5	0.1 ± 0.1	10.8 ± 6.1

Table 1. Light trap catches (moths/trap) and Magnet® kills (moths/50m) on nights coinciding with the flush count efficiency experiments. Species classified as "similar to *Helicoverpa*" were those of a size and colour which might have made them difficult to separate from *Helicoverpa* during flush counts.

The results from light traps and Magnet® kills indicate that during the first experiment the *Helicoverpa* spp. present were predominantly *H. punctigera*, and that there were few other moths which might have been confused with them during flush counting. During the second experiment, the light trap catches indicated that the two species of *Helicoverpa* were present in approximately equal numbers, and again, there were few moths likely to cause confusion. The other moths found on this occasion were mostly of a dark grey species tentatively identified as *Rictonis* sp., which was not seen during the daylight hours and was thought to either move into the field only at night, or shelter during the day in locations not accessible to flush counting, such as soil cracks. Moths likely to cause confusion in flush counting which have been identified in previous work in cotton include the sugarcane armyworm *Mythimna loreyminima*, common armyworm *Mythimna convecta*, and brown cutworm *Agrotis munda*. Very few of these were present in either of these experiments.

The "Other" moths recorded during flush counts (Fig. 2) were therefore not *Helicoverpa* spp. or anything similar. They were generally small moths, identified by closer inspection as family Pyralidae and (in the second experiment) a few rough bollworm, *Earias perhuegeli*.

2. Effects of time of day and operator

Analysis of the first experiment (27/01/2010), in which two operators (JE and PG) were involved, revealed a significant effect of operator ($F_{1, 70} = 4.57$, $P=0.036$), and of time of day ($F_{6,70} = 2.98$, $P=0.012$) on the numbers of moths observed, but no significant interaction between the two factors. These results are shown in Figs. 5a and 5b. There was a trend for increasing numbers of moths to be counted as the day progressed. The difference in operators was mainly because PG (a more experienced flush counter) was recording more moths than JE, especially during the middle part of the day.

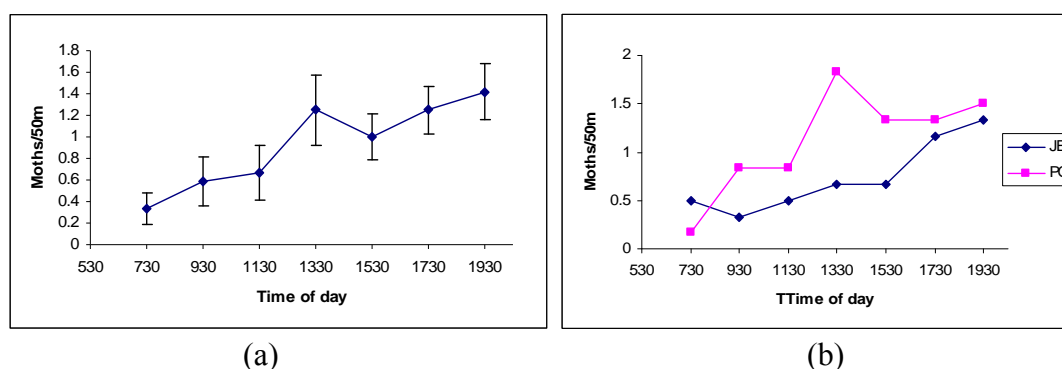


Fig 5. (a) Mean "*Helicoverpa*" moths flushed at two-hourly intervals on 27/01/2010, both operators combined. bars are s.e. of the mean. (b) Counts of "*Helicoverpa*" moths for individual operators (mean of 6) for the same period, error bars not shown.

For "Other" moths (i.e. recognisably not *Helicoverpa*) there was a significant effect of operator ($F_{1,70} = 10.72$, $P = 0.002$), but no significant effect of time of day, and no significant interaction. The effect of operator is shown in Fig. 6, and again the difference was due to higher counts by PG compared to JE.

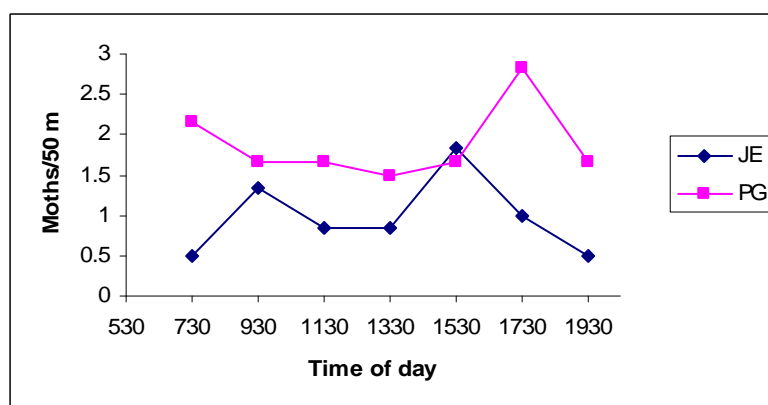


Fig. 6. Counts of "Other" moths for individual operators (mean of 6) at two-hourly intervals on 27/01/2010, error bars not shown.

On the second day, 12/02/2010, when three operators were involved, there were again significant effects of operator ($F_{2,63} = 3.69$, $P=0.031$) and time of day ($F_{6,63} = 3.44$, $P = 0.005$) on counts of "*Helicoverpa*" moths, but no significant interaction (Figs. 7a and 7b).

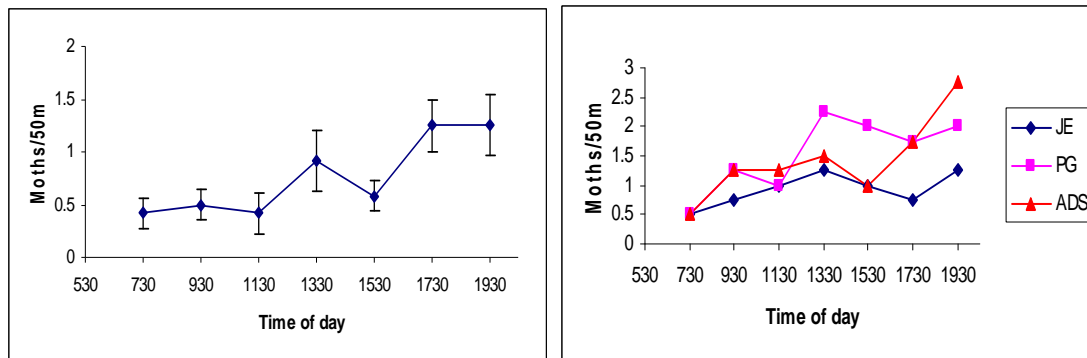


Fig. 7(a) Mean "*Helicoverpa*" moths flushed at two-hourly intervals on 12/02/2010, three operators combined. bars are s.e. of the mean. (b) Counts of "*Helicoverpa*" moths for individual operators (mean of 6) for the same period, error bars not shown.

The patterns were similar to the first day, with higher numbers of moths being counted in the afternoon, and the operator difference being primarily due to lower counts by JE compared to PG and ADS, especially later in the day.

For "Other" moths on this day, there were no significant effects of operator, time of day or the interaction. Numbers of "Other" moths were extremely low on this occasion.

Weather conditions during these two experiments are shown in Fig. 8.

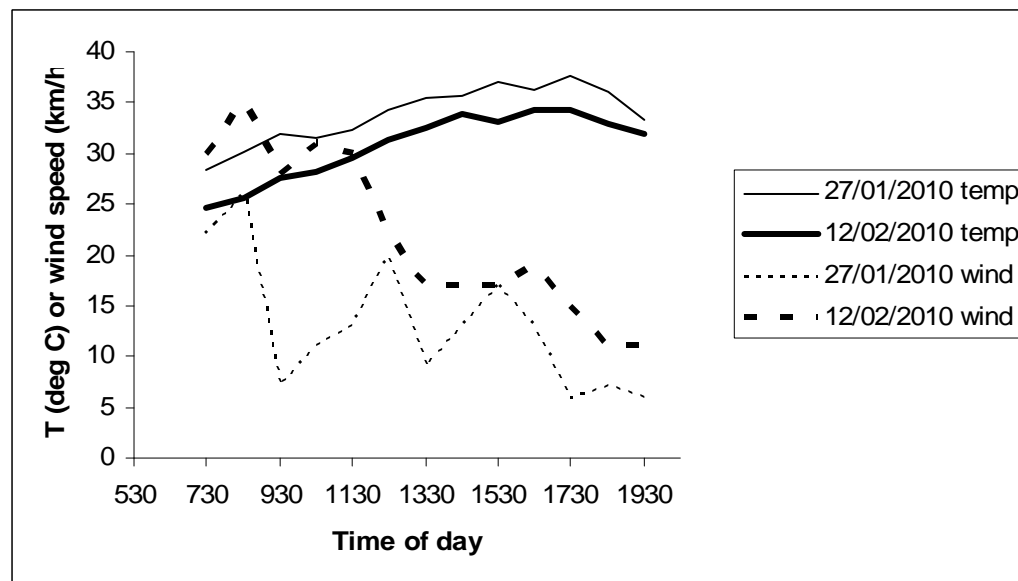


Fig. 8 Temperature and wind speed for the two days of the time-of-day experiments

Both days were fine and warm, with maximum temperatures of 34-38°C. Wind speed was high early in the morning, especially on 12/02/2010, but moderated during the day. The increase in flush counts as the day progressed might be in part due to the inefficiency of flush counting in high winds (discussed in Section 4.1 above), early in the day. It is unlikely to be due to lower temperatures, since even the early morning temperatures were well above the flight threshold of *Helicoverpa* spp., which is about 12°C for light trap catches (Gregg & Wilson 1991). All temperatures were comparable to or above those of the efficiency experiments (Fig. 4), when quite high efficiencies were recorded (Fig. 3). Additionally, these results provide no evidence that flush counting may be affected by heat torpor of moths, at least at temperatures up to 38°C.

The possibility that there is a circadian rhythm in diurnal activity in *Helicoverpa* spp. which leads to greater flush counts as the day progresses cannot be ruled out, but would need further investigation through more experiments similar to these, under different weather conditions.

The significant differences between operators in these experiments warrant comment, in view of the fact that no such differences were found during the flush count efficiency experiments (Section 4.1). They could be due to the different weather conditions. Operator differences were most pronounced in the hot and still conditions later in the day, compared to earlier in the same day, and compared to the conditions of the flush count efficiency experiments (compare Figs. 4, 5b, 7b and 8). In such conditions moths often appear more "flighty", and respond to flushing not only from the area impacted by the dirt, but from several meters ahead. Subsequent discussions between the operators indicated that the more experienced operators, PG and ADS, anticipated this effect and were looking further along the row than the less experienced JE, who was focusing on the area impacted by the dirt. This indicates the need to train operators in the technique under a range of weather conditions

3. Relationships between flush counts, egg numbers and pheromone trap catches

The flush count efficiency and time-of-day experiments described above were aimed primarily at validating the use of flush counting for research purposes in relation to Magnet®, especially for estimating the proportion of moths in a field which might be killed by a Magnet® application. However, the question arises of whether the technique could be used for more conventional pest management strategies, which are directed at the immature stages. It might be used either as a substitute for, or as an addition to, conventional bug-checking which estimates the number of eggs and larvae per meter through visual searches. In this role, the critical consideration is whether flush counts correlate with egg laying. In the case of other methods of estimating adult *Helicoverpa* numbers, such as pheromone traps, this correlation is often poor (Gregg & Wilson 1991, Daly & Fitt 1993).

To investigate this question, we took means of egg counts and flush counts (3 replicates) from each day they were done, at each location in the field, and correlated them with each other, and with the catch from the nearest pheromone trap. We were unable to find any statistically significant correlations between these variables. However, the numbers in all three measures were very low by comparison with previous experiments (eg Grundy *et al.* 2006, Del Socorro *et al.* 2010). Also, the correlations were sought between different areas in a 75 ha field, which might be too small for spatial differences in egg laying, related to moth numbers, to be apparent (ie, laying females might move throughout the field).

We therefore re-examined similar data from an experiment conducted in 2008/09, as part of Project 1.05.10, to see if such correlations could be found. In this experiment, three fields of Bollgard II® cotton (not treated with Magnet®) were monitored throughout the season using flush counts, egg counts and pheromone traps (*H. armigera* only). Two flush counts and two egg counts were done near each pheromone trap, at approximately weekly intervals between 16/12/2008 and 12/2/2009. Means of these two counts, and corresponding pheromone trap catches, were correlated with each other using regression analysis. For pheromone traps, no significant correlation could be established with either egg counts or flush counts. However, flush counts were significantly correlated with egg counts ($F_{1,37} = 34.9$, P

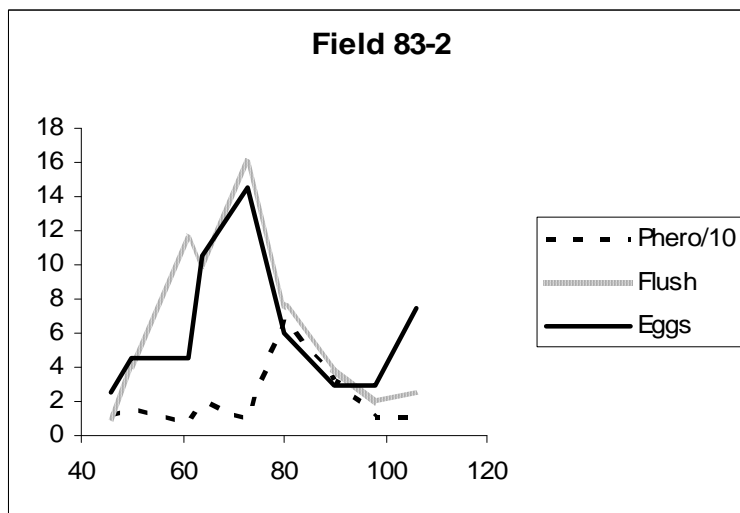
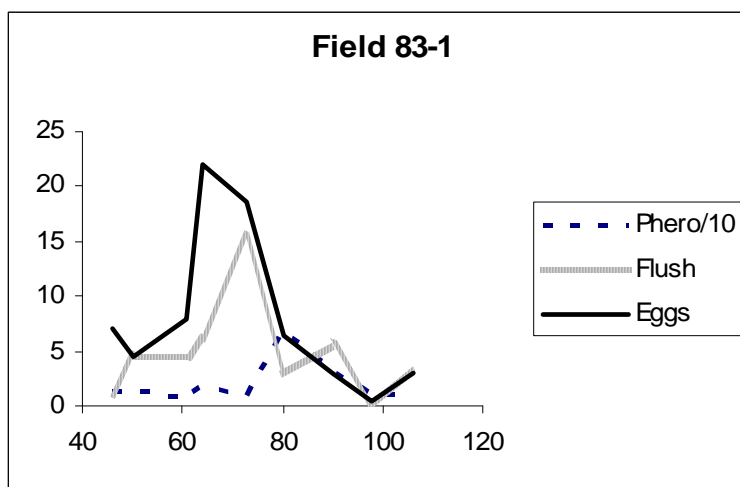
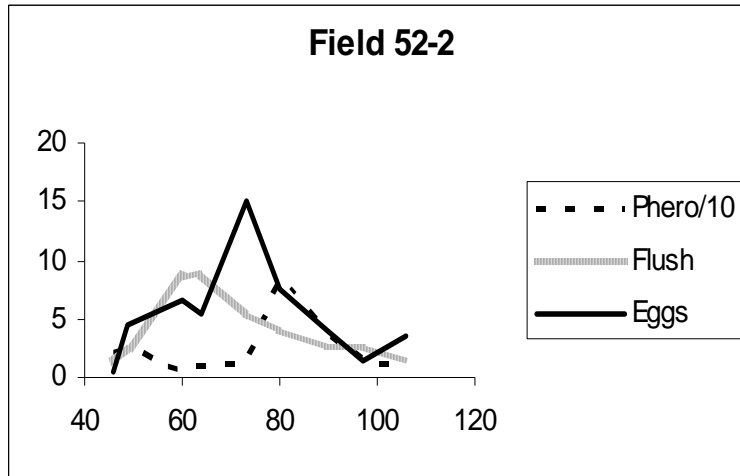


Fig. 10 Temporal patterns in egg counts, flush counts and pheromone traps from "Auscott", 2008-09. X-axis is days after November 1. Y axis is egg counts (per meter), flush counts (per 50 m) or pheromone trap catches (per night, divided by 10 to suit the scale).

5. Conclusion:

Despite difficulties due to low moth numbers, the work reported here has demonstrated that flush counting is a valid means of measuring the adult *Helicoverpa* population in a cotton field, for the purposes of experiments with control methods which target the adults, such as Magnet®. Under suitable weather conditions, it has an efficiency approaching 80%, which is high compared to visual methods of counting other pests in conventional bug-checking, and other methods such as beat sheeting and suction sampling (eg Garcia *et al.* 1982, Wilson & Room 1982, Stanley 1997, Deutscher *et al.* 2003). It is quick, taking about 30 seconds to complete a 50m transect. It is simple, requiring no specialised equipment. It requires only limited training, though practice is necessary to ensure its reliability. The method appears to be fairly robust in relation to weather conditions and the time of day, although there are some conditions in which it should not be used, for example in high winds or close to sunset. Operators may need training in how to use the method in varying conditions, for example, the need to look further forward when sampling in hot and still weather. Operators do need to be aware of the limitations of the technique. Foremost among these is the need to be aware of the presence of other moth species which might be hard to distinguish from *Helicoverpa* when seen only briefly, in flight, from a distance of several meters. This requires considerable practice. It can be assisted by using closer inspection of flushed moths, which can be done separately from the quantitative sampling transects. It can also be done using trapping or killing methods such as light traps or Magnet® treated strips, although the potential for differential attractiveness of these techniques for *Helicoverpa* compared to the potentially confusing species should be considered.

For practical pest management, there may be a place for flush counting. Some consultants already use the method in a crude and qualitative form, by throwing dirt at the crop near where they are sampling using other methods. This project has provided some guidelines for formalising that process, and for interpreting the results in terms of likely egg counts. The existence of a significant correlation with egg numbers (and therefore probably with eventual damage) suggests that it may be a valuable supplement to egg and larvae counts, perhaps providing early warning in some cases. It is unlikely, however, that consultants would entirely abandon conventional bug-checking in favour of flush counting, since the former provide a more direct measure of potentially damaging pest populations. .

6. Highlights:

The validation of flush counting as a technique for use in research which requires quantitative estimates of moth populations in cotton fields, for example, in determining the impact of control techniques which target the moth stage, such as Magnet®

7. Presentations and public relations:

None

8. References:

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9. Executive Summary:

(provide a one paragraph summary of the Summer Scholarship, suitable for posting on the Cotton CRC web site)

This small project aimed to validate the technique of flush counting to estimate the abundance of adult *Helicoverpa* spp. (cotton bollworm moths) in cotton fields. The work was necessary to enable the assessment of the impact of control measures targeting this stage of the pest, such as the attractant Magnet®, which are being developed in a related project, 1.05.10. It was found that flush counting (a simple technique involving counting moths flushed from the crop by throwing soil at it) was a highly efficient technique, robust in range of different weather conditions, and cheap, quick and simple to employ. Some limitations of the method were identified, but these can be overcome with adequate training of operators and the use of supplementary methods for identifying species which might be confused with *Helicoverpa* spp. When data from this and a previous project were combined, a good correlation was found between flush counts and egg density, which suggests that flush counting could be a useful supplement to traditional bug-checking methods for commercial pest management.