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THE DEVELOPMENT OF SAMPLING AND CONTROL MEASURES FOR MIRIDS IN COTTON

PROJECT NUMBERS UQ15C AND UQ20C (THE LATTER BEING A POSTGRADUATE SCHOLARSHIP TO MELINA M. MILES)

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"A final report prepared for the Cotton Research and Development Corporation"

Please note that this report should be read in conjunction with the thesis of M.M. Miles, which is attached as an addendum. All point covered in the present report are expanded and fully justified in the thesis.

1. Introduction.

Green mirids present a significant problem to the cotton industry in Australia. Whereas these bugs are believed to have been controlled incidentally in the past when broad spectrum insecticides were applied regularly against bollworms, they now warrant control in their own right. Green mirids are known for invading early season cotton and then "tipping out" terminals and damaging squares. Although such damage does not usually reduce yield, it does delay maturity by about a week (Chinajariyawong 1988). The risk associated with a delayed harvest is generally not acceptable to growers.

The mirids that invade cotton have been postulated to come from one of two sources, either from nearby crops (lucerne and safflower, for example) or from a distance on storm fronts. Interpretation of the ecology and pest status of the bugs has been seriously hampered by a lack of understanding of the species status of the various green mirid populations that are present in the field. For example, Chinajariyawong (1988) documented the presence of two species of similar-looking mirids in Queensland cotton, and suggested the presence of a third species.

The information available on cotton green mirids is rather anecdotal and information about the relationship of the pest to dryland cotton is poor. The general objective of these two projects was to address this lack of information and to build a sound understanding of green mirids and their control in relation to cotton. The specific objectives of the study are outlined in the following section.

2. OBJECTIVES:

1. To study sampling and control practices for mirids in irrigated and dryland cotton.

   * development of more accurate sampling techniques

   * insecticide trials to improve control

   * development of economic thresholds

2. To assess the importance of local alternate hosts in producing populations of green mirids that attack cotton.

   * assess mirid invasion of cotton from nearby lucerne and safflower

   * assess whether mirids invade cotton on weather fronts from distant sources

3. RESULTS AND DISCUSSIONS:

i) SAMPLING TECHNIQUES.

a) Methods: Alternative methods are available for mirid sampling in cotton, but so far no comparison or standardisation of methods has been attempted. Absolute counts were measured and, for comparison, counts were made by means of vacuum sampling and the most common commercial sampling method, which entails disturbing the mirids and counting them as they fly away or fall off the plant.

The relationship between the absolute and relative (vacuum and commercial) estimates varied between cotton season and with mirid life stage. Generally, estimates of abundance were better for adults than for nymphs, but variation was sufficient to disrupt all efforts to develop a conversion factor to adjust relative estimates to absolute estimates. For nymphs, the commercial sampling method is ineffective. The vacuum method was frequently accurate, as assessed against absolute counts, but it was not sufficiently accurate for a conversion factor to be developed.

Overall, the vacuum sampler is the more accurate method, but the variability evident in the sampling results indicates that sampling effectiveness is a function of more than just the method used.

Mirid distribution in cotton fields: Green mirids are distributed randomly in cotton fields, at least when taking samples at intervals of 20m

ii) INSECTICIDE TRIALS.

Field trials conducted at Biloela indicate that granular formulations are more effective against mirids than sprays.

Endosulfan EC was not effective against adults, whereas endosulfan ULV was generally effective. Omethoate was effective at lower rates of application than endosulfan.

Predator buildup in cotton was not affected by up to three sprays of organophosphates.

iii) DAMAGE POTENTIAL OF GREEN MIRIDS AND ECONOMIC THRESHOLDS.

a) Boll damage by mirids: Preliminary results indicate that mirids can damage bolls, but this aspect warrants further investigation before any link with the pest status of mirids can be made.

b) Estimates of threshold densities - field cage trials: Male and female mirids had a statistically similar impact on cotton.

Irrigated Siokra L22 (first square) compensated for damage caused by as many as 15 green mirids per meter^2.

* Lint yield per plant was not affected by mirid densities.

* Number of harvestable bolls was not reduced significantly by higher bug densities.

* Results obtained are the same as those obtained with irrigated DP90, by Chinajariyawong (1988).

Raingrown Deltapine 90 (first square) could not compensate for mirid damage, although the maximum mirid density was only 8/m^2.

* Lint yields and numbers of harvestable bolls were reduced significantly by bugs at the higher densities.

* Regression of lint yield against green mirid density indicated that one bug/m^2 may reduce yield by as much as 0.3 bales ha^{-1}.

* The economic injury level calculated from the above result comes to 0.03 green mirids/m of row. This is the density of bugs that could be tolerated if the bugs were to be continuously present in the crop for 32 days. This is much lower than the recommended thresholds and the thresholds used by consultants.

* The calculated loss of yield attributable to green mirids is 2.0 kg/ha.

Conclusions that relate to both the irrigated and raingrown trials.

* The amount of injury to the plant was not directly related to the density of bugs present.

* Densities of only 4 mirids/m^2 delayed boll opening by up to 14 days.

iv) LUCERNE AND SAFFLOWER AS SOURCES OF COTTON MIRIDS.

Two species of mirids occur in cotton in Queensland, but only one of them is found further south. The more widely distributed species is commonly known as the green mirid, and its correct scientific name is *Creontiades dilutus*. The second species, in Queensland, we refer to as the brown mirid (*Creontiades pallidifer*). It has never been known to reach high densities in cotton. All stages of each species, including the eggs, are distinguishable from the equivalent stage of the other species, and an expanded comparison between all stages is now available (Miles 1995).

A more contentious issue has been the question of the source of green mirids (*C. dilutus*) that invade cotton. The question has important practical implications in the monitoring of populations and the development of alternative control methods. Two alternative views are available, but they are not mutually exclusive.

1. *Creontiades dilutus* on cotton is only one of a complex of sibling species that may be largely host specific. In particular, the bugs on lucerne (also called *C. dilutus* because they are morphologically indistinguishable from the ones on cotton), and perhaps safflower represent a sibling species of *C. dilutus* that does not attack cotton. Green mirids are thought to invade cotton from central Australia, perhaps mainly from the channel country of western Queensland.

2. *Creontiades dilutus* is thought to invade cotton primarily from nearby crops, especially lucerne and safflower.

a) Why was the existence of two species suspected?

Two observations of the field ecology of green mirids suggested the presence of more than one species in the taxon *C. dilutus*.

First, green mirids are usually abundant in lucerne. When lucerne is mown the mirids leave, but they apparently do not invade nearby cotton, which would be predicted if we were dealing with a single species with a wide host plant range.

Second, even though green mirids may be abundant in lucerne growing near to cotton, invasion of cotton is frequently delayed. When invasions do take place, they simultaneously cover vast areas that are under cotton, and they coincide with weather fronts.

b) Testing for the existence of two species.

Two methods were used for testing the species status of green mirids from cotton, lucerne, safflower and channel country weeds, namely cellulose acetate electrophoresis and pheromone analysis.

Twenty two enzyme systems were screened for fixed allelic differences, but no alternative alleles were fixed in populations from different host plants. Such fixed differences, if present, would indicate the presence of two species. However, different species do not inevitably have fixed differences, so our results are no indication that the two populations are of the same species.

Therefore the polymorphic loci were analysed for allelic frequencies, to test whether samples (and therefore populations) were in Hardy-Weinberg Equilibrium, and to test if the populations were in genetical equilibrium with one another. Genetic distance analysis indicated little genetical differentiation between populations from different hosts, regardless of geographic separation. Indeed, the greatest similarity was found between cotton and lucerne populations collected in Biloela, and the greatest differentiation was between western Queensland populations (whatever the host plant) and those in Queensland and New South Wales crops.

The electrophoretic results are, therefore, equivocal. Although they do not demonstrate the existence of two species in the taxon *C. dilutus*, neither do they provide unequivocal evidence that we are dealing with only one species. Therefore, an alternative approach was initiated.

Sex pheromones were investigated because different congeneric species frequently use molecules or chemical mixtures that differ one from the other. At the start of this part of the study, nothing was known about the sexual communication of green mirids. Other heteropteran bugs are known, however, to use pheromones (including seven species of mirids) and acoustic communication. We could not investigate the latter because of the difficulties in getting the bugs to mate in a confined space.

Initially we established that virgin female green mirids do indeed produce a long-distance chemical attractant. Traps baited with virgin females brought in only male green mirids in the field. In contrast, male-baited traps caught nothing.

Gas chromatographic analysis of metathoracic gland extracts showed no differences between males and females, or between green mirids and brown mirids. The metathoracic gland therefore does not produce a sex pheromone, but an alarm pheromone whose major constituent is hexyl hexanoate. Alarm pheromones in bugs are not useful in distinguishing species.

Two methods were then used in efforts to detect sex pheromones, namely air-borne trapping of volatiles released by live bugs and solvent extracts of whole bugs. The former yielded only alarm pheromones whereas the latter revealed many compounds, but none that were different between males and females. In summary, the pheromone analysis did not resolve the species issue, although it has the potential to do so.

c) Conclusions.

* Electrophoresis revealed no fixed allelic differences, although differences in allelic frequencies were recorded. The results are consistent with there being only one species, but they cannot discount the existence of two species.

* The metathoracic gland extracts of females is exactly the same as that of male extracts. Hexyl hexanoate, being secreted by both sexes, is therefore an alarm pheromone.

* Trapping of air-borne volatiles and hexane extracts from whole bugs yielded no female-specific volatile that could have been a sex pheromone.

* Pheromones may yet prove suitable for testing the species status of different green mirid populations.

v) DO GREEN MIRIDS INVADE COTTON FROM CENTRAL AUSTRALIA?

Green mirid invasions into cotton were dated precisely, in two seasons, by means of regular sampling. At that time, lucerne populations were increasing. Lucerne populations showed no dip in numbers or decrease in population growth at the time that the cotton invasion took place. Yet enormous numbers of mirids would be required, from relatively restricted areas covered with lucerne, to achieve the high densities (average of about 35 mirids per 20 m of row) recorded in the 1992/1993 season.

Sticky traps placed in or near lucerne trapped green mirids at all times. Only once did sticky trap samples peak at the time of the cotton invasion, which suggests the invasion comes from elsewhere.

Alternative sources of green mirids were sought in central Australia. Although *C. dilutus* was collected on many plant species, it was numerous and breeding on relatively few of them, including blue parsnip, wild sunflower and annual verbine (Miles 1995). On annual verbine green mirids were present in vast numbers and the plant covered large areas of the channel country.

To check the feasibility of green mirids invading cotton from the channel country, daily weather charts were consulted to establish if synoptic systems could have influenced the movement of mirids into central Queensland at the time that the influx into cotton took place. However, results were ambivalent, perhaps because the assumptions made on behalf of the model were inappropriate. More information on the behaviour associated with migration may help in a more realistic interpretation of the potential for green mirids to invade cotton from the channel country.

4. CONCLUSIONS, RECOMMENDATIONS AND APPLICATION TO INDUSTRY.

INDUSTRY BENEFITS: Industry benefits in several ways from the research reported.

1) We provide the first comprehensive account of the ecology and effecrtiveness of sampling of green mirids. This has major implications for the design of future pest management strategies. In particular, our reservations about green mirids in lucerne (and perhaps safflower) possibly belonging to another species that does not attack cotton are relevant to any non-chemical means of manipulating these organisms. Before the results of this project, the lucerne mirids were simply treated as cotton mirids on another host.

2). We have confirmed that irrigated cotton compensates for mirid damage, but not to the extent that boll maturation is not delayed. Raingrown cotton, in contrast, cannot compensate for yield or time of maturation. We have identified important questions about green mirid behaviour in cotton that will help to refine threshold values.

3) A survey of consultants indicated that there is no uniform perception of whether the crop is being managed to maximise yield or to achieve maturation within a minimum time. Previously these two aspects have been treated together.

Our project aims were achieved in large measure, and we could confirm Chinajariyawong's (1988) major conclusions, as well as extend his work significantly. Nevertheless, several important gaps remain in our understanding of green mirids in cotton. Below we summarise our conclusions, and detail our recommendations for future research.

SAMPLING FOR GREEN MIRIDS: Sampling for green mirids presents two problems. First, all sampling methods underestimate the density of mirids that is actually present. Second, each method returns variable results, so we were unable to derive standard correction factors to convert sample values derived from one method into reliable estimates of absolute values. A reliable conversion factor would help considerably in monitoring mirids accurately so that established thresholds are used realistically.

Sampling trials to date indicate that the bugs are randomly distributed in cotton fields, that the vacuum method of sampling is most reliable, and that samples spaced at 20 m from each other are adequate for estimating density

Recommendations: Vacuum sampling is recommended for all density estimates, whether for research purposes or for commercial purposes. A standardised method should be recommended across the industry so that all sampling data are comparable.

Future research: 1. The reason for sampling being so variable relative to absolute methods of assessing mirid density warrants research. Is the variation a result of variable behaviour with time of day or among age classes of bugs, for example?

2. The distribution of bugs in cotton fields needs to be systematically investigated in different cotton-growing areas and at different times of the year, to confirm that the recommended sampling procedure is sufficiently accurate.

ECONOMIC THRESHOLDS: The field cage threshold trial using Siokra under irrigation returned results identical with those obtained by Chinajariyawong (1988) with irrigated Deltapine. Yield was not reduced significantly, but boll maturation was delayed significantly and by at least seven days with bug densities of 4/m of row.

In contrast, a raingrown trial with Deltapine was affected quite differently, with a significant loss of yield and delay in maturity at bug densities of 4/m. A threshold calculated with the prevention of yield loss in mind came to 0.03 mirids/m of row, but that is assuming that the mirids are present and causing damage for 32 days.

Recommendations: 1. Irrigated cotton can compensate readily for yield, but not inevitably for delay. Consultants surveyed were ambivalent about whether their recommendations are for yield or delay (Miles 1995). This issue needs to be assessed across the industry to determine how thresholds should be established, because different aims warrant different attitudes to threshold recommendation.

2. Raingrown cotton requires intervention against green mirids because they delay the crop as well as reduce yield.

Future research: 1. The calculated threshold is lower than current commercial thresholds, but it has been developed on the assumption that the individual bugs are present in cotton and feed continuously for 32 days. Retention times of adult bugs in cotton need to be assessed, probably independently for individuals that develop from eggs laid on cotton and individuals that invade cotton as adults. A more realistic threshold could then be developed.

2. The specific objectives of growers need to be established, so that recommendations can address the desired end product specifically.

SOURCE OF MIRIDS IN COTTON: Two potential sources of mirids have been postulated.

1.) Mirids are thought to invade cotton from nearby lucerne (Qld and NSW) and safflower (NSW) fields. Direct evidence to support this assertion is not available, and several points suggest this interpretation is incorrect. Specifically, mirids tend to invade large numbers of cotton fields simultaneously across an area, rather than some fields before others, as one would expect if the invasion originated in nearby lucerne fields. Also, the invasion of cotton takes place suddenly some time after cotton is suitable for the bugs. If the bugs came from nearby lucerne, for example, a gradual buildup would instead be expected.

2.) Mirids have been postulated to invade cotton from central Australia. Such an origin would explain the patterns of invasion outlined under point one, and is consistent with the common observation that bugs arrive on storm fronts. Large populations of green mirids have been found in the channel country of western Queensland, but attempts to connect the movement of appropriate weather fronts with invasion dates were inconclusive, perhaps because the assumptions about green mirid migratory behaviour that had to be made were inaccurate.

Because patterns of invasion of cotton by green mirids is inconsistent with the bugs having come from lucerne, the other way of investigating their source was to test whether two host plant associated sibling species are present. The results of the tests were consistent with their being only one species on all host plants (i.e. cotton, lucerne, safflower and native hosts in the channel country). However, the pheromone tests were not run to completion because none of the standard methods yielded an obvious sex pheromone for comparison among host-associated populations.

Recommendations: The status of the different host-associated populations should be treated as an open question.

Future research: 1. The pheromones of the different host-associated bugs should be investigated further, to test their species status. Other means of investigating species status of the different populations should also be tested, for example cuticular hydrocarbons and, if mating can be achieved in the laboratory, sound recording and cross-mating trials.

2. The pattern of mirid invasion of cotton should be investigated further to confirm that the appropriate patterns are being dealt with in interpreting the ecology of green mirids. Collections of samples across fields in a growing area and across growing areas should be co-ordinated to test that invasion is indeed simultaneous. In addition, standardised sampling across growing areas would help in interpreting the different perceptions of green mirid pest status between New South Wales and Queensland (Miles 1995).

5. COMMUNICATION OF RESULTS:

Publications:

i) MILES, M., PYKE, B. & WALTER, G.H. 1992. Sampling and control of mirids in cotton. *Proceedings of the 1992 Australian Cotton Conference. Broadbeach, August 1992: 289-296.*

ii) MILES, M.M., PYKE, B. & WALTER, G.H. 1993. Invasion of cotton by green mirids (*Creontiades* sp). *24th Scientific Conference & AGM of the Australian Entomological Society, Cairns.*

iii). MILES, M.M., PYKE, B., WALTER, G.H. & MALIPATIL, M. 1994. The mirid problem and options for management. *Proceedings of the 1994 Australian Cotton Conference Broadbeach, August 1994.* In press.

iv) MILES, M., WALTER, G.H. & PYKE, B. 1993. An update on green mirid research at the University of Queensland. *Australian Cotton Grower* **1 4**: 62-63.

v) MILES, M.M. PhD. Identification, pest status, ecology and management of the green mirid, *Creontiades dilutus* (Stål) (Hemiptera: Miridae), a pest of cotton in Australia. The thesis is currently being examined.

Workshops:

1) M.M. MILES & G.H. WALTER. Early season cotton pest workshop held at Gold Coast on 11 August 1992.

ii) M.M. MILES & G.H. WALTER. Early season cotton pest workshop held at Toowoomba on 10 & 11 May 1993.

iii) G.H. WALTER. Early season cotton pest workshop held at the Australian Cotton Research Institute on May 12-13, 1994.

iv) M.M. MILES & G.H. WALTER. Sucking pest workshop held at the Australian Cotton Research Institute on November 15-16, 1994.

BUDGET:

The total funds contributed by the CRDC amounted to:

1990/1991 - $	30 000
1991/1992 - $	35 609
1992/1993 - $	35 009
	1 000 (travel grant)
1993/1994 - $	11 384
1994/1995 - $	7 410
TOTAL	$120 412

REFERENCES CITED:

CHINAJARIYAWONG, A. 1988. The sap-sucking bugs attacking cotton: biological aspects and economic damage. Unpublished PhD thesis, The University of Queensland.

MILES, M.M. 1995. Identification, pest status, ecology and management of the green mirid, *Creontiades dilutus* (Stål) (Hemiptera: Miridae), a pest of cotton in Australia. Unpublished PhD thesis, The University of Queensland. The thesis is currently being examined externally.