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Cotton Research and Development Corporation

7/88 to 6/91

Project Title: Reappraisal and refinement of SIRATAC sampling procedures for insect pests of cotton, particularly *Heliothis* .

Project Number: CSE4C (formerly CS64L)

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A final report prepared for the Cotton Research and Development Corporation

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BACKGROUND

The CRDC funded project CSE4L commenced in November 1988. This final report details the work carried out on this project over the three year funding term.

The sampling systems for *Heliothis* and other pests currently used in SIRATAC were developed from intensive data sets collected from 1973/74 to 1975/76. The relationships derived from this data allow the estimation of pest densities by the conversion of the proportion of a sample of plants infested to mean number of insects per plant, and thence to mean number per metre. Data was collected on DP16 and DP61 cotton varieties with stand densities of 15-17 plants per metre. Since that time both varieties and stand densities have changed considerably. Also of importance is the fact that much of this original data was collected in unsprayed crops where the dispersion pattern of eggs and larvae may conceivably be different to that on sprayed crops.

This project was therefore initiated to allow a statistical re-evaluation of the sampling methods used by SIRATAC and to collect further data from crops using current varieties, commercial spray regimes and covering a range of plant densities. Findings from this work may be included within the personal computer based insect management system *Entomologic*.

OBJECTIVES

(i) To evaluate the reliability of current SIRATAC sampling procedures and conversion equations relating proportion infestation to mean number of insects per plant.

(ii) To develop sampling systems able to cope with a range of crop conditions including variable stand density and different cotton varieties.

(iii) Investigate by simulation the threshold procedures for *Heliothis* to allow for variable plant density and growth stage.

FIELD WORK: METHODOLOGY AND RESULTS

Over the last three growing seasons extensive sampling studies have been conducted to determine the distribution and abundance of *Heliothis* on different cotton varieties. A major emphasis of the field work has been the collection of data for the testing of the SIRATAC conversion equations (see below). In addition to this, supplementary field experiments were conducted in order to investigate the within field distribution of *Heliothis*, the implications of varying plant densities on the sampling procedure, the relative efficiency of various sampling procedures and the effect of observer bias on sampling results.

1. Conversion Equations

In order to collect data relating the proportion of a sample infested to the mean number of *Heliothis* per plant, regular sampling was conducted to record total numbers of *Heliothis* and numbers of plants infested within individual metres of plant row. The position of each individual egg or larvae on the plant was also recorded. This data has then been used to establish new conversion equations for comparison with the original SIRATAC equations. Data was collected separately for two commonly grown varieties (Siokra and Sicala) and thus differences between varieties with respect to *Heliothis* numbers was also investigated.

Detailed statistical analysis of this data is still continuing with advice from CSIRO statistician Dr R. Morton. Analysis to date has shown that new relationships, derived from recent data, are very similar in form to the original Siratac conversion equations. Newly calculated insect specific parameters have been found to be significantly different statistically from their original counterparts. The actual biological significance and implications of these differences will probably be very small. Further consideration of these differences form part of the on-going analysis.

Two sets of conversion equations are being developed, these reflect the two different types of sampling currently used, whole plant sampling or terminal sampling. Whole plants are sampled early in the season when the plants are relatively small. Later in the season, when samples are taken only from terminal portions of the plant, the conversion equation process becomes a little more complicated. The original Siratac conversions for terminal sampling involved complicated iterative processes to derive mean number of *Heliothis* from proportion of terminals infested. It has been decided to derive a new set of equations, using the recent data acquired, so that mean number of *Heliothis* per plant can be derived from proportion of terminals infested directly, rather than by using the iterative process.

Analysis to date has shown that differences between numbers of *Heliothis* found on the two varieties were negligible. This has allowed pooling of the results for the two varieties and the development of a single conversion equation. Time of season is also being included within the analysis so, when completed it will be known if a single equation will adequately describe the conversion relationship throughout the whole of the terminal sampling period. The incidence of medium and large larvae low down on the plant towards the end of the season has prompted discussion of the need for a specific late season conversion relationship.

Once statistical analysis of this area of research is complete, the results will be prepared and submitted for publication in a scientific journal. Copies of these journal articles will be forwarded to the CRDC as they become available.

2. Within Field Distribution of Pests

The broad scale distribution of *Heliothis* eggs and larvae within a 100 Ha field was investigated by weekly sampling of fixed grid points, distributed evenly across the whole field. This sampling regime allowed us to investigate the spatial distribution of *Heliothis* and to determine if these distributions change throughout the growing season. Sampling has been carried out in both Siokra and Sicala fields and the results were very similar for the two varieties. Analysis has indicated that significant small scale clumping of both *Heliothis* eggs and larvae does occur, but there was no consistency in the spatial distribution of these clumps in the field throughout the season. For example, higher numbers of *Heliothis* may have been detected in the tail drain region of the Southern end of the field during one of the weekly checks, but subsequent checks did not show this area to have higher *Heliothis* numbers than other areas of the field on a regular basis. The patchy distribution of *Heliothis* eggs and consequently larvae did not consistently favour any one area of the field.

3. Implications of Varying Plant Densities

Variable or low plant stand densities may arise from a number of different causes e.g germination failure, seedling disease etc. Field work during the 1989/90 season was aimed at determining if low plant densities require different sampling methods or different thresholds for *Heliothis* control than stands of higher plant density.

The studying of plant density effects was complicated by the ability of cotton plants to compensate for low plant stand density by increasing the fruit load per plant. Thus any given area of cotton may be capable of producing a similar yield despite varying plant stands. This was the case in this experiment, fruit counts indicated no significant differences in the number of bolls counted in the different plant density treatments (Table 2). Unfortunately the experimental plot was badly damaged by hail in mid February, so fruit counts and extensive insect sampling was not continued through to harvest.

There is a time lag before compensation occurs, and in this pre-compensation period early in the season, the insect load **per plant** is the important factor and thresholds should probably be expressed in these terms. Damage at this stage may prohibit low plant stands from adequately compensating at a later date, thus reducing the potential yield. By mid season (mid January) plants have compensated for their planting densities and (assuming adequate pest control) an average metre of cotton would be similar, both vegetatively and in terms of fruit load, despite varying planting densities. Thus later in the season, following compensation, the number of insects **per metre** is an appropriate measure of insect pressure.

The possible influence of varying plant density on the number and distribution of *Heliothis* eggs and larvae was investigated in replicated plots with low, medium and high plant densities. The variety used in this experiment was Siokra. Results are separated into early season counts (prior

to Jan 1), expressed as insects per plant (Table 1), and mid /late season counts (after Jan 1) expressed as insects per metre (Table 2).

Table 1. Early season *Heliothis* numbers on plots of different plant density.

Plant density	Mean plants per metre	Mean eggs per plant	Mean larvae per plant
Low	4.3	0.13 a	0.06 a
Medium	7.4	0.07 b	0.04 a
High	12.5	0.06 b	0.02 a

Within each column, means followed by the same letter are not significantly different at P = 0.05

Table 2. Mid/late season *Heliothis* numbers on plots of different plant density and numbers of squares per metre at peak count (23 Jan) and bolls per metre at peak count (6 Feb - last count prior to hail damage).

Plant density	Mean plants per metre	Mean eggs per metre	Mean larvae per metre	Mean squares per metre at peak	Mean bolls per metre at peak
Low	4.2	3.71 a	1.38 a	122.0 a	115.6 a
Med	6.8	1.53 b	0.85 a	135.6 a	131.1 a
High	10.7	3.06 ab	1.12 a	170.3 b	117.7 a

Within each column, means followed by the same letter are not significantly different at P = 0.05

Early in the season, there were differences in the mean number of *Heliothis* eggs per plant across the different density treatments. As might be expected, low density plots showed significantly higher mean egg numbers per plant than either the medium or high density plots. These differences were not reflected in significant differences in numbers of larvae, though there was a trend towards more larvae on the plants at low density. This relation between egg and larval numbers continued in the mid and late season checks, but further sampling at higher *Heliothis* pressure will be needed before definitive conclusions can be drawn about the effects

of plant density on *Heliothis* numbers. If it can be demonstrated that low density stands carry a significantly higher larvae load per plant then the question of different insect thresholds for these plants prior to compensation becomes important.

Within the context of this research project it was decided that resources could be better employed by looking at observer differences rather than repeating this work for another season. The preliminary results gained from the one season of field work were however valid and interesting. This field of work deserves further investigation.

4. Efficiency of sampling procedures

Current sampling methods are based on checking a minimum of 30 plants per 50 Ha. of cotton. The more widely distributed these plants, the better the representation of insect activity across the whole field. However, the time spent moving between sample plants must also be considered, especially in the commercial context where scouts have to sample many fields in a day.

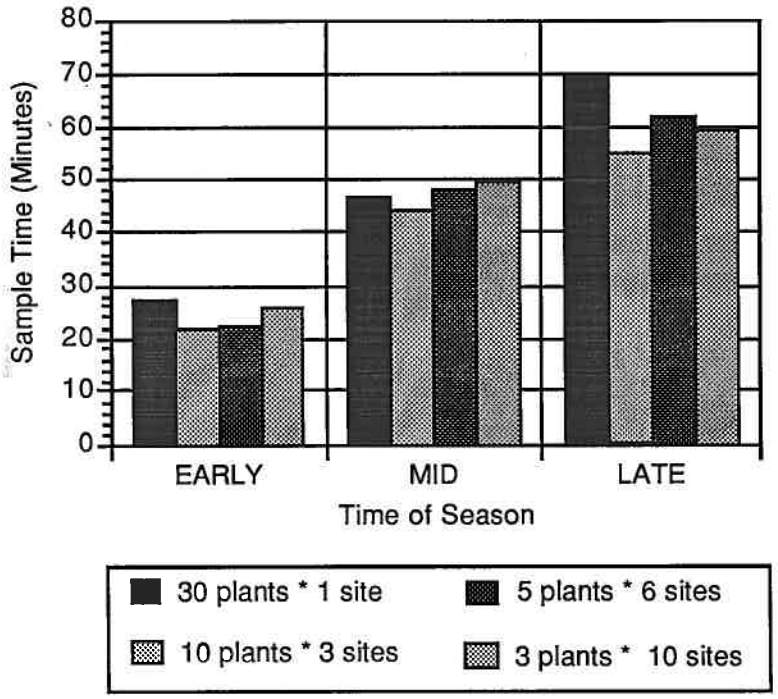
Comparisons were made between four sampling strategies involving different distributions of the 30 sample plants within a field. The studied distributions were: 30 plants at 1 site, 10 plants at 3 sites, 5 plants at 6 sites and 3 plants at each of 10 sites. The distance between sample sites was constant. The total number of *Heliothis* found within each 30 plant sample was recorded, along with the time taken to complete the sample.

Low overall *Heliothis* numbers present during the experimental period made statistical comparisons between the sampling methods difficult. Nevertheless analysis indicated that there were no significant differences between the number of *Heliothis* eggs or larvae (expressed as mean number per plant) found by the different strategies.

The timing data (Figure 1) indicates that, despite the time needed to walk between sample sites, dispersed sampling strategies did not take significantly longer to complete than did clumped ones. Results in fact indicate that spreading sample plants across several sites may reduce the overall sample time. The reasons for this are not clear, but the psychological hurdle of having to sample a large number of plants at a single site may

be daunting, especially late in the season, when the plants are large and more difficult to search. Moving between sample sites provides a break from the repetitive and often tedious task of insect scouting and so may help to keep the observer alert and more efficient.

FIGURE 1: Mean time taken to complete each type of 30 plant sample. Results shown are averages of two seasons' data.



5. Observer Differences

In order to quantify differences in the number of insects found by different observers, experiments were conducted to directly compare the findings of three experienced checkers. Field experiments were conducted on four separate days during February 1991. On each day, 30 plants were marked and the three observers all looked at the same plants. The plants were arranged in six groups of five plants and the observers moved through the groups in rotation. Thus each observer got a turn at being the first, second and third observer to sample a particular group of plants. Plants were checked in the usual way, and the number of *Heliothis* found were recorded along with their position on the plant. Whole plants were sampled throughout this experiment to eliminate problems with different interpretations of what is the terminal portion of the plant.

Results from these observer comparison experiments are summarised in Tables 3 to 6. Considering the three observers were checking exactly the same plants, the differences in the number of *Heliothis* found were surprisingly large. It is acknowledged that some *Heliothis*, especially eggs, may have been dislodged by previous observers, but it was thought that this effect would be acting fairly equally on all observers, so the overall comparisons of *Heliothis* numbers would still be valid.

Table 3. Number of *Heliothis* eggs found by each observer in a 30 plant sample.

		OBS A	OBS B	OBS C
Day 1	White eggs	33	47	30
	Brown eggs	26	16	11
	Total eggs	59	63	41
Day 2	White eggs	5	7	5
	Brown eggs	5	8	4
	Total eggs	10	15	9
Day 3	White eggs	6	14	6
	Brown eggs	12	14	5
	Total eggs	18	28	11
Day 4	White eggs	16	24	12
	Brown eggs	18	12	7
	Total eggs	34	36	19

Table 4. Number of *Heliothis* larvae found by each observer in a 30 plant sample.

		OBS A	OBS B	OBS C
Day 1	Very small larvae	0	2	0
	Small larvae	0	0	0
	Medium larvae	0	0	0
	Large larvae	0	0	0
	Total larvae	0	2	0
Day 2	Very small larvae	5	11	2
	Small larvae	3	7	2
	Medium larvae	1	1	0
	Large larvae	0	0	1
	Total larvae	9	19	5
Day 3	Very small larvae	8	9	5
	Small larvae	5	7	2
	Medium larvae	3	2	3
	Large larvae	1	3	2
	Total larvae	17	21	12
Day 4	Very small larvae	10	10	3
	Small larvae	1	6	0
	Medium larvae	0	0	1
	Large larvae	0	0	0
	Total larvae	11	16	4

Table 5. Number of plants infested with *Heliothis* eggs found by each observer in a 30 plant sample.

		OBS A	OBS B	OBS C
Day 1	White eggs	18	21	13
	Brown eggs	12	7	8
	White or brown	21	22	15
Day 2	White eggs	3	4	4
	Brown eggs	2	3	2
	White or brown	5	6	6
Day 3	White eggs	5	8	4
	Brown eggs	8	7	4
	White or brown	11	13	7
Day 4	White eggs	7	11	7
	Brown eggs	8	6	3
	White or brown	11	14	8

Table 6. Number of palnts infested with *Heliothis* larvae found by each observer in a 30 plant sample.

		OBS A	OBS B	OBS C
Day 1	Very small larvae	0	2	0
	Small larvae	0	0	0
	Medium larvae	0	0	0
	Large larvae	0	0	0
	Any larvae	0	2	0
Day 2	Very small larvae	4	7	2
	Small larvae	3	7	2
	Medium larvae	1	1	0
	Large larvae	0	0	1
	Any larvae	7	12	5
Day 3	Very small larvae	4	7	5
	Small larvae	4	6	1
	Medium larvae	2	1	2
	Large larvae	1	2	1
	Any larvae	8	13	8
Day 4	Very small larvae	8	6	3
	Small larvae	1	5	0
	Medium larvae	0	0	1
	Large larvae	0	0	0
	Any larvae	9	11	4

Table 3 details the number of *Heliothis* eggs found by each observer on each day. Comparing numbers of white and brown eggs found by each observer, the differences appear quite large. However, the total number of eggs found by each observer is more equitable. This indicates that some of the differences in numbers of specific life stages may simply be a matter of differences in classification. The same trend is evident in larval numbers found by each observer (Table 4). Differences in classifying *Heliothis* into the six arbitrary stages can be corrected by education and periodic reference to a standard to prevent "drift" in perception and classification.

What is more difficult to correct however, is the sort of variation shown by observer C, who consistently finds less *Heliothis* than the other observers. Once an observer has been taught the basics of insect scouting and identification, there is little more that can be done to help them actually find *Heliothis* . Experience is of course important in searching for and finding insects, but all three observers used in this comparison were experienced bugcheckers who were checking crops regularly, so inexperience would not be an important factor here.

When the SIRATAC sampling system is used, the observer simply records the proportion of the sample plants that had *Heliothis* present. It is expected that this sampling method, rather than having to record the total number of *Heliothis* present, would help reduce differences between observers. Tables 5 and 6, show the number of plants in the 30 plant sample found to have *Heliothis* eggs and larvae present. Surprisingly, distinct differences still exist between observers. This means that some observers were not simply missing the presence of multiple *Heliothis* on plants, but were missing the fact that some plants were infested with *Heliothis* at all. So,while the use of a presence/absence recording system helped reduce observer differences, they were not eliminated.

Following the sample results through to decision recommendations allowed us to assess the impact of observer differences on pest management practices. Each observer's counts were entered separately into both SIRATAC and Entomologic. The counts of all three observers produced spray recommendations for each of the sample days. The only differences found were in the type of chemical that the expert systems recommended. Ovicidal rate recommendations were made on several of the sampling days for the counts recorded by observers A and B, but not observer C. This reflected the differences in number of eggs found. If the numbers of *Heliothis* found had been closer to the action threshold levels then perhaps we would have seen some greater divergence in the spray recommendations. The present *Heliothis* action thresholds appear to be robust enough to cope with the levels of variation recorded between experienced observers during this study.

CONCLUSIONS

The field sampling techniques developed for SIRATAC have been accepted by much of the industry as a basic standard sampling procedure. This involves sampling a recommended 30 plants per 50 Ha of cotton. It must be noted that this level is well below that necessary for statistical precision and accuracy. However, in this project, with its statistical evaluation of SIRATAC sampling, we were not aiming to develop the most statistically correct system. Within the context of this project, the current sampling system has proved adequate in gaining samples indicative of the general *Heliothis* pressure in a field. The recommendation of larger sample sizes, on statistical grounds, would place extra pressure on the tight time schedules of field scouts and consultants, and thus would probably be disregarded. Thus it seems that the current field sampling procedures set down by the SIRATAC system are working well, in that they are accepted by the industry and provide an acceptable measure of *Heliothis* activity in the field.

The computer conversion equations used by SIRATAC to convert field counts of proportion infestation to mean number of insects per metre are still under review. It appears however that some slight changes in the mathematical parameters may be made. In addition to this it is hoped to develop a new direct conversion relationship for samples from the terminal portion of the plant. This new simplified relationship will replace the original iterative process and may be used within the Entomologic program.