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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 omethaote 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. larvae 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, mectins, 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

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

Active ingredient	g AI l^{-1} and formulation	Manufacturer	Application rate	
			ml l^{-1}	l ha^{-1}
Bacillus thuringiensis	Biological	Valent	20	2
Nucleopolyhedrovirus	Biological	Bayer Crop Science	5	0.5
Buprofezin	200 g l^{-1} EC	Syngenta	10	1
Pyriproxifen	500 g l^{-1} EC	Sumitomo	5	0.5
Indoxacarb	200 g l^{-1} SC	Du Pont	8.5	0.85
Spinosad	480 g l^{-1} SC	Dow AgroSciences	2	0.2
Fipronil	200 g l^{-1} SC	Bayer Crop Science	1.25	0.125
Emamectin benzoate	17 g l^{-1} EC	Syngenta	5.5	0.55
Abamectin	18 g l^{-1} EC	Syngenta	6	0.6
Diafenthiuron	500 g l^{-1} SC	Syngenta	6	0.6
Imidacloprid	200 g l^{-1} SC	Bayer Crop Science	2.5	0.25
Omethoate	800 g l^{-1} SL	Bayer Crop Science	1.4	0.14

EC, emulsifiable concentrate; SC, suspension concentrate; SL, soluble liquid.

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 Potter Precision spray tower was then used to apply 2 ml aliquots of insecticide to the upper and lower inner surface of each Petri dish as described by Holland & Chapman (1995) and Herron *et al*. (1998).

Agral® non-ionic wetter (nonyl phenol ethylene oxide 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 row (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). Application decisions were based on commercially accepted density thresholds for *Helicoverpa* spp. and *Creontiades* spp. as well as crop damage models for bud and fruit retention (Schulze & Tomkins, 2002; Johnson & Farrell, 2003). Insecticide applications on the sprayed plots were made at daybreak whilst wind was minimal to avoid insecticide drift into adjacent plots. A record of the insecticides applied to the sprayed treatment and the soft insecticide treatments is given in table 2. No pesticides were used on the crop area

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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.

Pest	Active ingredient	Rate	Treatments sprayed	Application date
2002/03 Experiment				
Helicoverpa	NPV	500 ml ha^{-1}	CS, SI, SI & Pp	13 Dec 2002
Helicoverpa	NPV	250 ml ha^{-1}	SI, SI & Pp	18 Dec 2002
Helicoverpa	Spinosad	200 ml ha^{-1}	CS	20 Dec 2002
Helicoverpa and mirids	Fipronil/NPV	40 ml ha^{-1} & 250 ml ha^{-1}	CS, SI and SI & Pp	9 Jan 2003
Helicoverpa	Spinosad	200 ml ha^{-1}	CS	9 Jan 2003
Helicoverpa	NPV	250 ml ha^{-1}	CS, SI, SI & Pp	14 Jan 2003
Helicoverpa	Indoxacarb	750 ml ha^{-1}	CS, SI, SI & Pp	20 Jan 2003
2003/04 Experiment				
Helicoverpa	NPV	500 ml ha^{-1}	CS, SI, SI & Pp	30 Dec 2003
Helicoverpa	NPV	250 ml ha^{-1}	SI, SI & Pp	5 Jan 2004
Helicoverpa	Spinosad	200 ml ha^{-1}	CS	5 Jan 2004

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 plant canopy were searched for eggs and small larvae because these instars are frequently found in those plant regions (Farrer & Bradley, 1985). Flowers and bolls throughout the plant canopy were also inspected for larger larvae. Larvae were recorded as small (2–10 mm), medium (11–20 mm) and large (> 20 mm). Numbers of *P. plagipennis* nymphs were recorded at the same time.

A beat sheet sampling method was used to assess the presence of *Creontiades* spp. and other insects. The sheet used was 1.5 m wide by 2 m long and made from yellow canvas. A 25 mm diameter piece of timber dowel (1.5 m long) was fixed to each end of the sheet to prevent the ends lifting in the wind. Samples were taken by placing the sheet behind the cotton plants to be sampled, along the inter-row and up over the adjacent row of cotton to create a 'wall' to catch flying insects. A 1-m long stick was used to shake 1 m of row onto the sheet for assessment. The cotton bushes were shaken several times from the base of the plants to the top. Dislodged insects were aspirated off the sheets with a domestic-styled hand-held vacuum appliance (Breville BHV2) and returned to the laboratory for thorough assessment. Beat sheet samples were made on four randomly selected 1 m row lengths of cotton plants in each treatment replicate.

Each crop was grown through to harvest. Heavy rain due to a hurricane depression delayed the harvest of the 2002/03 crop and resulted in significant yield losses due to boll rot and weather damage. The cotton was picked from the six central rows of each treatment replicate with an experimental two-row picker. The 2002/03 crop was picked on 10 April 2003 and the 2003/04 crop was picked on 11 March 2004.

The cotton picked from each plot was weighed and a sub-sample taken for ginning to determine the relative proportions of lint and seed. The yield from each plot was divided by the sub-sample gin turnouts for the proportion of lint and seed from which yield in bales of cotton lint (227 kg per bale) per hectare could be calculated.

Environmental conditions were recorded during the experiment with a Mark 4 weather station (Environdata, Warwick, Queensland).

Analysis of data

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.

Count data for *Helicoverpa* spp., *Creontiades* spp. and other insects at each sampling date were analysed using a repeated measurements analysis using the method of residual maximum likelihood (REML) with ante dependence covariate structure of order 1 using GenStat. This model was used to assess treatment effects for each experiment. Wald tests were used to assess overall treatment differences. Differences between treatments were determined by comparing predicted means using the standard error of differences.

Results

Insecticide compatibility

Significant differences were found between insecticides ($P < 0.05$, LSD 4.55), dose rates ($P < 0.05$, LSD 2.74) and the interaction between dose and insecticides tested ($P < 0.05$, LSD 9.11). Pyriproxifen, buprofezin, *Bacillus thuringiensis* and nucleopolyhedrovirus were non-toxic to *P. plagipennis* nymphs whilst indoxacarb was of very low toxicity. Spinosad, fiprinol, 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. Diafenthiuron, imidacloprid and omethoate were highly toxic to *P. plagipennis* nymphs even when applied at reduced rates (table 3). For intermediate

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Table 3. Percentage mortality (±SE) of first instar *Pristhesancus plagipennis* treated with various insecticides in laboratory bioassays.

Product	Percentage of recommended field rate tested			
	100	75	50	25
Bacillus thuringiensis	0	0	0	0
Nucleopolyhedrovirus	0	0	0	0
Buprofezin	0	0	0	0
Pyriproxifen	2.2±0.1	0	0	0
Indoxacarb	7±2.8	2±0.1	0	0
Spinosad	27±1.9	11±0.1	12±1.93	7±3.4
Fipronil	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 4. Percentage mortality (±SE) of each *Pristhesancus plagipennis* instar treated with various insecticides at the full recommended rate in laboratory bioassays.

Product	Percentage mortality of each *P. plagipennis* instar				
	I	II	III	IV	V
Indoxacarb	6±2.7	4±2.7	0	0	0
Spinosad	28±2.9	11±2.2	4±2.2	0	0
Fipronil	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

toxicity products (table 3) 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

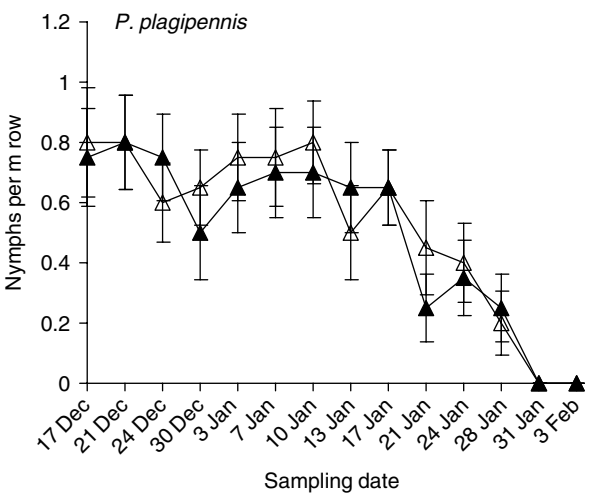

Fig. 1. Time series showing numbers per m row of *Pristhesancus plagipennis* nymphs sampled from all treatments for the 2002/03 experiment. The bars denote se. —▲—, *P. plagipennis*, Only; —△—, *P. plagipennis* & Soft Insecticides.

($P < 0.01$) 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

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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.

Treatment	*Creontiades* spp.		*Chrysodexis* spp.		Large *Helicovpera* larvae	
	Mean	% Pest Reduction	Mean	% Pest Reduction	Mean	% Pest Reduction
Untreated control	1.31	n/a	1.78	n/a	0.51	n/a
Pristhesancus plagipennis only	0.75	42.7	1.05	40.5	0.18	64.7
Soft insecticides	0.31	76.4	1.31	26.7	0.24	52.9
Soft insecticides and *P. plagipennis*	0.30	77.0	1.28	27.7	0.15	70.5
Conventionally sprayed	0.55	58.0	0.45	74.8	0.13	74.5
Standard error of differences	0.26			0.31	0.03	
Chi *P* value	< 0.001			< 0.01	< 0.001	

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

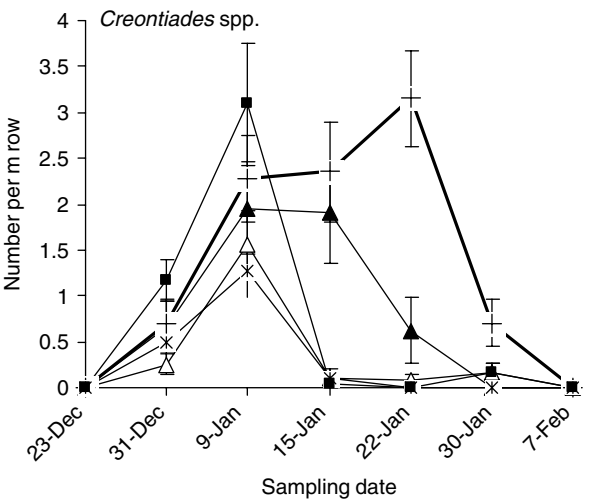

Fig. 2. Time series showing mean numbers per m row of *Creontiades* spp. sampled from all treatments for the 2002/03 experiment. The bars denote se. —▲—, *P. plagipennis* Only; —+—, Untreated Control; —△—, *P. plagipennis* & Soft Insecticides; —✳—, Soft Insecticides Only; —■—, Conventionally Sprayed.

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

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

Treatment	Lint yield (bales ha^{-1})	
	2002/03 Experiment	2003/04 Experiment
Untreated control	5.3a	9.34a
Pristhesancus plagipennis only	6.73b	9.86ab
Soft insecticides	6.91b	9.95ab
Soft insecticides and *P. plagipennis*	6.94b	10.90b
Conventionally sprayed	7.22b	10.56b
LSD at 5%	0.67	1.05

Treatment means marked with different letters are significantly different ($P < 0.05$).

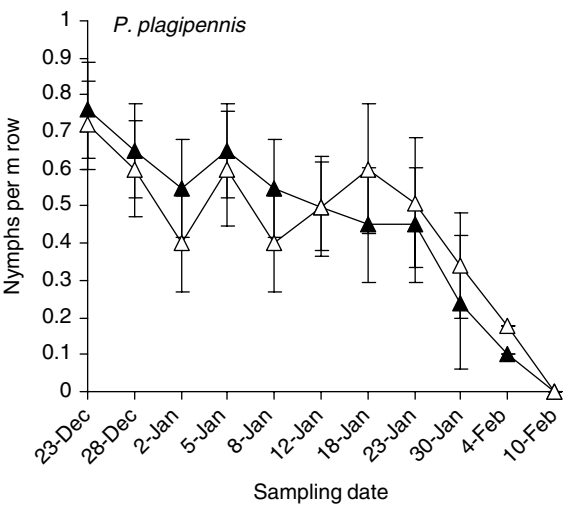

Fig. 3. Time series showing numbers per m row of *Pristhesancus plagipennis* nymphs sampled from all treatments for the 2003/04 experiment. The bars denote se. —▲—, *P. plagipennis* Only; —△—, *P. plagipennis* & Soft Insecticides.

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

Treatment	Large *Helicoverpa* larvae		Total *Helicoverpa* larvae	
	Mean	% Reduction	Mean	% Reduction
Untreated control	0.06	n/a	0.61	n/a
Pristhesancus plagipennis only	0.01	83	0.31	49.2
Soft insecticides	0.01	83	0.36	41.0
Soft insecticides and *P. plagipennis*	0	100	0.16	73.7
Conventionally sprayed	0	100	0.21	65.5
Standard error of differences	0.01		0.06	
Chi *P* value	< 0.001		< 0.001	

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

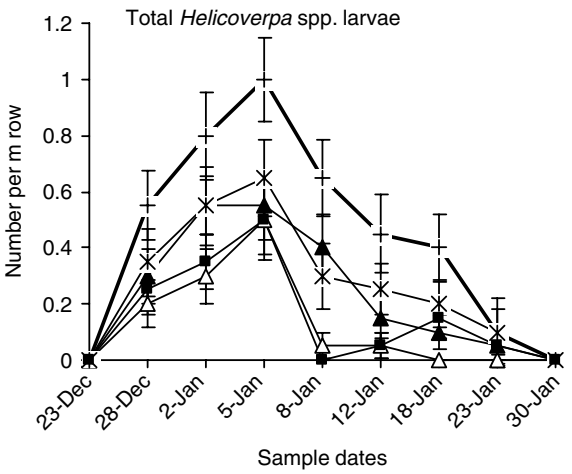

Fig. 4. Time series showing the mean numbers per m row of all *Helicoverpa* spp. larval instars sampled from all treatments for the 2003/04 experiment. The bars denote se. —▲—, *P. plagipennis* Only; —+—, Untreated Control; —△—, *P. plagipennis* & Soft Insecticides; —✳—, Soft Insecticides Only; —■—, Conventionally Sprayed.

densities further suggested that both the *P. plagipennis* combined with soft insecticides and the conventionally sprayed treatments provided complete control with no large larvae recorded in the plots (table 7).

The conventional and *P. plagipennis* with soft insecticides treatments yielded the same amount of lint, both of which were significantly more ($P < 0.05$) than the unsprayed control (table 6).

Discussion

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 omethoate, 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 & Vogeles' experiment (0.0053% vs. 0.5–0.125% a.i).

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 diluted 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*

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and *Helicoverpa* spp. by reducing the prevalence of *Chrysodeixis* 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 pesticide control dominates (Waage, 1996).

The cost of rearing *P. plagipennis* nymphs in the laboratory has been estimated at AUD$3.52 per 100 third instars (Grundy, 2001), which when released at the rates tested would equate to AUD$352 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 approximately AUD$300 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. plagipennis* 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 using 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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