Utilizing the assassin bug, *Pristhesancus plagipennis* (Hemiptera: Reduviidae), as a biological control agent within an integrated pest management programme for *Helicoverpa* spp. (Lepidoptera: Noctuidae) and *Creontiades* spp. (Hemiptera: Miridae) in cotton

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Abstract

*Helicoverpa* spp. and mirids, *Creontiades* spp., have been difficult to control biologically in cotton due to their unpredictable temporal abundance combined with a cropping environment often made hostile by frequent usage of broad spectrum insecticides. To address this problem, a range of new generation insecticides registered for use in cotton were tested for compatibility with the assassin bug, *Pristhesancus plagipennis* (Walker), a potential biological control agent for *Helicoverpa* spp. and *Creontiades* spp. Indoxacarb, pyriproxifen, buprofezin, spinosad and fipronil were found to be of low to moderate toxicity on *P. plagipennis* whilst emamectin benzoate, abamectin, diafenthiuron, imidacloprid and omethoate were moderate to highly toxic. Inundative releases of *P. plagipennis* integrated with insecticides identified as being of low toxicity were then tested and compared with treatments of *P. plagipennis* and the compatible insecticides used alone, conventionally sprayed usage practice and an untreated control during two field experiments in cotton. The biological control provided by *P. plagipennis* nymphs when combined with compatible insecticides provided significant (*P* < 0.001) reductions in *Helicoverpa* and *Creontiades* spp. on cotton and provided equivalent yields to conventionally sprayed cotton with half of the synthetic insecticide input. Despite this, the utilization of *P. plagipennis* in cotton as part of an integrated pest management programme remains unlikely due to high inundative release costs relative to other control technologies such as insecticides and transgenic (Bt) cotton varieties.

Keywords: mirids, predators, inundative, mass release

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Introduction

Arthropod predators and parasitoids are considered to be important pest mortality agents in Australian cotton production systems, although they are rarely capable of controlling Helicoverpa spp. (Lepidoptera: Noctuidae) unassisted (Fitt, 2000; Mensah, 2002). In recent years, there has been a shift towards integrated pest management strategies that include the use of more selective spectrum insecticides (Holloway & Forrester, 1998), Helicoverpa spp. biopesticides (Mensah et al., 2005) and sacrificial trap crops grown to divert pest species from cropping areas (Sequeira, 2001; Grundy et al., 2004); however, predator and parasitoid utilization in Australian cotton remains predominantly passive.

A conservational approach, through judicious insecticide selection, has been shown to increase the diversity and abundance of beneficial arthropods in cotton (Mansfield et al., 2006) and generally improve gross margins (Hoque et al., 2000). Bollgard® (Bt) varieties have been broadly adopted by the Australian industry during the last decade with an expectation that Bt varieties would provide a platform for vastly reducing pesticide usage from the conventional average of 12 applications per season (Doyle et al., 2002) and consequently increase the robustness of natural enemy complexes (Fitt, 2000; Wilson et al., 2006). However, despite the reduction in spraying for Helicoverpa spp. and associated expectations for improved predation and parasitism, secondary pests remain abundant in Bollgard® cotton crops and often require insecticide intervention (Wilson et al., 2006).

Although the conservation approach to biological control has provided a way for the Australian cotton industry to reduce insecticide dependence, agro-ecosystems are inherently changing environments and the abundance of natural enemies fluctuate due to many biotic and abiotic factors that are poorly understood (Stanley, 1997). The unpredictability of natural predators and parasitoids remains a key factor limiting their greater exploitation in Australian cotton pest management programmes (Johnson et al., 2000).

Although augmentation by mass release is one method that could be used to increase the reliability and effectiveness of predators and parasitoids within cropping systems (New, 2002), the potential of generalist predators, particularly predatory bugs, has been largely ignored in cotton production systems (King & Powell, 1992). However, in a monoculture environment where the main pests, Helicoverpa spp. and Creontiades spp. (Hemiptera: Miridae), are characterized by migratory behaviour and a multi-voltine lifecycle (Zalucki et al., 1986; Miles, 1995), generalist predators may have a survival advantage as their population dynamics are not solely dependent on any one pest species (Murdoch et al., 1985; Nyffeler et al., 1992).

The assassin bug, Pristhesancus plagipennis (Walker) (Hemiptera: Reduviidae), is a generalist predator of various insects in both orchard and field crops (Pyke & Brown, 1996; Smith et al., 1997). Several studies have suggested that P. plagipennis may be suited for augmentation against Helicoverpa spp. and Creontiades spp. with inundative releases resulting in reduced populations of these pests in cotton (Grundy & Maelzer, 2000, 2002; Grundy, 2004). Densities of one P. plagipennis nymph per metre row (10,000 nymphs ha⁻¹) were sufficient to reduce Helicoverpa spp. larva densities on cotton (Grundy & Maelzer, 2002; Grundy, 2004). However, it was evident during these experiments that release rates of one P. plagipennis nymph per metre row were insufficient to control Helicoverpa spp. during peak infestation events that can occur on cotton during some seasons (Fitt, 2000). The pre-emptive release of higher P. plagipennis numbers that might counter peak population events was shown to be an unsuccessful strategy with high losses of nymphs occurring from the plots during periods of low pest abundance due possibly to starvation and/or cannibalism (Grundy & Maelzer, 2000, 2002). A more reliable method may be one that utilizes the biological control afforded by P. plagipennis during periods of low to moderate pest abundance and allows for the use of compatible insecticides during peak pest invasion events.

Earlier insecticide compatibility studies suggested that P. plagipennis were tolerant of some organochlorine and carbamate insecticides (Grundy et al., 2000a). However, these insecticides are generally considered toxic to a range of other beneficial insects found in Australian cotton fields for which disruption can give rise to secondary pest problems (Wilson et al., 1998) and are, therefore, unsuitable for use within integrated pest management programmes that seeks to emphasize the conservation of natural enemies for biological control. Several selective new generation insecticides (e.g. spinosans, methetics, nicotinoids) have since entered the Australian marketplace, some of which have been identified as being less disruptive to a range of beneficial insects that occur in cotton (Deutscher et al., 2004) and, if compatible with P. plagipennis, may be suited for integration.

The objective of the present study was to identify the compatibility of a range of new generation insecticides with P. plagipennis nymphs and to then test an integrated field release strategy where P. plagipennis and compatible insecticides were combined and compared with unsprayed and conventionally sprayed cotton treatments.

Materials and methods

The P. plagipennis nymphs used in the experiments were progeny reared from adult bugs originally collected from the Coffs Harbour (23°16'S, 150°21'E) and Rockhampton (29°59' E, 153°08'S) regions of New South Wales and Queensland, respectively. Pristhesancus plagipennis used in each study were reared on a diet of Tenebrio molitor (Linnaeus) in a constant climate laboratory at 26 ± 1°C and 55–75% RH, with a 15:9 L: D photoperiod supplied by cool white 36 watt fluorescent tubes (Grundy et al., 2000b).

Insecticide compatibility

Four-day-old first instar P. plagipennis were used in each experiment, as earlier studies indicated that this stage was the most sensitive and, therefore, provided a ‘worst case’ test result (Grundy et al., 2000a). Pesticides that are found to be non-toxic using the assumptions of a ‘worst case’ test generally require no further testing on other stages (Hassan et al., 1994).

The active ingredient, formulation and manufacturer for each insecticide treatment are listed (table 1). The commercial formulation of each insecticide was tested at its maximum registered rate for the control of insect pests on cotton within Australia as well as at three dilutions (75, 50 and 25% of the recommended rate), as the application of insecticides at below label rates for the improved
conservation of natural enemies has become commonplace within the Australian cotton industry (Deutscher et al., 2004). For the laboratory tests, disposable 200 mm diameter Petri dishes were used as a standardized application target. The Petri dishes were modified by punching four 30 mm diameter holes into the lid of each container and gluing a piece of muslin gauze over the opening for ventilation. A diameter holes into the lid of each container and gluing a piece of muslin gauze over the opening for ventilation. A Potter Precision spray tower was then used to apply 2 ml condensate) (Crop Care, Australia) was added at the rate of 0.1 ml l\(^{-1}\) to each insecticide suspension before application because wetting agents are commonly mixed with pesticides to enhance spray coverage in Australia. Agral was also mixed with distilled water at the same rate and used as a control treatment.

The experiment was conducted on 11 August 2002. Three replicates of 30 nymphs were topically treated on the Petri dish plates with one of the four concentrations of each product. Before being treated, the nymphs were temporarily immobilized with carbon dioxide (CO\(_2\)) to allow easy handling and to slow the nymphs from escaping the open Petri dishes during application. After treatment, the Petri dishes containing the sprayed nymphs were placed in a constant climate laboratory under conditions used for rearing for 24 h. The nymphs were then transferred to clean Petri dishes and provided with T. molitor prey larvae, and those that successfully moulted to the second instar were recorded as having survived the treatment.

A second experiment was conducted to examine the tolerance of each nymphal instar to emamectin benzoate, spinosad, fipronil and indoxacarb. The full recommended rate of each product was applied to three replicates (30 nymphs per replicate) of each nymph stage using the same methods of application and assessment outlined for the first experiment.

### Field studies

Two experiments were conducted within a 2.5-ha irrigated field planted to cotton (cv. Sicot 71) during the summer of 2002/03 and 2003/04 near the township of Biloela, central Queensland (24°22'S, 150°06'E). In each experiment, treatment plots with dimensions 30 m × 10 m and 1 m row spacing were arranged in a randomized block design with five replicates of each treatment. The plots were separated by 6 m buffers, which consist of 2 m of bare earth adjacent to a 2 m strip of cotton on all sides.

Five treatments were compared in each experiment.

1. Third instar *P. plagipennis* released at one nymph per m\(^2\) (10,000 nymphs per hectare) with no other control inputs.
2. The same *P. plagipennis* release treatment combined with selected compatible soft insecticides.
3. A soft insecticide sprayed treatment to which the same compatible insecticides were applied at the same time as those applied with the soft insecticide and *P. plagipennis* treatment plots.
4. A conventionally sprayed treatment, which was managed with insecticides that would be generally applied by growers using a conservational approach (avoidance of broad spectrum insecticides).
5. A *P. plagipennis* nymph and insecticide free control.

*Pristhesancus plagipennis* nymphs were released in each experiment within a week of the first flowers appearing on the crop on 15 and 20 December 2002 and 2003, respectively. Nymphs for each treatment were released singularly onto the terminal shoots of the crop foliage using a camel-hair brush late in the afternoon after 1700 h during each experiment.

The sprayed treatments were managed with insecticides chosen in accordance to the Insecticide Resistance Management Strategy set by the Australian cotton industry for each season (Schulze & Tomkins, 2002; Johnson & Farrell, 2003).

### Table 1. Active ingredient (AI), formulation and recommended application rates of insecticides compared for their activity against *P. plagipennis*.

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>g AI l(^{-1}) and formulation</th>
<th>Manufacturer</th>
<th>Application rate m l(^{-1})</th>
<th>1 ha l(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus thuringiensis</strong></td>
<td>Biological</td>
<td>Valent</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td><strong>Nucleopolyhedrovirus</strong></td>
<td>Biological</td>
<td>Bayer Crop Science</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Buprophen</strong></td>
<td>200 g l(^{-1}) EC</td>
<td>Syngenta</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td><strong>Pyriproxifen</strong></td>
<td>500 g l(^{-1}) EC</td>
<td>Sumitomo</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Indoxacarb</strong></td>
<td>200 g l(^{-1}) SC</td>
<td>Du Pont</td>
<td>8.5</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>Spinosad</strong></td>
<td>17 g l(^{-1}) EC</td>
<td>Dow AgroSciences</td>
<td>2</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Emamectin benzoate</strong></td>
<td>18 g l(^{-1}) SC</td>
<td>Bayer Crop Science</td>
<td>1.25</td>
<td>0.125</td>
</tr>
<tr>
<td><strong>Abamectin</strong></td>
<td>17 g l(^{-1}) EC</td>
<td>Syngenta</td>
<td>5.5</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>Difenthiuron</strong></td>
<td>18 g l(^{-1}) SC</td>
<td>Syngenta</td>
<td>6</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Imidacloprid</strong></td>
<td>0.1 ml l(^{-1})</td>
<td>Bayer Crop Science</td>
<td>2.5</td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Fipronil</strong></td>
<td>800 g l(^{-1}) SL</td>
<td>Bayer Crop Science</td>
<td>1.4</td>
<td>0.14</td>
</tr>
</tbody>
</table>

EC, emulsifiable concentrate; SC, suspension concentrate; SL, soluble liquid.
Table 2. The insecticides applied to the conventionally sprayed (CS), soft insecticide only (SI) and soft insecticide with Pristhesancus plagipennis (SI & Pp) treatments during the 2002/03 and 2003/04 experiments.

<table>
<thead>
<tr>
<th>Pest Type</th>
<th>Active Ingredient</th>
<th>Rate</th>
<th>Treatments sprayed</th>
<th>Application date</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002/03 Experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helicoverpa</td>
<td>NPV</td>
<td>500 ml ha(^{-1})</td>
<td>CS, SI, SI &amp; Pp</td>
<td>13 Dec 2002</td>
</tr>
<tr>
<td>Helicoverpa</td>
<td>NPV</td>
<td>250 ml ha(^{-1})</td>
<td>SI, SI &amp; Pp</td>
<td>18 Dec 2002</td>
</tr>
<tr>
<td>Helicoverpa</td>
<td>Spinosad</td>
<td>200 ml ha(^{-1})</td>
<td>CS</td>
<td>20 Dec 2002</td>
</tr>
<tr>
<td>Helicoverpa and mirids</td>
<td>Fipronil/NPV</td>
<td>40 ml ha(^{-1}) &amp; 250 ml ha(^{-1})</td>
<td>CS, SI and SI &amp; Pp</td>
<td>9 Jan 2003</td>
</tr>
<tr>
<td>Helicoverpa</td>
<td>Spinosad</td>
<td>200 ml ha(^{-1})</td>
<td>CS</td>
<td>9 Jan 2003</td>
</tr>
<tr>
<td>Helicoverpa</td>
<td>NPV</td>
<td>250 ml ha(^{-1})</td>
<td>CS, SI, SI &amp; Pp</td>
<td>14 Jan 2003</td>
</tr>
<tr>
<td>Helicoverpa</td>
<td>Indoxacarb</td>
<td>750 ml ha(^{-1})</td>
<td>CS, SI, SI &amp; Pp</td>
<td>20 Jan 2003</td>
</tr>
<tr>
<td>2003/04 Experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helicoverpa</td>
<td>NPV</td>
<td>500 ml ha(^{-1})</td>
<td>CS, SI, SI &amp; Pp</td>
<td>30 Dec 2003</td>
</tr>
<tr>
<td>Helicoverpa</td>
<td>NPV</td>
<td>250 ml ha(^{-1})</td>
<td>SI, SI &amp; Pp</td>
<td>5 Jan 2004</td>
</tr>
<tr>
<td>Helicoverpa</td>
<td>Spinosad</td>
<td>200 ml ha(^{-1})</td>
<td>CS</td>
<td>5 Jan 2004</td>
</tr>
</tbody>
</table>

NPV, nucleo polyhedrovirus.

except for those sprayed treatment plots. In each experiment, pre-release pest insect counts were made prior to predator release and then every 3–7 days until the end of the experiment. The data were expressed as numbers of insects per metre row for each treatment.

Visual counts of Helicoverpa spp. eggs and larvae on the cotton plants were made on four randomly selected 1 m row lengths of cotton plants in each treatment replicate. The growing points and squares of the upper two-thirds of the cotton plants were made on four randomly selected 1 m row lengths of cotton plants in each treatment replicate. Beat sheet samples were made on four randomly selected 1 m row lengths of cotton plants in each treatment replicate. The data were expressed as numbers of insects per metre row for each treatment.

The nymph mortality data from the insecticide compatibility experiments was corrected for control mortality using Abbott’s formula (Abbott, 1925) and was analysed using ANOVA in GenStat (Payne et al., 1989). Least significant differences (LSDs) were calculated to determine treatment differences at \(P<0.05\). An angular transformation was considered for the mortality data but deemed unnecessary.

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Analysis of data

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Results

Insecticide compatibility

Significant differences were found between insecticides \((P<0.05, \text{LSD 4.55})\), dose rates \((P<0.05, \text{LSD 2.74})\) and the interaction between dose and insecticides tested \((P<0.05, \text{LSD 9.11})\). Pyriproxifen, buprofezin, Bacillus thuringiensis and nucleopolyhedrovirus were non-toxic to P. plagipennis nymphs whilst indoxacarb was of very low toxicity. Spinosad, fipronil, emamectin benzoate and abamectin were of low to moderately high toxicity, respectively, with each product having a significant dose response \((P<0.05)\) with reduced application rates. Diazinon, imidacloprid and omethoate were highly toxic to P. plagipennis nymphs even when applied at reduced rates (table 3). For intermediate
significant differences were found between insecticides ($P < 0.05$, LSD 3.54) and the different $P. plagipennis$ instars ($P < 0.001$, LSD 3.54) and the interaction between dose and insecticides tested ($P < 0.001$, LSD 7.93).

The susceptibility of $P. plagipennis$ nymphs to indoxacarb, spinosad, fipronil and emamectin benzoate decreased as nymphs became more developed, with fourth and fifth instars remaining relatively unaffected by direct exposure (table 4).

Field studies

2002/03 Experiment

$Helicoverpa$ spp. and $Creontiades$ spp. were abundant during the first experiment. $Helicoverpa armigera$ (Hübner) was the dominant species, with only low numbers (< 20%) of $Helicoverpa punctigera$ (Wallengren) observed. Green mirids, $Creontiades dilutus$ (Stål) were the dominant species encountered during sampling, with only low numbers (< 10%) of brown mirids, $Creontiades pallidifer$ (Walker), observed.

No significant ($P > 0.05$) differences in $P. plagipennis$ nymph densities were recorded between the $P. plagipennis$ alone and $P. plagipennis$ with soft insecticide treatments during the experiment (fig. 1).

The conventional, soft insecticide only and soft insecticide and $P. plagipennis$ treatments resulted in significantly ($P < 0.001$) reduced $Creontiades$ spp. populations compared to the control (table 5). A significant reduction ($P < 0.001$) in $Creontiades$ spp. numbers was also recorded in the $P. plagipennis$ only treatment compared to the control during the latter half of January 2003 (table 5, fig. 2).

Significant reductions in looper, $Chrysodeixis$ spp., densities were recorded in the conventional insecticide treatment ($P < 0.01$) compared to all other treatments (table 5). Significant ($P < 0.01$) reductions in looper densities were also recorded in both predator and soft insecticide only treatments compared with the untreated control (table 5).

Each of the treatments resulted in a significant reduction ($P < 0.001$) in large larvae densities compared to the untreated control with the conventionally sprayed and
combined *P. plagipennis*/compatible insecticide treatments providing the largest reduction in larval densities compared to the control (table 5). The assessment of treatment effects on crop yield were hampered by extremely adverse wet weather conditions in February, which coincided with the onset of boll opening in the plots and caused extensive yield losses due to boll rots and tight loch (>25%). The exception was the control treatment, where earlier insect damage had caused a later pattern of compensatory boll set. Despite the wet weather impacts, all treatments yielded significantly (*P* < 0.001) more lint than the control (table 6).

**2003/04 Experiment**

No significant (*P* > 0.05) differences in *P. plagipennis* nymph densities were recorded between the *P. plagipennis* alone and *P. plagipennis* with soft insecticide treatments during the experiment (fig. 3).

The 2003/04 experiment was subject to very low levels of pest pressure with no *Creontiades* spp. and low numbers of *Helicoverpa* spp. larvae recorded, of which *H. punctigera* was more prevalent (>60%). Significantly lower densities (*P* < 0.001) of *Helicoverpa* spp. larvae were recorded in all of the predator and insecticide treatments compared to the control (table 7, fig. 4). A comparison of late instar larvae

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**Table 5.** The repeated measures analysis predicted treatment means for *Creontiades* spp., *Chrysodexis* spp. and large *Helicoverpa* larvae densities per metre crop row for the 2002/03 experiment duration.

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Creontiades</em> spp.</th>
<th><em>Chrysodexis</em> spp.</th>
<th><em>Large Helicoverpa</em> larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>% Pest Reduction</td>
<td>Mean</td>
</tr>
<tr>
<td>Untreated control</td>
<td>1.31 n/a</td>
<td>1.78 n/a</td>
<td>0.51 n/a</td>
</tr>
<tr>
<td><em>Pristhesancus plagipennis</em> only</td>
<td>0.75</td>
<td>42.7</td>
<td>1.05</td>
</tr>
<tr>
<td>Soft insecticides</td>
<td>0.31</td>
<td>76.4</td>
<td>1.31</td>
</tr>
<tr>
<td>Soft insecticides and <em>P. plagipennis</em></td>
<td>0.30</td>
<td>77.0</td>
<td>1.28</td>
</tr>
<tr>
<td>Conventionally sprayed</td>
<td>0.55</td>
<td>58.0</td>
<td>0.45</td>
</tr>
<tr>
<td>Standard error of differences</td>
<td>0.26</td>
<td>&lt;0.001</td>
<td>0.31</td>
</tr>
<tr>
<td>Chi <em>P</em> value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The percentage pest reduction compared to the untreated control has been calculated and standard error of the differences and chi *P* value is given.

**Table 6.** Mean treatment lint yield (bales per hectare) for the 2002/03 and 2003/04 experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lint yield (bales ha&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2002/03 Experiment</td>
</tr>
<tr>
<td>Untreated control</td>
<td>5.3a</td>
</tr>
<tr>
<td><em>Pristhesancus plagipennis</em> only</td>
<td>6.73b</td>
</tr>
<tr>
<td>Soft insecticides</td>
<td>6.91b</td>
</tr>
<tr>
<td>Soft insecticides and <em>P. plagipennis</em></td>
<td>6.94b</td>
</tr>
<tr>
<td>Conventionally sprayed</td>
<td>7.22b</td>
</tr>
</tbody>
</table>

LSD at 5% 0.67 1.05

Treatment means marked with different letters are significantly different (*P* < 0.05).
The percentage pest reduction compared to the untreated control has been calculated and standard error of the differences and chi $P$ value is given.

The increasing robustness of developing nymphs to insecticide exposure as observed for indoxacarb, spinosad, fipronil and emamectin benzoate indicated that products found to be moderately toxic on first instar nymphs could be used several weeks post-release in the field when nymphs have developed into older instars. The increased tolerance of $P. plagipennis$ to insecticides with nymph development could allow for a broader range of insecticides to be used later in the season.

The field release experiments were conducted to test the use of $P. plagipennis$ as a biological control agent within an integrated programme that aimed to reduce pesticide inputs whilst maintaining crop yield. The release of $P. plagipennis$ combined with compatible insecticides provided equivalent pest insect reductions and crop yields compared to the conventional insecticide treatment, whilst reducing synthetic insecticide inputs (excluding nucleopolyhedrovirus biopesticides) by half. Significant reductions in pest densities were observed in the $P. plagipennis$ only plots although the biological control recorded was characterized by a time lag of several days as was observed for Creontiades spp. (fig. 2). As anticipated from the laboratory studies, no deleterious effects of fipronil and indoxacarb applications on $P. plagipennis$ densities were observed (fig. 1).

The impact $P. plagipennis$ on Helicoverpa spp. and Creontiades spp. in the 2002/03 experiment was possibly dulled due to high densities of largely uneconomic Chrysodeixis spp. larvae that served as substitute prey as indicated by the significant reductions ($P<0.01$) in this species recorded in the $P. plagipennis$ treatments (table 5). In retrospect, the use of indoxacarb in place of Helicoverpa specific nucleopolyhedrovirus biopesticides during this experiment may have enhanced the subsequent levels of biological control afforded by $P. plagipennis$ on Creontiades spp.

### Table 7. The repeated measures analysis predicted treatment means for large and total Helicoverpa larval densities per metre crop row for the 2003/04 experiment duration.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Large Helicoverpa larvae</th>
<th>Total Helicoverpa larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>% Reduction</td>
</tr>
<tr>
<td>Untreated control</td>
<td>0.06</td>
<td>n/a</td>
</tr>
<tr>
<td>$Pristhesancus plagipennis$ only</td>
<td>0.01</td>
<td>83</td>
</tr>
<tr>
<td>Soft insecticides</td>
<td>0.01</td>
<td>83</td>
</tr>
<tr>
<td>Soft insecticides and $P. plagipennis$</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Conventionally sprayed</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Standard error of differences</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Chi $P$ value</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

The tolerance of $P. plagipennis$ to a range of new generation insecticides as shown by the laboratory insecticide compatibility studies is advantageous when considering its release into cotton production systems characterized by frequent insecticide use (Murray et al., 2005). $Pristhesancus plagipennis$ tolerance to fipronil and indoxacarb provides compatible insecticides for the control of Creontiades spp. Previous registered options for Creontiades spp. control were predominantly organophosphate products, such as dimethoate and methoate, which were found to be highly toxic to $P. plagipennis$ during this and earlier experiments (Grundy et al., 2000a). The high toxicity of diafenthiuron to $P. plagipennis$ was unexpected as previous research on related predators such as Nabis, Geocoris and Orius spp. that commonly occur in Australian cotton fields had suggested low toxicity (10–20% mortality) to these predatory bug species (Deutscher et al., 2004). The high toxicity of imidacloprid to $P. plagipennis$ also contradicted earlier research suggesting this nicotinoid was non-toxic to $P. plagipennis$ (James & Vogele, 2001), although this discrepancy appears to be due to differences in the application rates tested, with lower concentrations of active ingredient (a.i.) used in James & Vogele’s experiment (0.0053% vs. 0.5–0.125% a.i.).

The table shows the effect of the treatments on the larvae densities. The bars denote the standard error of the differences and the $P$ value is given.
and Helicoverpa spp. by reducing the prevalence of Chryso- 
deixis spp. from the crop canopy. The full potential of the treatments in terms of yield 
impacts were not fully realised in either experiment due 
to adverse wet weather and resultant boll loss in 2002/03 
and very low pest densities in 2003/04 (the lint yield of 
the untreated control plots exceeded the best yields of 
the 2002/03 experiment). Despite these difficulties, it is 
notable that both experiments yielded equivalent quantities 
of lint from the P. plagipennis integrated with soft insecticides 
and the conventionally sprayed treatment plots, suggesting 
that an integrated biological control strategy could provide 
a comparable degree of economic control to a conventional 
insecticide dependant programme.

The present research, together with earlier studies 
(Grundy & Maelzer, 2000; 2002; Grundy, 2004), suggests 
significant potential for the use of P. plagipennis as an 
inundative bio-control in cotton, although the adoption of 
this predator as part of an integrated strategy is doubtful at 
this stage. The Australian cotton industry has generally 
relied upon single technology solutions such as pesticide use 
(Fitt, 1994), and more recently transgenic Bt cotton varieties 
(Fitt, 2000) against which alternative pest management 
options such as applied biological controls are unlikely 
to compete on a cost versus efficacy basis alone. Such a 
challenge to the uptake of a biological control is not unique 
to P. plagipennis or the Australian cotton industry but 
prevalent throughout first world agricultural systems where 
predator control dominates (Waage, 1996).

The cost of rearing P. plagipennis nymphs in the 
laboratory has been estimated at AUD3.52 per 100 third 
instars (Grundy, 2001), which when released at the rates 
tested would equate to AUD352 per hectare excluding 
shipping and physical release costs. In comparison, the 2005 
licence fee for Bollgard® transgenic cotton varieties 
that provide near complete Helicoverpa spp. control was approxi-
mately AUD300 per hectare sown (Barber, 2005), which 
still leaves a considerable margin for secondary pest control 
compared to the cost of P. plagipennis release and use of 
compatible insecticides.

The primary expenses associated with rearing P. plagi-
pennis were labour costs and the use of T. molitor as insect 
prey. Whilst considerable gains in labour efficiency could 
be expected with the commercial production of P. plagipennis, 
the use of T. molitor as a prey insect would remain expensive. 
The use of an artificial diet could circumvent the need for 
host prey insects as has been demonstrated by Cohen 
(1985) who used beef and hens egg based diets for the 
rearing of Geocoris punctipes (Say) (Hemiptera: Lygaeidae) 
and later Chrysoperla carnea (Stephens) (Neuroptera: 
Chrysopidae) (Cohen & Smith, 1998). These diets have 
since been demonstrated to have potential for rearing other 
predatory Heteroptera including various pentatomids (De 
Clercq & Degheele, 1993; Zanuncio et al., 1996; De Clercq 
et al., 1998) and Dicyphus tamaninii (Wagner) (Heteroptera: 
Miridae) (Iriarte & Castane, 2001). Basic experimentation 
with these described diets has suggested that P. plagipennis 
can also be reared from first instar nymphs to adults, 
although the fecundity of diet-reared insects was poor 
compared to those reared on T. molitor (P.R. Grundy, 
unpublished data, 2004). However, the acceptance and 
development of P. plagipennis on meat-based artificial diets 
suggests some potential to develop a suitable rearing 
substrate, which could significantly reduce the rearing costs 
for P. plagipennis and make it a more cost competitive pest 
control option for cotton.

Since P. plagipennis has a potential lifespan of 9–11 
months (James, 1994), the adults can continue living well 
after a crop such as cotton has been destroyed. Therefore, 
an alternate strategy for increasing the value of inundative 
P. plagipennis releases in annual summer field crops is to try 
and retain a proportion of the released predator populations 
on-farm between summer seasons (thus reducing predator 
release requirements each season) through the provision of 
specifically planted vegetative refuge habitats to provide 
prey and shelter during the normally fallow winter months. 
However, experiments examining the potential for such 
a strategy did not identify any vegetative refuge types 
suitable for retaining P. plagipennis for the period of six 
months or more between summer cotton crops (Grundy & 
Maelzer, 2003).

Without substantial advances in predator mass-rearing 
technologies, the cost of utilising inundatively released 
biocontrol agents, such as P. plagipennis, compared with 
increasingly sophisticated transgenic technologies is likely 
to prevent the uptake of this predator in cotton for 
the foreseeable future. Given the potential efficacy of 
P. plagipennis against larvae and bug pests, this bio-control 
may be better directed towards higher value crops such as 
citrus and berry fruits, where it has already been recorded 
as a potential mortality agent of bug pests (James, 1994; 
Coombs & Khan, 1998). Within such perennial systems, a 
lower cost inoculative rather than inundative release strategy 
might be effective for increasing predator numbers to gain 
effective biological control. The integrated pest management 
programmes utilized by these industries are already 
partially reliant on inoculative releases of various other 
beneficial insect species (Smith et al., 1997) and may be more 
conducive to the uptake of a predator such as P. plagipennis.

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