

# ASSASSIN BUGS AND COTTON IPM PROSPECTS AND LIMITATIONS

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## Summary

The Assassin Bug, *Pristhesancus plagipennis* is a natural enemy with considerable promise for controlling *Heliothis* (*Helicoverpa* spp.) and mirids in cotton. However, this promise is offset by several challenges that currently prevent its use as a commercial biological control agent. This paper will place the results of our research into context and speculate on the future of this predator and its place within cotton IPM programs

## Background

Assassin bugs are endemic to Queensland and parts of New South Wales. They feed on many different insects and are commonly referred to as generalist predators. However, it is this generalist behaviour that allows assassin bugs to survive and prosper in cotton crops particularly when the main pests, *Heliothis* and mirids, are characterised by migratory behaviour and intermittent presence.

Over the last few years we have been examining the potential of assassin bugs to play a role in cotton IPM programs for the control of *Heliothis* and mirids. This research has turned up many interesting aspects that would support the use of this predator as a mass released biological control in cotton.

## Insecticide Compatibility

For a mass-released biological control agent to succeed in cotton, it must be compatible with a suite of chemical products. These products are still required within an IPM program where the control of other pests such as whitefly is required or for when a mirid or *Heliothis* treatment may be warranted during periods of intense pest pressure.

The results from testing insecticides on assassin bugs provide insight as to the robustness of these predators. Tests have indicated that assassin bugs are compatible with Steward®, Admiral®, Applaud®, Tracer®, Regent®, Affirm® and all currently registered NPV and Bt biopesticides (Table 1). The flexibility afforded by being able to use these products with assassin bugs in the field is highly desirable from an IPM program perspective. Our tests have also shown that as assassin bug nymphs grow and develop, they become more robust in terms of their tolerance to insecticides (Table 2). This is of advantage as the season progresses and released nymphs develop as it allows the use of harder chemistry options.

**Table 1.** Mean mortality (%) and s.e. of first instar *P. plagipennis* treated with various insecticides in laboratory bioassays. Insecticides are listed in increasing order of toxicity.

Product	Percentage of Recommended Field Rate Tested			
	100	75	50	25
Bacillus thurengiensis	0	0	0	0
Nucleopolyhedrovirus	0	0	0	0
Buprofezin	0	0	0	0
Pyrproxifen	2.2 ± 0.1	0	0	0
Indoxacarb	7 ± 2.8	2 ± 0.1	0	0
Spynosad	27 ± 1.9	11 ± 0.1	12 ± 1.93	7 ± 3.4
Fiprinol	43 ± 2.8	25 ± 3.0	18 ± 1.1	14 ± 0.1
Emamectin benzoate	69 ± 8.4	47 ± 2.8	42 ± 8.1	16 ± 2.3
Abamectin	84 ± 1.1	61 ± 2.0	51 ± 1.9	41 ± 8.5
Diafenthiuron	100	100	91 ± 2.8	84 ± 1.1
Imidacloprid	100	100	96 ± 0.9	94 ± 1.0
Omethoate	100	100	100	100

**Table 2.** Mean mortality (%) and s.e. of each *P. plagipennis* instar treated with various insecticides at the full recommended rate in laboratory bioassays.

Product	Percentage Mortality of Each <i>P. plagipennis</i> Instar				
	I	II	III	IV	V
Indoxacarb	6 ± 2.7	4 ± 2.7	0	0	0
Spynosad	28 ± 2.9	11 ± 2.2	4 ± 2.2	0	0
Fiprinol	39 ± 5.5	29 ± 2.2	18 ± 4.4	9 ± 2.2	4 ± 2.7
Emamectin benzoate	65 ± 9.4	33 ± 3.8	11 ± 2.2	8 ± 1.9	4 ± 2.2

## Release Techniques

A significant challenge for using assassin bugs as a mass-released biological control in cotton is the logistics of evenly dispersing large numbers of insects over a crop, given that every hectare of conventional cotton can contain up to 10 km of row. Assassin bug nymphs are flightless and limited in their ability to spread. It is therefore critical that the nymphs are spread evenly and consistently throughout the field during release. Over the last season we have been testing different approaches that may overcome the challenges of broad-acre release.

So far, two methods have shown promise. The first is the release of nymphs mixed with a bulking agent such as vermiculite and then spread over a crop with a fertiliser spreader. This method results in significant nymph establishment in the crop and provides relatively even coverage. The main problem with this method is the constraints of handling large numbers of nymphs and passing them through fertiliser spreading equipment. This method would need to be fully integrated with a future commercial supplier from a packaging and handling perspective to be viable.

The second approach has been the release of eggs mixed in non-toxic foam and applied to foliage. The foam serves to adhere the eggs to the leaves until they hatch. Several tests of this approach during the last season showed that up to 50% of the released eggs could be accounted for as ensuing third instar nymphs in the crop several weeks post-release. The advantages of an egg release method compared to nymphs include ease of handling, packing and shipping, ability to store in the fridge for several days until needed, and eggs are significantly cheaper to produce from a mass-production perspective. Disadvantages associated with eggs include susceptibility to being washed off by rain post-release and the biological control time delay requiring earlier release intervention. Eggs are also less robust and therefore compensatory numbers need to be released. The cost benefit is difficult to assess until such time as assassin bugs are commercially available although estimates suggest that the release of twice as many eggs would be less costly than releasing nymphs.

### **Field Performance**

Releases of assassin bug nymphs in cotton have shown that the nymphs will persist and prey on *Heliothis* and mirids for a 6-week period. Several trials have been conducted on the Darling Downs and central Queensland in an attempt to determine how many assassin bugs are required to control pest insects in cotton. These trials suggest that the most appropriate release rate for assassin bugs is somewhere between 0.5-1.0 nymphs per metre row of cotton or 5000-10000 nymphs per hectare.

In terms of control we have found that assassin bugs have significant impacts on pest species as shown in the following figures. The treatments include an untreated control, assassin bugs released with no other controls (Assassin Bugs Only), assassin bugs combined with soft options where necessary (A/Bugs & Soft Options), the same soft options applied to plots without assassin bugs (Soft Options Only) and conventionally treated plots (Conventional) which were managed with conventional insecticides from an IPM perspective. These treatments were replicated 6 times within two experiments conducted at the Biloela Research Station during the 2002-03 and 2003-04 seasons. Each experiment was conducted in Sicot 71 conventional cotton grown on metre rows under trickle irrigation.

#### ***2002-03 Experiment***

Loopers were sampled whilst beat sheeting for mirids in the plots and Figure 1 shows that conventional chemistry caused the largest reductions in looper numbers. The soft only, soft options and assassin bugs and assassin bug only treatments also had a significant ( $P < 0.05$ ) impact in reducing looper numbers in the plots during the later half of January compared to the control.

The second figure shows the effects of the different treatments on mirids sampled at the same time as loopers. Again as expected the conventional treatment resulted in significantly lower numbers of mirids. The soft only and soft and assassin bug treatments also resulted in significantly reduced mirid populations compared to the control. Although

there was a delay in control, the assassin bug only treatment also resulted in significant reduction in mirid numbers compared to the control during the latter part of January (Fig 2).

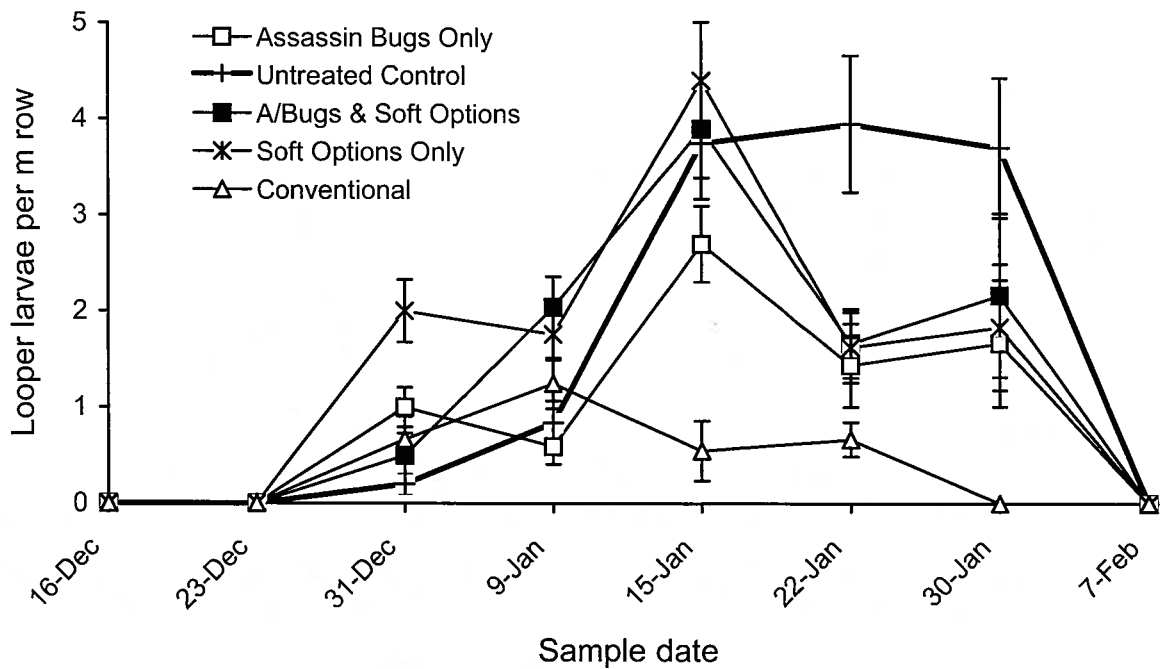
Figure 3 shows some interesting trends concerning the impact of the treatments on large *Heliothis* larvae. The untreated control had consistently higher levels of large larvae compared to the other treatments. Of particular interest is the larvae densities on 21 January where the conventional and assassin bug only treatments have the lowest numbers of large larvae. The soft options only and assassin bug and soft option treatments had higher numbers of larvae due to flaring caused by an earlier Fiprinol application for mirid on 9 January 2003 which resulted in much higher egg and neonate survival in these two treatments. The higher survival in the conventional treatment was subsequently controlled with an Indoxacarb application whereas the NPV application that was used in the soft options only and assassin bugs with soft options treatments was ineffective on the larvae despite early targeting. This example demonstrates that chemistry should be selected carefully with regard to impacts on other natural enemies not just assassin bugs.

The treatment impact on yield during this experiment was difficult to determine due to adverse weather conditions in February. The onset of boll opening in the plots coincided with an extended period of wet weather from ex-tropical cyclone "Beni" which crossed the coast north of Rockhampton. A total of 380 mm fell on the crop over a period of three weeks with very little in the way of clear weather occurring between rainy periods. As a result all of the treatments suffered extensive boll rot and tight loch (>20%) damage except the control, which was affected to a much lessor extent due to a later fruit set. Figure 4 shows the yield from the 2002-03 experiment. Despite the weather damage, all treatments yielded significantly ( $P<0.05$ ) more lint than the control.

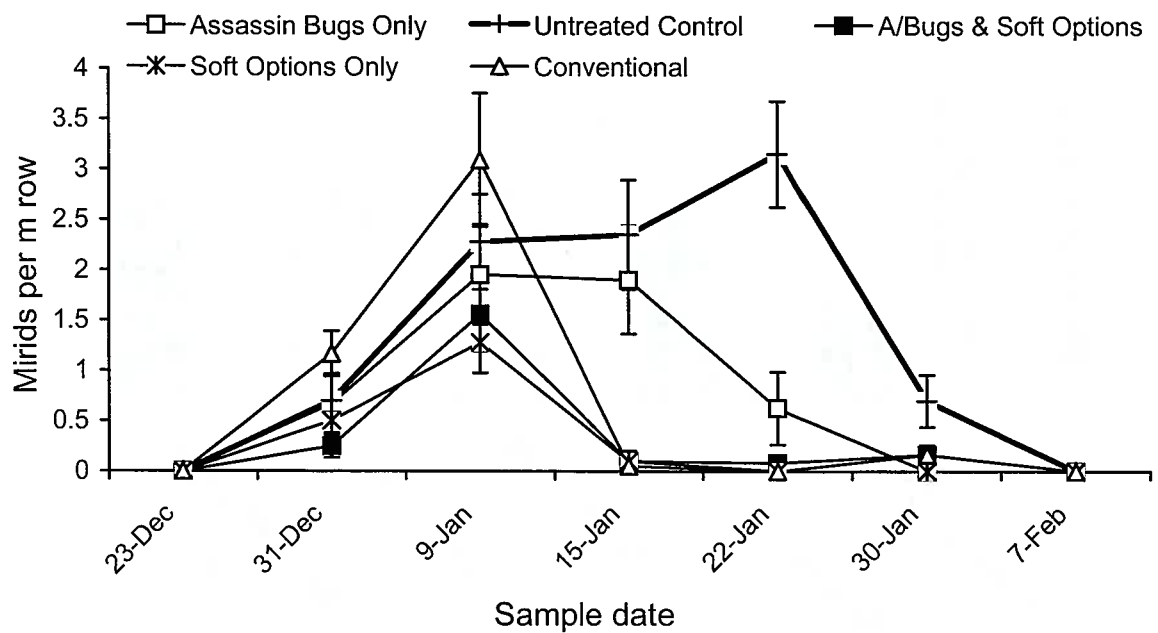
#### ***2003-04 Experiment***

A similar experiment was repeated during the 2003-04 season. This experiment was subject to very low levels of pest pressure with little in the way of mirid or *Heliothis* activity. Figure 5 from this experiment shows *Heliothis* densities in relation to the same treatments. In this trial the soft options were chosen with greater care than in the previous trial, which resulted in a greater additive effect although this is not reflected significantly due to the low overall levels of pest pressure.

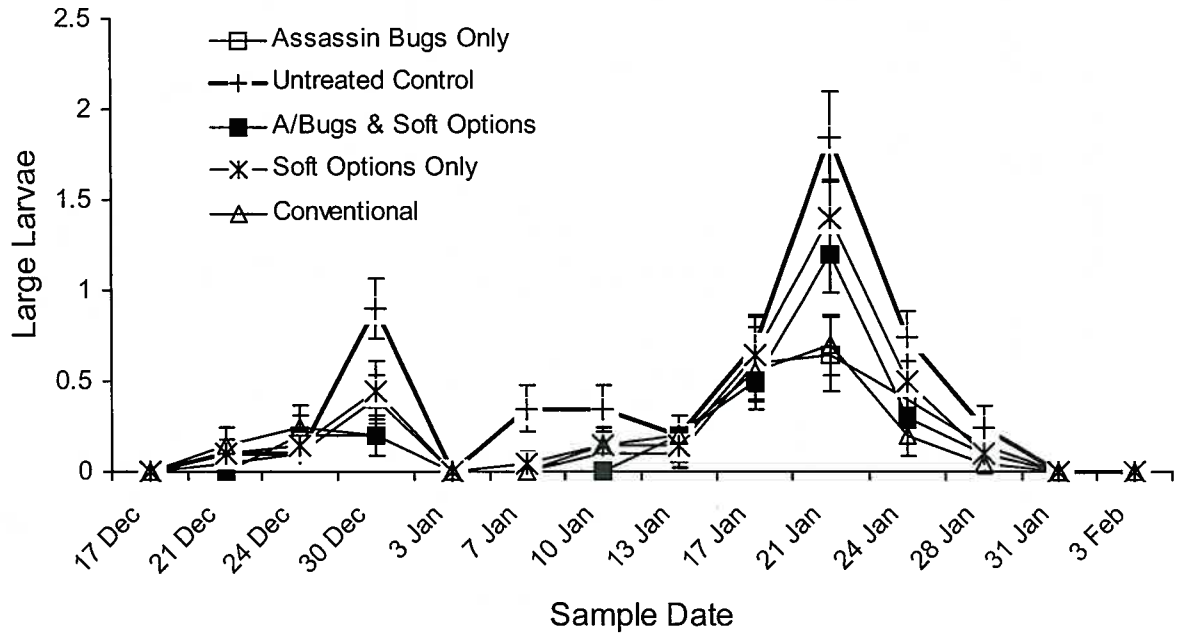
The cumulative treatment impact is perhaps better demonstrated by Figure 6 which shows the yields picked and ginned from the plots. This figure suggests a trend of cumulated benefit associated with combining soft options with assassin bugs. In this trial the untreated control yielded 3 bales which reinforces our observations of the crop being subject to very low levels of pest pressure during the 2003-04 season.



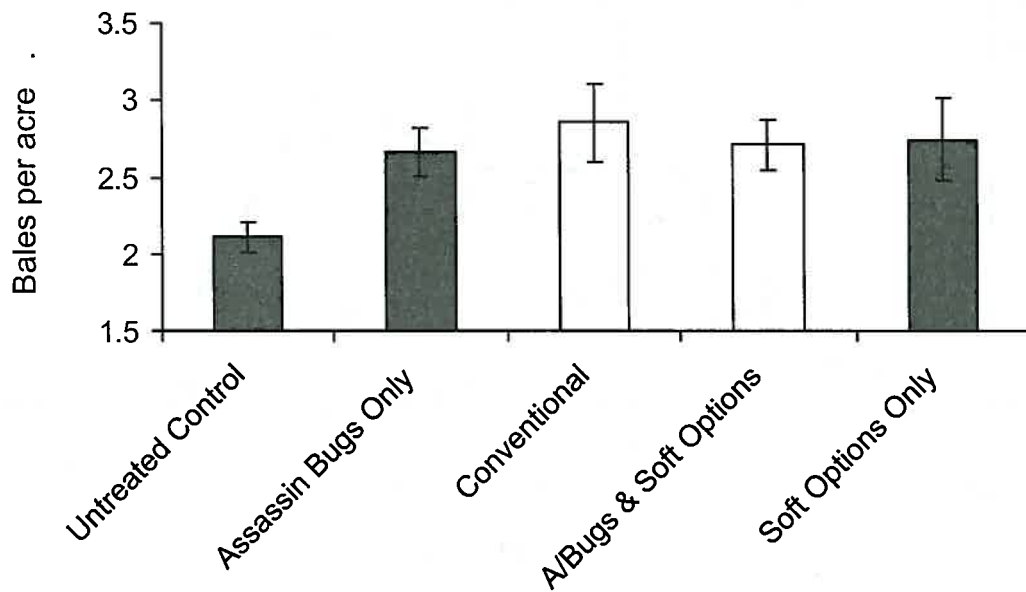
**Figure 1.** Time series showing numbers per m row of Looper larvae in cotton plots for the two assassin bug releases (with and without soft options), a conventionally sprayed treatment, soft option only treatment and untreated control. Assassin bugs were released on 17 December 2002. The bars denote  $\pm$  s.e.



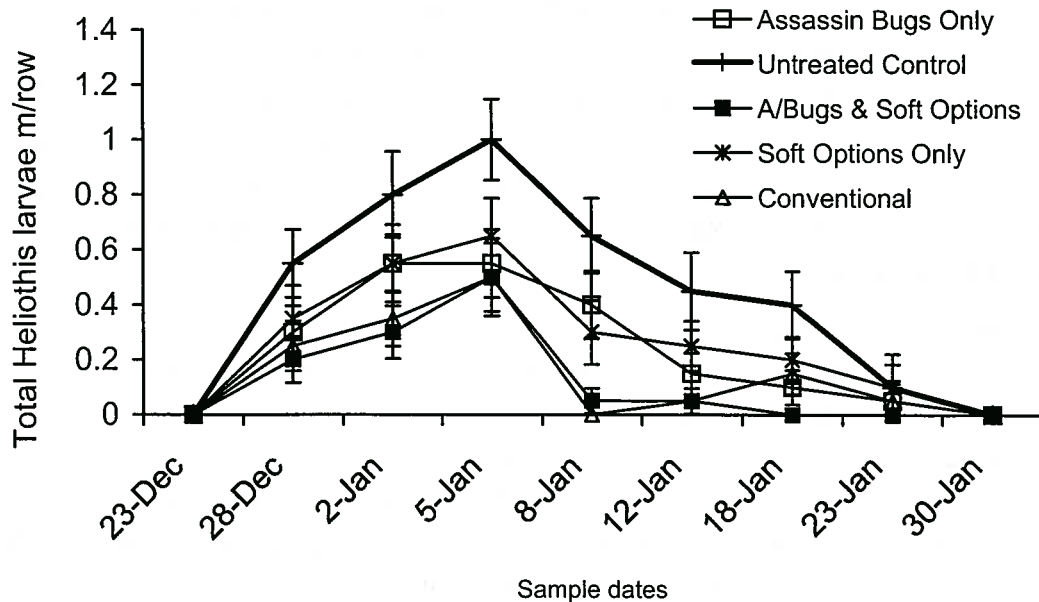
**Figure 2.** Time series showing numbers per m row of mirids in cotton plots for the two assassin bug releases (with and without soft options), a conventionally sprayed treatment, soft option only treatment and untreated control. Assassin bugs were released on 17 December 2002. The bars denote  $\pm$  s.e.



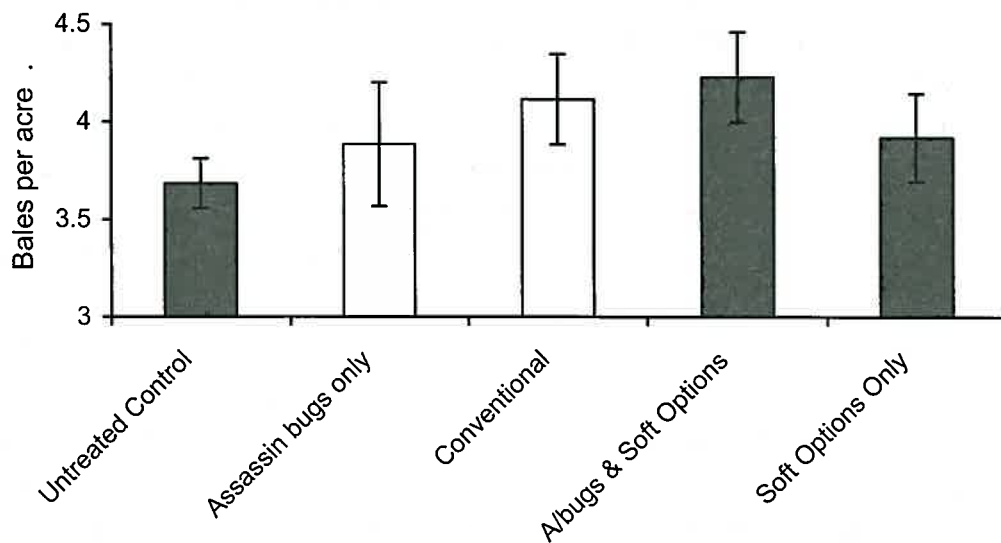
**Figure 3.** Time series showing numbers per m row of large *Heliothis* larvae in cotton plots for the two assassin bug releases (with and without soft options), a conventionally sprayed treatment, soft option only treatment and unsprayed control. Assassin bugs were released on 17 December 2002. The bars denote  $\pm$  s.e.



**Figure 4.** The picked and ginned yields from the two assassin bug releases (with and without soft options), a conventionally sprayed treatment, soft option only treatment and unsprayed control during the 2002-03 experiment. The bars denote  $\pm$  s.e.



**Figure 5.** Time series showing the total numbers of *Heliothis* per m row in the cotton plots for the two assassin bug releases (with and without soft options), a conventionally sprayed treatment, soft option only treatment and unsprayed control during the 2003-04 season. Assassin bugs were released on 20 December 2003. The bars denote  $\pm$  s.e.



**Figure 6.** The picked and ginned yields from the two assassin bug releases (with and without soft options), a conventionally sprayed treatment, soft option only treatment and unsprayed control during the 2003-04 experiment. The bars denote  $\pm$  s.e.

## Future Directions

Our research demonstrates that assassin bugs could play a valuable role within a conventional cotton IPM program for the control of *Heliothis* and mirids. Our results show that the control afforded by these predators on cotton both with and without soft options is sufficient to provide similar yields to conventionally sprayed cotton. The ability to combine these predators with a range of selected insecticide options makes them a more flexible biological control compared to many other natural enemies that are highly sensitive to most insecticides.

However, to take advantage of these predators would require augmentation on a massive scale. Assassin bugs are generally scarce within cotton agro-ecosystems and therefore the only reliable way to take advantage of this predator is to mass-rear and release them into cotton crops early in the season. Our current and earlier research suggests that assassin bugs can indeed be mass-reared for release onto broad-acre crops, however, the viability of such an approach will depend on commercial insectaries being able to produce large quantities of assassin bugs efficiently and cheaply enough.

Presently the rearing of these predators is achieved using mealworms as a food source, meaning a commercial insectary would have to breed two insects for the sale of one. The cost of production for mealworms is around \$25/kg not including the capital infrastructure investment required to house a mealworm facility.

A potential solution is to use an artificial diet to mass rear assassin bugs. An artificial diet would have significant advantages in that it supersedes the need for mealworms, allows greater production consistency and reliability, it is capital less intensive, more labour efficient and hence more cost-effective. To this end we have been investigating the potential of currently available artificial diets that have been developed by researchers in the United States and Europe for the production of other predatory bug species. Of these diets, a meat-based formulation that is composed primarily of a mix of beef liver, mince and hens eggs published by Cohen (1998) has shown some potential for rearing assassin bugs. This diet costs around \$4/kg to manufacture.

When formulated, we found assassin bugs will accept this diet and it was sufficient to support their growth through until the adult stage without any obvious impediment compared to nymphs reared on mealworms. However, the egg production from the assassin bugs reared on the diet was very low compared to those reared on mealworms and therefore it was not adequate to close the lifecycle completely in the insectary.

The acceptance of and development of assassin bugs on an artificial diet suggests that there is potential to use an artificial diet-based rearing system, although such a diet will need to be refined so that it is conducive to high egg production. Many research organisations are investigating improvements for artificial diets and it is likely that future developments in diet technology will pave the way for the mass-production of a range of natural enemies including assassin bugs. It is probable that improved natural enemy production capabilities

due to diet and other technologies will lead to the greater use of mass-released natural enemies, and they will become more commonplace just as mass-produced viruses such as NPV products Gemstar and Vivus have evolved to be a key IPM tool over the last decade.

Our research with assassin bugs demonstrates that these predators have significant field potential for the control of *Heliothis* and mirids in cotton. If these predators become commercially available in the future they may well play an important role in the production of cleaner and greener conventional cotton.

### **Acknowledgements**

We thank Gavin Mann (Farming Systems Institute, QDPI) and his team picked and ginned the cotton. This research has been funded by the Cotton Research and Development Corporation (DAQ122CC).

### **References**

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