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Potential for Pheromone Based Attract-and-Kill and Mating Disruption of the Green Mirid, *Creontiades dilutus* (Stål) (Hemiptera: Miridae)

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Abstract: Attempts were made at applying green mirid pheromones in a sprayable formulation for mating disruption and attract-and-kill in *Creontiades dilutus* (Stål), an emerging significant pest of cotton and other crops in Australia. In the mating disruption trials, a total trap shutdown for 2 days was observed. The short trap shutdown period is thought to have arisen from the formulation used. In the attract-and-kill work, efforts made to locate and count dead mirids for quantification did not work. Either the insecticide did not kill the mirids fast enough, resulting in their moving away from the treated row before dying, or the low numbers of mirids present made the sampling method ineffective. However, the trap results suggest that attract-and-kill for male green mirids remains a promising option. As with mating disruption, however, further work needs to be done on a long lasting formulation to overcome potential problems with reinvasion of treated fields.

Key words: Pheromone traps, mating disruption, attract and kill, green mirid, D-vac

INTRODUCTION

The green mirid, *Creontiades dilutus* (Stål) (Fig. 1) is found in many crops such as lucerne, potatoes, soybeans, mung beans, pigeon pea, stone fruits, sunflower and grapes (Hori and Miles, 1993; Malipatil and Cassis, 1997; Carver *et al*., 1991). It was identified as a sucking pest of Australian cotton in the early 1970's (Room, 1979; Bishop, 1980). Pre-mature abortion or deformation of fruits, leaf wilt and disease transmission are some of mirid plant damages.

Fig. 1: A photograph of an adult green mirid, *Creontiades dilutus* feeding on mung beans

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In conventional (non-transgenic) cotton in the past, the green mirid (GM) rarely reached economic levels because the populations were usually suppressed by insecticides sprayed to control the key pests, *Helicoverpa* sp. With an increase in the adoption of integrated pest management strategies in the cotton industry as well as the commercialisation of transgenic (Bt) cotton, broad spectrum insecticide use is being reduced and replaced by more selective insecticides. GM pest status is therefore increasing, and it is expected to become an important economic pest in cotton in Australia. Similarly in the US, data indicates resurgence of heteroptera as pests with the implementation of Bt-cotton (Layton *et al*., 1997; Layton, 2000).

Strategies in place for managing GM in Australian cotton involve the following: (a) destruction of all alternate hosts including native weeds at least two weeks before planting of cotton; (b) establishment and management of lucerne as a trap crop at least two weeks prior to cotton planting; this is to attract mirids away from the cotton; (c) monitoring mirid damage and thresholds (0.5 m^{-1} of both adult and nymphs in cool regions and 1 m^{-1} in warm season areas (Johnson and Farrell, 2003) to determine if control is needed; mirids are then sprayed only when insect and damage counts are at the threshold levels; (d) avoiding the use of broad-spectrum insecticides for control of green mirid which reduce the numbers of beneficials that feed on *Helicoverpa* and mites (Mensah and Khan, 1997).

The development of sex pheromone lures or other attractants for this species would complement current control methods. They might be useful for attract-and-kill, as monitoring tools of GM populations, or perhaps for mating disruption. Attract-and-kill might be suitable since GM have a relatively long adult pre-reproductive period (about 7 days; Khan, 1999), which would provide a long window in which males could be removed before mating, thereby reducing female oviposition. Also, since adult male mirids cause damage, reducing their numbers would be beneficial, independently of any reduction in oviposition.

This research describes our studies at manipulating green mirid populations in cotton using formulations of their sex pheromones in mating disruption and attract-and-kill techniques. A successful pheromone based formulation application against the green mirid would help reduce the use of insecticides in the cotton and other vegetable industries, cutting down cost and environmental degradation.

MATERIALS AND METHODS

Experiment 1: Attract-and-Kill Experiment Using Suction Sampling

The experiment was designed to investigate whether the mirid pheromone components identified through laboratory and field-trapping experiments could be used in a sprayable formulation for attracting male GM to specific rows of cotton, for possible application in an attract-and-kill system.

The trial was set up in a field of flowering faba beans at Carbucky, near Goondiwindi, Queensland in 2004. Magnet® base was used to formulate the treatments. Magnet® is a sprayable attract-and-kill formulation which contains plant volatiles and insecticides, and is being commercialised for use against *Helicoverpa* moths in Australian cotton (Gregg and Del Socorro, 2002). For the purposes of this experiment, the plant volatiles and insecticides were omitted. There were two treatments, Magnet® base alone and base containing the GM pheromone blend. The GM pheromone blend consisted of 1% hexyl hexanoate and 0.2% (E)-hexenyl hexanoate determined from field bioassays as optimum blend for the green mirid (Lowor *et al*., 2009).

Treatments were applied to 50 m strips of faba beans, arranged in a square pattern of four rows each containing one replicate of each treatment, with 50 m buffer strips between them. Rows were separated by 50 m. Formulations were applied to the tops of plants in each replicate by hand (shaken from a plastic bottle) at 500 mL per 50 m.

The treatments were sampled using a large backpack suction sampler (D-Vac), based on a Solo Mist Blower Port 423. This machine has a sampling efficiency of 50-60% for GM in cotton (Stanley, 1997). The nozzle was moved over the top of the plants at a slow walking speed and insects collected in a nylon bag, then transferred to plastic bags and frozen prior to counting. Treatments were sampled at 20, 31, 78 and 123 h post application. Twenty hour sample was done in mid-morning, on the day following application, but all subsequent samples were done at night, around 2200-2300 h. This was because trapping studies showed that most male mirids came to the pheromone in the early evening. On each sampling occasion, four control (untreated) 50 m sections were sampled from randomly chosen locations between the treated rows. The controls therefore represented sections from which insects had not been previously removed; whereas for the treated sections, most insects collected probably represent arrivals since the last sampling time.

Experiment 2: Attract-and-Kill 2 and Mating Disruption Trial in Cotton

Experiment aimed at testing formulations using mirid pheromones identified for attract-and-kill and for mating disruption. The trial was carried out in dryland cotton (pre-squaring stage) at Prospect, near Warra, Queensland. Two sites, about 400 m apart, were chosen for this experiment. These sites were labelled as field S3 (conventional cotton) and field WL4 (Bollgard II® or transgenic cotton). Both fields had double skip rows, that is, two rows planted and two rows skipped, which is a common configuration for dryland cotton cultivation in Australia. Field WL4 was used as another control. Trial layout and design was as shown in Fig. 2 and 3. Treatment blocks were demarcated as 290×300 m (8.7 ha) for field S3 as shown in Fig. 3. Buffer zones of 300 m were left between treatments. For field WL4, no treatments were imposed but pheromone traps were laid out in a similar pattern to field S3, except that the dimensions of this field were slightly smaller (Fig. 2).

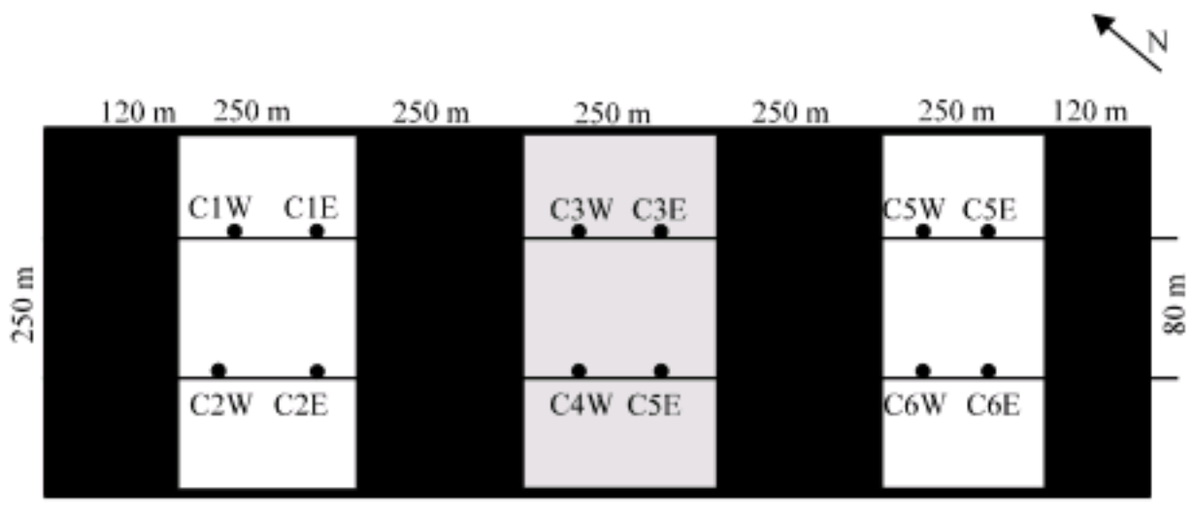

Fig. 2: Layout of field WL4 (control) showing location of pheromone traps. •: pheromone traps

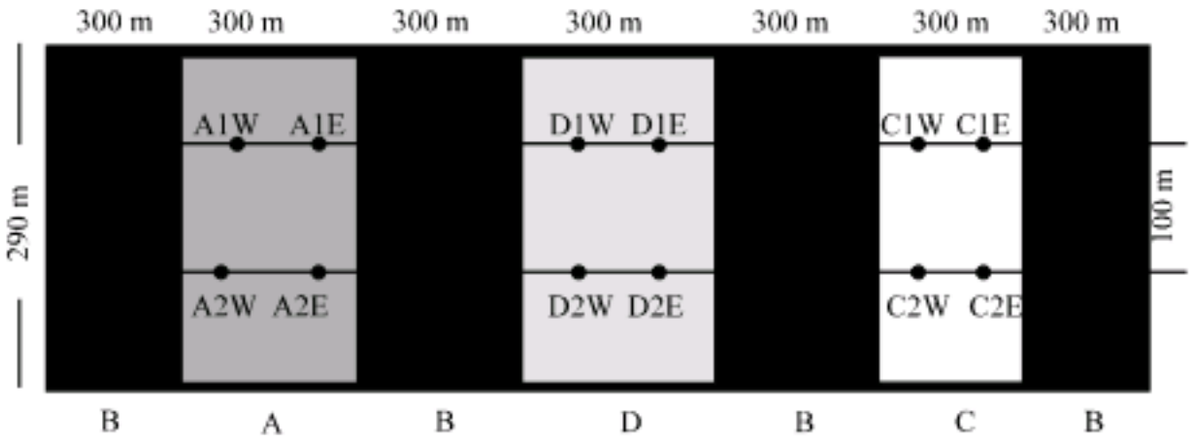

Fig. 3: Layout of field S3 (treated) showing location of heromone traps. •:pheromone traps, A: Attract-and-kill, D: Mating disruption, C: Control , B: Buffer zones

Each treatment plot had 4 pheromone traps (funnel design) containing mirid lures (5:1 hexyl hexanoate: (E)-2-hexenyl hexanoate at 2 mg loading) Traps were located one-third of the way in from each corner, in two rows (row 1 = Northern, row 2 = Southern) and coded for ease of identification as shown in Fig. 2 and 3.

Attract-and-Kill

For the attract-and-kill treatment, one row in every 32 (including skip rows) was treated with 500 mL per 100 m of 1.2% mirid pheromone mix prepared in a base similar to Magnet® (Gregg and Del Socorro, 2002), but omitting the sugar and food dye. A latex-based adjuvant and sticker (Bond®, Nufarm Aust. Limited, Laverton, Victoria, Australia) was then added to give a 1% concentration. Fipronil (Regent®, Bayer Australia Ltd., Pymble, NSW, Australia) was the insecticide sprayed onto the treated rows as a cover spray, immediately after the pheromone, at a rate of 1.25 mL a.i. per 100 m.

Mating Disruption

For mating disruption, one row in every 16 (including skip rows) was treated with 500 mL per 100 m of 2.4% mirid pheromone in the base described above. The pheromone quantity applied per hectare was therefore four times that in the attract-and-kill treatment (twice as concentrated, and applied to twice as many rows). However, there was no insecticide present.

Formulations were applied by spraying through a low-pressure electric pump mounted on a modified motorcycle using a nozzle designed for liquid fertiliser application. The treatments in field S3 were sampled using a large backpack suction sampler (D-Vac), as described earlier. Four samples were taken from 50 m strips of cotton that did not have any treatment application within the treatment blocks. Treatments were sampled at 0 day (pre-treatment), 1, 2, 3, 4 and 7 days post application.

Statistical Analysis

Statistical analysis of data were done using the R statistical package version 1.9.0 (R Development Core Team, 2004). Data were summarized using means and standard errors. Relationships between variables were determined using one-way analysis of variance on log (x+1) of the data followed by contrast to determine the least significant differences between means.

RESULTS AND DISCUSSION

Experiment 1: 'Attract-and-Kill' Experiment Using Suction Sampling

Mean numbers of male and female GM from each treatment on each sampling occasion is shown in Table 1. GM numbers were very low during this experiment. In the control sections they were always below 1 per 50 m. At the 20 h sample, there were no significant differences between treatments. This may have been because males that approached the pheromone during the night left again the next morning, before the sampling. For the 31 h sample, there was a tendency for more male mirids in the pheromone treatment ($F_{2,11}$ = 3.76, p = 0.065). In the 78 h treatments there was a significant difference between the treatments in the case of males ($F_{2,11}$ = 5.72, p = 0.025). For the females, however, there were no significant differences. For the males, the differences were mostly due to higher numbers in the pheromone treatment. This treatment was significantly different from all the others (Table 1). At the final sample time, 123 h, the trends were similar to earlier samples, but the differences were not statistically significant for the males ($F_{2,11}$ = 1.88, p = 0.208).

When catches were summed over all sample intervals, there was a significant difference for males ($F_{2,11}$ = 4.98, p = 0.035). Most of this was due to the pheromone treatment, which was significantly different from the others. Overall, this treatment yielded approximately 11 times the number of male GM compared to the control.

Table 1: Numbers of GM males and females collected by suction sampling from 50 m sections of treated rows in faba beans, Carbucky, Goondiwindi, Qld

Treatment	Hours post spray	Males	Females	Total
Control	20	0.00a	0.00a	0.00a
Base	20	0.00a	0.50a	0.50a
Pheromones	20	0.75a	0.00a	0.75a
Control	31	0.25a	0.25a	0.50a
Base	31	1.00a	1.25a	2.25ab
Pheromones	31	3.50b	0.75a	4.25b
Control	78	0.25a	0.00a	0.25a
Base	78	0.75a	0.00a	0.75a
Pheromones	78	3.00b	0.75a	3.75b
Control	123	0.25a	0.50a	0.75a
Base	123	0.00a	0.00a	0.00a
Pheromones	123	2.00a	0.00a	2.00a
Total control		0.75a	0.75a	1.50a
Total base		1.75a	1.75a	3.50a
Total pheromones		8.50b	1.50a	10.00b

Means within the same column for the same sampling time which are followed by common letters are not significantly different (p>0.05)

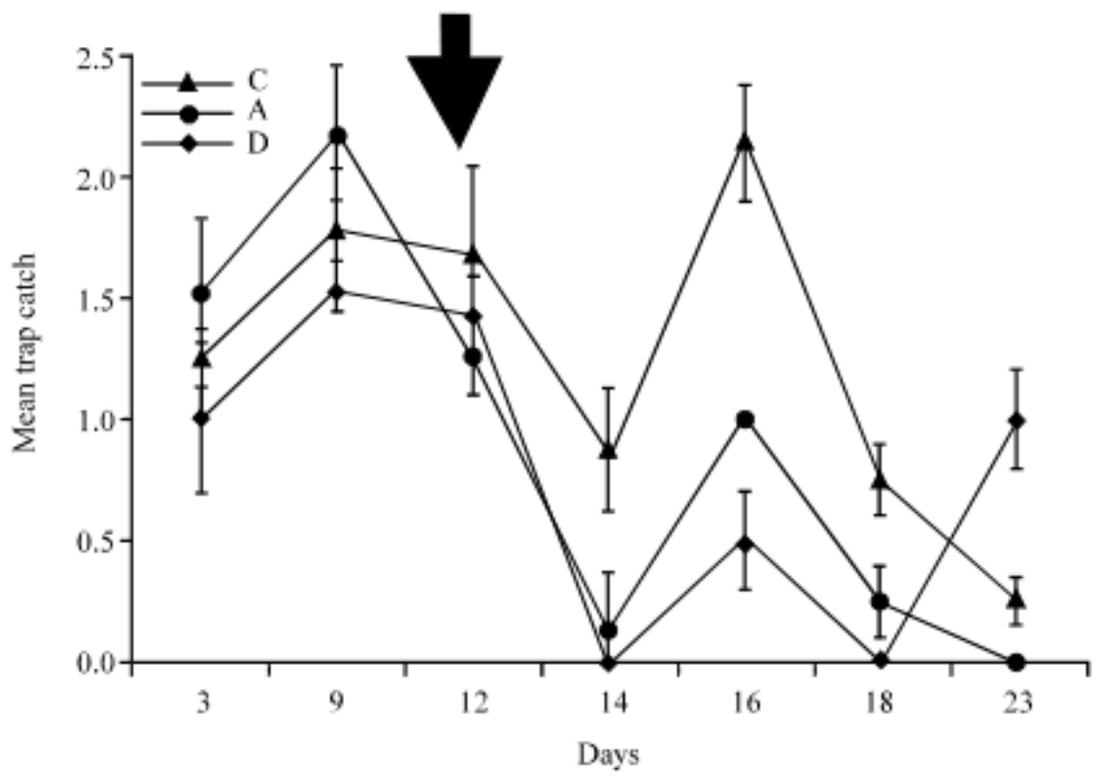

Fig. 4: Mean pheromone trap catches for the treated plots in field S3, Prospect, Warra, Qld. D-mating disruption treatment A-attract-and-kill treatment C-control. Black arrow indicates day of treatment application. Bars represent standard errors

This result indicates that there may be potential for attract-and-kill of GM using pheromones. As well as increased numbers in the suction samples, green mirids were frequently observed sitting immobile on the foliage close to deposits of the sprayed pheromone formulation. They were not observed to contact this material, however. Such observations were made during both daylight hours and by night (using a torch). These observations suggested that an appropriate strategy for attract-and-kill would be to treat the rows to which pheromone was applied with a cover spray of an insecticide effective against GM, such as fipronil. The data suggest that with an 11-fold concentration factor over 120 h (and this is conservative because new control sections were sampled at each time), most of the males in the population could be removed by treating only occasional rows, which would allow survival of most of the beneficial insects.

Experiment 2: 'Attract-and-Kill' and Mating Disruption in Cotton

Figure 4 and 5 show mean pheromone trap catches in field S3 (treated), both before and after the treatments and in field WL4 (control), respectively. In field S3, mirid trap catches were not

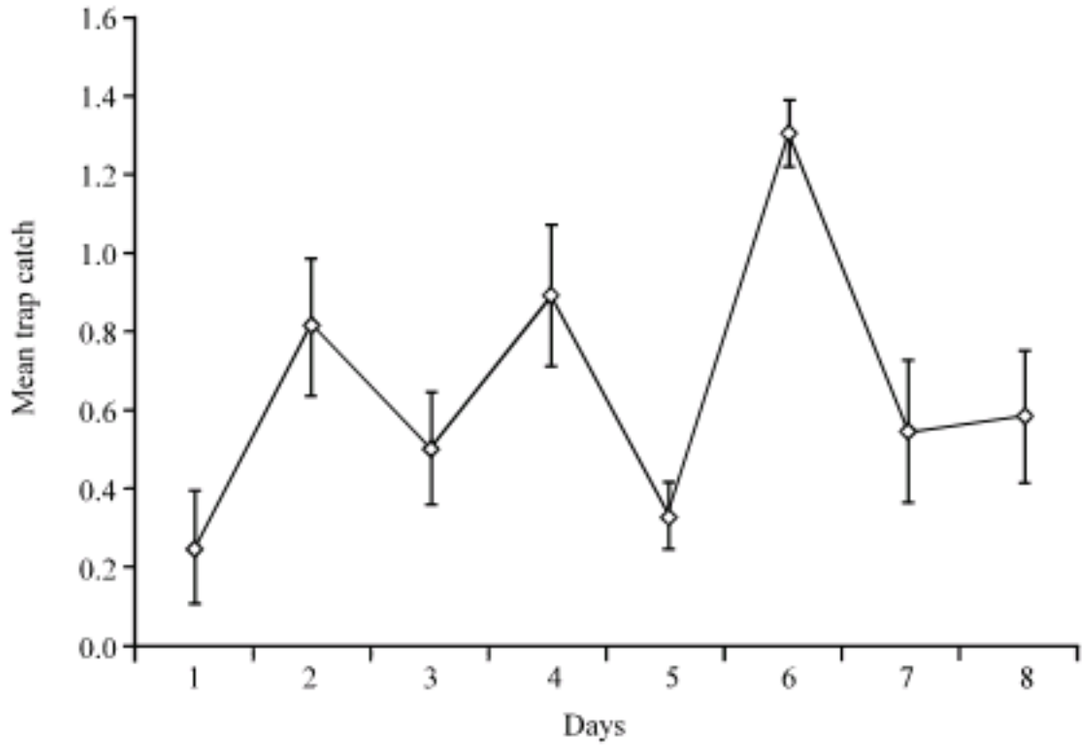

Fig. 5: Mean pheromone trap catches for the untreated field WL4, Prospect, Wara, Qld. Bars represent standard errors

significantly different ($p = 0.658$) between the sites marked for the various treatments from day 1 to day 12 (that is, during the pre-treatment phase).

Differences however, were observed between the control and the treatments after the application of the mating disruption and attract-and-kill formulations to field S3 on the 12th day. Catches of male GM in the control plot were significantly higher compared to the mating disruption and attract-and-kill plots for day 14 ($p = 0.003$, when no GM were found in any trap from either the attract-and-kill or the mating disruption treatment), day 16 ($p<0.001$) and day 18 ($p = 0.004$). There was however, no significant difference between the treatments on day 23 ($p = 0.06$). For field WL4, no significant differences existed between the summed trap catches from days 1 to 23 ($p = 0.358$). Further analysis revealed no significant variation, either along or across the field, on any day. This further strengthens the inference that the differences between plots within field S3 on days 14, 16 and 18 could have been due to the treatments applied.

The result suggests a two-day total trap shut down for the mating disruption treatment and a further partial shut down for at least another 4 days. It should be noted that the lures in the pheromone traps were replaced on day 14, 2 days after the treatments were applied. With the fresh lures, it is possible that a surface effect which involved a temporarily higher release rate of the pheromone could have taken place, overcoming the mating disruption application temporarily, hence the appearance of a few mirids in the pheromone traps in the mating disruption plot on days 16-18.

Trap shutdown following the application of pheromones is generally regarded as an indication that mating would be disrupted, since if males were unable to locate a point source of pheromone, they will also be unable to locate a female (Jones, 1998). Successful mating disruption however, does not always equate to reduced crop damage, since females, which have mated outside the treated area, may move in and oviposit (Betts *et al*., 1993). All these ecological questions need to be examined before it can be concluded that mating disruption is a viable option for mirid management. Also, the period over which trap shutdown occurs would need to be extended (perhaps by the use of controlled-release formulations) before the method is likely to become economically viable. Nevertheless, these results show promise for the use of mating disruption against GM, especially in view of the ready commercial availability of the two-pheromone components, and their low price compared to Lepidopteran

pheromone components. This experiment represents one of the few examples where trap shutdown has been demonstrated for a Hemipteran species, especially in a broad acre field crop (Zhang and Aldrich, 2003).

Lower trap catches were also recorded from the attract-and-kill treatment, after the formulation was applied (Fig. 4). This could have been because there were fewer males in the plot, because the insecticide applied to the treated rows had killed some. It could also have been because there was sufficient pheromone in the formulation to produce mating disruption, independently of the killing effect. An attempt was made to assess the impact of the insecticide by placing horticultural plastic along 5 m of treated row in three locations within the attract-and-kill plot and examining it at regular intervals for dead insects. No GM (males or females, or nymphs) was found. This may have been because the fipronil did not kill mirids quickly enough to prevent them moving away from the treated area before dying. It is therefore not possible to be sure of the mechanism for trap reductions in this treatment. However, the attract-and-kill formulation seemed to work over the same time frame as the mating disruption formulation, which suggests mating disruption as the most likely 147 possibility.

Figure 6 and 7 show the mean numbers of GM males and females respectively caught in the D-Vac sampling from the three treatments- D (mating disruption), A (attract-and-kill) and C (control).

Analysis of the data showed no significant differences between the number of males sampled with the D-Vac among the various treatments from day 12 (pre-treatment) to day 17 (5 days after treatment). The p values were: day 12 ($p = 0.98$), day 13 ($p = 0.20$), day 14 ($p = 0.80$), day 16 ($p = 1$), day 17 ($p = 0.28$). This could partly be explained by the low numbers of mirids in the experimental plots. It also suggests GM males were equally present in all the treatment plots. The differences observed in the trap catches in the attract-and-kill and mating disruption treatments could only then have come from the disruptive effects of the treatments applied. Similarly, no significant differences were observed in the number of females occurring per 50 meters of the treated fields on day 12 ($p = 0.94$), day 14 ($p = 0.32$), day 16 ($p = 0.87$) and day 17 ($p = 0.39$). However, there were significantly higher number of females in the control plot compared to the mating disruption plot ($p = 0.012$) on day 13 (1 day after treatment). It is possible that the females, unable to attract any males for mating because of the mating disruption treatment, moved into the buffer zones or control plots for a greater chance of finding mates.

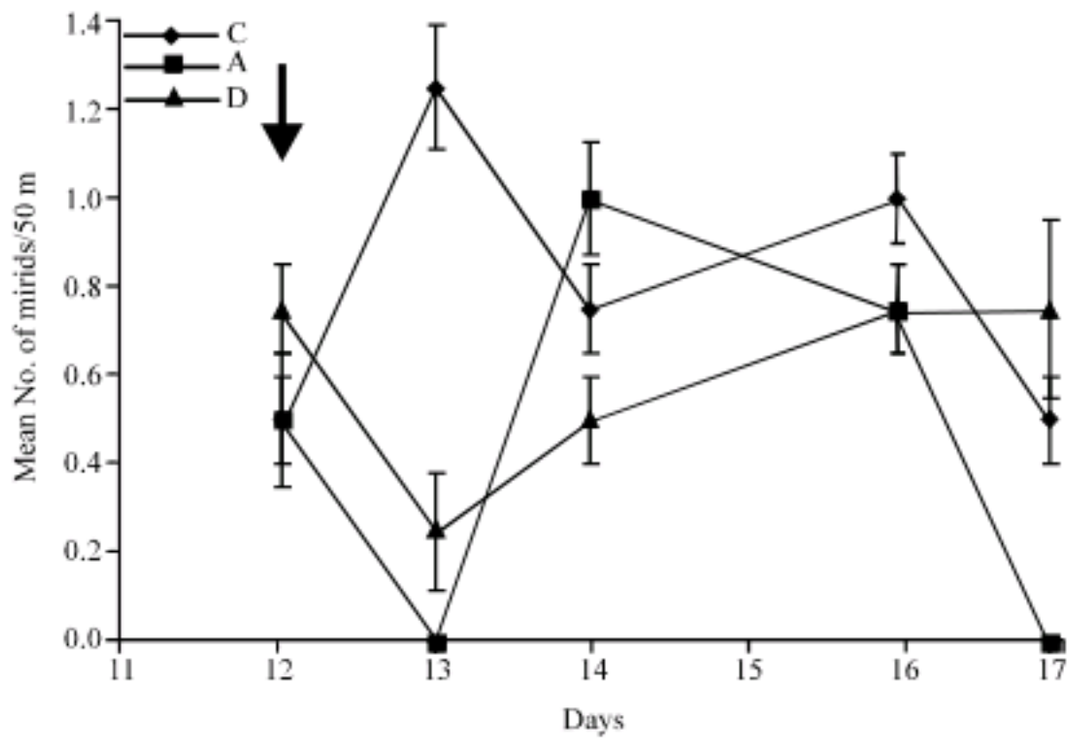

Fig. 6: Mean numbers of GM males per 50 m caught in D-Vac sampling, Prospect, Warra, Qld. D-mating disruption treatment A-attract-and-kill treatment C-control. Black arrow indicates day treatment was applied. Bars represent standard errors

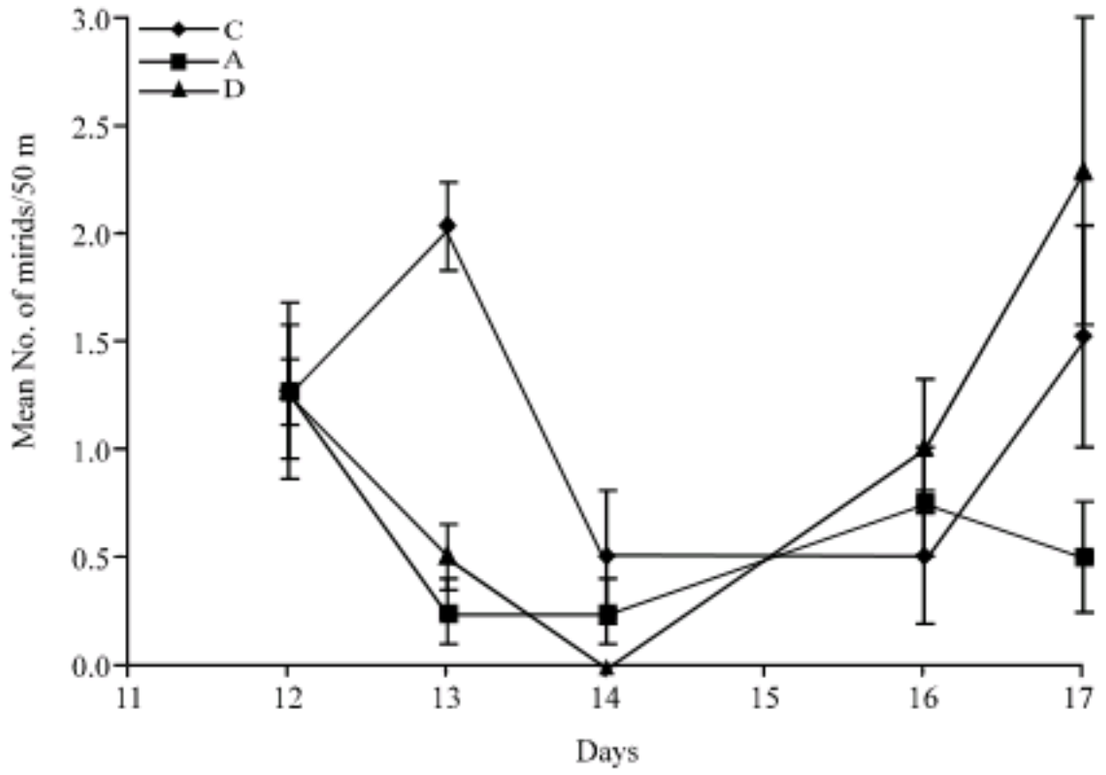

Fig. 7: Mean numbers of GM females per 50 m caught in D-Vac sampling, Prospect, Warra, Qld. D-mating disruption treatment A-attract-and-kill treatment C-control. Black arrow indicates day treatment was applied. Bars represent standard errors

CONCLUSION

The actual numbers of GM collected are probably an underestimate, since the suction sampler was only 50-60% efficient (Stanley, 1997). Nevertheless, the experiment showed a clear tendency of male (but not female) green mirids to accumulate in the rows treated with pheromone. If a contact foliar insecticide had been applied to these rows, it would have killed the males. We did not do this because dead mirids are very hard to find, especially at the densities present in this experiment. Instead we collected them live by suction sampling. Insecticides registered for control of GM in cotton, which have contact activity, include alpha-cypermethrin, beta-cyfluthrin, bifenthrin, chlorpyrifos-methyl, deltamethrin, dimethoate, endosulfan, fipronil, imidacloprid, lambda-cyhalothrin and omethoate (Johnson and Farrell, 2003). All these insecticides damage natural enemy populations and the ability to control GM by treating only occasional rows with them and allowing natural enemies to survive in the other rows would be a considerable advance in cotton IPM. Killing male GM would reduce damage to cotton directly (since the males themselves feed on the crop), and indirectly by removing potential mates for the females, thus reducing the next generation. The magnitude of the indirect effect would depend on the extent of multiple mating, and the ability of mated female GM to move into the crop from outside sources. Both of these factors are not well understood for GM at present.

Mating disruption may also be possible with this pest. Though there have been few examples reported in the literature, encouraging results have been reported for *C. verbasci* (McBrien *et al*., 1996, 1997) and *T. caelestialium* (Kakizaki and Sugie, 2004). Initial data obtained from the fieldwork done in Warra, Qld indicated a total trap shutdown for 2 days when the pheromone was used in a mating disruption experiment. The short trap shutdown period is thought to have arisen because of the formulation used. This needs further work to provide a slow and sustainable long-term release formulation for use in mating disruption. In the attract-and-kill work, efforts made to locate and count dead mirids for quantification did not work. Either the insecticide did not kill the mirids fast enough, resulting in their moving away from the treated row before dying, or the low numbers of mirids present made the sampling method ineffective. Taken together with the results from Experiment 1, however, the trap results suggest that attract-and-kill for male GM remains a promising option. As with mating

disruption, however, further work needs to be done on a long lasting formulation to overcome potential problems with reinvasion of treated fields.

ACKNOWLEDGMENT

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REFERENCES

Betts, M.D., P.C. Gregg, G.P. Fitt and M.J. MacQuillan, 1993. A Field Trial of Mating Disruption for *Helicoverpa* spp. in Cotton. In: Pest Control and Sustainable Agriculture, Corey, S.A., D.J. Dall and M.W. Milne (Eds.). CSIRO, Melbourne, ISBN-10: 064305426X, pp: 298-300.

Bishop, A.L., 1980. The potential of *Campylomma livida* Reuter and *Megacoelum modestum* Distant, (Hemiptera: Miridae) to damage cotton in Queensland. Austr. J. Expt. Agric. Anim. Husbandry, 20: 229-233.

Carver, M., G. Gross and T.E. Woodward, 1991. Hemiptera (Bugs, Leafhoppers, Cicadas, Aphids, Scale Insects etc.). In: The Insects of Australia, A Textbook For Students and Research Workers, Naumann, I.D., P.B. Carne, J.F Lawrence, E.S. Nielsen and J.P. Spradbery *et al*. (Eds.). Melbourne University Press, Carlton, ISBN: 0522 846 366, pp: 429-509.

Gregg, P.C. and A.P. Del-Socorro, 2002. Attractants for moths. Publication No. WO/2002/089577, World Intellectual Property Organisation, pp: 85. http://www.wipo.int/pctdb/en/wo.jsp?wo=2002089577.

Hori, K. and P.W. Miles, 1993. The etiology of damage to lucerne by the green mirid, *Creontiades dilutus* (Stål). Aust. J. Expt. Agric., 33: 327-331.

Johnson, A. and T. Farrell, 2003. Cotton Pest Management Guide 2003-04. Australian Cotton Cooperative Research Centre, Narrabri.

Jones, O.T., 1998. Practical Applications of Pheromones and Other Semiochemicals. In: Insect Pheromones and Their use in Pest Management, Howse, P., I. Stevens and O. Jones (Eds.). Chapman and Hall, London, UK., ISBN-10: 0412 444 100, pp: 263-352.

Kakizaki, M. and H. Sugie, 2004. Female sex pheromone and communication disruption in the rice leaf bug, *Trigonotylus caelestialium* (Kirkaldy) (Heteroptera: Miridae). Proceedings of XXII International Congress of Entomology, Aug. 15-21, Brisbane, Australia.

Khan, M.H., 1999. Aspects of the biology, ecology and management of the green mirid, *Creontiades dilutus* (Stål) in Australian cotton. Ph.D. Thesis, University of New England, Armidale, Australia.

Layton, M.B., M.R. Williams and S. Stewart, 1997. Bt cotton in Mississippi: The first year. Proceedings of Beltwide Cotton conference, Jan. 6-10, New Orleans, pp: 861-863.

Layton, M.B., 2000. Biology and damage of the tarnished plant bug, *Lygus lineolaris*, in cotton. Southwestern Entomol., 23: 7-20.

Lowor, S.T., A.P. Del-Socorro and P.C. Gregg, 2009. Sex pheromones of the green mirid, *Creontiades dilutus* (Stål) (Hemiptera: Miridae). Int. J. Agric. Res. (In Press).

Malipatil, M.B. and G. Cassis, 1997. Taxonomic review of *Creontiades* Distant in Australia (Hemiptera: Miridae: Mirinae). J. Aust. Entomol. Soc., 36: 1-13.

McBrien, H.L., G.J.R. Judd and J.H. Borden, 1996. Potential for pheromone-based mating disruption of the mullein bug, *Campylomma verbasci* (Meyer) (Heteroptera: Miridae). Can. Entomol., 128: 1057-1064.

McBrien, H.L., G.J.R. Judd and J.H. Borden, 1997. Population suppression of *Campylomma verbasci* (Heteroptera: Miridae) by atmospheric permeation with synthetic sex pheromone. J. Econ. Entomol., 90: 801-808.

Mensah, R.K. and M. Khan, 1997. Use of *Medicago sativa* (L.) interplantings/trap crops in the management of the grenn mirid, *Creontiades dilutus* (Stal) in commercial cotton in Australia. Int. J. Pest. Manage., 43: 197-202.

R Development Core Team, 2004. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN: 3-900051-00-3.

Room, P.M., 1979. Insects and spiders of Australian cotton fields. New South Wales Department of Agriculture, Sydney. pp: 69.

Stanley, J.N., 1997. The seasonal abundance and impact of predatory arthropods on Helicoverpa species in Australian cotton fields. Ph.D. Thesis, University of New England, Armidale, Australia.

Zhang, Q.H. and J.R. Aldrich, 2003. Male-produced anti-sex pheromone in a plant bug. Naturwissenschaften, 90: 505-508.