



FINAL REPORT 2018

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Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: CSE1501

Project Title: Keeping Pest Populations Lower for Longer: Connecting farms and natural systems.

Project Commencement Date: 01/07/204 **Project Completion**
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Date Submitted:

31 August 2018 _____

Part 3 – Final Report

Background

1. Outline the background to the project.

Native vegetation (NV) in agricultural landscapes provides a range of ecosystem services such as, erosion control, carbon storage, and habitat for wildlife and beneficial arthropods, all of which contribute to controlling insect pests. Research shows that the landscape context is important for managing pest populations and that high levels of pest suppression are associated with native vegetation and landscape complexity. The relative timing of pest and beneficial insects arriving in crops and how landscape context facilitates or hinders colonisation processes, is thought to be one of the key mechanisms for lower pest populations. Early removal of pests avoids future damage, and often more importantly, it avoids future damage by the pests' offspring. Similarly, late arrival of pests, or arrival in low numbers, can delay pests reaching economic injury levels (Bianchi et al, 2015, Costamagna et al, 2015, Macfadyen et al, 2015, Schellhorn et al, 2014).

Overseas and Australian research shows that the source of beneficials can be nearby and more distant, suggesting that both a linear strip of native vegetation and the broader landscape are important. In Australia, research shows that: crops, pasture and weeds are more likely to harbour pests, and native plants are more likely to support beneficials; beneficial insects are present on native plants when no crops are in the ground; they move from remnants into fields throughout the cropping season; and cotton pests near remnants can suffer higher attack rates by beneficials than on cotton crops far from remnants. However, in Australia evidence of impact is lacking, as well as knowledge of the mechanism for these patterns. This project builds on results from previous projects which investigated the role of native vegetation for supporting beneficial insects, and it seeks to measure the impact of these landscape features on pest suppression and link the outcomes with vegetation management guidelines. The overall goal is to promote healthy cotton farms and landscapes that are productive, profitable, and sustainable, by further understanding the role of natural assets for insect pest control. To achieve this we addressed two key objectives, to:

- identify patterns in pest populations, relationships with pest thresholds, and how these relate to landscape structure, and
- determine whether pest populations stay lower for longer and have a slower rate of increase as a function of distance (e.g. near vs far) and landscape structure (e.g. amount and location of natural areas, diverse habitats, crop and non-crop at 1 km, 1.5 km and 2 km from cotton fields).

To achieve these objectives, we tested the following hypotheses in cotton production landscapes:

- 1) differences in pest and beneficial numbers, and differences in 'measures of impact' are a function of landscape structure;
- 2) the rate of increase in pest populations (lower for longer) is not influenced as a function of distance from (e.g. near vs far) semi natural areas;
- 3) proportion of semi natural vegetation at a landscape level has no relation to insecticide application to manage pest populations; and
- 4) insect pests and beneficials are not influenced by local sources or broader context.

The approaches to achieve these objectives included analysing an extensive five year crop scouting data set to address hypotheses 1, 2 and 3; designing and conducting two field experiments (1 Biological control in NV vs Cotton and 2 Sentinel Plant pollination interactions) to address hypotheses 1, 2, 3 and 4; and a glass house (pest vs pollination) experiment, to address hypothesis 1.

Objectives

2. List the project objectives and the extent to which these have been achieved, with reference to the Milestones and Performance indicators.

Table 1: Summary of project objectives milestones performance indicator and completion / output

Objective Description	No	Milestone Description	Performance indicator	Method summary	Completed /Output
1 Project Start	1				
2 Identify patterns in pest populations, relationships with pest thresholds, and how these relate to landscape structure	2.1	Continue building relationships with crop consultants from four regions and acquire scouting reports.	Crop consultants to share scouting reports and identify field location on maps	Consultant contacted	Data sharing agreement negotiated and signed with Mathew Holding. Data obtained. Complete
	2.2	Enter scouting report data, geo-reference farms, generate metrics	Land use metrics generated for fields that meet the criteria for the study	Extensive period committed to checking scouting records and mapping of farm landscapes	Scouting records and for 170 data sets harmonised. Landscape details digitised
	2.3	Conduct analysis on patterns of landscape structure and pest economic injury thresholds and beneficial populations	Analysis completed, and hypotheses refined	Landscape metrics merged with crop scouting data. Analysis of crop scouting data. Complete	Results show relationships that relate timing of insecticide application, pest resurgence after spraying to surrounding area of NV.
3 Determine whether pest populations stay lower for longer, and have a slower rate of increase as a function of distance (e.g. near vs far) and landscape structure (e.g. amount and location of natural areas, diverse habitats, crop and non-crop at 1 km, 1.5 km and 2 km from cotton fields)	3.1	Identify field sites to conduct experiments that meet landscape structure criteria	Field sites identified	Approx. 30 growers contacted each season Letters sent invitation to participate in project	Ethics approval completed Fields ground truthed Sites chosen Landscape surrounding fields digitised Completed
	3.2	Collect field data on pest and beneficial insect movement, in-field data and adjacent NV condition and insect presence across 2 seasons.	Field data collected including insect movement, in-field sampling, and NV condition and insects.	Ex 1 Glass House pest (mirids) vs pollination and yield Complete Ex 2 Field Sentinel Plant Experiments Complete	Ex 1 Glass House Experiment completed yield assessment for treatments. Complete Ex2 Field Sentinel Plant Experiments Yield assessment. Pests, beneficials, and pollinators

				Ex 3 Field Biological control in semi-natural areas vs Cotton Complete	identified and counted from digital images of flower visits. Complete. Ex 3 Field Biological, control in semi-natural areas vs Cotton Complete
	3.3	Analyse data		A range of statistics were applied to analyse the data from specific Experiments 1-3 Completed See Section 4 of the report for detail	Data prepared and analysed Completed See Section 4 of the report for results
4 Determine the costs or savings of the number of sprays required or avoided for the different landscape conditions both as part of the crop-report analyses and field studies	4.1	Collect information from crop scouting reports on the number of above threshold sprays as a function of landscape structure	Costing from crop-scouting analyses completed and communicated	Inconsistent data from crop scouting records precluded data analysis in relation to economic threshold. Data analysed included Results = time to first spray time between sprays	See 2.3 results Any savings / costs are indicative only. For example, results support those crops adjacent semi-natural may require insecticide application less often.
	4.2	Collect information from study fields on the number of above threshold events and subsequent sprays as a function of landscape structure	Costing Field studies completed and communicated	Information collected as part of Milestone type of insecticide and frequency	See 2.3 results Any savings / costs are indicative only. For example, results support those crops adjacent semi-natural may require insecticide application less often.
5 Link studies and outcomes with options for vegetation management guidelines.	5.1	Experiment is designed to answer whether arrival is facilitated by local sources or the broader landscape context	Study Established	See Milestones 2.3 3.2, 3.3	The results support clear links See results from Milestones 2.3 3.2, 3.3
	5.2	Species of NV bordering crop is sampled for beneficial and pests	Pest and beneficial samples collected and identified with the native plant host	Pest and beneficial insects sampled and semi-natural area identified as part of 2.3, 3.2 , and 3.3	Results from miles stones 2.3, 3.2, and 3.3 show that effects of semi-natural area versus no native has been linked at a field and landscape level.

	5.3	Working with CRDC and NRM tech specialists discuss options for vegetation management guidelines to update on sustainable landscapes section of Cotton Pest Management Guide	Vegetation management guidelines provided	Worked with CRDC On cotton pest management guidelines for 2017 - 18	Contributed to 2017 2018 Cotton Pest management guidelines Extend results from project as they relate to semi-natural area quality and diversity. For example, contribute outcomes from the project to Cotton Pest management guidelines for next the 2018 19 season.
6 Package and communicate findings to various stakeholders	6.1	Work with NRM tech-specialists to disseminate results. Inform regional NRM and regional grower groups about the project	NRM tech-specialists, regional NRM groups, and grower groups are aware of our project	Presentations to NRM audiences and researchers	Local NRM Allora QLD April 2018 Restore, Regenerate and Revegetate (RRR) Conference held in Armidale from 5 February 2017
	6.2	Prepare popular press articles on the project and findings	Popular press articles published	Articles	Australian Cotton Grower July 2017
	6.3	Present findings at cotton grower meetings and Land Care meetings	Presentations given	Presentations	Completed: 1 x Cotton CRDC Webinar May 2018 2 X International Conferences (see also 6.1)
	6.4	Prepare fact/ action sheets for stakeholder groups	Fact sheets distributed and available for downloading	Worked with CRDC	Researcher profile and project featured in Spotlight Magazine Draft fact sheet attached to final report APPENDIX D
	6.5	Prepare a minimum of two peer reviewed scientific publications	Manuscripts prepared and submitted to CRDC for approval process	Draft Manuscripts	2 x Draft Manuscripts attached as part of final report APPENDICES A and B
7 Final Report					

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

Consultant Crop Scouting Data

A commercial consultant, Mathew Holding, provided his proprietary data from his consulting business, which was collected by himself and his employees who “scouted” the fields of his clients growing cotton. Fields were located within the Darling Downs cotton growing region in QLD and distributed in a region south from Cecil Plains Brookstead and Leyburn.

The data consisted of 170 individual files from 30 properties (or 12 growers) for five seasons, 2011-2016 (308 cotton fields), and consisted of insect counts and information with fine spatial and temporal resolution. In preparation for statistical analysis, all fields were geo-referenced using GIS software and to ensure that all data was consistent, 3500 individual scouting reports were re-formatted. During the process data checking, harmonising much of the spatial data was found to be incomplete. Therefore, additional ground-truthing was undertaken to check accuracy and complete missing spatial data sufficient to allow further analysis. The crop scouting data was merged with the spatial data and analyses conducted.

Field Experiments

In conjunction with analysing the scouting data, experiments were designed to determine the benefit of high percentage native vegetation (NV) in the landscapes surrounding cotton fields in addition to the effect of neighbouring NV. To address hypotheses 3 and 4 we designed and conducted field experiments across two cotton-growing seasons to answer the following:

- What are the specific functional groups of predatory insects, depending on their spatio-temporal activity (diurnal vs nocturnal and ground vs canopy dwelling) that contribute to pest control in cotton crops?
- What level of biocontrol provided by semi-natural habitats? and
- How does insecticide application affect the levels of pest suppression by beneficials or predators?

Field Sites and Mapping

All field experiments were conducted on growers’ fields. Letters were sent to approximately 30 growers before the growing season to introduce and explain the project as well as inviting growers to participate. This initial communication was followed up to confirm grower participation and request more specific information such as farm maps, crop varieties and planting dates.

Field experiments were conducted during two cotton growing seasons 2015-2016 and 2016-2017 in the Darling Downs area Nandi and North West of Dalby. The fields chosen were a minimum of 2 km apart, and within a 50 x 50 km region depending on the presence/absence of semi-natural field margins (Figure 1). Semi-natural margins consisted mainly of highly disturbed woody vegetation with predominantly mixed grass understory as linear strips or along creeks. The native vegetation (NV) or non-crop areas adjacent to cotton crops where we conducted our experiments was often characterised by highly disturbed areas with a sparse upper canopy. There was seldom any mid-story and the ground layer of semi-natural areas adjacent to the rivers were dominated by the exotic weed *Phyla canescens* or Condamine couch and mixed grasses dominated areas away from the river. Throughout the project we refer to these areas as semi-natural vegetation.

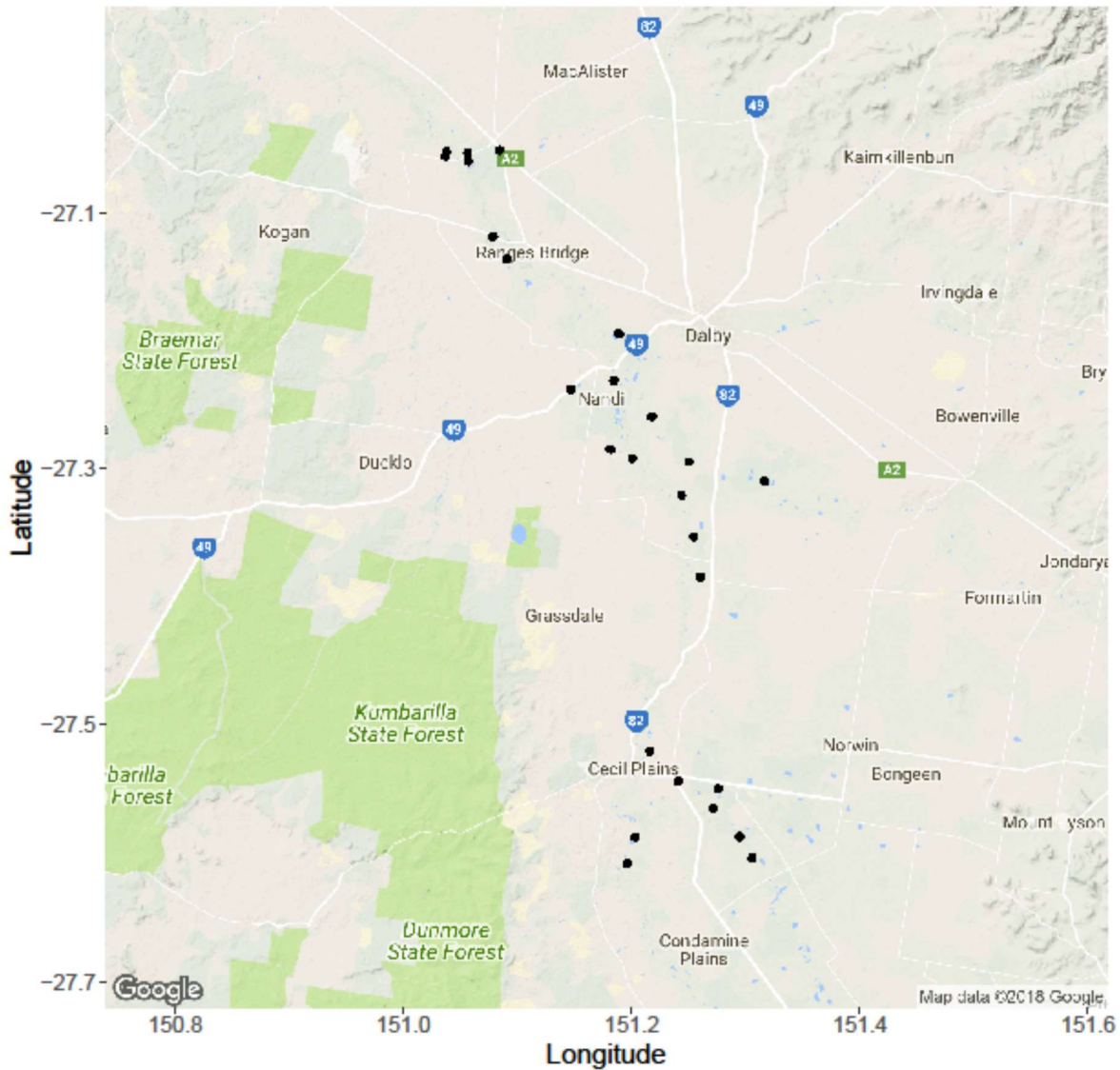


Figure 1 Experimental Field sites 2015-2016 and 2016-2017 (indicated as black circles)

Field boundaries of crop and non-crop habitat were digitised using the satellite image service base map in ArcGIS 10.2 (ESRI 2014), and landscape data and maps were generated. In addition to the landscape data (% semi-natural vegetation in a 1 km circle, distance from semi-natural vegetation) we also collected additional information on cotton boll retention, crop variety, insect spray dates, irrigation, crop phenology, weather, weeds and diseases.

Field Experiment – Biological control in semi-natural area vs Cotton

Results from a recent literature review suggested the importance of semi-natural margins for natural enemies of pests in Australian crops, while responses to landscape composition were inconsistent (Gagic *et al.* 2018). Therefore, we tested the effects of the presence/absence of semi-natural crop margins, rather than the amount of semi-natural area in the wider landscape. We selected eight cotton fields with, and nine without, a semi-natural margin in the first season. In the second season, we selected nine fields with and ten without semi-natural margin. No single field was used twice across two seasons. The sampling was conducted in both crop and neighbouring semi-natural habitats resulting in 53 (36 crop and 17 semi-natural) habitats in total.

To test the impact of functional groups of predators we ran a biocontrol experiment twice during 2015-2016 and once in the 2016-2017 season using sentinel egg cards of cotton bollworm (*Helicoverpa armigera*, Hübner) to quantify predation. Ten litre buckets were lined with cardboard for *Helicoverpa armigera* moths to lay eggs on. The card liner complete with eggs (laid over a 12 hour period) was removed and carefully cut to leave pieces of card with approximately 15 and 10 eggs per card in the first and second season, respectively. The pieces of card with the eggs were stapled to a larger card ~ 5 cm square. To quantify predation egg cards were exposed in the fields using treatments: 1. Strata (Ground vs Canopy), and 2. Exposure time (Day vs Night) in a fully crossed design. This was achieved by attaching egg cards to wooden stakes at the appropriate height (canopy or ground level) and replacing them after approximately 12 hours of day or night exposure (replacements between 7-9 am and 7-9 pm). To ensure that eggs within the canopy treatment were only accessible to flying predators, we used Tanglefoot™ to isolate the cards from ground-dwelling predators. We conducted the experiment at three positions within each field (cotton and semi-natural) 10 m apart and 10 m from the field edge. In both seasons, the experiment was conducted when cotton crops were flowering, which is when cotton crops are most susceptible to pest damage. In the first season, we repeated the experiment twice, in January and February, while in the second season the experiment was conducted once in January.

To collect data on predator abundance of the main ground-dwelling predators we used pitfall traps. Pitfall traps were constructed using plastic beakers filled with water and a drop of detergent (to reduce surface tension) and exposed for one week at approximately the same time when the experiments were conducted. Samples of pests and beneficials were also collected, identified from the dominant plants in the semi-natural bordering crop and in the cotton were sampled using beating box, and identified in the field. To reduce the impact of irrigation on our results, we conducted experiments at least three days after an irrigation event and placed the experimental treatments on the elevated parts of the field, within the plant rows.

A draft manuscript, detailing the pest (mirids) vs pollination - Sentinel Plant and Flower Visitation research can be found in (**APPENDIX A**); Vesna Gagic, Andrew Hulthen, Anna Marcora, Xiaobei Wang, Nancy Schellhorn. *Semi-natural area does not benefit biocontrol in insecticide sprayed crops*. In preparation for Proceedings of the Royal Society B.

Field Experiments - Sentinel Plants and Flower Visitation

Sentinel Plants

This experiment was designed to test what effect the combination of pollination and biocontrol had on cotton crop yield under field conditions. We exposed potted sentinel plants raised in the greenhouse using the same plant rearing protocol as the glasshouse experiments (see below). At the beginning of flowering, potted sentinel plants were transported and placed within semi-natural habitats 10 m from the cotton field edge at two positions 10 m apart. The plants were placed within semi-natural habitats, rather than within cotton fields, to avoid negative effects of insecticide spraying. Eight sentinel plants were placed in each semi-natural habitat (4 plants per position), thus totalling 96 plants. One of the four plants randomly received one of two treatments: pollination (2 level factor: with and without) or biocontrol measured using predator enclosure (2 level factor: with and without) in a fully crossed design. Pollination treatment consisted of all open or all bagged flowers on a plant. Plants were regularly watered and checked so that each newly formed

flower bud was bagged prior to opening. Predator exclusion treatment consisted of placing Tanglefoot™ at the base of the stem (protected by a sticky tape) to exclude ground-dwelling predators.

The cotton plants were regularly watered and exposed in the field for three weeks (2-22 January 2017). After this period plants were visually inspected and all arthropods (pests and their natural enemies) counted. Finally, plants were sprayed with insecticide and brought back to the greenhouse until yield and growth assessment was conducted as described above.

Flower visitation

We used digital cameras to record flower visitation rate in potted plants. Digital cameras were mounted, focussed on two flowers per habitat type per site and recorded visiting insects. The cameras took snapshots at 10 seconds intervals from 5 am until 7 pm. However, due to the time it took to reposition the cameras on plants and fields, individual flowers were often recorded 1-2 hours less than a full 14 hours. Although this method can underestimate flower visitation rate it is not likely to introduce bias in relative comparisons among treatments. The digital images were visually inspected to identify and record any floral visitors: 1. within flowers touching reproductive parts, 2. on the outer part of the flower (feeding on extrafloral nectaries). Flower visitors were identified to the highest possible taxonomic resolution, mainly genus. Additionally, we recorded presence/absence of ants on the plants within crop fields.

Infrared Field Cameras

Modified Redleaf RD1006 infrared trail cameras recorded the insects visiting cotton flowers “Flower visitation” and the insect predators consuming *H. armigera* eggs as part of the “Biological control” experiments. By default, these cameras only take images when triggered by moving objects that emit infrared, so this mode of detection doesn’t work with cold blooded animals like insects. Therefore, the cameras were modified by removing the housing and unscrewing the lens to focus close-up on small insects from a distance of 20-30 cm. The passive infrared capability was turned off, and time-lapse mode was set to take images continuously every 10 seconds for 24 hours. In the field, cameras were supported on a metal frame and the camera housing was secured ~20 cm and focussed on the egg cards. Twenty-four cameras recorded over 10,000 hours with a sampling rate of one frame every 10 seconds. Over 100 hours of recorded digital footage was replayed through Virtual dub software <http://www.virtualdub.org/> to identify and record the insect pollinators and predators active during the experiments.

Glass House Experiment - Pest (mirids) vs pollination

To test the interactive effects of mirid pest pressure (no mirids, low and high) and pollination on cotton yield quantity and quality an experiment was designed and conducted in a CSIRO glass house.

Cotton was planted in 25 mm diameter pots on 15/01/2016 (one plant per pot) which were fertilized and watered regularly. The temperature was constant at 20°C at night and 28-30°C during the day. In March 2016, at the beginning of cotton flowering, we transferred three plants per cage, and each cage was assigned pollination (2-level factor: with or without) and mirid (3-level factor: 0, 1 and 5 per plant) treatment in a fully crossed design (6 treatments in total). Varying pest pressure was used as a proxy for biocontrol. Each treatment was replicated five times, resulting in 30 cages and 90 plants.

In the mirid treatments, adult green mirids raised in laboratory conditions were released in the cages on 14/03/2016. Cages were checked regularly (each 2-4 days) to detect mortality of mirids, and when necessary, mirids were replaced to maintain appropriate treatment numbers. After 15 days, insecticides were sprayed to remove mirids and the cages removed. In the pollination treatment, throughout the flowering period (starting on 12/03/2016), newly open flowers were hand pollinated each morning using fresh pollen from 30 additional plants using a paintbrush. Cotton flowers are only open for one day. Plants were monitored on three occasions after the treatments were completed (March, June and August) for the

number of first position bolls (bolls closest to the plant stem that are formed first), plant height, node number and number of fruiting branches. At the end of the experiment, five first position bolls per plant were hand harvested starting from the first formed bolls at the lower plant parts and boll weight recorded.

A draft manuscript, detailing the Pest (mirids) vs pollination - Sentinel Plant and Flower Visitation research can be found in **(APPENDIX B)**; Vesna Gagic, Anna Marcora, Lynita Howie. *Additive and interactive effects of pollination and pest control on crop yield*. In preparation for Proceedings of the Royal Society B.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

How do areas of semi natural vegetation at a landscape level relate to insect pest management activities?

Relationships between land use, specifically the proportion of semi-natural vegetation, insect pests and management using insecticides at different spatial scales, were identified by analysing land-use information and scouting data. Analysing crop-scouting data for five seasons from multiple cotton fields in the Darling Downs and calculating proportions of land use up to two km around these fields included over 36400 observations, by field and date. The data was corrected and merged with landscape data and analysed to determine the relationship between land use, management activities, and insect pest populations. Analysis in R using Generalised Additive Models with Poisson distribution included the number of sprays, days between spray 1 and 2, landscape complexity, time of pest colonization, time when first sprayed. Generalised Additive Mixed Effects Models (gamm from R mgcv library) with Field ID in the random structure and bernoulli distribution was applied to test the presence and absence of mirids. A moving-average correlation structure with Season as a grouping factor to control for temporal pseudo replication ($p=2$, $q=0$). Day in year with a cubic spline smoother was applied to account for temporal trends. The two model statements used were:

- 1 Sprayed fields where the interaction between % NV and time since spraying using tensor product smooths was tested and
- 2 Unsprayed fields where the interaction between %NV and field area using tensor product smooths was tested

Results showed that the probability of mirids being present in cotton is likely to be less and remain so for longer in fields where there is 20% or more semi-natural habitat at a 3 km radius. Spraying reduced the probability of mirids in a crop. However, the likelihood of mirids increasing after spraying insecticide is greater where there is less native vegetation. The time between sprays is extended as the proportion of semi natural area increases (Figure 2). There is also a relationship between the size of the cotton field and proportion of native vegetation and probability of mirids. For example, the time between sprays increases with increasing native vegetation in small fields. Similarly, the likelihood of mirids being present in cotton increases as field size increases but where there is also less native vegetation.

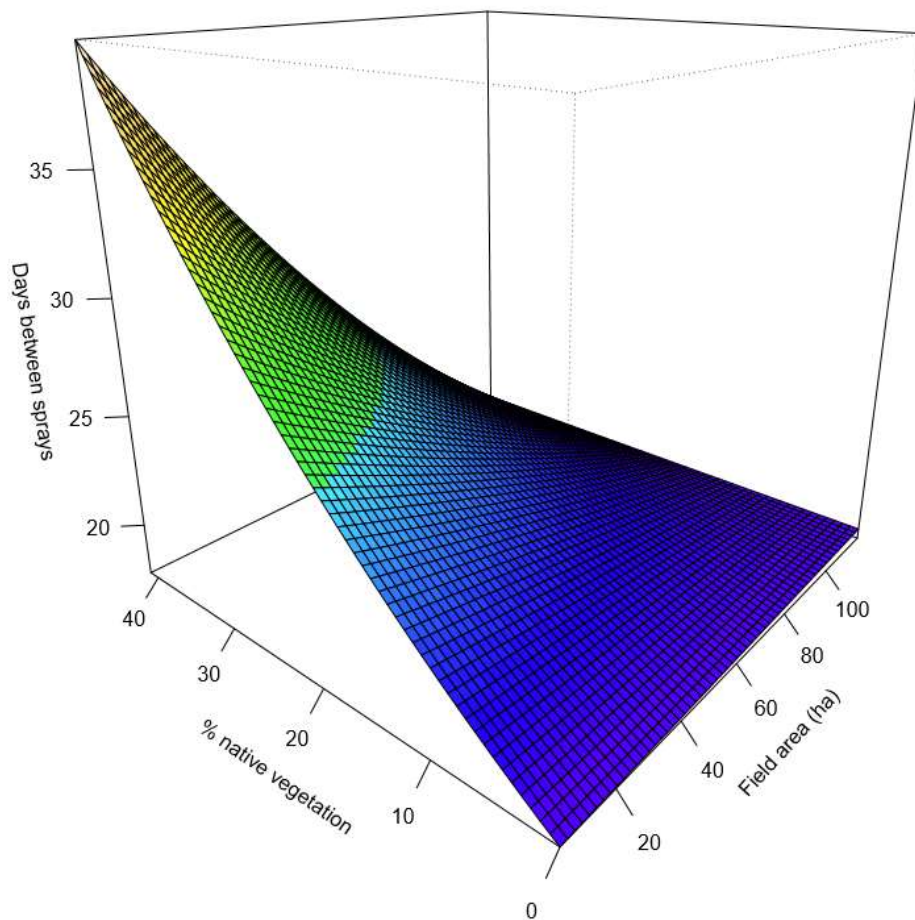


Figure 2. The effect of increasing % native vegetation and the days between insecticide sprays

Are pest and beneficial numbers, and differences in ‘measures of impact’ a function of habitat?

Data from the biological control in semi-natural area vs cotton experiment was analysed using a range of statistics to determine what explained the variation of predation by beneficial insects between crop and semi-natural areas.

Generalized Mixed Effects Models with negative binomial distribution were used to analyse the effects of the treatments on predator abundances and biocontrol. We calculated biocontrol as a number of removed eggs after the field exposure period and used log of initial egg numbers as an offset. These models were a better fit and produced more conservative estimates in comparison to the models with a binomial distribution. The explanatory variables for the biocontrol models were: Habitat type (3-level factor: crop with margin, crop without margin and semi-natural habitat), Diel time (2-level factor: day vs. night), Strata (2-level factor: ground vs. canopy) and their 2-way interactions. We constructed two separate models that included 1. Data from sprayed fields from both seasons and one additional explanatory variable Recovery time (continuous), 2. Data from the first season and one additional explanatory variable Insecticides (2-level factor: yes vs. no) for the fields that were sprayed and unsprayed prior to the experiment. Therefore, the effects of insecticides were tested as both presence/absence (first season) as well as the recovery time since they were sprayed (both seasons). Using non-linear relationship for Recovery time did not improve the models. Random structure included sampling position within a field (1:3), nested within site type (crop vs. semi-natural), and nested within landscape ID. The model that included both seasons additionally had sampling month (January vs. February) nested in the sampling season (2016 and 2017) in the random

structure. The analyses was conducted in R (version 3.5.0) using packages “lme4” (Bates et al. 2015) for analyses and “DHARMA” (Hartig 2018) for residual diagnostics.

A Mantel test was used to test whether field abundances of predators correlate with their predation efficiency recorded by camera. Because ants were the most commonly observed predator feeding on eggs, we constructed two separate models using only observed predation by ants in the second season and 1. The same explanatory variables as above, 2. Abundances of the two dominant morphospecies. This was in order to investigate to what degree the results from the above model were driven by ant predation and what the most important ant morphospecies are. To investigate the importance of different taxa for ground predation in the first season when predator-prey interactions were not directly observed, we used linear models as described above and abundances of the three taxa shown to be important predators (ants, earwigs and crickets) as explanatory variables. Finally, we tested the effects of our explanatory variables (excluding Strata and Diel time) on abundances of all ants and the dominant morphospecies using the same model structure as for biocontrol models (without an offset and without sampling position in the random structure).

To investigate how composition of predator-prey interactions changes with Habitat type, Diel and Recovery time, we conducted redundancy analyses on Hellinger transformed data, using “vegan” package (Oksanen et al. 2018). To test interaction turnover depending on the Habitat type and Diel time, we calculated multivariate dispersion (average distance of group member to the group centroid) using “betadisper” function (Anderson et al. 2006). The significance of the tests was assessed using permutation test with 999 permutations. Finally, we tested whether species richness of the predator taxa recorded to feed on eggs changes with our explanatory variables using linear models as described above.

Results:

Egg predation varied between 0 and 100% with mean 32% and 23% in the first and second season, respectively. Ground predation was higher than canopy predation in both sprayed (Ground: 1.725 ± 0.271 , $p < 0.001$) and unsprayed fields (Ground: 2.180 ± 0.269 , $p < 0.001$). In the unsprayed fields, ground predation was lower in crops without than crops with semi-natural margins (Ground x Without margin: -0.709 ± 0.281 , $p = 0.012$), while canopy predation remained unchanged. There was no effect of field margins on any functional group in the sprayed fields (Figure 3). Ground predation in semi-natural habitats was higher than in the neighbouring crop fields only if crops were sprayed (Ground x Semi-natural in sprayed: 0.684 ± 0.337 , $p = 0.042$ and unsprayed fields: 0.209 ± 0.495 , $p = 0.673$). Only ground predation increased with time after insecticide spraying (Ground x Recovery time: 0.342 ± 0.140 , $p = 0.014$). Diel time did not have an effect on predation in unsprayed fields. When crops were sprayed night predation was lower in the crops (Night: -0.861 ± 0.292 , $p = 0.003$), but not in semi-natural area (Night x Semi-natural: 0.672 ± 0.327 , $p = 0.039$).

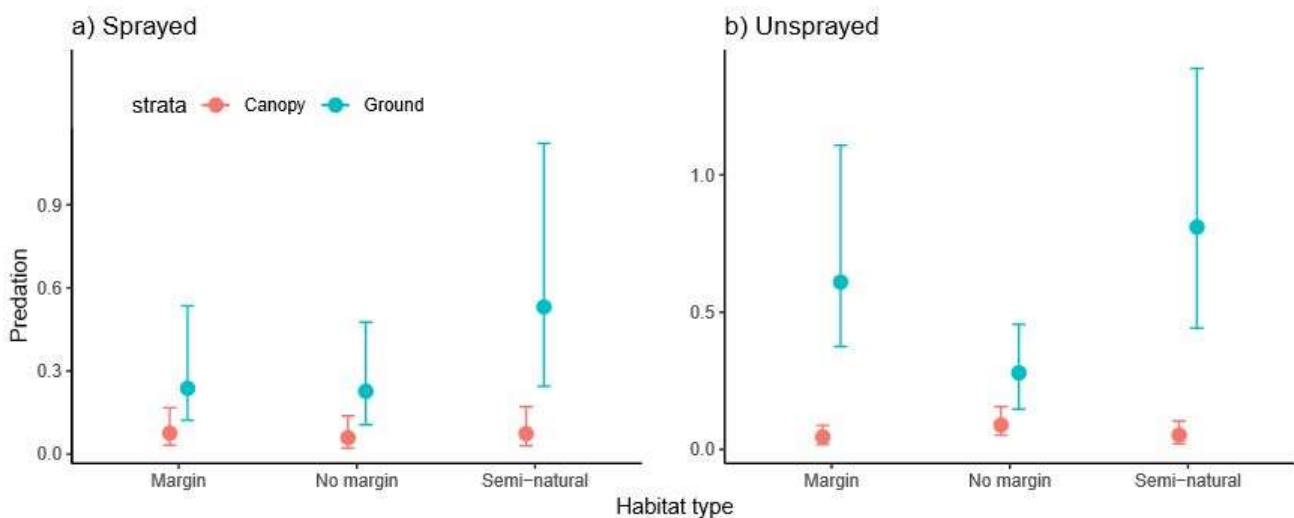


Figure 3. Model estimates with mean and bootstrapped 95% confidence intervals for the interactive effects

of habitat type (semi-natural habitat, crop with margin and crop without margin) and strata (Canopy vs. Ground) on egg predation in the a) sprayed and b) unsprayed fields.

Predator-prey interaction composition (Fig 3) differed with Diel time ($F_{1,28} = 4.30$, $p = 0.009$), habitat type ($F_{2,28} = 5.11$, $p = 0.001$) and their interaction ($F_{2,28} = 2.76$, $p = 0.022$), but not with time since insecticides were sprayed (Recovery time). Predation in the semi-natural habitats was strongly dominated by ants during both, day and night (94%). Ants were the most efficient predator group in the crops during day (Without margin= 79%, With margin= 65%), but during the night the dominant predators were earwigs (Without margin= 62%, With margin= 7%) and crickets (Without margin= 38%, With margin= 56%). Additionally, red and blue beetle contributed 34% day predation in the crops with margins.

Predation efficiencies across all predator taxa recorded by camera significantly correlated with their abundances in the field (Mantel: $r = 0.167$, $p = 0.013$). Ant abundances were higher in semi-natural habitats than in neighbouring crops (2.848 ± 0.643 , $p < 0.001$) and did not show change with the time since spraying. In the second model that compared sprayed and unsprayed fields, ant abundances decreased with spraying only in the fields without semi-natural margin (-1.514 ± 0.666 , $p = 0.023$).

Together these results provide strong support for the benefits of semi-natural field margins on predation in cotton production environments especially for ground-dwelling predators. This is consistent with numerous published studies. However, the results show that semi-natural field margins benefit pest control only in unsprayed fields where magnitude of predation was higher than in crops without margins and similar to that in the neighbouring semi-natural area. Insecticide spraying diminished the benefits of semi-natural area and recovery of predation function was slow even in presence of semi-natural field margins.

The second key finding is that although one dominant taxon is largely responsible for high biocontrol levels within a particular time and place (e.g. in crops with margins ants during day, but earwigs during night), a turnover of dominant predator-prey interactions maintains high biocontrol with increasing spatio-temporal scales (diel time and across fields). However, other factors not tested in this study, such as condition of semi-natural vegetation (e.g. grazing, weediness, structural complexity) could also influence canopy predation and could explain some variation in the effects of semi-natural area on ground predation. In comparison to unsprayed crops, the results show that in insecticide sprayed crops biocontrol is low, and recovery is slow after spraying and crops do not benefit from semi-natural margins. Therefore, to realise the benefits of semi-natural areas for the ecosystem service of biological control it will be necessary to consider in-field management activities in relation to landscape features simultaneously.

Interactions between ecosystem services, pest and beneficials, pollination and 'measures of impact' on yield in relation to habitat structure?

Linear Mixed-Effects models were used to test the interactive effects of biocontrol and pollination on cotton yield and the effects of cotton boll retention on plant compensatory mechanisms (plant height and boll weight). The first position boll retention was used as a proxy for cotton yield. The model for boll weight from the greenhouse study only used the last measured retention as a predictor. For arthropod abundances we analysed the most common groups in the count data (pest, ants, spiders, and pollen beetles) in relationship to the ant exclusion treatment using a Generalized Mixed-Effects model with Poisson distribution (except ants, that were modelled using Negative Binomial distribution). Ants were used as a response variable to confirm that the ant exclusion treatment was efficient. Additionally, to confirm that bagging of flowers did not affect abundances of ants due to restricting their access to plant nectaries we tested the effects of pollination exclusion in this model. The random structure in the models from the field study included field Position nested within Site ID, while models from the greenhouse experiment included Cage ID in the random structure. Models for boll weight had an additional term for Plant ID at the lowest nesting level and the model for boll retention in the greenhouse included Date in the random structure.

To test the effects of habitat type (crop vs. semi-natural) and ant presence on flower visitation 1. Within flowers (pollination) and 2. On the outer part of flowers (feeding on extra floral nectaries), we used Generalized Mixed-Effects model with Site ID in the random structure and Poisson distribution. The model residuals were visually inspected to validate the model assumptions and additionally, generalised linear models were checked for over dispersion. When necessary, we used Constant or Exponential variance function to model heteroscedasticity in the residuals. All analyses were conducted in R (R version 3.3.2, 2016-10-31), using packages “nlme” (Pinheiro *et al.* 2018), “lme4” (Bates *et al.* 2015), “MuMIn” (Barton 2018) and “DHARMA” (Hartig 2018). For plotting purposes we used packages “effects” (Fox 2009?) and “ggplot2” (Wickham 2009).

Results show that the dominant pests on the sentinel plants were thrips (51.7%) and jassids (19.2%). However, other more mobile pests, such as mirids could have remained undetected with the visual count method we used. In addition to ants (56%), the dominant predators on the plants were spiders (17%). The pollen beetles (*Carpophilus aterrimus*, Nitidulidae) were abundant in cotton flowers, but their ecological role is unclear. There is no strong evidence of their contribution to cotton pollination (Cunningham 2014), and since they feed on pollen they could potentially cause damage to flowers. The dominant pest abundances (sum of thrips and jassids) were substantially reduced in treatments without ant enclosure (-0.991 ± 0.244 , $p < 0.001$). Ant presence also significantly reduced spider abundances (-0.724 ± 0.225 , $p = 0.001$) and had no effect on abundances of pollen beetles. Abundances of ants on plants were not affected by the pollination treatment, but they were substantially reduced in the ant enclosure treatment (-3.4690 ± 0.489 , $p < 0.001$).

Cotton boll retention varied between 11.1% and 100%. Ant enclosure (-A) reduced boll retention by 12% when pollination was absent (-A: -12.050 ± 4.018 , $p = 0.004$, Figure 4b). In the presence of pollination, ant enclosure did not have a negative effect on boll retention (+P x -A interaction: 14.552 ± 5.661 , $p = 0.01$). Cotton boll retention decreased with increased flower beetle abundances (-2.534 ± 1.199 , $p = 0.038$). Boll weight did not change with changes in retention. Plant height was highly correlated with the total number of fruiting branches (Pearson's product-moment correlation = 0.63, $p < 0.001$) and decreased with increased retention (-0.173 ± 0.0487 , $p < 0.001$).

Results from analysing the flower visitation data showed that the most common observed taxa within 10 cm radius circle around flowers were hoverflies (42%), native bees (20%) and honeybees (11%). When considering only taxa observed in the flowers, touching reproductive flower parts, native bees were the most common visitors comprising the majority of observed visits within the semi-natural habitats (91%) and large proportion within crop habitats (40%). Honeybees were observed only in crops (near or on the outer parts of the flowers) except for one observation within a semi-natural habitat. They comprised 46% of observations on the outer parts of the flowers, and they were not observed within the flowers. The majority of the flowers opened between 7-8 am and fully closed by 5 pm. Flower visitation rate (touching reproductive flower parts) was higher in semi-natural than crop habitats (1.143 ± 0.431 , $p = 0.008$) and lower when ants were present (-1.240 ± 0.487 , $p = 0.011$). When considering visitors on the outer parts of flowers (excluding ants) the results were similar, but with stronger negative response to the presence of ants (-2.896 ± 0.731 , $p < 0.001$) and no difference depending on the habitat type.

Interactions between pests, pollination and ‘measures of impact’ on yield

Similar to analysing the data from the sentinel plant experiment (above) Linear Mixed-Effects models were used to test the interactive effects of biocontrol and pollination on cotton yield and the effects of cotton boll retention on plant compensatory mechanisms (plant height and boll weight). The first position boll retention was used as a proxy for cotton yield.

Analysis of data from the greenhouse experiment that tested interactive effects of mirid pest pressure (no mirids, low and high) and pollination on cotton yield quantity and quality. Cotton boll retention varied between 12.5% and 100%. Pollination (P) did not affect boll retention in the absence of pests (Figure 4a).

The results show that at high mirid pressure (5 mirids per plant) the yield loss is substantially lower when plants are pollinated. For example high mirid ($n=5$) treatment significantly reduced boll retention by 40% (-39.947 ± 3.030 , $p < 0.001$). However, pollination had low or no benefit to cotton yield quantity in treatments with low (1 mirid per plant) or no pest pressure.

In the greenhouse experiment, pollination did not contribute to cotton yield at low pest pressure. However, high pest abundances and in the presence of pollination, pest damage was significantly reduced by 23% (16.954 ± 4.284 , $p < 0.001$). Results show that pollination contributes to the retention of the first position boll at high mirid pressure. On the other hand, non-pollinated plants compensate for high mirid damage by increasing the number of second position bolls and the weight of first position bolls, but this is not sufficient to increase overall yield. This shows that managing pollination has the potential to be a useful approach to respond to high mirid pressure. It could provide an alternative option that reduces the frequency of insecticide application, which can flare other secondary pests such as whiteflies and cause sticky cotton.

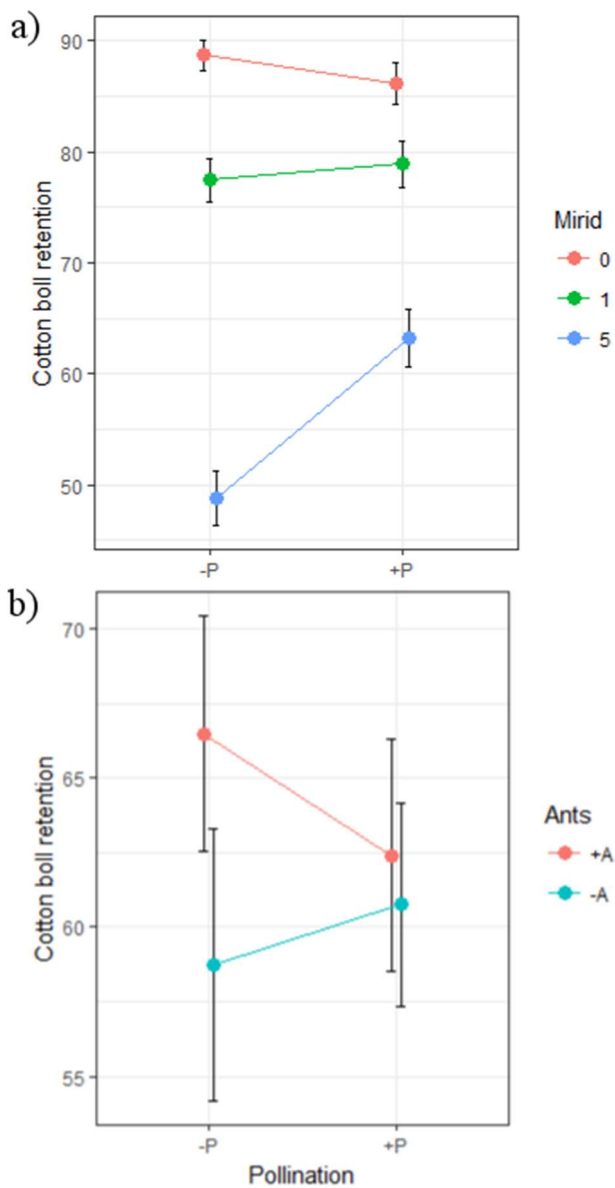


Figure 4. Interactive effect of pollination and biocontrol on cotton boll retention (mean \pm SE) from the a) greenhouse experiment and b) field study. Note that biocontrol was simulated in the greenhouse experiment by manipulating mirid pest abundances (0, 1 and 5 individuals per plant), while it was measured

in the field using ant enclosure treatment (“+A” presence and “-A” absence of ants). Pollination presence and absence is denoted as “+P” and “-P”, respectively.

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- Schellhorn, N.A., Bianchi, F.J.J.A., and Hsu, C. 2014. The movement of entomophagous arthropods in agricultural landscapes: Links to pest suppression. *Annual Review of Entomology*, 59:559-581.

Outcomes

5. Describe how the project’s outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

Please see the table (**Appendix C**) which list the planned outcomes identified in the project application, and the planned outcomes achieved to date.

6. Please describe any:-

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

Our technical advances are for the public good and new knowledge. No advance is commercially significant or has the potential for patents. However, others should be encouraged to modify and use the Infrared cameras.

b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and

None

c) required changes to the Intellectual Property register.

None Required

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

These results support the concept that native vegetation in conjunction with the judicious use of insecticides can keep insect pests lower for longer. The take home messages include:

- Twenty percent or more semi-natural habitat at a 3 km radius reduces the likelihood of mirids increasing after applying insecticide is greater where there is less native vegetation.
- At a local or field level, interactions between ecosystem services of predation and pollination showed that pollination could reduce yield loss due to insect pest damage, therefore, providing a level of “insurance”. That, when biocontrol is low (i.e. pest numbers are high), pollination can contribute to yield and reduce boll loss caused by mirids and other pests.
- Applying insecticides disrupts and reduces the benefit of adjacent semi-natural habitat for beneficial insects responsible for egg predation and pollination.

Native vegetation in cotton production systems can:

- Reduce and delay mirid colonisation and thereby reduce risk and the number of insecticide sprays in cotton fields, therefore increasing the time for beneficials to have an impact on pests.
- Increase predation in unsprayed cotton crops, adjacent semi-natural vegetation compared to fields without semi-natural vegetation.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

(b) for the future presentation and dissemination of the project outcomes.

Please see Appendix C for project outcomes

(c) for future research.

Recommendations for future research include:

Revise, develop and validate insect pest management actions such as spray thresholds, by incorporating the results from this project and delivering them in a way that facilitates ease of decision-making. For example, scouting protocols could include recording land use information such as proportion of native vegetation. Combining information such as crop location in relation to areas of semi-natural vegetation, yield and time of season could be used to establish dynamic thresholds. This could extend the opportunities for consultants and growers to maximise biocontrol and reduce insecticide applications.

Furthermore, while the results of the project show that areas of semi-natural vegetation have positive outcomes for insect pest management in cotton, it remains to be tested that managing these areas by reducing weeds, grazing and increasing vegetative diversity, could increase these benefits.

**9. A. List the publications arising from the research project and/or a publication plan.
(NB: Where possible, please provide a copy of any publication/s)**

Peer Reviewed Journal Publications:

- Vesna Gagic, Andrew Hulthen, Anna Marcora, Xiaobei Wang, Nancy Schellhorn. *Semi-natural area does not benefit biocontrol in insecticide sprayed crops*. In preparation for Proceedings of the Royal Society B in preparation (APPENDIX A)
- Vesna Gagic, Anna Marcora, Lynita Howie. *Additive and interactive effects of pollination and pest control on crop yield*. In preparation for Proceedings of the Royal Society B in preparation (APPENDIX B)
- Vesna Gagic, Bill Venables, Andrew Hulthen, Nancy Schellhorn. *Keeping insect pests lower for longer benefits of native vegetation at a landscape scale*. In Progress

Popular Press Industry Publications:

- Vesna Gagic and Cate Paull *Keeping insect pests lower for longer – benefits of native vegetation*. Draft CRDC Fact Sheet (APPENDIX D)
- Vesna Gagic Researcher Profile published CRDC Cotton Spotlight 2017
- Vesna Gagic and Nancy Schellhorn *Employing the natural farm assets to help control pests*. The Australian Cotton Grower June-July 2017 70
- Stacey Vogel and Vesna Gagic. *Pesticides and the environment* in CRDC Cotton Pest Management Guide 2017-18 pages 143 -144.

Workshop Conferences Presentations:

- Vesna Gagic presented *Balancing nature conservation goals with those of farmers* at the NRM Conservation Farmer Inc. and Condamine Alliance field day Allora QLD 27 April 2018.
- Vesna Gagic presented *Balancing nature conservation goals with those of farmers* for the CRDC Webinar 23 April 2018.
- Vesna Gagic presented *Balancing nature conservation goals with those of farmers* Denver November 7 2017 Entomology Society of America
- Vesna Gagic presented *Linking ecosystem services agrochemicals and yield* Feb 2017 Armidale RRR
- Vesna Gagic presented *'Keeping pest populations lower for longer: connecting farms and natural systems'* ICE 2016 XXV International Congress of Entomology in Orlando, Florida.
- Nancy Schellhorn, title: *'Resource exploitation: The role of movement, oviposition, and the landscape context'* presented ICE 2016 XXV International Congress of Entomology in Orlando, Florida.
- Nancy Schellhorn gave a plenary talk at the National Biological Farming Conference Convention Centre, Cairns, 28-30th October 2016. Title: *'Arthropods in agricultural landscapes: Challenging and supporting food and fibre production.'*

B. Have you developed any online resources and what is the website address?

None

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Native vegetation (NV) in agricultural landscapes provides a range of ecosystem services such as, erosion control, carbon storage, and habitat for wildlife beneficial arthropods, which contribute, to controlling insect pests. However, in Australia, evidence of impact is lacking, as well as knowledge of the mechanism for this pattern. This research builds on results from previous projects, which investigated the role of native vegetation for supporting beneficial insects and seeks to measure the impact of these landscape features on pest suppression and link the outcomes with vegetation management guidelines. The aim of the study was twofold, to understand the combined effects of semi-natural area and insecticide spraying on different predators, and to evaluate importance of these predators for biocontrol service provision in time and space in cotton production systems.

To achieve this we completed an analysis of an extensive data set provided by a commercial consultant, several field, and a glasshouse experiment. Results from these contributed to:

- Identifying patterns in pest and beneficial populations, relationships with pest thresholds, and how these relate to landscape structure and
- Determining whether pest populations stay lower for longer and have a slower rate of increase as a function of distance (e.g. near vs far) and landscape structure (e.g. amount and location of natural areas, diverse habitats, crop and non-crop at 1 km, 1.5 km and 2 km from cotton fields).

Collecting data at the landscape scale is expensive and time consuming. However, pest counts are regularly conducted by commercial consultants and agronomists and represent extensive and rarely available information. Obtaining and analysing a large data set from a commercial consultant was very valuable and allowed us to quantify the impact of semi-natural areas on biological control in relation to management activities. For example, results showed that the probability of mirids being present in cotton are likely to be less and remain so for longer in fields where there is 20% or more semi-natural habitat at a 3 km radius. Spraying reduced the probability of mirids in a crop. However, the likelihood of mirids increasing after spraying insecticide is greater where there is less native vegetation. There is also a relationship between the size of the cotton field and proportion of native vegetation and probability of mirids. For example, the time between sprays increases with increasing native vegetation in small fields. Similarly, the likelihood of mirids being present in cotton increases as field size increases but where there is also less native vegetation. Although the results support that, the presence of semi-natural areas could result in fewer sprays being required, a comprehensive cost benefit analysis was not included in this study.

Results from predation experiments showed that there are specific functional groups of predatory insects, depending on their spatio-temporal activity (diurnal vs. nocturnal and ground vs. canopy dwelling) that contribute to pest control in cotton crops. The results showed that there are not only differences in pest and beneficial numbers, and that their impact is not only related to landscape structure but is also related to management of insect pests, i.e the rate in increase of beneficial insects is reduced after the application of insecticides.

Pollination and biocontrol are ecosystem services provided by mobile arthropods and are generally affected by similar factors. Thus, they are likely to be simultaneously enhanced (by e.g. increasing semi-natural area surrounding crop fields) or reduced (e.g. insecticide application) by certain practices. Trade-offs are evident from the interactions between pollination and biocontrol effects on yield. However, results indicate that they cannot, and might not, need to be simultaneously delivered to enhance cotton yield, but that one service can

act as insurance when the other service is reduced. That is when biocontrol is low (i.e. pest numbers are high) pollination can contribute to yield and reduce boll loss caused by mirids and other pests. For cotton production systems to take full advantage of these services the findings underline the importance of considering and integrating multiple functions (pollination and biological control), within field management and surrounding landscape features simultaneously.

The results support the concept that native vegetation in conjunction with the judicious use of insecticides can keep insect pests lower for longer. Together with recommendations, they provide further evidence of: i) the importance of semi-natural vegetation in facilitating ecosystem services specifically how ii) the negative impact that insecticides have on these services and iii) the proportion and scale of semi-natural vegetation required to measure a significant benefit. Furthermore, while these results show areas of semi-natural vegetation have positive outcomes for insect pest management in cotton, it remains to be demonstrated whether managing existing native vegetation by reducing weeds, grazing and increasing vegetative diversity could increase the benefits reported here. The next step will be to incorporate these findings into insect pest management strategies including adjusting and testing pest thresholds and understanding if these results are transferable to other cotton pests. Doing this would give consultants and growers a broader range of options to maximise biocontrol and reduce insecticide applications to promote cotton production landscapes that are productive, profitable, and environmentally sustainable.