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Part 1 - Summary Details

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

CRDC Project Number: CSP1303

Project Title: Identification of beneficials attacking silverleaf whitefly and green vegetable bug

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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.

Integrated pest management in cotton emphasises conservation of beneficial species (mainly insects and spiders) to suppress pest populations and reduce the need to apply insecticides. Many beneficials encountered in cotton are 'generalists' allowing them to consume a range of prey, subsisting on non-

pest prey when pests are scarce. These generalists are very important in managing pest species especially when the pests are at low densities. Disruption of these generalist beneficial populations, and the resulting reduction in mortality on pest species, can lead to outbreaks of secondary pests. This is especially a risk for fast life cycle species such as mites and aphids but also for pests such as SLW and *Helicoverpa* and probably also mirids and green vegetable bug.

Given the significance of generalist predators in biological control (75% of studies quantifying the impact of generalist predators report a positive suppression on pest species), it is surprising that we have a poor understanding of which species have the greatest effect on pest abundance in Australian cotton. This is further compounded because a number of pest species are also generalist predators, such as 'phytophagous' thrips species, green mirids and apple dimpling bugs. The lack of information reflects the difficulty in measuring predation by observation and impracticality of remote methods (eg video). However, knowledge of the key beneficial species would be valuable so that they can be targeted for conservation in crops. Further, it can also focus research to understand how these predators survive within the agroecosystem which may lead to strategies to enhance their abundance (e.g. conservation of native vegetation remnants on farm).

This project used 'molecular diagnostics' to identify the key predators of silverleaf whitefly (SLW; *B. tabaci* MEAM1, commonly known locally as B-biotype) and green vegetable bug (GVB, *Nezara viridula*) both important pests in current Bollgard II dominated cotton systems. This involved collaboration with Professor James Harwood, University of Kentucky, who has pioneered this technique and has extensive experience with its application in agricultural systems.

Objectives

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

This project aims to identify the key predators of SLW and GVB bug in cotton systems. This information will allow more targeted sampling for these predatory species and the development of guidelines for their conservation within the crop. Further, it provides the basis from which to develop further studies of beneficials focusing on species that likely have greatest impact. Potential predator species can be collected from the field and analysed for the presence of DNA from the pest species, SLW or GVB, indicating that they have eaten these specific prey.

Methods, Results and Discussion

1. Development of target-specific primers.

The methodology relies on the availability or development of short synthetic oligonucleotide primers specific to DNA that is only found in the target pest organism (e.g. SLW or GVB) that can be used in a Polymerase Chain Reaction (PCR) assay to amplify a small region of the targets' DNA which can then be visualised as a specific sized band by agarose gel electrophoresis. The primers are generally chosen to the mitochondrial DNA of the pest rather than its nuclear DNA as there are many hundreds to thousands of copies of the mitochondrion in each cell of an organism so are more easily detected by PCR. If these primers are available and their specificity is confirmed, that is, they do not cross react with other species, then they can be used to detect the DNA of the target species in the gut/body of potential predatory species. This means that those predatory species actually consuming the target species can be identified.

Primers for GVB were already available from Prof Harwood's group and were derived from specimens in the USA, where this species is also a key pest. Primers for SLW were available from the published literature. However, in both cases it was necessary as a first step to confirm that these primers worked with GVB or SLW from Australia whose mitochondrial genomes had not previously been sequenced. Consequently, the first phase of this project was the collection of specimens of silverleaf whitefly (SLW) and green vegetable bug (GVB) from Australia to validate that the primers available worked correctly.

Collections of SLW and GVB were made through August of 2013; for SLW these were made from the Gwydir and Lower Namoi, and for GVB from the Gwydir, Upper Namoi and Lower Namoi. In addition, one collection of GVB and one collection of SLW were also made from our cultured colonies at ACRI so that we could include samples from all life stages in the validation process. At the sample sites there was no cotton growing so insects were taken from a wide variety of herbs and trees, naturally occurring endemics, introduced weeds and garden grown species. Altogether eighty seven GVB and fifty nine SLW were collected across the sample sites and from the colonies. GVB were taken in sweep nets or collected by hand from beneath bark. Whitefly were collected using a hand held aspirator. Each individual GVB or SLW was stored separately in alcohol, to avoid cross-contamination. Individuals of both species were catalogued, transferred immediately to 95% ethanol and kept in a cooled esky until frozen at -18 C°.

For both species a selection of individuals was sent to Prof Harwood's laboratory. Each selection comprised 20 of each species from a mix of life-stages, locations and hosts. The samples sent for evaluation included GVB collected from: green beans and peanuts, River Red Gum, Mediterranean Olive, Bay Laurel, Pinus sp and mixed herbaceous weeds. For SLW hosts were Sipima 280 cotton (glasshouse), Milkthistle, and Turnipweed. Samples were chosen to be fairly evenly distributed between the collection regions and colonies.

The GVB primers worked well but the published primers for SLW were not reliable and new primers were developed by Prof Harwood. This was challenging due to the presence of closely related *B. tabaci* Aus1 in Australia. A primer was successfully developed for SLW. The primer sequences were provided to Dr Danny Llewellyn and samples of SLW and GVB sent to him so he and his team (Ms Jackie Oliver) could ensure that the primers were reliable and to iron out any technique difficulties of transferring the methodology to Australia. This went well and the primers reliably amplified DNA of the correct species.

The next step was to confirm that the primers were sensitive enough to detected DNA of SLW or GVB in the guts of predators. This is an important step as the amounts of pest DNA in the gut of predators may be much lower than when testing the actual target species directly. To test if the primers were effective at detecting either SLW of GVB DNA in the gut of predators Tanya Smith fed a range of potential predatory species with either SLW or GVB, preserved them in alcohol and then sent them to Dr Llewellyn and his Team (Ms Jackie Oliver) to test. Jackie found that the primers worked but were not very sensitive which means there is a higher chance of identifying a potential predator as not having fed on SLW or GVB when in fact it may have. Dr Llewellyn and Ms Oliver then modified the primer sequences and assay conditions slightly for both species which improved sensitivity considerably.

2. Confirmation of the specificity of primers

This component of the study aimed to test if the primer developed were specific for the target organism (e.g. SLW or GVB). If the primers cross-react with other species this can cause problems because (i) the cross-reacting species may wrongly be identified as a predator of either SLW or GVB or (ii) these species may be consumed by other predators which will then show up positive for GVB or SLW DNA even though they may not have ever consumed these species themselves.

The first phase of developing a pest molecular diagnostic assay is normally to confirm specificity compared with closely related species, as these species are the most likely sources of cross reaction (they will have similar mitochondrial gene sequences). The second phase is to test much more widely against a wide range of species likely to be found in a cotton field. These studies, described below, were initially done in Prof Harwood's Lab for the initial primers developed by him and confirmed their specificity to the respective targets. This analysis was repeated in Dr Llewellyn's laboratory to ensure similar results were obtained in a different laboratory and that there were very few cross reactions. However, the subsequent finding that the initial primers were not very sensitive and that sequence

and assay modifications were required to make them more sensitive (see above) meant that this work had to be repeated with the newly designed assay as the changes could have altered the specificity of the primers. This is a technical challenge with such assays where you are trying to ensure both specificity and sensitivity at the same time, and when you do not really know which predator species are likely to be most important so optimising the primers and assay conditions becomes trial and error. Here, for brevity, we only report the results of the final series of testing with the modified primers and assay conditions.

Testing against related species

The related species for SLW are the Eastern Australian Native (*B. tabaci* AUS1) and the greenhouse whitefly (*Trialeurodes vaporariorum*). Completed testing showed the primers for SLW did not display any cross- reactions against these species, or indeed against GVB or any of its' related species.

The related species for GVB included horehound bugs (*Agonoscelis rutila*), apple dimpling bug (*Campylomma leibknechti*), glossy shield bug (*Cermatulus nasalis*), green mirid (*Creontiades dilutus*), green potato bug (*Cuspicona simplex*), brown shield bug (*Dictyotis caenosus*), spined predatory shield bug (*Oechalia schellenbergii*), red-banded shield bug (*Piezodorus hybneri*), green stink bug (*Plautia affinis*), brown shield bug 2 (*Poecilometis* spp.), cotton harlequin bug (*Tectocoris diopthalmus*), pale cotton stainer (*Dysdercus sidae*).

The primer for GVB did show some cross-reaction with the red-banded shield bug (Table 1). However, sequencing of PCR reaction products indicated that there were internal sequence differences that allow it to be distinguished from that of GVB.

Table 1. Species tested for cross reaction to silver leaf whitefly and green vegetable bug primers that are closely related to silver leaf whitefly or green vegetable bug

Species	Common Name	Times Tested by Llewellyn for Primer Cross Reaction	Positive Reaction to Silver leaf whitefly	Positive Reaction to Green vegetable bug
Nezara viridula	Green Vegetable Bug	4	N	Y
Bemisia tabaci AUS1	Bemisia tabaci EAN	1	N	N
Bemisia tabaci MEAM1	Bemisia tabaci B-biotype	20	Y	N
Trialeurodes vaporariorum	Greenhouse whitefly	1	N	N
Agonoscelis rutila	Horehound Bugs	1	N	N
Campylomma liebknechti	Apple dimpling bug	2	N	N
Cermatulus nasalis	Glossy shield bug	1	N	N
Creontiades dilutus	Green mirid	1	N	N
Cuspicona simplex	Green Potato Bug	2	Y[+]	N
Dictyotus caenosus	Brown Shield Bug	2	N	N
Oechalia schellenbergii	Spined Predatory shield bug	1	N	N
Piezodorus hybneri	Redbanded Shield Bug	2	N	Y*
Plautia affinis	Green Stink bug	2	N	N
Poecilometis sp	Brown shield bug 2	1	N	N
Dysdercus sidae	Pale Cotton Stainer	1	N	N

[+]gel run positive, sequences positive (potential technical contamination)

*gel run is positive, but sequencing results in 2 base pair mismatch

Testing against a wide range of species found in cotton crops
This involved collection of a wide range of invertebrates (insects, mites, spiders, millipedes) from within cotton crops and screening these for cross-reactivity against each assay. This can be a problem occasionally with molecular diagnostic assays and cross-reactivity may be across quite widely divergent species. Cross reactions that occur in relatively rare species are often not a problem, but a cross reaction to a common species, for instance if GVB cross reacted with leaf hoppers, would be a significant issue. Over 230 non-target species were collected from several cotton crops in the Namoi Valley in the 2013-14 season. They were either collected from beatsheet samples, sweep nets or in some instances opportunistically using an aspirator (see section below about the validity of these sample methods). Species which are known to be herbivorous were collected directly into individual tubes of 100% ethanol.

Species that we knew or suspected were predatory, including pest species such as green mirid and apple dimpling bug that are also herbivorous, had to be handled differently because their gut could already contain DNA from either SLW or GVB that they had eaten and this could give a false positive for cross-reaction. Consequently, potential predatory species were held unfed (starved) but with access to water in small containers to allow DNA from either SLW or GVB to be eliminated from their bodies before they were preserved in alcohol and tested. Insect predators were starved for 2 to 3 days while spiders, which tend to have a slower metabolism, were starved for at least a week.

Specimens also had to be taxonomically identified. Common species presented no problem, however, there were a wide range of other species present, especially many small diptera (flies) and spiders that required more specialist knowledge to classify. Identification to species level was not always possible, or necessary in the case of uncommon species, so in some cases 'species' were defined as morpho-types. Dr Mary Whitehouse (CSIRO) provided assistance with both identification of spiders and with grouping. Samples of at least two specimens of each species were then sent to Dr Llewellyn's laboratory for DNA analysis. Where a potential cross reaction was identified the amplified DNA products were sequenced to confirm if it was identical to SLW or GBV DNA – which would indicate a technical contamination of samples or assay components rather than a cross reaction. Contamination is always a potential problem when the same PCR assays are carried out over and over again in a laboratory as any aerosols can carry many more copies of the amplified sequences than are present in actual samples. Laboratory procedures are put in place to minimise such cross-contamination of assays but it can always occur.

The results obtained indicated relatively few instances of cross reactions for either SLW or GVB and those that did exist were with species that were generally rare in cotton, especially compared with SLW. This means that the potential for such cross reactions to significantly affect the results is very low. Further, as we have the capability to sequence the amplified products where there was a question mark over the result, this did not represent a significant problem.

Table 2. Species Tested for Cross Reaction to Silver leaf whitefly and Green vegetable bug primers that are not closely related to Silver leaf whitefly or Green vegetable bug. Potential predatory species were starved to ensure their gut was cleared of SLW or GVB DNA.

Species/Group	Common Name	Number tested	Positive Reaction to Silver leaf whitefly	Positive Reaction to Green vegetable bug
ARANEOMORPHAE	**SPIDERS**			
Oxyopes amoenus	Banded lynx spider	1	N	N
Araneidae	Black & white orb weaver spider 1503	1	N	N
Badumna sp	Brown house spider	2	N	N
Runcinia ?acuminata	Flower spider	1	N	N

Eriophora sp	Garden Orb Weaver	3	N	N
Uloboridae	Hackled orb weaver	2	N	N
Zosis sp	Hackled orb weaver (Zosis sp)	2	N	N
Desidae	Intertidal spider	1	N	N
Desidae 2	Intertidal spider 2	1	N	N
Cytaea sp	Jumping Spider	1	N	N
Simaetha sp	Jumping Spider	1	N	N
Plexippus sp	Jumping Spider	1	N	N
Bianor sp	Jumping Spider	2	N	N
Copocrossa sp	Jumping Spider	2	N	N
Salticidae 2603	Jumping spider 2603	1	N	N
Salticidae 5	Jumping spider 5	1	N	N
Salticidae	Jumping spider 6205	1	N	N
Salticidae 8	Jumping spider 8	1	N	N
Amaurobidae 2635	Lace webbed spider 2635	2	N	N
Amaurobius sp	Lace-webbed spider	1	N	N
Molycriinae	Long spinneret ground spider	1	N	N
Oxyopes sp 'dotty'	Lynx spider 'dotty'	2	N	N
Oxyopes sp	Lynx spider juvenile	2	N	N
Dictynidae 2	Mesh web weaver 2	1	N	N
Dictynidae 3	Mesh web weaver 3	1	N	N
erigonidae	Money spider	1	N	N
Cheiracanthium sp	Nightstalker	2	N	N
Cheiracanthium sp 2	Nightstalker 2	2	N	N
Cheiracanthium sp 4	Nightstalker 4	2	N	N
Cheiracanthium sp no leg spines (immature)	Nightstalker no leg spines (immature)	2	N	N
Araneidae	Orb weaver	2	N	N
Dolophones ?conifera	Orb weaver spider	1	N	N
Araneus circulisparssus	Orb weaver spider	2	N	N
Argiope extensa	Orb weaver spider	2	N	N
Argiopinae	Orb weaver spider	2	N	N
Argiope sp	Orb weaver spider	1	N	N
Araneidae Mary's morph 5	Orb weaver spider Mary's morph 5	2	N	N
Oxyopes molarius	Plain brown lynx spider	2	N	N
Leucauge dromedaria	Silver orb spider	1	N	N
Austracantha minax	Six spined spider	1	N	N
Miturgidae	Spider other	1	N	N
Cribellate sp	Spider other (Cribellate)	1	N	N
Araneomorphae 2648	Spider other 2648	1	N	N
Gnaphosidae 3 morph B D	Stealthy ground spider 3 morph B D	2	N	N
Gnaphosidae	Stealthy ground spiderling	1	N	N
Oxyopes gracilipes	Stocking lynx spider	2	N	N
Supunna picta	Swift ground spider	1	N	N
Achaearanea sp	Tangle web spider	2	N	N
Achaearanea sp 1	Tangle web spider 1	1	N	N
Achaearanea sp 1wau	Tangle web spider 1wau	1	N	N
Achaearanea sp 2	Tangle web spider 2	1	N	N
Achaearanea sp 3	Tangle web spider 3	1	N	N
Pisauridae	Water spider	1	N	N
Venatrix konei	Wolf spider	2	N	N
Venatrix fontis	Wolf spider	2	N	N
Hogna sp	Wolf spider	1	N	N
Lycosidae 206	Wolf spider 206	1	N	N
Lycosidae 5902	Wolf spider 5902	1	N	N
Lycosidae orange/black	Wolf spiderling	2	N	N

Cheiracanthium mordax	Yellow nightstalking sac spider	2	N	N
COLEOPTERA	**BEETLES**			
Anthicus sp	Ant-like flower beetle	1	N	N
Anthicus sp --	Ant-like flower beetle --	1	N	N
Anthicus hesperi	Ant-like flower beetle (hesperi)	1	N	N
Anthicus sp 1	Ant-like flower beetle 1	2	N	N
Anthicus sp d	Ant-like flower beetle d	2	N	N
Sericoderus sp	Beetle other	2	N	N
Chaetocnema sp	Brown Flea beetle	3	N	N
Microlestodes macleayii	Carabid beetle	5	N	N
Carabidae	Carabid beetle Small black	1	N	N
Harmonia conformis	Common spotted ladybeetle	4	N	N
Nitidulidae	Flower beetle	1	N	N
Typhaea sp	Fungus beetle	1	Y*	N
Scymnomorphus sp	Ladybeetle	1	N	N
Coccinellidae	Ladybeetle	1	N	N
Scyminae	Ladybeetle-Scyminae	2	N	N
Serangium scertetum	Ladybeetle-Serangium scertetum	1	N	N
Telsimia sp	Ladybeetle-Telsimia sp	1	N	N
Palaeomela sp	Leaf beetle	1	N	N
Ditropidus sp	Leaf beetle	1	N	N
Chryptocephalini 1	Leaf beetle 1	1	Y*	N
Diomus notescens	Minute two spotted ladybeetle	3	N	N
Stethorus sp	Mite eating ladybeetle	2	N	N
Corticaria sp	Mould beetle	2	N	N
Corticaria elongate	Mould beetle	2	N	N
Corticaria japonica	Mould beetle-C. japonica	2	N	N
Carpophilus sp (prev Aethina concolor)	Pollen beetle	2	N	N
Dicranolaius bellulus	Red and blue beetle	2	N	N
Nitidulidae 1	Rove beetle	6	N	N
Nitidulidae 4121	Rove beetle	1	N	N
Philonthus subcingulatus	Rove beetle	1	N	N
Staphylinidae	Rove beetle	2	N	N
Camptodes sp	Sap feeding beetle	1	N	N
Dicranolaius sp 1	small red and blue beetle	1	N	N
Micraspis frenata	Striped ladybeetle	2	N	N
Harmonia octomaculata	Three banded ladybeetle	2	N	N
Lema trivittata	three-lined Lema beetle	2	N	N
Coccinella transversalis	Transverse ladybeetle	2	N	N
Epilachna cucurbitae	Twenty eight spotted ladybeetle	3 tested by Harwood	N	N
Apion sp 885	Weevil-Apion sp 885	2	N	N
Curculionidae large red/brown	Weevil-Curculionidae	1	N	N
Hippodamia variegata	White collared ladybeetle	3	N	N
DERMAPTERA	**EARWIGS**			
Euborellia sp	Earwig	1	-*	N
Earwig 4013	Earwig 4013	1	N	N
Dermaptera	earwig 4209	1	-*	N
Labidura sp	Earwig	1	N	N
Parisolabus	Earwig-Parisolabus	1	N	N
DIPLOPODA	**MILLIPEDES**			
Diplopoda 1	Millipede	1	N	N
DIPTERA	**FLIES**			
Acalyptra	Acalyptrate fly	1	N	N

Lauxanidae	Acalyptrate fly	1	N	N
Asteiidae	Acalyptrate fly	1	N	N
Ephydridae 3	Alkali fly	2	N	N
Ceratopogonidae 1	Biting midge 1	3	N	N
Sepsidae	Black Scavenger Fly	1	N	N
Calliphoridae A-2	Carrion fly	2	N	N
Calliphoridae D	Carrion fly	2	N	N
Calliphoridae	Carrion fly	1	N	N
Calliphoridae AorB	Carrion fly AorB	1	N	N
Tipulidae 1	Crane fly 1	1	Y+	N
Tipulidae 2	Crane fly 2	1	N	N
Tipulidae 3	Crane fly 3	1	N	N
Sciaridae	dark-winged fungus gnat	1	N	N
Sciaridae 1	dark-winged fungus gnat 1	1	N	N
Chloropidae	Eye gnat	2	N	N
Chloropidae 1	Eye gnat 1	2	N	N
Chloropidae 3	Eye gnat 3	1	N	N
Chloropidae 4	Eye gnat 4		N	N
Chloropidae 4	Eye gnat 4	2	N	N
Muscidae	House Fly 5	1	N	N
Muscidae	House Fly 6	1	N	N
Muscidae	House Fly 6-1	3	N	N
Sphaeroceridae sp	Lesser dung fly	1	N	N
Sphaeroceridae sp 1	Lesser dung fly 1	1	N	N
Sphaeroceridae sp 2	Lesser dung fly 2	1	N	N
Bibionidae	March fly	2	N	N
Sciomyzidae	Marsh fly	2	N	N
Chironomidae 1	Midge 1	1	N	N
Chironomidae 2	Midge 2	1	N	N
Chironomidae 3	Midge 3	1	N	N
Chironomidae 4	Midge 4	2	N	N
Chironomidae 6	Midge 6	1	N	N
Chironomidae	Midge zebra	1	N	N
Platystomatidae	Picture winged fly	1	N	N
Ephydridae	Rock fly	1	N	N
Diptera	Slender fly	1	N	N
Heleomyzidae	Sun fly	1	N	N
Tachinidae	Tachinid fly	1	N	N
Tachinidae B-1	Tachinid fly B-1	1	N	Y*
HEMIPTERA/HOMOPTERA	**BUGS**			
Reduviidae	Assassin bug	1	N	N
Harpactorinae	Assassin bug-Harpactorinae	1	N	N
Geocoris sp	Big eyed bug	1	N	N
Bobilla sp	Bobilla sp	2	N	N
Creontiades pacificus	Brown mirid	1	N	N
Deraeocoris signatus	Brown smudge bug	3	N	N
Cletus sp	Cletus sp	1	N	N
Aphis gossypii	Cotton aphid	2	N	N
Nabis sp	Damsel bug	2	N	N
Pentatomidae	Eysacoris-like bug with moon mark	1	N	N
Balclutha sp 1	Grass leafhopper	1	N	N
Cicadellidae	Jassid	1	N	N
Oteana lubra	Jassid-Oteana lubra	2	N	N
Orius sp	Minute pirate bug	2	N	N
Leptocoris mitellata	Redeyed bug	1	N	N
Nysius vinitor	Rutherglen bug	2	N	N

Hemiptera	Seed bug	2	N	N
ID Oncocoris sp	Shield bug-Oncocoris sp	2	N	N
Melanacanthus scutellaris	Small brown bean bug	2	N	Y*
Austroasca viridigrisea	Vegetable leafhopper	2	N	N
HYMENOPTERA	**BEES AND WASPS**			
Gasterupiidae	Carrot wasp	1	N	N
Bethylidae 1	Cuckoo wasp 1	1	N	N
Cynipidae	Gall wasp	1	N	N
Apis mellifera	Honey bee	1	Y*	N
Encyrtidae	Parasitic wasp	1	N	N
Eupelmidae	Parasitic wasp	1	N	N
Ichneumonidae	Parasitic wasp	1	N	N
Braconidae 1	Parasitic wasp 1	1	N	N
Braconidae 4122	Parasitic wasp 4122	1	N	N
Braconidae metallic, red eyes	Parasitic wasp metallic, red eyes	1	N	N
Trissolcus sp	Parasitic wasp-Trissolcus sp	2	N	N
Megaspilidae	Parasitoid wasp	1	N	N
Megaspilidae 1	Parasitoid wasp	1	N	N
LEPIDOPTERA	**MOTHS**			
Helicoverpa armigera	Cotton bollworm	2	N	N
Achyra affinitalis	Cotton webspinner	1	N	N
Lepidoptera 2	moth 2	2	N	N
Micromoth 4144	Moth other	1	N	N
Helicoverpa punctigera	Native budworm	2	N	N
Earias huegeliana	Rough boll worm	2	N	N
Anomis sp 1	Semi-looper moth sp 1	1	Y*	N
Endotricha sp	Snout moth	1	N	N
*Pyraloide*a 1	Snout moth 1	1	N	N
Pyraloidea no beak	Snout moth no beak	1	N	N
Pyralidae 1	Snout moth-Pyralidae 1	1	N	N
MANTODEA	**MANTIDS**			
Micromis tasmaniae	Brown lacewing	1	N	N
Mallada signatus	Green lacewing	2	N	N
NEUROPTERA	**LACEWINGS**			
Mantodea	Mantid	1	N	N
Mantinae 3	Mantid 3	2	N	N
Orthodera sp	Mantid-Orthodera sp	2	N	N
ODONATA	**DRAGONFLIES**			
Agriocnemis heterostica	Dragonfly	1	N	N
ORTHOPTERA	**GRASSHOPPERS AND CRICKETS**			
Grillinae WL1	Cricket WL1	1	N	N
Stenopelmatidae	Jerusalem cricket	1	N	-
Caedicia sp 1	Katydid 1	2	N	N
SYMPHIPLEONA/COLLEMBOLA	**SPRINGTAILS**			
Collembola 2	Springtail 2	2	N	N
Isotomidae	Springtail-Isotomidae	1	N	N
THYSANOPTERA	**THRIPS**			
Desmothrips sp	Desmothrips sp	2	N	N
Thysanoptera	Thrips	5	N	N
Tubulifera	Thrips-Tubulifera	5	N	N
TROMBIDIFORMES	**MITES**			
Tetranychus urticae	Two spotted mite	2	N	N

*tested and sequenced positive, but not starved before testing
+tested and sequenced positive, starved prior to testing
-not tested or no result

3. Developing a strategy to sample potential predators and validation.

Once there was confidence that the primers were selective or that any known cross-reactions were unlikely to compromise the results the next phase was to develop a strategy to use the primers to assess predation in the field. The basic approach was to (i) collect a reasonable sample size of the likely predator species from cotton crops and (ii) at the same time sample those crops to quantify the abundance of the target prey species (SLW or GBB) and of all other invertebrates present. When this is done over several dates the changes in the proportion of predators showing a positive reaction to the target pest, for instance increasing as the abundance of the prey increases, can be analysed.

There were some challenges to carrying out this type of study. Firstly, lack of advance knowledge of the range of predator species for SLW and GVB meant narrowing the range to be targeted for more efficient sampling wasn't possible and secondly, the sampling technique used can increase or decrease the risk of contamination but there was no local information available prior to carrying out the experiment to test this.

These issues are discussed in more detail and reported on below.

Potential predator species (research in the 2013-14 season)
If the range of predatory species was already known for either SLW or GVB then it would be possible to make sampling and PCR analysis more efficient by targeting just those species. Unfortunately this information was not available so in the first summer of this project we sampled a wide range of potential predators in a range of crops at times when it is known the target prey is abundant. Sampling a range of crops ensures that there is broader diversity of potential predators collected, as each field may have a slightly different complex present at any given time. If a reasonable sample size of each of the potential predatory species is then processed for the presence of SLW or GVB DNA then it will be possible to arrive at a list of potential predators where predation has been confirmed. Then considering the abundance of each predator species and the frequency of positive recordings for SLW or GVB DNA it should be possible to narrow the range of predatory species for more detailed studies in later years.

In 2013-14 samples of potential predator species were collected from Block 17 at ACRI (three dates) as well as at three commercial farm fields (Belah; two dates, Togo; two dates and Edithville; one date) in the Namoi Valley. On each occasion we aimed to sample at least 20 individuals of each predator species found. However, due to the natural variability in abundance both between species and between locations this was not practical for many collection dates. Sampling was done using beatsheets, where individual metres of cotton were shaken onto the sheet and potential predators collected quickly using either hands or an aspirator modified to easily suck up and the expel individual invertebrates. These specimens were individually preserved in micro-centrifuge tubes in 100% ethanol and labelled for site and date. At the same time 4 sets of sweep net samples (each 20 sweeps of the crop) were taken and the invertebrates caught also preserved for later counting and identification. Visual samples of 80 leaves at the 5th node below the terminal were also made to score the abundance of SLW adults and nymphs, spider mites, thrips and aphids. These samples were later counted and species identified – with the same approach to deal with the less common species used as described above.

Twenty-four predator species tested positive for the presence of SLW DNA (Table 3; summarised across all nine sites for 2013-14). A 'predation' index was calculated by multiplying the abundance of the species at the site by the proportion positive for either SLW DNA (Table 3) or GVB DNA (Table 4). This attempt to identify species likely to be more important as predators of that pest. Some species such as red and blue beetles were very abundant and had relatively high positive scores (80%) and hence high 'index', others such as green lacewing larvae were much rarer though they had relatively high positive scores (38.5%) so ended up with low 'predation index'. Others such as thrips adults

(*Frankliniella* spp.) had relatively low scores (7.9%), but were very common in crops so had a high 'predation index'. There were a few surprises with a number of Diptera tested that showed 22% positive. These were Chloropids and Muscids which likely feed on decaying plant or animal material possibly explaining their positive reactions. Further, 5 brown shield bugs were also tested with 2 showing positive. This is hard to explain unless they have become contaminated while traversing leaves, accidentally penetrated and consumed SLW when feeding on leaf tissue or actually are occasionally predators.

GVB abundance was generally very low in 2013-14 and they were only found at one site (Table 4) and even then at low numbers. At this site only tangleweb spiders and 'Spiders other' showed a very low proportion scoring positive for GVB DNA.

Table 3. Potential predatory species tested for reaction to SLW primer, 2013-14. The mean number of SLW adults per 20 sweeps across all sample dates was 104.9).

Species / group	Common Name	Mean no. of arthropods per site per date (mean per 20 sweeps)	Total tested for SLW DNA	No. positive to SLW DNA	% positive to SLW DNA	Index
Araneomorphae	**Spiders**					
Araneidae	Orb weaver spiders	0.00	34	10	29.4	0.0
Clubionidae	Nightstalker spiders	0.53	79	9	11.4	6.0
Araneomorphae	Spiders Other	1.22	75	6	8.0	9.8
Desidae	Intertidal spiders	*	15	3	20	-
Dictynidae	hackled band-producing spiders	*	8	2	25	-
Lycosidae	Wolf spiders	*	21	1	4.8	-
Oxyopes spp.	Lynx spiders Total	2.66	201	14	7.0	18.6
Oxyopes spp.	Juvenile lynx	*	35	0	0	-
Oxyopes molarius	Plain brown lynx	*	126	10	7.9	-
Oxyopes amoenus	Banded lynx	*	33	2	6.1	-
Oxyopes gracilipes	Stockinged lynx	*	7	2	28.6	-
Thomisidae	Flower spiders	0.50	75	4	5.3	2.7
Salticidae	Jumping spiders	0.19	50	2	4.0	0.8
Theridiidae	Tangle web spiders	0.06	85	2	2.4	0.1
Hemiptera	**True bugs**					
Deraeocoris signatus	Brown smudge bugs	1.41	38	29	76.3	107.6
Nabis kinbergii	Damsel bugs	0.19	39	11	28.2	5.4
Dictyotus caenosus	Brown shield bugs	0.09	9	2	22.2	2.0
Cermatulus nasalis	Glossy shield bugs	0.06	7	1	14.3	0.9
Geocoris lubra	Big eyed bugs	0.34	33	3	9.1	3.1
Orius spp.	Minute pirate bugs	3.03	90	8	8.9	27.0
Creontiades dilutus Adults	Green mirid Adults	1.28	32	2	6.3	8.1
Campylomma liebknechti	Apple dimpling bugs	8.72	110	7	6.4	55.8
Oechalia schellenbergii	Spined predatory shield bugs	0.03	3	0	0.0	0.0
Coleoptera	**Beetles**					
Dicranolaius bellulus	Red & blue beetles	46.25	20	16	80.0	3700.0
Staphylinidae	Rove beetles	0.06	20	6	30.0	1.8
Micraspis frenata Adults	Striped ladybeetle Adults	0.06	17	4	23.5	1.4

Stethorus spp.	Mite eating ladybeetle Adults	0.38	20	3	15.0	5.7
Coccinella transversalis	Transverse ladybeetle Adults	0.47	37	4	10.8	5.1
Hippodamia variegata Adults	White collared ladybeetle Adults	0.19	10	1	10.0	1.9
Coleoptera	Herbivorous beetles	2.22	37	1+	2.7	6.0
Anthicus spp. Adults	Ant-like flower beetle Adults	0.00	23	1	4.3	0.0
Diomus notescens	Minute 2 spotted ladybeetle Adults	0.22	6	0	0.0	0.0
Neuroptera	**Lacewings**					
Mallada signata	Green lacewing Larvae	0.16	26	10	38.5	6.2
Thysanoptera	**Thrips**					
Thysanoptera	Thrips Adults	134.09	63	5	7.9	1059.3
Thysanoptera	Thrips Nymphs	37.78	19	0	0.0	0.0
Diptera	**Flies**					
Diptera spp.	Flies	28.43	14	2	14.3	-
Other Predators and Parasites	Other Predators and Parasites	0.56	26	3	11.5	6.4

Table 4. Potential predatory species tested for reaction to GVB primer, 2013-14. The mean number of GVB was 1.25 per 20 sweeps across all dates).

Species / group	Common Name	Mean no. of arthropods per site per date (mean per 20 sweeps)	Total tested for GVB DNA	No. positive to GVB DNA	% positive to GVB DNA	Index
Araneomorphae	Spiders					
Theridiidae	Tangleweb Spiders	0.25	37	2	5.4	1.3
Clubionidae	Nightstalker Spiders	2.25	29	0	0.0	0
Araneomorphae	Spiders Other	0.25	22	1	4.5	1.1
Oxyopes spp.	Lynx Spiders Total	1.25	16	0	0.0	0
Thomisidae	Flower Spiders	0	10	0	0.0	0
Salticidae	Jumping Spiders	0.25	2	0	0.0	0
Araneidae	Orb weaver spiders	0	2	0	0.0	0
Hemiptera	Bugs					
Deraeocoris signatus	Brown smudge bugs	2.5	37	0	0.0	0
Orius spp.	Minute pirate bugs	1.75	20	0	0.0	0
Campylomma liebknechti	Apple dimpling bugs	3.5	16	0	0.0	0
Nabis kinbergii	Damsel bugs	0.25	8	0	0.0	0
Geocoris lubra	Big eyed bugs	0	1	0	0.0	0
Coleoptera	Beetles					

Stethorus spp. Adults	Mite-eating ladybeetle Adults	0.25	17	0	0.0	0
Staphylinidae	Rove Beetle	0	6	0	0.0	0
Hippodamia variegata Adults	White collared ladybeetle Adults	0	4	0	0.0	0
Micraspis frenata Adults	Striped ladybeetle Adults	0	2	0	0.0	0
	Herbivorous Beetles	0.25	2	0	0.0	0
Anthicus spp	Anthicus Adults	0	1	0	0.0	0
Neuroptera						
Mallada signata Larvae	Green lacewing Larvae	0	1	0	0.0	0
Diptera						
	Diptera General	19.8	5	0	0.0	0

Unfortunately the results presented in Tables 3 and 4 were not available in time to help us select the species to be targeted for collection in 2014-15. This was due to the delays in having to repeat testing when new primers and assay conditions were developed as described above. So based on observations made during earlier SLW life history studies and GVB sampling studies a short-list of potential predators was selected (Table 5) and sampled.

Table 5. Species targeted for collecting in 2014-15

Species	Common name
Araneomorphae	Spiders
	Common nightstalker spider – adult female, male and juveniles
	Plain brown lynx spider - – adult female, male and juveniles
Hemiptera	True bugs
	Apple dimpling bug
	Brown smudge bug
	Damsel bug
	Big eyed bug
Coleoptera	Beetles
	Mite eating ladybeetle
	Transverse ladybeetle
	White collared ladybeetle
	Two spotted ladybeetle
	Red and blue beetle
Thysanoptera	
	Thrips nymphs

Validation of sampling strategies (research in the 2013-14 season)

Secondly, the method of collection of the predators is potentially important as it can be a source of physical contamination of collected specimens. For instance, when SLW are abundant they will be present on the undersides of many leaves, often at high densities, and adults will also be flying around in the crop, sometimes in small 'clouds' of individuals. At such densities nymphs of adults may become squashed on the rim of sweep nets, on the plastic of beat sheets or in the tubes of aspirators. This could release body contents that could contaminate other species that come into contact with these apparatus during the course of collecting the samples. This could lead to species that have not consumed SLW showing a 'positive' for SLW DNA and result in 'false positives'.

In 2013-14 we tested if contamination was a risk for beatsheet, sweep net and aspirator sampling. To do this we collected soft bodied vegetable leaf hoppers (jassids) and hard bodied Pollen Beetles (both non-predators) using sweeps, beatsheets or aspirator from a field with high Silverleaf Whitefly b-biotype populations and small numbers of Green Vegetable bug. We repeated sampling with each method until we had collected at least 200 individuals of each species for that method. Individuals were stored in Eppendorf tubes filled with 100% ethanol and put into storage -18^{o}C in Danny Llewellyn's Canberra laboratory. DNA was extracted from each individual and they were tested against both SLW and GVB primers. The results showed few instances of contamination (Table 6) although it could occur at a low level with any of the collection methods but was least by aspiration (though this is the slowest collection method).

Table 6 Comparison of collection contamination with Silver leaf whitefly DNA and Green vegetable bug DNA: Soft bodied (Vegetable leaf hoppers, *Austroasca viridigrisea*) and hard bodied (Pollen beetles, *Carpophilus sp.*) insects, using Sweep net sampling, Aspirators or Beat Sheets for insect collection. Numbers in brackets are number of samples confirmed by sequencing.

Method of collection	Insect Collected	Total Insects with No Silver leaf whitefly contamination detected	Total Insects with Silver leaf whitefly contamination detected	% Silver leaf whitefly Contamination Detected	Total Insects with No Green vegetable bug contamination detected	Total Insects with Green vegetable bug contamination detected	% of Insects with Green vegetable bug Contamination Detected
Aspirator	Pollen beetles	193	7 (5)	3.5%	78	0	0.0%
	Vegetable leaf hoppers	199	1	0.5%	200	0	0.0%
	Total Aspirator	392	8 (5)	2.0%	295	0	0.0%
Beat sheet	Pollen beetles	192	8 (6)	4.0%	200	0	0.0%
	Vegetable leaf hoppers	194	6	3.0%	197	3	0.0%
	Total Beat sheet	386	14 (11)	3.5%	397	3	0.8%
Sweep Net	Pollen beetles	193	7 (5)	3.5%	135	1	0.7%
	Vegetable leaf hoppers	196	4 (2)	2.0%	200	0	0.0%
	Total Sweep	389	11 (7)	2.8%	370	1	0.7%

Targeted predator collection across time (research in the 2015-16 season)
This component of the research aimed to make targeted collections of the predators listed in Table 5 sequentially from the same field. This allowed us to test if the proportion of individuals testing positive for SLW or GVB primers was correlated with the abundance of these pests or with other pest species. Predator species were collected from Block 18 at ACRI (three dates) as well as at three commercial farm fields (Havannah North: one date, Mollee; three dates and Togo; three dates) in the Namoi Valley. Sampling was done using beatsheets, where individual metres of cotton were shaken onto the sheet and potential predators collected quickly using either hands or an aspirator modified to easily suck up and the expel individual invertebrates. The aim was to collect at least 20 individuals of each species from each site on each date. In reality this proved impractical as the number that could be collected was strongly influenced by their abundance. These specimens were individually preserved in micro-centrifuge tubes in 100% ethanol and labelled for site and date. At the same time 4 sets of sweep net samples (each 20 sweeps of the crop) were taken and the invertebrates caught also preserved for later counting and identification. Visual samples of 80 leaves at the 5th node below the terminal were also made to score the abundance of SLW adults and nymphs, spider mites, thrips and aphids. These samples were later counted and species identified – with the same approach to deal with the less common species used as described above.

The data for the 2014-15 season for SLW is summarised in Table 7. The results confirm that a several spider species, especially the species of nightstalkers and lynx spiders, a range of bug, especially brown smudge, big-eyed, minute pirate and damsel bugs and a range of predatory beetles, especially red and blue beetles appear to be important predators of SLW. Interestingly two 'pest' species, apple dimpling bug and thrips are also important.

Table 7. Potential predatory species tested for reaction to SLW primer, 2014-15. The mean number of SLW adults per 20 sweeps across all sample dates was 243.

Species/Groups	**Common name**	Mean no. per site per date	No. Tested for SLW DNA	Total no. +ve	% +ve SLW DNA	Index
Araneomorphae	**Spiders**					
Clubionidae	Nightstalker spiders	0.20	138	16	11.6	2.3
Salticidae	Jumping spiders	0.88	85	8	9.4	8.2
Oxyopes spp.	Lynx spiders Total	3.60	385	31	8.1	29.0
Oxyopes spp.	*Lynx spiderlings*	*	226	16	7.1	*
Oxyopes molarius	*Plain brown lynx*	*	108	7	6.5	*
Oxyopes amoenus	*Banded lynx*	*	17	1	5.9	*
Oxyopes gracilipes	*Stockinged lynx*	*	34	7	4.9	*
Thomisidae	Flower spiders	0.00	15	1	6.7	0.0
Araneomorphae	Spiders Other Total	3.63	784	48	6.1	22.2
Badumna sp.	*House*	0.7	767	46	6.0	4.2
Araneomorphae	*Ground dwelling*	*	12	1	*	*
Araneomorphae	*Other*	*	5	1	*	*
Araneidae	Orb weaver spiders	0.08	22	1	4.5	0.3
Theridiidae	Tangle web spiders	0.53	6	0	0.0	0.0
Hemiptera	**True bugs**					
Deraeocoris signatus	Brown smudge bugs	0.68	35	27	77.1	52.1
Nabis kinbergii	Damsel bugs	0.38	53	9	17.0	6.4
Geocoris lubra	Big eyed bugs	3.15	8	1	12.5	39.4
Campylomma liebknechti	Apple dimpling bugs	11.56	224	18	8.0	92.9
Orius spp.	Minute pirate bugs	4.13	58	1	1.7	7.1

Oechalia schellenbergii	Spined predatory shield bugs	0.10	4	0	0.0	0.0
Cermatulus nasalis(	Glossy shield bugs	0.05	4	0	0.0	0.0
Coleoptera	**Beetles**					
Coccinella transversalis	Transverse ladybeetle Adults	0.05	11	3	27.3	1.4
Micraspis frenata	Striped ladybeetle Adults	0.18	14	3	21.4	3.8
Stethorus spp.	Mite eating ladybeetle Adults	0.38	31	5	16.1	6.0
Hippodamia variegata	White collared ladybeetle Adults	0.03	20	3	15.0	0.4
Diomus notescens	Minute 2 spotted ladybeetle Adults	0.08	14	2	14.3	1.1
Dicranolaeus bellulus	Red & blue beetles	17.38	242	30	12.4	215.4
Coleoptera	Herbivorous beetles	4.08	25	1*	4.0	16.3
Carabidae	Carabid beetles	0.05	6	0	0.0	0.0
Neuroptera	**Lacewings**					
Mallada signatus	Green lacewing Larvae	0.15	28	5	17.9	2.7
Thysanoptera	**Thrips**					
Thysanoptera	Thrips Nymphs	17.10	198	21	10.6	181.4

*This is a species of Sericoderus, a member of Corylophidae which are generally fungivores. So these positive probably represent scavenging rather than predation.

GVB abundance was generally very low in 2014-15 and they were only found at five of the 10 sites (Table 4) and even then at low numbers. Only 6 predator groups scores positive for GVB DNA, probably reflecting that these pests had low abundance.

Table 8. Potential predatory species tested for reaction to GVB primer, 2014-15. The mean number of SLW adults per 20 sweeps across all sample dates was 1).

Species/Group	**Common name**	Mean no. per site per date	Total Tested for GVB	Total no. +ve	% +ve for GVB	Index
Araneomorphae	**Spiders**					
Oxyopes spp.	Lynx spiders Total	2.90	158	5	2.5	12
Oxyopes spp.	*Lynx spiderlings*	*	84	1	1.1	*
Oxyopes molarius	*Plain brown lynx*	*	52	3	5.7	*
Oxyopes amoenus	*Banded lynx*	*	4	0	0	*
Oxyopes gracilipes	*Stockinged lynx*	*	18	1	5.5	*
Cheiracanthium spp.	Nightstalker spiders	0.25	88	1	1.1	0.3
Araneomorphae	Spiders Other	3.55	362	3	0.8	2.9
Badumna sp.	*House*	0.7	355	3	0.85	0.6
Araneomorphae	*Ground dwelling*	*	5	0	0	*
Araneomorphae	*Other*	*	2	0	0	*

Thomisidae	Flower spiders	0.00	7	0	0	0
Salticidae	Jumping spiders	1.25	66	0	0	0
Araneidae	Orb weaver spiders	0.10	4	0	0	0
Theridiidae	Tangle web spiders	0.50	4	0	0	0
Hemiptera	**True bugs**					
Nabis kinbergii	Damsel bugs	0.55	40	2	5.0	2.8
Campylomma liebknechti	Apple dimpling bugs	17.52	138	0	0	0
Geocoris lubra	Big eyed bugs	6.20	6	0	0	0
Deraeocoris signatus	Brown smudge bugs	1.30	34	0	0	0
Cermatulus nasalis	Glossy shield bugs	0.05	7	0	0	0
Orius spp.	Minute pirate bugs	2.70	17	0	0	0
Oechalia schellenbergii	Spined predatory shield bugs	0.15	3	0	0	0
Coleoptera	**Beetles**					
Micraspis frenata	Striped ladybeetle Adults	0.30	9	1	11.1	3.3
Dicranolaius bellulus	Red & blue beetles	21.20	120	1	0.8	18
Diomus notescens	Minute 2 spotted Ladybeetle Adults	0.05	8	0	0	0
Stethorus spp.	Mite eating ladybeetle Adults	0.50	7	0	0	0
Coccinella transversalis	Transverse ladybeetle Adults	0.05	6	0	0	0
Hippodamia variegata	White collared ladybeetle Adults	0.00	8	0	0	0
Carabidae	Carabid beetles	0.10	2	0	0	0
Neuroptera	**Lacewings**					
Mallada signatus	Green lacewing Larvae	0.20	24	0	0	0
Thysanoptera	**Thrips**					
Thysanoptera	Thrips Adults	22.35	24	0	0	0
Thysanoptera	Thrips Nymphs	8.30	77	0	0	0

5. Prey retention studies 2015-16

Different predator species may digest SLW or GVB at different rates. This could affect the results from PCR testing as species that have a short retention time of SLW or GVB DNA could be under-estimated while those with a long retention time could be over represented. To understand retention times we collected predator species from the field, starved then for 2-5 days for insects or 1 or 2 weeks for spiders, then fed them one SLW adult or nymph. Ten predators of each species tested were preserved at 0, 0.5, 1, 2, 4, 8, 18, 24, 36, 48, 72, 96 or 120 hours after feeding, with an additional unfed ten preserved as controls. These were then analysed for the presence of SLW DNA. This work was not done for GVB due to the low numbers of this pest encountered in the field during sampling which meant that the predation data would be too sparse.

The results from these studies were mixed. In terms of tracking retention by time useful results were obtained for Apple dimpling bug, Brown smudge bug and Red and blue beetle (Figure 1), which

indicated retention of less than 24 hrs for Apple dimpling bug and Red and blue beetle and less than 10 hours for Brown smudge bug. For the other species tested the results showed very few positive results, even though the analysis was repeated. These included mite eating ladybeetle and pale night stalker and lynx spiders. We are uncertain why this has not worked out despite exhaustive attempts to identify the problem. It means that we may be underestimating the amount of predation occurring in the field. Some of these difficulties may be in the feeding habits of some of the predators as some chew the whole SLW nymphs or adults where as others suck the internal contents leaving an empty shell. DNA present in larger chunks of the pest are likely to survive longer in the predators gut and hence be more easily detected in the assay.

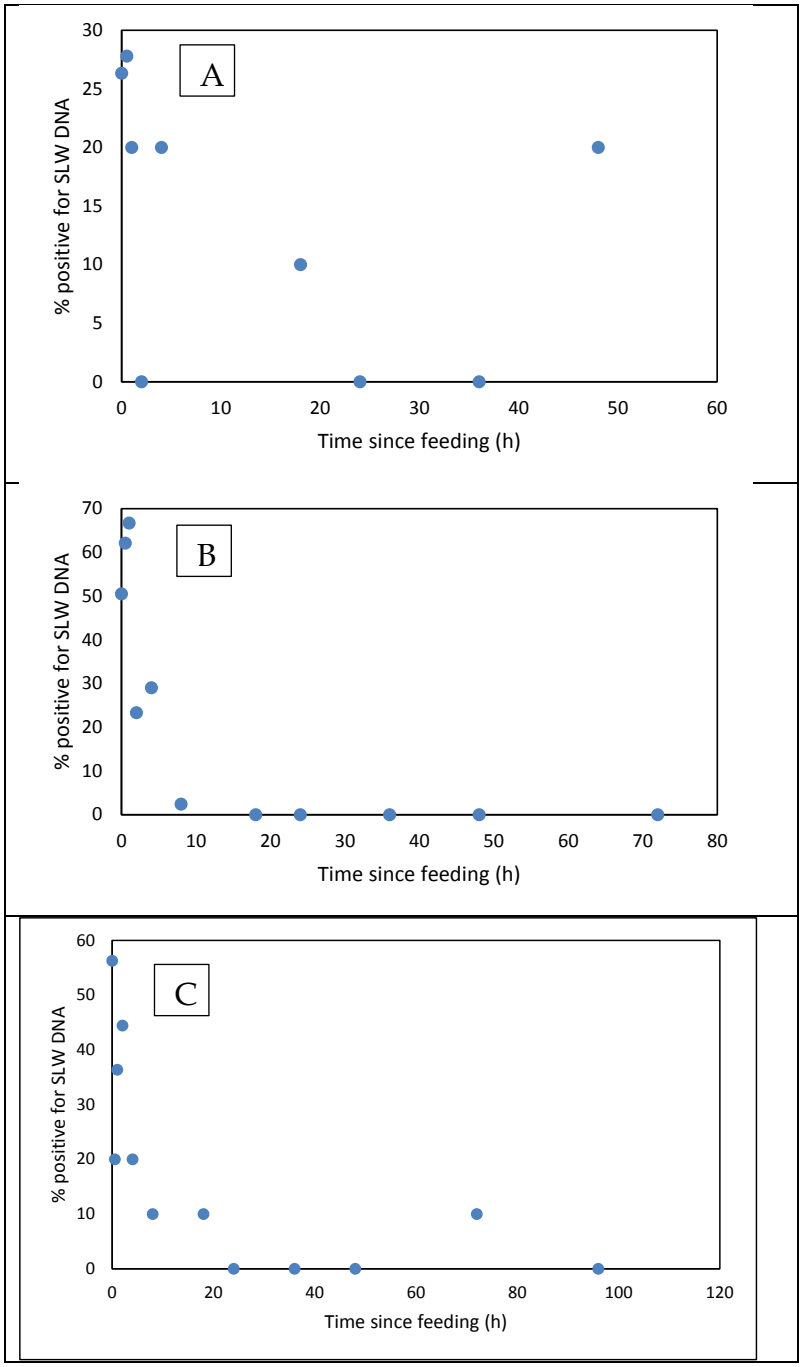

Figure 1. Retention period of SLW DNA for (A) Apple dimpling bug (B) Brown smudge bug and (C) Red and blue beetle.

Nevertheless, the process of completing the retention studies meant that there was detailed observation of feeding by the predators. This was particularly valuable in understanding the way that predatory bugs such as Apple dimpling bug and Brown smudge bug feed (Figures 2 to 4). These bugs

sometimes feed by penetrating the dorsal surface of younger (1st and 2nd instar) SLW nymphs (Fig 3b). However, more often they push their proboscis to the side of the nymph then insert their stylet in between the nymph and the leaf (Fig, 2c, d; Fig 3 d). They move the stylet around within the nymph and suck out the contents (Fig 3e). The nymph remains looked flattened and dried out as if they had died from dehydration (Fig 3f). Tanya noted that sometimes they were also dislodged from the leaf surface or were easily dislodged after having been fed on. Lacewing larvae similarly feed by piercing the whitefly nymphs with their mandibles and sucking out the contents, again leaving a dried out remains (Fig 4 c., d.).

We also made opportunistic observations of predation events and preferences for some species. We observed that Lynx spiders readily attacked adults SLW but not nymphs or eggs. Similarly Tangle web and Orb weaver spiders tended to eat adult SLW caught in webs. Surprisingly, a number of other predators also readily attacked and caught adult SLW including, Apple Dimpling bugs, Brown smudge bugs, Red and blue beetles and mantis nymphs. These predators were surprisingly fast moving when attacking SLW. Lacewing larvae, Mite-eating ladybeetles and damsel bugs were observed eating SLW nymphs and/or eggs. Red and blue beetles also ate SLW eggs and/or nymphs but reluctantly. Red and Blue beetles also ate prey very quickly so predation events would be easily missed. Similarly, bugs often rapidly withdrew their style from SLW larvae if disturbed and predation would be missed.

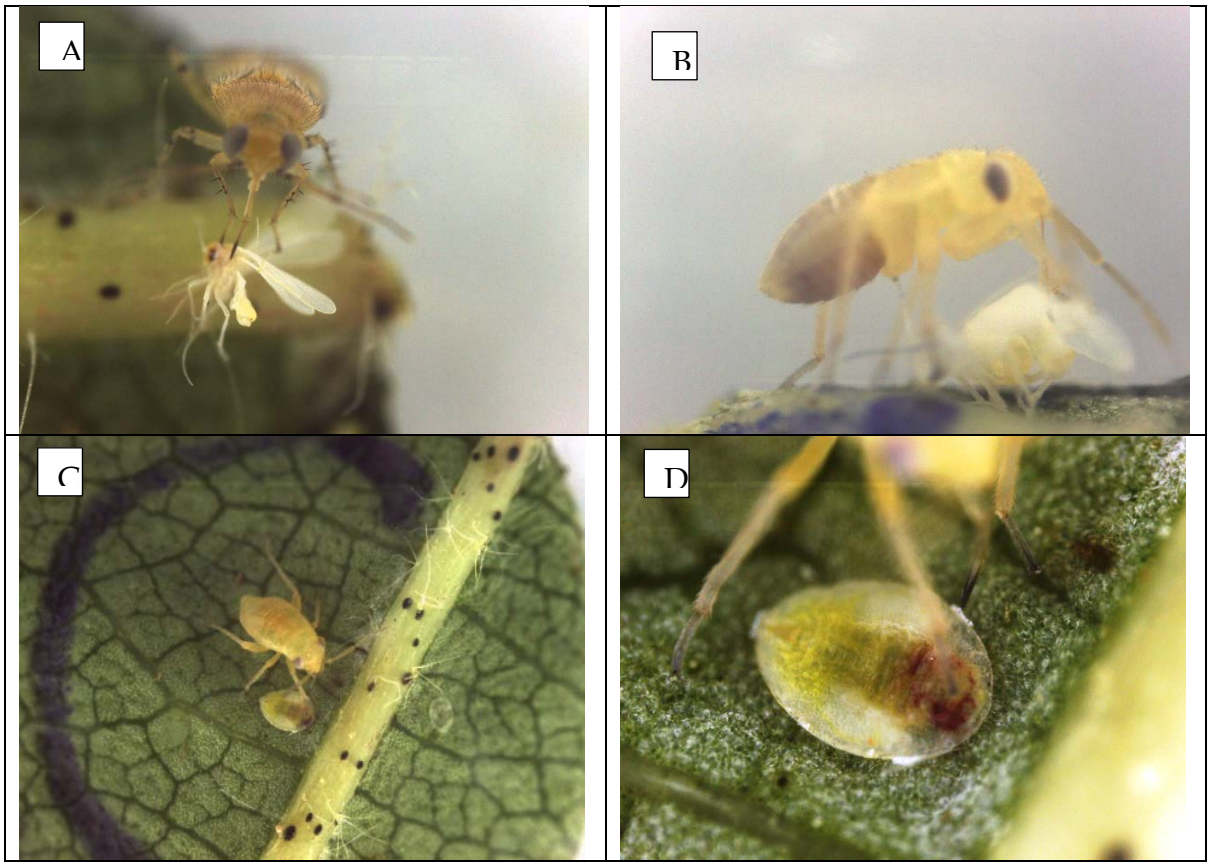

Figure 2. Predation on SLW adult by adult (A) and late nymph (B) of Apple dimpling bug and on SLW nymph by late nymph (C) Apple dimpling bug and (D) close up showing characteristic insertion of stylet underneath the side of late stage SLW nymph. ACRI February, 2016.

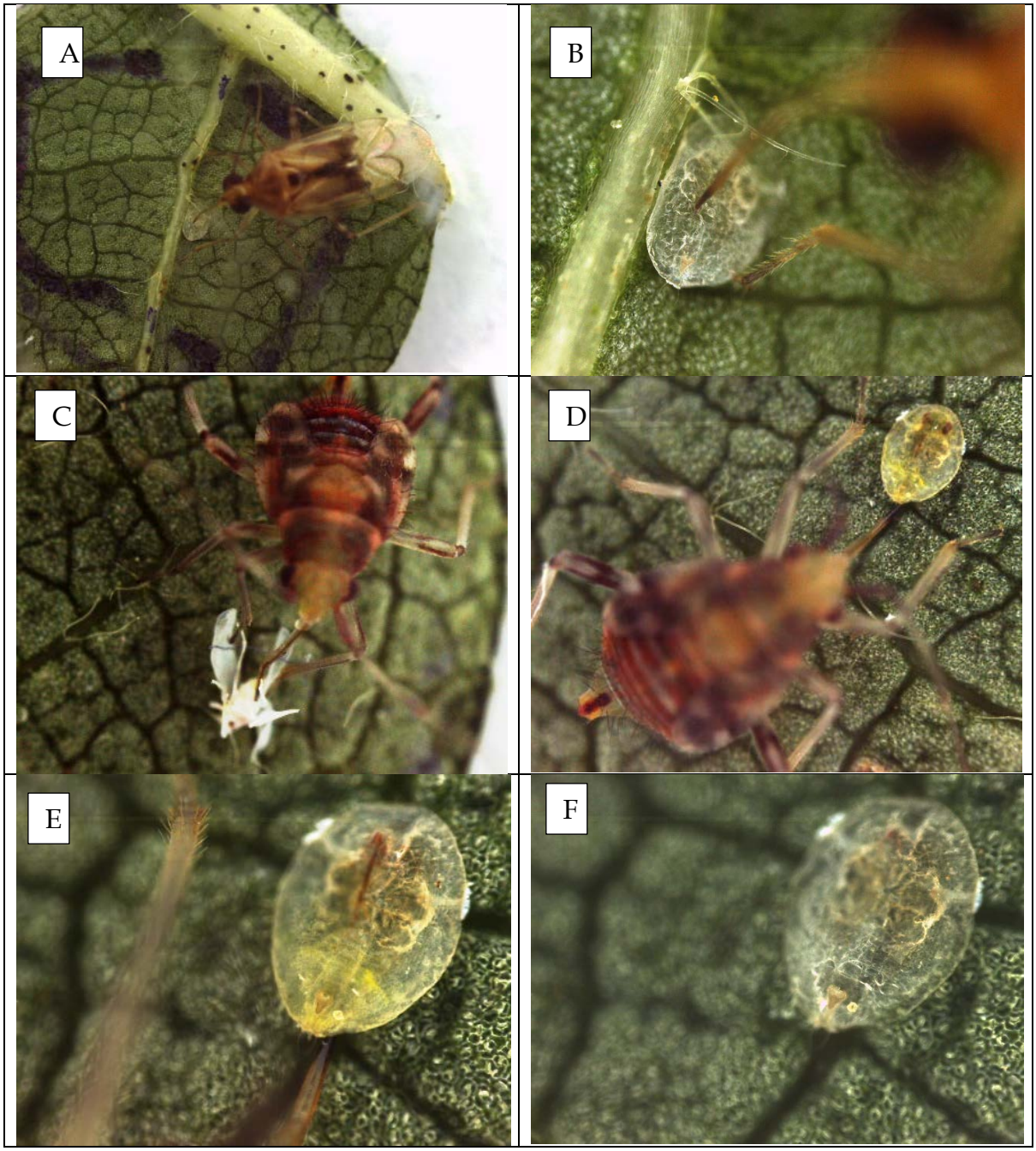

Figure 3. (A) Predation on young SLW nymph by adult Brown smudge bug and (B) close up of remains of SLW nymph. (C) Predation on SLW adult and (D) on SLW late stage nymph by late stage Brown smudge by nymph. (E) close up showing characteristic insertion of stylet underneath the side of late stage SLW nymph. The stylet can be seen inside the nymph near the upper middle area. (E) Characteristic 'empty' remains after extraction of contents by Brown smudge bug nymph. ACRI February, 2016.

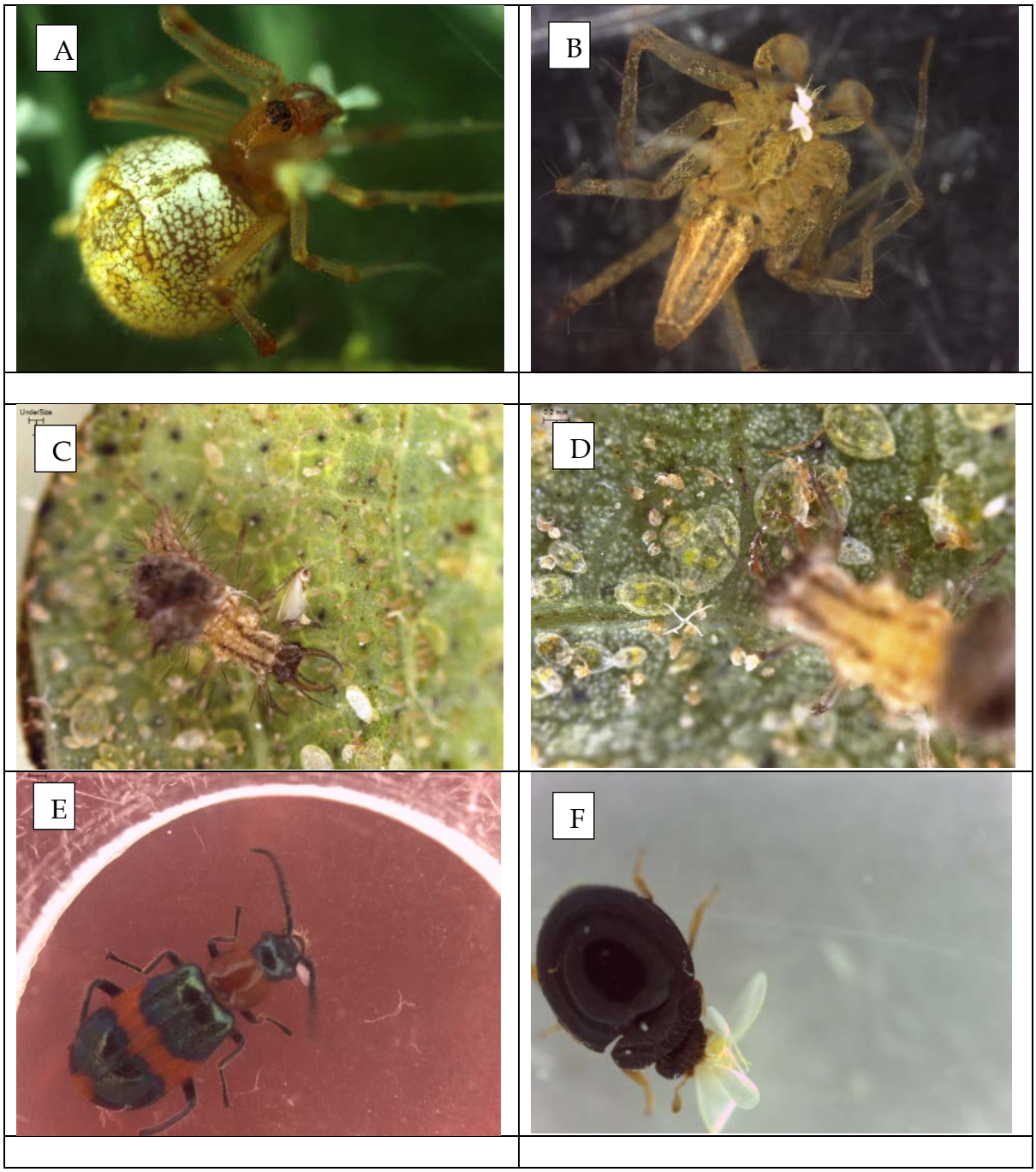

Figure 4. (A) Predation on young SLW adult by Tangle web spider and (B) Lynx spider. Predation on SLW nymph by Green lacewing larvae (C) and (D) close up showing penetration of SLW larvae by Green lacewing larvae with chelicerae. Predation on SLW adult by Red and Blue beetle (E) and Mite-eating ladybeetle (F). ACRI February, 2016.

Correlations between SLW abundance, predator abundance and predation.

Most of the predators identified for SLW are 'generalists' in that they don't specialise on a particular prey type. Ladybeetles often do specialise, for instance some species will feed virtually exclusively on aphids if aphids are abundant. However, when there are few aphids these ladybeetles will tend to feed on a range of prey to stay alive. Predators that are specific on particular prey tend to show a functional response as prey density increases. Usually this is an increase in predator density in response to increasing prey density. However, as most of the predators identified for SLW are generalists we do not expect to see a functional response in predator numbers to SLW abundance. As the beneficial species complex varied in abundance and composition between field and across time there are many gaps in the data where certain species were not present, or present at very low abundance. As a result, apart from Red and blue beetles and Apple dimpling bugs, we grouped beneficials into 'predatory bugs, predatory beetles, spiders and thrips. Red and blue beetles and

Apple dimpling bugs and Red and Blue beetles remained separate because their high abundance swamps other species if combined. Regressing the abundance of these groups against SLW density showed no relationship with total predatory bugs excluding Apple dimpling bug ($F_{1,15} = 0.12$, $P = 0.73$), total predatory beetles excluding Red and blue beetles ($F_{1,15} = 0.47$, $P = 0.50$), Red and blue beetles ($F_{1,15} = 0.19$, $P = 0.67$), total spiders ($F_{1,15} = 0.41$, $P = 0.53$) or Green lacewing larvae ($F_{1,15} = 1.03$, $P = 0.32$). There was a marginally significant positive association for Apple dimpling bugs ($F_{1,15} = 3.99$, $P = 0.06$) but if one unusual site (Edithville in the top left of the Fig 5c) is excluded this becomes highly significant ($F_{1,15} = 16.6$, $P = 0.001$). There was a marginally significant negative non-linear relationship with total thrips ($F_{1,15} = 4.39$, $P = 0.05$).

Analysing the relationship between the % of tested potential predators testing positive and SLW density must be done carefully as the number of individuals actually tested at a particular date strongly influences confidence in the outcome. We excluded dates where less than 4 individuals had been tested. The results showed no significant relationship for total predatory bug excluding Apple dimpling bug ($F_{1,12} = 0.06$, $P = 0.81$), Apple dimpling bug ($F_{1,13} = 0.11$, $P = 0.74$), predatory beetles excluding Red and blue beetle ($F_{1,10} = 3.04$, $P = 0.11$), Red and blue beetle ($F_{1,9} = 1.41$, $P = 0.27$) (Figure 5), Green lacewing larvae ($F_{1,2} = 0.81$, $P = 0.46$), total spiders ($F_{1,15} = 0.16$, $P = 0.69$) or thrips ($F_{1,15} = 0.89$, $P = 0.36$) (Figure 6). These results show that at these densities of SLW there was no obvious increase in consumption rate of predator species as the density of SLW increased from 0 to 16 per leaf (adults and nymphs).

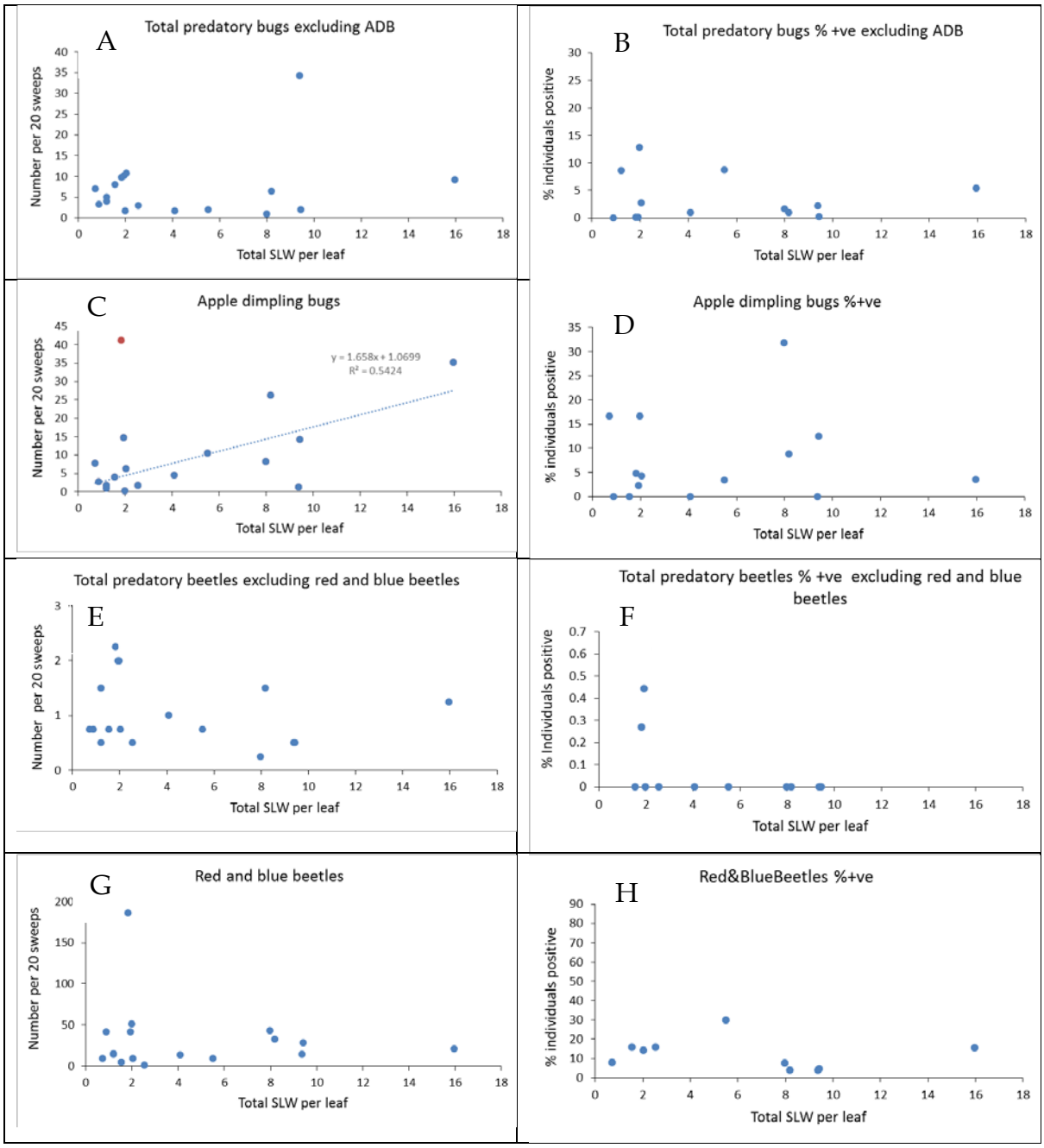

Figure 5. Relationship between predator density and SLW density for (A) predatory bugs (excluding Apple dimpling bugs), (C) Apple dimpling bugs (E) total predatory beetles excluding Red and blue beetles (G) Red and blue beetles and relationship between the % of predators showing positive for SLW DNA and SLW density for (B) predatory bugs (excluding Apple dimpling bugs), (D) Apple dimpling bugs (F) total predatory beetles excluding Red and blue beetles (H) Red and blue beetles.

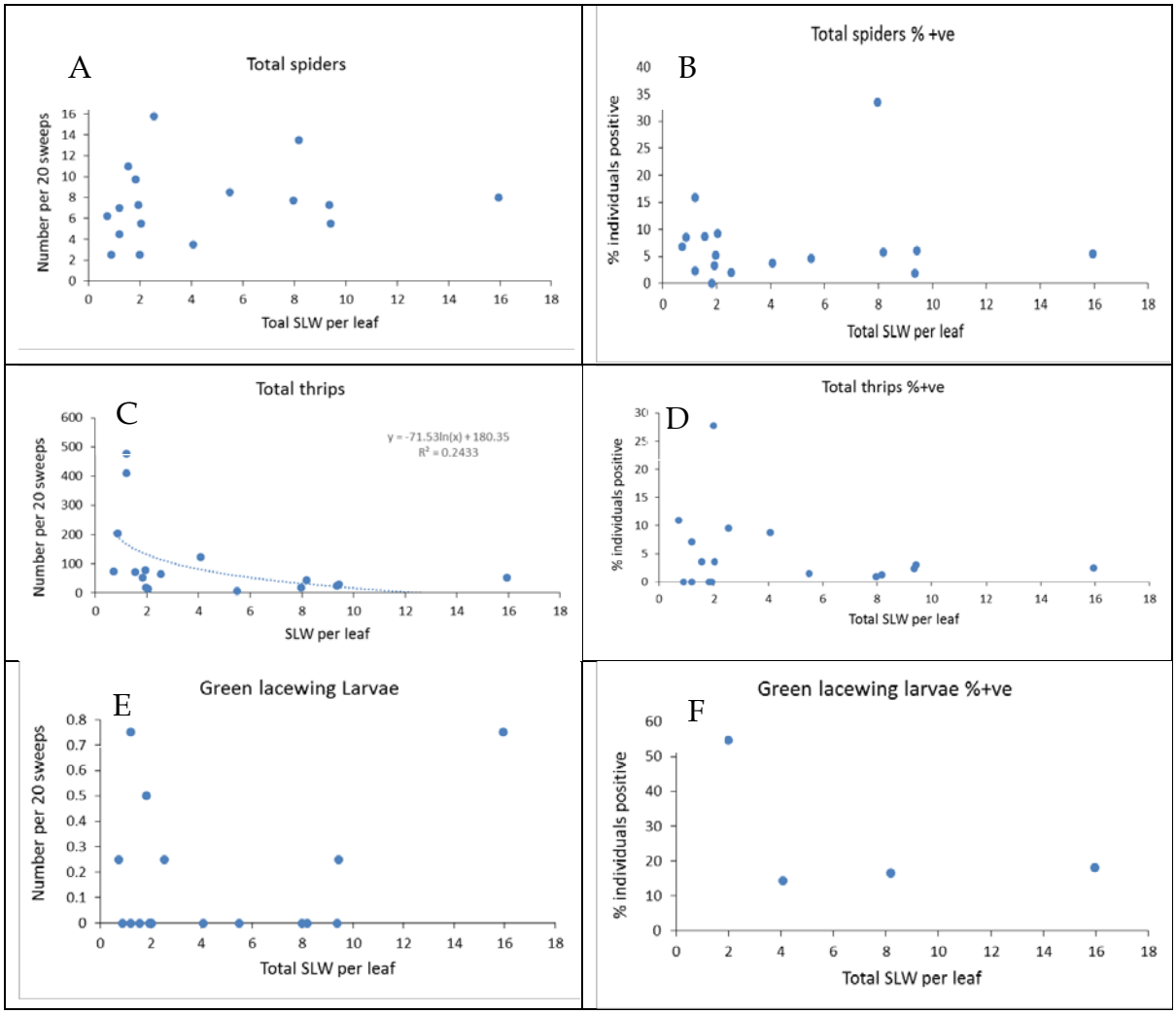

Figure 6. Relationship between predator density and SLW density for (A) total spiders (C) total thrips and (E) Green lacewing larvae and relationship between the % of predators showing positive for SLW DNA and SLW density for (B) total spiders (D) total thrips and (F) Green lacewing larvae.

Outcomes

3. **Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.**
   This project has developed primers for the specific detection of SLW and GVB. The specificity of these primers has been validated against closely related species and against a wide array of other insects and spiders found in cotton crops. Analysis of samples has confirmed that a range of predator species consume SLW. Data for GVB is incomplete due to low abundance in the field over the course of the project. Nevertheless, the outcomes of the project can be used to provide industry with better information about the beneficial species that attack SLW and this can be incorporated both in decisions about the need to spray this pest and in the choice of insecticide used when targeting both SLW and other pests. This information will therefore support implementation of better IPM in cotton crops.

4. **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
Primers were developed that were specific to SLW or GVB DNA.

c) required changes to the Intellectual Property register.
Register has been updated.

Conclusion

5. 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?
This project is one of the first attempts to use a molecular method to identify predators of key cotton pests. Unfortunately due to the low abundance of GVB during the project the results are of limited value for this pest. However, for SLW the project has identified a range of insect and spider species that consume SLW. In terms of key learnings there are several.

(a) The project was probably too ambitious for the time frame. As we didn't know what the predator species would be we had to screen widely to begin with and then narrow down to just a few predator species. This meant that it wasn't really practical to ensure that the primers used were sensitive across the range of predator species. As a result it seems to have performed well in some species but poorly in others. With the benefit of hindsight more time spent in validating the sensitivity of the primers across a wide range of species by feeding potential predators with just a single SLW adult or nymph would have delayed progress, but conversely identified issues with primer sensitivity early on so that the methodology could be modified. It is likely that in this study we have underestimated the predation rates by many predators because the primers were not sensitive enough to detect predation when low numbers of SLW were eaten, particularly in species who suck out the contents of the insect rather than chewing them whole as the latter would leave larger chunks in their guts that would digest more slowly.

(b) The retention studies proved valuable in understanding how the various predator species fed and what the remains of the insects looked like. This information would have been very helpful in earlier SLW life history studies where we followed the survival of SLW eggs and nymphs in the field and attempted to ascribe mortality to either death, predation, parasitism or missing. The studies here confirm that some predators consume the whole nymph, leaving no remains. So a portion of nymphs classified as missing in the life history studies were probably eaten. Similarly, other predators suck the contents from the nymph but leave the 'shell' which may appear to have simply died and dried out, so would be wrongly classified as death. Further, these 'sucked out' shells dislodge more easily from the leaf, often leaving no remains or sometimes a small leaf scar where the nymph had been. These observations help explain the high mortality of SLW nymphs on leaves with open bags compared with leaves with closed bags to exclude predators. The results will be important also in helping consultants and agronomists to better identify healthy versus damaged SLW in the field, and this is important in understanding population trends and predicting the potential need to control this pest.

(c) The project has identified a range of predatory species on SLW. Importantly these are not necessarily those that we would have thought would be important based on experience with other similar pest species such as aphids and mites. These included a range of spiders including lynx, nightstalker, flower, house, jumping and tangle web spiders. Among these the lynx spiders were especially adept at catching adult SLW in the retention studies. A range of predatory bugs especially Brown smudge, Damsel, Big eyed, and Minute pirate bugs. In addition Apple dimpling bugs, which are herbivorous and predatory, were important because they are very abundant. A range of predatory beetles were also identified as predators of SLW, including five species of ladybeetles. Red and blue beetles appear to be important predators because they are so abundant compared to most other insect predators. Green lacewing larvae were also identified. Perhaps the biggest surprise was that adult and larval thrips are predators of SLW. This was unexpected but thrips are important as they can be very abundant on leaves at times, even late in the cotton

season. Consultants have noted that SLW tend to be less abundant on leaves where thrips numbers are high.

(d) The research with GVB was hampered by low abundance throughout the cotton industry during the years of study. Nevertheless, we were able to identify some species positive for GVB DNA including Lynx, Night stalker, House and Tangleweb spiders, Damsel bugs, Striped ladybeetle adults, Red and blue beetles though the proportions scoring positive to GVB DNA were all, not surprisingly, low.

Extension Opportunities

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

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

With the experience we have it would be possible to revisit SLW predation by fine-tuning the PCR assay for greater sensitivity, though there is a risk that this also broadens the range of 'non-target' species that may also cross react with the primers used.

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

The outcomes of this research are valuable to industry. With assistance from the Cotton Info team it would be useful to provide information to industry on the range of predatory species and their mode of feeding. Combined with information on the selectivity of insecticides provided in the Cotton Pest Management Guide this will allow consultants and agronomists to identify if important SLW predators are present, both by counting them and by seeing signs of their impact on SLW nymphs, and to select control options with least negative effects on these predators.

(c) for future research.

New primers could be developed for key pests such a spider mites and especially mirids to better understand predation on these species.

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)

No publications yet. Lists of beneficial species recorded attacking SLW, stages attacked and images of predated eggs and nymphs will be included in the 2026/17 Cotton Pest Management Guide.

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

None developed

Silverleaf whitefly (SLW) and green vegetable bug (GVB) are important pests in the Australian cotton industry. However, the particular natural enemy species involved in suppressing population development of these pests is poorly understood. Such information would be valuable in developing strategies for the conservation of these natural enemies, especially in terms of insecticide selection, management decisions and understanding sources of the beneficials (e.g. other crops, native vegetation).

We used primers specific to either SLW or GVB DNA to identify the presence of SLW or GVB DNA in the gut of predators. Positive results would indicate that predator had consumed either SLW or GVB. We used a pre-existing primer for GVB and developed a new primer for SLW, however, both primers required refining to improve sensitivity. We tested these primers against a wide range of insects and spiders found in cotton crops and confirmed that the primers reacted with the appropriate DNA and did not cross react with that from other species. We also confirmed that the risks of contamination due to the sampling process was low, eg SLW DNA contaminating a sweep net or beatsheet leading to contamination of insects collected from the sheet.

Over two cotton seasons we collected a wide array of potential predator species and tested them for the presence of either SLW or GVB DNA. Twenty-four predator species tested positive for the presence of SLW DNA. These included a range of spiders (Night stalker, Lynx, Orb weavers, Tangle web and Jumping spiders), predatory bugs (Brown smudge bugs, Damsel bugs, Big-eyed bugs, Minute pirate bugs), “facultative” predatory bugs (Green mirids and Apple dimpling bugs), predatory beetles (Red and Blue beetles, 4 lady beetle species), Green lacewing larvae and “phytophagous” thrips adults and larvae (probably mostly *Frankliniella* spp.). GVB abundance was low, limiting the value of results. Nevertheless, a number of predators tested positive for GVB DNA including several spider species (Tangleweb, Lynx, Nightstalker and House spiders), Damsel bugs, Red and Blue beetles and Striped ladybeetle adults.

Detailed observations were made of predatory behaviour and the appearance of SLW nymphs after being attacked by different predators and these observations and images will be useful for identifying nymphs that have suffered predation in the field.

Attempts to correlate the abundance of predator groups with the abundance of SLW showed no significant relationships except for Apple dimpling bug where abundance increased as SLW abundance increase and total thrips (adults and larvae) which declined as SLW abundance increased. There were no significant relationships between SLW abundance and the proportion of any predator group testing positive for SLW DNA. This lack of correlation possible reflects the generalist nature of the predatory species, so they are not necessarily going to respond numerically in abundance to a single prey species.

A ‘predation’ index was calculated by multiplying the abundance of the species at the site by the proportion positive for SLW DNA. Across the two years of study Red and blue beetles, thrips adults and larvae, Brown smudge bugs, Apple dimpling bugs, Big-eyed bugs, Minute pirate bugs and Lynx and Night stalker spiders potentially have the biggest effect on SLW abundance. This study has provided the first step in using molecular techniques to identify beneficial species important for control of SLW for conservation in Australian cotton systems.