Australian Government
Cotton Research and Development Corporation

FINAL REPORT 2017

For Public Release

Part 1 - Summary Details

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

CRDC Project Number: UQ1403

Project Title: Multiple host use and gene flow in Green Vegetable Bug relative to cotton

Project Commencement Date: 01/10/2016 **Project Completion Date:** 01/04/2017

CRDC Research Program: 2 Industry

Part 2 – Contact Details

Administrator: Carol Pomfret (Finance Officer)
Organisation: The University of Queensland
Postal Address: Room 311 Gehrmann Building, St. Lucia 4072
Ph: 0733654856 **Fax:** - **E-mail:** c.pomfret@uq.edu.au

Principal Researcher: Dean Brookes (PhD Candidate)
Organisation: The University of Queensland
Postal Address: School of Biological Sciences, Goddard Building, St. Lucia 4072
Ph: 0421313635 **Fax:** - **E-mail:** dean.brookes@uqconnect.edu.au

Supervisor: Gimme Walter (Professor)
Organisation: The University of Queensland
Postal Address: School of Biological Sciences, Goddard Building, St. Lucia 4072
Ph: 0733652228 **Fax:** - **E-mail:** g.walter@uq.edu.au

Signature of Research Provider Representative: ____

Date Submitted: 30.06.17_____

Part 3 – Final Report

(The points below are to be used as a guideline when completing your final report.)

Background

1. Outline the background to the project.

The Green Vegetable Bug (*Nezara viridula*) is an invasive insect that has become a more significant pest of Australian cotton with the widespread use of *Bt* cotton, as it is unaffected by the toxin. Although this bug is found more regularly in cotton, it does not reach highly damaging numbers in every season. It is not clear why *N. viridula* is an inconsistent pest and so predicting pest pressure from *N. viridula* is difficult. This project aimed to address questions that concern the movement, host use, and species status of Australian *N. viridula*, with the aim of understanding when and from where it invades cotton, and thus predicting when it is likely to reach damaging densities.

A number of scientists have suggested that *N. viridula* might represent a complex of species, but this issue has not been resolved. If Australian *N. viridula* comprises more than one species then each will need to be studied independently. Observations about numbers of *N. viridula* early in a season, on non-cotton host plants, may not translate directly to high numbers of this insect in cotton if multiple species are present. Further, it is not certain how Australian *N. viridula* relate to global populations of this insect. Not all global research on this insect may be relevant if it was conducted on insects with a different genetic background. Prior to this project Australian *N. viridula* are thought to be European in origin, but proximity to Southeast Asia might mean the Asian lineage of this insect is present too.

The biology of *N. viridula* is not clearly understood in a number of areas that relate to the host species use and annual abundance of this insect. Little is known about the movement of *N. viridula* bugs across the Australian cotton industry, or the genetic relationships between bugs from different host plants and different regions. The sequence of host plants that *N. viridula* uses throughout a season has been investigated, but whether the observed populations represent a single species across these host plants has not been tested. The distance that individual *N. viridula* disperse within and between seasons also has implications for understanding its local abundance and when it might appear in cotton in large numbers. If *N. viridula* individuals travel the large distances between growing regions, then host plants far from cotton growing regions may impact the abundance of this pest in cotton. If, however, *N. viridula* bugs do not travel long distances then observed host use early in a season may translate directly to the abundance of this insect in cotton. Resolution of these points would provide insights into the variable pest pressure observed in this insect.

The PhD thesis that is associated with this project is supplied alongside this final report. The version that is supplied is currently in revision to fulfil the requirements of the external examination. The chapters that deal with *N. viridula* focus on its population genetics as that relates to host plant use, movement of the bugs locally and across regions, all in relation to cotton. The thesis is broader than this, however, but is submitted in its entirety, as the population genetics on the other species included do help in understanding host plant use relative to insect movement generally. This report complements the thesis by focusing on the aspects of the results that are most relevant to the Australian cotton industry, including additional information not included in the thesis.

Objectives

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

1. ***Establish sampling procedures***

- **1.1**: Engage with persons previously involved in *N. viridula* research

- **1.2**: Literature review of hosts and compile what data exists for these

This milestone aimed to develop a suitable sampling program for the project. Both milestones **1.1** and **1.2** were completed and a list of regions and host plants where *N. viridula* was likely to be found was generated. Contacts were made in each of the major cotton growing areas so that the seasonal abundance of *N. viridula* could be monitored independently in each region. Sampling was conducted at different times during each season and when insects were reported to be in high and low abundance.

2. *Sampling N. viridula*

- **2.1**: Exploratory sampling focusing on a few localities and hosts
- **2.2**: Intensive sampling focused at a few established localities
- **2.3**: Opportunistic sampling outside of focused regions

Sampling of *N. viridula* was highly successful despite pest pressure from this insect being low for the duration of the project. More than 800 adult insects were collected from more than 30 different host plant species across Australia. These bugs were collected from all over eastern Australia but most were collected from the cotton growing regions of Queensland and New South Wales, from Emerald in Queensland to Griffith in New South Wales (milestone **2.2**). The bugs were also obtained from Kununurra in Western Australia, Darwin in the Northern Territory, as well as Darwin and Lockhart River in northern Queensland (milestone **2.1**). Some bugs were supplied by other organisations, such as staff of the North Australian Quarantine Strategy (NAQS), when travel was not feasible to a location. No samples were found between growing regions (milestone **2.3**) despite suitable host plants being searched regularly while travelling. These samples were extensive enough that each of the objectives of this project could be addressed. Differences in host use across representative growing regions could not assessed in any detail, however, given the low number of insects overall.

3. *Review the literature for N. viridula*

- **3.1**: Compiled database of all *N. viridula* literature

A review of the literature of *N. viridula* was conducted with a focus on the host plant species that this bug uses. Any reported differences in regional host suitability or biology were noted. The primary purpose of this review was to develop a comprehensive understanding of host use to aid sampling efforts, and to create a reference list of publications to complete the various milestones of the CRDC project and PhD program. The review was successful in both of these cases.

4. *Conduct molecular research*

- **4.1**: Apply the molecular tools used in previous studies
- **4.2**: Develop the high resolution molecular tools for this study
- **4.3**: Apply and interpret the high resolution molecular tools
- **4.4**: Conduct temporal molecular analysis

The molecular tools used in previous studies were applied successfully and Australian *N. viridula* were compared with those from global populations (milestone **4.1**). These results were used to guide sampling (milestone **2**) and to decide which individuals should be genotyped with microsatellites (milestone **4.3**). Microsatellite markers (milestone **4.2**) were also developed to complement the use of the molecular tools that had been used previously (milestone **4.1**). About 650 individuals were genotyped with these microsatellite markers which allowed for successful completion of milestones **4.3** and **4.4**.

5. *Mating and host use trials*

- **5.1**: Based on molecular field data, assess differential host use
- **5.2**: Based on molecular field data, assess mating signals across different *N. viridula* populations

Analysis of the molecular results (milestone **4.3**) indicated that mating and host use trials would not be appropriate. This was because the discordance between the nuclear DNA and mitochondrial DNA results resolved milestone **5.2** and there was no clear path for testing milestone **5.1** (this will be discussed with more depth in the results section). A new milestone was therefore formulated (**4.4**) to address aspects of host use more relevant to the cotton industry, and which could be addressed within the scope of this project. Milestone **4.4** replaced milestone **5.1**.

6. *Publish research*

- **6.1**: Publish to scientific journals
- **6.2**: Posters and presentations at industry meetings
- **6.3**: Make molecular tools available for future research

A publication plan (milestone **6.1**) is detailed in section 9 below. The research outputs from this project were presented at two conferences (milestone **6.2**), the Australian Cotton Research Conference (Toowoomba, Queensland, 2015) and World Cotton Research Conference 6 (Goiânia - Goiás, Brazil, 2016). The molecular tools generated by this research will be made available, for completion of milestone **6.3**), as part of a publication that is in development. These molecular tools will also be available from the submitted PhD thesis once it has been accepted, as the University of Queensland will make the thesis openly available.

7. *Submit thesis*

- **7.1**: Submission of thesis

The PhD thesis associated with this project was submitted on May 2nd 2017. Examiner comments for the thesis have been received and were positive. The thesis is currently being revised according to the examiner's comments and the final version will be submitted within one month. A final version of the thesis will be submitted to the CRDC once the thesis has been revised.

Methods

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

Samples of *N. viridula* were collected from northern and eastern Australia with a focus on cotton growing regions. The locations that were sampled are illustrated in Figure 1 (black dots). These samples covered the major weed and crop host plants that *N. viridula* uses in Australia, and samples were obtained from cotton in each major region (including Kununurra and Townsville). For the duration of this study, most regions at most times during the year had very few *N. viridula*. Kununurra was an exception, with more *N. viridula* found here than in any other single sampling trip. The most reliable method of collecting *N. viridula* when they were in low number was to wait until after cotton was defoliated and then sample them from pigeon pea refuges. This strategy was also used for other crops where *N. viridula* was found to be in low density, with nearby weeds being sampled once the crop had become unsuitable for *N. viridula*.

Bugs from each of the sampled regions and from different host plants were analysed using molecular tools. These molecular analyses used three different gene regions (one mitochondrial gene and two nuclear genes) and 12 different microsatellite markers. Each of these markers provides different evidence about the genetic relationships of *N. viridula* populations. The three gene regions evolve slowly and allow for the evolutionary history of the insects to be investigated. The mitochondrial genes allow us to identify the lineage of the bugs, as

the mitochondrial genes are only inherited through the females. However, previous molecular research carried out on *N. viridula* only used these mitochondrial gene regions which can lead to biased results. Further, mitochondrial DNA alone provides only low resolution information about movement between populations. The approach taken in this project therefore also includes nuclear gene regions and microsatellites to allow for a more comprehensive analysis of the genetic relationship between *N. viridula* populations. The nuclear genes have a contribution from both the male and female during mating, so it is possible to test if there are different populations within a lineage. For instance, if a male with a European lineage mated with a female of Asian lineage the mitochondrial genes would identify the offspring as being of Asian lineage, but the nuclear genes could indicate that the offspring is related to both lineages. Microsatellite markers use small DNA repeats that change quickly, and by using several different microsatellite markers it is possible to compare individuals from different hosts or regions with a higher resolution. Similarities and differences in the pattern of these microsatellites can help to understand the amount of movement between populations. Hence, the inclusion of microsatellite markers, used on insects collected in different years, also allows for an assessment of whether *N. viridula* populations are relatively sedentary or whether they travel large distances between growing seasons.

The molecular results are presented in three sections. The population genetic structure of Australian insects was assessed in depth, to determine whether *N. viridula* differs across growing regions and across host plants. A temporal analysis of gene flow was conducted to determine how much movement occurs between *N. viridula* populations in different regions between years. The relationship between Australian *N. viridula* and global populations of this insect was assessed using phylogenetic analyses. The molecular results are covered briefly in the following results section, and with more depth in thesis itself.

Results

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

Population genetics of Australian N. viridula

Figure 1 shows the genetic relationship between populations of *N. viridula* from different Australian localities using different types of molecular evidence. The circles and horizontal bars (orange and blue) in the figures represent the mitochondrial DNA (maternally inherited) and this relates to the Asian and European lineages of the insect. Each bar inside the boxes (light grey and dark grey) represents the microsatellite data which allows for an assessment of mating between regions, across host plants, and between the lineages. The mitochondrial DNA indicates that both the Asian and European lineages of *N. viridula* (recognised earlier by overseas researchers) are present in Australia. The microsatellite data indicates that two *N. viridula* gene pools are present in Australia, one in eastern Australia (mostly light grey bars) and one in northern Australia (mostly dark grey bars). However, it is clear that the distribution of these northern and eastern gene pools do not match completely with that of the Asian and European lineages (the mitochondrial DNA). Gene flow has occurred between bugs of these different lineages in northern Queensland but it appears to have had only a limited impact on the nuclear genome. This can be seen clearly in Figure 2, which shows an enlarged version of the area of overlap between the mitochondrial DNA of both lineages, and where few dark grey bars (representative of the Asian lineage) are present. Eastern Australian *N. viridula* are a single population, irrespective of the mitochondrial DNA they possess.

Figure 3 shows an analysis that uses only microsatellite data from individuals collected over two years and from each of the host plants from which *N. viridula* was sampled during the project. The overlapping circles in the dot plot in the left of the figure indicate that there is gene flow between all regions, as does the relative balance of each of the bars within the boxes. As in Figure 1, these bars represent the genetic identity of an individual insect. Although there is no genetic structure across *N. viridula* collected from different host plants, there is a relationship with geographic distance. Figure 4 correlates the genetic and geographic distances across these same individuals and a significant relationship is found for insects collected in both years. This significant relationship indicates that the movement of *N. viridula* between growing regions is not frequent and that their populations

may remain relatively localised. This means that local host use will be the most relevant for predicting the late season abundance of *N. viridula* in cotton.

Figure 5 shows the relationship between Australian *N. viridula* and global populations of this insect based on all mitochondrial data that was obtained from this project and from genetic databases (data created by other authors). The results of this analysis indicate clearly that Australian *N. viridula* are related to both the Asian and European lineages of this insect.

More detailed results associated with these figures can be found in the PhD thesis supplied alongside this report.

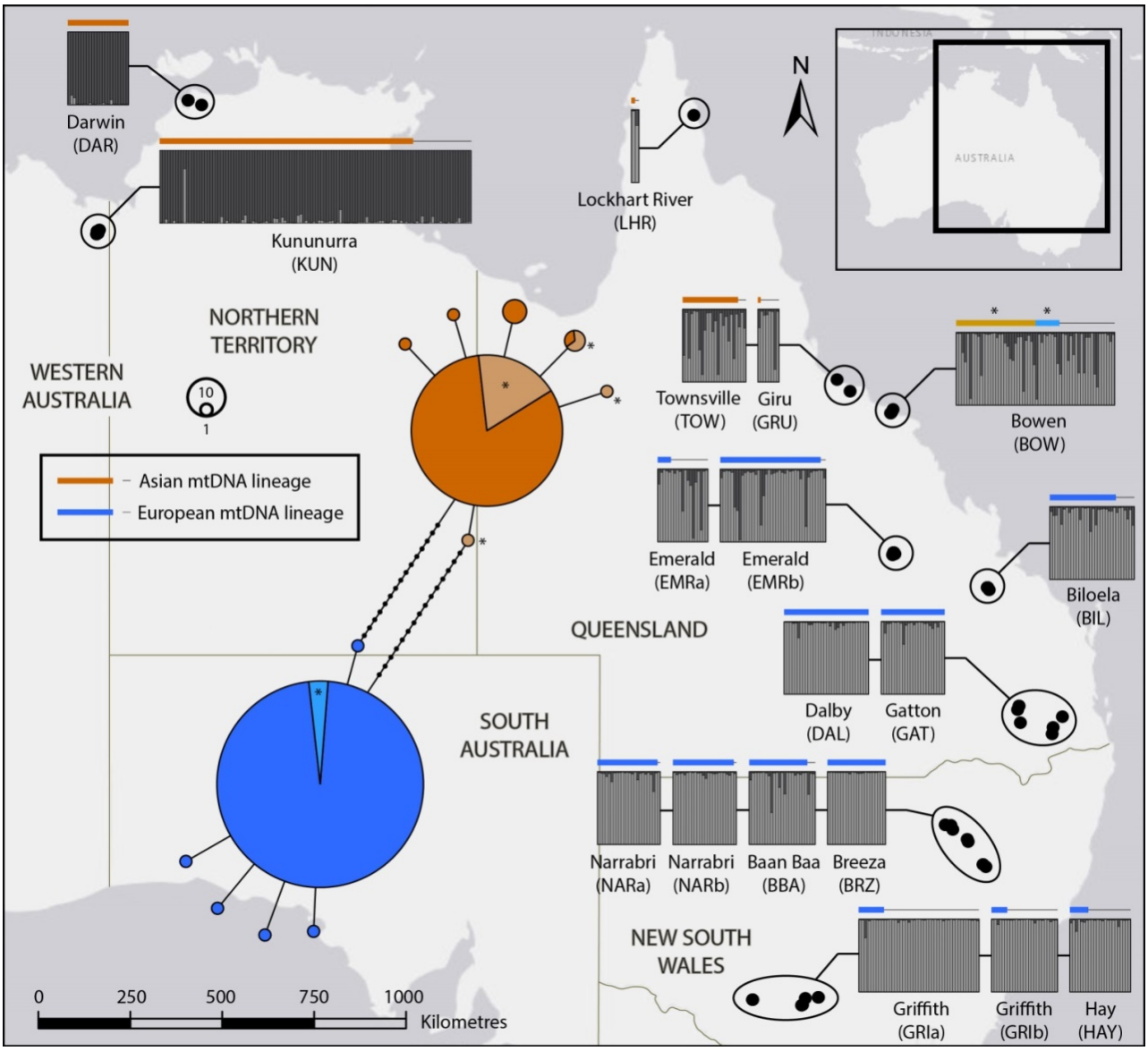

Figure 1. A map of *Nezara viridula* sampling sites with sampling locations represented using black dots. The circles define nearby sampling sites (black dots) into regions. Superimposed on the map are two sets of molecular data. The coloured circles and horizontal bars above the boxes relate to the mitochondrial lineages of *N. viridula*, the Asian and the European. For both of the analyses represented, the Asian mitochondrial lineage is shown in orange and the European in blue. Bowen is the only location in which bugs with both mitochondrial haplotypes were found together. Where haplotypes of both lineages were found in the same location, they are shown with an asterisk and a lighter shade of either orange or blue. The boxes display the microsatellite data.

Each bar within the boxes corresponds to a single individual and the colour, either light or dark grey, represents the probability that each individual is assigned to one of two populations.

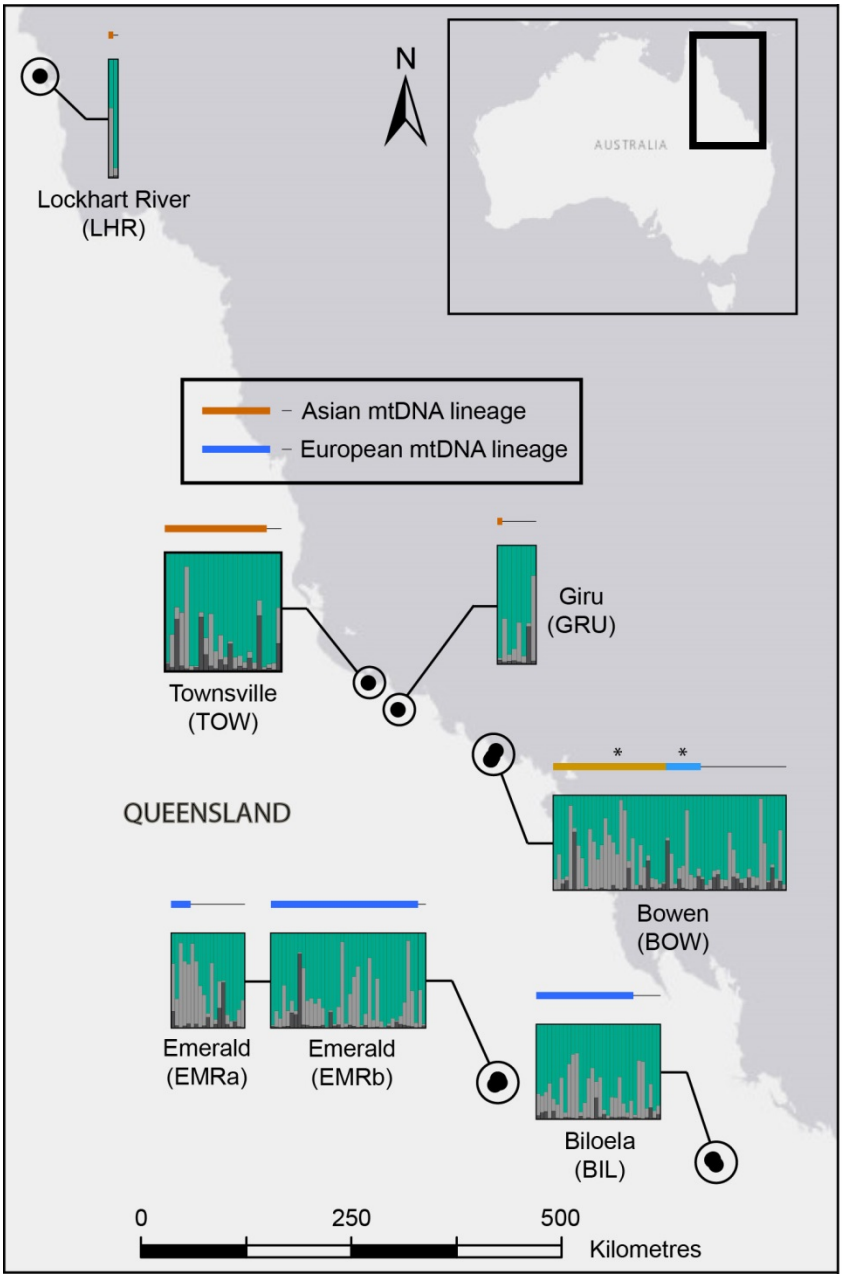

Figure 2. Map of north-east Australia showing the geographical area of overlap in the distribution of the two *Nezara viridula* mitochondrial lineages. Microsatellite data are displayed in the boxes. Dark grey bars represent assignment to the same population as most individuals from north-west Australia. Horizontal bars over the boxes indicate the mitochondrial haplotype of the corresponding individual. The Asian mitochondrial lineage is shown in orange and the European one in blue. Bowen is the only location in which bugs from both mitochondrial haplotypes were found together, and so these haplotypes are represented with an asterisk and a lighter shade of either orange or blue.

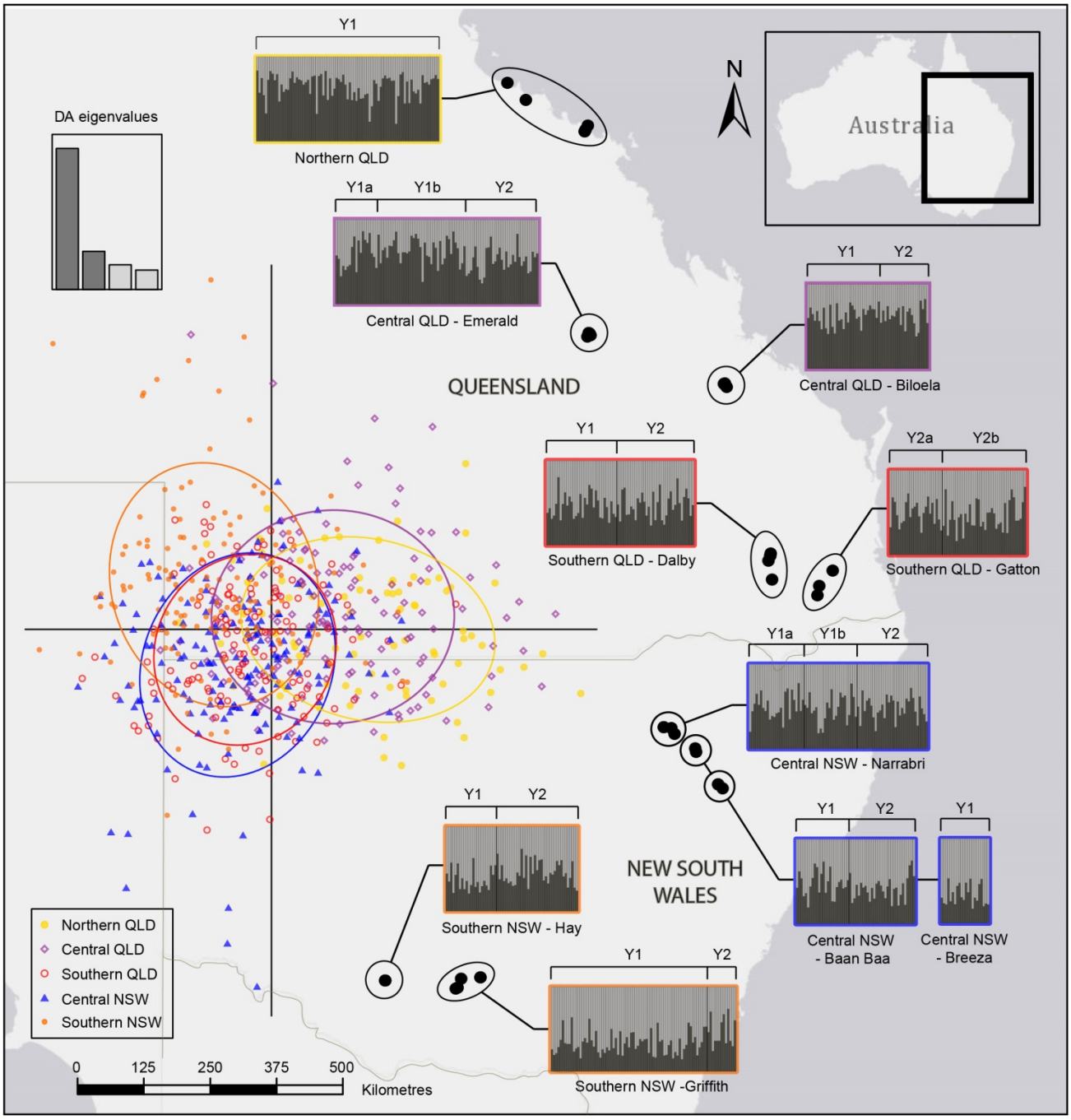

Figure 3. A map of genotyped *Nezara viridula* individuals grouped according to region and the season they were sampled. Populations represented by the STRUCTURE analysis boxes are labelled Y1 if collected during the 2014-2015 season, and Y2 if collected during the 2015-2016 season. A further label of 'a' or 'b' designates samples taken at different times within a season. Site names represent broad localities within regions. The scatter plot shows a Discriminatory Analysis of Principle Components (DAPC) with populations grouped according to region regardless of sampling time. The boxes represent the microsatellite data.

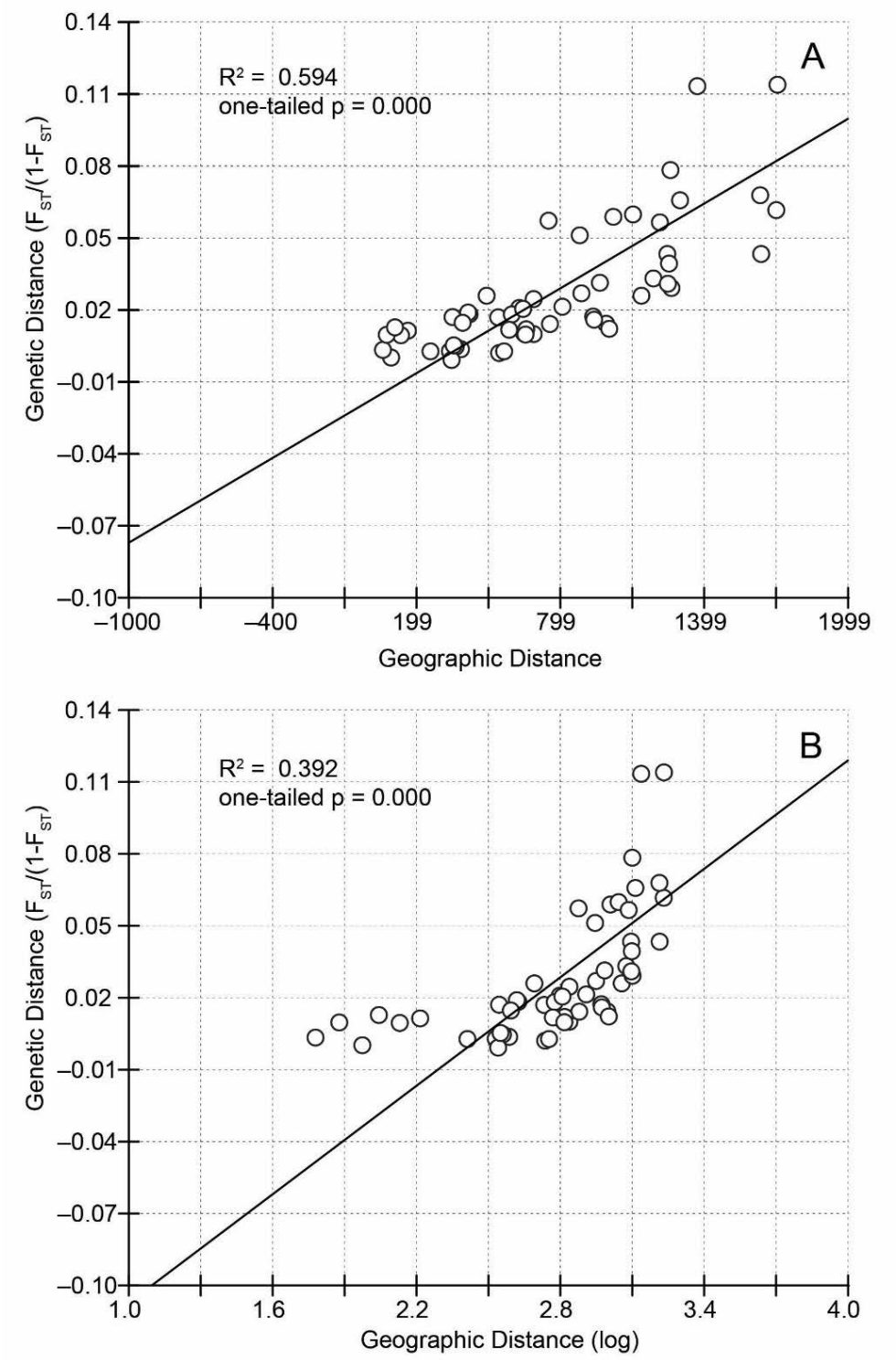

Figure 4. The geographic and genetic distance separating *N. viridula* populations are shown to be significantly correlated for eastern Australian populations. Individuals are grouped together into populations according to sample location and irrespective of time sampled. Geographic distance is used in Figure 4A and log transformed geographic distance is used in Figure 4B.

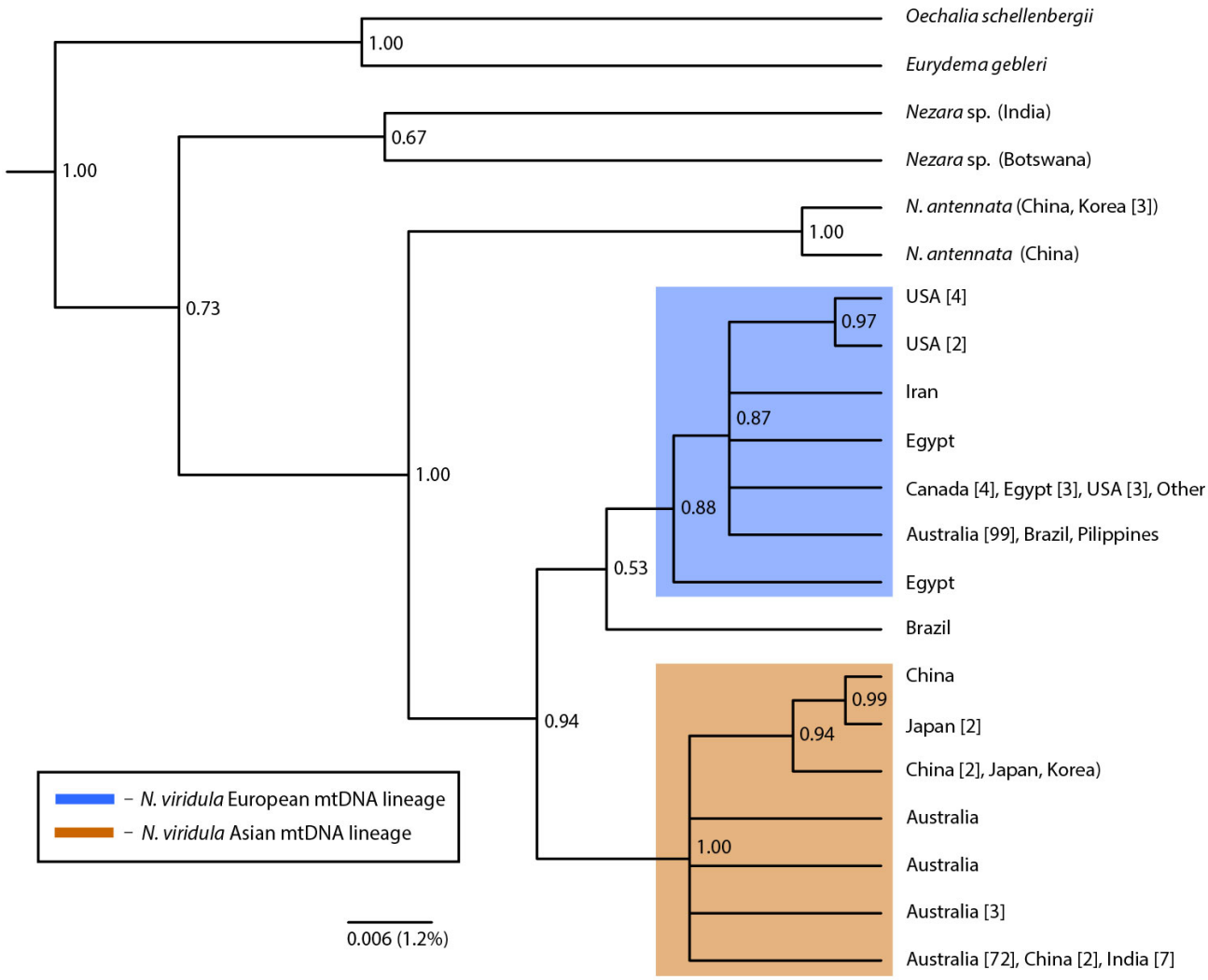

Figure 5. Tree representing the phylogeny of *Nezara viridula*, based on mitochondrial genetic data from all available individuals of both *N. viridula*, not only those generated during this project, and *N. antennata* (a *Nezara* species closely related to *N. viridula*). Only unique sequences were used and if the number of samples that shared a sequence is greater than one then the number is shown in square brackets. Two closely related insects, *Eurydema gebleri* and *Oechalia schellenbergii* are included to determine the root of the *Nezara* phylogeny. The label 'Other' denotes a haplotype of *N. viridula* common to individuals sampled from Greece, Guadeloupe, Galapagos, California, Japan, Italy and Brazil, from another data set (Kavar *et al.* 2006). Posterior probabilities are shown as node labels.

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.

Understand factors likely to result in local 'outbreaks'

The population genetics analysis of *N. viridula* from eastern Australia shows a pattern of isolation by distance (Figure 4) but no differentiation across the different host plant species from which *N. viridula* were sampled. This means that these insects become more genetically distinct from one another the further apart they are geographically and that there are no host-associated cryptic species in eastern Australian cotton growing areas. This pattern was consistent across the two years that *N. viridula* was sampled and this indicates that *N. viridula* are not regularly moving the large distances between cotton growing regions. Populations of this insect are therefore relatively localized. This is in contrast to other cotton pests, such as the green mirid, which appear to move large distances regularly and do not exhibit a pattern of isolation by distance.

If *N. viridula* bugs do not travel long distances regularly, then the host plants within cotton growing regions on which this bug feeds will be most important for predicting when pest pressure will be high. The weed and crop host plants that are most important in *N. viridula* reaching high numbers may differ for each cotton growing region. Although the molecular results indicate that movement between major growing regions is limited, it is not clear from the data exactly how far they do move. This point is relevant because these results apply to populations of *N. viridula* that were sampled during seasons when pest pressure was low. A similar pattern may not be evident during years when *N. viridula* has a high abundance.

Understand connectivity of N. viridula populations

The genetic results indicate that there are two different populations of *N. viridula* in Australia, and there is little movement between them. Specifically, there is an eastern population and a northern population. The *N. viridula* found in eastern Australia, from northern Queensland to southern New South Wales, are well connected genetically (Figure 3), even though there is some differentiation between them (Figure 4). The northern populations of *N. viridula*, those found in Darwin and Kununurra, are genetically distinct from the populations from eastern Australia. No gene flow appears to occur between the northern and eastern populations, although it appears to have occurred rarely at some point in the past as indicated by the presence of mitochondrial DNA from both lineages in northern Queensland *N. viridula* populations. These two populations, northern and eastern, are primarily associated with one of the two global lineages of *N. viridula*, but with some contribution of the Asian lineage to the eastern population (Figure 1). If cotton is grown regularly in Kununurra in the future then cotton entomologists should be aware that these northern populations may have differences in their biology, and this may well include their host species use, compared with insects found in eastern Australia.

Understand relevance of international N. viridula research

This project has revealed that both Asian and European lineages of *N. viridula* occur in Australia and that mating can occur between them. This can be seen in insects from the Bowen region (Figures 1 and 2), which possess mitochondrial DNA from both the Asian and European lineages of *N. viridula*, but which do not differ across their nuclear genome (they are all from the eastern Australian gene pool). The mitochondrial haplotypes of each lineage have not spread from this narrow area of contact. The lack of movement of the mitochondrial haplotypes is odd and we would expect to see the mitochondrial haplotypes found in all populations, rather than having a mostly separate distribution (Figures 1 and 2). When considering the relevance of international research, the most important consequence of this result is that we can only estimate the relationship between Australian and global populations using previously published molecular research. Only mitochondrial DNA was assessed for previous molecular research on *N. viridula*, and so direct comparisons cannot be made without assessing the nuclear genome of global *N. viridula* populations.

The results of this project provide a platform for the design of an international project on *N. viridula* species status, as we have illustrated how to overcome the difficulties involved with investigating the population genetics of *N. viridula*. Resolution of the relationship between global populations of *N. viridula* will require a comparison of the nuclear genome of global populations in addition to the mitochondrial genes that have already been assessed. Specifically, comprehensive sampling across continents and host plants, with simultaneous assessment of mitochondrial and nuclear markers, is necessary. These methods will be made available to other researchers through scientific publications.

6. Please describe any:-

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

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

c) required changes to the Intellectual Property register.

The technical developments from this study are primer combinations that allow for the amplification of the regions of *N. viridula* DNA that this project investigated. These primer combinations are two primer pairs for gene regions used in phylogenetic analysis, and 12 primer pairs for microsatellite analysis. Publication of these primers will be necessary for molecular research to be conducted on *N. viridula* populations outside of Australia, and they will be accessible through the publically available thesis and the publications that result from this project.

No changes to the intellectual property register are 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?

The *N. viridula* associated with cotton in eastern Australia are confirmed to be a single interbreeding population that derives from the European lineage of this insect, and was presumably introduced (accidentally) into Australia sometime after 1788. The Asian lineage of *N. viridula,* found in northern Australia, arrived on the continent at a different and uncertain time. Eastern Australian *N. viridula* can be confidently considered to comprise a single population, regardless of host plant. The results of the mitochondrial and nuclear DNA analyses indicate that the two global lineages of this insect can mate. Molecular markers of both types (mitochondrial and nuclear) must be assessed when comparing the genetic relationship between *N. viridula* populations.

The population genetic structure of eastern Australian *N. viridula* populations indicates that they do not disperse large distances over at least one year. The *N. viridula* found within cotton therefore originate from host plants within growing regions, rather than moving large distances into cotton. Movement and host use in *N. viridula* will therefore need to be investigated independently for each of the cotton growing regions, or at least where weed and crop hosts, as well as climatic conditions, differ significantly.

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.

(c) for future research.

Future research should focus on understanding the host use of *N. viridula* within independent cotton growing regions during years when this bug is at high densities.

Presentation and dissemination of the project's outcomes will be made through the publications listed below.

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)

Two publications will come from this project, and they are targeted at good journals, Molecular Ecology and Evolutionary Applications. These publications presently exist as thesis chapters but they have been written to publication standard. Both chapters are being developed into full manuscripts reformatted and edited for submission. They are entitled:

- Deep divergence within the herbivorous generalist bug *Nezara viridula* – global biogeography, invasion, host use, and secondary contact in Australia.
- Spatio-temporal population genetics of *Nezara viridula* in eastern Australia relative to cotton cultivation.

Two presentations were made using data from this project.

- Brookes, D.R., Hereward, J.P., Wilson, L.J. and Walter, G.H., 2015. Gene flow and host use in the Green Vegetable Bug, *Nezara viridula.* Paper presented at the *Australian Cotton Research Conference,* Toowoomba, Queensland.
- Brookes, D.R., Hereward, J.P., Wilson, L.J. and Walter, G.H., 2016. Gene flow and host use, relative to cotton, in *Nezara viridula* (the Green Vegetable Bug or Southern Green Stink Bug). Paper presented at the *World Cotton Research Conference 6*, Goiânia - Goiás, Brazil.

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

No online resources have been developed.

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.

The Green Vegetable Bug, *Nezara viridula*, has recently become a more significant pest of Australian cotton but it is not a problem every season. This project addressed aspects of the multiple host use and movement of *N. viridula* as they relate to cotton, as well as the genetic relationship between Australian *N. viridula* and the global populations of this pest.

Samples of *N. viridula* were collected from northern and eastern Australia and from a variety of weed and crop hosts, with an emphasis on cotton. The abundance of *N. viridula* was low for the duration of the project but about 800 adult insects were collected overall. Comprehensive phylogenetic and population genetics methods were used to address each of the questions. The methods developed during this study will be made available to other researchers through scientific publications.

The Australia populations of *N. viridula* come from two different evolutionary lineages, one European and one Asian. The former is distributed across eastern Australia and the other across northern Australia. At some point in the past some individuals of the Asian lineage have mated with individuals of the European lineage in northern Queensland but these events appear to have occurred only rarely. Across the different host plant species there are no genetic differences between *N. viridula* that would indicate separate host-specific gene pools.

The *N. viridula* in eastern Australia are more genetically distinct from one another the greater the geographic distance that separates the sampling localities from which they were collected. This slight genetic differentiation over geographic distance is present in insects collected across two years and this indicates that *N. viridula* populations remain relatively localised in the short term. This result indicates that the host plants available to *N. viridula* within each cotton growing region will be the most relevant for predicting the abundance of this insect in cotton. Pest pressure from *N. viridula* was low for the duration of the project and so this pattern may be different during seasons when *N. viridula* is present in high numbers. In years of high abundance host plants might be found between growing regions, and allow for the recruitment of *N. viridula* over a wider area.

Future research that addresses the host use of *N. viridula* should investigate populations from each cotton growing region independently, as local conditions, such as the crop and weed host plants used by *N. viridula* each season before cotton becomes attractive, will be the most relevant to late season numbers of this insect in cotton. A previous CRDC funded project has already addressed *N. viridula* host use in central New South Wales. If cotton is grown regularly in northern Australia then it would be prudent to treat the *N. viridula* population there as a separate entity, as there may be significant differences in their biology which could affect their host use and abundance in cotton. Any differences would therefore influence the development of management strategies.